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GLASNIK HEMIJSKOG DRUŠTVA, vol. 27, no. 1, pp. 5-8, 1962.

A CONTRIBUTION TO THE COMPLEXOMETRIC DETERMINATION OF METALS*

I. THE DETERMINATION OF COPPER

by

TIBOR A. KIŠ and VELIMIR D. CANIĆ

The complexes of copper (II), cobalt (II), iron (III), and some other metals which contain eriochrome black *T* are more stable than the corresponding complexes with *EDTA;* thus, the elements in question cannot be directly titrated in the presence of the above indicator because the latter is blocked (1).

The reaction of *EDTA*-copper complex with eriochrome black *T*, is rather slow and it is recommended to prevent the blocking of the indicator by rapid back-titration of the excess of *EDTA* (1, 2). On the basis of our experience, we came to the conclusion that the recommended method can be used only for the determination of small concentrations of copper with a rather great margin of error.

However, *Lucchesi* and *Hirn* have observed that the reaction of *EDTA*-cobalt complex (3) and iron (III) complex (4) with eriochrome black *T*, is retarded by the addition of some organic solvents, so that the excess of *EDTA* can be back-titrated with great accuracy. Our aim was to discover whether organic solvents exhibit a similar influence on the determination of copper, and whether higher copper concentrations could be thus determined with satisfactory accuracy. Therefore, we studied the effect of various amounts of ethanol and other organic solvents on the prevention of eriochrome black *T* blocking when the excess of *EDTA* is back-titrated in copper determination.

Some results obtained in the presence of ethanol are given in Table 1.

At the same copper concentration, the change of the color was more sharp parallel to the height of the concentration of ethanol. The 70% concentration of ethanol inhibited the blocking of the indicator to the same extent in the

* Communication delivered at the IXth Symposium of Chemists from PR Serbia, Belgrade, January 1961.

TABLE 1

Taken mg copper	Found mg copper	Error %	% Ethanol in the solution
6.68	6.72	+0.60	
	6.59	−1.35	
13.36	13.45	+0.67	30
	13.49	+0.97	
33.39	33.33	−0.18	
	33.42	+0.09	
	3.39		
3.34	3.41	+1.50	
	33.52	+2.10	
33.39		+0.39	50
	33.45	+0.18	
160.20	160.60	+0.25	
	160.40	+0.12	
3.34	3.37	+0.90	
	3.36	+0.60	
66.77	67.03	+0.39	70
	67.16	+0.58	
200.32	200.53	−0.15	
	200.02	+0.10	

presence of low and high copper concentrations. The sharpness of the transition of the color, at the above concentration of ethanol, was more pronounced in the presence of high copper concentration.

The blocking of the indicator in the presence of 70% concentration of ethanol was so effective that the titration could be performed after a period of twenty four hours, and the titration could be performed very slowly, near the equivalence-point, waiting five to six seconds after the addition of each drop. The indicator was not blocked, even after two minutes, and not even when only 0.5% *EDTA* excess remained so that the excess of zinc chloride in the overrun solution could also be slowly back-titrated with *EDTA*.

In the determination of iron, Lucchesi and Hirn (4) have prevented the blocking of eriochrome black T by the addition of various organic solvents which have small, medium, and great dielectric constants. The added solvents were: methanol, n- and iso-propanol, ethylene glycol, dioxan, acetonitrile, acetone, and formamide. We have established that a positive effect in the copper determination was obtained only with methanol, ethanol, and n-propanol, and isopropanol and ethylene glycol exhibited only a partial effect. Acetone, dioxan, and formamide had almost no influence on the blocking of the indicator (table 2). In experiments performed with the same concentration of copper and the same amount of various solvents, the change of the color was slightly sharper in the presence of methanol than with ethanol.

TABLE 2

Copper mg taken	Copper mg found	Error %	In the presence of 50 %
33.39	33.32	−0.21	methanol
	33.42	+0.09	Methanol
33.39	33.45	+0.18	*n* - propanol
	33.51	+0.36	*n* - Propanol
33.39	34.53	+3.41	isopropanol
	34.22	−2.49	Isopropanol
33.39	34.85	+4.37	ethyleneglycol
	34.60	+3.62	Ethyleneglycol

Experimental Part

Reagents used

Copper sulfate-pentahydrate p.t. "Merck"; zinc p.a. "Merck"; EDTA (disodium ethylenediaminetetracetate dihydrate) p.a. "Merck"; ammonia p.a. "Kemika" 25—27%; ammonium chloride p.a. "Kemika"; ethanol p.a. "Kemika", 96%; methanol p.a. "Reanal"; ispopropanol "Kemika"; n-propanol puriss. "Kemika"; acetone p.a. "Kemika"; ethylene glycol p.a. "Reanal"; formamide p.a. "Kemika"; eriochrome black T p.a. "Merck"; sodium chloride p.a. "Kemika".

Preparation of solutions

0.1 M EDTA solution. 37.225 g *EDTA* was dissolved in distilled water and diluted to one liter in a volumetric flask.

0.1 M Standard zinc chloride solution. Elementary zinc was used as the primary standard for the preparation of a standard solution of zinc chloride, and for the standardization of the *EDTA* solution. Zinc chloride solution was prepared from reagent grade zinc according to the procedure given by *Flaschka* (5). The oxide layer from the zinc surface was removed by dissolution in hydrochloric acid; and then the zinc was washed with distilled water, alcohol, and ether; 6.5380 g of purified zinc was dissolved in a slight excess of hydrochloric acid, and the solution was diluted to one liter. The solution was controlled by preparing several zinc chloride solutions by the same procedure, and titrating each with the same *EDTA* solution in the presence of eriochrome black *T*.

0.1 M Copper sulfate solution. About 25 g of copper sulfate pentahydrate were dissolved in water, a small amount of concentrated sulfuric acid was added, and the solution was diluted to one liter in a volumetric flask.

0.05 *M*, 0.01 *M*, and 0.005 *M* solutions of *EDTA*, zinc chloride, and copper sulfate were prepared by dilution of the corresponding 0.1 *M* solutions.

Buffer solution, pH 10. 70 g ammonium chloride was dissolved in 570 g concentrated ammonia (0.09) and diluted to one liter.

Eriochrome black T was mixed with dry powdered sodium chloride in a ratio of 1:100, and used as such.

0.1% *Ethanolic solution of eriochrome black T.* 0.1 *g* eriochrome black *T* was suspended in 100 *ml* 96% ethanol, one drop of buffer *pH* 10 was added, and all was dissolved by shaking. A fresh solution was prepared each time.

Standardization of solutions

0.1 *M* Copper sulfate solution was standardized electrogravimetrically by determining copper from a solution acidified with nitric and sulfuric acid and by rapid electrolysis with *Fischer's* electrodes (6).

0.1 M *EDTA* solution was standardized by complexometric titration of the standard zinc chloride solution at *pH* 10 in the presence of eriochrome black *T* (5).

Burettes, pipettes, and volumetric flasks were calibrated in the usual manner.

Apparatus

The following apparatus were used: *pH*-meter "Radiometer", type PHM 22 *p*, magnetic stirrer, 50 to 1000 turns a minute, made by Friedrich Geyer K.-G., type R 50, and electrolysis apparatus Laboratorni pristroje, type 278, Prague.

Procedure

A slightly acid copper solution containing as much as 200 *mg* copper is placed in a 600 *ml* beaker; then a measured volume of the standard *EDTA* solution of a corresponding concentration in excess, 10 *ml* buffer *pH* 10, and ethanol are added; ethanol is added gradually with constant stirring of the reaction mixture in such an amount that its concentration, at the end of the titration, is 70%. This is followed by the addition of the ethanolic solution of eriochrome black *T* until the appearance of a dark blue color. If a large amount of copper is present, the reaction mixture is dark blue due to the presence of the blue copper — *EDTA* complex; in such instances, the indicator is added until a very dark blue color is obtained. The reaction mixture is then titrated with constant stirring (magnetic stirrer, 100 turns in minute) with the standard zinc chloride solution, the concentration of which corresponds to that of the *EDTA* solution, using a bright background (fluorescent tube); the latter is required, especially in cases of high copper concentrations. The color of the solution changes from blue to red. At higher copper concentrations, the solution is titrated until the occurrence of a sudden transition to a blue-violet color.

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THE DETERMINATION OF MICRO AMOUNTS OF ELEMENTS BY CUPRIC FERROCYANIDE RING COLORIMETRY. I.

THE DETERMINATION OF URANIUM, MANGANESE, CADMIUM, COBALT, AND MERCURY (MERCUROUS AND MERCURIS IONS).

by

T. J. JANJIĆ, M. B. ĆELAP, and S. MARJANOVIĆ

The Weisz ring oven* (1), used in spot colorimetry, has been applied for the determination of a great number of elements. These determinations have been performed mostly by using a standard scale of silver sulfide (2, 3, 4). However, this scale can not be applied in the determination of some elements, such as uranium and mercury. Therefore, we studied the possibility of applying a standard cupric ferrocyanide scale** in the determination of micro amounts of elements. We established that uranium, manganese, and cadmium could be determined by a common standard scale, prepared from standard cupric sulfate solution, but the determination of cobalt and mercury (mercurous and mercuric ions) required separate cupric ferrocyanide scales made from standard solutions of corresponding ions.

The elements in question were determined by washing their salts into ring zones on the ring oven;; the rings were then dipped into a potassium ferrocyanide solution and were converted into corresponding ferrocyanides. The excess of the reagent was washed with water, and the filter paper was bathed in a cupric sulfate solution, whereby the ferrocyanides were converted into brown cupric ferrocyanide. For example:

$$2\,MnSO_4 + K_4[Fe(CN)_6] = Mn_2[Fe(CN)_6] + 2K_2SO_4$$

$$Mn_2[Fe(CN)_6] + 2CuSO_4 = Cu_2[Fe(CN_6] + 2MnSO_4.$$

The obtained rings were compared with the standard scale. The standard scale in the determination of uranium, manganese, and cadmium was prepared by washing various amounts of cupric sulfate into ring zones which were converted into cupric ferrocyanide rings by means of a potassium ferrocyanide solution.

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* Ringofen (in Germain), prstenasta peć (in Serbian).

** Ferric ferrocyanide scale was used for the determination of iron by H. Weisz; *Mikrochimica. Acta*, 1954, 785.

In the determination of cobalt and mercury (mercurous and mercuric ions), the corresponding standard scales were prepared by washing known concentrations of these ions into ring zones on the ring oven, and the obtained rings were converted into cupric ferrocyanide in the manner analogous to the preceding one.

The results were calculated in the usual way; factors used for uranium, manganese, and cadmium are as follows:

$$\frac{U}{Cu}=3.745; \quad \frac{Mn}{Cu}=0.865; \quad \frac{Cd}{Cu}=1.769.$$

The results obtained from nine successive determinations are given in Table 1. The obtained values deviate, on an

TABLE 1

Concentration (mg/ml)		Concentration (mg/ml)	
Calculated Uranium	Found Uranium	Calculated Cobalt	Found Cobalt
0.383	0.386	0.091	0.093
0.383	0.404	0.091	0.089
0.383	0.396	0.091	0.095
0.437	0.449	0.096	0.097
0.437	0.448	0.096	0.100
0.437	0.456	0.096	0.100
0.474	0.479	0.097	0.097
0.474	0.486	0.097	0.098
0.474	0.490	0.097	0.093
Manganese	Manganese	Mercury (Hg_2^{++})	Mercury (Hg_2^{++})
0.100	0.104	0.310	0.303
0.100	0.095	0.310	0.306
0.100	0.104	0.310	0.297
0.133	0.130	0.476	0.470
0.133	0.129	0.466	0.451
0.133	0.138	0.476	0.454
0.139	0.139	0.438	0.442
0.139	0.137	0.438	0.432
0.139	0.146	0.438	0.419
Cadmium	Cadmium	Mercury (Hg^{++})	Mercury (Hg^{++})
0.218	0.228	0.312	0.303
0.218	0.226	0.312	0.306
0.218	0.221	0.312	0.297
0.153	0.157	0.351	0.347
0.153	0.157	0.351	0.334
0.153	0.154	0.351	0.347
0.164	0.173	0.328	0.340
0.164	0.171	0.328	0.325
0.164	0.171	0.328	0.316

average, from those calculated as follows: uranium 2.9%, manganese 3.1%, cadmium 3.3%, cobalt 2.6%, univalent mercury 2.9%, and bivalent mercury 2.8%. The amounts of individual ions were about 10 γ.

It is evident from this that the results are within the limits of average error obtained in the determination of micro amounts of elements by other methods which are usually more complicated in regard to the apparatus and procedure.

The possibility of determining other elements by the use of a standard cupric ferrocyanide scale is under investigation.

Experimental Part

The solutions of investigated elements were prepared from the following salts: uranyl chloride, cadmium sulfate, cobaltous sulfate, mercurous nitrate, and mercuric nitrate. The volume of the capillary pipette was 2.6 μl. The salts were washed on filter paper, Schleicher & Schüll 589, Ø 5.5 cm (2) with hydrochloric acid (0.01N), except in cases of monovalent mercury, when nitric acid (0.05N) was used.

The concentration of the potassium ferrocyanide solution and that of cupric sulfate was 2 per cent; the filter paper was bathed in these solution for five minutes. The cupric sulfate solution used for the preparation of the standard scale contained 0.1 mg Cu/ml; the rings were made with 1, 2, 4, 6, 8, and 10 drops. In the determination of cobalt and mercury, the corresponding standard scales were prepared from the solutions of the salts of these elements, the concentration of which was equivalent to the concentration of copper in the cupric sulfate solution. The rings obtained on the filter paper were dried in an oven at 105°C. The standard cupric ferrocyanide scale was stable on a long period of standing.

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GLASNIK HEMIJSKOG DRUŠTVA, vol. 27, no. 1, pp. 13-21, 1962.

SOME NEW DERIVATIVES OF ISATIC ACID

by

DJ. STEFANOVIĆ, LJ. LORENC and M. MILJKOVIĆ

It has long been known that the action of aqueous alkalies on isatine ruptures its lactam ring, thus giving rise to alkaline salts of isatic acid (o-aminophenylglyoxylic acid) (1, 2, 3).

In the investigation of this reaction, *Dj. Stefanović* and his associates have established that two kinds of salts can be obtained in this way. The addition of an equivalent amount of 20% potassium hydroxide at temperatures below 40° exclusively produces yellow potassium salt of isatic acid; and the treatment of isatin with an excess of 40% aqueous potassium hydroxide under reflux, and the evaporation of the reaction mixture to dryness, all results in the formation of white potassium salt of anhydrobiisatic acid in an almost quantitative yield.

The isatic acid has not yet been isolated because of its instability, although contrary data can be found in the literature. In one of his papers, *O. L. Erdmann* (1) has reported that he succeeded in isolating the isatic acid by treating its lead salt with hydrogen sulfide. However, the analysis of the silver isatate, cited in this paper, indicates that the isolated compound was not the isatic acid but the anhydrobiisatic acid.

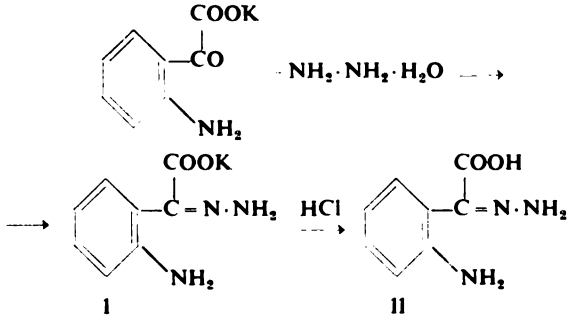
In contrast to the isatic acid, its alkaline salts are stable in aqueous and alcoholic solutions; they have been used for the syntheses of many important heterocyclic compounds. *Pfitzinger* has synthesized an entire series of substituted cinchoninic acids by heating the isatic acid with diketones in a strong alkaline solution (5, 6, 7, 8). The *Pfitzinger* reaction was subsequently applied in the syntheses of not only various quinolinic derivatives, but also of polynuclear compounds which contain nitrogen as a member of the ring (9,10).

In the study of the condensation reactions of isatic acid, we treated the dry potassium isatate with diketones, ureas, urethan, and guanidine, and have obtained various substituted cinchoninic acids and quinazoline derivatives (11, 12).

This paper covers further investigations of the chemical behavior of isatic acid, in which we have prepared some new

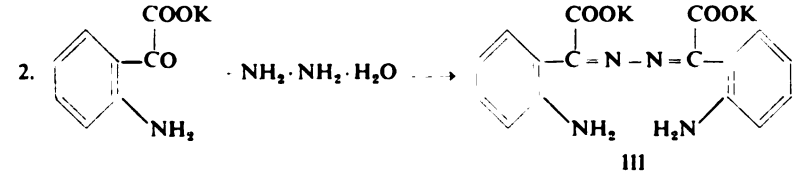
functional derivatives of isatic acid which have not yet been reported; these derivatives are intended to be applied in the syntheses of new heterocyclic compounds. All reactions described here have been performed with dry potassium isatate.

By heating the potassium salt of isatic acid with hydrazine hydrate, we obtained the hydrazone of potassium isatate which, on cold hydrolysis by means of dilute hydrochloric acid, furnished the hydrazone of isatic acid:



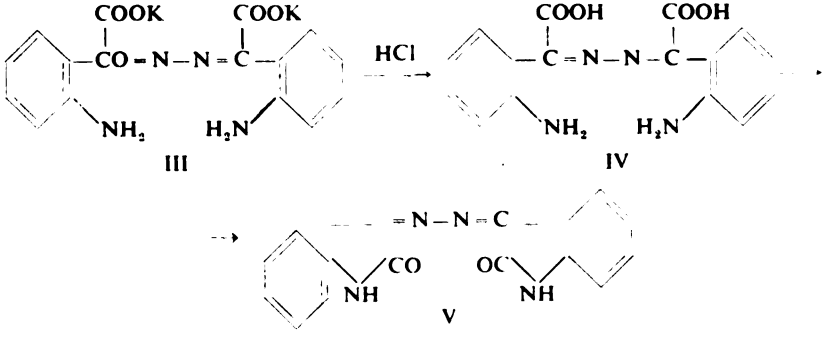
I II

However, with an excess of hydrazine hydrate we simultaneously obtained, in addition to the above hydrazone, the azine of potassium isatate according to the following scheme:



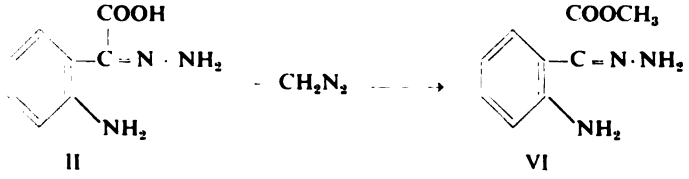
III

The azine of potassium isatate has been isolated in the form of intensely colored yellow crystals which, upon treatment with cold dilute hydrochloric acid, gave the azine of isatic acid. The latter contained two molecules of crystalline water and was identical with the azine described by *W. Borsche* and *R. Meyer* (13); it was converted into the diisatinazine upon heating:



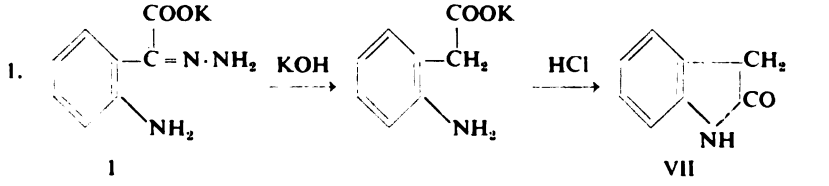
III IV V

The hydrazone of isatic acid can be esterfied by the action of diazomethane which gives rise to the hydrazone of methyl isatate:

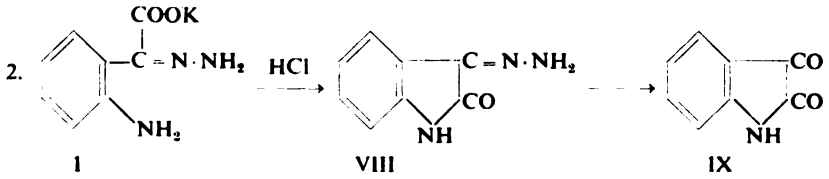


II VI

The alkaline hydrolysis of the hydrazone of potassium isatate afforded oxindole, but the acid hydrolysis by means of dilute hydrochloric acid first gave the hydrazone of isatin, and this was followed by the formation of the isatin itself:

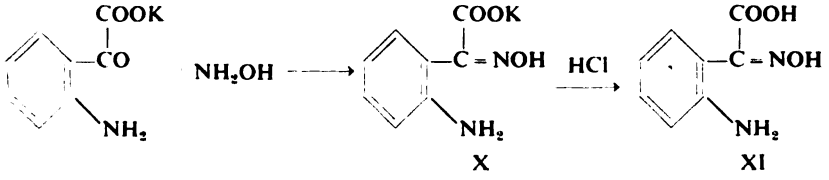


I VII



I VIII IX

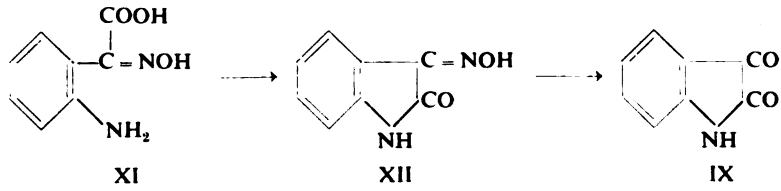
Refluxation of the potassium isatate with the alcoholic solution of hydroxylamine gave the corresponding oxime which, upon acidification with cold dilute hydrocloric acid, yielded the white oxime of isatic acid:



X XI

Although *Pfitzinger* (7) mentioned the existence of this oxime, claiming that it was formed as a byproduct in the condensation of isatic acid with acetaldoxime, it has not yet been isolated.

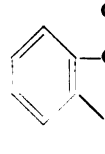
Similar to the corresponding hydrazone, the oxime of isatic acid, when heated with hydrochloric acid, first gave the oxime of isatin and then the isatin itself:



XI XII IX

Thirdly, we prepared the phenylhydrazone of the potassium salt of isatic acid by heating the alcoholic solution of the potassium isatate with phenylhydrazine in a water bath. The yield amounted to about 80%. However, when the potassium isatate was heated with phenylhydrazine at 150° in the absence of a solvent, no phenylhydrazone was obtained, but the isatic acid was decarboxylized and yielded an oxygen-free solid of pale yellow color which melted at 222 to 225°. The structure of this compound and its reaction will be discussed in a following paper.

Finally, we condensed the hydrazone of the potassium isatate with ethyl acetoacetate in two ways: at ordinary and at elevated temperature. In the former, we established that the carbonyl group of ethyl acetoacetate simultaneously reacts with the amino and the hydrazino group according to the following scheme:



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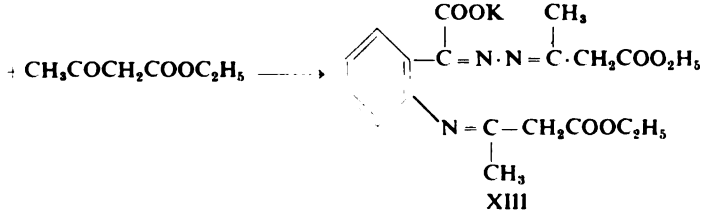
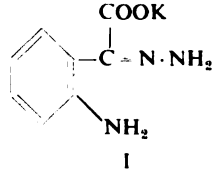


XIII

However, when this condensation is performed at an elevated temperature, it is very likely that the hydrazone of the potassium salt of 2-hydroxy-4-methyl-quinoline-8 (α)-ketocarboxylic acid will form; upon treatment with cold hydrochloric acid, this compound is easily hydrolyzed, and gives rise to the free α-ketoacid, as follows:



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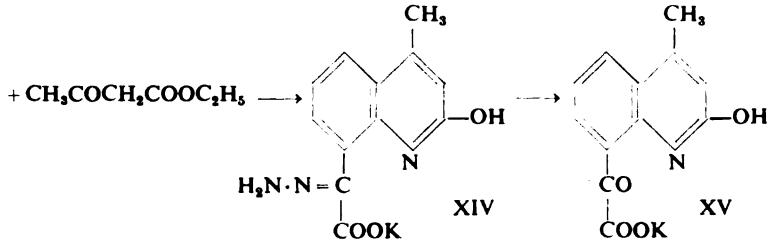

The structure of the obtained acid could be assumed on the basis of the following reactions: the presence of the carboxylic group was proved by the dissolution of the substance in saturated sodium bicarbonate solution with an evolution of carbon dioxide; and by esterification with anhydrous methanol in the presence of gaseous hydrogen chloride from which the corresponding methyl ester was obtained; the presence of the hydroxyl group was proved by the action of thionyl chloride on the methyl ester, from which the corresponding chloro derivative was obtained. The location of the hydroxyl group in the position 2, and not 4, was deduced from the high melting point (above 280°) which indicates a lactam bond, and also from the fact that the substance in question gave no colored reaction with ferric chloride. Finally, the carbonyl group was detected with 2, 4-dinitrophenylhydrazine. The molecular weight, calculated from analytical data and the determination of the equivalent weight, support the proposed structure.

Experimental Part

Hydrazone of potassium isatate (I) A mixture of 101.5 g (0.5 mole) potassium isatate and 71.4 g (1 mole) hydrazine hydrate was heated for one hour, with occasional stirring, at 120°. The solid obtained was washed with anhydrous ethanol and purified by crystallization in the following way: the washed substance was dissolved in the minimal volume of boiling water, and a ten-fold volume of boiling ethanol was added to the filtrate. On cooling, the white crystals of the hydrazone of potassium isatate separated. The yield was 70—75% (65—76 g).

Analysis	$C_9H_8N_3O_2K$	%N
Calculated		19.35
Found		19.54

Hydrazone of isatic acid (II). 4.6 g of purified hydrazone I was dissolved in 8 ml water, the solution was cooled to 5° and acidified with hydrochloric acid until slightly acid to kongo to give 3 g white solid precipitate (yield 79%), m.p. 218 to 219°. The obtained precipitate was washed with cold water until the filtrate did not give a positive chloride test.

Analysis:	$C_9H_9N_3O_2$		
	%C	%H	%N
Calculated	53.63	5.02	23.46
Found	53.48	5.09	23.33

Methyl ester of hydrazone of isatic acid (VI). — A suspension of 5 g hydrazone of isatic acid in 50 ml anhydrous ether was treated with an excess of ethereal solution of diazomethane. When the reaction was finished, the insoluble solid was filtered off giving 2.8 g methyl ester (53.9%), m.p. 106—108°. This was purified by crystallization from anhydrous methanol.

Analysis:	$C_9H_{11}N_3O_2$		
	%C	%H	%N
Calculated	55.95	5.69	21.76
Found	55.65	5.67	22.08

Azine of potassium isatate (III). A tenfold volume of ethanol was added to the filtrate obtained after the purification of hydrazine of potassium isatate; after several days, the intensely colored yellow azine of potassium isatate separated. This is a crystalline substance which is easily soluble in water; recrystallized from ethanol.

Analysis:	$C_{16}H_{12}N_4O_4K_2$
	%N
Calculated	13.90
Found	13.99

Azine of isatic acid (IV). The solution obtained by dissolving 2 g azine of potassium isatate in 15 ml water was acidified with hydrochloric acid. The precipitated acid was recrystallized from ethanol.

Analysis:	$C_{16}H_{14}N_4O_4.2H_2O$		
	%C	%H	%N
Calculated	53.03	5.01	15.46
Found	53.46	4.98	15.37

Diisatineazine (V). To the solution of 2.0 g azine of potassium isatate in 20 ml water, 10 ml hydrochloric acid (10) was added, and the reaction mixture was boiled for four hours. The intensely colored crystalline product thus obtained was purified by recrystallization from ethanol.

Analysis:	$C_{16}H_{10}N_4O_2$		
	%C	%H	%N
Calculated	66.20	3.47	19.30
Found	65.91	3.48	19.22

Oxime of potassium isatate (VII). The solution of 2.6 g (0.0375 mole) hydroxylamine hydrochloride in absolute ethanol was treated with an equivalent amount of alcoholic sodium methoxide solution, and the precipitated sodium chloride was filtered off; 5.1 g (0.025 mole) potassium isatate was added to the obtained solution, and the reaction mixture was heated to boiling for four hours. The alcohol was removed under reduced pressure, and the residue was purified by dissolving it in a minimal amount of boiling water and precipitating with a tenfold volume of boiling ethanol to give 4.5 g (82.5%) of a white crystalline substance.

Analysis	$C_8H_7N_2O_3K$
	%N
Calculated	12.84
Found	12.75

Oxime of isatic acid (VIII). An equivalent amount of hydrochloric acid was added to a solution of 1.0 g oxime of potassium isatate in 10 ml water cooled to 5°. The precipitated acid was filtered off and washed with cold water until the filtrate did not give a positive chloride rest. The yield was 81.0% (0.74 g), m.p. 185°.

Analysis:	$C_8H_6N_2O_3.H_2O$		
	%C	%H	%N
Calculated	48.48	5.05	14.14
Found	48.59	4.87	14.36

Condensation of hydrazone of potassium isatate with ethyl acetoacetate

A. At ordinary temperature (IX). A mixture of 22.0 g (0.1 mole) hydrazone of potassim isatate and 38.0 ml (0.3 mole) ethyl acetoacetate was allowed to stand at ordinary temperature for fifteen days with occasional shaking. The obtained product was washed with anhydrous ether to give 39.6 g (88.7%) of pale yellow crystalline solid (IX), which was further purified by recrystallization from ethanol.

Analysis:	$C_{20}H_{24}N_3O_6K$		
	%C	%H	%N
Calculated	54.49	5.45	9.53
Found	54.54	5.59	9.61

B. At elevated temperature (X, XI). A mixture of 21.7 g (0.1 mole) hydrazone of potassim isatate and 63.5 g (0.5 mole) ethyl acetoacetate was heated for one hour at 150° with occasional shaking. The obtained dark-brown solid was washed first with anhydrous ether in order to remove the excess ethyl acetoacetate, and then it was washed with anhydrous ethanol to give 17.3 g (61.2) pale-yellow colored potassium salt (X). The latter was dissolved in cold water, and the solution was filtered and acidified with hydrochloric acid. The precipitated acid (XI) (14.0 g) was purified by ester formation as described below. The pure acid melted at a temperature above 280°.

Analysis:	$C_{12}H_9NO_4$		
	%C	%H	%N
Calculated	62.40	3.90	6.07
Found	62.48	3.91	6.10

Esterification of 2-hydroxy-4-methyl-quinoline-8 (α)-ketocarboxylic acid (XI). Gaseous hydrogen chloride was passed through a suspension of 10.0 g acid (XI) in 75.0 ml anhydrous methanol; when the solution was saturated, the excess of hydrogen chloride and methanol was removed under reduced pressure. The obtained ester was purified by crystallization from anhydrous methanol with an addition of a small amount of active coal. The melting point of the pure ester was 234°.

Analysis:	$C_{13}H_{11}NO_4$		
	%C	%H	%N
Calculated	63.75	4.49	5.72
Found	63.88	4.49	5.81

Methyl ester of 2-chloro-4-methyl-quinoline-8 (α)-ketocarboxylic acid. A mixture of 2.5 g (0.01 mole) methyl 2-hydroxy-4-methyl-quinoline-8 (α)-carboxylate and 7.3 ml (0.1 mole) freshly distilled thionyl chloride was heated in a water bath until the reaction was finished. The excess

of thionyl chloride was removed by distillation under reduced pressure, and the solid residue was treated with water so that the dark red color of the reaction mixture was turned into an intense yellow. The yield was quantitative. The yellow colored chloro derivative was purified by crystallization from anhydrous methanol, m.p. above 150°.

Analysis:	$C_{13}H_{10}NO_3Cl$		
	%C	%H	%N
Calculated	59.35	3.80	5.33
Found	59.18	3.67	5.81

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THE CHEMICAL COMPOSITION OF A NITROGEN-FREE ORGANIC SUBSTANCE FOUND IN WALNUT FRUIT DURING CERTAIN PHASES OF THE VEGETATION PERIOD

by

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Introduction

The development of a plant is the result of the action of internal and external factors. The internal factors cause morphological and physiological changes in the plant organism, and their intensity depends upon external conditions, i.e., temperature, humidity; light, soil, etc. The cycle of living plant activity from the embryo to its physiological maturity and age thus comes into being; in the course of activity, the plant organism is exposed to numerous transformations which mainly manifest themselves in the successive appearance and disappearance of its various organs. Each organ of a plant passes through the corresponding phases of its development, which differ from each other in their morphological properties.

Numerous biochemical reactions occur in the plant during its development; they give rise to compounds which are indispensable to the building of various tissues, and for the maintenance of cell activity and metabolism, and their surplus is used as a reserve. It is known that the presence of individual compounds is characteristic of certain species which possess a hereditary character. Certain plants contain the same alkaloids, glycosides, etc., from generation to generation.

The question arises of whether it is possible to characterize the phases of the growth and maturation of certain plant organs on the basis of the presence of specific chemical compounds in individual plants in the course of their development. In other words, it is necessary to establish the changes in the chemical composition of the plant, i.e., its organs during the course of plant development. Reliable criteria for the establishment of morphological changes on the basis of chemical compounds present in the phase concerned have not yet been found. However, we are of the opinion that the chemical composition of the plant is closely related to the phases of its development, and that the morphological

criteria for the establishment of individual phases of the plant development should be extended with the biochemical criteria.

Starting from these conceptions, we have undertaken the chemical analysis of vegetative and reproducible plant organs of the walnut *Juglans regia L.* in the course of six successive vegetation periods. In order to study the chemical composition of the nitrogen-free organic substance of these organs, we determined the content of water, ascorbic acid, dehydroascorbic acid, reductones, strong reducing substances, sugars, pentosans, and cellulose, in each of the organs in question from the time of their earliest stage of development until their full formation.

The purpose of this work is that the study of chemical compounds in individual plant organs in the course of the vegetation period should yield more details about the type of compounds whose presence is characteristic of certain phases of organic development.

Experimental part

Material. Fruits for analyses were taken from one tree, from the same branches, and at the same time of day. In order to minimize errors caused by the variations of individual samples, analyses were performed with material of ten to twenty individual samples.

Extraction. The extraction of ascorbic acid, dehydroascorbic acid, reductones, and strong reducing substances was performed with a 3% trichloroacetic acid solution. The extraction was performed just after the collection of the fruits. Sugar was extracted with distilled water at 70—80°C. The material was homogenized with sodium chloride and treated with 12% hydrochloric acid for the determination of pentosans.

Methods. Ascorbic acid and reductones were determined by Tillmans' method (1), modified by *Levy* (2). *Emmerie-Eckelen's* method (3) was used in the determination of dehydroascorbic acid. Strong reducing substances were determined titrimetrically with 0.1 N $KMnO_4$ at 0°. Sugar was determined by *Bertrand's* method (4), crude cellulose by *Scharrer-Kürschner's* method (5), and pentosans were determined according to Tollens (6).

The content of these compounds was determined in each part of the walnut fruit during the course of the entire vegetation period. In order to obtain a clear insight into the variations of the content of these compounds from the initial to the final phase of the fruit development, we have performed these analyses from year to year, and at the same time of the vegetation period.

Results. The results obtained are given in the tables; all values refer to dry substance. Bearing in mind that the results refer to six vegetation periods, we have denoted the time the analyses were performed in weeks, not in dates; the time is designated by Arabic numerals, and the corresponding month is given in Roman numerals. In cases when the content of individual compounds ranged within limited values, the latter were represented with one number. Therefore, the tables contain such denotations as VII_4 and $VIII_{1,2}$. The tables contain the mean values of the content of individual compounds found in the corresponding time unit (a week).

Walnut fruit. The walnut fruit, from the beginning of its development until its fall, passes through several periods which are characteristic of all fruits in general.

In the *first phase* of the growing period, we investigated the whole fruit because its individual parts could not be dissected. This phase of the fruit development comprises the time period from the second week of May until the second week of June. There is an increase in the fruit weight from 0.2 g to 2.2 g during this period.

In the *second phase* of the growing period (up to the middle of July) the fruit reaches its maximal weight, an average of 320 g, and its volume is cca 29 cm^3. Two stages can be differentiated in this phase: the stage of endocarp differentiation (up to the fourth week of June), and the stage of endocarp hardening (up to the middle of July). In the stage of endocarp differentiation and in the initial part of its hardening stage, the fruit can be dissected into the succulent part, endocarp, developed endocarp, and the seed. In the course of further fruit development, i.e., in the stage of its hardening, the seed can be dissected into the kernel and the peel. We have investigated the above parts of the fruit separately from this stage until the end of the vegetation period.

These two phases of the growing period are followed by the phase of fruit maturation, in which we have continued our investigations until the fall of the succulent part of the walnut.

The entire fruit. The investigations were performed with the entire fruit, which weighed from 0.2 to 2.2 g and had a volume of 0.4 to 3.0 cm^3.

It is evident from Table 1 that the increase of the fruit weight results in an increase of the content of water, endiol compounds, strong reducing substances, pentosans, and reducing sugar, but the content of non-reducing sugar remains almost unchanged. These data show that in the vitamin C system (ascorbic acid and dehydroascorbic acid) one of its components, dehydroascorbic acid, is lacking in certain phases of the fruit development.

WALNUT FRUIT

TABLE 1

Months (Weeks)	Fruit development			Weight g	Volume cm^3	Investigated organ
$V_{2,3}$	Growing phase	initial		0.2—0,7	0.4—0.8	The entire fruit
V_4VI_1				1.9—2,2	2.0—3.0	
$VI_{1,2}$			Stadium of endocarp differentiation	4.2—9.1	4.0—8.5	1. Succulent part
VI_3				—16.0	—17.4	2. Endocarp
VI_4		intense growth	Stadium of Endocarp hardening	—23.4	—24.0	3. Developed endocarp
$VII_{1,2}$				27.1—32.0	27.5—29.0	4. Seed

(Cont.)

Months (Weeks)	Fruit development		Weight g	Volume cm³	Investigated organ
$VII_{3,4}$	Maturation phase		30.6—30.1	28.0—28.0	1. Succulent part of the fruit
$VIII_{1,2}$			25.8—25.8	26.0—26.0	2. Endocarp
$VIII_{3,4}$			25.3—25.1	25.0—25.0	3. Developed endocarp
$IX_{1,2}$			26.2—25.9	27.0—26.0	4. Peel
IX_3			23.9- 24.2	24.0—23.0	5. Kernel
$IX_4X_{1,2}$		Final phase	21.8—19.9		

Note: Months are denoted by roman figures and weeks by arabic ones.

The succulent part of the fruit. This part of the fruit was subjected to numerous investigations (7, 8, 9, 10, 11, 12, 13, 14, 15, and 16).

The succulent part of the fruit is characterized by several different morphological forms. We did not have the opportunity to differentiate all these forms, so we have analyzed this part of the fruit as a whole. The succulent part was analyzed from the beginning of June (green colored) when the endocarp could be dissected, until the end of September and the beginning of October, when it turns brown. The brown succulent part of the fruit was analyzed separately. In the final stage of the maturation phase, when the walnut is quite mature, this part of the fruit splits and falls.

The results given in Table 2 show the relation between the endiol compounds and the strong reducing substances, and between the crude cellulose and pentosans. The content of all endiol compounds (sum of ascorbic acid and reductones) is maximal in the stage of endocarp differentiation. The content of pentosans is doubled in the final phase of the fruit development. When the succulent part of the fruit is spontaneously separated from the endocarp and is brown colored, the content of endiol compounds and strong reducing substances is minimal.

Endocarp. There are only few data in the literature relating to the composition of endocarp in regard to vitamin C (8, 9, 17).

Endocarp can be dissected from the walnut fruit at the beginning of June, and at that time it represents a white, soft mass which becomes harder towards the end of June. By the middle of July, this mass is solid and its white color turns to light brown.

The results given in Table 3 offer very interesting data. The content of water is at its height, above 90%, in the stage of the development of the endocarp, when the latter can be dissected from other morphological parts of the fruit. But, some days later, in the initial phase of the endocarp hardening, the content of water decreases abruptly. The endocarp of mature fruits contains only cca 18% water. In addition, the stage in which the endocarp can be dissected from other

THE ENTIRE FRUIT

TABLE 2

Months (weeks)	Weight grams	Water %	mg %			Strong reducing substances 0.1 N $KMnO_4$ 1 g at 0°	Pentosans %	Sugars %	
			Ascorbic acid	Dehydroascorbic acid	Reductones			Reducing	Nonreducing
V_2	0.2	85.2	2728.4	1002.6	2746.3	60.0	7.8	5.4	4.2
V_3	0.7	85.7	6041.0	0	2567.4	55.5	8.3	8.8	4.0
V_4	1.9	86.7	8084.6	2987.9	6541.2	86.4	11.0	10.1	4.1
VI_1	2.2	87.5	13485.9	7905.4	4516.9	153.3	14.5	11.6	4.0

SUCCULENT PART OF THE FRUIT

TABLE 3

Months (Weeks)		Fruit development	Weight %	Water %		mg % Ascorbic acid		Dehydroascorbic acid		Reductones		Strong r. ducing substbances ml 0,1NKMnO4/lgat 0°		P. ntosans %	Cellulose %	Sugars % Reducing	Sugars % Nonreducing
			I	I	II*	I	II	I	II	I	II	I	II	I	I	I	I
VI$_1$	Second phase of growing	Stadium of endocarp differentiation	—	84.1		3207		6405		11507		81.2		2.0	9.4	10.8	—
VI$_2$			6.5	84.9		5067		188		5782		69.9		4.8	7.9	13.4	8.2
VI$_3$			7.0	85.5		6317		1519		5066		61.6		—	10.6	14.0	6.1
VI$_4$		Stadium of endocarp hardening	11.1	85.6		6368		528		3308		41.3		8.9	10.8	12.6	—
VII$_{1,2}$			12.7	86.3		6350		757		2707		37.1		7.6	8.6	10.7	10.2
VII$_{3,4}$			14.7	87.4		6631		882		2349		42.6		7.7	9.4	9.7	8.2
VIII$_{1,2}$			14.8	87.0		4518		908		1920		23.4		6.9	9.0	—	—
VIII$_{3,4}$			14.9	86.4		1804		315		1657		37.6		8.8	8.6	5.7	—
IX$_1$	Maturation phase		13.6	87.1		5219		292		2957		53.6		8.7	8.3	5.2	10.2
IX$_{2,3}$			18.9	87.8		5813		48.9		4004		47.7		15.5	—	2.1	14.3
IX$_4$		Final stadium	15.0	85.8	35	3687	0	49.6	123	2491	63	38.5	4.4	17.0	10.1	—	—
X$_1$			—	86.1	34	2796	0	158.3	83	1690	105	29.4	0.8	—	11.8	—	—

* II refer to brown colored succulent part of the fruit.

morphological parts of the fruit is characterized by a high content of ascorbic acid, which reaches a value of 22,000 mg %. In the course of further growing and ripening of the fruit, the ascorbic acid content falls abruptly, and the endocarp of mature fruits contains no ascorbic acid at all.

The maximal content of reductones is found in the phase which is characterized by a maximal content of ascorbic acid. In the final stage, the endocarp contains only minimal amounts of reductones.

The same results are obtained in regard to strong reducing substances. In fact, the parallelism of the content of ascorbic acid, reductones, and strong reducing substances is obvious. The maximal amount of dehydroascorbic acid appears in the stage of endocarp hardening, i.e., the stage in which the content of the above compounds decreases.

On the basis of the sum of ascorbic acid and reductone content, which can amount to more than one third of the dry endocarp substance, the question arises as to whether these compounds represent the starting material for the syntheses of other compounds in the phase of endocarp hardening.

In the phase of endocarp hardening, in which the endiol compounds disappear, the content of pentosans increases (in some cases, by 100%), but the dry endocarp substance of the mature fruits contains 50% pentosans. The content of cellulose in the endocarp constantly increases in the maturation period.

Accordingly, in the period of the growth and the maturation of the fruit, the endocarp contains structurally different compounds: in the initial growing period, it consists of endiol and strong reducing compounds; and in the final phase of the maturation period, the dry substance of the endocarp almost exclusively consists of pentosans and crude cellulose.

Seed (kernel and peel). In the literature there are only few data relating to ascorbic acid and dehydroascorbic acid in the seed of the walnut fruit. According to Daglish (9), the content of ascorbic acid in the seed of the walnut fruit is small. In our experience, this content is small but only in the phase of the fruit maturation; in the phase of endocarp differentiation it reaches a value of 3000 mg %. In the stage of endocarp hardening, the peel of the seed can be dissected from the kernel. The separated kernel is of a soft consistence, but it hardens rapidly in the course of further development. The chemical composition of the soft kernel differs from that of the hardened kernel. Therefore, the results obtained are classified into three groups which relate to the seed, the peel, and the kernel.

Seed. The seed of the fruit was investigated as an entity until the moment when the peel could be dissected from the kernel. This period lasted from the end of May until the end of July. Starting from this moment, the individual morphological parts of the seed were examined separately.

Similar to other organs found in the initial phase of the plant development, the seed contains exceptionally high amounts of endiol compounds in the initial phase.

Peel. There are no data in the literature relating to the examination of this part of the fruit. We have investigated the composition of the peel from the end of July, when it could be dissected from the kernel, until the end of the vegetation period.

The content of water and ascorbic acid in this part of the fruit is also at its height in the stage of endocarp hardening. The content of dehydroascorbic acid shows two pronounced maxima: in the phase in which the ascorbic acid content decreases rapidly, and in the phase in which the ascorbic acid disappears.

The highest amounts of reductones are found in the phase of endocarp hardening. In the latter phase, a special kind of reductones has been observed, those which react in their endiol form only in a solution

ENDOCARP

TABLE 4

Months (Weeks)		Fruit development	Weight g	Water %	mg %			Strong reducing substances ml 0,1N KMn_4/lg na 0°	Pentosans	Crude cellulose	Sugars %	
					Ascorbic acid	Dehydroascorbic acid	Reductones				Reducing	Nonreducing
VI_1	Second phase of growing	Stadium of endocarp differentiation	7.0	90.2	22055	0	4513	83.2	8.4	7.0	2.9	2.6
$VI_{2,3}$			6.9	89.6	19176	238	3925	64.8	10.6	7.4	11.8	13.7
VI_4		Stadium of endocarp hardening	7.8	86.2	10413	2748	1335	42.4	9.8	8.6	11.2	11.2
$VII_{1,2}$			7.9	76.6	6607	636	1043	15.4	18.0	9.0	6.8	5.8
VII_3	Maturation phase		8.3	64.9	2102	83	181	7.8	18.1	18.3	2.6	0.5
VII_4 $VIII_1$			7.5	53.3	1300	135	125	4.9	20.1	19.7	2.8	1.3
$VIII_{2,3}$			6.9	45.0	45.8	191	48	2.9	22.4	26.0	1,9	0.9
$VIII_4$ IX_1			5.9	43.2	4.2	212	40	1.7	16.3	—	1.6	3.5
$IX_{2,3}$			6.6	84.3	1.4	104	29	1.0	51 2	—	0.3	0.2
IX_4 X_1		Final stadium	—	18.0	—	25	20	0,7	—	—	0.0	0.0

PEEL

TABLE 5

Months (Weeks)	Fruit development		Weight *g*	Water %	mg %			Strong reducing substances *ml* 0,1 *N* $KMnO_4$/1 *g* 4 at 0°	Pentosans	Crude cellulose	Sugars %	
					Ascorbic acid	Dehydroascorbic acid	Reductones				Reducing	Nonreducing
VI_4 VII_1	Second phase of growing	Stadium of endocarp hardening	1.2	83.1	555.8	378.1	268.1	146.4	2.9	7.5	6.5	8.2
VII_2			1.1	81.1	142.5	1051.5	485.9	105.2	3.6	3.8	6.3	6.6
$VII_{3,4}$	Maturation phase		1.0	69.4	119.6	284.3	295.8	125.2	4.3	4.6	5.9	4.2
$VIII_{1,2,3}$			0.8	62.3	46.7	70.9	213.2	133.7	3.9	3.6	9.4	3.8
$VIII_4$			0.5	54.9	0	379.3	183.5	116.6	3.5	6.2	7.8	3.3
IX_1			0.5	51.4	0	450.4	322 7	131.9	4.4	5.8	1.8	1.0
$IX_{2,3}$			0.5	48.8	0	793.2	368.0	163.7	3.3	5.5	2.5	1.1
IX_4		Final stadium	0.4	40.6	0	658.9	362.4	133.1	3.5	6.3	1.5	0.2
$X_{1,2}$			0.3	29.1	0	85.9	392.0	117.5	3.6	7.7	0	0

TABLE 5

KERNEL

Months (Weeks)	Fruit development		Weight g	Water %	mg %			Strong reducing substances ml/ 0,1 N $KMnO_4$/1 g at 0°	Pentosans	Crude cellulose	Sugars %	
					Ascorbic acid	Dehydroascorbic acid	Reductones				Reducing	Nonreducing
VI_4	Second phase of development	Soft kernel consistence (stadium of endocarp hardening)		97.0	308.7	2940.4	4622.0	17.5	3.8	9.0	20.0	40.5
$VII_{1,2}$				95.4	1376.1	1028.5	332.1	9.2	3.9	8.9	7.4	13.8
$VII_{2,3}$	Maturation phase		3.2	89.5	236.1	164.1	123.4	3.5	3.1	3.1	4.6	5.9
VII_4 $VIII_1$			3.5	80.8	86.1	43.7	55.7	2.5	2.5	1.3	5.0	2.5
$VIII_{2,3}$			3.3	34.2	18.3	23.6	24.8	1.6	1 8	1.9	0.9	0.5
$VIII_4$			3.2	31.7	0	81.9	19.2	1.0	2.6	1.3	0.7	0.7
IX_1			3.8	29.2	0	37.0	19.9	1.3	2.5	1.1	0.6	1.9
$IX_{2,3}$			3 8	25.1	0	35.8	9.9	1.0	2.4	1.1	0.2	2.7
IX_4		Final stadium	3.9	24.2	0	50.8	14.1	1.2	2.5	1.1	0.2	1.8
X_1			4.3	21.1	0	17.0	9.0	0.6	2.1	1.1	0	3.0

of 22% hydrochloric acid. They appear in the final phase of the maturation of the walnut fruit (ten to fifteen days before the fall of the walnut). This part of the fruit contains the highest amount of strong reducing substances in the course of the whole vegetation period. If the content of these materials were calculated on the basis of ascorbic acid equivalent, more than 80% of the dry substance of the peel would be composed of compounds of ascorbic acid type. This exceptionally high content of endiol or peroxy compounds (19) indicates that the peel plays a specific role in the formation of the peroxide-free organic matter of the kernel. When the dry substances of the kernel are formed, i. e., when there is no increase in the kernel weight, the peel desiccates, and simultaneously the content of its strong reducing substances decreases abruptly.

The content of pentosans is almost constant in the course of the whole vegetation period, ranging at an average of 3 to 4%. This fact indicates that the formation of pentosans terminates in the peel during the initial phase of the fruit development.

In the investigation of the peel, it was observed that strong reducing substances are the precursors of the final organic matter of the walnut fruit, and the presursors of these are the endiol compounds.

Kernel. The data referring to the content of vitamin C in the walnut "seed" are very scanty (7, 9, 18). In view of the fact that the values given for vitamin C content are very low, it might be concluded that these data refer to the already hardened kernel.

Of all the parts of the fruit, the kernel, when soft, contains the maximal amount of water, over 97%. The transformation of the kernel into the solid state results in an abrupt decrease of water, and the content of water amounts only to 21% in the final stage of the fruit maturation.

The ascorbic acid content depends upon the state of the kernel matter. When the kernel is soft, the content of ascorbic acid reaches a value of 1500 mg %, but the transformation into the solid state (seven or eight days later) results in an abrupt decrease to less than 200 mg %. In the middle of August, the kernel contains no ascorbic acid.

The content of dehydroascorbic acid alters analogously to that of ascorbic acid. The soft kernel contains up to 3000 mg %, and the hardened kernel contains 120 mg% at the most.

The above data show that the total amount of vitamin C in the soft matter reaches a value of 5000 mg%, and the hardened kernel contains no ascorbic acid.

The highest concentration of reductones, which reaches a value up to 5000 mg %, is to be found in the soft kernel. On hardening, the reductone concentration falls to some tenths per cent. Similarly, the hardened kernel contains no strong reducing substances.

The soft kernel contains maximal amounts of water, endiol compounds, strong reducing substances, and sugar. On hardening, the chemical composition of the kernel rapidly changes, and the content of endiol compounds, reducing substances, and sugar is strongly decreased.

The study of the kernel matter in the course of the vegetation period offers useful data concerning the transformation of one type of compounds into the other.

Conclusions

These investigations were performed in the course of six successive vegetation periods. The conclusions given below are general and refer to all vegetation periods without exception.

1. Each of the compounds investigated reaches the maximal content value at the same time of the vegetation period, but the numerical values of these maxima are different and depend upon the weather conditions of the vegetation period concerned.

2. In the initial phase of the fruit development, the organic nitrogen-free matter (fat was not investigated) consists mainly of labile endiol compounds and strong reducing substances, but in the final phase, the main constituents are cellulose and pentosans, i.e., stable compounds. A more intense formation of stable organic compounds occurs in the phase in which the maximal fruit weight is attained. In this phase of the fruit development, the organic matter is substituted for the water which disappears.

3. All investigated parts of the walnut fruit contain the maximal amount of ascorbic acid during the phase in which the organ concerned contains the maximal amount of water.

4. The presence of two types of reductones has been established:

a) reductones which react similarly to ascorbic acid, and

b) reductones which react in an endiol form only in 22% hydrochloric acid solution. These compounds were found in the endocarp and in the peel.

5. Ascorbic or dehydroascorbic acid was found in some phases of the development of individual organs; these findings indicate that the acids in question do not always appear in the system ascorbic acid ⇄ dehydroascorbic acid.

6. The maximal amounts of reducing and non-reducing sugar were found in the phase of the fruit development. In the phase of the fruit maturation, the contents of these compounds decrease and the mature fruit contains practically no sugar. The only exception is the kernel which, when mature, contains non-reducing sugar.

7. The investigations of the kernel and the peel have shown that the precursors of the final organic matter of the kernel are endiol and strong reducing substances. Bearing in mind that the peel contains exceptionnally high, but simultaneously constant, amounts of strong reducing substances in the course of the whole vegetation period, i.e., until the final formation of the kernel, it is concluded that the peel plays a role of supplying the kernel with substances which bring about the formation of the final kernel matter.

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ENZYMATIC DEGRADATION OF SOME ORGANOPHOSPHORUS COMPOUNDS CONTAINING p-NITROPHENYL RADICAL IN PLASMA AND ORGANS OF VARIOUS ANIMAL SPECIES. I.

by

Z. BINENFELD

The earliest data on an enzyme capable of catalyzing the hydrolysis of organophosphorus compounds (OPC) were reported by *Mazur* (1). He observed the presence of an enzyme which degrades DFP in the plasma and tissues of rabbits. Subsequent work in this field (2—5) proved the existence of an enzyme which other than DFP, hydrolyzes paraoxon in the sera of a whole series of animals. *Main* (6) performed the purification of an enzyme which degrades paraoxon in sheep serum.

Iuchi and *Miyai* (7) studied the action of an enzyme which degrades parathion in various tissues of the dog, and they established that it differs considerably from the distribution of cholinesterase (ChE) and alkaline phosphates. The same was found with other enzymes of this kind, which are often named "phosphorylphosphatases". (8). The same authors (8, 9) also investigated the normal values of the enzymatic breakdown of parathion, and found significant variations.

It has recently become evident that the plasma and tissues of various animal species, including man, contain an enzyme which is capable of hydrolyzing OPC which contain p-nitrophenyl radical (11, 12, 13).

The aim of this work is a detailed study of the enzymatic degradation of some OPC of the type in question, in order to establish, if possible, some relation between their chemical structure and the enzymatic degradation; and to correlate their toxicity with their enzymatic hydrolysis.

Materials and Methods

The organophosphorus compounds used in this investigation are described in Table I.

Thanks are due to medical technicians Mrs. *Zorica Burić*, military medical captain, and Mrs. *Marija Pavliček*, civil servant, for their technical assistance in this work.

TABLE 1

	Synonim b. p.	$LD_{50}/\gamma/kg/*$
Ethyl p-nitrophenyl phosphate	armine, b. p. 144—5° 0.15 mm Hg	943/819.8/1085
Isopropyl methyl-p-nitrophenyl phosphate	i-armine, b. p. 155° 0.2 mm Hg	1.227/1.154-1 302.8
Ethyl methyl-p-nitrophenil phosphate	m-armine, b. p. 132° 0.1 mm Hg	839.9/793.2 889.3

* LD_{50} was determined with male white rats i. p.

The experiments were performed with aqueous solutions of these compounds because they do not undergo hydrolysis for a long period of time, at pH less than 7 (14, 15). Their solutions were not used in a bicarbonate buffer because they partially decompose when dormant. The stability of solutions was constantly controlled by means of spectrophotometric determination of p-nitro-phenol (14).

The following were used as enzyme preparations: plasma of rabbit, dog, sheep, rat, guinea-pig and man; kidney, heart, liver, lungs, brain, adrenal glands, and spleen of rabbit. Blood was taken with all precautionary measures in order to avoid coagulation (heparin), and then centrifuged. The organs were homogenized, and extracts were prepared in a bicarbonate buffer, which was the same for both plasma and organs. After homogenization, centrifugation was performed (3000 turns, during 15'). All calculations were made with respect to the separated liquid which was used in the experiments.

The enzyme activities were determined by the *Warburg* technique. Enzyme preparations, dissolved in a buffer consisting of 0.164 M sodium chloride and 0.0357 M sodium bicarbonate, were placed in the middle of the *Warburg* flask. The buffer was free of Mg^{++} ($MgCl_2$) because it was shown, in accordance with *Augustinsson's* results (8), that concentrations smaller than 0.002 M $MgCl_2$ do not influence the enzymatic degradation of compounds, but higher concentrations produce a significant inhibitory effect.

Substrate solutions (0.02 to 0.49 ml, depending upon the concentration) were placed in the wide side-arm of the apparatus.

Solutions were gassed with 95 N_2+5 CO_2. The temperature was equilibrated within 30'. The pH of the reaction mixture, after mixing enzyme and substrate, was 7.2—7.4. All experiments were made at 37°C, and readings were taken every 10' from 40' to one hour. The interim between the dilution procedure and the mixing of substrate and enzyme was usually one hour.

Liberation of CO_2 from the bicarbonate buffer was considered proof of a p-nitrophenol formation. The enzyme activity was expressed in two ways:

I. The unit of enzyme activity denotes microliters of CO_2 liberated in 30' by plasma or tissue (b_{30}). This value is equal to the total amount of CO_2 evolved in 30' (a_{30}), minus the amount of CO_2 in ml evolved during the same period of time by non-enzymatic hydrolysis or accidental contamination. Calculations were made on the basis of the initial slope of the curve.

II. The unit of enzyme activity is equal to the amount of CO_2 liberated in one minute by 1 ml plasma or 1 g tissue. These activities were also calculated from the initial slope of the curve with consideration given to non-enzymatic hydrolysis and contamination.

Results

Hydrolysis of armine, i-armine, and m-armine is catalyzed by the plasma of various animal species. In view of the fact that spontaneous hydrolysis of the examined compounds does not take place under given experimental conditions, the hydrolysis which was observed can be fully ascribed to the action

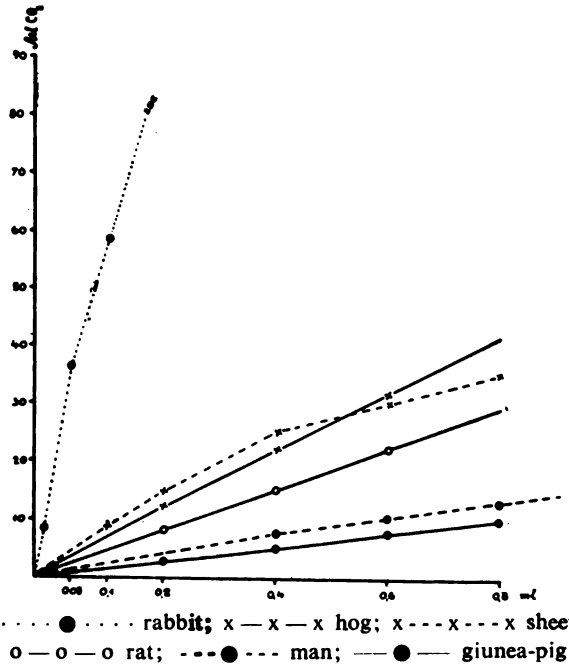


Figure 1

Enzymatic hydrolysis of armine by the plasma of various animals as a function of plasma concentration. Substrate, 2.2 10^{-3} M armine $CO_2 = b_{30}$

of an enzyme or enzymic system which is present in the plasma of various animal species. The reaction is continuous, and dependent upon the amount of plasma used (Figures 1, 2, 3).

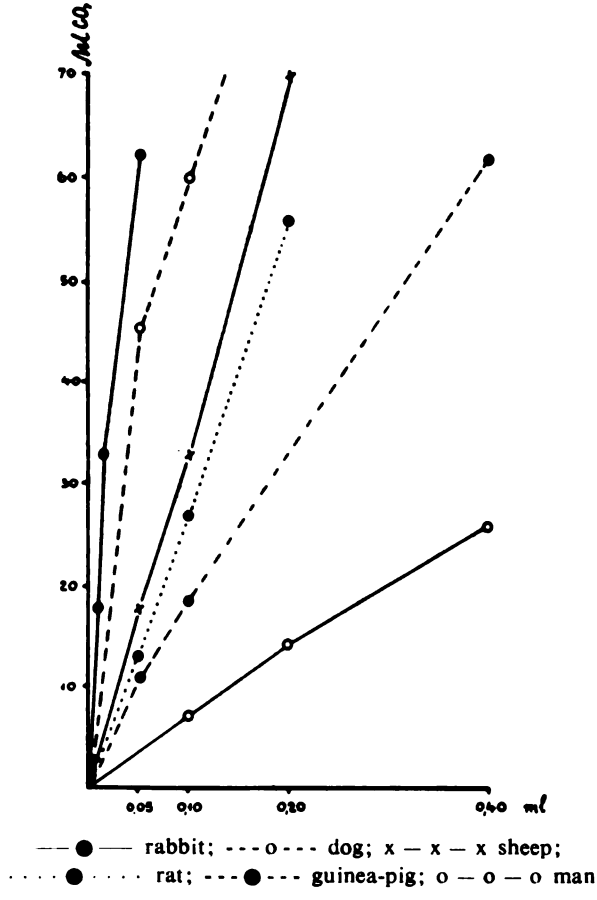


Figure 2

Enzymatic hydrolysis of m-armine by the plasma of various animals as a function of plasma concentration. Substrate, 2.2 10^{-3} M m-armine $CO_2 = b_{30}$

The following terminology will be used in this text: the enzyme which degrades armine will be called "arminase"; the one which degrades i-armine, "i-arminase"; and the one which degrades m-armine, "m-arminase."

Figures 4, 5, and 6 clearly illustrate that the reactions observed are concerned with the enzymatic hydrolysis of armine, i-armine, and m-armine, where plasma was the source of the enzymes.

The enzyme (or enzymes) in question clearly belongs to the group of so-called "phosphorylphospatases" because the

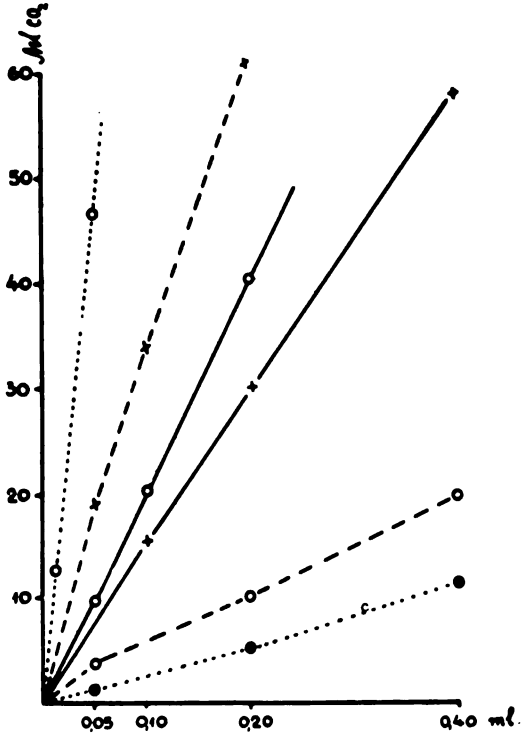


Figure 3

Enzymatic hydrolysis of i-armine by the plasma of various animals as a function of plasma concentration. Substrate, 1.7 10^{-3} M i-armine $CO_2 = b_{30}$

TABLE 2

The rate of enzymatic hydrolysis of m-armine, i-armine, and armine by plasma from various animal species

	µl CO_2/1ml plasma/1 minute		
	m-armine	i-armine	armine
rabbit	147	53.4	23.4
dog	30	6.35	0.83
rat	26.7	6.67	1.35
guinea pig	22	1.5	0.67
sheep	18.2	4.35	2.34
mouse	7.4	4.4	1.8
man	6.4	4	1.4

plasma of the examined animals also catalyzes the hydrolysis of other organophosphorus compounds (DFP, tabun, etc.).

In most of our experiments, the rate of substrate hydrolysis is proportional to the amount of plasma used. An example is given in the enzymatic degradation of m-armine in the presence of guinea pig plasma (Fig. 7).

TABLE 3

The rate of the enzymatic hydrolysis of armine, i-armine and m-armine by rabbit organs.

	$\mu l\ CO_2/1\ g$ organ/1 min.		
	m-armine	i-armine	armine
Kidney	10.3	1.33	0
Liver	6	2.63	1

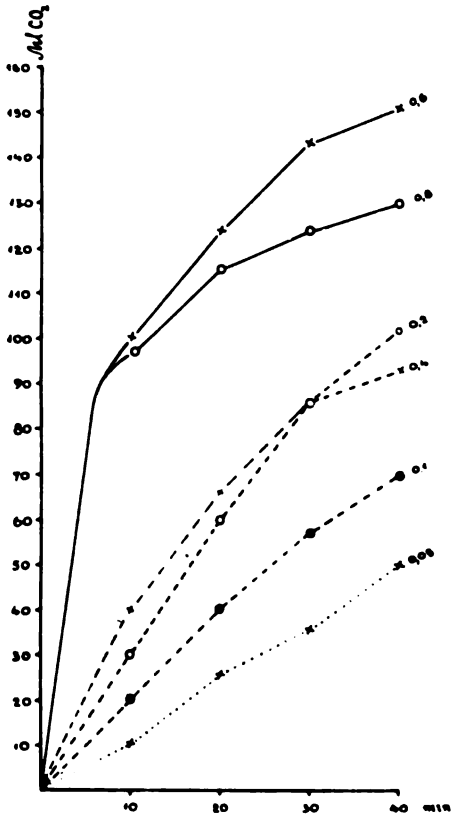


Figure 4

Enzymatic hydrolysis of m-armine by the action of rabbit plasma. Numbers (0.05 to 0.8) refer to ml plasma in the reaction mixture. Substrate, 2.2 10^{-3} M m-armine.

Parallel experiments on the rate of hydrolysis of armine, i-armine, and m-armine by plasma from various animal species have shown that the order is: (table 2)

m-armine: rabbit > dog > rat > guinea pig > sheep > mouse ≅ man

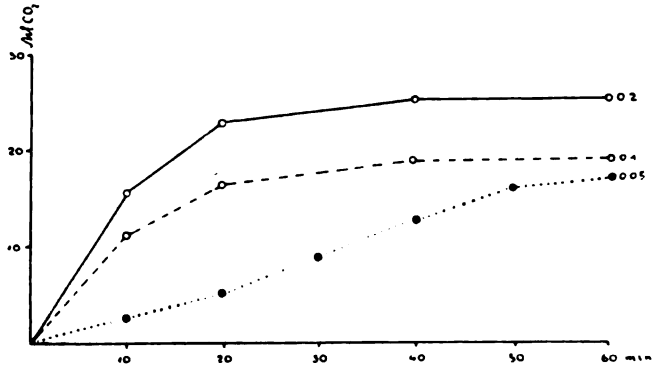


Figure 5

Enzymatic hydrolysis of i-armine by the action of dog plasma. Numbers (0.05 to 0.2) refer to ml plasma in the reaction mixture. Substrate, 1.7 10^{-3} M i-armine.

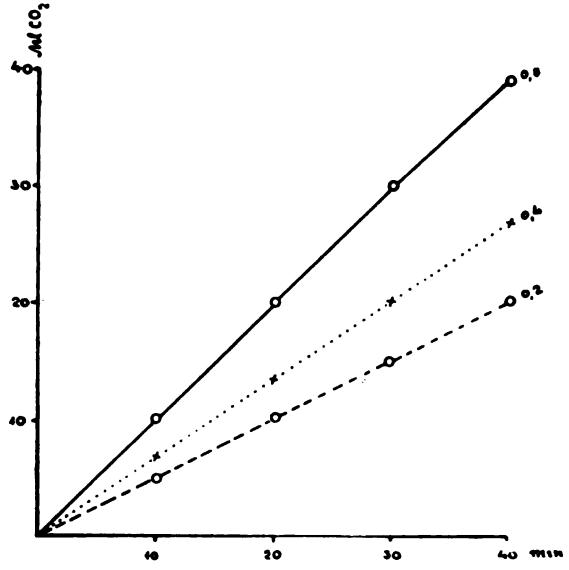


Figure 6

Enzymatic hydrolysis of armine by the action of dog plasma. Numbers (0.2 to 0.8) refer to ml plasma in the reaction mixture. Substrate, 2.2 10^{-3} M armine.

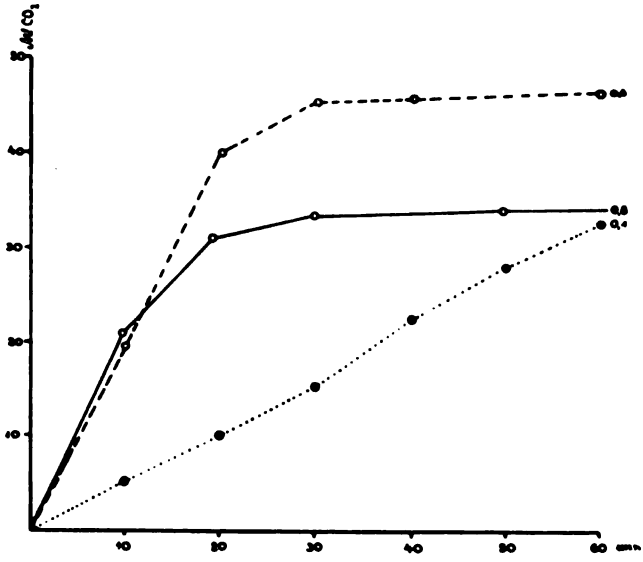
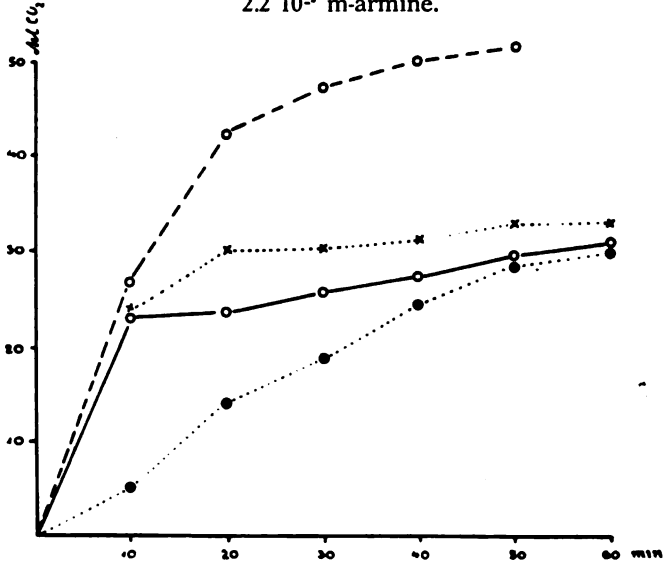


Figure 7

Enzymatic hydrolysis of m-armine by the plasma of guinea-pig. Numbers(0.1 to 0.8) refer to ml plasma in the reaction mixture. Substrate, 2.2 10^{-3} m-armine.



o - - - o - - - o paraoxon; x - - - x - - x i-armine;
o—o—o m-armine; o · · · · o · · · · o armine

Figure 8

Enzymatic hydrolysis of armine, m-armine, i-armine and paraoxon by the action of human plasma. Substrate, 2.2 10^{-3} M m-armine, armine, i-armine, and paraoxon. Enzyme source: 0.08 ml human plasma.

i-armine: rabbit > dog ≅ rat > mouse ≅ sheep ≅ man > guinea pig

armine: rabbit > sheep > mouse > man ≅ rat > dog ≅ guinea pig

It is evident from table 2 that there are considerable differences in the rate of enzymatic hydrolysis of different substrates (armine: m-armine: i-armine) by the same enzyme source. The rate of enzymatic hydrolysis is also different for each of the examined substrates in regard to various animal species.

The rabbit plasma was the most active of all three examined compounds; its greatest activity was in the case of m-armine. In some animal species, the rate of enzymatic hydrolysis of i-armine and m-armine was (in that order) 2 to 7 and 4 to 36 times greater than that of armine.

The supposed values for some animal species are sometimes very different from those actually found, especially when plasma is taken from various animals of the same species (fig. 8).

Figure 8 shows that human plasma, when taken as an enzyme source, gives a picture different from that which is shown in Table 2.

TABLE 4

The action "arminase" on the enzymatic distruction of armine in rabbit's organs in the presence of NaF

	μlCO_2/1 *g* tissue/one minute	
	Wi hout NaF	With NaF
Heart	0	4.4
Spleen	0	7.5
Lungs	1.4	19
Kidney	0	17
Liver	2.4	15
Brain	0	3.1
Adrenal glands	0	50

We examined the enzymatic degradation of armine by human plasma (both sexes) in a previous paper (12), and found very great individual differences; thus the activity of one member cannot be taken as a measure of species activity.

b_{30}-Values per 1 ml plasma vary within limits from 3.1 to 37 μlCO_2 for men, and from 1.1 to 42.7 μlCO_2 for women.

Various rabbit organs were used as an enzyme source in our investigations. Contrary to the findings of *Augustinsson* (8), who established measurable amounts of "tabunase" (enzyme degrading tabun) in a series of experiments, we succeeded in detecting only a slight activity of "arminase" in

rabbit liver and rabbit lungs. Traces of this activity could not be detected in the spleen, brains, kidneys, heart, and adrenal glands of rabbit. However, some of these organs are capable of hydrolyzing m-armine and i-armine (table 3).

In order to gain a better insight into the enzymatic degradation of armine, i-armine, and m-armine, we have studied the effect of Mg^{++} and the effect of sodium fluoride and eserine on that degradation.

At a concentration of 1×10^{-2} M, eserine inhibits "arminase" (35% inhibition) in rabbit plasma, but eserine concentrations of 1×10^{-3} M and 1×10^{-4} M have no effect. The same result was obtained with "arminase" in rabbit liver.

At a concentration of 1×10^{-1} M, sodium fluoride increases the activity of "arminase" by 100%, and Aldridge (5) showed that this sodium fluoride concentration inhibits "paraoxonase." A sodium fluoride concentration of 10^{-1} M in rabbit tissues has no inhibitory effect, but it does increase the degree of "arminase" activity so that the latter can be detected in the heart, spleen, kidney, brain, and adrenal glands, i. e., where we otherwise failed to determine it (table 4).

Discussion

The investigation of the enzymatic hydrolysis of OPC is an aid to understanding the behavior of these compounds in living organisms. The results obtained so far have not justified the claim of some authors (8) that the enzymatic hydrolysis is, in a way, responsible for the toxicity of OPC. Our previous observations (13), which indicated that the toxicity of armine, m-armine, and i-armine is not directly connected with their toxicity in organisms, were supported by the works of *O'Brien* (16). He has demonstrated that the increased amount of paraoxon in the mouse organism, caused by the inhibition of "paraoxonase" due to preventive application of SKF-525 A *in vivo*, did not increase the toxicity of paraoxon. Our experiments have shown that m-armine, which is the most toxic of the three examined compounds, is the most rapidly hydrolyzed in vitro. The possibility of different effects of "phosphorys-phosphatases" in various organs and tissues, and even in vital centers where we are still unable to determine the specific activities, should also be taken into consideration. In armine poisoning, the process of ChE inactivation is practically terminated after only 10 minutes (13), although a rather great amount of untreated poison remains in the organism. The fact that the action of "phosphorylphosphatases" consists of the degradation of the unreacted part of OPC was considered. This could be a working hypothesis for further investigations in this field

Our experimental results on the action of sodium fluoride and eserine on "arminase" differ from those which were ob-

tained by *Augustinsson* and *Aldridge* (5, 8) concerning "tabunase" and "paraoxonase"; these differences can be ascribed to various experimental conditions or to the existence of various enzymes which degrade paraoxon and tabun on one hand, and armine, i-armine, and m-armine on the other.

Comparisons of animal species in regard to the known activity of "phosphorylphosphatase" should be greatly corrected. This especially refers to those animal species of which the enzyme activity is very similar. Results obtained by means of plasma of one or only a small number of animals can lead to erroneous conclusions concerning the comparison of species. The comparison of species requires the statistical treatment of a great number of animals as a prerequisite condition. Comparisons between individual compounds, used as substrates, should always be made with the same enzyme preparation.

Great differences observed in the enzymatic degradation of structurally similar compounds are very likely to be the result of the effect of alkyl and alkoxy group on the rate of enzymatic hydrolysis of the compounds in question. A very small number of examined compounds does not permit definite conclusions. However, it has been observed that m-armine and i-armine, having a methyl group joined directly to phosphorus, are more rapidly hydrolyzed than armine, which contains an ethyl instead of methyl group.

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A CONTRIBUTION TO THE INVESTIGATION OF SELF-PURIFICATION OF WATERCOURSES CONTAMINATED WITH WASTE WATERS

I. THE CONTAMINATION OF THE WATERCOURSE NADEL

by

NATALIJA RADAK, VERA MITROVIĆ and DRAGOSLAV ŠINŽAR

The rapid growth of towns and industry has made necessary the solution of a series of problems, among which is the problem of regulation of canal and industrial waste waters. The undertaking of measures of precaution for the protection of natural waters undoubtedly represents an easier task than the sanitation of already devastated watercourses and standing waters. For these reasons, the study of the effect of waste waters on natural recipients is considered very important in all countries where industrialization is growing and, consequently, in Serbia. In regard to the fact that surface waters possess the ability to self-purify, the study of the dynamics of that process can yield the answer to the question of the capacity of natural waters to act as recipients for waste waters.

In this study, we made an attempt to fix the limits of the phases of self-purification of a watercourse, i.e., to mark the polluted zones on the basis of the contamination degree, which is decided upon by the absence of nontolerant, and the presence and quantity of tolerant, planktonic and benthonic organisms (1, 3, 4, 8).

In order to attain this aim, i.e., to determine the polluted zones of the watercourse under investigation, we have used both chemical and hydrobiological methods for the analyses of qualitative and quantitative compositions of net-plankton and bottom fauna. The use of both of these methods has made possible a comparison of the results obtained in chemical and hydrobiological analyses as contamination parameters.

The object of our investigation, the Nadel watercourse, is a recipient of organic waste waters of the starch factory "Jabuka" in Jabuka. This watercourse is very amenable to the study of the effect of waste waters upon the quality of water and its living population because only one source of contamination is located there, and the process of constant pollution with organic waste waters is of a rather long duration, almost sixty-five years.

Despite the fact that the Nadel watercourse has been the recipient of waste waters for such a long period, the problem of its pollution has not yet been examined.

Principal Data About the Watercourse Under Investigation and the Source of Contamination

The Nadel watercourse is about 25 km (Fig. 1) long and approximately 50 to 100 m wide; it originates at the "Crepajski vinogradi" (vineyards of Crepaja), where it is a natural recipient of flowing and pen waters; it flows into the Ivanovački

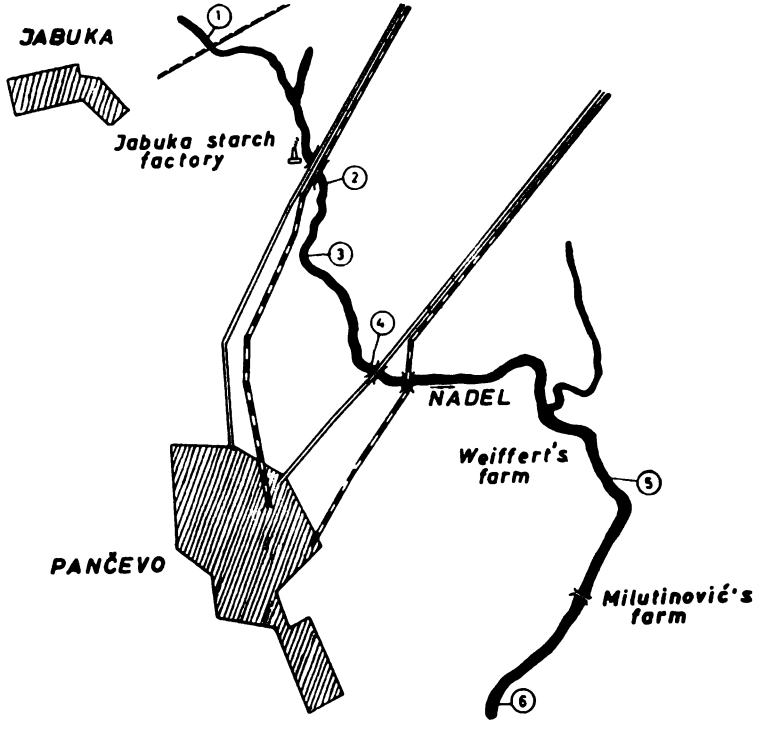

Fig. 1.

canal before its final flow into the Danube. Nadel uses the natural depression as its bed, and therefore its depth varies considerably. Its banks are overgrown with reed.*

The starch factory "Jabuka" began operations in 1897. It produces starch, syrup, oil, dextrin and approximately 14,000

* The rate of water is slow and amounts to an average value of 5 cm/sec in its free course.

tons of corn per annum. The abundance of raw material and the great demand for starch and starch products have made the factory operate continuously in three shifts all year long. This kind of industry requires a great quantity of water, and gives great quantities of waste waters. Used water is returned

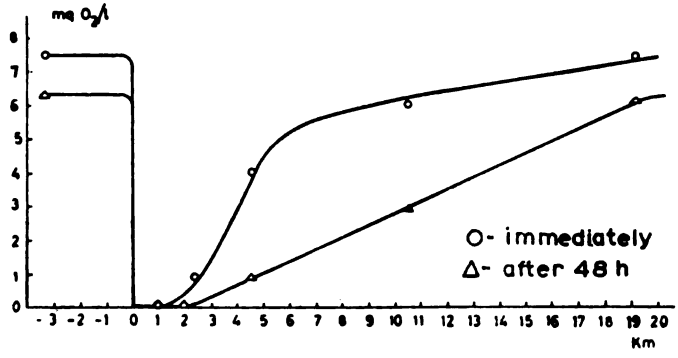

Fig. 2.

several times to the production process. The starch factory "Jabuka" does not possess the equipment for the purification of waste waters. Its waste waters contain organic materials, predominantly proteins, and sugar, lactic acid, cellulose, sulfurous acid, and some mineral salts. The starch industry "Jabuka" ejects by gravitation an average of cca 800 liters of waste waters per minute, the temperature of which is cca 30°C.

Methods of Investigation

a) Chemical methods. — The choice of chemical methods has been conditioned by the fact that the Nadel watercourse is contaminated with organic waste waters. We have examined the content of oxygen dissolved in water and its amount after a period of forty-eight hours; the consumption of potassium permanganate, nitrates, ammonia, phosphates, and decay reactions has also been examined. Moreover, we determined pH, alkalinity, dry residue, and iron content. All the analyses were performed by standard methods for the examination of water and sewage (7). Our practice was to perform all the chemical analyses either directly on the terrain or in laboratories shortly after the taking of samples (six hours at the latest) without any conservation in order to avoid the changes which rapidly take place in organic waste waters. According to already established practice, water samples were taken by means of a *Friedinger* bottle of 1 m, at a depth of 50 cm.

b) Hydrobiological methods; — Samples for qualitative and quantitative analyses of net-planktonic and benthonic organisms were taken by means of standard hydrobiological apparatus, i.e. by means of zoo and phytoplanktonic net No. 20 and 25 for qualitative analyses, and samples for quantitative analyses of plankton were taken by means of a

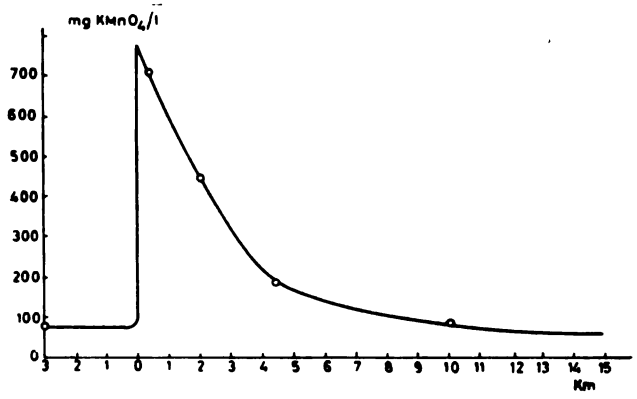

Fig. 3.

Friedinger bottle at a depth of 50 cm; samples of fauna were taken from the bottom by means of an excavator of the *Ekman* type. We used here the quantitative analysis of the population of planktonic and benthonic types because the *Kolwitz-Marsson* system for the establishment of contaminated zones in regard to organisms-indicators is, in our opinion, insufficient for the estimation of the degree of contamination. We have accepted the practice of American authors (2,9) by introducing quantitative hydrobiological methods.

Results of Investigation

The terrain investigations of the contamination of the Nadel watercourse with waste waters derived from the starch industry "Jabuka" were performed during April 1960. Laboratory works of the sample material were performed in the course of 1960/1961.

The aim of this study is not concerned with the seasonal aspects of organic water production, but exclusively with the investigation of the effect of waste waters on the recipient and territorial zoning of the watercourse in regard to the degree of contamination. Thus, we are of the opinion that an investigation covering a longer period of time is not necessary for the accomplishment of our task. The taking of samples during a longer period of time would be indispensible only in case of acute and sporadic contamination of the recipient, which,

however, is not the case with the Nadel watercourse; as already noted, the Nadel watercourse has been permanently contaminated over a period of sixy-five years.

The choice of location for sample-taking was based upon the results of preliminary chemical and biological analyses, and upon objective possibilities, such as the access to the watercourse and its sufficient depth. We fixed six locations where the investigation was performed.

Point 1 was located at 3.8 km upward from the starch factory, in fact, before the Nadel was contaminated. Points 2; 3, 4, 5, and 6 were fixed at 0.25, 2,5, 4.5, 10.5, and 18.5 km downward from the exit canal of the factory.

TABLE 1

Point	Temperature of water °C	Nitrates mg/l	Ammonia mg/l	Chlorides mg/l	Dry residue mg/l	Iron mg/l	Alcalinity ml n/10 HCL	Phosphates
1	12	0.24	+	43	1032	0.0024	16.57	0.005
2	14	0.32	+ +	61	1709	0.12	17.96	1.04
3	14	0.32	+ +	60	1396	0.0026	14.44	1.02
4	13	0.24	+ +	55	963	0.0021	15.36	0.084
5	13	0.068	trace	27.2	676	0.0021	11.48	0.1
6	13	0.060	+	25	491	0.0026	10.74	0.081

There is a complete review of results obtained in chemical and biological analyses at different points of the investigated watercourse affixed to Table 1 and Diagrams 1 and 2.

Point 1. In this section the bank zones of the Nadel watercourse are densely overgrown with reed, so that there is only a little water left free. The depth of water is also small (less than 1 m). The water is of a greenish-grey color, and free from any odor. The bottom is covered with large quantities of phyto-detritus.

The oxygen saturation amounts to 69.3 per cent. The consumption of potassium permanganate and the dry residue are considerably great, as are the alkalinity and chloride content. Carbon dioxide and ammonia are also present. The foregoing findings indicate the mineral character of the water and the rapid decomposition of phyto-detritus.

At this location, the zoo-plankton is qualitatively rich, but quantitatively very poor. The wide-spread groups of netplankton are *Rotatoria* groups (eleven kinds), and types of the

family *Brachionus* are the most numerous; other kinds are not numerous and are predominantly epiphytic. Two types of *Cladocera* were found, but neither of them are numerous. There are few *Cyclopide* belonging to *Copepoda* which contain numerous larva shapes. *Ostracoda* and *Nematoda* appear individually. The plant component of the netplankton is represented by *Diatomaceae* and fibrous *Chlorophyceae (Spirogyra)*. The numerousness of phytoplantonic kinds is very small, and this is caused by the fact that this section of the watercourse is overgrown to a great extent with microphytic vegetation.

The fauna of the bottom consists of *Chironomida, Heleida, Coleoptera* (larvae), *Oligochaeta, Hirudines,* and *Gasteropoda* We found 355.52 ind/m² in all.

The results of chemical analyses correspond to those of hydrobiological ones. In fact, the results of both analytical methods indicate that this section of the watercourse belongs to β-mezosaprogenic zone. Most of the identified kinds are indicators of this zone *(Brachinous calyciflorsus, Keratella cochlearis, Ceriodaphnia* sp. etc).

P o i n t 2. The water of this section is black colored, and has a thin, white, foamy surface layer. Its odor is penetrating and unpleasant, resembling that of decay. Methylene blue is decolorized after four hours. The mud is also black colored.

Water samples contain no oxygen. Carbon dioxide and ammonia are present in large quantities, and the consumption of potassium permanganate is very high. The dry residue is increased, but the alkalinity and the bicarbonate content is only a little more than that which was found in the previous location.

Planktonic and benthonic tests were negative. It is characteristic that there is no resistent organism, not even at this point of the watercourse. The negative results of the hydrobiological tests are in full agreement with chemical analyses, and it is therefore justified to designate this section of the watercourse as a septic part of the polysaprogenic zone.

P o i n t 3. The color of the water, which is reddish and has a penetrating odor, is the principal difference between points 2 and 3. The oxygen saturation amounts to 7.2 per cent. It was not possible to detect oxygen after a period of forty-eight hours. The consumption of potassium permanganate is decreased in comparison to that of the preceding point, but it is still rather high. Ammonia is present in large quantities, but the alkalinity is somewhat smaller. At this point, we have determined the total absence of netplanktonic organisms and bottom fauna. Hence, this section of the watercourse must be designated as a septic one.

P o i n t 4. The visible characteristic of this point is the yellow-greenish color of the water, which has an odor of decay.

Methylene blue is decolorized after thirty-six hours. The oxygen saturation amounts to 38.7 per cent; however, the oxygen content is only 0.91 mg/1 after forty-eight hours. The consumption of potassium permanganate constantly decreases, but it is still higher than that of non-contaminated water. The dry residue does not correspond to that of non-contaminated water.

Planktonic and benthonic tests indicate an abundance of organisms, but the number of different kinds is very poor. Four zooplanktonic types were found: *Brachionus urceolaris, B. caliciflorus, Rotifer vulgaris,* and *Protozoa Actinosherium eichorni.* The most numerous is the *B. urceolaris,* about 882ind/1, and other kinds only appear individually. Phytoplankton is rather poor from both a qualitative and quantitative point of view; the most numerous is *Euglena viridis.*

The bottom fauna consists of: *Oligochaeta* (3244ind/m²), *Heleida* (1022.12ind/m²), and *Chironomida* (88.88ind/m²).

This section of the watercourse can be designated as typically saprogenic. This conclusion is drawn on the basis of an abundance of the abovementioned organisms, the absence of planktonic *Crustacea,* and the scarcity of plytoplanktonic forms. The abundance of resistant forms of the bottom fauna supports the polysaprogenic character of this section on the Nadel.

Zoo- and phyto-planktonic types, found individually in the biological tests, indicate a tendancy to self-purification in the direction of smaller contamination; most of them belong to types which are indicators of α-mezosaprogenic zone.

P o i n t 5. This section of the watercourse is overgrown with reed; large amounts of *Lemna* can be found on its surface. The water is of grey-greenish color, and is not odious. Large amounts of phyto-detritus were observed at the bottom.

The oxygen saturation amounts to 58.6 per cent; the oxygen content is 3.21mg/1 after forty-eight hours. The consumption of potassium permanganate is highly decreased (about four times) as compared to the preceding location. The dry residue is also decreased, and only traces of ammonia can be detected.

The zoo- and the phytoplanktonic organisms are not numerous, but the number of zooplanktonic types is considerably greater (ten types in all) than that which was found in the preceding location. The most numerous group is *Rotatoria* with six types, whereas other kinds appear only individually. The appearance of *Copepoda* is worth being emphasized. The phytoplankton is composed of a low number of *Diatomaceae.* The bottom fauna is also not numerous. *Oligochaeta* and *Asellida* were also found.

Hydrobiological and chemical analyses show that the process of self-purification gradually comes into its final phase

here, and that the indentified organisms belong to α-mezosaprogenic and β-mezosaprogenic zones.

Point 6. The banks of the Nadel are overgrown with reed at this location. The appearance of submersed flora is characteristic. The water is greenish and free from any odor.

No proof of the process of decay could be found. The oxygen saturation amounts to 70.2 per cent; the oxygen content is 6.10mg/1 after forty-eight hours. The consumption of potassium permanganate is smaller than in the preceding location, and the same is true for the dry residue. Ammonia is detected, but the alkalinity is less than at all preceding points.

The zooplankton is qualitatively and quantitatively rather rich. Thirteen types of netplankton were found. *Copepoda* and *Ostracoda* quantitatively dominate, and β-ms Rotatoria qualitatively. Only one type of *Cladocera (Chydorus sphaericus)* was founds. Altogether, 365 ind/1 of zooplanktonic organisms was determined. Phytoplankton is various but quantitatively rather poor. *Chlorophyces, Diatomaceae,* and *Desmidoaceae* predominantly appear. The phytoplankton is also of a mezosaprogenic type.

The bottom fauna is quantitatively rather poor, 5932.24 ind/m², but is composed of various types. The appearance of larva of insects with gills *(Ephemeroptera, Odonata)* is characteristic. In addition, *Chiromida, Oligochaeta* and *Asellida* were found.

Such a structure of population at this point of the watercourse and the biological investigations indicate that the process of self-purification is finished; there are no essential differences between this location and the location which precedes the reception of the waste waters.

Discussion and Conclusions

The results of chemical investigations show the characteristics and the intensity of the contamination of the Nadel watercourse and the individual phases of the process of self-purification, and thus enable us to draw some conclusions about the characteristics of the Nadel watercourse as a whole. A deficiency of oxygen found in the section which was not contaminated with waters, an increased consumption of potassium permanganate, a high content of chlorides and phosphates, a high alkalinity, and the dry residue all indicate the mineral character of the Nadel's water and a great quantity of phyto-detritus. Differences in the quality of water taken above the confluence of waste waters and 10.5 and 15.8 km below the source of contamination are probably due to the effect of the septic zone of contamination because the waste waters have destroyed the matrophytes in the septic zone and have in-

hibited their development downward in the stream; they may also have changed the character of the bottom.

The consideration of the results of hydrobiological tests offers the possibility of discovering some characteristics of the contamination and self-purification of the Nadel. Unfortunately, we could not establish all the phases of these processes. The absence of a degradation zone is characteristic, as there is no gradual transition from pure to contaminated water. The appearance of the septic zone represents an exceptional phenomenon; the small distribution of polysaprogenic and α-mezosaprogenic zone is also exceptional.

It is necessary to emphasize that the massy appearance of *Brachionus urceolaris* in the plankton at Point 4 is not in agreement with other hydrobiological and chemical findings. According to the tables given by *Kolkwitz* and *Marsson*, and *Śramek* and *Liebmann*, this type is characteristic of α-mezosaprogenic and α-mezosaprogenic-β-mezosaprogenic zones. Thus its occurrence would conform with the data from the literature, but its full domination does not. However, one of the authors has established numerous populations of this kind in intensely polluted waters, such as those from the hemp plant in Bezdan, in very polluted parts of the river Begej, in the Vizelja watercourse below the confluence of waste waters from the slaughter-house and the dairy, etc. Therefore, we believe that this and some other types of *Brachionus*, under Yugoslav climatic conditions, can occupy a polysapro-α-mezosaprogenic place in the sapro system. It is, at any rate, necessary to perform further and deeper idioecological investigation of the types of this family.

The establishment of these zones and their distribution is confirmed by hydrobiological and chemical results. However, besides the statement of full agreement of results obtained by both methods, it should be stressed that the conclusions drawn from biological tests differ from those obtained by chemical analyses. The results of chemical analyses are the reflection of an instantaneous state of contamination, and the conclusions concerning the process of self-purification could not be inferred from them. However, hydrobiological tests show the dynamics and the tendency of self-purification, and, consequently, they can replace an entire series of periodical and successive chemical analyses.

Parallel consideration of chemical and hydrobiological analyses enables us to infer, on the basis of the classification of contaminated zones given by *Kolkwitz-Marsson* and *Suter*, the general characteristics and specificities of the contamination and self-purification of the Nadel wetercourse. The contamination with organic waste waters from the starch industry results in a complete alteration of the hydrochemical regime and, consequently, of the composition of the living

population, so that below the influence of waste waters there arises a short septic zone, next to it a short polysaprogenic zone which is followed by a mezosaprogenic zone, and finally at a distance of 18.5 km from the sourse of contamination, the effect of waste waters disappears, and the Nadel returns to the state which corresponds to chemical and hydrobiological waters above the starch industry. According to the obtained results, the places which are not influenced by waste waters must be designated as a β-mezosaprogenic zone. Consequently, the Nadel is characterized by the absence of the oligosaprogenic zone, the zone of pure water which should be the final result of the process of self-purification.

All the specificities of the Nadel watercourse can be explained by its long lasting contamination with organic waste waters from the starch industry, and by the very type of the watercourse. In fact, the Nadel represents a shallow plain watercourse of an insignificantly slow rate, whose banks are densely overgrown (the quantity of free water is small), and whose water is of a mineral character.

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CONTENTS

GLASNIK HEMIJSKOG DRUŠTVA, vol. 27, no. 2-3, 1962, pp. 65-74.

THE EFFECT OF ULTRASOUND OF FREQUENCIES 800 AND 3000 kHz ON THE DECONTAMINATION OF SOME SURFACES CONTAMINATED WITH IODINE-131

by

J. GLIGORIJEVIĆ, B. PAVLOVIĆ and D. NOVČIĆ

Other than the classical decontamination methods which are, in many cases, still irreplaceable, attempts have been made in recent years, to apply ultrasound for decontamination (1, 2, 3). According to the cited papers, which mainly offer the results of previous investigations, it is evident that the ultrasonic method increases the rate and the degree of the decontamination of metal surfaces in comparison to other decontamination methods. However, the above papers do not treat the effect of ultrasound on the decontamination of surfaces of other materials, and they do not discuss the effect of ultrasound on the decontamination of surfaces which are contaminated with various radioisotopes. We have investigated the decontamination effect of ultrasound on various metals, synthetic materials, textile, and glass contaminated with iodine-131.

Experimental Part

a) *Apparatus for the decontamination.* The apparatus consists of two cylindrical glass vessels which are coaxially fixed to the projector (Fig. 1). The inner vessel, the diameter of which equals that of the vibrator, is shut on the bottom with a rubber membrane, and is placed on the vibrator. It is filled with about 50ml water or some other solution, and in it are placed the objects on which the ultrasonic decontamination is to be examined. The outer vessel has a diameter which corresponds to that of the projector, and contains two openings by which water is let in and out; water is necessary for cooling the inner vessel, which is exposed to ultrasound.

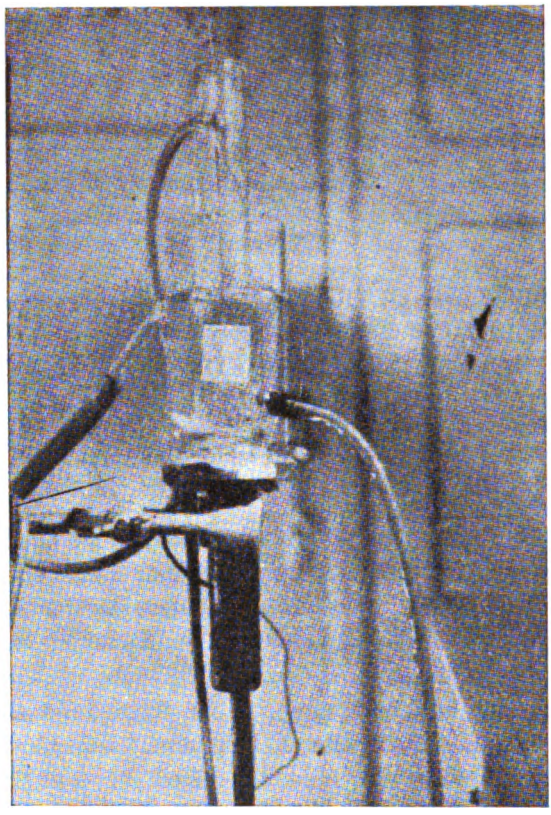


Figure 1

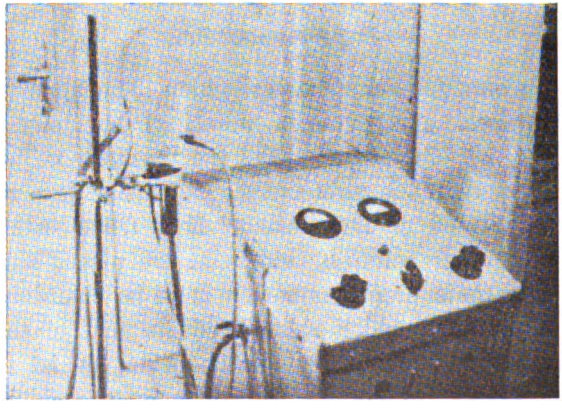


Figure 1a

b) *Ultrasonic genereter.* Experiments have been performed with piezoelectrical ultrasonic generator of home-made production, at frequencies of 800 and 300 kHz and at intensities 0.5, 1 and 1.5 W/cm², determined by help of the colorimetric method. The quartz vibrator, which has a diameter of 3 cm, is inserted into the metallic part of the projector. The projector is fixed to the handle-trasmitter of high frequencies and it must be kept horizontal during operation.

c) *Samples.* Rectangular metallic disks, 30 × 20 mm, of a thickness of 0.2 to 2 mm, have been used in the investigation of the action of ultrasound on the decontamination of surfaces contaminated with iodine-131. Experiments have been performed with aluminium, iron, zinc, lead, nickel, copper, and brass. Their surfaces were well cleaned and polished*. Synthetic materials were also cut in the form of rectangles. Plexiglas, pertinax, polyvinyl, and rubber have been examined. The samples of textile were cut into squares 15 × 15 mm; cotton cloth, woolen cloth, jersey cloth, and nylon cloth have been investigated. Glass used in the decontamination experiments was of tubular profile, fully cylindrical in shape, and flat.

d) *Iodine-131 and the contamination of samples.* The contamination was performed with an alkaline solution of KJ^{131} (pH 8). This solution was poured in drops on clean and greaseless material, and was dried with the help of an infrared lamp.

e) *Registration of the activities of contaminated and decontaminated samples.* The activities were measured by a GM ring-shaped counter tube, type TGC, attached to the scalar-SK-1 produced by PAE (Belgrade). The activity of the contaminated sample was measured before being placed into the inner vessel of the apparaturs. Immediately after the exposure to ultrasound, the activity of the decontaminated sample was recorded. From these data, we obtained the decontamination effect $D = \frac{A_0 - A}{A_0} 100$, where A_0 represents the initial activity and A represents the activity recorded after decontamination.

f) *Decontamination procedures.* Samples of contaminated materials were joines by a thread, and they hung freely in an upright position inside the inner vessel; they were placed at the desired distance from the vibrator, and were exposed to ultrasound for

* Discs with polished surfaces have given better a decontamination effect than the unpolished ones, and the results were reproducible.

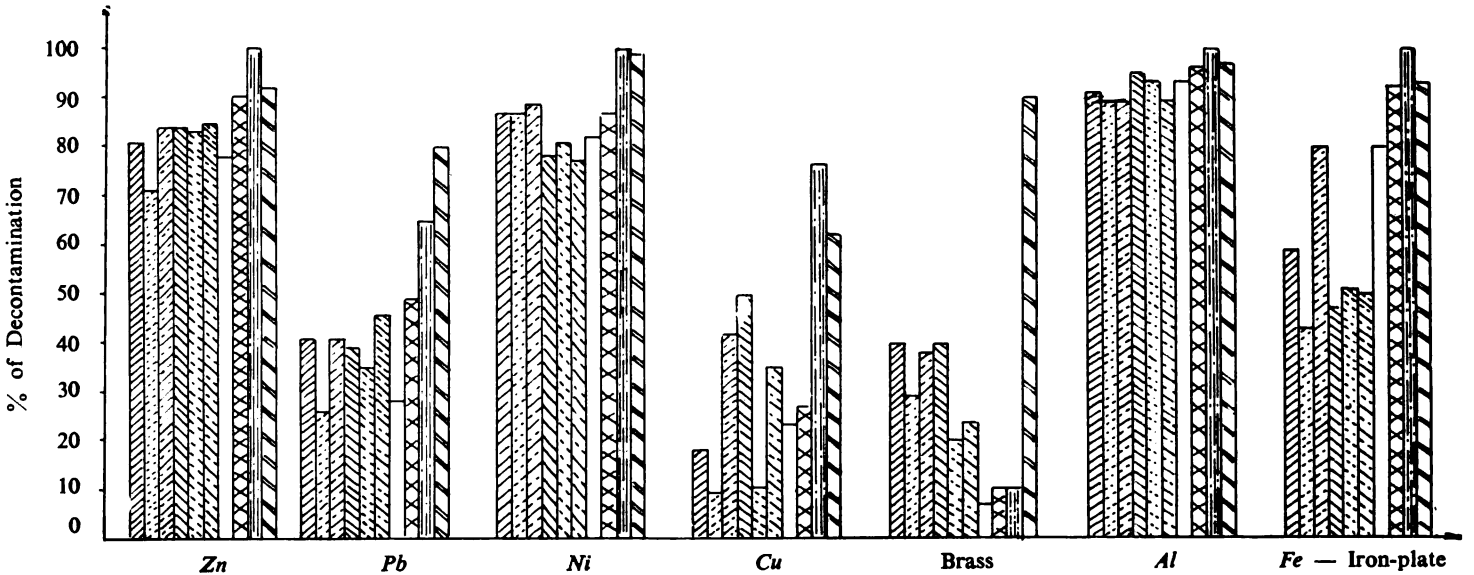


Diagram 1. Decontamination effects on metals

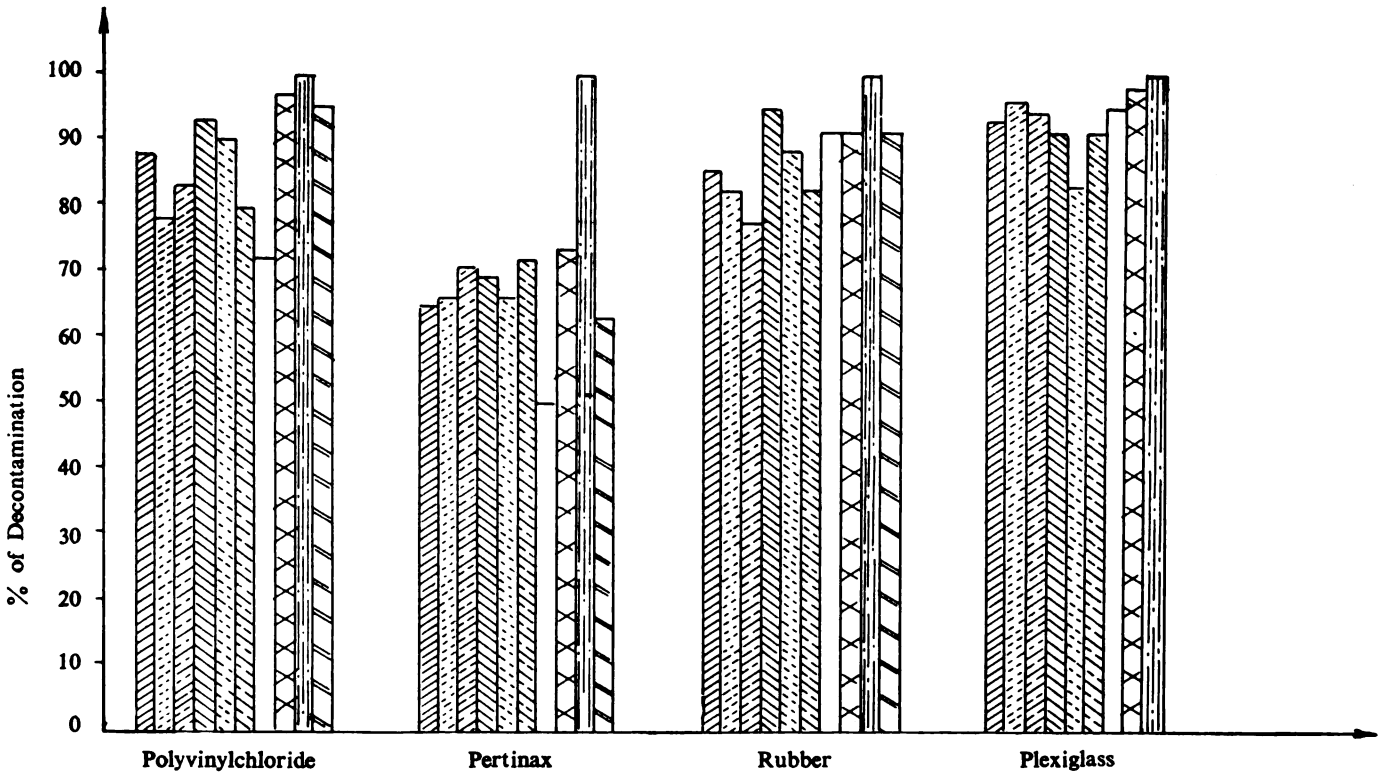


Diagram 2. Decontamination effects on artificial materials

five minutes. Water and detergent "Deteron" were used as fluids in which the exposures have been performed. The investigation was performed under static and dynamic conditions. When the decontamination was performed under static conditions, the amount of liquid in the inner vessel was not altered during exposures, but under dynamic conditions, the fluid flowed through the inner vessel.

Results of Investigation

The results obtained in the decontamination of various metals under different experimental conditions are given in Diagram 1. Although the ultrasonic decontamination effect was different for

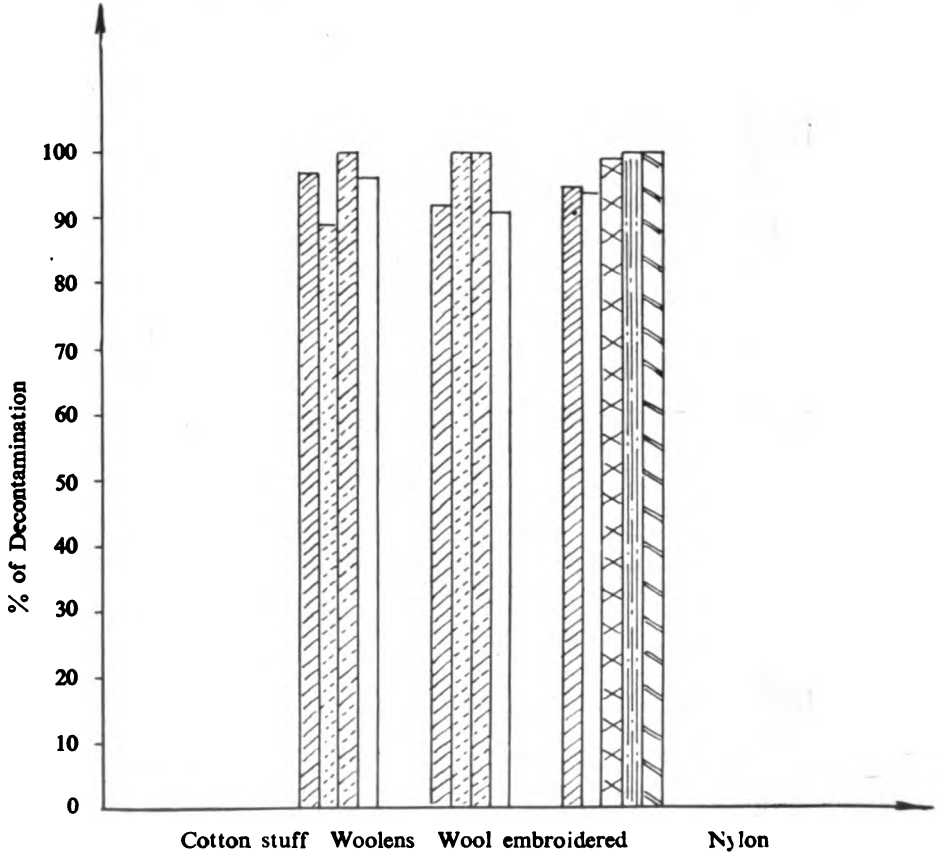


Figure 4. Decontamination effects on textile

each of the investigated metals, it has been found that the more the metal was decontaminated with water, the greater was the decontamination effect of the ultrasound. For example, the decont-

amination effect of water on copper was 23 per cent, and that of ultrasound, 50 per cent; but the decontamination effect of water on zinc was 78 per cent, and that of ultrasound, 90 per cent.

Diagram 2 shows the results obtained in the decontamination of some synthetic materials under various experimental conditions. It has been found that polyvinyl chloride splits when exposed to

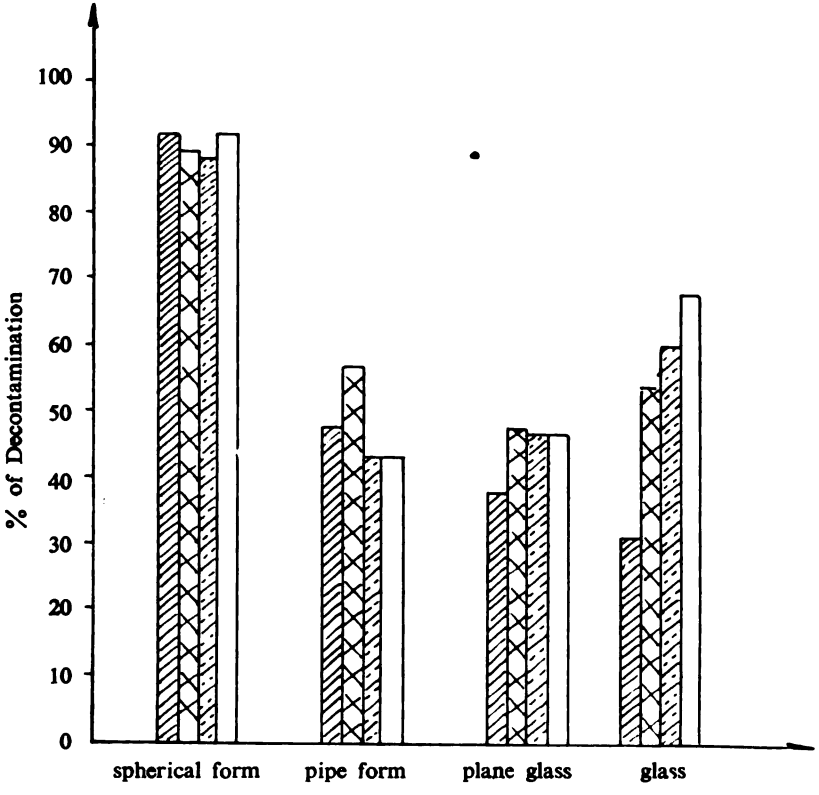


Figure 5. Decontamination effects on glass

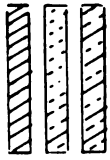
ultrasound of an intensity of $0 \cdot 5\,W/cm^2$. The areas of pertinax surface contaminated with iodine-131 are found to change color when exposed to the action of ultrasound.

The results obtained in the decontamination of textile are shown in Diagram 3. Under given experimental conditions, the decontamination effect of ultrasound is not considerably greater than that of water. The only exception was found with cotton cloth.

Diagram 4 gives the results obtained in the decontamination of glass; it shows that glass can be fully decontaminated from iodine-131 by the action of ultrasound.

Upon comparation of the results of the decontamination under static and dynamic conditions, we have established that there is no difference in the decontamination effect. Similarly, the exposures of longer than five minutes to ultrasound have not afforded better decontamination of samples. Accordingly, it is evident that the investigation of the decontamination with water after exposure to ultrasound could not give any effect.

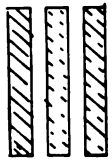
The improvement of the decontamination effect of ultrasound on all materials under investigation was obtained when exposures were performed in a 2 per cent solution of a detergent. In some



Legend for fig.

Decontamined with U-Z in water:

1. 800 *kHz*; 0,5 *W/cm²*
2. ,, ,, 1,0 ,,
3. ,, ,, 1,5 ,,



1. 300 *kHz*; 0,5 *W/cm²*
2. ,, ,, 1,5 ,,
3. ,, ,, 1,5 ,,

Decontamined with water for 1 hour

Decontamined with water 1 hour; then treated by ultrasound

Decontamined with ultrasound in water; material has been previously treated with the solution 1 % *KJ*

Decontamined with ultrasound in 2 % water solution of detergent

instances, this effect was greatly increased, as in the case of lead, copper, brass, and iron tin. However, the decontamination of pertinax gave contrary results. When each of these materials was decontaminated with a 2 per cent solution of detergent without being exposed to ultrasound, the decontamination effect was not always found to be greater than that obtained with water (4). Interesting phenomena have been observed with materials which were previously dipped into a solution of inactive KJ and then contaminated. In these cases, the action of ultrasound on the decontamination was further improved.

Interpretation of results

It is known that the decontamination of some surfaces is mainly due to the physical adsorption and, to a smaller extent, the chemisorption at the boundary surface of phases. Accordingly, the process of decontamination is similar to the phenomenon of desorption; therefore, when the contaminated surface is brought into contact with fresh amounts of liquid, the concentration of radioisotope in the boundary surface is decreased. However, a certain number of atoms or molecules located at the active surface centers cannot be removed by a simple desorption procedure. The removal of chemisorbed atoms and molecules is even more difficult because they are attached to the surface by chemical forces, not by physical ones, as in the case of physical adsorption. When the decontamination is performed by means of ultrasound, cavitation phenomena occurs at the boundary surface of phases. The cavitation predominantly exerts a mechanical effect on the surface, causing a greater rate of the liquid particles at the boundary surface liquid-solid, and consequently leads to a rapid removal of the radioisotope from the contaminated sample. The ultrasonic forces are not always of greater intensity than the chemical forces. Therefore, a number of atoms which are chemisorbed at the surface of the solid phase cannot be removed from the sample by the action of ultrasound. In this way, we can explain why the action of ultrasound of given frequencies and intensities has not brought about the decontamination of lead, copper, brass, iron tin, pertinax, and textile (woolen and nylon cloth).

Since lead and copper combine with iodine to give insoluble compounds, it is very likely that the surfaces of lead, copper, and brass (copper alloy) chemisorb iodine during contamination. However, iron does not give an insoluble compound with iodine; the

dissatisfactory decontamination effect of ultrasound in this case can be explained by assuming that iodine ions are bound to the iron surface by adsorption forces which are stronger than those of other examined materials. In regard to the action of iodine ions on pertinax, it is evident that chemical reactions occur at the pertinax surface because the color of contaminated areas is changed by the action of ultrasound. The molecules of wool and nylon contain a series of groups which can react with iodine ions. The reactivity of these groups can be increased by ultrasound, although the decrease of the decontamination effect in the case of nylon can be due to other factors. It is feasible that polymeric nylon chains, when exposed to ultrasound, are moved apart so that iodine can enter the structure of the nylon fiber.

The explanation of the phenomenon that samples dipped in a solution of inactive KJ and then contaminated are better decontaminated by the action of ultrasound is based upon the assumption that inactive KJ occupies the most active centers of the surface. The adsorption is known to occur first in the most active places. When these centers are occupied, only less active places are available for the contamination, or the adsorption may take place in the so-called secondary layer. It is evident that the desorption of ions from the secondary layer does not require forces equal to those which are necessary for the desorption from the primary layer.

The surface tension of the boundary surface of two phases has a definite value; the addition of detergent decreases this tension. Consequently, the attractive force between the solid and the ions of the adsorbed compound is also decreased. When such a solution containing detergent is exposed to ultrasound, the ultrasonic mechanic effect is more pronounced than when ultrasound acts upon water as a liquid phase.

It is necessary to emphasize that the above investigations have been performed at only two frequencies and with ultrasound of a small range of intensity so that adequate conditions have not been found for each material. There is no doubt that investigations covering a greater range of frequencies and intensities would make possible the adjustment of resonance conditions of ultrasonic oscillations and oscillation of the system; and a molecule at the surface of the solid — adsorbed molecule (or atom), which would give rise to different decontamination effects.

CONCLUSION

1. Ultrasound of frequencies 800 and 300 kHz and of intensities 0.5, 1 and 1.5 W/cm^2 affect the decontamination of some surfaces contaminated with iodine-131, and increase the decontamination rate in regards to decontamination with water.

2. The maximal decontamination effect of ultrasound is reached within a maximum of five minutes. The ultrasonic decontamination effects in an aqueous medium are:

a) glass	100	i) iron tin	80
b) plexiglas	96	j) pertinax	72
c) aluminium	95	k) nylon cloth	60
d) rubber	95	l) woolen cloth	57
e) polyvinylchloride	93	m) copper	50
f) cotton cloth	92	n) woolen jersey	48
g) nickel	89	o) lead	46
h) zinc	85	p) brass	40

3. It has been established that under the given experimental conditions the decontamination effect on textile is not satisfactory.

4. The ultrasonic decontamination effect on rubber and pertinax, obtained within five minutes, is not pronouncedly greater than the decontamination effect of water after one hour of washing.

5. The decontamination effect on polyvinylchloride is not satisfactory; moreover, polyvinylchloride splits when exposed to ultrasound of high intensity.

6. The decontamination of aluminium, nickel, and zinc by ultrasound gives satisfactory results.

7. The decontamination of lead, copper, and brass by the action of ultrasound does not afford satisfactory results.

8. The ultrasonic decontamination, when performed in a 2 per cent solution of detergent, affords better decontamination effect than when performed in water alone.

9. Exposures to ultrasound for a period longer than five minutes do not increase the decontamination effect, except in the decontamination of iron tin.

10. The decontamination effect is not dependent upon the shape of the sample under investigation, but upon the kind of material of which it is made and the manner in which its surfaces are worked up.

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MASS TRANSFER ONTO ANODICALLY OXIDIZED ALUMINUM UNDER CONDITIONS OF NATURAL CONVECTION

by

MITROVIĆ, M. V.

In a number of papers by S. Končar-Đurđević and coworkers as well as in our work (8), the adsorption method with a thin film of silica gel as adsorbent was used to study some flowing phenomena. In order to investigate the possibility of application of the aluminum oxide film, obtained by the anodic oxidation of aluminum as the adsorbent, we have studied in this work the most convenient methods of anodic oxidation, the materials to be adsorbed, the methods of determining the quantity of adsorbate, the time of adsorbtion, the temperature, and the quantity of the adsorbate transfered onto the unit surface of the adsorbent. Adsorption was studied in a motionless fluid, i.e. under the conditions of molecular diffusion and natural convection of the adsorbate from the bulk of the fluid onto the surface of the adsorbent.

The adsorbent obtained by the anodic oxidation of aluminum is suitable for the study of the mass transfer from solutions onto solid surfaces for the following reasons: (i) the adsorbent is in the form of a thin layer of defined thickness; (ii) the adsorbent is transparent, while the carrying metal is white, so that the quantity of the adsorbed matter can be determined by colorimetry in reflected light; (iii) the surface is mechanically very sturdy; (iv) the surface of the aluminum oxide can be of any desired roughness; (v) the aluminum oxide obtained by the anodic oxidation of aluminum is porous and has good adsorptive characteristics (9,11); (vi) it is possible, if the aluminum is of the same purity and all other conditions are equal, to obtain an adsorption layer of the same characteristics; (vii) according to our investigations, the adsorptive characteristics of the oxide layer do not change with time if the layer of the adsorbent is artificially aged.

Experimental Part

a) *Aluminum*

In this work aluminum foil of 0.4 mm thickness and 99.6% purity was used. For a small number of experiments the foil was mechanically polished, while for the rest it was sand blasted. This pretreatment was used since the measurement of the quantity of the adsorbed dye required the diffuse reflection of light from the surface. In addition, the surface of the metal, even after the anodic oxidation of the oxide, is greater, and this is more convenient for the determination of the adsorbed quantity of matter per unit of the projected surface. Before the anodic oxidation the foil was etched in 5% sodium hydroxide and concentrated nitric acid, to remove the grease and traces of other metals from the surface. The anodic oxidation was always done in 20% sulphuric acid, at 20 — 22°C, and with the current density of 1 A/cm^2. According to the data in the literature (9) and our experiments, these conditions appeared to be the most convenient. The oxidations lasted for 30 minutes. After the anodization, sheets made of foil were washed and dried for 30 minutes at 50°C. This caused the partial dehydratation of the oxide and a small decrease of adsorptivity, but on the other hand, the oxide layer retained constant adsorptive characteristics. Thus, the results of the measurements were independent of the duration of the experiment. We investigated the differences in the quantity of adsorbed dye per surface unit, immediately after this artificial aging of the oxide, and after 5 days. The differences we found were in all cases less than 1%.

b) *Adsorbate*

The adsorbate to be used for mass transfer determination should satisfy the following conditions: (i) the color of the adsorbate should be dark, preferably blue or black, in order to obtain the most reliable colorimetric determination; (ii) the adsorbate should be sufficiently chemically stable in order to get quantitatively in the solution after the dissolution of the oxide layer; (iii) adsorption of the adsorbate should be irreversible, in order to avoid losses of it in later manipulations with the adsorbent; (iv) the adsorbate should have a very small diffusion coefficient in water solutions in order to make adsorption process as slow as possible. We checked several special organic dyes for the dyeing of the anodically oxidized aluminum, made by "Dürand & Hygenin".

Using Fürt's method, we determined the diffusion coefficient of this dye. In water solution for a dye concentration of 0.5% the mean value of this coefficient was $1{,}40 + 0.05 \times 10^{-6} cm^2/sec.$, at 20°C. The diffusion coefficients of the same dye were also determined for the concentration of 0.1 and 0.01%, and the coefficient was the same for 0.1% solution, while for the 0.01% solution we could not obtain reliable results, even when the order of magnitude was correct. The diffusion coefficient we obtained is of the same order of magnitude as the literature values of the diffusion coefficients of colloidal water solutions.

c) *Adsorption procedure*

For the mass transfer study during the adsorption we used anodically oxidized aluminum plates with the following dimensions: $40 \times 40 \times 0.4$ mm. The dye was dissolved in distilled water. The plates were kept vertically hanging in the motionless solution, so that the adsorption took place evenly on both sides. In all the experiments the temperature was constant, being 10—20°C. Before the plates were introduced into the solution of dye, they were wet with water, in order to prevent a fast initial penetration of dye inside the oxide layer. The adsorption of dye on the wet plates was always more regular, even if the difference in adsorption between the wet and dry surfaces was not great. For adsorption times up to 1 minute, the differences were 3 — 7%. For times over 3 minutes we could not find any measurable difference. The concentrations of the dye solutions used were 1, 0.5, 0.1. 0.05 and 0.01%. Adsorption times varied between 15 seconds and 24 hours. It was impossible to work with shorter times than 15 seconds, since the errors due to the time necessary for introducing and taking out the plates from the solutions were great. After adsorption of dye, the plates were washed and dried in air at the normal temperature.

d) *Determination of the quantity of adsorbed dye*

In the determination of the quantity of transfered mass per unit surface of the adsorbent, we started with the assumption made in the work of S. Končar-Đurđević and coworkers (1—7): that the difference between the quantity of reflected light from the adsorbent before and after adsorption depends directly on the quantity of the adsorbed matter, if the adsorbent is white and if the light reflects diffusely. In these papers this assumption appeared to be partially correct, since the adsorbent they used (silica gel) had very small transparency. Thus, the dye adsorbed inside the layer gave a different

effect of light adsorption from that adsorbed on the surface of the layer. In our work we expected a smaller influence from this effect, since the oxide layer has good transparency (9) and the aluminum surface under the oxide reflects light well. Here, we used sand blasted surfaces in the measurements of the diffusely reflected light, since the quantity of reflected light from smooth surfaces depended considerably on the quality of the surface treatment of the aluminum, the incident angle of light, and the surface roughness. The influences of these factors are much smaller for sand blasted surfaces, and the errors due to irregularities of the plate surfaces were less than 2% for the plates before the adsorption of dye. In order to avoid these errors, too, before each series of experiments plates were selected so as to show deviations

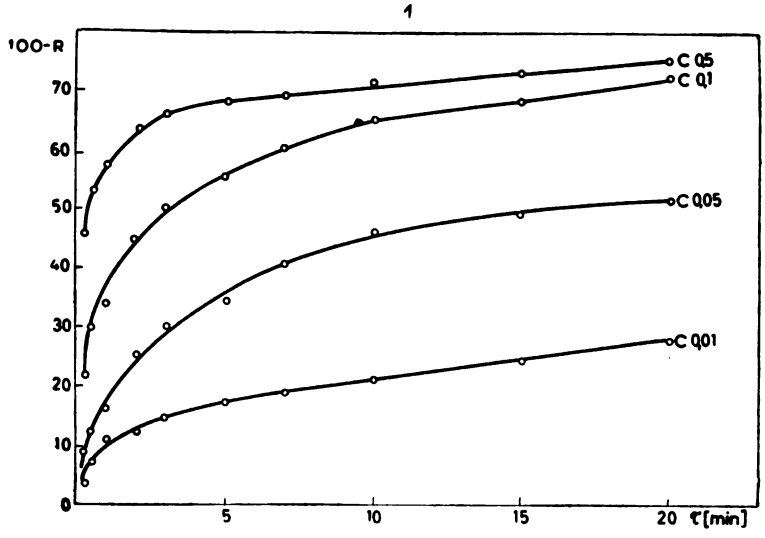

Figure 1.

Relatioship between the reflection of light from the aluminum plates and the time of adsorption, for various concentrations of adsorbate. (Weight percents)

in reflection of. 0.5% or less, from the mean reflection of the standard plate. The results obtained by colorimetry in reflected light, using the plate surfaces with the adsorbed dye, are given in Figure 1.

Verification of the assumption that the quantity of adsorbed light is proportional to the quantity of diffusely reflected light was done on the same specimens, by measurements of the quantity of the previously adsorbed dye in the solution, obtained by the quantitative dissolution of the oxide layer in 1% solution of sodium

hydroxide. In this operation, together with the oxide, the whole quantity of adsorbed dye went into the solution. The concentration of dye was determined by means of the Lange universal colorimeter. Since the change in the pH of the solution and the presence of aluminum ions in the solution slightly change the shade of the color, from black to violet, the same quantity of sodium hydroxide and approximately the same quantity of aluminum hydroxide were added to the solution of a known concentration, used

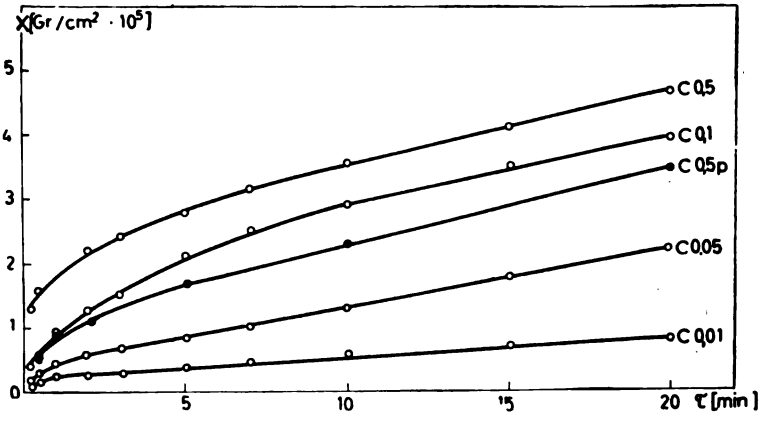

Figure 2.

Relationship between the quantity of adsorbate per surface unit of adsorbent and time of adsorption, for various concentrations of adsorbate. (C — weight percents).

for the calibration of the colorimeter. The results of measurements of the quantity of adsorbed dye by the method of dissolution of the oxide layer are given in Figure 2. Curve 0. 5p was obtained by the dissolution of the oxide from the polished surface, and it is translatorily shifted in respect to the curve obtained by the dissolution of the oxide from the sand blasted surface, for the same concentration of dye.

e) *Discussion*

From Figures 1 and 2, showing the dependence of the quantity of adsorbed matter on the time of adsorption and the concentration of dye in the solution, we see that the initial parts of the curves are nonlinear for all the concentrations of the adsorbate. This can be explained by the irregular adsorption in the beginning, when the adsorbate is penetrating into the body of the adsorbent, or while it is occupying the most active centers. Therefore, in our opinion, the linear parts of the curves, next to the nonlinear

parts, are the most appropriate for the study of the mass transfer by the adsorption method. These could be used in any system, i. e. in a motionless fluid or when the fluid flows over the surface of the adsorbent. We can assume in this case that the velocity of adsorption is constant and we can consider adsorption as a stationary process, the final velocity of which is determined only by the diffusion in the boundary layer between the adsorbent and the solution. The initial nonlinear parts of these curves always come into the measurement of the quantity of the adsorbed matter as a constant additive value, depending only on the concentration of the adsorbate in the solution, and on the quantity of the adsorbent. In our opinion, entirely stationary conditions for the mass transfer from the solution onto the surface would be obtained if before the experiments the specimens had adsorbed dye in quantities corresponding precisely to the linear parts of the curves. From Figures 1 and 2 we see that curves obtained by adsorption from 0.01 and 0.05% solutions of dye have the longest linear parts, and that, for the times used in these experiments, there is no tendency towards saturation of the adsorbent. Hence, in order to be able

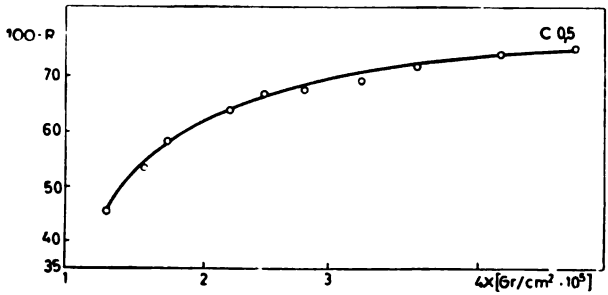

Figure 3.

Relationship between the light reflection from the aluminum plates and the quantity of adsorbed dye per surface unit of adsorbate, for a dye concentration of 0.5 %. (C — weight percents).

to take adsorption on aluminum oxide as a stationary process, it is necessary to work with as dilluted as possible a solution of adsorbate. However, this is limited by the sensitivity of the colorimeter and the quantity of impurities, which always appear during the dissolution of the aluminum oxide. Thus, the most convenient concentrations, in our opinion, are between 0.01 and 0.1%.

Figures 3 and 4 show the dependence between the quantity of reflected light and quantity of adsorbed dye per surface unit, for the particular points from Figures 1 and 2. The dependence

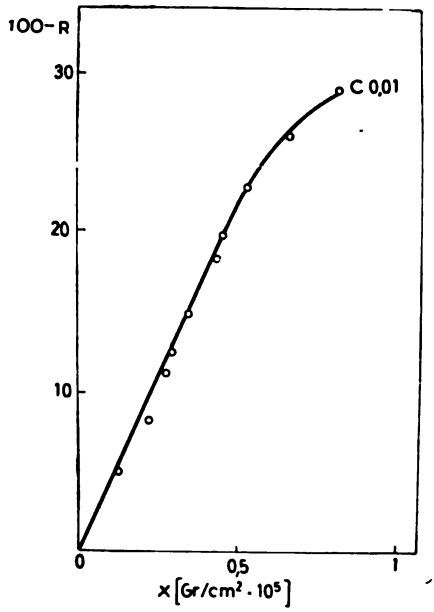

Figure 4.

Relationship between the light reflection from the aluminum plates and the quantity of adsorbed dye per surface unit of adsorbate, for a dye concentration of 0.01 %. (C — weight percents).

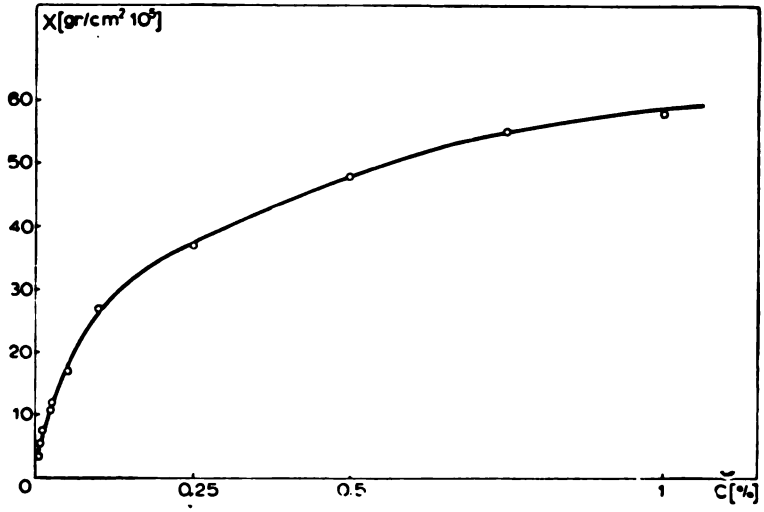

Figure 5.

Adsorption isotherm for the adsorption of ANOXYDAL black dye on anodically oxidized aluminum plates, at 20 °C. (C — weight percents).

is linear for the smaller quantities of adsorbed dye, while for the greater quantities of adsorbed dye colorimetric determination in the reflected light gave smaller values. This can be explained by the coverage of certain dye layers in the adsorbent and by the increased influence of the light reflection from the upper surface of the adsorbent. According to that, one can expect, when a dark color of dye is used in adsorption on aluminum oxide, that the method of measurement of the reflected light is reliable only for small quantities of transfered dye.

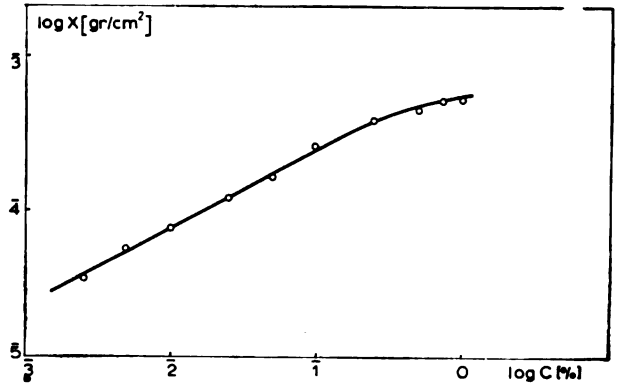

Figure 6.

Adsorption isotherm for the adsorption of ANOXYDAL black dye on anodically oxidized aluminum plates, at 20 °C, in logarithmic coordinate system. (C — weight percents).

Figure 5 shows the adsorption isotherm of the same dye for adsorption on anodically oxidized aluminum. It was determined at 20°C. The time of adsorption was 24 hours, sufficient for attaining equilibrium between the solution and the adsorbing oxide layer. Anodically oxidized aluminum plates of the same size and the same quality as in the previous experiments were used. The slope of the adsorption isotherm is great up to concentrations of approximatively 0.1%, while further on, it decreases relatively suddenly. In Figure 6 the same isotherm is given, but in log/log system. The obtained line is a straight line for the smaller concentrations of dye, showing that the shape of the isotherm corresponds to Freundlich's type of adsorption isotherms, as could be expected.

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THE DETERMINATION OF SMALL AMOUNTS OF COPPER WITH DITHIZONE IN PURE TIN METAL AND BEARING METAL

by

VASILIJE B. GOLUBOVIĆ and BELA B. KAŠANJI

The determination of small amounts of copper, present as a contaminant in pure tin metal, by the method of Schürmann and Blumenthal (1) is performed by d.ssolving 10—50 g of tin metal into a mixture of hydrobromic acid and bromine, treating the obtained solution in the usual way and precipitating the present copper, lead, zinc, bismuth, and silver with a 2 per cent solution of sodium sulfide. Without giving the details of the normal course of the analysis and further systematic treatment of the sulfides of these elements, copper is either electroanalytically (1) or photometrically (2) determined from the obtained solution.

This method of the determination of copper requires a rather long period of time, and we have therefore attempted to simplify the procedure by applying the well known method for the determination of copper with dithizone in silicate rocks, but with some necessary modifications (3).

Bearing in mind that the presence of large amounts of tin causes some difficulties in copper extraction (4), the dissolution of tin metal was not performed with a mixture of perchloric and nitric acid but rather with a mixture of hydrobromic acid and bromine. Unlike the procedure of Schürmann and Blumenthal (1), the analysis was performed with 1 g of sample, which, after being dissolved in the above mixture, was treated with nitric acid (1 : 1) and evaporated to dryness. This operation was repeated once with nitric acid and twice with hydrochloric acid. The obtained solution was diluted with 50 ml distilled water, and then a 5 ml 10% solution of sodium citrate and dropwise concentrated ammonia

were added until the appearance of the green color of thymol blue (pH 8.5). This treatment was followed by the preliminary extraction of copper with a 0.001% solution of dithizone in carbon tetrachloride (5) where iron remains in the basic citrate solution, and lead and copper are found in the carbon tetrachloride layer.

The extract is treated with 0.01 N hydrochloric acid or with 0.1 N to 0.5 N hydrochloric acid if silver, bismuth, and zinc are present, where the dithizonates of these metals which interfere with the copper extraction are decomposed, giving the corresponding chlorides in the aqueous phase. The extract contains only copper which is determined by an extractive titration of 1/25 aliquot portion of the original volume with a 0.001% solution of dithizone in carbon tetrachloride.

The results obtained in the determination of copper in two samples originating from India are given in Table 1.

TABLE 1

Sample	Sn %	Cu %	Taken Cu γ	Found Cu γ	Average value Cu γ	Difference γ
1	99.2	0.015	6.00	6.15 6.19 6.21	6.19	+0.18
2	99.905	0.022	8.80	8.80 8.65 8.55	8.66	−0.14

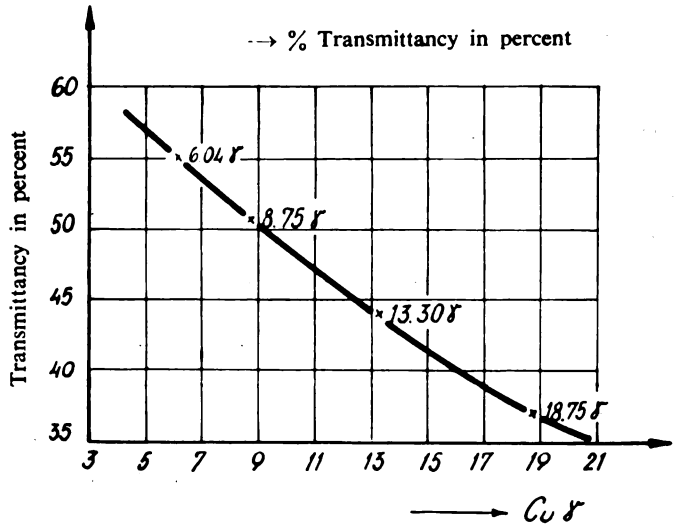

Figure 1.

The samples were treated analogously to the previous procedure in the spectrophotometrical determination of copper. After the preliminary extraction, the obtained cupric dithizonate was decomposed with a mixture of sulfuric and perchloric acid (6), the reaction mixture was then evaporated almost to dryness, and the cooled residue was dissolved in 0.1 N hydrochloric acid. The obtained solution was transferred to a volumetric flask and made up to 250 ml with the acid of the same strength. The analyses were performed with 10 ml of this solution of dithizone in carbon tetrachloride, and after necessary preparations (5), the measurements were performed with spectrophotometer model "Unicam" Sp. 600 in cuvettes of 1 cm^3, at a wavelength 510 μm. The standard copper curve (Fig. 1) was prepared with solutions of $CuSO_4 \cdot 5H_2O$ containing 8, 10, 13, and 16 γ Cu in 10 ml. The obtained results are shown in Table 2.

TABLE 2

Sample	Sn %	Cu %	Taken Cu γ	Found Cu γ	Difference γ
1	99.92	0.015	6.00	6.04	+0.04
2	99.905	0.022	8.80	8.75	−0.05

These results represent the mean values obtained from three successive measurements for each sample.

In addition to the foregoing, we have investigated the possibility of determining copper with dithizone in some other technical materials such as bearing metal. Although the existing procedures, volumetric (7), electrogravimetric, (1) and photometric (2) afford relatively good results, our opinion is that the above method of determining copper with dithizone is more simple and, to a certain extent, more rapid.

The procedure is as follows: 1 g of alloy in the form of turnings is dissolved in 20 ml concentrated sulfuric acid, the solution is cooled, and then 100 ml distilled water and 5 ml conc. hydrochloric acid are added; the reaction mixture is left to stand for one to two hours, whereby lead sulfate precipitates. After filtration, the filtrate is transferred to a volumetric flask and made up to 500 ml with distilled water; to 80 ml of this solution taken in a 250 ml beaker, about 1 ml hydrogen peroxide is added in order to oxidize the present iron, and the reaction mixture is boiled to remove the excess of peroxide. Next, 5 ml of a 10%

solution of sodium citrate is added to the cooled solution, and this is followed by the treatment identical for the determination of copper in pure tin metal; the solution obtained after the decomposition of cupric dithizonate and the excess of dithizone is transferred to a volumetric flask and made up to 100 ml with distilled water. Ten ml of this solution was taken for analysis.

The results obtained by extractive titration with two samples of bearing metal are given in Table 3.

TABLE 3

Sample	%	Cu %	Taken Cu γ	Found Cu γ	Average value Cu	Difference γ
1	Sn 18.35 Pb 67.64 Sb 12.00 Zn 0.66 Fe 0.005	1.33	13.30	13.26 13.21 13.22	13.21	−0.07
2	Sn 40.05 Pb 46.80 Sb 11.26 Zn — Fe 0.004	1.88	18.80	18.68 18.75 18.70	18.71	−0.09

The results of the spectrophotometric copper determination in these two samples of the bearing metal are obtained from the standard curve shown in Figure 1, and they are given in Table 4; this procedure is analoguous to the one preceding.

TABLE 4

Sample	Cu %	Taken Cu γ	Found Cu γ	Difference γ
1	1.33	13.30	13.30	0.000
2	1.88	18.80	18.75	−0.05

The analyses of pure solutions whose compositions corresponded to various types of bearing metal have shown that sample 2, having approximately the same composition as the alloy WW 42 DIN 1703, represents the maximal permissible concentration of tin which does not interfere with the given determination of copper with dithizone. In cases of higher tin concentrations, the samples should be treated similarly to pure tin metal, i.e., they should be dissolved in a mixture of hydrobromic acid and bromine.

All analyses were performed with pro analysis reagents.

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IODOMETRIC AND IODATOMETRIC DETERMINATION OF HYDRAZINE BY THE DEAD-STOP TECHNIQUE

by

MOMIR S. JOVANOVIĆ, DUŠANKA M. PETROVIĆ, and DOBRILA M. PAVLOVIĆ

The dead stop technique of end-point detection in a titration was accidentally discovered by Foulk and Bowden (1); it represents a special case of the application of bimetallic electrode systems to electrometric titrations.

The indicator circuit which is used in this simple method consists of a source of electric current, a rheostat, an electrical measuring instrument, and two identical platinum electrodes which are placed in the solution to be examined. It is believed that the phenomenon of dead-stop end-point can be partly explained by oxidation at the anode, and by reduction of the indicator system at the cathode (2). When the potential difference between these electrodes is low, a microelectrolysis of the aqueous solution occurs so that anode is enveloped in a cloud of oxygen, and the cathode in a cloud of hydrogen. The presence of oxygen at the anode and hydrogen at the cathode is considered to be a result of their absorption on platinum. However, if the solution contains a reducing agent, the anode will be depolarized, but the cathode will remain polarized. Under such experimental conditions, no current will flow through the solution at a potential difference of some tenth mV. The addition of an oxidizing agent, e. g., iodine solution at the moment when the last trace of the reducing agent has reacted, will result in the depolarization of the cathode (the anode will be depolarized by the presence of iodide ions), and a current will start to flow through the solution; its strength is increased by further additions of iodine.

As we have been interested for many years in the analytics of hydrazine, we have attempted to execute its determination by

iodometric (3) and iodatometric (4) methods, detecting the end-point of the titration by the dead-stop technique. Apart from our personal interest, we have undertaken this investigation because the end-point of the titration detected by means of starch (iodine method) deviated to a certain extent from the true end-point; but we failed to determine hydrazine with sufficient accuracy by the iodate method, which was reported in the literature.

Iodometric Determination of Hydrazine

Apparatus

The indicator circuit consists of two Ni-Cd cells in series, connected potentiometrically with a rheostat of 500 Ω. Thus, the desired potential difference between the indicator-electrodes could be applied; the electrodes were made of platinum plates, 10 × 5 mm. The detecting instrument was a millivoltmeter with a range of readings of 50 mV, connected as shown in Fig. 1. A voltmeter, being connected parallelly, was able to register minute alterations of the current strength which took place at the end-point of the titration.

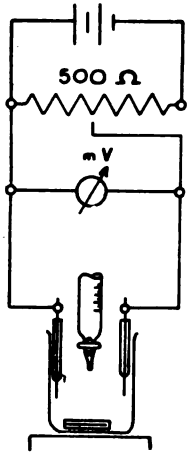

Figure 1

Analyses were performed in a 150 ml beaker, and the solutions were vigorously stirred with a magnetic stirrer.

Solutions

The solution of $N_2H_4 \cdot H_2SO_4$ (B. D. H., Analytical Reagent) was standardized by the widely applied iodometric method given by Stolle (3), from which it was found that 25 ml of $N_2H_4 \cdot H_2SO_4$ solution consume 22.90 ml N/50 iodine solution (F = 1.0886). The same iodine solution was used in electrometric determinations.

On the basis of the this data, it was established that:

$$T_{N_2H_4 \cdot H_2SO_4} = 0.6488 \text{ mg/ml}$$

Procedure

A measured volume of hydrazine sulfate solution was diluted to about 100 ml, and approximately 1g sodium carbonate was added. The electrodes were then placed into the solution, and the external circuit was adjusted so that the potential difference was 10 — 15 mV; next, the titration was started. As the end of the

titration approached, the needle of the detecting instrument started to show deflections, returning however, to its rest position when the reaction was terminated and the potentials at electrodes were equilibrated. At the end-point of the titration, the cathode was depolarized — the anode was polarized throughout the titration — so that a stronger current flew through the solution. This caused a permanent deflection of the detecting millivoltmeter needle from the value of its rest position, to a value close to zero.

Table 1 illustrates some of the results obtained from a series of thirty determinations.

TABLE 1

No	$N_2H_4 \cdot H_2SO_4$ ml taken	J_2 ml consumed	$N_2H_4 \cdot H_2SO_4$ ml found	Difference ml
1*.	1.55	1.44	1.57	+0.02
2*.	1.55	1.42	1.55	0.00
3*.	1.55	1.41	1.54	−0.01
4**.	7.70	7.12	7.76	+0.06
5**.	7.70	7.05	7.69	−0.01
6***.	15.40	14.20	15.48	+0.08
7***.	15.40	14.12	15.40	0.00
8***.	15.40	14.10	15.38	−0.02

* The average deviations of a series of ten determinations is +0.45%.
** The average deviation of a series of ten determinations is +0.23%.
*** The average deviation of a series of ten determinations is +0.15%.

Iodometric Determination of Chromate

During the investigation of the possibility of applying the dead-stop technique to the detection of end-point in iodometric titrations, we also studied the determination of chromate.

The potassium dichromate solution was prepared in the usual way, so that

$$T_{K_2Cr_2O_7} = 0.3394 \text{ mg/ml}$$

The experiments were performed in the previously described apparatus. The solution to be examined had a volume of 100 ml and contained a measured volume of standard potassium dichromate solution, and 20 ml concentrated sulfuric acid 1:4. After 1 *g* potassium iodide was added to the solution, it was allowed to stand for ten minutes, and then it was titrated with a standard N/50 thiosulfate solution. (F = 1.0200).

In the course of the titration, both indicator-electrodes were depolarized because of the presence of iodine and iodide ions. At the end-point of the titration, the depolarization of the cathode was stopped by the first superfluouse drop of thiosulfate, no free iodine remained. The anode was further depolarized by the presence of iodide ions. These conditions resulted in the decrease of the strength of the current flowing through the solution, and the needle of the parallelly-connected millivoltmeter deflected from a value close to zero to the value of applied potential of 10 — 15 mV.

Table 2 shows some of the results obtained from a series of thirty determinations.

TABLE 2

No	$K_2Cr_2O_7$ ml taken	$Na_2S_2O_3$ ml consumed	$K_2Cr_2O_7$ ml found	Difference ml
1*.	2.95	2.86	2.98	+0.03
2*.	2.95	2.83	2.95	0.00
3*.	2.95	2.82	2.94	−0.01
4**.	14.73	14.20	14.79	+0.06
5**.	14.73	14.10	14.69	−0.04
6***.	25.00	24.10	25.60	+0.10
7***.	25.00	24.00	25.00	0.00
8***.	25.00	23.95	24.95	−0.05

* The average deviation from a series of ten determinations is +0.35%.

** The average deviation from a series of ten determinations amounts to +0.16%.

*** The average deviation from a series of ten determinations amounts to +0.09%.

Iodatometric Determination of Hydrazine

Apparatus

In the following experiments, the millivoltmeter was replaced by a galvanometer, the sensitivity of which was 1 $\mu A/^0$, and which, of course, was connected parallelly. All other details of the equipment are the same as those previously described.

Solutions

The examined solution of hydrazine sulfate was standardized by means of an iodine solution, the end-point of the titration

being detected by the dead-stop technique, which gives excellent results, as already shown. It has been established that:

$$T_{N_2H_4 \cdot H_2SO_4} = 0.3528 \text{ mg/ml.}$$

Andrews (2) has found that potassium iodate, in solutions strongly acidified with hydrochloric acid, can be quantitatively reduced to iodomonochloride; Janieson (4) and Kurtenacker and Wagner (6) have used this reaction in the determination of hydrazine. It is believed that the following redox process takes place:

$$2\,N^{2-} - 4\,e = N_2^0$$

$$J^5 + 5\,e = 1/2\,J_2^0$$

$$1/2\,J_2 - e = J^+$$

Since the iodate molecule accepts a total of four electrons we wanted the potassium iodate (Merck p. a.) solution to be diluted as much as the hydrazine sulfate solution; therefore, we prepared a M/400 potassium iodate solution (F = 1.000).

Procedure

20 ml hydrochloric acid (d = 1,19) were added to a measured volume of hydrazine sulfate solution, and the solution was diluted to 100 ml. The electrodes were then placed into the solution, and a potential difference of 100 mV was applied by adjusting the external circuit. Since the anode was depolarized by hydrazine, whereas the cathode was polarized, the galvanometer needle was situated in its zero position. In the course of the titration, the cathode was depolarized due to the presence of free iodine, and the instrument needle was slowly leaving its zero position. As the end of the determination approached, further additions of iodate oxidized iodine were made to iodomonochloride, the pale yellow color of the solution started to disappear, and the instrument needle slowly returned to its previous position so that it was exactly in the zero position, at the very end of the titration. At that moment, the cathode was depolarized by the presence of iodomonochloride and iodate in excess but the anode was polarized, since there was no hydrazine (anodic depolarizor) left free.

Some of the results of a series of thirty determinations are given in Table 3.

TABLE 3

No	$N_2H_4 \cdot H_2SO_4$ ml taken	KJO_3 ml consumed	$N_2H_4 \cdot H_2SO_4$ ml found	Difference ml
1*.	2.83	3.10	2.85	+0.02
2*.	2.83	3.08	2.83	0.00
3*.	2.83	3.05	2.80	−0.03
4**.	14.17	15.42	14.19	+0.02
5**.	14.17	15.40	14.17	0.00
6**.	14.17	15.37	14.14	−0.03
7***.	28.34	30.84	28.37	+0.04
8***.	28.34	30.80	28.34	0.00
9***.	28.34	30.76	28.30	−0.04

* The average deviation from a series of ten determinations is 0.38.
** The average deviation from a series of ten determinations is 0.08.
*** The average deviation from a series of ten determinations is 0.06.

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BROMATOMETRIC DETERMINATION OF ANTIMONY BY THE DEAD-STOP METHOD

by

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The most widely applied and most inventively imagined volumetric method for the determination of antimony was suggested by S. Györy (1). Potassium bromate, in a solution acidified with hydrochloric acid, is a strong oxidizing agent, whereby it is reduced to bromide. The first excess of bromate oxidizes bromide into bromine at the end point of the titration. In Györy's method, the indicator was an azo-compound which is decomposed by free bromine. The author has also established that the appearance of bromine, or even chlorine, in the course of the reaction depends upon the concentration of the acid. However, it was later discovered that some other intermediary products can also be formed in dependence on the concentration of the acid (2).

One disadvantage of this method is the potential possibility of detecting the quasi end-point of the titration due to the appearance of a local excess of bromine. Many years later, Kolthoff (3) indicated the bad consequences of this disadvantage, and recommended, especially near the equivalence-point, a very slow addition of the bromate solution with a dilute potassium bromide solution, and methylorange as the indicator in order to establish whether or not the reaction mixture contains an excess of unreacted bromate. These difficulties are due to the irreversibility of the azo-compound which is used as the indicator. Therefore, various reversible indicators of organic and inorganic origin have been recommended by a number of different authors (4—9).

As we have been interested in electroanalytics of antimony for many years and have recognized all the advantages of potassium bromate as a primary standard, we wanted to avoid the disadvan-

tage of György's method by detecting the end-point of the titration electrometrically. We succeeded rapidly in our attempts by the use of the dead-stop technique.

Experimental

The potassium bromate (E. Merck, p. a.) solution was prepared in usual way: 1 ml potassium bromate solution corresponded to 1 mg antimony. As we considered the traces of present impurities, the solution was prepared so that

$$T_{KBrO_3} = 0.4575 \text{ mg/ml}$$

The analyses were performed with metallic antimony (Mallinckrodt, Analytical reagent, 99.79%) which was previously checked by the excellent electrogravimetric method of S. M. Jovanović (10) and was found to contain 99.70% antimony.

Determinations were performed in a 100 ml beaker and the solutions were vigorously stirred with a magnetic stirrer. In the dead-stop technique of the end-point detection, two small platinum electrodes made of platinum plates (10×5 mm) were used as electrodes-indicators. They were connected to a rheostat of 500 Ω through a galvanometer, the sensitivity of which was $\mu A/°$. The galvanometer was potentiometrically connected to two Edison cells in series.

A weighed amount of metallic antimony was dissolved by warming in 2—3 ml concentrated sulfuric acid, and the cold reaction mixture was diluted with about 40 ml water after dissolution. After the addition of 10 ml concentrated hydrochloric acid, the electrodes, which have a potential of about 15 mV, were immersed in the solution.

At the beginning and during the course of the titration, the cathode was polarized, but the anode was depolarized by the pressure of trivalent antimony, so that, at the low potential applied, practically no current flowed through the solution, or if there was a current flow, it amounted only to 1 μA. At the moment when the end-point of the titration was reached, the first excess of bromate liberated bromine, which depolarizes the cathode, but the anode remained depolarized by the presence of bromide ions. Due to these conditions between the electrodes, the current flowed through the solutions, and the needle of the detecting instrument showed a deflection to 5—7 μA.

The obtained results are shown in Table 1; the deviations have been calculated with consideration of the Mallinckrodt value of 99.79% antimony in the examined sample.

TABLE 1

No	mg Sb Taken	ml $KBrO_3$ Consumed	Sb % Found	% Error
1	4.650	4.620	99.35	−0.44*
2	4.30	4.320	100.46	+0.67*
3	9.550	9.480	99.26	−0.53*
4	10.300	10.350	100.48	+0.69*
5	23.550	23.400	99.36	−0.43*
6	30.150	30.300	100.40	+0.61*

* The numbers refer to maximal deviations out of a series of thirty determinations.

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THE DETERMINATION OF MICRO AMOUNTS OF ELEMENTS BY CUPRIC FERROCYANIDE RING COLORIMETRY.II.

The Determination of Copper, Zinc, Lead, Nickel, and Ferrocyanides

by

M. B. ĆELAP, T. J. JANJIĆ, and M. RISTIĆ

In a recent paper (1) we have described the method for the determination of uranium, manganese, cadmium, cobalt, and mercury (mercurous and mercuric ions) by cupric ferrocyanide ring colorimetry; the corresponding rings were obtained by means of a ring oven. Further studies on a wider application of this method for the determination of various ions have shown that this method can be used for the determinations of Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, and $[Fe(CN)_6]^{4-}$.

The first three ions were determined by means of a standard cupric ferrocyanide scale, which was used in the determination of uranium manganese, and cadmium. As already described (1. *c.*), this scale was prepared from standard cupric sulfate solution, and the determination was performed in a similar manner to that which was used in the previous paper.

Zinc concentration was calculated by using the conversion factor $\frac{Zn}{Cu} = 1.029$, but the results for lead were obtained by the use of an empirical factor, having a value of 4.720. The use of the stoichiometrical factor in lead determination afforded twofold smaller results, which indicates that lead, under given experimental conditions, does not join with potassium ferrocyanide to form a compound analogous to cupric ferrocyanide.

The above scale could not be used in the determination of nickel; therefore, a separate standard scale of cupric ferrocyanide was prepared from standard nickelous sulfate solution, similar to the determination of mercury and cobalt in the previous paper (1. c.).

Ferrocyanides were determined by washing their salts in ring zones on the ring oven, and bathing these rings in a cupric sulfate solution. The obtained ferrocyanide rings were then compared to those of the standard cupric ferrocyanide scale, obtained from a standard cupric ferrocyanide solution.

The results obtained from nine successive determinations are shown in Table 1. The error, on an average, was as follows: copper 4.4%, zinc 2.3%, lead 2.6%, nickel 3.5%, and ferrocyanides 1.0%. The amounts of individual ions were about 10 γ.

TABLE 1

Concentration		Concentration	
Calculated	Found	Calculated	Found
Copper	Copper	Copper	Copper
0.075	0.079	0.476	0.488
0.075	0.078	0.476	0.472
0.075	0.082	0.476	0.472
0.125	0.126	0.443	0.406
0.125	0.135	0.443	0.410
0.125	0.122	0.443	0.436
0.813	0.807	0.670	0.663
0.813	0.852	0.670	0.669
0.813	0.860	0.670	0.644
Zinc	Zinc	Nickel	Nickel
0.651	0.613	0.098	0.101
0.651	0.644	0.098	0.104
0.651	0.649	0.098	0.103
0.113	0.111	0.109	0.103
0.113	0.109	0.109	0.103
0.113	0.109	0.109	0.103
0.149	0.150	0.101	0.102
0.149	0.150	0.101	0.098
0.149	0.143	0.101	0.098
	Calculated Ferrocyanide	Found Ferrocyanide	
	0.307	0.304	
	0.307	0.306	
	0.307	0.304	
	0.290	0.306	
	0.290	0.303	
	0.290	0.295	
	0.255	0.289	
	0.255	0.258	
	0.255	0.256	

It is evident from this data that ferrocyanide ring colorimetry represents a general method which can be used for the determination of a great number of elements, provided they form sparingly soluble ferrocyanides which can be converted into cupric ferrocyanide by means of a cupric sulfate solution. This has been confirmed so far by the eleven following ions: UO_2^{2+}, Mn^{2+}, Cd^{2+}, Co^{2+}, Hg_2^{2+}, Hg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, and $Fe(CN)_6^{4-}$.

Experimental Part

The solutions of ions to be determined were prepared from cupric sulfate, zinc ammonium sulfate, lead nitrate, nickelous sulfate, and potassium ferrocyanide. The solutions were transferred to the filter paper by means of a capillary pipette of 3.1 μl volume, and the salts were washed with hydrochloric acid (0.01 *M*), nitric acid (0.05 *N*) being used only in case of lead nitrate.

The standard scale for the determination of nickel was prepared from nickelous sulfate (0.1 mg Ni/ml), and the scale for the determination of ferrocyanides was prepared from a potassium ferrocyanide solution (0.2 mg $[Fe(CN)_6]^{4-}$/ml). Other experimental conditions were given in a previous paper (1. c.).

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A CONTRIBUTION TO THE COMPLEXOMETRIC DETERMINATION OF METALS. II.

*The Determination of Copper, Nickel, Zinc and Magnesium**

by

VELIMIR D. CANIĆ and TIBOR A. KIŠ

Various alloys and other materials very often contain large amounts of some metals, but other metals are found only in smaller amounts or in traces. All these metals can be complexometrically determined from separate aliquot solutions. The aliquot solution containing a high percentage of metal to be determined is usually diluted, and the aliquot solution containing a smaller amount of metal to be determined is either slightly or not at all diluted. In this way we can modify the concentration of the metal in the solution to be titrated so that it lies between the maximal and the minimal values, as recommended by various authors. According to the literature, the maximal concentrations of copper, nickel, zinc, and magnesium which can be determined in the presence of various indicators range from 20 to 30 mg, and in case of zinc, up to 40 mg of metal per 100 ml of the titrated solution.

If alloys or metals are analyzed by titrating successively individual metals in one aliquot part, it is possible that some metals should be titrated in solutions of high concentrations. Therefore, it was of interest to investigate whether the titrations of metals with ethylenediaminotetraacetic acid (EDTA) can be performed in solutions which contain larger concentrations of metals than that which is mentioned above.

In direct complexometric titrations, the metal solution is titrated with EDTA in the presence of an appropriate indicator until the disappearance of the initial colortint. However, if the metal is determined by means of the backtitration method, the added excess of EDTA is backtitrated with the standard solution

* Part I., Bull. soc. chem., Beograd, 27, 5 (1962).

of the metal to be determined until the disappearance of the tint of the initial color.

Since the titrations are performed until an obvious color change is reached, it is expected that the results obtained in titrating the metal with EDTA would not conform to those obtained by the reverse procedure, i.e., in titrating EDTA with the corresponding metal solution.

Bearing in mind that the metal solution used in the back-titration method is usually standardized by titrating with EDTA, and not by the reverse procedure, we thought it would be of interest to study the titrations of the standard EDTA solution with various metal solutions, and to compare the results with those obtained by titrating the metal solutions with EDTA.

In this study, we titrated 0.1 M and 0.01 M solutions of copper, nickel, zinc, and magnesium with 0.1 M and 0.01 M EDTA solutions. The amounts of metals ranged up to 250 mg per 100 ml solution, except in the case of magnesium, where the quantities amounted to 120 mg per 100 ml solution. In the course of these investigations, we came to the following conclusions:

The titers of metals obtained by titrating high and low metal concentrations with EDTA in the presence of corresponding indicators are in accordance with each other;

The titers of metals obtained by the reverse procedure, i.e., by titrating EDTA with the same metal solutions in the presence of the same indicators, are also in accordance;

However, the titers of metals obtained by titrating the metals with EDTA are slightly higher than the titers obtained by the reverse procedure; and,

The titers of metals obtained by titrating EDTA with metals are in accordance with the results obtained by the usual gravimetric methods, except for copper, where the obtained results were slightly lower.

The inconsistency of the results obtained by titrating the metals with EDTA and by the reverse procedure might be due to the fact that the solution was not titrated until the change of the indicator color, but the titration was performed until the disappearance of the tint of the initial color; in this way, the solutions are overtitrated. Whether or not the above color change is the sole factor affecting the obtained results or the inconsistency of the result, or whether it might be ascribed to other factors is likely to be solved by means of potentiometric determinations which are under investigations.

Experimental

Copper, nickel, zinc, and magnesium were determined by directly titrating their dilute solutions with the standard EDTA solution, according to the usual procedures. Since the titration of concentrated solutions of these metals is encountered with many difficulties, the determination was performed by titrating neutral or slightly acid metal solutions close to the equivalence point, then adding the corresponding buffer and indicator solutions and continuing the titration. Concentrated solutions of copper sulfate and nickel sulfate which are colored were titrated in the presence of a bright background (fluorescent tube).

The reverse titrations, i.e., the titrations of the standard EDTA solutions with the same metal solutions of high and of low metal concentrations, were performed by procedures for the titration of metal with EDTA.

Preparation of standard solutions

"Merck" p.a. reagents were used.

0.1 M EDTA solution. Purified disodium ethylenediaminotetraacetate-dihydrate (EDTA), p.a. "Merck" (Titriplex III) was dried at 80° and used as the primary standard (1); 37.225 g EDTA was dissolved in redistilled water and diluted to one liter in a volumetric flask. The solution was kept in a paraffin waxed glass bottle (2a). The solution was controlled by preparing several EDTA solutions by the same procedure and titrating each with the same metal solution in the presence of the same indicators. The factor of the solution under consideration remained constant, even after several months (3, 4).

0.1 M Zinc solution. The primary standard for the preparation of standard zinc chloride solution was elementary zinc (5a). The solution of zinc chloride was prepared as previously described (6).

Approximately 0.1 M copper, nickel and magnesium sulfate solutions. Corresponding amounts of the salts were weighed, dissolved in distilled water, and diluted to one liter.

0.01 M EDTA solution and 0.01 M metal solutions were prepared by diluting the corresponding 0.1 M solutions. The solutions were controlled by preparing several solutions by the same procedure and titrating each by the same method. The factors of these solutions remained unchanged for several months.

Volumetric flasks were calibrated in the usual way. Temperature corrections for volumes of metal and EDTA solutions were also taken into consideration.

Apparata

The following apparatus were used: pH-meter "Radiometer", type PHM 22p, magnetic stirrer, 50 to 1000 turns per minute, made by Friedrich Geyer K. G., type R 50; and electrolysis apparatus, Laboratorni pristoje, type 278, Prague.

Determination of copper

a) *In the presence of murexide.* The solutions which contained up to 20 mg copper per 100 ml solution were titrated in the presence of ammonia and ammonium chloride (7a, 8a) by adding ammoniacal buffer pH 10 (5b), and in the presence of ammonia (2a). It was observed that the color change at the end point is very sharp when the titration is performed according to E. Kraus (8a). The results for copper were in accord, regardless of the method used. Kraus' method also enables the titration of high copper concentrations.

These methods were adapted for the titration of copper with EDTA.

b) *In the presence of pyrocatechol violet.* Copper solutions of lower concentrations (up to 25 mg per 100 ml solution) were titrated at pH 5.5—6.5 and also in the basic media. The titrations performed by both methods have shown that the change of the indicator color is sharper in the basic media. Sharp color changes were also observed in the titrations of solutions buffered with pyridine (8*b*, 11). Copper results obtained by these methods are in agreement. The above methods were adapted for the titrations of higher copper concentrations and for the titration of EDTA with investigated copper solutions.

c) *In the presence of variamine blue B.* Lower copper concentrations were determined in the presence of this indicator by the method of Wehber (2*d*, 12). It was found that this procedure can be adapted for the determination of higher copper concentrations, and also for the reverse procedure, i.e., the titration of EDTA with copper.

d) *In the presence of chrome azurol S.* Solutions which contain small copper concentrations were titrated according to the

method of Theis (2*b*, 13). We succeeded in applying this procedure for the determination of high copper concentrations and for reverse titrations.

Copper was determined electrogravimetrically (14a) and iodometrically for comparison (14*b*, 14*c*). The titers of copper obtained electrogravimetrically, iodometrically, and in the titration of copper with EDTA are in agreement; however, the results obtained in the titration of EDTA with copper sulfate were slightly lower; they amounted to 0.9% for higher concentrations, and to 0.3% for lower concentrations (Table 1).

Determination of nickel

a) *In the presence of murexide.* Solutions which contain up to 15 mg nickel per 100 ml solution were determined by titrating them with EDTA in the presence of small (2*e*) and large (8*c*) concentrations of ammonia, in ammoniacal solution in the presence of ammonium chloride (7*b*), and in an ammoniacal buffer pH 10 (5*c*). The reverse titrations were performed by corresponding procedures.

Solutions which contain higher nickel concentrations were titrated in neutral or slightly acid media in the presence of murexide with 0.1 EDTA until the green-yellow color of the solution turned to violet, thus indicating that the equivalence point is very close. Then, drops of ammonia were added until the violet color turned to light green yellow. Next, the titration was continued until the appearance of the violet color. The color change at the titration end point was more pronounced than in the presence of ammonium chloride by this procedure.

Since the reaction of nickel with EDTA at room temperature is rather slow, the titration should be performed very slowly in the vicinity of the equivalence point. The reaction is more rapid at 50—60 °C, but when the solution is warmed the titration should be performed very rapidly in order to avoid the decomposition of murexide (5*c*).

b) *In the presence of pyrocatechol violet.* Solutions which contain up to 30 mg nickel per 100 ml solution were titrated in basic media (2*f*, 8*d*, 9). The reverse titrations were performed analogously. When titrating solutions of higher concentrations, 30 ml instead of 10 ml ammonia-ammonium chloride buffer should be added. The color change at the equivalence point is not as sharp as in the titration in the presence of murexide.

TABLE 1

Titers of metal solutions, expressed in mg metal per 1 ml solution, were obtained by different methods. The results given are mean values of six to nine determinations of various amounts of metals. Amounts up to 50 ml 0.1 M and 0.01 M metal solutions and EDTA were analyzed.

	Copper		Nickel		Zinc		Magnesium	
Molarity	0.1	0.1	0.1	0.01	0.1	0.01	0.1	0.01
Titration of metal with EDTA in the presence of:								
Murexide	6.672	0.6674	6.204	0.6206	6.596			
Pyrocatechol Violet	6.671	0.6668	6.205	0.6205	6.599	0.6592	2.462	0.2463
Eriochrome Black *T*					6.592	0.6589	2.462	0.2462
Variamine Blue *B*	6.672							
Chrome Azurol *S*	6.672							
Titration of EDTA with metal in the presence of:								
Murexide	6.613	0.6668	6.177	0.6174	6.541			
Pyrocatechol Violet	6.609	0.6647	6.177	0.6180	6.545	0.6545	2.444	0.2445
Eriochrome Black *T*					6.546	0.6552	2.444	0.2444
Variamine Blue *B*	6.611							
Chrome Azurol *S*	6.609							
	6.671[1]		6.173[1]		6.538[3]		2.454[4]	
	6.669[2]						2.453[5]	

[1] Electrogravimetrically. [2] Iodometrically. [3] Solution prepared. [4] As magnesium pyrophosphate. [5] As magnesium sulphate.

Nickel was also determined electrogravimetrically by rapid and slow electrolysis of its ammoniacal solutions in the presence of ammonium sulfate (16).

The titers of nickels electrogravimetrically obtained are in agreement with those obtained by titrating EDTA with nickel sulfate, but the results obtained by titrating nickel sulfate with EDTA in the presence of the above indicator are approximately 0.5% higher (Table 1).

Determination of zinc

a) *In the presence of murexide.* The complexes of zinc with murexide are very labile; they are decomposed by ammonia in the presence of ammonium chloride at pH 8—10 (17). In order to avoid this decomposition, we titrated neutral zinc solutions close to the equivalence point, added murexide, and then neutralized the solution with dilute potassium hydroxide. This was followed by the addition of ammonia until the precipitate was dissolved and the solution turned to yellow due to the zinc-murexide complex formation. The titration was continued until an abrupt change of color to violet appeared. The reverse titrations, i.e., the titrations of EDTA with zinc chloride, were performed analogously. The obtained results were in agreement with those obtained in the presence of eriochrome black T and pyrocatechol violet.

Solutions of low (18) and high EDTA concentrations were titrated with zinc chloride in the presence of murexide at pH 8-8.5 in the absence of ammonia.

b) *In the presence of pyrocatechol violet.* Solutions which contain up to 40 mg zinc per 100 ml solution were titrated in the presence of an ammoniacal buffer (8*b*, 9, 15, 19). The solutions of higher concentrations could also be titrated by the addition of larger amounts of buffer. The reverse titrations were performed analogously.

c) *In the presence of eriochrome black T.* Zinc was determined in the absence of ammonium chloride, at room temperature, (5*a*), at 40° C, and in ammoniacal buffer at room temperature (2*g*, 7*c*, 8*e*) and at 40° C (7*c*). The results of these determinations were in agreement. It was observed that the color change at the titration end-point was somewhat sharper in the absence of ammonium chloride and at a higher temperature (when the solution was warmed to 40° C).

The table shows that the titer results obtained in the titration of zinc chloride with EDTA are 0.7% higher than those obtained

in the titration of EDTA with zinc chloride. The latter are in agreement with the titer of the standard solution (5*a*, 6).

Determination of magnesium

a) *In the presence of eriochrome black T.* Solutions which contain up to 20 mg magnesium per 100 ml solution were titrated with EDTA in an ammoniacal buffer at pH 10 at room temperature (7*a*); the titration was performed very slowly in the vicinity of the equivalence point. Since the reaction of magnesium with EDTA is rather slow, the titrations were also performed at 50—60° C (5*d*, 8*e*), but the transition of color was not very sharp. A sharper color change was obtained at 90° C. Sharper color transitions were obtained in the presence of mixed indicators: eriochrome black T and methylyellow (20, 21), tropeoline 00 (20. 22) methylred (23), and methylorange (21). The results of all these determinations were in agreement.

The titrations of higher magnesium concentrations, as well as the reverse titrations, i.e., the titrations of EDTA with magnesium, were performed analogously.

b) *In the presence of pyrocatechol violet.* Solutions of lower metal concentrations were titrated in the usual way (2*h*, 8*b*, 9, 15), but the higher concentrations and their reverse, the titrations of EDTA with magnesium, were performed according to corresponding procedures. The color transitions at the titration end point were not as sharp as in the case of titrations performed in the presence of eriochrome black T.

Magnesium was also determined gravimetrically in the form of magnesium pyrophosphate (24) and magnesium sulfate (25).

The table shows that the results obtained in the titrations of EDTA with magnesium sulfate are cca 0.7 % lower than the results obtained by titrating magnesium with EDTA, but the results obtained gravimetrically are found to be in the middle.

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THE STUDY OF THE REACTIONS OF HEXANITROCOBALTATES (III) WITH AMINO ACIDS. I.

The Preparation of Dinitrodiglycinecobaltates (III).

by

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Werner and Humphrey 1901 (1) by the action of ethylene diamine (En) on potassium hexanitrocobaltate, $K_3[Co(NO_2)_6]$, obtained a mixture of *cis* and *trans* dinitrodiethylenediamecobalt (III) nitrite, $[CoEn_2(NO_2)_2]NO_2$. Seven years later, Tschugaeff (2) similarly prepared sodium dinitrodimethylglyoximecobaltate, $Na[Co(DH)_2(NO_2)_2]$, from dimethylglyoxime (DH_2) and sodium hexanitrocobaltate, $Na_3[Co(NO_2)_6]$. In recent years, Janić and Ćelap, by means of paper chromatography have established that the action of aqueous ammonia on potassium hexanitrocobaltate gives rise to a mixture of *cis* and *trans* dinitrotetramminecobalt (III) nitrite*, $[Co(NH_3)_4(NO_2)_2]NO_2$.

From the reactions described, only four out of six nitro groups present in the complex of potassium and sodium hexanitrocobaltate are substituted by other adenda. In order to establish whether this behavior is constant in reactions of this complex with various compounds capable of substituting the nitro group of the complex, we have undertaken the study of the reactions of hexanitrocobaltates with aminoacid. The first reaction which we studied was that of glycocoll (GlH) with potassium and sodium hexanitrocobaltate.

By warming an aqueous solution of potassium glycocollate with potassium hexanitrocobaltate, we obtained a dark brown solution from which a brown crystalline compound separated on cooling (yield 70%). The chromatography of this compound, by means of

* At high concentration of ammonia, especially at lower temperature, hexamminecobalte (III) nitrite, $[Co(NH_3)_6](NO_2)_3$, is also formed.

two different solvents, has proved that it consists of one sole compound. The content of cobalt and potassium in the isolated compound was found to be 17.3% and 11.48%. By analogy with reactions already described, it might be expected that the reaction takes the following course:

$$K_3[Co(NO_2)_6] + 2\,H_2NCH_2COOK = K[Gl_2(NO_2)_2] + 4\,KNO_2$$

e. i., that the compound obtained is potassium dinitrodiglycinecobaltate. Accordingly, the content of cobalt and potassium in this compound should be 17.43% and 11.56%. Since these values are in full accord with those found experimentally, we draw the conclusion that the isolated compound has the above proposed structure.

In order to prove the above formula, we attempted to discover how many ions are formed by the disassociation of this compound. Therefore, we determined its molar conductivity and found that it amounts to 125 moi for 1/100 M solution. As the molar conductivity of an electrolyte giving two ions amounts to about 100, and that of electrolytes giving three or four ions amounts to 250 and 400 respectively, it is concluded that the compound in question is a binary electrolyte, i. e., that the above structure is correct.

In view of the fact that this compound is obtained in a 70% yield when $K_3[Co(NO_2)_6]$ reacts with an amount of glycocoll sufficient to substitute all six nitro groups, it might be inferred that this reaction occurs with the substitution of four out of six nitro groups, present in the molecule of $K_3[Co(NO_2)_6]$.

The obtained compound, after being washed with alcohol and dried in the air, loses an insignificant amount of weight when heated at 105°. It is insoluble in organic solvents, but easily soluble in water; at 23°C. 100 ml saturated solution contain 12.12 *g* salt, i.e., the saturated solution is 0.36 M.

The boiling of potassium dinitrodiglycinecobaltate with dilute mineral acids results in the decomposition of the substance, and it is reduced to cobaltous salt, as established by paper chromatography.

The action of silver nitrate on a concentrated solution of potassium dinitrodiglycinecobaltate precipitates silver dinitrodiglycinecobaltate, $Ag[CoGl_2(NO_2)_2]$, in the form of red crystals, the formula for which was established by determining the silver content (calculated 26.51% Ag; found 26.25%Ag). The silver salt is considerably less soluble than the potassium salt; at 20°C, 100 ml

saturated solution contain 2.32 *g* salt, i.e., the saturated solution is 0.057 M.

Since most of dinitrodiglycinecobaltates are more soluble than the corresponding potassium salt, their preparation could not be achieved by double decomposition of the potassium salt and a concentrated solution of easily soluble salts of corresponding metals (Na, Ca, Ba, Mg, Fe, Mn, Zn, Ni, Co, Hg, Pb, and Cu). Therefore, the sodium salt was prepared from sodium hexanitrocobaltate and sodium glycocollate in a manner analogous to that which was used in the preparation of the potassium salt.

Sodium dinitrodiglycinecobaltate, $Na[CoGl_2(NO_2)_2]$, is a crystalline, orange colored substance which loses its crystalline water at 105°C. The formula of this compound was confirmed by determining the cobalt content in anhydrous salt (calculated 18.30 % Co fund 18.14% Co). It is easily soluble in water; at 23°C 100 ml saturated solution contain 49.56 *g* anhydrous salt, i.e., the saturated solution is 1.54 M.

The action of an equivalent amount of hydrochloric acid on an aqueous solution of silver dinitrodiglycinecobaltate resulted in a yellow solution, which was chromatographically found to contain the free acid $H[CoGl_2(NO_2)_2]$. However, upon evaporation in a water bath, the acid was decomposed envolving nitrous gases; in fact, we failed to achieve its isolation in a pure state.

In view of the fact that the complex ion $[CoGl_2(NO_2)_2]^-$ contains two nitro groups and two glycyl rests as adenda, it might be expected, on the basis of the Werner coordination theory, that it would give rise to five geometrical isomers. Which of these possible isomers is the isolated product is a problem which will be subjected to further investigations. We also intend to study the reactions of hexanitrocobaltates with aminoacids other than glycocoll.

Experimental Part

1. *Potassium dinitrodiglycinecobaltate.* A solution of 4.75 *g* glycocoll and 2.2 g potassium hydroxide in 15 ml water was poured over 9 g potassium hexanitrocobaltate, and placed in a 100 ml beaker which was supplied with an upright condenser. The reaction mixture was heated to boiling for one hour with constant stirring. Unreacted $K_3[Co(NO_2)_6]$ was filtered off, and the filtrate was depositet on the cooling brown-red crystals of potassium dinitrodiglycinecobaltate. Concentration of the solution afforded an additional amount of the salt, to give a total yield of 70% (4.7 *g*).

The compound obtained was chromatographed by means of the following solvents:

1. 65 ml acetone + 30 ml water + 5 ml HNO_3 (sp.w, 1.42)(4)
2. 85 ml acetone + 15 ml water + lg potassium iodide

The chromatography with the first solvent lasted about six hours, and the second about three hours. The development was performed by immersing the paper in an ammonium sulfide solution. The following R_f—values were obtained: 0.76 (solvent 1) and 0.24 (solvent 2).

The determination of cobalt content was performed by complexometric titration: calculated, 17.43% Co; found, 17.33% Co. The potassium content was determined indirectly, by weighing the mixture $CoSO_4 + K_2SO_4$: calculated, 11.56% K; found, 11.48% K. The solubility was determined by weighing the dry residue which remained after evaporation of a measured volume of saturated solution ; at 23°C. 100 ml contain 12.12 *g* salt.

The measurement of molar conductivity was performed at 21°C, and the following values were obtained: N/100—83, N/250—95, N/500—96, N/1000—125 moi.

2. *Silver dinitrodyglycinecobaltate* was obtained in the form of brown-red crystals by the action of 0.5 M silver nitrate solution of a concentrated potassium dinitrodiglycinecobaltate solution. The crude product was recrystallized from hot water. Silver content of the salt dried at 105°C and was determined gravimetrically as silver chloride: calculated, 26.50% Ag; found, 26.25% Ag.

The solubility of the silver salt in water was determined analogously to that of the potassium salt; 100 ml saturated solution contain 2.32 *g* salt at 20°C.

3. *Sodium dinitrodiglycinecobaltate.* 1.1 g glycocoll and 0.6 *g* sodium hydroxide were added to a solution of 3 g sodium hexanitrocobaltate in 35 ml water. The reaction mixture was heated to boiling for one hour with an occasional addition of water in order to keep the volume of the solution constant, and a current of air was passed through the solution during boiling. On cooling, the yellow precipitate separated; it was filtered off, and the filtrate was evaporated to 10 ml. On standing, the latter solution deposited 0.8 *g* sodium dinitrodiglycinecobaltate in the form of brown-red crystals. They were recrystallized from hot water and chromatographed from solvent containing potassium iodide, as previously described (R_f—value 0.25).

The cobalt content of the anhydrous salt, dried at 105°C, was determined by complexometric titration. Calculated, 18.30% Co; found, 18.14% Co.

0.4780 *g* salt, washed with alcohol and dried in the air after being dried at 105°, weigh 0.4497 g, but the calculated weight of the substance containing one molecule of crystalline water should amount to 0.4527 g.

The solubility of $Na_3[CoGl_2(NO_2)_2]$ was determined in a manner analogous to that which was used in the case of potassium and silver salt respectively; at 23°C, 100 ml saturated solution contain 49,6 g anhydrous salt.

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CHEMICAL INVESTIGATION OF WHEAT

1.

DYNAMICS OF VARIOUS FORMS OF PHOSPHORUS IN WHEAT DURING ITS ONTOGENESIS. ACCUMULATION FORMS OF PHOSPHORUS IN RIPE WHEAT GRAIN. I. ORTHOPHOSPHATE AND TOTAL PHOSPHORUS

by

MIHAILO LJ. MIHAILOVIĆ, MIHAILO ANTIĆ, and DIMITRIJE HADŽIJEV

INTRODUCTION

Recent successes which have been achieved in crop production of domestic and especially high yielding varieties of wheat are well known. Up to date knowledge collected in Yugoslavia and abroad has enabled us to outline a production process of wheat, in which most of the production factors involved can be subjected to our direct control. A number of scientific and other papers were published dealing with these topics (1, 2, 3, 4, 5).

In these investigations, an important role was given to production levels accomplished by the heavy use of fertilizers (6,7), and to the relation of their use and corresponding yields. But, as often occurs, in the desire to obtain higher yields, more attention than needed was given to the major fertilizers, and to some other factors which result from the application of modern conditions of crop production.

These aspects are not sufficient for further increase of the production level with simultaneous profitable investments of fertilizer and other crop production techniques. Therefore, further endeavor is needed either to improve the genetical production capacity of wheat variety, or to decrease the heavy use of fertilizers to a more economical level, but without decreasing the crop yields.

Investigations of such kind require more knowledge about growth habits of wheat from the biochemical and biophysical viewpoints, i.e., from aspects which more slowly supply results owing to many experimental difficulties existing in these fields of investigation, but which offer an opportunity for a more complete solution of outlined problems.

In contributing to the solution of such problems, we have undertaken studies which should bring to a closer understanding (*a*) the role of phosphorus in wheat nutrition, (*b*) the uptake of phosphorus by wheat in its different stages of growth, (*c*) the manner of phosphorus incorporation in different compounds, and (*d*) the dynamics of various forms of phosphorus during wheat ontogenesis.

The content of total phosphorus in grain was also the subject of earlier investigations. However, these gave mostly controversial results. The values of 0.58 — 1.1% P_2O_5 are usually reported. In the contemporary domestic literature, the following results are to be found: Jekić (8) 0.98%; Lomejko (9) cites foreign authors; and Nikolić (10) gives 0.7 — 1.1%. Investigations of foreign authors gave these results: Mach and Herrmann (11) 0.58 — 0.90; Booth, Carter, Jones, and Moran (12) 0.43 — 1.3%; Webster (13) 0.98%; Peterburgskii (14) 1.04 — 1.55; and Alekseev, Baranov et al (15) 0.85% P_2O_5.

There are only a few data for the orthophosphate content in wheat grain. Webster's value of 48.1 mg% P_2O_5 for ripe grain is the one which is quoted, even in recent literature (16, 17, 18). The latest report from Kurmies (19) is only a confirmation of this statement

The total and the orthophosphate forms of phosphorus in the grain of wheat should theoretically depend upon a series of factors such as: biological properties of the varieties, available nutrients, climate, methods of applying fertilizers, plowing, sowing, and other cultivating measures, etc. The data at our disposal do not mention any of these factors' influence on these forms of phosphorus.

Investigations in this report deal with the problem of estimating, under the applied modern methods of crop production, the influence of increasing levels of major inorganic fertilizers on the uptake and accumulation of total and orthophosphate phosphorus in ripened wheat grain.

MATERIALS AND METHODS

A). *Field experiments*

Field tests were conducted on chernozem soil type on the Field Experimental Station of the Institute of Agricultural Research, Novi Sad. The soil was of neutral reaction, with 0 — 2% o calcium carbonate; 9.17 — 0.20% of organic nitrogen; 4.5 mg of P_2O_5 as available phosphoric acid, and with 28 mg of K_2O per 100 *g* of soil.

Tests were based on a random block system with three replications according to the following scheme:

Variety *San Pastore*

1. PK	5. NK	9. NP
2. PK + N_{80}	6. NK + P_{72}	10. NP + K_{80}
3. PK + N_{100}	7. NK + P_{144}	11. NP + K_{160}
4. PK + N_{160}	8. NK + P_{252}	12. NP + K_{240}

Domestic variety *Novosadska 1439*

1. ∅	8. ∅	15. ∅
2. PK	9. NK	16. NP
3. PK + N_{20}	10. NK + P_{30}	17. NP + K_{20}
4. PK + N_{40}	11. NK + P_{60}	18. NP + K_{40}
5. PK + N_{80}	12. NK + P_{90}	19. NP + K_{80}
6. PK + N_{160}	13. NK + P_{150}	20. NP + K_{120}
7. PK + N_{200}	14. NK + P_{210}	

The amounts of soil nutrients given into the base quantities of fertilizers were:

a. Domestic variety *Novosadska 1439* — 72 kg N, 172 kg P_2O_5 and 60 kg K_2O/ha.

b. Italian high yielding variety *San Pastore* — 100 kg N, 180 kg P_2O_5 and 80 kg K_2O/ha.

Applied fertilizers were in the following forms:

— N in the form of ammonium calcium nitrate, i.e. "nitromoncal" with 20.5% N, and in the form of ammonium sulphate with 21% N;

— P in the form of superphosphate with 17% P_2O_5.

— K in the form of 40% potassium salt.

From these fertilizers, nitrogen was the one which was applied by top dressings during the course of vegetation, and this contained 3/4 of the total N applied. The remainder consisted of ammonium sulphate which was applied with other fertilizers by the broadcast method, i.e., scattered over the soil surface before plowing, and then worked into the soil.

B). *Chemical determinations*

a. Applied method for the orthophosphate estimation. — Extraction of the plant material with trichloroacetic acid gives the acid soluble phosphorus compounds which contain sugar phosphates, the coenzyme fraction, phytin, all nucleotides, presumed poly- and metaphosphates, and total orthophosphate.

If such an extract is treated with ammonium molybdate, the orthophosphate is the only anion which builds the yellow heteropoly-molybdo complex. This reaction, being reverse, needs an excess of reagent. But, if the reducing conditions are not appropriate before developing the blue color, there is a noticeable reduction of excess reagent, and higher results are obtained. For this reason, it is necessary to first separate the heteropoly acid from the excess reagent by selective extraction with isobutanol, thus avoiding the need for special precautions.

The method of Berenblum and Chain is based on these facts, and is still in wide use for orthophosphate determination, especially in the presence of other phosphorus compounds (20).

In Schaffer, Fong, and Kirk's modification of this method, *n*-octyl alcohol is used for the heteropoly complex extraction (21).

According to Pons and Guthrie (22), the heteropoly complex is developed in isobutanol because the color is stable for over nineteen hours in this solvent.

Lueck and Boltz (23) investigated the optimum acidity needed for the heteropoly acid formation. They concluded that in the interval between 0.5 — 1.5 N there is no acidity effect on the formation of the complex acid. Before these authors, Besenblum and Chain (20) used 0.5 N, and Boltz and Mellon (24) 0.3 N acidities.

However, even the method of Berenblum and Chain, with certain improvements of the mentioned modifications does not give satisfactory results in the analysis of wheat extracts because some

other factors are ignored which might seriously influence the accuracy of the determination.

As proved by Weil-Malherbe (25), the addition of the molybdate reagent to the plant extract can cause catalytical acceleration of the hydrolysis of some phosphorus esters, thus increasing the orthophosphate content. Longer duration of the selective extraction under these conditions of acidities might bring about partial or complete decomposition of the present sugar phospates (fructoso-1, 6-diphosphate, glucose-1-phosphate, triosephosphates), polyphosphates and metaphosphates and might partially liberate phosphorus from nucleic acids and nucleotides (ATP, ADP, etc).

For this reason, in contrast to some other authors (26,27), but in agreement with Martin and Marton (28) and Anderson (29), the modification of Martin and Doty (30) was applied for orthophosphate determination. According to this procedure, the orthophosphate from the wheat extract was isolated in the form of the heteropoly acid by extraction with isobutanol-benzene, for fifteen seconds, and stannous chloride was used in a ternary solvent mixture under optimum acidity conditions for developing the blue color.

b. Preparation of sample for analysis — The wheat grain was milled in a laboratory mill (Raymond Combustion Ltd., London-Derby). The achieved particle size distribution was as follows:

Percentage from wholemeal grain	Particle size (in *mesh*)
8.92	60
24.11	77
23.91	110
13.20	155
12.85	190
17.01	240

For accurate results, the particle size should be at least 60 *meshes*,

c. Orthophosphate extraction from grain. — The extraction of orthophosphate was performed on milled samples containing 8—10 mg of total P_2O_5 with 50 ml of 0.75 N trichloroacetic acid for one hour on an end-over-end shaker (35 r/m). The suspension was then allowed to stand for a short time, the supernatant was decanted into centrifuge tubes of 50 ml and centrifuged at 6000 r/m for ten minutes. Finally, the supernatant was filtered through filter paper

(S & S white ribbon), and the first portion of the filtrate was discarded.

d. Extraction of heteropolymolybdophosphoric acid.— 5 ml of the filtrate was pipetted into a test tube of 25 × 200 mm (this volume corresponds to 0.005 — 0.045 mg of orthophosphate), and diluted with water to 15 ml. This solution was treated with 25 ml of an isobutyl alcohol-benzene solution (1 : 1 v/v), 5 ml of silicotungstate solution, and 5 ml of ammonium molybdate solution (5.7 *g* $Na_2SiO_3 \cdot 9\,HO_2$ + 79.4 g $Na_2WO_3 \cdot 2\,HO_2$ were dissolved in 500 ml of water, treated with 15 ml conc. sulphuric acid, refluxed for five hours, coolled, and finally diluted with water to 1000 ml; 50 *g* of ammonium molybdate were dissolved in 400 ml of 10 N sulphuric acid and diluted with water to 1000 ml). The stoppered test tube was shaken for fifteen seconds and then the phases were allowed to separate.

e. Development of the blue color and the calculation of orthophosphate— 10 ml of the isobutyl alcohol-benzene layer was pipetted into a 50 ml volumetric flask. In order to obtain a reproducible drainage of this solution, the pipet was washed with alcoholic sulphuric acid solution (20 ml conc. sulphuric acid in 980 ml of 99.5 % ethyl alcohol). The sample was then diluted to about 45 ml with the alcoholic sulphuric acid, and 1 ml of freshly prepared stannous chloride was added. The solution was diluted to volume with the same EtOH—H_2SO_4, shaken, and allowed to stand for thirty minutes. (Stannous chloride stock solution: 10 *g* of $SnCl_2 \cdot 6\,H_2O$ were dissolved in 25 ml of conc. HCl; the solution for reduction was prepared just before use: 1 ml of stock solution was diluted to 200 ml with 1 N sulphuric acid). The optical density of the blue color was read against the blank at $\lambda = 720$ mμ (Spectrophotometer Beckman Model DU).

Calculation of the concentration of the orthophosphate was made by using the calibration curve showed in Fig. 1. It is evident from the given curve that the intensity of the blue color in the interval 0.1 — 0.8 γ P/ml ($\log \frac{I_0}{I} = 0 - 0.5$) follows Lambert and Beer's law. If the wavelength at 725 mμ is used, then the corresponding curve also shown in Fig. 1 must be employed.

Two concentration scales are given on the abscissa. The first corresponds to the interval from 0 to 100 γ P, and is used for the above described procedure of analysis, and for the below given formula for calculation of the orthophosphate percentage. The sec-

ond is for the interval between 0—0.8 γ P/ml, if other aliquots are taken.

Absorption spectra of reduced heteropoly blue, developed from the orthophosphate extracted from wheat grain and from the chemically pure KH_2PO_4 (Merck p.a.), are given in Fig. 2. They are practically equal in the interval from 300 to 900 mμ.

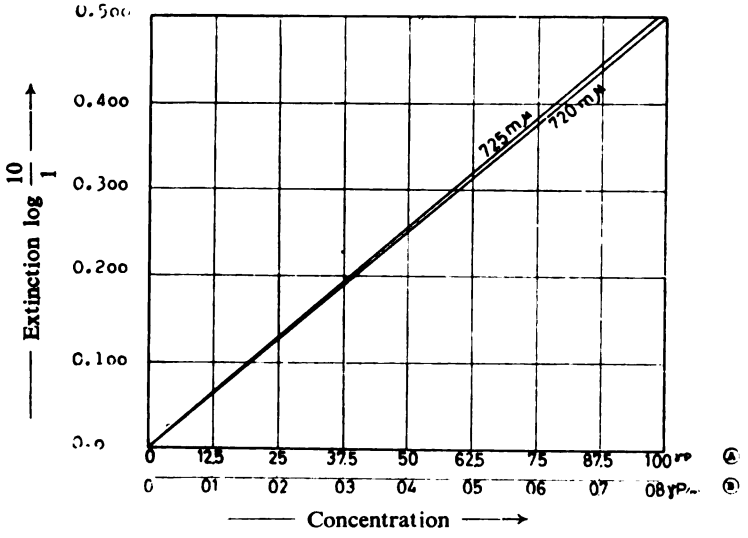

Figure 1.

Calibration Curve for Orthophosphate

For the calculation of the orthophosphate. the following formula was used:

$$\text{Orthophosphate in \% } P_2O_5 = \frac{\text{reading from calibration curve } A \times 2.29}{\text{weight of dry sample} \times 10^4}$$

If other aliquots are used the following formula should be applied:

$$\text{Orthophosphate in \% } P_2O_5 = \frac{\text{reading from calibration curve } B \times V_0 \times \frac{V_l}{V_p} \times \frac{V_s}{V_a} \times 2.29}{\text{weight of dry sample} \times 10^4}$$

whese V_0 = volume of volumetric flask

V_l = volume of the solvent mixture isobutyl alcohol-benzene used for selective extraction of heteropoly acid

V_p = volume of aliquots of heteropoly acid extract used for developing the blue color

V_s = volume of 0.75 N trichloroacetic acid used for orthophosphate extraction from milled wheat samples, and

V_a = volume of aliquots of trichloroacetic acid extract treated with molybdate reagent.

f. Total phosphorus determination.— A sample weighing 0.1—0.2 *g* was digested in a micro Kjeldahl flask with 2 ml of conc. perchloric acid and a few drops of 30% hydrogen peroxide. In the clear solution, the total phosphorus was estimated as heteropoly blue according to Allen's method (31).

g. *Potassium determination.* — For potassium determination, the methot of flame photometry with the corresponding interference filter was applied.

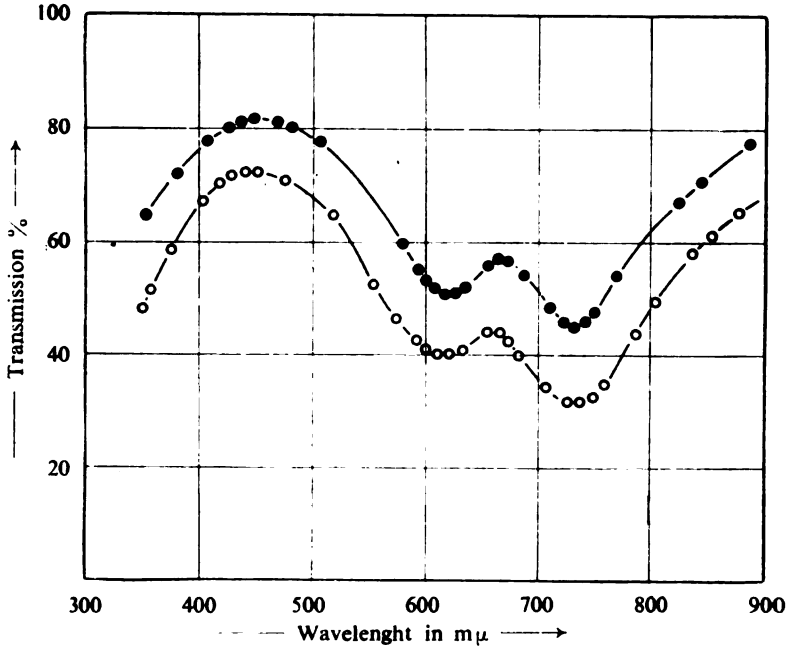

Figure 2

Absorption Spectra of Reduced Heteropolyblue

—●—●— Orthophosphate from KH_2PO_4 p. a. Merck

—○—○— Orthophosphate from wheat

RESULTS AND DISCUSSION

A). *Total phosphorus*

Total phosphorus in the wheat grain varied depending upon the applied doses of nitrogen, phosphorus, and potassium fertilizers, within the range of 805.1—976.5 mg% P_2O_5 in the high yielding variety, and from 621.6—975.0 mg% P_2O_5 in the domestic variety *Novosadska 1439*.

The influence of increasing amounts of nitrogen on the accumulation of grain phosphorus is given in Tables 1 and 2.

TABLE 1

The influence of increasing levels of nitrogenous fertilizers on the uptake of total phosphorus (in mg% P_2O_5) by the grain of wheat variety San Pastore*

Repetition	PK	PK + N_{80}	PK + N_{100}	PK + N_{160}
I	828.1	917.4	952.5	931.3
II	819.3	948.2	948.2	900.5
III	814.0	909.7	945.1	893.2
Mean value	820.5	925.1	948.6	908.3

* These results and those in the following tables are based on dry matter.

TABLE 2

The influence of increasing levels of nitrogenous fertilizers on the uptake of total phosphorus (in mg% P_2O_5) by the grain of wheat variety *Novosadska 1439*

Repetition	ø	PK	PK + N_{20}	PK + N_{40}	PK + N_{80}	PK + N_{160}	PK + N_{200}
I	906.6	949.0	821.4	880.0	962.0	809.0	939.8
II	905.0	1001.0	916.0	862.0	985.0	810.0	969.0
III	868.0	975.0	837.0	868.0	887.0	855.0	960.0
Mean value	893.2	975.0	858.1	870.0	944.7	824.7	956.3

It is evident from Table 1 that variable doses of nitrogen did not regularly influence the uptake of phosphorus in the high yield variety of wheat. Doses PK + N_{60} and PK + N_{100} showed a favorable influence, and the further increase of nitrogen (PK + N_{160}) decreased the uptake. However, it should be stressed that nitrogen had a general positive influence on phosphorus uptake by wheat grain.

If these results are compared with those obtained with the domestic variety *Novosadska 1439* (Table 2), a similar behavior was observed for this typical low yielding variety of wheat.

The extent of the influence of phosphorus from superphosphate upon the accumulation of total phosphorus in the grain of the high yielding variety is shown in Table 3, and its influence on the low yielding variety is presented in Table 4.

It can be seen from Table 3 that increasing levels of phosphorus from P_{72} to P_{252}, gave a positive correlation between doses

of applied phosphorus and its uptake. It is interesting to note that the grain on the plot where phosphorus was omitted accumulated more total phosphorus than the next plot, $NK + P_{72}$. This contradiction can be explained by the fact that wheat in the presence of nitrogen and potassium fertilizers uses available phosphorus directly from the soil.

TABLE 3

The influence of increasing levels of phosphate fertilizers on the uptake of total phosphorus (in mg% P_2O_5) by the grain of wheat variety *San Pastore*

Repetition	NK	$NK + P_{72}$	$NK + P_{144}$	$NK + P_{252}$
I	889.9	794.2	877.4	998.7
II	847.0	807.9	896.5	928.6
III	850.1	813.2	873.0	1002.0
Mean value	862.3	805.1	882.3	976.5

TABLE 4

The influence of increasing levels of phosphate fertilizers on the uptake of total phosphorus (in mg% P_2O_5) by the grain of wheat variety *Novosadska 1439*

Repetition	∅	NK	$NK + P_{30}$	$NK + P_{60}$	$NK + P_{90}$	$NK + P_{150}$	$NK + P_{210}$
I	613.0	652.0	709.0	856.0	903.0	937.0	917.0
II	644.0	580.0	690.0	628.0	767.8	944.5	900.6
III	778.0	632.7	642.0	806.4	794.0	930.8	951.0
Mean value	678.3	621.6	680.3	763.5	821.6	937.4	922.9

Similar results were obtained with the domestic, low yielding variety (Table 4). Here, the maximum uptake of phosphorus was achieved with the combination $NK + P_{150}$, and the minimum uptake with NK, i.e., on the plot where P was omitted. The uptake of phosphorus on this plot was even lower than in the control plot. This depression in the phosphorus uptake by the grain might be explained by a disturbance of the balance of nutrients due to the introduction of NK in the soil, which was richer in potassium but poorer in nitrogen and phosphorus.

The influence of increasing levels of potassium on the accumulation of total phosphorus in wheat grain is shown in Tables 5 and 6.

TABLE 5

The influence of increasing levels of potassium fertilizers on the uptake of total phosphorus (in mg% P_2O_5) by the grain of wheat variety *San Pastore*

Repetition	NP	NP + K_{80}	NP + K_{160}	NP + K_{240}
I	889.9	957.0	866.8	834.9
II	938.3	904.4	864.7	892.0
III	927.6	911.9	865.7	906.5
Mean value	918.6	924.4	865.7	877.8

TABLE 6

The influence of increasing levels of potassium fertilizers on the uptake of total phosphorus (in mg% P_2O_5) by the grain of wheat variety *Novosadska 1439*

Repetition	∅	NP	$NP + K_{20}$	$NP + K_{40}$	$NP + K_{80}$	$NP + K_{120}$
I	589.0	757.7	744.2	838.6	852.0	823.5
II	607.7	732.0	694.0	879.0	725.0	862.0
III	562.0	736.0	732.0	876.0	838.0	792.4
Mean value	586.2	741.9	723.4	864.5	805.0	825.9

It can be concluded from Table 5 that various doses of potassium had no definite influence on the uptake of total phosphorus by the grain of the high yielding variety. While the combination NP, i.e., the plot with potassium omitted, had an average of 919.0 mg% of total P_2O_5, the next plot, NP + K_{80}, showed an average increase of 0.63%. Further increase in applied fertilizer caused a decrease in the phosphorus uptake. The maximum dose of applied potassium (NP + K_{240}) slightly increased the content of accumulated phosphorus in comparison with the previous plot, and on the average this content was 878.0 mg% P_2O_5. It should be noted that this content was still lower than the one obtained without potassium fertilizer.

Results obtained with the domestic variety showed a similar behavior of the low yielding variety, although the total uptake was slightly lower.

According to the data given in Tables 1 to 6, it can be concluded that the results of the accumulation of total phosphorus in wheat grain, obtained in our experiments, are in accordance

with values reported by Nikolić (10), Jekić (8), Webster (13), and Alekseev et al. (15).

The minimal and maximal contents which were achieved under our conditions, with variable doses of N, P and K, are listed in Table 7. For purposes of comparison, results of similar investigations by other authors are also quoted.

TABLE 7

Total phosphorus in mg% P_2O_5

Values	Our results		Results of other authors	
	Domestic variety	Italian variety	Mach, Herrmann (11)	Booth, Carter, Jones, Moran (12)
Minimum	621.6	805.1	580	435.1
Maximum	975.0	976.5	900	1298
Mean value of all determinations	834.7	903.9	730	870

B. *Orthophosphate phosphorus*

The content of orthophosphate phosphorus in the grain of the low yielding variety varied with the treatments in the range of 36.0 — 48.6 mg% P_2O_5, but the control plot gave 32.0 mg% P_2O_5. Since these results are similar to those given by Webster in 1928 (13), in further discussion we shall consider only the results achieved with the high yielding Italian variety *San Pastore*, which behaved differently.

The orthophosphate content of the high yield variety of wheat varied from 36.2 — 54.0 mg% P_2O_5 with a mean value of 45.7 mg% P_2O_5.

Fertilizer treatment influenced the content of orthophosphate. While the influence of nitrogen is not clear, there is a definite correlation between phosphorus fertilizer and the orthophosphate uptake, increasing doses of phosphorus causes an increase in the orthophosphate content in the grain. The smallest content was observed in the plot NK (in the average 36.2 mg% P_2O_5); then the contents rose stepwise and reached the highest value with the

maximum dose of applied phosphorus, $NK + P_{252}$, giving 51.8 mg% P_2O_5. In percentage, this increase amounted to 43% (Tables 8 and 9).

The influence of potassium fertilizer is shown in Table 10. As can be seen, there is an increase in the orthophosphate content with increased amounts of potassium. It should be noted that this accumulation is more efficient than in plots where phosphorus was the variable. Thus, the maximum dose of potassium accumulates 4.5% more orthophosphate in the grain than the highest dose of phosphorus fertilizer.

However, in plots where NK and NP were applied i.e., where mutual influences of P and K were excluded, phosphorus was the element whose exclusion caused a more positive influence on the orthophosphate accumulation.

TABLE 8

The amounts of orthophosphate (in mg% P_2O_5), potassium (in mg%) and the ratio K/PO_4 (in millivales) in the grain of wheat-variety *San Pastore*, as affected by increasing levels of nitrogenous fertilizers

	PK			PK + N_{60}		
Repetition	Orthophosphate	K	K/PO_4	Orthophosphate	K	K/PO_4
I	45.9	431.1	5.68	48.5	422.7	5.27
II	42.3	401.5	5.73	51.7	397.2	4.65
III	44.6	408.9	5.55	52.9	409.4	4.69
Mean	44.3	413.8	5.65	51.0	409.8	4.87
	PK + N_{100}			PK + N_{160}		
Repetition	Orthophosphate	K	K/PO_4	Orthophosphate	K	K/PO_4
I	40.6	429.0	6.41	43.9	426.9	5.89
II	47.8	421.9	5.34	44.2	420.0	5.74
III	41.4	393.4	5.71	45.1	407.2	5.47
Mean	43.3	414.8	5.82	44.4	418.0	5.70

As a fraction of total phosphorus in the grain, orthophosphate represented, on the average, only 5% for both varieties of wheat. Except for phosphorus fertilizers, no clear correlation was established

to support the opinion that the amount of orthophosphate is in some way directly dependent on the content of total phosphorus in the grain.

TABLE 9

The amounts of orthophosphate (in mg% P_2O_5), potassium (in mg%) and the ratio K/PO_4 (in millivales) in the grain of wheat variety *San Pastore*, as affected by increasing levels of phosphorus fertilizers

	NK			$NK + P_{72}$		
Repetition	Orthophosphate	K	K/PO_4	Orthophosphate	K	K/PO_4
I	41.2	383.6	5.64	36.1	406.4	6.83
II	33.3	429.9	7.79	43.6	415.2	5.76
III	34.0	361.4	6.46	37.1	409.1	6.66
Mean	36.2	391.6	6.63	38.9	410.2	5.75

	$NK + P_{144}$			$NK + P_{252}$		
Repetition	Orthophosphate	K	K/PO	Orthophosphate	K	K/PO_4
I	51.5	419.8	4.94	53.6	413.2	4.67
II	40.2	383.2	5.78	48.4	439.1	5.50
III	46.5	415.2	5.41	53.3	426.9	4.85
Mean	46.1	406.1	5.37	51.8	426.4	5.01

The content of orthophosphate in the grain of the high yielding variety of wheat is similar to that of the domestic variety, and also to Webster's (13) and Kurmies (19) results. However, the amount of orthophosphate in the high yielding variety reaches or exceeds that in the low yielding variety only when higher doses of phosphorus and potassium fertilizers are applied, and in other cases (except in the combination $PK + N_{60}$) it is lower on the average by 6.9%.

Our results indicate that the term "inorganic phosphorus" in wheat grain, as often used, should also include orthophosphate phosphorus. However, the designation "inorganic phosphorus" as a synonym to orthophosphate phosphorus is not convenient or correct because other forms of phosphorus, such as pyrophosphates, metaphosphates and polyphosphates, are also included in the term "inorganic phosphorus". Though the presence of all these forms

in wheat grain is still doubtful and controversial, the term inorganic phosphorus cannot be identified with orthophosphate phosphorus even if it should be proved that other forms of inorganic phosphorus are absent from wheat grain*. According to Sulliven (32), besides orthophosphate, pyrophosphate is also present in wheat grain. Edelman, Shibko, and Keys (33), however, could not prove this form of phosphorus or the enzyme pyrophosphatase, not even in traces. Sullivan (32) also claims the presence of metaphosphate. However, Albaum et al. (34) have found this form of phosphorus only in Euglena. The presence of metaphosphates in extracts of higher plants is still doubtful to these and other authors.

TABLE 10

The amounts of orthophosphate (in mg% P_2O_5), potassium (in mg%) and the ratio K/PO_4 (in millivales) in the grain of wheat variety *San Pastore* as affected by increasing levels of potassium fertilizers

	NP			NP + K_{80}		
Repetition	Orthophosphate	K	K/PO_4	Orthophosphate	K	K/PO_4
I	40.9	407.2	6.01	48.2	451.5	5.85
II	46.8	427.7	5.54	44.7	420.2	5.68
III	46.5	450.0	5.86	47.5	415.6	5.31
Mean	44.7	428.3	5.80	56.8	429.1	5.61

	NP + K_{160}			NP + K_{240}		
Repetition	Orthophosphate	K	K/PO_4	Orthophosphate	K	K/PO_4
I	46.8	416.5	5.40	46.0	407.2	5.36
II	47.0	420.0	5.42	56.9	415.6	4.42
III	48.5	426.5	5.31	59.1	431.1	4.42
Mean	47.4	421.0	5.38	54.0	417.9	4.73

Relatively small amounts of orthophosphate as compared to total phosphorus, especially in the high yielding variety of wheat, seems rather unexpected because it is known that orthophosphate is one of the side products in the biosynthesis of starch (35) and

* Various forms of inorganic phosphorus, their structures and properties, and methods for their separation and identification will be the subject of one of our next papers.

sucrose (33) in wheat. Its accumulation in the grain by transport through conducting vessels without previous transformation into organic form is improbable. But still, it should be expected to find orthophosphate to such an extent, in ripe grain, as to justify the term "accumulation form". On the contrary, not a single dose of applied fertilizer, which efficiently led to high grain yields, had any noticeable influence on the increase of the accumulation of orthophosphate. Therefore, it appears that during intensive biosynthesis in the grain, an acceptor is present which binds nearly all the liberated orthophosphate in an organic form, not allowing its accumulation in noticeable amounts.

According to Kent-Jones and Amos (16), the orthophosphate in wheat grain is bound to potassium in the form of monopotassium phosphate and dipotassium phosphate. The acid reaction of wheat grain is attributed to the presence of the first salt. In order to obtain a clear picture of how much potassium is available to orthophosphate in the grain, Tables 8, 9, and 10 list both the contents of orthophosphate and potassium in the grain of wheat-variety San Pastore.

Accumulated potassium in the grain varied from 391.6 to 429.1 mg% potassium, depending on the applied fertilizer. The same Tables also contain the ratio of potassium expressed in milligram-equivalents (*mval*) to orthophosphate, expressed as PO_4 also in millivales. This ratio varied from 4.73 to 6.63, with a mean value of 5.5.

It can be concluded, from the given data in case all the present PO_4 should be neutralized as the potassium salt, that only about one fifth of the present potassium would be combined to the orthophosphate. But, if we accept the view (16) that orthophosphate is present as K_2HPO_4 and KH_2PO_4 in the proportion of 1:1, then orthophosphate binds approximately only one tenth of the present potassium in the grain of wheat.

No correlation was found to support the view that the amount of orthophosphate in grain depended in any way on the uptake of potassium by the same grain.

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CHEMICAL INVESTIGATION OF WHEAT

2*

DYNAMICS OF VARIOUS FORMS OF PHOSPHORUS IN WHEAT DURING ITS ONTOGENESIS. ACCUMULATION FORMS OF PHOSPHORUS IN RIPE WHEAT GRAIN. II. PHYTATE PHOSPHORUS

by

MIHAILO LJ. MIHAILOVIĆ, MIHAILO ANTIĆ and DIMITRIJE HADŽIJEV

INTRODUCTION

Since Pfeffer isolated phytin from the aleurone layer of wheat grain in 1872, this compound was, and still is, the subject of extensive investigations. These studies involve its chemical nature (1, 2, 3), synthesis (4), and quantitative methods of determination (5, 6, 7, 8, 9, 10, 11).

Although the general distribution of phytin in plant material is well known, particularly in cereals and oil seeds (12, 13, 14), little information is available concerning its role and biosynthesis. According to Courtois (1), phytin acts as a source of phosphorus and energy in the course of seed germination, and also as a reserve supply of calcium and magnesium.

In contrast to the insufficient knowledge of phytin metabolism in plant tissues, its physiological action, its influence on the resorption of calcium and iron, and its role in human nutrition were investigated in detail (15, 16). In connection with these investigations, papers dealing with phytin changes in flour production and baking have also appeared (3, 8, 15, 17).

From the aspect of proper animal feeding, phytin assimilation and its influence on calcium resorption have been the subject of recent research (18).

* Paper 1: Glasnik hemiskog društva (Beograd) 27, 65 (1962).

However, in these papers, especially in those referring to phytin accumulation in cereals and more particularly in the grain of wheat, contradictory data is often found. Courtois and Pérez (13) report for phytate phosphorus content in the whole grain the value of 190 mg% for hard wheat, and 160 mg% for soft wheat. Average results of other authors are: Pérez (9) 177 mg%; Pringle and Moran (8) 322 mg%; Menger (3) 277 mg%, Greaves and Hirst (19) 205 mg%; Webster (20) 303 mg%; Hay (21) 215 mg%; Schormüller and Würdig (14) 322 mg%; and Kurmies (11) 485 mg% phytate phosphorus. No original data on phytate phosphorus content have been reported in the domestic literature. According to these results, it appears that phytate phosphorus constitutes from 52 to 94% of the total phosphorus content of the grain.

If the variations caused by the applied methods are disregarded, then the differences in the results obtained by the cited authors can be attributed to various factors such as location, climate, wheat variety, fertilizers, and other measures of crop production. Thus, Young and Greaves (22) have shown that phytate phosphorus content varied between 152 and 328 mg% according to the wheat variety (i.e., in percentage of total phosphorus between 52—94%); and according to locality, soil treatment, and fertilizer, between 222 and 292 mg% (74—93% of total phosphorus).

Data about the influence of inorganic fertilizers (under modern crop production conditions) upon phytin biosynthesis, i.e., phytin accumulation in cereal, are extremely scarce. The recent papers of Schormüller and Würdig (14), and Kurmies (11) only confirm the necessity for further investigations along these lines.

In continuation of our studies on the chemistry of wheat, the present work was limited to investigations performed in view of establishing the influence of various amounts of nitrogen, phosphorus, and potassium fertilizers on the phytin formation in ripe wheat grain under the usual conditions of modern crop production.

MATERIALS AND METHODS

A) *Field experiments and preparation of samples for analysis*

Field tests were performed with the Italian high yielding variety of wheat *San Pastore* on chernozem soil type on the Field Experimental Station of the Institute of Agricultural Research, Novi Sad. Tests were based on a random block system with three replications, according to the following scheme:

1. PK	5. NK	9. NP
2. $PK + N_{60}$	6. $NK + P_{72}$	10. $NP + K_{80}$
3. $PK + N_{100}$	7. $NK + P_{144}$	11. $NP + K_{160}$
4. $PK + N_{160}$	8. $NK + P_{252}$	12. $NP + K_{240}$

The amounts of soil nutrients given into the base quantities of fertilizers were: 100 kg N, 180 kg P_2O_5, and 80 kg K_2O/ha.

Fertilizers were applied in the following forms:

N in the form of ammonium calcium nitrate ($NH_4NO_3 + CaCO_3$) with 20.5% N, and in the form of ammonium sulphate with 21% N;

P in the form of superphosphate, with 17% P_2O_5;

K in the form of 40% potassium salt.

Methods of introducing fertilizers in soil were described earlier (23).

All wheat grain samples were harvested and threshed in the same manner, and then stored in a cold and dry place. Before the chemical analysis, the grain was ground in a laboratory mill (Raymond Combustion Ltd., London-Derby). Particle size distribution in wholemeal grain was mentioned in our previous report (23).

B). *Chemical investigations*

a. Calcium determination. — Calcium content in ripe wheat grain was determined by flame photometry. Although a procedure for this determination was previously described (24), for many reasons it could not be applied in the present study. Wet or dry combustion of wheat grain results in a digest sample in which phosphorus is present mainly as orthophosphate, and partly as pyro- and metaphosphate. Preliminary investigations have shown that when such a solution is directly injected into the flame, a considerable depression of the intensity of calcium emission occurs. Errors which may result in neglecting this effect are shown in Fig. 1, 2, and 3.

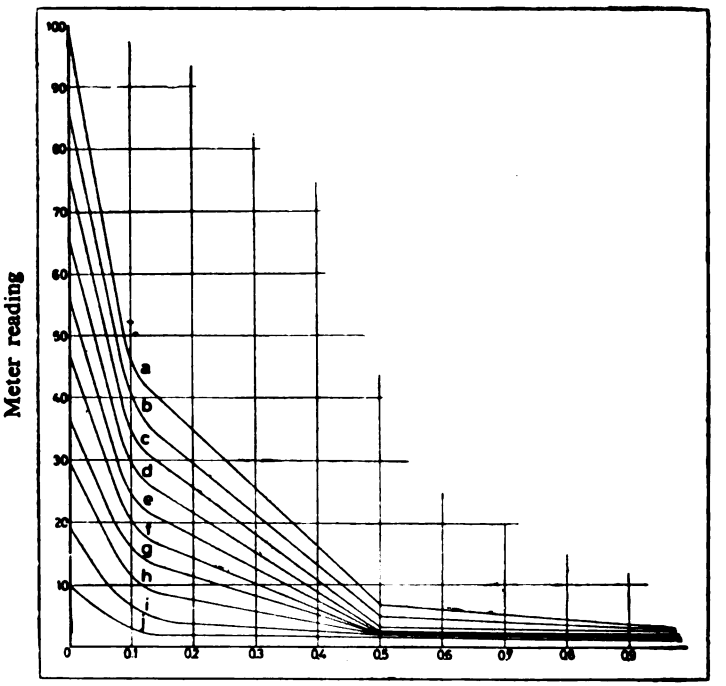
The influence of the orthophosphate ion on the depression of the flame emission of calcium was also reported by other authors (25).

If wet combustion of the grain is performed with concentrated sulphuric acid, then the interference caused by the presence of excess sulphate ions may also be important (26).

For these reasons, calcium was determined after a complete removal of the interfering anions. Instead of Gettkandt's method

(27), removal of the anions was effected by the anion exchange technique (28) in the following way:

Ash obtained by dry combustion of the grain was dissolved in hydrochloric acid and passed through a column of ion exchange resin Amberlite IR 4 B (Cl^-). The column was then washed at



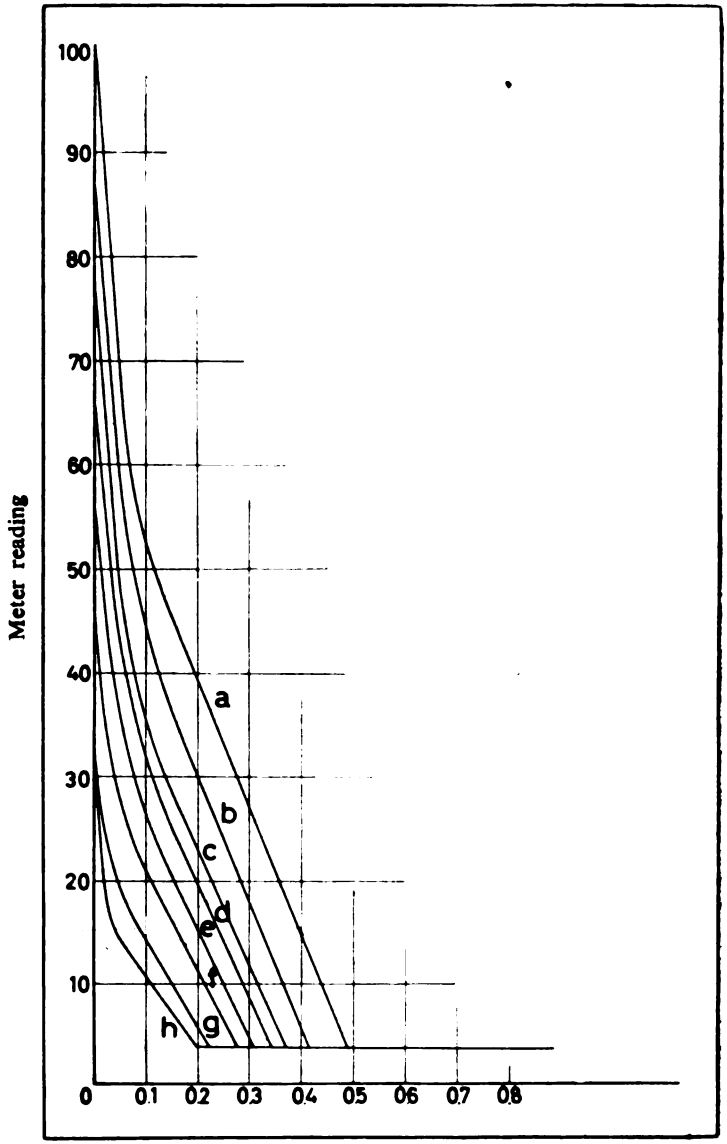
In solution present KH_2PO_4 g mol $\times 10^{-2}/l$

Figure 1.

Depression of calcium emission by orthophosphate. Spectrophotometer Lange Model 5. Galvanometer sensitivity 2, air pressure 0.4 atm.

a = 1 $\times 10^{-2}$ *gr. mol* CaO/b; $b = 0{,}9 \times 10^{-2}$; $c = 0{,}8 \times 10^{-2}$; $d = 0{,}7 \times 10^{-2}$; $e = 0{,}6 \times 10^{-2}$ $f = 0{,}5 \times 10^{-2}$; $g = 0{,}4 \times 10^{-2}$; $h = 0{,}3 \times 10^{-2}$; $i = 0{,}2 \times 10^{-2}$ $j = 0{,}1 \times 10^{-2}$.

least twice with 10 ml portions of boiled water (CO_2-free), the eluates were combined, diluted, and then injected into the flame photometer (emission at 422.7 mμ). [For this purpose the commercial resin Amberlite IR 4 B (OH^-) was previously converted to the (Cl^-) form: 30 ml of resin was treated with 75 ml of 3 N HCl and, after the foaming had ceased, the slurry was poured into a chromatographic column, 8 × 2 cm. When the excess of acid had drained off, the column was washed with CO_2-free distilled water

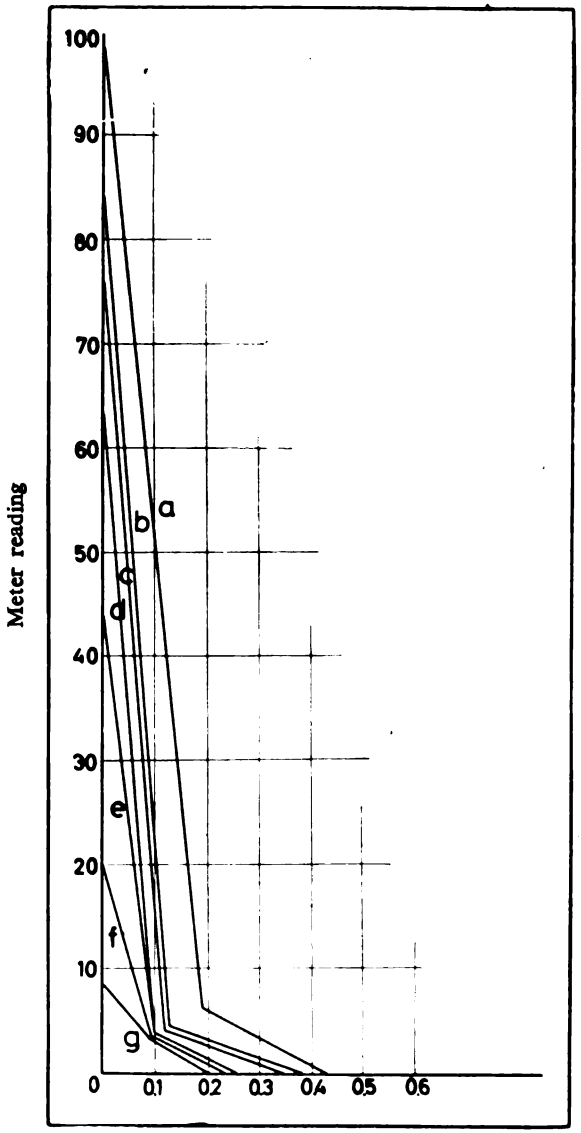


In solution present $Na_4P_2O_7$ g mol $\times 10^{-2}/l$.

Figure 2.

Depression of calcium emission by pyrophosphate. Spectrophotometer Lange Model 5. Galvanometer sensitivity 4, air pressure 0,4 atm.

a = 1×10^{-2} gr. mol CaO/l; $b = 0{,}9 \times 10^{-2}$; $c = 0{,}8 \times 10^{-2}$; $d = 0{,}7 \times 10^{-2}$; $e = 0{,}6 \times 10^{-2}$; $f = 0{,}5 \times 10^{-2}$; $g = 0{,}4 \times 10^{-2}$; $h = 0{,}3 \times 10^{-2}$.



In solution present $(NaPO_3)_n$ gr. mol $\times 10^{-2}/l$.

Figure 3.

Depression of calcium emission by metaphosphate. Spectrophotometer Lange Model 5. Galvanometer sensitivity 4, air pressure 0,4 atm.

$a = 1 \times 10^{-2}$ gr. mol CaO/l; $b = 0{,}9 \times 10^{-2}$; $c = 0{,}8 \times 10^{-2}$; $d = 0{,}7 \times 10^{-2}$.
$e = 0{,}6 \times 10^{-2}$; $f = 0{,}5 \times 10^{-2}$; $g = 0{,}4 \times 10^{-2}$.

until the chloride ions were absent in the washings, and the yellow color of the aqueous eluate had disappeared.]

b. Magnesium determination. — Spectrographical analysis was performed on a Hilger quartz spectrograph E 484, modified as follows: in place of the plate holder, a direct reading attachment was mounted for two optical channels, one for the magnesium line at 2802 Å and the other for the strontium line at 4077 Å, as an internal standard. Two exit slits were used to allow the light from these wavelengths to enter the photomultiplier tubes. The generated current then charged the capacitors. After exposure, the electric potentials which developed in these capacitors were proportional to the intensities of the magnesium and strontium spectral lines, and were measured by a zero reading electrometer. These readings were then compared with the readings of standard solutions.

Porous cup electrodes, made of carbon rods, 5.5 mm diameter (Grade SG-905-J Ship Carbon Co., Ltd., Great Britain) were used for the determination of all grain samples. The working electrode was 16 mm long with a bore of 3.2 mm, and a base thickness of 0.60 ± 0.01 mm. Before use, to improve its porosity, it was heated at 450° for two hours. The counter-electrode was prepared from the same grade of carbon, and had the form of a cone with an inclination angle of 70° at its apex.

The grain sample, ashed at 450° C, was treated with hydrochloric acid, and the undissolved silica was removed by filtration. The porous cup was then filled with this solution, the electrodes put at a spark gap of 2 mm and then placed 20 cm from the entrance slit of the spectrograph. The sparking lasted for fifty-six seconds.

With this modified method it was possible to determine the magnesium with accuracy, the results being unaffected by slight variations in base thickness of the electrode, counter-electrode shape, their position on the optical axis, length of the spak gap, and especially by changes in the chemical composition of the ash obtained from the wheat grain.

The accuracy of the reproducible results obtained by this method was $\pm 2.4\%$ (The colorimetric procedure using titanium yellow (20) is less accurate ($\pm 5.6\%$)). Preparation of the standard curve and construction modifications of the instrument used were described in detail elsewhere (30).

c. Total phosphorus determination. — The procedure for the colorimetric determination of the total phosphorus of grain was described in our previous report (23).

d. Determination of phytate phosphorus. 1. *Survey of some known methods.* Existing methods for the determination of phytate phosphorus can be classified into two major groups:

a) Methods for direct determination of phytate phosphorus;

b) Indirect methods for determination of phytate phosphorus, either by titration of phytic acid with ferric chloride in the presence of indicators (5, 31), or by potentiometric or colorimetric titration of the excess of ferric ions after the reaction between ferric chloride and phytic acid (10).

One must be careful and critical when selecting one of the described methods, because with uncertain results there is always a possibility of increasing the contradictory data which already exist in the literature. In this work, we have given preference to the first, direct method. However, several different procedures are also possible in this group of methods. One of these consists of the determination of phosphorus in the insoluble salts of calcium and ferric phytate. Another possibility is based upon the determination of the orthophosphate content before and after the action of the enzyme phytase. Since enzymatic hydrolysis is usually accompanied by a gradual breakdown of phytic acid, with formation of inositol phosphoric esters such as inositol mono-, di- and triesters, whose rates of further hydrolysis are exceptionally slow (32), this procedure was finally discarded and attention directed only to those methods in which phytate phosphorus is determined through insoluble salts of phytic acid (7, 8, 33).

2. *The method used for the determination of phytate phosphorus.* The method applied was that of McCance and Widdowson (7), with modifications described by Pringle and Moran (8), and Common (34), and it was checked for its analytical value by Schormüller, Höhne, and Würdig (10). For our investigations, the applied procedure was as follows.

A weighed sample, containing 7-12 mg of phytate phosphorus, was extracted with 100 ml of 0.5 N hydrochloric acid at room temperature for three hours in an end-over-end shaker of Wagner's type. The suspension was centrifuged and, if necessary, filtered through fluted filter paper or through a plug of cotton wool. Then 25 ml of clear filtrate was pipetted into a 50 ml volumetric flask and neutralized (to phenolphthalein) with a 25% sodium hydroxide solution. After acidifying the filtrate with a few drops of 0.5 N hydrochloric acid, the volume was made up to 50 ml. Twenty ml aliquots containing approximately 1 mg of phytate phosphorus were

then pipetted into 50 ml centrifuge tubes and 4 ml of a solution of ferric chloride in 1 N hydrochloric acid was added (1 g Fe^{3+}/li). The resulting mixture was then heated on a water bath for twenty minutes and cooled for the same amount of time. The precipitated gelatinous ferric phytate was separated by centrifuging at 3000 r/m for seven to ten minutes and, after discarding the supernatant, washed with 5 ml of 0.5 N hydrochloric acid. The content of the tube was again centrifuged, decanted, and finally allowed to drain, and the walls of the tube were dried with filter paper. The precipitate was then dissolved in concentrated sulphuric acid, transferred into a 50 ml Kjeldahl flask, and gently digested for one to two hours in the presence of a few drops of nitric acid.

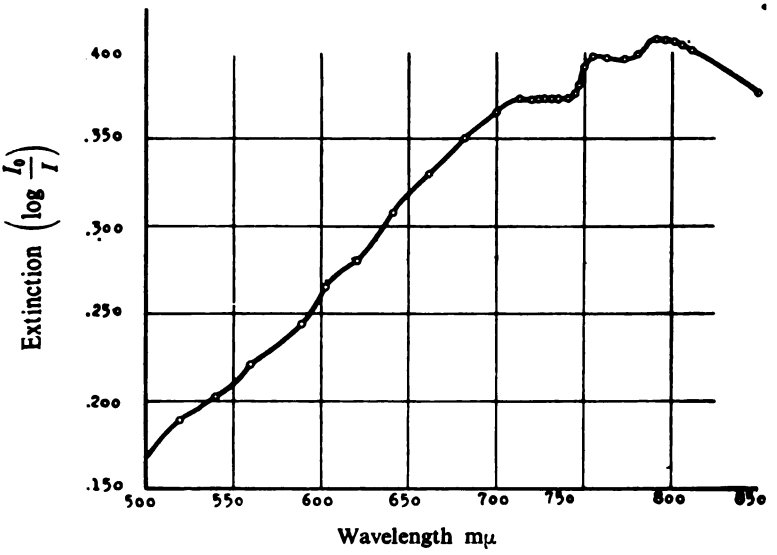


Figure 4.

Absorption spectrum of heteropoyblue developed according to Kent-Jones and Amos. Spectrophotometer Beckman Model DU

The quantitative determination of phosphorus was calculated colorimetrically by reading the intensity of the blue color of the reduced heteropoly-molybdophosphoric acid. Reduction in an aqueous solution of sulphite with hydroquinone as the reducing agent was performed according to Kent-Jones and Amos' procedure (35). A Beckman Model DU spectrophotometer was used, and readings were made at a wavelength of 725 mμ.

As can be seen from the absorption curve on Fig. 4, the absorbancies of heterpoly blue under these conditions of reduction

are nearly constant in the range of 712-740 mμ. Thus, calibration curves and readings within this range of the spectrum give practically identical results (Fig. 5).

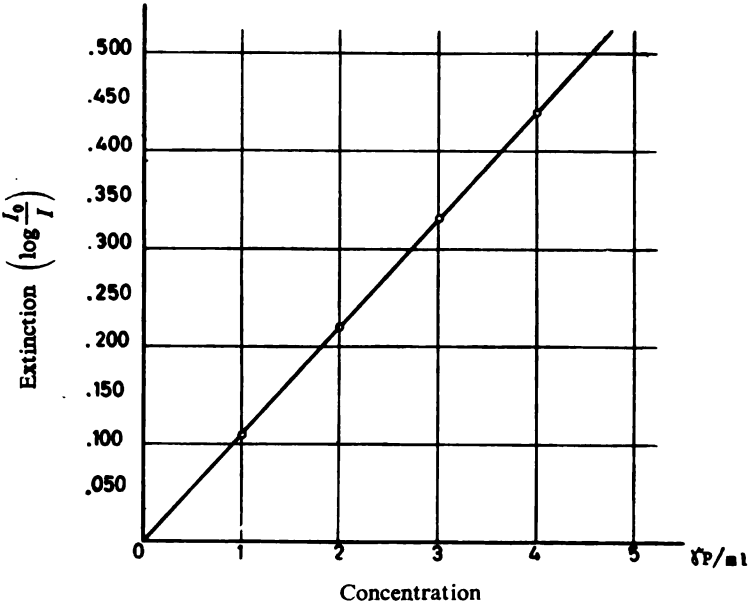


Figure 5

Calibration curve for phosphorus, λ = 725 mμ

RESULTS AND DISCUSSION

A) *Phytate phosphorus*

The content of phytate phosphorus in the grain of the high yielding variety of wheat varied according to the level of applied nitrogen, phosphorus, and potassium, in the range of 601.7 to 825.0 mg P_2O_5 per 100 g of grain.

The influence of increasing amounts of nitrogen fertilizer on phytic phosphorus accumulation in grain is represented in Table 1.

As can be seen, various doses of nitrogen fertilizer did not show a regular influence on the accumulation of phytate phosphorus. While the combination PK + N_{60} increased the amount in comparison to PK, a higher level of nitrogen, i.e., the combination with N_{100}, decreased the amount of phytate phosphorus. The next highest dose of this fertilizer did not change this accumulation. The observed variations of the content of phytate phosphorus expressed as a percentage of total phosphorus uptake by grain varied from 75.2 to 83.7%.

TABLE 1

The influence of increasing levels of nitrogenous fertilizers on the accumulation of phytic acid phosphorus in the grain of wheat, variety *San Pastore**

Level	Sample-plot	Phosphorus		
		Total (P_2O_5 mg %)	Phytic acid (P_2O_5 mg %)	% of phytic acid P based on total P
PK	1	828.1	602.96	76.0
	10	819.3	601.15	74.7
	19	814.0	601.03	75.0
	Mean	*820.4*	*601.7*	*75.2*
PK + N_{60}	8	917.4	773.5	84.2
	11	948.2	776.2	82.0
	20	909.7	770.9	84.9
	Mean	*925.1*	*773.5*	*83.7*
PK + N_{100}	2	952.5	773.6	81.2
	15	948.2	748.14	78.0
	21	945.1	731.02	77.4
	Mean	*948.6*	*750.9*	*78.9*
PK + N_{160}	5	931.3	821.2	88.2
	14	900.5	736.4	81.8
	17	893.2	692.7	77.6
	Mean	*908.3*	*750.1*	*82.5*

* These results and those in the following tables are expressed as percentage of dry matter.

The influence of phosphoric acid from superphosphate on the accumulation of phytate phosphorus in grain is given in Table 2.

As can be seen, by applying increasing doses of P, from P_{72} to P_{252}, a positive correlation was achieved between the applied level of fertilizer and the total phosphorus uptake in grain. Phytate phosphorus content also showed the same regularity: it increased with increasing amounts of applied fertilizer, from 645.2 to 825.0 $g\%$ P_2O_5. However, when these contents are expressed as a percentage of the total phosphorus uptake, the correlation loses its positive character. This especially concerns the plot NK, i.e., the level in which phosphorus was omitted, and where, contrary to the expectation that this accumulation should give the lowest content of phytate phosphorus, it accumulated nearly as much as the plot where the highest dose of P was applied, P_{252}.

The influence of the gradual increase of potassium fertilizer on the uptake of phytate phosphorus by grain is shown in Table 3.

It appears that variable doses of potassium have no regular influence on the phytate phosphorus uptake. While the plot NP,

TABLE 2

The influence of increasing levels of phosphate fertilizers on the accumulation of phytic acid phosphorus in the grain of wheat, variety *San Pastore*

Level	Sample-plot	Phosphorus		
		Total (P_2O_5 mg%)	Phytic acid (P_2O_5 mg%)	% of phytic acid P based on total P
NK	1	889.9	717.41	80.5
	12	847.0	718.17	84.8
	18	850.1	725.47	85.5
	Mean	*862.3*	*720.4*	*83.6*
NK + P_{72}	3	794.2	652.90	82.3
	6	807.9	653.74	81.0
	14	813.2	656.00	80.6
	Mean	*805.1*	*654.2*	*81.3*
NK + P_{144}	9	877.4	675.76	77.2
	16	896.5	712.62	79.5
	23	873.0	671.44	76.9
	Mean	*882.3*	*686.6*	*77.9*
NK + P_{252}	2	998.7	824.22	82.6
	10	928.6	815.67	88.0
	13	1002.0	835.13	83.4
	Mean	*976.4*	*825.0*	*84.7*

i.e., the combination in which potassium was omitted, gave, on the average, 717.75 mg% P_2O_5 (78.1%), the next plot NP + K_{80} yielded 685.1 mg% of phytate phosphorus, i.e., 74.4% based on the total phosphorus uptake. Further increase of potassium fertilizer caused a decrease in the accumulation of phytate phosphorus, the combination NP + K_{160} gave 659.4 mg%, and the combination NP + K_{240} gave 708.2 mg% P_2O_5, that is 76.2% and 80.7% respectively, based on the total phosphorus uptake.

B). *The contents of phytic acid, calcium, and magnesium*

The contents of phytic acid*, calcium and magnesium, expressed as mg% and as milligram-equivalents (mvales) are given in Tables 4,5, and 6.

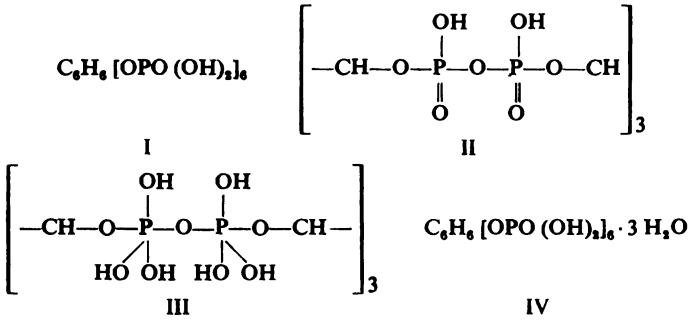
* According to Anderson (36) and Suzuki et al. (37), phytic acid was regarded as mesoinositol hexaorthophosphate (I). There are authors who simplify the structure of phytic acid and formulate it as the tripyrophosphate with the corresponding pyrophosphate linkage (II). However, phytic acid seems to be associated with 3 molecules of water and it combines with 12 metal equivalents, so that possibly the arrangement actually involved in this acid corresponds to

TABLE 3

The influence of increasing levels of potassium fertilizers on the accumulation of phytic acid phosphorus in the grain of wheat, variety *San Pastore*

Level	Sample-plot	Phosphorus		
		Total (P_2O_5 mg%)	Phytic acid	% of phytic acid P based on total P
NP	10	938.3	727.79	77.7
	12	927.8	725.27	78.0
	14	889.9	700.2	78.6
	Mean	*918.7*	*717.75*	*78.1*
NP + K_{80}	5	957.0	705.0	74.5
	8	904.4	671.22	74.2
	11	911.9	679.12	74.4
	Mean	*924.4*	*685.1*	*74.4*
NP + K_{160}	4	866.8	671.0	77.5
	7	864.7	650.0	75.2
	18	865.7	657.2	76.0
	Mean	*865.7*	*659.4*	*76.2*
NP + K_{240}	2	834.9	668.5	80.1
	13	892.0	731.1	82.0
	20	906.5	725.0	80.0
	Mean	*877.8*	*708.2*	*80.7*

formula (III), in which of 18 hydrogens available, only 12 are reactive. This structure is in formal agreement with the hydrate formula of phytic acid (IV), as proposed by Posternak (4).



According to Andrews and Herrarte (38), phytic acid dissociates with its first six hydrogen ions at p_k 2-5, and the remaining six hydrogen ions dissociate at p_k 6-11.

In our calculation of the content of phytic acid in wheat grain, the formula of mesoinositol hexaorthophosphate was used in its anhydrous form (Formula I) with a molecular weight of 660. This more adequately reflects the behavior or phytic acid in the process of neutralization (phytic acidphytate phosphorus in % $P_2O_5 \times 1.549$).

It is evident from the given tables, that the content of phytic acid varied from 931.9 to 1277.5 mg%, depending upon the amounts of applied fertilizers.

The average content of the accumulated phytic acid in the case of constant doses of PK and variable doses of nitrogen fertilizer was 1113.4 mg%; constant doses of NK and variable doses of phosphorus fertilizer, 1117.4 mg%; and constant doses of NP and variable doses of potassium fertilizer, 1081.4 mg%.

The nitrogen fertilizer showed the smallest influence on the uptake of phytic acid. By using increasing levels of this fertilizer, from N_{100} to N_{160}, the phytic acid content in the grain of wheat remained practically constant, with an average of 1162.05 mg%.

By applying increasing levels of phosphorus fertilizer, from P_{72} to P_{252}, a steady increase of the content of phytic acid was observed. However, while the change between the levels P_{72} and P_{144} was represented on the average only with 49.8 mg%, this increase between the levels P_{144} and P_{252} was more than fourfold, i.e., 214.3 mg% of phytic acid. The combination with the highest dose of phosphorus fertilizer (P_{252}) yielded the highest content of phytic acid (1277.5 mg%).

The content of phytic acid remained practically constant by applying increasing amounts of potassium fertilizer. Compared to the plot without K, the combination $NP + K_{80}$ decreased the content of this acid by 15.4 mg%. The highest level of this fertilizer (K_{240}) showed the same influence on the accumulation as the K_{80} plot, while, on the contrary, the level K_{160} decreased the phytic acid accumulation by 75.0 mg% (average value).

The content of phytic acid expressed in milligram-equivalents (mvales) varied in the range of 16.0 to 23.2 mvales, while the average value of all determinations was 20.0 mvales.

The investigations of the uptake of calcium and magnesium by wheat grain in dependence on variable levels of nitrogen, phosphorus, and potassium fertilizers gave the following results:

The content of calcium in the grain varied from 36.2 to 46.7 mg%, and that of magnesium from 136.2 to 159.9 mg%.

The calcium uptake by grain was nearly constant by applying increasing amounts of nitrogen fertilizer. The difference between the plots PK and $PK + N_{60}$ was only 3.8 mg%; between the plots N_{60} and N_{100}, 3.9 mg%; and, finally, between N_{100} and N_{160}, only 2.8 mg%. The variation of the content of magnesium under identical experimental conditions was also small, and reached its maximum value with 4.9 mg%.

By applying variable doses of phosphorus fertilizer, the content of calcium varied from 37.4 to 45.4 mg% and that of magnesium from 146.6 to 156.4 mg%, i.e., the changes for both elements were practically negligible.

Variable levels of potassium fertilizer also did not show a significant influence on the uptake of both elements. The calcium content varied in the range of 40.8-46.7 mg%, with an average of 44.0 mg% for all the estimations. A similar behavior was observed

TABLE 4

The amounts of phytic acid, calcium and magnesium and their ratio in milligramequivalents (*mval*) in the grain of wheat, variety *San Pastore*, as affected by increasing levels of nitrogenous fertilizers

Level	Sample-plot	Phytic acid		Ca		Mg		Ca + Mg	Difference $\Sigma mval_{phyt.a.}$ / $\Sigma mval_{Ca+Mg}$
		mg%	mval	mg%	mval	mg%	mval	Σmval	
PK	1	933.8	16.95	41.7	2.1	153.0	12.6	14.7	2.3
	10	931.1	16.9	31.9	1.6	152.9	12.6	14.15	2.75
	19	930.9	16.9	35.1	1.75	154.2	12.7	14.45	2.45
	Mean	*931.9*	*16.9*	*36.2*	*1.8*	*153.4*	*12.6*	*14.4*	*2.1*
PK + N_{60}	8	1197.9	21.8	48.3	2.4	153.2	12.6	15.0	6.8
	11	1201.2	21.9	35.0	1.75	154.55	12.7	14.45	7.45
	20	1193.5	21.7	36.8	1.8	149.9	12.3	14.1	7.6
	Mean	*1197.5*	*21.8*	*40.0*	*2.0*	*152.6*	*12.5*	*14.5*	*7.3*
PK + N_{100}	2	1197.9	21.8	44.1	2.2	152.0	12.5	14.7	7.1
	15	1157.3	21.0	48.2	2.4	153.9	12.6	15.0	6.0
	21	1131.9	20.5	39.5	1.97	151.0	12.4	14.4	6.1
	Mean	*1162.4*	*21.1*	*43.9*	*2.2*	*151.9*	*12.5*	*14.7*	*6.4*
PK + N_{160}	5	1271.5	23.1	47.8	2.4	158.4	13.0	15.4	7.7
	14	1140.7	20.75	43.9	2.2	158.2	13.0	15.2	5.55
	17	1072.9	19.55	48.3	2.4	153.7	12.6	15.0	5.5
	Mean	*1161.7*	*21.1*	*46.7*	*2.3*	*156.8*	*12.9*	*15.2*	*5.9*

in the uptake of magnesium, except on the plot where K was omitted, i.e., NP; here, the magnesium content had an average value of only 136.2 mg%, and on the other plots the magnesium uptake varied from 151.3 to 159.9 mg%, with 151.2 mg% as a mean value for all the estimations.

The sum of calcium and magnesium contents expressed in milligram-equivalents should also be indicated because it gives interesting results. This sum was practically constant, and amounted

on an average to 14.5 mvales for phosphorus fertilizer, 14.6 mvales for potassium fertilizer, and 14.7 mvales for nitrogen fertilizer. In this sum, the content of calcium is represented on the average by 2.1 mvales, and the remainder corresponds to the content of magnesium. This practically means that in the neutralization of phytic acid for one gram-equivalent of calcium, six gram-equivalents of magnesium are consumed, supposing that both alkaline-earth metals are exclusively bound to phytic acid. The excess of phytic

TABLE 5

The amounts of phytic acid, calcium and magnesium and their ratio in miligram-equivalents (mval) in the grain of wheat, variety *San Pastore*, as affected by increasing levels of phosphate fertilizers

Level	Sample-plot	Phytic acid		Ca		Mg		Ca + Mg	Difference $\frac{\Sigma mval_{phyt.a.}}{\Sigma mval_{Ca+Mg}}$
		mg%	mval	mg%	mval	mg%	mval	Σ mval	
NK	1	1111.0	20.1	40.1	2.0	148.9	12.2	14.2	5.9
	12	1112.1	20,1	35.0	1.75	154.9	12.8	14.55	5.55
	19	1123.1	20.3	37.1	1.85	150.3	12.4	14.25	5.95
	Mean	*1115.4*	*21.1*	*37.4*	*1.86*	*151.4*	*12.5*	*14.3*	*5.8*
NK + P_{72}	3	1011.34	18.4	40.1	2.0	145.7	12.1	14.1	4.3
	6	1012.6	18.4	48.3	2.4	153.2	12.6	15.0	3.4
	14	1016.1	18.5	37.3	1.9	141.0	11.6	13.5	5.0
	Mean	*1013.4*	*18.4*	*41.9*	*2.1*	*146.6*	*12.1*	*14.2*	*4.2*
NK + P_{144}	9	1046.6	19.0	43.9	2.2	146.0	12.0	14.2	4.8
	16	1103.3	20.0	48.3	2.4	156.2	12.8	15.2	4.8
	23	1039.9	18.9	43.9	2.2	149.0	12.25	14.4	4.5
	Mean	*1063.2*	*19.3*	*45.4*	*2.3*	*150.4*	*12.35*	*14.6*	*4.7*
NK + P_{252}	2	1277.1	23.2	41.5	2.4	157.8	13.0	15.4	7.9
	10	1261.7	22.9	37.3	1.9	157.5	12.95	14.8	8.1
	13	1293.6	23.5	37.0	1.85	153.8	12.65	14.5	9.0
	Mean	*1277.5*	*23.2*	*38.6*	*2.05*	*156.4*	*12.9*	*14.9*	8.3

acid, which is not bound to calcium and magnesium, and which amounts on the average to 5.4 mvales, could be attached to the alkaline metals. As reported in our previous paper (23), potassium accumulates in the grain to an extent which is just sufficient to neutralize the excess of phytic acid.

It is usually believed that sodium, as one of the alkaline metals, also participates in phytic acid neutralization. Our investigations showed, however, that under identical conditions of fertiliz-

ation, the grain of wheat incorporated this element up to 16.3 mg% only in a few cases (for instance, on the plot NP/10), and on the other plots this uptake was considerably lower and did not exceed on the average of 2.4 mg%. Doubtless, these results indicate that even if sodium participates in the phytin formation in wheat grain, this participation is negligible, i.e., in any case it amounts to less than one gram-equivalent of the available phytic acid.

TABLE 6

The amounts of phytic acid, calcium and magnesium and their ratio in milligramequivalents (*mval*) in the grain of wheat, variety *San Pastore*, as affected by increasing levels of potassium fertilizers

Level	Sample-plot	Phytic acid		Ca		Mg		Ca + Mg	Difference $\frac{\Sigma mval_{phyt \cdot a.}}{\Sigma mval_{Ca+Mg}}$
		mg%	*mval*	*mg%*	*mval*	*mg%*	*mval*	Σ *mval*	
NP	10	1127.0	20.25	41.3	2.1	129.6	10.6	12.7	7.6
	12	1123.0	20.2	44.0	2.2	125.4	10.3	12.5	7.7
	14	1084.0	19.7	43.9	2.2	153.7	12.6	14.8	4.7
	Mean	*1111.3*	*20.05*	*43.1*	*2.2*	*136.2*	*11.2*	*13.3*	*6.7*
NP + K_{80}	5	1091.0	19.8	50.6	2.5	160.6	13.2	15.7	4.1
	8	1039.5	18.9	41.2	2.05	158.6	13.0	15.05	3.85
	11	1157.1	21.3	48.2	2.4	253.9	12.6	15.0	6.3
	Mean	*1095.9*	*20.0*	*46.7*	*2.3*	*157.7*	*12.9*	*15.25*	*4.75*
NP + K_{160}	4	1040.0	18.9	48.2	2.4	155.6	12.8	15.2	3.7
	7	1007.0	18.3	37.3	1.9	162.0	13.3	15.2	3.1
	18	1018.0	18.5	37.0	1.85	162.0	13.3	15.15	3.35
	Mean	*1021.7*	*18.6*	*40.8*	*2.1*	*159.9*	*13.1*	*15.2*	*3.4*
NP + K_{240}	2	1035.0	18.8	43.9	2.2	138.3	11.35	13.55	5.25
	13	1132.0	20.3	44.0	2.2	164.8	13.5	15.7	4.6
	20	1123.0	20.2	48.3	2.4	151.0	12.4	14.8	5.4
	Mean	*1096.7*	*19.8*	*45.4*	*2.3*	*151.3*	*12.4*	*14.7*	*5.1*

For purposes, of comparison, our results and some data available from the literature are presented in Table 7. Concerning phytate phosphorus, our results are on the average higher than the results of earlier investigations, but they are in agreement with the recent results of Schormüller and Würdig (14).

The changes of the content of phytic acid phosphorus in wheat grain (our investigations) and in barley (14), depending upon inorganic fertilizers, indicate that more intensive biosynthesis of phytin occurs in both cereals when the phosphorus fertilizer is omitted

Simultaneosly, our results show that phytate phosphorus in wheat grain decreases by applying phosphorus fertilizer, in comparison to the plot NK; the increase was noticeable only when higher levels of phosphorus were applied. These findings are contradictory to Kurmies' (11) results.

TABLE 7

	Phytic acid phosphorus						Ca (mg %)			Mg (mg %)		
	mg % P_2O_5			Percentage of total phosphorus								
	Minimum	Maximum	Mean value	Minimum	Maximum	Mean value	Minimum	Maximum	Mean value	Minimum	Maximum	Mean value
Ours results	601.7	825.0	711.1	74.4	84.7	79.8	36.2	46.7	42.2	136.2	159.9	152.05
Mach & Herrmann (39)							28	164	93	115	188	182,9
Booth, Carter, Jones & Moran (40)	533.6	707.4	626.3	71.8	75.6		22	98	51	63	239	157

A comparison of our results with those obtained by Young and Greaves (22) could not be performed because the investigations of these authors were based on a different crop production technique, one which omitted the use of inorganic fertilizers, i.e., an obsolete technique under modern crop production conditions.

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ENZYMATIC DEGRADATION OF SOME ORGANOPHOSPHORUS COMPOUNDS WHICH CONTAIN *p*-NITROPHENYL RADICAL, IN THE PLASMA AND TISSUES OF VARIOUS ANIMAL SPECIES.* II.

by

Z. BINENFELD

It has been shown in a previous paper that the enzymatic hydrolysis of armine (ethyl p-nitrophenyl phosphate) and m-armine (ethyl methyl — p-nitrophenyl phosphate) occurs in the plasma and tissues of various animal species. In this study, we have attempted to establish by the method of mixed substrates, whether one or several enzymes are responsible for that reaction.

It has been established that the enzyme which hydrolyzes paraoxon also degrades DFP and tabun. Recently, Reiner et al. (3) have reported the same for armine and paraoxon. Therefore, we performed our experiments by using paraoxon (diethyl p-nitrophenyl phosphate) in addition to the above OPC.

The experiments were performed by Warburg's manometric method, which was described in the previous paper (1). The enzyme source was rabbit plasma (0. 02 ml); concentration of OPC was 1. 1×10^{-3} M per 2 ml reaction mixture; $\mu l\, CO_2$ (1') 1 ml were calculated from the initial slope of the curve (first 20').

Results

(Figures 1 to 6)

In experiments with mixed substrates, the initial reaction rate is slightly higher than in systems which contain only one individual substrate. However, this acceleration of the reaction rate was not such that we could accurately establish whether or not

* Thanks are due to medical technician, Mrs Zorica Burić, military captain, for her technical assistance in this work.

two enzymatic reactions took place simultaneously. The accelerated reaction rate is also not easily ascribed to the double molar concentration of the substrate mixture.

In the cases of armine and paraoxon a two-fold larger molar concentration (if we take 2.2 M instead og 1.1 M) does not increase the amount of liberated CO_2, but in the cases of m-armine and i-armine, the CO_2 increases to 100% and 40%, respectively. It is evident from Figure 1 that with armine and paraxon, the reaction includes the presence of one enzyme, and this is in accordance with the results of Reiner et al. (3). However it can be concluded from Figure 6 that two enzymes are involved in the reaction, but Figures 2, 3, 4 and 5 do not permit any definite conclusion about the number of enzymes.

If we attempt to illustrate the Figures 1 to 6 in numerical values (Table 1), it is clear that the experimental results which we obtained are insufficient to enable us to draw a definite conclusion.

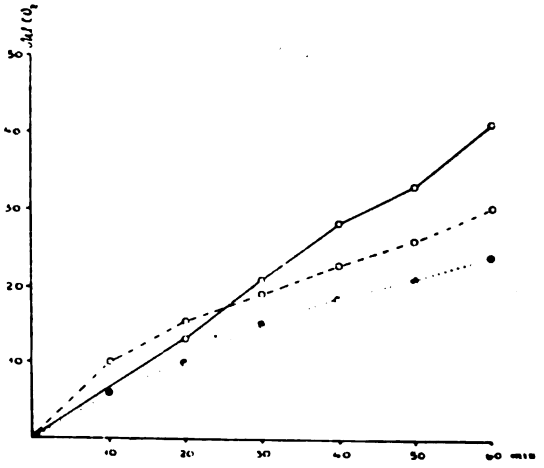
The experiments with mixed substrates cannot be taken as the sole indication of whether one or several enzymes are involved in the reaction because this can result in erroneous conclusions, as already shown by other authors (4).

It is also possible that the reaction is concerned with several enzymes, each of which predominantly attacks one definite substrate, but also degrades other substrates to a smaller extent.

The explanation of this problem requires further experiments.

TABLE 1

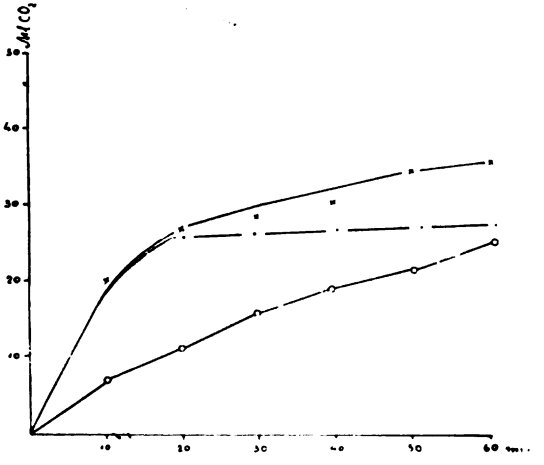
Fig.	Substrate	µl CO_2/1'/1ml	
		Found	Calculated
1	armine	27.5	
1	paraoxon	37.5	
2	i-armine	45	
3	m-aramine	67.5	
1	armine + paraoxon	35	65
2	armine + i—armine	62.5	72.5
3	m-armine + paraoxon	85	105
4	armine + m—armine	67.5	95
5	i-armine + paraoxon	62.5	82.5
6	i-armine + m—armine	97.5	112.5



o—o—o Paraoxon + Armin; o - - - o - - - o Paraoxon; ●···●···● Armin

Figure 1.

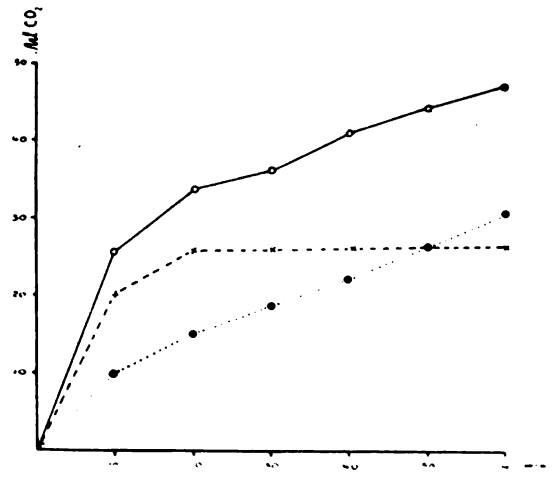
Enzymatic hydrolysis of paraoxon and armine, and a mixture of these substrates by rabbit plasma (0.02 ml). Substrate: 1.1 10^{-3}M paraoxon; 1.1 10^{-3}M armine; 1.1 10^{-3}M armine + 1.1 10^{-3}M paraoxon



× = × — × m-armin—Armin + m-Armin; ●—●=● m-armin—m-armin; o—o—o Armin

Figure 2.

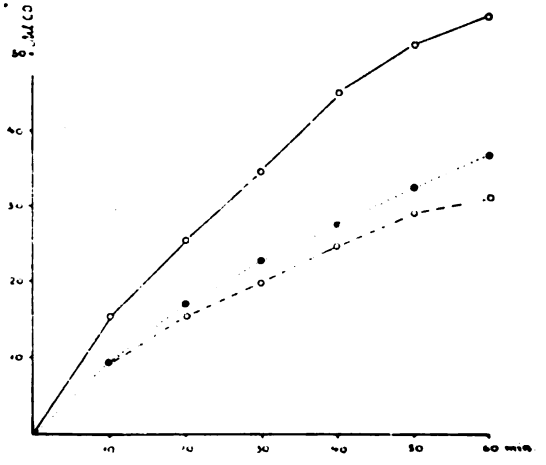
Enzymatic hydrolysis of armine and m-armine, and a mixture of these substrates by rabbit plasma (0.02 ml). Substrate: 1.1 10^{-3}M armine; 1.1 10^{-3}M m-armine; 1.1 10^{-3}M armine + 1.1 10^{-3}M m-armine



o—o—o Paraoxon + m-Armin; × · · · × · · · × Paraoxon; × - - - × - - - × m-Armin

Figure 3.

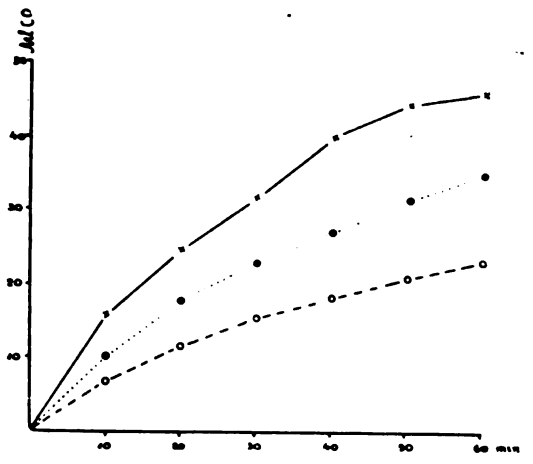
Enzymatic hydrolysis of paraoxon and m-armine, and a mixture of these substrates by rabbit plasma (0.02 ml). Substrate: 1.1 10^{-3}M paraoxon; 1.1 10^{-3}M m-armine; 1.1 10^{-3}M m-armin + 1.1 10^{-3}M paraoxon



o—o—o *i*-Armin + Paraoxon; ●···●···● *i*- Armin; o - - - o - - - o Paraoxon

Figure 4.

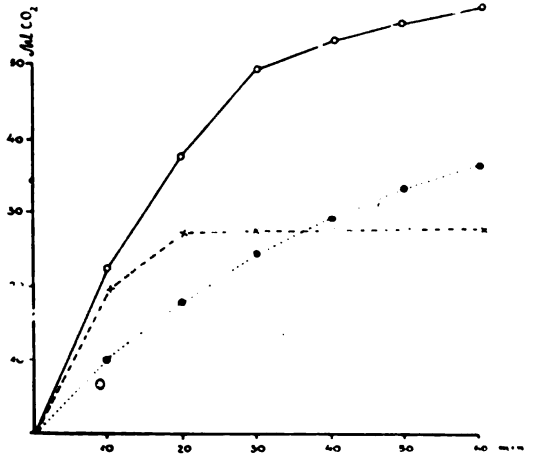
Enzymatic hydrolisys of paraoxon and i-armine, and a mixture of these substrates by rabbit plasma (0.02 ml). Substrate: 1.1 10^{-3}M paraoxon; 1.1 10^{-3}M i-armine; 1.1 10^{-3}M i-armine + 1.1 10^{-3}M paraoxon



× — × — × i-Armin ;●···●···● i-Armin; o - - - o - - - o Armin

Figure 5.

Enzymatic hydrolysis of armine and i-armine, and a mixture of these substrates by rabbit plasma (0.02 ml). Substrate: 1.1 10^{-3}M armine; 1.1 10^{-3}M i-armine; 1.1 10^{-3}M armine + 1.1 10^{-3}M i-armine



o—o— i-Armin + m-Armin; ●···●···● i-Armin; × - - - - - - - × m-Armin

Figure 6.

Enzymatic hydrolysis of i-armine and m-armine, and a mixture of these substrates by rabbit plasma (0.02 ml). Substrate: 1.1 10^{-3}M i-armine; 1.1 10^{-3}M *m*-armine; 1.1 10^{-3}M i-armine + 1.1 10^{-3}M m-armine.

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THE EFFECT OF SKF-525 A (DIETHYLAMINOETHYL-2:2 — DIPHENYLVALERATE HYDROCHLORIDE) ON THE ENZYMATIC DEGRADATION OF SOME ORGANOPHOSPHORUS COMPOUNDS IN RABBIT PLASMA. III*

by

Dr. mr. ph. ZLATKO BINENFELD, Lieutenant Colonel.

Compared to biochemical effects of organophosphorus compounds (OPC), little is known about the mechanisms of the degradation and detoxication of these agents in living organisms.

We know that the plasma and tissues of various animal species contain enzymes which degrade organophosphorus compounds (OPC) of various types. However, it has not yet been explained whether one or several enzymes are involved in these degradations. In regard to alkylalkoxy esters of p-nitrophenyl phosphoric acid, data reported in the literature indicate that the plasma and organs of various animal species contain an enzyme or enzymes which effect the enzymatic hydrolisis of these compounds (1, 2, 3).

Several papers have recently been published on the importance of SKF-525 A for the prevention and therapy of OPC poisoning. It has been shown that SKF-525 A blocks the "activation" of shradan, guthion, and parathion in mammalian liver preparations, (4). SKF-525 A in vivo protects against poisoning by shradan and guthion, but it fails to protect against parathion poisoning (6). O'Brien has demonstrated that SKF-525 A, when preventively given to mice, inhibits the degradation of injected paraoxon (7). Štern and Bošković (8) have shown that SKF-525 A in combination with atropine, PAM-2, and TMD-4 exhibits a strong protecting effect against armine poisoning.

These cases deal with the inhibitory effect of SKF-525 A on the conversion of OPC containing P = S group, into compounds

* Thanks are due to medical technician, Mrs Zorica Burić, for her technical assistance in this work.

with P = O group, and with the inhibition of enzymatic hydrolisis of compounds which otherwise differ one from another (paraoxon, atropine, oximes).

The aim of our work was to study the effect in vitro of SKF-525 A on the enzymatic degradation of OPC of p-nitrophenyl type in rabbit plasma, because among all the animal species, the greatest activity of enzyme degrading OPC was found in rabbit plasma.

Experimental Part

Materials and Methods. — In addition to previous studies of armine, i-armine, m-armine, and paraoxon (diethyl p-nitrophenyl phosphate), boiling at 149° (lmmHg) was used.

The compounds were dissolved in distilled water because they do not undergo hydrolysis for a long period of time, at pH less than 7.

SKF-525 A was dissolved in distilled water just before it was use.

The enzyme source was native rabbit plasma. It was kept in a refrigerator at + 4° C.

The enzyme activity was determined by the Warburg technique; 0. 02 ml plasma, SKF-525 A solution, and the corresponding amount of buffer consisting of 0. 146 M sodium chloride and 0. 0375 M sodium bicarbonate were placed into the Warburg flask; the side arm of the fask contained OPC solutions. The total volume of the reaction mixture was 2 ml.

Rabbit plasma was incubated with SKF-525 A for one hour. A mixture of 95% N_2 and 5% CO_2 was passed through the solution. The equalization of temperature lasted thirty minutes. The pH of the reaction mixture was 7.2 after mixing the substrate and enzyme. All the experiments were performed at 37°C, and readings were taken every ten minutes in the course of one hour. The enzyme activity was expressed in CO_2 microliters evolved by 1 ml plasma in one minute.

Results

SKF-525 A in the concentrations used in our experiments (Table 1) is stable in a buffer solution, both in the absence and presence of plasma. Similarly, SKF-525 A does not react directly with any of the OPC examined.

TABLE 1

Enzymatic degradation of armine, i-armine, m-armine, and paraoxon by rabbit plasma

Substrate	μl CO_2/1 ml/1'							
	—	%	SKF-525 A					
			1.10^{-5}M	%	1.10^{-4}M	%	1.10^{-3}M	%
armine	16.8	100	17	100	43	43	3.2	19
i-armine	51.2	100	50	99	34.4	67	19.2	37
m-armine	46.4	100	45.9	99	43.2	93	30.4	65.5
paraoxon	28.8	100	28.6	100	19.6	65	8.96	31

The concentration of armine, m-armine, and i-armine was 2.2×10^{-3}M; paraoxon concentration, 2.10^{-3}M.

It is evident from Table 1 that SKF-525 A in 1.10^{-5}M concentration has no effect on the enzymatic degradation of the examined OPC. The concentration of 1.10^{-4}M inhibits the enzyme within the limits of 7 to 53 per cent, depending upon the substrate. At the concentration of 1.10^{-3}M, the inhibition extends from 35.5 to 81 per cent. Regardless of the concentration, SKF-525 A most affects the enzymatic degradation of armine but its effect is least on the enzymatic hydrolisis of m-armine. The results are very close to each another with paraoxon and i-armine.

TABLE 2

Enzymatic degradation of a mixture of armine, i-armine and paraoxon by rabbit plasma

Substrate	μl CO_2/1 ml/1'			
		%	SKF-525 A $1.10^{-4}M$	%
armine + i-armine	52.4	100	38.3	73
paraoxon + m-armine	49.6	100	41.6	84
armine + paraoxon	42.2	100	29.6	80
paraoxon + i-armine	51.2	100	40.8	80
i-armine + m-armine	57.2	100	54.4	95
armine + m-armine	48	100	36.8	77

The concentration of armine, i-armine and m-armine was 1.1×10^{-3}M; paraoxon concentration, 1.10^{-3}M.

Preliminary experiments with mixed substrates (Table 2) showed that the concentration of 1.10^{-4}M of SKF-525 A produces practically the same inhibition (20—27) with all combinations of substrates, except for the mixture of i-armine + m-armine, which is only 5 per cent inhibited.

Discussion

In one of O'Brien's papers (7) he has demonstrated that the preventive application of SKF-525 A on mice which were poisoned by paraoxon results in a considerable accumulation of paraoxon in the organism. In his opinion, this is due to the effect of SKF-525 A on the enzyme, which degrades paraoxon (paraoxonase). Our results have shown that SKF-525 A in rabbit plasma inhibits the enzyme which degrades OPC and paraoxon as well. In our previous papers (9, 10), we have established that the activity of the enzyme in question varies with different substrates (armine, i-armine, m-armine, and paraoxon). The results which we have given indicate that the enzyme inhibition is also dependent upon the substrate. O'Brien's assumption (7) that SKF-525 A could be valuable for the detection and the differentiation of "paraoxonase" and related enzymes which degrade OPC seems, in view of our experiences, to be very probable.

Different activities of SKF-525 A with chemically related OPC justify further investigations in this field. and primarily investigations of various chemical constitution with OPC.

Our previous investigations on the application of mixed substrates (10) did not indicate whether one or several enzymes which hydrolyze OPC are present in the organisms; similarly, the present results do not give an explanation of this problem, although the inhibition is found to be smaller with mixed substrates than with individual substrates.

However, our results have offered proof of the effect of SKF-525 A on enzymes which break the bond between phosphorus and p-nitrophenyl grouping in molecules of the examined OPC. This effect on the breaking of the bond was assumed by O'Brien (7), and was based on his in vivo experiments with paraoxon.

Some additional experiments of ours in which we have established that 1.10^{-3}M and 1.10^{-4}M SKF-525 A concentration, in extract of rabbit liver, cause the inhibition of enzyme which degrades all four examid OPC, indicate that the action of SKF-525 A is not dependent on individual organs or sistems, and that SKF-525 A affects the enzyme regardless of the organ or system in which it is present. Further experiments with other tissues and other animal species including the use of SKF-525 A should contribute to the elucidation of the problem, whether various tissues contain one enzyme which degrades one OPC, and or one or several enzymes are responsible for the degradation of OPC of different chemical

constitutions. O'Brien's experiments in vivo, (7) have demonstrated: 1) that preventive injections of SKF-525 A to mice, poisoned by parathion, cause the increase of paraoxon concentration as compared to that found in mice which were not treated with SKF-525 A and 2) that LD_{50} of paraoxon is the same for all mice regardless of whether or not they were given SKF-525 A, and the inhibitory effect of SKF-525 A on "paraoxonase" in vitro supports our assumption that "paraoxonase" and related enzymes do not play a primary role in the toxicity of OPC.

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A NEW TEMPERATURE SCALE BASED UPON THE KINETIC THEORY OF GASES

by

DUŠAN M. RANČIĆ

The tremendous success of the kinetic theory of gases in the theoretical treatment of gas behavior is only a partial success in regard to the theoretical interpretation of temperature. The gas pressure as a function of the kinetic energy of N gas molecules in a volume V is expressed by the equation:

$$p = \frac{2}{3}\frac{N}{V}\frac{mv^2}{2} = \frac{2}{3}\frac{E}{V} \tag{1}$$

It is understood that the equation (1) refers to one molecule of gas. Its comparison with the equation of the state of ideal gases

$$pV = RT \tag{2}$$

gives the kinetic expression of the temperature:

$$T = \frac{2}{3}\frac{E}{R} \tag{3}$$

This treatment, unequivocally accepted in physical and physicochemical literature (1—6) is an adaptation of the kinetic theory of gase to the conceptions, i.e., conventions of thermodynamics, which treat temperature as an individual physical dimension. However, the kinetic theory of gases could treat the behavior of gases only on the basis of its mechanical conceptions. The equation (1) should be considered as a general equation of the state of ideal gases. Thus, it represents the theoretical expression of not only Boyle-Mariotte's law, but of Gay-Lussac's law as well, as we see from the following discusion of this equation.

If we observe one molecule of gas of total translational energy E_t, which at a constant volume can be increased to $E_t + 1$, the two corresponding gas states are given by:

$$pV = \frac{2}{3}E_t \quad \text{and} \quad p_1 V = \frac{2}{3}(E_t + 1) \tag{4}$$

The division of these equations by each other, and the corresponding transformation give:

$$p_1 = p + p\frac{1}{E_t} \tag{5}$$

This equation would be the kinetical expression of Gay-Lussac's law. If the energy of one molecule of gas is increased by unit measure, at a constant volume, the pressure increases by E_t-ieth fraction of its original value.

If E_t in equation 5 is replaced by E derived from equation 1, a somewhat different expression is obtained:

$$p_1 = p + \frac{p}{3/2pV} = p + \frac{2}{3}\frac{1}{V}$$

i.e., if the energy of one molecule of gas is increased by unit measure, at a constant volume, the pressure increases by 2/3 V-ieth fraction of the corresponding pressure unit.

Since the unity of the quotient $1/E_t$ in eguation 5 has the dimension of energy, this equation allows the energy to be expressed in any measure unit, including calories. This is, however, not the case with equation 6, in which the unity of the quotient $1/V$ must be expressed in terms of the CGS-system because V is always expressed in cm^3. The increase of energy by unity could be expressed in ergs, but the pressure unit would be expressed in dyn cm^{-2}. This requirement of equation 6 is in consistent corformity with the starting mechanical conceptions of the kinetic theory.

Similarly, we can observe the change of volume at constant presssure:

$$V_1 = V + V\frac{1}{E_t} \quad \text{and} \quad V_1 = V + \frac{2}{3}\frac{1}{p} \tag{7}$$

From this consideration of equation 1, it is evident that the kinetic theory of gases could treat the behavior of ideal gases and, to a certain extent, the behavior of real gases without introducing new dimensional parameters, but by assuming the starting state to be that of an ideal gas which has the rate and energy of individual molecules equal to zero, the result is that $p=0$ at any value of the gas volume. If however, we accept the necessity of expressing the state of energetic equilibrium between gases and liquids or solids as an equality of temperatures, the temperature of the corresponding matter could be given in Kelvin or Celzius degrees, or as the sum of translational energy of one molecule of

ideal gas with which the corresponding matter would be in energetic equilibrium. This new theoretical temperature scale based on the kinetic theory of gases would fit into the CGS system because the corresponding temperature degree would have the dimension $g\ cm^2\ sec^{-2}$, the magnitude of 1 erg, or even better of 10^8 ergs.

If we would keep and use the concept of the temperature in the kinetic theory of gases as identical to the translational energy of gas (retaining the symbol T but with the index kt), equation 1 would be expressed as:

$$pV = \frac{2}{3} T_{kt} \tag{8}$$

In the new kinetical equation of the state of ideal gases, there is no universal gas constant, its role in the corresponding thermodynamical equation is an energy unit. Nondimensional coefficient 2/3 derives form known treatment of equation 1.

If we assume that the unit of the theoretical temperature scale based upon the kinetic theory is 10^8 ergs, then one degree of the new scale corresponds to 3/2 Kelvin degrees, and the gas constant R must be expressed in 10^8 ergs per one Kelvin degree:

$$T°_{kt} = 3/2\ 8.315\ 10^7\ 10^{-8}\quad T°K = 1.2472\ T°\ K \tag{9}$$

According to the new temperature scale, water would freeze at $340.69°_{kt}$ and boil at $465.41°\ kt$.

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GLASNIK HEMIJSKOG DRUŠTVA, vol. 27, no. 2-3, 1962, pp. 173-175.

A TWOFOLD PERIODIC TABLE

by

IVAN BAJALOVIĆ

A great number of various forms of the Periodic Table of elements has been suggested since Mendeljejeff; and it might be assumed that this number will continue to increase in regard to the importance of the Periodic Table as a chemical system, and the possibility of its further improvement in view of the new data which is offered by the constant development of science.

Other than various forms of the Periodic Table based upon definite principles, and those which contain numerous data and constants which are dependent on the nature of the atom, the most frequently used forms are the short form which originated from Mendeljejeff, and the developed form given by Werner. Apart from the well-known good properties which will not be mentioned here, the disadvantage of both these forms is that the lanthanides and the actinides are not included. They could not be given their proper place in the short form because they would cut off the continuity of the groups. If they were enclosed in the developed form, they would augement the distance between the main groups, thus causing the lack of a survey of properties and analogies.

Our system is based upon the developed form, but it includes lanthanides and actinides. In order to avoid the above-mentioned disadvantage of the developed form, our system is twice bent so that the main groups, which involve the filling with ns and np electrons (n is the principal quantum number), are brought into direct connection in the first phase of the study. The first unfolding of the system reveals the inserted groups of elements which are filled with d electrons. In the second phase of the study, we obtain the developed Periodic Table, in which the main groups are separated. Finally, the third phase of the study gives rise to

the lanthanides which are situated between lanthanum and hafnium, and below them, the unended group of actinides which still more separates the main groups, and also separates scandium and ittrium from titanium and circonium. However, we do not consider these distances to be incovenient because they are formed only after the direct contact between the separated groups in the next phase of the study has been established. In our opinion, the study of the Periodic Table in three phases better emphasizes the analogies and differences between groups, especially those which are dependent upon their electronic configurations. The order of elements mainly corresponds to the order which is usually used in chemistry.

To make the dependence upon the electronic configuration more striking, filling the atoms with electrons is especially emphasized, and the groups in the Table are designated with capital letters according to the electrons which fill them.

In the first phase of the study, the SP-groups are placed in a single file with numbers from 1 to 8. The designation SP (and subgroup S and P) stems from the letters s and p given to electrons which fill the periphferal electronic levels of atoms in these groups. In the second phase of the study, when the system is unfolded for the first time, D-groups in which the n-1 level is filled with d-electrons are inserted between S and P groups. There are ten D-groups, but their order is somewhat changed; they range from D-3 to D-10, followed by D-1 and D-2. The third phase of the study involves fourteen elements of the F-group, in which f-electrons fill the n-2 level.

The number of SP-groups corresponds to the maximal positive charge of atoms, i.e., they are equal to the number of peripherial electrons which can form connections. The number of D-3 groups from D-2 up to D-7 also correspond to the maximal positive charge of atoms, but the number of other D-groups is greater than the maximal positive charge; the maximal positive charge of the D-1 group is greater than 1. F elements are not numbered in groups from 1 to 14, because they all belong to the D-3 group; accordingly, these numbers would not denote the maximal positive charge. Although a second s-electron comes into the D-2 group, the latter is placed with other D-groups because it involves the return of an ns electron which has already been in the S-2 group, and which passes as the tenth d-electron into the n-1 level of the D-1 group.

The capital letters of the first vertical row of the sistem denote the principal quantum number of each period. Below them

are given the principal quantum numbers (small figures) of preceding periods, the electrons of which are completed only in that period. The letters s, p, d, and f denote, as usual, electrons according to their subsidiary quantum number. The number of electrons which belong to each atom is represented on the left side of the atom.

In addition to the atomic number, simbol, and the atomic weight, the system also offers the reaction of corresponding oxides ( acid,  basic,  amphoteric,  basicamphoteric and  acidamphoteric; these data was taken from "Chemischer Handatlas" (Chemical Handatlas) by W. W. Meissner).

If the complete electronic structure of an element is desired, one should write down the structures of al preceding inert gases which are given in the system in the corresponding fields, and then the structure of the element in question should follows. For example: the structure of bismuth (83) is:

n^1		*s*	*p*	*d*	*f*
1	(He)	2			
2	(Ne)	2	6		
3	(Ar)	2	6	10	
4	(Kr)	2	6	10	14
5	(Xe)	2	6	10	
6	(Bi)	2	3		

The places given to hydrogen and helium are justified by the principle on which the given Periodic Table is based, i. e. only in regard to the order of filling with electrons. Their chemical behavior, which is different from the other members of the group concerned, is seen from their electronic configurations.

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CONTENTS

THE SPACE GROUP DETERMINATION OF ACENAPHTHENEQUINONE

by

JELICA MIŠOVIĆ and S. RAMASECHAN

The structures of certain organic aromatic molecules were examined with the aim of ascertaining whether or not the different phenyl (benzene) rings in them were coplanar. From this point of view, the structure of acenaphthenequinone would be of great interest to establish whether or not the two quinone groups would affect the coplanarity of the napthalene ring.

The space group of this substance has been determined by the present authors, but the structure of the compound has not been taken by them. Acenaphthenequinone was prepared from acenaphthene by oxidation with sodium bichromate in the presence of glacial acetic acid by a method described elswhere (1)

A good crystal showing no flaws was affixed to the end of a glass fiber and mounted on a goniometer head. A rotation picture and a Weissenberg photograph of the zero layer, and later of the first and second layer, were recorded. The hkO picture was also recorded by cutting the crystal and mounting it along another axis which could be easily detected under the polarising microscope.

The symmetry as revealed by the intensity distribution definitely showed that the crystal was orthorhombic with cell dimensions 7.66, 28.33 and 7.69 A° with 8 molecules per unit cell. The absent reflections also unequivocally fixed the space group as $P\ 2_1\ 2_1\ 2_1^2$ (2) (No. 19, International Tables for X-Ray Crystallography). Table 1 gives all the crystallographical data for this crystal.

TABLE I

Crystallographic data for acenaphthenequinone crystal.

$a = 7.66$ $b = 28.33$ $c = 7.69$ A° $\alpha = \beta = \gamma = 90°$

Thus, the axial ratios are: 0.2704 : 1 : 0.2714.
The number o molecules per unit cell is: 8.
Conditions limiting possible reflections:

hkl, 0kl, h0l, hk0	No conditions.	h00 h = 2n 0k0 k = 2n 00l l = 2n

Therefore space group is P $2_1\,2_1\,2_1^3$ (No. 19, International Tables for X-Ray Crystallography, 1952).

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A SPECTROPHOTOMETRIC METHOD FOR RAPID DETERMINATION OF MOLYBDENUM

by

JELICA MIŠOVIĆ and MILENA JOVANOVIĆ

Spectrophotometric investigations of complex compounds formed between molybdenic acid and complex cyanides indicate the formation of complexes of the type $Mo_2/Fe(CN)_6/_3$ (1). Since these complexes are colored, i.e., they absorb in the visible region of the spectrum, we have undertaken the study of quantitative estimation of molybdenum by spectrophotometrical measurement of the solutions of complex compounds obtained from molybdenum and complex cyanides.

The reaction of molybdenum with ferrocyanides occurs in strong acid solutions, whereby a brown-red precipitate in separated; in solutions of a lower degree of acidity, the precipitate is red colored (2). However, no precipitate is deposited in acetic acid solutions, but soluble brown-red complexes are formed it the molybdenum concentration is less than 5.5×10^{-1} g per 100 ml solution. Maximal absorption of these solutions falls into the region 420—450 mμ.

Spectrophotometric determination of the complexes is prevented in the presence of an excess of ammonium salts because the yellow precipitate of $(NH_4)_4 Fe(CN)_6 \cdot 2 MoO_3 \cdot 3 H_2O$ is deposited.

Our spectrophotometric study of the quantitative determination of molybdenum was based upon the formation of complexes of molybdenum under the above given conditions.

Experimental

Apparatus and reagents. All measurements were made on spectrophotometer UNICAM SP 600, using the cell of 1 cm. pH-values were obtained by means of Radiometer 22, Copenhagen.

Preparation of standard ammonium molybdate solution. This solution was made by dissolving 9.02034 g ammonium molybdate (p.a. "Merck") and diluting it to 500 ml. It was standardized by means of the gravimetric method. Corresponding solutions of smaller

concentration used as standards were made by diluting the above solution.

Preparation of potassium ferrocyanide solution. This solution was made by dissolving 105.6 g. $K_4/Fe(CN)_6/\cdot 3H_20$ and diluting the solution to one liter (cca 10%).

pH-values of the investigated solutions were adjusted by adding 10 ml of 2% acetic acid solution to 100 ml investigated solution, and controlling the values by means of pH-meter.

Procedure. The acidity of solutions which contain various molybdate concentrations was adjusted to pH = 4.1 (by adding 10 ml 2% acetic acid solution per 100 ml investigated solution), and to each of them was added 20 ml of 10% potassium ferrocyanide solution. The obtained solutions were permited to stand for ten minutes, and then their transmittance was measured spectrophotometrically at the wavelength of 520 mμ.

The concentrations of molybdenum were calculated from the calibration curve obtained by measuring solutions of known molybden concentrations.

Results and Discussion

Absorption curve. The absorption spectrum of the Mo-cyanide complex in the spectral region from 420 mμ to 750 mμ is given in Fig. 1.

Beer's law. Mo-cyanide complexes are in accordance with Beer's law (Fig. 2) in the range of molybdenum concentrations from 1×10^{-1} to 3.0×10^{-1} g Mo (100 ml the absorption measured at 520 mμ. The measurement of higher molybdenum concentrations (up to 4.5×10^{-1} g Mo /100 ml) can be effected by the addition of larger amounts of potassium ferrocyanide (30 ml of 10% potassium ferrocyanide solution per 100 ml investigated solution), and measuring the absorption at 590 mμ. It is also possible to measure smaller concentrations of molybdenum (down to 0.2×10^{-1} g Mo/100 ml) although they do not obey Beer's law, by making calibration diagrams. Accordingly, the concentrations of molybdenum that can be determined by this method at pH 4.1 range from 0.2×10^{-1} to 4.5×10^{-1} Mo/100 ml.

This method can be applied for spectrophotometric determination of molybdenum in the presence of vanadium (2).

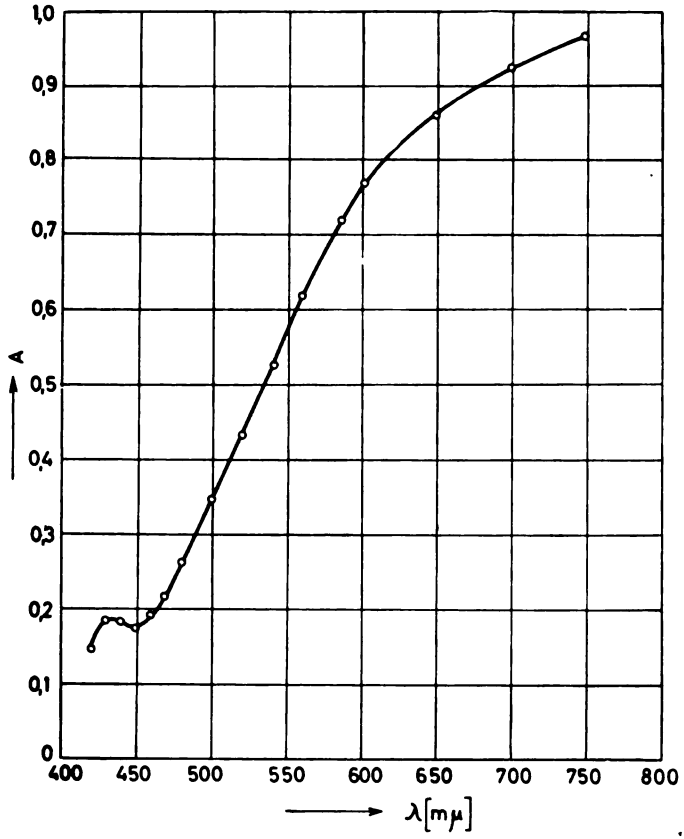

Fig. 1 Absorption spectrum of Mo-cyanide complex.

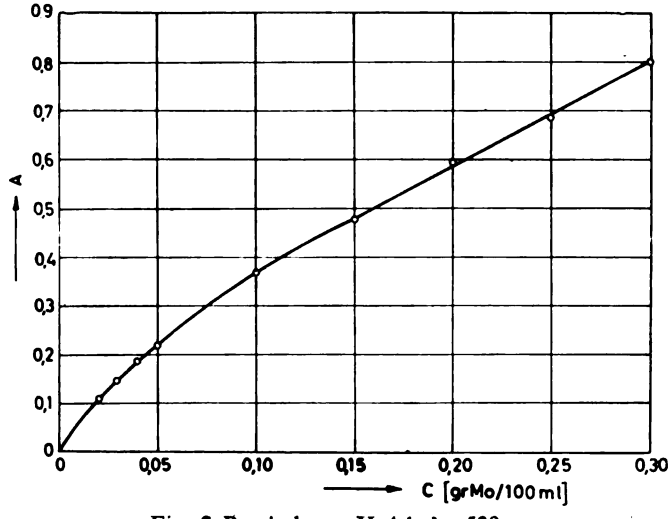

Fig. 2 Beer's law, pH 4.1, $\lambda = 520$ m μ

The effect of pH-values. Solutions which contain equal amounts of molybdate and potassium ferrocyanide were adjusted to various pH-values (in the range 3.28—4.32) by the addition of various amounts of acetic acid It was observed that a precipitate appears at pH-values less than 3.7 but the values above 4.32 are not convenient for this method of determination.

The curve in Fig. 3 shows that the absorption is affected by the change of pH, and therefore it is necessary to perform the measurements at the exact value of pH = 4.1.

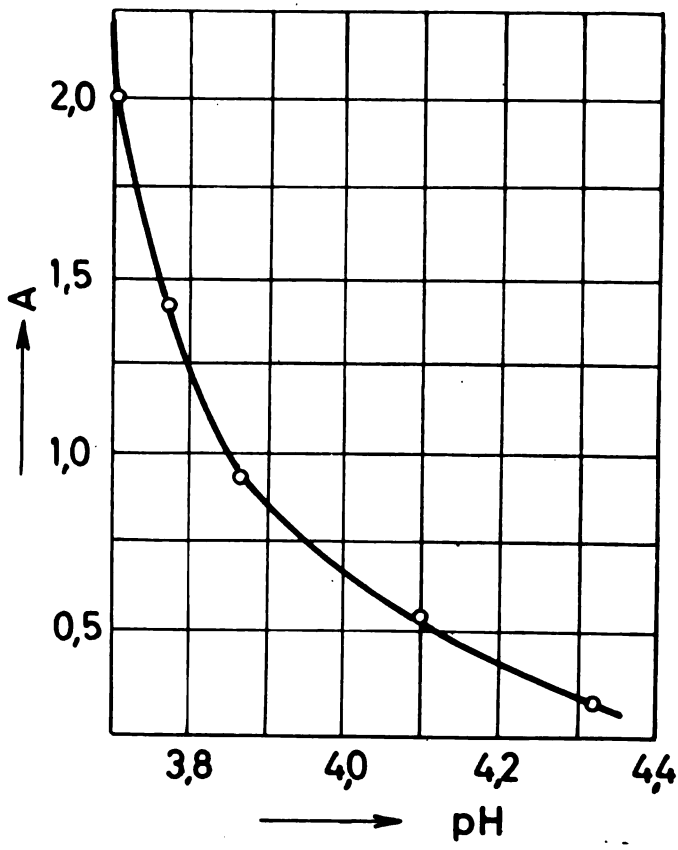

Fig. 3

The stabitity of the color in solution. Fig. 4 shows that the color of Mo-cyanide complex appears almost momentarily. The colored complex is stable for several hours after its formation.

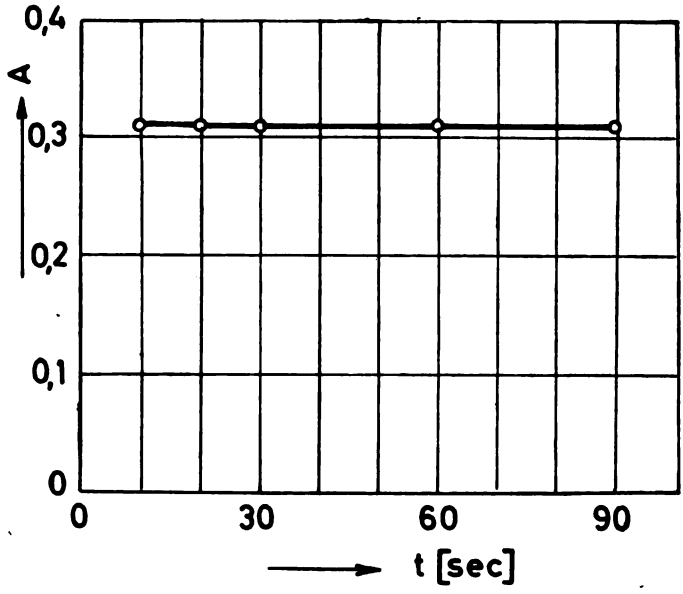

Fig. 4

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A MODIFICATION OF BIJVOET'S METHOD FOR SOLVING THE STRUCTURE OF NON-CENTRO-SYMMETRICAL CRYSTALS

by

JELICA MIŠOVIĆ

Since the diffraction of electromagnetic waves, either those of light, Roentgen, or some other nature, can occur only when the dimensions of the radiation and those of the diffracting object are of the same magnitude, the investigation of crystal structures could have been performed until now with the aid of X-rays, electrons, and slow neutrons.

The three dimensional periodicity of a crystal relates the scatterer to the scattered radiation by a Fourier transformation. To calculate the Fourier transform we need to know not only the intensities of the scattered waves but also the relative phases of the scattered X-rays. The latter data disappear when the intensities are registered on the film, an this fact is one of the essential obstacles which prevents the direct determination of crystal structures on the basis intensities obtained by roentgen diffraction.

In the crystal is considered to be a continuous and periodical distribution of electrons in space, then the intensity of the diffraction would depend on the density of electron distribution. The distribution of electron density can be represented by the Fourier series:

$$\rho(xyz) = \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} A_{hkl} \cos\left[2\pi(hx+ky+lz)-\alpha_{hkl}\right]$$

The coeficients of the Fourier series and the structure factor F_{hkl}, which is directly proportional to the intensity, relate to each other by the following simple expression

$$A_{hkl} = \frac{|F_{hkl}|}{V}$$

and α_{hkl} in the above equation represents the phase angle of the given reflection which originates from a surface with *hkl* indexes. The experiment affords only the intensities, i.e., the modulus $|F_{hkl}|$ of the structure factor. The phase α_{hkl} is not usually obtained by the experiment, but if it could be determined experimentally, the direct determination of the electron density in the unit crystal cell would be possible, thus the direct determination of the crystal structure would be achieved.

In the case of non-centro-symmetrical crystals, the determination of the phase angle is less complicated because for each atom in the position X_r, Y_r, Z_r there is the same atom in the position $-X_r$, $-Y_r$, $-Z_r$, thus α can possess two values: 0 and π; this means that $|F|$ can have either a positive (+) or a negative (—) sign. In the case of non-centro-symmetrical crystals, however, α can possess any value between 0 and 2π. This problem can be solved by many methods, and Bijvoet's technique is one of them.

In this work we have attempted to modify Bijvoet's method, which has been applied to non-centro-symmetrical isomorphous crystals with a heavy atom at the origin, in order for it to become applicable for solving the structure of non-centro-symmetrical isomorphous crystals with heavy atom at the general position. By the use of the vector diagram (Fig 1), which was given for the

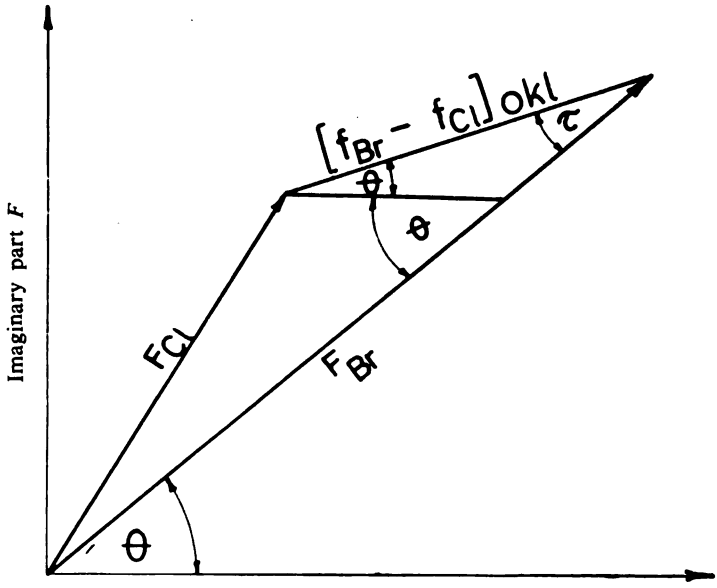

Fig. 1 Vector diagram

Table 1

	k = 2 *n*				1 − 2 *n*				
10 k	$\lvert F_{Br} \rvert$	$\lvert F_{Cl} \rvert$	$f_{Br}\, e^{-B\sin^2\theta/\lambda^2} - f_{Cl}\, e^{-B\sin^2\theta/\lambda^2}$	cos τ	∼ τ⁰	Φ⁰	Φ + τ⁰	Φ − τ⁰	θ calculated in degrees
2 0	38.72	22.49	14.81	1.057	0	0	0	0	8.5
4 0	8.64	1.90	8.65	.976	13	0	13	— 13	—10
6 0	5.00	1.47	4.18	.964	16	0	16	— 16	31.13
0 6	19.79	15.64	16.37	.641	50	180	180	180	180
0 8	24.44	12.18	15.23	.902	26	180	180	180	180
0 10	19.89	4.72	13.97	1.02	0	0	0	0	0
0 12	23.14	8.49	12.50	1.07	0	180	180	180	180
0 14	13.58	4.12	11.08	.964	16	0	0	0	0
0 18	4.49	1.68	8.24	1.152	0	180	180	180	180
0 20	9.36	4.26	6.99	.904	25	0	0	0	0
2 2	17.02	4.91	14.59	.693	16	180	196	164	172
2 4	16.88	10.75	14.20	.774	39	0	39	— 39	—20
2 6	6.02	4.41	13.58	1.230	0	0	0	0	—40
2 8	15.83	9.56	12.66	.797	37	180	217	143	184
2 10	17·88	10.63	11.66	.822	35	0	25	— 35	18
2 12	17.61	7.84	8.98	1.03	0	180	180	180	177
2 14	7.74	3.11	9.26	.949	19	0	19	— 19	—26
2 20	9.07	1.67	6.05	1.05	0	0	0	0	9
4 2	9.64	3.12	8.52	.948	19	180	199	161	179
4 4	5.10	2.12	8.34	1.07	0	0	0	0	4
4 6	4.31	2.93	7.93	1.06	0	0	0	0	13
4 8	6.40	2.90	7.50	.925	23	180	203	157	199
4 10	8.95	3.97	6.90	.906	25	0	25	— 25	13
4 12	10.03	3.87	6.37	.988	9	180	189	171	177
4 14	5.80	1.68	5.75	.958	17	0	17	— 17	—28
1 1	22.86	5.76	17.07	1.00	0	90	90	90	98.12
1 3	32.55	25.86	16.40	.618	52	—90	322	218	315
1 5	18.83	18.08	15.89	.468	62	90	152	28	61
1 7	19.90	15.68	15.03	.629	51	90	141	39	180
1 9	15.17	10.03	14.01	.766	40	—90	310	230	216
1 11	18.74	6.30	12.66	.994	6	90	96	84	94
1 13	21.09	6.12	11.39	1.11	0	—90	270	270	267
1 15	11.29	3.26	9.88	.96	16	90	106	74	99
1 17	5.49	2.37	8.54	1.04	0	—90	270	270	239
1 19	2.82	2.22	7.45	1.39	0	—90	270	270	289
1 23	10.30	2.26	5.33	1.17	0	—90	270	270	261
3 1	13.53	3.37	11.73	.97	13	90	103	77	94
3 3	13.48	9.60	11.53	1.03	0	—90	270	270	313
3 5	8.94	5.48	11.09	.87	29	90	119	61	60
3 7	3.03	1.79	10.42	1.81	0	90	90	90	80
3 9	9.18	2.29	9.76	.973	14	—90	284	256	252
3 11	12.06	3.80	8.87	.980	11	90	101	79	99
3 15	6.94	2.15	7.07	.953	18	90	108	72	110
3 17	4.58	2.08	6.30	.976	12	—90	282	258	248
3 19	5.61	1.93	5.47	.938	20	—90	290	250	299

general case, we were able to calculate the phase angle. The method was verified with two derivatives of diphenyl ketone: 2-amino-4'-bromo- and 2-amino-4'chloro- diphenyl ketone which belong to the non--centro-symmetrical space group $Pna2_1$. When the chlorine atom is replaced by the bromine atom, the structure factor is increased for Δf i.e., for the difference between the diffraction factors of chlorine and bromine. The imaginary part of the structure factor remains constant for structures which have a heavy atom at the origin. However, in the general case when a heavy atom is not at the origin, the imaginary part of F is also changed, and the diagram in Fig. 1 is obtained; the vector $\Delta f=(f_{Br}-f_{Cl})$ is not parallel to the axis which represents the true part of F. Let this vector make a right angle with the axis. Since the position of the atom which is replaced is known from the Patterson synthesis, the angle Φ resulting from the position of the bromine atom can be calculated. Next, the triangle OAB offers the calculation of the angle

$$\cos \tau = \frac{|F_{Br}|^2-|F_{Cl}|^2+|f_{Br}-f_{Cl}|}{2\,|F_{Br}|\,\Delta f}$$

Since $\cos \alpha = -\cos \alpha$, the term τ in the above expression can possess either a negative or a positive sign. The Fourier synthesis was performed with angles $\theta=\Phi+\tau$, and $\theta=\Phi-\tau$. The true position of the molecule can be rather easily obtained from projections which give the position of the molecule and its inversion pattern. The calculation of the structural factor with atomic parameters obtained from the foregoing projection resulted in the obtainance of the factor $R=0.23$, which is an excellent value for the work on solving the structure of non-centro-symmetrical crystals. This was proved by the same author in his final solution of this structure.

It should be emphasized, however, that the difficulties encountered in the determination of the precise value of the angle τ are due to

1. Experimental errors in the course of measurement and

2. Difference in scale factor of two isomorphous crystals. In spite of these difficulties, the obtained and calculated values did not differ significantly, as seen in Table 1.

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CONTRIBUTIONS TO METHACRYLACETONE STUDIES.

I. Synthesis.

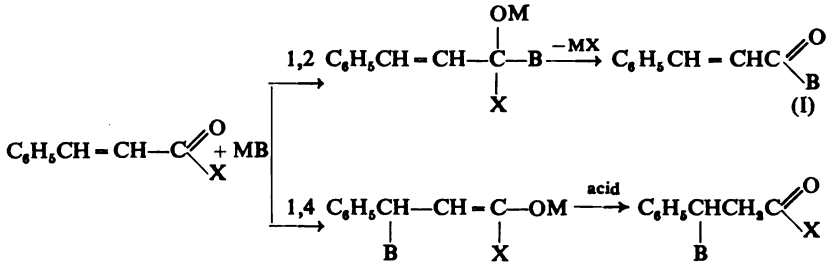
by

DJURO R. KOSANOVIĆ and ALEKSANDAR R. DESPIĆ

Introduction

The synthesis of methacrylacetone monomer (1-hexene 2-methyl 3,5 dione) as described by Despić and Kosanović (1) and by Teyssie and Smets (2) is based upon the Claisen condensation reaction between methacrylic acid esters and acetone in the presence of some basic catalyst, such as sodium methoxide, sodium amide, or metallic sodium. The quoted papers reported very low yields in pure methacrylacetone — as obtained via its copper chelate — up to 7%. Such results indicated that the Claisen condensation is not the predominant reaction in the case of methacrylacetone, but it is accompanied by a series of other reactions which do not take place in the case of syntheses involving saturated compounds. In such a situation, β-diketone is one of the many different reaction products. The purpose of this study was to investigate the possibility of increasing the yield in methacrylacetone by varying the conditions of synthesis, thus reducing the formation of unwarranted reaction products.

The literature on the reactions of unsaturated compounds under similar conditions indicated that, beside the Claisen condensation reaction, main competing reactions could be the addition at the double bond, and the ionic — as well as the radical polymerization. Using the cinnamic acid derivatives, Hauser et al. (3) found that the conditions of the Claisen synthesis favor two reactions, i. e., the 1,2 addition and the 1,4 addition, as illustrated below.



where *MB* is the basic reagent.

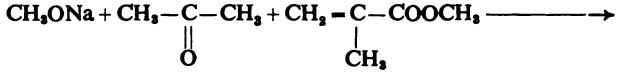
Hauser et al. concluded that the relative amounts of the 1,2 and 1,4 addition compounds formed depend on the rates of addition of the basic anion onto the carbonyl — or the β-carbon atom. The change in the substituent *X* should result in a change of the relative reaction rates since the steric and electronic effects should have a more pronounced influence on the 1,2 addition than on the 1,4 addition. Any substituent which allows an increase in the rate of the 1,2 addition must indirectly have a negative effect on the yield of the 1,4 compound. Such a conclusion may not be fully applicable to the case of methacrylacetone because methacrylic acid esters possess a much more reactive double bond, and do not contain the benzene ring which may influence the behavior of the cinnamic acid.

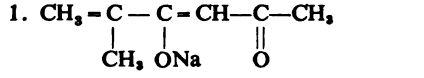
In the course of study of the Michael reaction, Koelsch (4) found that addition compounds are readily obtained from methacrylates or from acrylonitrile which are in contact with alcohols or alcoxy anions. Attempts to perform the Michael reaction with these compounds in alcohol solutions yielded β-alcoxy propionic esters or the corresponding nitriles. Similar results were obtained much earlier by Purdie and Marshall (5), and later by Hall and Stern (6) who reported that alcohols and phenoles are easily added onto the double bond of various acrylates in the presence of basic catalysts, particularly alcoholates and phenolates which yield propionic acid derivatives. Bieber (7) investigated the effect of various alcohols on different methacrylic acid esters in an alkaline medium, and found that methanol is the most reactive and the affinity to addition decreased with the increasing number of carbon atoms. Besides bmethanol, ethanol, utanol, iso-propanol, and terc.-butanol were used.

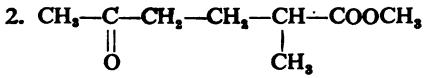
Ionic or radical type polymerization should also be considered when the Claisen synthesis is performed with unsaturated compounds. Although the anionic polymerization of methyl methacrylate and other vinyl compounds has been rather thoroughly studied recently (8, 9, 10, 11), relatively limited attention was given to the use of alcoxydes as the polymerization catalysts for vinyl monomers,

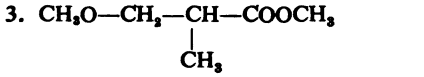
(9, 12, 13). Catalysts which are frequently used for anionic polymerization instead of alcoxides are: lithium and sodium organic compounds, lithium and sodium amides in liquid ammonia, metallic sodium, etc. upon consideration of the conditions required for polymerization with alcoxydes as catalysts, as well as the fact that sodium acetone can also act as a catalyst, we were able to forsee that in the course of the Claisen synthesis, beside the Claisen condensation and the other above-mentioned reactions, the anionic polymerization of both the methyl methacrylate and the produced methacrylacetone could take place. The radical polymerization of the methylmethacrylate and the methacrylacetone is also possible, particularly in the course of isolation and purification if the distillation is performed at elevated temperatures.

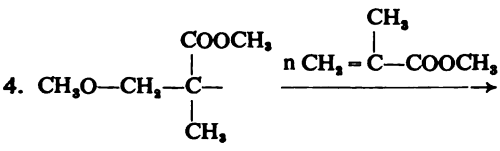
Summarizing these considerations, it can be deduced that the reaction between methyl methacrylate and acetone in the presence of sodium methoxide should be expected to proceed in many directions, according to the following scheme:

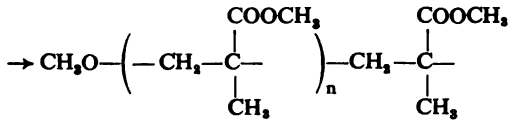


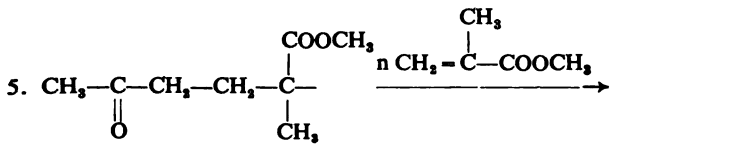


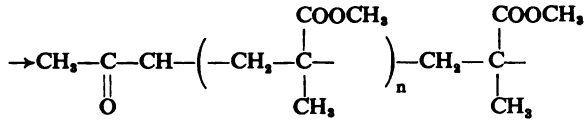












Having in mind that each of the reaction products can further react in some of the above manners, we realize that it is very difficult to determine the composition of the mixture at the end of the reaction period, although the detection and quantitative determination of certain components would give a good indication of the predominant role of some path in the reaction between methyl methacrylate and acetone in the presence of the basic catalyst.

The same difficulty applies to the problem of improving the yield of methacrylacetone, because no definite indication exists as to the effect of the physical and chemical factors on each of the above groups of reactions. Thus, an empirical investigation of the effect of varying the reactants, the temperature, and the time allowed for the reaction on the yield of methacrylacetone was undertaken.

Experimental

The apparatus for the Claisen synthesis was similar to that described earlier (1). The synthesis was performed as follows: 1 mole of sodium metal was placed into the reaction vessel containing 350—400 ml of anhydrous xylene; 1 mole of methyl-or ethyl-alcochol dissolved in 100 ml of xylene was then added in small portions to yield the respective sodium alcoxide upon heating. After this preparation was completed, the reaction mixture was cooled to a given constant temperature, and a mixture of 1 mole of acetone and 1 mole of methyl-methacrylate (or some other corresponding ester) was added dropwise under vigorous stirring for thirty minutes. When this was completed, stirring at a constant temperature was continued for a certain fixed time interval.

The reaction was discontinued by adding 600—700 of ice and water into the reaction mixture, and the latter was stirred as long as there some ice remained. The isolation of the methacrylacetone was performed in a novel manner, which was proved superior to the method applied earlier (1,2). After the Claisen condensation is finished and the ice and water added, two layers are formed: the uppper layer contains the organic solvent and the reaction products dissolved in it; the bottom layer consists of water containing sodium salt of the β-diketone, sodium methacrylate, and other waters-oluble by-products. Ammonium chloride was added to such a mixture in an amount equivalent to that of the sodium used in the reaction. (Instead of ammonium chloride, hydrochloric acid

may also be used). A small amount of concentrated ammonia was then added to achieve a pH value of 8.5, which is an optimum for the formation of the methacrylacetone copper chelate. When this was accomplished, one-eighth of the equivalent amount of crystalline copper sulphate was dissolved in the mixture and the latter was stirred for some time in order to allow the formation of the copper chelate and its almost quantitative extraction into the organic layer.

During the extraction, the pH-value was controlled and when it changed, ammonia was further added to pH 8.5 and stirring was continued until all of some solid phase was dissolved. After that, the dark-green colored organic layer was separated, and the extraction of the aqueous layer repeated two to three times with 50 ml portions of xylene... During the evaporation of xylene from the organic layer and from subsequent extractions, all the by-products of the reaction which were dissolved in it, and distilled over so that the crystalline methacrylacetone copper chelate was obtained in the residue. The latter was recrystallized from ethanol (10 gr of the chelate was dissolved in 200 ml of ethyl-alcochol at the b. p.)

The recrystallized methacrylacetone copper chelate had a m. p. of 167—168° C.

The results of the analysis were as follows:

Found:	C 53.8; H 5.9; Cu 20.5
Calculated for $C_{14}H_{18}O_4Cu$	C 53.57; H 5.78; Cu 20.25

In order to obtain pure methacrylacetone the copper chelate was decomposed in the manner described earlier (2), using 10% sulphuric acid cooled to 0° C. After the decomposition, methacrylacetone was extracted from the solution by ether. The extract was washed twice in water and dried on anhydrous sodium sulphate. The ether was evaporated and the methacrylacetone distilled in vacuum.

B. p. was 70° C at 25 mm Hg or 60° at 20 mm Hg.

Analysis:

Found:	C 66.1; H 7.8
Calculated for $C_{14}H_{18}O_4$	C 66.64; H 7.99

Results and discussion

The investigations of Hauser (3) and Ryan and Dunlea (14) indicated that the change in the substituent X (equation I) in the ester could affect the relative rates of formation of the addition

compounds 1.2 and 1.4. The authors predicted that the relative rate of the 1.4 addition should decrease if X is varied in the following order: —Cl, —OC_6H_5, —OCH_3, —$OC(CH_3)_3$, —$N(C_2H_5)_2$. This was confirmed by the experiments of the Claisen condensation with cinnamic acid esters.

In the present investigation, the first experiments attempting to increase the yield were oriented to the application of such conclusions to the methacrylacetone synthesis. Thus, the variation in the ester radical (methyl-, ethyl-, butyl-, phenyl-) as well as of the catalyst (sodium, sodium ethoxide, sodium methoxide, sodium amide), and the use of methacrylchloride was attemped, although

Table 1.

The yield in methacrylacetone as a function of time and temperature.

t°C	Time (hours)	Yield in the copper chelate (grams)	Yield in methacrylacetone (%)
0°	3	3,7; 3,8; 3,6; 3,9;	2,4; 2,4; 2,3; 2,5;
	4	9,3; 10,7; 9,5;	5,9; 6,8; 6,1;
	5.5	9,2; 9,4;	5,8; 6,0;
	7	9,0; 9,2; 9,4;	5,7; 5,8; 6,0;
	10	8,8; 9,0; 8,7;	5,6; 5,7; 5,5;
	12	8,4; 8,5; 8,3; 8,7;	5,3; 5,3; 5,2; 5,5;
	16	6,5; 7,4; 6,9;	4,1; 4,7; 4,4;
	20	7,0; 7,1; 6,7;	4,5; 4,5; 4,3;
	26	5,7;	3,6;
20°	2	5,6;	3,6;
	3	5,9;	4,4;
	4	8,0;	5,1;
	5.5	4,7; 4,8;	3,0; 3,1;
	23	4,3;	2,7;
	40	1,3;	0,8;
40°	1	3,9;	2,4;
	2	4,0;	2,5;
	3	7,8;	5,0;
	4	1,6;	1,0;
	5	0,9;	0,6;

doubts were justified on theoretical grounds as to the similarity of the cinnamic — and the methacrylic acid esters, and to the possibility to extrapolate the conclusions obtained with the former in the case of the methacrylic acid esters. The experimental evidence obtained proved that in no case could the improved yields in β-diketone be obtained, and that the factors influencing the relative rates of formation of the addition compounds 1.2 and 1.4 with the cinnamic acid esters do not influence the reaction comprising

methacrylic acid esters. The results are in complete agreement with those of Teyssie and Smets (2) who reported similar attempts with the same lack of success.

The failure of this set of experiments oriented further investigation towards a search of other possibilities of changing the ratio of the rates of various reactions which occur during this syntesis. The change in simple physical factors, such as the temperature and the time interval in which the reaction is allowed to proceed, as well as the molar ratio of the reactants, was taken as the subject of further investigations. Although the results of these experiments give an indication of some influence of these factors, no yield higher than 7% could be obtained.

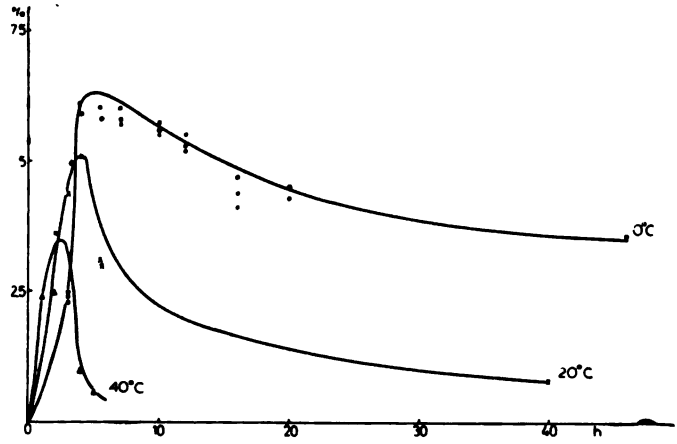


Fig. 1

Results of varying the time and temperature of the reaction, and of the influence of these factors on the yield in methacryl acetone are presented in Table 1 and the corresponding Fig. 1. Besides the experiments performed at 0°, 20° and 40° C, one synthesis was performed at — 10 C° during five hours, yielding 7.2 gr of the copper chelate or 4.5% of the theoretical yield.

The diagram in Fig. 1 shows considerable deviation of certain experimental points from the probable curves. However, the latter are considered to exhibit certain definite tendencies: they all possess maxima of the yield in the diketone for certain times of reaction. The decrease after the maximum is slower when the temperature is lower. The maxima in the yield-time curves are shifted towards longer times of reaction, the lower the temperature. The effect

of the reaction time after the maximum is reached is very small at 0° C. The results obtained at that temperature, at which the best yields are achieved, indicate that the minimum time which should be allowed in the syntheses should not be less than four hours, but also that times of the order of fifteen hours recommended for the Claisen type syntheses are not warranted because maximum yields are obtained between four and six hours. It is probable that longer reaction times stimulate the reactions with the β-diketone formed, and thus lead to the observed decrease in its yield. The reaction of the β-diketone is probably enhanced by the increase in the concentration of alcohol, which is liberated during the condensation and which can affect the double bond of the unsaturated diketone.

As can be seen from Fig. 1, the relative rate of formation of β-diketone as compared to other by-products is highest at 0° C, and at other temperatures some other reactions are favored which result in a decreased yield of the unsaturated β-diketone.

The change in molar ratios of acetone and methyl-methacrylate did not improve the yield of the β-diketone.

In order to obtain some indication about the participation of other possible reaction paths outlined earlier in this paper, an attempt was made to isolate some by-products from the water layer as well as from distillation products of the organic layer. This effort has not yet been completed, although methacrylic acid and the methyl ester of the 5 keto-2-methyl-caponic acid have already been isolated in limited yields. On the other hand, the fact that all of the organic layer could be distilled off, except the copper chelate, proved that the effect of polymerization during the synthesis is not significant.

During this investigation, a modified method was developed for the isolation of the methacrylacetone formed in the Claisen-synthesis. Previous work of Despić and Kosanović (1) as well as that of Teyssie and Smets (2) suggested a method of isolation comprised of a series of operations consisting of the separation of the layers, acidification, extraction by ether, washing of the ether extract, evaporation of ether, fractional distillation, and finally, making the copper chelate. The method described in this instance is much shorter, easier to effect, and results in a reduced loss of methacrylacetone during the isolation procedure.

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N-BENZOYLPHTHALIMIDE

I.

REACTION WITH ALCOHOLS

by

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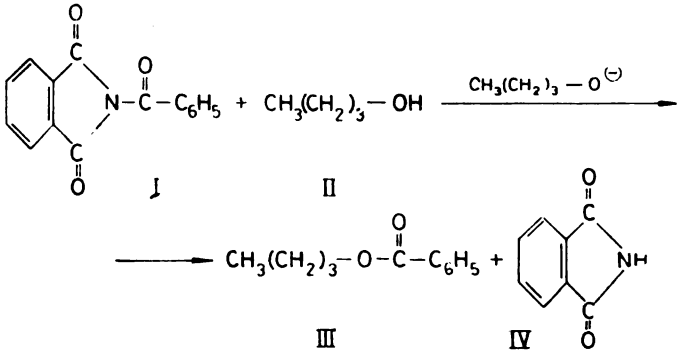
In contrast to N-acylphthalimides which have been studied to a certain extent (1, 2, 3, 4), the chemistry of N-aroylphthalimides is almost unknown. For this reason, and in connection with other studies, we examined in more detail some reactions of N-benzoylphthalimide. The only literature reports on N-benzoylphthalimide describe its preparation (it can be synthetized from benzoyl chloride and phthalimide in pyridine (5); by dehydration of N-benzoylphthalamic acid with acetyl chloride or benzoyl chloride (5); by heating phthaloyl chloride with benzamide or without toluene (6); by refluxing phthalimide, benzaldehyde, and tert-butyl peroxide in xylene with traces of copper salts (7), its partial saponification to N-benzoylphthalamic acid (5), and its reaction with potassium hydroxide (N-benzoylphthalimide when shaken in ether with powdered potassium hydroxide gave solid salts which contained one or two equivalents of base (1).

From these methods of synthesis the best yields (up to 97%) were obtained from the reaction between phthalimide and benzoyl chloride in anhydrous pyridine (5).

N-Benzoylphthalimide did not react at room temperature or in boiling benzene (at 80°), with primary n-butyl alcohol, even in the presence of acid catalysts such as p-toluenesulfonic acid. However, in the presence of a small amount of sodium butoxide, N-benzoylphthalimide (I) benzoylates n-butyl alcohol (II) readily (also at room temperature) to give n-butyl benzoate (III) and phthalimide (IV) (in over 90% yield).



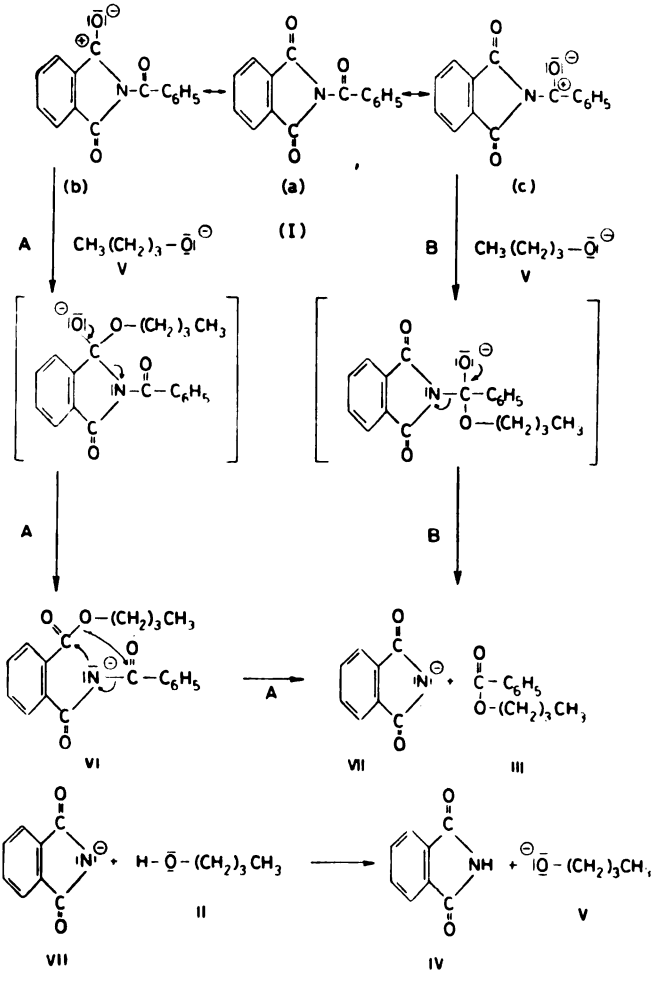
It is probable that benzoylation proceeds by nucleophilic attack of the alkoxide anion (V) on an imide ring carbonyl carbon of N-benzoylphthalimide (Ib), the phthalimide anion (VII) (i.e., the sodium salt of phthalimide) being an intermediate in the benzoylation reaction (path A). This anion (VII), as a very strong base, converted to phthalimide (IV) by abstraction of a proton from n-butyl alcohol (II), thus producing further amounts of n-butoxide ions (V) which will again react with N-benzoylphthalimide (I), etc.*

Although the initial attack by the alkoxide anion (V) can occur directly on the amide carbonyl carbon of the benzoyl residue in N-benzoylphthalimide (Ic; path B). the first alternative (path A), i. e. attack on the ring carbonyl, is preferred: (a) because, according to models, the carbon atoms of these groups are sterically less hindered than the carbonyl carbon of the benzoyl residue and (b) because of the analogy with reactions of phthalimide and N-alkylphthalimides with amines** The anion (VI) with a negatively charged nitrogen could again reform the imide ring with elimination of the alkoxide and benzoyl residues (in the form of n-butyl benzoate (III)) to produce the phthalimide anion (VII). However, the anion (VI) could also stabilizie in another way, i.e., by accepting a proton, and being converted to the n-butyl ester of N-benzoylphthalamic acid (VIII). This ester, as expected was not isolated because it is improbable that it would remain unchanged after heating or prolonged standing, even if it was formed as an

* Such a formulation is supported by the results of Rabjohn, Drumm, and Elliott (4), who have shown that acetylation of n-butyl alcohol with 4-methyl-N-acetylphthalimide proceeded readily when, instead of sodium butoxide, a small amount of sodium 4-methylphthalimide was present in the reaction mixture.

** See paper II on N-benzoylphthalimide.

intermediate product. It is known that esters of phthalamic and phthalanilic acids are unstable and lose alcohol and are converted to the corresponding phthalimides upon mild heating (e. g. in methyl alcohol) (8).* The ester (VIII) can decompose in two ways

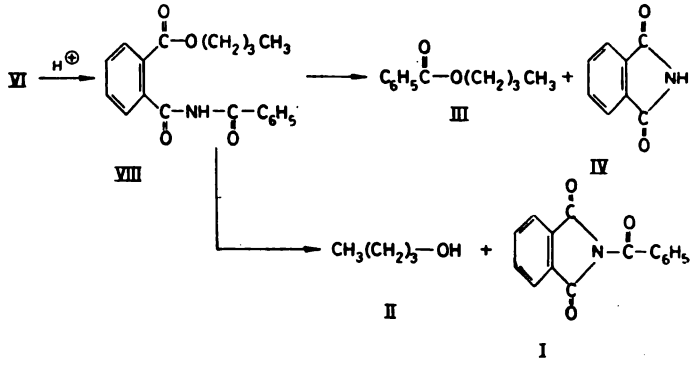


to give either n-butyl benzoate (III) and phthalimide (IV), or the starting products, n-butyl alcohol (II) and N-benzoylhphthalimide (I).

At higher temperatures, in boiling n-butyl alcohol (at 180°) or boiling decalin (at 190—195°), N-benzoylphthalimide benzoylates

* The free phthalamic acids and phthalanilic acid are also readily dehydrated (in boiling ethyl alcohol) to give phthalimides (9, 10, 11), and N-acetyl- and N-benzoylphthalamic acids show a similar behavior (in the presence of acetyl chloride) (5).

n-butyl alcohol after six to eight hours, even in the absence of sodium butoxide, phthalimide being isolated in yields of ca. 70%.



With sec-butyl alcohol, N-benzoylphthalimide reacted less readily than with the primary isomeric alcohol, so that after thirty-six hours at room temperature, in the presence of sodium sec-butoxide, only 31% of phthalimide was isolated (while the same reaction with n-butyl alcohol afforded 92% of phthalimide). After one hour in boiling benzene, in the presence of the corresponding alkoxides, sec-butyl alcohol and N-benzoylphthalimide gave 24% of phthalimide, whereas the primary alcohol (n-butyl alcohol) converted N-benzoylphthalimide to phthalimide in a 54% yield. In order to obtain a nearly quantitative conversion, the reaction with sec-butyl alcohol required five hours of heating (in benzene) (phthalimide was obtained in an 89% yield), and only two hours with n-butyl alcohol. However, in boiling decalin, the secondary alcohol behaves similarly to the primary isomer, and the reaction with N-benzoylphthalimide does not require the presence of the basic catalyst (alkoxide). The yields of phthalimide and sec-butyl benzoate were 62% and 35%, while under the same conditions, the primary alcohol gave 73% phthalimide and 38% n-butyl benzoate.

Finally, tert-butyl alcohol did not react with N-benzoylphthalimide in boiling benzene in the presence of a small amount of sodium tert-butoxide, even after eight hours of heating, although the tertiary alkoxide should be a somewhat stronger nucleophilic agent than the primary and secondary butoxides. However, at higher temperatures (boiling point of decalin), tert-butyl alcohol is partially benzoylated by N-benzoylphthalimide (in a 59% yield), even in the absence of the corresponding sodium alkoxide. All three alcohols behave similarly under these conditions.

The difference in reactivity of the three isomeric alcohols toward N-benzoylphthalimide is best explained by an increased steric hindrance in the folowing order: n-butyl alcohol < sec-butyl alcohol < tert-butyl alcohol. The three methyl groups on the α-carbon atom of tert-butyl alcohol have a considerable steric effect on the hydrohyl group and will strongly hinder the nucleophilic attack of the hydroxyl oxygen on the carbonyl groups of N-benzoylphthalimide, which are themselves hindered to a certain extent. Branching on the α-carbon decreases in the secondary, and particularly in the primary alcohol, thus facilitating the approach of hydroxyl oxygen toward the reactive centres in N-benzoylphthalimide.

Experimental part

All melting and boiling points are uncorrected.

N-Benzoylphthalimide, prepared from phthalimide and benzoyl chloride in anhydrous pyridine (5), melted at 168° after crystalization from ethyl alcohol.

A. *Reaction of* N-*benzoylphthalimide with n-butyl alcohol.*

a) A mixture of 5.0 g. (0.02 mole) of N-benzoylphthalimide and 1.5 g. (0.02 mole) of anhydrous n-butyl alcohol in 50 ml. of anhydroys benzene was heated under reflux for one hour. After evaporation of the solvent (35 ml.), the residue was cooled to 10° and the precipitate (5.0 g.) was recrystallized from ethyl alcohol to give 4.0 g. (98%) of unchanged N-benzoylphthalimide, m.p. 168°.*

b) The same reaction was repeated in the presence of 0.02 g. of p-toluenesulfonic acid, and N-benzoylphthalimide was again recovered in a quantitative yield.

c) The reaction was repeated in the presence of sodium butoxide (which was prepared by dissolving 0.01 g. (0.0004 g. atom) of sodium in n-butyl alcohol, before addition of benzene and N-benzoylphthalimide). After heating under reflux for one hour, the mixture was evaporated *in vacuo* at 35°, and the residue was dissolved in boiling ethyl alcohol. N-Benzoylphthalimide m. p. 166—168°, crystallized upon cooling and was obtained in a 30% yield (1.5 g.). The mother liquor was evaporated *in vacuo*, and the residue was treated with ether-petroleum ether (1 : 1; 50 ml.). The

* N-Benzoylphthalimide and phthalimide were always identified by m. p. and mixed m. p. determinations.

insoluble part was recrystallized from water to give 1.6 g. (54.4%) of phthalimide, m. p. 232—234° (12). From the solution in ether-petroleum ether there was obtained, after evaporation of the solvents and distilation of the residue, 1.5 g. (42.1%) of n-butyl benzoate, b. p. 245—250°/760 mm. (liter. b. p. 247—248°/760 mm. (13)). The product was redistilled, and the fraction boiling at 247—248° was analyzed.

Analysis:
Calculated for $C_{11}H_{14}O_2$ (178.2): C 74.13%; H 7.92%
Found : C 73.92%; H 7.95%

d) A mixture of 5.0 g. (0.02 mole) of N-benzoylphthalimide and 20 ml. of anhydrous n-butyl alcohol, to which 0.11 g. (0.005 g. atom) of clean sodium had been added, was allowed to stand for two hours at room temperature. After neutralizing with 20% aqueous acetic acid, n-butyl alcohol was removed *in vacuo* at 50—60° and the residue was treated, with stirring, with ether-petroleum ether (1 : 1). The precipitate (3.7 g.) was removed by filtration and shaken with cold 5% aqueous sodium hydroxide. The insoluble part (1.5 g.; 30% yield) melted at 168° after crystalization from ethyl alcohol, and consisted of unchanged N-benzoylphthalimide. Phthalimide, m.p. 231—232°, precipitated in a 51% yield (1.5 g.) upon acidification of the aqueous alkaline solution with 10% acetic acid.

e) A mixture of 5.0 g. (0.02 mole) of N-benzoylphthalimide in 25 ml. of anhydrous n-butyl alcohol, in which 0.05 g. (0.002 g. atom) of sodium had been dissolved. was allowed to stand for thirty-six hours at room temperature, with occasional shaking. After standing for another five hours at 0°, the mixture was filtered to give 2.5 g. of phthalimide, m.p. 231—234°. The filtrate furnished a further 0.2 g. of the same product, m.p. 230—232°, and the total yield of phthalimide was 91.8%. By fractional distillation of the filtrate,2.2 g. (61.8%) of n-butyl benzoate, b. p. 245—248° was obtained (13).

f) N-Benzoylphthalimide (25.1 g.; 0.1 mole) in 170 ml. of anhydrous n-butyl alcohol was heated under reflux (118°) for six hours. The mixture was cooled to 0° and, after standing for ten hours at this temperature, the precipitate (14.7 g.) was removed by filtration and extracted with boiling water. Phthalimide, m. p. 232—234°, separated upon cooling in 53.1% yield (7.8 g.). The solid which did not dissolve in boiling water was recrystallized from ethyl alcohol to give 6.0 g. (23.9%) of unchanged N-benzoylpthalimide,

m. p. 167—168°. The mother liquor from the original filtration was fractionated and, besides an excess n-butyl alcohol, furnished 5.6 g. (31.6%) of n-butyl benzoate, b. p. 244—248°/760 mm.

g) A mixture of 25.1 g. (0.1 mole) of N-benzoylphthalimide and 7.4 g (0.1 mole) of anhydrous n-butyl alcohol in 200 ml. of decalin was heated under reflux (190—195°) for six hours. After the working up as described in (f), 10.7 g. (72.8%) of phthalimide and 6.8 g. (38.2%) of n-butyl benzoate were obtained.

B. *Reaction of N-benzoylphthalimide with sec-butyl alcohol*

a) A mixture of 5.0 g. (0.02 mole) of N-benzoylphthalimide and 25 ml. of anhydrous sec-butyl alcohol, in which 0.05 g. (0.002 g. atom) of sodium had been dissolved, was allowed to stand at room temperature for thirty-six hours, with occasional shaking. After neutralizing with 5% aqueous acetic acid and evaporating the alcohol at 25—35° under 10^{-1} mm., the residue was treated with anhydrous ether-petroleum ether (1 : 2; 50 ml.). The precipitate was removed by filtration and shaken with a cold 5% aqueous sodium hydroxid (10 nl). Upon crystallization from ethyl alcohol, the insoluble solid gave 2.9 g. (58%) of unchanged N-benzoylphthalimide, m. p. 167—168°. The aqueous alkaline solution was weakly acidified with 10% acetic acid, cooled to 0°, and the solid which separated was collected by filtration and washed with cold water. 0.9 g. (30.6%) of phthalimide m. p. 230—232°, was obtained.

b) N-Benzoylphthalimide (5.0 g.; 0.02 mole), 1.5 g. (0.02 mole) of anhydrous sec-butyl alcohol, to which 0.01 g. (0.0004 g. atom) of sodium had been added, and 50 ml. of anhydrous benzene were heated under reflux for one hour. The mixture was then worked up as described in (B. a) to give 0.7 g (23.9%) of phthalimide and 3.3 g. (66%) of unchanged N-benzoylphthalimide.

c) The reaction was repeated with 3.0 g. (0.04 mole) of sec-butyl alcohol and 0.02 g. (0.0009 g. atom) of sodium, the mixture being heated under reflux for five hours. Upon cooling the benzene solution, phthalimide, m. p. 230—233°, separated in a 75.2% yield (2.2 g.). From the filtrate, which was worked up as in (B. a), we obtained a further 0.4 g. (13.6%) of phthalimide, m. p. 230—232°, the total yield thus amounting to 88.8%. The residue obtained from the ether-petroleum ether solution, after evaporation *(in vacuo)* of ether and petroleum ether, was distilled and furnished 1.9 g. (53.4%) of sec-butyl benzoate, b. p. 114—117°/16 mm. (liter b. p. 234.5—235.5°/757 mm. (14), 120°/20 mm. (15)).

Analysis:
Calculated for $C_{11}H_{14}O_2$ (178.2): C 74.13%; H 7.92%
Found : C 74.35%; H 7.98%

d) A mixture of 25.1 g. (0.1 mole) of N-benzoylphthalimide and 7. 4 g. (0.1 mole) of anhydrous sec-butyl alcohol in 200 ml. of decalin was heated under reflux for six hours, and then allowed to stand for five hours at 0°. The solid which separated was collected by filtration and extracted with boiling water. The insoluble part, after crystallization from ethyl alcohol, gave 7.2 g. (28.7%) of uchanged N-benzoylphthalimide, m. p. 166—167°, whereas from the aqueous solution, upon cooling, there precipitated 9.1 g. (61.9%) of phthalimide, m. p. 229—223°.

The original decalin filtrate was heater *in vacuo* to 60—80° in order to remove unreacted sec-butyl alcohol, and then separated by gas chromatography. We obtaned 35% sec-butyl benzoate, identified by its infrared spectrum.

C. Reaction of N-benzoylphthalimide with tert-butyl alcohol

a) N-Benzoylphthalimide (25.1 g.; 0.1 mole), 7.4 g. (0.1 mole) of tert-butyl alcohol, and 120 ml. of anhydrous benzene were heated under reflux for eight hours. After standing for twelve hours at room temperature, 24.9 g. (99.2%) of N-benzoylphthalimide, m. p. 166—168° was recovered.

b) The same reaction was repeated in the presence of sodium tert-butoxide (prepared by dissolving 0.18 g. (0.008 g. atom) of sodium in tert-butyl alcohol before the addition of benzene and N-benzoylphthalimide), and unchanged N-benzoylphthalimide, m.p. 166—168°, was recovered in a 97.2% yield (24.4 g.).

c) A mixture of 25.1 g. (0.1 mole) of N-bezoylphthalimide and 7.4 g. (0.1 mole) of tert-butyl alcohol in 200 ml of decalin was heated under reflux for eight hours, and then allowed to stand for twelve hours at room temperature. The precipitate was rewoved by filtration and extracted with boiling water. The insoluble part, after crystalization from ethyl alcohol, gave 7.5 g. (29.9%) of unchanged N-benzoylphthalimide, m. p. 166—167°, whereas from the aqueous solution, upon cooling, 8.6 g. (58.5%) of phthalimide, m. p. 230—232° crystallized.

The original decalin filtrate was heated *in vacuo* to 60—80° in order to remove unreacted tert-butyl alcohol, and then separated into components by preparative gas chromatography. Beside decalin,

33.5% of tert-butyl benzoate, b. p. 100—101°/15 mm. (liter. b. p. 94°/10 mm. (16) was obtained.

Analysis:
Calculated for $C_{11}H_{14}O_2$ (178.2): C 74.13%; H 7.92%
Found : C 74.05%; H 7.82%

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A NEW METHOD FOR THE PREPARATION OF SOME COMPLEXES OF MANGANOUS HALIDES WITH QUINOLINE

by

KOSTA NIKOLIĆ

First data relating to the synthesis of quinoline-manganohalide complexes originate from Reitzenstein (1); he assumed that the formula of the synthetized compoud was $2\ C_9H_7N + MnCl_2$. Borsbach (2) gave the procedure for the preparation of $C_9H_7N + MnCl_2 + + HCl + H_20$, and Taylor (3), after a more detailed study, proposed the formula $C_9H_7NH\,MnCl_3\,2H_20$ for the above compound. The crystallization of this compound from alcohol gave the precipitate containing one less molecule of water. The evaporation of the filtrate obtained after the separation of $C_9H_7NH\,MnCl_3 \cdot 2H_20$ yielded green crystals, the possible formula of which is $(C_9H_7NH)_2\,MnCl_4$ This compound was obtained in a very low yield, and it was difficult to isolate it in a pure state.

Within the investigation of these compounds, we wanted to discover a new method for the preparation of $C_9H_7NH\,MnCl_3\,2H_20$ and $(C_9H_7NH)_2\,MnCl_4$, and also to check the correctness of the proposed formulas by means of a U. V. spectra and the potentiometric method.

Experimental

Procedures for the preparation of $C_9H_7NH\,MnCl_3\,2H_20$

1. Manganese dioxide and quinoline are added to concentrated hydrochloric acid (sp. w. 1.15—1.16); the acid should be taken in excess. The solution obtained is then slowly evaporated in a water bath. It deposits a large yield of red crystals which exhibit the same properties as the compound described by Taylor and Borsbach. On further evaporation, the filtrate turns green, and on cooling it gives a viscous mass, which was extracted with hot ethanol. Green crystals are deposited from the ethanolic solution; they are

the same as those described by Taylor as $(C_9H_7NH)_2[MnC_4]$. Our method gives a very low yield.

2. By mixing equimolecular amounts of alcoholic solutions of quinoline hydrochloride ($C_9H_7N \cdot HCl$) and manganous chloride, the compound $C_9H_7NH\,MnCl_3\,2H_2O$ is obtained. The above compounds are obtained in a pure state by this simple and rapid method.

3. The dissolution of the compound $2\,C_9H_7N + MnCl_2$ in concentrated hydrochloric acid results in the formation of $C_9H_7NH\,MnCl_3\,2H_2O$.

Procedures for the preparation of the compound $(C_9H_7NH)_2\,MnCl_4$.

1. The compound $2\,C_9H_7N + MnCl_2$, obtained according to Reitzenstein, is dissolved in absolute ethanol, and a current of gaseous hydrogen chloride is passed through for five to six hours. The reaction mixture is then slightly evaporated and permited to crystallize.

2. A green crystalline product is obtained by passing gaseous hydrogen chloride for five to six hours through the alcoholic solutions of $C_9H_7NHMnCl_3 2H_2O$ and $(C_9H_7NH)_2MnCl_4$ respectively.

Both of these methods give high yields of pure product.

The Determination of the Structure

U. V. spectrophotometric measurements have shown that the spectra of ethanolic solutions of $C_9H_7NH\,MnCl_3\,2H_20$ and $(C_9H_7NH)_2\,MnCl_4$ are identical, and the spectrum of ethanolic solution of $2\,C_9H_7N + MnCl_2$ is identical with that of quinoline. The absorption spectra of the two former compounds are identical with the spectrum of ethanolic solution of quinoline, to which gaseous hydrogen chloride has been introduced, and identical with the spectra of aqueous solutions which contain quinolinium ion (C_9H_7NH). The electrolysis of aqueous solution results in the appearance of quinoline near the cathode due to reduction of quinolinium ion. This behavior proves the presence of the latter ion in the compounds concerned.

By the migration of ions in the electric field, we have established that the red color of $C_9H_7NH\,MnCl_3\,2H_2O$ solution is due to $[MnCl_3\,\overline{H_2O}]^-$ ions, and the green color of $(C_9H_7NH)_2MnCl_4$ can be ascribed to the presence of $[Mn\overline{Cl_4}]^-$ ions.

By potentiometrical titration, the aqueous solutions of M/10 $C_9H_7MnCl_3\,2H_2O$ and $(C_9H_7NH)_2MnCl_4$ and curves of the same shape have been obtained.

The first slope of the curve is due to the neutralization of $C_9H_7\overset{+}{N}H$ ion, and the second to the precipitation of manganous ions from the solution.

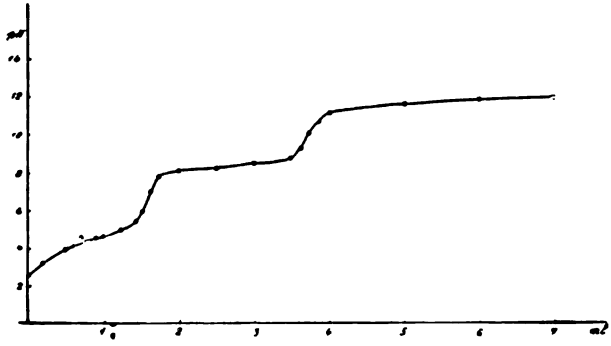

Fig. 1 Potentiometric titration curve of M/10 $C_9H_7NH\,MnCl_3\,2H_2O$ with 1 M KOH

On the basis of spectrophotometrical and potentiometrical measurements, and on the basis of their obtainance, we have come to the conclusion that the formula $C_9H_7NH[MnCl_3H_20]\,H_2O$ is the most convenient for the compound synthetized by Borsbach and Taylor. Measurements have also proved that the formula $(C_9H_7NH)_2[MnCl_4]$ given to the compound of Taylor is correct. Hydrolysis of the latter compound gives rise to the formation of $C_9H_7NH(MnCl_3H_2O)H_2O$; this behavior imposes the assumption that chlorine is linked coordinatively, and manganese is divalent. The divalency of manganese in the compound in question is supported by its obtainance from divalent manganous salts, and by its formation by the action of gaseous hydrogen chloride on manganoquinoline compounds.

Red crystals of the compound $C_9H_7NH[MnCl_3H_2O]\,H_2O$ exhibit an intense red fluorescence; and the compound $(C_9H_7NH)_2[MnCl_4]$ shows a green fluorescence; these properties can be used for rapid identification of the compounds under consideration.

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CHEMICAL INVESTIGATION OF WHEAT

3*

DYNAMICS OF VARIOUS FORMS OF PHOSPHORUS IN WHEAT DURING ITS ONTOGENESIS. RIBONUCLEIC ACID IN RIPE GRAIN OF DIFFERENT VARIETIES OF WHEAT

by

MIHAILO LJ. MIHAILOVIĆ, MIHAILO ANTIĆ, and DIMITRIJE HADŽIJEV

INTRODUCTION

Consideration of the role of nucleotides and ribonucleic acid (RNA) in various tissues of plants has gained increasing importance during the past few years.

This especially corresponds to the investigation of the correlation which exists between protein synthesis, i. e., growth, and RNA content (1). Böttger and Wollgiehn (2) studied this relationship on tobbaco leaves; Potapow and Maroty (3) on the meristems of root tips and shoot apices of bean seedlings; and Skoog and Woodstock (4) in the roots of some inbred lines of corn.

A stimulation for a further intensive study of the role of nucleic acid in the metabolism of higher plants are the recent reports from Hanson (5) and Smillie and Krotkov (6). These authors found that the leaf capacity for oxidative and photosynthetic phosphorylation followed the RNA content. Martin and Morton (7), upon investigation of the root of wheat, formed the conclusion that RNA associated with mitochondria were responsible for oxidative phosphorylation, and particles associated with microsome were involved in protein synthesis.

Recent reports from Oota et al. (8) Osawa and Oota (9), and Cherry et al. (10), particularly concern the nucleotide and

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* Paper 2: Glasnik hem. društva Beograd (Bull. soc. chim. Beograd), 27, 135 (1962).

ribonucleic acid metabolism in corn and other seedlings, and the same metabolism in their cytoplasmic fractions.

Although these investigations often comprised the embryo of seedlings, RNA in the whole grain (pericarp, aleurone layer, endosperm, and scutelum) was neglected. Kondo and Morita (11) recently reported that wheat glutenin is a nucleoprotein. This directed the investigations partly toward the whole grain.

Only one result is cited in the available literature for the content of RNA in ripe wheat grain. It refers to the Japanese variety with 118.1. mg RNA per 100 g of grain (12). This figure is also quoted in the recent biochemical literature (13). The lack of more results might be explained by the absence of a convenient and accurate method for the determination of RNA in seeds, and particularly in wheat grain.

The work reported in this paper was undertaken in order to select a method, and to prove its convenience in the analysis of wheat grain. With the chosen method, and by essentially accepting the criterion of Matsushita (12), the RNA content in the following varieties of wheat was determined: *Bankut 1205* and *Novosadska 1446*, both low yielding domestic varieties; *Abbondanza*, *Elia*, *Fortunato*, *Funo*, *Leonardo*, *Mara*, *Produttore*, and *San Luca*, all high yielding Italian varieties; and *Etoile de Choisy*, a French variety.

The results obtained should provide an answer to the question of which part of the phosphorus in the grain is bound to RNA, and thus partly complete our previous investigations on the phosphorus distribution in wheat grain, and to explain, if possible, the relationship between the RNA content and the genetical production capacities of the chosen varieties.

METHODS

a). *General estimations*

Phosphorus from the RNA hydrolysates was determined by reading the intensity of the blue color at 720 mμ through reduced heteropoly-molybdophosphoric acid. Ribose was estimated by Hurlbert et al's modification of Mejbaum's method (14), by determining the optical density at 660 mμ. Total nitrogen was determined by the Kjeldahl method in the usual way, with potassium sulphate and copper sulphate as catalysts in the proportion of 9 : 1.

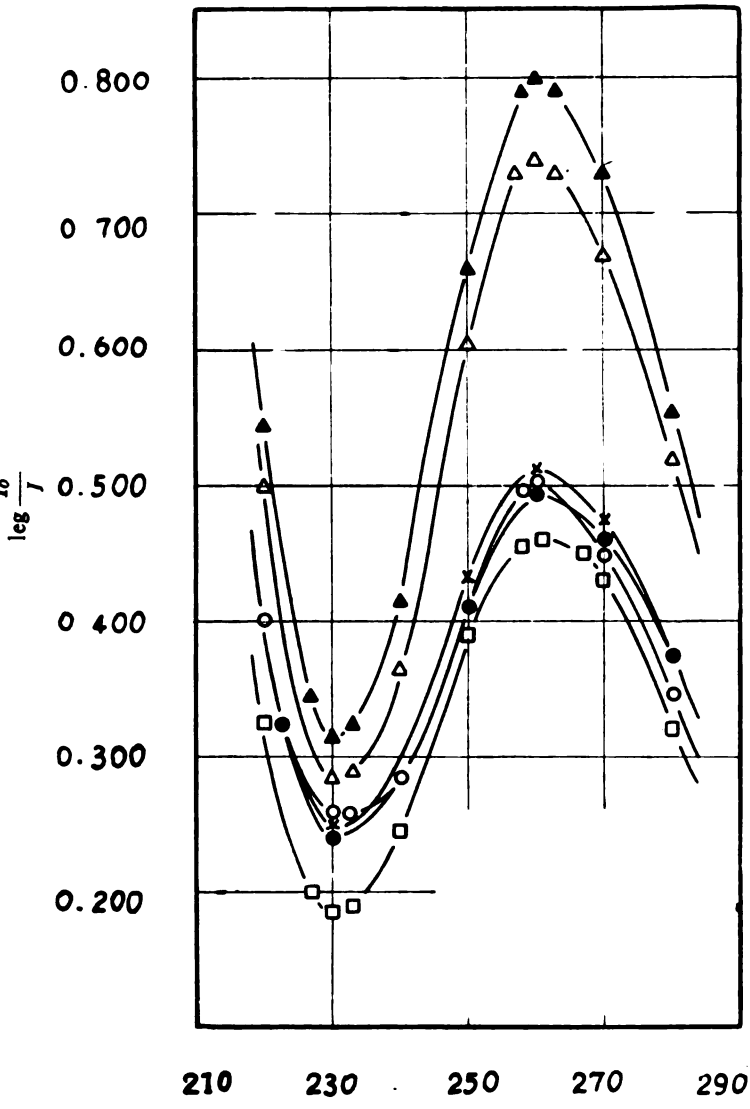


Fig. 1.

UV absorption spectra of ribonucleic acids isolated from root tips of some domestic varieties of corn seedlings

▲ Novisad golden dent, 26, 6 mg/lit RNA;
△ Novisad flajšman, 24, 28 mg/lit RNA;
× Novosadski white dent, 16, 96 mg/lit RNA;
○ Bankut bajša, 16, 63 mg/lit RNA;
● Vukovarski yellow dent, 15, 30 mg/lit RNA;
□ Šidski dent, 14, 93 mg/lit RNA

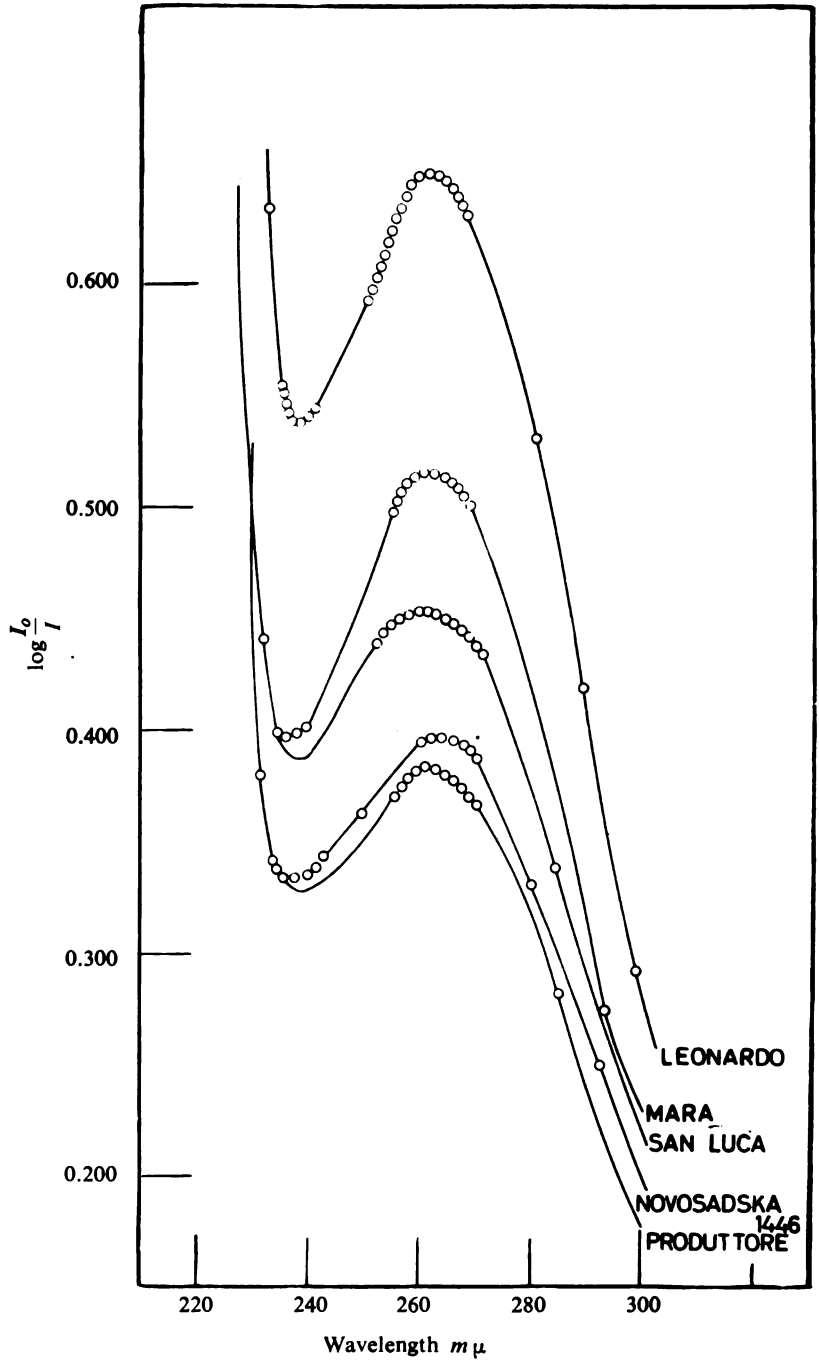


Fig. 2.

UV absorption spectra of ribonucleic acids isolated from ripened grains of some domestic and high yield varieties of wheats

b). *Examination of existing methods for RNA determination in wheat grain*

It is well known that nucleic acid can be extracted from plant or animal tissues by one of the following methods: 1) Schmidt and Thannhauser (15) 2) Schneider (16) and 3) Ogur and Rosen (17).

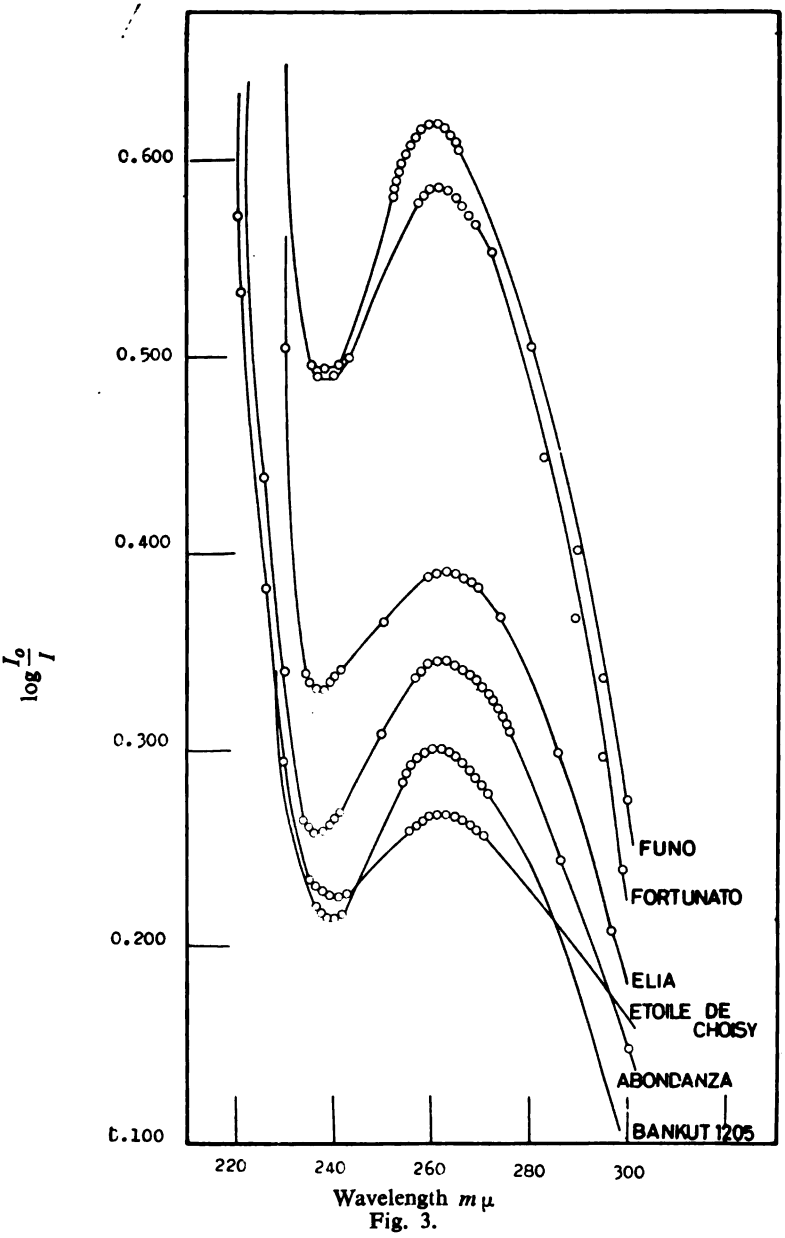


Fig. 3.

UV absorbtion spectra of ribonucleic acids isolated from ripened grains of some domestic and high yield varieties of wheats

While the first two methods have found wide application in the nucleic acid analysis of microorganisms and animal tissues, they are usually not applicable in plant material analysis (7, 12, 18).

When applied to wheat grain, Schmidt and Thannhauser's method (15) gives different results for RNA than those obtained through phosphorus or pentose determinations. Schneider's method (16) using heat, causes the dissolving of starch which is present in grain, and this usually results in a syrupy solution and gives unexpectedly high results by the orcine reaction.

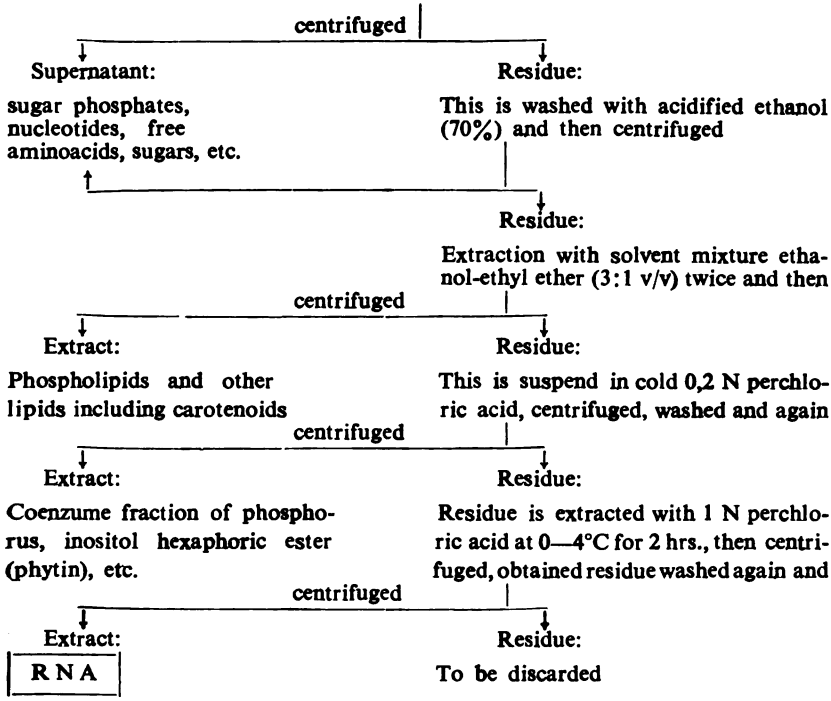
Ogur and Rosen's method (17) was primarily based on RNA and DNA (desoxyribonucleic acid) determinations in the meristem tips of roots of corn seedlings. This method, modified by Holden (18) was also used in analyses of green tobbaco leaves. Satisfactory results were obtained for both nucleic acids by applying it to the root tips of some domestic varieties of corn seedlings. UV spectra of isolated RNA, as shown in Fig. 1, agree with the absorption spectra of the purified commercial specimen of RNA, and show a distinctive minimum at 230 mμ, and particularly at 300 mμ; a maximum at 260 mμ; and a specific absorbancy ratio D^{260}/D^{286}. But when this method is applied to the ripe wheat grain, RNA is isolated with protein impurities (Folin-phenol reagent; see ref. 19), regardless of the investigated wheat variety. Protein presence is also obvious from the UV spectra given in Fig. 2 and 3.

All UV absorption spectra of isolated wheat nucleic acids showed distinctive shifts. Thus, the minimum absorption at 230 mμ is displaced to 240 mμ, and in some spectra there is an appearance of a convex shape of the curve between 270 and 290 mμ as compared to the opposite side of the absorption peak. It is known that protein hydrolysates give absorption maxima at about 280 mμ, and high absorbancy readings with a steep, sloping curve in the range of 230 mμ (20). It is obvious that the absorption spectra of RNA isolated from different varieties of wheat grain are the result of the superposition of the absorption of pure RNA and proteins present as impurities.

c). *Nucleic acid extraction*

RNA extraction and the removal of other interfering compounds is described in Scheme 1. It can be seen that essentially Ogur and Rosen's procedure was followed (17) with the difference that the phospholipids were extracted at a slightly lower temperature.

Milled grain (particle size below 60 meshes) is homogenised with 70% ethanol at room temperature and then



Scheme 1.— Extraction procedure for RNA from ripe wheat grain

d). *Quantitative determination of RNA*

Although RNA and protein absorptions are of mutual influence and similar in the ultraviolet region, it is still possible to calculate the proportion of proteins and RNA by reading the absorbancies at 280 and 260 mμ in the investigated solutions (12, 21).

If the absorbancy ratio for ribonucleic acid, D^{280}/D^{260}, is designated by "*A*", the absorbancy ratio for proteins, D^{260}/D^{280}, bu "*B*", the absorbancies of the protein and RNA mixture at the two wavelengths by D^{260}_{mix} and D^{280}_{mix}, the absorbancies of pure nucleic acid by D^{260}_{na}, and of proteins by D^{280}_{prot}, then, in a given wheat grain extract, the absorbancy value which derives exclusively from the present nucleic acid (D^{260}_{na}) is calculated from the following equations:

$$\text{I.} \quad D^{260}_{mix} = D^{260}_{na} + D^{280}_{prot} \times B$$

$$\text{II.} \quad D^{280}_{mix} = D^{280}_{prot} + D^{260}_{na} \times A$$

The value $D^{280}/D^{260} = A$ for purified RNA (Merck) is 0.616. For $D^{260}/D^{280} = B$ we accepted Matsushita's value of 0.963, obtained with the casein hydrolysate in 1 N perchloric acid, and which optically behaved as the hydrolysate of wheat grain protein (12).

When A and B in Equations I and II are replaced by these constants, one obtains:

I. $D^{260}_{mix} = D^{260}_{na} + D^{280}_{prot} \times 0.963$

II. $D^{280}_{mix} = D^{280}_{prot} + D^{260}_{na} \times 0.616$

By placing the value for D^{280}_{prot} from Equation II into equation I, one finally obtains:

$$D^{260}_{mix} = D^{260}_{na} + 0.963\left(D^{280}_{mix} - 0.116 \times D^{260}_{na}\right)$$

$$D^{260}_{mix} = D^{260}_{na} + 0.963 \times D^{280}_{mix} - 0.5932 \times D^{260}_{na}$$

$$D^{260}_{na} = \frac{D^{260}_{mix} - 0.963 \times D^{280}_{mix}}{0.407}$$

It can be seen from the final equation that the absorbancy for RNA in wheat grain extracts, in the presence of protein impurities, can be obtained by determining the absorbancy at two wavelengths, and introducing the values obtained into the given equation.

All readings were performed on a quartz spectrophotometer (Beckman Model DU). The calculation of RNA-P (phosphorus incorporated in RNA) was achieved by using the molar extinction coefficient for phosphorus (22) and the content of RNA was calculated from Schneider's factor (16).

RESULTS AND DISCUSSION

a). *Discussion concerning the applied method*

Wheat grain has a low content of RNA and a high content of starch and other sugars. It is for this reason that the methods of Schmidt and Thannhauser (15) and Schneider (16) could not be applied. Therefore, a modification of the method of Ogur and Rosen was used (17). Since perchloric acid shows a negligible absorption in the ultraviolet region, and dissolves RNA in the cold (0 – 4°C) without a noticeable hydrolysis of starch, the grain extracts of RNA were measured directly in the UV light. However, the extracts did not show absorption spectra which were specific for a pure

specimen of nucleic acid. The ratios D^{260}/D^{286}, D^{260}/D^{230} and D^{240}/D^{260} indicated the presence of impurities in all varieties of wheat. Therefore, a modification had to be used which would acount for the superposition of the UV absorbancies, caused by proteins as the major impurity.

By using the constant 0.616 for the ratio D^{280}/D^{260} for nucleic acid, and the constant 0.963 for the similar ratio for proteins, the determination of RNA in wheat grain gave results with a standard deviation of about 2.3%.

To illustrate the differences between corrected (according to the above-described procedure) and uncorrected measurements, the results for a few varieties of wheat are listed in Table 1:

Table 1.

The amounts of RNA-P and RNA (in mg %) in the grain of different wheat varieties, calculated from ultraviolet absorption data with and without correction for proteins present in the extract*

Variety	Moist gluten	Uncorrected RNA-P	Uncorrected RNA (mg%)	Corrected RNA-P	Corrected RNA (mg%)	Difference %
Produttore	26.85	14.10	150.02	5.90	62.81	+ 138.9
San Luca	28.10	16.02	170.45	7.14	76.01	+ 124.3
Fortunato	30.95	21.68	230.67	9.18	97.65	+ 136.1
Mara	30.10	17.97	191.20	9.51	101.17	+ 88.9
Funo	31.60	20.03	213.12	9.52	101.34	+ 110.3
Novosadska 1446	36.32	16.06	170.88	9.78	104.05	+ 64.2
Bankut 1205	37.25	15.97	169.92	9.95	105.90	+ 60.5

*These results and those in the following tables are based on dry matter. Uncorrected results are calculated without substraction of D^{230}.

It is evident from table 1 that the differences are high (60.5—138.9%) and, which is very important, they do not follow the gluten content of wheat grain, thus confirming that the quality, and not the quantity, of gluten influences the accuracy of the RNA determination.

It is well known that aromatic amino acids are responsible for the UV absorption of proteins or their hydrolysates. According to the above-mentioned observations, it can be presumed that the contents of phenylalanine, tyrosine, and tryptophane are variable in the gluten of the investigated varieties. The analysis of some wheat grains indicates that such small differences do, in fact, exist (23). Therefore, the question arises as to whether or not it is justified to accept and apply Matsushita's constant D^{260}/D^{280} for

proteins to different varieties of wheat. In order to obtain an answer, the RNA contents of a domestic and an Italian high yielding wheat variety, differing maximally in the quality of their gluten, were also colorimetrically estimated through phosphorus. The results of these control tests are listed in Table 2:

Table 2.

The amounts of RNA-P and RNA (in mg %) calculated from ultraviolet absorption data and from the colorimetrical determination of phosphorus.

Variety	By UV absorption (corrected) RNA-P (mg %)	RNA (mg %)	Colorimetrically RNA-P (mg %)	RNA (mg %)	Difference (%)
Produttore	5.90	62.81	6.46	68.73	9.5
San Luca	7.14	76.01	7·83	83.31	9.7
Mara	9.51	101.17	10.35	110.12	8.9
Novosadska 1446	9.78	104.05	10.78	114.75	10.3

The obtained results showed that nucleic phosphorus which was colorimetrically estimated was on an average of 10% higher as compared to the values obtained from UV absorption data and using the constant D^{260}/D^{280}. It is evident from these analyses that the quality of gluten in the low and high yielding varieties of wheat had no influence on the results.

The differences found in results between the two methods were expected. Earlier investigations showed that the RNA estimation in plant material colorimetrically through phosphorus usually gave higher values. This was attributed do the residual, phosphorus-containing, acid soluble fraction which was not completely removed in the previous steps of the extraction procedure. For this reason, we agree with the opinion of some other authors (7, 12, 24) that results achieved for RNA-P by UV absorption data are more reliable.

b). *RNA content in different varieties of wheat grain*

The contents of RNA-P, RNA, nitrogen, protein, and moist gluten in different varieties of wheat are given in Table 3.

The content of phosphorus incorporated in the RNA of ripened grain varied within the range of 5.9 – 9.95 mg P per 100 g of grain, depending upon the variety. The Italian high yielding variety *Produttore* had the lowest content (5.9 mg%), and the low yielding variety *Bankut 1205* had the highest content, 9.95 mg%.

The RNA content changed parallely, i. e. from 62.8 mg% for the variety *Produttore* to 105.9 mg% for the variety *Bankut 1205*.

Table 3.

The amounts of RNA-P, RNA, nitrogen, proteins and moist gluten in ripe grain of different varieties of wheat.

Variety	Moisture (%)	RNA-P (mg %)	RNA (mg %)	N (%)	Protein (N×6.25) %	Most gluten* (%)
Produttore	10.1	5.90	62.81	1.56	9.81	26.85
Elia	10.4	7.11	75.61	2.19	13.73	—
San Luca	10.4	7.14	76.01	1.66	10.42	28.10
Abbondanza	10.5	7.14	76.02	1.71	10.73	31.20
Etoile de Choisy	10.2	8.67	92.29	1.75	10.96	29.46
Fortunato	10.4	9.18	97.65	1.81	11.31	30.95
Mara	10.2	9.51	101.17	1.90	11.89	30.10
Funo	10.5	9.52	101.34	1.87	11.69	31.60
Leonardo	10.3	9.55	101.57	2.01	12.58	19.70
Novosadska 1446	10.9	9.78	105.05	2.03	12.73	36.32
Bankut 1205	10.2	9.95	105.90	2.16	13.51	37.25

*The gluten analysis (25) refers to flour of 60% extraction and were performed by Ing. D. Podkrajnik-Petrić, Institute for Food Technology, Novi Sad.

Comparing these results with the known variety characteristics of the investigated wheat, it is evident that the highest contents of RNA were found in the low yielding domestic varieties *Novosadska 1446* and *Bankut 1205* (on the average, 104.9 mg% RNA). whereas the Italian high yielding varieties had an average of 86.5 mg% RNA. However, there were still large differences within these varieties. While *Leonardo*, *Mara*, *Funo*, and partly *Fortunato* were similar to the domestic varieties in their RNA contents, this content in the varieties *Abbondanza*, *Elia*, and *San Luca* was rather low and amounted to an average of 75.8 mg%. In the variety *Produttore*, as previously mentioned, the content was exceptionally low, 62.8 mg RNA per 100 g of grain.

Protein determination in the grain of the same varieties gave the following results. The domestic varieties had an average of 13.1%; the high yielding varieties *Leonardo*, *Mara*, *Funo*, and *Fortunato*, 11.9%; *Abbondanza* and *San Luca*, 10.6%; *Elia*, an unexpectedly high percentage of 13.73; and the variety *Produttore* had the lowest content of proteins, 9.81%. The French variety *Etoile de Choisy* was similar to the Italian variety *Abbondanza* in its protein content.

The gluten content also varied in the investigated varieties, from 19.7 to 37.3%. The highest content was again found in the domestic varieties (on the average, 36.8%); and the Italian variaties *Mara*, *Funo*, and *Fortunato* had, on the average, 30.9%; *Abbondanza* and *San Luca*, 29.6%; and the variety *Produttore*, 26.8% of gluten. Unexpectedly, a very low gluten content was found in the variety *Leonardo* (19.7%). However, it should be mentioned that the gluten quality of this variety is far better than that of the other high yielding varieties of wheat.

Considering all the given results, it is obvious that a positive correlation exists between the amount of RNA and that of gluten in wheat grain. However, exceptions were found, thus limiting the general application of Brachet's hypothesis (1) and confirming the complex role of RNA in wheat grain and the need for further investigation of this problem.

From the aspect of phosphorus incorporation in different compounds of wheat grain, it might be concluded that the phosphorus content in RNA represents only 1.2% of the total phosphorus in the grain for the domestic variety, and 0.9% for the high yielding variety.

Further investigation are being performed essentially by applying the same methods for RNA determination, and these should answer the question as to whether the RNA content in the grain is a specific property of a variety, or if it varies (and to what extent) depending upon applied crop production measures.

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GLASNIK HEMIJSKOG DRUŠTVA, vol. 27, no, 5—6, 1962, pp. 241-252,

STATISTICAL QUANTUM INTERPRETATION OF THE EQUILIBRIUM CONDITION BETWEEN LIQUID AND VAPOR PHASES AT BOILING TEMPERATURES OF MONOATOMIC ELEMENTS

by

J. M. ŽIVOJINOV

The purpose of this work was to ascertain by means of statistical and quantum mechanics, the conditions of equilibrium between the liquid phase and its vapor at boiling temperature, i. e., conditions which should be valid fer the temperature interval from the triple point to the critical temperature of the monoatomic substances.

In a previous paper (1) we assumed that the particles of a substance in the liquid phase at boiling temperature are localized, i. e., thet the probability of particle distribution is given by the expression

$$w' = \frac{n'!}{n'_0! n'_1! n'_2! \ldots n'_r!} \tag{1}$$

where n' represents the total number of particles in the liquid phase; n'_0 the number of particles which are in the lowest energetic quantum state; n'_1 the same, but in the first higher quantum state, etc. In the case of elements which have monoatomic vapor, in the earlier paper (1) we made one more assumption: at boiling temperature particles of monoatomic elements are in the same monoatomic state, there are no associations. We are allowed, then to accept that in the liquid phase, too, particles possess only energy of translation. Thus, the partition function is given by the expression of the partition function which is valid for the ideal gas and vapor. In this case, it is given by

$$p' = e^{-\frac{\varepsilon'_0}{KT}} + e^{-\frac{\varepsilon'_1}{KT}} + e^{-\frac{\varepsilon'_2}{KT}} + \ldots + e^{-\frac{\varepsilon'_r}{KT}} \tag{2}$$

ε'_0 denotes the lowest, and, simultaneously, the zero energy level of particles in the liquid phase; ε'_1 the first higher energy level, etc.,

and K is the Boltzmann constant. Analogous to this expression, the partition function for the vapor phase is given by the expression

$$p'' = e^{-\frac{\varepsilon''_0}{KT}} + e^{-\frac{\varepsilon''_1}{KT}} + e^{-\frac{\varepsilon''_2}{KT}} + \ldots + e^{-\frac{\varepsilon''_r}{KT}} \tag{3}$$

which has already been used in the literature. Here also, this expression is valid for the case when the lowest energy level is at the same time the zero energy level, i. e., when $\varepsilon''_0 = 0$. ε''_1 denotes the first higher level, etc. Hower, if we accept that all the particles in the vapor phase are shifted over the zero energy level for a certain energy value η, taking this as the sublimation energy of particles at the absolute zero temperature, the following relations exist between the new energy levels and that previously mentioned:

$$\begin{aligned}
\bar{\varepsilon}''_0 &= \varepsilon''_0 + \eta \\
\bar{\varepsilon}''_1 &= \varepsilon''_1 + \eta \\
&\ldots\ldots\ldots \\
&\ldots\ldots\ldots \\
\bar{\varepsilon}''_r &= \varepsilon''_r + \eta \\
&\ldots\ldots\ldots \\
&\ldots\ldots\ldots
\end{aligned} \tag{4}$$

According to the equation (3), the new partition function for particles will now be (when the energy is measured from the new zero level, now being higher than the previous zero level for the value η)

$$\bar{p}'' = e^{-\frac{\bar{\varepsilon}''_0}{KT}} + e^{-\frac{\bar{\varepsilon}''_1}{KT}} + e^{-\frac{\bar{\varepsilon}''_2}{KT}} + \ldots e^{-\frac{\bar{\varepsilon}''_r}{KT}} + \ldots \tag{5}$$

i. e..

$$\bar{p}'' = e^{-\frac{\eta}{KT}}\left(e^{-\frac{\varepsilon''_0}{KT}} + e^{-\frac{\varepsilon''_1}{KT}} + \ldots + e^{-\frac{\varepsilon''_r}{KT}} + \ldots\right) \tag{6}$$

by substituting the terms in the brackets with the equation (3) we obtain:

$$\bar{p}'' = e^{-\frac{\eta}{KT}} \cdot p'' \tag{7}$$

In our problem, which is related to the equilibrium state between the liquid phase and its vapor at boiling temperature, particles in both phases are of the same nature. This equilibrium state is dynamic by nature, which means that a certain number of particles, under a constant temperature and pressure condition, can be transferred from the liquid phase into the vapor and vice versa, having at the same time the energy of the whole system (liquidvapor) constant.

We will study this equilibrium state from the viewpoint of statistical and quantum mechanic. Then, in order to have the condition for the constancy of the total energy fulfilled, we shall accept that there is an energy quantum state in the liquid phase, e. g., the level ε'_k, having the property of being equal to an energy quantum state of a level in the vapor phase, e. g., ε''_r; thus, $\varepsilon'_k = \varepsilon''_r$. If a particle is transferred from the energy level ε'_k in the liquid to the level ε''_r in the vapor, the total energy of the system will remain constant. However, the probability of distribution will change in the vapor phase as well as in the liquid phase. We treated this in detail in a previous paper (1). Therefore, we will present only the relations wich are necessary to solve the problem.

Between the probability of distribution of particles, W'^*, in the liquid after such a transfer of a particle from the level ε'_k and its previous value W', there is the relation

$$\frac{W'^*}{W'} = \frac{e^{-\frac{\varepsilon''_k}{KT}}}{p'} \tag{8}.$$

Before we give the relation between the changed, W''^*, and the original value of the distribution probability of particles, W'', in the vapor, after the arrival of a particle at the level ε''_k, we shall mention only that the distribution probability of particles in vapor (they are nonlocalized) is given by the expression

$$W'' = \frac{(n''_0 + p_0)!}{n''_0!\, p_0!} \times \frac{(n''_1 + p_1)!}{n''_1!\, p_1!} \times \ldots \times \frac{(n''_r + p_r)!}{n''_r!\, p_r!} \times \ldots \tag{9}.$$

Here, n''_0 is the number of particles (atoms) of the vapor distributed among p_0 energy quantum states; n''_1 is the number of particles per p_1 states, etc. In regard to the relation between the mentioned probabilities, it is:

$$\frac{W''^*}{W''} = \frac{n''_r + p_r}{n''_r} \tag{10}.$$

It is known from statistical mechanics that the term on the righthand side of this equation is equal to the following multiple:

$$\frac{n''_r + p_r}{n''_r} = e^{\lambda} \cdot e^{\frac{\varepsilon''_r}{KT}} \tag{11}$$

in which we accepted (1) that

$$e^{\lambda} = \frac{p''}{\bar{n}''} \tag{12},$$

Here we introduce one more important assumption, that $\bar{n}''$ is the number of particles being transferred from one energy

level to another, but under the condition that the total energy of the system remains constant. Our main aim in this work is to determine the value of this number for the particular chemical elements. In order to find a suitable expression for its determination, we shall use the following method. From the equation (10) using equations (11) and (12), we obtain the equation

$$\frac{W''^{*}}{W''} = \frac{p''}{\bar{n}''} \cdot e^{\frac{\varepsilon''_r}{KT}} \tag{13}$$

From equations (13) and (8) we can find the relation between the multiple of the changed distribution probability and its original value as

$$\frac{W'^{*} W''^{*}}{W' W''} = \frac{p''}{\bar{n}''} \frac{\bar{e}^{\frac{\varepsilon'^{k}}{KT}}}{p'} e^{\frac{\varepsilon''_r}{KT}} \tag{14}$$

Since $\varepsilon'_k = \varepsilon''_r$, the equation given above can be written as

$$\frac{W'^{*} W''^{*}}{W' W''} = \frac{\frac{p''}{\bar{n}''}}{p'} \tag{15}$$

The value of this expression can be larger, smaller, or equal to unity. From the physical viewpoint that means that during the evaporization

$$\frac{p''}{\bar{n}''} > p' \tag{16}$$

However, when the vapor particles condense, it is due to

$$\frac{p''}{\bar{n}''} < p' \tag{17}$$

In the case we are dealing with, where there is an equilibrium between the liquid phase and the vapor phase, neither direction of occurrence is privileged. Then, from equation (15)

$$\frac{p''}{\bar{n}''} = p' \tag{18}$$

From this equation we find that the number of particles $\bar{n}''$, which are being transferred in the described manner (at the constant total energy of the system) is given by the following expression:

$$\bar{n}'' = \frac{p''}{p'} \tag{19}$$

This expression shows that the number of particles for which we are looking is equal to the quotient of the partition functions

for the vapor and the liquid (vapor is saturated). In this case, particles can be transferred in the described manner without any change of W'^* W''^* and W' W'' values, i. e., the multiple of distribution probabilites of particles in the liquid and vapor will remain the same.

We will determine the value of this expression for certain monoatomic elements, such as argon (Ar), krypton (Kr), xenon (Xe) and neon (Ne). These have been chosen because all the necessary data for the equation (19) are known from earlier measurements (2). However, it is necessary to mention that both partition functions in the equation (19) are related to the same basic level. However, if we accept that all the particles in the vapor phase, at the absolute zero temperature, received a certain quantity of thermal emergy, the heat of sublimation, we may consider that all the particles in the vapor shifted their energy quantum state for the corresponding value of the heat of sublimation. The magnitude of this thermal energy will be denoted, as in the beginning of this work, with η. The new partition function related to the ground energy level, shifted from the zero level for η, has been given by equation (7). Introducing in equation (19) the values of partition functions for liquid and vapor, each one related to its own ground energy level, we obtain from equations (7) and (19):

$$\bar{n}'' = \frac{\bar{P}'' e^{-\frac{\eta}{KT}}}{p'} \tag{20}.$$

In the beginning of this work we introduced the assumption that, for monoatomic elements, the partition function is in fact the partition function for translational energy, P_{tr}. Then, equation (20) may be written in the form:

$$\bar{n}'' = \frac{\bar{P}''_{tr} e^{-\frac{\eta}{KT}}}{p'_{tr}} \tag{21}.$$

where $\bar{p}''_{tr}$ represents the translational partition function for particles in vapor, and p'_{tr} the same, but in liquid. It is known from quantum mechanics (3) that the translational partition function is given by the following expression:

$$P_{tr} = \left(\frac{2\pi \cdot m\, kT}{h^2}\right)^{\frac{3}{2}} g \cdot v \tag{22}.$$

in which m is the mass of the particles (here, atomic mass); k — Boltzmann constant; T — absolute temperature; h — Planck constant; g — statistical weight of the lowest electronic quantum state in the atom of the related element; and v — volume (usually specific volume).

By applying this expression for the partition function for the vapor phase, we obtain:

$$\bar{P}''_{tr} = \left(\frac{2\pi m KT}{h^2}\right)^{\frac{3}{2}} g \cdot v'' \qquad (23).$$

v'' being the specific volume of vapor. This expression is known in the literature (3). But, the new moment here is the application of equation (22) to the liquid phase, too (based on the introduced assumption). Applied to the liquid phase. the equivalent expression is:

$$\bar{P}'_{tr} = \left(\frac{2\pi m KT}{h^2}\right)^{\frac{3}{2}} g \cdot v' \qquad (24)$$

with v' representing the specific volume of liquid at the same temperature. Substituting $\bar{P}''_{tr}$ from (23) and P'_{tr} from (24) into (21), we obtain

$$\bar{n}'' = \frac{v''}{v'} e^{-\frac{\eta}{KT}} \qquad (25).$$

Accepting all the other values to be the same for both liquid and vapor. Since our assumptions are considered to be valid only for the equilibrium between the liquid and its vapor, the obtained relation should be valid for any monoatomic element in a temperature interval between its triple point and critical temperature. In order to analyze it more thoroughly, we shall apply it to the critical temperature. Since the specific volumes of liquid and vapor are then the same, equation (25) will have the form:

$$\bar{n}''_k = \frac{1}{e^{\frac{\eta}{KT_k}}} \qquad (26).$$

As all the magnitudes included in this above equation are constant for one particular element, the entire expression should be constant. The question now is what is the physical meaning of this number $\bar{n}''_k$ at the critical temperature. As both phases at the critical temperature are identical, the meaning of $\bar{n}''_k$ representing the number of particles being transferred from one phase into another has no sense. However since $\bar{n}''_k \neq 0$, there is some transfer of atoms from one energy level to another. While one atom from the energy level, e. g., ε''_j can drop to the lower level, ε''_l, at the same time an other atom will change its energy level from ε''_j to ε''_k (first higher). Thus, the total inner energy remains unchanged. In other words, we assume that at the critical temperature there is a possibility of only this and similar kinds of transfer. This possibility will be symbolically termed: one degree of freedom of transfer.

For the transfer of particles at temperatures lower than critical, we accept here that there are three "degrees of freedom" due to the coexistency of two phases. The "first degree of freedom" is comprised of the possibility of transfer from the level ε'_k in liquid to ε''_k in vapor, etc. The "second degree of freedom" is comprised of the possibility of transfer of a similar kind but only in one phase, e. g., in vapor, but the "third degree of freedom" has the same meaning but only for the liquid phase. That means that for all temperatures below the critical, $\bar{n}''$ from the equation (25) should be three times larger (due to the three possibilities of transfer) than $\bar{n}''_k$ from equation (26) for the critical temperature.

In order to check this, we shall find the numerical values for $\bar{n}''$ and $\bar{n}''_k$ for some monoatomic elements. To calculate this, the experimental data for the greatest number of temperatures lower than the critical will be introduced into the equation (25). Then the same will be done for the critical temperature. Let us start with argon. Its triple point is — 189.33° C (83.83° K), and critical temperature — 122.41° C (150.72° K (2). The lowest temperature for which we had data was — 181.15° C (92.01° K) (see data in Table 1). It is close to the triple point. We should

TABLE 1

The values of $\bar{n}''$ for argon (*Ar*)

t °C	T °K	$\rho' \frac{g}{cm^3}$	$\rho'' \frac{g}{mc^3}$	$e^{\frac{s}{RT}}$	$\overline{n''}$	$\Delta(3 \cdot \overline{n_k''})$	$\delta(3 \cdot \overline{n_k''}$
— 181,15	92,01	1,37396	0,00801	29322,00	0,005850	—0,000225	— 4,00
— 175,39	97,77	1,32482	0,01457	15906 67	0,005684	—0,000059	— 1,05
— 161,23	111,93	1,22414	0,03723	4701,00	0,006994	—0,001369	—24,33
— 150,76	122,40	1,13851	0,06785	2280,70	0,007357	—0,001732	—30,79
— 140,20	132,96	1,03456	0,12552	1234,11	0,006679	—0,001054	—18,74
— 135,51	137,65	0,97385	0,15994	968,35	0,006288	—0,000663	—11,79
— 131,54	141,62	0,91499	0,19432	798,60	0,005896	—0,000271	— 4,82
— 125,17	147,99	0,77289	0,29534	598,98	0,004369	+ 0,001256	+ 22,33
— 122,41 *kr.*	150,75 *kr.*	0,53078	0,53078	533,45	0.001875		

mention here that instead of a specific volume for all the substances we had experimental data for their densities. Denoting densities of vapor and liquid with ρ'' and ρ', respectively, we obtain from equation (25):

$$\bar{n}'' = \frac{\rho'}{\rho'' e^{\frac{\eta}{KT}}} \qquad (27).$$

We had data (3) for the heat of sublimation for one gram-mole (here one gram-atom of each element). Denoting the number of particles in this mass (Avogadro number) with N, and the heat of sublimation for one gram-atom with s, there is a relation

$$\gamma_1 = \frac{s}{N} \tag{28}.$$

When we replace this in equation (27), we obtain for $\bar{n}''$:

$$\bar{n}'' = \frac{\rho'}{\rho'' e^{\frac{s}{NKT}}} \tag{29},$$

i, e.,

$$\bar{n}'' = \frac{\rho'}{\rho'' e^{\frac{s}{RT}}} \tag{30},$$

R being the universal gas constant. For the critical temperature we obtain the analogous expression:

$$\bar{n}''_k = \frac{1}{e^{\frac{s}{RTK}}} \tag{31}.$$

When we replace data (2) for the lowest temperature for argon (—181.15°) into the equation (30), we obtain:

$$\bar{n}'' = 0.005850 \tag{32}.$$

That means that only about 0.6% of particles are being transferred.

For the critical temperature

$$\bar{n}''_k = 0.001875 \tag{33}$$

or, about 0.2%.

From (33) we see that $3 \cdot \bar{n}''_k = 0.005625$. Thus, the absolute error between that and $\bar{n}''$ from (32) is: $\triangle (3 \cdot \bar{n}''_k) = -0.000225$, while the relative error is $\delta (3 \cdot \bar{n}''_k) = -4\%$. Several values for $\bar{n}''$ from equation (30) are presented in Table 1 for the temperatures for which we had all the necessary experimental data (2). From Table 1 we conclude, firstly, that $\bar{n}''$ is nearly constant in the interval from the triple point to the critical temperature; and secondly, that it is three times larger than that at the critical temperature. The largest relative error (in respect to $3 \cdot \bar{n}''_k$, therfore denoted with $\delta (3 \cdot \bar{n}''_k)$ is for temperature — 150.76°C (122.40°K). Taking into consideration that the value for the heat of sublimation at absolute zero was obtained by extrapolation to this temperature

(for argon this value is 1880 cal/g.-atom), these deviations are not great. Since the triple point and the critical temperature are very low, densities in this interval are probably determined with greater error.

Taking all this into consideration, we can be temporarily satisfied with these results, and accept that the properties ascribed to a fluid at its boiling temperature are theoretically in accordance with its behavior, and secondly, in accordance with the physical properties of a real fluid, in this case argon.

The next substance for which $\overline{n''}$ was determined was krypton. Its triple point is — 157. 2°C (115. 96° K), and the critical temperature is — 63. 75° C (209. 41° K). The lowest temperature for which we had the experimental data (2) was — 129. 11° C (144. 05° K). The heat of sublimation at absolute zero for this element is 2540 cal/g.-atom (3). Using other necessary data from Table 2, and using equation (30) for the lowest temperature for krypton, we obtain:

$$\overline{n''} = 0.008290 \qquad (34).$$

TABLE 2

The values of $\overline{n''}$ for kryptom (*Kr*)

t °C	T °K	$\rho' \frac{g}{cm^3}$	$\rho'' \frac{g}{cm^3}$	$e^{\frac{s}{RT}}$	$\overline{n''}$	$\Delta(3 \cdot \overline{n_k''})$	$\delta(3 \cdot \overline{n''_k})$
— 129,11	144,05	2,2202	0,03739	7162,83	0,008290	—0,001603	—23,97
— 119,81	153,35	2 1363	0,05774	4181,10	0,008849	—0,002162	—32,33
— 109,46	163,70	2,0350	0,09004	2467,94	0,009158	—0,002471	—36,95
— 102,22	170,94	1,9574	0,12014	1772,76	0,009191	—0,002504	—37,45
— 92,32	180,84	1,8338	0,17576	1177,00	0,008865	—0,002178	—32,57
— 84,76	188,40	1,7255	0,23501	886,30	0,008284	—0,001597	—23,88
— 79,55	193,61	1,6379	0,29030	738,35	0,007642	—0,000955	—14,21
— 73,51	199,65	1,5161	0,3774	604,63	0,006644	—0,000043	+ 0,64
— 71,24	201,92	1,4590	0,4217	562,63	0,006149	—0,000538	+ 8,05
— 67,15	206,01	1,3171	0,5404	496,16	0,004912	—0,001775	+26,54
— 64,94	208.22	1,1926	0,6464	464,52	0,003970	—0,002717	+40,63
— 63,75 *kr.*	209,41 *dr.*	0,9085	0,9085	448,59	0,002229		

Analogous to that, we determined $\overline{n''}$ for several temperatures near the critical one. The results obtained are given in Table 2. For the critical temperature, from equation (31), we have

$$\overline{n''}_k = 0.002229 \qquad (35).$$

Thus. $3 \cdot \overline{n''}_k = 0.006687$. Now, the relative error between (35) and (34) is $\delta\ (3 \cdot \overline{n''}_k) = -23.97\%$. From Table 2 we see that the largest

relative error is for the temperature about one degree below the critical, i. e. for — 64.94° C (208.22° K).

The next substance we had to deal with was xenon. Its triple point is — 111.9° C (161.26° K), and critical temperature, 16.6°C (289.76° K). The lowest temperature for which we had the experimental data (2) was — 66.8°C (206.36° K), and the highest was near the critical, 16° C (289.16° K). The heat of sublimation for this element at absolute zero is 3850 cal /g. — atom (for other values see Table 3). For the lowest temperature from (30):

$$\overline{n''} = 0.003905 \qquad (36).$$

For the critical temperature for xenon from (31):

$$\overline{n''}_k = 0.001245 \qquad (37).$$

Thus, $3 \cdot \overline{n''}_k = 0.003735$. Here, the relative error is only — 4.55%. Other values are given in Table 3. The largest relative error is at — 44.9° C (230.26° K).

TABLE 3

The values of $\overline{n''}$ for xenon (*Xe*)

$t°C$	$T°K$	$\rho' \frac{g}{cm^3}$	$\rho'' \frac{g}{cm^3}$	$e^{\frac{s}{RT}}$	$\overline{n''}$	$\Delta(3 \cdot \overline{n_k''})$	$\delta(3.\overline{n_k''})$
—66,8	206,36	2,763	0,059	11993,33	0,003905	—0,000170	— 4,55
—60,0	213,16	2,699	0,079	8888,60	0,003844	—0,000109	— 2,95
—59,3	213,86	2,694	0,078	8627,80	0,004004	—0,000268	— 7,18
—42,9	230,26	2,605	0,103	4524,60	0,005590	—0,001855	—49,63
—39,3	233,86	2,506	0,139	3974,63	0,004536	—0,000801	—21,45
—30,3	242,86	2,411	0,180	2923,60	0,004581	—0,000846	—22,65
—25,25	247,91	2,297	0,235	2484,88	0,003934	—0·000191	— 5,11
—20,0*	253,16	2,292	0,238	2113,00	0,004558	—0,000823	—22,03
—10	263,16	2,169	0,313	1579,60	0,004387	—0,000652	—17,46
— 5	268,16	2,074	0,363	1376,96	0,004149	—0,000414	—11,08
0*	273,16	1,987	0,421	1207,30	0,003913	—0,000178	— 4,77
5	278,16	1,879	0,501	1061,87	0,003532	—0,000203	+ 6,44
10	283,16	1,750	0,602	938,94	0,003096	—0,000639	+ 17,11
12	285,16	1,677	0,662	894,94	0,002831	—0,000904	+ 24,20
14	287,16	1,592	0,740	853,56	0,002520	—0,001215	+ 32,53
15	288,16	1,528	0,779	833,80	0,002352	—0,001383	+ 37,03
16	289,16	1,468	0,844	814,64	0,002135	—0,001600	+ 42,84
16,6 *kr.*	288,16 *kr:*	1,155	1,155	803,40	0,001245		

The same has been done for neon, too. Its triple point is at — 248.59° C (24.57° K), and critical temperature at — 228.75° C (44.41° K). Both are at very low temperatures and it could be expected that the experimental errors for all the necessary elements are larger. The lowest temperature for which we had data (2) was — 247.92° C (25.24° K), and the heat of sublimation at absolute zero is 446 cal/g. — atom (3). The rest of the experimental data is given in Table 4. For the lowest temperature from equation (30):

$$\overline{n''} = 0.031760 \qquad (38).$$

TABLE 4

The values of $\overline{n''}$ for neon (*Ne*)

$t\,°C$	$T°K$	$\rho'' \frac{g}{cm^3}$	$\rho'' \frac{g}{cm^3}$	$\frac{s}{e^{RT}}$	$\overline{n''}$	$\Delta(3\cdot\overline{n_k''})$	$\delta(3,\overline{n_k''})$
—247,92	25,24	1,23824	0,00534	7299,33	0,031760	—0,012641	—65,92
—246,94	26,22	1,22215	0,00711	5235,12	0,032834	—0,012715	—71,73
—245,94	27,22	1,20421	0,00939	3822,00	0,033554	—0,014435	—75,50
—242,96	30,20	1,14960	0,02013	1693,56	0,033721	—0,014602	—76,37
—240,00	33,16	1,08832	0,03831	872,94	0,032543	—0,013424	—70,21
—237,04	36,12	1,01750	0,06742	500,74	0,030139	—0,011020	—57,64
—235,26	37,90	0,96728	0,09310	373,96	0,027783	—0,008664	—45,32
—234,01	39,15	0,92803	0,11592	309,50	0,025867	—0,006748	—35,29
—232,025	41,135	0,85421	0,16563	234,68	0,021976	—0,002857	—14,94
—230,07	43,09	0,74866	0,23935	183,32	0,017074	+0,002045	—10,70
—228,75 *kr.*	44,41 *kr.*	0'4835	0,4835	156,92	0,006373		

For the critical temperature from equation (31):

$$\overline{n''}_k = 0.006373 \qquad (39).$$

Thus, $3\cdot\overline{n''}_k = 0.0019119$. The relative error is $\delta\,(3\cdot\overline{n''}_k) = -65.92\%$. Values of $\overline{n''}$ for all other temperatures are presented in Table 4. It can be seen that the relative errors are very great in the whole investigated interval, but the smallest are near the critical temperature, i. e, at — 230.07° C (43.09° K), while for all other substances the error is greatest near the critical temperature. These deviations are considered to be due to experimental errors at low temperatures.

Thus, the analysis of the obtained results shows: firstly, that the value for $\overline{n''}$ is nearly a constant value for each of investigated substance, and secondly that it is approximately three times larger than the corresponding value for $\overline{n''}_k$ for the same substance.

In this work we applied a statistical quantum interpretation of the properties of liquids and their vapors at boiling temperatures (under the corresponding pressure). The most important factor is that we attributed certain physical properties to a fluid in a liquid state, which rigorously taken could be properties of only an imaginary "ideal fluid." By theoretical treatment we have found the relations between the numbers of particles transferred into the vapor phase under the condition of constancy of the total energy of the system. The conclusion was that this number at the critical temperature should be three times smaller than that at lower temperatures.

Finally, it seems of importance to us to point out that it is possible in this way to include in this scheme the behavior of the real fluids, as argon, krypton, xenon, and neon. It remains for a future work to attribute certain new physical properties to this "ideal fluid". This will better comprise the behavior of the real fluids, and at the same time to broaden the application of the contemporary scientific method of statistical and quantum mechanics to states of fluids other than the case of a monoatomic fluid at its boiling temperature.

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CURRENT EFFICIENCY IN THE PROCESS OF ELECTROLYTIC HYDROGENATION OF GERMANIUM

by

B. B. DJURKOVIĆ

The purpose of the process of electrolytic hydrogenation of germanium, using the electrochemical reactions on the cathode is to simultaneously deposit, germanium and hydrogen from the water solution which contains their ions. The deposition should be performed under conditions which allow the mutual reaction of these two species to form the compound GeH_4. Thus, the process of the electrolytic formation of germanium hydride consists of:

(i) the electrochemical reaction of the deposition of germanium ions at the cathode,

(ii) the electrochemical reaction of the evolution of hydrogen at the cathode,

(iii) the chemical reaction of formation of the compound GeH_4 between the substances formed at the cathode.

The optimal theoretical conditions for a complex process such as this should enable the simultaneous electrochemical reactions of the deposition of germanium and hydrogen at the rates, the ratio of which is determined by the stoichiometry of the compound GeH_4. Also, they should enable the occurrence of the quantitative chemical reaction of formation of this compoud. Under such conditions the current efficiency of the whole process would be 100%. In this case, 1 gr atom of germanium (72.6 gr Ge) and 4 gr atom of hydrogen (4 gr H) are necessary for the formation of 1 gr mol of GeH_4.

The simultaneous electrolytic deposition of germanium and hydrogen in different weight ratios, i. e., the ratio which is necessary for the formation of GeH_4 is feasible. Even in 1936, R. Schwarz (1) obtained the firm metalic deposit of germanium at the copper cathode while electrolysing the solution of 0.025 *n* GeO_2 in 3 n KOH with the current density of 2 m A/cm^2, voltage of 1.35 — 4.25 V, and at a temperature of 70 — 90^0 C. The author of this work also succeeded in obtaining under the specific

conditions only metalic germanium, working with small current densities and at the cathodes with a sufficiently high hydrogen overpotential. With the further increase of polarization of the electrode, when the potential of the cathode reached the value of hydrogen overpotential, the slow hydrogen evolution started. Also, as soon as the copper cathode, under suitable conditions, was covered with a layer of metallic germanium, the slow hydrogen evolution started without any further increase of the electrode polarization, and the electrolytic deposition continued with the simultaneous deposition of both types of ions. If the cathode was made more negative and the electrolysis was performed with higher current densities, the hydrogen evolution became more vigorous. In this way, by suitable selection of values of all factors which influence the electrochemical reaction of germanium and hydrogen deposition (e. g., concentration of their ions in the solution, cell voltage, cathodic current density, kind and shape of metal surface used as the cathode), it was possible to achieve conditions for the simultaneous deposition of both substances in the ratio necessary for the stoichiometric formation of the GeH_4 compound.

For the simultaneous reduction of germanium and hydrogen ions, their electrode potentials of deposition should be equal, i. e., $e_{H_2} = e_{Ge}$

By equalizing the expression for the electrode potential of hydrogen evolution at the overpotential W_{H_2} from the solution with hydrogen ion concentration C_{H+}, with the expression for the electrode potential of germanium deposition from the solution with the germanium ion concentration $C_{Ge}4+$, the following equation can be obtained, determining in the first approximation, conditions for the simultaneous deposition of both types of cations:

$$0.058 \log C_{H} + + W_{H_2} = 0.25 + 0.0145 \log C_{Ge}4+$$

It is possible, by choosing the electrolyte, to vary within certain limits the hydrogen and germanium ion concentrations. Also, by choosing the kind and shape of the cathode, as well as the electrolysis conditions (current density, temperature, etc) it is possible, within certain limits, to influence the hydrogen evolution overpotential, and in this way satisfy the condition of simultaneous deposition of both types of ions. However, for the electrolytic formation of germanium hydride more than only the simultaneous deposition of the components of the compound — germanium and hydrogen — in the appropriate ratio is necessary. As for any other reaction, components should be energetically prepared for the mutual influence (reaction) to give as a result a new substance, in this case the compound GeH_4.

Germanium, formed by the electrochemical reduction of its ions from the water solution, is of a different physical structure when obtained under the different conditions of electrolysis (current density, concentration of ions in the solution, kind of

the cathode, etc.). Under certain conditions germanium could be obtained as a more or less ductile deposit but under other conditions it could be porous, in the form of the fine powder, or the deposition of metallic germanium could cease. All these different forms of germanium deposits, obtained under the different conditions of electrolysis, are the consequence of its different energetic states in the moment of formation at the cathode.

Hydrogen, evolved at the cathode during electrolysis, is reactive in different way, depending upon the kind of metal used as the cathode. It is known that the most active electrolytic hydrogen is obtained when for the cathode are used metals at which the higher voltages are used for hydrogen evolution. It seems as that a part of the used surplus of energy is binded to hydrogen (except energy evolved as the heat), in this way increasing its chemical activity.

Thus, for the performance of the chemical reaction of the formation of germanium hydride from germanium and hydrogen formed at the cathode, the necessary energy of activation is determined by the change of the free enthalpy of the electro-chemical reaction performed at the cathode. Since the change of the free enthalpy of the electrochemical reaction is proportional to the electrode potential ($\Delta G = -zFe$) at which it performs, the necessary energy of activation of the chemical reaction of the formation of germanium hydride depends upon the value of the electrode potential at which germanium and hydrogen are formed. Therefore, for the electrolytic hydrogenation of germanium it is necessary to have not only the conditions of electrolysis for the simultaneous deposition of germanium and hydrogen, but also a certain minimum value of the cathode potential at which the reactions at the cathode would proceed with sufficient changes of the free enthalpies. These values should be sufficient as the low energy of activation of the hydride formation best enables proceeding of this chemical reaction.

The experimental results of the electrolytic hydrogenation of germanium from the alkoline water solutions at the copper cathode are consistent with this interpretation.

Supposing that the electrolytic hydrogenation of germanium proceeds with the following reactions:

$$Ge^{4+} + 4e = Ge^{0}$$

$$4H^{+} + 4e = 4H^{0}$$

$$Ge + 4H = GeH_4$$

or summarizing

$$Ge^{4+} + 4H^{+} + 8e = GeH_4.$$

The electrochemical equivalent of germanium is:

$$\eta Ge = \frac{72.6}{4 \times 26.8} = 0.6772 \text{ gr Ge/Ah},$$

and the electrochemical equivalent of germanium hydride is:

$$\eta\, GeH_4 = \frac{72.6 + 4 \times 1.008}{8 \times 26.8} = 0.3574 \text{ gr } GeH_4/Ah$$

The yield of germanium hydride during the electrolytical hydrogenation is determined after the thermal decomposition and by weighing the formed metallic germanium. Thus, for the calculation of the current efficiency for the electrolytic obtaining of GeH_4, the electrochemical equivalent of germanium is:

$$\eta\, Ge\ (GeH_4) = \frac{72.6}{8 \times 26.8} = 0.3386 \text{ gr Ge/Ah}$$

The current efficiency of the hydrogenation. i. e., of the electrolytic reduction of germanium ions and in the corresponding ratio of hydrogen ions, represents the quotient of the theoretical quantity of electricity needed for the production of a certain quantity of germanium hydride and the quantity of electricity really used. The theoretical quantity of electricity is calculated as the quotient of the obtained quantity of germanium after the thermal decomposition of its hydride and the electrochemical equivalent of germanium, valid for the electrochemical production of GeH_4 (0.3386 gr Ge/Ah).

The hydrogenation at the copper cathode was performed with the current density of 8 A/cm^2 varying the concentration of GeO_2 in 20% KOH, at room temperature during eight hours. The really used quantity of electricity was determined by means of a copper coulometer.

The obtained values of current efficiencies in this series of experiments are presented in Table 1.

Table 1. *Current efficiency in the electrolytic hydrogenation of germanium as a function of the initial germanium concentration in the electrolyte.*

Experiment No.	Concentration gr/1 GeO_2	$\eta_{Ge\ (GeH_4)}$ %
1	10	5.18
2	30	9.46
3	50	16.34
4	75	21.50
5	100	24.68
6	120	28.00
7	150	31.00

In the second series of experiments the electrolytic hydrogenation of germanium was also performed at the copper electrode, but with the electrolyte containing 10 gr/1 GeO_2 in 20% KOH and varying cathodic current densities. The obtained results of this series are presented in Table 2.

Table 2. *Current efficiency in the electrolytic hydrogenation of germanium as a function of the cathodic current density*

Experiment No	D_k (A/ cm²)	η_i Ge (GeH_4) %
1	1.5.	2.76
2	2.0	2.58
3	3.0	3.10
4	3.4	3.66
5	4.2	3.70
6	5.1	3.60
7	6.0	4.10
8	6.8	5.30
9	7.6	4.92
10	8.4	4.38
11	9.1	3.80

The obtained results clearly showed the increase of the current efficiency of the electrolytic hydrogenation with the increase of the initial concentration of germanium in the electrolyte. The increase of the current efficiency with the increase of the germanium concentration is nearly linear when represented graphically. (fig. 1).

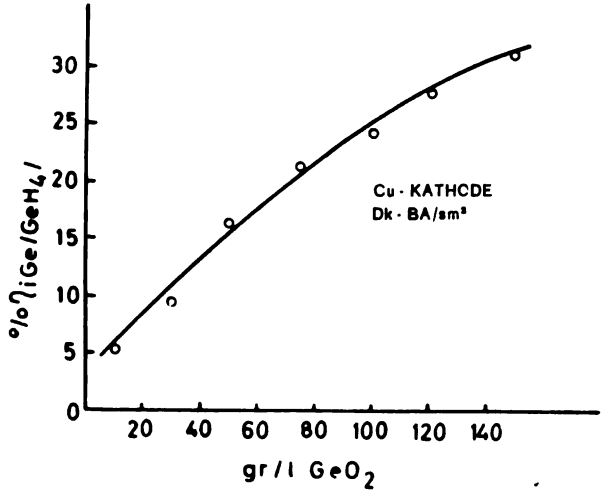

Fig. 1.

Fof the highest initial concentration of GeO_2 in the electrolyte, it reached a maximum value at the corresponding cathode. At the copper cathode, for the initial concentration of 150 gr/*l* GeO_2 after eight hours of electrolysis, it was 31.0%. Since the germanium concentration decreased during the electrolysis, the efficiency at the beginning was certainly higher.

The current efficiency of the electrolytic hydrogenation of germanium is also changes with the variation of the cathodic current density, but this change, as shown in the diagram (Fig. 2). is relatively small in the interval of current densities studied (1,5

—9.1 A/cm²). This series of experiments was performed with an electrolyte of relatively low initial concentration of germanium (10 gr/*l* GeO_2), and the current efficiencies were relatively small. However, as it can be seen, the increase of the cathodic current density slightly increased the current efficiencies, but only to a certain value. Further on, the increase of the cathode current density gradually decreased in the current efficiency.

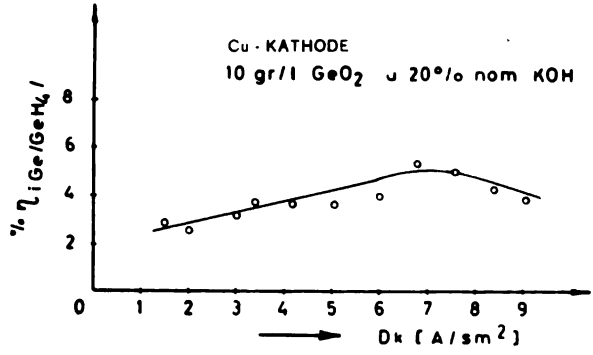

Fig. 2

According to the given results, and in accordance with the composed equation for the simultaneous reduction of germanium and hydrogen ions, the key factor which influences the current efficiency during the electrolytic hydrogenation of germanium at the copper cathode is the concentration of germanium in the electrolyte.

The change of the current density (under the present conditions of experiment) does not cause a large change of the current efficiency in the process of hydrogenation. That means that for the system used, the increase of the cathode potential over a certain value does not significantly influence the increase of the current efficiency, even up to a certain value and seen as a whole, it shows tendencies of the increase.

For the cases of the simultaneous deposition of metal and hydrogen, as is this one, and when the rate determining process is charge transfer, Esin (2) gives the equation for the current efficiency for metal deposition (η_{i_M}) as a function of cations activities (a_M and a_H), cathode potential (φ), and temperature (T):

$$\frac{\eta_{i_M}}{1-\eta_{i_M}} = \frac{K_M \cdot a_M z+}{K_H \cdot a_H+} e^{-\frac{(z_M \alpha_M - \alpha_H) F \cdot \varphi}{RT}}$$

Here, K_M and K_H are the complex electro-kinetic constants of metal and hydrogen ions, and α_M and α_H are the kinetic coefficients of the deposition of these cations.

It results from the given equation that the increase of the activity of metal ions and the decrease of the activity of hydrogen ions influence the increase of the current efficiency.

However, the change of the cathode potential could influence the current efficiency, in a different way depending upon the relation between $z_M \alpha_M$ and α_H. If $z_M \alpha_M > \alpha_H$ the increase of cathode potential causes the increase of the current efficiency of metal deposition, and when $z_M \alpha_M < \alpha_H$, the increase of cathode potential gives a decrease of the current efficiency as the effect.

The interpretation of the results shown in Fig. 2 using this equation implies the assumption that, for the system studied, in the wider interval of the cathode potential there are certain changes ot kinetic coefficients for these two kinds of cations. It seems that up to a certain value of the cathode potential the increase of the current density causes a slight increase of the current efficiency, and a further increase of the current density over this value gradually decreases the current efficiency of the hydrogenation of germanium.

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DERIVATIVE POLAROGRAPHIC TITRATION OF SOME BASES IN GLACIAL ACETIC ACID IN THE PRESENCE OF QUINHIDRONE

by

VILIM VAJGAND and TIBOR PASTOR

Derivative polarographic titration, originated by Reilly, Cooke, and Furman (1), has only recently been applied for the determination of some substances in nonaqueous solutions. Shain and Svoboda (2) have titrated weak organic acids with tetrabutylammonium hydroxide using acetone as the solvent in the presence of two polarized Pt-micro electrodes, and they observed that the titration end-point coincided with the maximum potential difference obtained in the course of the titration. Such a maximum was more enhanced than the potential jump in classical potentiometry. The effect of the polarization ef electrodes on the potential jump was studied by Rose and Shain (3), and by Harlow, Noble, and Wyld (4). The latter authors, when titrating potentiometrically weak acids in ethylenediamine in the presence of polarized Pt-electrode, obtained potential jumps two to three times as great as those obtained with glass electrodes. Svoboda (5) performed the determination of amines in the mixture of *m*-cresol and acetonitrile (1 : 1) with an 0.1 N solution of perchloric acid in *m*-cresol by the derivative polarographic method. However, when attempting the determination of the same amines in glacial acetic acid, which is most often used as the solvent, in nonaqueous titrations, he neither obtained reproducible results or a sharp maximum at the titrati n endpoint.

On the basis of known data, it is not possible to give a full explanation of the mechanism of the process which takes place at the polarized Pt-electrodes. These processes seem to be more complicated than assumed. In general, the anodic polarised Pt-electrode behaves similarly to the platinum-platinum oxide electrode, but the potential change at the cathode originates from the reduction of either the hydrogen ion or dissolved oxygen.

The platinum electrode is not convenient for the measurement of acidity of the solution because it is not fully reversible; its potential is dependent upon time, and the results are not reproducible. We have assumed that the addition of quinhidrone, which reacts reversibly in acid media, would eliminate these disadvantages of the Pt-electrode in glacial acetic acid.

EXPERIMENTAL

Our experiments were performed with the apparatus shown in Fig. 1. The current source was a steel 2.5 V accumulator. Electrodes of Pt-wire, 1.2 cm. long with a diameter of 0.5 mm, were sealed into glass rods so that their distance (2 cm.) remained constant during titration. We also used Pt-electrodes of 1 cm^2 surface, the distance between which could be arbitrarily changed. A resistance box ("Elektozveze MA 2110", from 1 kΩ to 11 MΩ) was used for the adjustment of the current strength and potential difference between electrodes. The potential difference was measured by means of "Radiometer" 22 p. In the potentiometrical determination

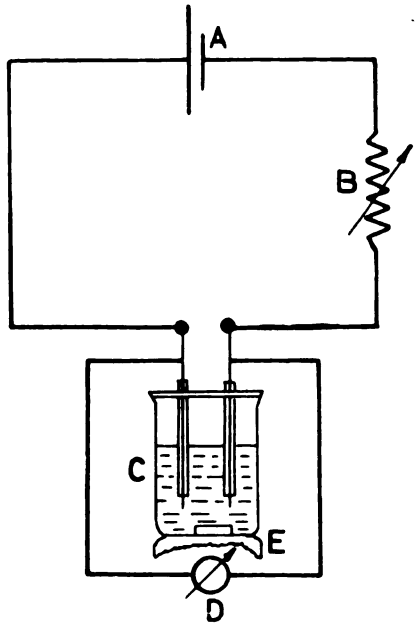

Fig. 1

A — storage battery
B — decade resistance box
C — cell with electrodes
D — pH-meter
E — magnetic stirrer

of the investigated substances a glass electrode (6) and a mercury-mercurous acetate ($Hg/Hg_2(CH_3COO)_2$) electrode which contains a saturated solution of sodium perchlorate in glacial acetic acid (7) was used; in the derivative polarographic titration the solution was saturated with quinhidrone ("Analar") prior to the titration.

The solvent used was glacial acetic acid, "Merck", p. a., s. w. 1.055—1.064, in admixture with acetic acid anhydride "Carlo Erba", s. w. 1.08. Standardization of 0.1 N perchloric acid solution was

performed with a solution of either sodium acetate or potassium hydrogen phtalate in glacial acetic acid. In titrations of hydrohalides of organic bases, 3 per cent mercuric acetate was added to the solution.

Preliminary investigations have shown that either the application of reversible electrodes or the presence of a reversible system in the solution can solve the problem of derivative polarographic titration in glacial acetic acid. The addition of quinhidrone fulfilled this requirement, but the detailed investigation demonstrated that in the presence of quinhidrone a sharp maximum at the titration end-point was obtained, and the results of the analyses were fully reproducible. The rate of potential establishment depends upon the titrated substance. The common feature of all systems investigated so far is that the potential is more rapidly established before the titration end-point, but more slowly at the equivalence point and subsequent to it. In order to ascertain which electrode process is characteristic of the titration, we have measured, during the course of a titration the changes of potential at both the anode and the cathode in regard to Z. K. E.*; (Fig. 2). We observed that the potential at the anode is slowly attained in the vicinity of the equivalence point and subsequent to it, but it increases constantly in the course of the titration, i. e., the electrode is increasingly positive; in most of titrated systems an enhanced potential jump appeared at the titration end-point. On the other hand, the potential at the cathode is rapidly attained and constantly decreases i. e., becomes more negative. An abrupt change of the potential also appears at the cathode, depending upon the titrated system. This change can take place upon addition of the same amount of the titrant, which gives rise to the potential jump at the anode before or after the addition. For example, in the titration of sodium p-aminosalicylate (Na-P. A. S.), the change of the cathode potential appears simultaneously with the rise of the anode potential; however, in the titration of quinine, it appears after the potential jump at the anode. It should be emphasized that the potential rise at the cathode is smaller than that at the anode. Accordingly, it is obvious that the shape of the titration curve (depicted in Fig. 2) depends upon the electrode processes at the anode and at the cathode, but the rate of the potential difference attainment depends only upon the process at the anode.

Our investigations have shown that the amount of acetic acid anhydride affects the magnitude of the potential jump at the titration end-point. The maxima obtained are more enhanced in the presence of an excess of acetic acid anhydride, and the potential jump at the anode amounts to 300 to 400 mV, per 0.01 ml 0.1 N perchloric acid solution. Therefore, we determined the content of water present in glacial acetic acid by the method of K. Fischer (8), and then we added acetic acid anhydride in an amount which made

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* The contact between the standard electrode and the investigated solution was effected by the use of an asbestos thread impregnated with glacial acetic acid.

the ratio of glacial acetic acid to anhydride 5:1. However, in titrating some substances, such as caffeine or caffeine-sodium benzoate, an excess of acetic anhydride was required.

The sharpness of the titration end-point is also affected by the magnitude of the platinum electrode surface. The increase of

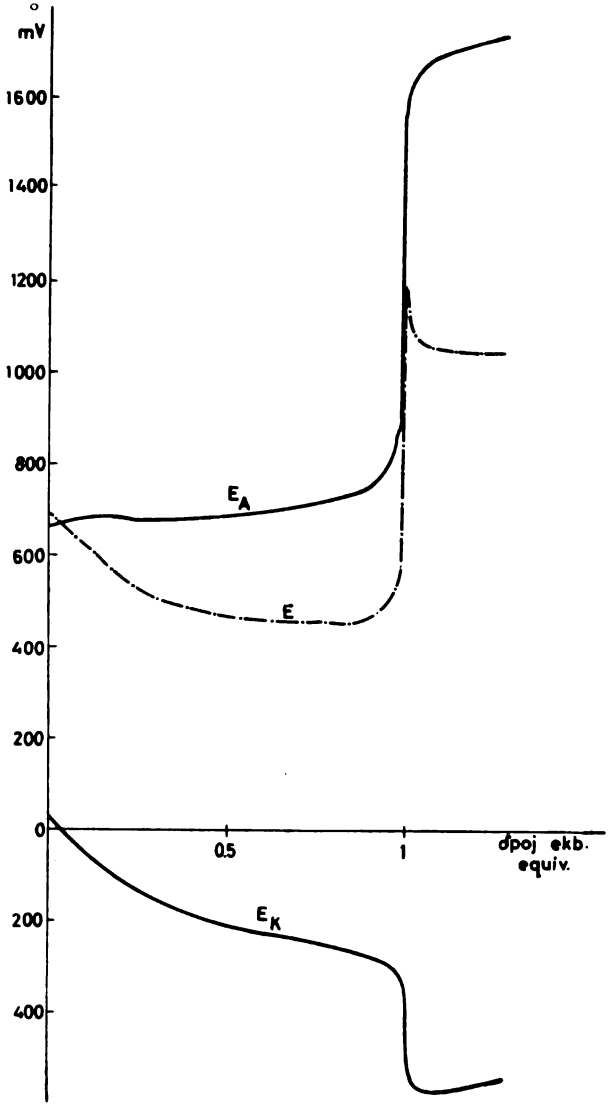

Fig. 2

Changes of anodic potential (E_a), cathodic potential (E_k)* and their difference (E) during the titration of $3 \cdot 10^{-2}$ M solution of antipyrine

* To avoid overhapping, the cathode potential curve is represented in the reverse manner.

the surface results in the decrease of the maximum height. However, no signifficant differences in maximum height were observed when using electrodes of 0.19 and 1 cm^2.

The amount of quinhidrone used neither affects the accuracy of the results or alters the shape of the titration curves. However, the investigations have shown that the maxima obtained in the absence of qinhidrone are significantly smaller, thus rendering the estimation of titration end-point more difficult; in addition, the obtained results are not in agreement.

In the investigation of derivative polarographic titration in glacial acetic acid, 0.1 to 0.2 g of substance* to be titrated, was weighed (with consideration to the procedures given in pharmacopeia), so that the concentrations of titrated solutions ranged from 0.03 M to 0.05 M. Preliminary investigations also indicated the possibility of titrating more diluted solutions. Good results were obtained in the titration of sodium hydrogen phtalate with 0.01 M perchloric acid solution.

The results obtained are presented in Table 1. It is evident from the table that the described method can be used in titrations of alkaline salts of organic acids and compounds which contain terciary nitrogen, and the results are of satisfactory accuracy and excellent reproducibility.

TABLE 1

Titrated substance	Taken *g*	Found by potentiom. titration *g*	Number of titrations by d. p. m. *g*	Found by d. p. t. *g*
Sodium *p*-amino salicylate	0.2000	0.1988	12	0.1987±0.0003
Sodium benzoate	0.1000	0.0981	10	0.0983±0.0003
Procaine *HCl*	0.1500	0.1493	13	0.1488±0.0002
Caffeine	0.1500		13	0.1482±0.0003
1-Phenyl-3-methyl-pyrazolone-5	0.1500	0.1481	9	0.1474±0.0001
Isoniazide	0.1000	0.0993	10	0.0992±0.0001
Antipyrine	0.1500	0.1492	10	0.1491±0.0002
Caffeine-sodium-benzoate	0.1500		8	0.1498±0.0001
Novalgin	0.2000		8	0.1957±0.0003
Aminopyrine	0.1500	0.1491	15	0.1492±0.0002

The investigated substances exhibited different behavior in the titrations (Fig. 3). Sodium acetate, potassium hydrogen phtalate. sodium *p*-aminosalicylate, sodium benzoate, procaine HCl, coffeine, 1-phenyl-3-methil-pyrazolone-5, isoniazide, and antipyrine gave only

* Pharmaceutical products, without any purification, were titrated.

one maximum corresponding to one equivalent of perchloric acid; aminopyrine gave rise to two maxima, the first of which was sharp and corresponded to one equivalent of perchloric acid, and the other, although higher, could not be used for the estimation

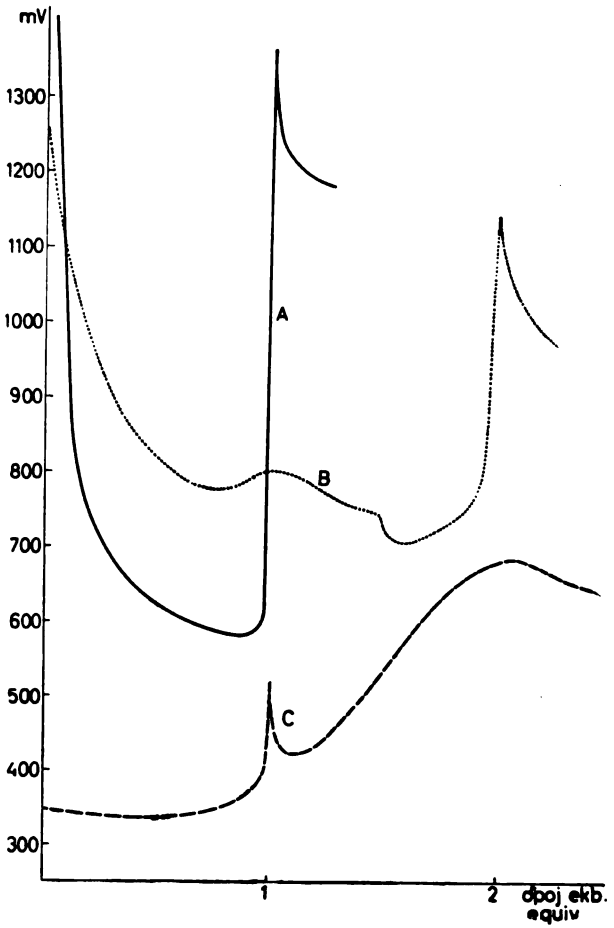

Fig. 3

Titration curves of: $A-10^{-2}$ *M* solution of caffeine (resistance 50 $K\Omega$), $B-10^{-2}$ *M* solution of novalgin (resistance 10 $K\Omega$) and $C-10^{-2}$ *M* solution of aminopyrine (resistance 80 $K\Omega$), with 0,1 *M* perchloric acid

of the titration end-point because it was not at all sharp. Coffeine-sodium benzoate and quinine gave only one maximum corresponding to two equivalents of perchloric acid. In the titration of novalgin, two equivalents of perchloric acid were consumed, but the shape of the titration curve depended upon the composition of the solvent.

The titrration in glacial acetic acid gave one maximum, but two maxima were obtained, in acetic acid anhydride, the second corresponds to the equivalence point.

The duration of the titration varied with different titrated systems (from thirty minutes to two hours), due to the different rate of potential establishment. The potential was most slowly attained in the titration of novalgin, but it is imperative to wait until the equilibium state at the electrodes is reached, otherwise the titration end-point cannot be determined. This difficulty was not encountered in titrations of other substances.

Thanks are due to chemist Milivoje Tošić and Milorad Ranković for their help in performing the experimental part of this work.

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FLUORESCENCE OF PLASMOCHIN AND ITS USE AS A FLUORESCENT INDICATOR

by

ILIJA BURIĆ and BOŠKO DRAŠKOVIĆ

Plasmochin differs from the greater part of quinoline derivatives by conditions under which it exhibits fluorescence, and also by its fluorescent spectrum (1,2).

When exposed to UV-rays ($\lambda = 3660$Å) plasmochin exhibitis a very weak yellow fluorescence in the visible part of the spectrum (3). The alcoholic solution of plasmochin shows a very intense fluorescence in the region from 4100 to 7100 Å. The fluorescent emission spectrum has a maximum at 5400 Å in the visible region (Fig. 1). Fig. 2 shows the fluorescent emission spectrum of

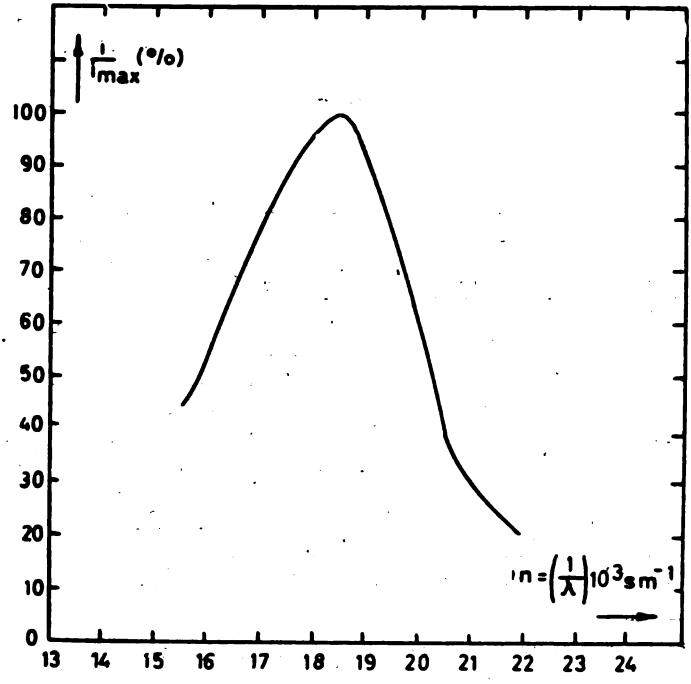

Fig. 1

plasmochin dissolved in 0.5 N sodium hydroxide solution; its maximum is shifted to 5350 Å*. The fluorescence intensity of the alcoholic solution of plasmochin depends upon its concentration.

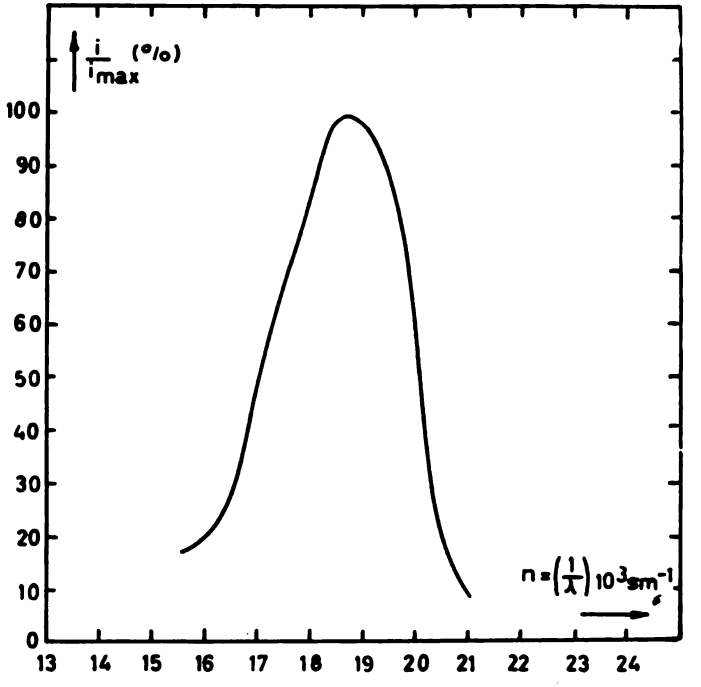

Fig. 2

The highest fluorescence is obtained when the concentration of the solution is 9.10^{-4}g/ml, whereby the fluorescent emission spectrum remains unchanged. The intensity of the fluorescence also depends upon the *pH* of the solution. Plasmochin shows fluorescence both in basic and acid media. The diagram in Fig. 3 shows the dependence of the fluorescent intensity upon the *pH*-values of the solution. In a solution of which the *pH*-values are greater than 4.5, the fluorescence is yellow and its intensity increases with the increase of the *pH*-value. However, in solutions where the *pH*-values are smaller than 3.6, the fluorescence is quenched in the visible region of the spectrum, although its intensity does not drop to zero. It is probable that the fluorescence is continued in the ultra-violet region of the spectrum. The curve which shows the dependence of the fluorescence intensity upon the *pH*-value of the solution has a shape typical to the color

* All spectra were obtained by means of a spectral photometer with photomultiplicator tube PHILIPS AVP 50.

change of colored indicators. In this case, the concentration of plasmochin was 9.10^{-4} g/ml (fluorescence optimum), and the intensity 100 was taken for sodium hydroxide solution having $pH = 10$ because the fluorescence is the most intense at this pH-value*. The minimal concentration of plasmochin which shows

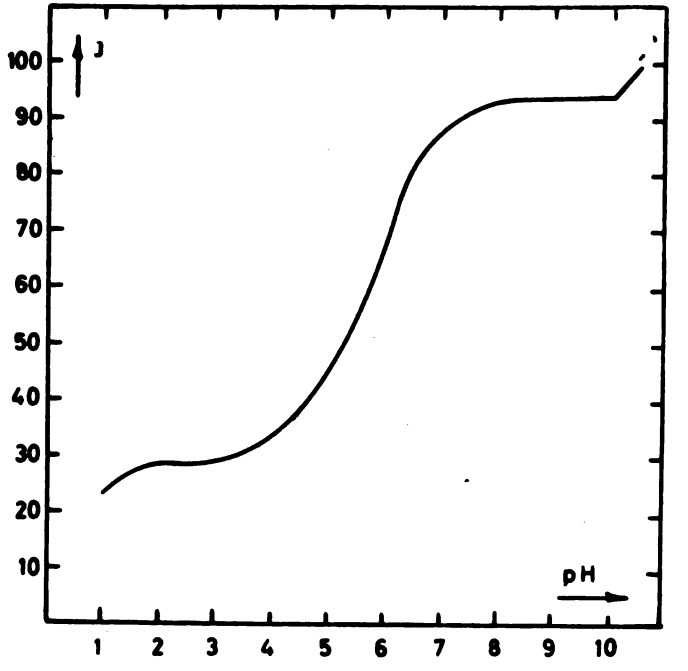

Fig. 3

fluorescence is 10^{-6} g/ml. The change of sodium hydroxide concentration affects the fluorescent emission spectrum of plasmochin. In solutions in which the sodium hydroxide concentration is less than 0.5. N, the fluorescence is strongly yellow; but in solutions in wich the concentration is higher than 0.5 N, the fluorescence is yellow-green. On longer exposures of solutions to UV-rays (more than thirty minutes), the intensity and the spectrum are altered — the intensely yellow fluorescence turns to pale-green.

The *pH*-Change of the solutions also affects the absorption spectrum of plasmochin. Fig. 4 shows that the absorption maximum in the short-wave length region of the spectrum is shifted towards shorter waves, and that it increases with the increase of the *pH*-value of the solution. The absorption maximum for the Hg-line of 3660 Å is increased with the increase of the *pH*-value, and this corresponds to the enhanced fluorescence at higher *pH*-values. In these experiments the concentration of plasmochin was 9.10^{-4} g/ml.

* The measurements were performed with a fluorometer for liquids type "KIPP".

THE USE OF PLASMOCHIN AS AN INDICATOR

The above fluorescent properties of plasmochin show that it can be used as a fluorescent indicator.

The analyses of the dependence of the change of intensity and of the fluorescent emission spectrum of plasmochin upon the *pH*-value of the solution indicate that its inversion point lies in the region *pH* 3.4 — 4.5. The titrations should be performed with an ethanolic solution of plasmochin, and the concentration in the solution to be titrated should be 10^{-4} g/ml. In all titrations in which this indicator can be applied, the inversion point is sharp and the transition from colorless to intensely yellow fluorescence is very clear.

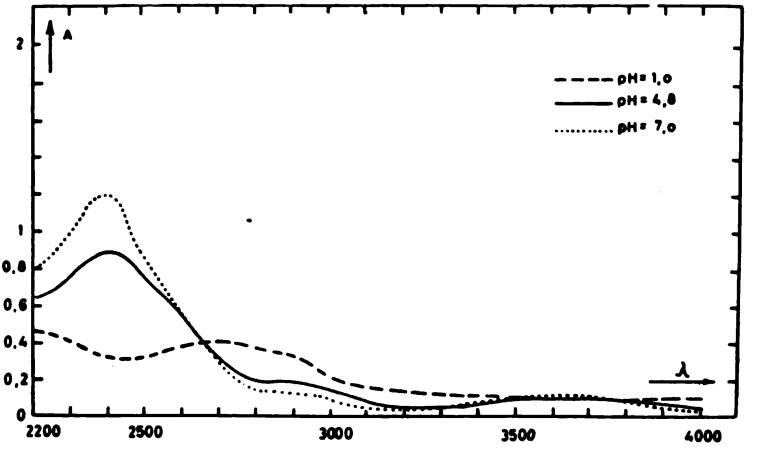

Fig. 4

This indicator can be successfully used in the titrations of strong acids and strong bases, as in the titration of the first dissociation of phosphoric acid. In all cases, it is better to titrate the acid with the base, because the transition is sharper than when reversed. The results obtained in the presence of plasmochin as a fluorescent indicator are in full agreement with those obtained in the presence of colored indicators (phenolphtalein, methyl orange, etc).

In regard to the inversion point of plasmochin, it cannot be applied in the titrations of weak mineral acids and organic acids such as acetic, lactic, citric, and oxalic acid, etc.

However, the application of plasmochin in iodometry makes it particularly interesting. Plasmochin can be used in iodometry, as a fluorescent indicator in all cases except in titrations of strongly acid solutions. The investigation was performed by titrating the N/10 iodine solution with the N/10 sodium thiosulfate

solution in the presence of starch as the colored indicator, and in the presence of plasmochin as the fluorescent indicator. The results obtained are given in Table 1.

TABLE 1

Indicator employed	ml of 0.10 N $Na_2S_2O_3$ solution per 10 ml iodine solution	Mean value
Plasmochin	20.20 20.15 20.20	20.18
Starch	20.20 20.20 20.20	20.20

In these experiments the 10 ml iodine solution was diluted with 20 ml water, and then the ethanolic solution of plasmochin was added in an amount so that the plasmochin concentration in the solution to be titrated amounted to 10^{-4}g/ml. The appearance of blue fluorescence indicated the titration end-point. The transition was clear and sharp. When plasmochin is added at the end of the titration, the titration end-point is indicated by the appearance of yellow fluorescence, in distinction to blue fluorescence which appears when plasmochin is added at the beginning of the titration. However, in both cases the consumption of sodium thiosulfate is the same. The difference exists only in the fluorescence color.

In addition to these titrations, we have determined bromine in bromine water, and chlorine in chlorine water. Potassium iodide was added to the saturated solution of bromine in water, and the liberated iodine was titrated with sodium thiosulfate. The end-point was detected by the appearance of yellow fluorescence. The same procedure was used in the titration in the presence of starch. The results obtained are shown in Table 2.

TABLE 2

Indicator employed	ml of 0.10 N $Na_2S_2O_3$ solution per 10 ml bromine water	Mean value
Plasmochin	14.00 14.00 14.10	14.05
Starch	14.00 14.00 14.10 14.30 14.10	14.10

The results obtained in the titration of iodine liberated from potassium iodide by chlorine water are given in Table 3.

TABLE 3

Indicator employed	ml of 0.01 $Na_2S_2O_3$ solution per 10 ml chlorine water	Mean value
Plasmochin	0.90	
	0.90	0.93
	1.00	
Starch	0.90	
	0.90	0.96
	1.10	

The results obtained in the determination of arsenous acid by iodine solution in the presence of plasmochin as the indicator were in agreement with those obtained in the presence of starch as the indicator.

Since rather small amounts of plasmochin are required in these titrations, it can be classified in the group of good fluorescent indicators.

The main advantage of plasmochin in regard to colored indicators lies in the possibility of its application in slightly colored and turbid solutions. It has the same advantage over starch, which cannot be used in colored solutions.

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DETERMINATION OF SMALL AMOUNTS OF ELEMENTS BY THE COMBINATION OF PARTITION AND PRECIPITATION PAPER CHROMATOGRAPHY

by

MILENKO B. ČELAP, TOMISLAV J. JANJIĆ, and DEJAN STOJKOVIĆ

In previously published papers (1-4), we have described the method for the determination of small amounts of elements by precipitation chromatography. This method can be used for the determination of elements in admixtures with other elements, provided the latter are not precipitated on impregnated filter paper strips. For example, bromides can be determined by means of paper impregnated with silver chloride in the presence of chlorides, sulfates, and carbonates. Some elements can be determined, even in admixtures with elements which are precipitated on impregnated paper, provided the compounds formed differ considerably in their solubility products. For example, bromides and iodides can be determined in admixtures because less soluble silver iodide is first precipitated, followed by the precipitation of more soluble silver bromide. This method cannot be used in other cases because all elements are precipitated together on impregnated strips, e. g., copper and iron are both precipitated on filter paper impregnated with nickelous ferrocyanide. The present paper describes the method for determination of these elements in admixtures by combining partition and precipitation paper chromatography.*

The determination was performed by first chromatographing some drops of the solution of elements to be determined by the ascending method, with appropriate solvent, on filter paper strips of 30 cm in length and 3 cm in width. Seven strips were required for each determination; the solution to be analyzed was transferred onto three strips, and the standard solution of elements to be determined was transffered onto the remaining four strips. After developing, one of the strips with standard solution was sprayed

* The determination of some ions by the combination of partition and precipitation paper chromatography was performed by A. Lewandowski and M. Owoc, Zeszyty naukowe Univers. im. A. Mickiewicza, Mat., fiz., chem., 3, 9 (1960).

with an appropriate reagent in order to establish the position of elements on the other strips. The zones of separated elements were then cut out, and the segments obtained (Fig. 1, A) were connected by means of cellophane adhesive tape with narrow filter paper strips (Fig. 1, B) impregnated with a corresponding reagent. Impregnation of strips, their development, spraying, and the calculation of results were performed in the same way as was described in previous papers (1. c.).

This method was applied in the determination of elements in the following solutions:

1. Copper and cadmium
2. Copper and zinc
3. Cadmium and uranium
4. Uranium and zinc
5. Copper in the presence of zinc, cadmium, and mercury
6. Uranium in the presence of alumium, iron, chromium and nickel.

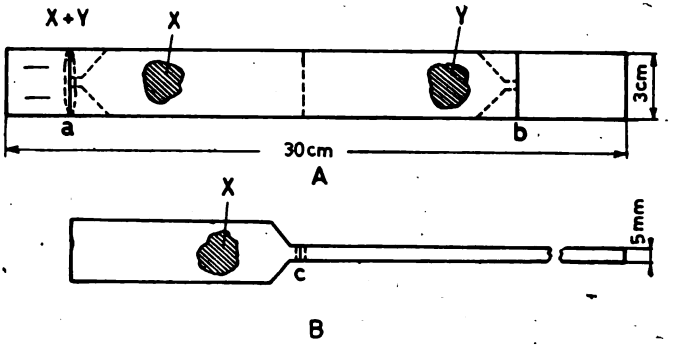

Fig. 1

The strips for the partition (A) and precipitation chronomatography (B)
a-starting point: b-solvent front: c-cellophane adhesive tape

The results obtained are given in Table 1. The table shows that the combination of partition and precipitation chromatography affords satisfactory results, which are similar to those obtained for individual elements by precipitation paper chromatography (1. c.). A method is thus given for the determination of elements in admixtures by the method of precipitation paper chromatography, provided they can be separated by partition chromatography.

TABLE 1

Concentration of solution *mg/ml*

Calculated	Found	Calculated	Found
Cu in the presence of *Cd*		*Cd* in the presence of *Cu*	
2.93	3.02	4.01	4.15
2.16	2.18	3.64	3.60
3.41	3.47	4.61	4.67
Cu in the presence of *Zn*		*Zn* in the presence of *Cu*	
4.45	4.46	4.58	4.58
3.94	4.10	4.06	4.22
3.68	3.50	3.80	3.78
Cd in the presence of *U*		*U* in the presence of *Cd*	
5.00	5.10	10.93	10.62
5.46	5.17	8.09	8.15
3.75	4.01	7.94	8.18
U in the presence of *Zn*		*Zn* in the presence of *U*	
9.08	9.08	2.61	2.56
7.52	7.82	2.67	2.75
9.50	9.10	2.87	3.02
Cu in the presence of *Zn*, *Cd* and *Hg*		*U* in the presence *Al*, *Fe*, *Cr* and *Ni*	
1.21	1.34	5.50	5.59
1.46	1.47	5.22	5.01
1.27	1.32	4.53	4.83

EXPERIMENTAL

Chromatographing was performed at 20° C, by the ascending method, on filter paper strips, Whatman No 1, 30 cm long and 3 cm wide.

The following solvents were used:

1. *n*-Butanol saturated with 3 N hydrochloric acid (5). R_f-values: Cu-0.17; U-0.23; Cd-0.84; Zn-0.93; Hg-0.93. This solvent was applied in the separation of copper from zinc and cadmium, cadmium from uranium, uranium from zinc, and for the separation of copper from zinc, cadmium, and mercury;

2. 85 ml i-Propanol + (ml water + 10 ml nitric acid sp. w. 1.42. R_f-values: U-0.64; Al-0.10; Fe-0.13; Cr-0.08; Ni-0.06. This solvent was used for the separation of uranium from aluminium, iron, chromium and nickel.

The time required for the development of chromatograms by means of solvent 1 and 2 was cca twenty and fourteen hours respectively. In both cases, the front traveled about 20 cm.

Copper, cadmium, and mercury were detected by means of dithizone; uranium and iron by means of ferrocyanide; aluminium and chromium by means of alizarine; and nickel by means of dimethyl glyoxime. Detailed experimental data were given in previous papers (1. c.).

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THE SEMIQUANTITATIVE DETERMINATION OF MICRO AMOUNTS OF IONS BY VISUAL COLORIMETRY OF SPOTS OBTAINED BY PARTITION PAPER CHROMATOGRAPHY. I. THE DETERMINATION OF MERCURY, COPPER, CADMIUM, ZINC, URANIUM, AND IRON

by

TOMISLAV J. JANJIĆ, MILENKO B. ĆELAP, and LJUBIŠA STOJKOVIĆ

The visual colorimetry of spots obtained by partition paper chromatography was first applied in the determination of uranium by Arden et al. (1), in 1949. These authors performed the determination by effecting the separation of uranium from other elements by means of an appropriate solvent, and then, they compared the spots detected by potassium ferrocyanide with those of the standard scale obtained by a standard uranium solution in the same way. The standard scale consisted of several spots which contained from 30 to 100 γ uranium. The experimental errors amounted to $\pm$ 30%.

In 1952, Weiss et al. (2) determined uranium, iron, and copper in their admixtures in a similar way; the average error in these determinations was $\pm$ 20%. The standard scale corresponded to amounts ranging from 5 to 80 γ uranium and from 10 to 160 γ copper and iron.

It is evident from this that the errors in the determination of elements by visual colorimetry of spots obtained by partition paper chromatography were rather great and amounted to some tens of per cents. For this reason we attempted to achieve a more precise determination by reducing the area of the starting point to its minimum, and by applying the colorimetric procedure given by Weiss in his ring colorimetry method (3).

The reduction of the starting point area was achieved by transferring a few drops of the solution to be analyzed by means of a capillary pipette to the filter paper strips at the position designated by B (Figure 1). The strips were then fastened onto a piece of folded aluminum foil so that the shorter part of the strip up to the position designated by A (Figure 1) was inserted into the aluminum block (Figure 2). The other part of the strip was left to hang freely over a fixed aluminum wire. The application

of the solvent by means of a capillary pipette to the strip at the position C brought about the concentration of the elements into a narrow line since the solvent evaporated at the edge of the aluminum block, which was heated with a micro burner to about

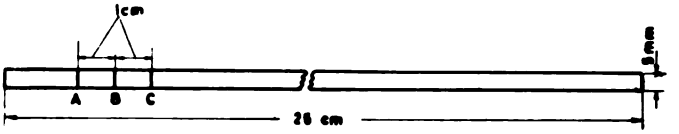

Fig. 1
Paper strip

110° C. In this way, three strips which contained various numbers of drops of the examined solution were prepared for each analysis. The standard scale for comparison was composed of 1,2,4,6,8, and 10 drops of the standard mixture of the elements to be determined. Then all strips were put together into a glass cylinder and were chromatographed by the ascending method by means of an appropriate solvent. The detection was performed with appropriate reagents after development.

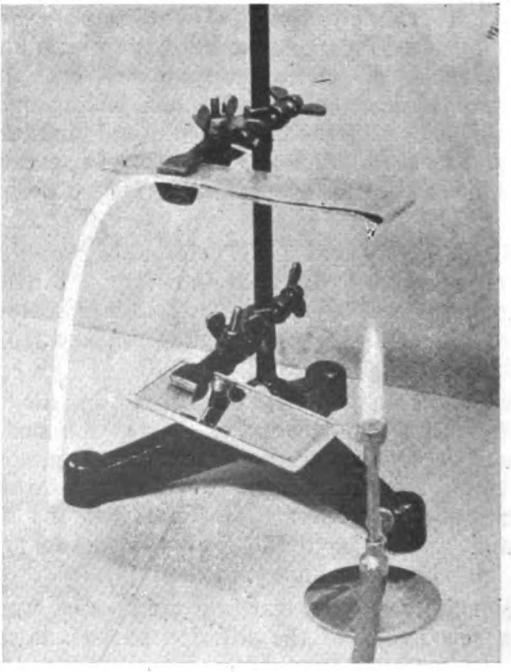

Fig. 2
Rectangular oven

The colorimetry of detected spots of separated elements was performed by comparing the intensities and areas of individual

spots of the examined solution with the intensities and areas of the spots of the corresponding elements on the standard scalle. The number of drops of the examined solution applied to all the three strips was summed up (K_A), and the sum of the corresponding drops of the standard solution (K_S) obtained by means of the standard scale was divided by the former value (K_A). By multiplying the concentration of the standard solution (C_S) with the quotient K_S/K_A, the concentrations of individual elements (C_A) in the examined solution were obtained:

$$C_A = C_S \frac{K_S}{K_A}$$

For example: in the determination of copper, one, two, and three drops of the examined solution were applied to the first, second, and the third strip, respectively. The comparison with the standard scale showed that the spot obtained with one drop of the examined solution corresponded to the spot obtained with two drops of the standard solution. The spot obtained with two drops of the examined solution corresponded to the spot obtained with four drops of the standard solution. The intensity of the spot obtained with four drops of the examined solution was more pronounced than the intensity of the spot obtained with four drops of the standard solution, but less pronounced than the intensity of the spot obtained with six drops of the standard solution, or it corresponded approximately to the intensity of the spot which would be obtained with five drops of the standard solution. Thus, $K_A = 6$ and $K_S = 11$. Since the concentration of the standard solution was 0.3 mg Cu/ml, the calculated concentration of the examined solution was:

$$0.3 \frac{11}{6} = 0.55 \text{ mg Cu/ml}$$

In this way the following pairs of elements were determined:

1. Mercury and copper in the presence of bismuth
2. Copper and cadmium in the presence of bismuth
3. Copper and zinc
4. Uranium and iron.

The amounts of individual ions required for one determination were: 40 γ mercury, 20 γ copper 30 γ cadmium, 10 γ zinc, 15 γ uranium, and 3 γ iron.

The results obtained are presented in Table 1. The table shows that the average deviations of nine successive determinations (are:) 2.8% for mercury, 2.1% for copper in the pesence of mercury and bismuth, 2.6% for copper in the presence of cadmium and bismuth, and 2.7% for copper in the presence of zinc. The average deviations for uranium, cadmium, and iron are 3.0%, 3.3% and 3.5% respectively; the deviation for zinc is only 1.9%.

TABLE 1

Concentration of the solution in mg/ml

Calculated	Found	Calculated	Found
Hg^{2+} in the presence of Cu^{2+} and Bi^{3+}		Cu^{2+} in the presence of Hg^{2+} and Bi^{3+}	
2.44	2.30	1.57	1.58
2.44	2.33	1.57	1.61
2.44	2.36	1.57	1.56
2.72	2.65	1.68	1.62
2.72	2.76	1.68	1.69
2.72	2.79	1.68	1.61
3.58	3.51	2.27	2.29
3.58	3.52	2.27	2.32
3.58	3.67	2.27	2.18
Cu^{2+} in the presence of Cd^{2+} and Bi^{3+}		Cd^{2+} in the presence of Cu^{2+} and Bi^{3+}	
1.57	1.64	1.75	1.75
1.57	1.57	1.75	1.67
1.57	1.69	1.75	1.75
1.57	1.57	1.58	1.58
1.57	1.53	1.58	1.49
1.57	1.56	1.58	1.64
1.42	1.42	2.30	2.41
1.42	1.47	2.30	2.43
1.42	1.46	2.30	2.40
Cu^{2+} in the presence of Zn^{2+}		Zn^{2+} in the presence od Cu^{2+}	
0.579	0.622	0.557	0.547
0.498	0.508	0.608	0.629
0.622	0.622	0.608	0.629
0.871	0.851	0.768	0.773
0.871	0.856	0.768	0.763
0.871	0.830	0.896	0.917
0.566	0.591	0.896	0.907
0.629	0.596	0.640	0.651
0.545	0.545		
UO_2^{2+} in the presence of Fe^{3+}		Fe^{3+} in the presence of UO_2^{2+}	
1.26	1.24	0.196	0.193
1.26	1.30	0.196	0.207
1.26	1.27	0.196	0.207
1.19	1.24	0.242	0.244
1.19	1.13	0.242	0.238
1.19	1.17	0.242	0.234
1.23	1.24	0.203	0.218
1.23	1.17	0.203	0.193
1.23	1.17	0.203	0.202

It is evident from this that the above described visull colorimetry of spots obtained by partition paper chromatography affords almost tenfold better results than any of the methods described so far. It involves the reduction of the starting point area and the application of the colorimetric procedure recommended in the ring colorimetry method.

The determinations of other elements by this method are under investigation.

EXPERIMENTAL

The investigated solutions were prepared from the following salts: mercuric chloride, cupric sulfate, cadmium chloride, zinc sulfate, uranium acetate, ferric chloride, and bismuth chloride. The solutions were transferred to the filter paper strips by means of a micro capillary pipette having a volume of about 1 μl (the exact volume of the capillary is not required when the same pipette is used for the application of the standard and the examined solutions). The Whatmann No 1 paper strips of standard width (5 mm) and about 25 cm length were cut out by means of a special knife with two edges (4). The chromatography was performed in a glass cylinder by the ascending method at a temperature of 20° C.

The investigated elements were separated by means of a solvent consisting of *n*-butanol saturated with 3 N hydrochloric acid solution (5), except uranium and iron, which were separated by means of a solvent mixture consisting of 85 ml isopropanol, 10 ml nitric acid (sp. w. 1. 42), and 5 ml water. The time required for the development amounted from two to three hours. The detection of mercury and bismuth was performed by means of ammonium sulfide. Copper in the presence of mercury and bismuth, and cadmium and bismuth, respectively, was also detected by means of ammonium sulfide. The detection of copper in the presence of zinc was done with a 1% potassium ferrocyanide solution (excess of potassium ferrocyanide was washed with water). Uranium and iron were detected in the same way. Cadmium was detected by first dipping the strips into the solution of ammonium sulfide, and after washing them they were dipped into a 1% silver nitrate solution and washed again. The detection of zinc was performed by first dipping the slrips into a 1% potassium ferrocyanide solution, and then, after washing them with water, they were dipped into a ferric chloride solution. The excess of ferric chloride was washed with 0. 01 *N* hydrochloric acid. The strips were dried in the air. The following R_f-values were obtained:

Cd^{2+}	0.90	Cu^{2+}	0.10
Hg^{2+}	0.88	UO_2^{2+}	0.61
Bi^{3+}	0.55	Fe^{3+}	0.10
Zn^{2+}	0.50		

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GLASNIK HEMIJSKOG DRUŠTVA, vol. 27, no 5—6, 1962, pp. 289-291

PERSULFATOMETRIC DETERMINATION OF HYDRAZINE BY THE DEAD STOP METHOD

by

MOMIR S. JOVANOVIĆ and MIRJANA P. KALINIĆ

The analytics of hydrazine has long represented a field of interest for one of us, and, among other investigations, we have proposed a new method for the volumetric determination of hydrazine. In a slightly alkaline solution, and in the presence of potassium iodide, hydrazine is titrated with a warm standard potassium persulfate solution; the titration end-point is indicated by the appearance of free iodine. The disadvantage of this method involves the constant possibility of hydrazine autooxidation in its warm alkaline solution, (2,3) and a subjectively erroneous observation of a slightly yellow iodine color at the moment of titration end-point. In order to avoid subjectiveness in the estimation of the titration end-point, we have applied the electrogravimetric detection, using the inventive and simple dead-stop technique. At the same time, we wanted to finish a small cycle (5) of attempts in determining hydrazine by this spectacular method, originated by Foulk and Bawden (6), more so, because our original method was in good agreement with another similar method (4).

EXPERIMENTAL

The apparatus employed consisted of one indicator circuit of platinum plates, 5×10 mm. The desired potential difference between electrodes, dipped into the solution to be investigated, was applied by moving a mobile contact of a potentiometrically connected rheostat of 500 Ω; in addition, the current circuit was connected to a galvanometer, the sensitivity of which was 1 $\mu A/^0$, and a millivoltmeter with a range of readings of 50 mV.

The determination was performed with an $N_2H_4 \cdot H_2SO_4$ (B. D. H., analytical reagent) solution standardized iodometrically according to Stolle (7) by the use of the dead stop technique. This method was recommended as the standard one by E. Merck (8); the disadvantage of starch as the indicator was avoided by electrogravimetrically detecting the titration end-point. By the use of this method, we have found that

25.00 ml N/10 $N_2H_4 \cdot H_2SO_4$ solution consume 25.00 ml N/10 J_2 (F = 1.0330) solution, from which it is evident that

$$T_{N_2H_4 \cdot H_2SO_4} = 3.3609 \text{ mg/ml.}$$

We also performed the standardization of $K_2S_2O_8$ solution, the titrant for the determination of hydrazine. This was performed by Kempf's method (9): the persulfate solution was acidified with sulfuric acid, and then a known excess of oxalic acid was added. In the presence of silver sulfate as catalyst, and at the temperature of a boiling water bath, the evolution of carbon dioxide from oxalic acid was soon terminated, and the unreacted oxalic acid was titrated with a standard permanganate solution.

It was found that the volume of 10.00 ml N/10 $K_2S_2O_8$ solution to which 25.00 ml N/10 $H_2C_2O_4$ (5F = 1.0058) were added, consumes 15.60 ml N/10 $KMnO_4$ solution for the unreacted oxalic acid. Accordingly, the factor of N/10 $K_2S_2O_8$ solution is

$$F = 0.9126.$$

In a previous paper (1) we have stressed that the final result of the determination is the same, whether the reaction between hydrazine and persulfate is direct or indirect, i.e., primarily liberated iodine secondarily reacts with hydrazine. Some observations obtained in the course of the present electrogravimetric measurements support the assumption that the reaction is indirect, at least in the presence of sodium bicarbonate. The aim of this work was not to give a detailed answer to the above probelm, which is the subject of a separate investigation. By adopting the conception of Leko (10) concerning the negative monovalent state of oxygen in peroxy bond, the summed equation which represents the oxidation of hydrazine with potassium persulfate is formulated as follows:

$$2N^{3-} - 4e = N_2^0$$
$$2 \times 2O^{1-} - 4e = 2 \times 2O^{2-}.$$

Accordingly, the ratio of reacting components is 2 moles of persulfate per 1 mole of hydrazine.

Attempts to determine small amounts of hydrazine sulfate (about 1 mg) by titrating it with a warm standard persulfate solution, after the addition of potassium iodide and sodium bicarbonate, have shown that autooxidation of hydrazine plays an important role in cases with small amounts. The following procedure is recommended in order to avoid the oxidation of hydrazine:

The investigated hydrazine sulfate solution should be used as the titrant instead of the persulfate solution. The volume of persulfate required for the reaction with an aliquot part of investigated hydrazine solution should be determined in a preliminary experiment. This volume of standard persulfate solution is poured into a glass of 150 ml, about 2 g potassium iodide is added, the

mixture is diluted to 100 ml, and warmed to about 80° C. The oxidation of iodide to iodine very rapidly occurs at this temperature. Then, about 1 g sodium bicarbonate is added and the indicator electrodes are dipped into the solution. The potential difference of 12 to 14 mV is applied by an external source. The solution is stirred with a magnetic stirrer, and titrated with the investigated hydrazine solution. Since the oxidation of hydrazine with iodine is instantaneous, its autooxidation does not occur at all. The indicator anode is depolarized by the presence of an excess of iodide before the addition of hydrazine, but the cathode is depolarized by the presence of iodine, and therefore the current strength which passes through the solution amounts to about 7 μA. The entire amount of iodine is consumed by hydrazine at the titration endpoint and therefore the cathode is no longer depolarized; the galvanometer needle returns to its zero position. Table 1 shows only some of the results obtained from a series of thirty determinations.

TABLE 1

3.369 ml N/100 $K_2S_2O_8$ solution (F = 0.9126) correspond to 1.000 mg of $N_2H_4 \cdot H_2SO_4$ (calculated value)

$K_2S_2O_8$ solution ml taken	$N_2H_4 \cdot H_2SO_4$ mg consumed	difference from calculated value	error %
3.369	0.998	−0.002	−0.20
3.369	1.001	+0.001	+0.10
16.85	5.007	+0.007	+0.14
16.85	4.972	−0.028	−0.56
33.69	10.013	+0.013	+0.13
33.69	9.996	−0.04	−0.4

Only the maximal deviations are shown.

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A CONTRIBUTION TO THE IODOMETRIC DETERMINATION OF NOVALGIN

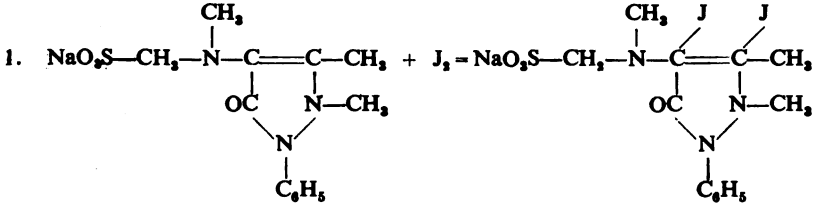
by

BORISLAV BUDIMLIĆ and DRAGICA DUMANOVIĆ

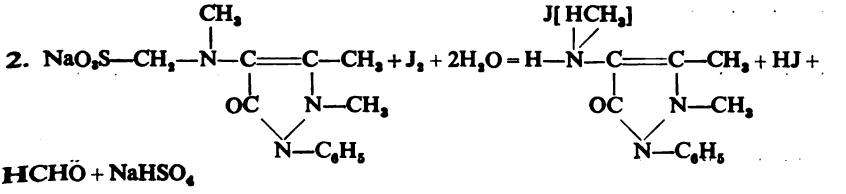
The known methods for the determination of novalgin, although rather numerous, are not fully reliable. The gravimetric methods which involve the determination of sodium chloride, sodium sulfate, and barium sulfate deserve mention. There are also two colorimetric methods (1). From the volumetric methods (2, 3, 4, 5, 6, 7, 8, 9, 11) we checked the iodometric ones (5, 6, 7, 8, 9, 11), since the latter methods are usually used for the determination of novalgin in pharmacopeas.

Armestar and Schultz (5) proposed an iodometric method for the determination of novalgin but they did not give a detailed procedure.

Wirth (6) has suggested a method which, in fact, was a modification of the known iodometric method for the determination of antipyrine. According to these authors, the reaction between novalgin and iodine consists of the addition of iodine to the double bond of the ring:



Rapoport and Shvartsburd performed determination by adding the iodine very slowly. They assumed that the reaction takes place as follows:



When checking the methods cited under (6) and (7), Shub and Kobzareva (8) quoted that they were unable to obtain reproducible results and recommended a method of their own. They used a rather small amount of water in regard to the amount of novalgin, explaining that the titration end-point was less sharp in dilute solutions. The titrations were performed by a rapid addition of iodine.

According to Wojahn (9), Wirth's method afforded too high results (101—102); in his opinion better results might be obtained by varying the amounts of acetic acid and by regulating the temperature and the rate of the titration. The latter author suggested a method in which special attention was paid to the rate of the titration. The solution of novalgin is to be titrated by dropwise adding the 0.1 N iodine solution, and the titration end point is determined by means of starch as the indicator; the blue color should persist at least for two minutes.

In the propositions for the III Supplement of Swiss Pharmacopea, there is a method in which the rate of the titration is more precisely defined (1.50 ml per minute). The end of the titration is determined as in the previous method.

The review of the above cited method is given in Table 1.

TABLE 1

Authors	Weighed amount of novalgin g	Added amount of water ml	Added amount of acid	Indicator	Rate of the titration
Armestar, M. A. Schultz, A.	0.25	50	CH_3COOH	starch	
Wirth,C.	0 25	50	3 ml dilute HCl	starch	rapid addition of iodine is not recommended
Rapoport, L. Shvartsburd, M.	0.15	10		methylene blue	slow addition of iodine
Shub, N. Kobzareva, N.	0.40	5—6			rapid
Wojahn, H.	0.20	50	5 ml 1/50 N HCl	starch	dropwise
III Suppl. of Swiss Pharmacopea N° 83	0.30	50	1 ml CH_3COOH	starch	1 drop per minute

If the above methods are examined in detail, it is evident that there are no essential difference among them. The only difference is that some authors (5, 6, 9, 11) used an acidic solution of novalgin, and the others (7,8) did not add any acid. Further, various authors use different amounts of water and novalgin for the analysis. Most authors used starch as the indicator (5, 6, 9, 11), some replaced it with methylene blue, and Shub and Kobzareva claimed that iodine itself is sufficient for the detection of the titration end-point.

Assuming that the equations (1) and (2) are correct, we are of the opinion that the reproducibility of the results should not depend up on the amounts of water and novalgin used nor upon the titration rate and the choice of indicator since such a dependence could not be justified by the equations (1) and (2). We have performed a great number of determinations under the same experimental conditions, except for the titration rate, and have established great variations (3 per cent). The obtained discrepancies and a rather great number of iodometric methods which are not essentially different indicate that the problem of the determination of novalgin by means of iodine is not fully examined.

In this work we attempted to give a contribution to the reaction mechanism of novalgin and iodine. As far as we know, there are two equations (6,7) which are basically different.

On the basis of our experimental results, the equation (1) which was suggested by Wirth (6), and which is given in newly published pharmacopea, is to be discarded. The following facts are not in conformity with the equation (1):

(1) In the course of the titration the SO^{-}_{4} ions are formed (their formation is the basis for the gravimetric method (barium sulfate) of novalgin (10);

(2) The concentration of hydrogen ions is increased in the course of the titration; and

(3) After the titration, the titrated solution contains formaldehyde in addition to SO^{-}_{4} and H^{+}.

These findings reject the equation (1) and support the equation (2). Apart from the given components, the equation (2) provides the possibility for the formation of the salt of 4-methylamino-antipyrine (1-phenyl-2, 3-dimethyl- 4-methylamino-5-pyrazolone). We have paid special attention to the latter compound since other components were easily identified.

In order to isolate 4-methylamino-antipyrine, Rapoport and Shvartsburd (7) applied the following procedure: when the reaction was over, the reaction mixture was made alkaline and was extracted with chloroform. The solvent was evaporated and the residue was recrystallized several times. The obtained crystals melted at 168—170°. The authors claimed that the obtained compound was 4-methylamino-antipyrine and identified it in the form of its formyl derivative, although they were unable to find its melting point in the literature.

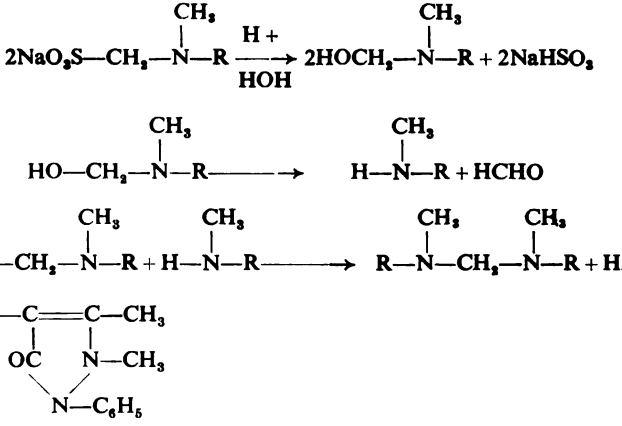
We found in the literature (12,13) that 4-methylamino-natipyrine is an oil which crystallizes on standing (m. p. 60° C). Therefore, we concluded that the substance isolated by Rapoport and Shvartsburd was not 4-methylamino-antipyrine, and we attempted to identify the substance melting at 168—170°.

We repeated the experiments of Rapoport and Shvartsburg and ob'ained a compound which, after several crystallization from benzene, melted at 171—175°.

If the reaction between novalgin and iodine occurs according to equation (2), then the separation of 4-methylamino-antipyrine should be effected from the alkaline solution. The data in the literature (15, 16, 17, 18, 19) indicate that under these experimental conditions bis (4-methylamino-antipyrine) methane, m. p. 174° (16, 17), 175° (18), 171—175° (15), is separated. On the basis of these data we assumed that the compound isolated by the above authors is in fact bis (4-methalamino-antipyrine) methane. This assumption was proved to be correct by the reproduction of two works referring to the preparation of the compound in question. Bis (4-methylamino-antipyrine) methane was obtained as a final hydrolytic product by the action of dilute hydrochloric acid on novalgin by Wagner (15) and Takahasni et al. (18, 19), and it was isolated as an intermediate product of the novalgin synthesis by the method of Isler (14).

Novalgin was hydrolized with 12.5% hydrochloric acid, and the reaction mixture was extracted with chloroform. The solvent was evaporated and the residue was recrystallized from benzene and water; m. p. 171—175° C.

The scheme for the hydrolytic degradation of novalgin according to Wagner is as follows:



The experimental conditions for the preparation of bis (4-methylamino-anatipyrine) methane according to Wagner are the same as those encountered in the separation, effected after the iodometric determination of novalgin, except for the sulfite ions which are oxidized to sulfate in the latter case.

Bis (4-methylamino-antipyrine) methane is obtained by the Isler's method by passing formaldehyde into a neutral solution of 4-methylamino-antipyrine. The reaction mixture is then extracted with chloroform and the crystals, obtained after the evaporation of the solvent, are recrystallized from benzene; m. p. 171—175°.

Both products, obtained by two different ways, possessed the same melting point and exhibited the same physical and chemical properties as did the substance obtained by Rapoport and Shvartsburd.

In conformity with the equation (2), our experiments confirmed the presence of formaldehyde, sulfate, and hydrogen ions. We also proved that the substance isolated by the procedure of Rapoport and Shvartsburd was not 4-methylamino-antipyrine but bis (4-methylanimo-antipyrine) methane. However, we do not know whether this substance was formed from 4-methylamino antipyrine and formaldehyde which are present in the reaction mixture, or it already existed as such.

It was of interest to establish which component was present in the reaction mixture. If 4-methylamino-antipyrine was formed, the consumption of iodine should have been much greater since it reacts with iodine liberating hydrogen ions, and gives a pink-colored solution. The presence of bis 4-methylamino-antipyrine) methane does not interfere with the iodometric determination.

It is known (18) that 4-methylamino-antipyrine and formaldehyde can exist side by side in acidic media; methane is separated in neutral and alkaline media bis (4-methylamino-antipyrine).

In the titration of novalgin with iodine, the reaction mixture is acid even in the methods (7,8) where no acid was added; *pH* of the solution during the titration ranged from *pH* 6 to *pH* 1.5.

The acidic media favors the formation of 4-methylamino-antipyrine, which reacts further with iodine. On the other hand, in the presence of an acid, novalgin is degradated (10) liberating SO_2, and the consumption of iodine is decreased by the loss of SO_2. The equation (2) does not take these facts into consideration.

This problem requires further investigation of the reaction mechanism. This is the only way to find a method which could give reproducible results regardless of the titration rate and the amount of novalgin and water used.

We thank Mrs B. Selivanovski for her technical help.

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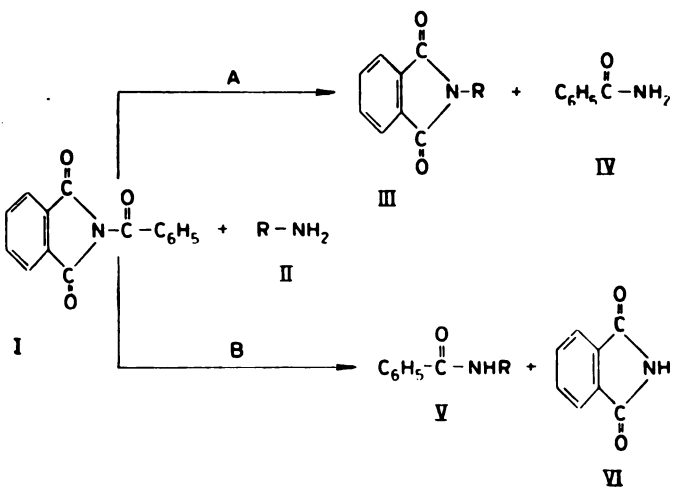
N-BENZOYLPHTHALIMIDE
II.* REACTION WITH AMINES

by

RUŽA BORISAVLJEVIĆ, JOVAN BOŠNJAK, RASTKO I. MAMUZIĆ
and MIHAILO LJ. MIHAILOVIĆ

In the continuation of our studies on the chemistry of *N*-benzoylphthalimide (1), the present paper reports results of the reaction with primary and secondary amines.

Primary amines (II) can react with *N*-benzoylphthalimide (1) in two ways: to give either *N*-substituted phthalimides (III) and benzamide (IV) (reaction A), or *N*-substituted benzamides (V) and phthalimide (VI) (reaction B). For this reason, we attempted to determine which products were obtained in these reactions, and whether or not amines of different glasses behaved similarly.



(*a*) R = C_6H_5 (*b*) R = *p*—HO—C_6H_4 (*c*) R = C_6H_{11}
(*d*) R = C_6H_5—CH_2 (*e*) R = HO—CH_2CH_2 (*f*) R = $CH_3(CH_2)_7$
(*g*) R = $CH_3(CH_2)_3$.

* Paper I: Glasnik hem. društva Beograd (Bull. Soc. Chim. Beograd) 27, 201 (1962).

Aromatic amines, aniline (II*a*), and *p*-aminophenol (II*b*), reacted with *N*-benzoylphthalimide (I) exclusively according to path A, giving *N*-phenylphthalimide (III*a*) and *N*-(*p*-hydroxyphenyl)-phthalimide (III*b*) in yields of 83% and 80% respectively; benzamide (IV) was also isolated in both cases, but phthalimide (VI: reaction B) was not present in the reaction mixture.

The reaction proceeded mainly according to A with cyclohexylamine (II*c*), giving *N*-cyclohexylphthalimide (III*c*) in a 72% yield, and only to a slight extent according to B, so that phthalimide (VI) and *N*-cyclohexylbenzamide (V*c*) were obtained in 10% and 5% yields, respectively. Benzylamine (II*d*) behaved similarly, and reacted with *N*-benzoylphthalimide (I) to give (reaction A) *N*-benzylphthalimide (III*d*) and benzamide (IV), in yields of 72% and 74%, respectively; and (reaction B) *N*-benzylbenzamide (V*d*) and phthalimide (VI), in yields of 6% and 10%, respectively.

N-benzoylphthalimide (I) reacted with 2-aminoethanol (II*e*) at room temperature, but in this case the amide benzoyl carbonyl of *N*-benzoylphthalimide was attacked by the base to a greater extent, so that besides *N*-(2-hydroxyethyl)-phthalimide (III*e*) (yield 58%; reaction A), 31% of phthalimide (VI) (reaction B) was also obtained. With *n*-octyl-amine (II*f*) and *n*-butylamine (II*g*), the ratio of reaction A an B was about 1 : 1, so that benzamide (IV) and *N*-alkyl phthalimide (III*f* or III*g*) (reaction A), as well as phthalimide (VI) and *N*-alkyl benzamide (V*f* or V*g*) (reaction B) were obtained in approximately equal yields. These primary aliphatic amines (*n*-octyl- and *n*-butyl-) reacted on standing with *N*-benzoylphthalimide as early as 0°.

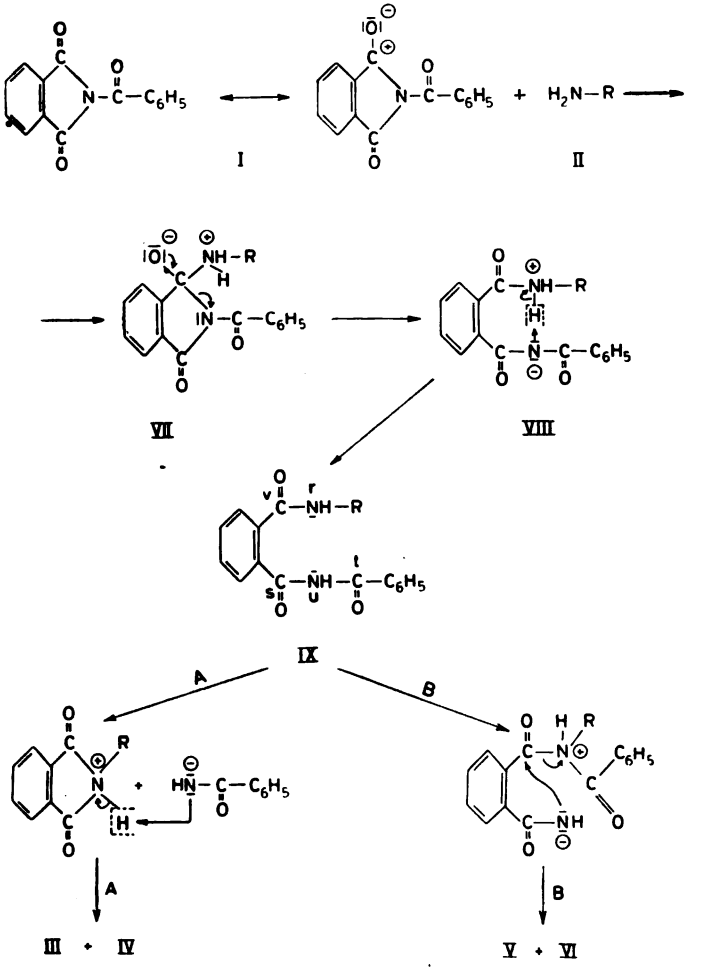
Although reactions A and B can be formulated as independent attacks of the nucleophilic amino group, either on the carbonyl carbon of the imide ring (reaction A) or on the carbonyl Carbon of the benzoyl rest (reaction B) of *N*-benzoylphthalimide, it seems more probable that both reactions proceed through a common intermediate, i. e., through the *N*-benzoyl-N'-substituted phthalamide (IX), which could arise from structures VIII and VII, respectively, by initial attack of the nucleophile (II) on the imide ring carbonyl group of *N*-benzoyl-phthalimide (I) (Scheme 1).

This phthalamide (IX) can decompose in two ways: (1) by attack of the lone electron pair of nitrogen (*r*) on carbon (*s*) the *N*-substituted phthalimide (III) is obtained, with elimination of a proton from nitrogen (*r*) and of the benzamide anion from carbon (*s*), in the form of benzamide (IV) (Scheme 1; reaction A); (2) by attack of the lone electron pair of nitrogen (*r*) on carbon (*t*) and by ring closure between nitrogen (*u*) and carbon (*v*), *N*-substituted benzamide (V) and phthalimide (VI) are formed (Scheme 1; reaction B).

A number of authors (2, 3, 4, 5, 6) have shown that phthalimides of type XI are intermediate products in the transamination of phthalimides of type X to phthalimides of type XII.

It has also been reported that phthalamides (XI and XIII) are often unstable (3, 4, 6) and can be converted to the corresponding

phthalimides (X or XII) under mild conditions (2, 6, 7), or even in the cold (3, 4). In the reaction of primary amines with *N*-benzoylphthalimide, we could not isolate the intermediate phthalamides (IX), even when the reactions were carried out at 0°, except in the case of cyclohexylamine (II*c*). A white voluminous precipitate was obtained with this amine (at 0°), whose composition corresponded to that of *N*-benzoyl-*N*-cyclohexylphthalamide (IX; $R = C_6H_{11}$), but

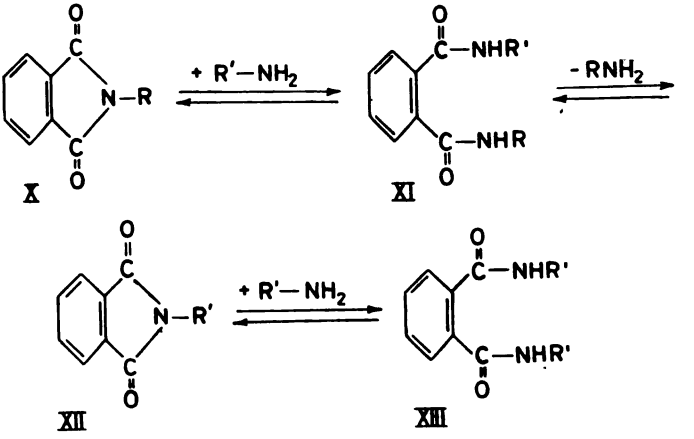


Sheme 1

which was unstable and, upon prolonged standing at room temperature, or upon heating in ethyl alcohol or benzene, decomposed according to A and B to give reaction products in the same ratios as in the normal reaction between cyclohexylamine an *N*-benzoylphthalimide.

Whatever the course of reactions A and B may be (direct attack on the amide and imide carbonyls of *N*-benzoylphthalimide or formation of the intermediate phthalamide (IX)), the results obtained can be rationalized in terms of steric and electronic factors in the following way:

The basic (and nucleophilic) properties of the primary amines used in the present study follow a decreasing order (8): cyclohexylamine > *n*-octylamine ≈ *n*-butylamine > 2-aminoethanol > benzylamine > > aniline > *p*-aminophenol; and the steric effect decreases in the following order (9): *p*-aminophenol ≈ aniline > cyclohexylamine > benzylamine > 2-amino-ethanol ≈ *n*-octylamine ≈ *n*-butylamine. According to models, it appears that the benzoyl carbonyl carbon of *N*-benzoylphthalimide is sterically more hindered than the imide-ring carbonyl carbon. Therefore, it is anticipated that the course of the reaction (A or B) would mainly depend upon the steric requirement of the used amine, and polar factors would be of less importance,



particularly since there should not be any noticeable difference in the electrophilic properties of the three carbonyl carbons of *N*-benzoylphthalimide. The results described have confirmed this assumption, i. e. that steric factors are more involved than polar factors (for instance, the similar ratios of corresponding products obtained in the reactions of *N*-benzoylphthalimide with aniline and cyclohexylamine, respectively, two bases of like steric effects but of widely different nucleophilic properties). However, the somewhat higher yield of reaction B, when cyclohexylamine was used instead of aniline, or when *n*-octylamine or *n*-butylamine were used instead of 2-aminoethanol (pairs of amines of similar steric requirements), is explained by the fact that polar factors do have a certain influence on the course of the reaction, the stronger nucleophiles more readily attack the hindered benzoyl carbonyl group of *N*-benzoylphthalimide. Unhindered amines with strong nucleophilic properties, such as *n*-octylamine and *n*-butylamine, attack both carbonyl carbons

(the open-chain benzoyl carbonyl carbon and the ring imide carbonyl carbon) of *N*-benzoylphthalimide to the same extent, so that the yields of reactions A and B are nearly equal.*

From the results obtained, we conclude that the yield of reaction A (transamination of *N*-benzoylphthalimide) will increase with increasing steric effect and decreasing nucleophilic characteristics of the primary amine (reaction A can be quantitative), and, inversely, that reaction B (formation of substituted benzamides) will be favored with decreasing steric requirements and increasing nucleophilic properties of the reaction amine (the yield of reaction B, in the best case, does not exceed much over 50%).

As much as steric factors are of major importance for the course of reaction between primary amines and *N*-benzoylphthalamide, so are polar factors significant for the rate of these reactions. While strong bases (cyclohexylamine, *n*-octylamine) reacted quantitatively after two to five hours at 0° and bases of medium strength (benzylamine, 2-aminoethanol) after five to ten hours, weak bases (aniline) required over twenty-four hour at 0° for a complete reaction.

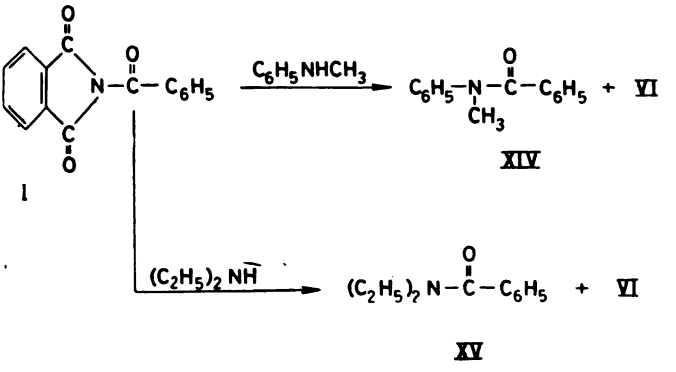
For comparison, *n*-octylamine was condensed with *N*-acetylphthalamide, giving phthalamide (VI) in a 34% yield (reaction B). Although steric hindrance exerted by the methyl group on the acetyl carbonyl carbon of *N*-acetylphthalamide is considerably smaller than the steric hindrance of the benzoyl phenyl group in *N*-benzoylphthalimide, an although *n*-octylamine shows only a negligible steric effect, the reaction with *N*-acetylphthalamide proceeded to a larger extent by attack of the base on the imide carbonyl carbon (reaction A) than on the amide carbonyl group (as compared to the reaction of *N*-benzoylphthalimide with the same amine). Such a behavior can be explained by assuming that the electron releasing methyl group from the acetyl remains will decrease the electrophilic properties of the adjacent carbonyl carbon, thus directing the attack of the nucleophilic reagent preferentially on the imide (ring) carbonyl carbon, which is more positive since it is attached to the electron which attracts the benzene ring. As already stated the difference in polarity of the imide and amide carbonyl carbons in *N*-benzoylphthalimide is probably small because all the carbonyl groups are directly attached to the benzene residue, and therefore *n*-octylamine, being a strong base with a small steric effect, will attack both carbonyl groups (amide and imide) to the same extent (giving nearly equal yields of reactions A and B).

It should be noted that compounds which contain both a hydroxyl and an amino group, such as *p*-aminophenol (II*b*) and 2-aminoethanol (II*e*), did not react with *N*-benzoylphthalimide with their oxygen function, but exclusively with the more nucleophilic

*) *Yanagi* (10) has found that strong bases, cyclohexylamine and ethylamine, reacted with *N*-vinylphthalamide by addition across the double bond of the vinyl rest, but weaker bases (aniline and 2-aminoethanol) caused transamination and gave *N*-phenylphthalimide (III*a*) and *N*-(2-hydroxyethyl)-phthalimide (III*e*), respectively. In this case, polar factors are more important than steric factors because the vinyl double bond is not sterically hindered.

amino grcup, affording *N*-substituted phthalimides (III) and *N*--substitıtted benzamides (V), as already described.

Secondary amines underwent simple benzoylation when allowed to react with *N*-benzoylphthalimide (I). Thus, *N*-methylaniline gave *N*-methylbenzanilide (XIV) and phthalimide (VI), and diethylamine afforded *N*, *N*-diethylbenzamide (XV) and phthalimide (VI).



The reaction with *N*-methylaniline was slow, and even after a prolonged heating in benzene, the yield in phthalimide amounted only to about 63%; but diethylamine reacted nearly quantitatively after two hours in boiling benzene, and proceeded even at 0°. This diffeı ence in the reaction rate can be attributed again to steric, and particularly polar, factors. The considerable steric effect and weak nucleophilic properties of *N*-methylaniline (8) will retard the reaction with *N*-benzoylphthalimide, but on the contrary, diethylamine will react more easily, because it is a strong nucleophile and sterically less hindered.

Similar to the primary amines, secondary amines can also react with *N*-benzoylphthalimide in two ways, but both reaction courses will give the same end products, i. e. *N*, *N*-disubstituted benzamides (XIV and XV) and phthalimide (VI): the amino group can directly attack the amide benzoyl carbonyl group or it can first react with the imide (ring) carbonyl (Scheme 1) to give the intermediate phthalamide (of type IX; instead of H the nitrogen (*r*) carries a substituent *R'*), with opening of the five-membered ring. This phthalamide (IX) can decompose in only one way (according to B) and produce the *N*, *N*-disubstituted benzamide (XIV or XV) and phthalimide (VI). When diethylamine was caused to react with *N*-benzoylphthalimide at 0°, (only in one run) a product which had the composition corresponding to *N*-benzoyl-*N'*, *N'*-diethylphthalamide (IX; $R = C_2H_5$, instead of H there is a second C_2H_5) was obtained, but this was very unstable, and decomposed on standing and very rapidly upon slight warming (in acetone or alone over 80°) to give the corresponding benzamide (XV) and phthalimide (VI).

The results of the reaction of *N*-benzoylphthalimide with alcohols (1) and amines (primary and secondary) suggest that in these cases there is an initial attack by a nucleophilic agent on an imide (ring) carbonyl group of *N*-benzoylphthalimide which is followed by decomposition of the so formed intermediate products. However, direct attack of the nucleophilic compound on the amide (benzoyl) carbonyl group of *N*-benzoylphthalimide would also partly account for the products obtained, so that it is possible that this reaction also proceeds to a certain extent.

EXPERIMENTAL PART

All melting and boiling points are uncorrected.

N-benzoylphthalimide, prepared from phthalimide and benzoyl chloride in anhydrous pyridine (11), melted at 168° after crystallization from ethyl alcohol.

Benzamide, m. p. 128° (12), phthalimide, m. p. 233° (12) and *N*-benzoylphthalimide, m. p. 168° (11) were always identified by the melting point and mixed melting point determinations (with authentic products).

Reaction of N-benzoylphthalimide with aniline

a) A mixture of 5.0 g (0.02 mole) of *N*-benzoylphthalimide 1. 9. g. (0.02 mole) of aniline and 100 ml of anhydrous, benzene* was heated under reflux for two and one half hours. After standing overnight at 10°, the solid was collected by filtration (6.2 g.) and recrystallized from ethyl alcohol to give 3.7 g. (82.9% yield) of *N*-phenylphthalimide, m. p. 207—209°. Upon two more crystallizations from the same solvent, the pure product melted at 210—211° (liter. m. p. 211° (13)) and did not depress the m. p. of authentic *N*-phenylphthalimide prepared from phthalic acid and aniline (14).

Analysis:
Calculated for $C_{14}H_9NO_2$ (223.2): C 75.32%; H 4.06%; N 6.28%
Found : C 75.01%; H 4.11%; N 6.50%

The ethanolic filtrate, after evaporation to dryness, gave 1.9 g. (82.6%) of benzamide, m. p. 126—127° (water).

b) The same amounts of reactants were dissolved in 50 ml. of tetrahydrofuran and allowed to stand for five hours at 0°. The precipitate (1.7 g.; 34%) consisted of unreacted *N*-benzoylphthalimide, m. p. 166—167°. From the filtrate, after twenty four hours at 0°, 1.9 g. of *N*-phenylphthalimide, m. p. 208—210° was obtained (42.6% based on total *N*-benzoylphthalimide or 64.8% based on *N*-benzoylpthalimide which remained in the solution after five hours, upon filtration of 1.7 g. of unreacted starting material).

* If not otherwise stated, all the solvents used in this work were *anhydrous*.

Reaction of N-benzoylphthalimide with p-aminophenol

N-Benzoylphthalimide (5.0 g.; 0.02 mole) and 2.2 g. (0.02 mole) of *p*-aminophenol in 50 ml. of dioxane were refluxed for four hours. The solvent was evaporated *in vacuo* to a small volume and, after cooling, the resulting solid vas removed by filtration. Upon crystallization from ethyl alcohol (with the addition of activated charcoal) and from acetic acid, 3.9 g. (79.5%) *N*-(*p*-hydroxyphenyl)-phthalimide, m.p. 293—294° (liter. m.p. 287—288° (15) and 295° (16)) was obtained. Il did not depress the m.p. of an authentic sample prepared from phthalic anhydride and *p*-aminophenol (16).

The residue, obtained by evaporation to dryness of the ethanolic filtrate, was recrystallized from water to give 1.2 g. (50%) of benzamide, m.p 128°.

Reaction of N-benzoylphthalimide with cyclohexylamine

a) A mixture of 10.0 g. (0.04 mole) of *N*-benzoylphthalimide and 4.0 g. (0.04 mole) of cyclohexylamine in 100 ml. of benzene was heated under reflux for four hours. As soon as the reactants were mixed, a voluminous white precipitate was formed, which changed to a different crystal form upon heating, more soluble in benzene. The solvent was evaporated *(in vacuo)* to dryness and the solid residue, after drying, was powdered and treated (with stirring) for a few minutes with a cold 5% sodium hydroxide (20 ml.). The alkaline solution was weakly acidified with 20% acetic acid, cooled to 0°, and the precipitate was removed by filtration. 0.6 g. (10.2%) of phthalimide, m.p. 230—232°, was obtained.

The part insoluble in alkali, upon crystallization from ethyl alcohol, furnished 6.6 g. (72.1%) of *N*-cyclohexylphthalimide, m.p. 167—169° [liter. m.p. 167—168° (17,18)]. After two crystallizations, the pure product melted at 169—170°.

Analysis:
Calculated for $C_{14}H_{15}NO_2$ (229.3): C 73.33%; H 6.59%; N 6.11%
Found : C 73.28%; H 6.43%; N 6.40%

The first ethanolic filtrate was concentrated to half its original volume and diluted with water. Upon standing, there crystallized *N*-cyclohexylbenzamide (along with some benzamide), which was recrystallized from dilute (50%) ethyl alcohol, to give 0.4 g. (5%) of pure product, m.p. 148.5—149° (liter. m.p. 149.5° (19)).

Analysis:
Calculated for $C_{13}H_{17}NO$ (203.3): C 76.79%; H 8.43%; N 6.89%
Found : C 76.50%; H 8.40%; N 6.51%

b) *N*-Benzoylphthalimide (5.0 g.; 0.02 mole) was dissolved in 50 ml. of warm tetrahydrofuran. Upon addition of cyclohexylamine (2.0 g.; 0.02 mole), a white voluminous precipitate was

formed, which was removed by filtration after the mixture had stood for five hours at 0° (yield 1.9 h. or 27.1%), and immediately recrystallized from acetone. Although *N*-benzoyl-*N*-'-cyclohexylphalamide, m.p. 155—156°, was soluble only with difficulty in acetone, its yield decreased noticeably after each crystallization and the filtrates were found to contain *N*-cyclohexylphthalimide and benzamide. *N*-Benzoyl-*N'*-cyclohexylphthalimide decomposed rapidly in boiling benzene to give the mentioned products, and decomposition took place slowly even upon standing at room temperature.

Analysis:
Calculated for $C_{21}H_{22}N_2O_3$ (350.4): C 71.98%; H 6.33%; N 7.99%
Found : C 71.60%; H 6.28%; N 8.29%

The original tetrahydrofuran filtrate was concentrated *in vacuo* (at 10°) to 25 ml., and after standing for twenty four hours at 0°, 1.5 g. (61.9%) of benzamide, m.p. 127—128°, was obtained.

Reaction of N-benzoylphthalimide with benzylamine

N-Benzoylphthalimide (10.0 g.; 0.04 mole) and benzylamine (4.3 g.; 0.04 mole) in 100 ml. of benzene were heated for two and one half hours under reflux. After standing overnight, the mixture was filtered, and the precipitate (4.6 g.) was treated (with shaking) with cold 5% sodium hydroxide. Acidification of the alkaline filtrate with 20% acetic acid gave 0.6 g. (10.2%) of phthalimide, m.p. 233—235°, and the alkali insoluble solid consisted of benzamide (3.6 g.; 74.3%) which melted at 126—128° after crystallization from water.

The original benzene filtrate was evaporated to dryness. The residue, recrystallized from ethyl alcohol, gave 6.5 g. of *N*-benzylphthalimide, m.p. 115—116°. By concentration of the mother liquor and crystallization of the precipitate (0.45 g.), a further 0.3 g. of material, m.p. 114—116° was obtained so that the total yield of *N*-benzylphthalimide amounted to 6.8 (71.7%). The pure product, after three crystallizations from ethyl alcohol, melted at 117° (liter. m.p. 116° (20)).

Analysis:
Calculated for $C_{15}H_{11}NO_2$ (237.3): C 75.93%; H 4.67%; N 5.90%
Found : C 75.79%; H 4.96%; N 5.99%

By further evaporation of the ethanolic filtrate, 0.9 g. of a precipitate which was recrystallized from dilute ethyl alcohol to give 0.5 g. (5.9%) of *N*-benzylbenzamide, m.p. 104—105° was obtained. After crystallization from benzene, it melted at 106° (liter. m.p. 105—106° (21)).

Analysis:
Calculated for $C_{14}H_{13}NO$ (211.25): C 79.59%; H 6.21%; N 6.63%
Found : C 79.48%; H 6·10%; N 6.84%

Reaction of N-benzoylphthalimide with 2-aminoethanol

A solution of 10.0 g. (0.04 mole) of *N*-benzoylphthalimide and 2.5 g. (0.04 mole) of 2-aminoethanol in 100 ml. of tetrahydrofuran was allowed to stand for forty-eight hours at room temperature. The solvent was evaporated *in vacuo* at 25-30°, and the residue was dried in high vacuum (10^{-2} mm.) over phosphorus pentoxide. It was then powdered and treated, by stirring, with cold 5% sodium hydroxide. The alkaline solution, after filtration, was weakly acidified with 20% acetic acid and cooled in ice to give 1.8 g. (30.6%) of phthalimide, m. p. 230—233°. The alkali insoluble solid, after crystallization from 90% ethyl alcohol, furnished 4.4 g. (57.6%) of N-(2-hydroxyethyl)-phthalimide, m. p. 126 — 127.° Recrystallized from ethyl alcohol, it melted at 127—128° (liter. m. p. 127—128° (22)).

Ánalysis:
Calculated for $C_{10}H_9NO_3$ (191.2): C 62.81%; H 4.74%; N 7.33%
Found : C 62.69%; H 4.81%; N 7.33%

Reaction of N-benzoylphthalimide with n-octylamine

a) A mixture of 12.55 g. (0.05 mole) of *N*-benzoylphthalimide and 6.45 g. (0.05 mole) of n-octylamine in 100 ml. of benzene was heated under reflux for one hour. After cooling at 10°, the precipitate (5.5 g.) was removed by filtration, dried, powdered, and treated with cold 5% sodium hydroxide (25 ml.). Benzamide (1.8 g.), m. p, 122—124°, remained undissolved; after crystallization from water, it melted at 126—127°. The alkaline solution was weakly acidified with 20% acetic acid, giving 2.9 g. of phthalimide, m p. 230—234° after concentration to a low volume. A further 0.4 g. of benzamide, m. p. 120—125°, was obtained from the filtrate.

The original benzene filtrate was evaporated to dryness (*in vacuo*), and the residue (13.7 g.) was treated with 50 ml. of petroleum ether. The undissolved solid (1.5 g.) was removed by filtration and gave, after fractional crystallization from ethyl alcohol and then from water-ethyl alcohol, 0.5 g. (4%) of unreacted *N*-benzoylphthalimide, m. p. 165—168°, 0.3 g. of phthalimide, m. p. 230—231°, and 0.4 g. of benzamide, m. p. 125—126°. Therefore, the total yield of phthalimide amounted to 3.2 g. (43.5%) and of benzamide to 2.6 g. (43%).

The petroleum ether filtrate was evaporated *in vacuo* to dryness, the residue (22 g.) dried over phosphorus pentoxide (under reduced pressure), and dissolved in 300 ml. of petroleum ether. 125 ml. of this solution (corresponding to 5 g. of product) was chromatographed on aluminium oxide (the column, length 35 cm., diameter 2 cm., was filled with 125 g. of Al_2O_3 (neutral, activity 1, type 507 C, Fluka)). Elution with petroleum ether gave 2.4 g of *N*-(*n*-octyl)-phthalimide, m. p. 48.5°; elution with benzene furnished 1.0 g. of *N*-(*n*-octyl)-benzamide, m. p. 38.5°, and elution with ethyl alcohol afforded a further 1.2 g. of crude *N*-(*n*-octyl)-benzamide, m. p. 35—40°. Therefore, the total yield (in 12 g. of residue)

of *N*-octylphthalimide was 5.8 g. (44.7%); and *N*-octylbenzamide, 5.3 g. (45.4%).

For analysis, *N*-(*n*-octyl)-phthalimide was recrystallized from petroleum ether, without a change of melting point (48.5°) (liter. m. p. 48—49° (23), 50—51° (24)).

Analysis:
Calculated for $C_{16}H_{21}NO_2$ (259.3): C 74.10%; H 8.16% N 5.40%
Found : C 74.13%; H 8.08%; N 5.66%

N-(*n*-Octyl)-benzamide was also recrystallized from petroleum ether and melted at 38.5°.

Analysis:
Calculated for $C_{15}H_{23}NO$ (233.3): C 77.20%; H 9.94% N 6.00%
Found : C 77.03%; H 10.09%; N 5.98%

b) When the reaction was repeated at room temperature (after twenty-four hours of standing), the same products were obtained in similar yields.

c) *N*-Benzoylphthalimide (5.0 g.; 0.02 mole) was dissolved in 65 ml. of warm tetrahydrofuran, and 2.6 g. (0.02 mole) of *n*-octylamine was added to this solution. The resulting solution was immediately cooled to 0°, and was evaporated to dryness after four hours at this temperature. The residue was treated with benzene (25 ml.), and the mixture worked up as described in (*a*). Phthalimide and benzamide were obtained in yields of 40.2% and 42.5%, respectively.

Reaction of N-benzoylphthalimide with n-butylamine

A mixture of 12.55 g. (0.05 mole) of *N*-benzoylphthalimide and 3.65 g. (0.05 mole) of *n*-butylamine in 100 ml. of benzene was heated under reflux for three hours. After standing for five hours at room temperature, the mixture was filtered and the precipitate and filtrate worked up as described for *n*-octylamine in (*a*). 3.2 g. (43.5%) of phthalimide, 2.6 g. (42.9%) of benzamide, and 0.3 g. (2.4%) of unchanged *N*-benzoylphthalimide were obtained.

After evaporation of petroleum ether, the dried residue (7.5 g.) was dissolved in 300 ml. of ligroin (b. p. 60—80°) -benzene (2:1 v/v), and the solution was chromatographed on aluminium oxide (the quality and amount of Al_2O_3 as well as the dimensions of the column were identical as described for *n*-octylamine (*a*.)) Elution with ligroin-benzene (2:1) gave 3.8 g. (36.5%) of *N*-(*n*-butyl)-phthalimide, m. p. 33.5-34.5° (from petroleum ether) (liter. m. p. 36.5° (25)).

Analysis:
Calculated for $C_{12}H_{13}NO_2$ (203.2): C 70.91%; H 6.45%; N 6.89%
Found : C 70.93%; H 6.44%; N 7.18%

Further elution with benzene and then with diethyl ether gave 1.8 g. of *N*-(*n*-butyl)-benzamide, and ethyl alcohol eluted

another 1.4 g. of the same product (total yield, 36.2%). *N*-Butylbenzamide did not solidify, even after prolonged standing (contradictory reports are to be found in the literature about this compound; according to same authors (26), the product melts at 68—70°, although we believe that the structure of "*N*-butylbenzamide" prepared by these authors is doubtful; accordind to other authors (27), *N*-butylbenzamide is an oil). Its constitution was confirmed by hydrolysis with 30% sulfuric acid and identification of benzoic acid, m. p. 121° (12), and of *n*-butylamine (through its picrate, m. p. 151° (12)).

Reaction of N-benzoylphthalimide with N-methylaniline

a) A mixture of 5.0 g. (0.02 mole) of *N*-benzoylphthalimide, 4.3 g. (0.04 mole) of *N*-methylaniline, and 30 ml. of benzene was heated under reflux for twenty hours. After standing overnight at room temperature, the precipitate was removed by filtration and recrystallized from water, affording 1.39 g. of phthalimide, m.p. 229 — 231°.

The benzene filtrate was evaporated to dryness and the residue was extracted with ether. The insoluble part was recrystallized from water, and gave a further 0.44 g. of phthalimide, m. p. 228—232°. The total yield of phthalimide was 1.84 g., i. e., 62.6%. The ether extract was washed with 10% hydrochloric acid (to remove unreacted *N*-methylaniline), dried over anh. K_2CO_3, and evaporated. The oily residue was subjected to distillation, and gave 2.0 g. (47.6%) of *N*-methylbenzanilide, b. p. 198—203°/16 mm. (liter. b. p. 331—332°/760 mm. (28); 195°/12 mm. (29)). It solidified after standing in petroleum ether, m. p. 58—59 (liter. m. p. 58° (30); 59° (29); 63° (31)).

Analysis:
Calculated for $C_{14}H_{13}NO$ (211.25): N 6.63%
Found : N 6.58%

b) When the reactants were heated for only eight hours, unchanged *N*-benzoylphthalimide was isolated in a 73.7% yield (3.7 g.).

Reaction of N-benzoylphthalimide with diethylamine

a) A mixture of 5.0 g. (0.02 mole) of *N*-benzoylphthalimide, 1.5 g. (0.02 mole) of diethylamine, and 25 ml. of benzene was refluxed for two hours. After cooling at 20°, the precipitated phthalimide, m. p. 229—232°, was removed by filtration (yield 2.7 g., i. e. 91.8%). The benzene filtrate was evaporated and the residue subjected to distillation. We obtained 1.9 g. (56.5%) of *N*, *N*-diethylbenzamide, b. p. 165—168°/25 mm. (liter. b. p. 280°/760 mm. (32); 173—175°/35 mm. (33)).

Analysis:
Calculated for $C_{11}H_{15}NO$ (177.2): N 8.47%
Found : N 8.28%

b) When the same amounts of reactants were dissolved in 60 ml. of tetrahydrofuran, and the solution was allowed to stand for five hours at 0°, we obtained a white precipitate (1.0 g.; 15.4%) of an undefined melting point which was directly analyzed.

Analysis:
Calculated for $C_{19}H_{20}N_2O_3$ (324.4): C 70.34%; H 6.22%; N 8.64%
Found : C 69.88%; H 5.95%; N 8.48%

Upon prolonged standing at room temperature, or upon attempted crystallization from benzene or acetone, *N*-benzoyl-*N'*, *N'*--diethylphthalamide decomposed, and yielded phthalimide, m. p. 231 — 233°.

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N-BENZOYLPHTHALIMIDE
III.*
REACTION WITH HYDRAZINES

by

JOVAN BOŠNJAK, RASTKO I. MAMUZIĆ and MIHAILO LJ. MIHAILOVIĆ

As shown in our previous papers (1,2), the reaction between *N*-benzoylphthalimide and alcohols or amines probably consists of an initial attack by a nucleophilic agent on an imide ring carbonyl group of *N*-benzoylphthalimide, which is followed by the decomposition of the so formed intermediate products.

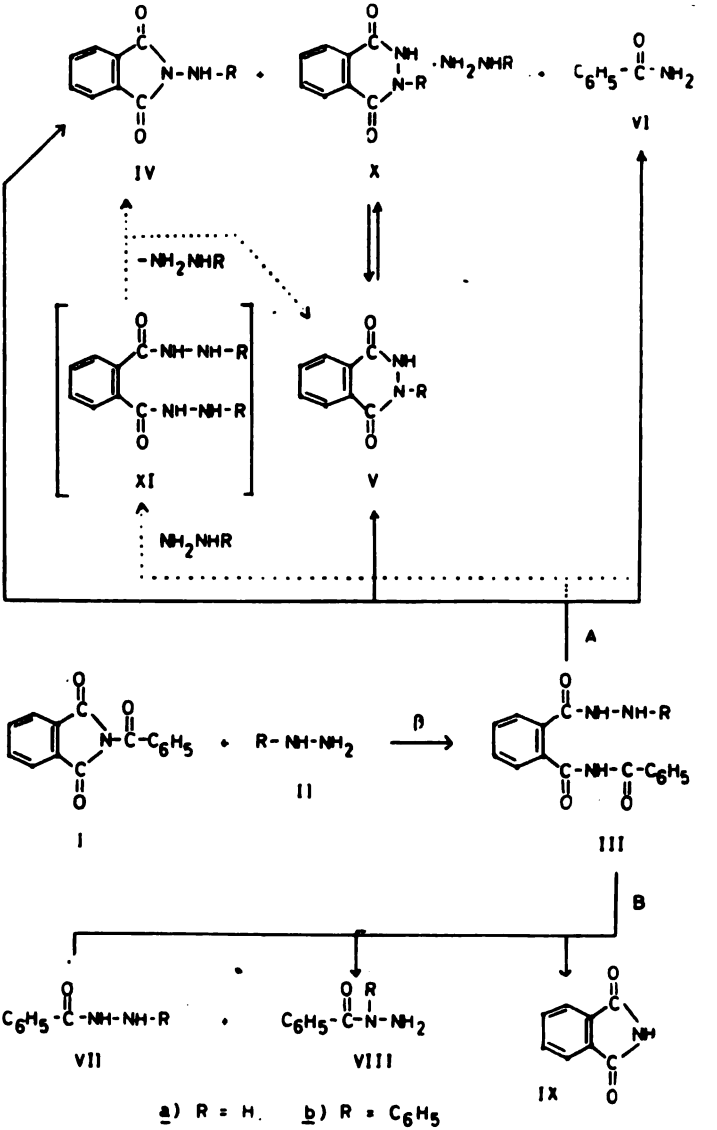
Similarly to amines (2), hydrazines (II) can also react with *N*-benzoylphthalimide in two ways, to give either *N*-aminophthalimides (IV), the isomeric 2,3-dihydro-1,4-phthalazinediones (V) and benzamide (VI) (reaction A); oı hydrazides of benzoic acid (VII and VIII) and phthalimide (IX) (reaction B) (Scheme 1).

Two mechanisms can be envisaged for reactions A and B: α) independent attacks of the nucleophilic hydrazine either on the carbonyl carbon of the imide ring (reaction A) or on the amide carbonyl carbon of the benzoyl rest (reaction B) of *N*-b nzoylphthalimide (I); and β) the formation for both reactions of a common intermediate — *N*-benzoylphthalamic acid hydrazide (III), which would arise by initial attack of the hydrazine (II) on the imide ring carbonyl group of *N*-benzoyl-phthalimide, and which could then decompose according to A or B (Scheme 1).

It is possible that both mechanisms (α and β) are involved in the reactions A and B, their extent depending on the nature of the reacting hydrazine. However, as products similar to III were isolated in the reaction between amines and *N*-benzoylphthalimide (I) (2), as well as between amines and phthalimide or *N*-substituted phthalimides (3), and because of similar results reported in the reaction of hydrazine (II*a*) with phthalimide (4) or *N*-alkylphthalimides (5), and phenylhydrazine (II*b*) with phthalimide (6,7) or *N*-substituted phthalimides (6, 8, 9), it seems more probable that reactions A and B mainly occur through *N*-benzoylphthalamic acids hydrazides (III) (Scheme 1).

* Paper II: Glasnik hem. društva Beograd (Bull. soc. chim. Beograd), 27, 303 (1962).

Hydrazine hydrate (II*a*) and *N*-benzoylphthalimide (I) in boiling ethyl alcohol gave 2,3-dihydro-1,4-phthalazinedione (V*a*) and benzamide (VI) in yields of 80% and 43% respectively; and phthalimide (IX), which is formed according to B, was obtained



Scheme 1

in low yield (10%). *N*-Aminophthalimide (IV*a*) was not isolated (reaction A) but this was to be expected because it is known that IV*a* is unstable and readily converts to the six-membered hydrazide (V*a*) (4).

N-Benzoylphthalimide (I) and phenylhydrazine (II*b*) reacted (in boiling ethyl alcohol) mainly according to A, and furnished *N*-anilinophthalimide (IV*b*) in 58% yield, accompanied by a small amonut (5%) of the isomeric 2-phenyl-2,3-dihydro-1,4-phthalazinedione (V*b*); but reaction B occurred only to a negligible extent, giving 3% of *N*-benzoyl-*N'*-phenylhydrazine (VII*b*) and 2% of the isomeric *N*-benzoyl-*N*-phenylhydrazine (VIII*b*).

When hydrazine hydrate (II*a*) and *N*-benzoylphthalimide (I) were allowed to react in ethyl alcohol, or in chloroform, a white voluminous precipitate was immediately formed, even at 0°, whose composition corresponded either to the hydrazine salt of 2,3-dihydro-1,4-phthalazinedione (X*a*) or to the dihydrazide of phthalic acid (XI*a*), and which, upon heating in dilute ethyl alcohol or water (or faster in the presence of acids), decomposed to give 2,3-dihydro-1,4-phthalazinedione (V*a*). Barber and Wragg (10) consider this compound to be a salt (as they consider the primary product from the reaction of *N*-benzylphthalimide and hydrazine hydrate to be the benzyl amine salt of the six-membered hydrazide (5)), although the possibility that this product could be the intermediate dihydrazide of phthalic acid (XI*a*) is not to be ignored.* In the case of phenylhydrazine (II*b*) the salt X*b* was not isolated, but that was to be expected because phenylhydrazine is a weak base.**

As the benzoyl amide carbonyl carbon of *N*-benzoylphthalimide (I) is sterically more hindered, and probably somewhat less electrophilic than the imide-ring carbonyl carbon atoms of I, it was expected that phenylhydrazine (II*b*), being a weak base ($pK_b = 8.8$ (12)) with considerable steric requirement, would more easily attack the imide-ring carbonyl group to give products according to A. Hydrazine (II*a*), on the other hand, is a stronger base ($pK_b = 5.5$ (12)) with a negligible steric effect, and should thus

* To the compounds obtained from phthalimide and hydrazine hydrate (4), phthalimide and phenylhydrazine (6,7), *N*-anilinophthalimide (IV*b*) and phenylhydrazine (II*b*) (6, 8, 9), the structures corresponding to derivatives of phthalic acid (of type XI) were assigned. Since these compounds are readily decomposed and give *various products* (not only products of type V), e. g., phthalic acid bis-*N*-phenylhydrazide (XI*b*) affording IV*b*, V*b* and phenylhydrazine (6, 8, 9, 11), and since phenylhydrazine, is a weak base and, cannot form salts of type X*b*, it is possible that in reaction A (Scheme 1) the primary formed hydrazides III are first converted to phthalic acid dihydrazides (XI), which would further decompose into five-membered (IV) and or six-membered derivatives (V). These compounds (V) can then eventually give salts of type X.

** If reaction A proceeds throguh the dihydrazide XI*b*, then the absence of this compound (at low temperatures) could be explained by the fact that the primary intermediate, i. e., *N'*-benzoylphthalamic acid *N*-phenylhydrazide (III*b*), should be formed with more difficulty than the unsubstituted *N'*-benzoylphthalamic acid hydrazide (III*a*) (because of steric and polar effects). Therefore, a higher temperature is required for the formation of III*b*, at which temperature, however, both III*b* and the dihydrazide XI*b* should undergo immediate decomposition. (As the *des*-benzoyl derivative of III*b*, i. e., phthalamic acid *N*-phenylhydrazide, decomposes readily in hot ethyl alcohol to give IV*b* and V*b* (6, 7), the hydrazide III*b* should be still more unstable. Similarly, phthalic acid bis-*N*-phenylhydrazide (XI*b*) is also converted upon heating (alone or in solvents) to IV*b* and V*b* (6, 8, 9, 11).)

react to a large extent (as compared to phenylhydrazine) according to B, i. e., attack of the banzoyl amide carbonyl carbon of *N*-benzoylphthalimide should be facilitated. However, as described above, with hydrazine the yield of reaction B was also low (10%), although it could be *a priori* expected that hydrazine hydrate would behave as 2-aminoethanol, both compounds having similar basic properties (12). As shown in our previous paper (2), 2-aminioethanol reacted with N-benzoylphthalimide according to B in 31% yield. An inspection of scale models affords an explanation of this difference in the behavior of the two bases. While in *N*-benzoylphthalamic acid hydrazide (III*a*)* the steric conditions are very favorable for closure of a six-membered ring (to give product V*a*), in the analogous phthalamide, formed as an intermediate from *N*-benzoylphthalimide and 2-aminoethanol (2), cyclization to the five-membered phthalimide ring is not favored to such an extent, so that the competing reaction B, i. e., attack of the nitrogen on the benzoyl amide carbonyl carbon, will occur in a somewhat higher yield.

In contrast to the favorable steric conditions and polar factors (greater nucleophilic properties of the nitrogen of the free amino group than those of the hydrazide nitrogen attached to the carbonyl group in III*a*) in *N*-benzoylphthalamic acid hydrazide (III*a*) for the cyclization to the six-membered hydrazide V*a*, the intermediate *N*-benzoylphthalamic acid *N*-phenylhydrazide (III*b*), because of the considerable steric effect of the phenyl group in the hydrazide rest, should undergo preferential ring closure to the five-membered derivative. This assumption was confirmed by the yields of the reaction products IV*b* and V*b*. (It appears that polar factors are of minor importance because on ground of electronic properties six-membered ring formation should be favored). Other reactions, e. g. the action of phenylhydrazine on phthalic anhydride, have also been reported to furnish the five-membered *N*-anilinophthalimide (IV*b*) in a higher yield than the corresponding isomeric six-membered 2-phenyl-2,3-dihydro-1,4-phthalazinedione (V*b*) (6, 7, 13).

Naturally, besides steric and polar factors, the stability of the reaction products will also influence their yield. Thus, as already mentioned, *N*-aminophthalimide (IV*a*) is easily converted to 2,3-dihydro-1,4-phthalazinedione (V*a*) (4), but the same transformation of the *N*-phenyl derivative (IV*b* → V*b*) requires much more drastic conditions (13, 14).

EXPERIMENTAL PART

All melting points are uncorrected.

N-Benzoylphthalimide, prepared from phthalimide and benzoyl chloride in anhydrous pyridine (15), melted at 168.° after crystallization from ethyl alcohol.

* If reaction A proceeds through the dihydrazide XI, then this discussion on cyclization conditions for III is also valid for the dihydrazide XI.

Reaction of N-benzoylphthalimide with hydrazine hydrate.

a) A mixture of 12.55 g. (0.05 mole) of N-benzoylphthalimide and 2. 7 g. (0.052 mole) of 98% hydrazine hydrate in 100 ml. of 96% ethyl alcohol was heated under reflux for four hours. After standing for six hours at 0°, the precipitate (7.7 g.) was collected by filtration and recrystallized from water to give 6.5 g. (80.2%) of 2,3-dihydro-1,4-phthalazinedione, m. p. 343-346° (liter. m. p. 341-344° (4), 342-346° (5)).

Analysis:
Calculated for $C_8H_6N_2O_2$ (162.1): C 59.26%; H 3.73%; N 17.28%.
Found : C 59.50%; H 4.01%; N 17.14%.

The mother liquor from the original filtration was concentrated and cooled, and the resulting phthalimide was recrystallized from dilute ethyl alcohol to give 0.7 g (9,5%) of pure product, m. p. and mixed m. p. 232-233° (16). The original ethanol filtrate was evaporated to dryness, and the residue was recrystallized from water. We obtained 2.6 g. (43%) of benzamide, m. p. 125-127°, which did not depress the melting point of an authentic sample of benzamide, m. p. 128° (16).

b) We added 0.50 g. (0.010 mole) of hydrazine hydrate (98%) in 5 ml. of ethyl alcohol to a stirred, ice cold solution of 1.26 g. (0.005 mole) of *N*-benzoylphthalimide in 20 ml. of chloroform. A white, voluminous precipitate was immediately formed. The mixture was stirred for twenty minutes at 0°, the precipitate collected by filtration and washed thoroughly with chloroform and ethyl alcohol. We obtained 1.5 g. (77.3%) of the hydrazine salt of 2,3-dihydro-1,4-phthalazinedione, which was dried *in vacuo* over phosphorus pentoxide for three days.

Analysis:
Calculated for $C_8H_{10}N_4O_2$ (194.2): C 49.48%; H 5.19%; N 28.85%.
Found : C 49.90%; H 5.19%; N 28.55%.

The hydrazine content was determined by the iodate method of Kolthoff (17), after decomposing the salt with hydrochloric acid (10).

Analysis:
Calculated for $C_8H_{10}N_4O_2$ (194.2): NH_2NH_2 16.45%.
Found : NH_2NH_2 16.80%.

The hydrazine salt of 2,3-dihydro-1,4-phthalazinedione reduced the Nessler reagent in the cold and melted above 300° (10), decomposing to 2,3-dihydro-1,4-phthalazinedione and hydrazine. The same dissociation occurred by repeated crystalization from boiling water or dilute ethyl alcohol and faster in the presence of acids.

Reaction of N-benzoylphthalimide with phenylhydrazine.

A mixture of 12.55 g. (0.05 mole) of *N*-benzoylphthalimide and 5.4 g. (0.05 mole) of phenylhydrazine in 100 ml. of 96%

ethyl alcohol was heated under reflux for four hours. Upon standing for ten hours at 0°, the solid which separated (10.3 g.) was removed by filtration and treated with 40 ml. of 10% sodium carbonate. After shaking vigorously, the suspension was filtered, and the precipitate washed with water until neutral. The carbonate insoluble yellow *N*-anilinophthalimide, m. p. 178-181°, was obtained in a 58.4% yield (7.0 g.). Upon crystallization from water, the pure product melted at 181-182° (liter. m. p. 181-182° (18)).

Analysis:

Calculated for $C_{14}H_{10}N_2O_2$ (238.2): C 70.58%; H 4.23%; N 11.76%.
Found : C 70.52%; H 4.24%; N 11.85%.

The sodium carbonate solution was weakly acidified with 20% acetic acid and cooled to 0°. After standing for twelve hours, the solid was collected by filtration and recrystallized from water to give 0.6 g. (5.3%) of the isomeric 2-phenyl-2,3-dihydro-1,4-phthalazinedione, m. p. 210-211° (liter. m. p. 211-212° (14)).

Analysis:

Calculated for $C_{14}H_{10}N_2O_2$ (238.2): C 70.58%; H 4.23%; N 11.76%.
Found : C 70.30%; H 4.12%; N 11.72%.

The original ethanolic filtrate from the mixture from which the isomeric phenylhydrazides had been removed was concentrated to a low volume, and the resulting solid (1.0 g.), m. p. 130-153°, was removed by filtration. Crystallization from dilute (50) ethyl alcohol afforded 0.3 g. (2.8%) of *N*-benzoyl-*N'*-phenylhydrazine, m. p. 165-166°. Upon crystallization from water, the pure product melted at 167-168° (liter. m. p. 168°).

Analysis:

Calculated for $C_{13}H_{12}N_2O$ (212.2): C 73.57%; H 5.70%; N 13.20%.
Found : C 73.28%; H 5.62%; N 13.00%.

The original ethanolic filtrate, from which *N*-benzoyl-*N'*-phenyl hydrazine had been removed, was evaporeted to dryness. The residue was dried *in vacuo* and extracted with anhydrous ether. The solution was saturated with carbon dioxide, filtered if necessary, and evaporated to dryness. The residue was treated with dilute sulfuric acid ($H_2O - H_2SO_4$ = 2:1 v/v) (with stirring), and the resulting solid was removed by filtration, washed with ethyl alcohol and ether, and recrystallized from water-ethyl alcohol. We obtained 0.3 g. (1.9%) of *N*-benzoyl-*N*-phenylhydrazine sulfate, m. p. 190° (liter. m. p. 191° (20)).

Analysis:

Calculted for $C_{13}H_{12}N_2O \cdot H_2SO_4$ (310.3): N 9.03%.
Found : N 8.90%.

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THE BEHAVIOR OF ASCORBIC ACID IN SOLUTION. II

by

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Our previous investigations (1, 2, 3, 4) have shown that *pH* and aging of the solutions are the factors which are responsible for the transformation of ascorbic acid* in solution.

However, on the basis of these investigations we could not acquire a deeper knowledge of either the routes of the transformation under given conditions or the nature of the transformation products.

Therefore, we attempted to apply the chromatographic analysis with the hope that it will help us to arrive at the desired information.

EXPERIMENTAL

These investigations were performed with 2% aqueous ascorbic acid solutions buffered at *pH* 3.9 and 6.9, respectively. The ascorbic acid used was produced by "Carlo Erba", p. a., m. p. 192°, $[\alpha]^{20} + 20.5°$.

The buffer system, prepared according to Britton and Robinson, consisted of phosphoric, acetic, and boric acid. It was very amenable to these investigations because of the presence of phosphate and acetate ions, which prevent the oxidation of ascorbic acid on the chromatograms.

The method of circular paper chromatography according to Ulmann's (5) procedure was employed. Ulmann's procedure was modified so that all operations, until the "detection" of the chromatograms, were performed in the absence of light. A second modification involved the "detection", which was performed with silver nitrate in the atmosphere of ammonia, and also with 2,4-dinitrophenylhydrazine, 2,3,5-triphenyltetrazolium chloride, and 2,6-dichlorophenolindophenol. In this way we were able to separate the compounds which contain a carbonyl group from those which contain an enediol group. The reaction with silver nitrate made it possible for us to distinguish the ascorbic acid from the dehydroascorbic acid.

*In further text, the transformation of ascorbic acid will be referred to as ascorbic acid.

In the course of these investigations, attention was given to some factors which could affect the obtained results. Such a factor is the light which catalyzes the autooxidation of ascorbic acid. Therefore, all operations were performed in the absence of daylight. The detection was performed by spraying the paper with the appropriate reagent, and drying it in a vacuum oven at 65—70° in order to accelerate the appearance of colored zones.

We observed that the temperature greatly influences the rate of the movement of components on the paper. Therefore, all experiments were performed at the temperature interval 22—25°.

Water used for the preparation of solutions was distilled three times from a "Pyrex" apparatus in order to avoid the presence of metal ions, particularly those of iron and copper which catalyze the oxidation of ascorbic acid by atmospheric oxygen.

Paper Whatman No. 1, diameter 16 cm, was used; we found that the best separations of compounds were achieved when the paper diameter was not greater than 20 cm.

The results of the chromatographic analysis of aqueous ascorbic acid solutions buffered at *pH* 3.8, and 6.9 respectively, and performed in the course of 120 hours are given in Table 1, 2 and 3.

TABLE 1

R_f— values of compounds found in the aqueous ascorbic acid solution
Solvent: butanol: acetic acid: water (4:1:5)

Reagent	The solution was left to stand for				
	1 h	24 h	48 h	96 h	120 h
DNPH	0.52	0.46 0.52	0.46 0.52	0.47 0.52	0.46 0.52
TTZ	0.47	0.46	0.46	0.47	0.47
TR	0.46	0.47	0.47	0.46	0.47
$AgNO_3$	0.47	0.46	0.47	0.47	0.46

DNPH — 2,4-dinitrophenylhydrazine
TTZ — 2,3,5-priphenyltetrazolium chloride
TR — Tillmans reagent (2,6-dichlorophenolindophenol)

TABLE 2

R_f — values of compounds found in buffered aqueous ascorbic acid solution, $_pH$ 3.8
Solvent: butanol: acetic acid: water (4:1:5)

Reagent	After standing of the solution for				
	1 h	24 h	48 h	96 h	120 h
DNPH	0.52 0.46 0.22	0.52 0.46 0.22	0.52 0.46 0.22	0.52 0.47 0.23	0.52 0.46 0.22
TTZ	0.47	0.47	0.46	0.47	0.46
TR	0.47	0.46	0.47	0.47	0.46
$AgNO_3$	0.47	0.46	0.47	0.47	0.57

TABLE 3

R_f — values of compounds found in buffered aqueous ascorbic acid solution *pH* 6.9
Solvent: butanol: acetic acid: water (4:1:5)

Reagent	After standing of the solution for				
	1 h	24 h	48 h	96 h	120 h
DNPH	0.52 0.46 0.22	0.52 0.46 0.21	0.52 0.46 0.22	0.52 0.46 0.22	0.52 0.46 0.22
TTZ	0.47	0.46	0.47	0.46	0.46
TR	0.47	0.47	0.47	0.47	0.47
$AgNO_3$	0.47	0.46	0.46	0.47	0.47

DISCUSSION

The chromatographic analysis of a freshly prepared aqueous ascorbic acid solution has shown that this solution contains ascorbic acid in its enediol form. In our experiments the R_f-value was 0.46—0.47, except for those chromatograms which were treated with 2,4-dinitrophenylhydrazine, where the R_f-value was 0.52, representing the R_f-value of dehydroascorbic acid phenylhydrazone. (The appearance of the colored zone of ascorbic acid phenylhydrazone is due to the fact that the "detection" was performed at 65—70°, so that ascorbic acid reacted with 2,4-dinitrophenylhydrazine giving the hydrazone, identical with dehydroascorbic acid hydrazone).

When chromatographing the aqueous ascorbic acid solution which was left to stand for twenty-four hours, the chromatograms which were sprayed with 2,4-dinitrophenylhydrazine gave rise to the appearance of a colored zone of a compound, formed by the transformation of ascorbic acid; its R_f-value was similar to that of ascorbic acid phenylhydrazone. Since the presence of the latter compound was not detected on chromatograms sprayed with either 2,3,5-triphenyltetrazolium chloride or 2,6-dichlorophenolindophenol, specific reagents for the enediol group, it may be concluded that the detected zone can be ascribed to a transformation product which contains no enediol group, but rather a carbonyl group instead.

The chromatograms of a freshly prepared solution buffered at *pH* 3.9, which were sprayed with 2,4-dinitrophenylhydrazine, in addition to ascorbic acid and its transformation product detected after standing of the solution for twenty-four hours, showed the presence of a new compound. This compound was not detected on other chromatograms of the same solution, indicating that it contains no enediol group. The amount of this compound is increased on further standing of the solution.

The chromatograms of a freshly prepared solution buffered at 6.9 were identical with those obtained with the solution of *pH* 3.8; the only difference is the rate of ascorbic acid transformation,

which is more rapid at *pH* 6.9. than at *pH* 3.8. On aging of the solution buffered at *pH* 6.9, the transformation is more intense than in the solution which was *pH* 3.8.

The formation of compounds which contain no enediol group indicates that the enediol group of ascorbic acid undergoes a transformation, but not in the direction of a dehydroascorbic acid formation, but in obtainance of other compounds which do not contain an enediol group.

The results of the chromatographic analysis are in full agreement with our previous findings, obtained in the titration of ascorbic acid solutions with Tillmans reagent and potassium permanganate. In our later works we have established that the consumption of the above reagents is decreased in the course of one hour both with buffered solutions and on standing of the aqueous solution. This phenomenon is due to the transformation of ascorbic acid and the formation of new compounds which do not contain an enediol group.

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A CONTRIBUTION TO THE INVESTIGATION OF ($\alpha+\beta_1$) THE BRASS HARDENING PHENOMENON

by

BRANKO I. BOŽIĆ and NADA P. VIDOJEVIĆ

The β-phase of the copper-zinc system which is stable at higher temperatures shows in a definite interval of composition — ($\alpha+\beta_1$) brass region — the decrease of copper solubility with increasing temperature, due to which a partial $\beta \rightarrow \alpha$ transformation occurs; the latter is followed by the separation of the α-phase, which is rich in copper. At a temperature of 453° C, the β-phase appears to be settled, whereby the atoms of copper and zinc take fixed positions in the β-lattice and form a well arranged β′-solid solution.

Theoretically, both phenomena, the separation from the solid solution and the arrangement of the disodered solid solution, can cause the increased hardness of the alloy.

In cases of brass, the $\beta \rightarrow \beta_1$, reaction cannot be supercooled. However, the rapid cooling of the β-phase secures the formation of a solid solution supersaturated with copper. Therefore, it was of interest to study the effect of hardening by precipitating the α-phase in dependence up on the percentage of zinc in the examined alloy.

MATERIALS AND METHODS

The investigations were performed with two following brass samples:

Alloy	Cu	Zn	Pb	Fe
Cu 58 Zn	57.5	42.5	—	—
Cu 60 Zn	60.04	39.95	0.005	traces

The dimensions of the samples were: ⌀ 11 mm, h = 6 mm.

The separation of the α-phase from the β_1, solid solution was controlled under microscope and by measuring the hardness and the electroconductivity.

The sample of the Cu 58 Zn alloy was corroded with an aqueous ammoniacal solution of copper ammonium chloride (10 g copper ammonium chloride in 120 ml water), and the characteristic structures were magnified 200 times and photographed. The samples of Cu 60 Zn brass were corroded with an ammoniacal 10% ammonium persulfate solution, and the selected structures were magnified 500 times and photographed.

The hardness of the samples was determined by the method of Vickers (30 kg), whereby six prints were taken.

The electric conductivity was measured with the "Sigmatest" apparatus; the measurements were repeated three times.

The thermal treatment of the samples involved two operations: a) the thermal dissolution and the hardening of the samples in order to retain the β_1-solid solution, and b) the thermal precipitation, or the aging of hardened samples.

The thermal dissolution of Cu 58 Zn brass was performed at $700 \pm 20°$ (air) in the course of half an hour, and Cu 60 Zn at $870 \pm 20°$ for the same period of time. The hardening in water succeeded in preventing the separation of the α- phase in the case of Cu 58 Zn brass, furnishing the homogeneous β_1-structure. However, a much more drastic cooling of Cu 60 Zn alloy in 10% aqueous sodium chloride solution could not hinder the appearance of a minimal amount of α-needles at the boundaries of the grain.

The thermal precipitation of hardened samples was performed over the temperature range from 200 to $575 \pm 10°$ C during 1 to 106 hours.

Table 1 shows the values of hardness and electroconductivity of both samples under various conditions.

TABLE 1

Alloy	Condition	HV kg/mm²	m/Ohm mm²
Cu 58 Zn	Initial state	146	15.9
	Heated at 700° for half an hour and cooled	80	16.0
	Hardened in water after being heated at 700° for half an hour	119	16.4
Cu 60 Zn	Initial state	82	15.1
	Heated at 870° for half an hour and cooled	62	15.0
	Hardened in aqueous 10% sodium chloride solution after being heated at 870° for half an hour	122	16.3

In both cases the increase of hardness was achieved by hindering the separation of the soft α-phase; the electrical conductivity is also simultaneously increased.

RESULTS

a) Thermal Precipitation of Cu 58 Zn

The thermal precipitation of hardened samples of Cu 58 Zn was performed at the following temperatures: 200, 250, 300, 380, 420, 470, 520, and 575° C. The duration period at higher temperatures amounted to three hours, and at the temperatures of 200 and 250° C it lasted up to 106 hours.

Structure of Cu 58 Zn alloy thermally hardened at 250 °C

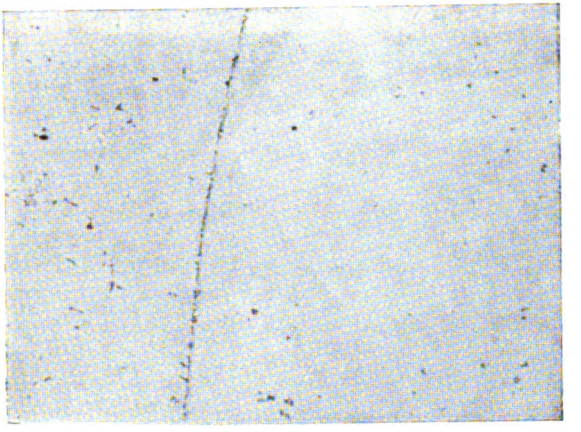

Figure 1 — 3h x 200

Beginning of the separation of α-phase plates

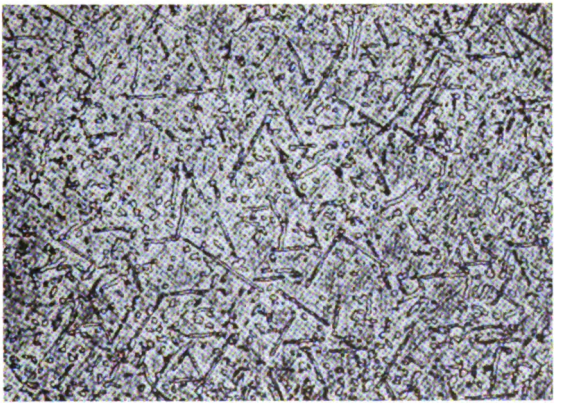

Figure 2 — 12h x 200

Further separation of α-phase plates

Etched with an aqueous ammoniacal solution of cooper ammonium chloride

Microphotographs 1 — 8 show that the transformation starts with the appearance of α-plates, the dimensions of which increase with increasing precipitation temperature. Very rough plates of the α-phase appeared at a temperature of 575° (5—8). The incubation period for the separation of the α-phase is prolonged

with the decreasing temperature. The amount of separated plates increases with the duration of heating, and the initially formed plates thicken. The structure of samples treated at 250° indicated a pronounced moderation of the α-phase separation (fig. 3 and 4) (2).

It has been observed, as a common feature for all the temperatures, that the separation of the α-phase is followed by the immediate formation of relatively rough plates.

Structure of Cu 58 Zn alloy thermally hardened at 250 °C

Figure 3 — 18h x 200
Marked aligned separation of α-phase

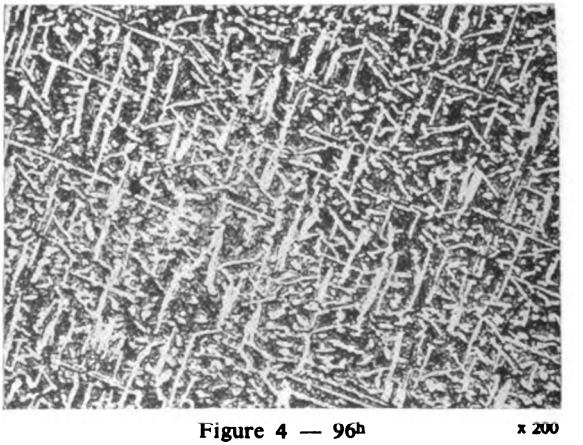

Figure 4 — 96h x 200
White, α-phase; black, β_1-phase
Etched as Fig. 2

The minimal increase of hardness, observed at the early stage of the α-phase separation at lower temperatures, indicated the weak effect of thermal precipitation. It might be said that the change of electrical conductivity shows no regularity.

b) Thermal Precipitation of Cu 60 Zn

The thermal precipitation of hardened samples of Cu 60 Zn was performed at the following temperatures: 200, 250, 300, 350, 400, and 500° C. The duration period amounted from five seconds to eighty-eight hours.

The study under the microscope showed that the thermal precipitation of samples of Cu 60 Zn brass was associated with

Structure of Cu 58 Zn alloy thermally hardened at 575 °C

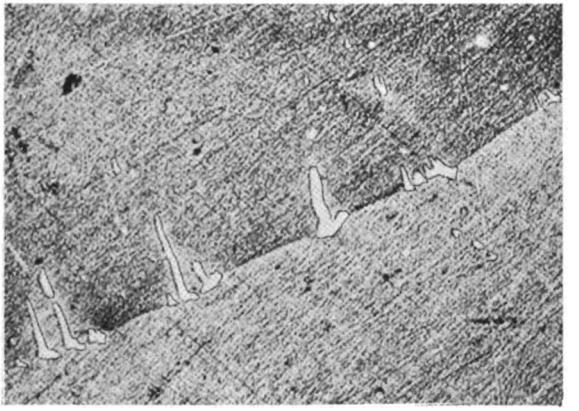

Figure 5 — 30^s x 200

Occurrence of α-phase at β_1 grain boundaries

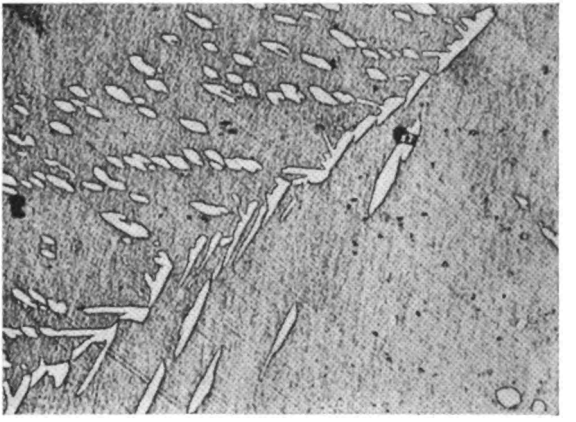

Figure 6 — 40^s x 200

Further separation of α-plates
Etched as Fig. 2

the separation of α-phase in the form of very thin plates. The lower the temperature of the treatment, the finer were the plates. The number of separated plates increased rapidly with time, so

that they embodied the β_1-basis. With a prolonged time of treatment at higher temperatures, the platey structure of the α-phase disappeared and islands of β_1-phase in the α-phasis were clearly observed. Microphotographs 9—14 show the characteristic structures of this brass obtained by thermal precipitation at 250 and 350° C.

The dependence of the hardness and the electrical conductivity upon the conditions of the precipitation, i. e., the temperature and

Structure of Cu 58 Zn alloy thermally hardened at 575 °C

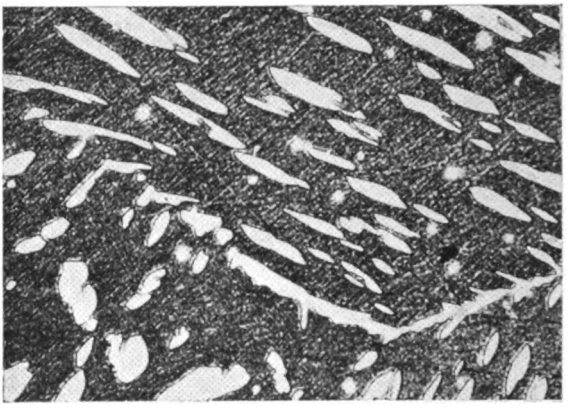

Figure 7 — 4 min. x 200

Growth and further separation of α-plates

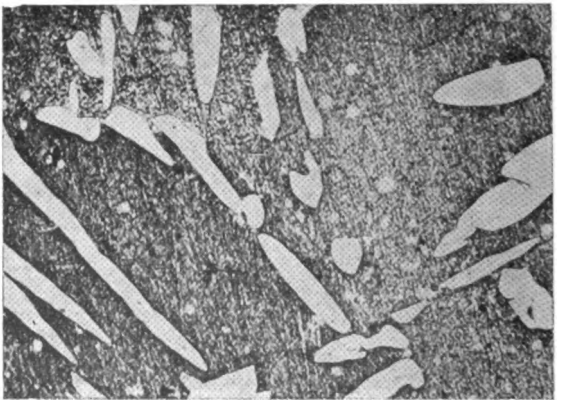

Figure 8 — 24 min. x 200

Coagulation of α-phase plates
Etched as Fig. 2.

the duration of treatment, are depicted in Diagrams 1 and 2. When connecting the change of the hardness with the transformation of the structure, we can state that the increase of hardness appears

at the moment when the platey α-precipitate was clearly observed in the basis of β_1-grain. Further separation of α-plates was followed by the increase of hardness until the moment when a definite degree of separation and dispersion of the α-phase was achieved; the hardness decreases further on. With increasing temperatures, the maximum of hardness is shifted towards shorter periods of treatment, and the value of maximal hardness decreases. At 350

Structure of Cu 60 Zn alloy thermally hardened at 250 °C

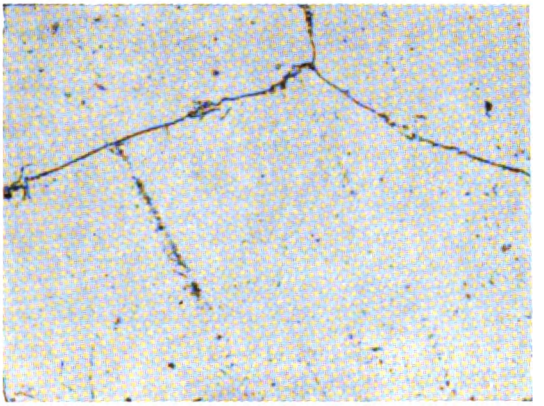

Figure 9 — 1 min. x 500

Beginning of the separation of α-phase at β_1 grain boundaries

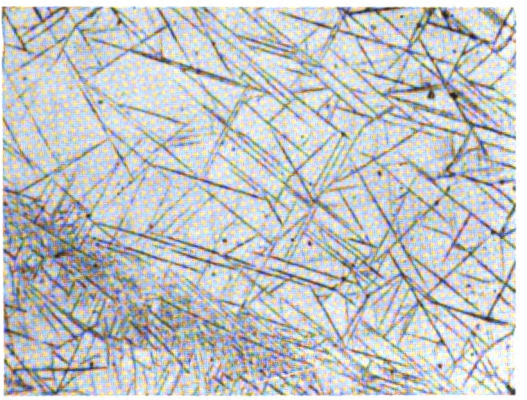

Figure 10 — 5 min. x 500

Further separation of α-phase plates

and 400° C, the transformation is associated with a small change of hardness, but at 500° C the hardness changes from the very moment of the alloy softening, due to the rapid transformation and the rapid growth of α-plates.

The conductivity of α-brass is smaller that that of the β-brass of the same concentration; therefore, the conductivity of the alloys falls with the separation of the α-phase. At lower temperatures, the curves which represent the dependence of the electrical conductivity upon time show a pronounced minimum, after which the conductivity increases (3,4).

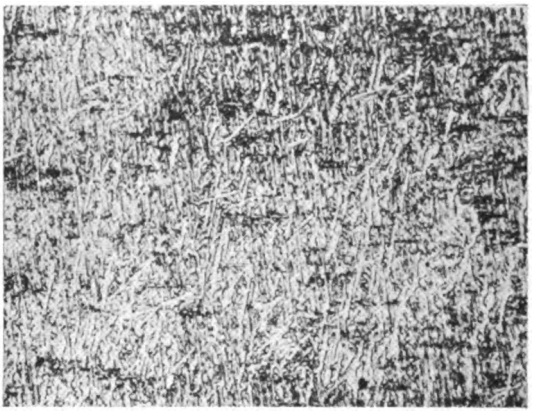

Figure 11 — 24^h x 500

α- and β_1 phases
Etched with 10% $(NH_4)_2S_2O_8$ in water + NH_4OH

Structure of Cu 60 Zn alloy thermally hardened at 350 °C

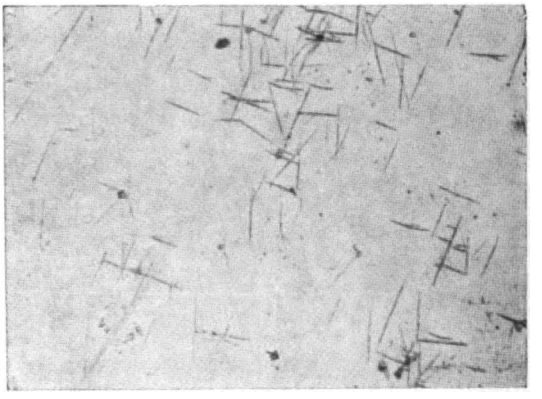

Figure 12 — 5^s x 500

Separation of α-plates in a β_1 base
Etched with 10% $(NH_4)_2S_2O_8$ in water + NH_4OH

DISCUSSION OF RESULTS

The change of hardness caused by the separation of the α-phase from a supersaturated β_1-solid solution of Cu 58 Zn and Cu 60 Zn alloys, respectively, shows that the hardening by

precipitation occurs only with Cu 60 Zn brass under the given experimental conditions.

The fact that the thermal precipitation of Cu 58 Zn brass has practically no effect on the hardness can be explained by; a) the amount of the separated α-phase which affects the hardness

Structure of Cu 60 Zn alloy thermally hardened at 350 °C

Figure 13 — 10^s x 500

Further separation of α-phase

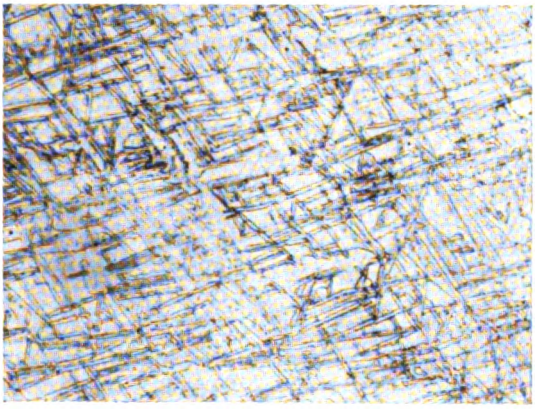

Figure 14 — 20^s x 500

α-plate network in β_1-phase mass
Etched with 10% $(NH_4)_2S_2O_8$ in water + NH_4OH

is much smaller in the structure of Cu 58 Zn alloy than in that of Cu 60 Zn brass; b) the separated α-phase in the case of Cu 58 Zn has a relatively rough structure even upon short treatment; such a structure might be due to the increased rate of growth of α-plates and to the slow formation of particles.

The appearance of the α-phase during the thermal precipitation of Cu 60 Zn is an exeptional case of precipitation. As with other

precipitation systems, we should also assume here the coherence of lattices of the α-and β_1-phases at the early stage of the α-phase separation; the tension of the β-lattice is thus increased, and

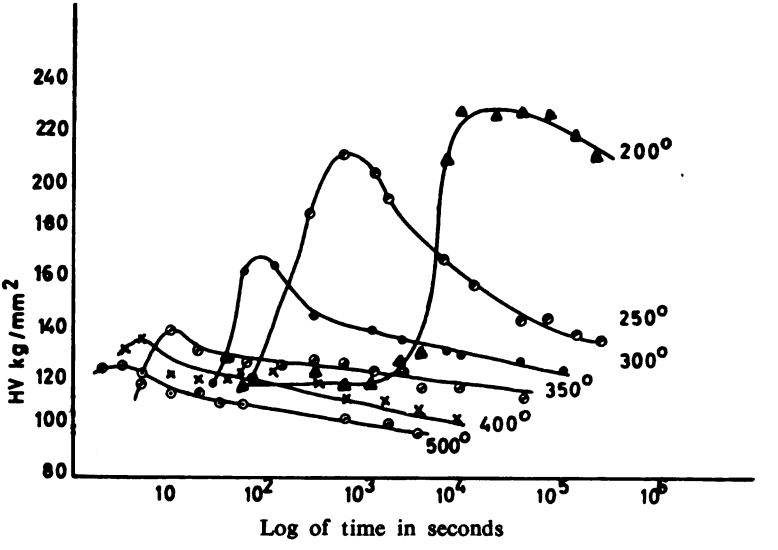

Figure 15

Dependence of hardness of Cu 60 Zn alley on temperature and duration of thermal treatment

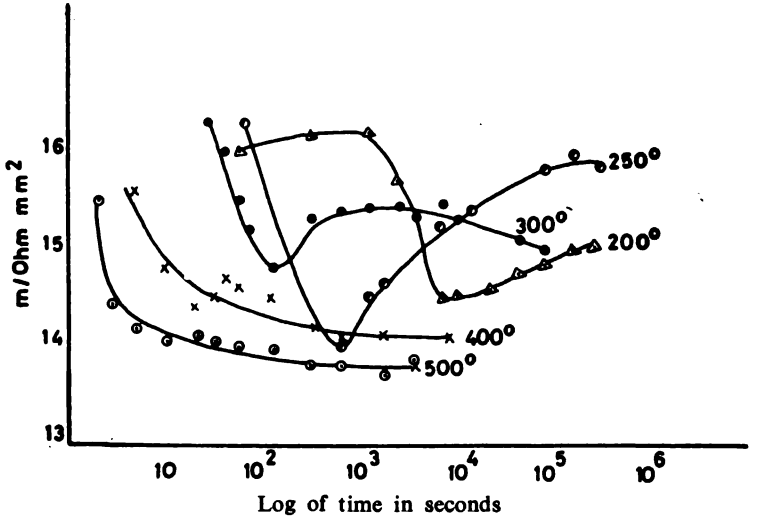

Figure 16

Dependence of electrical conductivity of Cu 60 Zn alloy on temperature and duration of thermal treatment

consequently the hardness is also increased. In cases in which the higher hardness is ascribed to the coherence of both lattices, the increase of hardness is observed before the precipitaion is visible

under the optical microscope. However, during thermal precipitation of Cu 60 Zn alloy, the increase of hardness is observed only when the precipitation can be seen.

The above findings were explained by R. D. Garwood (5) as originating from a small change of specific volume and small rate of particle formation. In fact, if the particles are considered to be centers of tension, it is obvious that a greater number of such particles will affect the increase of hardness.

According to E. Hornbogen, (4) it might be assumed that the appearance of particles of the α-phase at lower temperatures is in connection with the process which is similar to the martensitic transformation, whereby the concentrations of the α- and the β_1-phase are equal for a very short period of time. Hornbogen by means of x-ray investigations, established that no changes in the reflection of the β-lattice are observed until the first appearance of the needles of the α-phase, although these reflections are slightly enlarged due to induced tensions. On further aging there appear new reflections, and they can be explained by assuming the existence of two tetragonal interphases. We may consider that the spatial β-lattice is transformed into the planar α-lattice through two interphases, the first of which is similar to the β-lattice and the other to the α-lattice. Accordingly, the hardening is connected with the presence of interphases.

The abrupt fall of electrical conductivity, observed at the moment of the α-phase appearance, can be explained by the tension of the base, by the existence of interphases with strained lattices, and by higher electrical conductivity of the α-phase. Further increase of the conductivity might be ascribed to the coagulation of the separated phase.

The present investigations offer proof that the increased hardness of Cu 60 Zn can be effected by precipitating the α-phase from an supersaturated β′-solid solution. The interpretation of the mechanism of hardening requires further investigations.

On the basis of the above given results, obtained on aging of Cu 58 Zn and Cu 60 Zn alloys, we can draw the following conclusions:

1) The hardness of the Cu 58 Zn alloy cannot be increases by thermal precipitation of the α-phase from an supersaturated β_1-solid solution; and

2) The thermal precipitation of Cu 60 Zn alloy results in an enhanced hardness; for example, the increase of hardness even amounts to 100%, and this might be of great practical importance.

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A CONTRIBUTION TO THE STUDY OF THE EQUILIBRIUM DIAGRAM OF THE Fe — As SYSTEM

by

BRANKO I. BOŽIĆ and NADA P. VIDOJEVIĆ

As Yugoslav iron ores contain arsenic which, in the course of metal manufacturing, passes predominantly into iron and later accumulates in the steel, the problem of the effect of the increase of arsenic content on various properties of iron and steel is worth of full attention. In order to understand the influence of arsenic on the properties of steel and iron, and possibly to predict these properties to some extent, it is necessary to know the equilibrium diagram of the Fe — As system and the Fe — As — C system. According to the data in the literature, these systems have not yet been fully examined. We have undertaken this work in order to determine the solubility of arsenic in iron at ordinary temperature, and to ascertain the changes of solubility with temperature, i. e., to construct the solvus line. In further text we will treat the Fe — As system which contains 100 to 70% Fe, with which we have performed the investigation.

The diagram of the Fe — As system, given in Fig. 1, was constructed on the basis of the thermal analysis and the microscopic study of K. Friedrich (1); P. Oberhoffer and A. Gallaschnik (2), and on the basis of the x-ray investigations of G. Hägg (1). From the break of the liquidus curve at 1440° and 2.4% As, Oberhoffer and Gallaschnik have assumed that arsenic enlarges the γ-field, but they could not prove this assumption by the existence of peritectical at 1440°. On the other hand, F. Wever established a closed γ-field, but gave no detailed data. W. Jones (4) found that saturated γ-crystals contain from 3.25 to 4% As at the temperature of 1150°; his findings were based on the diffusion of arsenic in Fe—As alloys of various composition. Several intermetal phases were also established: Fe As, Fe_3As_2 (uncertain), and Fe_2As. Intermetal phase Fe_2As (40.10% As) has a tetragonal lattice and six atoms in the elementary cell; Fe_2As forms an eutecticum with α-solid solution which, according to Fridrich, has the following coordinates: composition — 70% Fe, temperature — 833—835°. The maximal solubility of arsenic in α-solid solution at the temperature of the eutectical is 8%. According to Oberhoffer and Gallaschnik (2)

the eutecticum lies at 69.7% Fe at a temperature of 827°, and the maximal solubility of arsenic in α-iron is 6.8%. From the shifts of x-ray interferences of α-iron containing a higher arsenic content, Hägg concluded that the solubility of arsenic at ordinary temperature is about 5%. Recently Sawamura (6) established that

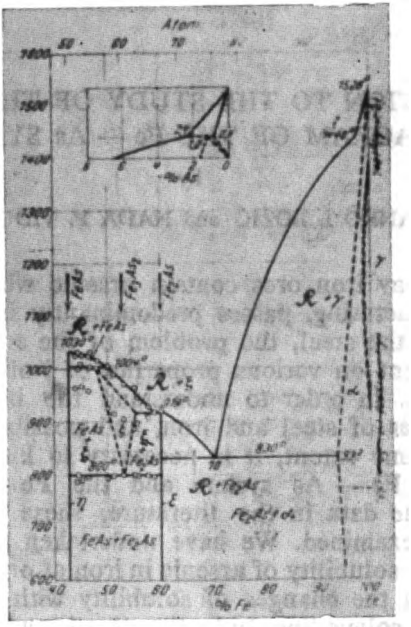

Figure 1. Diagram of state of the Fe—As System

the magnetic transformation temperature A_2 (768°) is constant for alloys which contain less than 4% As. The increase of arsenic content results in the decrease of the A_2-temperature until the value of 730°, this value being constant for all alloys which contain more than 10% As. It is evident from this that the limit solubility of arsenic in ferrite, at a temperature of 730°, lies at about 10% As, and this value is slightly smaller that the limit solubility (11% As) found in a previous author's investigation.

The above short review of the literature indicates that there is no unique opinion in regard to the solubility of arsenic at room temperature and its maximal solubility in α-iron; therefore, this paper represents an attempt to solve this problem.

MATERIALS AND METHODS

The investigations were performed with a series of alloys of different composition (containing from 0.023 to 30.14% arsenic). The starting material for the preparation of these alloys was carbonyl iron and arsenic of high purity; the melting was performed

in vessels of beryllium oxide in order to avoid any contamination of the alloys.

The determination of the solubility of arsenic at ordinary temperature was performed by measuring the microhardness of crystals of α-solid solution of arsenic in iron. The apparatus used

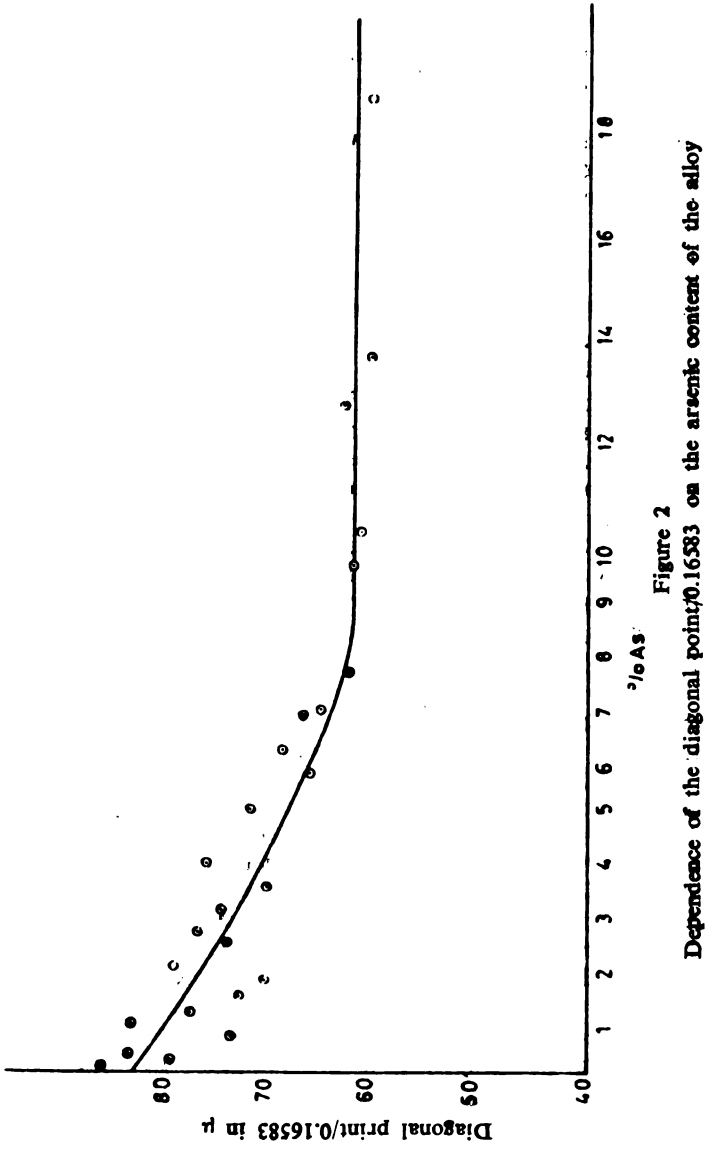

Figure 2
Dependence of the diagonal print/0.16583 on the arsenic content of the alloy

was "Reichert" (Reichert-Härteprüfer), loaded with 15 g. Each sample was measured ten to fifteen times, and the mean value is

given in the diagram in Fig. 2. As we were not interested in the absolute value of microhardness, but only in its changes, we did not calculate the microhardness, which represents a rather long procedure, and gave in Diagram 2 only the readings taken on the apparratus which represent a measure for the diagonal print (By multiplying the readings with the factor 0.16583, the diagonal prints in microns are obtained).

The determination of changes of arsenic solubility in α-iron and the verification of the eutecticum temperature was performed by the method of thermal dissolution and hardening from definite temperatures, and the dissolution of arsenic was followed microscopicaly. If the microstructure of an alloy after hardening from a definite temperature indicated the presence of two phases, the solubility at this temperature was surpassed. If only one phase is present, the hardening of the alloy is carried from a lower temperature, and so the first appearance of a secondary phase corresponds to the limit solubility of the given composition. According to the literature (7) the microscopic method gives results which are in good agreement with those obtained by determining the limit solubility by the x-ray method.

Thermal dissolution was performed in an oven with automatic temperature regulation, and the temperature variations were $\pm 0.5°$. The temperature in the oven was measured separately by a calibrated pirometer. In order to avoid the evaporation — the sublimation of arsenic during thermal treatment — the samples were sealed in tubes of quartz glass which contained the minimal amount of free space. The duration of exposures to different temperatures was determined experimentally, and ranged from four to forty-eight hours depending upon the temperature.

The samples were prepared in the usual way for the microscopic study. The corrodation was performed with a solution of 5 parts of conc. hydrochloric acid and 1 part of 10% chromium trioxide in water, thus the α-solid solution was covered with an oxide film which reflected light in the microscope; however, the Fe_2As phase remained unattacked. We should mention that the selection of a good corrodation agent was encountered with great difficulty due to a higher resistance of alloys which contain arsenic, against usual corrodation agents.

As an additional method for the determination of maximal solubility of arsenic in α-iron we applied the modified method of construction, Tammann's triangle. The surfaces which represent the eutecticum on microphotographs of alloys containing 18.39 and 13.51% As were cut out, and their weights were compared with those of microphotographs before cutting; from their ratio we calculated the percentage of eutecticum in the alloys in question. Starting from the assumption that the alloy which contains 30.14% As is of eutectical composition, we plotted the percentages of the eutecticum on the verticals of composition, starting from the eutecticum, and we obtained three points through which a straight line could be drawn. The cross point of this line and the eutectical repreesents the maximal content of arsenic in the α-iron solid

solution. However, this method is only of approximative value due to: a) the specific weights of the α-solid solution, and the Fe_2As phase are not identical so that their volumes are not equal to their weights, and b) the reduced solubility of arsenic in the α-solid solution at lower temperatures gives rise to the separation of the Fe_2As phase, which is difficult to differentiate from the eutecticum; thus, the amount of eutecticum which corresponds to the eutectical is not of satisfactory accuracy. The reduced percentage of arsenic causes greater errors, and therefore the alloys which contain 12.62 and 10.21% As were not taken into consideration.

RESULTS AND DISCUSSION

a) The determination of maximal solubility of arsenic in α-iron at ordinary temperature

The diagram in Fig. 2 shows the dependence of the readings (calculated on an apparatus for the determination of microhardness) upon the content of arsenic in alloy. The diagram is constructed on the basis of results obtained from thirty alloys of different composition. Although there are some deviations in results obtained with alloys which contain a low percentage of arsenic, the tendency of the decrease of the diagonal print (the increase of hardness) and its later constancy is obvious. The diagram in Fig. 2 shows that the constant magnitude of the diagonal print, i. e., hardness, ranges from 7.82 to 6.5% As, and it is concluded that the solubility of arsenic in α-iron at ordinary temperature falls into this region. This is in full agreement with results obtained by x-ray and microscopic analyses of the same samples (these analyses were performed in this Institute).

b) The determination of the dependence of the solubility of arsenic in α-iron upon the temperature

The results obtained in the investigation of the solvus line, the boundary between monophase, — and twophase $\alpha + Fe_2As$ — areas are depicted in Fig. 3. As seen from the diagram, the solubility of arsenic increases with the increase of temperature, and the extrapolation affords the maximal value of the solubility of arsenic, which lies at 10.15% As. As only few alloys which contain this transitional concentration were available, the solvus line could not be determined with several points.

The dissolution of arsenic in α-iron with increasing temperature with alloys containing 7.59 and 20.21% As, respectively, can be followed on Microphotographs 4 to 10. The microphotographs 8 to 10 show that the alloy which contains 10.21% As does not pass through the monophase α-area. It was established that the composition 10.21% As crosses the eutectical because the amount of Fe_2As phase does not decrease with the increase of temperature; and the Fe_2As phase separates in agregates of spherical shape, which indicates that the fusion of one part of the alloy has occurred.

From the construction of Tammann's triangle, the value of the maximal solubility of arsenic is 10% As, and the latter is very close to the value obtained by the microscopic method.

Small samples of alloy which contain 30.14% As (2x 2x 5 mm) and have sharp edges were first sealed in tubes of quartz glass,

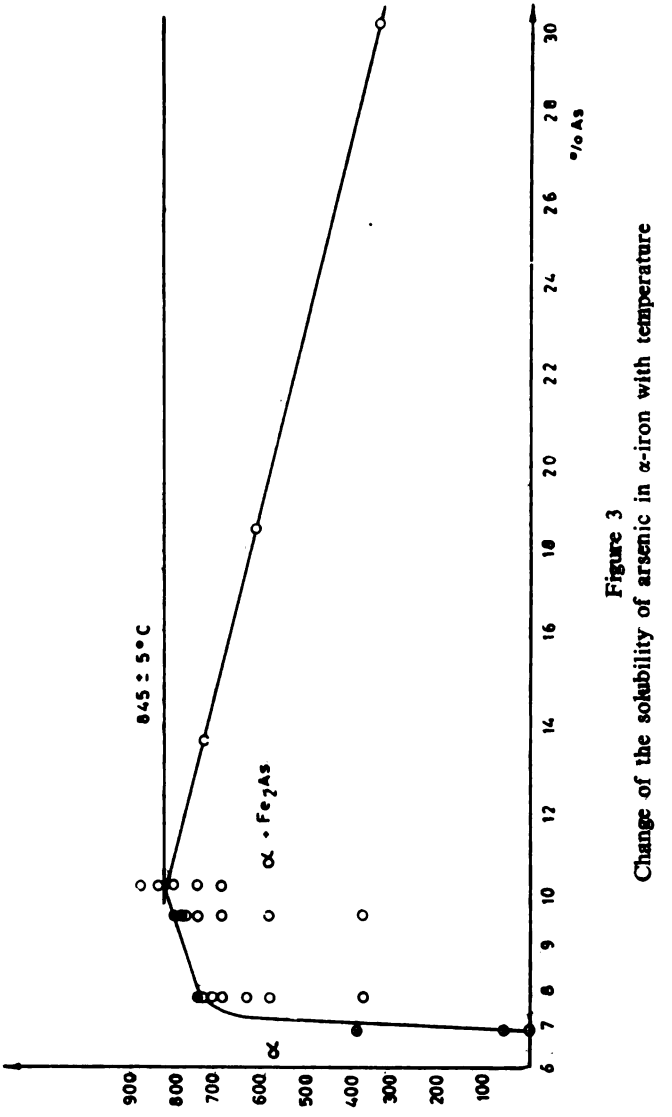

Figure 3
Change of the solubility of arsenic in α-iron with temperature

and then kept at 840, 845, 850, 855, and 860° C, respectively, for a period of one hour, and then they were hardened in water. It was established by means of a magnifying glass that the fusion started

at 854 ± 5°, and this temperature was adopted as the temperature of the eutectical. Microscopic investigations have shown that the alloy which contains 30.14% As is not quite homogeneous, but contains areas with primarily separated Fe_3As crystals so that the fusion occurred at the interval 845 — 850° when the whole mass was melted.

Structure of alloy with 7.59% As after thermal dissolution and hardening

Figure 4 — 7.59% As, 385°

Figure 5 — 7.59% As, 710°

Figure 6 — 7.59% As, 755°

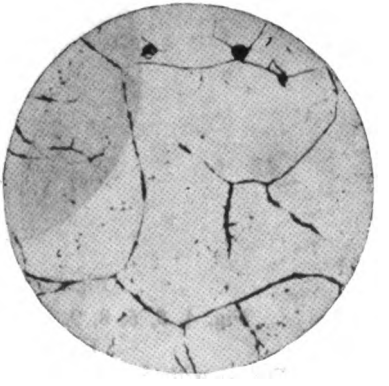

Figure 7 — 7.59% As 765°

Etched with sparts HCl (conc.) to 1 part 10% $CrCO_3$ in water
Magnified 200 ×

On the basis of these investigations, we came to the following conclusions:

1. By measuring the microhardness of the α-solid solution of arsenic in iron, we established that the maximal solubility of arsenic in iron at ordinary temperature lies between 6.82 and 7.59% As;

2. The solubility of arsenic in iron increases with the increase of temperature, as seen from the solvus line which was determined microscopically. The extrapolation shows that the maximal solubility of arsenic in α-iron is 10.15% As;

Structure of alloy with 10.21% As after thermal dissolution and hardening

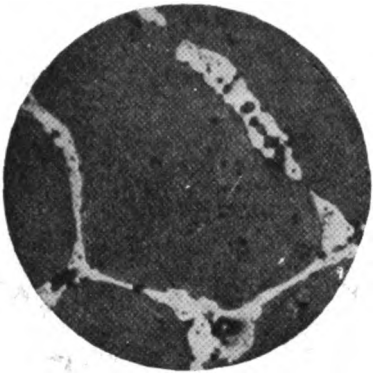

Figure 8 — 10.21 As, 845°

Figure 9 — 10.21% As, 820°

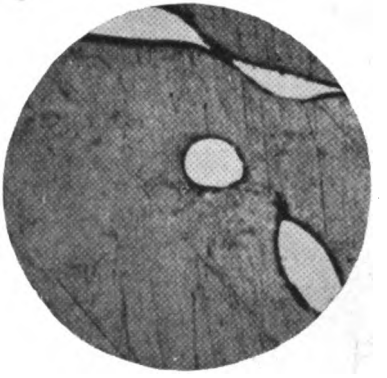

Figure 10 — 10.21% As, 855°

Fig. 4, 5, 6, 8, 9, 10: white — Fe_2As; Black — α-phase
Fig. 7: α grain boundaries
Etched with 5 parts HCl (conc.) to 1 part 10% $CrCO_3$ in water
Magnified 200×

3. The temperature of the eutectical reaction was determined by observing the initial fusion of the eutectical alloy; its value is 845 ± 5°, and should be taken as an approximative value due to the fact that the alloy is also nonhomogeneous as in regard to the applied method;

4. The experiments relating to the duration the thermal of dissolution of individual alloys at a definite temperature show that the diffusion of arsenic in iron is a rather rapid process,

and this is contrary to the observations in the literature which refer to an extraordinary slow diffusion of arsenic in iron;

5. On the basis of a high resistence of samples of Fe — As alloys against corrodation agents, it is concluded that arsenic exhibits a positive influence on the chemical resistence of iron in general.

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GLASNIK HEMIJSKOG DRUSTVA, Vol. 27. No. 7—8, 1962, pp. 355—366

THE APPLICATION OF BAKER-PHILIPPOFF'S FORMULA FOR THE DETERMINATION OF INTRINSIC VISCOSITY OF POLYMETHYL METHACRYLATE AND THE COPOLYMER DIMETHYL ITACONATE — STYRENE

by

RUŽA BORISAVLJEVIĆ, FRANJA EBEL, and ĐURO KOSANOVIĆ

The measurement of the viscosity of a polymer solution is one of the most convenient and frequently used method for the determination of the molecular weight of macromolecular compounds. If more precise results are required, especially in case of high molecular weights, it is necessary to measure η_{rel} at various concentrations, and than the intrinsic viscosity is determined

$$[\eta] = \lim_{c \to 0} \left(\frac{\eta_{sp}}{c} \right) \qquad (1)$$

by one of the usual methods. If less precise data are required, the value of η_{rel} at one concentration is determined, and $[\eta]$ is obtained by means of one of the well-known formulae given by Govaerts and Smets (1), Huggins (2), Martin (3), or Baker and Philippoff (4, 5). In our laboratories the determination of $[\eta]$ have usually been performed by the use of either Huggins or Govaert-Smets formulae.

Baker-Philippoff's formula is represented by the following expression:

$$[\eta] = \frac{\alpha}{c} \left[\sqrt{\frac{\log \eta_{rel}}{\alpha}} - 1 \right] \qquad (2)$$

where

$[\eta]$ = intrinsic viscosity
η_{rel} = relative viscosity
c = concentration in g/100 ml
α = constant

For the application of this formula it is necessary to determine the value of the constant α of the investigated polymer since it is dependent upon the system polymer-solvent. Although, Philippoff has assumed that the value of eight might be taken for the constant α, whatever system is investigated. Genung and Gage (6) have described the graphic determination of the constant α for the following systems: bleached sulfite pulp in cuen (cupriethylenediamine), cellulose acetat in the mixture of methylene chloride — methanol (90 : 10 weight parts); and cellulose acetate in acetone. By the use of the formula (2), the values for η_{rel} were calculated for various values of [η]), 0,7, 2, 3, 4, 7, 9), α (2, 4, 10, 25), and c (0,1, 0,25 0.50

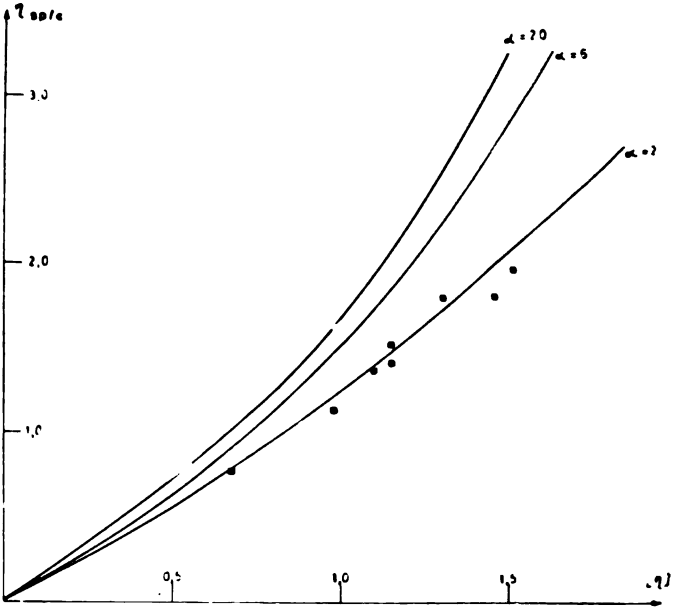

Figure 1. Graphical determination of the constant α for polymethylmethacrylate and the copolymer dimethyl itaconate — styrene. (c=0.25 g/100 ml)

concentration in g/100 ml). The corresponding diagrams were constructed on the basis of these calculated values for η_{rel}, and from these diagrams and experimental results the value of the constant α for the given system polymer-solvent was determined.

This work represents an attempt to apply Baker-Philippoff's formula to polymethyl methacrylate and the copolymer

Table 1

[η]	c gr/100 ml	α	η_{rel}	η_{sp}	η_{sp}/c
	0.10	2	1.0100	0.0100	0.100
		20	1.0100	0.0100	0.100
	0.25	2	1.0250	0.0250	0.100
		20	1.0253	0.0253	0.101
0.1	0.50	2	1.0506	0.0506	0.101
		6	1.0510	0.0510	0.102
		20	1.0512	0.0512	0.102
	1.00	2	1.1025	0.1025	0.1025
		6	1.1045	0.1045	0.1045
		20	1.1049	0.1049	0.1049
	0.10	2	1.0302	0.0302	0.302
		20	1.0305	0.0305	0.305
	0.25	2	1.0764	0.0764	0.3056
		6	1.0774	0.0774	0.3096
		20	1.0777	0.0777	0.3108
0.3	0.50	2	1.1556	0.1556	0.3112
		6	1.1597	0.1597	0.3194
		20	1.1612	0.1612	0.3224
	1.00	2	1.3225	0.3225	0.3225
		6	1.3401	0.3401	0.3401
		10	1.3430	0.3430	0.3430
		14	1.3457	0.3457	0.3457
		20	1.3469	0.3469	0.3469
	0.10	2	1.0506	0.0506	0.506
	0.25	20	1.0512	0.0512	0.512
		2	1.1289	0.1289	0.5156
		6	1.1300	0.1300	0.5200
		20	1.1327	0.1327	0.5308
0.5	0.50	2	1.2656	0.2656	0.5512
		6	1.2778	0.2778	0.5556
		20	1.2820	0.2820	0.5640
		2	1.5625	0.5625	0.5625
		6	1.6171	0.6171	0.6171
	1.00	10	1.6289	0.6289	0.6289
		14	1.6563	0.6563	0.6563
		20	1.6923	0.6923	0.6923

Table 1 (continued)

[η]	c gr/100 ml	α	η_{rel}	η_{sp}	η_{sp}/c
	0.10	2	1.0712	0.0712	0.712
		20	1.0723	0.0723	0.723
	0.25	2	1.1827	0.1827	0.7308
		6	1.1890	0.1890	0.7560
		20	1.1903	0.1903	0.7612
0.7	0.50	2	1.3806	0.3106	0.7612
		6	1.4057	0.4057	0.8114
		20	1.4148	0.4148	0.8296
	1.00	2	1.8226	0.8226	0.8226
		6	1.9392	0.9392	0.9392
		10	1.9671	0.9671	0.9671
		14	1.9799	0.9799	0.9799
		20	1.9898	0.9898	0.9898
	0.10	2	1.1025	0.1025	1.025
		20	1.1049	0.1049	1.049
	0.25	2	1.2656	0.2656	1.0624
		6	1.2775	0.2775	1.1100
		20	1.2821	0.2821	1.1284
1.0	0.50	2	1.5625	0.5625	1.1125
		6	1.6171	0.6171	1.2342
		10	1.6289	0.6289	1.2478
		20	1.6386	0.6386	1.2772
	1.00	2	2.2500	0.2500	1.2500
		6	2.5220	0.5220	1.5220
		10	2.5937	0.5937	1.5937
		14	2.6291	0.6291	1.6291
		20	2.6333	0.6333	1.6533
	0.10	2	1.1556	0.1556	1.556
		6	1.1597	0.1597	1.597
		20	1.1612	0.1612	1.612
	0.25	2	1.4102	0.4102	1.6408
		6	1.4387	0.4387	1.7548
		10	1.4451	0.4451	1.7804
1.5		20	1.4501	0.4501	1.8004
	0.50	2	1.8907	0.8907	1.7814
		6	2.0273	1.0273	2.0546
		10	2.0611	1.0611	2.1222
		14	2.0771	1.0771	2.1542
		20	2.0882	1.0882	2.1764

Table 1 (continued)

[η]	c gr/100 ml	α	η_{rel}	η_{sp}	η_{sp}/c
		2	3.0626	2.0626	2.0626
		6	3.8141	2.8141	2.8141
1.5	1.00	10	4.0455	3.0455	3.0455
		14	4.1501	3.1501	3.1501
		20	4.2480	3.2480	3.2480
		2	1.2100	0.2100	2.100
	0.10	6	1.2171	0.2171	2.171
		20	1.2202	0.2202	2.202
		2	1.5625	0.5625	2.250
		6	1.6171	0.6171	2.508
	0.25	10	1.6289	0.6289	2.516
		20	1.6923	0.6923	2.769
2.0	0.50	2	2.2500	1.2500	2.500
		6	2.5220	1.5220	3.044
	0.50	10	2.5937	1.5937	3.187
		14	2.6291	1.6291	3.258
		20	2.6533	1.6533	3.307
	1.00	2	4.0000	3.0000	3.0000
		6	5.6178	4.6178	4.6178
		10	6.1918	5.1918	5.1918
		14	6.4960	5.4960	5.4960
		20	6.7275	5.7275	5.7275

Theoretical relationship of [η], α, η_{rel}, η_{sp}, η_{sp}/c calculated from Baker–Philippoff's formula

dimethyl itaconate — styrene. By means of the above formula, the values of η_{rel} for various values of α and c were calculated and are given in Table 1. As it is shown in Table 1 the values of η_{rel} were most precisely calculated for [η] from 0.7 to 2.0 as only some of the investigated copolymers dimethyl itaconate styrene had [η] values smaller than 0.7 and the greatest part of the investigated polymethyl methacrylate and copolymer dimethyl itaconate styrene had the value for η between 0.7 and 2.

The diagrams shown in Figures 1, 2, and 3 were constructed on the basis of the values given in Table 1. By plotting the values of intrinsic viscosities [η] obtained by the extrapolation of experimental data into the corresponding diagrams,

it can be concluded, which value of α corresponds best for the given system polymer-solvent (polymethyl methacrylate — chloroform, and dimethyl itaconate — styrene — chloroform).

Experimental

In the present investigations chloroform "Kemika" was used, dried and purified by distillation, and methanol "Chemapol" without any purification since the latter represented only the precipating reagent for fractionation. The investigated copolymers and polymers were obtained by suspension polymerization in our laboratories, except *DIALUX*, *SUPERACRYL*, and *SIMPLEX*. *DIALUX* is the copolymer of dimethyl itaconate and styrene, made in Italy, and SUPERACRYL and SIMPLEX are polymethyl methacrylates made in Czechoslovakia and England respectively. The composition of the copolymer dimethyl itaconate — styrene was 46 : 54 (weight parts in %).

The measurements of viscosity were made with an Ostwald viscosimeter which was placed in a thermostat of 15 l at a temperature of 25.00±0.05°C. The measurements of viscosity, the calculations of η_{sp}, η_{rel}, and the extrapolation for the determination of [η] were performed as previously described (7).

Chloroform was used as the solvent for measuring viscosity and the average time of the solvent flow for the viscosimeter used was 70,1 second. Five measurements were performed for each copolymer and polymer, and the average value was calculated. Several measurements of η_{rel} at various concentrations were also performed for each polymer or copolymer, and [η] was determined by extrapolation to $\lim_{c \to 0} \left(\frac{\eta_{sp}}{c} \right)$. The extrapolation was performed statistically. The obtained [η] values were considered as true ones. All errors were calculated in regard to this value.

The concentrations of investigated solutions amounted in some cases to 1 per cent; although such high concentrations are not recommended in the literature (8), it was necessary to use them since the [η] values of some copolymers of dimethyl itaconate and styrene were very small. The increase of the concentration resulted in an increased accuracy of the measurement.

The fractioning of polymers was achieved by fractional precipitation, which was effected either by adding the precipitating reagent (methanol) or by evaporating the solvent (chloroform) as previously described (7). In both cases the system chloroform — methanol was used.

Results and Discussion

By plotting the values of $\eta_{sp}/_c$ against [η] (obtained experimentally at various concentrations), the diagrams given in Figures 1, 2, and 3, were drawn. They are constructed on the basis of Baker-Philippoff's equation, and a series of

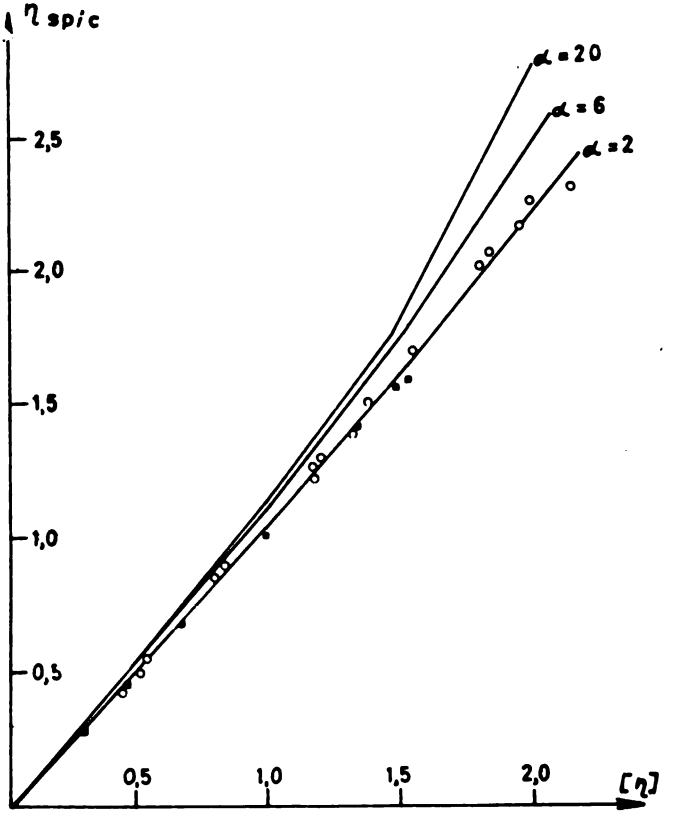

Figure 2. Graphical determination of the constant α for polymethylmethacrylate and the copolymer dimethyl itaconate — styrene (c=0.5 G/100 ml).

points representing the values of the constant α was thus obtained. The diagrams show that the best value for the constant α in both cases is α=2. By replacing the constant

α with the value of two in the formula (2), the following expression is obtained:

$$[\eta] = \frac{2}{c}\left[\sqrt{\frac{\log \eta_{rel}}{2}} - 1\right] \qquad (3)$$

The comparison of $[\eta]$values of polymethacrylate obtained by extrapolation with those calculated from Baker-Philippoff's and Huggins' formulae.

Table 2

Sample No.	c gr/100 ml	η_{rel}	$[\eta]_{extr}$	$[\eta]_{B-Ph}$	% Error	$[\eta]_{Hugg}$	% Error
1	0.50	1.655	1.170	1.145	—2.1	1.095	—6.3
2	0.50	2.215	1.935	1.955	+1.0	1.780	—8.0
3	0.50	1.750	1.315	1.291	—1.7	1.225	—6.8
4	0.50	2.114	1.780	1.817	+2.1	1.710	—3.9
5	0.50	1.695	1.192	1.208	+1.2	1.085	—9,0
1	0.25	1.310	1.170	1.155	—1.3	1.133	—3.1
2	0.25	1.545	1.935	1.946	—0.25	1.873	—3.3
3	0.25	1.352	1.315	1.301	—0.6	—1.276	—2.5
4	0.25	1.507	1.780	1.820	+2.3	1.762	—1.0
5	0.25	1.325	1.192	1.200	+0.5	1.186	—0.5

By means of the expression (3) the $[\eta]$ values of all investigated polymers and copolymers were calculated; they are shown with other values in Tables 2 and 3.

Table 3

The comparison of $[\eta]$ values of the copolymer dimethyl itaconate — styrene obtained by extrapolation with those calculated from Baker-Philippoff's formula

Sample No.	c gr/100 ml	$[\eta]_{rel}$	$[\eta]_{extr}$	$[\eta]_{B-PR}$	% Error
1	1.00	2.520	1.150	1.160	+1.0
2	1.00	1.574	0.490	0.494	+0.7
3	1.00	1.366	0.320	0.335	+1.4
4	1.00	2.760	1.310	1.322	+1.0
5	1.00	1.785	0.680	0.672	—1.3
1	0.50	1.660	1.150	1.154	+0,3
2	0.50	1.260	0.490	0.489	—0.2
3	0.50	1.170	0.320	0.326	+1.8
4	0.50	1.769	1.310	1.320	+0.8
5	0.50	1.360	0.680	0.664	—2.3
1	0.25	1.309	1.150	1.153	+0.3
2	0.25	1.126	0.490	0.489	—0.2
3	0.25	1.082	0.320	0.324	+1.2
4	0.25	1.356	1.310	1.316	+0.4
5	0.25	1.178	0.680	0.683	+0.4

All errors were calculated by assuming that $[\eta]_{extr}$ represents the true value.

On the basis of results given in Tables 2 and 3, it is evident that Baker-Philippoff's formula is much more suitable for the calculation of the intrinsic viscosity than the equation given by Huggins, in spite of the fact that Huggins' equation is usually used for the polymethyl methacrylate — chloroform system: and has been used in our laboratory until the present. There are no data in the literature which refer to the appli-

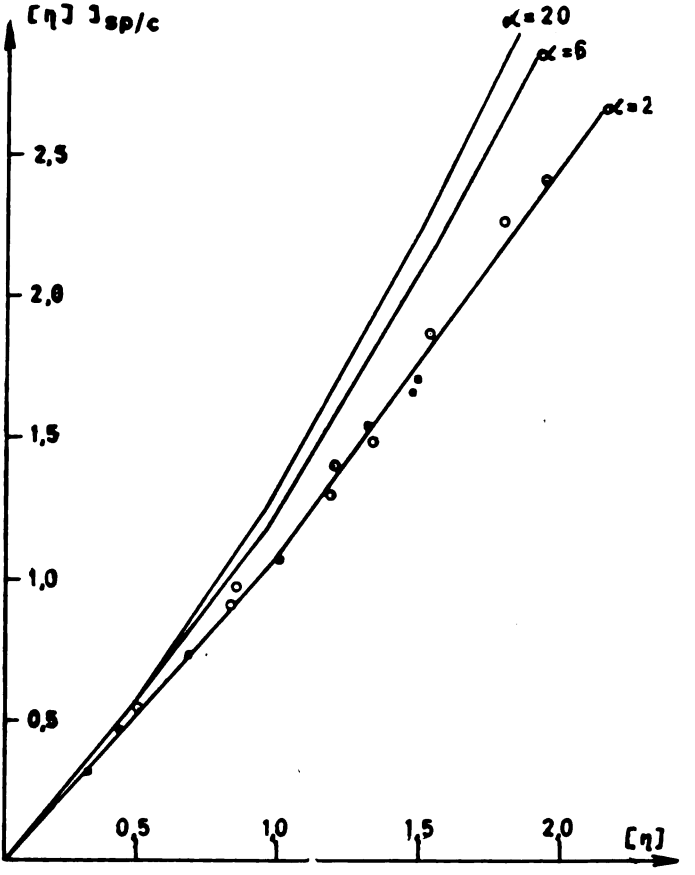

Figure 3. Graphical determination of the constant a for the copolymer dimethyl itaconate — styrene (c=1.00 g/100 ml).

cation of Baker-Philipoff's formula for the calculation of [η] for systems: polymethyl methacrylate — chloroform, and dimethyl itaconate — styrene — chloroform. Moreover, there are no data referring to the constants of the copolymer dimethyl itaconate — styrene; for other equations usually

used for the calculation of $[\eta]$ on the basis of a single η_{rel} measurement has not been reported.

From the diagrams in Figures 1, 2, and 3 the value of $[\eta]$ can be obtained graphically from a single measurement of η_{rel}, by measuring the viscosity for a definite concetration. In cases of polymers having a lower molecular weight, it is better to use higher concentrations (1 g polymer per 100 ml solvent) for a better accuracy; however, in case of polymers with high molecular weights, lower concentrations give very good results from (0.25 to 0.50 g per 100 ml).

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PHYSICO-CHEMICAL PROPERTIES OF 1-PHENYL-TETRAZOLE-5-THIOL

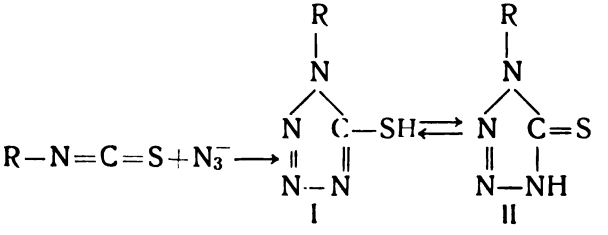
by

DUŠAN B. STEVANČEVIĆ

The synthesis of 1-phenyl-tetrazole-5-thiol was first accomplished by Freund and Hempel (1), and consisted of the action of nitrous acid on phenylthiosemicarbazide. Some time later Oliveri-Mandala and Noto (2,3) discovered that the reaction between phenylisothiocyanate and hydrazoic acid gives rise to the formation of a product which exhibits the same properties as the compound obtained by Freund and Hempel. However, they assumed that the reaction product had a thiocarbamyl azide structure.

The discrepancy in regard to the structure of the primary product obtained in these reactions was elucidiated by Lieber et al (4, 5). They established that both reactions give identical products, i. e., 5 — substituted aminothiotriazole derivatives which are isomerized in alkaline media into 1-substituted tetrazole-5-thiol derivatives, provided that the substituent is an aryl group.

The same authors demonstrated that phenylisothiocyanate reacts with azide ion in an aqueous solution and directly gives 1-phenyl-tetrazole-5-thiol according to the following scheme:



This divergence in the mode of the action of hydrazoic acid and the azide ion was ascribed to the effect of the solvent

and to the resonance interaction of phenylisothiocyanate and the azide ion which favorize the formation of the tetrazole nucleus.

Phenyl-tetrazole-5-thiol can exist in two tautomeric forms: the thiol form (I) and the thion form (II), as given above.

In our previous works (6, 7) we have suggested the possibility of applying 1-phenyl-tetrazole-5-thiol in analytical chemistry. However, the study of the reaction of this compound with metals requires a knowledge of its physico-chemical properties. Since these properties have not yet been published, we present here, the results of our investigations in this direction.

Experimental

REAGENTS

1-Phenyl-tetrazole-5-thiol (in further text PhTT) was synthesized by the procedure given by Lieber et al (5). The starting substances were: phenylisothiocyanate (BDH) and sodium azide (Merck). The yields of the reaction product were about 90 per cent. The elementary analysis of a sample of PhTT, recrystallized from ethanol, corresponded to the theoretical contents of individual elements (6).

The PhTT solutions were made with water distilled three times from which oxygen was removed by a current of purified nitrogen.

APPARATUS AND ACCESSORIES

Titrations and *p-H*-measurements were performed with the *pH*-meter Beckman model GS which has a glass-saturated calomel electrode system. The latter electrode was exterior, i.e., it was connected by means of an agar-agar bridge with the investigated solution. With the commercial electrode system in which the electrodes are directly dipped into the solution, PhTT, by diffusion and electromigration, passes into the calomel electrode and changes its potential. The elecrode system and the *pH*-meter were standardized with potassium hydrogen phtalate buffer (0.05 *M*). The titrations were performed in an atmosphere of purified nitrogen and at the constant temperature of $25 \pm 0.1^{\circ}C$.

The spectrophotometric measurements were made with a Beckman spectrophotometer, model DU.

The polarographic investigations were performed with the polarograph Radiometer PO-4. The potential can be registered with 50 mV/cm paper. The accuracy of recorded half wave potentials was $\pm$ 5 mV. The capillary constants were $t=3.05$ sec. at -0.5 V in 0.1 N potassiumchloride and 45 cm mercury. The mercury flow, $m=2.82$ mg/sec. The saturated calomel electrode was the reference electrode.

The amperometric titrations with rotating Pt-electrode were performed with an electrode of the "kuke" type, which rotates at a constant rate of 600 revolutions per minute by means of a synchronic rotator. The diffusion currents were measured with a microamperometer of 1.10^{-9} uA/mm sensitivity and an exterior critical resistance of 1500Ω.

Results

THE SOLUBILITY OF PhTT

The solubilty of PhTT in water at various temperatures and in perchloric acid solutions of various molarities was determined. The crystals of PhTT and the solution were equilibrated at a given temperature and in an atmosphere of nitrogen by means of stirring. Definite volumes of the saturated solution were samples from time to time by means ot an immersion filter of Jena G3 sintered glass, and the PhTT content was determined by amperometric titration with a rotating Pt-elecrode (vide infra).

The solubility of PhTT in water at 25, 30, and 35°C was found to be 5.0, 5.9, and 6.6 10^{-3}M, respectively; the solubility of PhTT in 0.1 N, 1 N, 2 N, and 4.8 N perchloric acid was 2.8, 3.8, 4.4, and 6.8 10^{-3}M, respectively. The increased solubility in a strong acid media might be accounted for by the protonozation of the compound.

Ultraviolet Absorption Spectra

The ultraviolet absorption spectra are depicted in Fig. 1; The ordinate represents the logarithms of molar extinction coefficients, and the abscissa represents the wave lengths in

mμ. The absorption curves refer to various *pH*-values and to the chloroform solution of the compound (dotted line). The concentration of all solutions was $C_{HR}=1.01.10^{-4}M$.

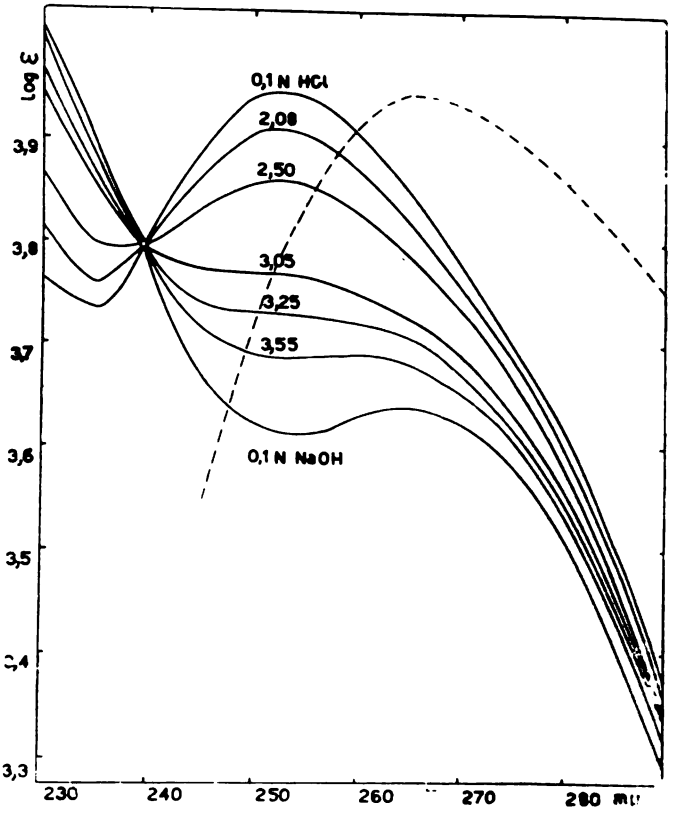


Figure 1. Ultraviolet absorption spectra of 1-phenyl-tetrazole-5-thiol. μ=0.1 (KCl+HCl)

It is evident from the absorption curves that the absorption of the compound is greatly dependent upon the Ph-values, due to the dissociation of the compound. The absorption maximum of the undissociated form (HR) lies at 253 mμ, and the molar extinction coefficient at that wavelength is $\varepsilon_{HR}=8800$.

The anion (R^-) has the maximal molar extinction coefficient $\varepsilon_R=4400$ at a wavelength of 265 mμ. Accordingly, the anion absorbs at higher wavelengths than the undissociated acid, which indicates a significant resonance stabilization of

the anion. This fact explains a rather pronounced dissociation of the compound in aqueous solutions.

The absorption maximum of the chloroform solution of PhTT lies at 265 mμ; the molar extinction coefficient at that wavelength is $\varepsilon_{265}=8800$.

Dissociation in Aqueous Solutions

On the basis of qualitative observations, Freund and Hempel (1) concluded that PhTT exhibits rather acidic properties. These findings were confirmed by absorption spectra.

Accordingly, the equilibium in aqueous solutions of PhTT can be expressed as:

$$HR+H_2O \rightleftharpoons (H_3O)^+ + R^-$$

The thermodynamic constant of this equilibium, the dissociation constant of PhTT, is given by this expression:

$$K_a=\frac{a_H\ a_R}{a_{HR}}=K_a^c\ \frac{Y_H\ Y_R}{Y_{HR}}$$

where a_H, a_R, and a_{HR} represent the activities, and Y_H, Y_R, and Y_{HR} represent the activity coefficients of hydrogen ion, anion, and the undissociated acid, respectively; K_a^c represents the concentration dissociation constant.

The dissociation constant was determined by pH-metic titrations and spectrophotometric measurements. In the former case, the solution of PhTT of the total concentration C_a was titrated with a standard solution of potassium hydroxide, and after each addition of potassium hydroxide the equilibrium activity of hydrogen ion (a_H) was determined by means of a standard pH-metric system. Bearing in mind the condition of the electroneutrality of the solution, the expression for the concentration constant acquires the subsequent form (in acid media, the concentration of OH ions is the magnitude of the second order in regard to other concentrations):

$$K_a^c=\frac{C_H\,(aC_a+C_H)}{C_a-aC_a-C_H},$$

where a is the degree of neutralization, and C_H is the concentration of hydrogen ion. If the concentration of hydrogen ion in this expression is replaced by the activity of hydrogen ion (a_H), the expression represents the apparent constant (K'_a) which can be calculated directly from the titration data since a_H is directly obtained by means of the pH-meter.

Table 1

The titration of 1-phenyl-tetrazole-5-thiol

Total concentration C_a=2.5 10^{-3}M. Initial volume 100 ml; titrated with 0.1001 N pottassium hydroxide. Ionic strength μ=0.1. Temperature 25±0.1°C.

v ml	pH	A_H 10^4M	C_H 10^4M	10a	C_a 10^3M	pK'$_a$	pK$_a^c$
0	2.86				2.500		
0.30	2.95	11.48	12.48	1.201	2.492	2.80	2.69
0.80	3.08	8.32	9.04	3.203	2.480	2.80	2.71
1.00	3.14	7.24	7.87	4.004	2.475	2.79	2.70
1.20	3.21	6.17	6.70	4.805	2.470	2.78	2.69
1.40	3.31	4.90	5.32	5.605	2.465	2.81	2.73
1.50	3.35	4.47	4.85	6.006	2.463	2.80	2.72
1.60	3.41	3.89	4.23	6.405	2.460	2.81	2.79
2.00	3.70						
2.45	5.19						
2.50	8.62						
2.60	9.83						
				Mean value:		2.80	2.71

Table 1 shows the data obtained in one titration along with the values for the apparent constant (pK'$_a$) and for the concentration constant (pK$_a^c$). The later constant was calculated from the previously given expression, the value of C_H being obtained from a_H and from the mean stechiometric factor of the electrolyte activity which was determined separately for the given experimental conditions.

The thermodynamic constant was determined according to Debye-Hückel law ($-\log y_\pm = Az_1z_2\sqrt{\mu}$) by extrapolation of pK'$_a$ — values, determined at various ionic strengths in the range $\mu > 0.1$ to the ionic strength μ=0. The extrapolation is depicted in Figure 2. The value of the thermodynamic constant obtained in this way was K_a=1.28 10^{-3}, i. e., pK$_a$=2.89.

The change of the free energy at the temperature of 25°C was

$F^0_{293} = 3942$ cal mole^{-1}.

The apparent dissociation constant at $\mu = 0.1$ was also determined from data obtained from absorption spectra.

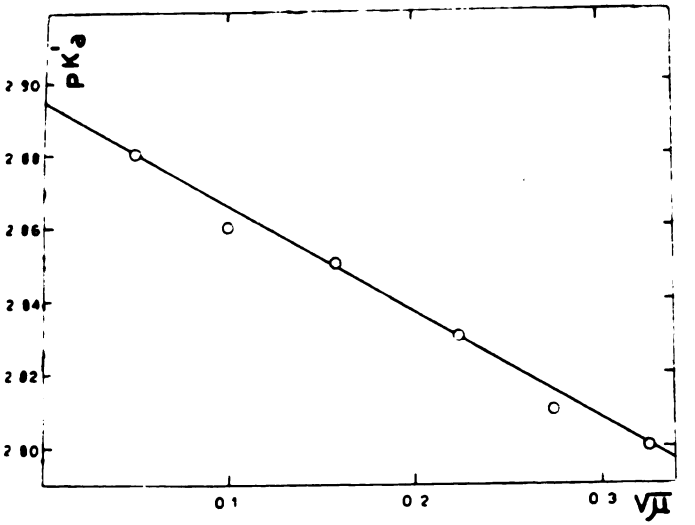


Figure 2. The determination of pK_a by extrapolating to $\mu = 0$.

The ratio between the anion concentration and the concentration of the undissociated molecule can be expressed by the aid of Lambert-Beer's law:

$$\frac{C_K}{C_{HR}} = \frac{\varepsilon_{HR} - \varepsilon}{\varepsilon - \varepsilon_R}$$

where ε_{HR} and ε_R are the molar extinction coefficients of the acid and the anion, respectively; ε represent the molar extinction coefficient at the given pH value. By introducing this term, the expression for the apparent dissociation constant acquires this form:

$$pK'_a = pH - \log \frac{\varepsilon_{HR} - \varepsilon}{\varepsilon - \varepsilon_R}$$

Figure 3 shows the graphic solution of this equation, and the corresponding measurements are presented in Table 2.

Table 2

The spectrophotometric determination of the ration C_R/C_{HR} and pK'_a. $\mu=0,1$; $\lambda=2350m\mu$; $\varepsilon_{HR}=8800$; $\varepsilon_R=41\cdot 0$

pH	ε	$\log \frac{\varepsilon_{HR}-\varepsilon}{\varepsilon-\varepsilon_R}$	pK'_a
2.08	8170	—0.75	2.83
2.21	7920	—0.60	2.81
2.50	7310	—0.31	2.81
2.87	6320	+0,06	2.81
3.05	5890	0.23	2.82
3.25	5380	0.44	2.81
3.55	4890	0.71	2.84

The value of the dissociation constant ($10^{-2.82}$) obtained spectrophotometrically is in agreement with the value obtained by the *pH*-metric titration.

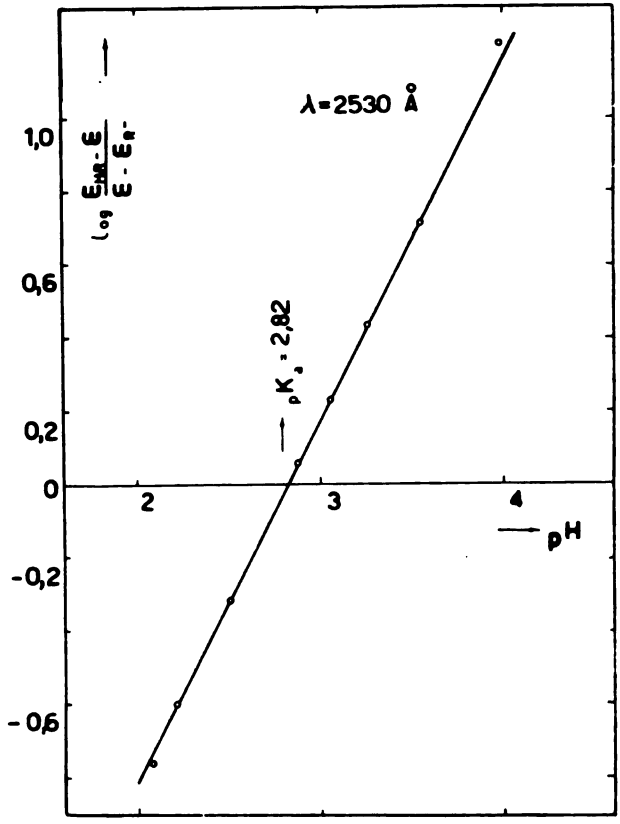


Figure 3. The spectrophotometric determination of the dissociation constant. $\mu=0.1$ (KCl+HCl)

Polarographic behavior of 1-Phenyl-tetrazole-5-thiol

We have studied the dependence of the half wave potential ($E_{1/2}$) of the anodic waves and their diffusion current (i_d) upon the pH-values. This dependence is depicted in Figure 4. The increased *pH* shifts the half wave potentials to more negative values. In 0.1 N perchloric acid the $E_{1/2}$ is —0.09 V.

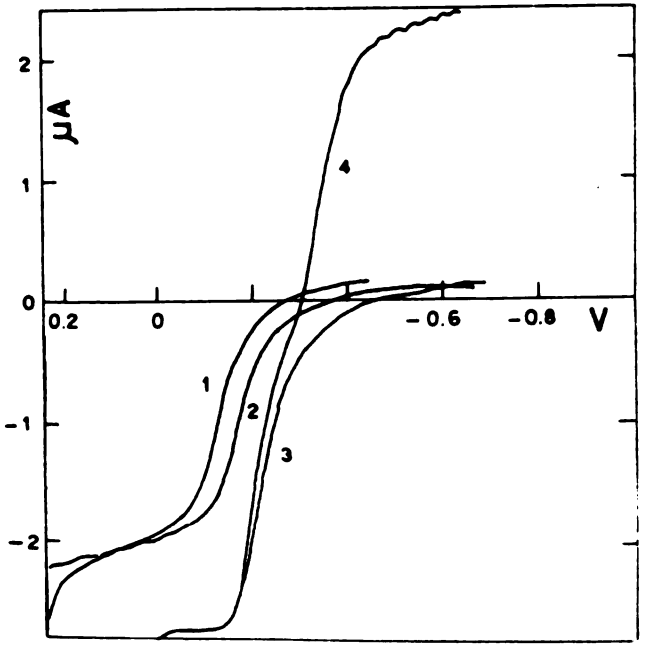


Figure 4. Anodic polarographic waves of PhTT. $C_{HR}=2.10^{-3}$M
1. 0.1 perchloric acid
2. 0.2 N acetate buffer pH 2.44
3. 0.5 N sodium acetate (oxygen free)
4. 0.5 N sodium acetate (in the presence of oxygen)

The ratio of the diffusion current and the concentration is constant in the limits of the experimental error and amounts to 1.20 μA/mmole l^{-1}. The capillary constant was $m^{2/3}t^{1/6}=$ $=2.392$ $mg^{2/3}sec^{-1/2}$, so that the constant of the diffusion current was I=0.51.

The catalytic action of PhTT was investigated in 2.10^{-3}M PhTT solutions in ammonia-ammonium chloride buffer. The addition of Co^{2+} ions has no effect on the formation of the catalytic hydrogen wave. Accordingly, pHTT is a thiol compound which lacks the ability to exhibit the catalytic effect which is characteristic of many other compounds of this type.

Methods for the Determination of 1-Phenyl-tetrazole-5-thiol

As a rather strong acid, PhTT can be determined by acidimetric titration. The determinations can be also performed by iodometric titrations, but more precise results are obtained when the excess of iodine is back titrated than by performing a direct titration. Higher amounts of PhTT can be determined by the precipitation method, i. e., by means of some metal ions, such as cadmium.

However, the best method for the determination of PhTT is the amperometric titration with a rotating Pt-electrode. This method has been applied for the determination of mercaptans by Kolthoff and Harris (8) it is based on the fact that the rotating Pt-electrode is a very sensitive indicator electrode for Ag ions, which react with mercaptans and give insoluble mercaptides.

The titrations of PhTT were performed in slightly acid solutions. The reference electrode was the saturated calomel electrode, and the potential difference between electrodes was high enough to produce measurable diffusion currents so that no exterior current source was used.

The amperometric titration has shown itself to be a very sensitive method; 10^{-5}M PhTT solutions were titrated with 10^{-3}M silver nitrate solution with an accuracy of $\pm$ 3 per cent.

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HIGH-FREQUENCY ALKALIMETRIC DETERMINATION OF HYDRAZINE SULPHATE

by

M. S. JOVANOVIĆ, M. M. PETROVIĆ, and M. B. KRSTIĆ

Due to the fact that hydrazine sulphate is a well defined salt and that in addition to the properties of a reducing agent it has those of a free acid, I. M. Kolthoff (1) recommended that it be used as a standard substance in alkalimetry. Although by neutralization with acids hydrazine forms mono- and di-acid salts, the salt known under the name of hydrazine sulphate ($N_2H_4 . H_2SO_4$) is extremely well defined. It crystallizes without water of crystallization, it is not hygroscopic (2) and therefore meets all the requirements for a standard substance.

However, in protolysis reactions, this salt reacts so that one mole of salt consumes only one gram-equivalent of base, so that the equivalent weight of this salt is M/1 and not M/2 as might be expected. The explanation for this behavior of hydrazine sulphate is found in the fact that the base is weaker than ammonia, as is also seen from the dissociation constants (3):

$$N_2H_4 + HOH = N_2H_5^+ + OH^- \qquad K_1 = 8.5 \cdot 10^{-7}, \text{ and}$$
$$N_2H_5^+ + HOH = N_2H_6^{2+} + OH^- \qquad K_2 = 8.9 \cdot 10^{-16}$$

Since the concentration of the $N_2H_6^{2+}$ ion is far lower it follows that in the neutralization reactions hydrazine sulphate can be treated as $N_2H_5 \cdot HSO_4$, so that in an aqueous solution the following dissociations take place:

$$N_2H_5 \cdot HSO_4 = N_2H_5^+ + HSO_4^- \text{ and}$$
$$HSO_4^- = H^+ + SO_4^{2-}$$

On the basis of the first inflection in the titration curve obtained by potentiometric titration with glass electrodes

Gilbert (4) found the value of K_1 to be equal to $3 \cdot 10^{-6}$. On the basis of the weakly pronounced second inflection he concluded that the value of K_2 should be 10^{-12} or even less.

It follows from these considerations that hydrazine sulphate can be determined alkalimetrically by using methyl red, as Kolthoff (1) recommended.

By making use of the observations of the authors mentioned above we wished to investigate the possibility of determining hydrazine sulphate with standard sodium carbonate and to detect the end-point of the reaction by using a high-frequency titration apparatus.

Experimental

A Radelkisz titrimeter, Budapest, designed by E. Pungor (5) was used in these experiments. Pungor's titration apparatus is of the so-called grid-dip type. It has one oscillatory high-frequency circuit and works at a frequency of 125 Mc/s. The frequency of the circuit varies with the changes that take the place in the solution during titration, because the container with the solution is the condenser of this circuit. Since the condenser is located between the grid and the anode of the electron tube, the change in the capacity will also cause changes in the intensity of grid current. These changes measured in μA can be followed on the dial of a microammeter. During titration the solution was agitated energetically by means of a magnetic stirrer attached to the titration apparatus.

To be quite safe, the hydrazine sulphate used in these experiments, although of sufficient purity (a product of "Pliva", Zagreb, p. a.), was recrystallyzed twice from aqueous solution. In reference to the reports of Sommer and Weis (2) mentioned above, we could conclude that after these precautionary measures the composition of the substance was $N_2H_4 \cdot H_2SO_4$.

The solution of hydrazine sulphate was standardized iodimetrically after Stollé (6). The error likely to occur in this method owing to the use of starch as indicator, was obviated by applying the dead-stop method of end-point detection (7). In this way it was established that 10.00 ml M/100 of the $N_2H_4 \cdot H_2SO_4$ solution consumes 3.981 ml N/10 of the I_2 solution (F=0.9702).

Whence

$$T_{N_2H_4 \cdot H_2SO_4} = 1.2820 \text{mg/ml}$$

A solution of hydrous carbonate (a product of C. Erba, Milano, r. p.), previously dried to a constant weight, was prepared by dissolving 5.2995 g of this product in exactly 10.00 ml of water, thus giving a

N/10 solution of Na_2CO_3 with F = 1.0000.

Since one of the main conditions of conductometric titration is the dosing of the concentrated solution of the titrant, in the cases when the added volume of the sodium carbonate solution was too small, this solution was made exactly ten times more dilute and then used in the experiment.

The titrations of hydrazine sulphate with a solution of sodium carbonate were carried out in a 150 ml container. Only a container of this size could be placed within the annular carriers of the titrimeter which together with the container and solution represent the condenser. Since the level of the liquid in the container had to be several millimeters above the upper ring, the total volume of the solution to be

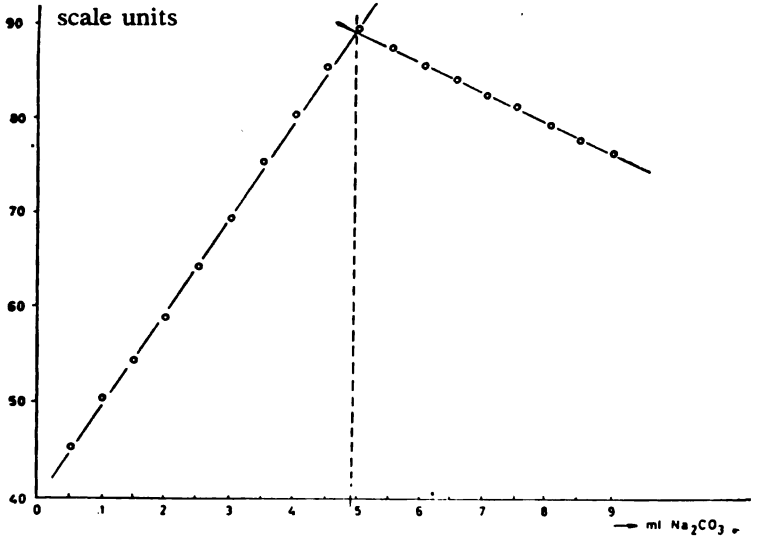

Fig. 1

determined was about 130 ml. This shows that special care should be taken to use a sufficiently concentrated solution of the titrant to keep the level of the water in the container from rising too high. Besides this, the water used for dilution must be kept at room temperature because the construction of the apparatus precludes the use of any kind of thermostat.

To be able to follow the course of the titration over the entire range of the dial, the most suitable sensitivity should be found by a preliminary "blind test". This sensitivity can then be used for the whole series of measurements.

By adding the titrant in equal small portions, and waiting each time for the current to stabilize we obtained the curves, one of which is represented in Figure 1. As is evident, the curve of the neutralization titration by the high-frequency method is quite similar to the curve obtained in a similar titration by the ordinary conductometric method of analysis.

The results obtained are recorded in Table 1.

*Table 1**

No	Taken $N_2H_4.H_2SO_4$ mg	Consumed Na_2CO_3 N/10 ml	Found $N_2H_4.H_2SO_4$ mg	Difference mg	Error %
1	12.82	0.983	12.79	—0.03	—0.23
2	12.82	0.984	12.81	—0.01	—0.07
3	12.82	0.986	12.83	+0.01	+0.07
4	12.82	0.988	12.86	+0.04	+0.31
5	6.41	0.491	6.39	—0.02	—0.32
6	6.41	0.492	6.40	—0.01	—0.15
7	6.41	0.493	6.42	+0.01	+0.15
8**	3.21	2.455	3.20	—0.01	—0.33
9**	3.21	2.470	3.21	0.00	0.00
10**	3.21	2.455	3.20	—0.01	—0.33

* The maximal deviations from thirty analyses are only shown.
** The analyses were performed using the N/100 potassium carbonate solution.

As the Table shows, the maximum error did not exceed — 0.94% and + 0.31%.

Since in analytical laboratories the ordinary conductometer is still far more often used than a high-frequency titrimeter, it was interesting to investigate the possibility of also using this instrument, the more so as there were hints of positive results. The investigations were carried out by using a conductometer of Yugoslav make (IEV, Ljubljana) with a magic eye as a null-point detector. It should be noted that the range of the variable compensating resistance had to be changed only when an amount of hydrazine sulphate of approximately 7 mg was being determined. Besides this, although in these investigations it was possible to use a larger beaker with a dip-type cell, titrations were in this case also carried

out in a 150-ml container, and the total volume of the solution was about 130 ml. The solution was sufficiently well agitated by means of a specially provided magnetic stirrer.

By using this conductance apparatus, working at a frequency of 1000 Mc/s and with an output voltage of 6V, we obtained the results recorded in Table 2.

*Table 2**

No	Taken $N_2H_4.H_2SO_4$ mg	Consumed Na_2CO_3 N/10 ml	Found $N_2H_4.H_2SO_4$ mg	Difference mg	Error %
1	12.82	0.984	12.80	—0.02	—0.17
2	12.82	0.982	12.78	—0.04	—0.33
3	12.82	0.985	12.82	0.00	0.00
4	6.60	0.508	6.62	+0.02	+0.33
6	6.60	0.507	6.61	+0.01	+0.15
5	6.60	0.505	6.57	—0.03	—0.45
7	3.30	2.540	3.31	0.01	0.30
8	3.30	2.530	3.29	0.01	0.30

* The maximal deviations from thirthy analyses are only shown. The analyses 4—8 were performed after the concentration of the examined solution had been altered, here it was $T_{N_2H_4 \cdot H\,SO_4} = 13.190$mg/ml. Besides, analyses 7 and 8 were done using $H/_{100}$ potassium carbonate solution.

The concentration of the hydrazine sulphate solution that we examined was altered in the analyses designated by numbers 11—30. In these analyses the concentration was

$$T_{N_2H_4 \cdot H_2SO_4} = 13.190 \text{ mg/ml}$$

As is evident from this series of analyses the maximum error did not exceed —0.45% and + 0.33%.

CONCLUSION

The alkalimetric determination of hydrazine sulphate was investigation by means of conductometric end-point detection. Both high-frequency and ordinary conductometers were used in these experiments. The maximum error did not exceed —0.94% and + 0.33%. In alkalimetric determinations the equivalent weight of hydrazine sulphate is equal to its molecular weight.

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COMPLEXOMETRIC TITRATION OF CHROMIUM (III) IN THE PRESENCE OF ALUMINUM, CALCIUM AND MAGNESIUM

by
V. B. GOLUBOVIC and M. J. LJUSTINA

Complexometric titration of chromium (III) has always presented a certain problem. According to the investigations of Schwarzenbach and Biedermann (1) and Schwarzenbach *et al* (2), chromium (III)-ion forms with EDTA three different complexes depending on the pH of the solution. Since chromium (III)-ions react with EDTA within a very wide pH range it follows that the complex formed is very stable (3). This is also reflected in its stability constant (pK=23). Of all other metals forming complexes with EDTA only thorium (IV) and iron (III) have higher stability constants (4).

Searching for reasons for such a high stability of the chromium (III)-EDTA complex, Ackermann and Schwarzenbach (5) assumed that it is a result of the formation of a covalent complex, while a negligible reactivity of chromium (III)-ions is explained by the fact that in an aqueous solution they are hydrated with six molecules of water. This is why practically no reaction takes place between chromium (III)--ions and EDTA at room temperature; only at 70°C a slow transformation takes place and the corresponding chelate is formed.

The low reactivity with EDTA and end-point detection (due to the intensive color of the complex) represent the main difficulties in the complexometric titration of chromium (III).

This was the reason why J. Kinnunen and B. Wennerstrand (6) resorted titration of the excess of EDTA with a solution of manganese sulphate with eriochrome black T as indicator. Since this reaction is slow the solution of chromium (III)-salt was allowed to boil for about fifteen minutes prior to the experiment. However, because of the intense color of the com-

plex formed, determinations are not possible even at concentrations higher than 8 mg of chromium (III) per 100 ml of solution. There is a similar case with the retitration of the excess EDTA with a solution of thorium (IV) nitrate (7).

The visual detection of the end-point of the reaction has been greatly facilitated by the methods of G. Doppler and R. Patzak (8), and R. Weiner and E. Ney (9). In both cases the retitration of the excess EDTA is conducted in a basic and not in an acid medium, because the chromoxo-complex is of a less intense color than the normal complex. In the first case (8) the retitration is carried out by a solution of zinc nitrate with eriochrome black T as indicator; in the second (9) by a solution of nickel sulphate with murexide. By the addition of certain compensatory dyes, such as Congo red and eriochrome green B (8), a sharp transition of color is obtained at the end-point of the reaction (from gray into violet).

Except for the paper of R. Patzak and G. Doppler (10) dealing with the determination of chromium (III) in the presence of aluminum and iron, to our knowledge, there are no other papers dealing with the problem of the titration of chromium (III) in the presence of other metals.

In this work tried to carry out complexometric titration of chromium (III) in the presence of aluminum, calcium, and magnesium, and to check this technique on some other solid materials (iron ore).

When considered separately, the titration of chromium (III) in the presence of aluminum presents no problem, since by varying the conditions of titration it is possible to attain a full selectivity for the determination of both metals (10). The difficulty lies in the fact that under the conditions for the determination of chromium (III) at pH 10 a reaction between EDTA and calcium and magnesium takes place at the same time.

Of the complexing agents only Tiron and ammonium fluoride are applicable in this case. Although R. Přibil (11) states that Tiron is suitable not only for masking aluminum, iron, and titanium, but also earth alkaline metals, no details for its application in these cases can be found in the literature. For this reason the use of ammonium fluoride appeared more suitable, since this compound forms with aluminum a very stable complex, and slightly soluble salts (13) with earth alkaline metals.

From the heats of formation of the fluorides referred to above (12) (AlF_3, 331.5 kcal; CaF_2, 289.2 kcal; MgF_2, 264.3 kcal; CrF_3, 171.3 kcal) it can be seen that the stability of fluorides decreases in the sequence Al — Ca — Mg — Cr, so that according to this there exists a theoretical possibility for complexometric titration of chromium (III) in their presence.

As had already been mentioned, chromium (III) and ammonium fluoride combine into chromium floride, a compound less stable than the corresponding fluorides of aluminum, calcium, and magnesium. From this compound EDTA replaces chromium (III) without any special difficulty and forms a chromium (III) complex.

Therefore, it has been found quite possible to determine chromium (III) complexometrically in the presence of aluminum, calcium, and magnesium by applying the technique of R. Patzak and G. Doppler (10), using ammonium fluoride as a masking agent (Table 1).

Table 1

Cr	Taken			Found Cr	Difference	Error
	Al	Ca	Mg	mg	± mg	%
	mg					
2.59	3.82	1.40	12.99	2.57	— 0.02	— 0.77
2.59	38.24	28.14	38.97	2.56	— 0.03	— 1.15
5.19	3.82	1.40	12.99	5.16	— 0.03	— 0.58
5.19	38.24	28.14	38.97	5.14	— 0.05	— 0.96
10.38	3.82	1.40	12.99	10.33	— 0.05	— 0.48
10.38	38.24	28.14	38.97	10.28	— 0.10	— 0.96

The data recorded in Table 1 refer to the mean values from five successive determinations of chromium (III) for the extreme values of the components added.

This technique gives satisfactory results with a chromium (III) concentration up to about 10 mg/150 ml solution. If this amount is exceeded the end-point of titration cannot reliably be determined because of the intense color of the solution.

This technique was also checked in the determination of chromium (III) in two samples of iron ore (magnetite) of Yugoslav origin. The data obtained are recorded in Table 2.

Table 2

No of Sample	Percentage		Cr %	Taken Cr mg	Found Cr mg	Difference ± mg	Error %
Sample 2	Fe	33.03	3.21	3.21	3.18	— 0.03	— 0.93
	Ni	0.63			3.20	— 0.01	— 0.31
	Si	9.06			3.22	+ 0.01	+ 0.31
	Mg	8.03			3.17	— 0.04	— 1.24
	Mn	0.12			3.18	— 0.03	— 0.93
	Al.	2.37					
	Ca	0.87					
Sample 2	Fe	26.30	1.85	1.85	1.84	— 0.01	— 0.54
	Ni	0.67			1.84	— 0.01	— 0.54
	Si	15.09			1.82	— 0.03	— 1.62
	Mg	9.31			1.82	+ 0.01	+ 0.54
	Mn	0.26			1.83	— 0.02	— 1.08
	Al	3.28					
	Ca	1.07					

Experimental

REAGENTS

EDTA, p. a. "Merck"; ammonium, p. a. "Pliva" (25—27%); amonium chloride, p. a. "Kemika"; ammonium fluoride, p. a. "Kemika"; hydrochloric acid, p. a. "Merck" (γ=1,17); Congo red "Chemapol"; eriochrome green B, "Merck"; eriochrome black T, "Merck"; potassium dichromate, p. a. "Merck"; aluminum chloride hexahydrate, p. a. "K. Erba"; metallic magnesium, p. a. "K. Erba"; magnesium chloride hexahydrate, p. a. "Pliva"; calcium chloride hexahydrate, p. a. "Kemika".

SOLUTIONS

A 0.01 M solution of EDTA was standardized by complexometric titration in a standard solution of $MgCl_2$ at pH 10, in the presence of eriochrome black T (11).

0.01 M solution of chromium (III) *sulphate* was obtained by dissolving 14.4805 g of potassium dichromate in 250 ml of redistilled water previously acidified with 13.9 ml of conc. sulphuric acid (γ=1.84). The reduction was carried out by gentle heating with a 30% solution of H_2O_2. The exceses H_2O_2 was removed by heating, and the solution after cooling trans-

ferred into a normal container and made up to one liter with redistilled water. The solution was standardized iodometrically.

A 0.01 M solution of zinc nitrate was prepared by dissolwing 2.9749 g of zinc nitrate hexahydrate in one liter of water. The solution was standardized gravimetrically.

The solutions of aluminum, calcium, and magnesium chlorides were prepared by dissolving adequate amounts of these salts, so that their concentration compared as follows:

Al:Cr	1:1.357
Ca : Cr	1:3.689
Mg:Cr	1:0.399

Acetate buffer. — 34.02 G of sodium acetate was dissolved in water. 17.5 Ml of 80% acetic acid ($\gamma=1.07$) was added and made up to 500 ml with distilled water.

Basic fluoride buffer. — 27 G of ammonium fluoride was dissolved in 174 ml of conc. ammonia (0.90) and diluted to 500 ml with water.

0.1% Solutions of eriochrome black T, eriochrome green B, and Congo red. — The solutions were always freshly prepared.

Burettes, pipettes, and calibrated containers were gauged in the usual manner.

PROCEDURE

To 5, 10, or 20 ml of dissolved chromium salt (previously diluted in the proportion 1:10) were added the corresponding amount of dissolved aluminum, calcium, and magnesium salts (Table 1), 3—5 ml of acetate buffer, and 1—2 g of ammonium fluoride. The solution was diluted with about 60—100 ml of water and heated to 70°C. Then, 40 ml of 0.01 M EDTA solution was added and the liquid allowed to boil for about 15 minutes. After cooling the solution to room temperature, 10 ml of fluoride buffer was added together with several drops of indicator and compensatory dyes. The excess EDTA was titrated in a solution of 0.01 M zinc nitrate until the transition of gray into violet.

The same procedure was used in the complexometric titration of chromium (III) in iron ore (Table 2). One gram of finely divided ore was after smelting with Na_2O_2 treated

in the usual manner, and the solution obtained removed into a 250 ml calibrated container. Each time 25 ml of this solution was taken for the analysis and titrated complexometrically according to the procedure described above. The results obtained from several successive titrations (Table 2) were compared with the results obtained iodometrically.

CONCLUSION

By using the Patzak-Weiner titration method chromium (III) was determined in the presence of aluminum, calcium, and magnesium. For a more precise end-point detection, the compensatory dyes eriochrome green B and Congo red were used in addition to the indicator eriochrome black T. Aluminum, calcium, and magnesium were masked by the addition of ammonium fluoride. Satisfactory results were obtained with chromium (III) concentration up to about 10 mg/150 ml of the solution.

This procedure was also checked in the determination of chromium (III) in two samples of iron ore (magnetite) of Yugoslav origin.

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N-BENZOYLPHTHALIMIDE

IV.*

PYROLYSIS AND REACTION WITH PHENYLMAGNESIUM BROMIDE

by

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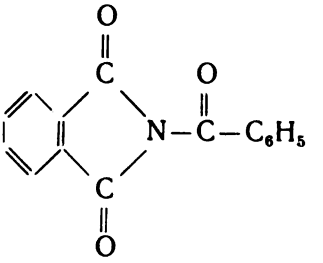
In the continuation of our study on the chemistry of N-benzoylphthalimide, (1, 2, 3) the thermal decomposition of this compound and its reaction with phenylmagnesium bromide have been studied in the present work.

By pyrolysis of N-benzoylphthalimide (I), at temperatures not exceeding 360°, several products were obtained: 11% of phthalimide (II); 34% of phthalic acid (III), 17% of benzonitrile (IV); and 18% of benzoic acid (V). From the phthalimide part of N-benzoylphthalimide, 45% of products (phthalimide + phthalic acid were isolated, and the benzamide rest of N-benzoylphthalimide gave 35% of products (benzonitrile + benzoic acid).

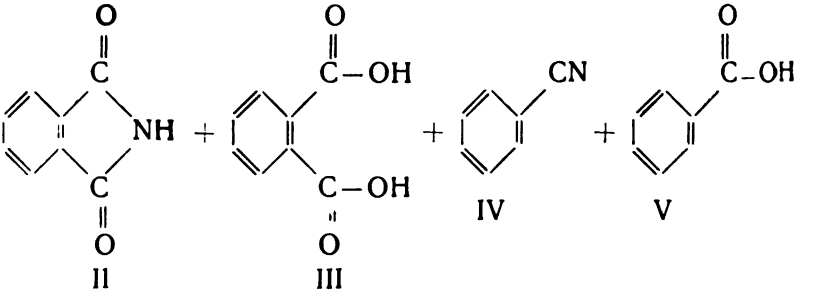
Although Hurd et. al., (4, 5) have shown that certain N-acylphthalimides of the general formula $C_6H_4(CO)_2N\text{-}COCH_2R$ give rise on pyrolysis to ketones, $(RCH_2)_2(CO)$, besides phthalimide and the acid $RCH_2\,COOH$ or its anhydride, in the case of N-benzoylphthalimide (I), upon heating to 360°, benzophenone was not found among the reaction products.

* Paper III: Glasnik hem. društva Beograd (Bull. soc. chim. Beograd), 27, (1962).



I

pyrolysis (up to 360°)

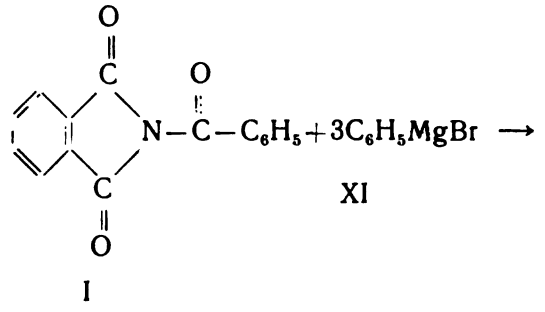


II III IV V

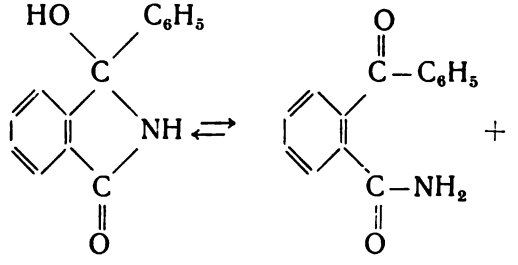
As it contains three amide carbonyl groups, N-benzoylphthalimide could add up to three moles of Grignard reagent in the first phase of the reaction. However, if the so formed addition complexes were to undergo decomposition with formation of ketones, prior to hydrolysis, these compounds with keto-carbonyl groups could further react with new amounts of organomagnesium salts. Scheme 1 shows the possible reaction products of such a Grignard reaction on the example of a simple amide.

When treated with a little more than three moles of phenylmagnesium bromide (XI), N-benzoylphthalimide (I) gave a mixture of products, from which the following compounds were isolated: 3-hydroxy-3-phenylphthalimidine (XII) in 52% yield, *o*-dibenzoylbenzene (XIV) in 6% yield; and triphenylcarbinol (XV) in 37% yield.* In addition, a small

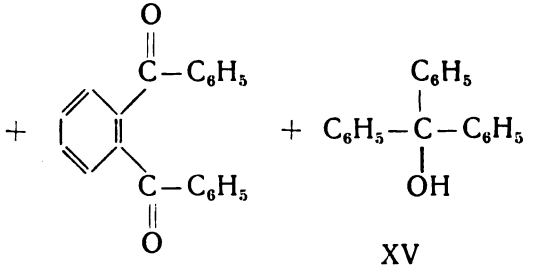
* A similar reaction with N-acetylphthalimide produced 47% of 3-hydroxy-3-phenylphthalimidine (XII); 17% of diphenylmethyl-carbinol (corresponding to triphenylcarbinol (XV) in the case of N-benzoylphthalimide); and 5% of *o*-dibenzoylbenzene (XIV) (6). Mustafa, Asker and Hishmat (7) have reported that N-benzoylphthalimide and phenylmagnesium bromide gave only 3-hydroxy 3-phenylphthalimidine (XII) and triphenylcarbinol (XV). Moreover the ratio of reaotants used and the yields of products were not clearly mentioned.



I XI



XII XIII

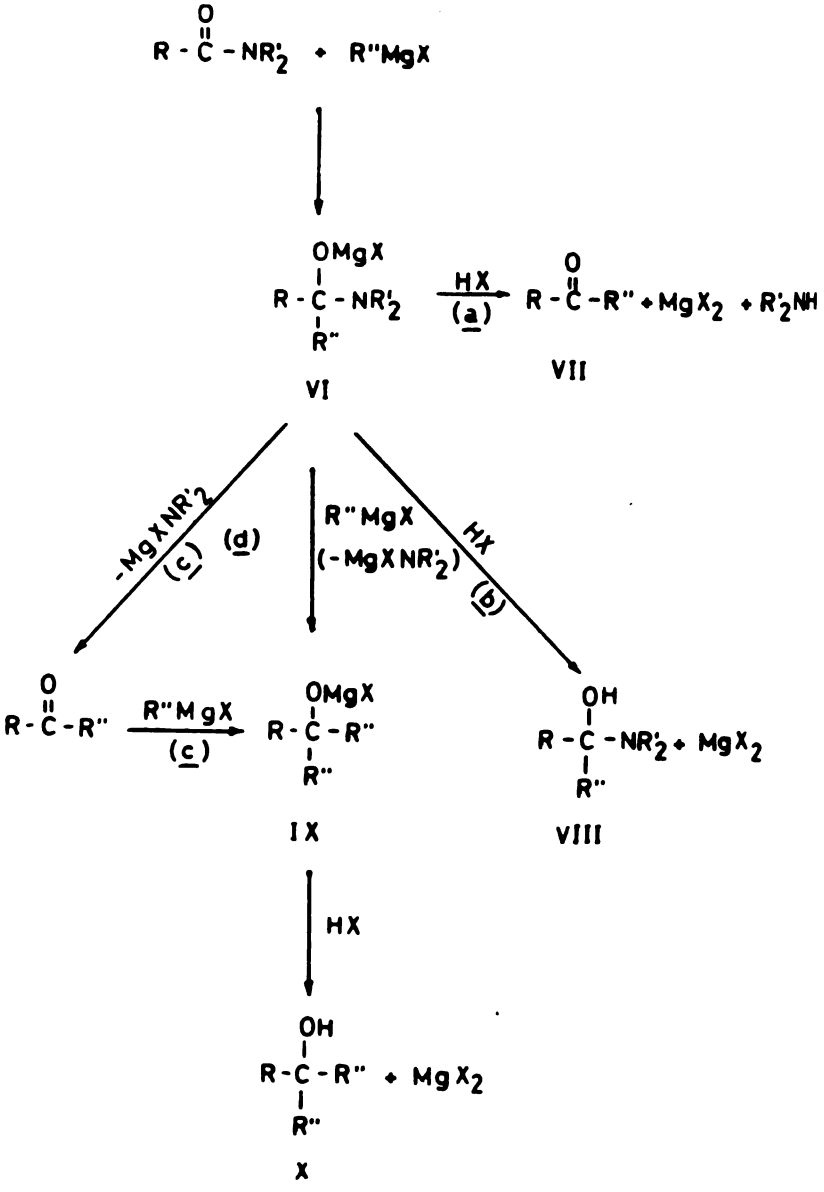


XIV XV

quantity of biphenyl was obtained (yield 5%), which probably resulted from the coupling of two phenyl radicals from the Grignard reagent. 3-Hydroxy-3-phenylphthalimidine (XII) can exist in two tautomeric forms, the amide form being represented by XIII (*o*-benzoylbenzoic acid amide).

It is obvious from Scheme 1 that the formation of the first two compounds (XII and XIV) occurs by addition of the Grignard reagent to one or both imide-ring carbonyl groups of N-benzoylphthalimide (I), followed by hydrolysis of the complex VI to compound VII (=XIII and XIV) and VIII (=XII), according to paths (*a*) and (*b*), respectively.

Similarly, triphenylcarbinol (XV) is formed by the addition of phenylmagnesium bromide to the benzoyl amide carbonyl group of N-benzoylphthalimide (I), and by conversion of



the initially formed complex VI to the addition product IX, according to (*c*) or (*d*), which is finally hydrolyzed to the tertiary alcohol X (=XV) (Scheme 1). If this alcohol

(triphenylcarbinol) is produced by reaction (*c*), benzophenone should appear as an intermediate. Although this ketone was not isolated, its absence does not prove that the reaction path (*c*) should be disregarded, and that the only possible route to triphenylcarbinol (X=XV) is that which is represented by (*d*), because it is well known that ketones readily combine with Grignard reagents to produce tertiary alcohols.

Experimental Part

All melting and boiling points are uncorrected.

N-Benzoylphthalimide, prepared from phthalimide and benzoyl chloride in anhydrous pyridine (8), melted at 168° after crystallization from ethyl alcohol.

PYROLYSIS OF N-BENZOYLPHTHALIMIDE.

N-Benzoylphthalimide (50 g; 0.2 mole) was placed in a distillation flask of 250 ml. and gradually heated (in a bath consisting of equal amounts of KNO_3 and $NaNO_3$). The temperature was slowly raised to 360° over a period of eight hours. Heating was discontinued when yellow vapors appeared at 360°. During the heating period 0.5 g. of benzonitrile passed over.

The content in the flask was steam distilled, and three liters of distillate were collected. The aqueous distillate was extracted with ether, and the ethereal layer, after drying (over anh. Na_2SO_4) and evaporation of the solvent, gave 2.9 g. of benzonitrile, b. p. 188—190° (liter. b. p. 191° (9). The total yield of benzonitrile amounted to 3.4 g. (16.5%). It was identified by quantitative hydrolysis to benzoic acid, m. p. 121° (9), and by conversion with hydrogen peroxide to benzamide, m. p. 128° (9).

The residue in the flask, after steam distillation, solidifed upon cooling. The solid was separated by filtration, and treated with a cold 5% sodium bicarbonate solution. The insoluble part was recrystallized from water and gave 3.1 g. (10.5%) of phthalimide, m. p. and mixed m. p. 232—233° (9). The bicarbonate solution was acidified with hydrochloric acid, and the resulting precipitate (m. p. 115—145°), upon repeated fractional crystallization from water, afforded 4.3 g. (17.6%)

of benzoic acid, m. p. 121° (9), and 11.2 (33.7%) of phthalic acid, m. p. 205—207° (dec.) liter. m. p. 206° (9). Phthalic acid was identified by conversion (upon slow heating) to phthalic anhydride, m. p. 128—130° (liter. m. p. 131°) (9).

REACTION OF N-BENZOYLPHTHALIMIDE WITH PHENYLMAGNESIUM BROMIDE

In a round-bottomed, three-necked flack equipped with a mechanical stirrer, reflux condenser, and dropping funnel, we placed 8.5 g. (0.354 g. — atom) of magnesium, one or two crystals of iodine, and, 150 ml. of anhydrous ether. Through the dropping funnel a solution of 55 g. (0.35 mole) of bromobenzene in 50 ml. of anhydrous ether was slowly added. After the addition was complete, the reaction mixture was heated under reflux until all the magnesium had dissolved. It was then cooled in an ice-salt bath, and 25.1 g (0.1 mole) of N-benzoylphthalimide was slowly added, in small portions to the solution of phenylmagnesium bromide (0.35 mole) in ether, with constant stirring. The reaction mixture was allowed to come to room temperature, was stirred for an additional hour, and was then treated with an ice-cold solution of 50 g. of ammonium chloride in 50 ml. of water. The ether layer was separated and the water solution was continuously extracted with ether. The ethereal solution were combined, dried over anhydrous magnesium sulfate, and concentrated to a volume of about 25 ml. The white 3-hydroxy-3-phenylphthalimidine which precipitated (10.5 g.; m. p. 157—160°) was removed by filtration, and the ethereal filtrate was evaporated to dryness. The residue, upon crystallization from toluene, gave another 3 g. of the same compound, m. p. 155—159°. Both fractions were combined and recrystallized from toluene, affording 11.7 g. (52%) of 3-hydroxy-3-phenylphthalimidine, m. p. 162—163° (liter. m. p. 165°). (10).

Analysis:

Calculated for $C_{14}H_{11}NO_2$ (225.2) : C 74.65%; H 4.92%; N 6.22%.
Found : C 74.39%; H 4.88%; N 6.35%.

This product was identified by mixed melting point determination with an authentic sample of 3-hydroxy-3-phenyl-

phthalimidine (10), and by hydrolysis to *o*-benzoylbenzoic acid, m. p. 125—126° (liter. m. p. 126°) (9).

The original toluene filtrate was evaporated to dryness, and the solid residue was subjected to steam distillation. From the distillate, by ether extraction in the usual way, we obtained 2.1 g. of product which gave, upon crystallization from ethyl alcohol, 1.38 g. (5.1%) of biphenyl, m. p. and mixed m. p. 69—70° (liter. m. p. 70°) (9).

The residue in the flask, which did not distill with steam solidified up on cooling. This precipitate, after filtration, was recrystallized from ethyl alcohol and gave a product, m. p. 137—149°, which upon repeated fractional crystallization from ethyl alcohol, furnished *o*-dibenzoylbenzene and triphenylcarbinol.

o-Dibenzoylbenzene, m. p. 146—147° (liter m. p. 147.5—148.5°) (11), was obtained in 6.3% yield (1.8 g.). It did not depress the melting point of an authentic sample of *o*-dibenzoylbenzene, which was prepared from phenylmagnesium bromide and phthaloyl chloride (11).

Analysis:
Calculated for $C_{20}H_{14}O_2$ (286.3) : C 83.90%; H 4.93%.
Found : C 83.68%; H 4.90%.

Triphenylcarbinol, m. p. 161° (liter. m. p. 162°) (9), was obtained in 36.7% yield (9.5 g). It did not depress the melting point of an authentic sample of triphenylcarbinol, which was synthesized from ethyl benzoate and phenylmagnesium bromide (12).

Analysis:
Calculated for $C_{19}H_{16}O$ (260.3) : C 87.66%; H 6.19%.
Found : C 87.64%; H 6.10%.

The authors are indebted to Mrs. Ružica Tasovac and Miss Radmila Dimitrijević of the Microanalytical Laboratory of our Institute for the analyses which they performed.

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REACTIVITY OF PYRIDINECARBOXYLIC ACIDS. I. REACTION KINETICS OF PICOLINIC, NICOTINIC, AND QUINOLINIC ACIDS, AND α- AND β- METHYL ESTERS OF QUINOLINIC ACID WITH DIPHENYLDIAZOMETHANE IN ETHANOL

by

DJ. M. DIMITRIJEVIĆ, Đ. D. TADIĆ, and M. D. MUŠKATIROVIĆ

In our earlier papers on the reactions of anhydrides and imides of quinolinic acid with amines (1) we showed that these reactions take place so that the amine reacts only with the alpha-carbonyl group of anhydrides, and mostly with the alpha-carbonyl group of imides forming the corresponding N-substituted alpha-quinoline amino acids, and alpha N-substituted quinoline amides.

This reaction can be explained by the fact that the alpha-carbonyl carbon atom, owing to its higher partial positive charge, is more electron-attracting than the beta-carbonyl carbon atom. The difference in charge between these two carbonyl carbon atoms is caused by the different negative inductive effect which they undergo due to an unequal distribution of electrons over the pyridine nucleus. As is known (2), the presence of a nitrogen atom in the pyridine nucleus results in the relative increase of electron density on the nitrogen and a relative decrease of electron density on the carbon atoms; this is greater at the 2-, 4-, and 6-positions than at the 3- and 5-positions. In other words this means that the partial positive charge is higher on the carbon atoms in the 2-, 4-, and 6-positions than on the carbon atoms in the 3- and 5-positions. In pyridine derivatives this results in greater electron migration to the nucleus in the substituents attached in the 2-, 4-, and 6-positions than to those attached in the 3- and 5-positions. Naturally, all this is true if we assume that the relative distribution of electrons over the pyridine nucleus even in the substituted pyridines (in this case in

anhydrides and imides of quinolinic acid) has not been essentially changed in relation to the unsubstituted pyridine, and this can be taken as very probable because of the dominant influence of the nitrogen atom as a member of the pyridine ring.

If the same considerations are applied to pyridine carboxylic acids, we reach the conclusion that the carboxyl groups in the 2-, 4-, and 6-positions will show a greater tendency to release protons than the carboxyl groups in the 3- and 5-positions owing to the greater inductive effect which they undergo.

Adequate experimental determinations to establish this tendency will undoubtedly enable us not only to check the above conclusion, but will also provide a new method for the study of the relative distribution of electrons over the pyridine nucleus.

Taking the example of pyridine monocarboxylic acids (picolinic, nicotinic, and isonicotinic acids) for which there is also spectral evidence that the presence of a carboxyl group does not essentially change the original distribution of electrons over the unsubstituted pyridine nucleus (3), the above conclusion on the tendency of their carboxyl groups to release protons should mean that picolinic and isonicotinic acids are stronger acids than nicotinic acid.

However, the present results of the determination of dissociation constants as a standard for measuring the strength of the pyridine monocarboxylic acid mentioned above, obtained by potentiometric titration (4) and spectrophotometric examination of their aqueous solutions (5), are not in agreement with these anticipations.

The highest value for the dissociation constant was obtained for nicotinic acid, which should mean that it is a stronger acid than picolinic and isonicotinic acids.

As shown by Jaffé (6) and Stephenson and Sponer (3) this disagreement between experimental results and the theoretical predictions mentioned above is a result of incorrect interpretation of the thermodynamic dissociation constants of these acids K_a and K_b, made by earlier authors (5). In calculating the thermodynamic dissociation constants on the basis of the measured apparent dissociation constants K_1 and K_2 the earlier authors defined the latter as if the undissociated molecular species of these acids consist of neutral, uncharged

molecules, without paying attention to the possibility of the formation of zwitterions.

However, Jaffé (6) concluded and Green and Tong (7) and Stephenson and Sponer (3) proved in their spectrophotometric examinations that the above pyridine monocarboxylic acids, which in aprotic solvents and in absolute alcohol are really found in the form of undissociated neutral uncharged molecules (3), in an aqueous solution at the isoelectric point are mostly found in the zwitterion form. Relying on this in their calculation of thermodynamic dissociation constants of these acids Stephenson and Sponer (3) obtained the values whose relative ratio is in agreement with that expected on the basis of the above discussed theoretical assumptions.

It should also be noted that their calculations of the dissociation constant are only approximately correct because they neglected the presence of the neutral uncharged particles among undissociated molecules, taking the undissociated particles exclusively as zwitterions. The fraction of neutral, uncharged molecules, although comparatively small, is different in these three acids, and as shown by Stephenson and Sponer (3) can be only approximately evaluated by spectrophotometric measurements.

Because of this we were of the opinion that for the comparison of the influence of electric effects which carboxyl groups of pyridine carboxylic acids undergo, it will be interesting to investigate some other reaction of these acids in which the reactivity of their carboxyl groups would be also directly dependent on the influence of electric effects which these groups undergo, so that the rate constant of this reaction could serve as a quantitative standard for the relative ratio of these effects. As has already been emphasized, these effects vary with the position of the carboxyl group in relation to the nitrogen atom of the pyridine nucleus.

The reaction with diphenyldiazomethane (*DDM*) was selected as the reaction most adequate for the solution of the problem.

DDM reacts with acids in non-hydroxylic solvents and decomposes by liberating nitrogen and forming the corresponding benzhydryl ester (8—10). The rate of this reaction could easily be measured by determining the amount of nitrogen liberated during the reaction. It was established in this way that the rate of the reaction depends on the strength of the acid (8).

A more precise method of measuring the rate of the decomposition of *DDM* which consists in following the decrease of its concentration by the spectrophotometric method (also applied by Roberts and co-workers (11-15) enabled us to study the kinetics and mechanism of this reaction in greater detail. In ethanol as a solvent the reaction is more complex because *DDM* also reacts with ethanol forming larger or smaller amounts of ethylbenzhydryl ether, depending on the nature of the acid which also acts as a so-called general acid catalyst (12—14). The reaction in ethanol is strictly first order for *DDM* at any acid concentrations, while in the wide range of acid concentrations it is also first order for the acid — so that the over-all rate of the reaction is second order (12—14). If the acid is present in large excess so that kinetics of pseudo-first order is observed, the slopes of the straight line plots obtained by the presentation of the logarithm of the *DDM* concentration *vs.* time are strictly dependent on the initial concentration of the acid (13, 14).

According to the mechanism of this reaction as proposed by Roberts and co-workers (14) if the acid takes part in the reaction directly, the slow step determining the rate of reaction is the migration of protons from the acid to the diazocarbon,

$$X-H + \underset{\displaystyle C_6H_5}{\overset{\displaystyle C_6H_5}{\overset{|}{C}}}{-}N{=}N \xrightarrow{\text{slow}} X^- \quad . \; . \quad H-\underset{\displaystyle C_6H_5}{\overset{\displaystyle C_6H_5}{\overset{|}{C}}}{-}N{\equiv}N \xrightarrow{\text{fast}} X-\underset{\displaystyle C_6H_5}{\overset{\displaystyle C_6H_5}{\overset{|}{C}}}{-}X + N_2$$

and also, if the acid acts as a general acid catalyst (the catalitic action of undissociated acid) according to the generally accepted view (16), the release of protons is again a step determining the rate of reaction. It follows that the rate of this reaction can be taken as a standard of the tendency of the given acid to release protons.

Indeed, by studying the reaction kinetics of *DDM* with a series of *m*- and *p*-substituted benzoic acids Roberts and co-workers (11) established a parallelism between the reactivity of these acids and their acidity. They came to the same conclusion by measuring ionization constants and the reactivity of a series of 4-substituted bicyclo (2. 2. 2.) octane-1--carboxylic acids toward *DDM* (15). These measurements were made to establish and compare the electrical effects provoked by the presence of different substituents in the

benzene nucleus, and in the system of bicyclooctanes, respectively, which the carboxyl groups of these acids undergo. Thus when deciding on this reaction our aim was the same as that of the scientists mentioned above.

Although our original aim was to compare the reactivity of all the three pyridine monocarboxylic acids with *DDM* under conditions in which the reactions of these acids with *DDM* take place as reactions of the pseudo-first order, i. e. when the concentration of the acid is ten times that of *DDM*, so that the slopes of the linear plots in the diagram of the *DDM* concentration plotted against time, at equal initial acid concentrations, could be used as a standard for the comparison of their reactivity. Because of the very low solubility of isonicotinic acid in ethanol our examinations had to be limited to picolinic and nicotinic acids. In addition, the reactivity of quinolinic acid and both of its half-esters: the alpha- and beta-methyl esters of quinolinic acid with *DDM* were studied under the same conditions.

Experimental

The course of the reaction was followed with a *Hilger Uvispek* spectrophotometer by measuring the rate of decrease in the *DDM* concentration using the method described by Roberts and co-workers (11). As a standard for a change in the *DDM* concentration the change in absorption was measured at 525 mμ with time. All measurements were made at $30 \pm 0.2^{\circ}$C. All the solutions were thermostated for at least one hour before the measurements started. Twenty-five milliliters of 0.06 E solution of the acid and 25 ml 0.006 E solution of *DDM* were mixed. Zero-time was taken as the moment of mixing of the solutions. The solvent used was absolute ethanol. Because of the high rates of the reactions observed, the course of the reaction was followed in one sample of the solution. A constant temperature during the reaction was maintained by thermostating the spectrophotometric cell. A possible change in the *DDM* concentration caused by the evaporation of the solvent was prevented by covering absorption cells. Since in all these measurements the acid concentrations were ten times higher than the *DDM* concentration, the kinetics observed was of pseudofirst order. The diagram of the logarithm of absorption A plotted *vs.*

time resulted in a straight line whose slope represents the rate constant of the reaction of the pseudo-first order.

$$\frac{\log A_0 - \log A}{0.4343 \cdot t} = k$$

To make the values plotted on the ordinate positive in all cases, the value *log* A+2 was plotted instead of the logarithm of absorption. This, however, did not affect the slope of the straight lines obtained experimentally. By dividing the pseudofirst-order rate constants by the initial acid concentration we obtained the values for the second-order rate constants. The influence of the solvent on the absorption was eliminated by taking the absorption of the solvent as the relative zero in each reading. Determinations of the rate of the reaction for each acid examined were repeated until perfect reproducibility was attained. Before each new determination the acid was recrystallized.

Materials

The absolute ethanol used in these experiments, free of acids and ketones, was obtained by the ordinary procedure (17).

Diphenyldiazomethane was synthetized according to the method described in *Organic Syntheses* (18).

The pyridinecarboxylic acids examined were supplied by the firm *Leight*. They were purified by recrystallization either from alcohol or water.

Monomethyl esters of quinolinic acid were synthetized according to Kirpal (19).

Results and discussion

PICOLINIC ACID

t min	1	2	3	4	5	6	7
A	0.233	0.209	0.180	0.157	0.138	0.117	0.102
log A+2	1.37	1.32	1.26	1.20	1.14	1.07	1.01

t min	8	9	10	11	12	13	14
A	—	0.075	0.064	0.058	0.050	0.043	0.038
log A+2	—	0.88	0.81	0.76	0.70	0.63	0.58

From these results the pseudofirst-order rate constant was calculated statistically:

$$k' = 0.143 \text{ min}^{-1}$$

By division by the initial concentration of the acid in the reaction mixture (0.03 M) the second-order rate constant was obtained:

$$k_2 = 4.77 \text{ liter mole}^{-1} \text{ min}^{-1}$$

NICOTINIC ACID

t min	4	5	6	7	8	9	10	11	12	13	14
A	0.360	0.317	0'277	0 242	0.210	0.185	0.151	0.141	0.124	0.110	0.097
log A+2	1.55	1.50	1.55	1.38	1.32	1.26	1.18	1.15	1.09	1.04	0.99

$k' = 0.132 \text{ min}^{-1}$ or,
$k_2 = 4.4 \text{ liter mole}^{-1} \text{ min}^{-1}$

QUINOLINIC ACID

t min	1	2	3	4	5	6
A	0.319	0.181	0.100	0.055	0.031	0.019
log A+2	1.50	1.26	1.0	0.74	0.49	0.28

$k' = 0.568 \text{ min}^{-1}$ or,
$k_2 = 37.86 \text{ liter mole}^{-1} \text{ min}^{-1}$
$k_2 = 18.93 \text{ liter mole}^{-1} \text{ min}^{-1}$

ALPHA-ESTER OF QUINOLINIC ACID

t min	2	3	4	5	6
A	0.212	0.134	0.082	0.052	0.033
log A+2	1.33	1.13	0.92	0.72	0.52

$k' = 0.466 \text{ min}^{-1}$ or,
$k_2 = 15.53 \text{ liter mole}^{-1} \text{ min}^{-1}$

BETA-ESTER OF QUINOLINIC ACID

t min	1	2	3	4	5
A	0.245	0.110	0.047	0.020	0.01
log A+2	1.39	1.04	0.67	0.30	0.0

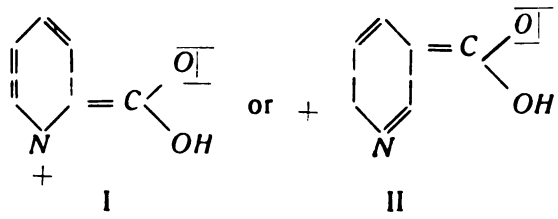
$k' = 0.810 \text{ min}^{-1}$ or,
$k_2 = 27.0 \text{ liter mole}^{-1} \text{ min}^{-1}$

It is evident from our results showing the rate of the reaction of picolinic and nicotinic acids with *DDM* that picolinic acid reacts faster. This means that the carboxyl group in the alpha position of the pyridine nucleus is more reactive than the carboxyl group in the beta position. This leads us to the conclusion that the alpha-carboxyl group undergoes a stronger inductive effect than the beta-carboxyl group. This is in turn explained by a higher partial positive charge on the alpha-C-atoms, which completely agrees with our earlier conclusion in connection with the results of the reactions of the anhydrides of quinolinic acid with amines (1) and also with the theoretical predictions of the electric effects induced by the presence of the nitrogen atom in the pyridine ring, and with the calculated values for the electron density distribution in the pyridine ring (2).

Furthermore, these results strengthen Jaffé's conclusion (6) that the interpretation of the thermodynamic dissociation constants of these acids given by earlier authors (5), who calculated them on the basis of their spectrophotometric measurements, was incorrect. Our measurements disagree with theirs, confirming at the same time the conclusion reached by Stephenson and Sponer (3) that picolinic acid is stronger than nicotinic acid. These authors came to this conclusion by calculating the dissociation constants of these acids on the assumption that in an aqueous solution they are present in zwitterion form. Thus our results confirm indirectly the view on the zwitterion structure of pyridine monocarboxylic acid in water solution.

As might be expected, the second-order rate constant of the reaction k_2 of *DDM* with quinolinic acid is higher than the corresponding constants of picolinic and nicotinic acids, because quinolinic acid is dibasic. However, even when calculated at the same concentration of carboxyl groups (k_2'') this constant is still considerably higher than the rate constants of the reaction of any of the monocarboxylic acids than we have examined.

This behavior can be explained by the assumption that in addition to the inductive effect tending to increase the acidity of picolinic and nicotinic acids, i. e. to increase the reactivity of their carboxyl groups, there is also the resonance effect in which among other the following resonating structure can take part:



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The resonating structures counteract the inductive effect and reduce its action.

In quinolinic acid steric effects hinder coplanar configuration between the pyridine nucleus and carboxyl groups and thereby also the resonance effect in which the above structures can participate, so that the inductive effect is fully pronounced, and consequently also the acidity, i. e. the reactivity of the carboxyl groups, is fully manifested.

In a similar way we can explain the higher reactivity of carboxyl groups in the half esters of quinolinic acid in comparison with the reactivity of the carboxyl groups of picolinic and nicotinic acids. In these half esters the steric effects also hinder the coplanar configuration between carboxyl groups and the pyridine nucleus as well as the resonance effect which would reduce the influence of the inductive effect so that in these half esters the reactivity of carboxyl groups is also fully manifested.

Our earlier statement that the alpha-carboxyl group undergoes a stronger inductive effect than the beta-carboxyl group is here confirmed in an even more striking way. A much greater difference in values for k_2 between the alpha- and beta-esters of quinolinic acid than between nicotinic and picolinic acid can be explained by a greater participation of the resonating structure (I) in picolinic acid than the resonating structure (II) in nicotinic acid.

Comparison of the values of the rate constants for the reactions of quinolinic acid (k_2'') and its half esters (k_2) when the concentration of carboxyl groups is equal shows that the value for quinolinic acid lies between the corresponding values for its half esters. This is also in agreement with the predictions, because k_2'' in quinolinic acid reflects in fact the statistical mean reactivity of both carboxyl groups.

CONCLUSION

The rates of reactions of picolinic, nicotinic and quinolinic acids and also of the α- and β-methyl esters of quinolinic acid with diphenyldiazomethane (*DDM*) in ethanol as a solvent have been measured by following the decrease in the *DDM* concentration with a spectrophotometer. The second-order rate constants for the reactions of all the acids mentioned above have been determined. A theoretical interpretation of the results was offered.

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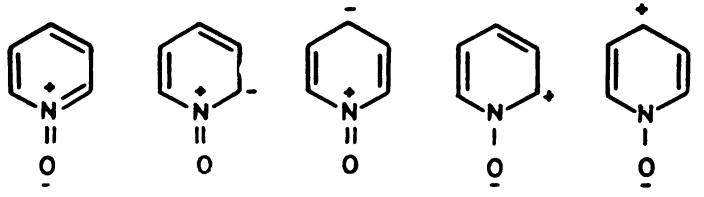
REACTIVITY OF PYRIDINECARBOXYLIC ACID 1-OXIDES. I. THE REACTION OF PYRIDINE MONOCARBOXYLIC ACID 1-OXIDES AND DIPICOLINIC ACID 1-OXIDE WITH DIPHENYLDIAZOMETHANE

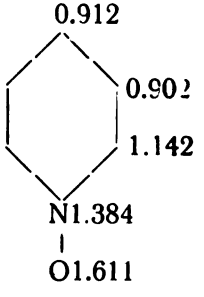
by

Ž. D. TADIĆ, DJ. M. DIMITRIJEVIĆ, and M. MIŠIĆ

Distribution of electrons and reactivity of individal positions of the pyridine 1-oxide nucleus have been studied by many scientists. Determination of the dipole moments (1, 2) and the possibility of electrophilic and nucleophilic substitution (3, 4) have shown that the nucleus of pyridine 1-oxide, depending on the substituents and the nature of the agent used, can be polarized in both directions, i. e. that the $\geqslant N^+ - O^-$ group can have the function of an electron donor and an electron acceptor. On this basis the following resonating structures have been proposed as possible:



By calculating Coulomb's integral Jaffé (5) gave the following values for electron distribution at various positions of the nucleus. These are in accord with the resonating structures cited above.



The study of the infra-red spectra of the substituted pyridine 1-oxides (6, 7) show that the electron density is higher at the 4-position than at the 3-position, which is in agreement with the theoretically calculated values for electron densities (5), dipole moments (1, 2), UV spectra (10) and the data on the chemical reactivity (3, 4). On studying the spectra of cyanopyridine 1-oxides A. R. Katritzky (7) concluded that depending on the positions in the nucleus the electron density decreases in the following order $4 \gg 3 \sim 2$. However, it was emphasized that the ratio between electron densities at the 3- and 4-positions could also be expected from other considerations, while the deduction of any conclusions whatever for the 2-position is very unsafe owing to the possible influence of steric and inductive effects.

Recent determinations of nuclear magnetic resonance spectra of 4-substituted pyridine 1-oxides (8) do not give a clear picture. While the first results show that electron density is higher at the 2-position than at the 3-position (which is not in agreement with the calculated values (5, 9) later measurements cited in the same paper give the order $2 < 3 \sim 4$ agreeing with other papers of the same authors (6, 10, 11).

Pyridinecarboxylic acid 1-oxides have been very little investigated. Except for the papers dealing with the synthesis of monocarboxylic acid (12, 13, 14, 15), the determination of their spectra (22), and several separate studies of esters (2, 16) almost no other data can be found in the literature.

The results obtained from investigation of the rate of the reaction between pyridinecarboxylic acid and diphenyldiazomethane (17) and the confirmation of the theoretical assumptions on the distribution of electrons over the pyridine nucleus have pointed to the possibility of obtaining interesting data on the nucleus of pyridine 1-oxide by using a similar reaction. It was to be expected that a definite difference in the electron density over the pyridine 1-oxide nucleus and the pyridine nucleus would cause a difference in the acidity of pyridinecarboxylic acid 1-oxides due to the position of the carboxyl groups in the nucleus and also a difference in their acidity in comparison to the corresponding pyridinecarboxylic acid. We believed that by relying on the experience of scientists who have studied the reactions of pyridinecarboxylic acids with diphenyldiazomethane (17) in ethanol as a solvent (17) and by using the same techniques we should be able to

deduct conclusions on the reactivity of pyridinecarboxylic acid 1-oxides based on the values of the reaction rate constants. Since the velocity of this reaction increases with decreasing electron density on the carbon atom to which the carboxyl group is attached (17, 18)), it is possible to obtain in this way an insight into the relative distribution of electrons.

In these investigations the rate constants for the reactions of diphenyldiazonmethane with picolinic acid 1-oxide, nicotinic acid 1-oxide, isonicotinic acid 1-oxide, and dipicolinic acid 1-oxide have been determined.

Experimental

The reaction rates of the acids that we examined were determined spectroscopically according to the method applied by Roberts and co-workers (18) for benzoic acid. This method was also used in our earlier paper (17). The decrease of diphenyldiazomethane concentration (0.003 E) in the mixture with the investigation carboxylic acid 1-oxide at a concentration of 0,03 E was measured. The reaction mixture of this composition was obtained for picolinic acid 1-oxide and dipicolinic acid 1-oxide by mixing 25 ml 0.006 E solution of diphenyldiazomethane in absolute ethanol and 25 ml 0.06 E solution of the acid also in absolute ethanol. Owing to the somewhat lower solubility of nicotinic acid 1-oxide and isonicotinic acid 1-oxide, 10 ml 0.015 E solution of diphenyldiazomethane and 40 ml 0.0375 E solution of the acid were used. Because of the high reaction rate, all the observations were made on one sample. Readings were made at a wavelength of 525 mμ and at a temperature of 30 ± 0.2^{0}C. The instrument used was of the *Hilger-Uvispek* type for the UV and visible ranges with a built-in thermostat. The reaction rate for each acid was determined by repeating the measurements until the results were entirely identical. Before each new measurement the acids were recrystallized.

Since in all these measurements the acids were ten times as concentrated as diphenyldiazomethane, the observed kinetics was of the pseudofirst order. The diagrams of the logarithm of optical density plotted *vs.* time shown in Figure 1 represented a straight line. From the expression for the pseudofirst order rate constant we obtain

$$\frac{\log A_0 - \log A}{0.4343 \cdot t} = k'$$

The slope of this straight line gives the rate constant of the reaction of pseudofirst order. To make the values plotted on the ordinate positive in all cases the value *log* A was increased by the constant value 2, which, however, did not influence the slope of the straight lines obtained experimentally. The corresponding second-order rate constants k_2 were obtained from the calculated constants of pseudofirst order by division by the initial acid concentration.

(1) PICOLINIC ACID 1-OXIDE

t Min	1	2	4	6	8	10	12	14
A	0.556	0.548	0.535	0.521	0.507	0.494	0.480	0.468
log A+2	1.745	1.738	1.728	1.717	1.705	1.694	1.682	1.671

From these data the pseudofirst-order rate constant was calculated statistically.

$$k' = 0.00132 \text{ min}^{-1}$$

By division by the initial concentration of the acid in the reaction mixture (0.03 E) the second-order rate constant was calculated:

$$k_2 = 0.044 \text{ mole}^{-1} \text{ liter min}^{-1}$$

(2) NICOTINIC ACID 1-OXIDE

t min	1	2	3	4	5
A	0.338	0.206	0.118	0.066	0.039
log A+2	1.530	1.314	1.072	0.820	0.590

$k' = 0.546 \text{ min}^{-1}$
$k_2 = 1.82 \text{ mole}^{-1} \text{ liter min}^{-1}$

(3) ISONICOTINIC ACID 1-OXIDE

t min	1	2	3	4	5	6	7	8
A	0.382	0.285	0.196	0.135	0.093	0.063	0.045	0.033
log A+2	1.582	1.455	1.292	1.130	0.968	0.808	0.654	0.518

$k' = 0.360 \text{ min}^{-1}$
$k_2 = 1.16 \text{ mole}^{-1} \text{ liter min}^{-1}$

(4) PICOLINIC ACID 1-OXIDE

t min	1	1.5	2	2.5
A	0.075	0.030	0.015	0.011
log A+2	0.875	0.475	0.175	0.040

$k' = 1.61$ min^{-1}
$k_2 = 53.6$ mole^{-1} liter min^{-1}

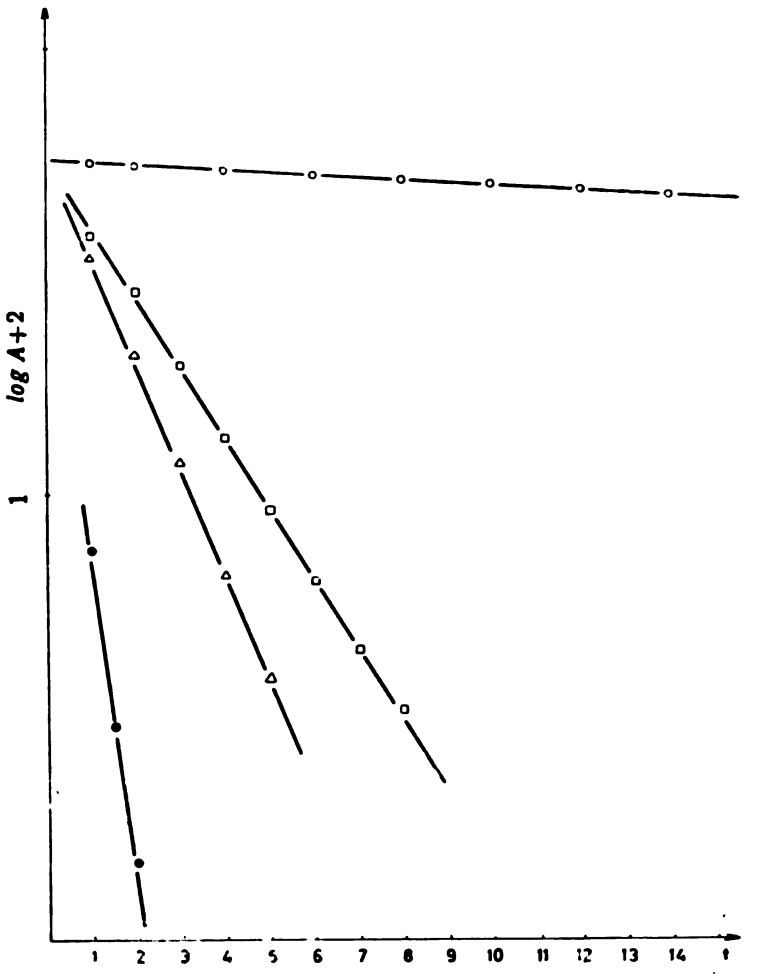


Fig. 1

The diagram in Figure 1 shows the straight lines obtained by the statistical study of experimental data.

Materials

Absolute ethanol, free of acids and carbonyl compounds was obtained by the standard procedure (19).

Diphenyldiazomethane was synthesized according to the technique described in *Organic Syntheses* (20).

Pyridine monocarboxylic acid 1-oxides were synthetized in the manner found in the literature (12, 13, 14, 15), recrystallized from water and then several times from absolute alcohol.

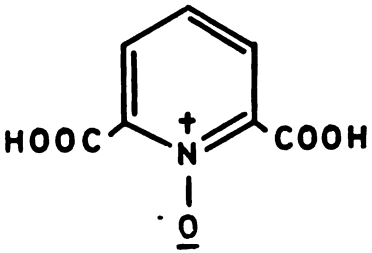
Dipicolinic acid 1-oxide was synthesized according to the method developed in our laboratory and recrystallized from water and absolute alcohol.

Discussion

It is evident from the rate constants obtained for the reactions of pyridine monocarboxylic acid 1-oxides with diphenyldiazomethane that the reactivity of the carboxyl group in the 3-position (nicotinic acid 1-oxide) is approximately 1.5 times as great as the reactivity of the carboxyl group in the 4-position (isonicotinic acid 1-oxide) and approximately 400 times as great as the reactivity of the carboxyl group in the 2-position (picolinic acid 1-oxide). Since the decrease in electron density at the corresponding position in the nucleus causes an increase in acidity, or in the case of ethanol solution an increased reactivity of the carboxyl group, according to our measurements, the electron density over the nucleus should decrease in the following order $2 \gg 4 > 3$. This conclusion is in agreement with the values calculated theoretically, and for the 3- and 4-positions also with the experimental results of the electrophilic and nucleophilic substitution, as well as with the measured dipole moments. However, no agreement could be established with the results of measurements of the infra-red spectra and the nuclear magnetic resonance spectra. It is interesting to note that Jaffé (5) considered the result calculated for the electron density at the 2-position disappointing because of disagreement with data on electrophilic substitution, and cited the possible reasons for errors. This result, however, agrees with our measurements.

The exceptionally low reactivity of the carboxyl group in the α-position of the pyridine 1-oxide nucleus can be a

consequence not only of a relatively high electron density but also of some other effect which should be taken in consideration. The carboxyl group reacting with diphenyldiazomethane is in this case in the ortho-position relative to the $\geqslant N^+—O^-$ group, so that, in addition to electrical effects, a certain interaction between these two groups and a possible steric effect may be assumed as the reason for the reduced reactivity of the carboxyl group. The inactivity in the α-position with respect to the electrophilic and nucleophilic substitution and a general uncertainty in deducing conclusions for this position is explained in the literature by the influence of steric and inductive effects (3, 4, 6, 7). To establish whether in this case a definite effect is in question, we measured for comparison the rate of the reaction between dipicolinic acid 1-oxide and diphenyldiazomethane under the same conditions as above. Since the $\geqslant N^+—O^-$ group is assumed to interfere with the reaction of both carboxyl groups we should expect the same steric effects in this acid as in picolinic acid 1-oxide. A very high rate constant for this acid shows that the low reactivity of picolinic acid 1-oxide is not due to steric effect. However, a possible interaction between the carboxyl



and $\geqslant N^+—O^-$ groups might be limited to only one of the carboxyl groups, so that the other be free to enter into reaction. We believe that further investigations of pyridine carboxylic acid 1-oxides and their half esters will solve this problem.

CONCLUSION

Rate constants for the reaction of picolinic, nicotinic, and isonicotinic acid 1-oxides and dipicolinic acid 1-oxide with diphenyldiazomethane in ethanol as solvent were determined. The obtained rate constants afford evidence that

the electron availability at the pyridine 1-oxide ring positions decreases in the following order $2 \gg 4 > 3$. An exceptionally high rate constant for dipicolinic acid 1-oxide indicates that the low reactivity of picolinic acid 1-oxide is not due to steric effect.

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CONDENSATION PRODUCTS OF α- AND β-ACETYLPYRIDINE WITH ETHYL OXALATE

by

J. JANCULEV and B. PODOLESOV

In connection with our earlier works (1, 2) we have synthesized ethyl esters of α- and β-pyridineoyl pyruvic acids by condensing (3) α- and β-acetyl pyridine (4, 5) with diethyl oxalate in proportion 1 : 1 mole, in the presence of sodium ethylate as condensation agent.

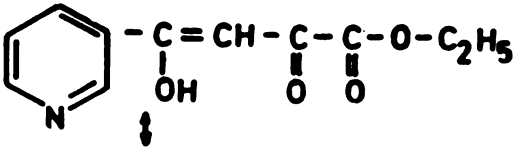
By decomposing the α- and β-sodium salts of the condensation products with dilute hydrochloric acid we obtained the α-ethyl ester of pyridineoyl pyruvic acid in the form of colorless crystals melting at 72—73°C, and the β-ethyl ester of pyridineoyl pyruvic acid, also in the form of colorless crystals, melting at 68—69°C (in the literature 66°C (6)).

When, however, the decomposition of the β-sodium salt was carried out with glacial acetic acid in aqueous solution and at elevated temperature, pink crystals, melting at 187—189°C, were separated. This phenomenon could not be observed in the decomposition of the corresponding α- and γ-sodium salts. It seems likely that this difference in behavior depends on the different properties of the substituents in the α- and γ-positions with respect to the properties of the substituents in the β-position of the pyridine nucleus. On longer standing the physical properties of the β-compound, melting at 68—69°C changed completely: the colorless and glittering crystals became gradually pink, the solubility in n-hexane disappeared, and the compound could be recrystallized from water; thereby its m. p. rose to 187—189°C. The product obtained showed no depression in milting point when mixed with the β-ethyl ester of pyridineoyl pyruvic acid, melting at 187—189°C, obtained from the β-sodium salts by decomposition with glacial acetic acid. The results of elementary analysis also showed that both compounds were identical. It is pos-

sible that the β-ester, melting at 68—69°, passes over from a labile into a more stable form.

The IR spectra* of both compounds were identical. They exhibited an associated —OH valence vibration at 3,450 cm^{-1}, the absorptions of the chelated OH-groups of β-diketones at 2,600—2,500 cm^{-1}, the absorptions of the immonium structure of the tertiary bases at 2,125 cm^{-1}, the C=O valence vibration at 1,775—1,680 cm^{-1}, the C=O absorptions of the enolized β-dicarbonyl group between 1,625 and 1,540 cm^{-1}.

The study of the IR spectra revealed that both substances are present in enolized and chelated forms. The chelation involved not only one carbonyl group but also the tertiary nitrogen of the ring.



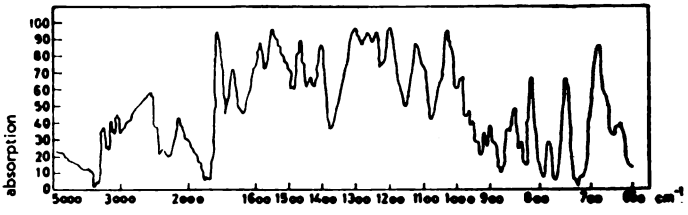
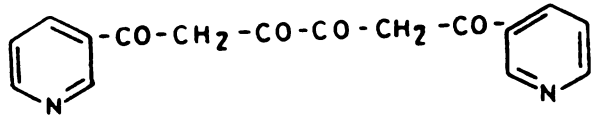


Fig. 1. — The infra-red spectrum of the β-ethyl ester of pyridineoyl pyruvic acid. (Taken with a *Beckman spectrophotometer Model IR 4* by using the *KBr-technique.)*

The condensation of two moles of β-acetyl pyridine with one mole of ethyl oxalate in the presence of sodium ethylate as condensation agent gave rise to 1,6-Di (β-pyridyl)-1, 3, 4, 6-hexanetetrone.

The tetraketone was identified by means of quinoxaline and dipyrazol derivatives.



* Thanks are due to Dr. M. Hanack, Pharm. Chem. Institute of the University of Tübingen, for measurement and interpretation of the IR spectra.

Experimental

Ethyl ester of α-pyridineoyl pyruvic acid. A mixture of 4.84 g (0.04 mole) α-acetylpyridine and 5.84 g (0.04 mole) ethyl oxalate was added dropwise into a cold ethereal solution of sodium ethylate prepared from 1.84 g (0.04 mole) absolute alcohol (7) and 0.92 g (0.04 mole) sodium. After three days the reaction mixture was filtered off yielding 8.5 g of crude sodium salt of the α-ethyl ester of pyridineoyl pyruvic acid. The sodium compound was made into a paste by the addition of 40 ml water and acidified with dilute hydrochloric acid to pH 7. The thick precipitate obtained was filtered off, dried on a porous plate, and recrystallized from n-hexane. Colorless needles melting at 72—73°C were obtained. The yield was 3 g or 34.2%.

Anal. Subst. 10.80 mg: CO_2, 23.54 mg, H_2O, 4.47 mg.
Subst. 3.02 mg: N_2, 0.166 ml (24°; 759 mm).
Calc'd for $C_{11}H_{11}O_4N$ (221.20): C, 59.72; H, 5.01, N, 6.33.
Found: C, 59.49; H, 4.63; N, 6.30.

Ethyl ester of β-piridineoyl pyryvic acid. The light yellow sodium salt of this ester was prepared with β-acetylpyridine in the same way as above. The compound was sparingly soluble in water. The yield was 10 g.

The sodium compound (2 g) was stirred in 10 ml of water and acidified with hydrochloric acid to pH 7. The bulky precipitate was filtered off and dried. After recrystallization from n-hexane long colorless needles, melting at 68—69°C, separated. The yield was 0.7 g or 40.0%.

Anal. Subst. 21.60 mg: CO_2, 47.60 mg; H_2O, 9.70 mg.
Subst. 4.06 mg: N_2, 0.235 ml (28°; 759 mm).
Calc'd for $C_{11}H_{11}O_4N$ (221.20): C, 59.72; H, 5.01, N, 6.33.
Found: C, 60.10; H, 5.02; N. 6.55.

On the other hand, when this sodium compound was dissolved in hot glacial acetic acid and on cooling diluted with water, a bulky precipitate was formed. It crystallized from water in pink needles melting at 187—189°C. The yield was 3.7 g or 74.7%.

Anal. Subst. 4.138 mg: CO_2, 9.051 mg; H_2O, 1.852 mg.
Subst. 1.499 mg: N_2, 0.215 ml (26°; 745 mm).
Calc'd: C, 59.72; H, 5.01; N, 6.33.
Found: C, 59.69; H, 5.00; N, 6.29.

1,6-Di-(β-pyridyl)-1,3,4,6-hexanetetrone. In a 200 ml round-bottomed flask supplied with a reflux condenser and calcium chloride tube, 80 ml of absolute ether and 2.3 g (0.1 mole) of thin sodium slices were placed and then more ether added in order to cover the sodium completely. Next, 4.6 g (0.1 mole) absolute alcohol (7) was added dropwise through the condenser, and the mixture was allowed to stay for 12 hours at room temperature. The reaction mixture was then cooled with ice-cold water and a solution of 21.1 g (0.1 mole) β-acetylpyridine and 7.3 g (0.05 mole) freshly distilled oxalate in 30 ml absolute ether was added dropwise for two to three minutes while the mixture was shaken. To avoid overheating, the flask should be cooled in ice water if necessary. After the completion of the reaction the mixture was allowed to stand for three to four days at room temperature. During this time its color gradually changed from yelow to green. The sodium salt was filtered by suction and washed twice with 20 ml absolute ether to remove the unreacted ethyl oxalate and β-acetyl pyridine. The dark yellow sodium salt was dried in a desiccator.

The sodium salt was dissolved in water and the solution made neutral with glacial acetic acid. The separated crude tetraketone was filtered off and dried on a porous plate. The yield was 7.5 g crude tetraketone or 50.6%

The tetraketone was readily soluble in acids, alkalis, and sodium carbonate solution. An alcoholic solution of tetraketone gives with a drop of ferric chloride solution a brownish red color. Recrystallization from glacial acetic acid gave yellow needles melting at 210°C with decomposition.

Anal. Calc'd for $C_{16}H_{12}N_2O_4$ (296.28): C, 64.84; H, 4.08; N. 9.46.
Found: C, 64.97; H, 4.01; N, 9.58.

Quinoxaline derivative of 1,6-Di-(β-pyridyl)-1,3,4,6-hexanetetrone. The quinoxaline derivative was obtained by heating equimolecular amounts of 1,6-Di-(β-pyridyl)-1,3,4,6-hexanetetrone and o-phenylenediamine in the presence of glacial acetic acid for one hour. On cooling needle-like crystals were obtained. After recrystallization from absolute alcohol the orange red crystals melted at 202—203°C.

Anal. Calc'd for $C_{22}H_{16}N_4O_2$ (368.38): C, 71.72; H. 4.38; N. 15.21.
Found: C, 71.70; H, 4.28; N, 15.31.

Dipyrazol derivative of 1,6-di-(β-pyridyl)-1,3,4,6-hexanetetrone. To a solution of 0.2 g 1,6-di-(β-pyridyl)-1,3,4,6-hexane-

tetrone in glacial acetic acid or ethanol an equimolecular amount of hydrazine hydrate was gradually added and the reaction mixture was constantly shaken and cooled. The flask was then supplied with a reflux condenser and warmed on a water bath for an hour and a half; then, the reaction mixture was poured into ice-cold water. After vigorous shaking 5,5'-di-(β-pyridyl)-dipyrazolyl (3,3) od 3,3'-di-(o-pyridyl)-dipyrazolyl (5,5') precipitated in the form of flakes. After recrystallization from acetoacetic ester the yellow precipitate melted at 330°C. The yield was 0.17 g.

Anal. Calc'd for $C_{16}H_{12}N_6$ (288.31): C, 66.65; H, 4.20; N, 29.15.
Found: C, 66.26; H, 4.51; H, 29.20.*

CONCLUSION

The ethyl ester of α-pyridineoyl-pyruvic acid was obtained by the condensation of α-acetyl pyridine and diethyl oxalate in the proportion 1 : 1 in the presence of sodium ethylate. The colorless, needlelike crystals melted at 72—73°C. The condensation of equimolecular amounts of β-acetylpyridine and diethyl oxalate in the presence of sodium ethylate gave a sodium compound of the ethyl ester of β-pyridineoyl pyruvic acid. After decomposition of the sodium compound with dilute hydrochloric acid and crystallization from n-hexane the product in the form of colorless needles melted at 68—69°C. If recrystallized from water, pink needle-like crystals were obtained. After being stored for 18 months the product melted at 187—189°C. If the decomposition of the sodium compound of the ethyl ester of β-pyridineoyl pyruvic acid was brought about by heating with glacal acetic acid and recrystallized from water, the product also had the form of pink crystals and melted at 187—189°C. When the products of these two reactions were mixed, they showed no depression in melting point, i. e. m. p. remained 187—189°C. The IR spectra of these two compounds were identical. On the basis of the IR spectra it could be concluded that the ethyl ester of β-pyridineoyl pyruvic acid, m. p. 187—189°C, has the enolic form.

The condensation of β-acetyl pyridine and diethyl oxalate in the proportion 2 : 1 in the presence of sodium ethylate

* We wish to express our indebtedness to E. Stevčeska (Mrs) of the same Institute for making the microanalyses.

yielded 1,6-di-(β-pyridyl)-1,3,4,6-hexanetetrone. For the identification of tetraketone its quinoxaline and dipyrazol derivatives were prepared.

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* Unreliable item. (Translator's note.)

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FATTY MATERIALS FROM THE NUT SEED *JUGLANS REGIA* L. IN THE PROCESS OF GERMINATION

by

MILAN O. MIRIĆ and ZORICA V. ĆUPIĆ

The metabolism of plant fatty materials has been extensively studied, but the mechanisms of the underlying biochemical reactions have not yet been fully explained. This might be understood because classical methods of fat analyses are rather inaccurate, complicated, and longlasting; therefore, many problems which refer to the biochemistry of lipids were left unsolved. The introduction of new physicochemical methods, and particularly the development of chromatographic methods, have offered the possibility of finding an answer to these problems in a much shorter time and with much more accuracy.

In this work we have attempted to obtain more detailed data about the degradation of nut oil in the process of germination by determining the ratio between individual fatty acids, unsaponifying materials, and vitamin E.

Experimental

Materials. The investigations were performed with nut fruits (*Juglans Regia* L.) which were left to germinate in moist sand at 25°C. The first germs appeared after twenty-three days. The analyses were performed on the twenty-eigth, thirty-fifth, and fifty-second days. Further investigations were not performed because the stalk was very well-developed and contained well-shaped leaves.

The analyses were performed with nut kernels which were dried in a vacuum drying oven (12 mm/Hg) at 50°C; the oil was extracted with petroleum ether (b. p. 40—60°) in a Soxleth apparatus, and the extracted mixture was measured.

Methods. The acid number and the nonsaponifying components were determined by the usual analytical methods. Vitamin E content was determined by the method of Emerie and Engel (1). The methyl esters of fatty acids were analyzed by means of gas chromatography, according to James and Martin (2), using the technique of Farquhar and coworkers (3). The stationary phase was diethylene glycol succinate 20% on "chromosorb" in steel spiral colomns of 3.08 m length and 4 mm in diameter; the mobile phase was pure nitrogen, and the flow rate was 60 ml per minute. The temperature of the column was 195°C. The apparatus "Aerograph-A-90", containing a catharometer as detector, was used.

Results

Table 1 shows the percentages of oil, nonsaponifying components, vitamin E, total fatty acids, and the values of the acid number. The percentages of the dry kernel matter, dry germ matter, and dry stalk matter are presented in Table 2. Table 3 offers the percentages of individual fatty acids.

Table 1

Date of the analysis	Oil %	Acid number	Total fatty acids %	Nonsaponifying components %	Vitamin E mg %
26. IX. 1961 Immediately after gathering	66.1	0.36	95.4	0.98	16
24. IV. 1962 Immediately after being left to germinate	64.3	0.59	94.7	0.97	10
On the twenty-eighth day of germination	53.2	4.5	94.5	1.02	9
On the thirty-fifth day of germination	41.7	5.1	92.0	2.7	60
On the fifty-second day of germination	31.8	0.8	91.4	3.3	76

Table 2

Date of the analysis	Kernel %	Germ and the stalk %	The height of the stalk in cm
Before germination	100	0	0
On the twenty-eighth day of germination	85.5	14.5	4.0 leafless
On the thirty-fifth day of germination	67.0	32.9	10—12 with fairly well-developed leaves
On the fifty-second day of germination	58.1	41.9	37—40 with well-developed leaves

Table

The ratio of individual fatty acids in %

Date of the analysis	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
26. IX. 1961 Immediately after gathering	14.8	2.2	12.7	64.5	4.9
24. IV. 1962 Immediately after being left to germinate	14.1	2.6	12.9	64.5	5.3
On the twenty-eight day of germination	11.8	1.3	17.8	65.0	3.2
On the thirty-fifth day of germination	13.5	2.1	15.2	63.8	5.2
On the fifty-second day of germination	11.3	2.3	16.7	63.3	5.8

Discussion

Very little is known about the chemical composition of fatty materials from the seeds of various plants in the process of their germination. Determinations of acid, saponification, and iodine number were performed but the obtained values varied in rather broad limits. It was not possible, on the basis of such uncomplete data, to observe all the chemical changes that take place in the process of seed germination.

There are only few data which relate to the composition of fatty materials from the nut seed in the course of germina-

tion, and for the most part these data represent the values of acid and iodine number with the changes of oil content (4, 5, 6).

The results of our analyses indicate that the oil content is only slightly decreased in the course of fruit storage (Table 1). However, during germination, a considerable loss of oil occurs, and the oil content falls to one-half of its original value on the fifty-second day. Similar results were reported by Skraup and Weber (5). In the course of storage, the acid number is only slightly increased, but during germination its value augments for the first thirty-five days, and then it diminishes. Desveaux and Kogane-Charles (7) obtained similar results with castor oil. The decrease of oil content in the course of germination is followed by the diminuation of the total kernel dry matter (Table 2), but the dry matter of the germ and the stalk rapidly increases.

The content of nonsaponifying component in oil which has been storaged for several months (Table 1) is almost constant; it is also constant in the oil of germinating nuts until the stalk is developed. When well-shaped leaves begin to appear, there is an increase of unsaponifying components which amounts to even 300%. Such an increase is perhaps only "apparent" due to the reduced gluceride content.

The content of vitamin E abruptly increases at the moment when the amount of nonsaponifying components is increased. No data are reported in the literature about vitamin E content in the course of germination, and our findings might be of interest for further investigation of the role which vitamin E plays in the process of germination.

The results illustrated in Table 3 show that the ratio of individual fatty acids is constant in the nonmetabolized part of the oil. These data indicate that selective mobilization on individual fatty acids does not occur in metabolic processes; fatty acids are uniformly consumed. Contrary to our findings, Weber (6) claimed that the degree of oil saturation varies in the course of germination. We have shown in our previous work that the ratio of individual fatty acids is constant during the germination of maise.

In this work we have also performed a partial analysis of fatty materials extracted from the germ. The isolated oil was found to differ from that of the kernel. It was established

that the germ contains 0.95 petroleum soluble materials which consists of 44% fatty acids and 32% nonsaponifying components. The analysis of methyl esters of isolated fatty acids shows that the acid mixture contains 32.9% palmitic, 6.7% oleic, 44.3% linoleic, and 6.3% linolenic acid. In fact, the germ forms its characteristic mixture of fatty acids; its degree of unsaturation is smaller than that of fatty acids extracted from the kernel.

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THE EFFECT OF INSULIN ON THE METABOLISM OF PROTEINS, LIPIDS AND GLUCIDES. VII. POLAROGRAPHIC ACTIVITY OF BLOOD SERUM PROTEINS IN SCHIZOPHRENIC PATIENTS, IN DEEP HYPOGLYCEMIC COMA EFFECTED BY INSULIN

by

J. J. BOJANOVIĆ, LJ. M. ŠEVALJEVIĆ, M. O. ĆORBIĆ

In investigating the changes in the blood serum proteins of patients in deep hypoglycemic coma effected by insulin we found a significant increase in the concentration of serum proteins, accompanied by a change in the relative ratios of individual electrophoretic fractions (1, 2, 3). We also established that the changes in the concentration of the α-amino acids and polypeptides of the serum are significant (1, 3). In order to obtain some insight into the nature of protein changes in insulin shock, we polarographically examined blood serum proteins prior to the injection of insulin and then in the deep coma provoked in schizophrenic patients. In these examinations native and denatured sera were used. The purpose of these experiments was to obtain information on whether any changes occur in the structure of the protein molecule of the blood serum and what is their extent. These changes might be expected as a consequence either of the specific character of the process bringing about the change in concentration of total proteins and the changes in the electrophoretic patterns, or of the direct action of insulin or other factors during shock.

Material and Methods

The investigations were made with sera of eight metabolically healthy shizophrenic patients (women aged 20–30) treated at the Neuropsychiatric Clinic of the Faculty of Medicine, Belgrade. The number of insulin shocks received prior to investigations varied from three to nine. The regime to which the

patients were subjected, the conditions under which the insulin shocks were effected, the drawing of blood and the preparation of sera were described in earlier papers (1, 4).

For these experiments the blood was taken just before the injection of insulin and two and a half hours after the application, i. e. twenty minutes after the patient had fallen into a deep coma. Polarographic investigation were made with native and denatured sera. By native serum is meant the serum obtained in the usual manner under conditions described above. Denatured serum was obtained by treating native serum with alkaline hydroxide. To 0.2 ml of native serum 0.3 ml of 0.3 N KOH was added and heated at 25°C for 30 minutes. The denaturation of the serum was made directly before the experiment.

Polarographic analyses. — The examinations were carried out with a *Radiometer Copenhagen PO 3h* apparatus. An aqueous solution of NH_3, NH_4Cl, and $[Co(NH_3)_6]Cl_3$ was used as basic electrolyte. In this solution the concentration of ammonia was 1 *N*, of ammonium chloride 0.1 *N*, and of cobalt hexamine chloride 0.001 M. The voltage of the current was 1—2 V, the sensitivity of the apparatus 1/5000, and the drop-time of mercury 3.5 seconds. The height of polarographic waves was measured from the plateau of the second cobalt wave to the maximum of the second protein wave and expressed in *mm*.

Total proteins. — The values for total proteins were obtained from the difference in the values for total nitrogen and the nitrogen of non-protein substances determined by the micromodification of *Kjeldahl*'s method (5).

Results and Discussion

The investigation of the polarographic activity of sera in metabolically healthy schizophrenic patients showed that the height of the second polarographic wave of the native serum in deep hypoglycemic coma is greater than before the injection of insulin. This increase is small but statistically significant (Table 1). The polarographic activity in the denatured serum is 52% higher than in the native serum (a highly significant change). However, there are no differences in the values for the denatured sera before insulin injection and in hypoglycemic coma.

Table 1

POLAROGRAPHIC ACTIVITY OF NATIVE AND DENATURED BLOOD SERUM PROTEINS IN SCHIZOPHRENIC PATIENTS (WOMEN) BEFORE INSULIN INJECTION AND IN DEEP HYPOGLYCEMIC COMA

Values Before/ in Coma	No of Cases	Polarographic Activity Heigh of the Second Polarographic Wave in mm								Increase in Polarographic Activity of Denatured in Relation to Native Proteins in %	Level of Significance
		Native Proteins				Denatured Proteins					
		Mean Value and Standard Deviation	Standard Error	Coefficient of Variation in %	Level of Significance	Mean Value and Standard Deviation	Standard Error	Coefficient of Variation in %	Level of Significance		
Before Coma	8	33.6 ± 0,92	± 0.33	2.74	—	51.1 ± 1.64	± 0.62	3.25	—	—	—
In Coma	8	34.2 ± 1.03	± 0 36	3.01	P~0.05	51.1 ± 1.55	± 0 58	3.05	P > 0.05	51.99 P < 0.001	P<0.001

The increease in the polarographic activity of native sera in hypoglicemic coma which we have established might have been expected because the amount of total proteins was increased (Table 2). However, this does not exclude the consideration of other factors which could affect the presence of polarographically active groups. The established differences in the polarographic activity of the native serum before the injection

Table 2

Values for total protein in blood serum of eight shizophrenic patients (women) before insulin injection and in deep hypoglycemic coma

Values Before/ in Coma	No of Cases	Range of Values	Mean Value and Standard Deviation	Standard Error	Coefficient of Variation in %	Increase in %	Level of Significance
Before Coma	8	8.03—6.19	7.22±0.61	0.21	8.45	—	—
In Coma	8	8.65—7.28	7.81±0.42	0.15	5.38	8.17	P<0.05

of insulin and in hypoglycemic coma could indicate the changes in the secondary and tertiary structure of the molecule of blood proteins. This hypothesis is strengthened by the fact that the denaturation of serum proteins probably cancels out the difference in the structure of the protein molecule provoked either by the direct action of insulin or the conditions caused by its presence. Although these results allow no definite conclusions to be made they may serve as an orientation for more detailed and specific investigations concerning this problem.

We wish to point out that the values for total proteins obtained in these experiments are in agreement with the results published in earlier reports (1, 3).

CONCLUSION

The polarographic activity of sera in eight metabolically healthy schizophrenic patients (women) was investigated. The sera were taken before insulin injection and in deep hypoglycemic coma.

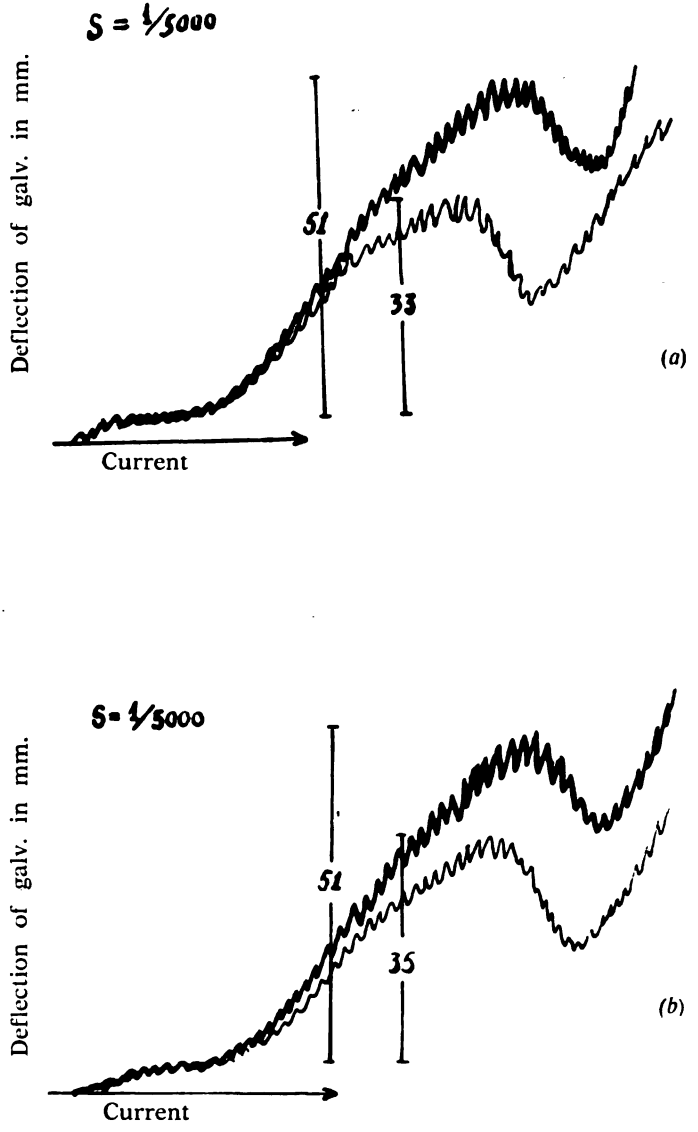

Fig. 1. — Polarographic waves of native and denatured blood serum proteins in schizophrenic patients (women). (a) Before insulin injection; (b) in deep hypoglycemic coma.

The heights of polarographic waves of native serum proteins measured before insulin injection and in deep hypoglycemic coma show small but significant differences, demonstrating a higher activity of proteins in the insulin shock. By the denaturation of serum proteins *in vitro* the differences in polagraphic activity disappeared. It was assumed that

the differences in the polagraphic activity of native sera are due to the changes in the secondary and tertiary structure of the protein molecule, provoked either by the direct action of insulin or by the conditions caused by its presence.

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PREALBUMINS, I. THE ISOLATION AND THE ELECTROPHORETIC AND POLAROGRAPHIC CHARACTERIZATION OF PREALBUMINS IN GUINEA PIG AND DOG BLOOD PLASMA

by

J. J. BOJANOVIĆ, LJ. M. ŠEVALJEVIĆ, D. B. PAVLOVIĆ, and DJ. J. PANJEVIĆ

The electrophoretic fraction of proteins which at pH 8.6 is faster than albumins and is known as the prealbumin fraction has been found in normal human serum and in the serum of some animal species in comparatively small quantities. However, it has been established that a fraction faster than the albumin fraction can be induced by means of some agents, such as heparinoid substances, or under given coditions of electrophoresis (6, 7, 9, 11). The examinations of the quantitative relations between individual fractions of heparinized blood plasma proteins, effected by small amounts of heparin *in vitro*, have shown that the amount of the prealbumins formed depends not only on the amount of heparin added, but also on the age of the animal (1 and 4). To study this relation in greater detail and contribute to a better knowledge of the nature of blood proteins and proteins in general, we have undertaken further electrophoretic investigations of the prealbumins of heparinized guinea pig plasma and oxalated dog plasma, as well as the polarographic examination of isolated prealbumins.

Material and Methods

These investigations were carried out with heparinized blood plasma of forty-one guinea pigs and thirteen dogs. In guinea pigs several age groups were considered: the animals were two, four, six, and twelve, and between twenty-four and thirty-six months old. Heparinized plasma was obtained by the addition of 450—650 γ of heparin per one ml of blood *in vitro*, as has been described in an earlier report (1).

Prealbumins were isolated from blood serum by preparative electrophoresis with a *Shandon* apparatus in a borate buffer at pH 8.6, at 450 V and 17—20 mA, the time of separation being 48 hours. About 3 ml of the serum was placed on Whatman No. 1 paper, 40 x 40 cm in size. Under these conditions prealbumins eluted before albumins forming a several millimeter wide zone which after the application of naphthalene black 12 B turned into a pale color and directly preceded the intensively colored wide albumin zone.

The electrophoretic analysis according to the *Labhard* and *Saub* method (10) was done with a *Kern* apparatus with dialyzed plasma under conditions described in an earlier report (1).

The relative mobility of electrophoretic fractions was calculated from the electrophoregrams taking into account the length of the fraction movement, the fall in potential per centimeter, and the time in which the fraction moved over the measured path. Besides the mobility of prealbumins, the mobility of albumins was also measured, so that it was possible to compare the values prealbumins as to have an insight into possible changes in the mobility of albumins due to the presence of prealbumins.

Polarographic analyses were made with a *Radiometer Copenhagen PO 3h* apparatus, wiht a sensitivity of 1/3000. The drop-time of mercury was 3.5 seconds at 1—2 V. Polarographic investigations were made on native and denatured prealbumins at concentrations of 12, 20, 40, 60 and 80 mg%. The concentration of proteins was determined by a micromodification of *Kjeldahl's* method (2) and the desired concentration obtained by dilution with water. Prealbumins were denatured with N/3 KOH for 30 minutes at a temperature of $25 \pm 0.1^{\circ}C$. The basic electrolyte consisted of an ammonium solution of cobalt hexamine chloride adjusted by ammonium chloride.

Results and Discussion

The prealbumins inducted by heparin have been described mainly as an electrophoretically homogeneous fraction of proteins. In our experiments we obtained in most cases only one fraction faster than the albumin fraction (Fig. 1) except in the examination of the heparinized plasma of a group of young guinea pigs (two and four months old) when in 50%

(a)

(b)

Fig. 1. — Electrophoretic patterns of heparinised blood plasma proteins in young (a) and old (b) guinea pig; the prealbumin fraction PA_2 is present

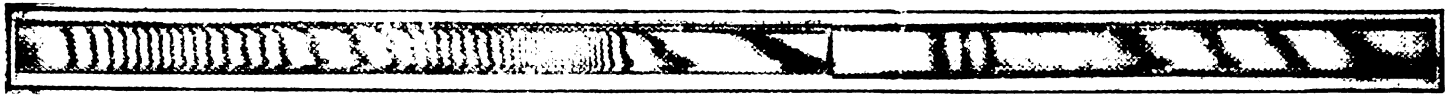

Fig. 2. — Electrophoretic patterns of heparinised blood plasma proteins in young guinea pig; the prealbumin fractions PA_1 and PA_2 are present

(a)

(b)

Fig. 3. — Electrophoretic patterns of sera proteins in dog; the prealbumin fraction PA_2 (a) and PA_1 (b) are present

of the cases we established two prealbumin fraction (Fig. 2). Such a qualitative relation between the action of heparin and the formation of prealbumins is associated with the state of proteins in circulation. This is agreement with a fact already observed, i. e. that the same amounts of heparin give rise to different amounts of prealbumins in the blood plasma of guinea pigs of different age (1).

According to Schultze et al. (11), the mobility of isolated unpurified prealbumins of human serum at pH 8.6 is approximately twice as high as the mobility of albumins, while the mobility of the purified prealbumins is lower and only about 20% higher that the mobility of albumins. Under our experimental conditions, at pH 8.6, the relative mobility of the prealbumin fraction (designated by PA_2) of heparinized plasma in guinea pigs two, four, and six months old was 86—89% higher than the mobility of albumins, and about 78% higher than in guinea pigs 12 months old. The difference is the greatest in guinea pigs aged 24 to 36 months, because the mobility of prealbumins is 95% higher than the mobility of albumins (Table 1). In young guinea pigs, in the cases

Table 1

Relative electrophoretic mobility and the relation between the mobilities of prealbumins and albumins of heparinized guinea pig blood plasma and dog sera

Animals	Age in Months	Electrophoretic Mobilities*			Mobility Ratio		
		Albumins	PA_2	PA_1	PA_1/A	PA_2/A	PA_2/PA_1
Guinea	2	5.39	10.03	—	1.90	—	—
Pigs		6.00	10.59	21.44	1.76	3.60	2.06
,,	4	4.66	8.82	—	1.89	—	—
		5.58	11.19	24.30	1.70	3.69	2.12
,,	6	5.33	9.99		1.87		
,,	12	5.06	9.01		1.79		
,,	24—36	5.25	10.25		1.95		
Dogs		5.64	9.07		1.60		

when two prealbumin fraction occur, fraction PA_2 has a considerably lower relative mobility, because in the blood plasma of guinea pigs two months old it is only 76% higher than the mobility of albumins, while in guinea pigs four months old it is 70% higher. The faster prealbumin fraction PA_1

* Electrophoretic mobilities: $x10^{-5}\ cm^2\ v^{-1}\ sec^{-1}$
Michaelis buffer at p_H 8.6, μ=0.1

which occurs only in young guinea pigs has considerably higher mobility than the prealbumin fraction PA_2, i. e. it is 275% higher than the mobility of albumins in guinea pigs two months old, and 269% higher than in guinea pigs four months old. Nevertheless, it must be borne in mind that the mobility of albumins in blood plasma is also different in animals of different age. The mobility of albumins and prealbumins is the lowest in the plasma of guinea pigs four months old, in the cases when the ratio of relative mobility between albumins and prealbumins is the highest. When two prealbumin fraction occur, the albumins show a higher mobility, especially in the plasma of guinea pigs four months old in which the slower prealbumin fraction (PA_2) has a maximum mobility. The faster prealbumin fraction (PA_1) has about 100% higher mobility than the slower prealbumin fraction (PA_2) in guinea pigs two months old, and 117% in guinea pigs four months old.

The comparison between the contents of individual fractions of proteins in the heparinized plasma of guinea pigs of different age (1) and their relative mobilities shows a striking positive correlation of these values for prealbumins and albumins.

Besides the mobility of prealbumins of the heparinized guinea pig plasma, the relative mobility of prealbumins of the oxalated dog plasma has also been determined. By electro-

Table 2

The content of prealbumins in heparinized blood plasma in guinea pigs of different ages

Age in Months	Number of Cases	Prealbumins		
		M	SD	SE
2	9	7.0	±3.00	±1.00
4	9	6.3	±2.22	±0.74
6	8	3.7	±2.77	±0.93
12	7	2.7	±1.22	±0.45
24—36	8	6.6	±2.66	±0.94

phoretic investigations of proteins in oxalated dog plasma prealbumins were established in 33% of the cases (Fig. 3). Under given experimental conditions the prealbumins occurred in these cases spontaneously and their presence in the electrophoretic spectrum of proteins was not induced by the

preliminary action of heparin, borate buffer, or any other agent. It has been established that the relative mobility of the prealbumin fraction PA_2 is approximately the same as the mobility of prealbumins of the heparinized plasma of adult guinea pigs (12 months old), while the mobility of albumins is higher. The mobility of prealbumins in dog plasma is about 60% higher than the mobility of albumins, so that the difference is considerably smaller than in guinea pigs.

The changes taking place owing to the action of heparin on blood proteins and the degree of these changes are evidently associated with the state of protein molecules of animals of different age. The occurrence of one or two prealbumin fractions and the differences in their mobility in relation to the age of the animal indicate not only the quantitative changes in blood proteins which take place during its life (1, 3), but also the qualitative changes which we assume to be considerable and specific for a particular age, and certainly for particular animal species. It should be noted that the formation of prealbumins induced by small amounts of heparin (450—650 γ per 1 ml of blood) is accompanied by quantitative changes in the α-globulin fraction, mainly in the α_2-globulin fraction, and to a certain extent also in fibrinogen, while in other fractions, under the same experimen-

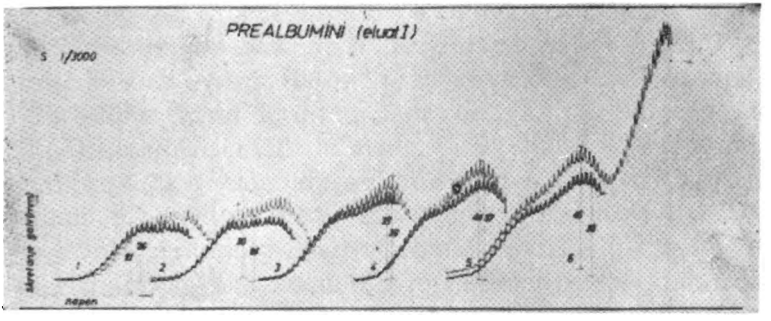

Fig. 4. Polarographic waves of native (thin line), and denaturated (bold line) blood sera prealbumins in guinea pigs, in concentrations: 1) 12 *mg%* 2) 20 *mg%*; 3) 40 *mg%*; 4) 60 *mg%*; 5) 80 *mg%* The initial potential: 1.25 *V*

tal conditions, no considerable changes could be observed (4). However, no pronounced correlation could be observed in the quantitative relation between these changes and the percentage of prealbumins formed.

For a closer study of the nature of prealbumins we examined polarographically the isolated prealbumin fraction

obtained from guinea pig sera by a continuous paper electrophoresis employing a borate buffer at pH 8.6. The polarographic analysis of these prealbumins showed that the polarographic waves of prealbumins are double at all the concentration that we have examined (12, 20, 40, 60, and 80mg%), and that the concentration at which the first and the second wave intersect is about 20 mg%. The wave heights of the native and denatured prealbumins increase with a concentration between 12 and 80 mg%. Within this concentration range the waves of native prealbumins are higher than the waves of detaured prealbumins (Fig. 4). The mean values and standard deviations of the heights of polarographic waves are recorded in Table 3.

The increase in the wave heights of native prealbumins at the concentrations of 12 and 20 mg% and 20 and 40 mg% is statistically significant ($p=0.025$ and $p=0.05$) while the differences at the concentrations of 40 and 60 mg% and 60 and 80 mg% are statistically nonsignificant ($p=0.70$ and $p=0.10$). The differences in the wave heights of denatured prealbumins are statistically significant only at the concentrations of 12 and 20 mg% ($p=0.001$) while at the other concentrations they are nonsignificant.

On comparing the polarographic behavior of the isolated protein fractions of the sera (albumins, and α_2-, β_1-, β_2-, and γ-globulins) it was established that only albumins within a certain concentration range (12—100 mg%) have a greater polarographic activity in the denatured form, while all the globulin fractions are more active in the native form (5). The fact that the native prealbumins are also polarographically more active than the denatured prealbumins is a characteristic in which prealbumins approach globulins. However, a close relationship between prealbumins and globulins is also manifested in a pronounced similarity in polarographic behavior between prealbumins and γ-globulins (Fig. 5) because these two fractions have similarly shaped waves, and at low concentrations (12, 20 and 40 mg%) the heights of their waves are practically the same.

Although prealbumins and γ-globulins are two electrophoretic fractions showing the greatest differences in mobility, their closely resembling polarographic activity leads us to the conclusion that they are related. This statement is in agreement with the data given by Göre and co-workers (8)

Table 3

MEAN HEIGHTS (IN mm) OF THE POLAROGRAPHIC WAVES OF NATIVE (N) AND DENATURED (D) PREALBUMINS AND γ GLOBULINS IN GUINEA PIG SERA

Protein Concentration in mg%	12		20		40		60		80	
	N / D		N / D		N / D		N / D		N / D	
	M±SD	SE	M±SD	SE	M±SD	SE	M±SD	SE	M±SD	SE
Prealbumins	25.2±3.00	±1.50	30.7±2.37	±1.18	38.2±5.44	±2.72	40.0±6.87	±3.43	43.6±2.34	±1.35
	20.2±1.00	±0.50	25.6±1.58	±0.91	31.0±5.74	±2.87	33.8±5.97	±2.98	37.6±2.54	±1.46
γ-Globulins	25.1±7.15	±2.26	30.7±4.77	±1.50	31.8±3.13	±0.59	30.5±2.89	±1.18	28.0±3.67	±1.64
	21.0±5.37	±1.90	27.0±3.77	±1.19	27.0±2.74	±0.86	24.3±4.06	±2.34	24.0±2.64	±1.52

that prealbumins resemble globulins in the composition of their amino acids.

The investigations of polarographic activities of the prealbumins in the sera of guinea pigs of different age are also important for a polarographic characterization of prealbumins (5). It was established in these investigations that the polarographic waves of young (aged one month) and old guinea

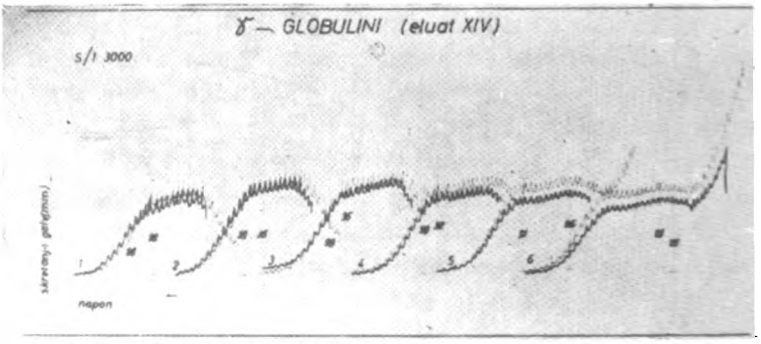

Fig. 5. Polarographic waves of native (thin line), and denatured (bold line) blood sera γ-globulins in guinea pigs, in concentrations: 1) 12 *mg%*; 2) 20 *mg%*; 3) 40 *mg%*; 4) 60 *mg%*; 5) 80 *mg%*
The initial potential: 1.25 *V*

pigs (aged 24—36 months) have the same form and the same relation in the native and denatured condition. Nevertheless, in a certain number of cases, the prealbumins in young guinea pigs, considerably more frequently than in old guinea pigs, showed some polarographic characteristics of albumins, i. e. a greater polarographic activity in the denatured than in the native condition.

CONCLUSION

The investigations of prealbumins of heparinized blood plasma of guinea pigs showed that their relative mobilities are dependent upon the age of the animals used in the experiments. The relative mobilities are in a positive correlation with the contents of these fractions in the blood plasma of guinea pigs of different age.

The heparinized blood plasma of young guinea pigs (two and four months old) contained in fifty percent of the cases two prealbumin fractions, one of which (PA_1) had a considerably higher mobility.

The oxalated adult dog plasma contained in thirty-three percent of the cases prealbumin fractions having approximately the same mobility as the relative mobility of the prealbumin fraction (PA_2) of the heparinized blood plasma of adult guinea pigs (twelve months old).

The polarographic examinations of isolated prealbumins showed a very pronounced similarity between the polarographic behavior of prealbumins and globulins, especially γ-globulins.

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COMBINED SILICONE WATER REPELLENTS

by

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Silicones (organosilicone polymers) are among the best water repellents for textille fabrics; they make the fabric water reppelent without decreasing its porosity for gases. In addition, the fabric impregnated with silicones is silky, it easily recovers from wrinkling, and it is resistant to dirt; fatty spots can be easily removed by washing or dry cleaning and the stability of the fabric is only slightly decreased.

Early attempts to produce water repellency with organosilicone compounds were performed with organochlorosilanes, but those procedures could not be applied to fabrics because of liberated hydrogen chloride which could not be completely removed and which damaged the textile fiber (1, 2, 3, 4).

However, in the course of time a considerable number of organosilicone compounds came into use and were successively applied to make the fabric water repellent. Those compounds were either monomers or polymers. The composition of monomers was characterized by the presence of at least one easily hydrolizing group which was hydrolized on the fabric, and by selfpolycondensation afforded cross linked organopolysiloxanes which made the fabric water repellent. The monomers were usually compounds of these types: $R_nSi(OCOR')_{4-n}$ and R_nSiX_{4-n} (R = alkyl or aryl group, R' = hydrogen or hydrocarbon residue, X = CN or CNS group); in addition, organoalkoxy and aryloxy silanes, substituted and nonsubstituted silaneamines, etc, (2, 4) were also used.

Compounds which were applied to the fabric in the form of polymers were linear or cross linked organopolysiloxanes of low molecular weight; they underwent final condensation on the fabric, and produced siloxane films which were firmly fixed to the fiber. The best representatives of those compounds were organohydrogenpolysiloxanes. By the

action of heat in the presence of a catalyst, the hydrogen which was directly linked to the silicone was oxidized, formed oxygen cross linakges (1), and was eventually bound to the reactive groups of the textile fiber (5). The most widely used were organohydrogensiloxanes (methyl) in which Si /H $\geq$1, and mixtures of linear and cross linked organohydrogenpolysiloxanes and diorganopolysiloxanes, polysilazanes, and metal salts of organosilicic acid (2, 6).

Since the final condensation and cross linking usually required a thermal treatment, the preparation of water repellent textile fabrics was performed with polysiloxanes which were condensed at 160—120°C within four to ten minutes. However, the thermal treatment can produce a negative effect on the mechanical properties of the textile fiber. Therefore, the problem of lower temperature treatment is still under investigation.

The decrease of the required temperature and the shortening of the treatment period could be achieved by the addition of a catalyst; the usual catalysts were the esters or salts of organic acids of *Ti, Zr, Pb,* and *Zn,* (5, 7) and the salts of aliphatic and naphtenic acids of *Fe, Co, Mn, Zn* and *Al.* (2). However, the problem of cold polycondensation has not yet been solved and the best results have so far been achieved with butyl titanate.

In the course of our recent works in the field of chemistry and technology of organosilicone compounds, we have observed that fabric treated with hydrogenpolysiloxane in combination with aluminum ethylate acquired water repellency even in the cold; this effect could not be achieved when the fabric was treated with only one of the above compounds. On the basis of this fact, we have begun the study of the mechanism of this reaction, and we have attempted to ascertain the necessary conditions for the achievement of the optimal effect. Therefore, we investigated the influence of the concentrations of both components and the effect of the succession of their application to the fabric, both on the value of the obtained water repellency and on its stability in washing and dry cleaning.

Experimental

The experiments were performed with cotton cloth — balloon fabric of these characteristics:

density of warp 56 wires per cm length
density of woof 26 wires per cm length
cotton weave, the highest degree of cross-weave
kind of weave 70/2, cotton, warp and woof

Since the fabric had to be free of grease and in the "loom state" (without being submitted to finishing operations), the experiments were performed with undyed and dyed fabric which contained no grease and which has been prepared for finishing operations; the samples were cut in 22 cm length squares.

The applied methylhydrogenpolysiloxanes were obtained by the hydrolysis of a mixture of dimethyldichlorosilanes and monomethyldichlorosilanes (molar ratio 4:3); aluminum methylate was not purified (9), and was dissolved in petroleum (fraction 80—120°C).

The fabric was either separately immersed into the solution of methylhydrogenpolysiloxane and aluminum ethylate, or immersed in their mixture. The immersion period was one minute (any prolongation of the immersion is denoted in the tables). The fabric was then squeezed by means of calender rolls so that the fabric contained 60 per cent of the water repellent solution. The solvent was evaporated at room temperature after each immersion. If the fabric was of dark shade, white spots of aluminum hydroxide were left which could be removed by brushing, and the achieved hydrophobic effect was only slightly decreased.

The estimation of the water repellency was performed by the method of hydrostatic pressure according to Veitch and Jarell (10); the hydrophobic effect is expressed by the value of the hydrostatic pressure at the moment whe the third drop of water passes through the fabric. The diameter of the apparatus funnel was 17.5 cm., and the rate of the level tube raising was 10 cm. per minute; the latter rate was kept constant in all experiments. In order to get an idea of the obtained hydrophobic effect, we measured the hydrostatic pressure which an untreated fabric can stand, and it amounted to only 45 mm.

The experiments were performed with 2, 1, 0.5, and 0.25 solutions of methylhydrogenpolysiloxanes and aluminum methylate. Each operation was performed with four samples, and the mean value was taken as the final result. The obtained data are given in the tables.

Tables 1 and 2 illustrate the values of water repellency of a dyed fabric depending upon the succession of immersion in solutions.

Table 1

Solutions: 2% aluminum ethylate and 2% organohydrogenpolysiloxane

Water repellents Procedure	Water repellency expressed in mm H_2O		Decreased of water repellency after brushing in %
	before brushing	after brushing	
Si	45	45	0
*Si**	150	150	0
Al	it is wetted	it is wetted	—
*Al**	immediately	immediately	—
Al—Si	420	405	3.6
Si—Al	450	425	5.5
(Al Si)	433	399	7.8
(Al Si) — Si	338	327	3.3
(Al Si) — Al	452	432	4.4
Al — (Al—Si)	440	412	6.4
Si — (Al Si)	401	374	8.3
(Al Si) — (Al Si)	396	378	4.5
Al — Si — Al	460	438	4.8
Si — Al — Si	403	390	3.2

Legend: *Si* — immersion of the fabric into methylhydrogenpolysiloxane solution

X — subsequent thermal treatment at 100°C for five minutes

Al — immersion of the fabric into aluminum ethylate solution

(Al Si) — immersion of the fabric into a mixture of aluminum ethylate and methylhydrogenpolysiloxane solutions

Table 2

Solutions: 1% aluminum ethylate and 1% organohydrogenpolysiloxane

Water repellents Procedure	Water repellency expressed in mm H_2O		Decreased of water repellency after brushing in %
	before brushing	after brushing	
Al — Si	388	384	1.1
Si — Al	400	381	4.8
(Al Si)	395	377	4.6
(Al Si) — Al	435	428	1.9
Al (Al — Si)	423	395	6.5
Si (Al Si)	390	378	2.8
Al — Si — Al	422	410	2.9
Si — Al — Si	368	360	2.2

It is evident from these results that the best water repellency was achieved when the final immersion of the fabric was performed in an aluminum ethylate solution. Although the percentages of the decreased water repellency after brushing are rather high, the final values of the achieved hydrophobic effects are the greatest. By comparing the results given in Tables 1 and 2, we may state that the value of the achieved water repellency decreases with the decreasing concentration of the corresponding water repellents; this statement is confirmed by the results shown in Table 3.

Table 3

Solutions: 0.25% aluminum ethylate and 0.25% organohydrogenpolysiloxane

Water repellents Procedure	Water repellency expresed in mm H_2O
Si — Al	335
Si — Al — Si — Al	203
Al — Si — Al — Si	297

As already mentioned, the results given in Table 3 confirm that the value of the hydrophobic effect is decreased with the decreasing water repellent concentration, even in cases where many immersions were performed.

The experiments in which the combination of solutions of different concentrations was used were performed with undyed fabric. We have also investigated the effect of previous washing and drying of the fabric and the influence of the subsequent thermal treatment (ironing) on water repellency. The obtained results are illustrated in Table 4.

Table 4

Water repellents Procedure	Unwashed fabric	Water repellency expressed in mm H_2O	
		previously washed and dried fabric	subsequently ironed fabric
2%*Si*—0.5%*Al*	90	95	100
0.5%*Si*—2%*Al*	270	245	260
(0.5%*Si* 2% *Al*)	390	405	400
(2%*Si* 0.5%*Al*)	260	275	240

As shown in Table 4, good results were obtained with the solution consisting of a mixture of 0.5 % methylhydrogen-

polysiloxane and 2% aluminum ethylate. The table also indicates that previous washing and drying of the fabric and the subsequent thermal treatment very slightly affect the value of water repellency.

In order to determine whether the duration of the fabric immersion period affects the value of water repellency, we performed a series of experiments, the results of which are shown in Table 5.

Table 5

Water repellents Procedure	Water repellency in mm H_2O				
	after 1'	5'	15'	30'	60'
(2% *Al Si*)	390	480	460	420	420
2%*Si* (1 min) — 2%*Al*	380	150	—	—	—

The results in Table 5 indicate that the prolongation of the fabric immersion period into a mixed solution results in an increase of water repellency up to a definite limit, and this is then followed by a gradual decrease; however, on prolonged immersion into an aluminum ethylate solution, the value of water repellency abruptly decreases.

In order to estimate the values of water repellency obtained by means of methylhydrogenpolysiloxane combined with aluminum methylate, we performed experiments with silicone water repellents produced by "Rhodorsil" (Hydrofugeant 85 T + Renforcateur GY) and "Imperial Chemical Industries (Product No 81537) which are recommended for the achievement of water repellency in cold, and which are considered to be the best silicone water repellents. The fabric was treated according to the given instructions. The comparisons of results are shown in Table 6. The table indicates that the results obtained with aluminum ethylate are significantly better.

Table 6

Silicone water repellents	Water repellency expressed in mm H_2O	
	undyed fabric	dyed fabric
"Rhodorsil"	215	180
"ICI"	280	—
Methylhydrogenpolysiloxane combined with aluminum ethylate	390—480	440—460

On the basis of the assumed mechanism of aluminum ethylate action, it might be expected that organopolysiloxanes which do not contain silanic hydrogen would give the same efect; therefore, we performed some tests with linear blocked methylpolysiloxanes, the viscosities of which were 100 cst and 500 cst, respectively; these products were made by "Wacker" (AR 100 and AR 500). Tests were also performed with tetraethoxysilane. The undyed fabric was first immersed into a 2% solution of an organosilicone compound and them into 2% aluminum ethylate solution. The obtained results are given in Table 7.

Table 7

Silicone water repellents	Water repellency expressed in mm H_2O	
	immediately after drying	after 24 hours
2% *Si* — 2% *Al*	380	380
2% *AR* 100 — 2% *Al*	30	330
2% *AR* 500 — 2% *Al*	70	380
2% Tetraethoxysilane —2%*Al*	210	210

These results indicate that blocked methylpolysiloxanes produce maximal water repellency only after twenty-four hours; however, with methylhydrogenpolysiloxanes and with tetraethoxysilane, the maximal value is obtained immediately after drying. In our opinion, the result obtained with blocked methylpolysiloxanes is of great interest, both from practical and theoretical points.

The resistance of water repellent protection obtained with aluminum ethylate was examined by the following standard procedures:

1. Usual washing of fabric — conditions same as those in household.

2. Drastic washing — the fabric was kept with constant stirring into a 0.5% solution of anhydrous soap for ninety minutes at 71°C (11).

3. Drastic washing — the solution of soap was previously neutralized with formic acid.

The effect of usual washing on the water repellency of the fabric is shown in Table 8, and that of drastic washings is shown in Tables 9 and 10. The tables include foreign silicone water repellents.

Table 8

The effect of usual washing on water repellency

Water repellents Procedure	Fabric	Water repellency expressed in mm H_2O		Decrease of water repellency in %
		before washing	after washing	
"Rhodorsil"	undyed	215	60	72
"Rhodorsil"	„	225	60	73.5
"Rhodorsil"	dyed	180	70	61
2% *Al* — 2 *Si*	„	420	180	57
2% *Si* — 2% *Al*	„	450	160	64.5
2% *Si* — 2% *Al*	undyed	380	80	79
(2% *Al Si*)	dyed	433	110	74.5

Table 9

The effect of drastic washing on water repellency

Water repellents Procedure	Fabric	Water repellency expressed in mm H_2O		Decrease of water repellency in %
		before washing	after washing	
2% *Si* — 0.5% *Al*	undyed	90	40	55.5
0.5% *Al* — 2% *Si*	„	270	130	52
0.5% *Si* 2% *Al*	„	390	185	52.5
(2% *Si* 0.5% *Al*)	„	260	145	44

Table 10

The effect of drastic washing with soap neutralized with formic acid on water repellency

Water repellents Procedure	Fabric	Immersion period in minutes	Water repellency expressed in mm H O		Decease of water repellency in %
			before washing	after washing	
"Rhodorsil"	undyed	5	215	90	58
"Rhodorsil"	„	5	225	130	42
"ICI"	„	5	280	180	36
2% *Si* — 2% *Al*	dyed	1	450	145	68
(2% *Al Si*)	„	1	433	220	49
2% *Si* — 0.5% *Al*	undyed	1	90	70	22
0.5% *Si* — 2% *Al*	„	1	270	200	26
(0.5% *Si* 2% *Al*)	„	1	390	200	49
(2% *Si* 0.5% *Al*)	„	1	260	80	69
(2% *Si Al*)	undyed	1	390	210	46
(2% *Si Al*)	„	5	480	180	62.5
(2% *Si Al*)	„	15	460	220	52
(2% *Si Al*)	„	30	420	140	66.5
(2% *Si Al*)	„	60	420	90	78.5

It is evident from these tables that the water repellency achieved by the application of silicone water repellents combined with aluminum ethylate in the cold is unstable in washing, but the percentage of its decrease is of the same order as that which results in the application of other commercial products; however, the absolute values of the retained water are higher in experiments using aluminum ethylate.

The stability of water repellency in dry cleaning was examined by immersing the fabric in ligroin (80—120°C) for ten minutes with slow stirring. The obtained results are shown in Table 11.

Table 11

The effect of washing in ligroin on water repellency of fabric

Water repellents Procedure	Water repellency expressed in mm H_2O	
	before dry cleaning	after dry cleaning
"Rhodorsil"	215	220
"Rhodorsil"	225	225
"Rhodorsil"	180	195
"ICI"	280	290
2% *Si* — 0.5% *Al*	90	100
0.5% *Si* — 2% *Al*	270	265
(0.5% *Si* 2% *Al*)	390	395
(2% *Si* 0.5% *Al*)	260	260

This table shows that water repellent protection is not decreased in dry cleaning; moreover, in some cases it is increased.

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THE PROCESS OF AGING OF LOW CARBON STEEL FOLLOWED BY THE CHANGE IN ELECTRICAL RESISTANCE

by

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The change in electrical resistance of steel with very low carbon and nitrogen content after quenching and during aging is in accordance with the fact that all deviations from an ideal lattice increase the electrical resistance of the metal. Consequently, the maximum value for electrical resistance is recorded immediately after the alloy is quenched, when, owing to lattice deformation, the presence of carbon and nitrogen atoms in the supersaturated solid solution of α-iron causes maximum impediment to the movement of electrons. During aging, the precipitation of solute atoms and the formation of a comparatively ordered structure reduce the electrical resistance to a certain constant value. On the basis of this, it is possible by measuring the changes in electrical resistance to follow the aging process after quenching, or to be more precise, to determine the carbon and nitrogen contents in the solid solution in α-iron.

The theoretical explanation that the yield point and in part strain hardening occur as a consequence of the process of segregation of carbon and nitrogen atoms to dislocations has given rise to the assumption that the same process should also bring about a change in electrical resistance. This assumption was confirmed experimentally by the work of A. H. Cottrell and A. T. Churchman in 1949 (1). Their experimental result proved that the change in electrical resistance has an activation energy equal to the activation energy of the process of diffusion of carbon and nitrogen atoms in α-iron, and this confirms the fact that the change in electrical

resistance is concerned with the migration of these atoms. They also established that strain aging takes place without an incubation period, and that the early stage of aging obeys the $t^{2/3}$-law, t referring to the time of aging.

The purpose of our investigations was to determine the changes in electrical resistance on the specimens of low-carbon steel (0.05%C) in relation to temperature and time, due to quench and strain aging.

Experimental

Measurements of electrical resistance were made on specimens of low-carbon steel of the following composition: *C*, 0.05%; *Si*, 0.056%; *Mn*, 0.28%; *S*, 0,011%; and *P*, 0.002%. The specimens had the form of a 250 mm — long wire, 2 mm in diameter.

Quench aging. — The specimens were quenched from 720°C in a 10% aqueous solution of *NaCl*. They were aged in oil bath for 0, 5, 10, 20, and 40 minutes, and 1, 2, 4, and 6 hours at 50, 90, 120 and 150°C.

Strain aging. — The specimens were quenched from 720°C in a 10% aqueous solution of *NaCl*. Immediately after quenching the specimens were deformed by tension to 6% elongation and aged under the same conditions as above.

The specimens were aged by heating in oil bath. The temperature was constant to ± 1°C. The electrical resistance was measured by means of a Thomson bridge using a galvanometer with a sensitivity of 10^{-9}A. The strength of the current fed through the specimens only during measurements was 0.6 A. The standard resistance of 0.01 ohm was used as reference.

The change in electrical resistance of the test specimen was expressed in relation to the value of the resistance of the standard specimen in which the process of aging was completed. The standard specimen was identical in chemical composition and form with the test specimen; it was quenched from 720°C and aged at 150°C for 85 hours. Thus the stabilized values of electrical resistance were an indication that all the changes had taken place.

The measurements of electrical resistance on test and standard specimens were in oil bath.

Results

The change in electrical resistance in test specimens was computed by the following equation:

$$\Delta R/R_o = \frac{R_x - R_o}{R_o} \cdot 100\%$$

where R_x is the resistance of the test specimen for a given time at a given temperature; R_o, the resistance of the standard specimen after the completion of the aging process at the same temperature.

Table 1 shows the following:

Table 1

No of Specimen	Elongation %	Aging Temperature °C	$\Delta R/R_o$ in %			Time Required to Attain Maximum Values τ min
			After Quenching and Quenching with Deformation	Final Records	Total Change	
1	—	50	1.497	1.497	0	>360*
2	—	90	1.500	0.659	0.842	>360*
3	—	120	1.395	0.456	0.938	>360*
4	—	150	1.300	0.359	0.942	>360*
5	6	50	7.450	6.270	1.18	~360*
6	6	90	7.360	6.180	1.16	60
7	6	120	7.630	6.740	0.89	40
8	6	150	7.280	6.370	0.91	20

(a) In column 4, the changes in electrical resistance ($\Delta R/R_o$) due to quenching, and, for strained specimens, the changes due to quenching and straining.

(b) In column 5, the final values of $\Delta R/R_o$ for specimens 1, 2, 3, 4, and 5 obtained after 360 minutes, i.e., those which did not stabilize, during that time, and the values of $\Delta R/R_o$ for specimens 6, 7, and 8 obtained for time τ cited in column 7, i. e. those which did not change in further treatment to the end of the 360-minute period.

(c) In column 6, the total change in electrical resistance; and

(d) In colum 7, the time required for the electrical resistance to attain a constant value.

* In test specimens 1—5 maximum values were not attained during the 360-minute period.

It is interesting to consider the changes in the carbon content in the solid solution occurring simultaneously with the change in electrical resistance. Relying on the data found in the literature (1, 2, 3) that a change of 2.5% in electrical resistivity, corresponds to a change in carbon content of 0,01% C, it is possible to calculate the relative change in the percentage of carbon in the solid solution in the test specimens by reference to the standard specimens. Without taking these results as absolutely accurate it is possible to calculate that the quenching brought about a relative increase in the carbon content (0.006%C on an average). This leads to the conclusion that the quenching procedure was not sufficiently effective. The differences in values for $\Delta R/R_0$ in individual specimens are due to the impossibility of maintaining constant conditions.

By accepting the above correlation between the changes in electrical resistance and the percentage of carbon in the solid solution, it is possible to calculate the percentage of carbon precipitated during the 360-minute period and the percentage of carbon which remained in the solid solution after this period of aging. These results show that the aging process in undeformed specimens was not completed even after 360 minutes. In this case, this method of calculation was not applicable to the deformed specimens because the change in the values for $\Delta R/R_0$ was effected not only by maintaining the supersaturated solid solution but also by cold work. Therefore, only the total change in the carbon content during aging can be calculated on the basis of the data recorded in column 6.

The aging curves for undeformed and deformed specimens are shown in Figures 1 and 2. From these curves we can conclude that:

(1) The process of aging in undeformed specimens was not completed during the 360-minute period at any of the temperatures considered, although at 150°C it approached a constant value.

(2) The process of aging was accelerated by the rise in temperature.

(3) Deformation accelerated considerably the process of aging so that the time required for the stabilization of electrical resistance was ~ 360, 240, 40 and 20 minutes for the

temperatures of 50, 90, 120, and 150°C, respectively. The course of the curves for the deformed specimens is also somewhat different when compared to that of the undeformed specimens.

Discussion

The course of the aging curves for undeformed specimens agrees qualitatively and quantitatively with the data obtained by other authors (3). The flat portions of the curves indicate an incubation period, while the bends indicate a change in electrical resistance. This decrease in resistance is brought about by the precipitation of carbon atoms from the supersaturated solid solution. Since the undeformed specimen has no internal "reserves" of energy, the precipitation of carbon from the solid solution depends only on the external source of energy, i.e. on the heating. This explains the considerably slowed-down process of aging in comparison to deformed specimens. Thus, a 360-minute period is not sufficient for the completion of the aging process.

Cold work induces dislocations into the crystal lattice of the metal. The dislocation field has energy of a higher order because strain energy is mostly stored here. Owing to the difference in energy, foreign atoms (in this case carbon and nitrogen atoms) segregate into dislocations thus reducing the energy of the system. External heat acceleratel this process. The diffusion of carbon and nitrogen atoms into the dislocation field brings about their more favorable arrangement with respect to the scattering of electrons, i.e. reduces the electrical resistance of the metal. Consequently the cold work treatment is bound to accelerate the aging process, as is evident from the data we recorded. The curves for strain aging do not indicate an incubation period and the maximum rate of the change in $\Delta R/R_0$ is obtained at the very beginning of the aging process. Thus, at 50°C, 25% of the total change in $\Delta R/R_0$ occurs after 30 minutes, 50% after 120 minutes, while the final stabilization of resistance is not attained even after 360 minutes. At 90°C, 25% of the change occurs after 5 minutes, 50% after 10 minutes, while a change takes place after 240 minutes.

Therefore, the above results agree qualitatively with the theory of strain aging, according to which this process starts without an incubation period and obeys the $t^{2/3}$-law in the beginning but later deviates from it and progressively slows down.

The comparison of changes in mechanical properties (4) and electrical resistance during aging of the specimens made of the stock alloy treated under the same conditions shows that the maximum values for mechanical properties were obtained after 1,200, 60, 20, and 10 minutes at temperatures

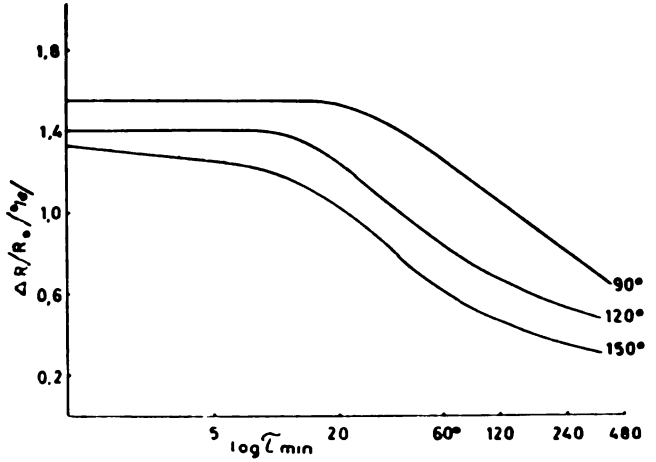

Fig. 1. — The change in electrical resistance of underformed specimens plotted as a function of time.

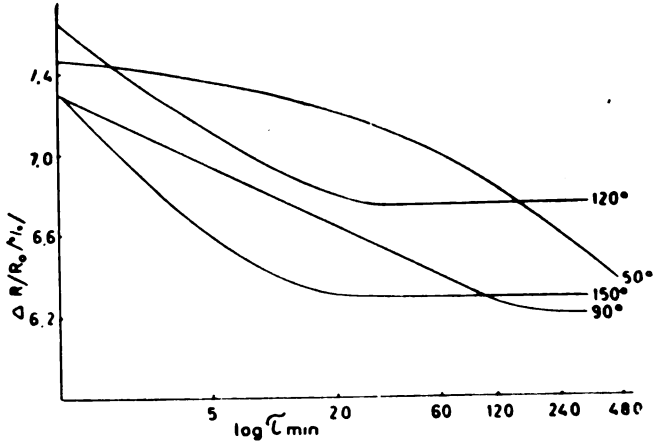

Fig. 2. — The change in electrical resistance of deformed specimens plotted as a function of time.

of 50, 90, 120, and 150°C, respectively, i. e. before the stabilization of values for $\Delta R/R_0$. This is in perfect agreement with the experimentally established fact that the precipitation of one carbon atom per dislocation is sufficient to attain the

maximum values for yield point and hardness, while the value of electrical resistance changes until an equilibrium concentration in the solid solution has been reached.

By investigating simultaneously the changes in mechanical properties and electrical resistance and eliminating all the factors which reduce the reliability of the values obtained for the changes in electrical resistance, it would be possible to establish the percentage of carbon precipitated at the moment when the maximum values for mechanical properties are reached. This would also make it possible to establish the density of dislocations.

CONCLUSION

The results of these investigations show a general qualitative agreement with the experimental works of other authors and agree with the results anticipated theoretically. To use quantitatively the data obtained by the method of measuring the electrical resistance it is necessary to secure steel of known composition (including also the nitrogen content) and apply a technique eliminating errors during measurements. Under these conditions the above method could be usefully employed as a comparative quantitative method for other methods of investigation.

Acknowledgement. — We wish to express our indebtedness to M. Jovanović for taking measurements and helping in the experimental work.

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ABSTRACTS

I.1.

RADIOCHEMISTRY AT THE "BORIS KIDRIČ" INSTITUTE AT VINČA

Z. DIZDAR

Boris Kidrič Institute of Nuclear Sciences, Vinča

A brief survey of the work done in the field of radiochemistry at the Institute, and the problems which are dealt with in connection with the tasks in this field contained in the development program of nuclear energy in Yugoslavia are presented. This paper also discusses the conditions under which such work is possible and its progress in future.

I.2.

DEPARTMENT OF RADIOCHEMISTRY OF THE "RUDJER BOŠKOVIĆ" INSTITUTE, ZAGREB 1956 — 1962

M. MIRNIK

Rudjer Bošković Ins.itute, Department of Radiochemistry, Zagreb

A brief description of the development of the Department of Radiochemistry since its foundation in 1956 is presented, including the information concerning its tasks, working program, and results obtained.

I.3.

PRODUCTION OF RADIOACTIVE ISOTOPES IN THE INSTITUTE "BORIS KIDRIČ" IN VINČA

Č. TEOFILOVSKI

Boris Kidrič Institute, Hot-laboratory Department, Vinča

Construction and the start of the nuclear reactor and the laboratory for high activity in Vinča created the conditions for the production of various radioactive materials for use in medicine, industry, and research work. Data concerning the production level for the last two years and trends of development are referred. Emphasis is made on basic techniques used in the production of radioactive isotopes, and a series of problems which arise in this production are discussed.

I.4.

PRODUCTION OF RADIONUCLIDES IN ZAGREB CYCLOTRON

P. STROHAL, M. VLATKOVIĆ, M. J. HERAK, R. DESPOTOVIĆ and M. MIRNIK

Rudjer Bošković Institute, Department of Radiochemistry, Zagreb

The results of the work on the production of radionuclides in the Department of radiochemistry in the Institute »Rudjer Bošković« are reported. General characteristics of the Zagreb cyclotron are given. Its deuteron beam makes it possible to complete the program of radionuclide production in our country. Various types of cyclotron targets used are described, as well as apparatus and radiochemical separation processes for the following radionuclides: Na-22, Na-24, Fe-55, Zn-65 and As-74. The production of Fe-59, Mn-54 and Co-56, 57, 58 is planned. First results of the work on the synthesis of inorganic labelled compounds ($NaCl^{36}O_4$) are reported.

WORK ON REPROCESSING IN THE "BORIS KIDRIČ" INSTITUTE

I. GAL

Boris Kidrič Institute, Hot-laboratory Department, Vinča

A brief survey of the purpose and methods of reprocessing with particular reference to the program of the »Boris Kidrič« Institute at Vinča is presented.

In the new Hot-laboratory building of the Vinča Institute, room is provided for laboratory studies of methods for the treatment of irradiated fuel by a wet procedure. For this purpose, in the course of 1962, construction of two special cells was completed in which manipulation and treatment of radioactive fuel will be possible at the activity level of the order of one curie.

This paper presents some details of these cells, the equipment, and the technological process developed in them. Some problems and difficulties faced with in work in this field are also presented, as well as the need for further investigation for the improvement of the chemical technological processes and the development of appropriate equipment.

I.6.

RADIATION CHEMISTRY AT THE "BORIS KIDRIČ" INSTITUTE AT VINČA

I. DRAGANIĆ

Boris Kidrič Institute, Department of Radiation Chemistry, Vinča

General remarks, scientific and practical aspects. Activity at the »Boris Kidrič« Institute of Nuclear Sciences at Vinča.

I.7.

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T. TASOVAC

Boris Kidrič Institute, Department of Radiological Protection, Vinča

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I.8.

ISOTOPE SCHOOL AT THE "BORIS KIDRIČ" INSTITUTE AT VINČA

O. MLADJENOVIĆ

The Boris Kidrič Institute, Isotope School, Vinča

History of the school. Program of lectures contained in the curriculum for theoretical and practical training in both the fundamental and the specialization parts of the course. Method of lecturing. A review of the books used in the course. Present location and equipment of the school. Statistical account of the previous courses.

THE SEPARATION OF URANIUM, PLUTONIUM AND FISSION PRODUCTS FROM THE HNO_3 SOLUTION ON ZIRCONIUM PHOSPHATE (PART TWO).

SEPARATION ON COLUMNS

A. RUVARAC and I. GAL

Boris Kidrič Institute, Hot-laboratory Department, Vinča

Taking into account the results obtained in Part One of this work, the purpose of this part was to obtain optimal conditions for the separation of UO_2^{++}, Pu^{4+}, and long-lived fission products (^{90}Sr, ^{95}Zr, ^{137}Cs, ^{144}Ce, and ^{106}Ru) from a nitric acid solution on zirconium phosphate columns.

Experiments have shown that the best results are obtained by adsorption from an 0.5 N nitric acid solution. During this process, uranium passes through the column followed by ^{90}Sr, ^{144}Ce and ^{106}Ru, while Pu^{4+} remains on the column with ^{137}Cs and ^{95}Zr. The traces of the remaining uranium are removed by washing the column with an 0.5 N nitric acid solution. The elution of Pu^{4+} is done with 8 N nitric acid, and ^{137}Cs is also eluted. The yield of Pu^{4+} obtained in these experiments amounts from 93 to 95 percent.

II.2.

THE SEPARATION OF Pu FROM U, Fe AND FISSION PRODUCTS ON THE ZrP_2O_7 COLUMN

D. CVJETIĆANIN and N. MILIĆ

Boris Kidrič Institute, Hot-laboratory Department, Vinča

The separation of Pu^{4+} from UO_2^{++}, Fe^{3+}, ^{95}Zr — ^{95}Nb, ^{90}Sr — ^{90}Y, ^{144}Ce, ^{106}Ru, and ^{137}Cs from nitroacid solutions on the ZrP_2O_7 column was investigated. We studied the influence of the particle size of the exchanger, the HNO_3 concentration, and the flow rate on the quantity of the separation. The following decontamination factors were obtained:

$$DF_U > 150,\ DF_{Fe} \sim 5 \text{ and } DF\beta \sim 300.$$

Conditions were also determined for the interseparation of some fission products (^{90}Sr, ^{90}Y and ^{137}Cs) as well as coloidal Pu (IV) and Pu^{4+} from nitroacid solutions.

Coloidal Pu (IV) was identified by the method of nuclear emulsions and by extraction with DBP.

II.3.

INVESTIGATION OF THE POSSIBILITY OF SEPARATION OF EUROPIUM FROM URANIUM BY THE EXTRACTION METHOD

M. HERAK and V. JAGODIĆ

Rudjer Bošković Institute, Department of Radiochemistry and Department of Structural and Inorganic Chemistry, Zagreb

The process of the extraction of europium by a petroleum solution of monooctylester of α-anilinobenzylphosphonic acid was followed by means of Eu-152 tracer. The conditions for the extraction of uranium were also investigated. The investigations were performed at various pH-values using a number of mineral acids. Conditions for the successful separation of europium from uranium have been established.

Europium complexes of monooctylester of anilinobenzylphosphonic acid were determined by radiometric analysis. The possibility of reextraction of the uranium bound to this reagent is described.

II.4.

THE EXTRACTION OF Zr AND Nb OXALATE COMPLEXES WITH TRI-N-HEPTYL AMINE AND TRI-ISO-OCTYL AMINE IN XYLOL

M. ŠUŠIĆ and Z. MAKSIMOVIĆ

Boris Kidrič Institute, Department of Reactor Materials
and Hot-laboratory Department, Vinča

The behavior of Zr and Nb oxalate complexes when extracting with tri-n-heptyl and tri-iso-octyl amine in xylol was investigated. The behavior of uranium and other fission products was also observed. The extraction of Zr and Nb was investigated as a function of oxalic acid and amine concentrations and the presence of other salts as well as of the initial pH of the aqueous phase.

EXTRACTION AND SEPARATION OF METAL IONS BY MEANS OF PHOSPHINIC ACIDS

F. KRAŠOVEC

Jožef Stefan Institute, Ljubljana

While the use of various esthers of ortho phosphoric acid for extraction and separation of metal ions has been a subject of numerous investigations, there are no literature data about the use of phosphinic acids for this purpose. The present work gives the results of the study of extraction of UO_2^{++}, Y^{3+}, Ce^{3+} by means of dibutyl- and diphenyl phosphinic acids. With previously determined dissociation, distribution and dimerization constants of the acids, it was possible to establish the chemical composition of extracted complexes, the reactions which occur during the extraction process, and the equilibrium constants of these reactions. By following the course of extraction of particular metal ions as a function of the beginning concentration of the reagents in the organic phase, or the H^+ concentration in the aqueous phase, conditions for their mutual separations have been established.

II.6.

QUICK RADIOCHEMICAL SEPARATION OF CESIUM

L. KOSTA, P. GORENC

Jožef Stefan Institute, Ljubljana

The isotopes of rubidium, formed in relatively large quantities in the fission of uran, make impossible the application of methods for the radiochemical separation of cesium described in the literature. By the application of ion exchange reactions on the combination of the anionic exchanger of the Dowex type and ammonium phosphormolybdate, we are able to separate cesium from uran and all other fission products in an 8-12 minute period. The separation factor of cesium is a hundred times larger than that of rubidium, and by subsequent precipitation of cesium with silicotungstic acid, it increases by more than ten times.

The technique of irradiation method of isolation of ^{139}Cs and use of gamma spectrometer for detection of isotopes with very short half-life periods are described.

II.7.

CHROMATOGRAPHIC SEPARATION OF METAL IONS ON PAPER TREATED WITH TRIOCTYL-PHOSPHATE

N. CVJETIĆANIN, J. ČVORIĆ and I. PALIGORIĆ

Boris Kidrič Institute, Hot-laboratory Department, Vinča

The behavior of uranium, thorium, and some other metal ions and long-lived fission products was investigated on paper treated with trioctyl-phosphate. Hydrochloric acid, nitric acid, sulphuric acid and perchloric acid in concentrations ranging from 0.1 to 10 N were used as the eluents. For the investigated ions, R_f values were given as a function of the concentrations from which possible interseparation of certain cations could de seen.

II.8.

TRACER LEVEL SEPARATIONS OF CYCLOTRON TARGET COMPONENTS BY MEANS OF CONTINUOUS ELECTROPHORESIS

Z. KONRAD-JAKOVAC, V. JOVANOVIĆ, B. KLJUČARIČEK and Z. PUČAR

Rudjer Bošković Institute, Zagreb

The applicability of the continuous electrophoretic separation of cyclotron target components for the preparation of carrier free radionuclides was studied. The process of separation consists of the irradiation of the target in the cyclotron, the dissolution of the irradiated target, and its continuous electrophoretic separation. For this purpose, these tracer level separations were performed:

1. Na-Mg-Al to obtain Na^{22} and Al^{26} from a magnesium target,
2. Pb-Bi-Tl to obtain Bi^{207} and Tl^{204} from a lead target, and
3. Cd-In to obtain In^{111}, In^{114} from a cadmium target.

1. Qualitative and quantitative study of the electrophoretic separation of Na-Mg-Al was performed in 0.02 M oxalic acid at pH 5.5. Optimal conditions for the separation are 500 V at a current of 100 mA. A method for electrolytic dissolution of a magnesium target was developed with the aim of diminishment of the high concentration of magnesium, which interferes in the separation of Na^{22} and Al^{26} from magnesium.

2. The continuous electrophoretic separation of a lead target for obtaining Bi^{207} and Tl^{204} was also qualitatively and quantitatively studied on the tracer level. The separations were performed in 0.05 N HBr, at 200 V and a current of 170 mA.

3. A mixture of Cd^{115}-In^{114} in the electrolyte consisting of 0.1 N KJ and 0.01 N HBr was separated by continuous electrophoresis, applying 200 V and a current of 280 mA.

Conditions for maximum separation capacity for these processes were investigated. With the conditions mentioned above, it is possible to separate the whole cyclotron target within 10-12 hours.

II.9.

STUDY OF ADSORPTION — DESORPTION PROCESSES BY USE OF RADIONUCLIDES

M. J. HERAK and M. MIRNIK

Rudjer Bošković Institute, Department of Radiochemistry, Zagreb

The adsorption—desorption processes were studied on solid-liquid systems. The effect of these processes on the formation of larger aggregations, grown from finely dispersed particles, was studied. The effect of macromolecules on adsorption-desorption processes in the formation of solid phase from electrolytic solutions has also been studied. This study was performed in order to establish regularities which control the processes of precipitation affected by »contaminants«, and the regularities which govern the processes of decontamination.

II.10.

STUDY OF THE PROCESS OF HETEROGENEOUS EXCHANGE BY THE RADIOACTIVE TRACER TECHNIQUE

R. DESPOTOVIĆ and M. MIRNIK

Rudjer Bošković Institute, Department of Radiochemistry, Zagreb

The process of heterogeneous exchange was studied by means of the radioactive tracer technique. Results obtained in this study are reported. Radionuclides Ag^{110} and I^{131} were used as radioactive indicators. The mechanisms of exchange were studied on silver iodide, which was used as a two-phase "solid-liquid" model system. A certain number of parameters, such as concentration and type of the constitutional ion in the solution, age of the observed precipitate, and charge of the coagulating ion were varied. The results obtained indicate facts which can affect the exchange process in view of its application for the isolation of radionuclides.

II.11.

DETERMINATION OF COMPLEX SOLUBILITY OF SPARINGLY SOLUBLE SUBSTANCES BY THE METHOD OF RADIOACTIVE INDICATOR

R. DESPOTOVIĆ and M. MIRNIK

Rudjer Bošković Institute, Department of Radiochemistry, Zagreb

General conditions to be fulfilled in setting experiments for measuring the radioactivity of the liquid phase of the two-phase system "solid-liquid" are defined. The calculation procedure is described for the results thus obtained. As an example, results of the measurements of the complex solubilities of silver iodide in various concentrations of sodium iodide with two different concentrations of cobalt nitrate at 20^0 C are given. This method is discussed and the results are compared to the known similar results. A possible suitable modification of this method is suggested.

II.12.

THE SEPARATION OF IODINE, IODATE, AND PERIODATE BY PAPER CHROMATOGRAPHY

I. PALIGORIĆ and J. ČVORIĆ

Boris Kidrič Institute, Hot-laboratory Department, Vinča

A procedure has been developed for the separation of the oxidation states of iodine by paper chromatography with an aim of observing the radiochemical purity of ^{131}J.

A mixture of amyl alcohol, pyridine, and concentrated ammonia in a 6:14:20 proportion was used as the solvent for he separation of $^{131}J^-$, $^{131}JO_3^-$ and $^{131}JO_4^-$.

II.13.

STUDY OF INTENSITY OF UPTAKE AND EXCRETION OF ^{131}J BY YOUNG SIMMENTHAL CATTLE

M. CAR and T. FILIPON

Faculty of Agriculture, Zagreb

In the study of the correlation between the thyroid gland activity and increase in weight, and also on the efficiency of nutrition in feeding cattle, comprehensive investigations were made of organically bound iodine in blood serum, as well as of the uptake of ^{131}J on erythrocytes and on resins. In order to explain the metabolism of inorganic iodine the uptake of ^{131}J was tested in the thyroid gland, PBI and the urinary »T« test were performed. The results obtained are presented.

II.14.

EXCHANGE MECHANISM OF CHLORINE IN CIS- AND TRANS-CHLORONITROBIS (ETHYLENEDIAMINE) COBALT (III) ION WITH RADIOACTIVE $^{36}Cl^-$ IN METHANOL

M. ORHANOVIĆ and S. AŠPERGER

Rudjer Bošković Institute, Department of Physical Chemistry, Zagreb

Exchange rates of chlorine in cis- and trans-chloronitrobis (ethylenediamine) cobalt (III) ion with radioactive $^{36}Cl^-$ in absolute methanol were determined. For the separation of ionic chlorine (LiCl*) of relatively high activity from the complex cation, the increase in activity of which was measured, the acetate form of the ion-exchange resin IRA-400 was used, since the usual separation with silver nitrate appeared to be unsuitable.

It has been found that the increase in LiCl* concentration by four times affects the increase of the constant of exchange rate of chlorine in the cis- and trans-complex by only a few percents. These results are similar to those obtained for the exchange of chlorine from the above complexes with thiocyanate ion, and they permit the conclusion that the substitution of weak nucleophilic reagents occurs through the methanolysis of the complex. Methanolysis is the slower, reaction rate determining process. It is followed by a fast exchange of coordinatively bound methanol with the nucleophilic reagent.

III.1.

THE REACTION CROSS SECTION MEASUREMENTS BY THE ACTIVATION METHOD

P. STROHAL, N. CINDRO and Z. KOLAR

Rudjer Bošković Institute, Department of Radiochemistry and Department of Nuclear Physics, Zagreb

The total cross sections for (n, 2n), (n, p), and (n, alpha) reactions induced by 14 MeV neutrons have been measured. The results are compared to the experimental and theoretical values obtained for (p, n), (p, d) and (p, 2n) reactions induced by 11 MeV protons. Measurements were performed either by the beta counting technique or by means of gamma counting. Most of the targets were treated radiochemically in order to obtain better radiochemical purity. About fifty cross sections were measured in the region around the atomic number Z 40.

III. 2.

DETERMINATION OF CROSS SECTION RATIOS FOR NUCLEAR ISOMERES

Z. KOLAR, P. STROHAL and N. CINDRO

Rudjer Bošković Institute, Department of Radiochemistry and Department of Nuclear Physics II, Zagreb

The ratios of cross sections for both metastable and basic states of nuclear isomers were measured by the activation method. Isomers were produced by neutron induced nuclear reactions. Radiochemical methods were used for the separation of zinc from gallium, germanium, and copper. The determination of the cross section ratio for the reaction Cs^{133} (n, p) Xe^{133} was performed on the non-separated sample by beta counting and by gamma counting using a 100-channel analyzer. These reactions were studied:

Ga^{69} (n, p) Zn^{69}
Ge^{72} (n, alpha) Zn^{69}
Cs^{133} (n, p) Xe^{133}

By comparison of experimental ratios of the cross sections with their theoretical values, conclusions concerning the mechanism of the investigated reactions can be drawn.

III. 3.

VALENCE FORMS OF ^{32}P PRODUCED BY NEUTRON IRRADIATED $(C_6H_5)_3PO_4$

O. JOVANOVIĆ-KOVAČEVIĆ

Boris Kidrič Institute, Hot-laboratory Department, Vinča

The valence forms of ^{32}P produced by neutron irradiated $(C_6H_5)_3PO_4$ were investigated by the method of ascending paper chromatography in dependence upon the conditions of irradiation in the reactor and the further treatment of the irradiated material.

The investigations were performed with solutions obtained by the extraction of ^{32}P from $(C_6H_5)_3PO_4$ irradiated with water and hydrochloric acid.

III. 4.

REACTION OF THERMAL ANNEALING AND OF ISOTOPIC EXCHANGE IN THE NEUTRON IRRADIATED $Ca(JO_3)_2$

S. KAUČIĆ and M. VLATKOVIĆ

Rudjer Bošković Institute, Department of Radiochemistry, Zagreb

The mechanism of thermal annealing of radio-iodine, formed by the bombardment of $Ca(JO_3)_2$ with thermal neutrons, was studied. Thermal annealing of the irradiated samples in the temperature range from 100° to 250°C showed the usual increase in the retention, as observed earlier with the products of Szilard-Chalmers reactions.

A series of systems $Ca(JO_3)_2$ with the built-in »impurity« ^{131}J in the form of J^- was also prepared and the isotopic exchange of JO_3^-—(J_2, J^-) was studied at higher temperatures (170° to 400°C). The results are discussed in view of the possibility that isotopic exchange reactions may participate in the processes of the return of recoil atoms to the chemical form of the target.

III.5.

RECOIL PROCESSES IN OXY-ANIONS FOLLOWING NEUTRON IRRADIATION

S. R. VELJKOVIĆ

Faculty of Science, University of Beograd

Recoil processes following neutron irradiation in some oxy-anions were investigated. The target material was in crystalline form. The possible effects of the particle size and of the present ocluded impurities were studied.

The processes were considered in the light of diffusion kinetics. The nature of the hot spikes was discussed with special concern for the heat transfer and the thermal expansion of the lattices. An attempt was made to correlate the heat and mass transfer in activated regions.

The results for the recoil processes in most halogens and some transition metal oxy-anions indicate the necessity of introducing more parameters concerning crystalline lattices into the present simplified kinetic picture of hot reactions. The considerations are limited to the high temperature regions and to processes with high energies of activation.

III.6.

VALENT STATES OF ^{131}J IN IRRADIATED TeO_2

J. STEVOVIĆ and LJ. JAĆIMOVIĆ

Boris Kidrič Institute, Hot-laboratory Department, Vinča

Valent states of ^{131}J, obtained by irradiation of TeO_2 in the reactor, have been studied as a function of irradiation time and subsequent treatment of the irradiated substance over the temperature range from 80 to 170°C. For the separation of valent states of iodine, the ion-exchange paper chromatography, and precipitation methods were used.

III. 7.

INVESTIGATION OF CHEMICAL FORMS OF RUTHENIUM IN SOLUTIONS OF HYDROCHLORIC ACID

T. ĆERANIĆ, R. NIKOLIĆ, Z. MAKSIMOVIĆ and R. DRAŠKOVIĆ

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We investigated the solution of commercial Ru (III) chloride in hydrochloride acid by spectrophotometric, ion exchange, and extraction methods with the use of ^{106}Ru as the tracer. The purpose of these investigations was to obtain ^{103}Ru of high specific activity.

We investigated the dependence of the chemical forms of ruthenium as a function of the concentration of the acid and the aging time of the solution. Ruthenium is found in several chemical forms in these solutions. These forms are not in chemical equilibrium, and the processes are irreversible for a definite concentration of hydrochloric acid and a definite time interval. The chemical forms of ruthenium in hydrochloric acid solutions change with time, and the concentration of each component also changes.

III.8.

A STUDY OF THE PHYSICO-CHEMICAL STATE OF FISSION PRODUCTS IN THE HEAVY WATER SYSTEM OF THE *RA* REACTOR

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The purpose of the investigations was to get a better insight into the state of fission products in the heavy water system of the RA reactor; this is very important in regard to possible bursting of aluminium claddings on the fuel element.

The investigations were performed with metallic uranium, which is used as the fuel element in the RA reactor. It has been found that most of the fission products are adsorbed on the particles of uranium dioxide (UO_2), and that some of them are found in water, probably in the colloidal form. Fission products are also deposited on the stainless steel of which the heavy water system of the reactor is made. We also investigated the possibility of purifying water which contains fission products by using ion exchange resins.

III.9.

DISTRIBUTION OF TRITIUM IN 4-OCTANE PREPARED BY CATALYTIC HYDROGENATION

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Rudjer Bošković Institute, Zagreb

4-Octane was prepared from disodium-acetylide and propyl-bromide in liquid ammonia. This acetylene was reduced to the corresponding olefin by hydrogenation with tritiled hydrogen in the presence of Lindlar catalyst. In order to check the specificity of labelling, the prepared radioactive 4-octane was ozonized, and the resulting ozonide was oxidized with hydrogen peroxide. The resulting butyric acid was purified and tested for radioactivity. The results show that 8.82% of tritium in 4-octane was not bound to double bond carbons. According to the obtained results, as well as to the literature data, it is concluded that catalytic hydrogenations are not suitable for specific labelling of compounds with hydrogen isotopes.

III.10.

RATE OF EXCHANGE OF TRITIUM IN DIETHYL METHYL MALONATE-t AND DIETHYL METHYL-d_3-MALONATE-t IN BUFFERED AQUEOUS SOLUTIONS

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According to theoretical data about the larger + I effect of deuterium or tritium as compared to that of protium, as well as according to some experimental data in the literature, it could be assumed that a secondary kinetic isotopic effect may occur in the reactions of carbanionic type, if the carbon in the vicinity of the formation of negative charge is labeled by a hydrogen isotope. To check this assumption, diethyl methyl malonate and the methyl-deuteriated analogue were prepared. The residual acidic hydrogen was partly substituted for tritium. These labeled compounds were permited to undergo the exchange of tritium for protium in buffered aqueous solutions at constant temperature. This reaction yields carbanion as an intermediate. The rate of exchange was followed by extracting the organic substance in definite time intervals and measuring the activity drop of the samples. The data obtained in this way were statistically treated by means of the electronic computer IBM 7090 in Oak Ridge, USA. The results show that, within the limits of error, the rate of exchange of tritium for protium in both cases is the same, i. e., that the vicinal deuterium, in regard to the acidic tritium, does not affect the reaction rate.

III .11.

DETERMINATION OF IMPURITIES IN ALUMINUM BY MEANS OF THE ACTIVATION ANALYSIS

C. KLOFUTAR and L. KOSTA

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The isotopes ^{46}Sc, ^{60}Co, ^{65}Zn, ^{59}Fe, and ^{115}Cd with the medium half-life periods can be used for the determination of these elements in aluminum. Since they have nearly the same energies of gamma radiation, the direct use of gamma spectrometry is not possible. The described scheme, based on the ion exchange reactions, enable the quick separation of the base and their mutual separation.

Suggestions for the separation and determination of some impurities (Mn, Ga, Cu, Na) with shorter half-life periods are given.

III. 12.

THE DETERMINATION OF COPPER IN SOME MATERIALS BY RADIOACTIVATION ANALYSIS

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The radioactivation analysis was used for the determination of microgram quantities of copper in aluminium, magnesium, steels, zirconium, titanium, and the ashes of plants.

Samples of these materials were irradiated with thermal neutrons in the RA reactor, and dissolved in suitable acids from which copper was extracted with a chloroformic solution of 1-phenyltetrazole 5-thiol. The content of copper was determined by measuring the activity of ^{64}Cu in the organic phase and comparing it to standard samples which were irradiated under the same conditions.

III.13.

THE USE OF THE NEUTRON ACTIVATION ANALYSIS FOR THE DETERMINATION OF IMPURITIES IN SOME REACTOR MATERIALS

O. BOJOVIĆ and O. BRADIĆ

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An account is given of the methods developed and introduced for the determination of:

1. Manganese, chloride, and some rare earths in graphite
2. Copper, manganese, and some rare earths in magnesium
3. Some rare earths in sodium flouride
4. Hafnium in zirconium and zirconium oxide.

Some experience gained in the use of these methods are presented.

III.14.

THE DETERMINATION OF TRACES OF HAFNIUM IN METAL ZIRCONIUM AND ZIRCONIUM OXIDE BY THE NEUTRON ACTIVATION ANALYSIS

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A method has been developed for the direct determination of traces of hafnium in metal zirconium and zirconium oxide by the activation analysis.

Samples are irradiated in the nuclear reactor for 2 - 4 hours at a thermal neutron flux of 7.10^{12} n/cm²/sec. The samples are cooled until the decay of ^{179}Hf and ^{180}Hf, and the intensity of the peaks of ^{175}Hf and ^{181}Hf are measured on the spectrometer at 340 and 480 keV.

Concentrations above 100 ppm Hf are determined without standard addition, but with standard addition the lower sensitivity limit of the method is 40 ppm Hf.

III.15.

THE DETERMINATION OF THE CONTAMINABILITY OF STEEL WITH ^{137}Cs FROM AQUEOUS SOLUTIONS

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The contaminability of steel was simultaneously determined by two methods: the method of contact with a solution containing a contaminant, and the method of mixing. The steel samples whose contaminability was determined had the form of a disc, 2.5 cm in diameter, and was polished. The value of contaminability was determined from the activity of the contaminant in dependence upon the time of contact between the steel and the aqueous solution of ^{137}Cs and on the pH of the solution.

IV.1.

THE OBTAINING OF CARRIER-FREE ^{32}P FROM NEUTRON IRRADIATED ALPHA-SULPHUR

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Boris Kidrič Institute, Hot-laboratory Department, Vinča

In order to achieve the production of carrier-free ^{32}P, a simple method was developed for the extraction of ^{32}P from irradiated elementary sulphur.

Crystalline alpha-sulphur obtained by the crystalization of flowers of sulphur from a mixture of organic solvents (xylene, toluene, chloroform, and trichloroethylene) was used as the starting material. The advantage of using crystalline alpha sulphur as a target is in that it completely dissolves after neutron irradiation in decaline and other organic solvents, thus rendering possible an efficient extraction of ^{32}P.

The extraction of ^{32}P from alpha-sulphur is performed at the temperature of $95 \pm 1°C$ with the use of decaline as the solvent and hydrochloric acid as the extraction agent. The yield of ^{32}P is about 85%. The slightly yellowish ^{32}P extract is purified by the adsorption of the impurities on active charcoal at an elevated temperature.

The ^{32}P obtained as described above is in the form of orthophosphate and it has the chemical and radioactive purity required (>99% ^{32}P). The loss during the purification of ^{32}P amounts to 5%.

IV. 2.

THE OBTAINING OF CARRIER-FREE ^{32}P FROM NEUTRON IRRADIATED $MgSO_4$. Ith ADSORPTION OF ^{32}P ON MgO

O. JOVANOVIĆ-KOVAČEVIĆ and R. NIKOLIĆ

Boris Kidrič Institute, Hot-laboratory Department, Vinča

Conditions for the adsorption of ^{32}P on MgO in dependence upon the concentration of the solution of irradiated $MgSO_4$, the time of contact between ^{32}P solution and the adsorbent, the temperature, and the quantity of the adsorbent were investigated, and optimal conditions were determined for the separation of submicro quantities of ^{32}P (activity of 100 mC) from submicro quantities of ^{35}S and macro quantities of ^{32}S.

The analysis of the chemical purity, the determination of the radioactive purity, and the analysis of the chemical form of the ^{32}P obtained have shown that ^{32}P has the necessary chemical and radioactive purity ($>99\%$ ^{32}P) and that it is present in the form of orthophosphate.

IV. 3.

THE OBTAINING OF CARRIER-FREE ^{32}P FROM NEUTRON IRRADIATED $MgSO_4$. II PROCEDURE, APPARATUS AND THE SHIELDING BOX

O. JOVANOVIĆ-KOVAČEVIĆ

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A simple and efficient procedure has been developed for obtaining carrier-free ^{32}P from irradiated $MgSO_4$. The procedure consists of these phases:

1) the dissolution of irradiated $MgSO_4$ in water,
2) the adsorption of produced ^{32}P on MgO,
3) the separation of MgO from the $MgSO_4$ solution by filtration and by washing it with water,
4) the dissolution of MgO in diluted HCl,
5) the purification of the obtained solution of carrier-free ^{32}P by passing it through the cation ion-exchange column.

By this procedure, a yield of about 95% ^{32}P is obtained.

The solution obtained, in which carrier-free ^{32}P is present in the form of $H_3{}^{32}PO_4$, has high chemical and radioactive purity (>99% ^{32}P).

This paper presents the scheme of the apparatus and the shielding box in which carrier-free ^{32}P is produced at an activity of 100 mC.

IV.4.

THE OBTAINING OF ^{51}Cr OF HIGH SPECIFIC ACTIVITY

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In order to produce ^{51}Cr as a chromate, possibilities for obtaining it by irradiating chromic oxide in the nuclear reactor were investigated.

Hydrated and dehydrated chromic oxides were irradiated, and the dependence of the yield of ^{51}Cr as a chromate was determined as a function of irradiation time, the percentage of water contained in chromic oxide samples, and acceptors such as Na_2CO_3, $10H_2O$, and $(NH_4)_2S_2O_8$.

While treating the irradiated samples, the dependence of $^{51}CrO_4^{2-}$-yield was investigated as a function of solvents, the time of treatment, and temperature.

IV.5.

AN ACCOUNT OF THE SYNTHESIS OF LABELLED COMPOUNDS AT THE "BORIS KIDRIČ" INSTITUTE OF NUCLEAR SCIENCES

V. JEZDIĆ and J. RAJNVAJN

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The synthesis of labelled compounds is part of one of the fields of research which have developed in the course of the past several years at the »Boris Kidrič« Institute. Work in this field was initiated with the aim of providing solid grounds for work with labelled compounds and enabling permanent synthesis of a certain number of labelled compounds, which will be used in both fundamental and applied research. This paper presents a survey of the achievements in the field of the synthesis of labelled compounds starting with providing a basis of fundamental labelled compounds to rather complicated syntheses.

IV.6.

SYNTHESIS OF 5-BROMOURIDINE

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Investigations in the field of nucleic acids are much more reliable and accurate if labelled compounds are used for various investigations. One of the well-known antimetabolites is also 5-bromouridine. With the purpose of studying its effect, a method was developed for the synthesis of 5-bromouridine with radioactive carbon built in the pyrimidine ring.

Taking thiocarbimide, over certain intermediary products (thiouracil, uracil, 5-bromouracil, mercury salt of 5-bromouracil, 1-/2', 3', 5'-tri-O-benzoil-β-D-ribofuranozil/-5-bromouracil), we obtained 5-bromouridine with a high yield. The method has been developed for work from 1 to 5 mmoles.

IV.7.

SYNTHESIS OF OROTIC ACID

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In biological processes, orotic acid (uracil-6-carbonic acid) appears as an intermediary product when uracil and uridine are produced. It is very easily incorporated in nucleic acids and is therefore added as a precursor in various biological investigations. Orotic acid with built-in radioactive carbon is broadly used in biological investigations, and for this reason the development of a procedure for its synthesis has been approached.

This paper presents a procedure which is to be used for the synthesis of orotic acid-6-^{14}C. In regard to the procedures previously described, this procedure is characteristic for its simplicity and a 20% increase in the yield. The procedure can be used within the range of 5 to 10 mmoles.

IV.8.

A CONTRIBUTION TO THE STUDY OF BIOSYNTHESIS OF VITAMIN B_{12} BY USE OF RADIOACTIVE COBALT ^{60}Co

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In the study of the biosynthesis and resorption of vitamin B_{12} in chickens, radioactive cobalt ^{60}Co was used.

Cobalt was introduced perorally in the form of a solution of $^{60}CoCl_2$ and then detected in the vitamin B_{12} that was isolated from the liver and from the content of the digestive system.

The extraction of the vitamin from the liver was performed by autoclave treatment of the homogenized liver at pH 4.5 with an addition of KCN. After filtration of the extract, vitamin B_{12} was adsorbed on active coal, and then eluted with 65% ethanol. After evaporation in vaccuo, vitamin B_{12} was brought to the aqueous solution and then purified by repeated extraction with organic solvents. Finally, after passage through the aluminium column, the purity of the aqueous solution was tested spectrophotometrically by the extinction ratios at 361 and 550 mμ, and the radioactivity of the isolated vitamin was then measured.

To find out whether it is possible to isolate the total amount of vitamin of the liver by the applied method, investigations were performed using radioactive vitamin B_{12}, tagged with ^{60}Co. The radioactive vitamin B_{12} was also introduced perorally, and then isolated from the liver in the same manner as with the animals that were treated with cobalt 60.

IV.9.

WET PROCEDURE FOR THE OXIDATION OF ORGANIC MATERIALS AND PREPARATION OF SAMPLES FOR RADIOACTIVE MEASUREMENTS

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The activity of the ^{14}C-organic material is measured in such a way that the radioactive material is converted into CO_2 by oxidation with some strong oxidation agent or by burning it in a current of oxygen, and then directly measured in the gaseous phase; or the CO_2 obtained is converted into $BaCO_3$ and then measured in the solid phase.

The oxidation of organic compounds by the wet procedure worked out in this method is based on the quantitative determination of carbon in $BaCO_3$. The method is often used for the measurement of ^{14}C-organic compounds and radioactive biological material. This method, which has been worked out for samples containing 0.1 to 15 mg of carbon, is considered to be a synthesis of the work done so far in this field complemented with many useful details (work in the stream of nitrogen, small blank runs, treatment of precipitates by centrifugation, etc.).

RADIOLYSIS OF AQUEOUS SOLUTIONS OF OXALIC ACID

I. Effect of pH (1 — 13) on the Mechanism of Radiolysis, Induced by ^{60}Co Gamma Radiation in Presence of O_2

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It has been shown that radiation chemical yields of products of decomposition of oxalic acid decrease with the increase of pH. The irradiated samples were subjected to the measurements using spectrophotometric and gas chromatographic methods.

The reaction kinetics was discussed. The proposed mechanism and the experimentally obtained G-values were used for calculation of radiation chemical yields of primary products of water radiolysis (H, OH, H_2 and H_2O_2) in a wide range of pH.

$$G_{OH} = \frac{1}{2} G(CO_2) \qquad G_{H_2} + G_{H_2} + G(H_2O_2)$$

$$G_{H_2} = G(H_2) \qquad G_{H_2O_2} = \frac{1}{2}\left\{G(H_2O_2) + G(H_2) - \frac{1}{2} G(CO_2)\right\}$$

Values for radiation chemical yields of primary products of water radiolysis thus obtained were used for calculation of radiation chemical yields of water decomposition at various pH-values. It was shown that with the increase in pH from 1.4 to 10, G_{H_2O} decreases from 4.4 to 3.3. This decrease as well as the decreased yields of free radicals account for the decreased decomposition of the substance dissolved in water.

At pH higher than 10, an appreciably decreased decomposition was established. This could not be expected with regard to the actual knowledge about the physical and physicochemical stage of water radiolysis. In connection with this fact, certain assumptions are made concerning the reaction mechanism of the solutions with pH higher than 10.

V.2.

RADIOLYSIS OF AQUEOUS SOLUTIONS OF OXALIC ACID

II. Some Electrochemical Measurements on Pt and Dropping Mercury Electrodes During Irradiation With Gamma Rays of ^{60}Co

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It is well known that the passage of radiation through an aqueous solution, i.e., radiolysis products thus formed, bring about ehanges in the oxidation-reduction properties of the medium, which can be followed by electrochemical methods. However, it is uncertain to what extent molecular radiolysis products (H_2 and H_2O_2), and to what extent free radicals (H, OH and HO_2), are involved. Therefore, measurements were performed on a system of known radiation chemical behavior since its molecular radiolysis products were previously determined by spectrophotometric and gas chramatographic methods.

Effects expected for defined conditions (pH of the solution, dose rate, total dose absorbed, tupe of electrode) were calculated on the basis of known reaction mechani m, known G-values, and measurements in absence of radiations. The calculated values were comrared to these measureo during irradiation.

Diffusion currents on the Pt electrode were measured in order to ob erve H_2, H_2O_2, and O_2. Some measurements indicate cventual participation of H radicals in the diffusion current.

Polarographic measurements with dropping mercury electrode permit a recording of the molecular oxygen and hydrogen peroxide.

These experiments prove the usefulness of electrochemical measurement for the study of thee radiolysis mechanism in aqueous solutions beacause of the possibility of direct, continuous measurement of molecular products (H_2, O_2, and H_2O_2) during irradiation.

The agreement of experimental and theoretical values is satisfactory, although deviations in some cases are considerable (ten percent and more). In certain cases, basic radiation chemical yields used in our calculations do not exceed this order of precision. The foregoing experiments will show to what extent, by imrrovement ot techniques, it will be possible to attain better accuracy and reproducicility.

V.3.

RADIOLYSIS OF AQUEOUS SOLUTIONS OF OXALIC ACID

III. Some Electrochemical Measurements in the Course of Irradiation by X-rays: Potential Changes of a Pt electrode as a Function of the Presence of Different Gases (N_2, O_2 and N_2O) and the Change in pH-values (1 to 12)

D. OVCIN and I. DRAGANIĆ

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Potential changes of the platinum electrode in oxalic acid solutions were recorded at various pH values, in the presence of various gases (N_2, O_2, or N_2O). Radiation conditions were chosen (relatively low absorbed doses) so that the molecular products H_2 and H_2O_2 were present in such concentrations, and that potential changes possibly induced by them could be practically neglected.

A fairly steep potential drop was observed in the presence of N_2, very near the potential of the saturated hydrogen electrode for the given conditions. In the presence of O_2 and N_2O, potential changes were differently and weakly expressed.

The possibility of interpretation of the experimental results by the reaction of the H radicals with the platinum electrode was discussed. In this reaction, the recombination of the H radicals to the molecular hydrogen should occur, producing the hydrogen potential, which could not be expected in view of the absorbed dose and the $G(H_2)$ for the given system. In the presence of O_2 and of N_2O, which react very efficiently with H radicals and remove them from the solution, considerable potential changes could not be expected.

The weakness of the present interpretation was also indicated.

V.4.

REACTIONS OF THE RADICAL IN SOLUTIONS OF SULPHURIC ACID

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During the irradiation of de-aerated aqueous solutions of the system vinylpryidinesulfate (VP)-ferric sulfate-sulphuric acid with gamma rays from cobalt-60, these radiation chemical reactions occur.

$$H_2O \text{—}\omega\text{→} H,\ OH,\ H_2,\ H_2O_2$$
$$H\ (\text{or}\ OH) + VP \longrightarrow R\cdot$$
$$R\cdot_{k-1} + VP \longrightarrow R\cdot_k$$
$$R\cdot_k + Fe\ (III) + H_2O \longrightarrow R_kOH + Fe\ (II) + H^+$$
$$H + Fe\ (III) \longrightarrow H^+ + Fe\ (II)$$
$$H_2O_2 + Fe\ (II) \longrightarrow OH + OH^- + Fe\ (III)$$

The radiation chemical yield is $Ge\,(FeII) = G\,(H) + G\,(OH)$. However, by increasing the sulphuric acid concentration in the range from 0.01 N to 1 N, G (FeII) decreases from 7.2 to 5.7. For interpretation of the observed effects of sulphuric acid possible mechanisms of radiation chemical reactions are considered.

It is concluded that in sulphuric acid solutions the reactions of the radical OH with bisulfate and sulfate ions should also be accounted for. The resulting products (bisulfate and sulfate ion radicals) oxidize the ferrous ions to ferric ions.

V.5.

SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF HCl IN NONAQUEOUS SYSTEMS AND ITS APPLICATION IN THE RADIATION CHEMISTRY OF ORGANIC LIQUID SYSTEMS

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A very sensitive and exact method for the spectrophotometric determination of minute amounts of HCl directly in organic liquid systems was developed and thoroughly studied. The sensitivity of the method permits a recording of radiation chemistry processes of low efficiency and in the range of low doses where the action of radiation upon the reaction product and concentration relations is negligible. The accuracy of the method makes possible the detection and determination of small changes in yield which are brought about by small differences in the chemical con_titution of the components of mixed systems.

V.6.

DETERMINATION OF GASES EVOLVED DURING THE IRRADIATION OF ION EXCHANGERS

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The cationic ion exchangers of the sulphonic acid type were irradiated, either in the form of the sodium salt or free acid, both in the dry condition and in the presence of the defined quantities of water, by a series of doses of 60, 120 and 180 Mrad. On the base of the analysis of the irradiated sample, soluble products of the radiolysis and radiolytic gases, the total balance of the decomposition during the irradiation is worked out. The following gases were determined: H_2, SO_2, CO_2, and CO. G values for the formation of gases are different for different forms of ion exchangers. In most cases, SO_2 is the main gascosus product of the decay, while its quantities are not linearly proportional to the magnitude of a dose.

Dowex 50W-X10, dry: $G(H_2) = 2.9 \times 10^{-2}$, $G(SO_2) = 0.21$
36.6% H_2O 9.1 0.00067

CHEMICAL DOSIMETRY OF HIGH DOSES OF REACTOR RADIATIONS

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The problem of a simple and reliable determination of doses absorbed in the materials which have been exposed in reactors is rather complex because of the nature of reactor radiations. Various types and energies of radiations, and induced radioactivity and relatively high dose rates complicate the theoretical and experimental determination of absorbed doses in the irradiated sample. The experimental work is rendered still more difficult by uneasy operations, activation during irradiations, limited volumes, etc. The complexity of calculating results still more restricts the choice of methods.

Among the current methods (ionization, calorimetry) chemical methods were chosen which are based on the known ratio of the dose absorbed and the number of chemical changes in the system. Experimental difficulties are smaller with these methods, and if a suitable system is chosen (average mass number of the unknown sample and of the dosimeter to be very near each other), a practically direct result of the absorbed dose in the irradiated sample can be easily obtained.

The aqueous oxalic dosimeter has been chosen for our measurements because of the large dose range (up to 1.6×10^8 rad) that can be measured and in view of the ease of handling and the absence of the induced radioactivity. Five series of determinations were performed in longer and shorter time intervals. The obtained reproducibility of results was very good. The agreements with calorimetric and neutron flux measurements ascertain the reliability of the method. In order to measure absorbed doses higher than 10^8 rad, the possibility of the use of solid systems was investigated. Their advantage is the formation of smaller amounts of gases than in aqueous solutions, and the possibility of use of a higher concentration of the substance. Experiments performed so far with solid oxalic acid and with some oxalates have permitted a precise choice of the system, an approach to technical realizations, and the obtainance of preliminary data concerning the dose region in which the dosimetry could be done.

V.8.

DESIGN AND APPLICATIONS OF CALORIMETERS IN THE STUDY OF RADIATION CHEMISTRY OF AQUEOUS SOLUTIONS

B. RADAK

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A knowlcdge of G-values is necessary for consideration of reaction mechanisms in radiation chemistry. Direct measurement of the absorbed radiation energy is therefore of the utmost importance. Calorimetry is in general an ideal technique for such measurements. In this paper, an attempt is made to systematically survey the advances in this field both abroad and in our country.

Radiation chemical calorimetry — theoretical backgrounds. Applications of time equation for the temperature of the thermally active body are the basis of all types of calorimetry. Isothermal calorimetry, heat cycling, differential calorimetry, measurements of short-time processes, adiabatic calorimetry.

Calorimeters. Three calorimeters for work with aqueous solutions were chosen. Two of them were elaborated in the Institute »Boris Kidrič« in Vinča.

— Calorimeter for the direct measurement of G-values in the field of gamma radiations. Applications, description of the adiabatic apparatus, results.

— Isothermal calorimeter for mixed reactor radiations. Specific difficulty here is the work in the two component fields. Description of the apparatus and of its principle (isothermal calorimeter with thermistor). Results of measurements on the reactor RA in Vinča.

— Differential calorimeter for radiation chemical measurements in the field of reactor radiations. Specific difficulty — direct measurement of G-values in reactor field. Principle of work and description of the differential calorimeter. Results of measurements on the reactor RA in Vinča and comparison with other measurements.

V.9.

EXPERIMENTAL SOURCE OF GAMMA RADIATION IN THE INSTITUTE "RUDJER BOŠKOVIĆ"

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The design of the apparatus and of its protection is described. The source of radiation consists of eight rods of ^{60}Co disposed to form a vertical cylinder of 113 mm in diameter. The source of radiation is of the closed type (the source is motionless), and is intended for experimental irradiation of solid samples and biological objects where homogeneous distribution of doses per volume is necessary. The protection of the surroundings from radiation is complete so that this apparatus can be installed in any laboratory, and its handling does not require previous extra training.

V.10.

SYNTHESIS AND DECAY OF MACROMOLECULES BY MEANS OF IRRADIATION

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We are able to produce various forms of modified starch and dextrose by means of irradiation and without the influence of any other reacting species, which can be used directly in practice. Gamma rays can cause the formation of unstable products of decay, able to combine with the »activated« macromolecules, thus forming the new molecular combinations.

SOME PHENOMENA RELATED TO THE IRRADIATION OF FOOD STUFF BY GAMMA RAYS

M. BLINC

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The irradiation of potato and onion by gamma rays in doses of 10,000 REP resulted in the reduction of germination during winter and spring, without changing the organoleptic properties. A good effect was achieved by irradiating pork, veal and beef, and fish and poultry, after they were treated by a 0.5% solution of sodium glutamate and stored at a temperature of 1° to 2°.

The irradiation of the sugar beat by a dose of 10,000 REP prevents the development of fungial infection, and the sugar content during several months of storage is thus kept at a higher level. Sugar beet seed exhibited an increased germination capacity after being irradiated by 10,000 - 15,000 REP.

Gamma ray irradiation can also be used for certain kinds of vegetables in special doses of 50,000 to 10^6 REP in relation with storing at 2° - 4°.

The irradiation of wheat flour with doses of 30,000 to 100,000 REP appeared effective in respect to improving the properties of dough, bread, and glue. Spectro proton magnetic resonance indicates that doses up to 100,000 REP do not have unwarranted effects on organoleptic properties of the food stuff.

VI.1.

THE RADIOACTIVE TRACER METHOD USED FOR THE INVESTIGATION OF THE INFLUENCE OF THE CONDITIONS OF THE DIESEL ENGINE OPERATION ON THE SCRAPING OF THE PISTON RING

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The radioactive tracer method was used for the study of the scraping of the piston ring. By measuring the increase in radioactivity in the lubricant which, during the experiment, continuously circulates between the engine and the chamber for measuring the radioactivity, we determined the scraping of the labelled piston ring. The piston ring was labelled along the scraped surface with 7 uniformly arranged insets whose total radioactivity was 105 μC ^{60}Co. The insets were made of an alloy of grey cast iron with 10% of cobalt and they were activated by irradiation in the reactor. The scraping of the piston ring was investigated in dependence upon the number of rotations of the shaft, the engine load, the temperature of the cooling water, and the sulphur content in the fuel.

APPLICATION OF ^{60}Co ISOTOPE IN CONTROL OF ENDURANCE OF BLAST FURNACE LINING

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The application of isotopes in blast furnaces is confronted with several problems: selection of proper kind of isotope, finding the proper places for building in of isotopes in the lining, building in of isotopes, measurements of radiations at the chosen control points, and a critical evaluation of results.

Radioactive isotope ^{60}Co was used as the tracer. It was built in at 44 control points in doses of 0.06 - 8.4 mC, all together 141.96 mC. By means of that, the continual control of the wall thickness and the maintenance of the inner profile of the furnace was made possible. Maintenance of the inner profile makes the technological control of the process easier, and a better endurance of the lining is simultaneously achieved. Both effects have the influence on the economical aspect of the entire process.

VI.3.

A STUDY OF THE MOVEMENT OF UNDERGROUND WATERS IN GROUNDS OF VARIOUS POROSITY USING THE RADIOACTIVE TRACER METHOD

V. VUKMIROVIĆ and A. FILIP

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Water in wells is labelled with radioactive iodine-131, and the dependence between the decrease in activity and time is determined from the decrease in activity due to underground water flow. An attempt has been made to determine the filtration rate from this dependence. The experiment was performed in two neighbouring wells which are made in materials of various characteristics (sand and gravel).

VI.4.

A STUDY OF THE MOVEMENT OF DRAGGED DEPOSITS IN THE VELIKA MORAVA BY USING SAND LABELLED WITH RADIOACTIVE ^{51}Cr

V. VUKMIROVIĆ and A. FILIP

Jaroslav Černi, Institute for Water Regulation, Beograd, and
Boris Kidrič Institute, Vinča

The movement of dragged deposits in small waters of the Velika Morava has been studied by means of radioactive tracers. Sand taken from the river bed was labelled with radioactive ^{51}Cr. The movement of the labelled sand placed on the bottom of the river was observed by measuring the radioactivity of the river bed. The experiment resulted in tracing the movement of the deposit, and in obtaining an insight into the rate of the movement in the bottom of the river during small waters.

V1.5.

PROBLEMS OF PROTECTION IN THE URANIUM MINES

R. NIKOLIĆ, M. KAČAREVIĆ, Z. OSTROGOVIĆ, M. RADOVIĆ
and Ž. CANIĆ

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Problems of protection against radiations in uranium mines are discussed. Dangers and precautions for the safety of personnel are considered. Methods for the detection of radiation are surveyed, and suggestions are given for avoiding the effect of radone, uranium dust, and other harmful factors which arise during the exploitation of uranium ore.

VI.6.

PROBLEMS OF PROTECTION IN THE PRODUCTION OF URANIUM

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Institute of Nuclear Raw Materials, Beograd

Problems of protection against radiation, concerning safe work while handling uranium ores, are considered. Possible dangers during the work, and methods for setting up protection systems are discussed. Methods of radiation control of working area are reviewed, and suggestions are given for the protection of the personnel and the surroundings from radiation and contamination.

VI.7.

PROBLEMS CONCERNING INTERNAL CONTAMINATION IN HANDLING URANIUM ORES

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The problem of the protection of personnel during work with uranium ores is considered. The harmful affect of uranium, the possibility of its penetration into the human organism, and its secretion are discussed. Methods of detection of uranium content in biological materials are given, and the results of systematic control of exposed personnel are reported. As a result of these investigations, suggestions are made for personal protection of the personnel while working with uranium.

VI.8.

SOME PROBLEMS OF THE METROLOGY OF RADIOACTIVE SOURCES

DJ. BEK-UZAROV and D. PALIGORIĆ

Boris Kidrič Institute, Department of Analyses and Metrology, Vinča

A general survey of the development in the field of metrology of radioactive isotopes and radiations is given. A brief survey is also given of the achievements concerning the problems of measurements according to the present needs. Possibilities are offered for further development, with special reference to the application of isotopes in science and industry in Yugoslavia.

VI.9.

AN ANALYSIS OF PURE BETA EMITTERS BY MEANS OF THE LIQUID SCINTILLATION COUNTER

LJ. DOBRILOVIĆ, DJ. BEK-UZAROV, V. GRADOJEVIĆ and D. PALIGORIĆ

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The liquid scintillation counter was used as a beta spectrometer. Its proportionality as a spectrometer was shown, and the sensitivity of the method was determined by artificial contamination with pure beta emitters.

A DEVICE FOR ADJUSTING A DEFINITE DEAD TIME ON THE *GM* COUNTER

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Various quenching units in GM argon alcoholic counters were examined and developed.

A very convenient device was designed which proved to be the most suitable for satisfactory results in work and the simple way of connecting it to standard electronic equipment.

VI.11.

CRITICAL SURVEY OF METHODS OF RECKONING CENTRIFUGAL CLASSIFIERS IN THE TECHNOLOGY OF URANIUM (INCLUDING AUTHORS OWN METHOD)

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A survey of contemporary systems of centrifugal classifiers in the technology of uranium. A survey of contemporary methods of reckoning these devices (critical comment). Authors own method of calculation.

THE NEW STANDARD FOR ATOMIC WEIGHTS

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The International Union of Pure and Applied Chemistry (IUPAC) (in Montreal 1961) and the International Union of Pure and Applied Physics (one year earlier) have adopted a unified scale for atomic weights, instead of the two scales (chemical and physical) that were previously in use.

Prior to this convention the atomic weight of the usual (»natural«) oxygen O = 16 has been taken as a chemical standard, whereas the mass of the oxygen isotope $^{16}O = 16$ has been taken as a physical standard. In the new unified scale the dominating carbon isotope $^{12}C = 12$ is adopted for standard.

After a historical introduction which deals with previous standards for atom weight determinations, the advantages of the new standards are stated, and differences between the new and the earlier atomic weights are pointed out. Emphasis is also made on new values for Avogadro number, gas constant, molecular volume, and electric charge of one gram-equivalent (1 Faraday).

B.1.

THE SCOPE AND SIGNIFICANCE OF ORGANIC REAGENTS IN ANALYTICAL CHEMISTRY

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The reacting groups in organic analytical reagents. The analytical methods based on organic reagents.

The complexometric titration, and the use of new indicators in various analytical determinations.

C.1.

SPECTROCHEMICAL ANALYSIS IN YUGOSLAVIA AND ABROAD

S. RISTIĆ

Faculty of Sciences, University of Beograd

A short but critical review on the spectrochemical activities in the world and in Yugoslavia is given by using the published Meggers-Scribner's **Indexes,** and also the other published and unpublished materials in order to give a full account of the principal development stages for the important Spectrochemical Methods of Analysis.

In view of the intention to hold the XIth International Spectroscopy Colloquium in Belgrade (Yugoslavia), a special effort was made to emphasize the principal aspects and some correlations in the development stages of spectrochemical activities, both in the world and in Yugoslavia.

D.1.

AGEING PROCESS OF LOW CARBON STEELS
(studied by means of changes in electrical resistivity)

B. BOŽIČ, D. MIHAJLOVIĆ and D. JOVANOVIĆ

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The precipitation and strain ageing processes on low carbon steel samples containing 0.05% C were studied.

The first series of samples was quenched after 700°C in water containing 10% NaCl. Then the samples were aged at 50°, 90°, 120°, and 150°C for 0, 5, 10, 20, and 40 minutes, and for 1, 2, 4, and 6 hours. During the period of ageing, the electrical resistivities of samples were measured. The second series of samples was treated in the same manner, but after quenching, and prior to ageing, the samples were deformed by tension with deformation degree of 6%.

The results obtained prove that the method used is very sensitive and quite suitable for the study of ageing processes. The results obtained are consistent with those theoretically expected.

AGEING PROCESS OF LOW CARBON STEELS
(studied by investigation of mechanical properties)

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The precipitation and the strain ageing processes on low carbon steel samples containing: C — 0.05 %, Si — 0.056 %, Mn — 0.28 %, S — 0.01 % and P — 0.002 % were studied.

For precipitation ageing these conditions were applied: quenching after 700° C in water containing 10 % NaCl, ageing at 50°, 90°, 120°, and 150°C, for 0, 5, 10, 20, and 40 minutes, and for 1, 2, 4, and 6 hours. These conditions were applied in the case of strain ageing: after quenching at 700° the samples were deformed by tension, with degree of deformation 6 %, the ageing process being performed under the same conditions as in the case of precipitation ageing.

Changes in properties due to ageing were studied by investigation of tensile strength, micro hardness, and micro structure. The results show that the changes in tensile strength, tensile limit and micro hardness, as functions of temperature and of duration of treatment, for both types of ageing correspond to the theoretically expected values, and that the investigation methods used are sufficiently sensitive for the study of ageing processes. However, microstructural changes can be detected by means of the optical microscope only in the last stage of ageing. Hence, this method is unsuitable for the study of ageing processes through all phases.

E.1.

MODERN METHODS OF BLEACHING SEMICHEMICAL PULP

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Applications of bleached and unbleached semichemical pulp. Importance of semichemical pulp as a substitute for pulp. Modern methods of bleaching semichemical pulp and its prospective applications in the production of paper. Semichemical pulping in Yugoslavia.

F.1.

THE DETOXIFICATION OF TOWN GAS

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The town gas supply of the major part of this country relies on the production of fuel gas from lignite by its gasification under pressure in the high Lurgi process plants. Because the gas derived from Lurgi high-pressure gasification systems contain, after conventional purification treatment, more than 25% vol. of carbon monoxide, it might be anticipated that the reduction of the toxicity of Yugoslav Lurgi gas by the reduction of its carbon monoxide content would be necessary.

The removal of carbon monoxide from industrial gases became a technological problem of prime importance with the advent of catalytic processes for the manufacture of synthetic ammonia, and it is now being developed only for that purpose. Thus, among the many methods proposed for the carbon monoxide removal from synthesis gas streams in the detoxification of town gas streams cannot be used directly, while others are not absolutely suitable (low-temperature techniques, oxidation of carbon monoxide). The applicability of the conversion of carbon monoxide in hydrogen and carbon dioxide, methanation, i e., the Fischer-Tropsch synthesis of hydrocarbons from carbon monoxide and hydrogen, and the absorption of carbon monoxide in copper-ammonium salt solutions have been considered only in connection with the carbon monoxide removal from town gas.

In the past several years, much has been done towards the choice of a technologically and economically acceptable method for the detoxification of domestic fuel gas. With these studies, partially performed on a full industrial scale, it was found that the process based on the catalytic conversion of carbon monoxide by action of water vapor, so-called water gas shift reaction, is the most suitable. Consequently, it appears that the shift conversion can also be the most applicable technique for the reduction of Yugoslav town gas toxicity.

F.2.

BRIKETIERUNG DER GETROCKNETEN FEINKOHLE AUS DER FLEISSNER-TROCKNUNG

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RBK "Kolubara", Vreoci

Labor- und halbindustrielle Versuche ausgeführt in DDR gaben gute Ergebnisse welche auf die Möglichkeit der industriellen Briketierung der Feinkohle ohne Bindemittel hinweisen.

Eine Beschreibung der ausgeführten Labor- und halbindustrielle Versuchen mit entsprechenden Angaben ist gegeben.

G.1.

GLASS STRUCTURE AND PROPERTIES OF GLASS

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A knowledge of glass and of its properties is important, not only for the glass industry, but also for the entire ceramic industry.

Structural changes and/or inhomogeneities influence the properties of glass. Therefore, properties of glass and their dependence upon the structural changes in glass, as viscosity, thermal dilatation, and chemical stability and density are considered.

G. 2.

CERAMICS IN NUCLEAR ENGINEERING

M. M. RISTIĆ, M. ŠUŠIĆ, M. STEVANOVIĆ and D. JOVANOVIĆ

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Possibilities for the use of ceramics in nuclear reactors are pointed out, and several problems involved are considered. Properties of uranium oxide and carbide are described, and problems of production of these materials are considered. Several problems concerning nuclear fuel of the cermet type are discussed.

Ceramic materials exhibit hardness and corrosion resistance at generally high melting points, but low thermostability and strength. Their application in nuclear reactors depends upon the adequate design and successful production of particular ceramic objects.

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