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DERIVATIVE POLAROGRAPHIC TITRATION OF BASES IN GLACIAL ACETIC ACID IN THE PRESENCE OF ANTIMONY OR QUINHYDRONE ELECTRODES

by

V. VAJGAND and T. PASTOR

In our earlier report we presented a method by which it is possible to determine bases in acetic acid by derivative polarographic titration, using quinhydrone electrodes. Our work was later extended to the investigation of the behavior and properties of other electrodes in derivative polarographic and biamperometric (dead-stop) titrations. In this report we present the results obtained by the use of antimony electrodes in derivative polarographic titration.

Antimony electrodes often used for pH determinations and for potentiometric titration of acids and bases in an aqueous medium, are sometimes also used for titrations in non-aqueous solvents. More detailed investigations have been made in neutral and alkaline solvents while Tomiček and Heyrovsky (2) and Shkodin and Karkuzaki (3) have also carried out potentiometric titrations in glacial acetic acid. The first two authors have established that the potential at antimony electrodes is more positive in acetic acid than in water and that it is necessary to remove water from acetic acid by the addition of acetic anhydride. Shkodin and Karkuzaki have noticed that at the antimony electrodes the potential is established rapidly and that the titration curve of strong bases (pyridine, sodium acetate) obtained in their presence is more symmetrical than the curves obtained in the presence of other electrodes. By examining the influence of water and acetic anhydride on the jump in potential at the end-point of the reaction of very weak bases we have established that in the presence of 0.5 to 1% of water the potential jump vanishes completely, the first 10—20% of anhydride has the greatest effect on the potential jump, while the highest jumps are obtained with 85 — 95% anhydride.

By experimenting with bimetallic electrode systems in a nonaqueous medium Novak (4) has established that in acetic acid containing 30% acetic anhydride the best results are obtained with the antimony-graphite electrode system (graphite has a constant potential.

Enoki and Mirisaka (5) have applied antimony electrodes in the derivative polarographic titration of acids and bases in aqueous and alcoholic solutions, and Bishop and Short (6,7,8) in an aqueous solution. The latter authors have studied in great detail the processes at the electrodes and various influences on the change in potential during titration.

It is evident from this that antimony electrodes have so far been used mostly for titration in an aqueous medium and less frequently in a non-aqueous medium. In this work we have studied the possibility of their application in derivative polarographic titration of organic bases and alkaline salts of organic acids in acetic acid with perchloric acid. We have also compared the properties of antimony and quinhydrone electrodes and the results obtained by these electrodes.

EXPERIMENTAL

The apparatus used was described in our earlier paper (1). Two "Radiometer" antimony electrodes were employed.

Glacial acetic acid p.a. "Fluka" mixed with acetic anhydride, p.a. "Kemika", was used as solvent. For the titration of hydrochloride of organic bases a 3% solution of "Kemika" mercuric acetate was added to the solution.

For the titration of liquid amines (triethylamine, triheptylamine, etc) the solution was prepared in a normal volumetric flask by weighing the specified amounts of amines on an analytical balance. From this solution weighed volumes were used for titration.

The potentiometric titration was carried out in the presence of a glass electrode connected to a reference $Hg/Hg_2(CH_3COO)_2$ electrode (9). A fiber-type saturated calomel electrode was used for measuring the anode and cathode potentials.

The 0.1N $HClO_4$ solution was standardized by a sodium acetate solution prepared by dissolving suitable amounts of sodium carbonate, p.a. "Merck", previously dried at 270° C.

Antimony electrodes are suitable in the titration of tertiary amines and alkaline salts of organic acids. The end-point of the reaction is marked by the maximum in potential difference obtained during titration. The peak of this maximum depends on the strength of the base titrated. Thus, for example in titrating diethylaniline ($K = 4.5 \cdot 10^{-8}$ in water) in a mixture of acetic acid and its anhydride (1 : 1) a change in potential difference of about 140 mV per 0.1 ml of the 0.1 N $HClO_4$ solution added was obtained at the equivalence point while for caffein, under the same conditions ($K = 4.1 \cdot 10^{-14}$ in water) and the same amount of the titration agent, the change in potential difference at the equivalence point was only 15 mV. The sharpness of the end-point of the reaction also depends on the composition of the solvent used, as illustrated by the following examples: In the titration of brucine with acetic acid and its anhydride mixed in the proportion 6 : 1 the potential difference obtained at the equivalence point was 50 mV for 0.1 ml of 0.1 N $HClO_4$, whereas if the proportion between the acid and anhydride was 1 : 1 the difference was about 120 mV. A similar relation was also found in other titrated systems.

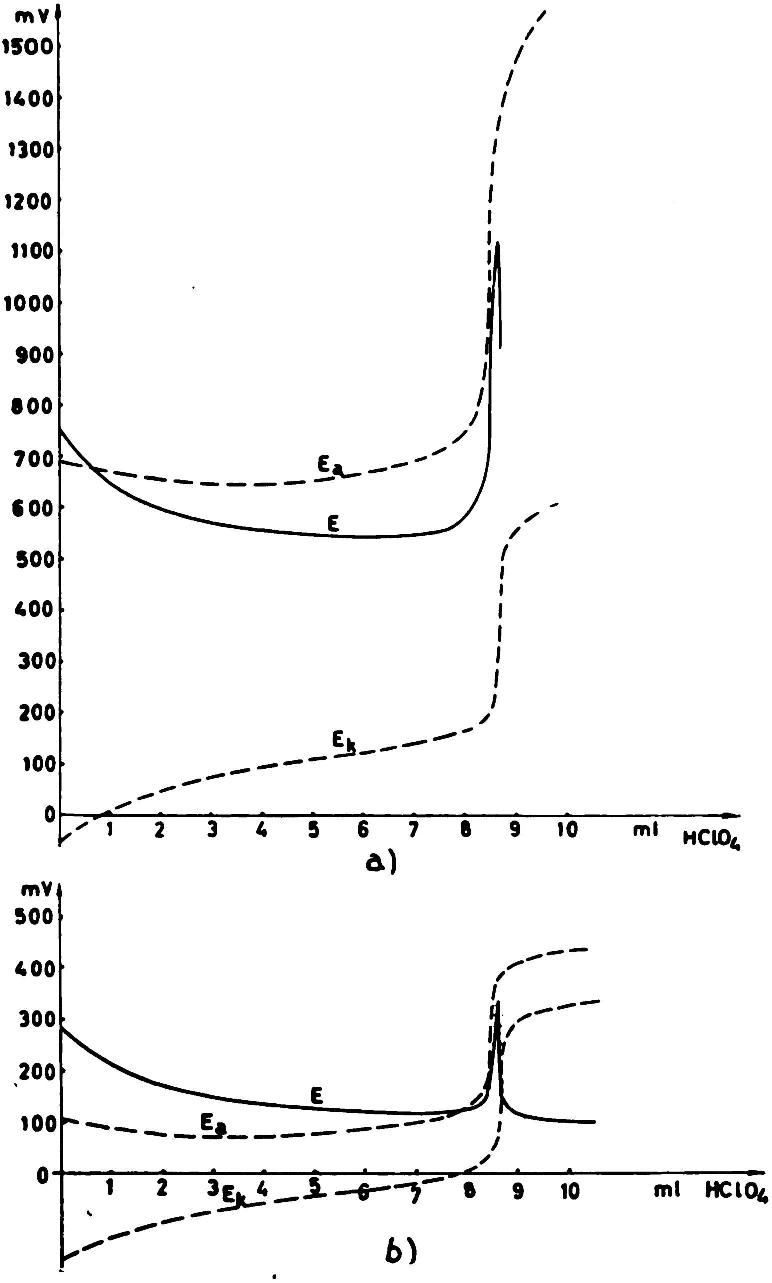
mV
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
E_a
E
E_k
1
2
3
4
5
6
7
8
9
10
ml
HClO_4
a)
mV
500
400
300
200
100
0
E
E_a
E_k
1
2
3
4
5
6
7
8
9
10
ml
HClO_4
b)

Figure 1

To study the titration curves we measured the change in potential at the anode and cathode against a saturated calomel electrode. These curves have a bilogarithmic form characteristic of potentiometric titrations at constant current (Fig. 1). The end-point of the reaction calculated from the change in the anode potential occurred somewhat earlier than the equivalence point, while the end-point calculated from the change in the cathode potential occurred somewhat later than the equivalence point so that the difference between these two end-points was about 0.02—0.03 ml of 0.1 N $HClO_4$. This is explained by the occurrence of concentration polarization due to the flow of constant current, which causes an increase in the concentration of hydrogen ions at the anode and a decrease at the cathode. The curve obtained by the derivative polarographic titration represents the differences in the anode and cathode potentials during titration. This difference is greatest at the equivalence point.

The comparison of the results obtained by the derivative polarographic titration with antimony and quinhydrone electrodes shows that the end-point is more sharply pronounced in the presence of quinhydrone electrodes (Fig. 1). The advantage of the antimony electrodes lies in the fact that the potential is established almost instantly. During titration of the halogen salts of organic bases in the presence of mercuric acetate the antimony electrodes amalgamate and at these amalgamated electrodes the potential is not established so fast.

The results obtained by derivative polarographic titration of sodium benzoate with antimony electrodes are recorded in Table 1, while the titrated systems and the results obtained are listed in Table 2.

TABLE 1

No.	Taken g	Consumed $HClO_4$ ml	Found g	Solvent
1.	0.1000	6.91	0.0996	10 ml CH_3 COOH + + 10 ml $(CH_3CO)_2O$
2.	0.1000	6.94	0.1000	,,
3.	0.1000	6.94	0.1000	,,
4.	0.1000	6.94	0.1000	,,
5.	0.1000	6.91	0.0996	,,
6.	0.1000	6.92	0.0997	,,
7.	0.1000	6.94	0.1000	,,

Mean value $= 0.0998 \pm 0.0002$*

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* ± 0.0002 is the average deviation

TABLE 2

Substance Titrated	Taken* g	Found by Potent. Titration g	Derivative Polarographic Titrations: In the Presence of Antimony Electrodes: No. of Titr.	Found g	In the Presence of Quinhydrone Electrodes: No of Titr.	Found g	Solvent
Sodium formate	0.0500	0.0499	3	0.0501 ± 0.0001	4	0.0500 ± 0.0001	20 ml CH_3COOH
Isopropyl phenazone	0.1500	0.1485	3	0.1848 ± 0.0000	6	0.1485 ± 0.0000	,,
Diethylaniline	0.1484	0.1464	3	0.1463 ± 0.0001	4	0.1464 ± 0.0001	,,
Sodium salicylate	0.1000	0.0997	2	0.0996 ± 0.0001	2	0.0997 ± 0.0000	10 ml CH_3COOH + + 10 ml $(CH_3CO)_2O$
Triethylamine	0.0838	0.0813	2	0.0813 ± 0.0001	2	0.0813 ± 0.0001	,,
Potassium acetate	0.0859	0.0839	3	0.0834 ± 0.0000	2	0.0838 ± 0.0000	,,
Brucine	0.2000	0.1988	2	0.1987 ± 0.0001	2	0.1987 ± 0.0001	,,
Triheptylamine	0.2969	0.2826	2	0.2826 ± 0.0005	3	0.2824 ± 0.0001	,,
Tributylamine	0.1953	0.1609	3	0.1605 ± 0.0003	4	0.1608 ± 0.0002	,,
Sodium benzoate	0.1000	0.0999	7	0.0999 ± 0.0003			,,
Antipyrine	0.1500	0.1506	6	0.1501 ± 0.0003			20 ml CH_3COOH
Sodium p-aminosalicylate	0.2000	0.1999	6	0.1998 ± 0.0005			20 ml CH_3COOH
Isoniazid	0.1000	0.0998	11	0.0999 ± 0.0002			,,
Aminopyrine	0.1500	0.1501	8	0.1499 ± 0.0003			10 ml CH COOH + 10 ml 3% Hg $(CH_3COO)_2$ in CH_3OOH
Procaine hydrochloride	0.3500	0.1498	6	0.1497 ± 0.0002			

* The determinations were carried out with pharmaceutical products. Their purity was checked by potentiometric titration (1).

The values recorded in Tables 1 and 2 show that the results obtained in these experiments are in very good agreement and afford excellent reproducibility.

CONCLUSION

The derivative polarographic titration of a series of bases with perchloric acid in a mixture of glacial acetic acid and acetic anhydride, by using two antimony and two quinhydrone electrodes, is described. The following substances were determined: sodium formate, isopropyl phenazone, diethyl aniline, sodium salicylate, triethylamine, potassium acetate, brucine, triheptylamine, tributylamine, sodium benzoate, antipyrine, sodium p-aminosalicylate, isoniazid, aminopyrine, and procaine hydrochloride.

Measurements of the anode and cathode potentials against a saturated calomel electrode showed that the change in the anode potential at the equivalence point is much more rapid with quinhydrone than with antimony electrodes, whereas the potential at the antimony electrodes is established much more quickly. The cathode potential jump at both pairs of electrodes is sufficiently great and the potential is established almost instantly, In derivative polarographic titrations the 'advantage of the antimony electrodes lies in that the titrations require a shorter time and afford excellent reproducibility.

The results obtained by potentiometric titration and by derivative polarographic titrations in the presence of antimony or quinhydrone electrodes are in very good agreement.

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A CONTRIBUTION TO METHACRYLACETONE STUDIES
II. PHYSICAL PROPERTIES OF THE MONOMER

by

A. R. DESPIĆ and DJ. R. KOSANOVIĆ

INTRODUCTION

Methacrylacetone monomer (1-hexene 2-methyl 3,5-dione) has been comparatively recently synthesized (1, 2, 3, 4) and so far no systematic study of its physical and chemical properties can be found in the literature. The two main papers on the synthesis of this compound contained scanty information, sufficient to prove its existence but insufficient for its complete physical and chemical characterization. Since this compound might be of theoretical interest because of the interaction between the double bond and the chelating diketone group in its immediate vicinity, and also of practical interest because of its use as a starting material in obtaining a new plastic and a new selective ion exchanger, it was considered useful to examine in greater detail the possibility of isolating and purifying the compound and to determine accurately the essential parameters which might be necessary in the synthesis, further study, and use. All these considerations determined the scope of the present paper. For a more precise characterization of this compound, its melting point, the infra-red spectrum, and refractive index were determined, while the density, viscosity, and vapor pressure were determined as the parameters required for the development of a large-scale synthesis.

Since the pure monomer is obtained by the decomposition of pure copper chelate, the infra-red spectrum of this compound has been determined and its solubility in various organic solvents investigated in detail in order to establish the optimal conditions for its extraction and recrystallization.

The investigations of its polymerizability, acidity and complexing properties will be reported on another occasion.

EXPERIMENTAL

1. The methacrylacetone monomer was obtained by the decomposition of twice recrystallized copper chelate according to the procedure described earlier (4) and the decomposition product destilled at reduced pressure. The product was then purified by recrystallization and its melting point determined.

The monomer is liquid at room temperature so that its recrystallization was carried out at a low temperature in a mixture of dry ice and acetone. A ground-glass stopper fitted with an alcohol thermometer and a slip joint stirrer attached to the recrystallization test tube prevented the access of moisture. Recrystallization was carried out by multiple freezing and melting with the separation of crystals by withdrawing the melt into a separate branch of the test tube. The system was not opened until a constant melting temperature was attained. The melting point was determined by plotting the cooling curve during this process.

The measurements were made with an accuracy to ± 1.0° C.

2. A *Perkin-Elmer spectrophotometer Model 13U* with a NaCl prism was used for the determination of the infra-red spectra of the recrystallized monomer and the copper chelate; the cappillary film technique was applied to the former, and the pellets technique to the latter.

3. The refractive index was determined by an *Abbé* refractometer as a mean value from five readings with an accuracy to ± 0.0002 against the light of sodium lamp. The instrument was gauged with a calibrated prism, and the temperature maintained constant to ± 0.02° C.

4. The density was measured with a pycnometer of 20 ml capacity. In each phase the temperature was maintained constant to ± 0.02° C by keeping the apparatus in an ultrathermostat for thirty minutes.

5. The vapor pressure was determined as a function of temperature by the *Smith-Menzies* isoteniscope method. The pure monomer exhibited a tendency for comparatively fast polymerization, especially at elevated temperature, even when some copper powder was added into the isoteniscope to inhibit the process. Because of this, special care was taken to evacuate the isoteniscope as quickly and efficiently as possible. The agreement between the values for vapor pressure obtained at one and the same temperature (first, as the temperature of the thermostate was raised and, then, as it was lowered) was taken as a proof that the polymerization during measurements did not advance significantly. The measurements were made to an accuracy of ± 0.5 mm Hg, while the temperature was maintained constant within 0.05° C.

6. The viscosity was measured with on *Ostwald* viscosimeter of 8 ml capacity. The flow time of water was 237 sec. The maximum deviation in flow time during ten measurements was 0.1 sec. The temperature was maintained constant to 0.05° C.

7. The solubility of the copper chelate was determined by the synthetic method. Since the solution was strongly colored, the formation of the "first", and the dissolution of the "last", crystal was observed in a flat, 3-mm thick test tube in a glass thermostat against a strong source of light. Increasing amounts of copper chelate were introduced gradually into the weighed quantity of the solvent. Agitation was provided by intensive convection brought about by the change in temperature. The dissolution temperature was fixed when an agreement between the observed dissolution temperature and crystallization temperature was achieved within 0.5° C. With an increase in solubility the temperature range had to be shifted to lower values since the intense coloring of the concentrated solutions at elevated temperatures made the observations inaccurate.

RESULTS AND DISCUSSION

The mean melting temperature obtained from the cooling curves of several experiments is

$$m.p. = -25 \pm 1\,^\circ\mathrm{C}$$

The precision of this determination was limited by the fact that the readings were considerably affected by the depth to which the test tube was immersed in the cooling mixture (because of the long, uninsulated neck of the alcohol thermometer).

The infra-red spectrum of recrystallized monomer of methacrylacetone is shown in Figure 1a. The absorption maxima at 1,724 cm^{-1}, 1,672 cm^{-1}, 1,618 cm^{-1}, 1,553 cm^{-1}, 1,443 cm^{-1}, 1,406 cm^{-1}, 842 cm^{-1} and 760 cm^{-1} correspond to overtones, fundamental oscillations, or combination tones of $CH_2=C(CH_3)-$, $C=C$, $C=O$, $CO+CH$, CH_3-, $CH_2=$, $CH_2=C$ and CH bonds respectively, thus confirming the assumed structure of this compound (6).

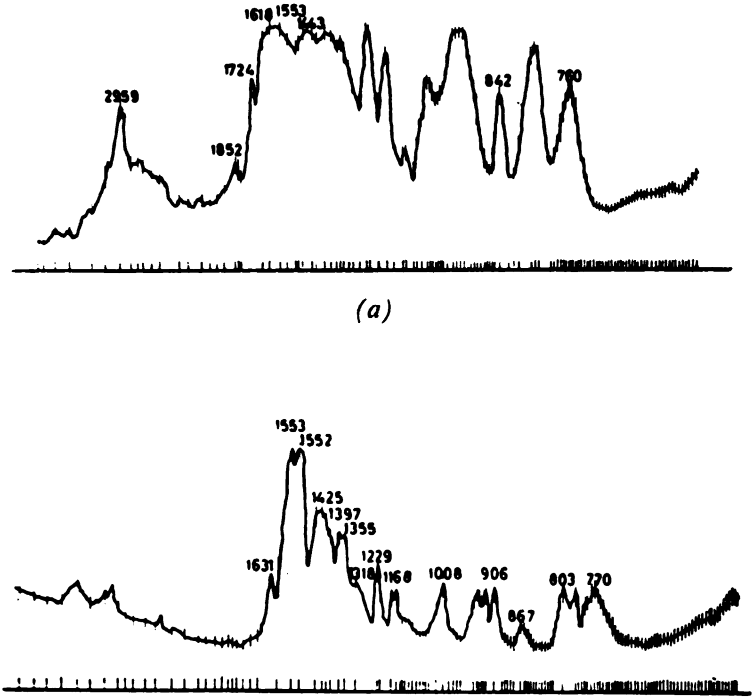

Fig. 1. — The infra-red spectrum of methacrylacetone monomer (a), and of its copper chelate (b).

From the value of refractive index

$$n_D^{20} = 1.5070$$

and the density at the same temperature, the value for molecular refraction was computed by the Lorentz-Lorenz equation:

$$R_M = 34.36 \text{ cm}^3/\text{mole}$$

The value calculated by summing up the table values for atomic refractions for individual groups of molecules (7) is 34.08 cm^3/mole for the keto-form of methacrylacetone, and 35.12 cm^3/mole for the enolic form. The position of the experimental value between the two values computed from atomic refractions indicates the presence of both possible forms of methacrylacetone in comparable quantities. This however, contradicts the conclusion reached by Teyssié and Charette (6).

The density of methacrylacetone is very close to the density of water, which makes this substance easy to emulsify and dissolve. Measured at 20, 25, and 40° C and compared to water at 4° C the density is:

$$d_4^{20} = 0.9904$$

$$d_4^{25} = 0.9830$$

$$d_4^{40} = 0.9711$$

The temperature dependence of the vapor pressure is shown in Table 1. The slope of the straight line of log p as a function of $1/T$ shown in Figure 2 was computed by the least square method. This slope rendered 6.550 cal/mole as the value of the latent heat of vaporization of methacrylacetone.

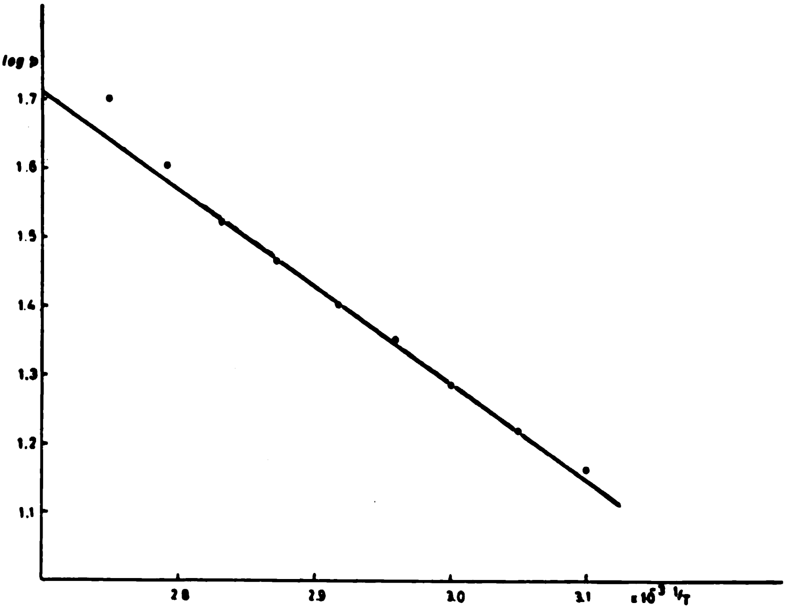

Fig. 2. — Vapor pressure of methacrylacetone as a function of temperature.

TABLE 1

Vapor pressure of methacrylacetone as a function of temperature

T° C	50	55	60	65	70	75	80	85	90
P_{exp}	14.5	16.5	19.2	22.5	25.2	29.2	33.2	40.2	50.5
p_{calc}	13.9	16.3	19.2	22.0	25.3	29.4	33.4	33.1	44.0

The values computed for each temperature reading from the slope of this curve are listed in Table 1 as $p_{calc.}$ It can be seen that with the exception of the two highest temperatures these values agree with the experimental data within experimental error. The deviations between the boiling points expected from the vapor pressure function as determined above and the distillation temperatures directly measured and reported by Teyssié and Smets (2) or Despić and Kosanović (1) are within the usual discrepancy between results obtained by the two methods.

The viscosity of methacrylacetone was measured at temperatures of 25 and 40° C. Its values are:

$$\eta_{25} = 1.045 \pm 0.005\, cP$$

$$\eta_{40} = 0.885 \pm 0.005\, cP.$$

The increase in viscosity above these values can be taken as a sensitive indication of the beginning of polymerization within the system.

The copper chelate obtained and recrystallized as described in an earlier work (4) produced an infra-red spectrum shown in Figure 1b. The introduction of cupric — ions into the chelate molecule caused a shifting of frequencies of $C=O$ and $C=C$ bonds to regions of longer wave length, while other absorption bands remained within the range of the corresponding groups.

Temperature dependence of the solubility of copper chelate is shown in Figure 3: for methanol (1); ethanol (2); acetone (3); benzene (4) — toluene (5); and xylene (6). The solubility in chloroform was so high that it could not be determined by the method used in this work owing to the excessively intensive color of the solution. For all the solvents the logarithm of solubility was plotted as a function of the reciprocal value of the absolute temperature. Straight lines were obtained without exception like the one shown in Figure 4 referring to toluene. Table 2 gives the values for the solubility of methacrylacetone copper chelate at 20° C, the values of slopes for the relation between the solubility in various solvents and temperature, as computed by the least square method, as well as the boiling temperatures of the solvents and the theoretical values of solubility at these temperatures calculated from the data given in the first three columns.

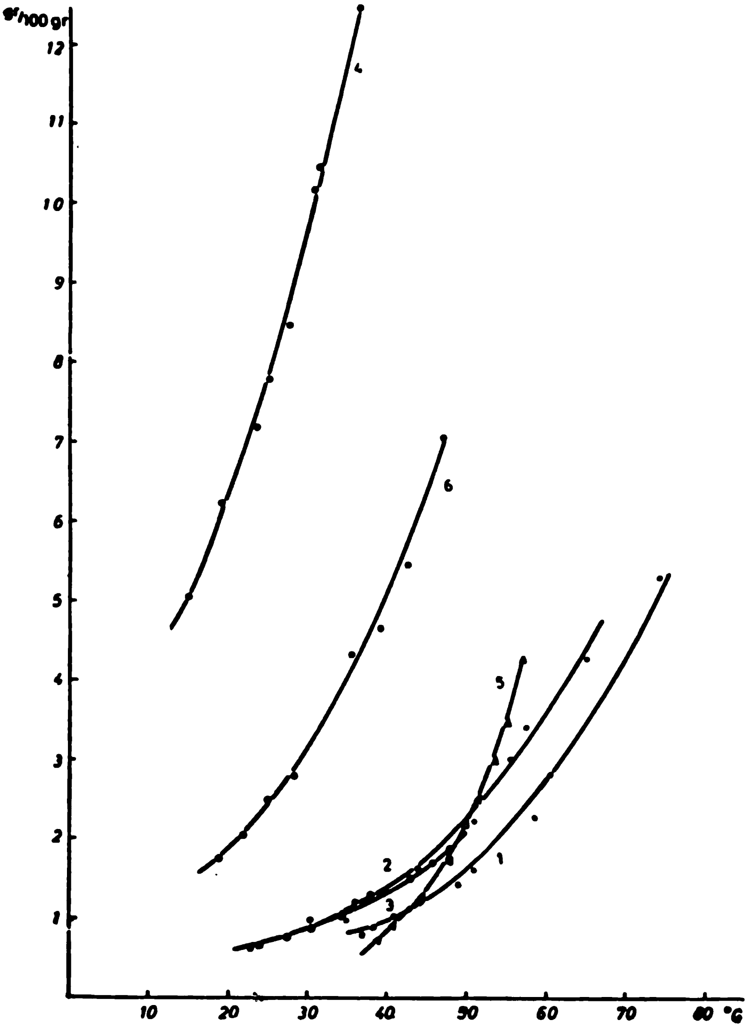

Fig. 3. — Temperature dependence of the solubility of methacrylacetone copper chelate in various solvents.

TABLE 2

Solubility of methacrylacetone copper chelate in various organic solvents

Solvent	c_{20} (g / 100 g)	m	*b.p.* °C	$c_{b.p.}$ (g/100 g)
Methanol	0.301	— 2.315	64.7	3.30
Ethanol	0.523	— 2.027	78.3	7.35
Acetone	0.544	— 1.834	56.5	2.71
Benzene	6.34	— 1.669	80.1	40.7
Toluene	0.0965	— 4.295	110.6	95.1
Xylene	1.885	— 1.967	140	79.1

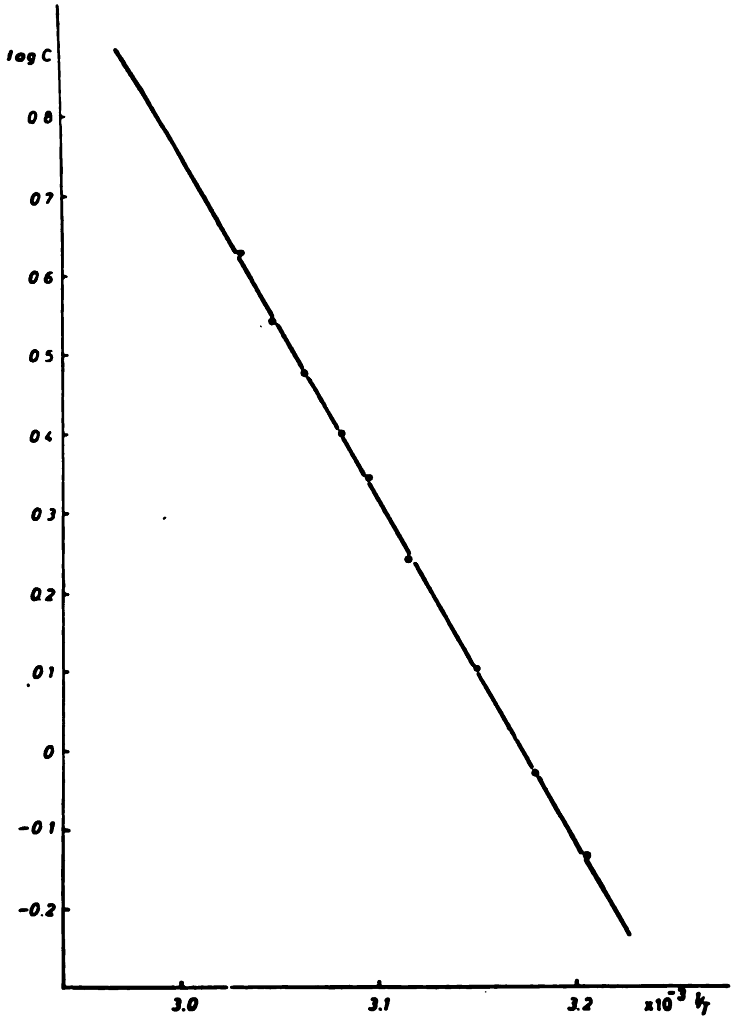

Fig. 4. — A characteristic *log* C *vs.* 1/T curve for the solubility of methacrylacetone copper chelate.

To calculate the solubility of the compound under investigation at the boiling temperatures of benzene, toluene, and xylene, it was necessary to convert the weight percentages obtained at 20° C into mole fractions and on calculating the mole fractions at the boiling temperatures to convert them back into weight percentages. However, since this form of the equation of isochore is limited in its applicability to dilute solutions, these extrapolated values must be considered approximate.

It is evident from these results that the most adequate solvents for the extraction of copper chelate from aqueous solutions at room temperature are chloroform and benzene because of the considerable solubility of the chelate in these chemicals. Taking the temperature coefficient of solubility into account it can be concluded that the recrystallization is most efficient from toluene. At its boiling temperature this solvent dissolves about one thousand times more chelate than at room temperature, at which a very small amount of dissolved matter remains in the solution.

Acknowledgements. The authors wish to express their indebtedness to Dr. Jeremić for obtaining and interpreting the infra-red spectra. Acknowledgement is also due to S. Jovanović and M. Dimovski for their help in the experimental work.

CONCLUSION

The physical properties of pure methacrylacetone monomer have been studied and the following parameters determined: the melting point (*m.p*), refractive index (n_D^{20}) density (d_4^{20}, d_4^{25}, and d_4^{40}), vapor pressure ($p_{exp.}$) as function of temperature, and viscosity (η_{25}, and η_{40}). The infra-red spectrum of the monomer has been taken and interpreted (Fig. 1a). This spectrum and the calculated value of the molecular refraction confirm the assumed structure of the compound.

The infra-red spectrum of copper chelate has also been recorded (Fig 1b) and its solybility in methanol, ethanol, acetone, benzene, toluene, and xylene determined as a function of temperature (Fig. 3). On the basis of these relations the theoretical values for solubility at the boiling temperature of these solvents has been calculated. It has been established that chloroform and benzene are the most suitable solvents for extraction, and toluene the most suitable for recrystallization.

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SEMIQUANTITATIVE DETERMINATION OF SMALL AMOUNTS OF MERCURY, PALLADIUM, LEAD, COPPER, CADMIUM, URANIUM, IRON, AND ZINC BY PRECIPITATION CHROMATOGRAPHY ON PAPER

by

M.B. ĆELAP, T.J. JANJIĆ, and D. RADANOVIĆ

A procedure for the semiquantitative determination of small amounts of elements on impregnated filter paper strips was described in our earlier papers (1—4). The procedure consists in washing out the elements to be determined by the method of descending

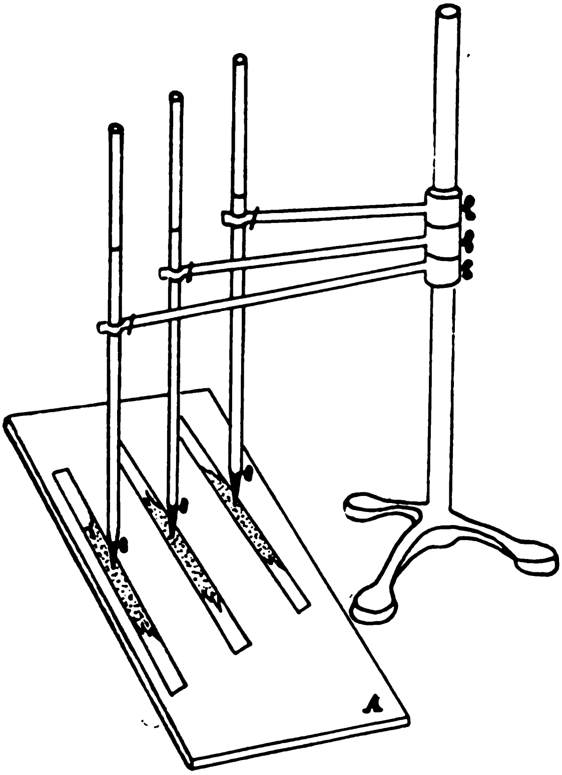

Figure 1
A — Glass-Plate

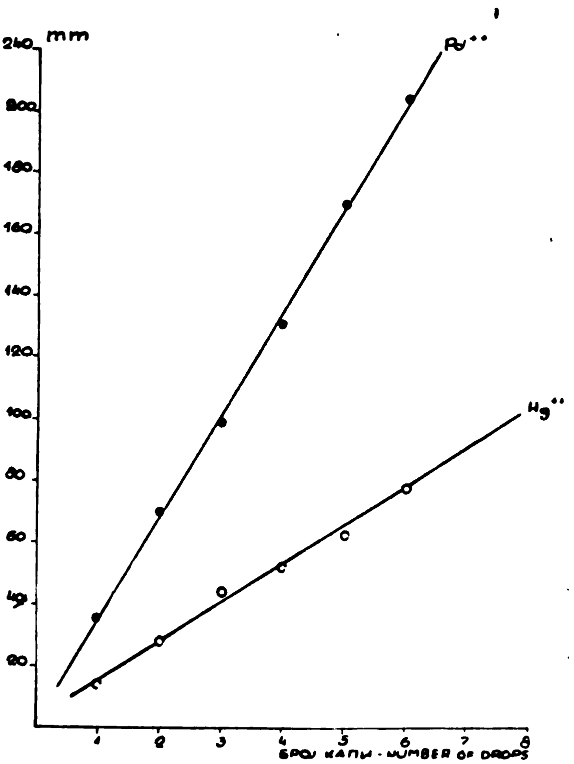

Figure 2

chromatography from the untreated to the impregnated part of the strip on which the precipitation takes place. Since the length of the double decomposition spot on the strips of standard width (5 mm) is proportional to the amount of the element to be determined, we can calculate the concentration of the solution analyzed by comparing this length with the length of the double decomposition spot of the given amount of the same element. One determination takes twelve hours, except in the case of arsenates, phosphates, and chromates, when it takes five hours.

To simplify and accelerate the procedure described earlier, we have modified it in this work by placing the investigated and the standard solutions in the middle of the impregnated strip (the unimpregnated part of the strip is not necessary in this case) and by adding the developing solution directly to the center of the starting point. For this purpose microburettes were used as shown in

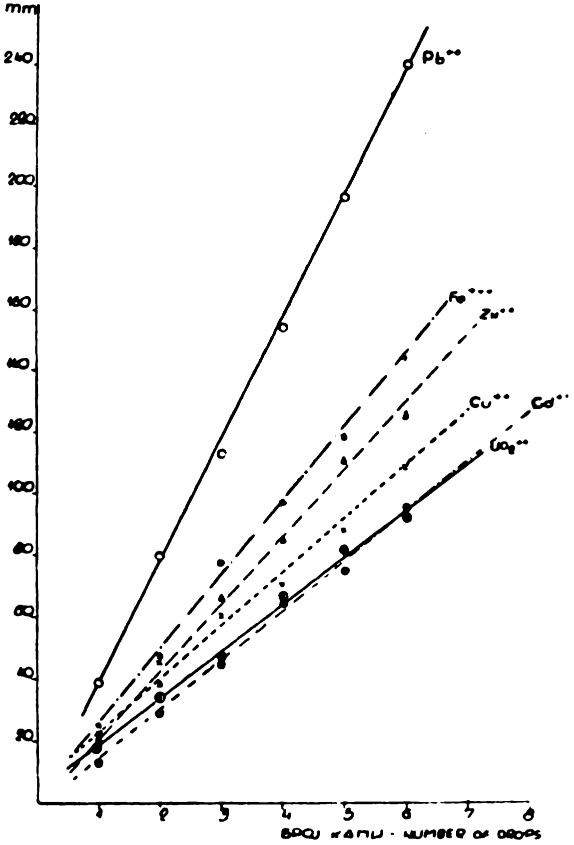

Figure 3

Figure 1. In this way we have succeeded in simplifying the procedure used earlier by eliminating the untreated part of the strip, and in shortening the time required for one determination from twelve hours to about half an hour, except for uranium. For this element the determination takes one hour and a half.

This procedure was checked by the determination of mercury and palladium on filter paper strips impregnated with cadmium sulphide, while lead, copper, cadmium, uranium, iron, and zinc were determined on paper strips impregnated with nickel ferrocyanide.

The accompanying diagrams on Figures 2 and 3 show that the length of the double decomposition spot in this method of development varies directly with the amount of the substance deposited. The results obtained in six successive determinations for each element are listed in Table 1.

TABLE 1

Concentration of the Solution mg/ml			
Calculated	Found	Calculated	Found
Mercury (Hg^{2+})		Palladium	
16.0	16.0	6.42	6.63
16.0	16.3	6.42	6.74
16.0	16.3	6.42	6.42
14.4	14.8	7.49	7.70
14.4	14.4	7.49	7.59
14.4	14.7	7.49	7.70
Lead		Copper	
15.7	15.8	2.23	2.10
15.7	15.7	2.40	2.30
15.7	15.4	1.86	1.98
17.3	16.3	1.86	1.90
17.3	17.6	2.28	2.17
17.3	17.8	2.52	2.40
Cadmium		Uranium	
3.60	3.80	5.16	4.92
3.60	3.79	4.92	4.92
3.60	3.76	3.84	4.10
4.72	4.54	4.56	4.80
4.72	4.65	3.60	3.42
4.72	4.56	4.62	4.40
Iron		Zinc	
1.26	1.27	4.18	4.25
1.34	1.40	4.18	4.30
1.30	1.29	4.18	4.34
1.11	1.07	5.23	5.23
1.26	1.19	5.23	5.15
1.19	1.19	5.23	5.16

As is evident from this table, in the determination of mercury the values obtained deviated by 1.5% on an average from those calculated. Each determination was carried out with about 0.5 mg of ions.

In the determination of palladium the results deviated by 2.5% on an average. Each determination was carried out with about 0.3 mg of the ion.

The mean error in the determination of lead was 2.2% and the amount of ions required for one determination was about 0.5 mg.

A somewhat larger mean error, i.e. 4.7%, was obtained in the determination of copper. The determination was carried out with about 0.1 mg of the metal.

Cadmium was determined with a mean error of 4.0%. The determination was carried out with 0.1 mg of this ion.

The determination of uranium was carried out with 0.1 mg of the metal. The mean error was 4.4%.

TABLE 2

Cation	Salt Used	Impregnating Reagents*	Developing Solution	Time of Developing in min	Means of Detection
Hg^{2+}	$HgCl_2$	(a) 0.05N $Cd(CH_3COO)_2$ (b) H_2S	1.10^{-2}N HCl	20	—
Pd^{2+}	$PdCl_2$	The same as for Hg^{2+}	1.10^{-2}N HCl	15	—
Pb^{2+}	$Pb(NO_3)_2$	(a) 0.1N $NiSO_4$ (b) 0.2N $K_4[Fe(CN)_6]$	2.10^{-3}N HNO_3	30	H_2S
Cu^{2+}	$CuSO_4$	The same as for Pb^{2+}	0,5% NH_4NO_3	30	—
Cd^{2+}	$CdCl_2$	The same as for Pb^{2+}	2.10^{-3}N HN_3O_3	25	(a) H_2S (b) 1% $AgNO_3$
UO_2^{2+}	$UO_2(NO_3)_2$	(a) 0.15N $NiSO_4$ (b) 0.3N $K_4[Fe(CN)_6]$	1% NH_4NO_3	90	—
Fe^{3+}	$FeCl_3$	(a) 0.05N $NiSO_4$ (b) 0.1N $K_4[Fe(CN)_6]$	1.10^{-2}N HNO_3	30	—
Zn^{2+}	$ZnCl_2$	The same as for Pb^{2+}	2.10^{-3}N HNO_3	25	Dithizone in chloroform

* Whatman No. 1 paper was used in all the cases considered.

The percentage of deviation between the results obtained in the determination of iron and those calculated is 2.6 on an average. The amount of iron required for one determination was only about 0.03 mg.

The mean error obtained in the determination of zinc was 1.9%. The determination was carried out with 0.1 mg of the metal.

Other experimental data are recorded in Table 2.

As is evident from the above, the procedure described is applicable to the determination of small amounts of elements (several tenth of a milligram) with an error of a few percentage points. It follows that the results obtained are almost of the same accuracy as those obtained by the considerably more complex and longer procedure that has been used up to now.

CONCLUSION

A procedure for rapid determination of small amounts of mercury, palladium, lead, copper, cadmium, uranium, iron, and zinc by precipitation paper chromatography has been presented in this paper. The determination takes about half an hour with a mean error of several percentages. The experiments were carried out with a few tenths of a milligram of the individual ions.

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CONDENSATION REACTIONS OF 2-THIOTHIAZOLIDINE-4-ONE (RHODANINE) WITH AROMATIC N, N-BISAMIDES IN THE PRESENCE OF BORON TRIFLUORIDE ETHERATE

by

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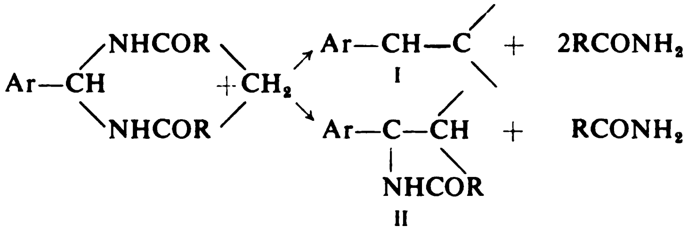
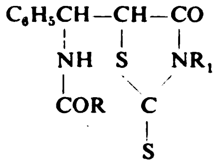
By condensing aromatic N, N-bisamides with the compounds containing an active methylene group we can obtain two kinds of condensation products according to whether one or both hydrogen atoms of the methylene group enter into the chemical reaction:



The importance of these condensations consists in obtaining the acylaminoaryl-substituted derivatives (II) because the arylidene derivatives (I) are obtained by direct condensation of aldehydes and compounds containing an active methylene group. Reports on the condensation products of type II have been published in numerous papers of Gj. Stefanović and his co-workers (1). The condensations were carried out in the presence of acetic anhydride or glacial acetic acid, or the constituents were melted directly. But under these experimental conditions 2-thiothiazolidine-4-one (rhodanine) condensed with aromatic N, N-bisamides giving high yields of arylidenerhodanine (I) (2).

We have investigated the condensations of aromatic N, N-bisamides with rhodanine and its derivatives in the presence of boron trifluoride etherate. Under these experimental conditions we succeeded in obtaining the acylaminoaryl-substituted derivates of rhodanine. The condensations were carried out in benzene as solvent, and the starting constituents and boron trifluoride etherate were used in equimolecular amounts. The reaction mixture was heated at the boiling point of benzene for one to four hours. The reaction products always contained smaller or larger amounts of arylidene derivatives

TABLE 1



R	R_1	m.p.	Molecular Formula	Found			Calculated		
				C %	H %	N %	C %	H %	N %
CH_3—	H—	214°	$C_{12}H_{12}O_2N_2S_2$	—	—	9.50	—	—	9.94
C_6H_5—	H—	170°	$C_{17}H_{14}O_2N_2S_2$	59.26	4.20	8.04	59.63	4.12	8.18
CH_3—	C_6H_5—	204°	$C_{18}H_{16}O_2N_2S_2$	60.45	4.68	8.24	60.67	4.53	7.86
C_6H_5—	C_6H_5—	188°	$C_{23}H_{18}O_2N_2S_2$	65.27	4.50	6.80	65.02	4.34	6.70
CH_3—	*m*-$CH_3C_6H_4$—	205°	$C_{19}H_{18}O_2N_2S_2$	61.29	4.95	7.60	61.61	4.90	7.56
CH_3—	*p*-$CH_3C_6H_4$—	207°	,,	61.49	4.90	8.03		,,	
C_6H_5—	*m*-$CH_3C_6H_4$—	190°	$C_{23}H_{18}O_2N_2S_2$	—	—	7.03	—	—	6.48
C_6H_5—	*p*-$CH_3C_6H_4$—	214°	,,	—	—	6.44	—	—	6.48

which were separated on the basis of their different solubilities in organic solvents. The yields of 5-acylaminoaryl rhodanine averaged about 50% and depended on the time of heating.

In this work benzylidene-N, N-bisacetamide and benzylidene-N, N-bisbenzamide were condensed with 2-thiothiazolidine-4-one and N-phenyl-, N-m-tolyl, and N-p-tolyl- 2-thiothiazolidine-4-ones. The melting points and the analyses of 5-(α-acylamino)-benzyl-3-aryl-2-thiothiazolidine-4-ones are given in Table 1.

The attempts to condense the aliphatic N, N-bisamides (methylene ethylidene-, and propylidene-) with 2-thiothiazolidine-4-one and its derivatives did not give positive results.

EXPERIMENTAL

The equimolecular amounts of the constituents (O. 1 mole) were partly dissolved by heating in anhydrous benzene (100—150 ml). The equimolecular amount (0,1 mole) of boron trifluoride etherate was added to the reaction mixture and this heated for one to four hours. After heating benzene was removed by distillation and the remaining reaction mixture poured into water. After they had been kept in icy water and rinsed with it, the reaction products solidified. The products were purified and separated by crystallization from diluted or 95% ethanol. In all the cases that we examined the acylaminoaryl derivatives were less soluble than the arylidene derivatives and separated readily.

CONCLUSION

By condensing benzylidene-N, N-bisacetamide and benzylidene-N, N-bisbenzamide with 2-thiothiazolidine-4-one and N-phenyl-, N-m-tolyl-, and N-p-tolyl- 2-thiothiazolidine-4-ones, respectively, in the presence of boron trifluoride etherate, the corresponding 5 (α-acetylamino) benzyl- and 5-(α-benzoylamino) benzyl-2-thiothiazolidne-4-ones were obtained.

Alkylidene N, N-bisamides do not react with 2-thiothiazolidine-4-one and its derivatives in the presence of boron trifluoride etherate.

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REACTIONS OF AROMATIC BISAMIDES*

by

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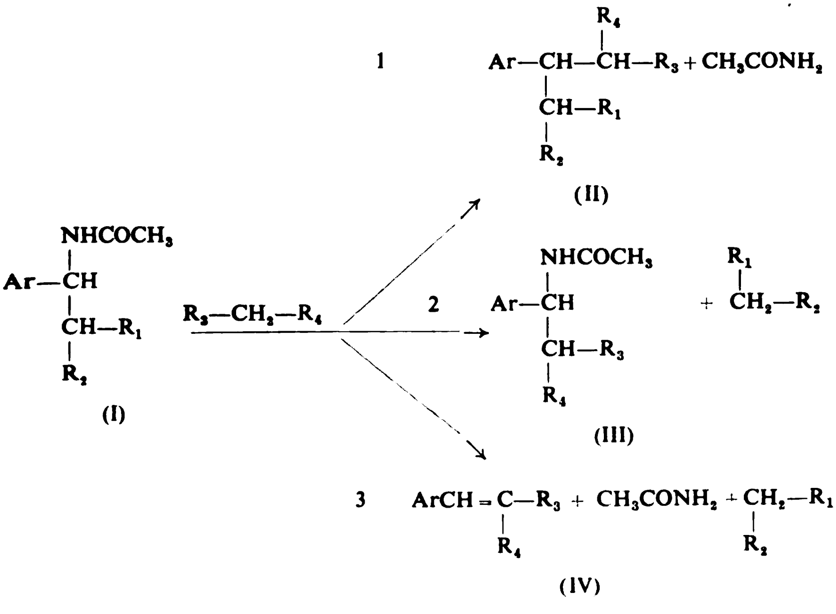
and M. STEFANOVIĆ

As is known from the earlier papers of Dj. Stefanović and co-workers (1), the aromatic N, N — bisamides react with compounds containing active hydrogen atoms and yield two types of condensation products depending on whether one or both bisamide residues are substituted.

In connection with these works it was of interest for the course of the "bisamide reaction" to investigate how the acylamino compounds of type I react with the compounds containing active hydrogen atoms. It could be assumed that the reaction would take place according to one of the following schemes:



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*See reference 7.

TABLE 1

Acylamino Compounds	Constituent Containing Active Hydrogene Atoms	Time of Heating (Hours) at °C	Product Obtained	±*	Yield %
α-carbethoxy-β-acetylamino--β-arylethyl methyl ketone (A)	Rhodanine	3, at 140—150°	Benzylidene-rhodanine (E)	+	85.5
Diethyl ester of β-phenyl-β-acetylamino-ethane-α, α-dicarboxylic acid (B)	N-dimethylaniline	2, at 120°	α-acetylamino-4-dimethylaminodiphenylmethane (D)	+	31.2
1 (α-acetylaminobenzyl)--naphthol-2 (C)	„	3, at 130°	(D)	+	86.5
(A)	„	4, at 130°	(D)	+	62.5
(A)	β-naphthol	3, at 130°	(C)	+	51.8
(D)	„	6—12, at 150°	(D)	—	—
(D)	Ethyl acetoacetate	12, at 140°	(D)	—	—
(C)	„	12, at 140°	(C)	—	—
(A)	Ethyl cyanoacetate	18, at 150°	(A)	—	—
(A)	Ethyl malonate	18, at 150°	(A)	—	—

* (+) The reaction takes place
(—) The reaction does not take place

In order to establish according to which type of scheme the reaction will take place, we have investigated the reactivity of several synthesized acylamino compounds (I) toward rhodanine, N-dimethylaniline, β-naphthol, ethyl acetoacetate, ethyl cyanoacetate, and ethyl malonate. The reactions of acylamino compounds with active compounds were carried out at temperatures ranging from 130—150° C either without condensation agent or in the presence of acetic anhydride.

The results obtained are recorded in Table 1.

As is evident from Table 1, the reaction products are compounds of type III, while the mixed derivatives (II) in which the second bisamide residue is replaced by the residue of the reactant having active hydrogen atoms are not obtained under these experimental conditions. In one case, the reaction of rhodanine with α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone gave only a compound of type IV.

On analyzing the results recorded in Table 1 it can be concluded that the active compounds R_3—CH_2—R_4 are not equally reactive toward acylamino compounds. Thus, e.q., N-dimethylaniline replaced β-naphthol, ethyl acetoacetate, and ethyl malonate in their acylamino compounds forming α-acetylamino-4-dimethylaminodiphenyl-methane, while ethyl acetoacetate did not react with acylamino compounds of type I. By their reactivity toward acylamino compounds estimated on the basis of the duration of the reaction and the products obtained, the compounds with active hydrogen atoms can be arranged in the following order: rhodanine, N-dimethylaniline, β-naphthol, ethyl acetoacetate, ethyl cyanoacetate, and ethyl malonate.

Compounds with active hydrogen atoms R_3—CH_2—R_4 arranged according to the descending values of their dissociation constants are listed in Table 2.

TABLE 2

Compound with Active Hydrogen Atoms	Temperature °C	K_d	References
Rhodanine	25	$3 \cdot 10^{-6}$	2
N-Dimethylaniline	18	$1.3 \cdot 10^{-9}$	3
β-Naphthol	20	$3.5 \cdot 10^{-10}$	3
Ethyl acetoacetate	25	$2 \cdot 10^{-10}$	3
Ethyl cyanoacetate	—	—	—
Ethyl malonate	25	$1.78 \cdot 10^{-16}$	3

The accompanying table of dissociation constants for the active compounds shows that the order is in perfect agreement with our results.

EXPERIMENTAL

Displacement of α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone with rhodanine. A mixture of 5 g (0.018 mole) α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone and 2.4 g (0.018 mole) rhodanine was heated for 3 hours at 140—150°C. On cooling, the mixture was poured into water affording 3.5 g (85.5%) of crude benzylidene rhodanine, m.p. 187°C. After recrystallization from alcohol it melted at 196—197°C. This benzylidene rhodanine did not show a depression in melting point when mixed with benzylidene rhodanine synthesized by the condensation of benzylidene-N,N-bisacetamides with rhodanine (4).

Displacement of α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone with N-dimethylaniline. A mixture of 5 g (0.018 mole) α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone and 2.2. g (0.018 mole) N-dimethylaniline was heated for 4 hours at 130°C. On treating the cooled mixture with ether a yield of 3 g (62.5%) α-acetylamino-4-dimethylaminodiphenylmethane was obtained in the form of white crystals melting at 150°C. Recrystallized from ethanol the product melted at 153°C. It did not show a depression in melting point when mixed with α-acetylamino-4-dimethylamino-diphenylmethane obtained by the condensation of benzylidene-N,N-disacetamide with N-dimethylaniline (5).

Displacement of diethylester of β-phenyl-β-acetylamino ethane-α, α-dicarboxylic acid with N-dimethylaniline. A mixture of 5 g (0.016 mole) diethylester of β-phenyl-β-acetylamino ethane-α, α-dicarboxylic acid and 2.5 g (0.02 mole) N-dimethyl-aniline was heated for 2 hours at 120°C. α-Acetylamino-4-dimethylaminodiphenylmethane was isolated and identified as above. The yield was 1.5 g (31.2%) (5).

Displacement of α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone with β-naphthol. A mixture of 5 g (0.018 mole) α-carbethoxy-β-acetylamino-β-arylethyl methyl ketone and 2.6 g (0.018 mole) β-naphthol was heated for 3 hours at 130°C. On cooling, the mixture was treated with ether yielding 2.8 g (51.8%) of crude 1 (α-acetylamino-benzyl) — naphthol-2 in the form of white crystals, m.p. 220°C. Crystallized from ethanol the product melted at 234°C and did not show a depression in melting point when mixed with 1 (α-acetylamino-benzyl)-naphthol-2 obtained by the condensation of benzylidene-N,N-bisacetamide with β-naphthol (6).

Displacement of 1(α-acetylamino benzyl)-naphthol-2 with N-dimethylaniline. A mixture of 5 g (0.017 mole) 1(α-acetylamino-benzyl)-naphthol-2 and 6 g (0.05 mole) N-dimethylaniline was heated 30 minutes at 210°C and then for 3 hours at 130°C. After the cold mixture was treated with ether it yielded 3.8 g (86.5%) α-acetylamino-4-dimethylaminodiphenylmethane, m.p. 153°C. This product did not show a depression in melting point when mixed with α-acetylamino-4-dimethylaminodiphenylmethane obtained by the condensation of benzylidene-N,N-bisacetamide with N-dimethylaniline (5)

CONCLUSION

By heating acylamino compounds with N-dimethylaniline, β-naphthol, ethyl acetoacetate, ethyl cyanoacetate, and ethyl malonate at 130—150° C, condensation products of type III were obtained. Only with rhodanine these compounds have yielded a product of type IV.

The compounds with active hydrogen atoms have been arranged according to their reactivity estimated on the basis of the duration of the reaction and the yield obtained. This order is in agreement with the values of the dissociation constants of these compounds, i.e. the reactivity decreases with the decrease in the K_d values (rhodanine > N-dimethylaniline > β-naphthol > ethyl acetoacetate > > ethyl cyanoacetate > ethyl malonate).

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EXAMINATION OF BELITE CEMENT CLINKER

by

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It is well-known that cement comes from ground clinker. This means that the structure of clinker is responsible for the properties of cement and its behavior during hydration. Examination of the constitution of clinker has shown it to be a mixture in which minerals are not found in their pure state. The main constituents of clinker are:

— tricalcium silicate 3 $CaO \cdot SiO_2$ or $Ca_3O \cdot SiO_4$ — abbr · C_3S
— dicalcium silicate 2 $CaO \cdot SiO_2$ — abbr · C_2S
— tricalcium aluminate 3 $CaO \cdot Al_2O_3$ — C_3A
— tetracalcium aluminate ferrite $4CaO \cdot Al_2O_3 \cdot Fe_2O_3$ — C_4AF.

These constituents are for the clinker of portland cement where tricalcium silicate, or alite, is dominant. Its presence in amounts of 60 to 70% is responsible for certain hydraulic characteristics, the development of significant amounts of hydration heat, and corresponding mechanical properties.

The object of our work was to examine the constitution of the clinker of belite cement, whose mineral structure differs from that of portland cement. Belite cement gets its name from belite, the dominant mineral in this clinker. Due to the mineral structure, cement gotten from ground belite clinker has low hydration heat, and corresponding hydraulic and mechanical properties.

The mineral structure of clinker has a significance which must not be neglected. Quantitative chemical analysis of clinker is not enough for a knowledge of its structure, i. e. the location and spatial relations of individual components. Quantitative chemical analysis together with quantitative mineral analysis give data on the constitution of clinker.

X-ray diffraction can also be used for qualitative identification and quantitative determination of the minerals of clinker, but was not used on this occasion. The examination was carried out by means of quantitative chemical analysis, and by qualitative microscopic analysis in reflected light.

The preparation of the mixing materials and the process of baking play a very important role in the technological processing of clinker. It must also be pointed out that these two processes are in mutual relation to each other and depend on the composition of the mixture of materials. The mixture is composed on the basis of an estimate. This estimate is based on the quantitative chemical analysis of all the materials which participate in the production, including the industrial fuel. Fine grinding, homogenization and granulation of the materials of the mixture are also important in the preparation process.

While the clinker is baking and the temperature is going up, the first reaction after the evaporation of the free water is the decomposition and dehydration of the clay and the decomposition of the magnesium carbonate. That happens at about 600°C. From 800 to 900°C the limestone decomposes.

Johnston has given the vapor pressure of $CaCO_3$ at different temperature (1):

Temperature in °C	*Pressure in mm* Hg
500	0.11
700	25.30
750	68.00
800	168.00
850	373.00
898	760.00
900	773.00
950	1490.00

At 898°C the vapor pressure of $CaCO_3$ is the same as atmospheric pressure, which explains the quick progression of the decomposition of $CaCO_3$ at that temperature.

At 1100°C the creation of dicalcium silicate begins. From 1100 to 1350°C the unstable beta form is created.

According to Lea and Desch, a high temperature and quick cooling do not cause inversion of dicalcium silicate. The alpha form resists inversion into the gamma form more than the beta modification and is obtained when a temperature of 1420°C is reached. (1) Some authors maintain that the limiting temperature is 1436°C. (2) This means that the alpha form remains without change when the system is brought to room temperature, if the temperature of the alpha-beta inversion was previously reached. It seems that the transition into the gamma form is inevitable if dicalcium silicate is pressent in beta form before the cooling begins.

The creation of tricalcium silicate begins at about 1350°C. At this temperature the transition of the low temperature beta modification to the high temperature alpha modification of tricalcium silicate is characteristic (3).

Dicalcium silicate and tricalcium silicate, in addition to being the most important constituents of clinker, are found in slag, incombustible materials and industrial glass.

The study of the compounds which make up the system $CaO \cdot SiO_2$ started in the 19th century. Many researchers have studied the phase diagram of this system, and Rankin and Wright gave the form accepted today in 1915. Figure 1 shows a complex diagram which should be studied part by part.

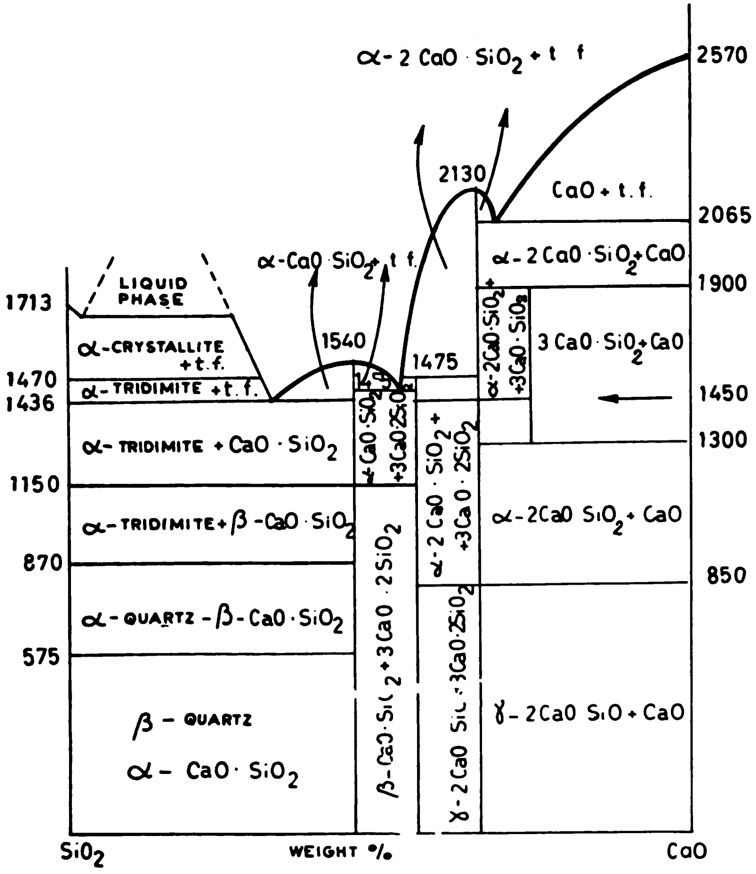
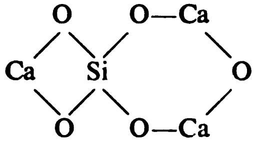


Figure 1
The binary system CaO. SiO_2

1. System CaO—$2CaO \cdot SiO_2$
2. System $2CaO \cdot SiO_2$—$CaO \cdot SiO_2$
3. System $CaO \cdot SiO_2$—SiO_2.

Tricalcium silicate, $3CaO \cdot SiO_2$, or according to Jeffrey (1952) $Ca_3O \cdot SiO_4$ (7), is found in the clinker of portland cement in significant percentages and is called alite. It is not in the clinker in its pure state but contains small quantities of MgO and Al_2O_3. It crystallizes into polygonal crystals whose average dimension is 50 microns in a monoclinic system. The dimensions of the elementary cell are: a = 33·08 Å, b = 7.07 Å, c = 18.56 Å, and the angle is 94°10'.

Mayer has worked out the structural formula of tricalcium silicate which explains the hydraulic properties of this mineral, since chemically it represents an anhydride:

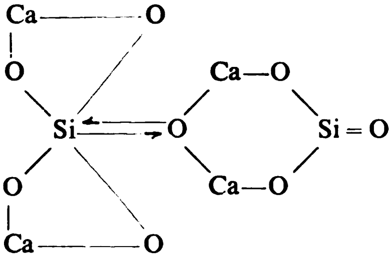


The formula $Ca_3O \cdot SiO_4$ should remind one that the structure of tricalcium silicate is built around the tetrahedron SiO_4. The crystalline lattice has 5 oxygen positions around the Ca and one oxygen position on one side of the tetrahedron. On the other side of the tetrahedron there is no oxygen, so that the OH group can easily be attached. This explains the quick hydration of tricalcium silicate. Dicalcium silicate does not have this structure, and the OH group cannot be attached. This allows much less hydration.

Dicalcium silicate $2CaO \cdot SiO_2$ or C_2S is found in the clinker of portland cement in amounts of 15 to 38% (8), depending on the kind and purpose of the cement.

Because of its polymorphous modifications and its proneness to pass into the gamma form (when it changes in volume and becomes 10 to 12% larger, which is commonly called "flowering of clinker") it has been the subject of much research. There are four forms of C_2S: alpha, alpha', beta and gamma, although some authors refer to a beta' as a fifth form.

Mayer believed that dicalcium silicate exists in two forms which are in equilibrium at a high temperature:



orthosilicate metasilicate

Orthosilicate is stable at low temperatures, and metasilicate is stable at high temperatures.

The properties of spontaneous disintegration which lead to the enlargement of volume are ascribed to orthosilicate.

Because of the internal forces between the crystals, the inversion of the beta form to the gamma form is accompanied by a 10% increase in volume. Cracks result from this tension and finally a fine powder, the "flowering of the clinker." The specific gravity changes from 3.28 to 2.97, and the index of refraction from 1.715 to 1.642. The optical character becomes negative.

In their paper published in 1906, Day and Shepherd (2) spoke of three modifications of C_2S:

$$\text{alpha} \rightleftarrows 1410^0\text{C} \rightleftarrows \text{beta} \rightleftarrows 675^0\text{C} \rightleftarrows \text{gamma}$$

The polymorphism of C_2S can also be seen in the following comparison (2):

	d	n_g	n_p
alpha 2 $CaO \cdot SiO_2$	3.27	1.737	1.715
beta 2 $CaO \cdot SiO_2$	3.28	1.735	1.717
gamma 2 $CaO \cdot SiO_2$	2.97	1.654	1.642

On the basis of crystallochemical hypotheses and x-ray analysis of powder samples at different temperatures, Bredig set up a diagram which explains the polymorphism of C_2S. This diagram is shown in Fig. 2.

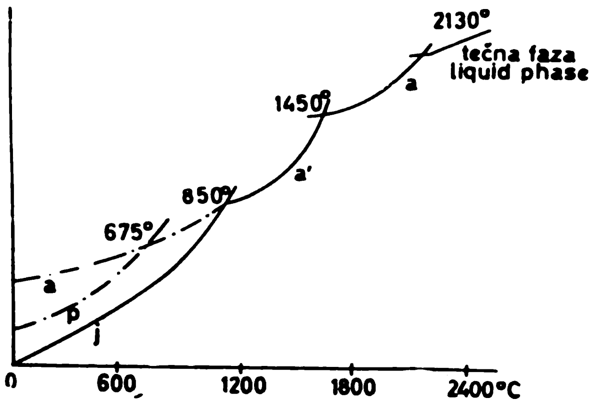


Figure 2

Bredig's scheme of the polymorphous transformation of C_2S

In the diagram can be seen the hexagonal alpha form, between 2130°C (the melting temperature) and 1450°C, and the rhomboid alpha' form, between 1450 and 850°C. The beta form is metastable with respect to the gamma form. It can be obtained from the alpha' form at temperatures of 675°C. The region of its metastable state lies below this temperature. The passage of the alpha' into the gamma form at about 850°C coincides with Bredig's prediction and accompanied the growth of specific volume (about 12%).

If foreign atoms are introduced into the crystalline lattice, the dicalcium silicate can be stabilized. This can be acheived with small amounts of Cr_2O_3, B_2O_3, P_2O_5, K_2O or Na_2O as stabilizers. The stabilization of C_2S in clinker can be obtained with the presence of iron oxide and other oxides. Due to this property to form compact solutions with oxides, up to 4.6% of the iron oxide of cement clinker dissolves, forming a compact solution. (3)

It crystallizes into a monoclinic system and under a microscope appears as circular crystals of average diameter of about 30 microns, (9) or between 10 and 60 microns, (8) as shown in Fig. 7.

Tricalcium aluminate $3CaO \cdot Al_2O_3$ or $Ca_3Al_2O_6$ appears in the form of darker crystals in the ferrite phase. The size and regularity of the crystals depends on the method of cooling. It crystallizes into a cubic system ($a = 15.22Å$). The position of the atoms in the crystalline lattice is not yet completely determined.

Tetracalcium aluminate ferrite $4\ CaO \cdot Al_2O_3 \cdot Fe_2O_3$ or C_4AF, is formed in clinker when the aluminate module is above 1. It has a light color and is found in clinker in amounts of 10 to 12%.

The role of the microscope in the observation of the minerals of clinker is augmented by other means of evaluating the technological processes which the clinker of cement undergoes. The arrangement of crystals shows the homogeneity of the mixed materials, the crystalline form shows the duration of the reaction at high temperatures, the size and appearance shows the method of cooling.

The presence of CaO and MgO can be established using the microscope. The presence of CaO is determined by an excessive amount of total CaO. It is known by estimate how much will react with SiO_2 giving $3\ CaO \cdot SiO_2$, and with Al_2O_3 giving $3\ CaO \cdot Al_2O_3$. The free CaO has a cubic system and an index of refraction 1.836.

Free MgO is found in clinker in the form of periclase. It also has a cubic system, and has a refractive index of 1.736. The identification of MgO suggests either insufficient homogenization or slow cooling. This is illustrated in the following photographs:

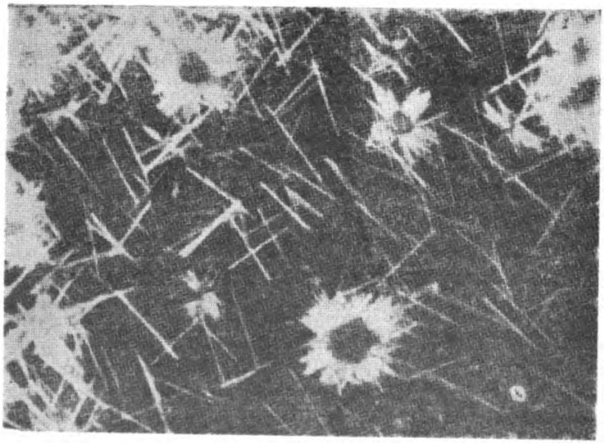


Figure 3
White's test for free CaO

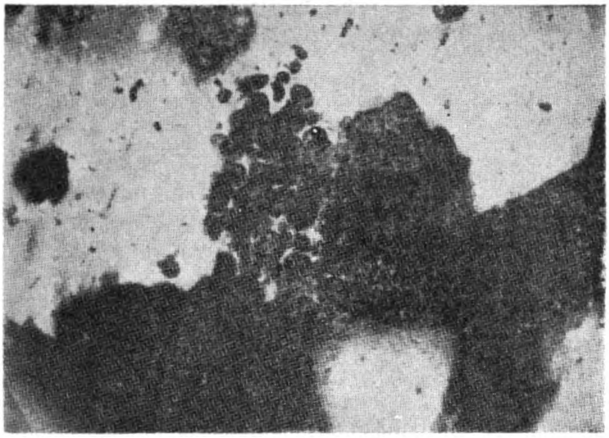


Figure 4
Free CaO in grain form

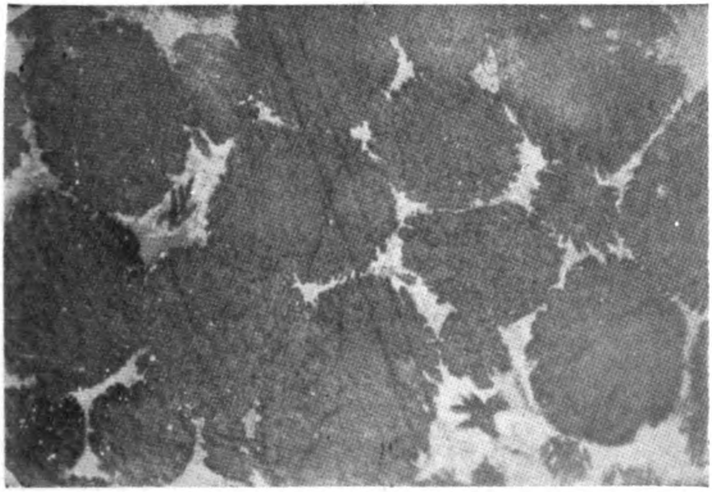


Figure 5
Clinker with a higher content of C_2S. Slowly cooled, with irregular boards.

Figure 1 shows the presence of free lime in the form of calcium phenolate, proved by White's test. Figure 2 also shows the free CaO in the characteristic cluster form. The polished form is cultivated by water.

The following figure shows slowly cooled clinker, which is seen by the irregular boards. Figure 6 is a microphotograph of quickly cooled clinker of portland cement with large crystals of tricalcium silicate.

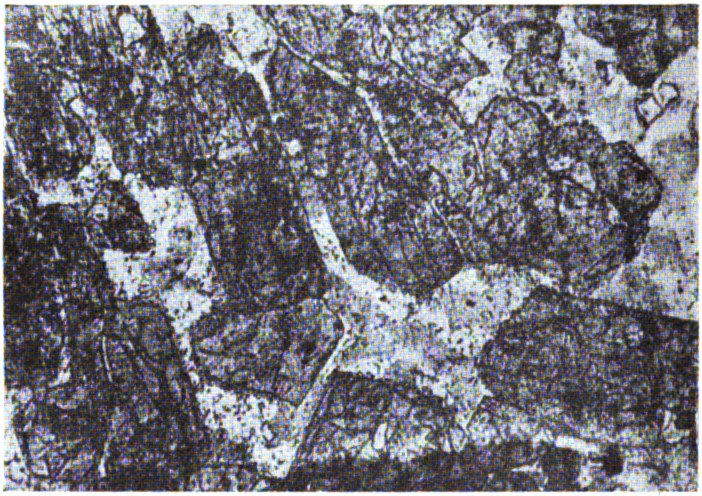


Figure 6

Clinker with a higher content of C_3S. Quickly cooled, with big crystals of C_3S, C_3A and C_4AF.

EXPERIMENTAL WORK

The aim of this work was to resolve the confusion regarding the different constitutions of portland and belite cements. A quantitative chemical analysis of the clinker of belite cement was made. Comparing this with the chemical analysis of the clinker of portland cement shows no striking difference. It was not possible to determine how much SiO_2 reacted with CaO and which minerals resulted.

Chemical analyses showed the structures of clinkers to be the following:

	clinker of belite cement	*clinker of portland cement*
SiO_2	24.86%	20.88%
Al_2O_3	4.70	7.96
Fe_2O_3	4.35	3.48
CaO	64.05	62.68
MgO	2.20	0.80
SO_3	0.20	0.35
Si_m	2.76	1.83
Al_m	1.08	2.29
H_m	1.89	1.94
St	80.40	90.00

The microscopic investigations were made with a microscope provided with an integration table for the quantitative determination of the mineral structure of clinker. Samples of clinker were poured into methyl methacrylate to which a catalyst had been added. After solidification they were polished,

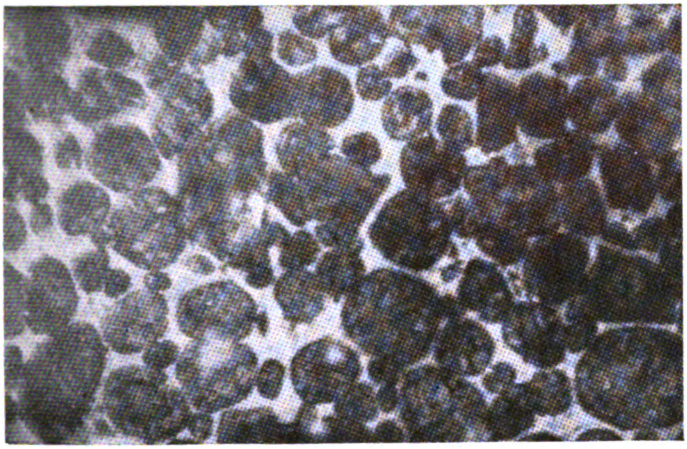


Figure 7
Clinker of belite cement.

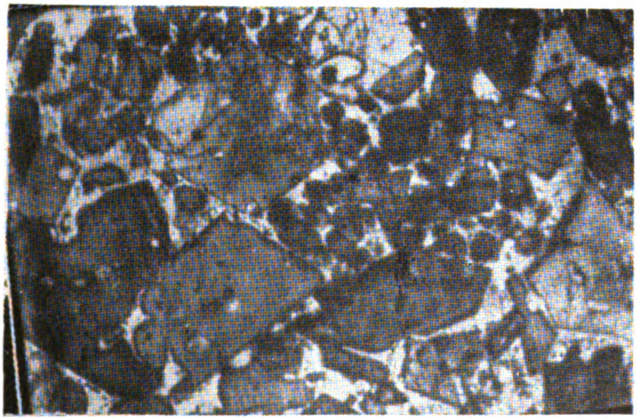


Figure 8
Clinger of portland cement

cultivated and observed. With the microscope the difference in mineral structure was immediately determined, still by quantitative analysis, since the clinker of belite cement contained mainly dicalcium silicate and that of portland cement tricalcium silicate.

The results of the quantitative microscopic analysis show that the clinker of belite cement contains a lagre amount of dicalcium silicate. This can also be concluded from the microphotographs. Since dicalcium silicate is thought to be belite, the type of cement which we have examined is called belite. It is concluded that the baking temperature of the clinker of belite cement was 1430°C. As can be seen in the figure, crystals of dicalcium silicate prevail. The arrangement of the crystals as well as the uniformity of their dimensions shows that the mixed materials are sufficiently homogeneous, and that thus the reaction of the components of the materials was satisfactory.

Swift's counter can be used in place of the integration table for quantitative mineral analysis. The minerals can be observed in transmitted light as well as in reflected light. Since it is difficult to prepare samples for examination in transmitted light, reflected light is customarily used.

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CONTENTS

GLASNIK HEMIJSKOG DRUŠTVA, Vol. 28, No. 2, 1963, pp. 65—72.

THE EQUATION OF STATE OF THE SATURATED VAPOR OF MONOATOMIC ELEMENTS

by

J. ŽIVOJINOV

The purpose of this paper is to present the equation for the state of the saturated vapor of monoatomic elements on the basis of statistical and quantum mechanics and classical thermodynamics. To achieve this, the author first made use of the result obtained in her earlier work [1]. In that paper, on the basis of statistical and quantum mechanics, the author came to the following relation:

$$\frac{V''}{V' \cdot e^{\frac{s}{RT}}} = \frac{3}{e^{\frac{s}{RTc}}} \qquad (1)$$

applicable in the cases of the equilibrium state between the liquid and gaseous phases of monoatomic elements for the temperature range between their triple and critical temperature. Here V'' and V' refer to the specific volume of vapor and liquid at boiling temperature T°K, s to the heat of sublimation at 0°K, and T_c to the critical temperature.

From classical thermodynamics the relation (Van der Waals' equation) was used:

$$ln \frac{p_c}{p} = \frac{r}{RT_c} \left(\frac{T_c}{T} - 1 \right) \qquad (2)$$

in which p_c is the critical pressure; p, the pressure at boiling temperature T; and R, the universal gas constant. As for r the author has found [2] that in monoatomic elements this value corresponds to the heat of vaporization at a pressure of 1 atm. From these two equations, the author has obtained in this work the equation for the state of the saturated vapor in the following way. From equation (1) it follows that

$$ln \frac{V''}{3\,V'} = \frac{S}{RT_c} \left(\frac{T_c}{T} - 1 \right) \qquad (3)$$

From this equation and equation (2) the author has found that

$$pV'' = 3p_c\ V' \cdot e^{\frac{S-r}{RTc}\left(\frac{Tc}{T}-1\right)} \tag{4}$$

This is the equation for the state of the saturated vapor of monoatomic elements.

In the second part of this paper this equation was used in the determination of critical pressure of some monoatomic elements such as xenon (Xe) and argon (Ar) to see whether it gives a good relation between the fundamental quantities of the state: p, V and T.

For the element xenon, at critical temperature $T_c = 289.76°K$, pressure $p_c = 58.22$ atm [3]. From equation (4) critical pressure is

$$p_c = \frac{pV''}{3V' \cdot e^{\frac{S-r}{RTc}\left(\frac{Tc}{T}-1\right)}} \tag{5}$$

$\left(\text{For this element } r = 3{,}021 \ \frac{\text{cal}}{\text{g—atom}} \text{ and } s = 3{,}850 \ \frac{\text{cal}}{\text{g—atom}}\right)$

Since the values for this element's specific densities ρ'' and ρ' (not for their specific volumes of vapor and liquid) are known from the literature [3], and also since it is known that the relation between these values is reciprocal, equation (5) can also be written in the form

$$p_c = \frac{p\,\rho'}{3\,\rho'' \cdot e^{\frac{S-r}{RTc}\left(\frac{Tc}{T}-1\right)}} \tag{6}$$

As is evident from Table 1, we have the experimental data [3] for all the values figuring in equation (6), obtained at three different temperatures. By inserting the experimental data for the lowest temperature, i.e. for $T = 213.16$ °K, in equation (6) we get

$$p_c = \frac{8.57 \text{ atm} \cdot 2.699 \ \frac{g}{cm^3}}{3 \cdot 0.079 \frac{g}{cm^3} \cdot 2.718282^{\,1.44003 \cdot 0.35935}} \tag{7}$$

i. e.

$$p_c = 58.13 \text{ atm} \tag{8}$$

It can be concluded from this that equation (6), and therefore also equation (4), give a good relation between the quantities of state mentioned above. That is to say, the difference between the experimental value for the critical pressure of xenon and the value obtained from equation (6) is only $\delta p_c = +0.2\%$. In exactly the same way the critical pressure of xenon was determined for other two temperatures, by inserting the values from Table 1. These results were also recorded in this table. As is evident from Table 1 the difference is the greatest for temperature $T = 253.16$ °K and amounts to $\delta p_c = -19.2\%$.

TABLE 1

Critical pressure for xenon (Xe)

$p_c - 58.22$ atm[3]

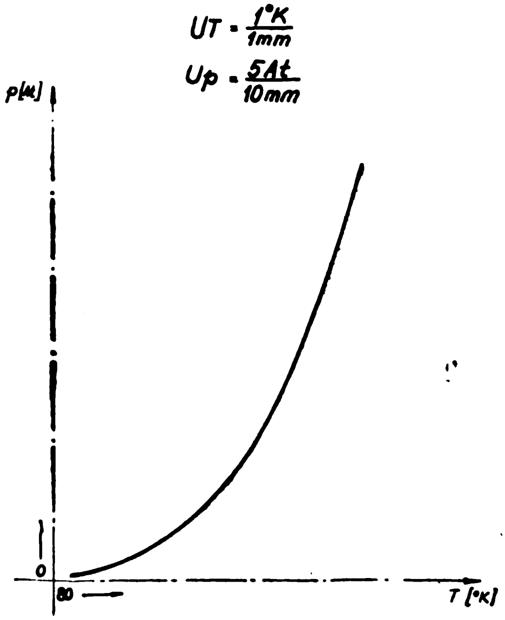
T °K	p atm	$\rho' \frac{g}{cm^3}$	$\rho'' \frac{g^{(3)}}{cm^3}$	$\frac{s-r}{e^{RTc}}\left(\frac{T_c}{T}-1\right)$	p_c atm from Equation (6)	δp_c %
213.16	8.57	2.699	0.079	1.67900	58.13	+0.2
253.16	26.73	2.292	0.239	1.23180	69.37	−19.2
273.16	41.24	1.987	0.421	1.09180	59.44	−2.1

Argon (Ar) was the next monoatomic element to which this method was applied. Its triple point is at temperature $T_t = 83.83°$ K, and its critical temperature at $T_c = 150.72°$ K, when $p_c = 47{,}996$ atm. As is evident from Table 2, for this chemical element we have experimental data [4] for boiling temperatures and the corresponding pressures for the temperature range between its triple and critical

TABLE 2

Pressure of the saturated vapor of argon (Ar)[4]

T °K	p atm
83.97	0.6739
85.0	0.780
90.0	1.321
95.0	2.13
100.0	3.23
105.0	4.69
110.0	6.59
115.0	9.00
120.0	11.98
125.0	15.61
130.0	19.99
135.0	25.2
140.0	31.3
145.0	38.5
150.0	46.8
150.72	47.996



temperature. Therefore, for this element we can use equations (4) or (6) for this entire temperature range. It should be noted that the experimental data for the densities of vapor and liquid are also known for this range (Table 3), but not for the same temperatures [5]. The pressures of saturated vapor for the temperatures listed in Table 3 were determined in the following way. First, Diagram 1 was drawn on the basis of experimental data from Table 2, and then the pressures for the above temperatures were calculated from it. This data was also recorded in Table 3. The heat of vaporization for argon at a pressure of 1 atm is $r = 1{,}558 \frac{\text{cal}}{\text{g-atom}}$ [6], and the heat of sublimation at 0°K is $s = 1{,}880 \frac{\text{cal}}{\text{g-atom}}$ [3]. According to equation (6), on the basis of the data recorded in Table 3, for the lowest temperature, i. e. for $T = 92.01$ °K,

$$p_c = \frac{1.64 \text{ atm} \cdot 1.37396 \frac{g}{cm^3}}{3 \cdot 0.0081 \frac{g}{cm^3} \cdot 2.718282^{1.07552 \quad 0.63808}} \tag{9}$$

i. e.

$$p_c = 47.2 \text{ atm} \tag{10}$$

It can be established again that equation (6) gives good results for critical pressure, because the value obtained in this way and the experimental values show a difference of $\delta p_c = +1.7\%$. In the same way equation (6) was used for a number of temperatures near the critical temperature of argon, and the results listed in Table 3. As is evident from this table, the differences between the theoretical and experimental values for critical pressure first rise with temperature (to a temperature of 122.40° K, when $\delta p_c = -24.5\%$), and then fall. For the temperature nearest to the critical, i.e. for $T = 147.99$ °K, the difference rises again and amounts to $p_c = +22.7\%$.

TABLE 3

Critical pressure for argon (Ar)

$p_c = 47.996$ atm

T °K	p atm Diagram 1	$\rho' \frac{g}{\text{cm}^3}$	$\rho'' \frac{\text{g}^{[5]}}{\text{cm}^3}$	$\frac{s-r}{eRTc}\left(\frac{T_c}{T}-1\right)$	p_c atm from Equation (6)	$\delta p_b\%$
92.01	1.64	1.37396	0.00801	1.98630	47.2	+ 1.7
97.77	2.74	1.32482	0.01457	1.79041	46.4	+ 3.4
111.93	7.42	1.22414	0.03723	1.45167	56.0	−16.7
122.40	13.70	1.13851	0.06785	1.28253	59.7	−24.5
132.96	23.00	1.03456	0.12552	1.15449	54.7	−14.0
137.65	28.32	0.97385	0.15994	1.10751	51.9	−8.1
141.62	33.20	0.91499	0.19432	1.07157	48.6	−1.3
147.99	43.40	0.77289	0.29534	1.02000	37.1	+ 22.7

Finally, we shall use Van der Waals' equation for the state which refers to real gases and gives the relation between the pressure, the volume and the temperature, and is usually written in the form

$$\left(p+\frac{a}{V^2}\right)(V-b)-RT \tag{11}$$

in which a and b are constant values for each chemical substance. We know from thermodynamics that at critical temperature the first and second derivatives of the equation for the state with respect to the volume are equal to zero. It follows from equation (11) that at the critical temperature

$$p_c=\frac{RT_c}{V_c-b}-\frac{a}{V^2{}_c} \tag{12}$$

On condition that the first derivative is equal to zero we get

$$\frac{RT_c}{(V_c-b)^2}\,(V_c-b)^2=\frac{2a}{V_c^3} \tag{13}$$

and on condition that this second derivative is also equal zero we get

$$\frac{RT_c}{(V_c-b)^3}=\frac{3a}{V_c^4} \tag{14}$$

From equations (13) and (14) it follows that

$$V_c=3b \tag{15}$$

and from this and equation (13)

$$T_c=\frac{8a}{27\,Rb} \tag{16}.$$

Finally, by substituting the values for V_c and T_c from equations (15) and (16) into equation (12) we get

$$p_c=\frac{a}{27\,b^2} \tag{17}.$$

It follows from the equation for the state that for a mole of ideal gas

$$\frac{p\cdot V}{R\cdot T}=1 \tag{18}$$

and from this equation, according to equations (15), (16), and (17) for Van der Waals' gas at critical temperature

$$\frac{p_c \cdot V_c}{R \cdot T_c} = \frac{3}{8} \qquad (19),$$

and whence

$$p_c = \frac{3}{8} \cdot \frac{RT_c}{V_c} \qquad (20).$$

We shall now use this expression to obtain the values for the critical pressures of xenon and argon to be able to correlate these results and the results obtained from equation (6) and see which give better agreement with the experiment. By using equation (20) for xenon we obtain

$$p_c = \frac{3 \cdot 848 \dfrac{kp\ \text{m}}{\text{K-atom}\ °K} \cdot 289.76\ °K}{8 \cdot 131.3 \cdot \dfrac{1}{1154}\ \dfrac{\text{m}^3}{\text{K-atom}}} \qquad (21),$$

i.e.

$$p_c = 80.99 \frac{kp}{\text{cm}^2} \qquad (22),$$

or,

$$p_c = 83.66\ \text{atm} \qquad (23)$$

The difference between this value and that obtained by experiment is now $\delta p_c = -43{,}7\%$, i.e. twice as great as the greatest difference with which the critical pressure for this element was determined by equation (6).

As to argon, its critical pressure obtained from equation (20) is

$$p_c = \frac{3 \cdot 848 \dfrac{kp\, m}{\text{K-atom}\ °\text{K}} \cdot 150.72\ °\text{K}}{8 \cdot 40\ \dfrac{1}{530.78}\ \dfrac{\text{m}^3}{\text{K-atom}}} \qquad (24),$$

i.e.

$$p_c = 63.60 \frac{kp}{\text{cm}^2} \qquad (25),$$

or,

$$p_c = 65.70\ \text{atm} \qquad (26).$$

Now the difference is $\delta p_c = -36.9\%$, i.e. it is one and a half time greater than the greatest difference by which the critical pressure for argon was determined by equation (6).

It can be concluded from these results that equation (4) (obtained on the basis of statistical and quantum mechanics and classical thermodynamics) which represents the equation of state of saturated vapor is applicable in this form in the field of saturated vapor of monoatomic elements, because relation (1) holds only for monoatomic elements.

CONCLUSION

The starting point of this work is the relation valid for the equilibrium state between the liquid phase and its vapor. This relation, which refers to two fundamental quantities of state: temperature and volume, was obtained by the present author in one of her earlier papers and holds only for monoatomic elements for the temperature range between their triple and critical temperatures (equation (1). Then, from Van der Waals' equation [equation (2)], figuring two fundamental quantities: temperature and pressure, and equation (1) the author has obtained the equation of state figuring three fundamental quantities: temperature, pressure, and volume [equation (6)]. The critical pressures for the monoatomic elements xenon and argon have been determined by this equation. After this, the critical pressures of the same elements have been determined on the basis of values for critical temperature, pressure, and volume obtained by Van der Waals' equation, and these were substituted into the equation of state of ideal gas. From the equation obtained (for Van der Waals' gas) the critical pressures of the two elements have been determined. It has been established that the results for the critical pressures of these monoatomic elements obtained by the equation proposed in this paper are much better than those obtained by the equation taken from classical thermodynamics [equation (20)].

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DETERMINATION OF TERTIARY AMINES AND SALTS OF ORGANIC ACIDS IN ACETIC ACID BY THE DEAD-STOP METHOD

by

VILIM VAJGAND and TIBOR PASTOR

In previous works (1,2) we described derivative polarographic titration of tertiary amines in glacial acetic acid. In those works the behavior of antimony and quinhydrone electrodes was examined, especially the speed with which potential is established on the electrodes and the changes of anode and cathode potential. The work was further extended to examine the possibility of using the electrodes to determine the end-point of titration by the dead-stop method. Because of its simplicity and precision this is a valuable method for analysis in nonaqueous media.

In spite of its good qualities the dead-stop method has not been widely used in titrations of acids and bases. The first attempt in this field was made by Mann (3), who titrated acids and bases in the presence of quinhydrone electrodes. He applied this method to determine the acid and saponification number of oils in a mixture of alcohol and benzene. He also obtained good results in the titration of organic acids and bases in the presence of quinhydrone when he replaced the platinum electrodes with aluminum electrodes. However with this method the results are not satisfactory for solutions which contain chlorides, nitrates, nitrites and strong oxidizing agents. Enoki and Mirisaka (4) further extended the dead-stop method by carrying out titration in the presence of antimony electrodes.

Of the above mentioned electrode materials, quinhydrone and antimony acted favorably during titration in glacial acetic acid because of their reversibility, rapid establishment of equilibrium and also because of their inertness towards a great number of organic substances.

EXPERIMENTAL

The examination was carried out with the apparatus shown schematically in Fig. 1. The voltage needed for polarizing the electrodes was provided by 2V lead accumulators connected to a 100 Ω potentiometer. The changes of current during the titration were measured with a Radiometer type GVM 13b multiflex galvanometer, whose sensitivity was changed with a parallel connected potentiometer. Besides the electrodes described in earlier works, (1,2)

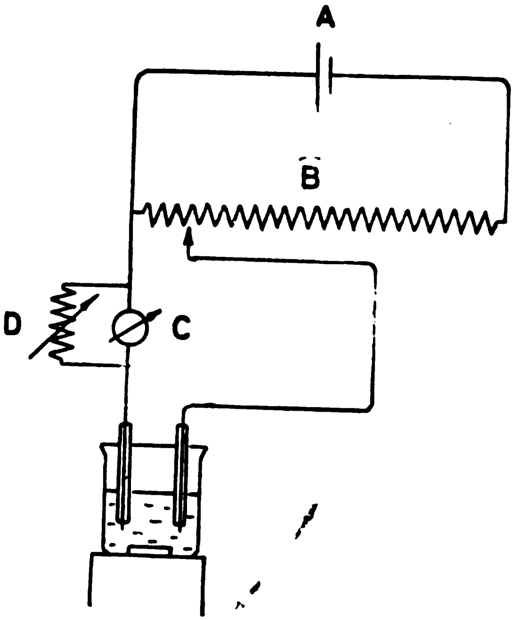

Figure 1

A Lead Acumulator
B 100 Ω Potentiometer
C Spotlight Galvanometer
D 1 *K* Ω Potentiometer

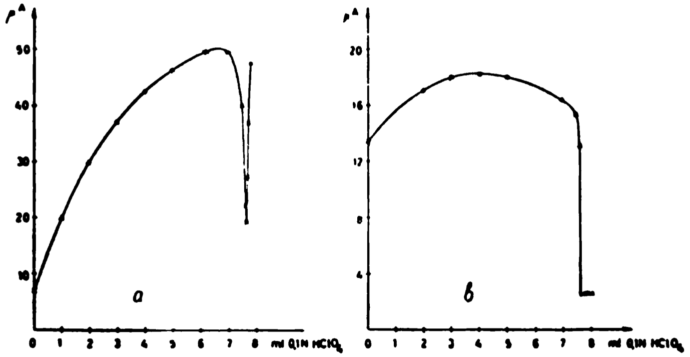

Figure 2

Titration curves of $3 \cdot 10^{-2} M$ solution of antipyrine with two antimony (a), and with two quinhydrone electrodes (b) small tungsten and aluminum electrodes in the presence of quinhydrone were used during the titrations.

The preparation of the solvents, standard solution of perchloric acid, and the samples for titration were described in a previous work. (2)

The curves obtained by the dead-stop titrations in acetic acid are similar to the curves obtained in aqueous solutions: in the presence of antimony electrodes the end-point of the titration is indicated by minimum current (Fig. 2, graph a). The minimums obtained are clearly expressed, but their sharpness depends to a great extent on the composition of the solution used. With an increased amount of anhydride of acetic acid in the solvent mixture the end-point is

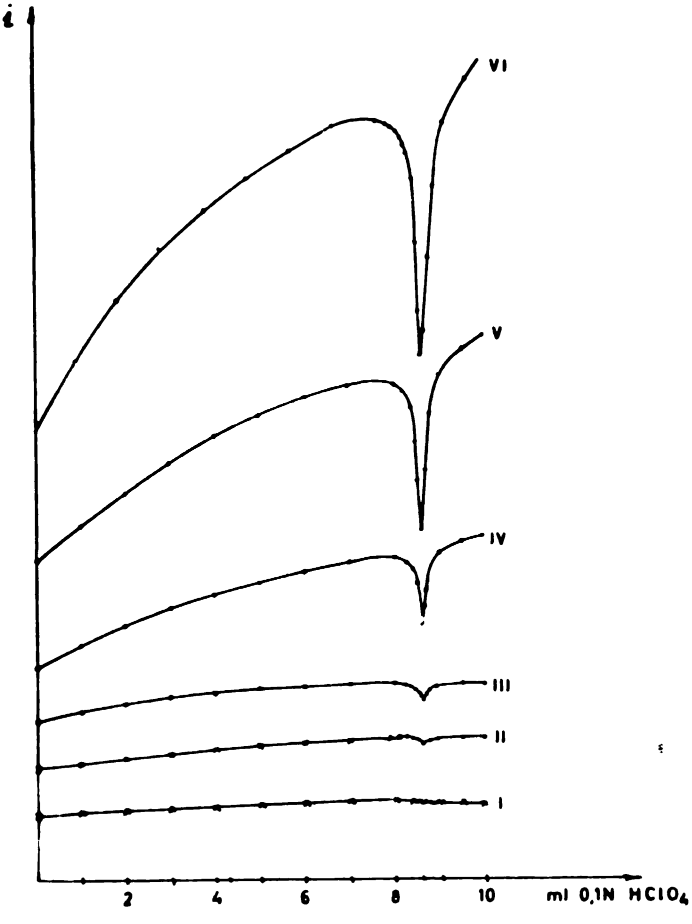

Figure 3

Titration curves of $3 \cdot 10^{-2}$ M solution of antipyrine: I in pure glacial acetic acid, II, III, IV, V and VI in solutions containing 10, 20, 40, 60 and 80%, respectively, of acetic acid anhydride more sharply defined. This is illustrated by the curves of the titrations of antipyrine in a mixture of acetic acid and its anhy-

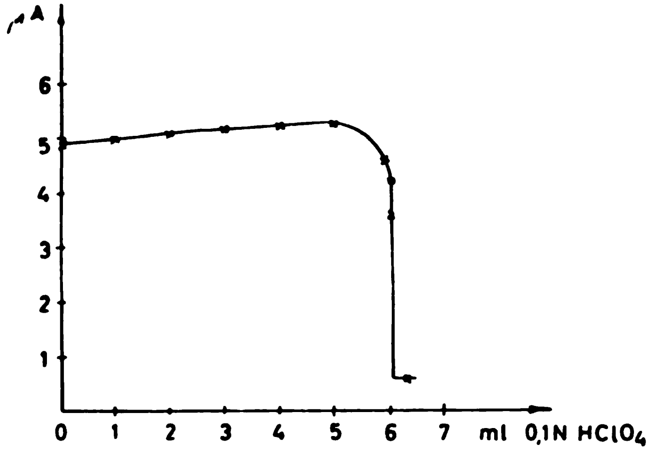

Figure 4
Titration culves of 3·10^{-2}M solution of sodium salicylate in the pres:nce of quinhydrone with two tungsten electrodes

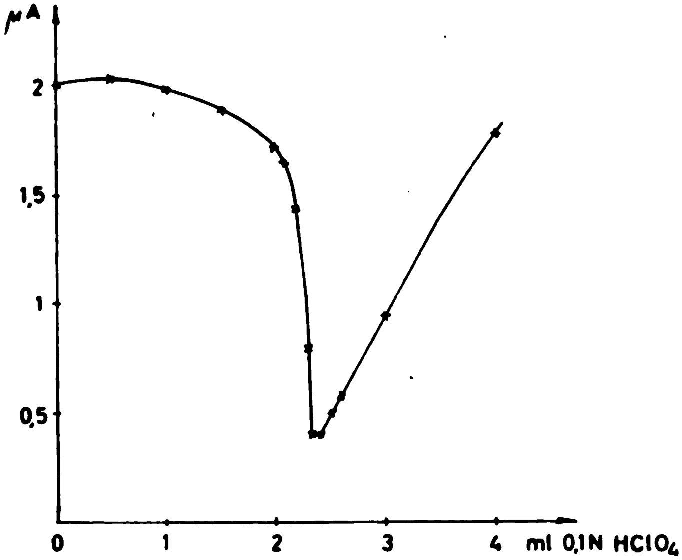

Figure 5
Titration curves of 10^{-3} M solution of narcotine in presence of quinhydrone with two aluminum electrodes

dride in different proportions (Fig. 3). A similar effect is observed when quinhydrone electrodes are used. The curves obtained from titrations carried out in the presence of quinhydrone electrodes are different from curves obtained when antimony electrodes were used. From the shape of the curve in Fig. 2, graph b, it can be seen that the current decreases suddenly at the end-point of titration and remains constant after it. During titration with quinhydrone electrodes the current is stable to near the equivalence point. At the equivalence point it changes slowly (to get constant current it is necessary to wait 5 to 20 minutes). After the titration end-point, the addition of each new amount of titration agent effects a small rise in current, but after some time the current slowly decreases to a constant value. Because of this titrations with quinhydrone electrodes require a rather long time. In order to remove this deficiency, tungsten and aluminum electrodes were tried in place of the platinum electrodes. However this change of electrodes did not yield the desired results. With the tungsten electrodes, which give titration curves of similar shape as the platinum electrodes (Fig. 4), the stationary state at the equivalence point is attained even more slowly, while with the aluminum electrodes the end-point of the titrations is not sharply defined. (Fig. 5).

The positive properties of antimony electrodes during dead-stop titration in glacial acetic acid are the stability of the current and the sharply defined end-point. However these electrodes also have some negative properties. For example, during titrations of hydrohalides of organic bases in the presence of mercury acetate an amalgam of antimony is formed, so that the electrodes are corroded very fast. During titration of strychnine nitrate the current fluctuates all the time up to the vicinity of the end-point of titration and the curve of the titration has a regular course only near the end-point and after it, when the current becomes stable. Antimony electrodes give good results in titration of iversal (p-benzoquinone amidinohydrazone thiosemicarbazone) in spite of the formation of a precipitate (which is formed during the titration and is dissolved after the titration end-point), whereas titration of this substance with quinhydrone electrodes does not succeed.

The potential difference between the electrodes was varied from 40 to 100 mV. It is also possible to apply a smaller potential difference if a more sensitive galvanometer is used. The current measured during the titrations was only 0.1 to 50 μA because of the high resistance of the titrated systems.

Solutions whose concentration was $7 \cdot 10^{-3}$ to $3 \cdot 10^{-2}$M were successfully titrated. The titration of theobromine ($K_b = 1.3 \cdot 10^{-14}$) and novalgin could not be perfomed by this method. The titrated systems and the results obtained are shown in Table 1.

TABLE 1

Titrated substance	Taken g	Found by potent. titr. g	Titrations by dead-stop method				Solvent
			in the presence of *Sb* electrodes		in the presence of quinh. electrodes		
			Nr. of titr.	Found g	Nr. of titr.	Found g	
Antipyrine	0.1500	0.1476	4	0.1472 ± 0.0002	3	0.1474 ± 0.0004	10 *ml* CH_3COOH+ 10 *ml* $(CH_3CO)_2O$
Sodium benzoate	0.1000	0.0983	3	0.0979 ± 0.0001	4	0.0978 ± 0.0001	— „ —
Iversal*	0.1000	0.0987	3	0.0991 ± 0.0001			— „ —
Nicotine	0.0811	0.0797	4	0.0799 ± 0.0001			— „ —
Strychnine nitrate	0.1000	0.0983	4	0.0984 ± 0.0002			— „ —
Potassium acetate	0.0633	0.0634	4	0.0633 ± 0.0001	4	0.0633 ± 0.0001	— „ —
Isoniazid	0.1000	0.0986	4	0.0983 ± 0.0002			— „ —
Sodium *p*-amino salicylate	0.1500	0.1475	4	0.1475 ± 0.0002	4	0.1479 ± 0.0002	— „ —
Sodium acetate	0.0822	0.0822	3	0.0818 ± 0.0001	3	0.0817 ± 0.0000	— „ —
Sodium salicylate	0.1000	0 0997	6	0.00995 ± 0.0001	5	0.0995 ± 0.0001	— „ —
Nicotinamide	0.1000	0.9998	3	0.0987 ± 0.0001	5	0.0985 ± 0.0002	— „ —
Cinchonine	0.1000	0.0942	4	0.0939 ± 0.0002	6	0.0937 ± 0.0001	10 *ml* CH_3COOH+ 10 *ml* $(CH_3CO)_2O$
Narcotine	0.1000	0.0987	3	0.0983 ± 0.0003	4	0.0984 ± 0.0002	— „ —
Piridoxine —*HCl*	0.1500	0.1473			4	0.1479 ± 0.0003	10*ml* 3% $Hg(CH_3COO)_2$ + 10 *ml* $(CH_3CO)_2O$
Endoiodin***	0.1500	0.1479			3	0.1478 × 0.0001	— „ —

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* *p*—benzoquinone amidinohydrazone thiosemicarbazone
** 5-hydroxy-6-methyl-3,4 pyridinedimethanol
*** 1,3-bis (trimethylamino)-2-propanol diiodide

The table shows that the dead-stop method gives results of excellent reproducibility.

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REACTIVITY OF PYRIDINECARBOXYLIC ACIDS II. REACTION KINETICS OF PICOLINIC, NICOTINIC, AND ISONICOTINIC ACID WITH DIPHENYLDIAZOMETHANE IN ETHANOL

by

DJ. M. DIMITRIJEVIĆ, Ž. D. TADIĆ, and M. D. MUŠKATIROVIĆ

In our first paper on the reactivity of pyridinecarboxylic acids (1) we pointed out that the purpose of our investigation was to study the reaction of these acids with diphenyldiazomethane (DDM) — and to establish the reactivity of their carboxyl groups depending on the position of these groups in relation to the nitrogen atom of the pyridine nucleus in order to make indirect deductions on the basis of their reactivity, on the strength of the electrical effects which they undergo, and thus obtain an insight into the relative distribution of electrons over the pyridine nucleus.

Since pyridinecarboxylic acids are insoluble in nonhydroxylic solvents, the reactions were carried out in ethanol. According to the investigations of Roberts and co-workers (2, 3, 4) DDM reacts also with ethanol, the acids acting as catalysts, so that the rate of the reaction of DDM with acids in ethanol is in fact equal to the sum of the reaction rates of DDM with acids and its reaction with ethanol. Since the rates of these two reactions depend on the strength of the acid, the overall rate constant of both reactions can be taken as a standard of the reactivity of the carboxyl group.

To simplify the interpretation of experimental data the conditions during the measurements of reaction velocities were adjusted so that the two reactions mentioned above were of the same order.

The reaction of DDM with ethanol as solvent was of pseudofirst order due to the large excess present. Because of this in all our measurements acids were used in large excess to make the other reaction also of the pseudofirst order. However, owing to the lower solubility of isonicotinic acid in ethanol, we could not carry out measurements with this acid under the same conditions as with picolinic and nicotinic acids, i.e. this acid could not be present in large excess over DDM and have the concentration between this acid and DDM in the proportion 10:1 as was the case with the other two acids. Therefore we had to restrict our measurements to the rate of the reaction of DDM with picolinic and nicotinic acids, i.e. to limit our investigations to the reactivity of the carboxyl groups in the α- and β-positions.

To examine also the reactivity of the carboxyl group in the γ-position and compare it with the reactivity of the carboxyl groups in the α- and β-positions we determined the reaction rates of DDM with all the three pyridine monocarboxylic acids (isonicotinic acid included) in ethanol at an initial concentration of the acids of 0.006 E, which, considering its solubility in ethanol, was suitable for isonicotinic acid, the least soluble of the acids investigated. Since this concentration was very low, it was not possible to work with ten times lower initial concentration of DDM at which the reaction would be of the pseudofirst order for DDM. Therefore the initial concentration of DDM was also 0.006 E, and this means that at the outset of the reaction the acid and DDM were present in equimolecular proportion.

The reaction kinetics of DDM with ethanol remains even under these conditions of the pseudofirst order, but the reaction of pyridinecarboxylic acids with DDM, on which our investigations are based, develops according to second-order kinetics. It was, therefore, necessary to find a mathematical expression embracing both reactions and at the same time making it possible to separate the value of the rate constant for the reaction of DDM with acids from the rate constant for the reaction of DDM with ethanol. It is evident that this required a thorough study of the mechanism of the reaction of DDM with pyridinecarboxylic acids in ethanol.

As is known (3,5) the reaction of DDM with carboxylic acids in ethanol takes place in two stages. The first which determines the rate of the reaction consists of the transition of protons from some donor to DDM thus forming the corresponding diazonium ions (DDMH$^+$). The second stage represents the reaction of these diazonium ions with one of the possible reactants. Thus all the reactions were divided into the reactions of formation and the reactions of decomposition of diazonium ions.

THE REACTIONS OF THE FORMATION OF DIAZONIUM IONS

The first possibility is that the proton-donor is a molecule of carboxylic acid:

$$\mathrm{DDM} + \mathrm{RCOOH}^{(a)} = \mathrm{DDMH}^+ + \mathrm{RCOO}^- \qquad (1)$$

The second possibility is the formation of diazonium ions from DDM and ethyloxonium ions as proton-donors:

$$\mathrm{DDM} + \mathrm{C_2H_5OH_2}^+ = \mathrm{C_2H_5OH} + \mathrm{DDMH}^+ \qquad (2)$$

Ethyloxonium ions were formed in the reaction between pyridinecarboxylic acid and ethyl alcohol:

$$\mathrm{RCOOH} + \mathrm{C_2H_5OH} \rightleftarrows \mathrm{C_2H_5OH_2}^+ + \mathrm{RCOO}^- \qquad (3)$$

(a) $R = C_5H_4N$

THE REACTIONS OF DECOMPOSITION OF DIAZONIUM IONS

The diazonium ion formed can react with the anion of pyridinecarboxylic acid forming its benzhydryl ester (Es), or with the molecule of ethyl alcohol forming benzhydryl ethyl ether (Et).

$$DDMH^+ + RCOO^- = Es + N_2 \tag{4}$$

$$DDMH^+ + C_2H_5OH = Et + N_2 + H^+ \tag{5}$$

There is also a possibility that DDMH$^+$ reacts with the unchanged molecule of pyridinecarboxylic acid and also with the DDM molecule. However, the participation of these reactions is very small when compared with the two reactions mentioned above so that it can be neglected in our considerations (2).

On the basis of reactions (1) and (2) the formation rates of DDMH$^+$ can be represented by the following expressions:

$$\left(\frac{dC_{DDMH^+}}{dt}\right)_1 = k\ C_{DDM}\,C_{RCOOH}$$

and

$$\left(\frac{dC_{DDMH^+}}{dt}\right)_2 = k'\ C_{DDM}\,C_{C_2H_5OH_2^+}$$

The concentration of ethyloxonium ion, after the addition of acid and before the addition of DDM, can be derived from the equilibrium constant of the reaction in which it is formed (reaction (3)).

$$K = \frac{C_{RCOO^-} \cdot C_{C_2H_5OH_2^+}}{C_{RCOOH}\ C_{C_2H_5OH}}$$

since $C_{RCOO^-} = C_{C_2H_5OH_2^+}$

and $C_{C_2H_5OH} = \text{const.} = A$

then $C_{C_2H_5OH_2^+} = (K \cdot A \cdot C_{RCOOH})^{1/2}$ (a)

The overall rate of the formation of DDMH$^+$ represents the sum of the rates of reactions (1) and (2)

$$\frac{dC_{DDMH^+}}{dt} = \left(\frac{dC_{DDMH^+}}{dt}\right)_1 + \left(\frac{dC_{DDMH^+}}{dt}\right)_2$$

$$\frac{dC_{DDMH^+}}{dt} = C_{DDM}\left[k\ C_{RCOOH} + k'\ (K \cdot A \cdot C_{RCOOH})^{1/2}\right]$$

If it is assumed that the transition of protons in the reaction between pyridinecarboxylic acids and DDM directly, or through alcohol, represents the slow phase of the reaction, then the rate

---

(a) This expression is approximately accurate until, owing to the reaction with DDMH+, the RCOO− concentration is essentially changed.

of decomposition of diazonium ions is equal to the rate of their formation, and this, or either of them, is equal to the rate of DDM decomposition.

$$\frac{dC_{DDMH^+}}{dt} = -\frac{dC_{DDM}}{dt}$$

or, if we substitute $k'\ (K \cdot A)^{1/2} = k''$

$$-\frac{dC_{DDM}}{dt} = k\, C_{DDM} C_{RCOOH} + k''\, C_{DDM}\, C^{1/2}_{RCOOH}$$

Since the reaction is carried out at equal initial concentrations of acid and DDM, at $t = 0$, the above equation becomes

$$\left(-\frac{dC_{DDM}}{dt}\right)_{t=0} = k\, C^{2}_{DDM} + k''\, C^{3/2}_{DDM}$$

This equation holds only for very small values for K when the change in the RCOOH concentration is negligible owing to the protonization of alcohol, so that the RCOOH concentration can be considered equal to stechiometric concentration. It holds until the moment when the change in the RCOO$^-$concentration becomes significant due to its reaction with DDMH$^+$, or until a greater decrease in DDM concentration is observed due to its side reaction with ethyl alcohol.

The value of $-\frac{dC_{DDM}}{dt}$ represents the slope of the tangent of the curve obtained experimentally. If we substitute

$$-\frac{dC_{DDM}}{dt} = D,$$

then $$D_{t=0} = k C^{2}_{DDM} + k'' C^{3/2}_{DDM}$$

or $$\left(\frac{D}{C^{3/2}_{DDM}}\right)_{t=0} = k\, C^{1/2}_{DDM} + k'' \qquad \text{(I)}$$

Considered from a strictly mathematical standpoint, the above equation is valid only for the outset of the reaction when $C_{DDM} = C_{RCOOH}$. However, we can carry out an approximation and apply it to the entire range in which the slope of the tangent of the experimental curve $(C = f(t))$ has a constant value. The graphical presentation of this equation (I) made it finally possible to separate the rate constants for the reactions of acids from the rate constants for the reaction of ethanol wiih DDM by determining the initial reaction rates for equal concentrations of pyridinecarboxylic acids and DDM in ethanol. The value of the constant is obtained from the slope which this straight line has when D is determined in the range close to the outset of the reaction.

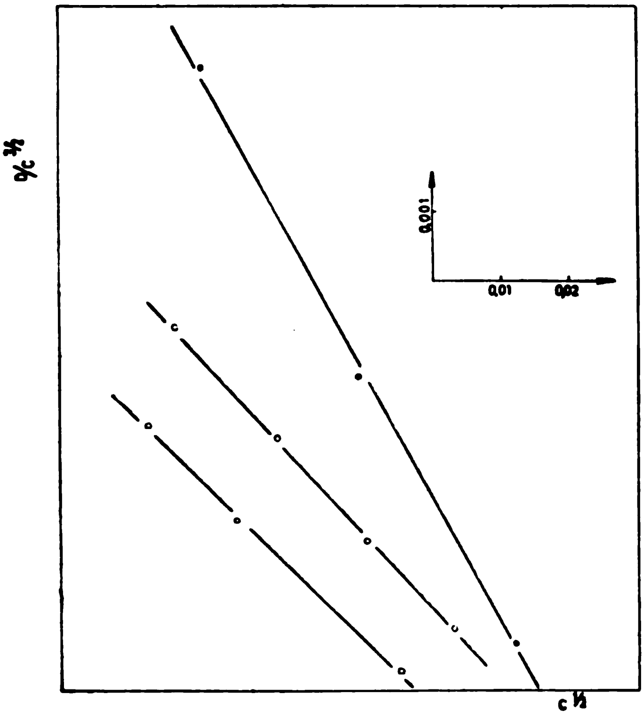

Fig. 1. — Plot of $\frac{D}{C^{1/2}}$ vs. $C^{1/2}$ (Equation (I))

—○— Picolinic acid
—□— Nicotinic acid
—●— Isonicotinic acid

EXPERIMENTAL

The procedures and the preparation of the materials for determination were the same as in our earlier work (1). The solvent used was absolute ethanol. The rate of the reaction was followed with a *Hilger Uvispek* spectrophotometer by measuring the decrease in absorption at 525 mμ with time. All measurements were made at 30±0.2°C.

Experimental Data

The determination of the reaction rate constants in all three cases was based on readings taken up to the moment when the slope of the tangent of the experimental curve ceased to have a constant value.

TABLE 1
Picolinic acid

Time t min	Absorption A	*DDM* Concentration C g liter^{-1}	$D^{(a)}$	$\frac{D}{C^{3/2}}$	$C^{1/2}$
2	0.541	0.560	0.01	0.0239	0.748
4	0.520	0.540	0.01	0.0252	0.735
6	0.500	0.520	0.01	0.0267	0.727
8	0.482	0.500	0.01	0.0283	0.707
10	0.465	0.482	0.009		
12	0.449	0.465	0.0085		
14	0.435	0.451	0.007		
16	0.421	0.438	0.0065		
18	—	—	—		
20	0.392	0.409	0.0055		

(a) Slope of the tangent of the experimental curve

The statistically calculated rate constant for the reaction has a value of

$k = 0.1080$ liter min^{-1} g^{-1}

TABLE 2
Nicotinic acid

Time t min	Absorption A	*DDM* Concentration C g liter^{-1}	D	$\frac{D}{C^{3/2}}$	$C^{1/2}$
2	0.542	0.563	0.009	0.0213	0.750
4	—	—	—	—	—
6	0.508	0.527	0.009	0.0235	0.726
8	0.490	0.508	0.009	0.0249	0.713
10	0.474	0.493	0.008		
12	0.458	0.478	0.0075		
14	0.441	0.460	0.009		
16	0.427	0.443	0.0085		
18	—	—	—		
20	0.400	0.415	0.007		

$k = 0.096$ liter min^{-1} g^{-1}

TABLE 3

Isonicotinic acid

Time t min	Absorption A	DDM Concentration C g liter^{-1}	D	$\frac{D}{C^{3/2}}$	$C^{1/2}$
2	0.529	0.550	0.0162	0.0397	0.742
4	0.499	0.517	0.0162	0.0436	0.719
6	0.469	0.485	0.0162	0.0481	0.696
8	0.444	0.460	0.0125		
10	0.420	0.435	0.0125		
12	0.398	0.412	0.0115		
14	0.377	0.391	0.0105		
16	0.358	0.370	0.0105		
18	0.340	0.351	0.0095		
20	0.323	0.333	0.009		

$k = 0.1820$ liter min^{-1} g^{-1}

DISCUSSION AND RESULTS

Our earlier investigations of pyridinecarboxylic acids (1) on the basis of the rate of their reactions with DDM showed that the α—carboxyl group in the pyridine nucleus is more reactive than the β—carboxyl group. This conclusion was reached by calculating the values of the rate constants for the reactions of the α—pyridine and the β—pyridine carboxylic acids with DDM in ethanol. The calculated constants represented the sum of the rate constants for the reactions of the acids with DDM and the rate constants of DDM with ethanol, with pyridinecarboxylic acid as catalyst. Since the rates of both reactions depend on the ease with which the given acid releases protons we did not separate the rate constants for the reaction between the acid and DDM, so that the overall constant was taken as a standard of the reactivity of the acid in question.

Since in the present work we experimented under a new set of conditions under which the reaction was of second order, we obtained the values of the rate constants for the reaction of pyridine monocarboxylic acids with DDM, eliminating the influence which the reaction between the solvent and DDM had on their values.

From the values obtained for the second-order rate constants k we can see that the reactivity of these acids, i.e. their carboxyl groups, decreases in the following order:

$$k_{\text{isonicotinic}} > k_{\text{picolinic}} > k_{\text{nicotinic}}$$

These results confirm the results obtained in our first work, i.e. that the carboxyl group in the α—position (picolinic acid) is more reactive than the carboxyl group in the β—position (nicotinic acid), and indicate by far the highest reactivity for the carboxyl group in the γ—position (isonicotinic acid).

Table 4 shows the values that we have obtained for the second-order rate constants together with the values for the relative electron density on the corresponding carbon atoms of the pyridine nucleus calculated by Longuet—Higgins and Coulson (6), and also the values for the thermodynamic dissociation constants K_a of these acids. These latter were calculated by Stephenson and Sponer (7) on the basis of the determined apparent dissociation constants of these acids (8) on the assumption that these acids occur in the aqueous solution prevailingly in the zwitterion form.

TABLE 4

Electron density at the respective pyridine ring *C*-atoms

Position of the Carboxyl Group in the Pyridine Nucleus	Second-Order Rate Constant k	Electron Density	Thermodynamic Dissociation Constant K_a
α-position Picolinic Acid	0.108	0.849	$6.61 \cdot 10^{-2}$
β-position Nicotinic Acid	0.096	0.947	$0.646 \cdot 10^{-2}$
γ-position Isonicotinic Acid	0.182	0.822	$1.20 \cdot 10^{-2}$

On comparing the order of our values for the rate constants of the reaction with DDM with the order of values for electron density on the corresponding carbon atoms of the pyridine nucleus we can establish a qualitative agreement in both cases. The carboxyl groups attached to the carbon atoms with lower electron densities, which consequently undergo a higher negative inductive effect, are more reactive than those attached to the carbon atoms with higher electron densities.

Thus our experimental results confirm qualitatively the theoretically calculated relations between electron densities over the pyridine nucleus.

When we further compare our results with the calculated thermodynamic dissociation constants K_a of these acids reported by Stephenson and Sponer (7), we can see that there is an agreement in the results for nicotinic acid, which according to our and their results, is the weakest, i.e. the least reactive, while for picolinic and isonicotinic acids there as a discrepancy between our results and the results obtained by the authors mentioned above.

While according to the results obtained by these authors picolinic acid is the most reactive, in our experiments isonicotinic acid was the most reactive. In our opinion this discrepancy in the results can be explained by the fact that in calculating thermodynamic dissociation constants on the basis of the determined apparent dissociation constants it was assumed that these acids occur in aqueous solution entirely in the zwitterion form, thus neglecting the participation of the neutral uncharged molecules which may be considerable and very hard to determine experimentally.

In our experimental data the reaction rate of isonicotinic acid is strikingly high when compared with that of picolinic acid. This will probably be explained by more detailed experiments.

CONCLUSION

Rate constants for the reactions of pyridine monocarboxylic acids with diphenyldiazomethane (DDM) in absolute ethanol were determined. Due to the acid to DDM concentration ratio the reactions were of the second order. On the basis of the values obtained for the rate constants of the reactions the following order of reactivity of these acids was established:

$$k_{\text{isonicotinic}} > k_{\text{picolinic}} > k_{\text{nicotinic}}$$

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THE AGING OF MILD STEEL FOLLOWED BY CHANGE IN MECHANICAL PROPERTIES

by

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Because of its practical and theoretical importance the aging of mild steels has been intensively investigated. The mechanisms of quench and strain aging are generally studied separately, although these processes do not take place completely independently one from the other.

The first reports date from 1930. E. S. Davenport and E. C. Bain (1935) published a report on the changes in hardness during aging. General characteristics of quench aging were given by R. L. Kenyon and R. S. Burns in 1940. Intensive studies were continued during the 1940—1947 period. In these works quench aging, and later also strain aging, were investigated by studying the changes in the mechanical and physical properties of steel. This data has been useful in the characterization of these processes, but they were of little help in explaining either the formation of a second phase, or hardening due to aging. From 1947 on, the works of A. H. Cottrell on the influence of solute atoms on the behavior of dislocations (1), and those of A. H. Cottrell and B. A. Bilby (2) and F. R. N. Nabarro (3) have laid the first theoretical basis for the explanation of the phenomenon of aging. These works initiated a period of rapidly growing interest in the imperfections of the crystal lattice and their influence on the properties of the metal. In addition to its practical interest, the study of the aging processes represents a more and more interesting method for checking the theories which explain the mechanism of aging. Changes in mechanical properties have remained the subject of greatest interest, and the comparison of results obtained by its study with the results obtained by the study of the change in electrical resistivity made it possible not only to find a confirmation of Cottrell's first postulates but also to supplement them. All this is discussed in full detail in a paper by D. V. Wilson and Russell, 1960 (4).

The precipitation process is caused by the thermodynamical instability of the multicomponent phase. Considered generally, if metal A contains atoms of metal B in a supersaturated solid solution, atoms of metal B will tend to precipitate in the form of a second phase, usually as a compound of metal A and metal B. This phase forms by successive processes of nucleation and growth, and the rate of this process is controlled by the diffusion of metal B through metal A. At a low temperature the rate of diffusion is very low, so that the supersaturated state of the alloy can be maintained for a certain time.

Quench aging of mild steels is caused by supersaturation with carbon and nitrogen. This paper will deal only with the influence of carbon, although the influence of nitrogen can be followed in a similar way. If steel with a low carbon content (0.05%) is rapidly cooled from 720°C, austenite with a face-centered cubic lattice is transformed into ferrite with a body-centered cubic lattice in which the solubility of carbon is extremely low (less than $10^{-5}\%$ at room temperature) in contrast to a considerably higher solubility of this element in austenite. The excess of carbon retained in the crystal lattice of ferrite tends to precipitate. This process of precipitation is conditioned by the diffusion rate of carbon. The precipitated carbon can be in equilibrium (usually metastable) with carbon dissolved in α-iron in four different forms: (a) as graphite; (b) as Fe_3C; (c) as ε-carbide, i.e. $Fe_{2,4}C$; (d) as an unhomogeneous solid solution, i.e. in the form of an elastic bond of carbon atoms with dislocations. Each type of bond corresponds to a certain equilibrium solubility of carbon in α-iron. Since the solubility of carbon at room temperature is very low it is possible to represent it by Van t'Hoff's equation according to which

$$\text{Solubility} = C_0 e^{-\Delta H/RT} \text{ or,}$$
$$ln \text{ of solubility} = lnC_0 - \Delta H/RT$$
$$log \text{ of solubility} = logC_0 - 0.4343\Delta H/RT = log C_0 - 1/T \cdot \Delta H/4.575$$

Since in the given case ΔH has a positive value, the solubility will increase with the increase in temperature. The coefficient of diffusion of carbon in α-iron is given by the following expresion:

$$D = D_0 e^{-Q/RT}$$

where $D_0 = 0.02$ cm²/s, and $Q = 20,100$ Cal/mole

The precipitation of carbon from a supersaturated solid solution in α-iron takes place in two stages. Carbide with an approximate formula of $Fe_{2,4}C$ (ε-carbide) precipitates first. It precipitates not because it constitutes a more stable phase, but because the formation of a heterogeneous nucleus is probably facilitated by a greater similarity between the crystal lattices of the matrix and the phase being precipitated.

ε-Carbide has a close-packed hexagonal lattice in which carbon atoms have a more or less ordered arrangement in octahedral interstices between iron atoms. The number of carbon atoms corresponds approximately to the formula of carbide. These atoms have the widest possible spacing. The distance between the planes (1011) in carbide is very similar to the distance between planes (101) in the crystal lattice of ferrite. The similarity is greatest when $c/a \sim 1.05$ because then the arrangement of atoms in these two planes is exactly the same. Consequently, the formation of a coherent nucleus does not require a greater strain energy.

Since ε-carbide is metastable when compared to cementite (Fe_3C), the increase in temperature increases the tendency to form this more stable compound. The techniques of most aging processes make it impossible to establish two stages of the process. However, the results of the investigation of this process with light and electron microscopes within the temperature range of 100—600°C have been published. The precipitation of particles of the size ranging between 300 and 500 Å takes place after one hour at 100°C, when the maximum hardness occurs. After sixteen hours these particles have a clearly pronounced discoidal form and are so large that they can be measured under an electron microscope. Their thickness reaches 500 Å and their diameter 2,000—3,000 Å. At temperatures of 200°, 300°, and 400°C the particles retain the same form and precipitate at sub-grain boundaries; only at higher temperatures they precipitate at grain boundaries. Only at 500° and 600°C are the particles spherical and resemble ordinary cementite. However, there are papers reporting the occurrence of cementite at a temperature of over 300°C. The advance in the field of electron microscopy will undoubtedly provide an answer to this question.

This precipitation of the excess carbon from a solid solution is accompained by an increase in hardness, yield strength, and ultimate tensile strength, while ductility and notch toughness decrease. These phenomena are known as precipitation hardening.

Hardening is caused by the interaction of carbon atoms and dislocations. It can be assumed that the precipitation of carbon from a homogeneous supersaturated solution takes place through the formation of an unhomogeneous solid solution, i.e. by the concentration of carbon atoms in regions more favorable from the point of view of energy — around lattice imperfections. Thus the earliest stage of precipitation hardening impedes the movements of dislocations. If carbon atoms are assembled at one point in sufficient amounts, the ε-carbide lattice will form in the quickest possible way, and a finely dispersed second phase will precipitate. At higher temperatures the second phase precipitates directly. The presence of this phase sets up strains in the metal, so that, for instance, the specific volume of this phase always differs from the volume of the parent metal. These strains in the interaction with dislocations impede the movement of dislocation which results in the hardening of the metal. Once a precipitate nucleus reaches a certain size it will grow by accretion of new solute atoms because of the mutual attraction of the precipitate and solute atoms. During aging, hardness, yield strength, and ultimate tensile strength increase for a certain time simultaneously with the size of the second phase particles, but later they decrease. Hardness usually reaches its peak when the mean distance between precipitate particles is 20— 50 atomic spacings. With further growth of the second phase particles this distance increases while these mechanical properties decrease, and the alloy is over-aged. Mott assumes that a dislocation can "wriggle" through between these precipitate particles at a smaller stress than that necessary to overcome the internal stress due to their presence in the lattice. There is a minimum radius of curvature of dislocations for a given strain. Maximum hardness is attained when the mean distance between precipitate particles λ is of the same order of magnitude as the minimum radius of curvature of dislocations r_c. In a finely dispersed second phase, or in single solute atoms, $\lambda < r_c$, but in this case the strain of the lattice is also smaller and consequently the hardening effect decreases. When $\lambda > r_c$, the mean distance between particles is so great that dislocations move by just bulging through them leaving a dislocation ring around each particle. The stress required to set the dislocation in motion is smaller and in this case the alloy is over-aged. The rate of growth of the precipitate particles varies with temperature; at higher temperatures the effect of hardening decreases and its maximum values are shifted to shorter time intervals, or it completely vanishes.

STRAIN AGING

Precipitation hardening can be considerably accelerated by cold work after quenching.

Strain aging is a well-known phenomenon observed in low carbon steels occurring either in quenched and immediately deformed steel or in low carbon steel which has only been deformed. It is a consequence of the interaction between solute atoms and the dislocations present in the lattice. In the stress field of an elastic dislocation there will be regions in which the strain energy of a foreign atom in solid solution is greater than the energy of the same atom dissolved in an unstrained lattice and also regions in which this energy is less. Foreign atoms tend to diffuse into regions where their energy is lowest and to reduce in this way the free energy of the system. In the Fe-C system, the intersticial solute carbon atoms segregate in the expanded region of the dislocation causing a non-uniform distribution of carbon atoms in the solid solution. The aggregated atoms anchor the dislocation so that the force required to set it in motion is considerably greater than before anchoring. If sufficiently great force is applied, the dislocation breaks away from the atmosphere formed by foreign atoms, the so-called Cottrell atmosphere, and can continue its motion under the influence of a smaller force. The yield strength of low carbon steels is based on this phenomenon.

It is evident from this that the segregation of carbon atoms into dislocations gives rise to the phenomenon of strain aging. Iron alloys with a very low carbon content strained immediately after quenching do not show a sharp yield point because all the dislocations are free of carbon atoms arranged uniformly in the supersaturated solution. After resting the quenched metal for several hours at a temperature at which the diffusion of carbon is sufficiently great, yield point returns because the dislocations have gradually become locked by carbon atoms, and thus the metal has become harder.

The same effect is observed in plastic deformation of iron alloy with a low carbon content (within the range of solid solution). If we load the alloy to the yield point at room temperature, unload it and reload it the yield point will not return. However, if we leave the alloy for several hours at a sufficiently high temperature the yield point does return. The explanation is the same as in the previous case: immediately after the first loading the dislocations are freed of carbon atoms so that there is no reason for yielding. When carbon atoms are allowed again to segregate into the dislocation the yield point reoccurs.

Thus the mechanism of strain aging differs somewhat from the mechanism of quench aging. In fact strain aging is based on the influence of a larger number of dislocations caused by cold work (the concentration of dislocations increases at 23% elongation from $10^8/\mathrm{cm}^2$ to $10^{12}/\mathrm{cm}^2$. The solute carbon atoms migrate by the shortest way to the dislocations that have formed and thus accelerate the aging process. This means that the rate of aging increases with the increase in the percentage of deformation if the concentration of carbon is sufficiently high.

The change in properties accompanying strain aging shows the same trend as in quench aging, although in some details essential differences exist. First, strain aging takes place only after the deformation of the alloy, regardless of the fact whether it is in the quenched or annealed state. Secondly, in the stage of over-aging hardness and ultimate tensile strength decrease much more slowly than in quench aging. These observations are in agreement with the accepted mechanism of aging due to the locking of dislocations.

The original statement that the carbon atoms segregated into dislocations are so firmly locked in strain aging that no precipitation can take place was later modified. It has recently been established that the segregation of carbon atoms into dislocations can under appropriate conditions continue through the formation of atmospheres and finally have the form of a fine precipitate. This modified conception is still controversial. Nevertheless, the explanation of results for the initial rate of aging is not complicated and there is an almost general agreement that the early concentration obeys the $t^{2/3}$-law as established by Cottrell-Bilby's equation (2). However, the later course of the process is controversial and the differences in the approach still exist. According to the recent interpretation of Wilson and Russel (4) strain aging depends primarily on the formation of Cottrell atmospheres, while according to the results obtained by the investigation of mechanical properties after the early stage of the aging process the increase in ultimate tensile strength is associated with a form of precipitation hardening.

It is evident from the study of the development of the theory of strain aging that the basic mechanism of the process has been given a very good explanation and has been supported by abundant experimental data. However, it seems that experiments made with pure alloys of well-known composition are necessary for a final confirmation and explanation of the details concerning the kinetics of the process. Investigations are being made along these lines, and the correlation between the stage of aging and the change in physical properties of the metal appears to be a promising subject of study.

In the Institute for Physical Metallurgy and the Metallurgy of Iron and Steel of the Faculty of Technology of Belgrade, attempts were made to follow the changes in the properties of a Fe-C alloy (0.05% C) relative to temperature and time by the method of alternating bending. The results obtained so far show that this method can be usefully applied. Nevertheless, these results are only qualitative indices and we believed that at this stage of the work it would be very useful to follow the changes in the same material by other methods simultaneously. The study of changes in mechanical properties and changes in electrical resistance were selected as comparative methods.

The purpose of the investigations discussed in this paper was to establish the changes in yield point, ultimate tensile strength, and hardness in the above alloy in quench and strain aging. The experimental conditions were the same for all the methods used in comparative investigations. We also wanted to check the possibility of carrying out these investigations with new and adapted equipment in this Institute.

EXPERIMENTAL

The composition of the steel investigated: C, 0.050%; Si, 0.056%; Mn, 0.28%; S, 0.011%; and P, 0.002%.

Form of test piece: 180-mm long wire, 2 mm in diameter.

Initial state: soft annealed.

Quench aging. — The specimens were quenched from 720°C in a 10% aqueous solution of NaCl. After quenching the specimens were heated to 50, 90, 120, and 150°C for 0, 5, 10, 20, and 40 minutes, and for 1, 2, 4, and 6 hours.

Strain aging. — The specimens were quenched from 720°C in a 10% aqueous solution of NaCl and strained by tension to 6% elongation immediately after quenching. The deformed specimens were heated under the same conditions as above.

Test pieces were heated to the quenching temperature in a furnace without a protective atmosphere. Oil bath was used for aging after quenching, or after quenching and cold work. Deformation by tension was performed on a tensile testing mashine.

As soon as this treatment had been completed, yield point, ultimate tensile strength and hardness were determined on the specimens and their microstructure was examined at the same time. Because of the size of the specimens the change in hardness was determined by the measurement of microhardness under a load of 10 g. In these experiments special care was taken to keep the working conditions as constant as possible.

RESULTS

The relation between the changes in yield point and ultimate tensile strength relative to time and temperature for the samples treated under conditions for quench aging are shown in Figure 1, while for those treated under conditions for strain aging are presented in Figure 2. The time τ in which the above properties reach their maximum, the values of the maxima and the increase in values expressed in percentages of the values immediately after quenching, and quenching and deformation are given in Tables 1 and 2.

TABLE 1

CHANGE IN *YP* AND *UTS* IN QUENCH AGING

Aging Temperature °C	Time Required to Attain Maximum Values τ min	*UTS* kg/mm^2		*YP* kg/mm^2	
		Max.	Increase %	Max.	Increase %
As Quenched		32.90		27.75	
50	1,200	38.23*	18	36.75*	26.7
90	60	39.16	19	33.07	19.25
120	20	38.35	16.5	33.42	20.08
150	10	37.79	14.8	33.60	21.7

* The values for *UP* and *UTS* at 50° were attained after twenty hours of aging, but they are not maximum values.

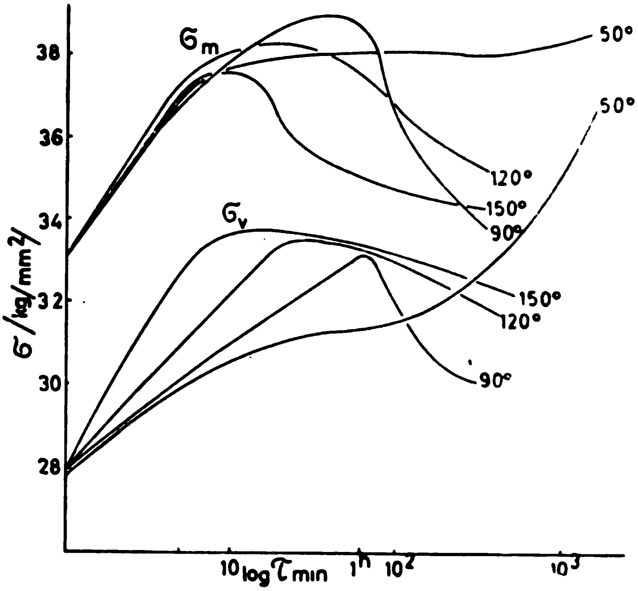

Fig. 1. — Dependence of *YP* and *UTS* on temperature and time in quench aging.

σ_m = *UTS* σ_v = *YP*

TABLE 2

CHANGE IN *YP* AND *UTS* IN STRAIN AGING

Aging Temperature °C	Time Required to Attain Maximum Values τ min	*UTS* kg/mm²		*YP* kg/mm²	
		Max.	Increase %	Max.	Increase %
As Quenched and Deformed		31.60		28.93	
50	720	47.20*	49.9	45.15*	56.1
90	60	46.85	48.1	44.87	55.0
120	20	43.95	39.0	42.95	48.5
150	10	43.25	37	42.70	47.6

* The values for *YP* and *UTS* at 50° C were attained after twelve hours of aging, but they are not maximum values.

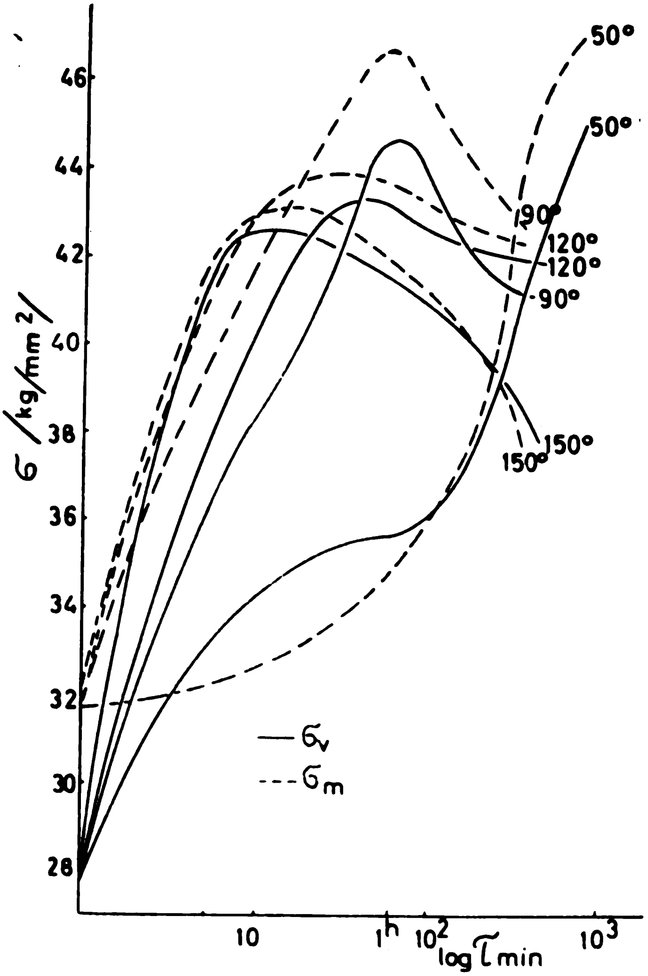

Fig. 2. — Dependence of *YP* and *UTS* on temperature and time in strain aging.

σ_m = *UTS* σ_v = *YP*

These data show:

(1) At a temperature of 50°C the process of aging was not completed in either case.

(2) With an increase in the aging temperature the time τ required to attain maximum values for YP* and UTS* shifts to lower values.

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* *YP* = Yield point; *UTS* = Ultimate tensile strength

(3) The highest values for YP and UTS are obtained at the lowest temperature and they decrease with an increase in temperature. There are deviations with respect to YP in quench aging: the maximum values for YP at 90, 120, and 150°C are almost identical.

(4) The hardening process indicated by the increase of values for YP and UTS related to the initial state (expressed in percentages) is twice as fast in strain aging as in quench aging.

(5) In deformed specimens the increase in values for YP compared to the increase of values for UTS is much faster in undeformed specimens. In deformed samples these values are in some cases very similar.

The change in microhardness is shown in the diagram on Figure 3. This diagram shows that

(1) Hardness changes with tensile properties.

(2) At 50°C the maximum was not reached even after 1,200 minutes.

(3) In quench aging, maximum values were attained for the same time as in measuring tensile properties. In strain aging the maxima shifted to a shorter time. The difference in maximum hardness is smaller, except for aging at 90°C.

As was expected, the examinations of microstructure (x 1,000) did not make it possible to establish any changes in the structure of the specimens.

DISCUSSION

The established increase in the rate of hardening relative to temperature is in accordance with the assumption that both aging processes depend on the rate of diffusion of carbon atoms. The relation between the logarithm of the rate of aging (with respect to YP and UTS) and the reciprocal values of the absolute temperature shown in the diagram on Figure 4 is linear. This satisfies equations (1) and (2) according to Nabarro:

$$\tau = x^2 \cdot 2D \qquad (1)$$

$$D = D_0 \cdot e^{-Q/RT} \qquad (2)$$

or,

$$\tau = x^2 \cdot 2D_0 \cdot e^{-Q/RT}$$

where τ is the time necessary to attain a given concentration at distance x. By extending the straight line obtained by experimental data the value of τ for $t = 50°C$ can be read. As the diagram shows, its value is about fourteen hours, and this is in agreement with the experimental results of other authors. The circles in the diagram (Fig. 4) refer to the results for the change in hardness obtained by Bain and Davenport. The agreement between these results and the results of our experiments is remarkable.

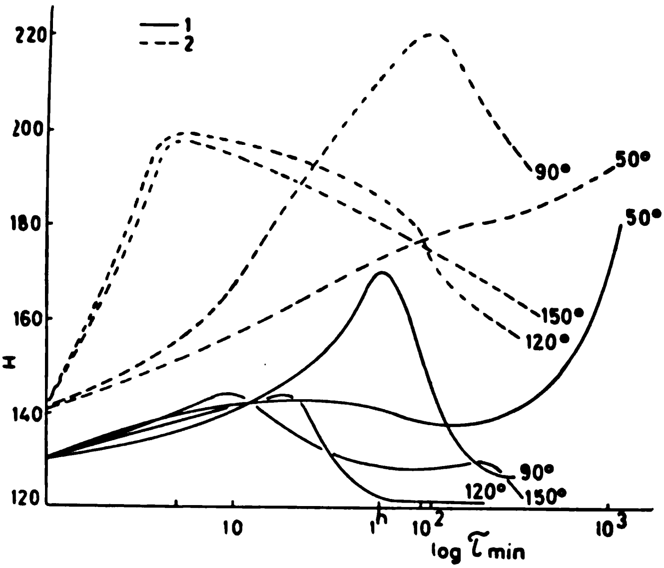

Fig. 3. — Dependence of microhardness on temperature and time. (1) In quench aging; (2) in strain aging.

The acceleration of the process caused by deformation is very pronounced. The fact that maximum values are obtained for the same time as in quench aging overshadows this effect to some extent. However, if the time intervals required to attain the same values for YP and UTS in quench and strain aging are compared, it can be noticed how much faster hardening occurs in the latter.

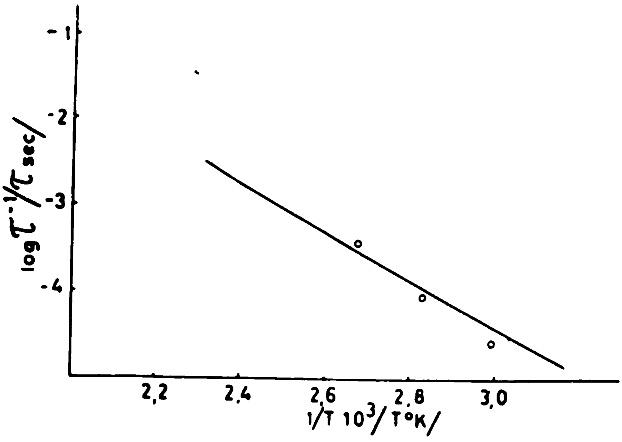

Fig. 4. — Dependence on temperature T of the rates τ^{-1} of strain aging and quench aging.

The increase in the rate of hardening results from the increase in the number of dislocations in the metal during work treatment.

Since yield strength is a property dependent on the locking of dislocations by the segregation of foreing atoms, the increase in the number of dislocations at this concentration of carbon will result in a sudden increase of yield point value so that it approaches the values for UTS.

A decrease of values after the maximum has been reached is noticable in both cases. However, while in quench aging the decrease is considerable so that the values are sometimes close to the values in the initial stage, in strain aging the values are reduced by only about ten percent. These differences in behavior are explained by the fact that in quench aging the size of the precipitated particles is changed, the lattice energy reduced, and hardness and tensile strength are decreased due to over-aging. In strain aging the strain around dislocations is reduced by the diffusion of carbon atoms into the expanded part of the dislocation field. The deformation remains, but the strain has been removed so that there should be no tendency for a further rearrangement of segregated atoms, i.e. for the unlocking of dislocations. A certain decrease of values is attributed to the presence of a stable second phase of extremely small dimensions precipitated into dislocations.

The decrease in hardness in strain aging is greater than expected. However, we believe that in this case the method of measuring of microhardness was not quite adequate for the investigation of changes in hardness, and therefore the results must be taken with caution. In further investigations standard methods for measuring hardness should be used.

A shortcoming of this experiment is that the nitrogen content of the investigated steel was not determined. As is known, nitrogen also plays a part in the aging process so that the results that we have obtained cannot be interpreted as the influence of carbon only. For a more reliable interpretation of results the process of aging should be followed on a pure alloy of known composition.

In spite of this shortcoming the experimental results provide the data on the behavior of steel of given composition during aging under specified conditions so that they can be used for comparison with the results of investigations by other methods.

CONCLUSION

The process of quench and strain aging were investigated on low carbon steel containing 0.05% C.

Quench aging. — After quenching from 720°C in a 10% aqueous solution of NaCl the specimens were aged for 0, 5, 10, 20 and 40 minutes and 1, 2, 4, and 6 hours at 50, 90, 120, and 150°C.

Strain aging. — After quenching from 720°C the specimens were deformed by tension to 6% elongation and aged under the same conditions as above.

The change in mechanical properties (yield point, ultimate tensile strength, and hardness) relative to temperature and time agrees with that expected theoretically. As is evident from the results, the methods applied are sufficiently sensitive to follow the process of aging.

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RADIOCHEMISTRY AT THE BORIS KIDRIČ INSTITUTE OF NUCLEAR SCIENCES, VINČA

by

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Work on radiochemistry at the Boris Kidrič Institute started in 1948. These were the first steps in the field of nuclear sciences in Yugoslavia. Of the many laboratories at Vinča today, in 1948 there were only two: the physics laboratory and the radium department. In this department a group of chemists, mostly graduates, led by Prof. Pavle Savić, got their first experience in radiochemistry. They worked with radium and mesothorium and their decay products. They became acquainted with already known methods for isolation and purification of these products and worked on their further development. (1,2) The beginning was therefore limited to *natural* radioactivity, because the technical equipment of the Institute was meager. Owing to this the physicists were only able to develop detection techniques (3, 4, 5, 6,) and to investigate various sorts of radiation (7, 8) and nuclear reactions caused by charged particles (9, 10).

The situation was somewhat changed after the setting up of neutron sources using radon. In 1950, eight neutron sources were constructed, in 1951 — ten, in 1952 — eleven. Their intensity was mostly about 1 curie. The first neutron-induced nuclear reactions were made using these sources. This enabled the chemists to expand their work to the field of *artificial* radioactivity (11, 12) and made possible the application of artificial radioisotopes in other research fields of the Institute — for example, in physics, where work on nuclear physics problems became possible, and in biology, where the distribution of radioactive phosphorus in living organisms could be investigated using P 32 obtained by irradiation of carbon disulphide with a (Rn + Be)—source (13).

Although this work was of immediate value, its main aim was the training of scientists for the tasks, then already outlined, for the exploitation of nuclear energy in Yugoslavia in the near future.

Possibilities for work in the field of artificial radioactivity were further expanded by a Cockroft-Walton accelerator set up in 1962. With this machine it was possible to obtain fairly large quantities of radioactive nuclides, which made possible the investigation of the procedures for their separation and purification and for obtaining them in a carrier-free form (14).

The construction of a high-power research reactor in 1959 was a turning point in the work on radiochemistry. The potential possibilities created by the erection of the reactor were enormous. Nuclear reactors have advanced radiochemistry generally, giving numerous opportunities for new investigations and presenting many new problems.

The use of the reactor made possible large-scale production of radioactive isotopes. These were the first real results of many years work and investment in the field of nuclear energy. The radiochemists at the Institute showed themselves not only capable of handling these complex problems (15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29) but also of solving many difficult technical problems such as the constructions of special laboratories, protective and manipulative equipment, etc. Since 1961, the Yugoslav production of radioisotopes has become more and more capable of supplying the needs of the country in quantity and variety. To show the effort the Institute has made in achieving this, it should be pointed out that in 1961, the first year of production, 220 C of various isotopes were produced while in 1962 the production exceeded 650 C.

Since the countries which use isotopes extensively have benefitted considerably from them — last year the Soviet Union saved 220 million dollars in this way — it is certain that Yugoslavia also can only gain. The advantage of the use of isotopes is already obvious although as yet there have been no exact analyses or documentation of the savings.

The aim of the Institute in the field of radiochemistry was directed in the beginning towards mastering the production of radioisotopes. Since then, however, its basic task has been to give radioisotopes the place which belongs to them in an advanced industry. After the initial period, which was very difficult, the prospects became much better. The number of users of our isotopes is slowly but steadily increasing.

To further the wider application of radioisotopes, the Institute itself has worked on the solution of some practical problems. Thus, it cooperated with the Torpedo motor factory in Rijeka on the study of wear in internal combustion engines using radioactive cobalt (30). Similar research is in preparation also at the 27 Mart automobile and tractor parts factory in Novi Sad. Arrangements have been made with the Filip Kljajić factory in Kragujevac for the checking in the production of driving chains using radioisotopes. The corrosive action of some motor oil additives has been investigated using radioactive iron.

Isotopes have also been used in hydrology. Thus, in cooperation with the Jaroslav Černi Institute for Water Resource Development, river sediment transport and the movement of underground waters have been investigated. Sediment transport investigations were made using radioactive chromium, both on the model of the

Velika Morava at the Jaroslav Černi Institute's laboratory and on the river itself (31). In the investigations of the movement of underground waters radioactive bromine and radioactive iodine were used.

The second field opened up by the reactor was the reprocessing of the irradiated reactor fuel. Test-tube scale work on this was started in 1959. Investigations have been made of the extraction of uranium, plutonium and the most important fission products from nitric acid and hydrochloric acid solutions using tributyl phosphate diluted in kerosene (32, 33) and of the effect of diluents (34) and temperature (35) on the extraction of uranium. Beside extraction with organic solvents, ion exchange has also been extensively investigated. Work has been done using organic resin exchangers and inorganic ion exchangers which are much more stable under irradiation, such as MnO_2 (36), SiO_2 (37), Ti and Zr phosphate (38, 39, 40) and Zr pyrophosphate (41).

The building of a special laboratory for work on high activity material in 1962 allowed work to be transferred to a much higher level, for the time being to the activity level of 10 C. The extensive laboratory experience obtained in this field has made possible the design of a semi-industrial plant for reprocessing irradiated uranium which will not only be used for process research on a technological scale but will also make it possible to reprocess irradiated uranium from the existing reactor at Vinča.

Plutonium, the nuclear fuel of future power stations, has also been a subject of research at the Institute for some time. So far the work has consisted of developing the most suitable procedures for purifying nitrate solutions of plutonium obtained in reprocessing. Here too, both extraction using organic solvents (42) and ion exchange (43) have been used. The new hot laboratory will also make possible a considerable extention of this work, which because of the extremely high radiotoxicity of plutonium requires very strict protection measures. Already in this year's program it is planned to obtain some pure plutonium salts which will later serve as raw material for obtaining nuclear pure plutonium metal.

In the last few years the Institute has also done a considerable amount of work on fission products, on the one hand because of their importance in the reprocessing of irradiated uranium, and on the other, because of the possibility of isolating and using some of them. Research on fission products is also essential because of the necessity for their safe disposal, and so that proper decontamination measures can be taken, particularly with regard to the key parts of the reactor (44.45, 46).

Of course work in all the fields mentioned must be continually radiometrically and analytically checked. At the Institute radiometric methods of detection, measurement and analysis of radionuclides have been developed over a number of years and are now widely used. Alpha, beta and gamma spectrometry have been especially developed. Chemical and physico-chemical methods of analysis, although in principle the same as those for nonactive material, have specific characteristics, since they must be as simple and as quick as possible, even at the price of lower sensitivity, and must be adapted for work at distance. The hot analysis group has worked out numerous methods both for work on artificial radioisotopes and on the reprocessing of irradiated uranium. Particularly noteworthy are the procedures for determining Te in I 131 (47) and for determining the valence states of P (48) and I (49) by paper chromatography. An example of a method which has been developed for checking the reprocessing of irradiated uranium is the determination of uranium in a high activity medium by measuring the density using a modified Mohr's balance.

Study of methods for decontaminating various materials also falls within the sphere of radiochemistry. The decontamination of radioactive liquid wastes is an important field of work at the Institute (50), all the more so as the time approaches when this problem will have to be solved in practice. Even now regular operation of the reactor and the beginning of work in the new hot laboratory, with considerable quantities of active wastes, require a practical solution of this problem.

Another important aspect of decontamination, which is also included in the field of radiochemistry, is human decontamination, both internal and external, as well as the investigation of various excrements for content of radionuclides (Pu, fission products).

The use of radioisotopes as tracers in research is an important field of radiochemistry. At Vinča, radioisotopes have been widely used in this way from the very beginning. As early as 1952, in a report at the Second Meeting of Chemists of Serbia (51), Prof. Pavle Savić's chief concern was to advocate to this application, which at that time was quite new in Yugoslavia. Radioactive tracers have been used in the development of analytical methods (52, 53, 54, 55, 56, 57, 58, 59), for solving various physico-chemical (60, 61) and biological problems.

A field which has recently received considerable attention at the Institute is hot atom chemistry, which deals with the state of an atom when it has undergone a nuclear transformation, and the reactions of such an atom (62, 63, 64, 65). The aim of this work is to clarify the principles on which the mainly empirical procedures for producing radioactive isotopes are based, and thus to improve the existing procedures or to develop new, more rational ones.

Training at the Institute was initially obtained entirely by practice. Later various courses were introduced, among which courses in the handling of radioisotopes were particularly important. These courses have been held continually since 1952 and have developed into a very valuable institution of a special type. Of course, the great benefit which workers in this field have gained from training in laboratories abroad should not be neglected. Not only have they acquired new knowledge but also collaborated in the work of those laboratories. Numerous collaborative publications have emerged from this work (42, 43, 66), as well as friendly and very valuable connections between Yugoslav and foreign scientific workers.

Learning and training, the first radiochemists soon became teachers themselves. Now they lecture at the universities, supervise postgraduate studies in radiochemistry, and give courses in the handling of radioactive material. So far, over 600 persons have passed through these courses. Apart from teaching, the scientists of the Institute also transmit their knowledge and experience by practical work in the laboratories, whose doors are open to anyone, either from industry or anywhere else who wants to be trained or to solve some research problem. With their wide experience in the designing of radioisotope laboratories and manipulation devices they have often given their help where required. Also, by lectures and other means, they popularize and advance radiochemistry in Yugoslavia.

Here we have said nothing about the numerous fields which are dealt with in the Institute and which are more of less closely connected with radiochemistry, such as radiation chemistry, metrology and measuring instrumentation, nor even about nuclear chemistry in a broad sense, which treats the nuclear properties of radionuclides and their scheme and energy of decay, the angular distribution of emitted particles, nuclear reactions, effective cross-sections, etc.

Even within these narrow limits, the activity in radiochemistry at the Boris Kidrič Institute is great and significant. It may be said without exaggeration that the development of the Institute, the results it has achieved and the facilities at its disposal put it among the important radiochemistry centers in the world.

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GLASNIK HEMIJSKOG DRUŠTVA, Vol, 28, No. 3—4, 1963, pp. 115—122

SPACE GROUP DETERMINATION OF DOUBLE SALT
$(NH_4)_2SO_4 \cdot 3NH_4NO_3$

by

JELICA MIŠOVIĆ and R. RAMASECHAN

Schreinemakers and Hoern$^{(1)}$, De Waal$^{(2)}$ and Massink$^{(3)}$ investigated the formation of double salts in the system $(NH_4)_2SO_4 - NH_4NO_3$ and reported the existence of two double salts having the compositions $(NH_4)_2SO_4 \cdot 2NH_4NO_3$(*D* 1 : 2) and $(NH_4)_2SO_4 \cdot 3NH_4NO_2$(D 1 : 3). Since the formation of such complexes might be of technical importance, particularly in fertiliser industry, some attention was given to this matter by Robertson, Bragg and others$^{(4)}$. After X-ray studies these authors reported that the double salts had definite crystal structures which differed from the crystal structures of the components $(NH_4)_2SO_4$ and NH_4NO_3. They also suggested that the (D 1 : 2) complex belongs to the triclinic class probably with two molecules per unit cell. This was denied however, by Janecke, Eisser and Brill$^{(5)}$ who studied the symmetry and unit cell dimensions of the (*D* 1 : 2) complex by morphological and X-ray methods. They argued that this salt belongs to the bipyramidal class of the orthorhombic system with

$$a:b:c - 1\cdot 106 : 1 : 3\cdot 804$$

$$a = 11\cdot 2_2\,\text{Å} \qquad b = 10\cdot 1\,\text{Å} \qquad c = 38\cdot 5\,\text{Å}$$

$$\alpha = \beta = \gamma = 90^\circ$$

and 16 molecules per unit cell.

The three principle refractive indices were

$$n\alpha = 1.519,\quad n\beta = 1.528,\quad \text{and}\quad n\gamma = 1.534$$

The birefringence was not high. However, they stated categorically that the (*D* 1 : 3) complex is not formed.

This view was then proved erroneous by Sokolov$^{(6)}$, Bokii and Burovaia$^{(7)}$ who made an extensive study of the (*D* 1 : 3) complex and established that it was a definite compound (double salt) crystallising in a class quite different from that of $(NH_4)_2SO_4$ or

NH_4NO_3 or (D 1 : 2). The authors concluded that the (D 1 : 3) belongs to the prismatic class of monoclinic systems with

$$a:b:c — 1\cdot0409:1:0\cdot8443$$

$$\beta = 92°42'$$

The recent detailed work of Krishnaswamy and Aravamudan(8) on the solubility isotherms of the system $NH_4NO_3 = (NH_4)_4SO_4$ also definitely established the existence of both the (D 1 : 2) and (D 1 : 3) complexes, and crystals prepared with the help of these authors in the General Chemistry Department of the Indian Institute of Science were made available for the present study. A study of the solubility isotherm of this system indicates that an aqueous solution containing 62.6 percent NH_4NO_3 and 13.3 percent $(NH_4)_2SO_4$ would yield about 25 percent of the complex salt (D 1 : 3) on attainment of equilibrium.

The chemical analysis of the samples showed within experimental error a stoichiometric composition of $(NH_4)_2SO_4 \cdot 3NH_4NO_3$.

The crystals formed were in the main extremely small (0.2 mm x 0,05mm), and so larger crystals were grown by gradual controlled evaporation of water under isothermal conditions at 30° in a thermostatically controlled water bath. Quite large crystals (1 cm x 0.1 cm) could be grown in this manner. Each time the individual crystals were examined under the polarization microscope to see whether they were homogeneous and each batch was analyzed chemically. This was necessary since the crystals absorb moisture at a fairly rapid rate and the double salt undergoes a gradual decomposition on absorption of moisture. It was necessary therefore to perform all the operations of mounting, centering, etc., in a dry box.

A good crystal showing perfect extinction and no signs of twinning was selected and inserted into a fine Lindemann capillary. This capillary was then mounted on the stem of the goniometer head which was put on a Weissenberg goniometer (Unicam). The needle axis was adjusted to coincide with the axis of rotation. Using copper K radiation an oscillation photograph about the needle axis (unique) and a zero layer Weissenberg photograph for an oscillation of 200° were made. From these it was established that the crystal belongs to the monoclinic system, with

$$a = 12.34\ \text{Å} \quad b = 5.94\ \text{Å} \quad c = 9.95\ \text{Å} \quad \text{and} \quad \beta = 92°48'$$

The axial ratios therefore are

$$a:b:c = 2.077:1:1.675$$

These values are practically twice the ratios reported by Bokii and Burovaia(7) after morphological studies.

The density of the compound was determined by the flotation method and was found to be $1.75 + 0.002$. The volume of the unit cell $V = abc \sin\beta = 7.285 \times 10^{-22}$ cm³ and the molecular weight of the (D 1 : 3) complex is 373.2. The number of molecules per unit cell is thus

$$\frac{6.022 \times 10^{23} \times 7.285 \times 10^{-22} \times 1.75}{372.3} = 2.06 = 2$$

To establish the exact space group it is necessary to make Weissenberg photographs giving the $h01$, $h11$, $h21$, and $hk0$ reflections. Equi-inclination Weissenberg photographs of the lst ano 2nd layer were made, the crystal being oscillated about the b axis (unique axis). The photographs showed no systematic absences.

TABLE 1

Crystallographic data for ammonium sulphate 3 ammonium nitrate.

$a = 12.34 \quad b = 5.94 \quad c = 9.95$ Å

$\alpha = \gamma = 90° \quad \beta = 92°48'$

Axial ratios:

$$a : b : c = 2.077 : 1 : 1.675$$

Number of molecules per unit cell: 2

Linear absorption coefficient: 30.66 cm^{-1}

Condition limiting possible reflections:

$hk1$ No conditions.
$hk0$ No conditions.
$h01$ No conditions.
$h00$ No conditions.
$0k0$ $k = 2n$
001 No conditions.

The space group is $P\ 2_1/m$ (No.11) or $P\ 2_1$ (No.4). (International Tables for X-ray Crystallography(9).

The zero layer c axis Weissenberg ($hk0$) was photographed and the only systematic absences were with axial reflections $0k0$. Here the $0k0$, $k = (2n+1)$ were absent indicating the presence of a two-fold screw axis paralell to the b axis.

The table gives the data obtained for this crystal. From this one sees that the crystal could either belong to the space group $P2_1$ (No.4) or to $P2_1/m$ (No.11), according to the International Tables for X-ray Crystallography(9).

The only known methods of distinguishing between these groups is by Wilson's statistical method(10) for the detection of the center of symmetry in crystals, and Howell, Phillip and Roger's(11,12) statistical survey method. For Wilson's method it is necessary to have fairly accurate data on the intensity. .Since in this particular case $P2_1$ and $P2_1/m$ belong to two monoclinic, the $k01$ projection will be centro-symmetric in both cases while the $hk0$ projection will be noncentro-symmetric in the case of $P2_1$ and centro-symmetric in the case of $P2_1/\mathrm{m}$. Consequently, it would be quite sufficient to obtain (*hk*0) intensity data. However, the shape of the crystal was far from cylindrical and it would not have been possible to apply accurate absorptioncorrec-Hence, it was decided to get intensity data not only for the *hk*0 reflections but also for *h*11, *h*21, etc.

Intensity data using the multiple film technique of Robertson⁽¹³⁾ was collected for *hk*0, *h*01, *h*11 and *h*21. A suitable intensity scale was prepared using one of the fairly strong reflections in the *h*01 group. The spots were all indexed by means of the equatorial Weissenberg chart and the intensities were measured on all three films using the intensity scale. The film absorption factor was found to be 2.75 for normal incidence (on zero layer Weissenberg) and it correspondingly increased to 2.77 for the first layer and 2.85 for the second layer due to the obliquity factor. Using these factors the data was reduced. A slight excess of weighting was introduced for readings that lay in the range most sensitive for visual measurement.

A reciprocal lattice was drawn and the sin 0 values were read off by measuring the distance from the origin to the reciprocal points and dividing by two.

For the zero layer the Lorentz and polarisation correction was read off from Buerger's Tables⁽¹⁴⁾. For the higher layers the *L.P.* correction given by the formula

$$1/Lp = \left(1 - \frac{\zeta^2}{4\sin^2\theta}\right)^{1/2} \cdot \frac{\sin^2\theta}{1 - \cos^2 2\theta}$$

was used.

The second factor for any reflection in a higher layer can be read off from Buerger's Table⁽¹⁴⁾. The first factor can be determined from a curve of

$$\left(1 - \frac{\zeta^2}{4\sin^2\theta}\right)^{1/2}$$

against $\sin^2\theta$ drawn for each layer. The product of the two factors gives the actual $1/L.P.$ factor to be used. The intensities are not given in this paper. The validity of the statistical method depends on the divisions of $\sin^2\theta$ ranges being fine and each range containing approximately the same number on reflections which must be sufficiently large for statistical significance. These conditions generally conflict; if the ranges are too fine, the number of each group may be too small. It was decided, if it were possible to satisfy both conditions, to discover whether a finer or coarser division made any significant difference in the results of the statistical theory.

Thus the *hk*0 reflections were divided into two sets of ranges — for the first set the $\sin^2\theta$ ranges were in steps of 0—0.2, 0.2—0.4 and 0.4—0.6, and for the second set, 0—0.1, 0.1—0.2, 0.2—0.3, etc. In the first case there were 22.21 and 26 reflections in each range and in the second (i.e., finer division) there were 12, 10, 11, 10, 11 and 15 reflections in each range. These, of course, are the actual numbers of reflections whereas the effective number is less since the axial reflections lying on a reciprocal lattice vector, common to two unit cells, have a weight of only one-half. The mean value of the intensity for any range was determined and the numbers of reflections that lay within 0.1, 0.2, 0.3... 1.0 of this mean intensity (IM) were counted for each range, due care being exercised to give the proper weight to axial reflections. Tables 2 and 3 give the complete set of results for the two sets of ranges. The two distribution curves obtained for these and the theoretical curves for both the centro and noncentro-symmetric distributions are given in Fig.1. One finds the division of the reflections into the larger or smaller range does not affect the final results much and the final curve appears to be very close to the centro-symmetric distribution.

TABLE 2.

Values of N(Z), in percentages, for hk0 reflections of Ammonium sulphate 3 Ammonium nitrate

Range	0.1	0.2	0.3	0.4	0.5	0.6z	0.7	0.8	0.9	1.0
I	21.62	37.84	48.65	48.65	59.45	65.77	67.57	78.38	81.08	81.08
II	26.31	31.58	36.84	39.47	39.47	50.00	60.53	60.53	60.53	65.79
III	30.61	32.65	32.65	40.72	40.82	65.31	65.31	65.31	65.31	77.55
Mean	26.18	34.02	39.38	42.98	46.58	60.96	64.47	68.07	68.97	74.80

TABLE 3

Values of N(Z), in percentages, for hk0 reflections Ammonium sulphate 3 Ammonium nitrate

Range	0.1	0.2	0.3	0.4	0.5	0.6z	0.7	0.8	0.9	1.0
I	21.05	42.10	52.63	52.63	52.63	68.42	68.42	68.42	68.42	78.95
II	22.22	22.22	33.34	44.44	44.44	55.56	55.56	55.56	55.56	55.56
III	20.00	30.00	45.00	45.00	45.00	55.00	55.00	55.00	65.00	65.00
IV	33.33	33.33	33.33	33.33	33.33	44.44	55.56	66.67	66.67	66.67
V	23.81	28.81	23.81	23.81	52.38	52.38	52.38	71.43	71.43	71.43
VI	21.43	35.71	39.28	39.28	53.57	53.57	75.00	75.00	75.00	75.00
Mean	23.64	31.21	37.89	39.75	46.89	54.90	60.32	65.34	67.01	68.77

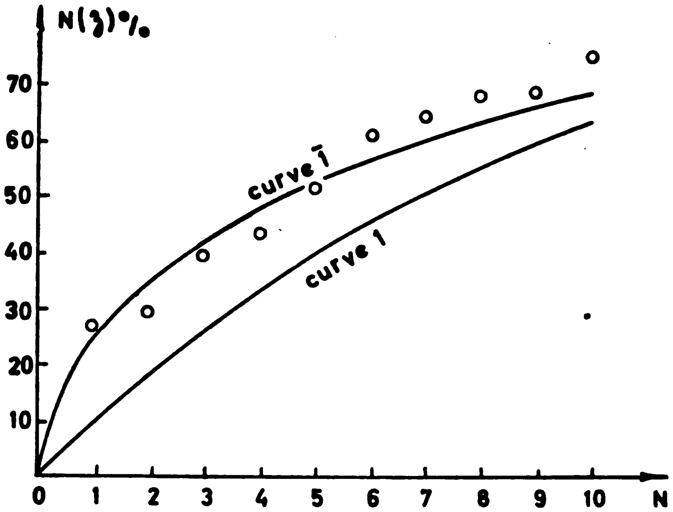

Fig. 1

Distribution for $(NH_4)_2SO_4 \cdot 3NH_4NO_3$, *hk0* reflections, 3 ranges, compared with theoretical curves 1 and $\bar{1}$

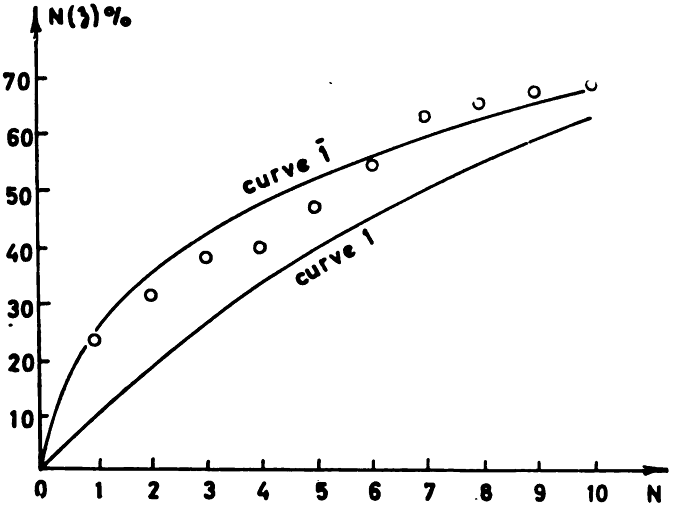

Fig. 2

Distribution for $(NH_4)_2SO_4 \cdot 3NH_4NO_3$, *hk0* reflections, 6 ranges, compared with theoretical curves 1 and $\bar{1}$

In a similar manner the *h*11 and *h*21 reflections were divided into ranges and the distribution curves drawn. There seems to be little doubt that, at least according to Wilson's statistics, the crystal $(NH_4)_2SO_4 \cdot 3\ NH_4NO_3$ belongs to centro-symmetric space group $P2_1/m$. It is however worth-while remembering that in at least two cases the results obtained by this statistical method do not correspond to the actual symmetry of the crystal. (Robertson⁽¹⁵⁾, Trotter⁽¹⁶⁾).

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A CONTRIBUTION TO THE STUDY OF THE REACTION KINETICS OF THE Al—TiO_2 SYSTEM

by

MOMČILO M. RISTIĆ

From the papers published so far it is well known that aluminum and titanium dioxide react exothermically, that the reactions are quick and they take place between 800 and 900° (1,2,3). Our studies of the thermochemistry of this system(4) have shown how the course of the reaction depends on the initial ratio between aluminum and titanium dioxide, principally with respect to the compounds formed in these reactions. It has been shown that the reaction of the reduction of titanium dioxide with aluminum proceeds via Ti_2O_3 and TiO to elementary titanium.

Taking account of these results we have studied the problem of the thermodynamical equilibrium criteria and the reaction kinetics of the Al-TiO_2 system.

THERMODYNAMICAL EQUILIBRIUM CRITERIUM

In developing the theory of similarity in the field of physico-chemical processes Diakonov (5) has shown that the deviation of the system from thermodynamical equilibrium is defined by the thermodynamical equilibrium criteria. This criteria represents the ratio of the internal rates of the process investigated. If the resulting rate of the process is considerably lower than the rate of setting up of equilibrium then the thermodynamical equilibrium criteria is unity, i.e. it represents a continuous sum of states of thermodynamical equilibrium. If the thermodynamical equilibrium criteria is much higher than unity, then the system is not in equilibrium.

Hence for the reaction of the reduction of TiO_2 with aluminum which takes place via the reactions(4)

$$2Al + 6TiO_2 = Al_2O_3 + 3Ti_2O_3 \quad (1)$$

$$2Al + 3Ti_2O_3 = Al_2O_3 + 6TiO \quad (2)$$

$$2Al + 3TiO = Al_2O_3 + 3Ti \quad (3)$$

the thermodynamical equilibrium criteria may be calculated as follows:

for reaction (1) $K_1 = 2 \cdot 5 \times 10^{95}$

„ (2) $K_2 = 3 \cdot 2 \times 10^{38}$

„ (3) $K_3 = 5 \cdot 0 \times 10^{19}$

From this it may be concluded that in all cases the systems are not in equilibrium. It is obvious that the non-equilibrium is greatest in the reduction of TiO_2 to Ti_2O_3 and least in the reduction of TiO to Ti.

GENERAL CONCLUSIONS ON THE KINETICS OF THE PROCESS

Equations characterizing the kinetics of the main processes by which the reaction of the reduction of TiO_2 with aluminum takes place can be obtained using the thermodynamical equilibrium criterion and the general equation of similarity of physico-chemical phenomena$^{(5)}$.

The kinetics of reaction (1) is characterized by the general expression

$$\frac{dc_{Al_2O_3}}{dt} = -k_1 \cdot \varphi\ (C_{Al_2O_3}, C_{Ti_2O_3}) \left[\frac{1}{2{,}5 \cdot 10^{95}} - 1\right] \qquad (4)$$

where

$C_{Al_2O_3} = Al_2O_3$ concentration

$C_{Ti_2O_3} = Ti_2O_3$ concentration

k_1 = rate coefficient of the reverse process

φ is a homogeneous function of the concentrations

t = time

The number in brackets may be taken as —1, so that

$$\frac{dc_{Al_2O_3}}{dt} = -\ k_1 \cdot \varphi\ (C_{Al_2}{}^{O}{}_{3,} C_{Ti_2O_3}) \qquad (5)$$

It was proved earlier (6) that the reverse reaction of that represented by equation (1) is infinitely slow. Hence from (5) it may be concluded that under certain conditions reaction (1) may be very fast. This agrees with the theoretical study of S. Koikova *et al.* (7) of the kinetics of defect production in TiO_2.

The same results are obtained for reaction (2) and (3) whose kinetics are characterized by

$$\frac{dc_{Al_2O_3}}{dt} = -k_2 \cdot \varphi(C_{Al2O3}, C_{Ti}O) \qquad (6)$$

$$-\frac{dc_{Al_2O}}{dt} = -k_3 \cdot \varphi(C_{Al_2O_3}, C_{Ti}) \qquad (7)$$

Let us consider this problem in terms of reactions which characterize defect production in TiO_2 and Al oxidation with oxygen extracted from TiO_2.

The occurrence of anionic vacancies in TiO_2 may be expressed by (8)

$$2Ti^{4+} + O^{2-} = 2Ti^{3+} + 1/2O_2$$

As in this case reaction takes place between oxygen and aluminum, the general reaction may be expressed by

$$6Ti^{4+} + 3O^{2-} = 6Ti^{3+} + 1^1/_2O_2 \qquad (8)$$

$$2Al + 1 \cdot 5\, O_2 = Al_2O_3 \qquad (9)$$

At the temperature of the reaction between aluminum and titanium dioxide, in view of the irreversibility of reaction (9) there is a constant shift of the equilibrium of reaction (8) towards the occurrence of anionic lattice defects. However, if the aluminum melts at this temperature and gets into close contact with the titanium dioxide (if the latter is finely dispersed) it does not oxidize according to the parabolic law which is usually valid for the interval 350–450°(9), but goes considerably faster. This was shown earlier(4) by thermogravimetric investigation of the oxidation of aluminum.

It is evident, therefore, that reactions (8) and (9) are interrelated, and temperature has a positive influence on anionic defect production.

These studies are in agreement with the analysis based on the general equation of similarity of physico-chemical processes. The same results are obtained if the further phases of the reactions between Al and TiO_2 which take place according to equations (2) and (3) are analysed.

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THE THERMOCHEMISTRY OF THE REACTION OF THE Al—TiO_2 SYSTEM

by

DEJAN DELIĆ and MOMČILO M. RISTIĆ

In the reaction of aluminum and titanium dioxide TiO_2 is reduced and Al oxidized to Al_2O_3(1). Papers published to date which deal with the reduction of TiO_2 with aluminum have treated this problem mainly from the standpoint of practical metallurgy, i.e. the production of titanium by thermite reaction with aluminum or the alloying of titanium with aluminum.

In extracting Ti and Nb from titanium-niobium ores, C. Kreimer(2) found that in the reduction of TiO_2 with aluminum the yield of titanium is lower than that of niobium obtained by the reduction of Nb_2O_5. From this he concluded that the total reaction of the reduction proceeds in two phases:

$$3TiO_2 + 2Al = 3TiO + Al_2O_3$$
$$3TiO + 2Al = 3Ti + Al_2O_3$$

The reduction of TiO is exothermic whereas the reduction of TiO to titanium is endothermic.

The reduction of TiO_2 with aluminum for obtaining Al—Ti alloy has been theoretically treated by Kubaschewski(3). The study indicates the possibility of obtaining the Al—Ti alloy thermite reaction. It should be pointed out that the earlier procedures(4) for obtaining the Al—Ti alloy are based on the reaction

$$3TiO_2 + 4Al = 2Al_2O_3 + 3Ti + 146 \text{ kcal.}$$

In dependence on the Al/TiO_2 ratio the corresponding alloy is obtained. The present work deals with the colorimetry of the reaction of the Al—TiO_2 system and the corresponding thermodynamics.

PROPERTIES OF THE AL AND TiO_2 POWDERS

Powders produced by E. Merck were used. The Al powder contained 99.99% Al, while the chemical analysis of the TiO_2 powder was:

TiO_2	99.90%
Cl^-	0.01%
SO_4^{2-}	0.01%
PO_4^{3-}	0.05%
Fe	0.05%

The specific surfaces of the powders were determined by the BET method and were for the Al powder

$$P_{Al} = 1.0 \pm 0.5\ m^2/g$$

for the TiO_2 powder

$$P_{Tio2} = 7.6 \pm 0.1\ m^2/g$$

Electron-microscopy showed that the TiO_2 powder was composed of large and small agglomerates of round particles about 0.15 μ in diameter. The Al powder was composed of thin irregular flakes several microns in diameter.

PREPARATION OF SAMPLES

The mixtures of Al and TiO_2 were prepared by adding first the required amount of Al powder and then the corresponding amount of TiO_2 to a given amount of ethyl alcohol. After homogenization and alcohol evaporation the samples were pressed into tablets in a hydraulic press.

A series of samples were investigated. Their compositions are given in Table 1.

TABLE 1

The compositions of the investigated combinations of the Al—TiO_2 system

Combination	Weight % Al	Weight % TiO_2	Molar ratio Al/TiO_2
AT—01	10	90	0,331
AT—02	20	80	0.741
AT—03	30	70	1.274
AZ—04	40	60	1.986
AT—05	50	50	2.970
AT—06	60	40	4.453
AT—07	70	30	6.90
AT—08	80	20	11.90
AT—09	90	10	26.70

CALORIC EFFICIENCY OF THE REACTION

For the investigation of enthalpy changes of the Al—TiO_2 reactions we used a calorimeter with a calorimeter bomb as used for the investigation of solid fuels. To fulfil the conditions of atmospheric inertness and supply the amount of heat reguired for reaction activation(5), we modified the method as follows:

— the calorimetric vessel was filled with oxygen, as usual, at a pressure of 1 atmosphere;

— as a large amount of heat is released in the reaction between oxygen and Mg, this reaction was used for the activation;

— the amount of Mg always corresponded to the amount required to bind the oxygen from the vessel.

Results of these investigations are given in Fig. 1.

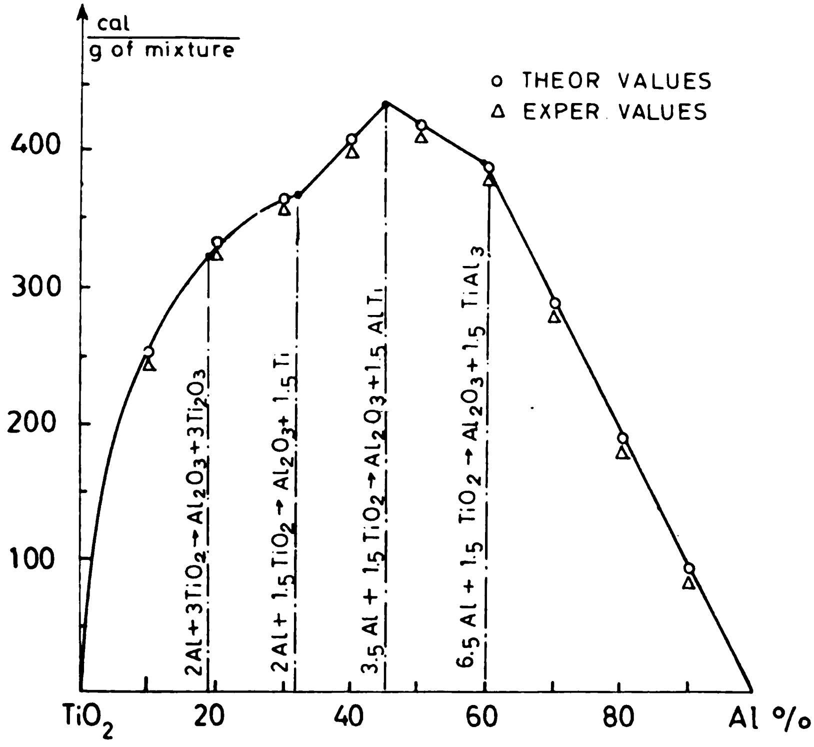

Fig. 1 Changes of entalpy of the *Al* — TiO_2 system reactions

Let us assume that the reaction of the reduction of TiO_2 with aluminum can take so that:

a) the reduction of TiO_2 proceeds via lower oxides to TiO without any further reaction;

b) the reduction of TiO_2 proceeds to elementary Ti without the formation of intermetal compounds;

c) the excess of aluminum may react with the reduced titanium producing intermetal compounds AlTi and Al_3Ti.

The basic product of the reaction is Al_2O_3.

The enthalpy changes found show good agreement with assumption c).

DISCUSSION

Figure 2 shows a graph of the enthalpy changes with respect to one mole of Al_2O_3. It is obvious that the least amount of heat per mole of Al_2O_3 is released in the reaction in which Al_2O_3 and elementary titanium, are produced.

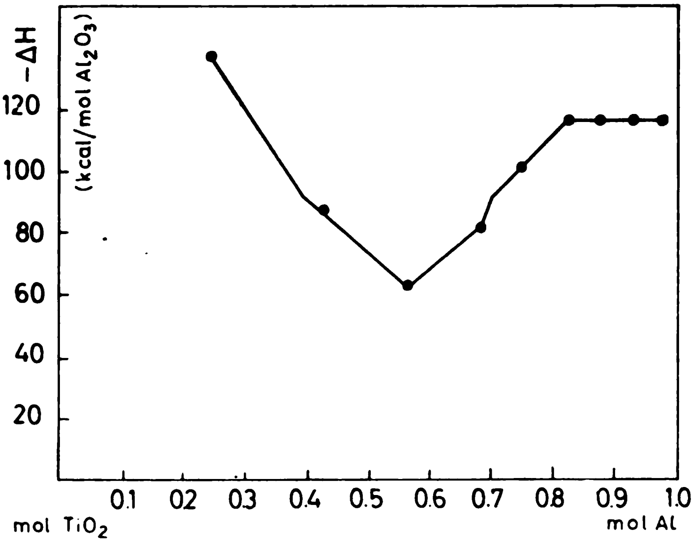

Fig. 2. Changes of enthalpy of the *Al—TiO₂* system reactions with respect to 1 mol of *Al₂O₃*

In Al — TiO_2 reactions all the systems are in the state of spurious equilibrium at 293°K although formally, since the reactions do not proceed by themselves, one may conclude that they are in equilibrium. Detailed investigations have shown that the reaction temperatures are 1128± 5°K$^{(5)}$. The presence of an energy barrier proves the chemical inertness of the system investigated.

If all the investigated reactions are considered to be reductions of TiO_2 with aluminum, then in general this reduction may be represented by the following equations:

$$2Al + 6TiO_2 = Al_2O_3 + 3Ti_2O_3 \quad (1)$$
$$2Al + 3TiO_2 = Al_2O_3 + 3TiO \quad (2)$$
$$2Al + 3Ti_2O_3 = Al_2O_3 + 6TiO \quad (3)$$
$$2Al + 1.5TiO_2 = Al_2O_3 + 1.5Ti \quad (4)$$
$$2Al + Ti_2O_3 = Al_2O_2 + 2Ti \quad (5)$$
$$2Al + 3TiO = Al_2O_2 + 3Ti \quad (6)$$

The change of the isochornal thermodynamical potential is

for reaction (1)	−130.8 kcal
,, (2)	— 92.2 ,,
,, (3)	— 52.8 ,,

for reaction	(4)	—59.4 kcal
„	(5)	—35.6 „
„	(6)	—27.0 „

From these data it is clear that all the reactions analysed are possible. However, it is clear that reaction (1) must take place first. As soon as Ti_2O_3 is formed the reaction may proceed either according to equation (3) or equation (5). From the change of the isochornal thermodynamic potential it follows that the reaction proceeds according to equation (3). Therefore, the last phase of the reaction takes place according to equation (6). This is shown in Fig. 3

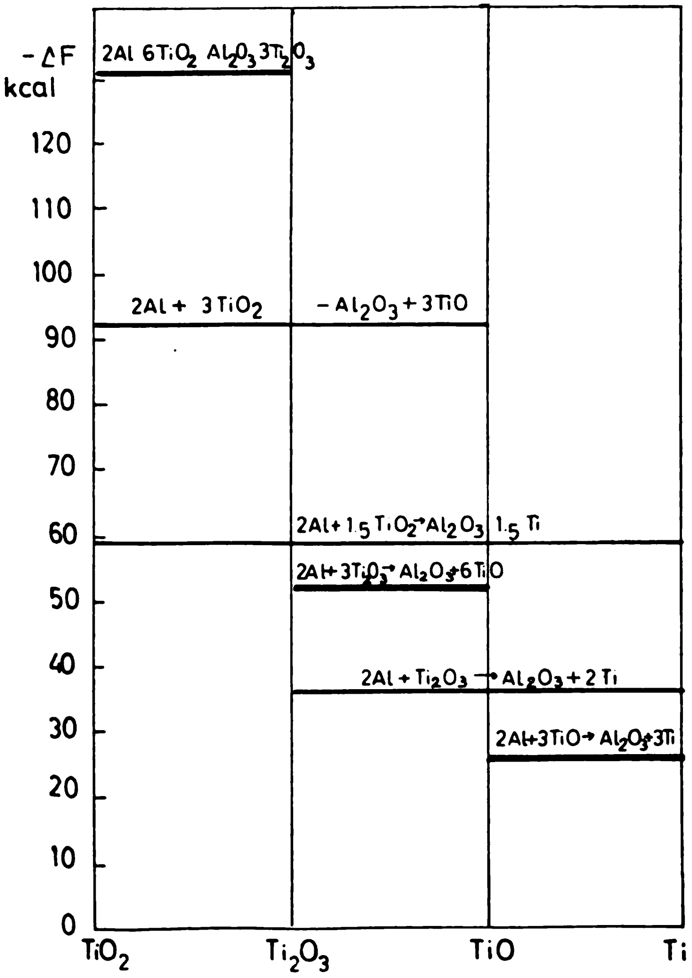

Fig. 3. Changes of isochornal thermodynamic potential of the *Al—TiO*$_2$ system reactions

From the termochemical standpoint this shows that G. Kreimer ⁽²⁾ is wrong when he states that the reaction of the reduction of TiO with aluminum is endothermic.

A general thermochemical conclusion on the direction of the reaction of the system can be made from the hypothesis of Kubaschewski ⁽³⁾ who found that the entropy change in the Al-Ti system is negligibly small compared to the enthalpy change. The calculated changes of the isochornal thermodynamic potential for particular reactions (Table 2) show that it magnitude increases with increasing amount of aluminum in the combination.

TABLE 2.

Changes of the isochornal thermodynamic potential of reactions of the Al-TiO_2 system for the combinations studied

Combination	— Δ F (kcal/mol Al)
AT — 01	65,5
AT — 02	42,6
AT — 03	30,4
AT — 04	20,6
AT — 05	23,9
AT — 06	16,0
AT — 07	10,7
AT — 08	6,2
AT — 09	2,8

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DISSOLUTION OF METALS IN CONCENTRATED SOLUTIONS OF SODIUM HYDROXIDE

by

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In the production of artifical cellulose fibers very pure sodium hydroxide is needed(1) because in the fabrication of viscose various impurities have a detrimental effect. For solving the problem of purifying sodium hydroxide it is necessary to determine the solubility of various components in sodium hydroxide solutions.

Analysing our hydroxides we found that in electrolytic sodium hydroxide the impurities were mostly iron and manganese, which get into the hydroxide by corrosion of the evaporation or storing, vessels and calcium and magnesium from the water used for decomposing of the sodium amalgam(2). Sodium hydroxide also contains a large amount of carbonates because of absorption of carbon dioxide from the air.

In this work the solubility of various ingredients depending on the concentration of sodium hydroxide is determined experimentally.

EXPERIMENTAL WORK

Seven series of sodium hydroxide solutions, in concentrations from 20 to 50% NaOH, were made*.) Each series consisted of 7 solutions, the concentrations being 20%, 25%, 30%, 35%, 40%, 45%, 50%. Two liters of each solution were made. One series was left without additions so that the amounts of various components in dependence on the concentration of sodium hydroxide could be followed. To the series for the investigation of the solubility of iron and manganese, the following were added:

Series 1: — strips of sheet-iron

Series 2: — powdered iron (ferrum reductum p.a.) — 1 g

Series 3: — ferromanganese with 98% Mn — 1 g

Series 4: — manganese sulfate — 2 g

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*) All the sodium hydroxide solutions were made with electrolytic alkali and crystal electrolytic sodium hydroxide produced by the Elektrobosna factory at Jajce.

To determine the solubility of calcium and magnesium in the sodium hydroxide solutions, saturated calcium bicarbonate and magnesium bicarbonate solutions were added. In this way conditions similar to those in industry were obtained because iron and manganese get into soda by corrosion of metal vessels. Calcium and magnesium come from water, in which they exist mostly in the form of bicarbonates.

Carbonates were not added. The maximum concentration of carbonates in the sodium hydroxide solutions from absorption of carbon dioxide from the air was determined.

The prepared sodium hydroxide solutions were shaken every day for a period of 15 days, and after that they were left to clear. After a month of clearing, the quantities of added ingredients dissolved in the clear part of each solution (iron, manganese, calcium, magnesium, and carbonates) were determined.

Methods of analysis: iron was determined colorimetrically with a Hellige comparator. Manganese was determined by the ammonium persulfate method (3) for colorimetric determination of small quantities of manganese. Calcium and magnesium were determined complexometrically. The sodium hydroxide and carbonates contents were determined according to Varder using phenolphthalein and methylorange as indicators.

In the next experiment all the sodium hydroxide solutions were diluted to a concentration of 20%. They were left to clear for a month and the content of various ingredients was then determined.

RESULTS AND DISCUSSION

The results are given in Table 1 and in Figs. 1, 2 and 3. Each point on the graph represents the maximal content of one of the ingredients in a given concentration of sodium hydroxide, determined after saturation and clearing of the solution.

TABLE 1.

% Na OH	20	25	30	35	40	45	50
%Fe. 10^3	0.3	0.33	1.4	2.4	3.4	5.5	6.3
%Mn. 10^3	2.6	2.65	2.72	2.78	3.00	5.48	5.94
%Na_2CO_3 (in pure Na OH	0.10	0.13	0.27	0.47	0.57	0.61	0.47
%Na_2CO_3 (in the presence of Fe)	0.10	0.13	0.27	0.47	0.56	0.61	0.47
%Na_2CO_3 (in the presence of Mn)	0.10	0.13	0.27	0.46	0.56	0.61	0.47

The quantity of iron dissolved increases with increasing concentration of sodium hydroxide, as can be seen from the graph in Fig. 1.

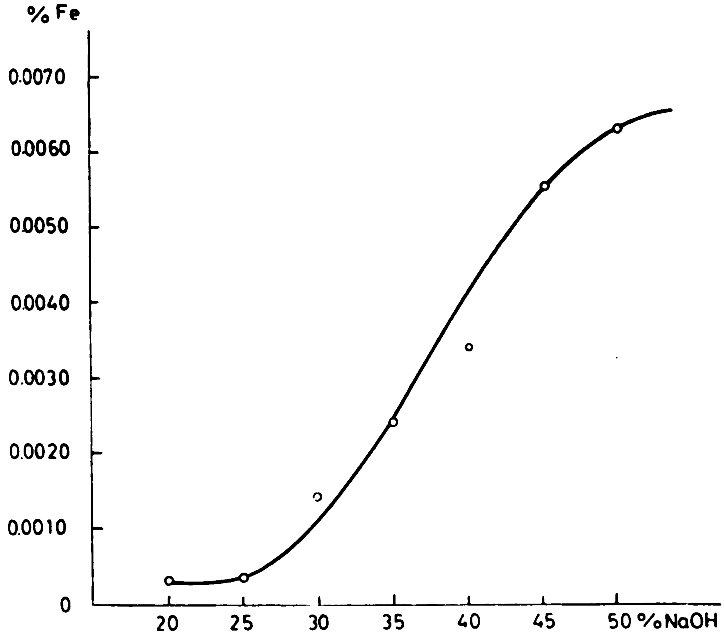


Fig. 1

The greater the concentration of electrolytic alkali the greater the increase of the concentration of iron. In the dissolving of iron in sodium hydroxide the following reactions occur (5):

1) $Fe + NaOH + H_2O = HO-Fe-ONa + H_2$

2) $HO-Fe-ONa + H_2O = Fe(OH)_2 + NaOH$

3) $Fe + 2NaOH + H_2O = HO-Fe(ONa)_2 + 1.5\ H_2$

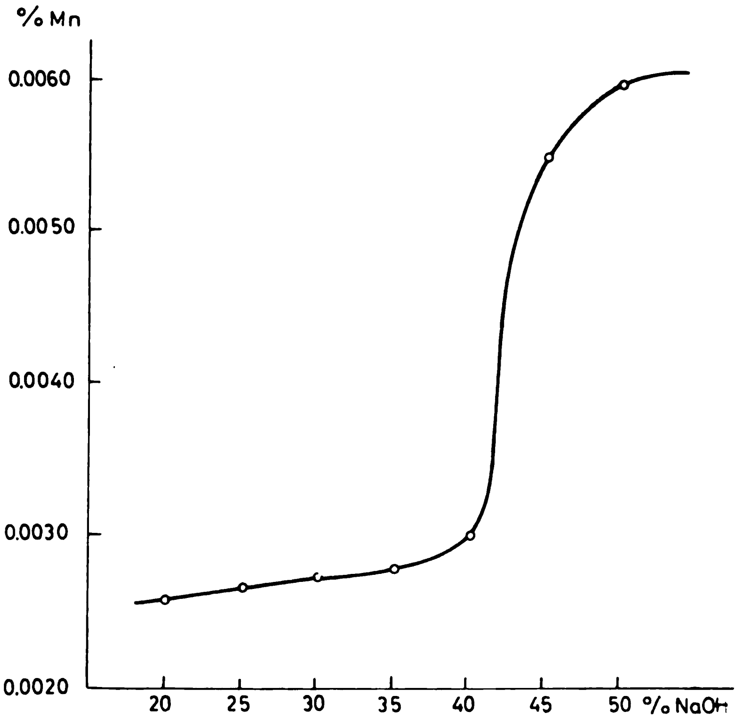
4) $HO-Fe(ONa)_2 + 2H_2O = Fe(OH)_3 + 2NaOH$

Similar forms were determined in another work(2). Assuming that bivalent iron is always formed first, we think that reaction(3) is possible only under the influence of some means of oxidation. In this case it was oxygen absorbed in the sodium hydroxide solution.

The quantity of dissolved manganese also increases with increasing concentration of sodium hydroxide, as can be seen from the graph in Fig. 2.

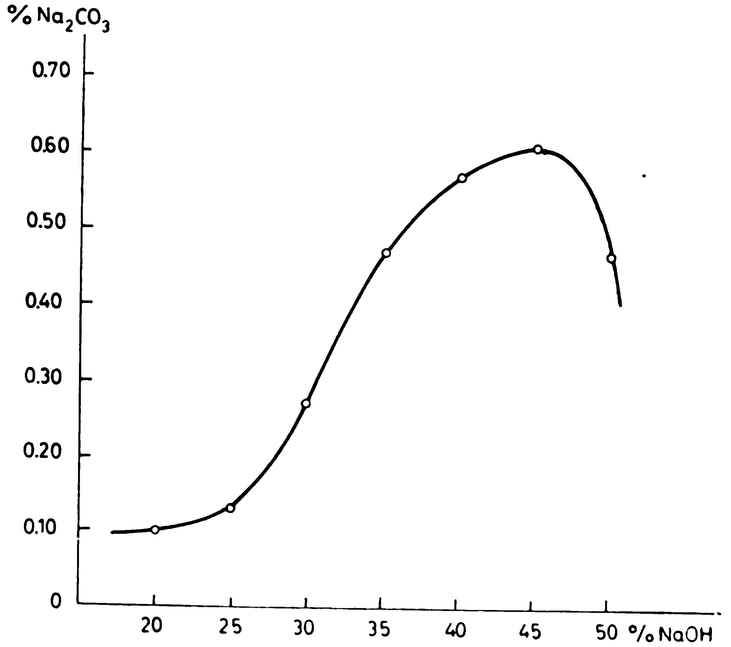
Up to 40% NaOH the increase of the concentration of manganese is relatively small and approximately linear. From 40% to 45% NaOH it increases suddenly, while over 45% NaOH it is again small.

The concentration of iron and manganese in the solutions are of the same order of magnitude and the graphs of the dependen-



% Mn
0.0060
0.0050
0.0040
0.0030
0.0020
20
25
30
35
40
45
50 % NaOH

Fig. 2



% Na_2CO_3
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
20
25
30
35
40
45
50 % NaOH

Fig. 3

ce of these concentrations on the concentration of sodium hydroxide are similar. This similarity indicates a certain analogy in the behavior of iron and manganese in concentrated solutions of sodium hydroxide. The fact that after a sufficiently long time an equilibrium concentration of the iron or manganese dissolved by corrosive processes is reached indicates that by these processes a larger quantity of these metals is dissolved than that which is allowed by the products of solubility of the corresponding compounds (ferrite, manganite etc.). It also indicates that these equilibrium concentrations correspond to the solubilities of these compounds.

The amount of carbonates in the solutions increases with the increase of the concentration of sodium hydroxide to a maximum at about 45%, after which it decreases. The presence of iron and manganese does not influence the maximum concentration of carbonates in the solution. The concentration of carbonates for all three cases is shown in Fig. 3.

The results show that the solubility of the ingredients investigated is least in the most dilute, i. e. in the 20% solution. This concentration is the most widely used in the fabrication of viscose.

Analysis of the 20%-solutions obtained by dilution of more concentrated solutions showed that the content of dissolved ingrediends decreased to the same value independent of the initial concentration of alkali and the quantity of impurities in it. This decrease of the concentration is greater than that which would be caused just by dilution. The surplus of the dissolved matter settles out in the form of $Fe_2O_3 \cdot nH_2O$ or $MnO_2 \cdot H_2O$. The more concentrated the solution from which the solution is made, the greater the quantity of the sediment. All this confirms the supposition that the solubility oı the corresponding compounds is the important factor.

The amounts of dissolved calcium and magnesium in the sodium hydroxide solutions could not be determined precisely by complexometry titration because these quantities were very small. They were of the order of 10^{-7} and were at the sensitivity threshold of the reaction of the indicator with magnesium. The consumption of solution of 0.01 M complexon III was about 0.5 ml for 40 g Na OH. This value exactly corresponds to that calculated from the solubility products of magnesium hydroxide, assuming that in the solutions of sodium hydroxide concentrated in this way the law of mass action is valid. The second reason why complexometry titration could not be applied in these determinations is the inactivity of the indicator eriochromblack T, i.e. ill-defined boundaries between the colors. This is due to the great concentration of sodium chloride produced by neutralization of sodium hydroxide. By titration of small quantities of magnesium (same consumption of complexon: 0.5 ml) in a pure water solution, i.e. without sodium chloride, a clear sharp boundary between the colors of the indicator is obtained.

Therefore we can conclude that the quantities of dissolved iron and manganese whose presence has a negative influence in the use of sodium hydroxide in the fabrication of viscose increase with increasing concentration of sodium hydroxide. We may also conclude that by the dilution of more concentrated solutions to a concentration of 20%, the amounts of dissolved iron and manganese decrease to definite values independent of the initial concentration of the alkali.

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COMPLEXOMETRIC DETERMINATION OF METALS IV*

DETERMINATION OF ALUMINUM**

by

VELIMIR CANIĆ and TIBOR KISS

By the method we proposed in our earlier work(1) we determined aluminum by titrating the excess of ethylenediaminetetraacetic acid (EDTA, H_4Y) with a solution of zinc in an acetic buffer at pH 7 in the presence of Eriochrome Black T as the indicator. We prevented blocking of the indicator by adding ethanol to the titrated solution(1–5).

To develop our work further it was also necessary to investigate retitrations with a zinc solution(6–8), magnesium(9) or manganese(10) in an ammoniacal medium and at pH 10. In these retitrations the following difficulties are encountered: the AlY^-complex is rather unstable at pH 10(11) and, therefore, it decomposes at the equivalence point when there is no excess of EDTA. The EDTA destroys the red Eriochrome Black T-zinc complex and the solution is turned blue by the liberated indicator. Because of this phenomenon it is difficult to determine the end-point of the titration(11)

Attempts to eliminate this difficulty by quick titrations at room(6–8) or at lover temperature(10,12) have not quite been successful(11).

Since in the presence of ethanol more suitable relationships of the stability constants of the AlY^- to ZnY^{2-} complex(13) are established and probably the reaction of the decomposition of the AlY^-complex is depressed, we titrated in the presence of about 70% of ethanol. In this case the color changes at the end-point of titration were sharp and reversible, and the results were satisfactory.

In the presence of ethanol we successfully titrated the excess of EDTA with a solution of magnesium or manganese as well.

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*III comunication, Chemist-Analyst 52: 111, 1963

** Report at IX Meeting of Chemists of Serbia in Beograd, January 1961.

EXPERIMENTAL

Reagents.— A standard 0.1000 M EDTA solution was prepared by dissolving 37.225 g of purified (14,15) disodium ethylenediaminetetraacetatedehydrate (Merck) in water and diluting to 1000 ml.

Standard 0.1000 M solutions of aluminum(16), zinc (17,18), magnesium and manganese were prepared by dissolving 0.1000 moles of a corresponding metal (99.99%) in hydrochloric acid and diluting to 1000 ml.

0.0500 and 0.0100 M standard solutions were prepared by diluting 0.1000 or 0.0500 M of the solution.

A solution of the pH 10 buffer was prepared by dissolving 13.5 g of ammonium chloride p.a. in a little water, adding 88 ml. of a concentrated ammonium solution and diluting to 250 ml.

All the solutions were prepared with water purified with ion-exchangers(19) and kept in polyethylene bottles(14,20,22).

The indicator mixture was prepared by spraying 0.2 g of Eriochrome Black T with 100 g of sodium chloride p.a.

Other reagents: 0.2 % solution of Eriochrome Black T in 96% ethanol, 1 M ammonium solution, 0.1 % solution of Methyl red in 50 % ethanol, ascorbic acid, hydroxylamine chlorhydrate, 96 % ethanol, methanol, 2-propanol, acetone and formamide.

PROCEDURE

A weighed amount of standard EDTA solution of the appropriate concentration was added to a weak acid solution containing 1 to 130 mg of aluminum. One drop of Methyl red solution was added and an ammonium solution added dropwise until the color turned yellow. Then 2 ml of a pH 10 buffer solution was added with constant mixing of ethanol so that at the equivalence point the titrated solution contained about 70 % of it. After the addition of the indicator mixture (or a fresh solution of the indicator) it was retitrated with a solution of zinc, magnesium or manganese of the same concentration as the added EDTA solution, until the

TABLE 1

Retitrated with a solution of :

EDTA Molarity	Zinc			Magnesium			Manganese		
	mg Al			mg Al			mg Al		
	Weighed	Found	Error%	Weighed	Found	Error%	Weighed	Found	Error%
0.0100	1.079	1.084	+0.5	1.349	1.341	—0.6	1.079	1.074	—0.5
	1.349	1.345	—0.3	2.428	2.435	+0.3	1.349	1.357	+0.6
	2.428	2.445	+0.7	2.698	2.703	+0.2	2.698	2.695	—0.1
0.0500	5.396	5.418	+0.4	6.745	6.726	—0.5	5.396	5.474	+0.4
	6.745	6.758	+0.2	12.14	12.19	+0.4	6.745	6.758	+0.2
	12.14	12.08	—0.5	13.49	13.48	—0.1	13.49	13.45	—0.3
0.1000	13.49	13.57	+0.6	24.28	24.11	—0.7	24.28	24.35	+0.3
	26.98	26.93	—0.2	26.98	27.11	+0.5	26.98	26.79	—0.7
	134.9	134.3	+0.3	134.9	135.7	+0.6	134.9	134.5	—0.3

blue color turned red. When titration was done with a manganese solution then about 0.1 g of ascorbic acid or hydroxylamine chlorhydrate was added to the titrated solution before titration.

The results for aluminum were calculated in the usual way and some of them are given in Table 1.

DISCUSSION

The presence of ethanol made it possible to obtain a reverse color change of the indicator at the equivalence point, i.e., even an excess of zinc in the pretitrated solutions could be titrated with EDTA.

Methanol, 2-propanol, acetone or formamide can also be successfully used instead of ethanol.

In determining aluminum by retitration, the titrated solution must be boiled after the addition of the excess of EDTA(10,12) because the reaction of aluminum with EDTA at room temperature is slow. If an organic solvent is added boiling is unnecessary because the same results are obtained no mater whether the solution is boiled or not.

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A CONTRIBUTION TO THE STUDY OF BENZOTHIAZOLE, BENZIMIDAZOLE AND BENZOXAZOLE.

I. THE REACTION OF QUINOLINIC ACID ANHYDRIDE WITH *O*-AMINOTHIOPHENOL

by

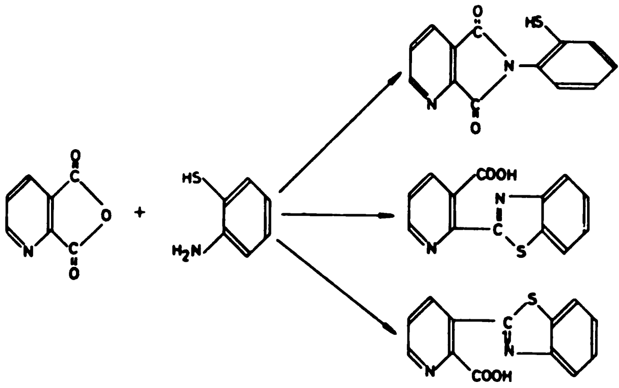
BORIVOJE L. BASTIĆ and MIROSLAV V. PILETIĆ

Pyridylbenzothiazoles have been studied to a considerable extent. They are obtained by the reaction of the corresponding picolines, aromatic nitro, nitroso, or amino compounds in the presence of elementary sulphur (1, 2, 3, 4, 5, 6, 7, 8, 9), or by the reaction of thiopicolineanilides and the corresponding aromatic amino compounds (10). Porter (11) synthesized them using *o*-aminothiophenol and thio derivatives of the corresponding acids, Wallenfels (12) used *o*-aminothiophenol and the aldehydes of the pyridine series. Zubarovski(13) has synthetized benzothiazoles by heating *o*-aminothiophenol with picolinic, nicotinic, and isonicotinic acid in sealed tubes to high temperatures.

Pyridylbenzothiazoles have been studied as drugs, antituberculous agents, fungicides and useful intermediary products.

A review of the literature reveals that benzothiazoles obtained from pyridinedicarboxylic acids have not been investigated so far· We prepared these compounds by the reaction between *o*-aminothiophenol and the anhydrides of pyridinedicarboxylic acids, whereby we wanted to synthesize the corresponding benzothiazoles and their derivatives and to study the course of the reaction itself. The present paper deals with the reaction of *o*-aminothiophenol with the anhydride of 2,3-pyridinedicarboxylic acid (quinolinic acid).

By condensing *o*-aminothiophenol with the anhydride of quinolinic acid we obtained a condensation product melting at 215°C; its formula was found to be $C_{13}H_8N_2O_2S$. This compound may be assumed to have one of the following structures:

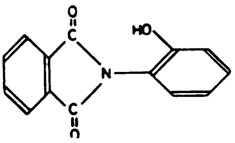


(I)

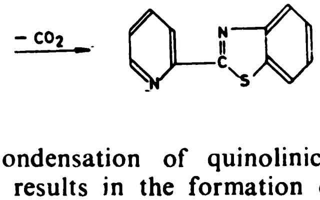
(II)

(III)

The possibility of I is supported by the fact that by condensation of phthalic acid anhydride with *o*-aminophenol Ladenburg⁽¹⁴⁾ got a compound of the following structure:



However, when the obtained compound, m.p. 215°C, was heated above its melting point, carbon dioxide was evolved and a new product, melting at 135°C, was obtained. This experimental fact would support formulae II and III for the compound melting at 215°C. Further investigations have shown that compound IV, m.p. 135°C, obtained by the decarboxylation of the compound melting at 215°C, is identical with the compound obtained by Zubarovski and Voronina⁽¹³⁾ in the condensation of picolinic acid and *o*-aminothiophenol; the mixed melting point of these compounds showed no depression.



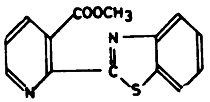
(IV)

Thus the condensation of quinolinic acid anhydride with *o*-aminothiophenol results in the formation of a compound melting at 215°C, the structure of which is given by formula II. This shows that the reaction proceeds with an opening of the ring of quinolinic acid anhydride in such a way that the group in the ortho position to nitrogen of the pyridine ring reacts. Such a course of the reaction might be expected on the basis of the similar condensation reactions of quinolinic acid anhydride, and the reaction product of the above condensation is 2-(benzothiazyl-2)-3-pyridine-carboxylic acid.

Some derivatives of this compound have been synthesized as well.

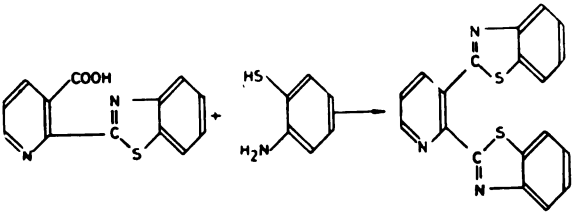
The silver salt of the formula $C_{13}H_7N_2O_2SAg$ was prepared and isolated in the usual way.

By the action of diazomethane the corresponding methyl ester with the formula $C_{14}H_{10}N_2O_2S$, melting at 117°C, was obtained:

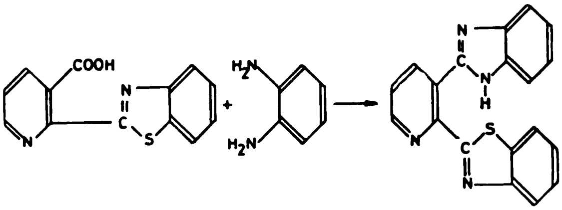


2-(benzothiazyl-2)-3-pyridinecarboxylic acid did not react with methyl iodide and from the reaction mixture the starting compound was isolated. This behavior was not unexpected since it is already known(13) that, contrary to the other two isomeric pyridylbenzothiazoles, 2-(pyridyl-2)-benzothiazole does not react with alkyl halides.

By condensing 2-(benzothiazyl-2)-3-pyridinecarboxylic acid with *o*-aminothiophenol (in excess) in a sealed tube at 180 — 200°C we obtained a new product, melting at 193°, whose formula was found to be $C_{19}H_{11}N_3S_2$. Analysis showed that it was formed by reaction between *o*-aminothiophenol and the free carboxylic group of the above acid. The obtained product was 2,3-bis-(benzothiazyl-2)-pyridine:



We also condensed 2-(benzothiazyl-2)-3-pyridinecarboxylic acid with *o*-phenylenediamine to see if the reaction between the free carboxylic group and *o*-phenylene diamine would give a compound containing pyridine, benzothiazole and benzimidazole rings at the same time. The reactants were heated at 200° in an open vessel, and from the reaction mixture, after cooling, we succeeded in isolating a compound of the formula $C_{19}H_{12}N_4S$, melting at 223°. Analysis of the product confirmed our hypothesis. The condensation product was identified as 2-(benzothiazyl-2)-3-(benzimidazyl-2)-pyridine.



The condensation reactions of other pyridinecarboxylic acids with *o*-aminothiophenol, *o*-aminophenol and *o*-phenylenediamine are under investigation.

EXPERIMENTAL

All the melting points are uncorrected.

The anhydride of quinolinic acid was prepared from quinolinic acid[(15)].

1. *Condensation of o-aminothiophenol with quinolinic acid anhydride*

In a 100 ml flask connected to vertical condenser 3 g *o*-aminothiophenol and 3.6 g quinolinic acid anhydride (equimolecular amounts) were mixed with 10 ml toluene, whereby considerable heat was evolved. The mixture was then refluxed in paraffin oil bath at 110° for half an hour; the cooled reaction mixture was filtered off and the precipitate was washed twice with 2.5 ml toluene and four times with 5 ml ether 4.3 g (64%) of light green-yellow crystals were obtained; m.p. 185 — 200°.

The raw product was heated with 200 ml ethanol until almost completely dissolved, then filtered, treated with charcoal, and left to stand.The separated crystals were filtered off and washed with ethanol, furnishing 2.4 g (36%) of product melting at 215°, which was only slightly colored. The addition of water to the filtrate furnished an additional amount of about 0.4g of the product.

Analysis:

Calculated for $C_{13}H_8N_2O_2S$: C 60.92%; H 3.15%; N 10.93%

Found : C 61. 1%; H 3.1%; N 10.5%

2. *Decarboxylation of compound melting at 215°*

In a 100 ml flask conected to vertical condenser 0.4 g of compound of m.p. 215° was heated at 220 — 230° in paraffin oil bath for one hour. The reaction mixture melted at about 210° with evolution of bubbles of carbon dioxide. After one hour the temperature was raised to 230 — 235° and the heating was continued until the evolution of bubbles ceased (about half an hour). When the reaction was over the white crystalline sublimate collected at the upper part of the flask was scraped off with a scalpel; m.p. 133 — 135°; yield: 0.2 g (60%).

Since the melting point of the decarboxylation product corresponded to 2-(pyridyl-2)-benzothiazole, we synthesized the latter product by the method of Zubarovski and Voronine(13); the mixed melting point of the sythesized substance and the decarboxylation product showed no depression.

Analysis:

Calculated for $C_{12}H_8N_2S$: C 67.90%; H 3.80%; N 13.20%
Found : C 67.7%; H 3.7%; N 13.2%

3. Silver salt of 2-(benzothiazyl-2)-3-pyridinecarboxylic acid

About 100 mg of compound of m.p. 215° was dissolved in hot ethanol and a small amount of concetrated ammonia was added. The reaction mixture was heated to boiling and a silver nitrate solution (water and ethanol) was added dropwise. The separated brown-grey pricipitate was filtered off and washed with ammoniacal ethanol

Analysis:

Calculated for $C_{13}H_7N_2O_2SAg$: Ag 29.7%
Found : Ag 30.1%

4. Methyl ester of 2-(benzothiazyl-2)-3-pyridinecarboxylic acid

In a 100 ml flask with a ground glass stopper a suspension of 0.5 g 2-(benzothiazyl-2)-3-pyridinecarboxylic acid in 30 ml absolute methanol was cooled in an ice bath to below 0°; to the cold solution a slight excess of diazomethane was added in 4 — 5 portions with constant shaking. The mixture was left to stand in a cold place and was then evaporated in vacumm to 40% of its original volume. On cooling a white precipitate was separated, filtered off and washed with a small amount of absolute methanol; the yield was 0.19 g. The addition of an equal volume of water to the filtrate furnished further 0.28 g crystals. The total yield was 0.47 g (90%); m.p. 117°.

Analysis:

Calculated for $C_{14}H_{10}N_2O_2S$: C 62,20%; H 3.74%; N 10.36%
Found : C 62. 0%; H 3.4%; N 10.3%

5. The reaction of 2-(benzothiazyl-2)-3-pyridinecarboxylic acid with methyl iodide

A mixture of 0.3 g of compound of m.p. 215° and 1 ml methyl iodide was sealed in a tube and heated on a water bath for two and a half hours. After cooling, methyl iodide was evaporated and the residue was treated with hot ethanol, filtered and left to stand. The filtrate deposited 0.24 g crystals melting at 215°. The mixed melting point with the starting compound showed no depression.

6. 2,3-bis-(benzothiazyl-2)-pyridine

A mixture of 0.5 g 2-(benzothiazyl-2)-3-pyridinecarboxylic acid and 0.75 ml *o*-aminothiophenol (in excess) was sealed in a glass tube. The sealed tube was heated at 180 — 200° in an oil bath for 4 hours and then left to stand. On cooling clear drops of water were observed on the inner walls of the tube. The content of the tube was dissolved in 20 ml etanol. After treatment with active charcoal the filtrate was left to stand. The separated crystals were filtered off, m.p. about 165°; yield 0.15 g (23%). The addition of water to the filtrate furnished a white precipitate which was not further investigated.

The crystals melting at 165° were dissolved in 8 ml absolute methanol, the hot solution was filtered and left to stand. The separated crystals melted at 193°.

Analysis:
Calculated for $C_{19}H_{11}N_3S_2$: C 66.06%; H 3.21%; N 12.17%
Found : C 66.2%; H 3.1%; N 12.5 %

7. Condensation of 2-(benzothiazyl -2)-3-pyridinecarboxylic acid with o-phenylenediamine

A thoroughly mixed mixture of 0.5 g *o*-phenylenediamine and 0.5 g 2-(benzothiazyl-2)-3-pyridinecarboxylic acid was heated in a glass tube in parafin oil bath for one hour. The evolution of bubbles was observed at 180° and drops of water appeared on the walls of the tube. The reaction mixture was then left to stand, and a dark-colored solid mass formed at the bottom of the tube. The mass was first suspended in ethanol by gradual addition of 70 ml ethanol and then heated to boiling and treated with active charcoal. On cooling the filtrate gave no precipitate but the addition of an equal volume of water caused the separation of crystals. The reaction mixture was heated to boiling and filtered; on standing crystals melting at 223° were deposited with a yield of 0.12 g (20%). The separated crystals were recrystallized from 10 ml absolute methanol;

Analysis:
Calculated for $C_{10}H_{12}N_4S$: C 69.50%; H 3.68%; N 17.05%
Found : C 69.9%; H 3.7;% N 16.6 %

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A STUDY OF THE NITROGENOUS SUBSTANCES OF HELLEBORUS

by

MILOŠ MLADENOVIĆ and OLGA STEFANOVIĆ

In studying the nitrogenous substances of Helleborus, Keller(1) unsuccessfully attempted to isolate alkaloids from the underground organs of *Helleborus niger* and *Helleborus viridis*. Later, Schöbel(2) succeeded in isolating nitrogenous substances from *Helleborus viridis* and these he identified as alkaloids: celiamine, sprintilamine and sprintiline. Čučković(3) however, failed to isolate alkaloids from *Helleborus atrirubens*.

In biochemical studies of the glucide of *Helleborus odorus* and *Helleborus atrirubens* we obtained a crystalline substance from the alcohol which had been used for the stabilization of the drug. Because this substance yielded positive reaction on nitrogen we suspected it to be an alkaloid. However, more detailed investigations showed that there are no alkaloids in either *Helleborus odorus* or *Helleborus atrirubens*. We continued to investigate the nitrogenous substance assuming that it might be some aminoacid, especially since aminoacids and their amides are often found in the underground organs of plants. It should be pointed out that this substance has been obtained in investigations of other varieties of Helleborus. Our investigations have shown that it is asparagine that is involved.

Having isolated and identified asparagine, we were interested in finding out whether there were any other aminoacids in the alcoholic solution from which we had isolated the asparagine. We used the dimensional paper chromatography developed by Boissonas(4) and modified by Kofrany(5) for this investigation. In *Helleborus odorus* we identified: asparaginic and glutamic acid; serine; arginine; glycine; histidine; threonine; alanine; proline; methionine + valine and leucine + isoleucine. In *Helleborus atrirubens* we identified: asparaginic and glutamic acid, lysine, arginine, histidine, threonine, alanine, proline, methionine + valine and leucine + isoleucine.

EXPERIMENTAL PROCEDURE

The underground organs of Helleborus were first cleaned and ground and then stabilized with 96% alcohol by slightly boiling for 15 minutes. After cooling the alcohol was filtered and left in a cool place for 15 days. Even after 24 hours there were tiny crystals at the bottom, and over a longer period the deposit of crystals increased.

The isolated substance contained nitrogen. The non-purified crystals were long plates and melted and decomposed at about 215°. The crystals were purified by repeatedly dissolving the substance in water and precipitating it with concentrated alcohol. The crystals purified in this manner melted at a temperature of about 230° (Fischer's block).

The substance was readily soluble in water and had an acidic reaction. From the aqueous solution it precipitated with 96% alcohol. $[\alpha]^{20} = -5°$, ($c = -2\%$, H_2O). The value of the angle of rotation depended on the *pH* of the medium (in a neutral medium $\alpha = -0.10°$, in a base 0.15, and in an acid 0°). The substance yielded a biuretic reaction. With two or three drops of sulphuric acid and some resorcin it became yellowish-green and dilution with water and saturation with ammonia produced fluorescence(6).

For analysis the substance was dried in a high vacuum at 100° and the following results were obtained:

C 36.59% H 6.31% N 21.60%

For waterless asparagine:

C 36.36% H 6.11% N 21.20%

All the data obtained indicated that the substance isolated is asparagine.

Further testing confirmed that it was asparagine. We treated it with 12.5% hydrochloric acid and obtained *L*-asparaginic acid after boiling for 3 hours. After cooling the solution, adding a fixed amount of 6% ammonia, and leting it stand for several hours we obtained crystals of asparaginic acid which melted at 276° (Fischer's block). The substance hardly dissolved in cold water but with an increase of temperature it dissolved more readily. It was insoluble in alcohol and concentrated acetic acid but readily soluble in the presence of mineral acids and bases $[\alpha]_D^{20} = +24°$ ($c = 1\%$, HCl).

SEPARATION OF AMINOACIDS OF HELLEBORUS BY PAPER CHROMATOGRAPHY

Glass cylinders 25 cm in diameter and 52 cm long were used as the chambers.

The separation was performed on Whatman No. 1 paper. The size of the paper for the separation of slow aminoacids was 40 × 40 cm and for fast acids 23 × 27 cm.

As the solvents for the slow aminoacids we used a mixture of n-butanol-glacial acetic acid and water (70 : 15 : 15) for one direction and phenol saturated with water for the other.

As the solvents for the separation of fast aminoacids we used a mixture of tertiary butanol, methyl ethyl ketone and water (5 : 3 : 2) for one direction and a mixture of tertiary butanol, methanol and water (4 : 5 : 1) for the other.

After the separation, the chromatograms were dried in a stream of air at 60°. They were developed with ninhydrin. After spraying the chromatogram with a 0.1% solution of ninhydrin in methanol and drying in a drying oven at 60° violet spots appeared in the places where aminoacids had separated. The alcoholic extracts of *Helleborus odorus* and *Helleborus atrirubens* were investigated at the same time. Their chromatograms are shown in Figs. 1 and 2.

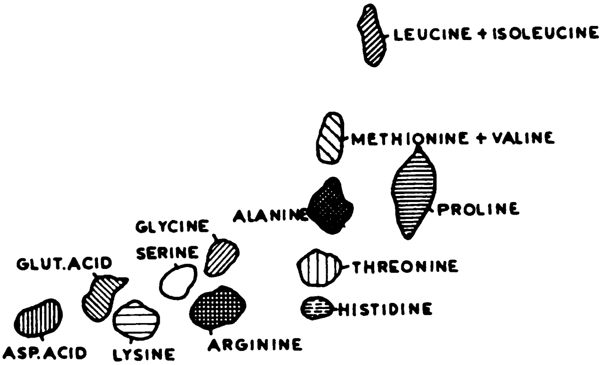

Fig. 1. — Chromatogram of aminoacids from *Helleborus odorus*

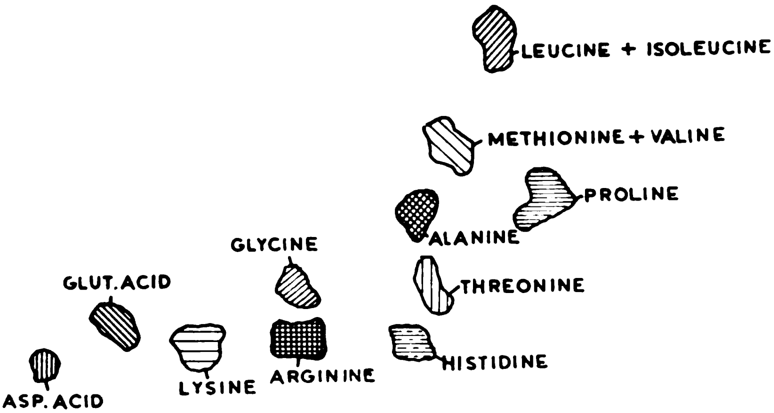

Fig. 2. — Chromatogram of aminoacids from *Helleborus atrirubens*

The chromatograms show that the only difference is that the aminoacid serine is found in *Helleborus odorus* but not in *Helleborus atrirubens*.

The following aminoacids were identified in the extract of *Helleborus odorus*: asparaginic, glutamic, lysine, serine, arginine, glycine, histidine, threonine, alanine, proline, methionine + valine and leucine + isoleucine. The following aminoacids were identified in *Helleborus atrirubens*: asparaginic, glutamic, lysine, arginine, glucine, histidine, threonine, alanine, proline, methionine + valine and leucine + isoleucine.

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A STUDY OF THE CARBOHYDRATES OF DOMESTIC SPECIES OF HELLEBORUS

by

OLGA STEFANOVIĆ and MILOŠ MLADENOVIĆ

In our investigations of the chemical composition of underground organs of *Helleborus odorus* and *Helleborus artirubens* we made an investigation of glucide. In a recent paper(1) we reported a biochemical investigation of glucide, in which we found that there is saccharose in the root extract of Helleborus, and deduced the presence of stachyose and raffinose as well on the basis of the enzymolitic reduction index. We assumed further that apart from these glucides there are other mono- and oligosaccharides, either free or bound to the agluconic component of the glucoside of Helleborus. We then tried to confirm the presence of the glucides indicated by the biochemical method and to identify any other glucides present by chromatography, employing both the column and the paper chromatographic methods.

EXPERIMENTAL PROCEDURE

Isolation of saccharose by column chromatography

The root and rhizome of *Helleborus* atrirubens were extracted with 96% alcohol and passed through a chromatographic column of Merck aluminum oxide standardized according to Brockmann. The chromatographic column was then eluted with methanol and after evaporation of the methanol, solid, transparent, prismatic crystals were obtained which were easily soluble in water but hardly in concentrated alcohol. The aqueous solution of the isolated substance did not yield characteristic reactions of reduction sugars until after hydrolysis with hydrochloric acid. The aqueous solution showed optical activity characteristic of saccharose and after hydrolysis it rotated to the left. All the reactions and the physical properties show that saccharose is involved. From 78.75 g of dried extract 2.32 g i.e. 3% of sacharose is obtained.

The isolation of pure, crystalline saccharose confirmed the earlier biochemical finding of it in the underground organs of *Helleborus atrirubens*.

Carbohydrates were isolated from *Helleborus odorus* in the same way but only very small quantities of crystalline saccharose were obtained from this type of hellebore by eluting the chromatographic column.

Identification of carbohydrates in a mixture by paper chromatography

After isolating saccharose by eluting the cromatographic column with ethanol, we eluted it with water. A yellow eluent was obtained which became a brown residue and smelled like caramel after the evaporation of water. The aqueous solution of this eluent yielded positive reaction with Fehling's solution before and after inversion. This shows that there are other carbohydrates in the mixture too.

A 30 g mixture of carbohydrates, 38% from *Helleborus atrirubens* and only 20% from *Helleborus odorus* was obtained from the dried extract of the drug (78.75 g).

The paper chromatography developed by Fischer and Dörfel(2) was used to identify the carbohydrates in the mixture. This was done on Whatman No.1 paper. The mobile phase was a mixture of pyridine, ethylacetate and water (11 : 40 : 6). The following carbohydrates were identified in *Helleborus atrirubens* on the basis of the Rg values: saccharose; galactose; fructose and raffinose. There was another spot on the chromatogram which according to the Rg values could be identified as lyxose or methylgalactose.

Another spot was assumed to be some other carbohydrates which could not be separated by this method.

The following carbohydrates were identified in the mixture of carbohydrates obtained from the extract of *Helleborus odorus*: raffinose, saccharose, galactose, fructose, and rhamnose.

The only differences between the carbohydrates is that whereas there is rhamnose in *Helleborus odorus* there is methylgalactose or lyxose in *Helleborus atrirubens* instead.

The presence of rhamnose in the root and rhizome of the Helleborus studied was confirmed by the paper chromatographic method. Its presence had been debuced from the enzymolitic reduction index obtained from the investigations of Helleborus extracts.

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A NEW GLUCOSIDE IN THE LEAF OF THE MEDLAR-TREE, (*MESPILUS GERMANICA* L.) I. – ISOLATION OF GLUCOSIDE, INVESTIGATION OF ITS STRUCTURE AND ITS CHEMICAL AND PHYSICAL PROPERTIES

by

SAVA G. STANIMIROVIĆ and DARINKA L. STANIMIROVIĆ

Investigating saccharides in the leaf and fruit of the medlar-tree(1) we have found by paper chromatography that only the leaf contains an appreciable amount of a compound whose Rg = 2.62. The compound yielded a positive reaction on the chromatogram with the alkaline reagents 3.5-dinitrosalicylic acid(2), silver nitrate(3) and triphenyltetrazolium chloride(4), but not with other reagents used to detect saccharides: anilinhydrogenphthalate(5), p-anisidinehydrochloride(6), naphthoresorcinol(7), urea(8), and orcinol(9).

The compound was isolated in a crystal state and was found to be a new, as yet unknown, glucoside.

This paper presents the procedure for the isolation of this glucoside, its physical and chemical properties, and the results from the investigations of its structure.

EXPERIMENTAL PROCEDURE

Extraction and isolation of glucoside.—Very soon after picking the medlar leaves are weighed, cut into pieces and dipped into boiling 96% ethanol(10). An amout of ethanol is used such that its highest concentration, allowing for the water from the leaf, is about 80 percent. The sample is ground to a fine pulp with Ultra Turax TP 18/2 and then filtered through Buchner's funnel. The precipitate on the funnel is washed with 80% ethanol and the ethanol is removed from the filtrate by distillation at about 45° under reduced pressure. The precipitate settled in the aqueous solution is eliminated by filtering through a G — 4 sintered glass funnel.

The filtrate is passed down a Duolite $C_{20}(H)$ (11) cation exchanger column, and then down an Amberlite IRA — 400 (CO_2) anion exchanger column(12). The dimensions of the columns used depend on the amount of the extract. The eluate thus obtained contains a mixture of sugar and glucoside. Under reduced pressure it is evaporated at 60° to syrupy consistency and then the glucoside isolated

We isolated the glucoside on a charcoal-celite column(13). For this purpose we used BDH activated decolorizing charcoal washed with hydrochloric acid, and Celite 535 (Malinkrodt). The syrup obtained from about 120 g of medlar leaves was adsorbed on a charcoal-celite column (4x17 cm) and eluted first with water then with 5% ethanol and finally with 10% ethanol. 100 ml fractions were investigated by paper chromatography. 1200 ml of water was used for the elution of the monosaccharides, inositol and sorbitol. 1100 ml of 5% ethanol was used for the elution of saccharose and a non-identified substance. Absolutely pure glucoside was eluted with 1500 ml of 10% ethanol. Fractions with glucosioe were put together, the solution was evaporated to a small volume by distillation at 60° under reduced pressure, filtered through a G—4 sintered glass funnel to remove the celite and charcoal and finally evaporated to dryness over a water bath. After repeated crystallization from absolute ethanol and drying over phosphorus pentoxide under reduced pressure, we obtained glucoside of a constant on the basis of the boiling point from the crude crystal mass.

The glucoside rotates the plane of polarized light $[\alpha]_D^{20}-46°$ (water, C 3.06). It melts first at 74° then at 140 — 141° (Kofler's microscope).

Elementary analysis: C 46.41 %
H 7.04 %

calculated for $C_{12}H_{22}O_9$: C 46.45 %
H 7.15 %

The molecular weight of the glucoside determined cryoscopically was 274 and on the basis of the elementary analysis 310.29.

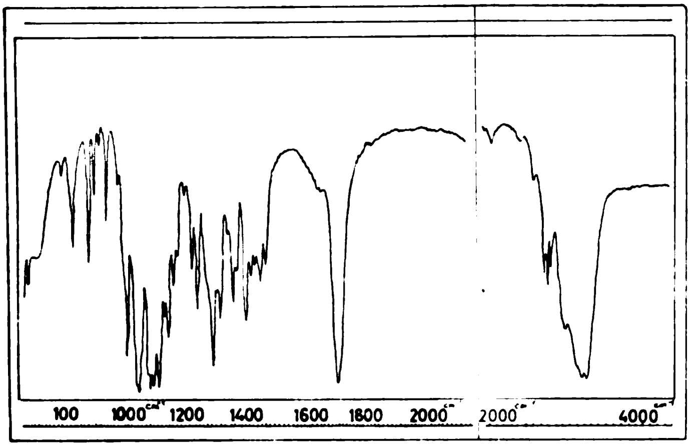
The glucoside was dried under reduced pressure at 78° until constant weight, weighed, left to stand for 24 hours under ordinary conditions and then weighed once more. It was found that by drying it loses and on standing it again binds 5.9 % of water.

Elementary analysis of waterless glucoside: C 49.49 %
H 6.76 %

calculated for $C_{12}H_{20}O_8$: C 49.31 %
H 6.84 %

The molecular weight of waterless glucoside determined by elementary analysis was 292.12.

In the ultra-violet part of the spectrum glucoside did not show any characteristic band, whereas in the infra-red (Fig.1) it showed bands (in potassium chloride) at the following wavelengths: 1715 cm^{-1} (ester function), 3420 cm^{-1} (hydroxyl function), 1360 cm^{-1} (the methylene group in conjugation with carbonyl function).



Fig, 1

Hydrolysis of glucoside.—We took 53.9 mg of waterless glucoside and hydrolized it with 10 ml 1 N sulphuric acid in a sealed tube at 60° for 4 days (so that the destruction of sugar would be reduced to minimum).

After removing the sulphuric acid with an Amberlite IRA-400 (CO_3) exchanger we examined one portion of the hydrolysis product by paper chromatography(14). The chromatogram contained only one spot(3). By its reactions on saccharides (5,7) and test substances we identified glucose.

We determined glucose by the method of Somogyi(15). in the other portion of the hydrolysis product after neutralizing the sulphuric acid with a sodium hydroxide solution. The glucoside contained 56.7% glucose.

Oxidation of glucoside with periodic acid.—90 mg of waterless glucoside dissolved in 3 ml of water was oxidized with 15 ml 0,05 M periodic acid for 16 hours. After oxidation we determined the amount of periodic acid consumed in 6 ml of the solution (which equals 30 mg of glucoside) using a bicarbonate solution of arsenic trioxide and a solution of iodine(16). We found that 3.9 ml of the periodic acid solution had been consumed, i. e. two moles of periodic acid per one mole of glucoside.

After treating an other 6ml of the solution, with ethylene glycol, we determined the amount of formic acid produced with 0.01 N sodium hydroxide(17). 9.7 ml 0.01 N formic acid had been produced, i.e· one mole of formic acid per one mole of glucoside.

The excess of periodic acid and the built-up iodic acid was removed from the rest of the solution with an Amberlite IRA-400 (CO_3) exchanger. After removing the exchanger we tested the solution for formaldehyde by treating it with chromotropic acid dissolved in sulphuric acid(18). The reaction was negative.

Deter mination of the number of hydroxyl groups in glucoside.—We acetylized 121 mg of waterless glucoside using a 3 ml solution of acetic anhydride in dry pyridine(19). The consumption of onhydride was determined with 0.5 N sodium hydroxide, 32.7 ml was used for the control test and 29.4 ml for the analysis. This means that one mole of glucoside contains four hydroxyl groups.

Determination of the number of methyl groups in glucoside.— We oxidized 82.8 mg of waterless glucoside with a 5 ml solution of chromic anhydride in sulphuric acid in a sealed tube at 120° for 8 hours(20). The built-up acid was separated from the reaction mixture by distillation with water vapor and titrated with 0.01 sodium hydroxide. We used 14.74 ml of sodium hydroxide, i.e. about 52% of the theoretical value calculated for one methyl group per molecule of glucoside.

Identification of the produced acid.— Under reduced pressure we evaporated the neutralized distillate to a small volume and treated it with a Duolite C_{20} $(\overset{+}{H})$ exchanger. We converted the solution of the free acid to diethylamine salt and chromographed it on paper(21'22). We found that the distillate contained acetic acid, i.e.., the glucoside contained a methyl group.

Lactonic titration.— We dissolved 41.3 mg of waterless glucoside in 5 ml of distilled water in a 50 ml flask and added 15 ml 0.1 N sodium hydroxide. After 15 hours we titrated the excess of sodium hydroxide with 3.59 ml 0.1 N solution of sulphuric acid in the presence of phenolphtalein. We then added 5 ml 0.1 N sulphuric acid to the neutralized solution. After 48 hours we retitrated sulphuric acid with 3.59 ml 0.1 N sodium hydroxide(23). This means that one mole of sodium hydroxide was used to neutralize one mole of glucoside.

Properties of glucoside.— Colorless, bitter, odorless crystals easily soluble in water, slightly in ethanol and pyridine. Hardly soluble in dioxane and insoluble in ether and chloroform.

Glucoside produces positive Tollen's (slower than glucose, faster than saccharose) and Molisch's tests and reaction on esters and lactones(24). It does not produce a positive Legal test. In an alkaline medium it easily hydrolyses, liberating glucose: by titrating the solution of glucoside with the Somogyi alkaline copper reagent for 20 minutes in a boiling water bath glucoside completely hydrolyses, enabling determination of the glucose liberated. It is probably for this reason that glucoside produces positive reaction with the alkaline reagents used for the determination of saccharides.

DISCUSSION

Cryoscopic determination of its molecular weight and an elementary analysis showed that the molecular formula of the glucoside is C_{12} H_{22} 0_9 (molecular weight 310.29). Since it loses 5.9 percent of water by drying, and binds the same amount on standing in ordinary conditions it must crystallize as a monohydrate. This is also confirmed by the elementary analysis of the waterless glucoside. Its molecular formula is $C_{12}H_{20}O_8$ (molecular weight

292.12), i.e., it differs from the above molecular formula by one molecule of water.

Chromatographic investigations of the glucoside product of hydrolysis showed that its sugar component is glucose. The determined molecular weight shows that it can contain only one remainder of glucose. The finding of 56.7 percent of glucose in a molecule of glucoside also confirms this since it gives the molecular weight of glucoside as identical with that already determined. These results also confirm that the molecular formula of aglucone is $C_6H_{10}O_3$ (molecular weight 130.29).

Since one molecule of glucoside contains four hydroxyl groups and it forms one molecule of glucose, with acid hydrolysis it follows that all the four hydroxyl groups belong to the remainder of glucose. This implies that the remainder of aglucone does not contain any free hydroxyl group, i.e., the only hydroxyl group of aglucone is the one already engaged in the glucoside bond.

Since two moles of periodic acid were required for the oxidation of one mole of glucoside and one mole of formic acid was liberated, it can be concluded that periodic acid oxidizes the glucose remainder and three out of four hydroxyl groups are in the vicinal position. Since formaldehyde was not formed during this oxidation it means that none of the three vicinal hydroxyl groups of glucose is primary and therefore, it must be the remainder of a glucopyranose, i.e., the glucoside is a glucopyranoside(25).

The results also suggest that the aglucone remainder also does not contain any other vicinal groups which can be oxidized by periodic acid (α-diketone, α-dialdehyde, etc.).

The glucoside did not react with 2.4-dinitrophenylhydrazine, nor with Schiff's reagent which indicates that the aglucone remainder does not contain even a common ketone or aldehyde group. The absorption band in the infra-red at 1715 cm^{-1} indicates the presence of lactonic function. The glucoside does not contain free carboxyl function since its reaction in aqueous solution is neutral. Similarly it produces positive reaction on esters and lactones. Since one mole of sodium hydroxide was consumed, in the neutralization of one mole of glucoside, it follows that the remainder of aglucone is lactone. Lactonic titration also showed that the lactonic ring is stable: it was not possible to titrate glucoside directly with 0.1 N sodium hydroxide. It was possible however to titrate the excess of the added acid in an acidified neutralized glucoside solution without repeated interference of the built-up lactone in the color change of the indicator.

Since the ultra-violet spectrum of the glucoside does not show characteristic absorption it means that it is saturated. Nor does the infra-red spectrum show the absorption which corresponds to a double bond. The negative Legal test and the positive test on ammoniacal solution of silver nitrate also show that the aglucone remainder is a saturated lactone.

The stability of the lactonic ring indicates that the remainder of aglucone is a five-membered or six-membered lactone. Therefore, having in mind its molecular formula, the glucoside would be expected to contain the alkyl group too. We have demonstrated that oxidization of the glucoside with chromic acid yields acetic acid, which means that the aglucone remainder contains the methyl group. It is not possible to determine the number of methyl groups in the aglucone reminder however, from the theoretical value of 52% calculated for one methyl groyp in a molecule of glucoside.

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A NEW GLUCOSIDE IN THE LEAF OF THE MEDLAR—TREE (*MESPILUS GERMANICA* L.) II.— ISOLATION OF AGLUCONE, DETERMINATION OF ITS STRUCTURE AND THE STRUCTURE OF GLUCOSIDE

by

DARINKA L. STANIMIROVIĆ and SAVA G. STANIMIROVIĆ

From the results we obtained by studying glucoside we were not able to determine the structure of aglucone and hence of glucoside. If we determined the type of lactone, on the basis of the molecular formula of glucoside we could determine the number of methyl groups: the six-membered lactone could contain only one and the five-membered lactone two methyl groups. We should also determine the position of methyl groups and the glucoside bonds. We therefore isolated aglucone and determined its structure. From the structure of aglucone can determine the structure of glucoside.

EXPERIMENTAL PROCEDURE

Acid hydrolysis of glucoside.— 2.4 g of glucoside was hydrolyzed with 400 ml 1 N sulphuric acid over a boiling water bath with a condenser for three hours. By paper chromatography we determined that the glucoside was completely hydrolyzed. We extracted aglucone from the acid product of hydrolysis by continual extraction with ether for 48 hours. We washed the ethereal extract with a small amount of saturated sodium chloride solution, dried it with waterless sodium sulphate and filtered. Ether was removed by distillation. The obtained yellowish oil was twice distilled in high vacuum at 50° (0.001 mm) and a colorless oily liquid was obtained which did not crystallize with cooling to —20° or standing for a long time.

Preparation of enzymes from medlar leaves.— We cut 300 g of medlar leaves into fine pieces, extracted with 900 ml of distilled water and filtered. From the water extract the enzyme was precipitated with 3.6 l 95% ethanol. The precipitate was filtered, washed with 95% ethanol, then with ether, and dried under pressure over sulphuric acid(1). We obtained 3 g of crude enzyme.

Enzymatic hydrolysis of glucoside.— 0.5 g of glucoside dissolved in 500 ml of water was hydrolyzed with crude enzyme at 35°. The hydrolysis was followed by paper chromatography. It was completed after 96 hours. We extracted aglucone from the product of hydrolysis and purified it by the method by which we extracted aglucone from the acidic product of hydrolysis.

In a diluted solution (10 mg glucoside in 100 ml water) emulsin hydrolyzed glucoside for 48 hours.

The pure aglucone obtained by acid hydrolysis is identical with the aglucone obtained by enzymatic hydrolysis. The isolated aglucone does not rotate the plane of polarized light. n_D = 1.4885 — 1.4886. It shows absorption in the ultraviolet spectrum (Fig. 1) λ max 214 cm^{-1}, ε 9.601. In the infra-red (Fig. 2) aglucone shows absorption bands at 1383 cm^{-1}, 1640 cm^{-1} and 1725 cm^{-1}.

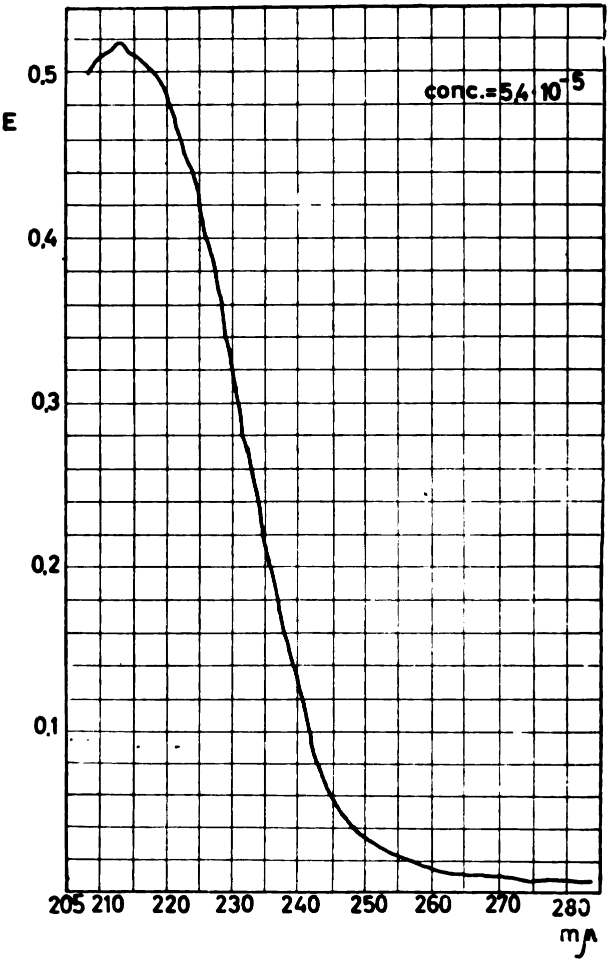


Fig. 1

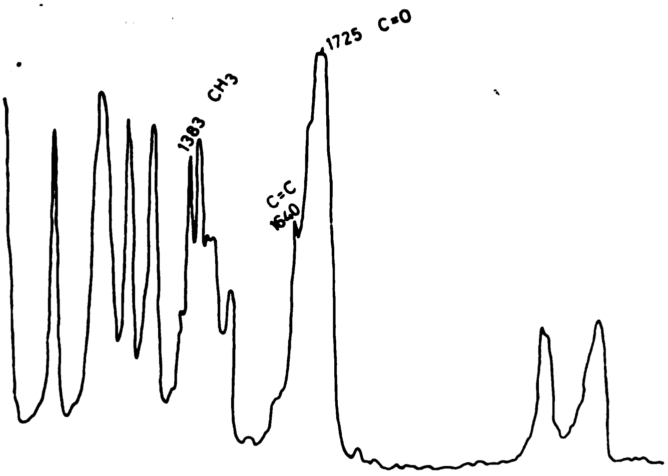


Fig. 2

Elementary analysis of aglucone C 63.27%
H 7.31%
calculated C 64.27%
H 7.19%

The molecular weight of aglucone is 112.12.

Lactonic titration.— We added 5 ml 0.1 N sodium hydroxide to a 17.85 mg aglucone solution in 5 ml of water. After it had stood for 15 hours we retitrated sodium hydroxide with 3.41 ml 0.1 N sulphuric acid. The obtained molecular weight of aglucone was 111.5.

Aglucone gives a positive reaction on lactones(2) and in Legal's and Raymond's test(3). It does not produce a positive reaction on silver ammonium nitrate solution(4). Aqueous solution of aglucone (32.45 mg + 10 ml water) gies a neutral reaction (4,5).

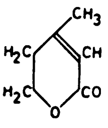
DISCUSSION

The elementary analysis and lactonic titration of aglucone show that its molecular weight is 112.12 and its molecular formula $C_6H_8O_2$. From the molecular weight and the formula of glucoside the molecular weight of aglucone should be 130.29 and its molecular formula $C_6H_{10}O_3$. Since the isolated aglucone contains one less water molecule we consider that anhydration took place during hydrolysis. This anhydration is performed either with acid or enzymatic hydrolysis because in both cases the same anhydrated product is obtained. Therefore, it follows that the isolated aglucone is an unsaturated lactone.

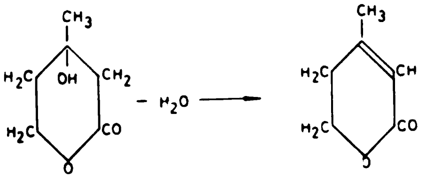
Maximum absorption in the ultra-violet 224 cm^{-1} shows that the aglucone is an α, β —unsaturated aglucone (6). The absorption band 1640 cm^{-1} in the infra-red shows that the unsaturated bond is in conjugation with the carbonyl group(7). A positive Legal's test and a negative test on silver ammonium nitrate solution, and the fact that the agueous solution of aglucone gives a neutral reaction also prove that aglucone is an α, β —unsaturated lactone. We underline that glucoside does nos yield a positive Legal's test, but produces a silver mirror with a silver ammonium nitrate solution. The absorption band of aglucone in the infra-red at 1725cm^{-1} shows that aglucone is a six-membered α, β —unsaturated lactone(7'8).

The oxidation of glucoside with chromic acid, and the absorption band of aglucone at 1383 cm^{-1} skow that aglucone also contains a methyl group(9). The molecular formular and the fact that aglucone is a six-membered α, β —unsaturated lactone show that it contains only one methyl group.

The results presented here show that the lactone is isomeric with parasorbic acid (δ — Δ hexanolactone)(10), which is isolated from plant substance, and is identical with δ -lactone 5-hydroxy-3-methyl-2-pentenic acid:



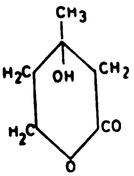
This lactone is obtained by direct synthesis(11) and by anhydrating synthetic lactone of mevalonic acid(12'13):



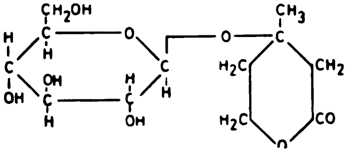
To obtain lactone of mevalonic acid, and not unsaturated δ-lactone 5-hydroxy-3-methyl-2-pentenic acid, we also hydrolyzed glucoside with enzymes. We wanted to avoid a possible catalytic effect of the acid on anhydration. Since we obtained the same product, in this way too anhydration of aglucone during hydrolysis must be spontaneous.

The fact that emulsin hydrolyzes this glucoside proves that it is a β-glucoside.

Since glucoside is a saturated compound and its infra-red spectrum shows that there is a methylene group in the α-position in aglucone, it follows that aglucone is anhydrated on account of the hydrogen of this group and the hydroxyl group of aglucone of the neighboring β-C atom which was engaged in the glucoside bond. This means that the agluconic component of this glucoside is the lactone of mevalonic acid (mevalolactone):



The structural formula of the glucoside is:



.e. mevalonolactonyl-β-glucoside.

Mevalonic acid, i.e. its lactone is extracted from marc(14). It is produced during fermentation by the action of yeast enzymes. Although it has been confirmed that lactone is the starting material in the building up of polyisoprenic compounds widespread in plants, we do not know whether it has been isolated from plant material.

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CHEMICAL INVESTIGATION OF WHEAT

5*

SOME CHROMATOGRAPHIC INVESTIGATIONS OF WHEAT LIPIDS

by

MIHAILO LJ. MIHAILOVIĆ, GEORGE ALLAN GARTON,
MIHAILO ANTIĆ and DIMITRIJE HADŽIJEV

Wheat lipids have recently been shown to contain simple and complex lipids. While the simple lipids are mainly the well known triglycerides, the complex lipids include phospholipids, various glycolipids and other phytoglycolipids(1,2,3).

By means of counter-current distribution, Mason and Johnston(4) have recently shown that acetone insoluble flour lipids can be separated into fractions, the phospholipids (of the serine, ethanolamine and choline types) amounting to 50% and the glycolipids, with galactose predominating as the sugar component, amount to 42%. The phytoglycolipid(1) content was much lower, on the average only 4% of the acetone insoluble lipids.

Although the fractionation of flour lipids by means of the counter-current distribution technique was successful, this method has not found wide application in the analysis of wheat lipids. However, the recently introduced methods for separating complex lipids by silicic acid column chromatography and by thin-layer chromatography represent rapid but nevertheless precise analytical procedures, which are now in general use(5,6).

Because of the polar nature of their molecules, the phospholipids in wheat grain are present mainly as lipoprotein complexes, while only a small proportion are present in the free form(7). Although these complexes have been investigated, they are still the source of many difficulties in the analysis of lipids derived either from gluten or flour of various extraction percentages.

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In view of the above difficulties, the aims of the present work were as follows: *(a)* to find a convenient method for further investigation of the isolated lipids in the extraction and purification of the lipids of whole meal flour or its gluten; *(b)* to analyze the separated lipids in detail by modern analytical methods, and *(c)* if possible, to interpret the results achieved from the aspect of the varietal-technological characteristics of the wheat samples investigated, particularly in relation to their baking quality.

MATERIALS AND METHODS

a) Extraction of total lipids

Ground wheat grain (10 g samples), of particle size less than 60 mesh, was extracted with 50 ml of methanol for 24 hours at room temperature, with occasional shaking. Two volumes of chloroform were then added and the mixture brought to gentle boiling for 4—10 minutes. After filtration through a Büchner funnel the residue in the funnel was washed with hot chloroform (50 ml). The crude extract was evaporated to dryness on a water bath and the residue extracted with chloroform. By this procedure the bulk of the impurities, such as starch and proteins, was removed. For further purification the chloroform solution was evaporated to dryness, the residue treated several times with methanol and the solution again evaporated to dryness. The lipids were finally reextracted with chloroform and separated from the residual proteins by filtering on a folded Whatman No. 1 filter paper. The clear solution was concentrated to a small volume, then made up to 10 ml with a solvent mixture of chloroform and methanol (2 : 1 v/v), and the resulting solution of crude lipids was washed with a 0.002 N aqueous solution of $MgCl_2$, according to the method of Folch, Lees and Stanley(8)

b) Extraction of free lipids

The free lipids present in the whole meal flour or in the corresponding gluten were extracted with anhydrous ethyl ether at room temperature. For this the sample was first dried in vacuo over phosphorus pentoxide.

c) Extraction of bound lipids

The bound lipids in the wheat grain or gluten were extracted with hot methanol for 5 hours, from samples from which the free lipids had previously been removed. The extract was evaporated to dryness, the residue dissolved in ethanol-free chloroform and filtered from undissolved proteins, starch and other impurities. The clear lipid solution was brought to dryness, the residue dissolved in boiling methanol and the solution again evaporated to dryness. After this treatment with methanol had been repeated several times, the residue was finally extracted with chloroform, the solution separated from traces of liberated proteins, evaporated to dryness and the residue dried and weighed.

d) Fractionation of total lipids

The lipid solution, purified according to the method of Folch *et al.*,⁽⁸⁾ was brought to dryness and the residue redissolved in 5 ml of pure, ethanol-free chloroform. This solution was chromatographed on a mixture of silicic acid and hyflo super cel (3 : 1). The silicic acid (Mallinckrodt, chromatographic grade, 100 mesh) used for the separation was first washed with boiling methanol and acetone, and activated by heating overnight at 120°. The hyflo super cel was also first washed in the same way, dried in air and then in an oven at 105° for 2 hrs. The quantity of lipids applied to the column (diameter 2.5 cm) was 30 mg per gram of silicic acid.

The fractions of free fatty acids, neutral lipids and some other products* were eluted together with pure chloroform. The phospholipids were then removed using a mixture of equal volumes of chloroform and methanol⁽⁹⁾. Since silica is slightly soluble in methanol, the collected phospholipid fractions were distilled to dryness, and the residual phospholipids redissolved in pure chloroform and separated by filtration from insoluble silica. The combined solutions were then transferred with a Pasteur pipette into a 5 ml flask, evaporated to dryness, and the flask containing the residue filled with nitrogen and weighed.

The collected chloroform eluates were combined, distilled to dryness and the residue redissolved in ethyl ether saturated with water. The free fatty acids were extracted from this solution in a separatory funnel by gentle shaking with 0.5 *N* aqueous KOH⁽⁹⁾. After repeated extractions, the alkaline extracts were combined, acidified with 10 *N* sulphuric acid and the liberated fatty acids extracted with ethyl ether. The combined extracts, after being washed with distilled water, were concentrated to a small volume, transferred to a flask of 5 ml and finally evaporated to dryness. Traces of water in the residue were removed by adding ethanol and distilling the azeotropic mixture formed, while the excess of added ethanol was removed in vacuo at 45—50°. The flask with its content was then filled with nitrogen and weighed.

The ether layer, from which the free fatty acids had been removed, was washed with water, concentrated to a small volume, transferred with a Pasteur pipette into a 15 ml flask and then brought to dryness and weighed. This residue was then saponified with 0.5 *N* ethanolic KOH, under gentle reflux for 2 hrs. The

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* "Other products" means free and esterified sterols, pigments such as lutein, carotene, etc.

saponification product was diluted with water, acidified with 10 *N* sulphuric acid and the liberated acids extracted three times with ethyl ether. The combined ether extracts were re-extracted with 0.5 *N* aqueous KOH as mentioned above, and the separated fatty acids were further treated in the same way as the fraction of free fatty acids. These fatty acids were designated as fatty acids bound to neutral lipids. The ether solution, containing ether soluble non-saponifiable products, was washed with water, concentrated to a small volume, transferred with a Pasteur pipette to a 5 ml flask, evaporated to dryness as described for free fatty acids, and finally weighed. The difference between the original weight of this lipid fraction and the weight of the ether soluble non-saponifiable products was designated as the fraction of neutral lipids. It should be mentioned that all distillations and evaporations to a small volume or to dryness were carried out at temperatures as low as possible and in a nitrogen atmosphere.

e) Determination of phospholipid nitrogen and phosphorus

The phospholipids, isolated from 10 g of whole meal flour or gluten obtained from the same flour sample, were dissolved in chloroform and the solution made up to 10 ml in a volumetric flask. For the estimation of phosphorus an aliquot of 1 ml was taken and after evaporation of the solvent the residue was digested with 1 ml conc. perchloric acid and a few drops of conc. nitric acid. The combustion product was diluted with distilled water to 25 ml, and in aliquots containing about 10 γ of P (2 ml), phosphorus was determined according to the method of Berenblum and Chain(10), or in larger aliquots by the method of Harris and Popat(10).

Nitrogen was estimated from a 1 ml aliquot of the original phospholipid solution by a semimicro Kjeldahl method, in the presence of a selenium catalyst. Liberated ammonia was distilled into 2 percent boric acid in an apparatus according to Markham.

f) Preparation of methyl esters

The methyl esters of the fraction of free fatty acids and of the fraction of neutral lipids were prepared by refluxing the acids for two hours with an excess of methanol, in the presence of 1% conc. sulphuric acid as catalyst. The reaction mixture was then diluted with water, extracted with ethyl ether, and the combined extracts washed with 0.5 *N* aqueous K_2CO_3 and water. The ether solution was evaporated to dryness and the flask containing the esters filled with nitrogen and weighed.

The methyl esters of the fatty acids bound to the phospholipid fraction were prepared by methanolysis of this fraction with 6 *N* hydrochloric acid in absolute methanol (1 ml of this solution per 20 mg of phospholipids), the reaction mixture being refluxed for 5—6 hrs(9).

Until required for further analysis, all the esters were stored as chloroform solutions at 0°.

g) *Separation of fatty acid methyl esters*

The separation and subsequent quantitative estimation of individual methyl esters were done by running a gasliquid chromatogram on a column of acid washed Embacel (60—100 mesh), with ethylene glycol succinate-polymer (EGS) as the polar liquid phase, and argon as the carrier gas. The temperature of the column was 170°. A PYE chromatograph was used with a Sr-90 (20 mC) radioactive source mounted into the detector system.

The stationary phase was synthetized from 11.9 g of ethylene glycol, 22.7 g of succinic acid and 10 g of *p*-toluenesulphonic acid, in the usual way. The low molecular compound were removed in vacuo, the resulting polymer was allowed to solidify, and was then dissolved in chloroform and filtered to remove any insoluble material. Embacel was added to this solution in a proportion of 8 : 2, and after thorough stirring, the mixture was heated on a water bath to remove chloroform. The residue was dried overnight in an oven at 105° and thed used to fill the standard glass column (4 mm × 80 cm).

h) *Silicic acid fractionation of the phospholipid fraction*

The silicic acid fractionation of glycolipids and phospholipids was performed according to Fisher(2), on columns of chromatographic grade Mallinckrodt silicic acid.

Free sterols, sterol esters, mono-, di- and triglycerides and some pigments, such as carotene, were first removed from the column by using ethyl ether as eluent.

The phospholipids and some other fractions were qualitatively separated on a thin layer of silicic acid, activated according to Stahl (Merck) in the usual way and with solvents described elsewhere(6).

i) *Analysis of the lipoprotein complex*

The purified extract of total lipids (from 20 g of whole meal flour) was refluxed for 24 hours with 100 ml of a 21% solution of hydrochloric acid in water-methanol (1 : 1 v/v). The hydrolysis product was evaporated to dryness in vacuo at 60°. The residue was dissolved in 50 ml of water and again brought to dryness. This step was repeated until hydrochloric acid was completely removed. Finally, the residue was dissolved in 10 ml of 10% isopropanol in water, the layer containing the fatty acids was removed, and the residual clear solution was again evaporated to dryness. The residue was dissolved in 0.3 ml of 10% isopropanol in water, and 10—20 microliters of this solution were applied to

Whatman paper No. 1. The chromatogram was developed for 20 hrs, using the descending technique and Hausmann's solvent system⁽¹¹⁾. The amino acids were detected by spraying the chromatogram with a reagent consisting of a 0.2% solution of ninhydrin in acetone which contained 2% collidine, and by allowing the paper to dry overnight. By this method of detection the spots acquired colors specific for each amino acid. The quantitative comparison of the amino acids was made planimetrically, from graphs obtained with the Spinco Analytrol Model RB densitometer, using a 550 mμ interference filter, a neutral density filter 0.6, and Cam B—2.

RESULTS AND DISCUSSION

The disadvantages of solvents such as *n*-butanol, petroleum ether, ethyl ether or ethanol, which are used in various methods for the extraction of total lipids, are well known(12). Of these solvents, water saturated *n*-butanol is the most satisfactory, especially since it readily decomposes the lipoprotein complexes(13). But because of its high boiling point, this solvent may cause oxidation and polymerization of linoleic acid and other unsaturated acids, and is therefore not convenient when further fatty acid analysis is necessary.

In our investigations the application of the chloroform-methanol solvent mixture, in the proportion originally proposed by Folch *et al.*(8), resulted in the complete extraction of lipids from whole meal flour or its gluten. But since a high amount of starch and proteins was extracted together with the lipids, it was necessary to evaporate the extract to dryness and to re-extract the residue with chloroform. However, the lipids isolated under these conditions still contained a variable amount of protein impurities. Therefore this residue was vigorously treated with hot methanol (after the removal of chloroform). Nevertheless, the residue obtained from methanol and dissolved in chloroform still contained a certian amount of lipoprotein complex: however, this amount was no longer variable but constant and characteristic for a given wheat variety.

The purification of lipids with the mixture chloroform-methanol-water can also be useful, since in this way nonlipid phosphorus is removed from the extract.

The amounts of total lipids and some of their fractions in whole meal flour and gluten of some wheat varieties, determined according to the methods described above, are listed in Tables 1,2 and 3.

As evident from Table 2, ether-soluble free lipids isolated from gluten contain only a small amount of phosphorus. This indicates that practically all the phospholipids in gluten are present in the bound from, *i. e.* as a lipoprotein complex.

The results obtained by the extraction of bound lipids with chloroform-methanol and the subsequent elimination of protein

TABLE 1

The contents of total lipids and some of their fractions present in the grain of wheat varieties of good baking quality*

Lipid fractions (in *mg* per 100 *g* of grain)		Wheat varieties			
		Bankut 1205	***Novosadska 1439***	***Novosadska 1446***	***Stara Banatska***
Moisture content of the grain (in %)		11,4	10,4	11,3	11,2
Neutral lipids		958,9	1388,0	920,4	1226,3
Ether soluble non-saponifiable products		103,9	131,7	105,9	121,6
Phospholipids		650,1	657,3	617,5	391,9
Fatty acids	free	624,1	182,0	753,2	296,2
	bound to neutral lipids	693,1	1323,0	462,5	1144,0
	bound to phospholipids	426,5	446,4	349,5	265,8
Total lipids		2337,0	2359,0	2397,0	2036,0

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* In this and the following tab'es all the results are based on dry matter.

TABLE 2

The content of lipid franctions in the gluten of some low yielding (Yugoslav) and high yielding wheat varieties

Phosphorus forms and lipid fractions		Wheat variety							
		Bankut 1205	*Novosadska 1993*	*Rumska Crvenka*	*Abbondanza*	*Autonomia*	*Etoile de Choisy*	*Produttore*	*Elia*
		in % of dry gluten							
Total *P* of gluten		0,452	0,453	0,318	0,440	0,360	0,240	0,297	0,391
Acid soluble *P*		0,240	0,294	0,240	0,246	0,192	0,122	0,153	0,229
Phospholipid *P*	total	0,122	0,108	0,036	0,19	0,081	0,045	0,071	0,079
	free	0,009	0,005	0,005					
	bound	0,113	0,103	0,031					
Total lipids		10,80	10,21	4,20	10,13	6,04	9,83	9,84	10,26
Free lipids		2,75	2,65	1,39	2,7	1,57	3,14	3,24	2,87
Bound lipids		8,05	7,55	2,81	7,3	4,47	6,69	6,60	7,39
Phospholipids		3,56	3,16	1,05	3,3	1,75	2,75	2,46	2,82
		in % of total P of gluten							
Acid soluble *P*		53,1	63,6	75,5	56,0	53,0	50,2	51,5	58,7
Phospholipid *P*	total	27,0	23,4	11,3	27,1	22,5	19,19	24,0	20,0
	free	2,1	1,2	1,6					
	bound	24,9	22,2	9,7					
		in % of total lipids							
Free lipids		25,5	26,0	33,1	26,5	26,0	32,0	32,5	28,0
Bound lipids		74,5	74,0	66,9	73,5	74.0	68,0	67,5	72,0

TABLE 3

Nitrogen and phosphorus contents of wheat grain phospholipids and whole wheat grain

Wheat variety	Phospholipid-*N* (as % of grain phospholipids)	Phospholipid-*P* (as % of grain phospholipids)	Phospholipid ratio *N/P*	Phospholipid-*N* (in *mg* per 100 *g* of grain)	Whole grain total *N* (in %)	Phospholipid-*N* (as % of grain total *N*)	Phospholipid-*P* (in *mg* per 100 *g* of grain)	Whole grain total *P* (in %)	Phospholipid-*P* (as % of grain total P)
Bankut 1205	1,00	1,70	1,30	6,50	2,75	0,24	11,05	391,0	2,83
Novosadska 1439	1,25	1,83	1,51	8,22	2,62	0,31	12,03	411,5	2,92
Novosadska 1446	1,28	1,76	1,61	7,90	2,71	0,29	10,88	377,8	2,88
Stara Banatska	1,26	1,82	1,53	4,94	2,76	0,18	7,13	415,6	1,72

impurities, indicate that in wheat varieties of good baking quality about 50% of bound lipids are actually phospholipids.

The high content of acid-soluble phosphorus present in gluten is also significant. As shown recently(14), this form of phosphorus in wheat grain is highly influenced by crop production technique and is mainly present in the form of phytic acid. It is probable that this phytic acid form of phosphorus is also present in gluten in the form of a complex, *i.e.* bound to proteins, and that it is partially dissolved by vigorous lipid extraction. As can be seen from Table 4, crude lipid extracts, washed by the procedure of Folch *et al.*(8), lose on average 17 per cent of non-lipid phosphorus*. Similar losses when crude lipid extracts were purified by passage through a cellulose powder column(2) were reported recently.

The analysis of wheat lipids showed that the amount of the phospholipid fraction present in whole meal flour was very similar to the amount found in the gluten of the same weight of flour. This result is of considerable interest, because it indicates that the available phospholipids in the grain of wheat varieties of good baking quality are mainly engaged in the formation of lipoprotein complexes, which represent one of the components responsible for the elastoplastic properties of flour dough(15).

As can be seen from data given in Table 5, this results is supported in part by the farinological analyses of some of the wheat varieties investigated.

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* With the exception of the variety *Rumska Crvenka*

TABLE 4

"Loosses" observed after the purification of crude lipid extracts according to the procedure of *Folch et al* (8)

Variety	Before purification		After purification		Loss	
	Lipids (in *mg*)	Phospholipid-*P* (in *mg*)	Lipids (in *mg*)	Phospholipid-*P* (in *mg*)	Lipids (in %)	Phospholipid-*P* (in %)
Bankut 1205	188,6	0,880	171,1	0,724	9,26	17,77
Novosadska 1446	225,4	0,982	214,8	0,824	4,71	16,07
Novosadska 1993	177,0	0,845	175,5	0,697	0,84	17,50
Rumska Crvenka	175,2	0,192	151,4	0,180	13,69	6,25

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* With the exception of the variety *Rumska crvenka*

TABLE 5

Mean farinological values of flours prepared from some wheat varieties*

Farinological characteristics	Wheat variety			
	Bankut 1205	*Novosadska 1439*	*Novosadska 1446*	*Rumska Crvenka*
Year of crop	1958—1959	1958	1958—1959	1959
Wet gluten (%)	36,4	35,0	36,3	28,4
Dry gluten (%)	12,7	11,0	12,2	9,5
Gluten extensilibity**	strong			very strong
Water absorption (%)	65,7	67,3	68,2	
Dough development in mins.	3,25	2,50	4,10	1,75
Dough stability in mins.	1,50	1,00	1,50	1,25
Softening grade *(F.u)*	70	105	60	155
Quality index	65,0	53,2	70,3	35,6
Quality grade***	B-1****	B-2	A-2	C-1
Energy in cm^2	72,8	29,3	70,1	
Resistance to extension in *C* units	225	120	255	
Extensibility in *mm*	171	163	164	
Resistance/extensibility	1,37	0,73	1,67	

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* Analyses correspond to the flour of 60-70% extraction

** Estimated by *Kranzkosmin* method

*** Graded in decreasing order of baking quality, *viz.* A-1, A-2, B-1, B-2, C-1 and C-2

**** The quality grade of the variety *Stara Banatska* was also B-1

In order to establish possible qualitative differences in various lipid fractions and correlate them with varietal characteristics (particularly baking quality), an attempt was made to separate the wheat lipids by paper chromatography, using Whatman No. 1 paper impregnated with silicic acid and the solvent system described by Marinetti and Stotz(16). As this procedure failed to give satisfactory results, either with a total lipid sample or with individual lipid fractions previously separated on a column of silicic acid and hyflo super cel (3 : 1), an attempt was made to separate the lipids by replacing the acetic acid in the solvent system of Marinetti *et al.*(16) with pyridine(16) or methanol, according to the modification of Hack and Leatherman(16). As adequate separation was not achieved, even by the application of much smaller amounts of lipids and by using the very sensitive reagent protoporphyrin(17), our further attempts were concetrated on the separation of lipids by thin layer chromatography on silicic acid. By this procedure satisfactory results were obtained for all lipids fractions, and some of these results are illustrated in Fig. 1.

The separation obtained for the fractions of neutral lipids, phospholipids, and ether soluble non-saponifiable products on thin layers of silicic acid, showed that in these fractions there were no significant differences which could be assigned to the wheat variety and its technological properties.

The results obtained for the fatty acid composition of the fractions of free fatty acids, neutral lipids and phospholipids are given in Table 6.

As evident from Table 6, of the total acids present in each fraction, approximately 74—81 per cent are unsaturated acids, linoleic acid being predominant. Of the saturated fatty acids, palmitic acid predominates in all fractions while the acids with a lower number of carbon atoms are present only as minor constituents.

The detection of fatty acids with an odd number of carbon atoms in all the lipid fractions of the wheat varieties investigated is of considerable interest. As can be seen from the gas-liquid chromatograms given in Figure 2,3 and 4, their biosynthesis seems to be favored to practically the same extent as the biosynthesis of lauric, myristic or palmitoleic acid.

Of the fatty acids found in wheat grain, those which are present in the phospholipid fraction are undoubtedly of primary significance for dough quality. In the presence of water during the doughing process, phospholipid molecules, with their hydrophilic phosphoric acid groups and hydrophobic fatty acid chains, form highly oriented bimolecular leaflets, which bind to the protein platelets in gluten, thus producing the lipoprotein complexes responsible for the elastoplastic properties of dough(15). Of these acids, linoleic acid is present in large excess. The doublet of its metastable form (peak 11 on the gas-liquid chromatogram in Fig. 4), detected among fatty acids, is identical in its nature and position with the doublet recently discovered by Coppock and Daniels(20).

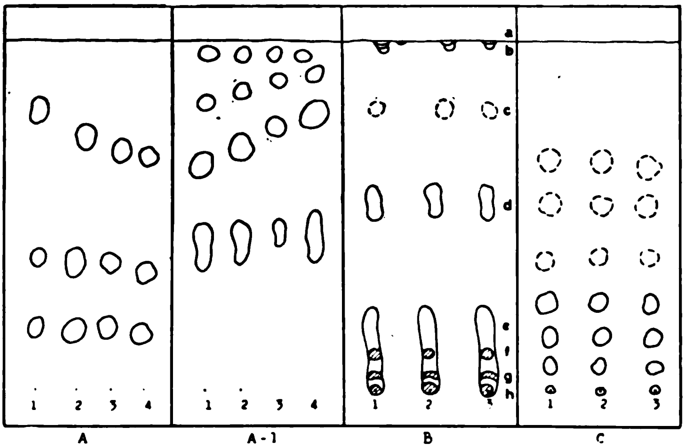

Fig. 1

Separation of the individual lipid fractions on a thin layer of silicic acid

A. Phospholipid fraction. On plates applied: 0.06—0.1 γ as chloroform solution.
Solvent used: chloroform-methanol-water (65:25:4 *v/v*).

Spraying reagent: 0.2% ninhydrin in acetone (*Rf* values of spots: 0.16, 0.35, and 0.73)

A—1. Indentical conditions as for A, except that the spraying reagent was that of *Hanes & Isherwood* (6)

1. *Autonomia* 2. *Bankut 1205* 3. *Stara Banatska* 4. *Novosadska 1446*

B. Fraction of neutral lipids. On plates applied: 500 γ as chloroform solution. Solvent used: ethyl ether-petroleum ether (BDH., b. p, 40—60°), 10:100 (*v/v*). The spots were detected in UV light, after spraying the chromatogram with a solution of 0.2% 2', 7' — dichlorofluorescein in 95% ethanol, and then with 50% sulphuric acid

(*Rf* values: *h* — phospholipid traces, 0.04; *f* and *g* — free sterols, 0,05 and 0.1; *e* — triglycerides and free fatty acids; *d* — triglycerides, 0.55; *c* — traces of methyl esters, 0.79; *b* — unsaturated sterol esters, 0.94; *a* — saturated sterol esters, 1.00)

1. *San Pastore* *Bankut 1205* *Novosadska 1446*

C. Fraction of ether soluble non-saponifiable products. On plates applied: 50 γ as chloroform solution. Solvent used: ethyl ether-petroleum ether (BDH, b. p. 40—60°) (20:100 *v/v*). The spots were detected by spraying the plates with 50% sulphuric acid, and heating the plates at 80° for 15 mins. (*Rf* values: 0.12, 0.23, 0.33, 0.38, 0.56, and 0.67)

1. *Bankut* 2. *Fortunato* 3. *Novosadska 1446*

Our investigations showed that the appearance of this form of linoleic acid is exclusive to the phospholipid fraction of wheat flour. This fact and the role attributed to the metastable form of linoleic acid (oxygen transfer in the oxidation processes taking place in the course of baking) would suggest the possibility of a partial correlation existing between the contents of linoleic acid, *i. e.* its metastable form, and the baking quality of wheat varieties.

TABLE 6

Fatty acid composition of the fractions of free fatty acids, neutral lipids and phospholipids, expressed in percentage of the total acids present in the given fraction. Separation achieved by gas-liquid chromatography

Fatty acid methyl ester (*C* atoms: double bonds in acid)	Retention times	Fatty acid	Fatty acids of the fraction of free fatty acids				Fatty acids of the fraction of neutral lipids				Fatty acids of the fraction of phospholipids			
			Bankut 1205	*Novosadska 1439*	*Novosadska 1446*	*Stara Banatska*	*Bankut 1205*	*Novosadska 1439*	*Novosadska 1446*	*Stara Banatska*	*Bankut 1205*	*Novosadska 1439*	*Novosadska 1446*	*Stara Banatska*
12:0	0.16	*Lauric*	0.4	0.9	*tr**	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*
14:0	0.33	*Myristic*	0.3	1.1	0.2	0.7	0.4	*tr*	0.2	*tr*	*tr*	*tr*	*tr*	*tr*
15:0	0.41	*Pentadecanoic*	0.2	*tr*	0.1	*tr*	0.4	*tr*	0.1	*tr*	*tr*	*tr*	*tr*	*tr*
16:0	0.55	*Palmitic*	18.3	28.6	21.8	22.5	19.5	15.6	17.1	16.0	17.6	18.0	18.4	20.2
16:1	0.73	*Palmitoleic*	0.2	*tr*	0.2	*tr*	0.3	0.3	0.7	0.3	*tr*	*tr*	*tr*	*tr*
17:0	0.78	*Heptadecanoic*	0.2	*tr*	0.3	*tr*	*tr*	*tr*	0.2	*tr*	*tr*	*tr*	*tr*	*tr*
18:0	1.00	*Stedric*	1.6	4.2	1.3	3.1	1.9	1.0	1.1	1.1	1.7	1.3	1.2	2.2
18:1	1.19	*Oleic*	16.4	13.9	16.7	14.2	19.2	18.3	17.0	17.9	11.0	11.3	10.9	11.0
18:2	1.54	*Linoleic*	58.2	48.2	55.5	55.3	54.3	60.7	60.6	60.2	67.1	66.3	66.8	63.2
18:3	2.11	*Linolenic*	4.2	3.1	3.9	4.2	4.0	4.1	3.0	4.5	2.6	2.9	2.7	3.4

* *tr – traces*

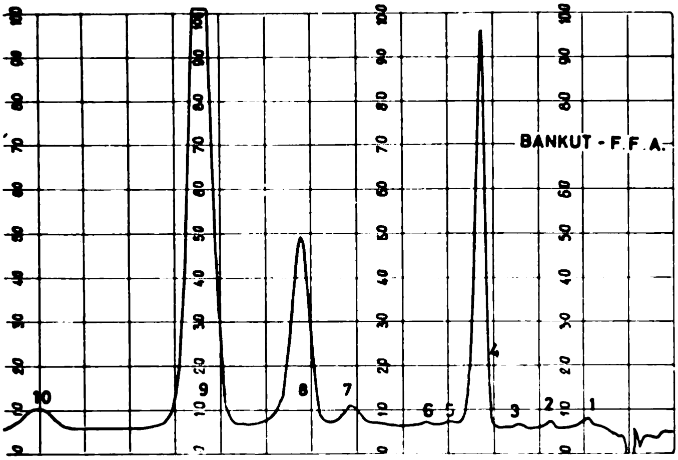

Fig. 2

GLC of fatty acids of the fraction of free fatty acids from the grain of wheat variety *Bankut 1205*

Experimental data of separation: Column, acid washed Embacel/*EGS*, 8:2; column temperature 170°; argon flow rate 50 *cc/min*; detector voltage 1250 V; recorder sensitivity x 3; chart speed 30"/*h*; volume of esters applied 0.05 microliter

1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Heptadecanoic acid; 7. Stearic acid; 8. Oleic acid; 9. Linoleic acid; 10. Linolenic acid

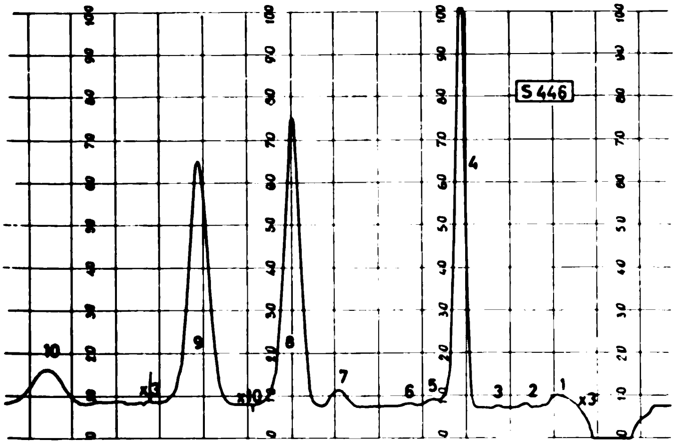

Fig. 3

GLC of fatty acids of the fraction of neutral lipids from the grain of wheat variety *Novosadska 1439*

Experimental data of separation as mentioned in Fig. 2, with the following exceptions: recorder sensitivity for linoleic acid x 10 and applied esters volume 0.1 microliter.

1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Heptadecanoic acid; 7. Stearic acid; 8. Oleic acid; 9. Linoleic acid; 10. Linolenic acid.

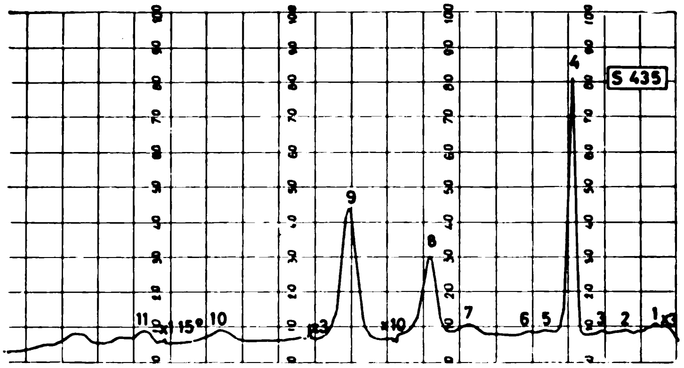

Fig. 4

GLC of fatty acids of the phospholipid fraction from the grain of wheat variety *Bankut 1205*

Experimental data of separation as in Fig. 3, but after linolenic acid methyl ester the recorder sensitivity was x 1 and the chart speed 15"/hr

1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Heptadecanoic acid; 7. Stearic acid; 8. Oleic acid; 9. Linoleic acid; 10. Linolenic acid; 11. "Mestable" linoleic acid

Although this study included two quality grades, A—2 (*Novosadska 1446*) and B—2 (*Novosadska 1439*), this correlation could not be confirmed (Table 6). However, further investigations of wheat varieties of different quality grades, now in progress, suggest the possibility of such a correlation.

In order to establish further possible differences in the polar lipid fractions of whole meal flour, which might be related to the different baking quality of the wheat varieties the phospholipid fractions of the flour of quality grades A—2 (*Novosadska 1446*) and C—2 (*San Pastore*) were separated on a silicic acid column. Elution diagrams of these separations are given in Figure 5.

As evident from the elution diagrams, the stepwise increase of methanol in ethyl ether separates the phospholipid fraction into six well defined subfractions. Although some glycolipids are found in nearly all peaks, they are mainly concentrated in peak *B* (galactose reaction with anthrone reagent). The lipoprotein complex is chiefly present in peak *D*, while smaller amounts are also to be found in peaks *B* and *C* (ninhydrin reaction after hydrolysis). The other peaks consist chiefly of phospholipid phosphorus.

A comparison of the elution diagrams of the two wheat varieties (Fig. 5) shows that a certain difference exists in the composition of peaks *B* and *D*. These peaks are richer in lipoprotein complexes in the variety *Novosadska 1446*, whereas in the *San Pastore* variety they are richer in glycolipid content. These findings of increased and decreased amounts of lipoprotein complexes in whole meal flour of good and poor baking qualities are in fairly good agreement with the values obtained for phospholipid nitrogen and phosphorus and their atomic ratio (N/P) (see Table 3).

The variable amount of the lipoprotein complex present in the flour of different wheat varieties is a fact of considerable importance. Therefore, the next step should be to find out if the composition of these complexes varies with the wheat variety? Total hydrolysis of the isolated complex and separation of the liberated amino acids by paper chromatography (see Fig. 6) gave practically identical chromatograms for different quality grades investigated. On all chromatograms the following amino acids were present: alanine, asparagine, glutamic acid, arginine, cystine, glycine, histidine, leucine + isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine.

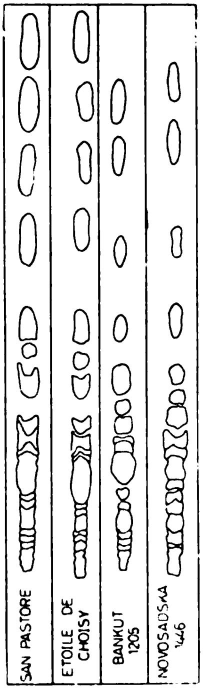

Fig. 6

Chromatograms of amino acids present in the hydrolyzate of the complexes isolated from whole meal flour of the wheat varieties *Bankut 1205*, *Etoile de Choisy*, *Novosadska 1446* and *San Pastore*

Paper used, Whatman No. 1; solvent system, *n*-butanol-formic acid-water (75:15: 10 *v*/*v*). Developed by the descending technique at room temperature for 20 hrs. [See Materials and methods (*i*)]

Earlier investigations have shown that acid hydrolysis of the lipoprotein complexes furnishes at least nine amino acids(18). Bottomley *et al.*(19) and Bourdet and Herard(7) have found that there are more of these acids and that the amounts of glutamic acid and proline are lower and those of cystine and lysine higher in these complexes than in the total hydrolysates of gluten. Our results indicate that the amino acid composition of these complexes expressed as percentage of the total amino acids present (similarly to the expression used for the fatty acid content), is practically constant, regardless of the variety investigated.

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SEMIQUANTITATIVE DETERMINATION OF MICROQUANTITIES OF IONS BY VISUAL COLORIMETRY OF SPOTS OBTAINED BY PARTITION PAPER CHROMATOGRAPHY

II. THE DETERMINATION OF NICKEL, COBALT, MANGANESE, CALCIUM, STRONTIUM, AND BARIUM

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We recently reported on the determination of microquantities of mercury, copper, cadmium, zinc, uranium and iron by this method.(1) We have further investigated the applicability of this method and in this paper we will report on determination of microquantities of nickel cobalt, manganese, calcium, strontium, and barium. Nickel, cobalt and manganese, or calcium, strontium and barium, were first separated by ascending chromatography on narrow strips of filter paper*) and were then detected with the appropriate reagents. The spots of separated elements obtained were compared with the standard scale spots in the way described in our first report.

For the separation of nickel, cobalt and manganese**) a mixture of 87 ml of acetone, 8 ml of hydrochloric acid (sp.g. 1.19) and 5 ml. of water(2) was used. The developing time was 2 hours. Detection was done with a 1% — alcohol solution of salicylaldoxime and dimethylglyoxime after which the strips were exposed to ammonia vapor. A red spot for nickel, a green spot for manganese and a dark spot for cobalt were obtained. As this reaction is not senstitive enough for the detection of cobalt it is better to detect it with a 1%—solution of alpha-nitroso-beta-naphthol in 50%— — acetic acid (a dark red spot is obtained). The following R_f-values were obtained: nickel 0.06, manganese 0.4 and cobalt 0.8.

As a solvent for the separation of calcium, strontium and barium, a mixture of 85 ml of absolute ethanol and 15 ml of water(3) was used. Developing took about 2 and a half hours. Detection was done by soaking the strips with the separated chlorides of these elements in a 0.1 N solution of silver nitrate. After this the strips were rinsed with water and put into a solution

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*) For determination of nickel, cobalt and manganese the strips were 0.5 cm wide while for the alkaline-earth metals they were 1 cm wide. The strips were of the Whatman No. 1 paper.

**) Solutions of all elements determined here were made by dissolving the corresponding chlorides.

of ammonium sulfide. Where there were separated elements black spots of silver sulfide appeared. These R_f-values were obtained: barium 0.28, strontium 0.45 and calcium 0.63.

The results of nine successive determinations of each element are shown in Table 1. The mean deviation for nickel was 3.6 %, for cobalt it was 2.4 %, for manganese 3.8 %, for calcium 4.5 %, for strontium 3.8 % and for barium 3.5 %. Since one determination needs only about 10 γ of each ion the results are satisfactory. The accuracy is the same as that obtained by other micromethods. By this method, nickel, cobalt and manganese, or calcium, strontium and barium in a mixture can be determined at the same time, which is an advantage over many other micromethods for the determination of these elements. So far 12 elements have been determined by this method, but by this its possibilities have been only fractionally exploited. Its further possibilities are being investigated.

TABLE 1

Concentration of solution mg/ml			
Calculated	Found	Calculated	Founrd
Ni^{2+} in the presence of Co^{2+} and Mn^{2+}		Ca^{2+} in the presence of Sr^{2+} and Ba^{2+}	
0.615	0.648	0.40	0.37
0.615	0.575	0.40	0.39
0.519	0.542	0.40	0.38
0.519	0.495	0.36	0.34
0.519	0.524	0.36	0.38
0.599	0.578	0.36	0.35
0.599	0.599	0.44	0.47
0.599	0.597	0.44	0.46
— — —	— — —	0.44	0.44
Co^{2+} in the presence of Ni^{2+} and Mn^{2+}		Sr^{2+} in the presence of Ca^{2+} and Ba^{2+}	
0.289	0.289	0.92	0.87
0.289	0.289	0.92	0.86
0.289	0.287	0.92	0.90
0.261	0.255	1.10	1.04
0.261	0.268	1.10	1.01
0.261	0.241	1.10	1.07
0.230	0.229	1.06	1.05
0.230	0.223	1.06	1.09
0.230	0.218	1.06	1.08
Mn^{2+} in the presence of Ni^{2+} and Co^{2+}		Ba^{2+} in the presence of Ca^{2+} and Sr^{2+}	
0.131	0.137	2.01	2.02
0.131	0.142	2.01	2.02
0.131	0.140	2.01	2.07
0.137	0.132	2.07	2.06
0.137	0.135	2.07	2.08
0.137	0.132	2.07	2.10
0.120	0.117	2.27	2.07
0.120	0.121	2.27	2.11
0.120	0.122	2.27	2.07

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MECHANISM OF THE ELECTROLYTIC FORMATION OF GERMANIUM HYDRIDE

by

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In the electrolysis of germanium from aqueous alkaline solutions, at a definite cathode potential, above the catholite, there is evolved, in addition to hydrogen, a very volatile germanium compound—germanium hydride. By passing the obtained mixture of gases—hydrogen and germanium hydride—through a quartz tube heated to above 360°, the complete thermal decomposition of germanium hydride into hydrogen and metallic germanium takes place; the metallic germanium deposits on the walls of the tube in form of metallic powder. By heating the metallic germanium above its melting point it fuses and on cooling polycrystalline germanium of a very high purity is obtained.

The problem of the mechanism of the electrolytic separation of germanium from aqueous solution, and of the electrodeposition of germanium in general, have been very little examined so far. Winkler [1'2] was the first to attempt the quantitative electrolytic separation of germanium from aqueous solutions. Some years later Hall and König (3), and Schwarz (4) attempted to obtain a firm metallic deposit of germanium at a copper cathode. However, as soon as the cathode was covered with a layer of germanium, further deposition of the metal soon stopped. Land (5) carried out the electrolysis of germanium with a platinum anode and platinum cathode, the latter being first covered with freshly obtained electrolytic copper. Duiring the electrolysis, the metal was deposited at the cathode in the form of a fine silver-grey precipitate. Microscopic investigations proved that germanium was deposited uniformly and firmly an the copper in the amount needed to cover the whole active surface of the cathode.

We have also succeeded in obtaining pure metallic germanium with small current densities and with a sufficiently high hydrogen overpotential at the cathodes under certain conditions.

While studying the process of the electrolytic formation of germanium hydride under various experimental conditions, we came to the following observations:

1. the formation of germanium hydride does not take place until the evolution of hydrogen at the cathode is clearly visible, regardless of the kind of the cathode and the electrlolyte employed;

2. In case the evolution of hydrogen in the course of the germanium electrolysis is visible, no germanium deposit is found at the cathode after the electrolysis.

In order to find the optimal conditions for the electrolytic formation of germanium hydride and to study the mechanism of the process itself, we investigated the dependance of the current efficiency in hydride formation upon the cathode current density for some metals and the dependance of the current efficiency upon the initial concentration of germanium in the electrolyte. The current efficiency of hydride formation was taken as the ratio of the theoretical quantity of electricity required for the reduction of a given quantity of germanium and the stechiometrical quantity of hydrogen necessary for the formation of GeH_4, to the amount of electricity actually consumed. This current efficiency represents a criterion for the share of germanium and hydrogen reduction in the whole process of the electrolysis; this is one of the factors necessary for the explanation of the essential features of the mechanism of the electrolytic formation germanium hydride.

Some of the results are shown graphically in Figs. 1 and 2. With increasing of the cathode current density the current efficiency at copper, zinc and lead electrodes increases, reaches a maximum, and then decreases gradually. The maximal current efficiency is almost the same for all kinds of cathodes but comes at different current densities. The differences in the current efficiency between various metals are considerably greater away from the maximum.

The change of the current efficiency in dependance with the density at a germanium cathode differs considerably from that at copper, lead and zinc cathodes. There is no maximum but instead a constant increase of the current efficiency with increasing current density (in the current density range studied).

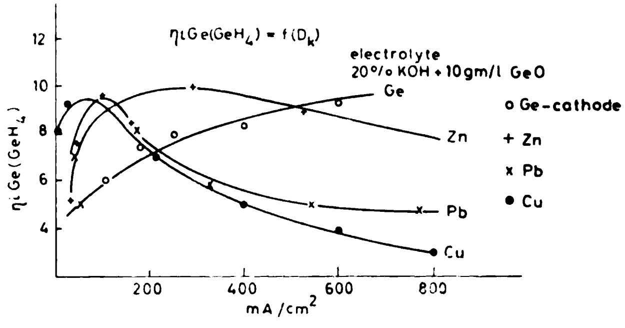

Fig. 1

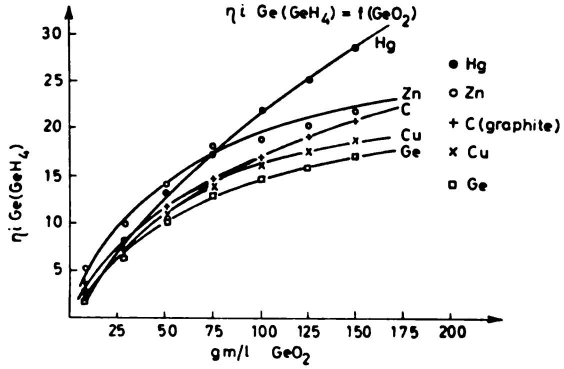

Fig. 2

The strongest influence on the current efficiency is exercised by the concentration of germanium in the electrolyte. The increase of the initial germanium concentration increases the current efficiency for all the kinds of electrodes investigated — copper, zinc, mercury and graphite. However, the rate of the current efficiency increase is different for different metals. A more rapid increase is found with metals exhibiting a higher hydrogen overpotential.

Broadly speaking, the formation of germanium hydride as a result of the simultaneous electrolytic reduction of germanium and hydrogen ions can be achieved in several ways. However, for the formation of germanium hydride in the electrolysis of an aqueous solution of germanium, it is necessary (although not sufficient) that the following two requirements be fulfilled:

1. The atoms of germanium formed by electrochemical reactions at the cathode should not form a crystal lattice but should enter into the reaction of hydride formation immediately after the charge transfer:

2. the atoms of hydrogen formed by electrochemical reactions at the cathode should not recombine to give H_2 molecules, but should (as absorbed atoms) enter immediately into reaction with the germanium atoms.

From our results we may draw the conclusion that the process of the electrolytic reduction of hydrogen proceeds mainly at the active surface of the metal of the cathode, i.e. at the primary cathode, and only to an insignificant extent at the secondary cathode, i.e. at the germanium which is deposited during electrolytic reduction reactions at the cathode. The hydride formation and the current efficiency of the process itself, for different cathodes show evident differences. Had the electrolytic reduction of hydrogen taken place at germanium, the reaction would have been the same regardless of the metal used as the base for the germanium.

, According to the well known mechanism of the electrolytic separation of hydrogen, only the Volmer reaction is of importance in the proces of hydride formation. In fact, the reaction of the hydride formation should take place as a continuation of the Volmer reaction:

$$H_2O + M + e = MH + OH^- \qquad (1)$$

Immediately after the charge transfer, the hydrogen atoms which are adsorbed on the electrode should enter into reaction with germanium atoms liberated immediately after their charge transfer. If we assume that the concentration of hydrogen atoms is sufficiently high, the overall reaction of the hydride formation would be as follows:

$$nMH + Ge = GeH_4 + (5n-4)MH + 4M \quad (2)$$

Since the collision of one germanium atom with at least four adsorbed hydrogen atoms in unlikely to occur at one place, we assume that this reaction proceeds stepwise. The intermediary products are lower hydrides, which then react with adsorbed hydrogen atoms until the compound GeH_4 is formed. The molecules of germanium hydride which are adsorbed and are sufficiently stable to exist. They are desorbed from the surface of the electrode and leave the reaction layer.

From the above it follows that the atoms of germanium formed and adsorbed at the electrode may react in two different ways:

1. they may diffuse through the free metal surface of the electrode first to the boundary of a metal grain, and then up that border to a place favorable for their incorporation into the crystal lattice;

2. they may enter into reaction with hydrogen atoms adsorbed on the free metal surface of the electrode, and by stepwise reaction give rise to stable germanium hydride, GeH_4.

According to the already mentioned works of some authors (3,4,5) and in accordance with the results of our investigations, the atoms of germanium formed by the electrolytic reduction form a firm metallic coating — crystalline phase — only when they are separated at the free surface of the primary cathode under strictly specific conditions. The atoms of germanium formed under these specific conditions of electrolytic reduction can find favorable places on the free surface of the primary cathode and they are first incorporated et the boundaries of the metal grains, initiating the formation of the crystal lattice. When the active surface of the primary cathode is covered with a layer of metallic germanium formed further diffusion and incorporation of newly formed germanium atoms into the crystal lattice is more difficult. In our opinion further deposition of germanium is difficult mainly because of too low a hydrogen overpotential at the electrolytically deposited germanium. So long as the active surtace of the primary cathode contains free places which are not covered with germanium, the overpotential of hydrogen is sufficiently high to bring about only the electrolytic reduction of germanium at low current densities.

As soon as the active surface of the cathode is covered with germanium, the character of the cathode changes and the hydrogen overpotential at the newly formed cathode is so small that further electrolysis proceeds almost exclusively by the evolution of hydrogen at the cathode. Along with the reduction of hydrogen, further reduction of germanium ions will take place only if the potential of the cathode is made sufficiently negative. However, the germanium atoms formed by electrolytic reduction under the new conditions are no longe able to diffuse freely and to find suitable places for incorporation into the crystal lattice. On their way they are exposed to the action of adsorbed hydrogen atoms, react with them and give rise to germanium hydride. The lattice energy of germanium is 42 kcal per gram atom, whereas the formation of germanium hydride from germanium and hydrogen atoms requires 39.7 kcal per mole.

From the above data it follows that the atoms of germanium formed by eletrolytic reduction of germanium solutions are more likely to react with hydrogen atoms, which are present in sufficient concentration, and form germanium hydride, than to incorporate by surface diffusion into the crystal lattice of germanium.

The hydrogen atoms formed by electrolytic reduction of aqueous solutions may react in three different ways.

1. two adsorbed hydrogen atoms may recombine to give hydrogen molecules (Tafel reaction $MH + MH = H_2 + 2M$).
2. the electrolytic reduction of hydrogen at a place where another hydrogen atom is already adsorbed may produce a hydrogen molecule (Heyrovsky reaction $MH + H_2O + e = H_2 + M + OH^-$);
3. hydrogen atoms may react with germanium atoms which are also formed at the cathode and give germanium hydride.

From our experimental results, especially the current efficiency data we believe that in practice the formation of hydride involves all three reaction paths.

The appearance of germanium hydride above the catholite confirms the third reaction path. It has already been shown that the formation of germanium hydride can be achieved by reaction between germanium and hydrogen atoms only when they are simultaneously formed by the electrolytic reduction under certain conditions.

Relatively low current efficiency in the electrolytic formation of germanium hydride proves that a considerable amount of hydrogen formed by electrolytic reduction does not react with germanium but leaves the reaction medium in the form of hydrogen molecules; this is possible only by the reaction paths 1 and 2. The second path (Heyrovsky reaction) is more probable in case the electrode surface is covered to a high degree with hydrogen atoms, while path 1 (Tafel reaction) follows the Volmer reaction, which is more probable in case the electrode surface is poorly covered with hydrogen atoms. Under conditions which favor neither one reaction or the other, both take place and their contributions to the final reaction products depend upon the experimental conditions.

For the process of hydrogenation preceded by the electrolytic reduction at the active surface of the primary cathode the third reaction path is optimal. In the Tafel and Heyrovsky reactions hydrogen atoms produce hydrogen molecules which are of no interest for the process of hydrogenation. Since the formation of hydrogen by the Tafel reaction requires 102.6 kcal per mole, this reaction is less favorable energy wise than the reaction of germanium hydride formation. However, for the formation of this compound a high germanium concentration is essential and this is not easily achieved by electrolysis. When the germanium concentration is not sufficiently high, the contribution of the Heyrovsky reaction is greater and therefore the current efficiency is lower.

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A CONTRIBUTION ON THE HYDROMETALLURGICAL PRODUCTION OF MERCURY FROM MERCURY ORES OF THE ŠUPLJA STENA AREA — AVALA

by

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At present mercury is mainly produced pyrometallurgically by roasting and distilling mercury ores or mercury concentrates with an excess of oxygen in cylindrical rotatory furnaces, and subsequent condensation of mercury vapors.

The oxidative roasting of the raw material at a temperature from 700 to 750° results in the decomposition of mercuric sulphide and the formation of metallic mercury, which then intensively evaporates and is carried over with other gaseous products to the condensing system.

This procedure has a number of great disadvantages, such as: the small capacity of the furnace, the difficulty of hermetically closing the furnace and the condensing system (which leads to considerable losses of mercury and danger to the workers), the difficulty of regulating the function of the furnace automatically, the agressivness of the gaseous products (requiring frequent changing of the gas outlet system) etc.

At the same time with the exploitation of mercury deposits the natural reserves of rich ores are diminishing and this imposes an orientation to the exploitation of lower—grade ores. For these reasons it is indispensable to find new, more effective highly productive and economically profitable processes for the treatment of mercury ores.

In recent years there have been extensive studies and investigations in USSR on the applicability of roasting mercury ore in a fluidized bed, to find the most rational construction of the furnace and method of heating and to determine the main technological and economic indicators of the process(1). Roasting in a fluidized bed in shacht-type furnaces (kilns), although in the chemical process identical with roasting in cylindrical furnaces gave considerably better processing. The system was hermetically closed and the process completely automatized. The percentage yield of mercury was increased by from four to five percent. The productivity of the furnace was increased by from five to nine times, since the time of keeping the ore in the furnace was reduced from sixty to forty minutes. The reduced time of roasting was made possible by intense turbulence, which permitted considerable decrease of external diffusion resistence of the physico-chemical processes and improved the heat exchange.

Along with the improvement of the technology of mercury production by pyrometallurgical procedures, investigations have been made on hydrometallurgical production of mercury, based on the known solubility of mercuric sulphide in sodium sulphide solution:

$$HgS + Na_2S = HgS \cdot Na_2S$$

From the obtained double salt which passes into the solution, mercury is precipitated with metallic aluminum,

As early as 1932, Plaskin and Fishkova investigated the possibility of applying these methods to the processing of Rusian ores, and established the effects of individual factors on the percentage of mercury extraction(2). They obtained satisfactory results since under the given experimental conditions of leaching, the percentage of mercury extraction was over 95 percent.

A few years later, Ivković(3) confirmed the results of Plaskin and Fishkova by leaching the ore from the village Mišić (Dalmatia), and established that under the given experimental conditions the extraction of mercury was successful.

The latest works of Stickney and Town(4) have shown that the applicability of the hydrometallurgical procedure depends upon the mineralogical structure and the chemical composition of the ore. The leaching of poor ores with aqueous sodium sulphide solutions which contain sodium hydroxide resulted in from 60 to 90 percent, extraction of mercury, and the mercury extraction from flotation concentrates amounted to about 95 percent.

The aim of our investigation was to determine the possibility and the optimal conditions of hydrometallurgical treatment of Yugoslav mercury ores from Šuplja Stena, Avala.

The deposits of this ore are formed from thermal solutions at low temperatures. The ore contanis mercury in the from of aggregates of cinnabar, particle size from 0.06 to 0.8 mm, and some native metal and calomel.

It has been established that the ore contains from 0.12 to 0.32 percent of mercury. The reserves are estimated to be sufficient for 10 — 15 years exploitation.

The poor quality of the ore and the low reserves suggested the idea of hydrometallurgical processing, since in this way many disadvantages and great financial investments which are associated with the usual pyrometallurgical procedure might be avoided.

EXPERIMENTAL

To determine the optimal conditions for the leaching of the ore from Šuplja Stena, the effects of the following factors on the percentage of mercury extraction were investigated:

a) the amount of sodium hydroxide added

b) the concentration of sodium sulphide solution and the leaching time

c) the ratio of liquid to solid phase

d) the ore particle size i. e. its granulometric composition.

Before leaching the ore was crushed to the desired size and then dried at 100°C. The ore prepared in this way contained 0.31 prercent mercury.

The leaching solution was prepared from crystalline sodium sulphide, $Na_2S \cdot 7H_2O$, p. a. The concentration of the solution was calculated for anhydrous sodium sulphide.

In order to prevent the oxidation of sodium sulphide and the hydrolysis of the double salt during leaching, a definite amount of p. a. sodium hydroxide was added to the leaching solution. The leaching was carried out by agitating the solution moderately with an electrical agitator at a constant rate.

After a definite leaching time the pulp was filtered off and the residue on the filter was first washed with one percent sodium sulphide solution and then with water. The washed precipitate was dried at 100°C and then analyzed.

The percentage of mercury extraction was determined by analyzing the residue according to the method given by Eschi(6)

a) *The effect of the amount of sodium hydroxide added on the extraction of mercury*

To determine the optimal amount of sodium hydroxide leaching was caried out with 5 percent sodium sulphide solution to which the following amounts of sodium hydroxide were added: 0.1, 0.2 and 0.3 percent of the leaching solution. With all solutions the leaching times were 1, 2.5, 5, and 7,5 hours. The ore particle size was 100% — 60 mesh. The results are given in Fig. 1 and Table 1.

The results show that the maximal extraction of mercury was achieved with 5 percent sodium sulphide solution to which 0.2 percent sodium hydroxide was added. In further experiments, therefore, 0.2 percent sodium hydroxide was used as the optimal quantity.

b) *The dependance of mercury extraction upon the concentration of sodium sulphide solution and leaching time.*

Ore of particle size 100% — 60 mesh was leached with the optimal amount of sodium hydroxide (0.02%) in 2.5, 5, 7.5 and 10 percent solutions of sodium sulphide. The leaching times for all the concentrations vere; 1, 2.5, 5. and 7.5 hours as in the preceeding experiments.

TABLE 1

The dependence of mercury extraction upon the amount of sodium hydroxide and the leaching time. L:S = 2:1; ore particle size 100%—60 mesh; leaching temperature 20° C; mercury cotent 0.31%.

Experiment No.	NaOH added %	Leaching time hrs	Distribution of mercury %	
			in the residue	in the solution
1	0.1	1.0	26.33	73.67
2	0.1	2.5	21.31	78.69
3	0.1	5.0	16.94	83.06
4.	0.1	7.5	12.30	87.70
5	0.2	1.0	1.69	98.31
6	0.2	2.5	1.38	98.62
7	0.2	5.0	7.07	93.93
8	0.2	7.5	7.09	93.91
9	0.3	1.0	13.67	86.33
10	0.3	2.5	12.30	87.70
11	0.3	5.0	11.60	88.40
12	0.3	7.5	9.91	90.09

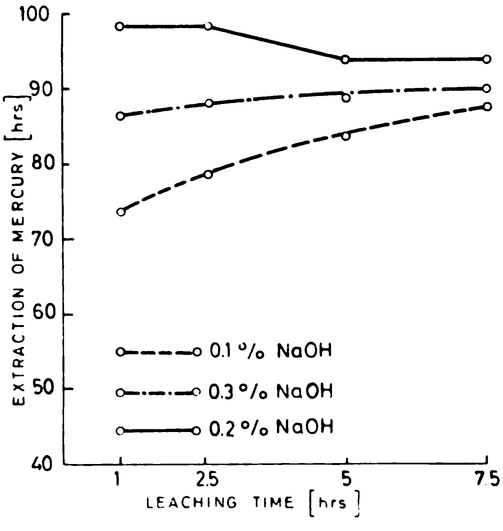

Fig. 1. The dependance of mercury extraction upon the amount of sodium hydroxide in leaching with 5 percent sodium sulphide solution L:S = 2:1

The data given in table 2 and Fig. 2 show that the maximal extraction of mercury, 98.31 percent, was achieved with 5 percent solution. Further increase of the concentration had almost no effect.

In case the leaching lasts longer than one hour the percentage of mercury extraction can be considered constant for all concentrations except for 5 percent, with which prolongation of the

TABLE 2

The effect of sodium sulphide concentration and the leaching time with the optimal amount of sodium hydroxide on the percentage of mercury extraction. L:S = 2:1; ore particle size 100%—60 mesh; temperature 20°C; mercury content 0.31%

Experiment	Concentration of Na_2S in Sol. %	Leaching time (hrs)	Distribution of mercury (%) in the residue	in the solution
1	2.50	1.0	79.90	28.10
2	2.50	2.5	67.71	32.29
3	2.50	5.0	63.40	36.60
4	2.50	7.5	60.74	39.26
5	5.00	1/2	15.32	84.68
6	5.00	3/4	10.00	90.00
7	5.00	1.0	1.69	98.31
8	5.00	2.5	1.38	98.62
9	5.00	5.0	7.07	93.93
10	5.00	7.5	7.09	92.91
11	7.50	1.0	1.39	98.61
12	7.50	2.5	1.07	98.93
13	7.50	5.0	1.07	98.93
14	7.50	7.5	0.80	99.20
15	10.00	1.0	4.19	95.81
16	10.00	2.5	4.00	98.00
17	10.00	5.0	1.38	98.62
18	10.00	7.5	4.19	95.81

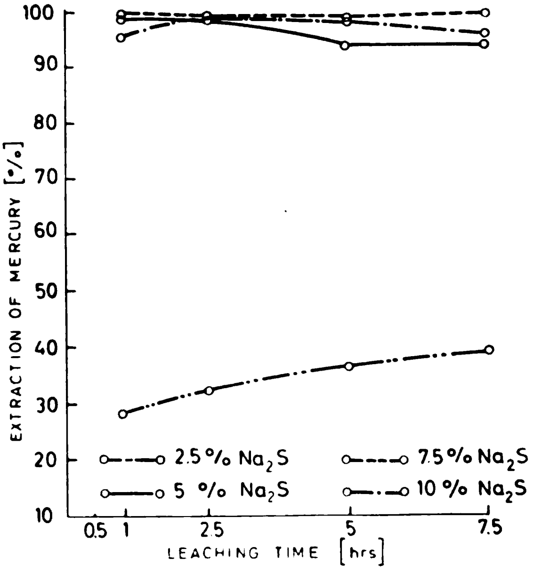

Figure 2. The dependance of mercury extiaction on the concentration of sodium sulphide solution and leaching time

leaching time results in a decrease of mercury extraction. Thus the results show that the formation of the soluble double salt of mercuric sulphide and sodium sulphide and its subsequent dissolution proceed rather fast with ore from Šuplja Stena. One hour can be considered as the optimal leaching time and the optimal sodium sulphide concentration is 5 percent (the extraction of mercury is considerably lower when the leaching time is less than one hour).

c) *The effect of the ratio of the liquid to the solid phase on the extraction of mercury*

It is known that in leaching of many ores increase of the *L:S* ratio increases the extraction of the useful component. In our study we wanted to determine whether the extraction (98.31%) achieved with 5 percent solution and *L:S* = 2:1 could be increased this ratio or whether the same percentage might be obtained with a smaller value of the *L:S* ratio. Also the question was raised whether it is possible to achieve the optimal mercury extraction obtained with 5 percent sodium sulphide solution by leaching with a solution of lower concentration but with an *L:S* ratio higher than 2:1. Therefore leaching experiments were made with 5 percent solution and with *L:S* ratios 1:1 and 4:1, and with 2.5 percent solution and *L:S* = 4 : 1.

TABLE 3

The effect of the L:S ratio and the leaching time on the extraction of mercury in leaching with 2.5 and 5 percent sodium sulphide solutions containing 0.2 percent sodiumhydroxide. The ore particle size 100%—60 mesh; mercury content 0.31%.

Experiment No.	Concentration of Na_2S in sol. (%)	L:S	Leaching time (hrs)	Distribution of mercury (%)	
				in the residue	in the solution
1	2.5	4:1	1.0	17.50	82.50
2	2.5	4:1	2.5	15.00	85.00
3	2.5	4:1	5.0	9.69	90.00
4	2.5	4:1	7.5	10.00	90.31
5	5.0	1:1	1.0	50.24	49.76
6	5.0	1:1	2.5	49.00	51.00
7	5.0	1:1	5.0	46.40	53.60
8	5.0	1:1	7.5	42.80	57.21
9	5.0	4:1	1.0	1.72	98.28
10	5.0	4:1	2.5	1.26	98.74
11	5.0	4:1	5.0	4.19	95.81
12	5.0	4:1	7.5	4.32	95.68

The results (Table 3 and Fig. 3) show that with leaching with 5 percent sodium sulphide solution and L:S = 4:1, the extraction of mercury was slightly increased only when the leaching time was longer than 2.5 hours. For shorter periods (from one to 2.5

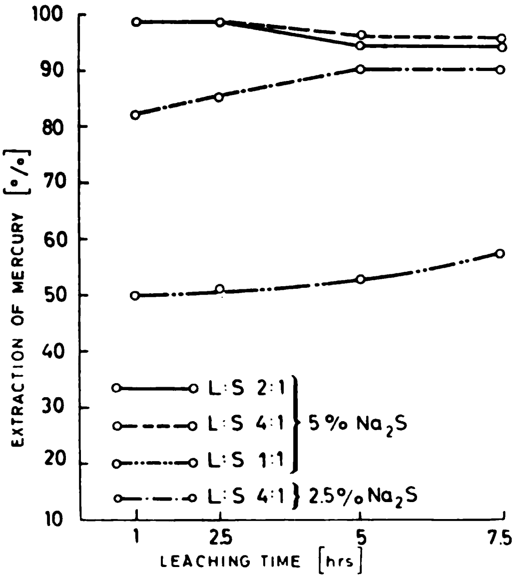

Figure 3. The dependance of mercury extraction on the ratio *L:S*, the concentration of the solution and the leaching time.

hours) the extraction was the same as in leaching with L:S = 2:1. Similarly, in leaching with 2.5 percent solution and L:S = 4:1 extractions better than 90 percent could not be achieved, even with prolonged leaching time. Accordingly it may be concluded that the optimal liquid to solid phase ratio in the leaching of ore from Šuplja Stena is 2:1.

d) *The effect of the ore particle size*

In all the above experiments the ore particles were 100%—60 mesh.

Since absolute extraction is not likely to be achieved, and considering the obtained extraction of 98.31 percent as satisfactory, we investigated the effect of ore particle size on extraction in order to find out whether the same percentage of extraction could be obtained with particles of greater size, whereby the economics of the process could be increased. Leaching experiments were made with ore of particle sizes 100%—24 mesh and 1 mm. The results are given in Table 4 and Fig. 4.

From the results it is evident that with the larger ore particles the extraction of mercury was considerably decreased. The extractions achieved with 100%—24 mesh and 1 mm were smaller than that obtained with 100%—60 mesh, at all times of leaching. It is

TABLE 4

The effect of the granulometric composition and the leaching time on the percentage of mercury extraction under optimal experimental conditions (0.2 percent added sodium hydroxide, 5 percent sodium sulphide solution, L:S = 2:1)

Experiment	The ore particle size	Leaching time (hrs)	Distribution of mercury (%)	
			in the residue	in the solution
1	24 mehk	1.0	16.33	83.67
2	„	2.5	14.80	85.20
3	„	5.0	13.00	87.00
4	„	7.5	13.00	88.00
5	1 mm	1.0	24.34	75.66
6	„	2.5	19.00	81.00
7	„	5.0	13.67	86.33
8	„	7.5	11.00	89.00

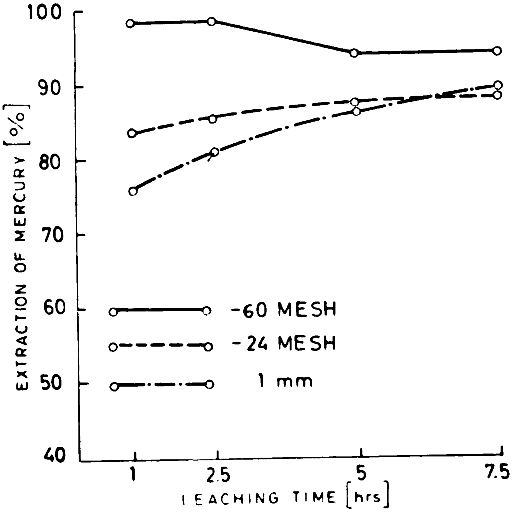

Figure 4. The dependance of mercury extraction on the ore particle size under optimal conditions

characteristic that the differences in extraction percentages are higher the shorter the leaching time, i. e. the differences decrease as the leaching time increases.

CONCLUSIONS

Investigations on the leaching of ore from the area of Šuplja Stena, Avala, have given satisfactory results. Favorable mineralogical structure and chemical composition of the ore allow efficient extraction of mercury with sodium sulphide solution under the right conditions, whereby a high percentage of extraction is achieved.

The following have been established:

1. The optimal extraction of 98.31 percent is achieved with 5 percent sodium sulphide solution with the addition of 0.2 percent sodium hydroxide and a leaching time of one hour. Shorter leaching time gives considerably lower extraction (84.86 percent in 30 minutes, and 90.00 percent in 45 minutes); after longer periods of extraction remains practically the same.

2. The optimal liquid to solid phase ratio is 2:1. Increasing this ratio has no effect on the extraction for shorter leaching periods and for leaching periods above 2.5 hours the increase of the extraction is insignificant.

3. The optimal ore particle size is 100%—60 mesh.

Along with the investigation of the optimal conditions for percolation leaching, further work will be directed towards determining the optimal conditions for agitating and percolation leaching of the concentrates obtained by flotation of ore from Šuplja Stena, and for the separation of mercury from the solution.

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GLASNIK HEMIJSKOG DRUŠTVA, Vol. 28, No. 5-6 1963, pp. 237-244

STATISTICAL INTERPRETATION OF THE RADIOACTIVITY OF SEDIMENTS IN THE THERMAL SPRINGS OF NIŠKA BANJA

by

BOŠKO PAVLOVIĆ

The land surrounding thermal springs of Niška Banja represents sedimental layers of travertine tufa about 10 meters thick, according to geological researchers(1). It has been established that in some places these layers are as much as 22 meters thick. The sedimental layers of tufa were formed by waters of the Glavno vrelo (Main Spring). On the surface there is a layer of humus 0.2 to 1.2 meters thick.

While measuring the radioactivity of the spring waters and the surrounding land, it was found that the concentration of radon in the gases of the spring waters, as well as the gases of the tufa varies within wide limits(2,3). The most radioactive layers were found near the stream along which the water flows away from the Main Spring. Figure 1 shows the places where borings were made and where the radioactivity of the tufa gases was measured.(2)

Figure 1 suggests a certain relation between the radioactive tufa gases and the distance of the radioactive stream from the Main Spring. However the actual influence of this water on the radioactivity cannot be determined. The increase of radioactivity in some places might result from many factors. Examining the various possibilities, it is clear that over a period of time the level of the stream rises because of sedimentation, which causes the water to overflow its boundaries, thus changing the course of the stream. It is also possible that the overflowed water sometimes forms pools. The silt of such pools could hold or adsorb a greater percentage of radioactive elements than nearby tufa. Where such pools formed the radioactivity would be greater. The third possibility is that the sediments do not have the same structure. In the course of decades, and even centuries, the water had different chemical properties with respect to the content of diluted salts and the quantity of radioactive elements. Thus the sedimented tufa had a different structure than that which sedimented during recent centuries. It can also be supposed that in certain sediments with large amounts of hydrated iron, manganese, aluminum and silicon oxides, radioactive elements accumulated in greater measure than in pure tufa.

To determine the validity of the hypothesis that there is a relation between the radioactivity of the terrain and the distance of the stream, a probability calculation was carried out. This was done by determining whether the distribution of radioactivity at the borings could belong to the same basic group, or whether the results obtained could be the consequence of some normal distribution. To carry out the calculation, the terrain near the stream was divided into ten zones, as is shown in Figure 1. Only in zones from 1 to 4 and from 1′ to 4′ are there places where the boring instruments extracted gas with a radioactivity of 40 m μ C/lit.

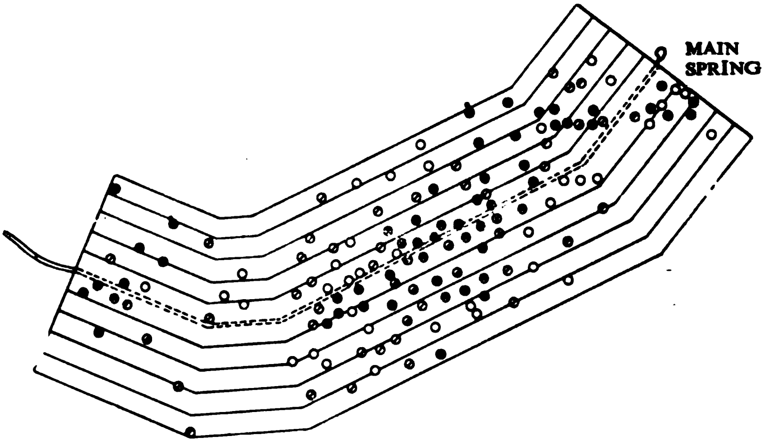

Figure 1

= = = =	Stream	⊖	40—60 m μ C/lit
○	over 100 m μ C/lit	+	20—40 m μ C/lit
●	80—100 m μ C/lit	⊕	5—20 m μ C/lit
◒	60— 80 m μ C/lit	O	less than 5 m μ C/lit

The number of places, by zones, where the radioactivity of the gases near the streams was measured, is given in the following table:

n_1	x_i'	n_2	x_i''
1 zone 1	11 places	1 zone 3	3 places
2 zone 1	6 places	2 zone 3	3 places
3 zone 2	3 places	3 zone 4	0
4 zone 2	3 places	4 zone 4	1 place

From this data

$$t = \frac{\overline{x_i'} - \overline{x_i''}}{s} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} = 1{,}96$$

where: $\bar{x}_i' = \frac{\sum x_i'}{n_1}$; $\bar{x}_i'' = \frac{\sum x_i''}{n_2}$ and

$$s = \sqrt{\frac{\sum (x_i' - \bar{x}_i')^2 + \sum (x_i'' - \bar{x}_i'')^2}{n_1 + n_2 - 2}}.$$

From the table for the probability function (t), it is found that for $t = 1{,}96$, probability $P \approx 0{,}05$ i.e. very low. Thus in zones 1,1′, 2,2′, 3,3′, 4,4′, the concentrations of radioactive elements in the sediment belong to the same group, which means the nearness of the stream strongly affects the radioactivity of the terrain.

Figure 1 shows that from 143 measurements it was established that at 36 places the radioactivity of the gases was less than 5 mμ C/lit, at 42 places from 5 to 20 mμ C/lit, at 36 places 20 to 40 mμ C/lit, at 14 places 40 to 60 mμ C/lit, at 5 places 60 to 80 mμ C/lit, at 5 places 80 to 100 mμ C/lit, and at 5 places more than 100 mμ C/lit. To determine the correlation between the distance of the stream and the radioactivity of the terrain, the radioactivities of the gases were classified as follows:

class 1:	activity	of	gases	from	0	to	20 mμ C/lit
class 2:	”	”	”	”	20	”	40	”
class 3:	”	”	”	”	40	”	60	”
class 4:	”	”	”	”	60	”	80	”
class 5:	”	”	”	”	80	”	100	”
class 6:	”	”	”	”	over	”	100	” .

In Table 1 these classes are designated by x_i, and the zones on either side of the stream by y_j. Zones 1 and 1′ are included in 1, 2 and 2′ in 2, and so on.

TABLE 1

y_j	x_i	1	2	3	4	5	6	sum	x_i
1		23	11	5	2	5	5	51	2.41
2		14	10	3	3			30	1.83
3		18	8	5				31	1.58
4		14	3	1				18	1.28
5		9	4					13	1.31
sum		78	36	14	5	5	5	143	
y_j		2.64	2.42	2.14	1.60	1.00	1.00		

In Fig. 2 are represented the functions $y = f(x)$ and $x = \varphi(y)$. It can be seen that here there may exist linear correlation, or more probably a non-linear correlation.

From the data in Table 1 the coefficent of co.relation was calculated:

$$r = \frac{\mu_{11}}{\sigma_x \sigma_y} = -0.346$$

where:

$$\mu_{11} = \frac{1}{N} \sum_i \sum_j f_{ij} x_i y_{ij} - \bar{x} \cdot \bar{y},$$

$$\sigma_x = \frac{1}{N} \sum_i f_i x_i^2 - \bar{x}^2 \quad \text{and} \quad \sigma_y = \frac{1}{N} \sum_j f_j y_j^2 - \bar{y}^2.$$

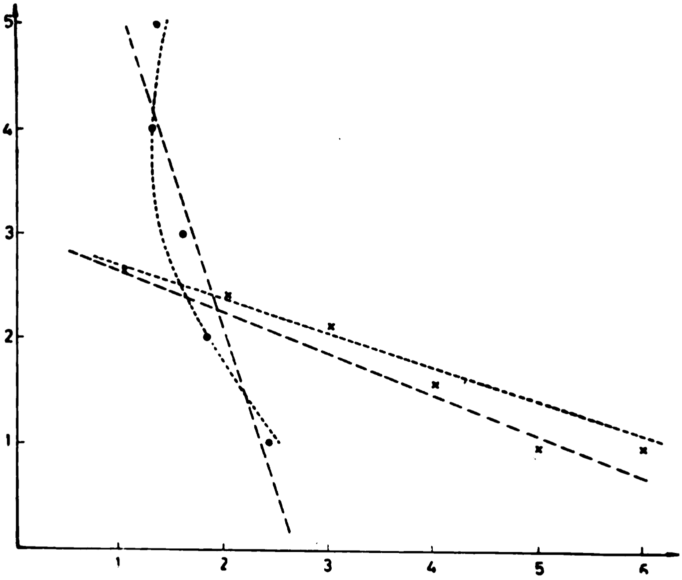

Fig. 2

For good correlation we must have that

$$-1 \leq r < -0.5 \quad \text{or} \quad 0.5 < r \leq 1$$

Thus there is a weak linear correlation between the radioactivity of the tufa and its distance from the stream. However, from the negative sign of the coefficient of correlation it can be concluded that the radioactivity falls with the distance from the stream.

The equations of the correlation directions are:

1. $$y - \bar{y} = \frac{\mu_{11}}{\sigma_x^2} (x - \bar{x})$$

$$y = -0.356\, x + 3.05.$$

2. $$y - \bar{y} = \frac{\sigma_y^2}{\mu_{11}} (x - \bar{x})$$

$$y = -2.97\, x + 7.94.$$

These equations of the correlation directions are shown in Fig. 2. by the dashed line.

Since it was concluded that there was no linear correlation between the radioactivity and the distance of the places where samples were taken from the stream, the attempt was made to show the relations of these quantities by means of non-linear correlations. If the equation of non-linear correlation is:

$$Y = b_o + b_1 x + b_2 x^2 + \cdots\cdots + b_k x^k,$$

then the coefficients b_o, b_1 and b_2 can be calculated from the equations

$$1.\quad \frac{1}{N} \sum_i \sum_j f_{ij} y_{ij} - b_o - b_1 \frac{1}{N} \sum_i f_i x_i - b_2 \frac{1}{N} \sum_i f_i x_i^2 = 0.$$

$$2.\quad \frac{1}{N} \sum_i \sum_j f_{ij} x_i y_{ij} - b_o \frac{1}{N} \sum_i f_i x_i - b_1 \frac{1}{N} \sum_i f_i x_i^2 - b_2 \frac{1}{N} \sum_i f_i x_i^3 = 0$$

$$3.\quad \frac{1}{N} \sum_i \sum_j f_{ij} x_i^2 y_{ij} - b_o \frac{1}{N} \sum_i f_i x_i^2 - b_1 \frac{1}{N} \sum_i f_i x_i^3 - b_2 \frac{1}{N} \sum_i f_i x_i^4 = 0$$

provided that the field results can be correlated with a second degree curve.

From the solution of these equations,

$$b_o = 3.062; \qquad b_1 = -0.348 \text{ and } b_2 = 0.005$$

and

$$Y = 3.062 - 0.348\, x + 0.005\, x^2.$$

From these equations we get the values of Y for each value of x_1:

x_i	Y_i	x_i	Y_i
1	2.719	4	1.750
2	2.386	5	1.447
3	2.063	6	1.154.

Thus, the correlation coefficient will be:

$$R_{yx}^2 = 1 - \frac{S_y^2}{\sigma_y^2} = 0.1024 \text{ i. e. } R_{yx} = 0.32$$

where:

$$S_y^2 = \frac{1}{N} \sum_i \sum_j f_{ij} (y_{ij} - Y_i)^2 = 1.564.$$

Since the absolute values for the correlation coefficient r and the index of correlation R_{yx} are close, it can be concluded that the correlation between the radioactivity of the tufa and the distance from the stream is more complex than it seemed at first glance. For a good correlation it is necessary that $R_{yx} \rightarrow 1$, since then $S_y^2 = 0$, according to laws of mathematical statistics which were used to calculate this correlation. Thus all points y_{ij} would lie on the curve

$$Y = 3.062 - 0.348\,x + 0.005\,x^2.$$

This curve is represented by dots in Fig. 2.

The index of correlation R_{xy} can be calculated in an analogous manner as the index of correlation R_{yx}. It is necessary to find the values of the coefficients in the following equations.

$$1.\quad \frac{1}{N}\sum_i \sum_j f_{ij}\,x_{ij} - c_o - c_1\frac{1}{N}\sum_j f_j\,x_j - c_2\frac{1}{N}\sum_j f_j\,y_j^2 = 0$$

$$2.\quad \frac{1}{N}\sum_i \sum_j f_{ij}\,x_{ij}\,y_j - c_o\frac{1}{N}\sum_j f_j\,y_j - c_1\frac{1}{N}\sum_j f_j\,y_j^2 - c_2\frac{1}{N}\sum_j f_j\,y_j^3 = 0$$

$$3.\quad \frac{1}{N}\sum_i \sum_j f_{ij}\,x_{ij}\,y_j^2 - c_o\frac{1}{N}\sum_j f_j\,y_j^2 - c_1\frac{1}{N}\sum_j f_j\,y_j^3 - c_2\frac{1}{N}\sum_j f_j\,y_j^4 = 0.$$

The solutions of these equations are

$$c_o = 3.547; \quad c_1 = -1.193; \quad \text{and} \quad c_2 = 0.154,$$

so that

$$X = 3.547 - 1.193\,y + 0.154\,y^2.$$

From these equations, the value of X for each value of y_i may be calculated:

y_j	X_j	y_j	X_j
1	2.508	4	1.239
2	1.777	5	1.432
3	1.354		

Hence

$$S_x^2 = \frac{1}{N}\sum_i \sum_j f_{ij}\,(x_{ij} - X_j)^2 = 1.460,$$

and the index of correlation,

$$R_{xy}^2 = 1 - \frac{S_x^2}{\sigma_x^2} = 0.105, \text{ i. e. } R_{xy} = 0.325.$$

The values for r, R_{yx} and R_{xy} lead to the conclusion that there is only a partial correlation between the radioactivity of the tufa and the distance from the stream. Many factors suggest that a greater concentration of radium is found in sediments near the stream along which flows the water from the radioactive Main Spring. This results from a subsequent accumulation of radium in the tufa. Dry remains of spring water contain only 13.6×10^{-12} g Ra/g, whereas the samples of tufa contain as much as 180×10^{-12} g Ra/g tufa. If $CaCO_3$ and Ra^{++} sedimented proportionally during the formation of the tufa, then the fall in the concentration of radium in the sediments around the stream could not be explained if the subsequent accumulation of radium from the water was not taken into consideration. This radium was most probably adsorbed by certain components of the tufa.

With respect to the vertical distribution of radioactivity in the tufa, certain regularities were observed. The higher, younger layers of tufa have significantly greater radioactivity than deeper, older layers. From the vertical distribution of the radioactivity, the age of the tufa and the speed of sedimentation were calculated[3]. This data agreed with geological studies. Since the time factor was taken into consideration to explain the fall of radioactivity in sediments in the vertical direction, it seems the time factor should also have been taken into consideration when analyzing the horizontal distribution of the radioactivity. However, the past dislocations of the stream's path are not known. In addition, the duration of the stream's overflow and the duration of the pools which were possibly formed are also unknown. Thus, when the horizontal distribution of radioactivity in the sediments was being studied, i. e. when the correlation between the radioactivity of the tufa and the distance from the spring was being sought, this time factor could not be taken into consideration. Thus a regularity, in the sense that the layers near the stream are younger and those far away older, could not be determined, and the age of the sedimental layers cannot be calculated according to laws which are valid for the vertical distribution of radioactivity. This was also confirmed by the analysis of the vertical distribution of the concentration of radioactive tufa gases: when the thermal spring terrain was bored, it was found that the radioactivity of the tufa fell regularly with depth.

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THE DISTRIBUTION OF RADIOACTIVITY OVER FRACTIONS OF SEDIMENTS WITH DIFFERENT SPECIFIC GRAVITIES

by

BOŠKO PAVLOVIĆ

Research on the distribution of radioactive elements in rock samples has shown that the radioactivity is localized in small fractions of the rock,(1, 2, 3) and mainly in "accessory" minerals.(4, 5, 6) In sedimentary rocks there are always heavy mineral fractions which are usually carriers of radioactive elements. It can thus be assumed that the radioactivity of the sediments originates in these accessory minerals.[4] Most sediments contain a certain amount of clay which results from the transformation of heavy accessory minerals. During this transformation chemical influences can lead to a redistribution of radioactive elements in the clayey minerals. It can thus be expected that some of the radioactive elements in sediments are related to some light mineral fractions which are generally not carriers of radioactive elements. It can also be expected that some of the radioactive elements will be adsorbed on the colloidal material (hydrated Fe_2O_3, MnO_2, Al_2O_3, SiO_2) in the sediment.

The distribution of radioactivity was examined in fractions with different specific gravities. Travertine from Niška Banja, from Fojnička Banja and from Debarska Banja, and sand from Banjsko (near Strumica) were used. Some of the research presented in this paper appeared in earlier publications.(7, 8)

EXPERIMENTAL PART

It was found by quantitative chemical analysis that the travertines from Niška Banja, Fojnica and Debarska Banja contained:

	CaO	Al_2O_3	SiO_2	Fe_2O_3	MnO	Organic material
Niška Banja	to 48.8%	to 4.5%	to 9.5%	to 2.7%	to 0.6%	to 3.8%
Fojnica	to 38%	to 6.1%	to 20%	to 9.8%	to 1.9%	
Debarska Banja	to 36.7%	to 6.7%	to 7.1%	to 0.2%	traces	

The travertine with the most uniform structure was found in Debarska Banja.

Samples of these travertines and of the sand from Banjsko were qualitatively spectrographically analized in region 2700 to 4400 Å. Table 1 shows the results of the spectrochemical analysis.

TABLE 1

Spectrochemical Analysis of the Travertines and Sand

element	travertine			sand	element	travertine			sand
	NB	*DB*	*F*	*S*		*NB*	*DB*	*F*	*S*
Li	—	—	—	—	Zr	—	—	—	—
Na	+	+	+	+	Th				—
K	—	—	—	—	Si	+	+	+	+
Rb	—	—	—		Ge	—	—	—	—
Cs	—	—	—		Sn	—	—	—	—
Be				—	Pb	—	—	—	+
Cu	+	+	+	+	V	+	+	+	+
Ag	—	—	—	—	Sb	—	—	—	—
Au	—	—			Bi	—	—	—	—
Mg	+	+	+	+	Cr	+	—	+	+
Ca	+	+	+	+	Mo	—	—	+	—
Sr	+	+	+	+	W	—	—	—	—
Ba	+	+	+	—	U	—	—	—	—
Ra	—	—	—	—	Mn	+	+	+	+
La	—	—	—	—	Fe	+	+	+	+
B	+	+	—	—	Co	—	—	—	—
Zn	—	—	—	+	Rh	—	—	—	—
Al	+	+	+	+	Ni	—	—	—	+
Ga	—	—	—	—	Ti	+	+	+	+
Pt	—	—	—	—					

(+) Identified; (—) Not identified; ( ) Not determined

Special attention was paid to detecting such rare elements as U, Ra, Bi, Pb La, but they were not spectrographically detected.

Since most of the mass of travertine is composed of $CaCO_3$, all $CaCO_3$ was removed with 10% HCl, thus concentrating the other minerals in the sediment and making mineral analysis possible. A large percentage of calcite was also found in the Banjsko sand, and for the mineral analysis of this sand the same method for removing $CaCO_3$ was used. The results of the mineral analysis of the Niška Banja travertines are given below.

Calcite makes up most of the mass rock; in some cases it is associated with a certain amount of iron;

quartz follows calcite in amount, and appears in the form of grains with different sizes and irregular shapes;

clayey minerals are present in the same quantity as quartz;

feldspar is present in somewhat smaller amounts than quartz, and takes the form of irregular-shaped grains;

muscovite is a relatively pure mineral which appears in very small flakes;

biotite is present in somewhat smaller amounts than muscovite and appears in larger flakes;

amphibole, relatively rare, takes the form of prismatic grains;

tourmaline: only a few prism-shaped grains of an intensive polychromic blue were observed;

epidote, very common, appears in irregular-shaped grains;

apatite: a few, usually round, grains were observed;

zircon is relatively common and takes the form of grains of different sizes and often irregular shapes;

The results of the Fojnica travertine were as follows:

calcite makes up almost the entire mass of the travertine;

quartz follows calcite in amount, and takes the form of irregular-shaped grains;

clayey minerals are relatively rare;

feldspar is relatively rare and takes the form of very small grains;

muscovite is relatively common and takes the form of very small flakes;

biotite is relatively common and in the form of small flakes;

amphibole: only a few prismatic grains were observed;

tourmaline: only a few grains;

rutile: only a few very small grains;

epidote appeared quite often in small irregular-shaped grains;

zircon appeared in grains of different shapes and sizes, and in frequency it folowed quartz, feldspar, amphibole, biotite and muscovite;

iron hydroxide probably took the form of limonite and was distributed in the calcite and quartz.

The results of the mineral analysis of the Debarska Banja travertine are given below;

calcite makes up most of the travertine;

aragonite is the most essential component of the travertine after the calcite;

gypsum appeared in the form of round grains;

magnesite took the form of irregular grains and was not equally represented in all the samples;

quartz was the most dominant mineral after calcite and aragonite; its grains often contained fragments of other minerals;

barite: a few grains of different sizes were found;

feldspar was very common and took the form of grains of different sizes and shapes;

muscovite was very common, in the form of different-sized blades which contained other minerals;

biotite was present in smaller amounts than muscovite and its flakes contained fragments of different minerals;

amphibole took the form of irregular shaped grains;
tourmaline: only a few grains;
anatase: a few small grains;
rutile: a few small grains;

epidote was a very common mineral and appeared in very small irregular-shaped grains;

apatite: only a few grains;
zircon appeared in grains of different sizes;
orthite: only a few small grains.

The results of the mineral analysis of the Banjsko sand are given below:

In addition to calcite, which made up 50% of this sand, there was also a certain amount of clay and humus. The mineral structure of the sand was as follows:

calcite appeared in the form of large and small grains; a certain amount of iron was associated with it;

quartz followed calcium in representation; it appeared in the form of very nonuniform large grains which often contained inclusions of other minerals, sometimes zircon;

feldspar was present in smaller quantities than quartz and appeared in grains of different sizes and shapes;

muscovite appeared in blades of different sizes which often contained some magnetite;

biotite was as common as muscovite and often contained zircon and fragments of some other minerals;

amphibole was widely distributed and took the form of very uniform grains;

epidote was a relatively common mineral which appeared in prismatic grains or sometimes in irregular-shaped grains;

sphene was a relatively rare mineral, a few grains were found;

magnetite a few grains of differet sizes were separated with a hand magnet;

iron hydroxide appeared in traces distributed among the calcite and quartz, most probably in the form of limonite;

zircon was a very common, in grains of different sizes and shapes; its representation followed that of quartz, feldspar, amphibole and mica.

For the sake of comparison, the mineral analyses of the different samples of travertine and sand are shown together in Table 2.

On the basis of the mineralogical structure of the travertines and the sand it was determined that all the samples have some mineral components which could be carriers of radioactive elements. The most likely carriers of uranium, and possibly thorium, are zircon, apatite, sphene, orthite, biotite[3]. These minerals have greater specific gravities than the minerals that make up most of the mass of the travertines and sand. In order to determine the radioactivity of the different fractions, fractionization of the components of these minerals was carried out with respect to their specific gravities.

TABLE 2.

Mineralogical analysis

mineral	specific gravity p/cm^3	travertine			sand Banjsko
		Niška Banja	Fojnica	Debarska Banja	
calcite	2.70—2.73	+	+	+	+
aragonite	2.93—2.94			+	
gypsum	2.20—2.40			+	
magnesite	2.90—3.10			+	
guartz	2.50—2.80	+	+	+	+
clayey minerals and humus		+	+		+
feldspar	2,54—2.58	+	+	+	+
muscovite	2.76—3.10	+	+	+	+
biotite	2.80—3.20	+	+	+	+
amphibole	2.90—3.20	+	+	+	+
tourmaline	2.94—3.24	+	+	+	
epidote	3.25—3.50	+	+	+	+
apatite	3.17—3.23	+		+	
orthite	3.0 —4.0			+	
rutile	4.27		+	+	
anatase	3.83—3.93			+	
sphene	3.40—3.60				+
zircon	4.50—4.70	+	+	+	+
limonite	3.40—3.95	+	+		+
magnetite	4.50—5.20				+
barite	4.30—4.60			+	

Each sample of travertine or sand was suspended in water. The suspension was then passed through a system of DIN meshes for wet sifting. The dimensions of the mesh openings were:

1. 0.75 mm	3. 0.15 mm	5. 0.10 mm
2. 0.20 mm	4. 0.12 mm	6. 0.0075 mm

The sunspension was poured on to the mesh; then water was poured on until the water above the remains on the mesh was clear: this was the sign that all the smaller particles had passed through. The first mesh was then taken away, and the remains were removed and placed to dry. This was done for each mesh, and in this way six fractions were gotten. After drying, these fractions were prepared for further treatment. Particles which were so small that they passed through mesh number 6, namely those with diameters of less than 7.5 μ, were thrown away.

Indexes from 1 to 6 were added beside the provenience of the sample.* These indexes refer to the mesh in which the fraction remained. For example, S_1 means that the particles are from Banjsko and that they remained in the first mesh, and thus have dimensions greater than 0.75 mm.

Among the NB and F travertine samples the greatest number of particles remained in meshes 3, 4, 5, and 6. The most numerous fractions of the DB travertine samples were 2, 3, and 6. In the samples of S sand, the most numerous fractions were 2 and 6.

Fractions 1 to 6 were then separated on the basis of their specific gravities. Each fraction was shaken in acetylenbromide to separate the mineral components whose specific gravities were greater from those whose specific gravities were smaller than 2.96 p/cm^3, which is the specific gravity of acetylentetrabromide.** Centrifugation was carried out to stratify the suspension. Light fractions were designated with an index *L* and heavy fractions with and index *T*. Fraction *L* contained the following mineral components: calcite, aragonite, clayey minerals feldspar and gypsum, and limited amounts of muscovite, biotite, amphibole and magnesite. The *L* fractions made up the greatest part of the mineral mass of the travertines and the sand. Fraction *T* contained the following mineral components: epidote, apatite, zircon, rutile, orthite, anatase, sphene, barite, limonite, magnetite and tourmaline, and limited quantities of muscovite, biotite, amphibole and magnesite. In the NB and F travertine samples, fraction *T* made up less than 1% of the total mass. In the DB samples, the presence of fraction *T* was somewhat larger than 1%. The *T* fraction was significantly larger in the sand S than in the travertines.

After the travertine and sand samples were separated according to their size (1 to 6) and according to their specific gravities (*L* or *T*), magnetic separation was carried out on each component.*** Magnetite and some other iron minerals were separated this way. The minerals separated by means of a magnet were marked Fe. With this treatment it was possible to separate a few grains of magnetite and similar ferromagnetic particles only in sample S.

Further analysis was carried out on fractions of different sizes, fractions with mineral components of different specific gravi-

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* In the remainder of the text, the travertine sample from Niška Banja will be designated by NB;

The travertine sample from Fojnica will be designated by F;

The travertine sample from Debarska Banja will be designated by DB;

The sand sample from Banjsko (near Strumica) will be designated by S.

** Some NB fractions were not separated with acetylentetrabromide, but rather with bromoform, whose specific gravity is 2.88 p/cm^3.

*** Samples of NB and S were separated with an electromagnet, whereas an ordinary hand magnet was used for DB and F samples.

ties,* and fractions gotten by magnetic separation. The initial weight of the samples taken for analysis was 250 grams. However, after fractionization the total weight of the different fractions was very small, of some no more than a few centigrams. Thus in order to make qualitative analysis of the radioactivity of the individual fractions, only photonuclear techniques could be used.

Fractions whose radioactivity was examined were placed on the shelves of specially built boxes. Ilford D_1 photonuclear plates were placed over the boxes, about 1 mm above the sample. One plate was not placed above a sample; this plate served as a reference. The tracks of alpha and beta particles from disintegrated radioactive atoms in the fractions were read in relation to the reference. The plates were kept above the fractions for at least four days. After the plate was removed, if there were statistically more traces on it than on the reference plate it meant that the fraction under that plate was radioactive.

Table 3 shows the results of this analysis.

TABLE 3

The Radioactivity of various Fractions of Travertine and Sand Samples. Qualitative Analysis by Photonuclear Techniques.

fraction and components	travertine samples				Sand sample S
	NB	F	DB	F	
1	L	nr	nr	n.d.	n.d.
	T	—	nr	n.d.	n.d.
	Fe	—	—	—	n.d.
2	L	r	nr	r	r
	T	r	r	r	r
	Fe.	—	—	—	r
3	L	r	r	r	nr
	T	r	r	r	r
	Fe	—	—	—	r
4	L	r	r	r	nr
	T	r	r	r	r
	Fe	—	—	—	r
5	L	n.d.	n.d.	r	nr
	T	n.d.	n.d.	r	n.d.
	Fe	—	—	—	r
6	L	n.d	n.d.	r	n.d.
	T	n.d	n.d.	—	n.d.
	Fe	—	—	—	n.d.

n.d. radioactivity of fraction not determined
nr not radioactive
r radioactive
— sample does not contain the fraction.

---

* I. l. fractions whose specific gravities were above or below 2.96 p cm^3. However, some fractions of the NB sample were separated according to whether their components had specific gravities above or below 2.88 p/cm^3, since this separation was carried out with bromoform.

The results show that both heavy and light mineral components are radioactive in the travertines. In other words, samples of travertine from three different places, separated into six fractions according to size, and further separated in terms of light and heavy components, made up a total of 25 different fractions which were examined for radioactivity. Of these 25 fractions, only 4 were inactive, i. e. had the same number of traces as the reference plate. Three of these inactive fractions contained light mineral components, only one heavy components.

It was noted that in the Banjsko sand the radioactive elements were associated mainly with heavy fractions and with the fractions separated by magnet.

Those of fractions 1 to 6 which were quantitatively larger were diluted and the radium content was analyzed. This was done to determine if the radium concentration depends on the size of the grain in each fraction in relation to the radium concentration in the basic, unfractionated sample. Table 4 shows the results of these measurements. These results show that the radium concentration does not depend on the grain size; the radium concentration in individual fractions in relation to that in the unfractionated sample varies within limits 0.89 to 1.08. This would correspond to an error of ±10%, which is expected when determining radium.

TABLE 4

Relation of Radium Concentration in Individual Travertine and Sand Samples to Radium Concentration in the Unfractionated Sample

fraction	travertine or sand sample			
	NB	DB	F	S
1	0.94	n.d.	1.06	n.d.
2		0.90		0.89
3	1.08	0.95	1.03	1.06
4	1.05	0.99	0.92	
5	1.05		0.97	
6	n.d.	0.93	n.d.	1.01

INTERPRETATION OF EXPERIMENTAL RESULTS

The difference in the chemical structure of travertines from different regions can be understood in terms of the fact that these sediments form at different rates and in different circumstances, i. e. from spring waters which have different chemical structures and temperatures. However, all these travertines have in common a high calcium carbonate content, whether in an amorphous, noncrystalline form, or in crystalline form (such as calcite and aragonite). The presence of other compounds is considerably smaller.

Spectrochemical analysis have shown that natural radioactive elements are present in travertines in such small concentrations that they cannot be spectroscopically detected.

When travertine is formed by spring waters with calcium carbonate, radium may be taken up too, since the spring waters contain radium concentrations of 10^{-10} to $10^{-12}\%$. The resulting travertine becomes radioactive.* However, it is an open question whether the radioactivity in the travertine is a consequence of this type of chemical process, or whether the radium and other radioactive elements are related to components of a different origin. Satisfactory answers to this question were not found in the literature where, on the contrary, it is expressly stated that the distribution of radioactivity in sedimentary rocks, including travertines, has not been examined.[9]

Sediments always contain a certain amount of clay which is formed by the transformation of heavy mineral fractions (the so-called accessory minerals). It may therefore be assumed that the radioactivity of the sediments originates from these accessory mineral components which, in most cases, are carriers of radioactive elements.

On the other hand, physical an chemical factors which affect the accessory mineral components during the process of transformation may lead to a redistribution of the radioactive elements from heavy to light mineral fractions. It is necessary to take this factor into account when analyzing the distribution of radioactivity in individual fractions. The mineral analysis of travertine shows that the fractions contain heavy mineral components which could be carriers of radioactive elements.

The Banjsko sand, as all other sands, was not formed by sedimentation because of the action of chemical factors, as is the case with the travertines. It was formed by strictly physical phenomena. Suspended particles of sand thrown out by spring waters sediment because the particles have a larger specific gravity. Sand is also formed by the mechanical pulverization of stones over which water passes, in which case it could be expected that the radioactivity of the sands would have its source in radioactive mineral components such as uranium and thorium. This would be analogous to the situation which is found in analysis of the radioactivity of magmatic and metamorphic rocks[10,11]. It has been determined that the radioactivity of rock, lava and meteorites originates from mineral components which are carriers of uranium and thorium[2,6]. It was found that the Banjsko sand contained some accessory minerals as well as a certain amount of clay and humus. It can thus be

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* Analysis of the U: Ra ratio in the travertine showed that the uranium concentrations are far below what they should be in travertines with radioactive equilibrium for the radium concentration which was measured[7,8].

supposed that the radioactivity of this sand has its source in these accessory mineral components, as was the case with the radioactivity of the travertines.

The experimental results conclusively show that there is an important difference between the distribution of radioactivity in individual fractions of travertine, which are sediments formed from water by chemical sedimentation, and the sand, which is a sediment formed by mechanical pulverization of rock or mechanically from a water suspension. The results presented in Table 3 show that by qualitative photonuclear analysis of light and heavy fractions of travertine, only four out of 25 fractions were found to be non-radioactive. It may be asked whether this data proves the hypothesis that the distribution of radium in travertine is such that both light and heavy fractions are radioactive. Since there was a total of $n = 25$ measurements, according to this hypothesis all 25 fractions should be radioactive, namely 13 fractions with light and 12 with heavy specific gravities. However, 21 fractions were found to be radioactive, 10 light and 11 heavy fractions. Thus among the light fractions there was a divergence $l_L = 13 - 10 = 3$, and in the heavy fractions there was a divergence $l_T = 12 - 11 = 1$.

The number of data is small for a rigorous statistical interpretation. Nevertheless this data does show, if only roughly, whether or not the hypothesis of the distribution of radium between light and heavy fractions can be accepted from a mathematical and statistical standpoint.

In order to calculate the probability that the divergence will be within the limits $\pm l$, the probability formula valid for small numbers of samples may be used. From that formula, the probability for the light fraction is

$$P_L = 2\varphi\left(\frac{l}{\sqrt{npq}}\right) = 2\varphi\left(\frac{3}{\sqrt{25 \cdot \frac{3}{13} \cdot \frac{10}{13}}}\right) = 0.848$$

and for the heavy fraction

$$P_T = 0.396.$$

The results show that the divergence is within the allowed limits. It follows that there is a large probability that radium is distributed among the light as well as the heavy mineral fractions in travertine.

From the results shown in Table 4 it can be deduced that the travertine grain size has no significant effect on the radium distribution. Nevertheless it may be expected that small grains will adsorb a larger quantity of radium since they have a greater total surface per unit of mass. The results lead to the conclusion that the radium in travertine is not related to a particular mineral component nor a particular particle size, but rather that it is distributed among all the components. It must nevertheless be mentioned that it remains an open question whether or not somewhat larger quantities of radium are associated with the accessory mineral components which participate in the travertine with minimal quantities. This problem was not examined in this study because of the difficulty of quantitative analysis of radium content in small samples of fractions which contain heavy mineral components.

With respect to the distribution of radioactive fractions in sand S, it was found that all the heavy fractions, and those separated magnetically, were radioactive, and among the light fractions only one out of four was radioactive. It follows that in sand S the radioactive elements are predominantly connected to heavy mineral components, most probably zircon, sphene and biotite. These components are also mentioned in the literature as possible carriers of radioactive elements. It can also be concluded that in sand S the radioactive elements are limitedly connected to magnetite and similar ferromagnetic mineral components which are separated by means of a magnet. On the other hand the light mineral fractions of sand S were inactive which shows that in this case there was no redistribution of radioactive elements from heavy to light mineral components nor was there adsorption, which is possibly what had taken place in the travertines.

CONCLUSION

The experimental results show that there is a significant difference between the distribution of radioactive elements in components of travertine and the distribution in components of sand.

It was observed earlier[7] that all the components of NB travertine are more or less radioactive. The results of the present study show that this is also the case for travertines from other regions. It was found that in travertines radium is not in equilibrium with uranium[7,8]. When examining the distribution of radioactive elements between the travertine components, one could actually speak of the distribution of radium among the light and heavy components of the travertine. Since all the travertine components are radioactive, it follows that, when the travertine is formed, the radium is distributed among all the components and thus is not found only in the heavy components, i. e. those which are usually the carriers of radioactive elements in other rocks. In sand S, on the contrary, the radioactive elements are predominantly associated with heavy mineral components. A similar phenomenon was observed earlier in various magmatic and metamorphic rocks.

The different distributions of radioactivity among fractions of travertine and sand with different specific gravities could be explained in two ways. Adsorptive connections may be the reason why radium is associated with all the mineral components of the travertine. On the other hand the radium may have been built into the crystalline lattices of minerals which are not primarily radioactive; radium may have entered these components of travertine and clay during the transformation of accessory minerals, carriers of radioactive elements. With respect to sand S, it might be concluded that there were no changes which led to the redistribution of natural radioactive elements from mineral components with high specific gravities to those with low specific gravities, either by connecting or by building of these radioactive elements into the crystalline lattices of the light components. It may also be concluded that the light sand components do not adsorb radioactive elements from the natural water which impregnates the Banjsko sand and which contains less than 10^{-6} gU/lit and less han 10^{-12} gRa/lit.

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A CONTRIBUTION ON FLUORESCENCE QUENCHING IN QUINOLINE AND ITS DERIVATIVES WITH ORGANIC SUBSTANCES

by

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INTRODUCTION

Concentrated fluorescence guenching in quinoline and some of its derivatives with inorganic substances is described in reference (1). Since the results were in good agreement with the Stern-Volmer equation, we tried to check it with some organic substances as quenchers. Ii is not necessary to write here about the Volmer equation, which is derived by considering the specific reaction which occurs between the quencher and the excited molecules of the fluorescing substance. The formula is

$$I = \frac{I_0}{1 + kc},$$

where I_0 is the fluorescence intensity without the quencher and I the intensity for a qiven concentration of the quencher, c is the quenching constant, and k is the reciprocal of the quencher concentration (c_H) with which the initial intensity falls to one half.

We found that the majority of organic substances considerably influence the fluorescence intensity in quinoline and its derivates. Wę tested the following characteristic quenchers: pyrogallic acid, pyrocatechol, resorcin, orcin, hidroquinone, ortho-aminophenol and fluoroglucine in different media. Of the numerous quinoline derivatives whose fluorescences were quenched with these quenchers, we studied β—naphthaquinoline, whose fluorescence was one of the most intense.

EXPERIMENT

The experimental conditions of fluorescence intensity measurement were the same as in our first work in which we used inorganic salts as the quenchers, i. e. the optimum concentration of quinoline was 2.10^{-4} g/cc. It should be pointed out that the spectral distribution of the emission does not change on addition of the quencher, so it was sufficient to measure only the change of intensity. Fluorescence intensity changes were measured with a Kipp-type fluorimeter.

The results are shown graphically. The relative intensity is plotted on the ordinate and the corresponding concentration of the quencher on the abscissa. The intensity of the fluorescing substance without the presence of the quencher was taken as the standard (100).

Fluorescence quenching in quinoline in a decinormal solution of suphuric acid is shown in Fig. 1.

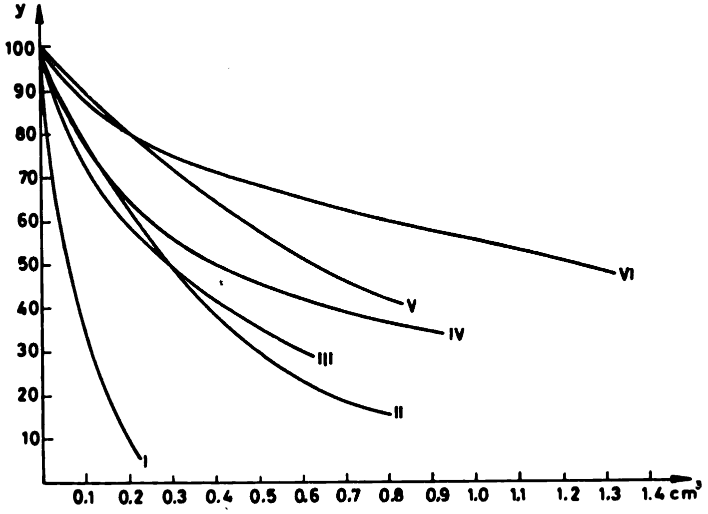

Fig. 1

The curves of fluorescence quenching in guinoline in an *n*/10 sulphuric acid solution

Ortho-aminophenol has the most intense quenching affect. Then follow hydroquinone, orcin, etc. The weakest quenching is produced by resorcin. From the graphs and from Table 1 it can be seen that the quenching follows Volmer's equation. The results for the intensity are in good agreement with the equation. Table 1 shows the experimental and calculated values for pyrogallic acid, resorcin and fluoroglucin. These quenchers were chosen because of their different quenching constants (pyrogallic acid has the highest and resorcin the lowest).

Figure 2 gives curves of fluoroscence quenching in quinoline in decinormal acetic acid with the same quenchers. Pyrogallic acid is the strongest quencher here (curve I).

TABLE I

Quinoline (in n/10 sulphuric acid; t = 20° C)

Quencher	Concentr. c	Experiment I	Calculated I	$k = 1/c_H$
Pyrog. acid	0.1	85	86.2	1.6
,,	0.2	75	75.7	,,
,,	0.3	65	67.5	,,
,,	0.4	60	60.9	,,
,,	0.5	55	55.5	,,
,,	0.6	50	51.0	,,
,,	1.0	38	38.0	,,
Resorcin	0.1	86	97.0	0.5
,,	0.3	80	89.2	,,
,,	0.8	68	75.7	,,
,,	1.3	58	58.1	,,
Fluoroglucin	0.1	88	90.1	1.2
,,	0.2	77	80.5	,,
,,	0.3	71	73.5	,,
,,	0.5	57	62.0	,,
,,	1.2	40	41.6	,,

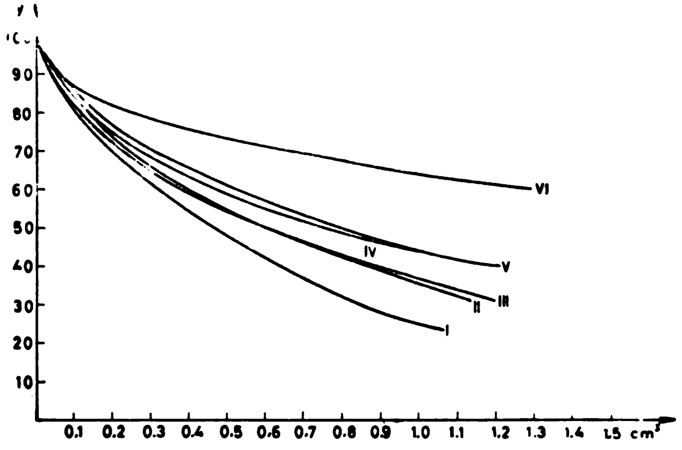

Fig. 2

The curves of fluorescence quenching in quinoline in acetic acid.

From Figs. 1 and 2 and Tables I and II it can be concluded that in both cases Volmer's equation is satisfied, and the kind of quenching is the same. The difference is only in the quenching constants, which are tabulated in Table III and which shows that the speed "rate" of quenching depends on the medium.

TABLE II

Quinoline (in n/10 acetic acid; $t = 20°$ C)

Quencher	Concentr. c	Experiment I	Calculated I	$k = 1/c_H$
Pyrog. acid	0.1	35	38	16.6
,,	0.2	18	23	,,
Resorcin	0.1	88	92	0.8
,,	0.3	76	80	,,
,,	0.6	65	67	,,
,,	1.0	55	55	,,
,,	1.5	45	45	,,
Fluoroglucin	0.1	81	82	2.3
,,	0.2	65	70	,,
,,	0.3	56	62	,,
,,	0.5	48	49	,,
,,	0.8	36	36	,,

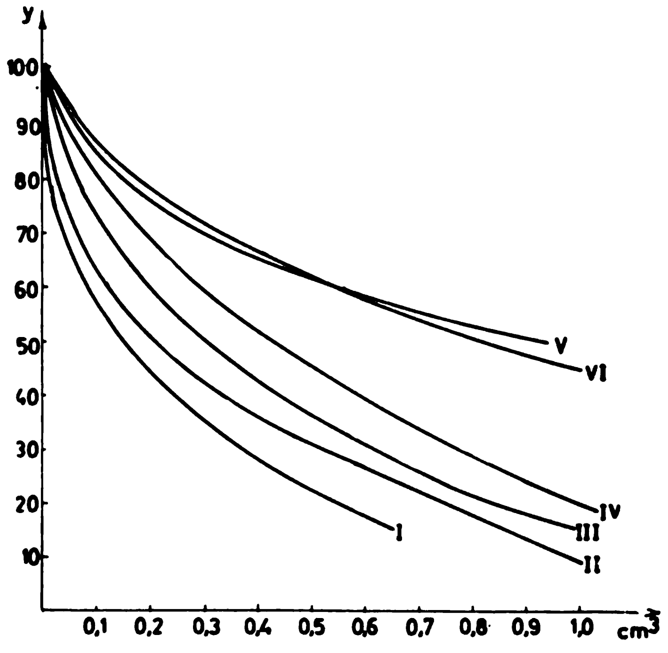

Fig. 3

The curves of fluorescence quenching for β-naphthaquinoline in $n/10$ sulfuric acid.

TABLE III

Quenching constants of quinoline in sulphuric acid and acetic acid

Quencher	Medium	c_H	k
Pyrogalic ac‘d	Sulphuric acid	0.62	1.6
,,	Acetic acid	0.06	16.6
Hydroquinone	Sulphuric acid	0.62	1.6
,,	Acetic acid	0.30	3.3
Fluoroglucin	Sulphuric acid	0.78	1.2
,,	Acetic acid	0.42	2.3
Pyrocatechol	Sulphuric acid		
,,	Acetic acid	0.28	3.5
Orcin	Sulphuric acid	0.85	1.1
,,	Acetic acid	0.62	1.6
Resorcin	Sulphuric acid	1.9	0.5
,,	Acetic acid	1.20	0.8

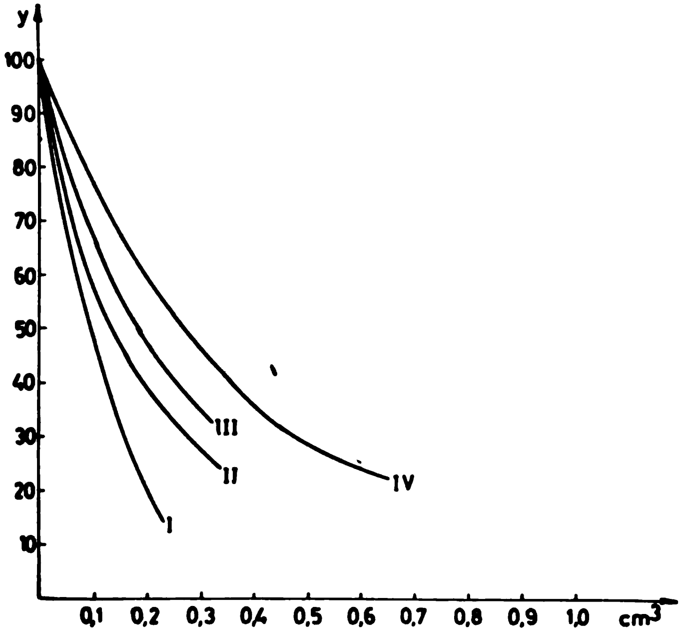

Fig. 4

The curves of fluorescence quenching for β-naphthaquinoline in acetic acid.

Fluorescence quenching in β — naphthaquinoline with the above quenchers in sulphuric and acetic acid is shown in Figs. 3 and 4. An optimal concentration of the fluorescing substance was taken here too. Best fluorescence quenching by resorcin is obtained in sulphuric and by pyrocatechol in acetic acid. It can be seen from Table IV that as in the case of quinoline the calculated and experimental values are in good agreement.

TABLE IV

β — *naphthaquinoline (in n/10 sulphuric acid at* $t = 20°$ *C*

Quencher	Concentr. c	Experiment. I	Calculated I	$k = \frac{1}{c_H}$
Resorcin	0.1	59	58.5	7.1
	0.2	45	41.3	
	0.3	33	31.9	
	0.4	—	25.9	
	0.6	18	19.0	
Pyrocatechol	0.1	90	89.2	1.2
	0.2	80	80.6	
	0.3	72	72.0	
	0.5	62	62.5	
	0.8	51	51.0	
	1.0	44	45.4	

β — *naphthaquinoline (in n/10 acetic acid at* $t = 20°$ *C)*

Quencher	Concentr. c	Experiment. I	Calculated I	$k = \frac{1}{c_H}$
Pyrocatechol	0.1	45	47.4	11.3
	0.2	21	31.0	
Orcin	0.1	73	72.4	3.8
	0.2	53	56.8	
	0.6	23	30.4	

To study the quenching process in quinoline and its derivatives, measurements of the degree of polarization, mean half-life of the molecules in the excited state and other relevant constants are under way.

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SPECTROPHOTOMETRIC STUDY OF IRON (III) COMPLEX OF MANDELHYDROXAMIC ACID

by

JOLANDA HOJMAN, BRANISLAVA STANKOVIĆ, ŽIVKA DUŠIĆ
and VERA MEDENICA

Hydroxamic acids have found a wide application as precipitating agents in guantitative determination of a great number of metal ions⁽¹⁾. Colorimetric methods for the determinantion of some metals (Fe, V, Mo, U, Mn)⁽²⁾ are well known; these methods are based upon the formation of soluble colored complexes. The colored reaction of ferric ion with hydroxamic acids plays an important role in the determination of various carboxylic acid derivatives in organic analysis⁽³⁾.

The iron (III) complex of some hydroxamic acids such as aceto-, benzo-, salicylo- and nicotinohydroxamic acid has been examined spectrophotometrically⁽⁴⁾. It was found that the color and composition of the complex depend on the pH of the solution. The equilibria between these complexes are very complex and have not been thoroughly investigated.

Our preliminary experiments with mandelhydroxamic acid as a precipitating reagent have shown that this acid behaves similarly to mandelic acid. However, on account of the presence of the —CONHOH group, it gives an intense color reaction with ferric ion, thus differing from mandelic acid. At $pH < 2.3$ the color of the solution is violet-reddish, then it turns orange until $pH < 4.65$, and for $pH > 5.07$ it is orange-yellow. The colored complex formed in slightly alkaline solution is exceptionally intense.

No data on the reaction of the ferric ion with mandelhydroxamic acid are reported in the literature. We have studied the mechanism of this reaction, the equilibria between various complexes in solution in dependence on pH, and their composition. We used different spectrophotometric methods, as for example: graphical analysis of absorption curves of the complex for different pH's of the solution, logarithmic analysis of absorbance curves for different concentrations of the metal ion or the ligand⁽⁵⁾, Job's method⁽⁶⁾, and the mole ratio method⁽⁷⁾.

EXPERIMENTAL

Reagents: Mandelhydroxamic acid was prepared by the procedure of Jones and Neufler(8), and was recrystallized from alcohol with addition of petroleum ether; m.p. 143.8° C (lit. 143.3° C)

Iron (III) solution was standardized by gravimetric and volumetric methods.

2 M $NaClO_4$ solution was used for adjustment of ionic strength.

Apparatus: Beckman Model DU Spectrophotometer; cells of 1 cm; Radiometer 22 pH-meter.

RESULTS AND DISCUSSION

Absorption curve of mandelhydroxamic acid (Fig. 1)

The absorption curve of mandelhydroxamic acid shows three maxima, at 253, 259 and 265 m μ. At higher pH-values the absorption is greater but no shifting of λ_{max} is observed. In acid solutions, at pH 1.10, the absorption curves of mandelhydroxamic acid and mandelic acid solutions of the same concentration differ very little at the 265 m μ absorption band.

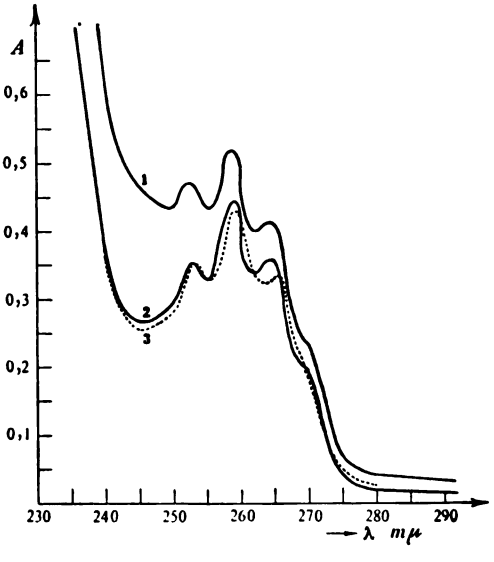

Fig 1

Absorption curve of mandelhydroxamic acid, $c = 2 \times 10^{-3}$ M; $\mu = 0.20$ ($NaClO_4$). Curve 1: mandelhydroxamic acid, pH 6.00; Curve 2: mandelhydroxamic acid, pH 1.10; Curve 3: mandelic acid, pH 1.10.

Effect of pH on the absorption curve of a solution of ferric ion and mandelhydroxamic acid

Figure 2 shows the absorption curves of solutions with different pH-values. The measurements were performed on solutions containing a large excess of mandelhydroxamic acid (HR). All the solutions had the same ferric ion and hydroxamic acid concentrations, and differed only in pH.

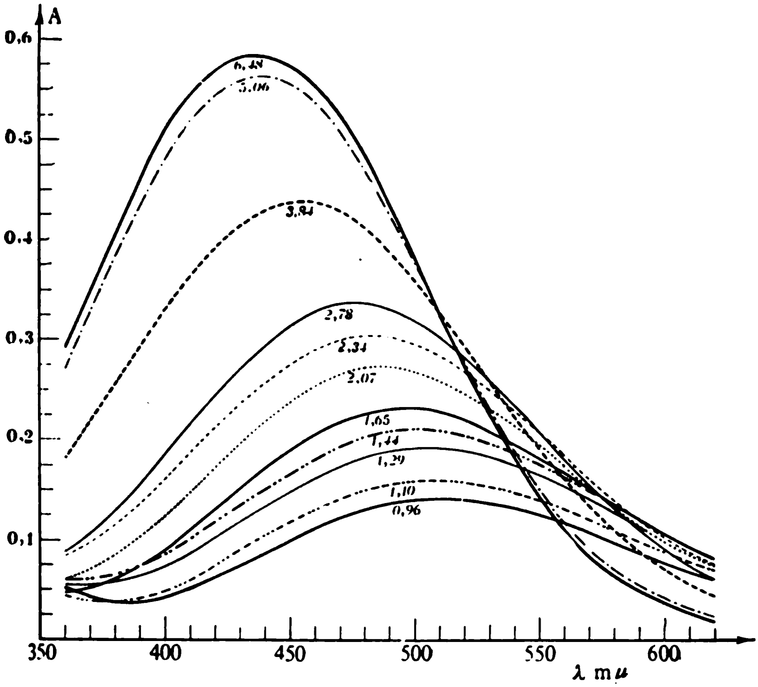

Fig. 2

Effect of pH on the absorption curve of solutions of Fe^{3+} and mandelhydroxamic acid $c_{Fe} + 3 = 10^{-2}$ M; $\mu = 0.20$ ($NaClO_4$). The number on the curve denotes pH.

The absorption curves of complex solutions with different pH-values show two isobestic points indicating the presence of three kinds of colored complex. All the curves for solutions of pH-values between 1.29. and 3.20 intersect at the first isobestic point at λ 570 mμ (isobestic point of violet-reddish and orange complexes). The curves of the solutions with pH > 3.2 pass through the second isobestic point at λ 570 m μ (isobestic point of orange and orange-yellow complexes).

Increasing pH shifts the absorption maximum towards shorter wavelengths with simultaneous increase of the absorbance, although the concentrations of ferric ion and mandelhydroxamix acid are constant. At longer wavelengths the absorbance decreases continuously, more rapidly in alkaline solutions. Mandelhydroxamic acid does not absorb in the visible region.

Dependence of λ_{max} on solution pH (Fig. 3)

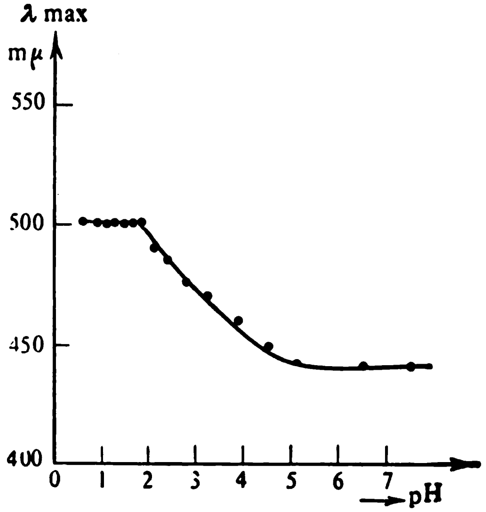

Fig. 3

Dependence of λ_{max} on solution pH $c_{Fe^{+3}} = 10^{-2}$ M; $\mu = 0.20(NaClO_4)$.

The curve of the λ_{max} shift against pH has two horizontal branches. At pH 0.55 λ_{max} is 500 mμ and till pH 1.78 it changes only insignificantly. With increasing of pH, it gradually shifts towards shorter wavelengths until pH 5.07, when the curve is again horizontal with λ_{max} 440 mμ. Further increase of pH to 9.54 causes no shift.

Dependence of absorbance on pH (Fig. 4)

To determine which complex is present at a given pH we studied the dependence of absorbance on pH at the following wavelengths:

a) 500 mμ — maximum of violet-reddish complex in strongly acid solution

b) 440 mμ — maximum of orange-yellow complex in slightly alkaline solution

c) 570 mμ — first isobestic point

d) 510 mμ — second isobestic point

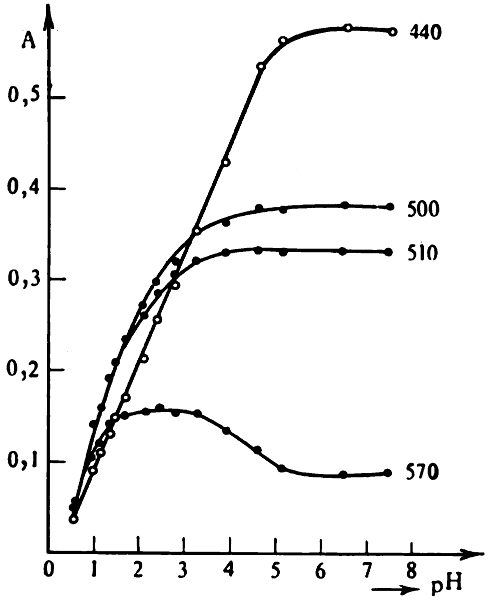

Fig. 4

Dependence of absorbance on pH, $c_{Fe^{+3}} = 2 \times 10^{-4}$ M; $c_{HR} = 10^{-2}$ M; $\mu = 0.20$ ($NaClO_4$).

At these wavelengths the absorbance-pH curves are not the same. This is not unexpected since complexes of different colors have different molar absorptivity.

In the pH range 0.55—0.96 the ascending branch of the curve is very steep at all wavelengths. With further increase of pH, the curves at 500 and 510 mμ slowly flatten, becoming horizontal at pH>4,: the curve at 440 mμ goes horizontal more sharply at pH˙5.07. The curve at 570 mμ has two horizontal sections. The first begins at pH>2.07. Then, at pH>3.20 the curve falls continuously, flattening to horizontal at pH 5.07 and remaining so with further pH increase.

Comparison of these curves shows the following:

a) The first complex (violet-reddish complex) is formed momentarily in a strongly acid solution.

b) The second complex (orange complex) is never present alone in the solution, but it is equilibrated with the first complex (pH 2.07—3.20) or the third (pH 3.20—5.07).

c) The third complex (orange-yellow) is formed and present in solutions of pH in the range 5.07—9.29. All the curves in this pH-range have a horizontal section.

The hydroxamic acid (HR) forms complexes with ferric ions liberating protons according to the following scheme:

$$Fe^{3+} + HR \rightleftarrows FeR^{2+} + H^+$$

$$FeR^{2+} + HR \rightleftarrows FeR_2^+ + H^+$$

$$FeR_2^+ + HR \rightleftarrows FeR_3 + H^+$$

We estimated the number protons liberated by logarithmic analysis of the ascending parts of the absorbance-pH curves (fig. 4), using the dependence of log ($c_{FeR_n}/c_{Fe^{+3}}$) on pH:

$$\log(c_{FeR_n}/c_{Fe^{3+}}) \sim \log\frac{A}{A_0-A} = n\,pH + \log K'$$

where A_0 is the absorbance at the point nearest the horizontal branch.

Logarithmic analysis of the dependence of $\log\frac{A}{A_0-A}$ *on* pH (Fig. 5)

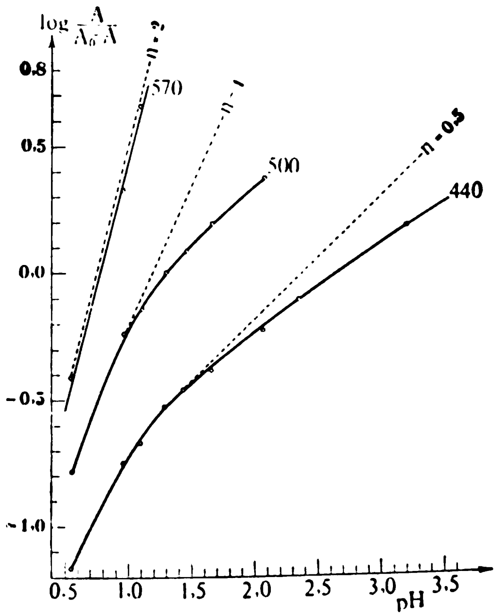

Fig. 5

Logarithmic analysis of log $\frac{A}{A_0-A}$ against pH.

$c_{Fe^{3+}} = 2 \times 10^{-4}$ M; $c_{HR} = 10^{-2}$ M; $\mu = 0.20$ (NaClO$_4$)

At wavelengths 500 and 440 mμ the dependence of $\log \frac{A}{A_0 - A}$ on pH is linear only in strongly acid solutions. At pH$>$1.29 the curve starts to be concave towards the pH-axis. The slope of the tangent at pH$<$1.1 is between 1.17 and 0.94, indicating that the number of protons liberated is one ($n = 1$). At higher pH-values the slope of the tangent is considerably smaller, and at pH $>$ 1.65 n is between 0.5 and 0.4. The same results were obtained at the wavelengths of 450, 470 and 510 mμ. The decrease of n with increasing pH may be explained by the dissociation of mandelhydroxamic acid in high pH solutions, whereby the anion R^- takes part in the reaction as a ligand.

At wavelength 570 mμ the dependence of $\log \frac{A}{A_0 - A}$ on pH is linear in the pH-range 0.55—1.69, the slope of the tangent changes insignificantly and n is between 1.97 and 1.71. The first and second complexes have the same molar absorptivity at 570 mμ, since the first isobestic point lies at that wavelength. n-values indicate that a small amount of complex of the composition HR/Fe = 2/1 is formed even in acid media (pH 1.69).

The composition of the complex of ferric ions with mandelhydroxamic acid was investigated by logarithmic analysis of the dependence of absorbancy on the concentrations of ferric ion and mandelhydroxamic acid, on the basis of thc following expression:

$$\log c_{\mathrm{Fe}_m\mathrm{R}_n} \sim \log A = m \log c_{\mathrm{Fe}} + n \log c_{\mathrm{R}} - \log K'$$

where K' is the dissociation constant of the complex $\mathrm{Fe}_m\mathrm{R}_n$.

Logarithmic analysis determination of the number (m) of central metal ions

Absorbance of solutions of the same pH (1.69) in the presence of a constant excess of hydroxamic acid ($c_{\mathrm{HR}} = 4 \times 10^{-3}$ M was measured. Ferric ion concentration varied from 5×10^{-5} to 7×10^{-4}M. Figure 6 shows the dependence of $\log A$ on $-\log c_{\mathrm{Fe}}$.

At all the wavelengths studied, the dependence of log A on $-\log c_{\mathrm{Fe}^{+3}}$ is linear in the whole concentration range. At 500 and 570 mμ the slope of the tangent is 0.94—0.98; at 440 mμ it is slightly less, 0.88—0.93. These values are close to 1, indicating that the iron coefficient (m) in the violet-reddish complex is one.

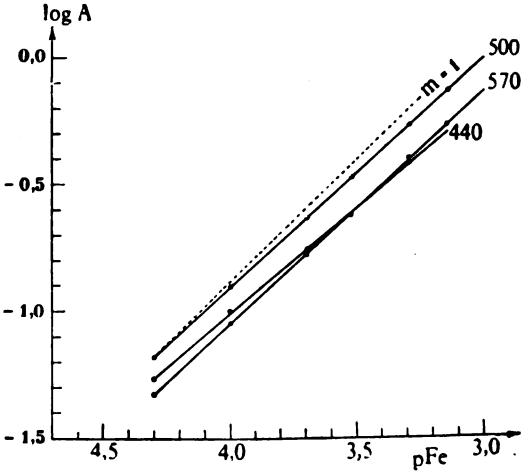

Fig. 6

Logarithmic dependence of log A on $-\log c_{Fe}$; $c_{HR} = 4 \times 10^{-3}$ M; $c_{Fe^{3+}} = 5 \times 10^{-5} - 7 \times 10^{-4}$ M, pH = 1.69 ± 0.02; $\mu = 0.20$ (NaClO$_4$).

Logarithmic analysis for the determination of the number (n) of ligands

Absorbance of solutions of pH 1.69 in the presence of a constant excess of ferric ion (2×10^{-3} M) was measured. Mandelhydroxamic acid concentration (c_R) varied in the range 5×10^{-5}—6×10^{-4} M. The results are shown in Fig. 7.

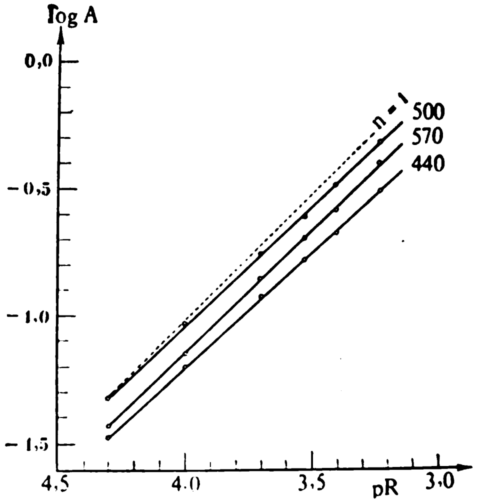

Fig. 7

Logarithmic analysis of the dependence of log A on $-\log c_R$; $c_{Fe^{3+}} = 2 \times 10^{-3}$ 2M; $c_R = 5 \times 10^{-5} - 6 \times 10^{-4}$ M; pH = 1.69 ± 0.02; $\mu = 0.20$ (NAClO$_4$)

The results obtained at 440, 500 mμ and 570 mμ show that the dependence of $\log A$ on $-\log c_R$ is linear. The slope of the tangent lies between 0.92 and 0.97, indicating $n=1$.

Job's curve

By logarithmic analysis of solutions it was found that the reaction of mandelhydroxamic acid with ferric ion in acid media produces the complex FeR^{2+}; the composition of this complex was demonstrated by Job's method of equimolecular solutions.

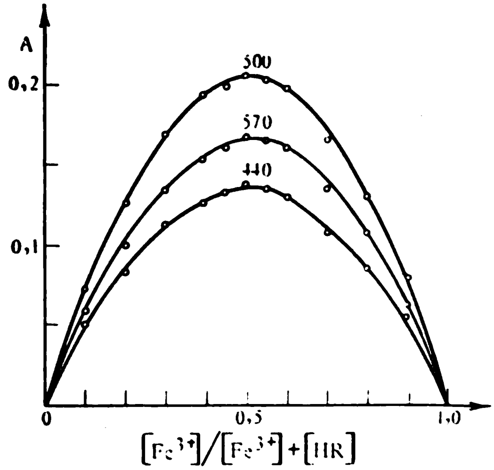

Fig. 8

Job's curves of equimolecular solutions. Total concentration 1×10^{-3} M; $\mu = 0.20$ ($NaClO_4$); pH = 1.69 ± 0.02.

From Fig. 8. it can be seen that in acid solutions hydroxamic acid forms a 1 : 1 complex with ferric ion.

From Job's curves for nonequimolecular solutions at pH 1.69 we determined the stability constant of the complex FeR^{2+}.

The maximum of Job's curve for nonequimolecular solutions at a fivefold higher concentration of hydroxamic acid has the same value at various wavelengths (440, 500, 510 and 570 m μ); $x_{max} = 0.375$. At a tenfold higher concentration of hydroxamic acid the maximum is also constant $x_{max} = 0.333$. The stability constant of the complex FeR^{2+} was calculated from Job's equation:

$$K = \frac{(p-1)(1-2x_{max})}{c_{Fe^{+3}}[(1+p)x_{max}-1]^2}$$

where $p = c_{HR}/c_{Fe^{3+}}$. The results are shown in Table 1.

TABLE 1

$c_{Fe^{3+}}$	p	x_{max}	pH	μ	log K
6×10^{-4} M	5	0.375	1.69 ± 0.02	0.20	3.028
6×10^{-4} M	10	0.333	1.69 ± 0.02	0.20	2.857

Mean value log $K = 2.94 \pm 0.086$

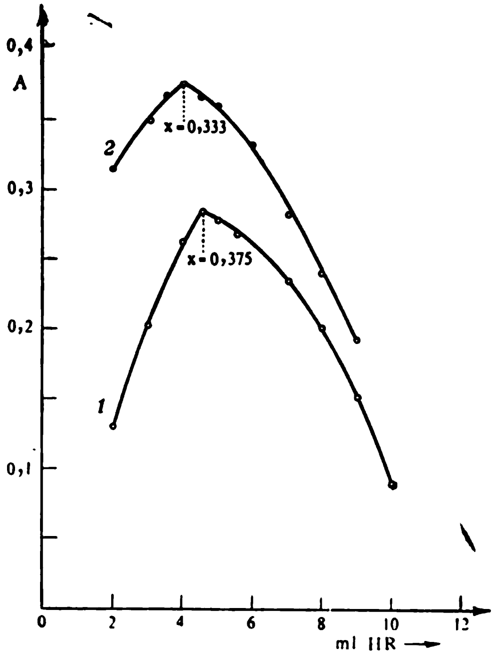

Fig. 9

Job's curves of nonequimolecular solutions. Total volume in each experiment 12 ml. Curve 1: $c_{Fe^{+3}} = 6 \times 10^{-4}$ M; $c_{HR} = 3 \times 10^{-3}$ M. Curve 2: $c_{Fe^{+3}} = 6 \times 10^{-4}$ M; $c_{HR} = 6 \times 10^{-3}$ M; pH $= 1.69 \pm 0.02$; $\mu = 0.20$ ($NaClO_4$); $\lambda = 500$ mμ; $t = 25 \pm 1°$ C.

In acid solutions mandelhydroxamic acid and ferric ion form a complex which is slightly more stable ($K = 8.9 \times 10^2$) than that of benzohydroxamic, acid whose stability constant, according to Christian, is 1.4×10^2 ($\mu = 0.20$).

Mole ratio method

Photometric titration of acid equimolecular solutions (pH = 1.69) at a constant ferric ion concentration also confirmed that the composition of the complex is 1 : 1. However the curves obtained in slightly acid (pH = 3.82) solutions did not show a sharp breakoff so the composition of the orange complex could not be determined.

In photometric titration of ecuimolecular solutions, pH 6.34, we obtained curves with a breakoff at the mole ratio HR : $Fe^{3+} = 3:1$. The measurements were carried out at 440 and 510 mμ. The results are shown in Fig. 10.

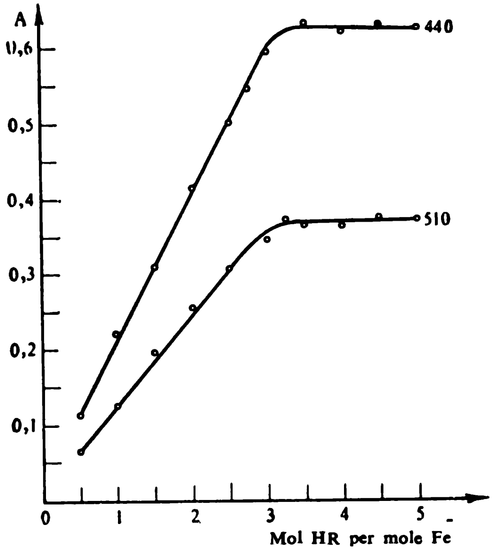

Fig. 10

Mole ratio method. $c_{Fe^{3+}} = 2.5 \times 10^{-4}$ M; pH = 6.34 ± 0.03; μ = 0.20($NaClO_4$)

The results of the mole ratio method prove that the composition of the orange-yellow complex is FeR_3.

Logarithmic analysis determination of the number (m) of central metal ion at pH 7.35

The number of ligands (*n*) in the complex formed in slightly acid (pH 3.8) and slightly alkaline solutions cannot be determined by logarithmic analysis of the absorbance. In these experiments ferric ion concentration is considerably higher than mandelhydroxamic acid concentration and the ferric ion which is not bound in the complex hydrolizes to form ferric hydroxide.

However in the presence of a great excess of mandelhydroxamic acid (4×10^{-3} M), the solutions of the complex at pH 7.36 are stable in the whole range of ferric ion concentration (5×10^{-5}—5×10^{-4} M). We studied the dependence of log A on $-\log c_{Fe}$ by measuring the absorbance of these solutions. The results are shown in Fig. 11.

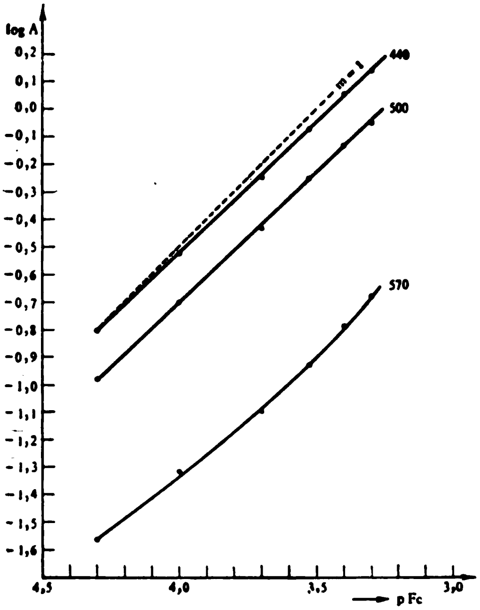

Fig. 11

Logarithmic analysis of the dependence of log A on $-\log c_{Fe}$. $c_{HR}=4\times$ $\times 10^{-3}$ M; $c_{Fe}=5\times 10^{-5}$—5×10^{-4} M, $\mu=0.20$ ($NaClO_4$); pH $=7.36\pm 0.03$

The results obtained at 500 and 440 mμ show a linear dependence of log A on $-\log c_{Fe^{+3}}$ in the whole concentration range, with a tangent slope 0.91—0.97. Consequently, the iron coefficient (m) of the orange-yellow complex is 1, as found by mole ratio method.

The slope of the curve obtained at 570 mμ is 1.10 if $c_{Fe^{3+}}$ $>2\times 10^{-4}$ M. In case $c_{Fe^{3+}}<2\times 10^{-4}$ M, it is 0.71—0.78. This may be explained by the small molar absortivity of the complex at this wavelength; on the other hand the slope value is very sensitive to experimental error in the measurement of absorbance of low ferric ion concentrations.

Beer's law

From our results we concluded that the determination of iron with mandelhydroxamic acid can be performed spectrophotometrically using the complex FeR_3 which is formed in neutral and slightly alkaline solutions. The intense orange-yellow color of the complex which is produced immediately is stable, and the absorbance remains constant for several hours.

To determine whether the color obeys Beer's law, we measured the absorbancy in slightly alkaline solution buffered with 0.1 M

$NaHCO_3$—Na_2CO_3 (pH 8.91). Mandelhydroxamic acid concentration was 1.5×10^{-2} M in each experiment, so that the reagent was present in great excess even at the maximal iron concentration.

The absorbance was measured at 440 mμ, since earlier investigations showed that mandelhydroxamic acid does not absorb at this wavelength.

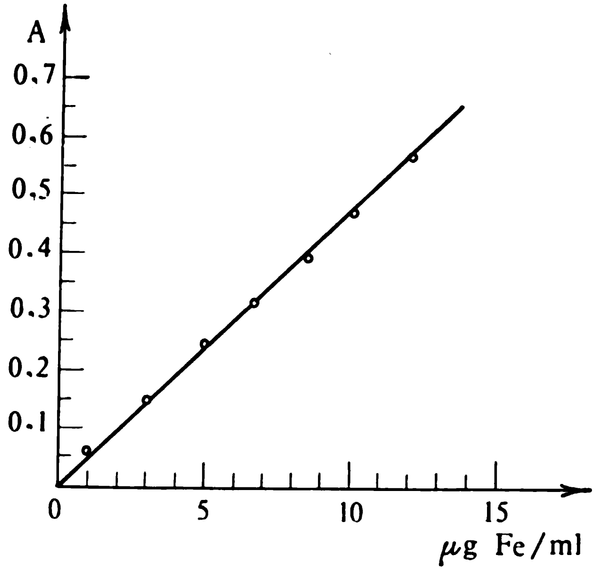

Fig. 12

Calibration curve. $c_{HR} = 1.5 \times 10^{-2}$ M; 0.1 M $NaHCO_3$—Na_2CO_3; pH 8.91; μ = 440 mμ

From the results shown in Fig 12 it may be seen that the reaction follows Beer's law and that the dependence of the absorbance on the concentration is linear in the range from 0.5 to 15 μg Fe per ml. From the calibration curves the molar absorptivity (a_M) at 440 mμ is 2662 ± 31, and the sensitivity (after Sandell) is 0.021 μg Fe per ml.

We examined the effect of some ions whose presence affects the color. It was found that vanadium (V), molybdenum (VI) and uranium (VI) seriously interfere with iron determination if their ratio to iron is greater than 1 : 4 while titanium interferes even in tenfold smaller concentration.

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COMPLEXOMETRIC DETERMINATION OF METALS
IV — DETERMINATION OF LEAD

by

VELIMIR D. CANIĆ and TIBOR A. KISS

Lead can be determined by direct titration with disodium ethylenediamine tetra acetic-acid (EDTA)* in the presence of one of the following as indicators: eriochrome black T(1–3); variamine blue B(4); xylenol orange(5–8); methyl thymol blue(8,9); a mixture of copper sulphate and PAN(10); PAR(11); dithizone(12); eriochrome red B(13); a mixture of copper sulphate and naphtylazoxine(14); pyrogallol red(15); bromine pyrogallol red(15); brilliant Congo blue Bel(16); or mixtures of vanadium complexes and diphenyl carbasone(17). The titrations are done in a weak acid or base medium. In a base medium lead is held in the solution by potassium sodium tartrate(1,2) or triethanolamine(18).

Vřestál and Havíř(19) titrated lead with EDTA in the presence of pyrocatechol violet as the indicator at pH 5.5 in a solution buffered with hexamethylenetetramine. This determination in a weak acid medium lasted for quite a long time.

It is possible to determine lead in a simpler way in the presence of pyrocatechol violet, by titrating at pH 10 in a solution containing an ammonia buffer and potassium sodium tartrate. Under these conditions the complex of lead with potassium sodium tartrate, pyrocatechol violet and EDTA is sufficiently stable for the end point of titration to be easily indicated.

EXPERIMENTAL

Reagents: Standard 0.1000 M EDTA solution was prepared by dissolving 37.225 g purified(20,21) disodium ethylene diamine tetra-acetic acid (Merck) and diluting to 1000 ml.

Standard 0.1000 M lead nitrate solution was prepared by dissolving 20.721 g pure (99.99%) lead in nitric acid and diluting to 1000 ml.

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*Communication III — Glasnik Hemijskog društva in press.

Standard 0.0500 M and 0.0100 M EDTA and lead nitrate solutions were prepared by proper dilution of the 0.1000 M and 0.0500 M solutions.

The titers of the standard 0.1000 M, 0.0500 M and 0.0100 M lead nitrate solutions were checked by direct titration with EDTA in the presence of pyrocatechol violet at pH 5.5(19) and eriochrome black T at ph 10(1).

The pH 10 buffer was prepared by dissolving 13.5 g ammonium chloride p.a. in a little water, adding 88 ml concentrated ammonium and diluting to 250 ml.

Other reagents: 0.1% aqueous solution of pyrocatechol violet and 1 M potassium sodium tartrate solution.

All the solutions were prepared with vater purified on ion exchangers(22) and kept in polyethylene bottles(20,23,24).

PROCEDURE

Approximately 100 ml weak acid solution containing up to 20 mg lead was heated to 40° C, 2 ml potassium sodium tartrate, 2 ml pH 10 buffer and 2 or 3 drops of pyrocatechol violet added and then it was titrated with EDTA until blue changed into a reddish violet color.

If the concentration of lead is higher, the heated weak acid solution is titrated to near the equivalence point and then the above amounts of potassium sodium tartrate, the buffer and the indicator are added to the solution and titration completed to a sharp color change. Results are shown in Table I.

Table I

EDTA molarity	Lead mg		Relative error in %
	taken	found	
0.0100	2.072	2.078	+ 0.29
	8.288	8.280	— 0.10
	10.36	10.32	— 0.39
	20.07	20.07	— 0.05
0.0500	41.44	41.64	+ 0.48
	52.80	52.62	— 0.34
	72.52	72.59	+ 0.10
	82.88	82.80	— 0.10
0.1000	103.6	103.3	— 0.30
	200.7	200.3	— 0.20
	528.0	528.5	+ 0.09
	1036.0	1040.0	+ 0.39

Results of titration of lead with EDTA at pH 10 in presence of pyrocatechol violet

DISCUSSION

In the presence of a high concentration of tartrate the color change of the indicator is turbid. To avoid this, it is titrated first in a weak acid medium to near the end point thus binding most of the lead to the EDTA complex and the remaining, smaller amount is retained in the solution at pH 10 with some tartrate. In this way up to 100 mg of lead and 100 ml of the titrated solution can be titrated and a sharp color change of the indicator obtained.

If the titrated solution is heated to 40° C, the color change of the indicator is sharper. This color change is more visible if the titrated solution is steadily mixed with a magnetic mixer and a fluorescent tube placed behind the solution.

Our results have shown that the color change of the indicator under these conditions is sharper than if titration is done at pH 5.5.

Since many metals are determined by retitration of the added excess of EDTA which is often titrated with a standard solution of lead, it was interesting to find out whether EDTA with lead can also be titrated at pH 10 in the presence of potassium sodium tartrate and pyrocatechol violet. Experiments have proved it is possible.

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REACTION OF TROPINONE WITH ACETYLENE

PREPARATION OF 3—ETHYLTROPANE—3—ol

by

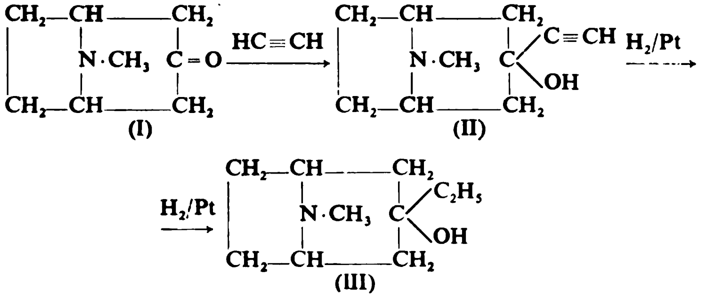
VUKIĆ M. MIĆOVIĆ, SLOBODAN MLADENOVIĆ,
and MILUTIN STEFANOVIĆ

Ketones react with acetylene in the presence of base catalysts giving two types of reaction product: a) tert-ethynylcarbinols $R_1R_2(OH)CC{\equiv}CH$, when one mole of ketone reacts with one mole of acetylene, and b) symmetrical tertiary acetylene glycols R_1R_2 $(OH)CC{\equiv}CC(OH\ R_2R_1$ when two moles of ketone react with one mole of acetylene⁽¹⁾.

While the ethynylation reaction of aliphatic, aromatic and alicyclic ketones has been extensively investigated and therefore a great many procedures and patents for the preparation of tert-ethynylcarbinol and tert-acetylene glycol are well known, the action of acetylene on ketones of the pyridine series has not been sufficiently studied. By performing ethynylation in liquid ammonia with metallic sodium as a catalyst, D. Papa and co-workers⁽²⁾ in 1954 synthesized several tertiary carbinols of the pyridine and piperidine series, but the yield of the reaction products was not been given. A more detailed study of the action of acetylene on pyridine ketones was made by Gautier, Miocque and Lafontaine⁽³⁾. They got good yields (up to 70%) of a great number of tert-ethynylpyridols.

In 1936 Unkovskii, Mohir and Urinović⁽⁴⁾ performed ethynylation of 1-methyl-4-piperidine and 1.3-dimethyl-4-piperidine, and obtained a yield of up to 70% of a mixture of geometrical isomers of corresponding ethynylpiperidols.

Within the scope of our studies of the reactions of ketone ethynylation we investigated the action of acetylene on tropinone⁽¹⁾ which by structure is a ketone of the piperidine series, condensed with one pyrrolidine ring.



Ethynylation of tropinone was performed according to Cella(5) and Stavely(6) in an ether-benzene solution with tertiary potassium-amylate as catalyst. Unreacted tropinone was separated by distillation, and the crude reaction product was obtained in the form of a dark brown oil (13%) which still contained a little unchanged ketone. Manifold chromatographic purification on a column with aluminum oxide as the adsorbent yielded a previously unknown crystalline product to which a 3-ethynyltropane-3-ol structure (II) is ascribed on the basis of the elementary analysis and the infrared spectrum (infrared spectrum of product II has pronounced maxima at 3480 and 3230cm^{-1} for the vibration of the —OH, i.e. the $-C{\equiv}CH$ group, and no maximum for the vibration of the $C=O$ group). Hydrogenation of product II in an alcohol solution using Pt as the catalyst yielded 3-ethyltropane-3-ol (III). The structure of this product was also confirmed by elementary analysis and infrared spectrum (maximum for vibration of the —OH group at 3475 cm^{-1}; no maximum for vibration of the ethynyl group).

There are several factors which prevent the reaction of ethynylation of tropinone:

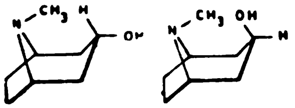
— the electrophile character of the carbonyl group in tropinone is weakened because the nitrogen atom in the bridge has nucleophile properties; however, as the reaction of ketone ethynylation is a nucleophilic addition of the acetylide anion to the electrophilic carbonyl group of ketone(7), the course of the reaction of acetylene with tropinone is made considerably difficult;

— the carbonyl group in tropinone is sterically hindered, which reflects on the yield of the 3-ethynyltropane-3-ol.

To prevent deactivation of the carbonyl group under the influence of the free electron pair on the nitrogen atom, we first tried to synthesize the N-oxide of tropinone(8). The addition of acetylene to the N-oxide of tropinone and the reduction of the reaction product with weak reducing agents ought to yield 3-ethynyl-tropane-3-ol. Likewise, with this N-oxide other nucleophillic additions could also be made.

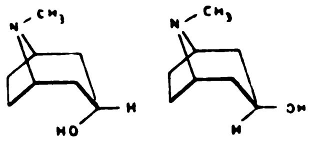
However, with organic peracids, i.e. perbenzoic, p-nitroperbenzoic and monoperphthalic, on tropinone, we mainly obtained salts of tropinone with peracids and some benzoate or-p-nitrobenzoate and phthalate of the N-oxide of tropinone. We were not able to isolate pure N-oxide of tropinone or one of its salts.

The reaction of acetylene with ketones is not stereospecific(9), and a mixture of corresponding geometrical isomers is always obtained. The question arises as to the conformation of the piperidine ring in the 3-ethynyltropane-3-ol (II) and the space relation of the hydroxyl or ethynyl groups to the nitrogen atom in the bridge. According to Fodor(10) the piperidine ring in tropine and ψ-tropine has the "C" conformation (formula *A* in the scheme). Fodor's formulation has been confirmed by measurements of the dipole moments of tropine and ψ-tropine, and by a study of the infrared absorption spectra of both isomers which shows that there is an intramolecular hydrogen bridge(11) in ψ-tropine. Bose and co-workes(12) and Archer and Lewis(13), however, propose the "Z" conformation for both isomers (formula *B* in the scheme) by analogy with the "Z" conformations of almost all cyclohexane compounds



tropinone ψ-tropinone

A

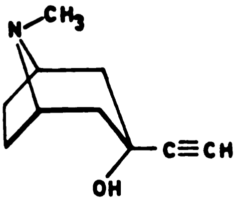


tropinone ψ-tropinone

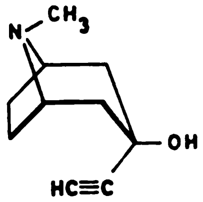
B

and pyranozides. According to these authors the existence of the hydrogen bridge in ψ-tropine is due to the mobility of the piperidine ring so that in ψ-tropine the "C" and "Z" conformations are in a dynamic equilibrium, while the "C" conformation is predominant.

The infrared absorption spectrum of 3-ethynyltropane-3-ol (II) shows that the hydroxyl group is not associated (sharp maximum for the vibration of the -OH group at 3480 cm^{-1}). Therefore, the piperidine ring probably has the "Z" conformation so that there can only be two isomers. The isomer structure II a is ascribed to



II a



II b

isolated 3-ethynyltropane-3-ol, i.e. ethynyl group is oriented equatorially and the hydroxyl group is axial. The adsorption properties of product II confirmed this structure on chromatographic purification on an aluminum oxide column. It was found that product II weakly adsorbs on aluminum oxide so that it can be eluted even with a benzene-chloroform mixture (7:3) (in more non-polarized fractions only unreacted tropinone is isolated). However, if a more polarized group (in this case the hydroxyl group) is equatorially disposed, product II could be more strongly adsorbed on aluminum oxide. Isomer II b could not be isolated.

It is well known that some ethynylcarbinols show significant physiological activity(2). The 3-ethynyltropane-3-ol (II) is thus also expected to have an interesting physiological action.

The study of the reaction of ethynylation of ketones from the tropa-alkaloids and others is the subject of our further investigations.

EXPERIMENTAL

Tropinone is obtained by saponifying atropine-sulphate(14) and oxidizing tropine with chrome trioxide in glacial acetic acid(15) and then purifying by distillation in vacuo, t.t. 42°C

Reaction of tropinone with acetylene

Ethynylation of tropinone was done according to slightly modified methods of Cella(5) and Stavely(6). 30.5 g (0.35 moles) of tertiary amyl alcohol is put into a roundbottom 250 ml four-neck flask and mechanically mixed. The flask has a mechanical mercury mixer (central neck), an upright condenser (which is protected from moisture by a calcium-chloride tube at its top), a dropper and an inlet tube for acetylene. The reaction vessel is put into a cold water bath and during several minutes 3.1 g (0.08 moles) of finely crushed metallic potassium, which is first cleaned well and dried, is added. After all the potassium has reacted, the mixture in the reaction vessel is diluted with 70 ml anhydrous ether and then acetylene is introduced (about 120 bubbles per minute). The acetylene is first purified and dried by passing throught two wash bottles containing chromium sulphuric acid, one wash bottle with a saturated solution of calcium chloride, two towers with anhydrous $CaCl_2$, and one tower with solid KOH. After half an hour, a solution of 10 g (0.07 moles) of tropinone in 50 ml of anhydrous benzene is added from the dropper. The adding lasts 30 minutes, and then acetylene is passed throught the reaction mixture for two more hours. During the whole process the temperature of the reaction mixture is maintained at about 5° C. The reaction mixture is allowed to stand at room temperature overnight and then the alcoholate is decomposed with about an equal volume of cold water. After the top ether-benzene layer has been separated in a separatory funnel, the water layer is continually extracted with ether. The combined extracts are then dried over anhydrous $MgSO_4$, filtered and the solvent is removed by distillation in vacuo, first on a water and then an oil pump. 2.1 g of brown oil (18.2%) is obtained.

The oil is chromatographically purified on a column of alkaline aluminum-oxide (Merck; Brockman 1) as the adsorbent. From the benzene-chloroform (7:3) fraction, 1.5 g (13%) of colorless oil is obtained which shows a tendency to crystallize.

The infrared spectrum showed the oil still contained some unreacted tropinone (maximum for vibration of the C = O group at 1640 cm^{-1}), so the oil was rechromatographed and from the benzene-chloroform fraction (7 : 3) a colorless oil was obtained which became solid on cooling. By crystallization from the benzene-hexane mixture crystalline 3-ethynyltropane-3-ol, t.t. 162° C was obtained.

Analysis:

Calculated for $C_{12}H\,_{5}ON$ (165.23) : C 72.69%; H 9.15%; N 8.48%

Found : C 72.41%; H 8.91%; N 8.59%

Infrared spectrum:

maxima at 3480 and 3230 cm^{-1} (for the -OH and $-C{\equiv}CH$ groups)

Hydrogenation of 3-ethynyltropane-3-ol in 3-ethyltropane-3-ol

A solution of 500 mg (0.003 moles) of 3-ethynyltropane-3-ol in 10 ml of ethanol plus 10 mg of platinum black as catalyst is hydrogenated under normal pressure. In ten minutes 134 cc (0.06 moles) of hydrogen is absorbed and then the introduction of hydrogen is stopped. After the catalyst has been separated by filtering, ethanol is removed by distillation in vacuo, and a quantitative yield of 3-ethyltropane-3-ol is obtained—a colorless oil—which is purified by distillation.

Analysis:

Calculated for $C_{10}H_{19}ON$ (169.26):C 70.96%; H 11.32%; N 8.28%

Found :C 70.30%; H 10.79%; N 8.85%

Infrared spectrum: maximum at 3475 cm (for the -OH group)

Reaction of tropinone with peracids

Perbenzoic, p-nitroperbenzoic and monoperphthalic acids were used. Their activities were first determined by titration.

An ethereal solution of 10 g (0.07 moles) of tropinone was mixed with 0.1 mole of an ethereal solution of peracid. Because of the reaction ether began to boil slowly. After standing for 24 hours at room temperature, a crystalline residue was obtained which was filtered, crystallized from alcohol (it has no definite melting point) and then analyzed. The crystalline substance obtained was chiefly a mixture of tropinoneperbenzoate with some N-oxide-tropinone-benzoate (or p-nitrobenzoate and phthalate, depending on the peracid used). Our attempt to isolate pure N-oxide of tropinone by treatment with sodium hydroxide and by continual extraction with ether failed and always only tropinone has been isolated.

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NATURE OF OIL-SHALE KEROGENS. OXIDATION WITH POTASSIUM PERMANGANATE IN ACETONE SOLUTION.

III.-OXIDATION OF KIMMERIDGE (DORSET, ENGLAND) OIL-SHALE KEROGEN

by

DRAGOMIR VITOROVIĆ and MILICA DJURIČIĆ

The earlier papers reported on the behavior of Colorado and Aleksinac oil-shale kerogens towards an acetone solution of potassium permanganate(1,2). In continuation of these studies, the action of potassium permanganate in acetone solution on an English type oil-shale kerogen (Kimmeridge, Dorset) has been investigated; the influence of different factors on the balance of organic carbon and the nature of degradation products has been investigated. This oil-shale was chosen because in the earlier investigations(3) it was found to be more easily degraded with an alkaline solution of potassium permanganate and because in oxidation products there are aromatic compounds which make it different from the types we investigated earlier. Besides, the Kimmeridge oil-shale has a rather high content of sulphur, about 9%, a small carbonate content and a large content of an organic substance.

Down and Himus(3) treated the Kimmeridge oil-shale for 57 hours with an excess of permanganate in a 3% alkaline solution and got the following balance of organic carbon: 49.8% carbon dioxide, 8.3 % volatile acids, 31.0 % oxalic acid, 10.5 % nonvolatile acids (mostly aromatic), 0.6% unoxidized part. From their results they consider the Kimmeridge oil-shale kerogen to be to a certain extent aromatic, but the percentage of carbon that oxidizes in benzene-carboxylic acids does not exceed ten. By oxidizing a large quantity of this oil-shale, they obtained a sufficient amount of the degradation product and in it they identified the acetic, oxalic, succinic, benzoic, o-phthalic, p-phthalic, and mellitic acid.

Dancy and Giedroyc(4) also investigated the Kimmeridge oil-shale kerogen by degrading it with permanganate, gaseous oxygen and hydrogen-peroxide. They also found that the organic substance of this shale easily oxidizes and thought it might have an unsaturated aliphatic or cyclic structure.

The present paper reports the results of oxidative degradation of Kimmeridge oil-shale with an acetone solution of potassium permanganate, and the influence of the changing the amount of permanganate and the heating time on the balance of organic carbon. The experimental conditions were varied according to the usual scheme(1): portions of 20 grams of enriched shale with an organic carbon content of 9.01 g were oxidized with 100, 200 and 300 g of potassium permanganate in 400 ml of acetone with heating for 16, 32, 48 and 64 hours (including the time taken for adding the permanganate). The oxidation and separation of acid and neutral products were performed according to the method described earlier(1). The acid and neutral products and unoxidized parts were analyzed and hence the balance of organic carbon was made.

It was pointed out earlier that the behavior of the Colorado and Aleksinac oil shales was quite similar. However, the results obtained with the Kimmeridge shale under similar conditions show that the changes following variation of the experimental conditions differ from those of the other two; the Kimmeridge shale oxidizes more easily and the products contain a lot of oxygen. However, even under the most drastic conditions, some organic carbon remains in the unoxidized part or it is obtained in the form of a product which is insoluble in ether.

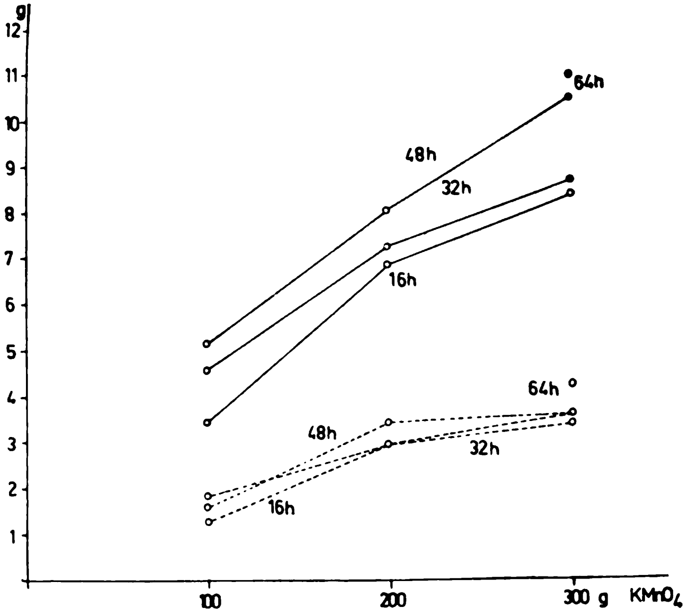

Fig. 1

Yields and carbon contents of acid products obtained by oxidizing Kimmeridge shale with different amounts of potassium permanganate for 16, 32, 48 and 64 hours

___________ Yield of acids

----------- Carbon content

REACTION PRODUCTS

a) *Acid products.* — When the shale is treated with different amounts of permanganate (100, 200, and 300 g) for a period of 16 hours, the yield of acid products rapidly rises with increasing amount of permanganate (3.34, 6.83, and 8.40 g), as can be seen in Fig. 1.

When the shale is treated with 100, 200, and 300 g of permanganate for 32, 48, and 64 hours more degradation products are obtained than for 16 hours with the same amount of permanganate; the yield increases almost regularly with an increasing amount of oxidizing agent, which is not the case with the Colorado and Aleksinac shales.

Figure 1 also shows data on the content of carbon in acid degradation products calculated on the basis of the elementary analysis (dashed lines).

The acid product yield as a function of heating time is shown in Fig. 2. From the results the following conclusions can be drawn:

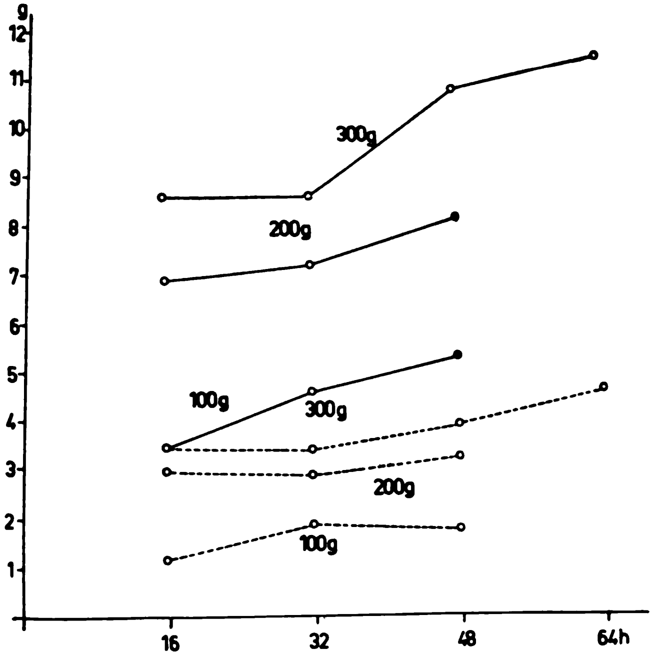

Fig. 2

Yields and carbon contents of acid products obtained by oxidation with 100, 200 and 300 g of potassium permanganate for different periods of time

__________ Yield of acids

- - - - - - - - - - - Carbon content

1. In oxidation with 100 and 200 g of permanganate for different periods of time (16,32 and 48 hours) the yield increases but the ogranic carbon content does not change proportionally (dashed line).

2. In oxidation with 300 g of permanganate, as distinct from the results obtained by oxidizing the Colorado and Aleksinac oil-shale under the same experimental conditions, the yield of acid degradation products gradually increases with heating time (8.40, 8.48, 10.48 and 10.95 g); the carbon content also increases but more slowly.

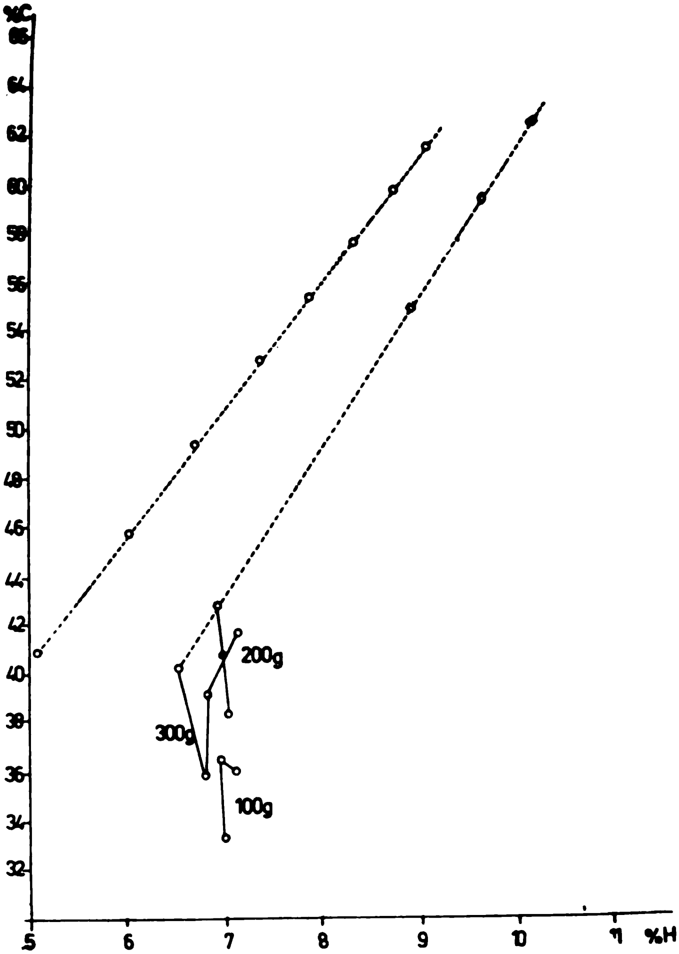

Fig. 3

C/H-contents of acid products obtained by oxidation with 100, 200 and 300 g of potassium permanganate for 16, 32, 48 and 64 hours

The continual increase of the amount of carbon in the form of acid products under ever more drastic oxidation conditions, shows that the kerogen is progressively degraded.

Change of the nature of the acid products is followed by changes in the content of carbon, hydrogen, nitrogen and sulphur, and of the acid numbers, equivalents and iodine numbers.

The C,H — content of acid products under different experimental conditions does not change regularly.

The graph in Fig. 3 show the results of elementary analyses of acid products and the C, H values of the defined monocarboxylic and dicarboxylic fatty acids. It is characteristic that the carbon content in acid products is very low compared to that in the defined acids, and lower than that in the corresponding products of the Aleksinac and Colorado shales.

Depending on the experimental conditions, 13.32 — 48.61% of the original carbon content was obtained in the form of acid products.

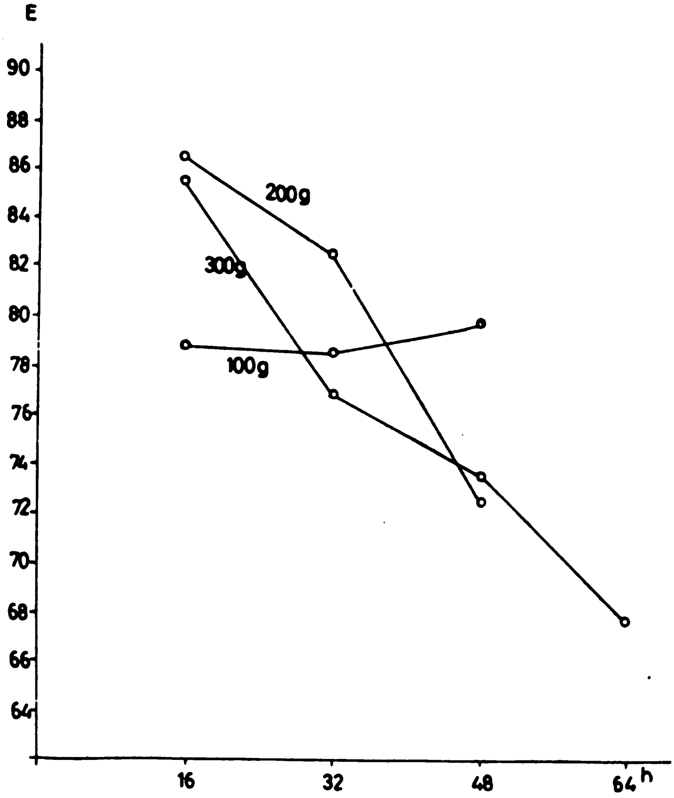

Fig. 4

Equivalents of acid oxidation products

The changes of the acid numbers and the equivalents of the acid products were mostly not regular, except for the products obtained by oxidation with 300 g of permanganate for 16, 32, 48 and 64 hours: the acid numbers increase regularly with heating time, and the equivalents decrease. The equivalents are shown in Fig. 4. It is obvious that the equivalents are lower than those of similar products of the Colorado and Aleksinac shales and that they correspond to the equivalents of acids with a smaller number of carbon atoms.

The iodine numbers are very low, from 0 to 0.63.

The sulphur which is found in all fractions is the most abundant in products of treating the shale with 300 g of permanganate (up to 2.3%).

b) *Neutral products.* — From 20 g of enriched oil-shale with 9.01 g organic carbon content, portions of about 0.2-0.5 g of a neutral product (1.5-4% of the original carbon content) were obtained. The yield does not change regularly with the change of the oxidation conditions. The carbon content is 63 - 76% and hydrogen 9 - 11%. The changes in the composition are not regular either. The C/H ratio of the neutral products varies from 6.8 - 7.2.

The neutral products contain a considerable amount of sulphur, 2.0 - 5.8%.

c) *Unoxidized part.* — From the elementary analysis the amount of organic carbon which remained unchanged or was degraded into products insoluble in ether was calculated. Like the Colorado and Aleksinac oil-shales, a large amount of organic carbon remains in the unoxidized part (38 - 88%). Nevertheless, it can be concluded that this shale oxidizes more easily than the other two. Organic carbon gradually oxidizes with an increasing amount of permanganate and longer heating. However, it can be concluded from the results that with the reduction of the excess of permanganate the organic substance is further degraded: the greater the excess of permanganate the longer the reduction time and the smaller the carbon content in the unoxidized part. This phenomenon was discovered earlier too and it will be further discussed in a future publication.

EXPERIMENTAL PROCEDURE

A sample of Kimmeridge (Dorset) oil-shale was crushed in a ball mill (—100 mesh, Tyler 0.149 mm). The concentrate was obtained in the conventional way by treating the shale with hydrochloric acid (1 : 1). From 100 g of shale we obtained 86.97 g of concentrate. Composition of the concentrate: C—45.03%, H—4.74%, or C—68.12%, H—7.39% calculated for the burnt out part.

Potassium permanganate was dried and ground into a fine powder; acetone was purified by boiling with potassium permanganate and then dried with anh. calcium chloride and redistilled.

For all tests portions of 20 g of enriched shale with an organic carbon content of 9.01 g were used. The experimental conditions were varied according to the scheme used in the earlier works⁽¹⁾.

Altogether ten experiments under different conditions were made. Control tests were made under identical conditions: a certain amount of acetic acid was obtained in the controls, on the basis of which a corresponding correction of the yield was made.

In all the experiments we used the procedure described earlier and used for the investigation of the Colorado and the Aleksinac shales(1,2): solid potassium permanganate is added to a gently boiling mixture of shale and acetone; the adding is adjusted so that the reaction is not violent. All the permanganate is added within 7 or 9 hours. After a definite period of heating the bulk of the acetone is removed and the cold mixture is diluted with water. The excess of permanganate and manganese dioxide are reduced with sulphur dioxide and then the oxidation products are extracted with ether from the acidified mixture. The acid oxidation products are extracted from the neutral products with a 5% solution of potassium hydroxide. The apparatus was the same as in the earlier experiments.

a) *Acid products.* — After being acidified with hydrochloric acid (1 : 1) the acid products are extracted with ether. The ether solution is dried and the solvent redistilled. The yields of the acid products are shown in Table 1.

TABLE 1

Yields (in grams) of acid oxidation products (20 g of enriched shale with 9.01 *g of organic carbon*

Heating period hours	Amount of permanganate, g		
	100	200	300
16	3.34	6.83	8.40
32	4.42	7.11	8.48
48	5.06	7.94	10.48
64	—	—	10.95

The results of C, H-analysis of acid products are shown in Table 2.

TABLE 2

C/H-contents of acid oxidation products

Heating period hours	Amount of permanganate, g		
	100	200	300
16	35.95 7.21	42.68 7.00	41.59 7.36
32	36.12 7.03	40.70 7.11	38.99 6,87
48	33.09 7.08	38.33 7.23	35.86 6.77
64	—	—	40.01 6.59

From the analysis, the carbon content in acid products was calculated and expressed as a percentage of the original amount of organic carbon (Table 6).

The acid values and equivalents were determined by titration with an n/10 solution of potassium hydroxide. The acid values are shown in Table 3.

TABLE 3

Acid values of acid oxidation products

Heating period hours	Amount of permanganate, g		
	100	200	300
16	710,2	648.2	656.2
32	712.6	678.9	728.2
48	704.1	771.4	760.8
64	—	—	825.7

The iodine values were determined according to Hanus's method and sulphur by Gasparini's method.

b) *Neutral products.* — Neutral products were isolated from the ether solution after the extraction of acids. The solution was dried with anh. sodium sulphate and ether was redistilled. The yield of neutral products under different conditions is shown in Table 4.

TABLE 4

Yields (in grams) of neutral oxidation products (20 g of enriched shale with 9.01 g of organic carbon)

Heating period hours	Amount of permanganate, g		
	100	200	300
16	0.2081	0.5594	0.3150
32	0.1260	0.5155	0.2045
48	0.2639	0.3000	0.2800
64	—	—	0.4891

The results of the C,H-analyses of neutral products are given in Table 5 and the carbon content expressed as a percentage of the original amount of carbon is shown in Table 6.

c) *Unoxidized parts.* — From the C, H-analysis the content of carbon in the unoxidized part was determined. It is expressed as a percentage of the original amount of carbon (Table 6).

As was pointed out before, the balance of organic carbon (Table 6) was calculated from the analysis of acid and neutral products and of the unoxidized parts.

TABLE 5

C/H contents of neutral products

Heating period hours	Amount of permanganate, g		
	100	200	300
16	72.13	63.13	69.10
	10.09	8.81	9.82
32	72.64	73.04	70.30
	10.17	10.49	9.74
48	70.29	67.70	69.19
	9.66	9.44	9.67
64	—	—	75.95
			11.14

TABLE 6

Balance of organic carbon obtained by oxidizing enriched Kimmeridge shale with an acetone solution of potassium permanganate under different conditions (20.00 g of the shale with a content of 9.01 g of organic carbon)
(a-acid product; n-neutral product; p-unoxidized part; t-total)

Heating period hours	Amount of permanganate, g		
	100	200	300
16	a 13.32%	a 32.41%	a 38.74%
	n 1.66%	n 3.88%	n 2.44%
	p 86.68%	p 38.07%	p 38.96%
	t 101.66%	t 74.36%	t 80.14%
32	a 18.98%	a 32.08%	a 36.74%
	n 1.00%	n 4.22%	n 1.55%
	p 87.10%	p 60.60%	p 44.73%
	t 107.07%	t 96.90%	t 83.02%
48	a 18.54%	a 33.74%	a 40.73%
	n 2.11%	n 2.22%	n 2.11%
	p 88.57%	p 64.24%	p 47.68%
	t 109.22%	t 100.20%	t 90.52%
64	—	—	a 48.61%
			n 4.11%
			p 48.92%
			t 101.64%

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CHEMICAL INVESTIGATION OF WHEAT

7*

GRAIN LIPIDS OF WHEAT IN RELATION TO FERTILIZER TREATMENT

by

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ANTIĆ and DIMITRIJE HADŽIJEV

INTRODUCTION

The first reports on the influence of phospholipids on the elastoplastic properties of flour dough(1,2) gave rise to the question of the possibility of applying modern crop production techniques to intensify the accumulation of lipids in wheat grain, and thus to influence the baking quality of wheat flour. This problem, of exceptional economic and theoretical importance, has not yet been fully answered, mainly because of experimental difficulties encountered in the separation and isolation of wheat grain lipids. Among these difficulties are:

a) The presence of a very stable lipoprotein complex in wheat grain. The liberation of lipids from such a complex and their quantitative determination require special analytical procedures.

b) Older methods of lipid extraction included some of the phosphoproteins, the free orthophosphate, the phytin and the phytic acid-protein complex. Therefore phospholipid phosphorus estimates in such crude lipid extracts could not provide reliable and accurate results.

Recently Kurmies(3) overcame some of these difficulties by using methanol as a lipid solvent, and in his experiments with increasing levels of phosphorus fertilizers he obtained a constant value for the content of phospholipid phosphorus in ripened wheat grain. Wenzel(4) had arrived at a similar result somewhat earlier.

Although these authors' data may be considered accurate, drawing the conclusion that fertilizer treatment does not affect

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* See paper 6(6)

wheat grain lipids would not be quite justified(5). The results of these authors were obtained either from a small number of tests under unspecified crop production measures (Kurmies) or under laboratory conditions of growth (Wenzel). Other studies on this problem have not been reported.

Wheat grain lipids are very complex products(6). Beside triglycerides, glycolipids and classical phospholipids of the phosphatidyl-ethanolamine, phosphatidyl-choline, phosphatidyl-inositol and phosphatidyl-serine types, wheat grain also contains phytoglycolipids and cerebrosides with phytosphyngosine, dihydrosphingosine, dehydrophytosphingosine and phytosphingosine-anhydro base as the basic components. Since nitrogen is incorporated in these molecules, it is obvious that the investigation of the influence of inorganic fertilizers on the accumulation of phospholipids and lipids in general should include nitrogen fertilizers, in addition to phosphate fertilizers. The complex nature of wheat grain lipids and the partially known pathways of lipid biosynthesis(7) suggests that increasing doses of these two fertilizers might influence the biosynthesis of phospholipids. This study deals with the following problems:

By applying modern crop production techniques in the presence of constant levels of PK and increasing levels of N or in the presence of constant levels of NK and increasing levels of P is it possible (*a*) to influence the accumulation of total lipids and the distribution of individual lipid fractions (*i. e.* the fractions of free fatty acids, neutral lipids, ether soluble non-saponifiable products and phospholipids) in wheat grain; (*b*) to affect the composition of individual lipid fractions; (*c*) to influence the percentage of fatty acids such as lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids within the given fractions and particularly in the phospholipid fraction; (*d*) to intensify the biosynthesis of fatty acids with an odd number of carbon atoms, such as tri-, penta-, and heptadecanoic acids, *i. e.* acids whose occurrence in wheat was recently discovered(8, 9, 10).

These investigations might determine whether or not increased accumulation of lipids can improve the baking properties of wheat. Therefore, *San pastore* wheat was selected, since this variety was graded by farinological and lipid analyses as a high yield wheat of very poor baking quality.

MATERIALS AND METHODS

The grain of the Italian high yield wheat *San Pastore* from 1960 crop was used. Field tests were based on a random block--system with five replicates and were conducted at the Experimental Farm of the Institute of Agricultural Research, Novi Sad. The soil characteristics, the scheme of the random block-system and the forms of nitrogen and phosphorus fertilizers applied were described in an earlier work(11).

For chemical analyses, materials from three replicates were used. The preparation of grain samples, the extraction of total lipids, their fractionation on a column of silicic acid and hyflo super cel (3 : 1), the preparation of fatty acid methyl esters and their separation by gas-liquid chromatography have also been previously described (10). Some experimental data concerning the separation on fatty acids present in the individual lipid fractions by means of gas-liquid chromatography are given in Figures 1-4.

TABLE 1

The contents of neutral lipids, free fatty acids, non-saponifiable products, phospholipids and total lipids in wheat grain, in relation to increasing levels of nitrogenous fertilizer**.*

Fertilizer treatment	Plot	In *mg* per 100 *g* of dry grain				
		Neutral lipids	Free fatty acids	Ether-soluble non-saponifiable products	Phospholipids	Total lipids
PK	I	847	589	137	582	2056
	II	716	524	122	563	1925
	III	748	593	131	582	2054
	Mean	*770*	*535*	*130*	*576*	*2012*
PK + N_{60}	I	851	414	173	594	2032
	II	658	568	108	444	1778
	III	796	544	153	503	1997
	Mean	*786*	*509*	*145*	*514*	*1936*
PK + N_{100}	I	686	535	155	555	1931
	II	797	539	148	539	2023
	III	779	553	167	547	2046
	Mean	*754*	*542*	*157*	*547*	*2000*
PK + N_{160}	I	714	657	134	551	2056
	II	688	536	140	553	1917
	III	705	672	142	505	2024
	Mean	*702*	*622*	*139*	*536*	*1999*

*In the present work the grain of the high yielding Italian variety *San Pastore* was used.

**In this and the following tables all results are for dry material.

The qualitative analysis of the fraction containing ether soluble non-saponifiable products was carried out on a thin layer of silicic acid. The thickness of the layer of silica gel G (activated according to Stahl, Merck), applied on glass plates 20 × 20 cm, was 250-275 microns. The investigated mixture, dissolved in chloroform, was applied on the plates in amounts of 50 micrograms, and the spots were developed by the ascending technique with the solvent mixture diethyl ether-petroleum ether (BDH, b. p. 40-60°) (20:100 v/v). Spots were detected by spraying the plates with 50 per cent sulphuric acid, followed by heating at 80° for 5-15 minutes (in a drying oven).

RESULTS AND DISCUSSION

A. Influence of increasing levels of nitrogen fertilizer

The amounts of neutral lipids, free fatty acids, ether soluble non-saponifiable products, phospholipids and total lipids in relation to increasing levels of nitrogen are listed in Table 1.

As can be seen in Table 1, the content of total lipids in wheat grain was practically constant, amounting on the average to 2.0 % (for dry grain). The individual lipid fractions were also unaffected by the fertilizer treatment; their average amounts (in per cent of dry grain) were as follows: neutral lipids, 0.7-0.8 %; free fatty acids, 0.5-0.6 %; non-saponifiable products, 0.13-0.16 %; phospholipids, 0.5-0.6 %.

Expressed as a percentage of total lipids present in the grain, the amount of ether soluble non-saponifiable products was 5 %, in good agreement with results reported for wheat germ oil(12).

All these results indicate that with increasing levels of nitrogen fertilizer, under modern crop production techniques, it was not possible to change the accumulation of total lipids nor the contents of the individual lipid fractions in wheat grain, including the phospholipid fraction. Thus the rest of this study concentrates on the qualitative composition of wheat lipids. Some usual data for the phospholipid fraction are given in Table 2.

As can be seen from Table 2, the content of nitrogen incorporated in the phospholipid fraction was independent of the applied levels of nitrogen fertilizer and amounted on the average to 0.9 % (calculated on isolated phospholipids). The amount of phosphorus was slightly higher, with an average value of 1.7 %, but was also unaffected by fertilizer treatment. For that reason the atomic ratio N/P was practically constant, with a mean value of 1.2. According to a report in the literature this ratio varies from 1.02. to 1.30(13).

The results for the amount of phospholipid nitrogen, expressed as a percentage of the total nitrogen present in wheat grain, were more interesting. When lower levels of nitrogen fertilizer were used this amount was practically constant, with an average

TABLE 2

Nitrogen and phosphorus contents of wheat grain phospholipids and whole wheat grain, in relation to increasing levels of nitrogencus fertilizer.

Fertilizer treatment	Plot	Phospholipid-N (as % of grain phospholipids)	Phospholipid-P (as % of grain phospholipids)	Phospholipid N/P ratio	Phospholipid-N (in *mg* per 100 *g* of grain)	Whole grain total N (in %)	Phospholipid-N (as % of grain total N)	Phospholipid-P (in *mg* per 100 *g* of grain)	Whole grain total P (in %)	Phospholipid-P (as % of grain total P)
PK	I	0.95	1.62	1.29	5.54	1.94	0.28	9.44	419.6	2.25
	II	0.71	1.55	1.01	4.00	1.85	0.22	8.73	409.2	2.13
	III	0.94	1.69	1.23	5.47	1.87	0.29	9.85	442.5	2.23
	Mean	*0.87*	*1.62*	*1.19*	*5.00*	*1.89*	*0.26*	*9.34*	*423.8*	*2.20*
PK + N_{60}	I	0.96	1.69	1.26	5.70	2.05	0.28	10.05	448.8	2.24
	II	0.99	1.75	1.25	4.40	1.95	0.23	7.78	452.1	1.72
	III	0.90	1.73	1.15	4.54	1.97	0.23	8.70	449.2	1.94
	Mean	*0.95*	*1.72*	*1.22*	*4.88*	*1.99*	*0.25*	*8.84*	*450.0*	*1.97*
PK + N_{100}	I	0.92	1.67	1.22	5.10	1.82	0.28	9.26	410.3	2.26
	II	0.91	1.62	1.24	4.90	1.89	0.26	8.73	387.2	2.26
	III	0.90	1.64	1.22	4.93	1.96	0.25	8.97	339.3	2.64
	Mean	*0.91*	*1.64*	*1.23*	*4.98*	*1.89*	*0.26*	*8.98*	*378.9*	*2.38*
PK + N_{160}	I	0.91	1.65	1.22	5.02	2.07	0.24	9.10	410.0	2.22
	II	0.89	1.62	1.22	4.92	2.23	0.22	8.95	431.2	2.08
	III	0.87	1.69	1.14	4.40	2.18	0.20	8.54	402.0	2.12
	Mean	*0.89*	*1.65*	*1.19*	*4.78*	*2.16*	*0.22*	*8.86*	*414.4*	*2.14*

value of 0.26 %. When the highest dose of nitrogen fertilizer was applied, *i. e.* plot PK + N_{160}, the total nitrogen in the grain increased while the phospholipid nitrogen (per cent of total N) had a tendency to decrease (0.24-0.20 %), which indicates that the uptake of nitrogen was intensified, but that it was incorporated in the protein of the grain and not in the phospholipid fraction.

Phospholipid phosphorus present in 100 g of grain (in mg) changed only negligibly, from 8.8 to 9.3 mg per 100 g of grain (mean values). Phospholipid phosphorus, expressed as a percentage of the total phosphorus present in whole wheat grain, amounted on the average to 2.2 %.

The qualitative and quantitative fatty acid composition of the fraction of free fatty acids is given in Table 3.

TABLE 3.

Fatty acid composition of the fraction of free fatty acids from wheat grain, in relation to increasing levels of nitrogenous fertiliser.

Fertilizer treatment	Plot	The amount of free fatty acids (in mg per 100 g of grain)	As percentage of total acids											
			Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Saturated acids	Unsaturated acids
PK	I	489	0.4	0.1	tr*	23.9	tr	tr	1.0	12.6	57.1	4.9	25.4	74.6
	II	524	0.4	0.3	tr	17.0	tr	tr	0.9	13.0	63.2	5.2	18.6	81.4
	III	593	0.3	0.3	tr	16.6	tr	tr	1.1	14.2	62.4	5.1	18.3	81.7
	Mean	*535*	*0.37*	*0.23*	*tr*	*19.2*	*tr*	*tr*	*1.0*	*13.3*	*60.9*	*5.0*	*20.8*	*79.2*
PK+N$_{60}$	I	414	0.3	0.2	tr	21.5	tr	tr	1.2	13.2	58.8	4.8	23.2	76.8
	II	568	0.4	0.3	tr	19.1	tr	tr	1.0	14.8	59.5	4.9	20.8	79.2
	III	545	0.6	0.5	tr	18.9	tr	tr	1.6	14.3	58.3	5.8	24.6	78.4
	Mean	*505*	*0.43*	*0.33*	*tr*	*19.8*	*tr*	*tr*	*1.27*	*14.1*	*58.9*	*5.1*	*21.9*	*78.1*
PK+N$_{100}$	I	535	0.3	0.3	tr	22.2	tr	tr	1.7	15.3	53.3	6.9	24.5	75.5
	II	539	0.4	0.2	tr	19.9	tr	tr	1.0	13.4	59.9	5.2	21.5	78.5
	III	535												
	Mean	*542*	*0.35*	*0.25*	*tr*	*21.05*	*tr*	*tr*	*1.35*	*14.3*	*56.6*	*6.1*	*23.07*	*77.0*
PK+N$_{160}$	I	657	0.3	0.2	tr	24.0	tr	tr	1.2	12.9	56.4	5.0	25.7	74.3
	II	536	0.3	0.3	tr	21.2	tr	tr	1.0	13.9	58.4	4.9	22.8	77.2
	III	672	0.4	0.3	tr	19.9	tr	tr	1.3	13.6	58.9	5.6	21.9	78.1
	Mean	*622*	*0.33*	*0.27*	*tr*	*21.7*	*tr*	*tr*	*1.2*	*13.5*	*57.9*	*5.1*	*23.5*	*76.5*
Mean values for all levels		*552*	*0.37*	*0.27*	*tr*	*20.44*	*tr*	*tr*	*1.25*	*13.8*	*58.6*	*5.3*	*22.3*	*77.7*

* In this and the following tables tr = traces

Since all the wheat samples were treated identically from harvest until the moment of analysis, data on the fraction of free fatty acids could provide information on the enzymatic activity of the lipases of wheat grain in relation to the crop production technique applied. However, as is evident from Table 3, increasing the level of nitrogenous fertilizer did not cause a selective lipolytic change of the lipase activity.

Of the free fatty acids about 78 % are unsaturated acids, linoleic acid being dominant (on the average 59 %). The amounts of oleic and linolenic acids are 14 and 5.3 % (mean values) respectively.

TABLE 4.

Fatty acid composition of the fraction of neutral lipids from wheat grain, in relation to increasing levels of nitrogenous fertilizer.

Fertilizer treatment	Plot	The amount of fatty acids bound to neutral lipids (in mg per 100 g of grain)	As percentage of total acids											
			Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Saturated acids	Unsaturated acids
PK	I	617.1	0.2	tr	tr	13.5	tr	tr	0.7	14.9	64.3	6.4	14.4	85.6
	II	557.7	0.2	tr	tr	13.9	tr	tr	0.6	13.5	66.8	5.0	14.7	85.3
	III	532.4	0.3	tr	tr	15.1	tr	tr	0.7	14.5	63.2	6.2	16.1	83.9
	Mean	*569.1*	*0.23*	*tr*	*tr*	*14.2*	*tr*	*tr*	*0.7*	*14.3*	*64.8*	*5.9*	*15.1*	*84.9*
PK + N_{80}	I	631.4	0.1	tr	tr	18.6'	tr	tr	0.5	16.0	60.3	4.5	19.2	80.8
	II	443.2	tr	tr	tr	14.0	tr	tr	0.7	14.5	65.0	5.8	14.7	85.3
	III	522.4	0.1	tr	tr	17.9	tr	tr	0.5	12.7	64.2	4.6	18.5	81.5
	Mean	*532.3*	*0.1*	*tr*	*tr*	*16.8*	*tr*	*tr*	*0.6*	*14.4*	*63.2*	*4.9*	*17.5*	*82.5*
PK + N_{100}	I	520.0	tr	tr	tr	14.3	tr	tr	0.9	15.9	62.6	6.3	15.2	84.8
	II	549.0	0.2	0.1	tr	13.7	tr	tr	0.7	14.4	65.5	5.4	14.7	85.3
	III	550.0	0.3	0.3	tr	14.7	tr	tr	0.8	14.3	63.4	6.2	16.1	83.9
	Mean	*539.7*	*0.2*	*0.1*	*tr*	*14.2*	*tr*	*tr*	*0.8*	*14.9*	*63.8*	*5.9*	*15.3*	*84.7*
PK + N_{160}	I	579.9	tr	tr	tr	15.5	tr	tr	0.6	14.5	63.4	6.0	16.1	83.9
	II	550.0	tr	tr	tr	16.5	tr	tr	0.7	15.3	61.2	6.3	17.2	82.8
	III	414.0	tr	tr	tr	15.0	tr	tr	0.6	14.2	64.3	5.8	15.7	84.3
	Mean	*514.6*	*tr*	*tr*	*tr*	*15.7*	*tr*	*tr*	*0.6*	*14.7*	*62.9*	*6.0*	*16.3*	*83.7*
Mean values for all levels		*538.9*	*0.2*	*tr*	*tr*	*15.2*	*tr*	*tr*	*0.7*	*14.6*	*63.6*	*5.7*	*16.1*	*83.9*

The most abundant of the saturated fatty acids in the fraction of free fatty acid is palmitic acid, with an average amount of 20 %. Stearic acid makes up 1.2 % while the lower fatty acids, such as myristic and lauric acids, are present in fractions of one per cent, *i. e.* 0.3 and 0.4 % respectively. It should be noted that the ratio of unsaturated to saturated acids in the fraction of free fatty acids was practically unaffected by increasing the level of nitrogenous fertilizer.

Finally, as can be seen from the gas-liquid chromatog ams (Figs. 1 and 2), only acids up to C-18 were present in measurable amounts in the fraction of free fatty acids.

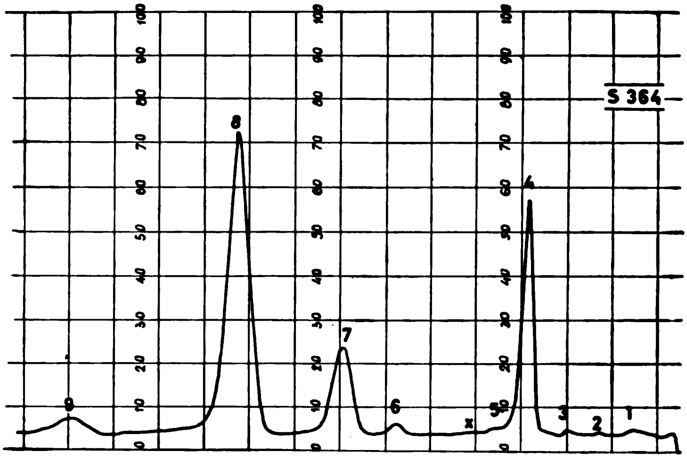

Fig. 1

GLC of fatty acids in the fraction of free fatty acids from the grain of the wheat variety *San Pastore* (plot NK + $P_{252}/2$). Experimental data of separation: column, acid washed Embacel/EGS, 8 : 2; column temperature 170°; argon flow rate 50 cc/min; detector voltage 1250 V; recorder sensitivity × 3; chart speed 30"/hr; volume of esters applied: 0.05 microliter. 1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Stearic acid; 7. Oleic acid; 8. Linoleic acid; 9. Linolenic acid.

Table 4 shows the composition and content of fatty acids in neutral lipids expressed as a percentage of the total acids present in this fraction, in relation to increasing levels of nitrogen fertilizer.

The content of fatty acids in neutral lipids did not change noticeably as a result of increasing levels of nitrogen fertilizer, and amounted on the average to 0.5-0.6 % (based on dry grain). The composition of the fatty acids of this fraction is similar to the composition of the previous fraction (see Table 3). The unsaturated acids are present in large amounts, particularly linoleic acid (average content 64 %). The amounts of oleic and linolenic acids are much lower *i. e.* 15 and 5.7 % (average) respectively.

Saturated fatty acids are represented by 16 % of the total acids present in this fraction, 15 % being palmitic acid. The content of stearic acid is slightly less than 1 %, while the lower fatty acids are present in very small amounts or only in traces. The changes due to increasing the level of nitrogen fertilizer are very small and probably not significant. A typical gas-liquid chromatogram of the acids present in the fraction of neutral lipids is given in Figure 3.

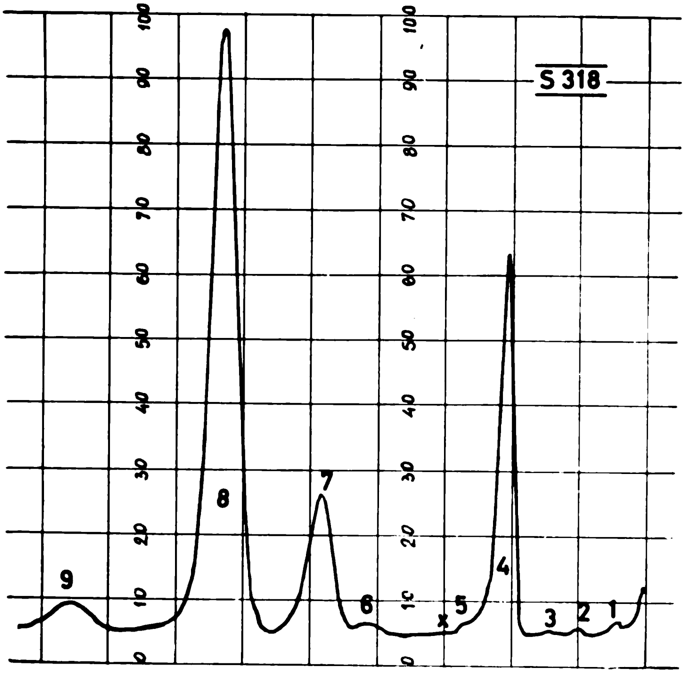

Fig. 2

GLC of fatty acids in the fraction of free fatty acids from the grain of the wheat variety *San Pastore* (plot PK/12). Experimental data of separation as for Fig. 1. Applied ester volume: 0.1 microliter. 1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Stearic acid; 7. Oleic acid; 8. Linoleic acid; 9. Linolenic acid.

Table 5 shows the influence of increasing levels of nitrogen fertilizer on the amount and the composition of the fatty acids bound to the phospholipid fraction.

The content of fatty acids bound to phospholipid varied from 352 to 389 mg per 100 g of grain (for dry grain). In the gas-liquid chromatogram (Fig. 4) of the fatty acid methyl esters of this fraction a double peak appears after the position of linolenic acid. This peak corresponds to the "metastable" form of unsaturated acids., *i. e.* essentially linoleic acid. The role of this partially polymerized form of linoleic acid and its exclusive presence in the fatty acids of the phospholipid fraction has been mentioned in earlier works(10, 14).

Of the fatty acids present in the phospholipid fraction, linoleic acid is again present in a large amount (66 %). Other unsaturated acids, such as oleic and linolenic acids, are present in much smaller amounts, *i. e.* 11 and 3.3 %, respectively. Of the saturated fatty acids, palmitic acid predominates, while the other saturated

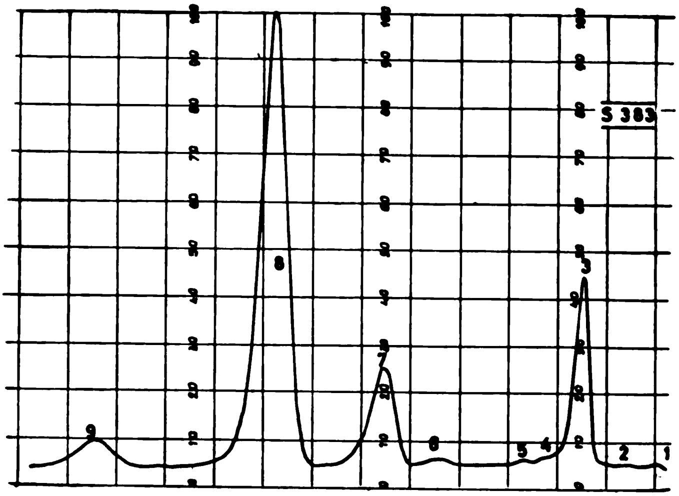

Fig. 3

GLC of fatty acids in the fraction of neutral lipids from the grain of the wheat variety *San Pastore* (plot NK/12). Experimental data of separation as in Fig. 2. 1. Myristic acid; 2. Pentadecanoic acid; 3. Palmitic acid; 4. Palmitoleic acid; 5. Heptadecanoic acid; 6. Stearic acid; 7. Oleic acid; 8. Linoleic acid; 9. Linolenic acid.

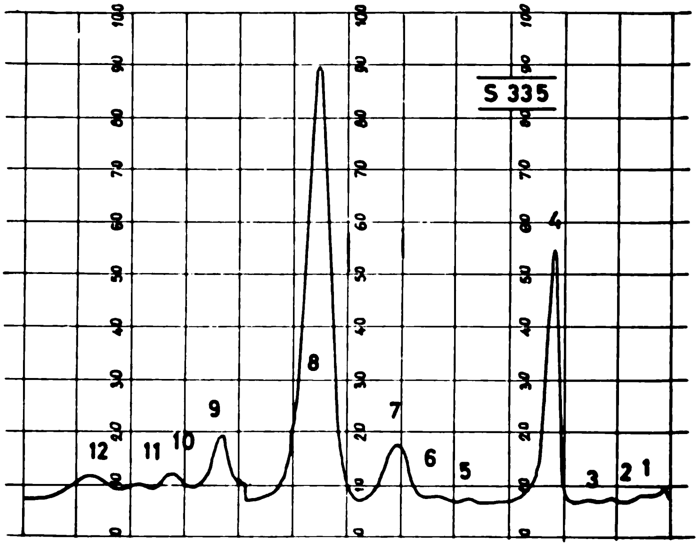

Fig. 4

GLC of fatty acids of the phospholipid fraction from the grain of the wheat variety *San Pastore* (plot PK/10). Experimental data of separation as mentioned in Fig. 2., with the following exceptions: recorder sensitivity after linoleic acid × 1, chart speed 15"/hr; applied ester volume; 0.05 microliter. 1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Heptadecanoic acid; 6. Stearic acid; 7. Oleic acid; 8. Linoleic acid; 9. Linolenic acid; 10. and 11. "Metastable" form of linoleic acid.

TABLE 5.

Fatty acid composition of the fraction of phospholipids from wheat grain, in relation to increasing levels of nitrogenous fertilizer.

Fertilizer treatment	Plot	The amount of fatty acids bound to phospholipids (in *mg* per 100 *g* of grain)	As percentage of total acids											
			Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Saturated acids	Unsaturated acids
PK	I	364	0.3	0.2	tr	18.9	0.3	tr	0.6	10.4	65.7	3.6	20.0	80.0
	II	342	tr	tr	tr	19.3	0.2	tr	0.3	11.1	66.1	3.0	19.6	80.4
	III	398	0.4	0.3	tr	17.3	tr	tr	0.5	12.1	64.1	5.3	18.8	81.5
	Mean	*368*	*0.2*	*0.2*	*tr*	*18.5*	*0.2*	*tr*	*0.5*	*11.2*	*65.3*	*3.9*	*19.4*	*80.6*
PK + N_{60}	I	420	tr	tr	tr	12.0	0.2	tr	0.7	12.8	70.0	4.3	12.7	87.3
	II	270	tr	tr	tr	20.9	0.5	tr	0.9	10.7	65.2	1.8	21.8	78.2
	III	366	0.4	tr	tr	16.8	0.2	tr	0.8	11.1	67.2	3.5	18.0	82.0
	Mean	*352*	*0.1*	*tr*	*tr*	*16.6*	*0.3*	*tr*	*0.8*	*11.5*	*67.5*	*3.2*	*17.5*	*82.5*
PK + N_{100}	I	406	tr	tr	tr	15.7	0.3	tr	0.8	10.6	68.2	4.4	16.5	83.5
	II	370	0.4	tr	0.3	20.8	0.7	tr	0.9	10.6	63.8	2.5	22.4	77.6
	III	390	0.2	tr	tr	18.5	tr	tr	0.7	8.5	69.2	2.9	19.4	80.6
	Mean	*389*	*0.2*	*tr*	*0.1*	*18.3*	*0.3*	*tr*	*0.8*	*9.9*	*67.1*	*3.2*	*19.4*	*80.6*
PK + N_{160}	I	406	0.4	0.3	tr	19.6	tr	tr	0.8	10.5	65.7	2.7	21.1	78.9
	II	324	0.5	0.3	tr	21.8	1.1	tr	0.9	8.9	64.0	2.5	23.5	76.5
	III	398	0.6	tr	tr	17.2	1.1	tr	0.8	12.6	64.3	3.4	18.6	81.4
	Mean	*376*	*0.5*	*0.2*	*tr*	*19.5*	*0.7*	*tr*	*0.8*	*10.7*	*64.7*	*2.9*	*21.1*	*78.9*
Mean values for all levels		*371*	*0.3*	*0.1*	*tr*	*18.2*	*0.4*	*tr*	*0.7*	*10.8*	*66.2*	*3.3*	*19.3*	*80.7*

acids are present in small amounts or in traces. As in other lipid fractions, increasing the level of nitrogenous fertilizer did not noticeably affect the total amount of fatty acids nor the percentages of individual acids in the phospholipid fraction.

B. Influence of increasing levels of phosphorus fertilizer

The results pertaining to the qualitative and quantitative composition of the lipids of wheat grain, harvested from plots where constant doses of NK and increasing doses of P fertilizer were applied, were practically the same as those obtained in the previous experiments, when increasing doses of N in the presence of constant levels of PK were applied. Higher doses of P fertilizer caused a higher uptake of phosphorus by the wheat grain. This phosphorus, however, was not incorporated in the phospholipid fraction but mainly in phytic acid(10).

Slight and insignificant changes were observed in the percentages of fatty acids in the fractions of free fatty acids and neutral lipids, as a result of increasing levels of phosphorus fertilizer. The average amounts of fatty acids in these fractions, expressed as a percentage of total acids present, are listed in Table 6.

TABLE 6.

Fatty acid composition of the wheat grain fractions of free fatty acids and neutral lipids, in relation to increasing levels of phosphorus fertilizer.

Fraction	Plot	As percentage of total acids in the given fraction											
		Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Saturated acids	Unsaturated acids
Free fatty acids	NK	0.4	0.3	tr	18.6	0.3	0.3	2.0	14.2	58.5	5.4	21.6	78.4
	NK + P_{72}	0.4	0.1	tr	17.6	0.2	0.4	1.7	14.5	59.2	5.9	20.2	79.8
	NK + P_{144}	0.3	0.2	0.1	17.4	0.3	tr	1.5	14.4	60.5	6.3	19.5	80.5
	NK + P_{252}	0.3	0.3	0.2	19.3	0.4	0.3	1.4	14.7	58.1	5.0	21.8	78.2
	Mean	*0.3*	*0.2*	*0.1*	*18.2*	*0.3*	*0.3*	*1.7*	*14.4*	*59.1*	*5.4*	*20.8*	*79.2*
Neutral lipids	NK	0.2	0.3	0.1	14.8	0.7	tr	0.7	13.3	64.7	5.2	16.1	83.9
	NK + P_{72}	0.1	0.1	0.1	14.8	0.8	tr	0.8	13.9	64.3	5.1	15.9	84.1
	NK + P_{144}	tr	0.1	0.1	15.1	1.0	tr	0.9	13.9	63.2	5.7	16.2	83.8
	NK + P_{252}	tr	0.1	0.2	14.0	0.7	tr	1.0	14.4	64.6	5.0	15.3	84.7
	Mean	*0.1*	*0.2*	*0.1*	*14.7*	*0.8*	*tr*	*1.0*	*13.9*	*64.2*	*5.2*	*15.9*	*84.1*

The presence of fatty acids with an odd number of carbon atoms (penta- and heptadecanoic acids) is of particular interest. As evident from the given Tables (3—6) and gas-liquid chromatograms (Figs. 1-4), their appearance in various lipid fractions of grain is characteristic for all plots where different levels of fertilizer were applied. Although the amounts of these acids were designated as "traces", they were not negligible and amounted approximately to 0.05 % of the total acids present in the corresponding fraction. (The planimetric angular method of calculating yields from the resulting peaks on the gas-liquid chromatograms is convenient for serial analyses, but is not accurate for such small amounts of acids. For that reason the amounts of these acids were designated as "traces".) Increasing levels of nitrogen and phosphorus fertilizer did not visibly influence the extent of biosynthesis of fatty acids with an odd number of carbon atoms. Their appearance in all saponifiable lipid fractions of grain of different wheat varieties(6, 10) and their constant presence in the grain of one variety, regardless of the crop production measures applied, suggest that in wheat not only Knopp' s β-oxidation system but also the α-oxidation system is operative(16).

a) $RCH_2CH_2COOH + 2\,H_2O_2 \rightarrow RCH_2CHO + CO_2 + 3\,H_2O$

b) $RCH_2CHO + DPN^{*} + H_2O \rightarrow RCH_2COOH + DPNH^{*} + H^{+}$

According to our previous(6, 10) and present results it appears that the processes involved in the α-oxidation system occur constantly in wheat, but to a much smaller extent compared to the β-oxidation system. The exclusive occurence of C-15 and C-17 acids as well as the recently detected C-13 acid(8, 9), indicates that the peroxidative decarboxylation of the fatty acids in wheat is limited to fatty acids with 14-18 carbon atoms. This finding agrees with the mechanism recently established by Stumpf and Bradbeer(17) for the α-oxidation system, and may be regarded as further experimental proof that this system is more widely represented in plant tissues.

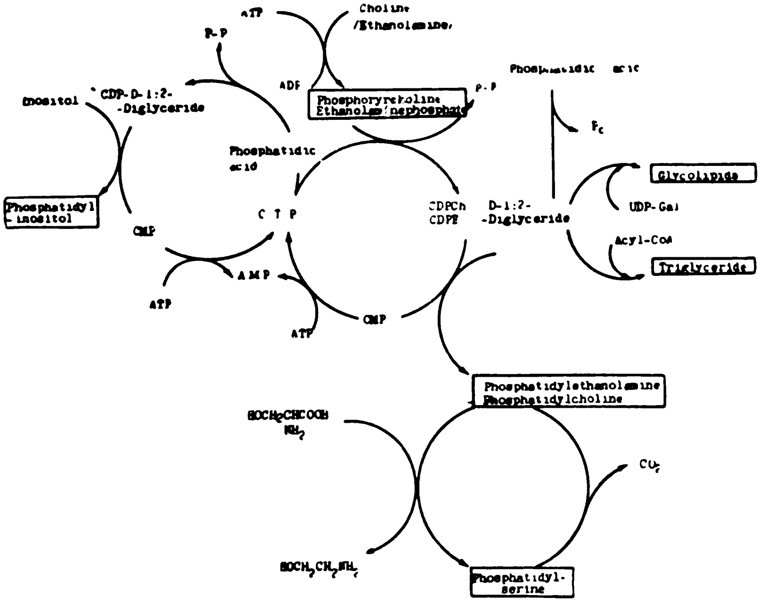

Fig. 5

Biosynthesis of glycerophosphatides

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* DPN — diphosphopyridine nucleotide (formerly called coenzyme I); DPNH — reduced DPN

It should be noted that the amounts of phospholipid nitrogen and phosphorus and therefore their atomic ratio N/P remained constant in all the plot samples, from $NK+P_{72}$ to $NK+P_{252}$. These data indicate the absence of any qualitative change within the phospholipid fraction when increasing levels of phosphorus fertilizer were applied.

If the pathway of the biochemical synthesis of lipids is considered from the aspect of classical phospholipids, as shown in Figure 5, our results might be explained by the fact that in relation to increasing levels of phosphorus fertilizer phosphoryl-choline has the role ascribed to it by Maizel, Benson and Tolbert(7) rather than the role of a reaction component of cytidine triphosphate (CTP)(7, 16, 18).

The total absence of an increase in the biosynthesis of phospholipids in wheat grain could also be ascribed theoretically to the limiting action of CTP or of the enzymatic system which forms phosphatidic acid.

At the same time, the unchanged amounts of nitrogen in the isolated phospholipid fractions indicate that increasing levels of phosphorus fertilizer did not produce any qualitative or quantitative change of the phytosphingolipids or the cerebrosides of wheat grain.

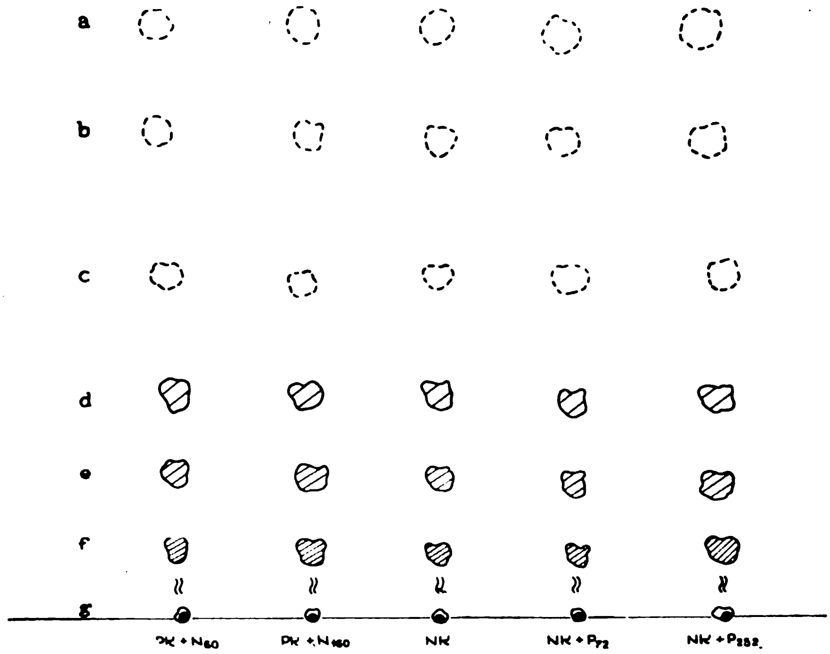

Fig. 6

Thin layer chromatogram of the fraction of ether soluble non-saponifiable products. Solvent system used: ethyl ether-petroleum ether (BDH, b. p. 40—60°), 20 : 100 v/v.

Finally the fraction of ether soluble non-saponifiable products needs to be explained. As described above, when increasing levels of nitrogen fertilizer were applied, the content of these products changed only slightly and had an average amount of 0.14 % (see Table 1). Since the gravimetric determinations are only accurate to 0.01 %, slight variations in the content of this fraction are probably not the result of fertilizer (nitrogen or phosphorus) treatment. Since a correlation might exist between this fraction and the baking quality of wheat flour, and because of the recent findings of Caldarera, Ronca and Lenaz(8) concerning the presence of considerable amounts of this fraction in the lipids of gluten of some Italian wheat varieties, qualitative analyses were performed to establish any change which might have occurred in this fraction as a result of fertilizer treatment. As can be seen from the thin-layer chromatogram (Fig. 6), the areas and color intensities of the separated components(12, 19, 20) indicate that such a change did not occur.

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IDENTIFICATION OF ORGANIC ACIDS AND SACCHARIDES IN THE FRUIT AND LEAF OF THE MEDLAR TREE (MESPILUS GERMANICA L.)

by

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There are few data available in the literature on the chemical composition of the fruit of the medlar-tree. It is well known that the medlar fruit contains glucose, fructose, malic and boric acid, and sorbitol(1,2). There are no data at all in the literature on the chemical composition of the leaf. In our earlier investigations we found that during rotting the medlar fruit contains small amounts of xylose(3), and besides malic acid there are a great number of organic acids which we did not identify. We isolated a glucoside from the medlar leaf, for the first time, which was proved to be mevalolactonyl-β-glucoside(4,5).

Accordingly, we thought it interesting to study in more detail the qualitative composition of the leaf and fruit of the medlar tree regarding the content of saccharides and organic acids.

The extraction and identification of saccharides from a mixture of plant material may be performed by paper chromatography and with specific staining reagents with which some groups of saccharides are differentiated(6,7). Although there are some monosaccharides which cannot be isolated by paper chromatography they are seldom found together in plant material. The same is true for oligosaccharides. For more sure identification in such cases it is necessary to isolate these saccharides first and then investigate them separately. This is done by chromatography on the column of starch(8), cellulose(9), activated charcoal(10) or ion-exchanger(11).

Paper chromatography has also yielded good results in the identification of organic acids. There are many cases, however, which reduce the certainty of such identification: one acid forming a tail which masks the presence of another; some acids producing several spots on the chromatogram; in more complex mixtures several acids often have the same or about the same speed. Besides, the number of organic acids found in plant material is relatively large. For these reasons chromatographic analysis on paper is only the first step in their identification(12). More reliable results are obtained by mixing and chromatographing a corresponding test acid with a mixture of the acid investigated. The obtaining of one spot with two different systems of solvents is a more reliable demonstration of a given acid(13). For this purpose, too, it is better to isolate the acids from the mixture or to fractionate them into less complex mixtures with acids which can be well separated by paper chromatography. However, it is best to isolate each acid from the mixture and analyze it separately.

Acids are isolated from mixtures mostly by chromatography on a silica gel column(14,15), ion-exchange column(16,17), or preparative chromatography on paper(18,19).

EXPERIMENTAL PROCEDURE

Leaves and fruit from a medlar-tree in Beograd were used. The experiments were carried out during a whole vegetation period to observe possible qualitative changes in the content of organic acid and saccharides.

The saccharides were analyzed in purified extracts of the leaf and fruit(4).

The acids which were fixed on the anion exchanger column during their isolation from the saccharides were eluted with a 1 N ammonium carbonate solution. To remove the excess ammonium carbonate the eluate was evaporated under reduced pressure (60-70°) and passed through a cation-exchange column. The solution of free acids was evaporated in a water bath at 60° to the desired volume, in which the acids were analyzed.

Identification of the saccharides

The saccharides were first isolated by descending chromatography on both paper and column. Whatman No. 1 paper was used. The solvent was ethylacetate: pyridine : water (40 : 11 : 6)(21). The chromatogram was developed by passage of the dry paper first through a solution of silver nitrate in acetone(22) and then, after drying, through a solution of potassium hydroxide in ethanol(23). Along with the extracts pure (p. a.) saccharide samples were chromatographed too.

Figure 1 shows a chromatogram of saccharides and related compounds from the medlar leaf and fruit, and of the mixture of saccharides chromatographed as a test.

The same saccharides were found in the leaf and fruit throughout the whole vegetation period.

Comparison of the locations of the spots of the leaf and fruit with those of the test substance has shown that the medlar leaf and fruit contain: saccharose, sorbitol, glucose, fructose, xylose and traces of two unidentified saccharides. The leaf also contains a glucoside (Rg 2.62) which was identified as mevalolactonyl-β-glucoside(4,5).

Glucose and xylose were determined on the chromatogram also by color reaction with anilinehydrogenphtalic acid$^{(6)}$ and fructose and saccharose by color reaction with urea$^{(7)}$. After chromatographing saccharose was eluted from the paper with water and

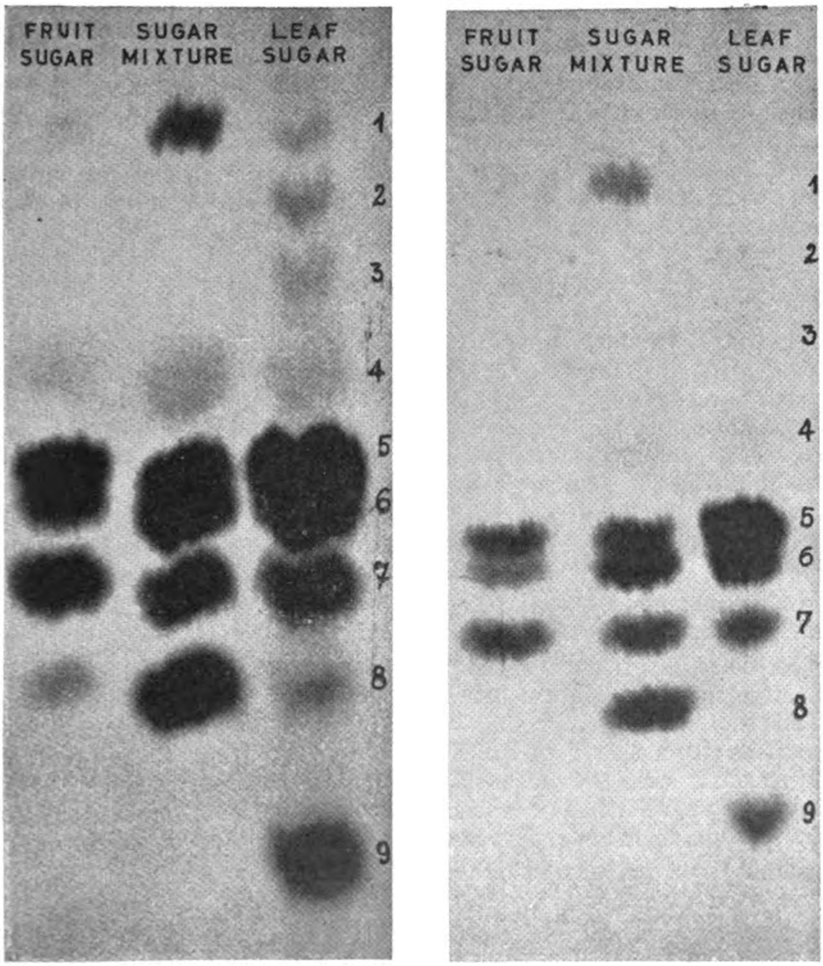

Fig. 1

Larger concentrations

Smaller concentrations

1. Inositol
2. unidentified
3. unidentified
4. saccharose
5. sorbitol
6. glucose
7. fructose
8. xylose
9. mevalolactonyl-β- glucoside

then hydrolyzed with hydrochloric acid. By chromatographing the products of hydrolysis from which the acid was first removed with an anion exchanger(24) we determined glucose and fructose.

We isolated sorbitol from purified extract of the leaf and fruit and determined it by converting it to crystal sorbitolhexaacetate. The dry extract (of the leaf or fruit) was chromatographed on a charcoal-celite column(10). 100 ml fractions containing sorbitol and monosaccharides, obtained by eluting the column with water, (fractions were paper chromatographed) were combined and treated with yeast(25). After removing the yeast the solution was evaporated at 60° and chromatographed on a charcoal-celite column once more (10). The fractions with pure sorbitol were combined, evaporated and the residue dried in a vacuum drying oven at 60° and treated with an anhydride of acetic acid in pyridine(26). The precipitate was recrystallized from ethanol. The melting point of the product was 98—99° which corresponds to sorbitolhexaacetate(27).

Identification of the acids

Acids of the medlar leaf and fruit were qualitatively investigated first by descending chromatography on Whatman No. 1 paper using the solvent system n-butanol:formic acid:water (10:2:15)(28). A mixture of test acids was chromatographed simultaneously. Acids on the chromatogram were identified with a solution of silver nitrate in acetone(22).

Figure 2 shows a chromatogram of the acids of the medlar leaf and fruit and a mixture of test acids. Qualitatively the same chromatograms were obtained throughout the whole vegetation period. The figure shows that the acid chromatograms of the leaf and fruit are qualitatively identical.

The upper part of the chromatogram shows the presence of a large number of non-separated acids. The test acid chromatogram also shows that with this system of solvents citric acid does not separate from shikimic acid. The question also arises of the presence of isocitric acid which does not separate from citric acid(29). For this reason we tried to isolate acids from the leaf and fruit or to fractionate them into less complex mixtures on an anion resin column by gradient power elution(30). Dowex 2 × 8 ($\overline{\text{Cl}}$, 300 mesh) was used. We first activated the resin with a solution of 1 N sodium acetate (negative reaction on $\overline{\text{Cl}}$) and washing the prepared column (20 cm high, 6 cm diameter, capacity 10.6 milliequivalents) with distilled water until negative reaction of the effluent. Then 12.7 milliequivalents of the acids being tested was passed. The acid effluent was collected for further investigation. The acids were eluted from the column with a formic acid solution whose concentration was continually increased from 0.1 N to 0.5 N(31) using the apparatus shown in Fig. 3. First 100 ml (or more if necessary)

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* In this way Busch's apparatus was considerably simplified(30)

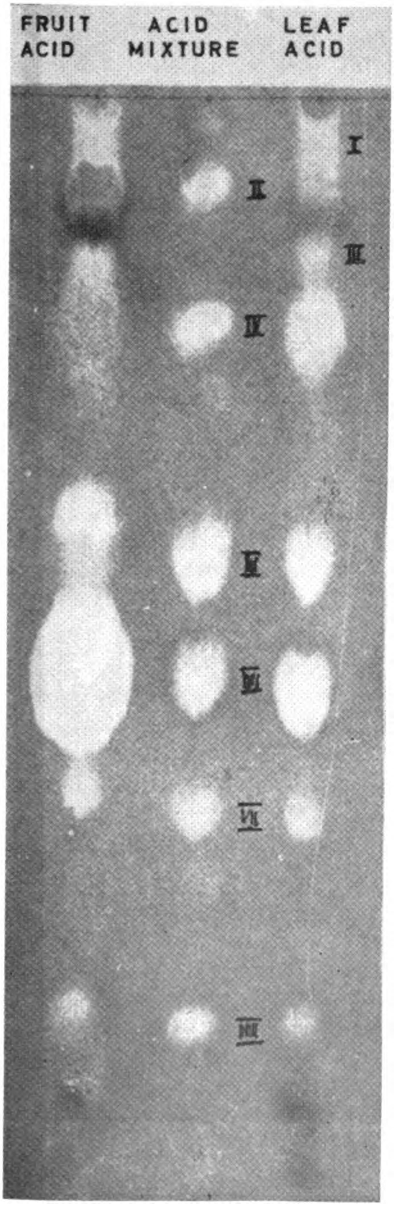

Fig. 2

I — glucose — 6 — phosphate
II — galacturonic acid
III — unidentified acid
IV — quinic acid

V — citric and shikimic acid
VI — malic acid
VII — malonic acid
VIII — succinic acid

of 0.1 N formic acid is poured into flask *B* which is stoppered tightly with a stopper through which the separating tube of funnel *A* and the connecting tube *C* pass (⌀ 0.5 cm). Faucet *F* is closed. 0.1 N of formic acid is poured into the separation funnel *A*, faucet *F* opened slowly and kept so until connection tube *C* is filled with the liquid. The faucet is closed. The solution is removed from the funnel and the funnel filled again with 0.5 N formic acid. The connection tube has a rubber stopper on its end with which it is connected to tube *E* containing the resin column above which there is some water. The faucet of funnel *A* is opened, the electromagnetic mixer *G* is turned on with a suitable stirring rate. Faucet *D* is also opened and the number of drops that pass through it each minute is fixed.

We collected the eluate in 4 ml fractions (flow rate 16 drops per minute). We collected 220 fractions. All the fractions were analyzed by paper chromatography.

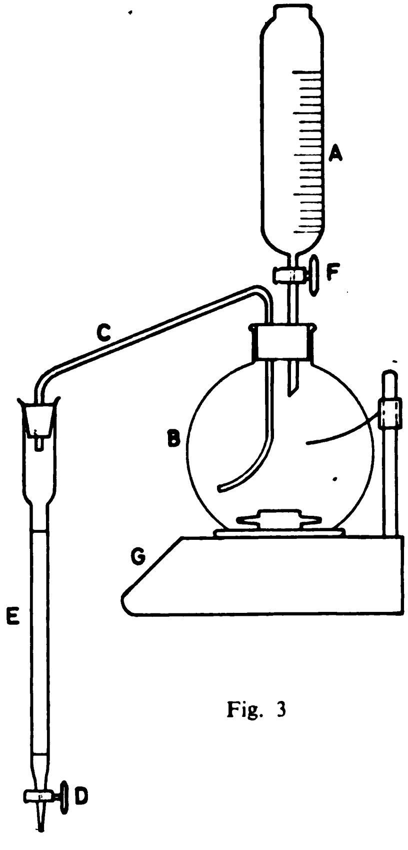

Fig. 3

The fractions containing only one acid were combined and evaporated to dryness over a water bath at 60°. The following acids were thus identified: quinic acid in fractions 6—9, succinic acid in fractions of 24—29 and malic acid in fractions of 30—65. Malonic acid was identified in fractions of 65—115 and citric acid was isolated from fractions of 115—150.

Identification of the acids isolated.

Quinic acid. — The dry amorphous residue obtained by treating the solution with activated charcoal was dissolved in 80 ml hot absolute ethanol 20 ml of benzene was added and the mixture distilled at about 70° to a small volume. The crystalline residue was dried in a dessicator under reduced pressure over phosphorus pentoxide.

Elementary analysis: C 42.82%, H 6.5%, calculated: C 43.29%, H 6.22%, $[\alpha]_D = -44°$.

This acid was also identified by specific reaction: a drop of the solution of the obtained product was mixed with 0.75 ml of a 0.01% solution of naphthoresorcinol in 96% sulphuric acid and heated for 40 minutes at 90° over a water bath. A green color formed which is characteristic of quinic acid(32).

Succinic acid. — A little of the dry residue obtained by evaporating the solution treated with activated charcoal was mixed in a test tube with one drop of 0.5% ammonium chloride and several milligrams of zinc powder and eveporated to dryness. The open end of the test tube was covered with a filter paper moistened with a 5% solution of dimethyl-p-benzaldehide in 20% trichloracetic acid in benzene. The bottom of the test tube was heated over a microflame for about one minute. A reddish-violet color appeared, characteristic of succinic acid(33).

Succinic acid was also demonstrated by means of sublimated resorcinol and sulphuric acid(34).

Malic acid. — Some dry crystalline residue was treated with 1 ml of a 0.0025 g β-naphthol solution in 100 ml of 96% sulphuric acid and heated for a short time over a water bath. A yellow color which fluoresced blue appeared characteristic of malic acid(35).

Malonic acid. — Fractions 65—115 contained several acids localized on the upper part of the chromatogram, and on the lower part there was a rather intense spot whose location corresponded to malonic acid. Malonic acid was demonstrated by specific reaction: a mixture of acids was chromatographed on paper along with a sample of p. a. malonic acid. After drying the paper was sprayed with a 4% solution of p-dimethylaminobenzaldehyde with the addition of one crystal of waterless sodium acetate ethanol and dried at 140° for 2 minutes. Two greens spots in parallel appeared on the chromatogram: one from malonic acid and the other from the chromatographed mixture. This reaction is characteristic of malonic acid(36).

Citric acid. — Fractions 115—150 contained several different acids. These fractions were combined, evaporated to dryness to remove formic acid, dissolved in 15 ml of water and chromatographed on a Dowex 2 × 8 $(\overline{\text{HCOO}})$ column (8 × 0.6 cm). The elution was performed with a solution of formic acid of continually increasing concentration (200 ml of water was mixed with a 0.5 N formic acid solution). 175 fractions of 4 ml each were collected. By paper chromatography fractions 111—130 were found to contain only one acid, whose Rf indicated citric. These fractions were combined, treated with activated charcoal and evaporated to dryness.

Some of the dry residue was mixed with about 100 mg of urea in a test tube and heated for two minutes in a glycerin bath at 150°. After cooling three drops of water were added to the hardened mass. The solution showed blueishgreen fluorescence in ultra-violet light, which is characteristic of citric acid. On the addition of a solution of hydrochloric acid (1 : 4) the fluorescence vanished(37).

The acidic effluent obtained when the acid extract was passed first time through the column (about 20% more acid was passed than the capacity of the column) was chromatographed on paper. The chromatogram showed three spots. One spot was identified as quinic acid; the second was localized above and the third below quinic acid. The location of the last corresponded to shikimic acid. The solution was evaporated to dryness, dissolved in water and chromatographed on a Dowex 2 × 8 $(\overline{\text{HCOO}})$ column. Elution was performed by the procedure used for the isolation of citric acid. 62 fractions were collected. Fractions 4—11 contained all three acids while fractions 19 to 62 contained only the acid which was located on the paper chromatogram above quinic acid (Rf × 100 = 7.5).

Glucose-6-phosphate. — Fractions 19—62 were combined, the solution treated with activated charcoal and evaporated to dryness. Some of the dry residue was dissolved in water and poured dropwise on chromatographic paper. After drying the paper was sprayed with a reagent consisting of a mixture of perchloric acid, ammonium molybdate and hydrochloric acid solution(38), dried again and exposed to ultra-violet light for 10 minutes. At the place where the solution was spotted there appeared a blue spot characteristic of phosphorylated compounds(39). The same acid treated with aniline hydrogenphthalate(40) yielded positive reaction upon reducing saccharides.

The rest of the dry residue was hydrolyzed for one hour in a boiling water bath with a 1 N hydrochloric acid solution. The hydrochloric acid was removed with water vapor and the solution chromatographed on paper. The chromatogram revealed the presence of glucose and phosphoric acid. Glucose-6-phosphate was thus demonstrated.

Shikimic acid. — Fractions 4—11 containing all three acids were evaporated to small volume. One part of this solution was chromatographed in parallel with the other part mixed with a sample of shikimic acid p. a. After development both chromatographs showed only one spot, at the location of shikimic acid.

This procedure was also used for the identification of all acids isolated and determined by specific reactions.

In addition to this, by using Amberlite IRA-400 $(\overline{OH})$, we isolated another acid in a crystalline state which while standing gradually darkened. It did not yield positive reaction on phosphorus, lactones and uronic acids. On the chromatogram developed by the system of solvents we used, this acid appears in the form of a short tail which begins little above and ends below the spot of quinic acid (Fig. 2). A dark spot localized above this acid also appears by chromatographing this acid after it had stood for a time.

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GLASNIK HEMIJSKOG DRUŠTVA, Vol. 28, No. 5-6, 1963, pp. 337-340

A MODIFICATION OF GROSSMAN'S METHOD FOR THE DETERMINATION OF NON-ESTERIFIED FATTY ACIDS IN BIOLOGICAL FLUIDS

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The determination of non-esterified fatty acids (NEFA) in blood has an important place in biochemical and clinical research. Several methods(1, 2, 3) based mainly on Davis' titrimetric method(4) have been used in the last few years. However, for our investigation the method of Grossman and co-workers(5) proved most suitable. While working on the determination of NEFA in human and animal plasma and sera we introduced some modifications that improved the precision and simplified the technique, as confirmed by our control investigation of NEFA in human plasma and the papers published earlier(6, 7, 8).

METHODS AND CONDITIONS OF WORK

The modifications of the method of Grossman and co-workers concern the evaporation of petroleum ether (ligroin), the use of a more dilute base (0.01 N), a different indicator (phenol red), and different apparatus for elimination of CO_2 and titration.

For the determination of NEFA in serum the required amount of blood was taken with a cooled syringe rinsed with heparin if plasma was to be obtained. The separation was done in cooled test tubes by centrifuging. The plasma and serum thus obtained were stored in a refrigerator adjusted to 5° C, and from there samples for each determination were taken.

Extraction. — 0.2 ml of 0.2 M phosphate buffer at pH 6, 2 ml of plasma (or serum), and 2 ml of 95% alcohol were placed into a test tube for centrifuging of about 15 ml capacity provided with a ground glass stopper. After mixing 3 ml of distilled petroleum ether (b.p. 30—60° C) were added, the liquids well mixed for one minute, and centrifuged (for a short time) until the layers separated. The upper layer of petroleum ether was taken off with a syringe with a long needle. The extraction with petroleum ether was repeated twice, and then all the petroleum ether extract collected in a glass dish was introduced into a vacuum desiccator placed over a water bath at approximately 50° C, and evaporated to dryness under reduced pressure.

Titration. — The dry residue was dissolved by the addition of 95% alcohol and warming over a water bath of about 40° C. The alcohol solution was quantitatively transferred (taking care that the total amount did not exceed 2.5 ml) into a special test tube (Fig. 1 B) of about 5 ml capacity in which titration was done by adding a 0.01 N NaOH solution from a microburette in the presense of phenol red indicator. The solution of sodium hydroxide was prepared just before work started and its factor was determined with an 0.01 N HCl solution. During titration a stream of CO_2-free air was passed through the solution. This was made possible by the special design of the test tube supplied with a built in capillary.

The complete equipment for titration and elimination of CO_2 is shown in Fig. 1. The apparatus is operated in the following way: 2 ml of alcohol with a drop each of an indicator (phenol red) and a 0.01 N solution of NaOH are placed in a titration test tube and exposed for some time to the action of atmospheric CO_2. If the apparatus works well, it is sufficient to pass the air from the system through this liquid for a few minutes to give it its former pH (red color). The control bottle (Fig. 1 C) is used for the same purpose.

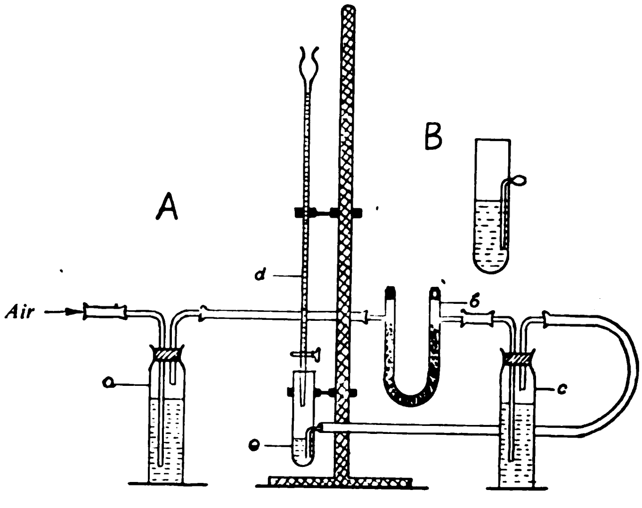

Fig. 1

Apparatus for Titration and Elimination of CO_2 A — A schematic representation of the entire apparatus. Air enters the system through a pipe immersed in a bottle filled with a 50% solution of KOH. (a) From this bottle it passes through a U-tube with soda lime (b) and through the control bottle containing water colored with a few drops of phenol red (c) It enters the titration test tube (e) fixed under a microburette (d).

B — The titration test tube with built in capillary enabling the air from the apparatus to pass through the solution during titration.

At the beginning and end of each series of determinations a control test is made in which the plasma is replaced by water. This test should give a negative result.

RESULTS

To establish the error with this modification of the technique a series of ten control determinations of NEFA in human plasma was carried out. All the blood required was taken from the same person at one time. The values obtained ranged from 300—345 μ Eq/L. The mean value was 314.5 μ Eq/L with $SD = \pm 12.1$ μ Eq/L; the variation coefficient was 3.8%.*

DISCUSSION

The modifications introduced into Grossman's method consist in the following:

(1) The evaporation of petroleum ether is carried out at 35—40° C (instead of 70° C) and at reduced pressure, which accelerates the procedure and gives more reliable results.

(2) A 0.01 solution of NaOH is used instead of a 0.02 N solution which gives greater precision of titration.

(3) Phenol red is used as indicator instead of thymol blue because it has a sharp and distinct color change at the end of neutralization and its color is easily distinguishable from the somewhat yellowish shade which an alcohol solution of plasma lipids often has.

(4) A special device enables simple production of CO_2-free air and its continuous passage through the titrating solution.

The results of the analyses of NEFA were within the limits of or near the normal values obtained by different authors(2—9). The error in this case is well indicated by the standard deviation. The small variation coefficient indicates low error of this procedure.

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* Standard deviation was calculated from $SD = \sqrt{\frac{\Sigma(y-\bar{y})^2}{n-1}}$

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SPECTROGRAPHIC DETERMINATION OF IMPURITIES IN Ti and TiO_2

by

JELENA S. MATIĆ

INTRODUCTION

The increasing use of titanium and its alloys in contemporary technology is the main reason for intensive analysis of methods and treatments for its purification. Together with the perfection of the extraction process, demands for developing corresponding methods of analysis increase. According to the literature,(1) various methods are used for determining impurities in titanium, its alloys, and in titanium dioxide. The spectrochemical method is most commonly used. Interstitial oxygen, nitrogen, carbon and hydrogen, and significant amounts of Fe, Mg, Ca, Al, Sn, Pb, Mn, Ni, Cu and Mo, are among the elements found in titanium. Fe is regularly present as a substitutive dissolved element(2).

Because of their known properties, spectrochemical methods are among the most commonly used. The most elementary method of spectrochemical analysis of titanium alloys, direct spark excitation, was used by Moiseyev and co-workers(3) ,Bogdanova and Kudelya(4), Kudelya(5), Tsimbal and Lichko(6) and Poehlman(7). With regard to the preparation of standards these methods are not particularly convenient. In order to extract a sample it is necessary to melt titanium in a vacuum or in some inert atmosphere, since at the melting temperature (1800°C) it reacts with O_2 and N_2 from air. Methods based on the analysis of solutions are used because the preparation of standars is less complex while almost the same sensitivity and accuracy are obtained. Peterson and Enns(8) and Peterson(11, 12) applied analysis of solutions by using porous electrodes. Lewis and Carpenter(9) and Heller and Lewis(13) applied the same method by using rotating discs. Arc excitation methods can be used for direct analysis of oxides and for the analysis of metals transformed to oxides ny suitable treatment. This was carried out by Standen(10), Melamed(14), Melamed and Saltikova(15), Filimonov and co-workers(16), Karabash and co-workers(17). Nachtrieb and Johnson(18) and Rozsa(19) also used the method of arc excitation, preparing the oxide sample for analysis by melting it with potassium pyrosulfate. Vainshtein and co-workers(20) analyzed titanium with a plasma burner. Ivanov and co-workers(21) analyzed titanium dioxide by discharge in a hollow cathode.

A modified method of Standen(10) is described in this work. This method is based on fractional distillation with sodium chloride as carrier. The advantages of the method are its simple experimental technique and relatively great sensitivity.

EXPERIMENTAL TECHNIQUE

Preparation of Standards. Two series of standards were synthesized in order to get standard samples with a titanium base. The first series contained the following elements: Fe, Cr, Mn and Pb. The second series contained Cu, Ni and Sn.

For each standard in a series 100 mg of titanium metal were dissolved by long heating below the melting point in dilute H_2SO_4 (3 ml conc. H_2SO_4 on 20 ml H_2O). Chloride solutions of the elements for which the standard was prepared were added to the clear solution. This solution was evaporated to dryness, and the dry remainder was heated to 800°C and transformed to oxide. The resulting mixture was mixed with sodium chloride in the ratio of 1 : 2.

Determination of the Evaporability of the Impurities. To decrease the spectral intensity of the base and the background blackening, the exposure time must be carefully chosen in to analyzing elements with complex spectra by means of the fractional distillation method. Standen's data(10) on the exposure time were confirmed by the "moving plate" technique(22). Standard 0,1% mixtures of Fe, Pb, Mn, Cr, Sn, Ni and Cu in titanium dioxide were recorded, and it was determined that before the sodium evaporates (this time being taken as the exposure time), lead, tin and copper evaporate completely. Nickel, manganese, iron and chromium remain in limited quantities in the resulting metal pearl even after exposure.

Figure 1 shows evaporability curves in density — time coordinates for a highly evaporable element (Pb—2833.07 Å), for an element with low evaporability (Cr—3021.56 Å) and for titanium (Ti—2599.9 Å). The dependence of the evaporation of the base on the presence of sodium in the arc plasma is clearly shown by the evaporability curve for titanium. The end of the evaporation of the sodium was accompanied by a sudden evaporation of the titanium due to increased arc temperature Since the titanium lines were used as internal standard lines, the reproducibility of the results depends directly on the accurate determination of the evaporation time of the sudium.

The change in electrode current, which appears as a consequence of structural change in the sample, was used as the indicator that the sodium had evaporated. This change can be seen one to two seconds before the complete disappearance of the sodium.

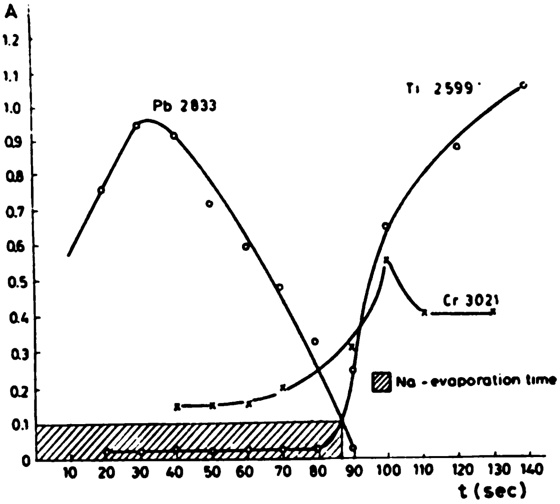

Figure 1
Density — time curves for Pb, Cr and Ti

Spectrography. Table 1 shows the operating conditions.

TABLE 1

Operating conditions

Spectrograph	Hilger E-478
Spectral region	2400 — 3320 Å
Slit width	0,015 mm
Excitation source	d. c. arc, 14 A, 220 V
Electrode system	Ringsdorff-Werke, Graphite, anode in form a cup, cathode with a flat end, dimension as in Fig. 2
Quantity of sample	15 mg (5 mg oxide sample + 10 mg² NaCl)
Analytical gap	9 mm
Exsposure time	To the end of sodium evaporation (aproximatily 90 sec.)
Photographic plate	Ilford Ordinary N-30
Developer	Ilford, ID-2, 3.5 min at 20°C

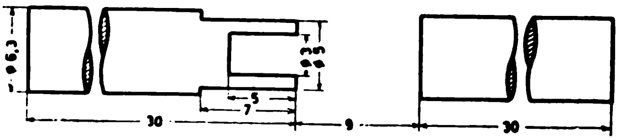

Figure 2
Form and size of the electrodes

Results. Table 2 shows analytical line pairs and the concentration range for the curves are given.

TABLE 2

Analytical line pairs

Element	Analytical line pair, Å	Concentration range, %
Iron	Fe 2599.4/Ti 2599,9	0.01 — 0.25
Chromium	Cr 3021.6/Ti 2928,3	0.01 — 0.25
Lead	Pb 2833.1/Ti 2841,9	0,0025 — 0.25
Nickel	Ni 3050.8/Ti 2965,7	0.01 — 0.5
	Ni 3037.9/Ti 2965,7	0.5 — 1.0
Manganes	Mn 3044.6/Ti 2965,7	0.05 — 1.0
Tin	Sn 3034.1/Ti 2965,7	0.027 — 0.1
Copper	Cu 2492.1/Ti 2599,9	0.25 — 1.0

The working curves for determining the iron, chrome, lead, nickel, manganese, copper and tin are drawn on a log-log graph.

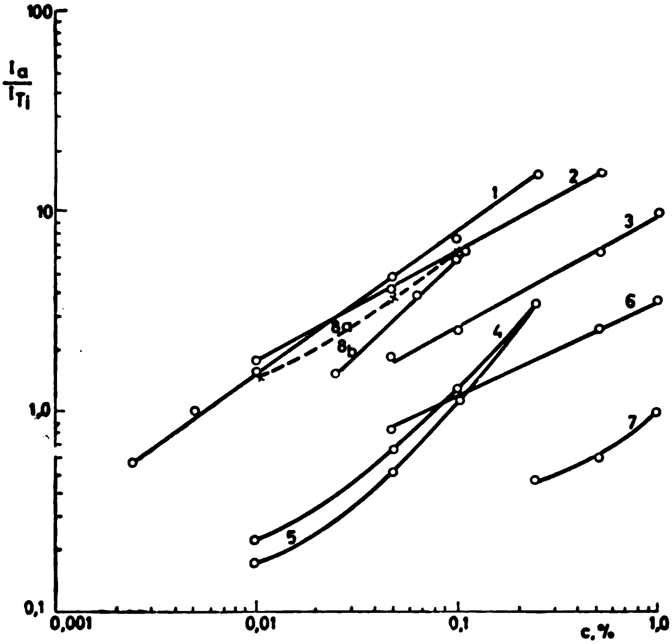

Figure 3

Working curves for Fe, Cr, Pb, Ni, Mn, Cu and Sn in TiO_2
1-Pb 2833/Ti 2841; 2-Ni 3050/Ti 2965; 3-Ni 3037/Ti 2965;
4-Cr 3021/Ti 2928; 5-Fe 2599,4/Ti 2599,9; 6-Mn 3044/Ti 2965;
7-Cu 2492/Ti 2599; 8_a-Sn 3034/Ti 2965; without correction and
8_b-Sn 3034/Ti 2965 with correction for the Sn
content of the basic sample

The concentration is on the abscissa, and the ratio between the intensities of the analytical lines and the lines of the internal standard on the ordinate. Figure 3 shows the working curves used to determine the Fe, Cr, Pb, Ni, Mn, Co and Sn in the TiO_2. The initial samle of titanium from which the standard was made contained a certain percentage of tin. The additive method$^{(23)}$ was used to determine the percentage. Figure 3 shows the working curve for determinig tin 8_a without correction and tin 8_b with correction for the tin content of the basic sample.

To give an idea of the precision of the method, the coefficients of varriation are calculated for each sample, and the results are summarized, in Table 3.

TABLE 3

Precision of the method

Element	Mean ($\bar{X}$) conc. in %	Coefficient of variation (V)	Number of determinations (n)
Fe	0.05	11.5	8
	0.25	11.8	7
Cr	0.1	17.8	7
	0.25	17.6	6
Ni	0.05	12.6	5
Pb	0.05	19.4	5
	0.01	17.6	6
Mn	0.05	21.4	6

a) The coefficient of varriation V is calculated from the following relation:

$$V = \frac{100}{\bar{X}} \sqrt{\frac{\sum (X_i - \bar{X})^2}{n-1}}$$

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A METHOD OF OBTAINING SPHERICAL METAL POWDER PARTICLES

by

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The shape of metal particles is an important factor where homogeneous packing of metal powder or high powder densities are required, in classification of metal powder, and in many other procedures for obtaining some special properties of a product, as e.g. in making metal filters, fuel cell electrodes, etc. This study aimed to investigate the possibility of transforming commercially available non-spherical fine metal powders (Cu, Ni, bronze and Raney-alloys) into metal spheres of the corresponding size, by a special heat treatment. This consists of passing a stream of gas, compressed air or oxygen, carrying fine metal powder particles, through a gas burner at the exit of which a fuel gas is mixed with the carrier gas and burnt.

Spherical particles are usually obtained by two methods: by thermal decomposition of carbonyl compounds, or by atomizing molten metals.

Thermal decomposition of metal carbonyls has found commercial application only in the production of iron and nickel spherical metal powders. The carbonyls (nickel tetracarbonyl $Ni(CO)_4$ and iron penta carbonyl $Fe(CO)_5$ are decomposed at the corresponding temperatures and under suitable pressures. Iron particles of 0.1 to 10 microns and nickel of 0.01 to 12.5 microns are obtained depending on the rate of cooling.

Pulverization of molten metals is the most generally employed method of obtaining spherical metal powders. There are two basic variations of this process. In the first the molten metal is heated to slightly above its melting temperature and is poured out into water or onto a metal plate. The particles obtained are rather coarse. This can be avoided however, by directing the molten metal against a stream of compressed air, water vapor, gas or water.

The second variation consists of leading a thin stream of molten metal through a nozzle at whose exit there is a stream of compressed air, gas or water which atomizes the metal into fine liquid balls that are suddenly frozen. The size distribution of the particles and their shape can be varied to a large extent by changing the size and shape of the nozzle, the temperature, and thus the viscosity and surface tension of the metal and the flow rate of the atomizing fluid. Non-spherical particles can also be obtained by this method.

Recently, two attempts were made to obtain spherical particles by passing non-spherical powders through a heated space. In the first case(1) metal powder particles were passed through a heated vertical furnace, while in the second case(2) an argon plasma torch was used to heat the particles to their melting temperatures. Refractory materials and many metals in powder form were converted into spherical shape by these two methods. Another attempt was reported in the literature(3) but no details were given as to the method.

EXPERIMENTAL PROCEDURE

The procedure involved passing particles of irregular shape — dendritic, flakes and acicular — through a gas flame. During passage the particles were melted so that the surface tension of the molten metal, which is normally very high, transformed them into liquid spheres of a fairly regular shape. On leaving the flame the particles were frozen and the shape retained.

Two apparatuses were constructed. In the first one stream of the carrier gas (air) was conducted through a fluidizing column at a flow rate sufficient to carry up the metal powder particles. The stream was then passed through a burner made of a tube coil which had orifices on the inner side through which butane was fed. Butane and air burnt forming a flame through which metal powder particles fell down into a beaker (Fig. 1). The results with this apparatus were rather poor. The percentage of spherical powder particles was very low, ranging from 5 to 15%. Besides the low yield, a large amount of the particles got stuck on the coil, choking the orifices and stopping the process.

In the second apparatus, presented in Fig. 2., the mixture of carrier gas and powder was formed in a mixing chamber. This chamber was connected to a vibrator to achieve a uniform fluidization of the particles. The flow of butane, air, oxygen, and a mixture of oxygen and air was measured by a set of flowmeters. The exit tube of the mixing chamber was connected with the inner tube of the burner. This tube had an exchangeble nozzle at its end. The fuel gas was introduced through the outer tube of the burner. The oxygen-butane mixture formed a flame whose composition and temperature could be adjusted by varying the butane and air (or oxygen) flows. On their way through the flame metal powder particles were melted and, by the action of the surface tension, transformed into liquid spheres.

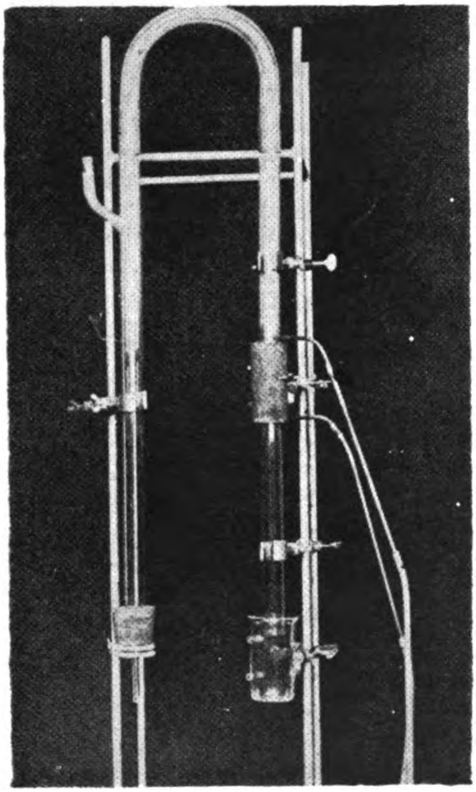

Figure 1

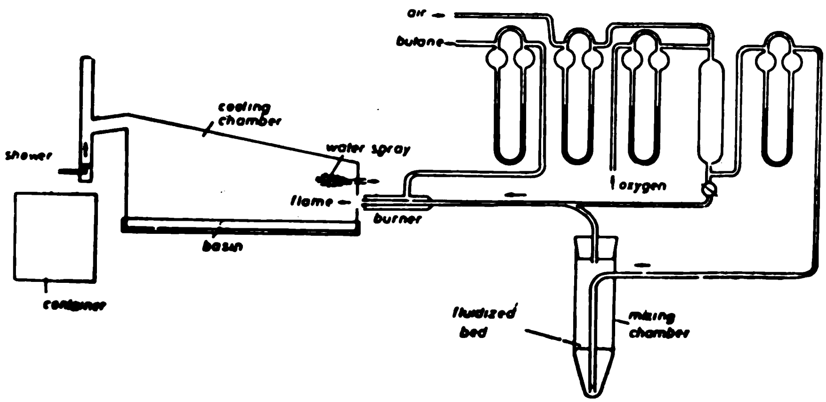

Figure 2

RESULTS

A chart of the flame temperature is given in Fig. 3. Curve 1 shows the temperature distribution along the axis of the flame for a butane-oxygen ratio of 1 : 1.4 using only air without additional oxygen. Curve 2 shows the temperature distribution for a butane to oxygen ratio of 1 : 2.4 where the best spheroidization of copper particles was achieved. It is evident that the high temperature zone in the first case was only 10 cm long, while in the second case it was almost 20 cm.

The burnt gases carrying the treated particles were forced into the cooling chamber where the powder spheres were frozen and collected by free fall into a water basin at the bottom of the chamber. Even with this set-up losses of the finest particles and the oxidation rate were very high, so that an additional water spray was introduced above the inlet of the flame thereby greatly improving the process. The finest particles were collected by a water shower at the exit of the cooling chamber.

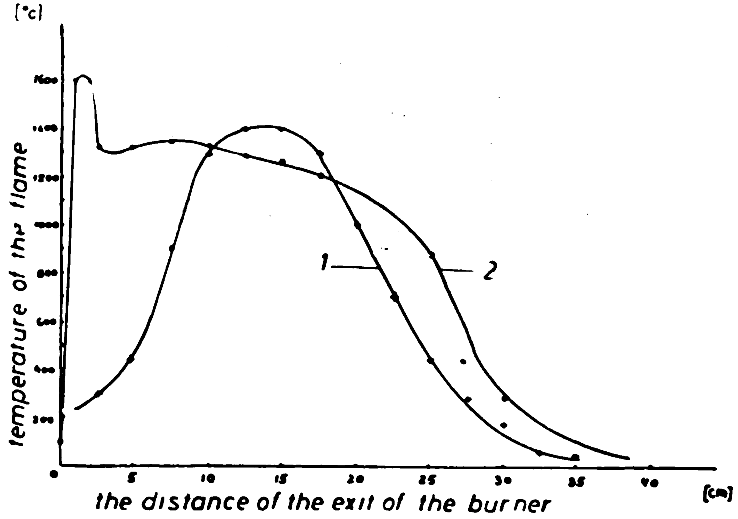

Figure 3

Tables 1 and 2 show the yield of spherical particles of electrolytic copper powder obtained with different butane to oxygen ratios. The optimal ratio seems to be 1 : 2.4 (sample No. 5) when 97% of the particles had a spherical shape.

Table 2 shows that by increasing the content of butane in a constant mixture of air and oxygen the percentage of spherical nickel particles could be increased. It is interesting to note that by introducing an additional water spray above the inlet of the burnt gases a very low oxygen content in the treated particles could be achieved (Sample No. 10).

TABLE 1

Electrolytic copper powder. Original particles 50 — 60 microns

Sample No.	Orifice diameter mm	Flow rate l/min			Ratio C_4H_{10}:O_2	Spherical particles in %	Oxygen content in powder %
		C_4H_{10}	Air	O_2			
1	—	—	—	—	—	0.0	0.02
2	3	1.9	13.6	0.0	1:1.4	13.0	1.23
3	3	1.1	8.1	5.3	1:6.3	62.0	2.41
4	3	3.0	10.8	2.9	1:1.7	66.5	1.88
5	3	3.0	7.9	5.7	1:2.4	97.0	2.17

TABLE 2

Nickel powder obtained by reduction of oxide. Original particles smaller than 40 microns

Sample No.	Orifice diameter mm	Flow rate l/min			Ratio C_4H_{10}:O_2	Spherical particles %	Oxygen content in powder %
		C_4H_{10}	Air	O_2			
6	—	—	—	—	—	0.0	0.45
7	3	2.0	7.9	5.7	1:3.6	81.3	16.42
8	3	2.5	7.9	5.7	1:2.9	87.1	15.61
9	2	2.5	7.9	5.7	1:2.9	89.3	14.85
10	3	3.0	7.6	5.7.	1:2.4	99.1	1.31*

*Run with an additional water spray

Tables 3 and 4 show that about the same ratio of butane to oxygen (1:2.5) gave optimum spheroidization of bronze and Raney-iron particles as in the previous experiments with copper and nickel powders.

TABLE 3

Bronze (Cu 95%, Sn 5%) obtained by atomization. Original particles 40 — 50 microns

Sample No.	Orifice diameter mm	Flow rate l/min			Ratio C_4H_{10}:O_2	Spherical particles %	Oxygen content in %
		C_4H_{10}	Air	O_2			
11	—	—	—	—	—	0.0	0.07
12	3	3.0	9.5	5.7	1:2.5	51.3	0.32
13	3	3.0	10.0	3.0	1:1.7	56.4	0.37
14	3	3.0	13.0	1.5	1:1.4	71.2	1.10
15	3	3.0	7.0	6.1	1:2.5	95.3	1.18

TABLE 4

Raney iron (Fe 50.6%, Al 49.7%) obtained by milling. Original particles 20 — 40 microns

Sample No.	Orifice diameter mm	Flow rate l/min			Ratio C_4H_{10}:O_2	Spherical particles %	Oxygen content in powder
		C_4H_{10}	Air	O_2			
16	—	—	—	—	—	0.0	—
17	3	2.0	13.6	0.0	1:1.8	0.3	—
18	2	3.0	3.6	10.0	1:3.6	69.3	—
19	3	3.0	6.1	5.7	1:2.3	82.7	—
20	3	3.0	5.7	7.9	1:3.0	90.1	—

The change of apparent and tap densities and of the flow rate of particles against percentage of spheres in the treated powder mixtures are shown in the following four diagrams (Fig. 4—7). Increasing content of spherical particles gave an increase of apparent and tap densities. The same happened with the flow rate of the treated powders, with the exception of the very fine nickel powder whose flow rate in all cases was nil.

Photographs of the untreated copper powder are shown in Fig. 8 of the copper powder with 66.5% spherical particles in Fig. 9 and of copper powder with 97.0% spherical particles in Fig. 10

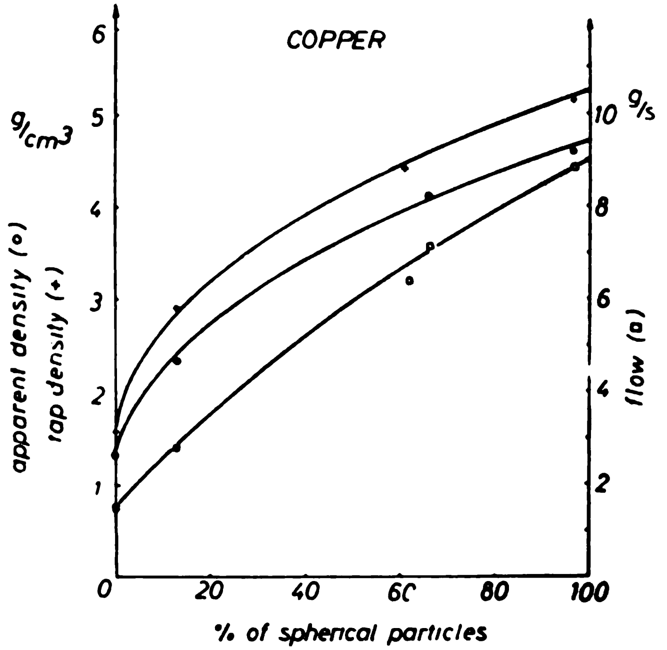

Figure 4

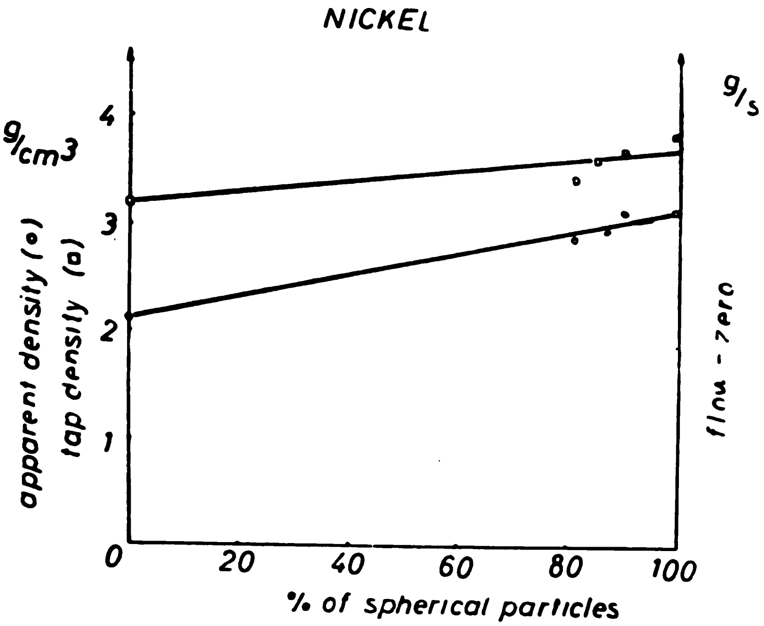

Figure 5

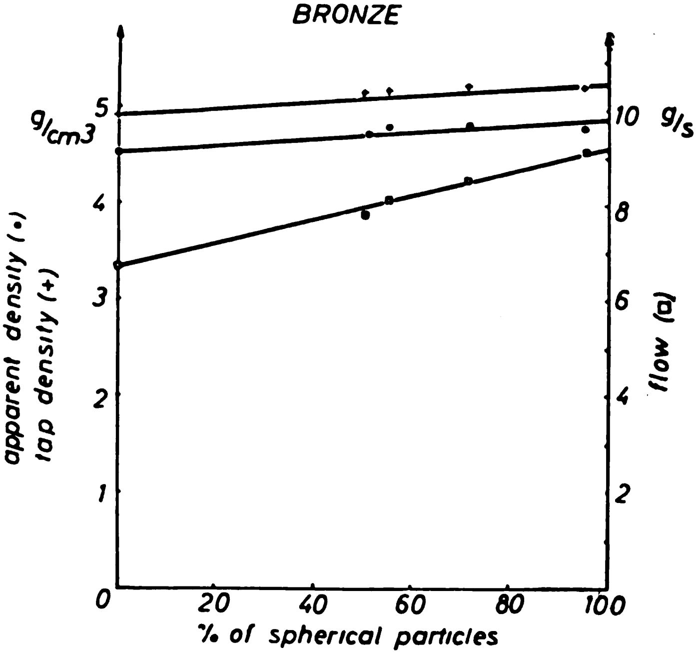

Figure 6

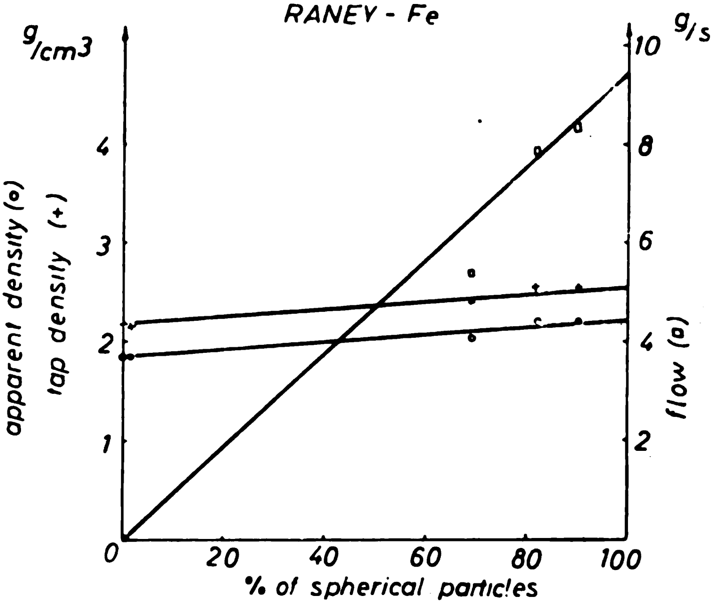

Figure 7

In conclusion it can be said that by the method described it is posible to obtain very regular spherical powders starting from irregular ones, with much better packing performances that the original.

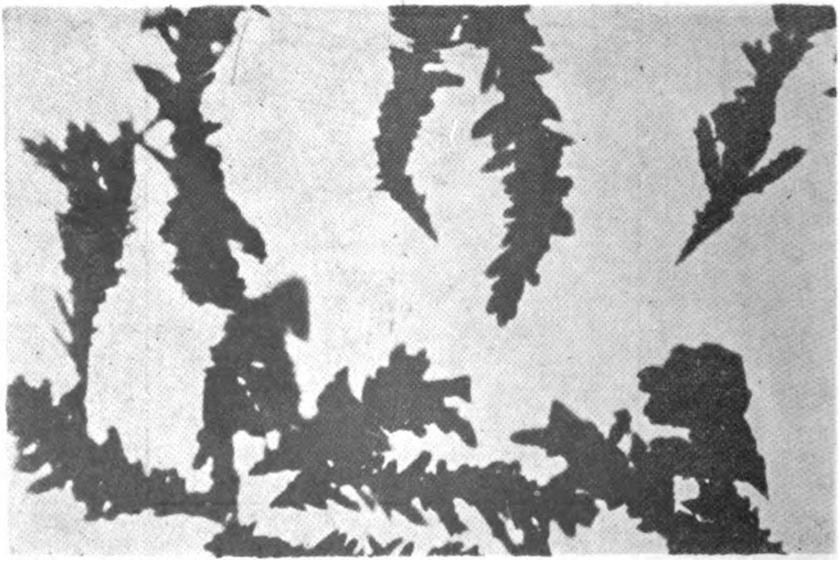

Figure 8

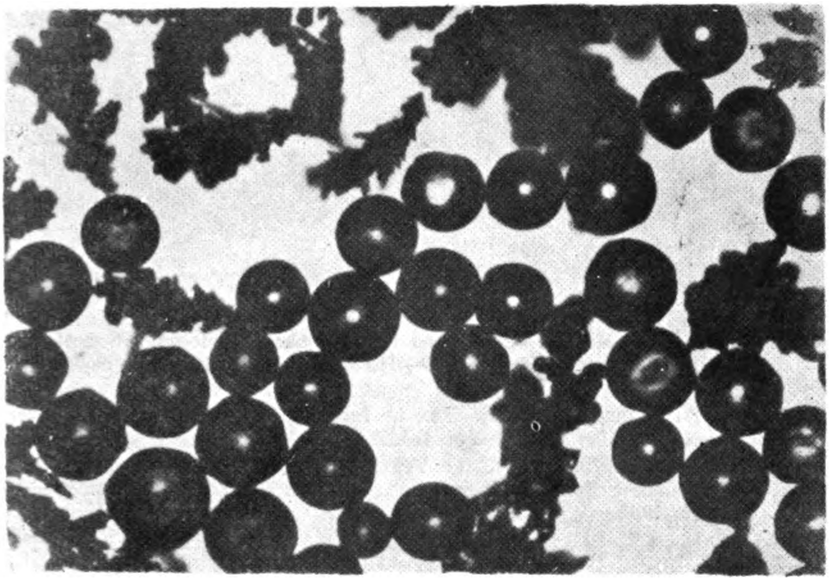

Figure 9

The arrangement used had a fairly high working capacity, about 600 g per hour of completely spheroidized powder. As compared to other torch arrangements its advantage lies in that fuel and oxygen are mixed outside the burner and thus the danger of flame penetration into the burner nozzle is excluded.

The method is very simple and can be used for the spheroidization of very fine metal powders.

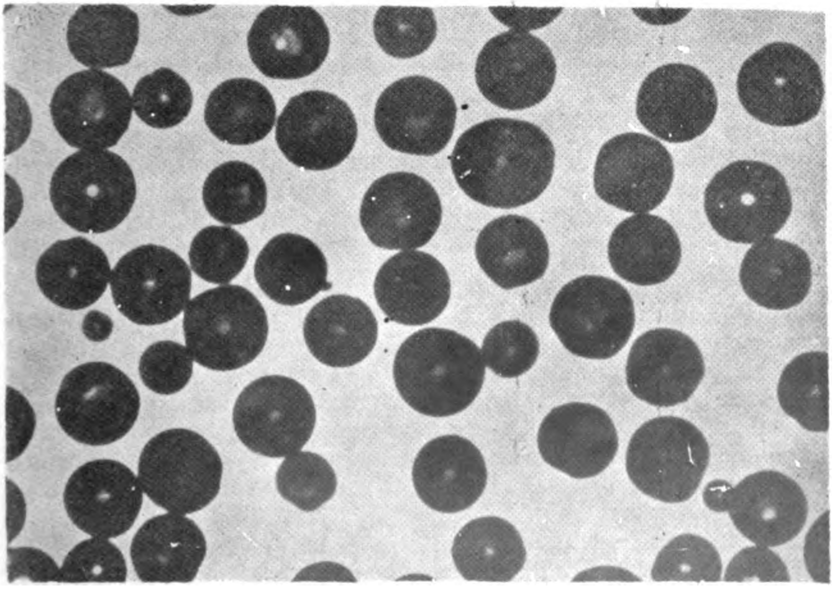

Figure 10

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SPECTROCHEMICAL ANALYSIS OF H_2O — D_2O MIXTURES BY MEANS OF BAND SPECTRA*

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In connection with their research work on the properties of the OH and OD molecules in electrical discharge and in gas reactions, and on the possibilities of using band spectra for the analysis of isotope mixtures, Jäger and Mikkeleit(1) published a paper in 1962 on spectrochemical analysis of H_2O — D_2O mixtures for the concentration range of 1 to 86.5% of D_2O with a relative error of 25% at 1% of D_2O. The investigation results presented in this report represent a continuation of this work which includes the study of the concentration range of 1% down to that of natural water and even less. They are the results of an attempt to establish spectroscopically the smallest possible concentration change of D_2O content in water of natural composition with the lowest D_2O concentration, so that the possibilities offered by this can be applied to biological, geological, physico-technological and chemical processes, where such concentration, changes occur.

While spectrochemical determinations of H_2 — D_2 mixtures have been carried out since the thirties(2—6), detailed research on the spectrochemical analysis of H_2O — D_2O mixtures has only recently been reported, by Donzow and Striganov(7), Broida, Morowitz and Selgin(8), Morowitz and Broida(9), and Freis, Higgins and Morowitz(10).

The analysis of H_2O — D_2O mixtures by means of lines emitted by OH — and OD — electronic band systems during electrical discharge in water vapor was carried out by Jäger and Mikkeleit(1), and also in the present work. The principle of the method is also based on the utilization of the isotopic effect in the electronic rotation-vibration band system of OH — and OD — molecules. In analyses of H_2O — D_2O mixtures published so far(7,8) some authors used the atomic lines H_α and D_α, others H_β and D_β as analytical

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* Communicated at the XIth Colloquium Spectroscopicum Internationale — Beograd (Yugoslavia), September 30th — October 4th (1963).

lines. Donzow and Striganow⁽⁷⁾, and Broida, Morowitz and Selgin⁽⁸⁾ excited the H_2O—D_2O mixtures to be analyzed, i.e. the water vapor to be analyzed, by means of high frequency discharge and estimated the D_2O content by the measurement of the intensity ratio between the atomic lines of hydrogen and deuterium.

In our earlier investigations⁽¹⁾ we used a medium-sized quartz spectrograph for the analysis of H_2O — D_2O mixtures. However, its dsipersion did not suffice to resolve the band heads of the O,O — vibrational transition of the $^2\Sigma + {}^2\pi_i$ of the electronic band system of the OH—and OD—molecules at 306.4 nm, so that we had to use the rotation lines of the satellite branch S_{R21} of extremely low intensity. However, with a 2 m plane grating spectrograph we succeed in resolving the band heads of the O, O— vibrational transition and in analyzing the lines of the highly intense OD—Q_1 and OH—R_2 branches. A high frequency generator proved to be the most suitable excitation source and it produced the mos intense discharge at a power of 250 W and a frequency of 27.12 Mc/s. The basic setup of the quartz capillary discharge tube used in the analysis can be seen in Fig. 1. This shows the sample-containing vessel (2) kept at —16±0·3°C, the capillary discharge tube (5) (diameter of capillary 1—2 mm) with the external electrodes used once with a water cooling jacket, another time with air cooling, the three-lens optical illumination equipment of the spectrograph (7, 8, 9, 12); and the cooling trap (10) located in front of the pump to recondense the vapors passing trough the discharge capillary. The apparatus was operated at a pressure of 2 Torr while the vapor pressure of the H_2O—D_2O mixture was 1 Torr (corresponding to the temperature of —16° C of the sample-containig vessel). The residual pressure was due to the presence of air by whose addition

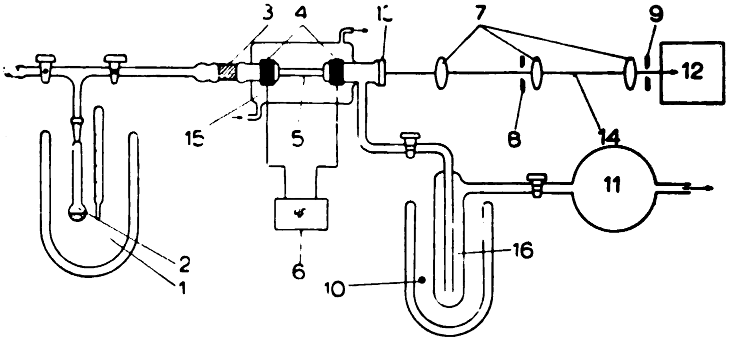

Fig. 1. — A schematic presentation of th apparatus for the H_2O/D_2O analysis.
(1) Refrigerating m'xture (= 16° C); (2) sample-containing vessel;
(3) standard ground joint (14,5); (4) external electrodes;
(5) capillary discharge tube; (6) HF-generator; (7) condenser lenses;
(8) screening aperture; (9) slit; (10) ethanol/dry ice; (11) manostat;
(12) spectrograph; (13) qnartz window; (14) optic axis;
(15) cooling jacket; (16) cooling trap.

it was possible to obtain a considerable increase in intensity. When all the requirements for the analytical lines were considered, the OD—Q_1 (5) and OH—R_2 (16) lines proved the most suitable combination.

On the basis of the absorbance (blackening) obtained ($S = 0.5$) of the analytical line used for D_2O OD—Q_1 (5) at the shortest exposure time — under the established optimum analytical conditions of pressure, temperature, addition of foreign gas, capacity of the generator, and material of the discharge tube — it was concluded that the detection limit of D_2O in H_2O was below the content of D_2O in natural water

As already established for analyses involving hydrogen (Botschkowa(11)), glass discharge tubes proved to be of little or no use for the analysis of H_2O—D_2O mixtures. Because of the wall effects occurring in them the tubes did not emit constant intensities of OH and OD lines. Figure 2 shows comparative curves taken with a glass discharge tube; the curves 1 and 2 were taken at an interval of two, the curves 1 and 3 at an interval of ten days. When quartz capillary discharge tubes were used the wall effects

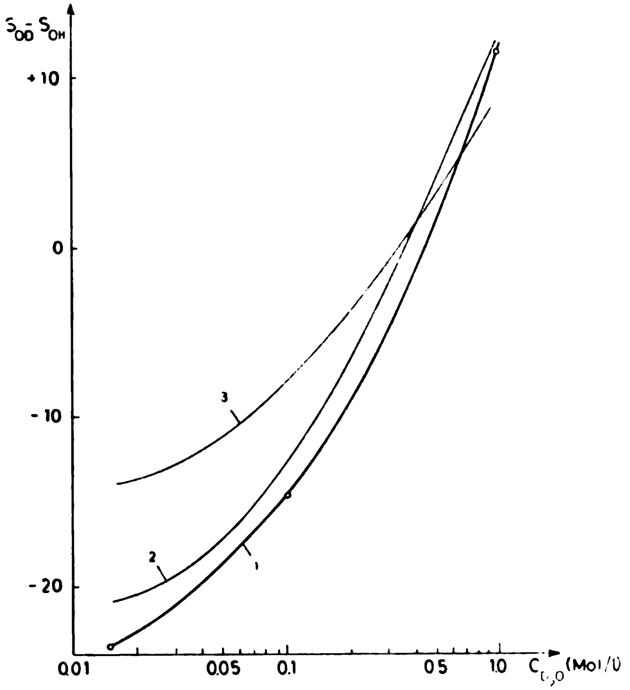

Fig. 2. — Comparative curves (obtained with glass discharge tube) Dependence of $S_{OD} - S_{OH}$ on log of the D_2O concentration.
S_{OD} : Absorbance of the OD line $Q_1(5)$
S_{OH} : Absorbance of the OH line $R_2(16)$

were reduced, as expected, but did not disappear completely. The difference in absorbance $S_{OD}-S_{OH}$ increased somewhat with time. Newertheless, at various concentrations, the increase was almost the same so that the comparative curves were parallel and only a little shifted apart. Therefore, when water mixtures are investigeted, it is advisable to plot a comparison test (e.g. with natural water) whenever possible. Figure 3 shows a comparative curve obtained with a quartz discharge tube.

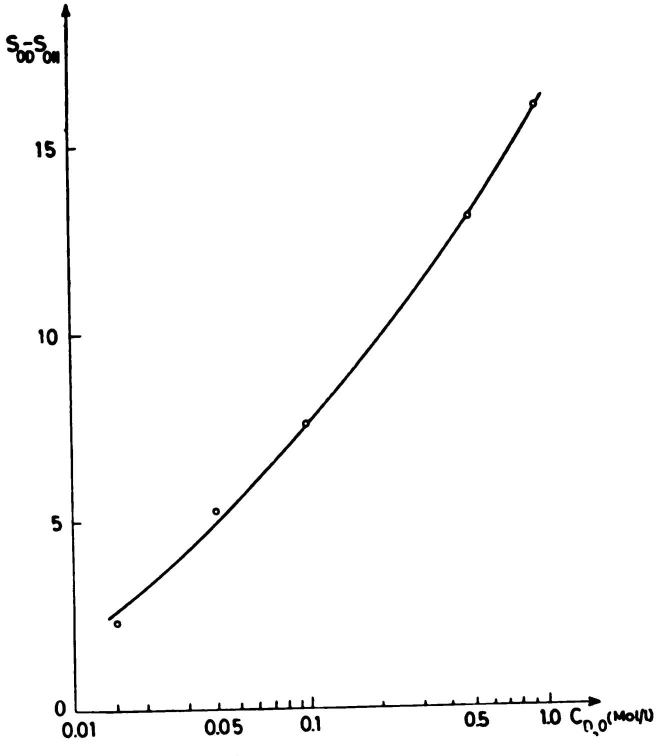

Fig. 3. — Comparative curve (obtained with quartz discharge tube) Dependence of $S_{OD}-S_{OH}$ on the log of the D_2O concentration.

The reproducibility obtained was ± 0.0006 Mol% D_2O at the concentration of natural water, and this corresponds to a relative error of 4%. The relative error at 1 Mol% of D_2O was 1%.

The data in Table 1 show the accuracy and sensitivity obtained with various methods of the isotopic analysis of water. If we compare the most accurate methods with one another, we can say that in the precision float method, like all other methods of investigation utilizing the difference in density of two isotopes, it is not possible to take into account the differences which might appear in the

TABLE 1

Methods used in the analysis of water isotopes and data on their accuracy and sensitivity

Method	Absolute Error (Mol% D_2O) At Limit Concentration	Limit Concentration	Amount of Substance	References
Viscosity	± 2			(12)
Vapor Pressure	± 0.25		0.5 mg	(12,13)
Thermal Conductivity	± 0.1	1 Mol%	0.5 mg	(12,14)
IR Spectroscopy	not very accurate		20 — 200 mg	(12,14,15)
Mass Spectrometer	± 0.0002	Nat. Water	0.1 ml	(12,16)
Radiochemistry	± 0.0006	„ „	0.1 ml	(17,12)
Pycnometrit Method	± 0.003	„ „	25ml	(12)
Falling Drop Method	± 0.002	„ „	0.05 ml	(12)
Interferometer Method	± 0.01	„ „	1 ml	(12)
Float Method	± 0.001	„ „	3 ml	(12)
Microfloat Method	± 0.003	„ „	0.05 ml	(12)
Precision Float Method	± 0.0002	„ „	40 ml	(12)
Nuclear Resonance	± 0.01			(12)
Spectroscopy:				
JÄGER, MIKKELEIT	± 0.25	1 Mol%	0.05 ml	(1)
APITZSCH	± 0.3	1 Mol%	0.05 ml	(18)
DONZOW, STRIGANOW	± 0.08	2 Mol%	0.02 ml	(7)
BROIDA and CO-WORK.	± 0.0002	0.01 Mol%	0.05 ml	(8,9,19)
DUNKEN, MIKKELEIT, HAUCKE	± 0.0006	Nat. Water	0.05 ml	

concentration of oxygen isotopes. On the other hand, with spectroscopic methods it is definitely possible to take these differences into account. When compared with the emission spectroscopic method, the mass spectroscopic and radiochemical methods have the disadvantage of

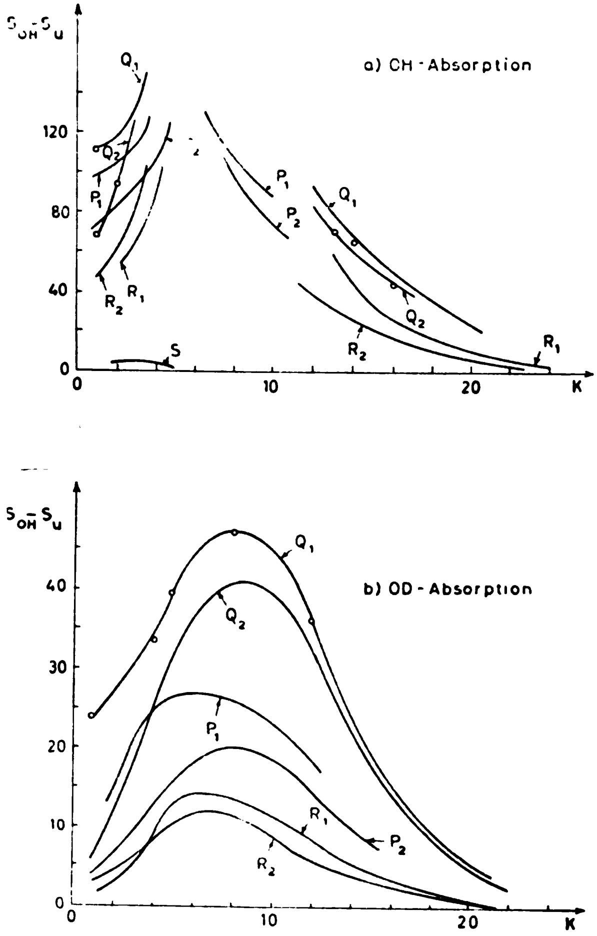

Fig. 4. — Intensity distribution in the O,O-vibrational transition of the OH and OD molecules in absorption.
K: Rotation quantum number
S_{OH}: Absorbance of the OH rotation lines
S_{OD}: Absorbance of the OD rotation lines
S_U: Absorbance of the background

a) OH absorption
(City gas flame, bright part)

b) OD absorption
(Hydrogen/air flame with 40% D_2)

being very expensive because of the apparatus used. They also impose great restrictions in connection with the preparation of samples, i.e. they require samples of a high degree of purity. In addition, in the mass spectroscopic method the substance to be analyzed, in our case a H_2O-D_2O mixture, must first be decomposed into its gaseous constituents. Accordingly, there remains the method of Broida and co-workers mentioned at the beginning of this article which, although somewhat more accurate than the procedure which we have worked out, was not used because of the following reasons:

(1) In the presence of air there is an increase in the intensity of the deterium line D_β, but in spite of this we found nitrogen bands in the region of the analytical lines, and with the grating spectrograph that we used, also OH lines of the second order.

(2) At low deuterium concentrations Broida and co-workers compared the deuterium line with a ghost of the hydrogen line H_β. The grating at our disposal did not produce this ghost, and certainly not every grating would produce a ghost of suitable intensity. However, the difference in intensity between the hydrogen and deuterium lines is so great that even the hydrogen line reduced to 5% by the usual step filters was still so intense compared with the deuterium line that it did not seem very suitable for measurement, while the absorbances i.e. the intensities of lines which we used had a more favorably ratio.

(3) The blazed wavelength of the grating was 260 nm in the second order, so that the intensity of the O, O-vibrational transition of the electron band system of OH and OD was also favorable.

(4) With their method Broida and co-workers were not able to carry out a simultaneous analysis of oxygen isotopes of water by using one spectrogram, and certainly not by using one and the same spectrum, as was our intention.

Work on the application and improvement of the above method concerning primarily the analyses of H_2O-D_2O mixtures with a D_2O content below that of the natural D_2O concentration is in progress.

It was attempted to carry out a H_2O-D_2O analysis, i.e. an H_2-D_2 analysis using the rotation lines of the O, O-vibrational transition of the $^2\Sigma^+-{}^2\pi_i$ electron band system of OH and OD by a method analogous to that of "atomic absorption". This method made it possible to establish a minimum of 5% D_2 in H_2 while on the other hand far less than 1% of H_2 could be estimated. Next, we established the intensity distribution of the O, O-vibrational transition of OH and OD in absorption. As evident from Fig. 4, it was proved thereby thet at approximately the same concentration the absorption of the OD molecule was far weaker than that of the OH molecule when the "absorbers" were two different flames fed by hydrogen with the same concentration of H_2, i.e. od D_2. The reasons for this difference in the behavior of OD and OH with regard to absorption have not so far been explained.

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MICROESTIMATION OF COBALT IN BIOLOGICAL MATERIAL USING α—FURILMONOXIME

by

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and

NIKOLA STOJANOVIĆ

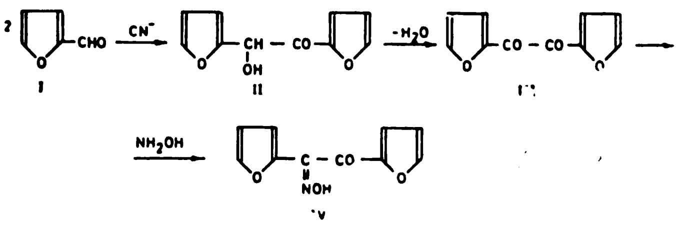
Cobalt was first discovered as a constituent of biological material in 1841, when Legrip⁽¹⁾ found it in *Lathyrus odoratus*. In the next seventy years it was proved that small amounts of cobalt occur in all plants that grow in soil containing cobalt(2, 3, 4). Cobalt is also found in almost all human and animal organs(5,6). According to present-day knowledge cobalt has no physiological role in the higher, but even in relatively small concentrations (1—2 mg CoO per liter of water in which plants are cultivated) it decreases the yield of many cultures and its effect is toxic(7, 8). On the other hand, the physiological role of cobalt in the animal organism is well known and studied, particularly in the case of ruminants, for which it has been proved that lack of cobalt causes serious illnesses and even death(9, 10, 11). This is now explained since it is known that vitamin B_{12} is a cobalt complex(12) and lack of this microelement leads to disorder of those functions in the organism which depend on vitamin B_{12}.

Although a great many colorimetric, polarographic, spectrographic and other methods(13—33) of determining micro amounts of cobalt are well known today, many of these connot be used for the determination of cobalt in biological, particularly plant material, either because they are not sensitive enough to determine amounts of 1—2 μg% of cobalt (dry material), or cobalt cannot be determined because other elements in the material interfere with the determination or make it impossible.

Studying salts produced by furfural derivatives with metals Martinek and Havorka(34) found that α-furilmonoxime with cobalt in a pyridine solution produces a characteristic pink-colored complex salt. They found that the complex when once built-up, is very stable, even in solutions of pH 1—2, while complexes of other metals (Fe^{++}, Ni^{++} and Cu^{++}) break up even at pH 3—4. This property of the cobalt complex allows its extraction with an organic solvent from acidic solutions and colorimetric determination of the intensity of the pink color. Martinek and Havorka developed a colorimetric method for the determination of cobalt in steel and industrial nickel. They determined cobalt contens of 0.01% to 10.03%, with a relative error from 0.5% to 2.56%.

We thought that Martinek and Havorka's method, in view of its high sensitivity, might be modified for determining micro-quantities of cobalt in silage. First we synthesized α-furilmonoxime, and then experimentally found the most suitable conditions for colorimetric determination of cobalt in dilutions of 0.1 to 5 μg of cobalt in a 10 ml solution, since according to the literature this ampunt of cobalt is found in 2 to 5 g of dry plant material.

We got α-furilmonoxime by starting from furfural (I). By acyloin condensation(35) we first obtained furoin (II) and oxidized it to furil (III) with ammonium-nitrate in the presence of cupric acetate(36). Then with hydroxylaminechlorhydrate we transformed furil to α-furilmonoxime (IV). We modified the procedure given by Martinek and Havorka(34) for isolating α-furilmonoxime from the reaction mixture because by this procedure the oily layer of α-furilmonoxime did not separate even after the reaction mixture had stood for several days. We used ether to extract the α-furilmonoxime from the reaction mixture and then proceeded as described in the experimental section of this paper.



INVESTIGATION OF THE INFLUENCE OF TEMPERATURE AND STANDING ON THE COLOR INTENSITY OF THE COMPLEX COBALT

In the same way as for plotting the calibration curve (see Experimental) extracts of the complex of Co^{+++} and α-furilmonoxime were made in a chloroform-pyridine solution, for dilutions of 0.1 to 5 μg of cobalt in a 10 ml solution, and the influence of temperature in the range 15° to 30° C and the standing time on the color intensity were investigated. It was found that the color intensity does not change in this temperature interval and that the color is stable for fifty minutes and then begins to fade, the quicker the more dilute the solution.

INVESTIGATION OF THE INFLUENCE OF OTHER IONS

In addition to minimal contents of cobalt in plant material there are also other elements in various quantities, so we made a series of measurements of the color intensity of the complex in the presence of ions which might interfere. The investigations were performed as follows: into a 25 milliliters separatory funnel we measured 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the initial cobalt solution (which contained 1 μg cobalt per ml) and to each of these solutions we added that quantity of ions of potassium, sodium, magnesium, calcium, iron, manganese, zinc, aluminium, copper, chromium or nickel, which, according to the literature is the average maximum found in one gram of hay. Cobalt was then determined in these solutions in the same way as for the calibration curve (see Experimental). If 3 ml of saturated NaF solution is added to the solution, and after color developing the solution is acidified with 3 ml of HCl (1 : 1), good values for cobalt are obtained (maximum error 2.06%).

EXPERIMENTAL

Melting points were not corrected. Colorimetric determinations were made on a Beckman DU spectrophotometer in corex-cells, with a 1 cm thick absorption layer.

Preparation of furoin(35). Starting from 400 g of furfural, 147.3 g (36.82%) of furoin in the form of yellow needle-like crystals was obtained, m.p. 134—135°C.

Preparation of furyl(36). Starting from 115.2 g of furoin, 100.8 g (88.4%) of furyl in the form of yellow needles was obtained, m. p. 164°C.

Preparation of α-furylmonoxime. Into a four-liter flask with a mechanical mixer and a dropping funnel, 38 g of hydroxylaminec hydrochloride was dissolved in 60 ml of cold distilled water. 300 ml of 96% ethyl alcohol and 100 g of pure furyl was added to this solution and the mixer activated. The suspension was cooled with a salt-ice mixture to −5°C and 200 g of 30% aqueous solution of KOH was added dropwise. The rate at which KOH was added was adjusted so that the temperature of the reaction mixture, did not exceed 0°C. After all the KOH solution had been added, the orange colored reaction mixture was diluted with two liters of water and crystals of unreacted furyl were separated by filtration (22 g of furyl was regenerated). The filtrate was acidified with acetic acid (1 : 1) until the congo-acid reaction, and the white emulsion formed was left to stand overnight. Since layers did not separate, the reaction mixture was extracted three times, each time with 200 ml of ether. The ethereal solution was washed with water until all the acetic acid was washed out and then dried with Na_2SO_4. The yellow ethereal solution was separated, concentrated to a small volume under reduced pressure at room temperature and left in a cool place to crystallize. The dirty-white crystal mass obtained was recrystallized from a benzene-ligroin mixture (2: 1). 68.1 g of α-furilmonoxime was obtained in the form of a yellowish-white crystalline substance, m. p. 97—98°C. The yield was 74.8% (calculated with respect to the amount of furil which reacted).

Hay burning. Two grams of hay crushed and dried to constant weight in a desiccator over P_2O_5 was weighed in a quartz bowl. It was then burnt in an electric furnace at 420—450°C, with occassional stirring with a thick platinum needle. Burning was continued to constant weight of the ash. 2 ml HCl (1: 1) was poured over the slightly stained ash and the whole gently heated to dryness over a water bath. The addition of HCl and evaporation was repeated twice more so that the oxides would be converted to chlorides. After the third evaporation, 5 ml of distilled water was added te the dry residue, the mixture heated to boiling and filtered through a small filter into a 25 ml separatory funnel. The quartz bowl and the filter were quantitatively washed with another 5 ml of water.

Colorimetric determination of cobalt

Reagents: a) 10% pyridine solution of α-furilmonoxime
b) 5% KOH solution
c) saturated NaF solution
d) hydrochloric acid
e) chloroform, "BDH"
f) pyridine, "BDH"
g) $Co(NO_3)_2 \cdot 6H_2O$, "Merck"

All reagents were p. a. and the organic solvents were dried in the usual way and distilled.

Plotting of the calibration curve. To plot the calibration curve which was used for all determinations of cobalt in this work we used a cobalt nitrate solution containing 1 μg par 1 ml. Of this solution 0.10, 0.25, 0.50, 0.75 .... to 5 ml was put into a 25 ml separatory funnel, 10 ml of distilled water was added, and then 3 ml of saturated NaF solution and 1 ml of 10% pyridine solution of α-furilmonoxime. The mixture in the separatory funnel was well shaken and left to stand for 10 minutes at room temperature. While standing the solution turned red by the formation of the complex of Co^{+++} and α-furilmonoxime. After adding 3 ml HCl (1: 1) to the solution it was shaken and extracted four times, each time with 2 ml of chloroform. The colored chloroform solution (which is usually turbid) was poured into a 10 ml volumetric flask and about 2 ml (up to the mark) of pyridine was added; a clear solution was obtained. The absorption of the solution was read at 415 mμ at room temperature using a mixture of chloroform and pyridine (8: 2) as the reference solution. Using the extincition values obtained for the different dilutions we plotted the calibration curve shown in Fig. 1.

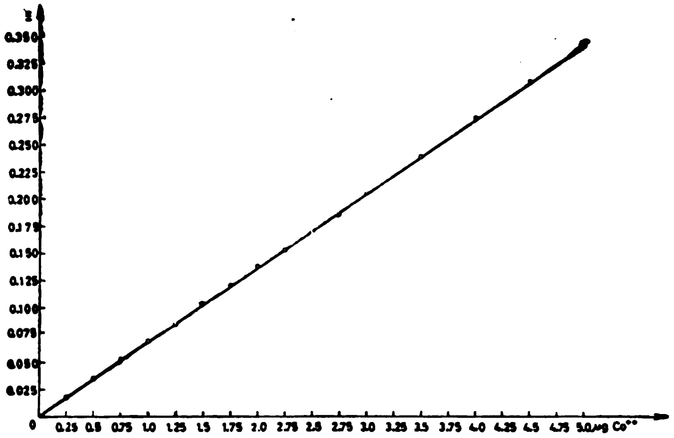


Fig. 1

TABLE 1

Plant	Taken *g*	Found μg *Co*	μg % *Co*
Hay I	2.0064	0.145	7.2
	2.1230	0.157	7.4
	2.0002	0.144	7.2
	2.0018	0.142	7.1
Hay II	2.0000	0.180	9.0
	2.0006	0.182	9.1
	2.0076	0.187	9.3
	2.0090	0.183	9.1
Hay III	2.0173	0.178	8.8
	2.0160	0.177	8.8
	2.0008	0.168	8.4
	2.0010	0.178	8.9
Hay IV	2.0001	0.148	7.4
	2.0032	0.152	7.6
	2.0040	0.146	7.3
	2.0002	0.148	7.4
Alfalfa I	2.0086	0.225	11.2
	2.0008	0.220	11.0
	2.0060	0.221	11.0
	2.0004	0.226	11.3
Alfalfa II	2.0000	0.196	9.8
	2.0092	0.197	9.8
	2.0130	0.195	9.7
	2.0024	0.196	9.8
Alfalfa III	2.0027	0.232	11.6
	2.0002	0.224	11.2
	2.0014	0.225	11.2
	2.0100	0.227	11.3
Silage	2.0008	0.188	9.4
	2.0014	0.197	9.8
	2.0008	0.196	9.8
	2.0000	0.182	9.1

Determination of cobalt in dry hay. Ten milliliters of a weakly acid solution of the ash obtained by burning about 2 grams of dry hay is put in a 25 ml separatory funnel. A 5% aqueous solution of KOH is carefully added to the solution up to pH 5—6. The procedure is then as described above for plotting the calibration curve.

By the method described cobalt was determined in dry hay from four different places (denoted by I, II, III, IV, in Table 1), in alfafa (I, II, III), and in silage. The results calculated for 100 g of plant material dried in a desiccator over P_2O_5 are shown in Table 1.

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A METHOD OF PREPARING 4.4'-BIS-METHYLAMINOBENZOPHENONE

by

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and DJORDJE M. DIMITRIJEVIĆ

In order to synthesize 4,4'-bis-methylaminobenzophenoxime, which was needed to determine the reaction rate of Beckmann's isomerization of some symmetric p-aminosubstituted aromatic ketoximes(1), it was necessary to synthesize 4,4'-bis-methylaminobenzophenone. The only synthesis of this compound which has been described starts with 4,4'-bis-dimethylaminodiphenylmethane and uses the cyanogen bromide reaction of von Braun(2). However, this synthesis does not yield satisfactory results and an attempt was made to get this compound by other means.

After a series of attompts the ketone was finally gotten by the action of methylamine on 4,4'-dichlorobenzophenone in the presence of CuO, NH_4NO_3 and $KClO_3$ at increased temperature and pressure. This was carried out in an autoclave:

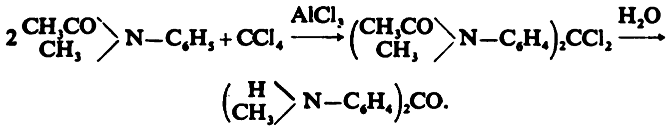
$$ClC_6H_4-CO-C_6H_4Cl \xrightarrow{CH_3NH_2} (CH_3)HN-C_6H_4-CO-C_6H_4-NH(CH_3)$$

in a manner analogous to getting 4,4'-diaminobenzophenone from 4,4'-dichlorobenzophenone and ammonia(3).

The separation of the pure compound from the resinaus raw product which appears in large quantities (90%) presents certain problems. Results can be obtained only by repeatedly passing a chloroform solution of the raw product trough a chromatographic column filled with Al_2O_3. The melting point of this compound was 126.5—127°, although according to von Braun it is 134°. The IR-spectrum was recorded and compared with the IR-spectra of benzophenone, 4,4'-diaminobenzophenone and 4,4'-bis-dimethylaminobenzophenone for more accurate identification. In addition to general similarity with the spectra of these ketones, the absorption maximum of the compound was 2.9 μ, which belongs to the valence oscillation $\nu-NH$.

In addition to this successful synthesis, an attempt was made to synthesize this ketone differently. The attempt was not successful byt several interesting observations were made.

The attempt was made to synthesize 4,4'-bis-methylaminobenzophenone by condensing N-methylacetanilide with carbon tetrachloride in the presence of $AlCl_3$, analogous to Fierz and Koechlin's[4] condensation of acetanilide with CCL_4. The product of the condensation was hydrolized according to the following schema:



$$2\begin{matrix}CH_3CO\\CH_3\end{matrix}\!\!>N-C_6H_5+CCl_4\xrightarrow{AlCl_3}\left(\begin{matrix}CH_3CO\\CH_3\end{matrix}\!\!>N-C_6H_4\right)_2CCl_2\xrightarrow{H_2O}\left(\begin{matrix}H\\CH_3\end{matrix}\!\!>N-C_6H_4\right)_2CO.$$

It was found that the condensation did not take place. Variation of the condensation conditions (increase of temperature, increase of the quantity of $AlCl_3$) did not lead to any results either.

The inhibition of the Friedel-Crafts reaction, provoked by the presence of a methyl group on the nitrogen atom of an acetanilide molecule, can be ascribed to the methyl group's inductive effect of releasing electrons compensating the carbonyl group's inductive effect of electron attraction. The character of the nitrogen atom approaches that of the nonsubstituted group which, as a substituent in the benzol ring, prevents the Friedel-Crafts reaction[5].

A further attempt was made to get 4,4'-bis-methylaminobenzophenone in a way analogous to the preparation of 4,4'-diaminobenzophenone[6] by condensing N-methylaniline with formaldehyde, acetylizing, and oxidizing the resulting 4,4'-bis-acetylmethylaminodiphenylmethane, and finally removing the acetyl group by hydrolysis. This attempt led to the unexpected conclusion that the oxydation of 4,4'-bis-acetylmethylaminodiphenylmethane could not be carried out under any conditions. The original substance was obtained whether the oxidation was carried out with chromic acid or with selenium dioxide.

Finally the attempt was made to get 4,4'-bis-methylaminobenzophenone by methylating 4,4'-bis-acetylaminobenzophenone followed by saponification of 4,4'-bis-acetylmethylaminobenzophenone. The attempt was made to methylate 4,4'-bis-acetylaminobenzophenone under conditions given for the methylation of acetanilide.[7] First of all an appropriate sodium salt has to be prepared and brought into reaction with methyliodine. Once again it was unexpectedly shown that under the given conditions 4,4'-bis-acetylaminobenzophenone does not react with metalic sodium.

EXPERIMENTAL PART

1. *Synthesis of 4,4'-bis-methylaminobenzophenone from 4,4'-dichlorobenzophenone and methylamine*

This synthesis was carried out in a manner analogous to Newton and Groggins'[3] synthesis of 4,4'-diaminobenzophenone. Fifty g (0.2 moles) of 4,4'-dichlorobenzophenone with 450 ml of 33% water solution of methylamine and 4.5 g (0.056 moles) of

CuO, 6 g NH_4NO_3 and 0.75 g $KClO_3$ were heated at 200° for 30 hours in a 1 liter autoclave. After the heating, a yellow, resinous product was separated. This product darkened on standing and was highly sensitive to light. The yield of the raw product was 90%. The 4,4′-dichlorobenzophenone that did not react was separated when the resinous reaction product was dissolved in weak hydrochloric acid. The reaction product was again precipitated by saturating the reaction mixture with ammonia. The flaky precipitate which was separated became gummy again when filtered. After dissolving the precipitate in alcohol and precipitating with water, the substance was no longer resinous, byt it was not yet crystalline. Light yellow needle-shaped crystals m. p. 126.5—127° (according to the literature(2) 134°) were gotten by dissolving the substance in chloroform and repeatedly passing the solution through a chromatographic column with Al_2O_3, followed by recrystallization from xylene of the mass which remained after the evaporation of the solvent. Found: C—74.86%; H—7.00%; N—11.44%. Calculated for $C_{15}H_{16}ON_2$: C—74.97%; H—6.71%; N—11.66%. IR-spectrum, characteristic bands: 2.93, 3.0, 6.21, 11.90 and 12.0 μ.

2. *Attempt to Condense N-methylacetanilide with Carbon Tetrachloride.*

This was carried out in a manner analogous to Fierz and Koechlin's(4) condensation of acetanilide with carbon tetrachloride. When powdered N-methylacetanilide (10 g) was mixed with powdered dry $AlCl_3$ (12 g), an exothermic reaction led to the melting of the initial products. The initial molten mixture of N-methylacetanilide and $AlCl_3$ was cooled to 30—33°, and then carbon tetrachloride (10 g) was added to the highly viscous mass. The expected separation of gaseous HCl was not observed. When water was added a violent reaction took place. Crystals were separated from the water solution. After recrystallization from water they melted at 100—101°. The melting point of the mixture of the crystals with initial N-methylacetanilide did not show depression.

The use of increased quantities of $AlCl_3$ (18 g and 24 g) did not lead to more satisfactory results. Raising the temperature of the reaction up to near the boiling point of the carbon tetrachloride did not lead to this condensation either.

3. *The Synthesis of 4,4′-bis-acetylmethylaminodiphenylmethane and the Attempt to Oxidize It in 4,4′-bis-acetylmethylaminobenzophenone*

a) 4,4′-bis-acetylmethylaminodiphenylmethane resulted from the acetylation of 4,4′-bis- methylaminodiphenylmethane, which was gotten by condensing N-methylaniline with formaldehyde according to the procedure of von Braun.(8) The reaction mixture of 85.6 g N-methylaniline and 30 g of 40% formalin was heated at 100° for 10 hours in a flask with a reflux condenser. After the reaction the reaction mixture was alkalized and the initial substances which did not react were removed by steam distillation. The separated raw 4,4'-bis-methylaminodiphenylmethane was re-distilled under reduced pressure; boiling point 230°/7 mm. The main fraction crystallized on standing, and the crystals (25 g) melted at 51—53°.

The acetylation of 4,4'-bis-methylaminodiphenylmethane was carried out in an manner analogous to Rivier and Farine's(6) acetylation of 4,4'-diaminodiphenylmethane. Ten g of 4,4'-bis-methylaminodiphenylmethane were dissolved in an adequate quantity of glacial acetic acid. Ten g of acetic acid anhydride were added, and the reaction mixture was heated on a water bath during one hour. The cooled reaction mixture was poured into water and the separated raw crystals were re-crystallized from *n*-heptane. Boiling point of the purified product: 123—124.2°. Found: C—73.50%; H—7.09%; N—9.03%. Calculated for $C_{19}H_{22}O_2N_2$: C—73.68%; H—7.02%; N—9.34%.

b) Attempts to oxidize 4,4'-bis-acetylmethylaminodiphenylmethane were carried out with chromic acid and selenium dioxide. The unchanged original substance was the only outcome of all the trials. The expected ketone did not appear at all.

7.4 g of 4,4'-bis- acetylmethylaminodiphenylmethane were dissolved in 24 ml of glacial acetic acid. The oxidation was carried out with 8.6 ml of chromic acid solution, by heating to 90°(6). (Chromic acid solution: 100 g of CrO_3 were dissolved on a water bath in 50 ml of distilled water. Then glacial acetic acid was added to a total of 240 ml.)

3 g diacetyl derivative was dissolved in 10 ml of glacial acetic acid. The oxidation was carried out with 5 g of CrO_3 in 15 ml of distilled water. The reaction was carried out at 90°(9).

2.8 g diacetyl derivative was dissolved in 7 ml of glacial acetic acid. The oxidation was carried out with 5.3 g of CrO_3 in 7 ml of glacial acetic acid at the boiling temperature of the solvent. 2 g of CrO_3 were then added(10).

3.7. g of powdered diacetyl derivative was mixed with 1.6 g of SeO_2 and the mixture was heated at 170—180° for half an hour(11).

A mixture of 3.7 g of powdered diacetyl derivative and 1.6 g of sublimed SeO_2 was heated for 3 hours in 25 ml of dioxane and 10 ml of distilled water until black flakes appeared. The solution was concentrated by evaporating the solvent. The separated crystals were dissolved in dioxane. The selenium was removed by introducing sulphur dioxide into the solution. After the evaporation of the solvent, an oil remained. The crystallization of the oil did not succeed.(11, 12)

3.7 g of diacetyl derivative were melted in a flask and 1.6 g of sublimed SeO_2 were added. The reaction mixture was heated at 170—180° for two hours(11).

4. *The Attempt to Get an Na-derivate of 4'4'-bis-acetylaminobenzophenone*

This was carried out in a manner analogous to the attempt to get the Na-derivate of acetanilide(7). 13.2 g of 4,4'-bis-acetylaminobenzophenone and 2.5 g of metalic sodium were heated for 6 hours at 130° in 100 ml of xylol dried over sodium. No reaction was observed. The heating was continued for another 12 hours, but there was no reaction then either. This could be seen by the pieces of sodium which did not react.

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SYNTHESIS OF 4,4'-BIS-METHYLAMINOBENZOPHENONE FROM MICHLER'S KETONE

by

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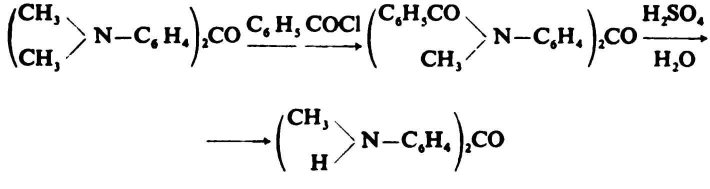
In a previous work⁽¹⁾ we described a new synthesis of 4,4'-bis-methylaminobenzophenone starting from methyl amine and 4,4'-dichlorobenzophenone. With respect to the technique and the yield, the described synthesis proved better than the only other one published⁽²⁾ so far which uses von Braun cyanogen bromide reaction. In the opinion of the author himself, this latter synthesis did not give satisfactory results. In the same paper we described a series of unsuccessful attempts to synthesize the desired ketone by some other route.

An unsuccessful attempt, which was not mentioned in the previous paper⁽¹⁾, was made to remove two methyl groups attached to nitrogen atoms from Michler's ketone and peplace them by hydrogen. However, further study of this problem gave positive results, which we wish to report in the present work.

We got the idea of preparing 4,4'-bis-methylaminobenzophenone from 4,4'-bis-dimethylaminobenzophenone (Michler's ketone) by replacing one methyl group at each nitrogen atom with a benzoyl group and then hydrolytically remowing the benzoyl groups from the observation of Nathanson and Müller⁽³⁾ that heating Michler's ketone with benzoyl chloride at 190°C results in the formation of 4,4'-bis-benzoylmethylaminobenzophenone.

The proposed synthesis can be schematically represented in the following way:



$$\left(\begin{matrix}\mathrm{CH_3}\\ \mathrm{CH_3}\end{matrix}>\mathrm{N-C_6H_4}\right)_2\mathrm{CO}\xrightarrow{\mathrm{C_6H_5COCl}}\left(\begin{matrix}\mathrm{C_6H_5CO}\\ \mathrm{CH_3}\end{matrix}>\mathrm{N-C_6H_4}\right)_2\mathrm{CO}\xrightarrow[\mathrm{H_2O}]{\mathrm{H_2SO_4}}$$

$$\longrightarrow\left(\begin{matrix}\mathrm{CH_3}\\ \mathrm{H}\end{matrix}>\mathrm{N-C_6H_4}\right)_2\mathrm{CO}$$

The first difficulties appeared in attempts to separate the product of benzoylation of Michler's ketone. According to the procedure given by Nathanson and Müller(3), the benzoylation was performed by using 1 mole of Michler's ketone and 2 moles of benzoyl chloride. Instead of the expected product of m.p. 204°C which would be 4,4'-bis-benzoylmethylaminobenzophenone, we obtained a glassy red mass, which after being powdered, washed with sodium carbonate solution and recrystallized several times from alcohol, yielded a product which melted in a wide range from 80 to 100°C.

Since purification of the product by recrystallization failed, we attempted to hydrolize the crude product and to isolate 4,4'-bis-methylaminobenzophenone. The hydrolysis was carried out by heating the product with dilute sulphuric acid (1 : 1). In the course of heating the separation of white crystals which sublimated from the reaction mixture was observed. They were identified as benzoic acid. Since the excess benzoyl chloride had been removed by washing with sodium carbonate solution, this observation led us to conclude that the reaction of Michler's ketone and benzoyl chloride proceeded with the benzoylation of the ketone. However, instead of the expected 4'4'-bis-methylaminobenzophenone (m.p. 134(2)), we isolated a substance melting at a rather higher temperature (184°C), which differed from the expected ketone as well as from the Michler's ketone (m.p. 173°C) in its composition and properties.

Since we had grounds to believe that the reaction takes place and that the reaction mixture contains 4,4'-bis-methylaminobenzophenone, we first attempted to isolate it by crystallization. Since this failed, we tried chromatographic separation. However, this method gave positive results either and the isolateted substances melted in a wide temperature range.

Despite a series of negative results we were convinced we were on the right track and instead of further efforts at separating the product of hydrolysis, we attempted chromatographic separation of the benzoylation products. We succeeded in isolating a substance melting at 199—201°C, which was identified as 4,4'-bis-benzoylmethylaminobenzophenone. The maximum yields of the pure compound were 30 percent, starting from Michler's ketone.

The hydrolysis of pure 4,4'-bis-benzoylmethylaminobenzophenone by means of dilute sulphuric acid (1 : 1) yielded the desired 4,4'-bis-methylaminobenzophenone, which was recrystallized from alcohol furnishing crystals of m.p. 127°C. Thus the synthesis of the desired ketone from Michler's ketone was shown to be possible.

In the benzoylation of Michler's ketone with benzoyl chloride in the ratio 1 : 1, under the same experimental conditions, the reaction was not complete and the formation of a considerable amount of resinous product was observed. The purification of the reaction product and its chromatographic separation yielded, in addition to the unreacted 4,4'-bis-dimethylaminobenzophenone (Michler's ketone), a small amount of 4-benzoylmethylamino-4′-dimethylamino-cenzophenone, which was isolated in the form of light yellow brystals melting at 143—144°C.

This compound and 4,4′-bis-methylaminobenzophenone and its dibenzoyl derivative were identified by elementary analysis, and the last two also by infra-red spectroscopy and mixed melting point determination (with authentic products). For this, 4,4′-bis-methylaminobenzophenone was prepared by the procedure described in our previous paper(1), and the corresponding dibenzoylderivative by benzoylation with benzoyl chloride.

In our opinion the series of unsuccessful attempts to synthesize the desired ketone may be ascribed to the fact that the replacement of the methyl group with the benzoyl group is not complete (this reaction has not yet been investigated in detail) and the products (including hydrolytic products, can only be separated by chromatography.

Attempts to perform the benzoylation using larger amounts of benzoyl chloride or to effect the acetylation of Michler's ketone were also unsuccessful.

1. BENZOYLATION OF MICHLER'S KETONE, HYDROLYSIS OF THE REACTION PRODUCT AND ATTEMPTS TO SEPARATE THE PRODUCTS OF HYDROLYSIS

In a 250 ml flask a mixture of Michler's ketone (27 g; 0.1 mole) and benzoyl chloride (28 g; 0.2 mole) was heated in an oil-bath at 190°C for 3 hours(3). The hot reaction mixture was poured from the flask and it solidified into a red glassy mass. This was powdered and washed with sodium carbonate solution to remove unreacted benzoyl chloride. Recrystallization from alcohol gave a resinous red mass melting at 80—100°C. Several recrystallization did not alter the melting point and the product always separated in the form of a resin.

The product of benzoylation of Michler's ketone (5 g) was mixed with 25 ml of dilute sulphuric acid (1 : 1) and the reaction: mixture was gentlty heated over a wire-gauze. In the course of heating white crystalline needles (benzoic acid, m.p. 120—121°C) deposited on the walls of the flask. The heating was continued until no further separation of sublimated benzoic acid was observed. The product, which was initially insoluble in dilute sulphuric acid, was finelv completely dissolved, giving a brown colored solution, After careful removal of benzoic acid, the solution was diluted with distilled water and saturated with ammonia. The deposited resinous mass was recrystallized several times from dilute alcohol, and then it was purified by dissolution in chloroform and chromatographing the chloroform on a colomn of aluminum oxide. The isolated yellow amorphous mass was recrystallized from xylene, yielding yellow crystals, m.p. 187°C.

Analysis:

Calculated for $C_{15}H_{10}ON_2$:	C 74.97%; H 6.71%; N 11.66%
Found:	C 76.69%; H 7.33%; N 11.00%

The results of elementary analysis correspond neither to 4,4'-bis-methylaminobenzophenone nor to Michler's ketone (calculated for $C_{17}H_{20}ON_2$: C 76.08%; H 7.51%; N 10.44%). Moreover, the separated crystals differed from crystals of Michler's ketone in shape, color and solubility. A mixture of the separated crystals, m.p. 187°C, and crystals of Micher's ketone, m.p. 173°C, melted at about 160°C (depression).

2. BENZOYLATION OF MICHLER'S KETONE AND SEPARATION OF THE BENZOYLATION PRODUCT

a) *Preparation of 4-benzoylmethylamino-4'-dimethylaminobenzophenone.* A mixture of Michler's ketone (12.5 g; 0.05 mole) and benzoyl chloride (7 g; 0,05 mole) was heated at 190°C for 4 hours. On cooling an orange-red glassy mass was obtained: It was powdered, washed with sodium carbonate solution and dried. Recrystallization furnished a resinous mass melting at 104-111°C.

The dried product (30 g) was dissolved in 30 ml of anhydrous benzene and the solution was chromatographed on a column of aluminum oxide. The following solvents were used for separation: petroleum ether, benzene, methylcyclohexane, ether and alcohol. The benzene fraction yielded crystals which melted at 166—167°, but after recrystallization from alcohol the melting point was raised to 170°C. In admixture with crystals of Michler's ketone (173°) the melting point was 171°C (no depression). From the ether fraction light yellow crystals of m.p. 140—141°C were isolated. After crystallization in benzene and precipitation with petroleum ether the melting point was 143—144°C.

Analysis

Calculated for $C_{23}H_{22}O_2N_2$:	C 77.06%;	H 6.19%;	N 7.81%;
Found:	C 77.15%;	H 6.20%;	N 8.30%.

The infra-red spectrum showed characteristic band at 6.09, 6.28, 6.93, 7.31, 7.59, 10.78, 12.09, 12.96 and 13.98 μ.

b) *Preparation of 4,4'-bis-benzoylmethylaminobenzophenone.* A mixture of Michler's ketone (12.5 g; 0.5 mole) and benzoyl chloride (14 g; 0.1 mole) was heated at 190°C for 4 hours. The cooled orange-red glassy mass was powdered, washed with sodium carbonate solution and dried: m.p. 80—100°C. The yield was 22 g.

The dried product (3 g) was dissolved in 30 ml of anhydrous benzene and the solution was chromatographed on a colomn of aluminum oxide. The following solvents were used: benzene, *n*-hexane, methylcyclohexane, ether and alcohol. After removal of the solvent the first fraction (benzene) yielded a small amount of resin and crystals melting at 163—165°C. From the next four fractions (benzene-hexane) a total of 0.95 g of crystals melting at 187°C were separated. The other fractions (ether and alcohol) furnished only resinous products (1.8 g) which melted in the range from 130 to 140°C. From these resinous products no crystalline substance was isolated.

The crystals obtained from the fractions benzenehexane were twice recrystallized from alcohol. About 0.5 g of pale yellow needles melting at 199—201°C was obtained.

Analysis

Calculated for $C_{20}H_{24}O_3N_2$:	C 77.66%;	H 5.39%;	N 6.24%;
Found	C 77.17%;	H 5.46%;	N 6.48%.

The infra-red spectrum showed characteristic bands at 3.23, 3.37, 6.10; 6.26, 7.31, 7.62, 7.48, 7.84. 8.50, 9.05, 10.73, 11.55, 12.59, 14.10 and 14.35 μ.

The mixture of the purified product (m.p. 199—201°) with 4,4'-bis-benzoylmetnylaminobenzophenone (m.p. 199—201°) obtained by benzoylation of 4,4'-bis-methylaminobenzophenone showed no depression of the melting point.

3. HYDROLYSIS OF THE PRODUCT OF BENZOYLATION OF MICHLER'S KETONE

Pale yellow crystals, m.p. 119—201° (1.2 g), obtained by separation and purification of the products of benzoylation of Michler's ketone, were heated with 20 ml of dilute hydrochloric acid (1:1) at 45°C for 2 hours(5). The solution was cooled, the separated crystals of benzoic acid were filtered off, and the filtrate was made alkaline with 10% sodium hydroxide solution. The precipitate was filtered off, dissolved in alcohol and decolorized with active char coal. After separation of the char coal, the clear solution was diluted with plenty of distilled water. On standing overnight crystals of m.p. 126°C separated. Two recrystallizations from dilute alcohol furnished a product melting at 127°C.

Analysis:

Calculated for $C_{15}H_{61}ON_2$:	C 74.97%;	H 6.72%;	N 11.66%;
Found:	C 74.50%;	H 6.87%;	N 11.07%.

The infra-red spectrum showed characteristic bands at 2.97, 3.41, 3.49, 6.20, 6.66, 10.13, 11.0 and 12.06 μ.

4. SYNTHESIS OF 4,4'-BIS-BENZOYLMETHYLAMINOBENZOPHENONE

In an erlenmayer flask with a ground glass stopper a mixture of crude 4,4'-bis-methylaminobenzophene (2.4 g; 0.01 mole), benzoyl chloride (3.23 g; 0.023 mole) and sodium hydroxide (0.4 g dissolved in 4 ml of water) was vigorously shaken for 10 minutes(6). Crude benzoyl derivative separated in the form of a white precipitate. The alkaline reaction mixture was diluted with water and the reaction product filtered off, washed with water and dried. The yield was 5 g, m. p. 195—197°. Two recrystallizations from alcohol furnished colorless needles melting at 199—201°C.

Analysis:
Calculated for $C_{20}H_{24}O_3N_2$: C 77.66%; H 5.39%; N 6.24%;
Found: C 77.28%; H 5.55%; N 6.41%;

5. FURTHER ATTEMPTS TO BENZOYLATE AND TO ACETYLATE MICHLER'S KETONE

A mixture of Michler's ketone (7 g) and benzoyl chloride (14 g) in molar ratio 1:4 was heated at 190° for 4 hours. After cooling a dark red resinous mass was obtained; it solidified slowly but no crystalline substance could be isolated either by crystallization or by chromatographic separation.

A mixture of Michler's ketone (7 g) and benzoyl chloride (28 g) in molar ratio 1:8 was heated at 190° for 4 hours. The product did not solidify even on standing for several days. The purification of the syrupy dark-colored mass gave no positive results.

A mixture of Michler's ketone (7 g) and acetyl chloride (18 g) was reflyxed for 4 hours. On cooling grey-green crystals melting at 125—160°C separated. The crystals were washed with sodium carbonate solution and dried. They were purified by chromatography of their benezene solution on a column of aluminum oxide. The purified crystals melted at 169—170°C. The mixed melting point with Michler's ketone showed no depression.

A solution of Michler's ketone (7 g) in acetic acid anhydride (60 ml) to which acetyl chloride was added (4 g) was heated at 190°C for 4 hours. After cooling and neutralization, the separated crystals were purified chromatographically; m.p. 170—171°C. The mixture with crystals of Michler's ketone showed no depression of the melting point.

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ISOLATION OF COLCHICINE FROM SEEDS OF COLCHICUM MACEDONICUM KOSS.

by

BOJAN D. PODOLEŠOV

Colchicum macedonicum is a Macedonian species of *Colchicum* which grows at a height of 2100—2400 m. It has so far been found only on the Jakupica massif.

Colchicine has been established to be the main constituent of the species of the genus *Colchicum*(1) so far investigated. Recently Beer *et al*(2) and Šantavy and Reichstein(3) discovered in addition to colchicine, the presence of some new substances; these components were found in small amounts but the authors succeeded in establishing their structures.

The aim of our investigation was to find out whether colchicine is present in *Colchicum macedonicum*, and if so, in what amount, and to establish whether other similar substances are present as well. We examined seeds of *Colchicum macedonicum* collected in July 1958 in the area of Begove Pole on the Jakupica massif.

The seeds were dried at room temperature, then finely ground and extracted in a Soxhlet apparatus with petroleum ether (b.p. 40—60°C) for 40 hours. About 9% of fatty oil was obtained. By a similar procedure, Šantavy and Reichstein(3) obtained 4—6% of fatty oil from *Colchicum automnale L.*

In order to isolate colchicine the degreased seed-powder was extracted with 80% ethanol with frequent shaking. The evaporation of ethanol left a residue which was dissolved in water. Colchicine was isolated from the aqueous solution by the method of Šantavy and Reichstein(3). The aqueous solution was first extracted with ether to remove nonpoisonous components. The aqueous phase was then shaken with chloroform to extract poisonous substances. The chloroform extracts were further purified by chromatography on aluminum oxide. After chromatographic separation and crystallization from ethylacetate-ether acout 0.4% of colchicine melting at 155—156°C was obtained. From a mixed melting point determination, optical rotation and colored reactions it was concluded that the substance isolated from *Colchicum macedonicum* was in fact colchicine.

The chromatographic separation showed the presence of a small amount of a new substance which could not be further investigated because of the small quantity.

Here we wish to express our thanks to Dr. K. Micevski for information about the places where *Colchicum macedonicum* could be collected.

EXPERIMENTAL

One hundred grams of finely ground seeds was extracted with petroleum ether (b. p. 40—60°C) in a Soxhlet apparatus for 40 hours. The petroleum-etherial extracts were then shaken three times with 50 ml of water to remove the extracted colchicine, and the petroleum-etherial solution was dried with sodium sulphate. The evaporation of the solvent yielded 8.8 g (8.8%) of yellow-brown oil. The degreased seed-powder was extracted at room temperature by shaking ten times with 200 ml of 80% ethanol. The ethanolic extracts were evaporated in vacuum (water bath) and the residue was dissolved in 60 ml of water. The aqueous solution was extracted with five 50 ml portions of ether and the combined ethereal extracts were then washed with 30 ml of water and dried over sodium sulphate. After removal of the ether there remained 1.2 g (1.2%) of a dark colored residue. The aqeuous solution was then saturated with sodium chloride and extracted with eight 50 ml portions of chloroform. The chloroform extracts were washed with 30 ml of water, dried with sodium sulphate and evaporated to dryness, leaving 0,65 g (0.65%) of residue. The residue was further purified by dissolving it in 50 ml of anhydrous benzene and chromatographing the benzene solution on a column filled with 15 g of neutral aluminum oxide (Merck-Brockmann). The column was then eluted with the following solvents: 50 ml of anhydrous benzene, 50 ml of benzene-ether (1:1), 50 ml of ether, 300 ml of chloroform and 100 ml of chloroform methanol (90:10). On evaporation of the chlorofom fraction 550 mg of a substance was obtained. Two recrystallizations from enthylacetate ether furnished 400 mg (0,4%) of colorless crystals (in the form of needles) melting at 155—156°C (lit. 154—156°C); on standing in air they tyrned slighthy yellow. The mixed melting point with an authentic specimen of colchicine showed no depression.

Specific rotaion $[\alpha]_{D}^{23} = -121.8^\circ$ (lit. -121.6°)

$$\alpha_{D}^{23} = --1.08^\circ \quad 1 = 1 \quad c = 1\ 2564 \text{ (chloroform)}$$

Colchicine was also identified by means of Zeissel's colored reactions⁽⁴⁾: 1) the addition of a few drops of mineral acid to the solution of colchicine results in the development of a yellow color; 2) the addition of concentrated nitric acid gives rise to a dark violet color which later turns to yellow; 3) the solution of some mg of colchicine in a few ml of water is made acidis with hydrochloric acid and the reaction mixture is heated on a water-bath for fifteen minutes; the addition of a few drops of ferric chloride solution to the cooled reaction mixture giwes a green color. Nonhydrolized solutions of colchicine give no reaction with ferric-chloride solution.

The evaporation of the chloroform-methanolic fraction furnished a small residue which was not further investigated.

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THE EFFECT OF INSULIN ON THE METABOLISM OF PROTEINS, LIPIDS AND GLUCIDES. V. THE ACTION OF SUCCESSIVE HYPERINSULINEMIAS ON BLOOD SERUM PROTEINS*

by

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and KOSARA B. NIKOLIĆ

Research on the action of insulin on nitrogenous components of blood serum taken from schizophrenic patients has shown that the administration of large quantities of this hormone cause deep disturbances in the metabolism of these components. In addition to significant quantitative changes of total proteins, polypeptides and free α-amino acids in deep insulin comas, changes have also been found in the distribution of individual eletrophoretic protein fractions(1, 2). Apart from the problem of the nature of these changes, there is the question of whether a long-term successive treatment with insulin produces similar changes. Two phases of insulin action were taken into consideration: the effect which appears in deep coma, and the effect observed several hours after insulin administration. This present paper reports on investigations of insulin action in the both these phases, on the total proteins, polypeptides and free α-amino acids in the blood serum of schizophrenic patients, subjected to insulin shocks every day for four weeks.

MATERIAL AND METHODS

Blood was taken from fifteen schizophrenic patients (female) aged from 19 to 29 treated at the Department of Neurology and Psychology of the School of Medicine in Beograd, Yugoslavia. Insulin was administered intramuscularly every day except Saturdays and Sundays. The insulin dose was determined individually and ranged from 90 to 150 units. Before the first shock the patients were treated with small amounts of insulin.

Our investigations covered the four week period of insulin shock therapy, in which twenty successive shocks were given. Blood

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* A part of this paper was communicated at the Ist Congress of Pure and Applied Chemistry, Zagreb, Yugoslavia, 1960.

was taken first just before the injection of insulin, and then two-and-a-half hours after (twenty minutes after the onset of coma).

The delayed effect of insulin was studied several hours after administration. Serum samples were taken just before the first and 24 hours after the ninth and the nineteenth shock.

The determination of total proteins i.e. total nitrogen and nonprotein nitrogen, polypeptides, i.e. nitrogen of total α-amino acids of deproteinized serum, and nitrogen of free α-amino acids, were described in a previous paper(1).

RESULTS AND DISCUSSION

Insulin Effect in Successive Shocks

The changes of total proteins, polypeptides and free α-amino acids in the serum are in agreement with the previously observed changes in shocks(1), but it was found that these changes differ quantitatively in different periods.

In the first and especially in the tenth shock a great increase of the total nitrogen and the total protein concentrations was observed in all cases and the changes were statistically highly significant. However, in the twentieth shock these changes were negligibly small and statistically insignificant. In the first and the tenth shock, the total nitrogen and total proteins changed in the same sense while in the twentieth shock 62% of cases showed an increase of total nitrogen and only 46% an increase in total proteins, in the other cases there was either no change or the concentration of total nitrogen and proteins decreased.

The fact that the increase of total protein concentration was small in the twentieth insulin shock and that it was observed in less than a half of the cases, shows that the strong anabolic effect of insulin on the proteins in the circulation fails to appear after many successive hyperinsulinemias.

It is of interest to note that before the tenth shock, in which the greatest increase of total protein cencentration was found, the level of proteins in the circulation was the lowest.

The total polypeptide concentration decreased in all the shocks observed. The decrease was the greatest in the first hypoglycemic coma; it was statistically significant and was observed in all cases. In the tenth shock the decrease was about 60% smaller than in the first and was observed in 83% of cases. The change was not statistically significant. In the twentieth shock the decrease was about 35% smaller than in the first and was observed in 91% of cases. The change was statistically significant.

The changes of polypeptide concentration and the altered stimulating effect of insulin on the anabolism of proteins, are undoubtedly consecutive and connected with altered metabolic processes in the liver.

The decrease of the concentration of nonproteinic substances in insulin shocks was almost the same in all periods of the therapy, but the greatest decrease was in the tenth shock. All the changes were statistically significant. In all the cases the changes had the same sense in the first and tenth shocks, but in the twentieth variations were found only in 15% of cases.

The concentration of nitrogen of free α-amino acids decreased in successive shocks in all cases. However, hypoaminoacidemia was the highest in the first and the lowest in the tenth shock. The changes were great and statistically significant.

From the results obtained it is evident that the effect of insulin which favors the elimination of amino acid from the circulation. loses intensity in the course of successive treatment with insulin shocks.

Delayed Effect Observed 24 Hours After Insulin Administration

The direct effect of insulin which is manifested immediately upon insulin injection is followed by a delayed effect which is evident in 6 to 72 hours. This effect is considered to involve processes which establish the optimal functions in the tissues. This statement is based upon experiments *in vitro*, mainly with liver slices of rabbits suffering from diabetes; this effect is therefore considered due to the direct action of insulin on processes in the liver.

The contents of proteins, polypeptides and free α-amino acids in blood serum taken from shizophrenic patients twenty four hours after insulin administration showed that the direction and degree of changes involved in the delayed insulin effect varied in dependence on the duration of the therapy. Thus, it was found that after nine successive insulin shocks the concentration of total nitrogenous substances and total proteins decreased in 86% of cases. The changes were small and statistically insignificant. It is of interest that the concentration of polypeptides decreased simultaneously; the change was statistically significant. However, on further administration of the same quantities of insulin, the concentration of total nitrogenous materials, total proteins and polypeptides increased. After ninteen shocks the concentrations of total nitrogen and total proteins were higher than before the first shock (in 61% of cases); the change was statistically insignificant. Meanwhile the polypeptide values were higher than after nine shocks (increase in 90% of cases). However, the polypeptide concentration did not reach the level found before the beginning of therapy with insulin shocks; the change was statistically significant.

These variations of the mean values of proteins and polypeptides may be ascribed to different metabolic rates, especially to different rates of protein synthesis. Krahl *et al*(4) have shown that insulin stimulates the synthesis of liver proteins *in vitro*, especially in the presence of glucose. Doubtless there are several factors which affect the synthesis and the state of proteins in the circulation in insulin shock therapy. Above all, we should take into consideration that long successive administration of large quantities of insulin (and glucose, two-and-a-half hours after insulin administration) affects the processes which depend on the action of this hormone as well as other active factors in prote in synthesis. However, the question arises of the direction of protein sythesis caused by successive hyperinsulineminas and of the rate of protein catabolism.

It is of interest that in analogous experiments with animals (rabbits) the level of total proteins changes in inverse correlation with the level of blood sugar, which is found to be the most pronounced after nine successive insulin shocks. After nineteen shocks (when the mean total proteins value is greatest) the blood sugar value is the lowest for the whole period of hyperinsulinemia.*

It should be noted that after long application of insulin shocks, in the period when the level of total proteins is high, the strong anabolic effect of insulin on blood proteins in deep insulin coma disappears, i.e. it seems that the direct effect of insulin on blood proteins is absent.

The great and statistically significant decrease of the polypeptide concentration after nine successive shocks also points to an alteration of metabolic processes in the liver caused by successive hyperinsulinemias. However, the hypothessis of a diminished rate of amino acid incorporation into polypeptides during this period of the therapy would not be in agreement with the delayed effect of insulin, established in an *in vitro* system, where insulin was found to enhance the synthesis of polypeptides(3).

The content of free α amino acids varied during successive administration of large quantities of insulin. The changes of their level were small and statistically insignificant. After nine successive shocks the free α-amino acids increased in 83% of cases, and this is in reciprocal correlation with changes of total proteins and polypeptides. After ninteen shocks they decreased to lower than before the first shock.

From the results it is evident that the changes in the period denoted as the period of delayed insulin effect, depend on the duration of the therapy. The state of total proteins, polypeptides and free α-amino acids after a long successive insulin shock treatment is similar to the state brought about by the direct effect of insulin in the initial stage of insulin therapy.

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* Unpublished data obtained in our laboratory,

TABLE 1

Statistically calculated results for total nitrogen, nitrogen of nonprotein substances and total proteins in blood serum of schizophrenic patients (women) before the injeciton of insulin and in insulin chocks

Subject of investigation	Values before/in shock	Number of cases	Range of Values	Mean value — Standard deviation	Standard Error	Coefficient of variation in %	% of decrease or inrease *	Level of significance *
Total nitrogen in mg%	Before first	15	1332.8—1052.2	1232.8 ± 68.75	17.18	5.58	+ 7.9	$p<0.001$
	In first	15	1438.9—1198.4	1330.2 ± 72.15	18.62	5.42		
	Before tenth	7	1285.2—1153.6	1205.7 ± 42.96	16.23	3.56	+ 10.1	$p<0.001$
	In tenth	7	1402.8—1274.0	1327.8 ± 43.71	16.52	3.29		
	Before twentieth	13	1351.3—1122.8	1260.6 ± 62.45	17.32	4.95	+ 2.2	$p<0.05$
	In twentieth	13	1363.6—1248.8	1287.9 ± 33.35	9.25	2.59		
Nitrogen of nonprotein substances in mg%	Before ~~first~~	15	56.68—35.84	42.74 ± 5.80	1.50	13.57	+ 14.53	$p<0.01$
	In first	15	44.24—28.56	36.53 ± 5.05	1.30	13.82		
	Before tenth	7	50.96—38.64	43.20 ± 5.24	1.98	12.13	+ 17.68	$p<0.05$
	In tenth	7	42.56—30.24	35.56 ± 4.98	1.88	14.00		
	Before twentieth	13	51.58—30.52	39.81 ± 6.62	1.83	16.63	+ 13.34	$p<0.05$
	In twentieth	13	44.52—23.80	34.50 ± 5.89	1.63	17.07		
Total proteins in 9%	Before first	15	8.06—6.22	7.43 ± 0.45	0.12	6.06	+ 8.34	$p<0.001$
	In first	15	8.75—7.30	8.05 ± 0.49	0.12	6.09		
	Before tenth	7	7.77—6.96	7.30 ± 0.32	0.12	4.38	+ 10.68	$p<0.001$
	In tenth	7	8.58—7.78	8.08 ± 0.27	0.10	3.34		
	Before twentieth	13	8.23—6.79	7.63 ± 0.39	0.11	5.11	+ 2.62	$p>0.05$
	In twentieth	13	8.35—7.56	7.83 ± 0.23	0.06	2.94		

% of decrease or increase and level of significance are given in relation to values before shock

TABLE 2

Statistically calculated results of njtrogen of total α-amino acids, nitrogen of free α-amino acids and polypeptide nitrogen in blood serum of schizophrenic pateints (women) before the injection of insulin and in insulin shocks

Subject of investigation	Values before/in shock	Number of cases	Range of values	Mean values — standard deviation	Standard error	Coefficient of variation in %	% of decrease or increase *	Level of significance *
Nitrogen of total α-amino acids in deproteinized blood serum in mg%	Befor first	11	30.82—17.08	22.08 ± 3.86	1.16	17.48	−34.19	$p < 0.001$
	In first	11	20.04— 7.84	14.53 ± 3.22	0.97	22.16		
	Before tenth	6	23.14—17.56	19.61 ± 2.18	0.89	11.12	−22.28	$p < 0.01$
	In tenth	6	17.72—12.44	15.24 ± 1.88	0.77	12.34		
	Before twentieth	11	26.00—15.14	19.07 ± 3.16	0.95	16.57	−24.12	$p < 0.01$
	In twentieth	11	22.66— 9.40	14.47 ± 4.32	0.30	29.85		
Nitrogen of free α—amino acids in mg%	Before first	11	11.52— 6.54	8.06 ± 1.51	0.45	18.73	−42.18	$p < 0.001$
	In first	11	7.78— 3.11	4.66 ± 1 53	0.46	32.83		
	Before tenth	6	9.55— 6.85	8.52 ± 1.18	0.48	13.85	−36.03	$p < 0.001$
	In tenth	6	6.57— 4.66	5.45 ± 0.74	0.30	13.58		
	Before twentieth	11	11.23— 5.62	7.56 ± 1.53	0.46	20.24	−31.48	$p < 0.01$
	In twentieth	11	8.79— 3.18	5.18 ± 1.54	0.46	29.73		
Polypeptide nitrogen in mg %	Before first	11	19.30—10.48	14.02 ± 2.77	0.84	19.76	−29.60	$p < 0.01$
	In first	11	13.47— 4.18	9.87 ± 2.50	0.75	25.33		
	Before tenth	6	13.59— 8.30	11.09 ± 1.79	0.73	16.14	−11.81	$p > 0.05$
	In tenth	6	11.71— 7.78	9.78 ± 1.36	0.56	13.91		
	Before twentieth	11	14.77— 9.15	11.51 ± 2.13	0.64	18.51	−19.20	$p = 0.05$
	In twentieth	11	14.11— 5.25	9.30 ± 3.39	1.02	36.34		

% of decrease or increase and level of significance are given in relation to values before shock

TABLE 3

Statistically calculated results of nitrogenous substances in blood serum of schizophrenc pateints (women) 24 hours after the administration of insulin

Subject of investigation	Values before/24 hours after shock	Number of cases	Mean value	% of decrease or increase in relation to values before the first shock	Level of significance in relation to values before the first shock	% of decrease or inreace in relation to values 24 hours after the ninth shock	Level of significance in relation to values 24 hours after the ninth shock
Total nitrogen in mg %	Before the first	15	1232.8	—	—	—	—
	After the ninth	7	1205.7	− 2.20	$p>0.05$	—	—
	After the nineteenth	13	1260.6	+ 2.25	$p>0.05$	+ 4.55	$p>0.05$
Nitrogen of nonprotein substances in mg %	Before the first	15	42.74	—	—	—	—
	After the ninth	7	43.20	+ 1.08	$p>0.05$	—	—
	After the nineteenth	13	39.81	− 6.85	$p>0.05$	− 7.85	$p>0.05$
Total proteins in g %	Before the first	15	7.43	—	—	—	—
	After the ninth	7	7.30	− 1.75	$p>0.05$	—	—
	After the nineteenth	13	7.63	+ 2.69	$p>0.05$	+ 4.52	$p>0.05$
Nitrogen of total α—amino acids in deproteinized blood serum in mg %	Before the first	11	22.08	—	—	—	—
	After the ninth	6	19.61	−11.19	$p>0.05$	—	—
	After the nineteenth	11	19.07	−13.63	$p=0.05$	− 2.75	$p>0.05$
Nitrogen of free α—amino acids in mg %	Before the first	11	8.06	—	—	—	—
	After the ninth	6	8.52	+ 5.71	$p>0.05$	—	—
	After the nineteenth	11	7.56	− 6.20	$p>0.05$	−11.27	$p>0.05$
Polypeptide nitrogen in mg %	Before the first	11	14.02	—	—	—	—
	After the ninth	6	11.09	−20.90	$p=0.05$	—	—
	After the nineteenth	11	11.51	−17.90	$p<0.05$	+ 3.79	$p>0.05$

CONCENTRATIONS OF TOTAL NITROGENOUS SUBSTANCES, TOTAL PROTEINS AND TOTAL NONPROTEINIC NITROGENOUS SUBSTANCES IN BLOOD SERUM OF SCHIZOPHRENIC PATIENTS (WOMEN) IN THE FIRST, TENTH AND TWENTIETH INSULIN SHOCK

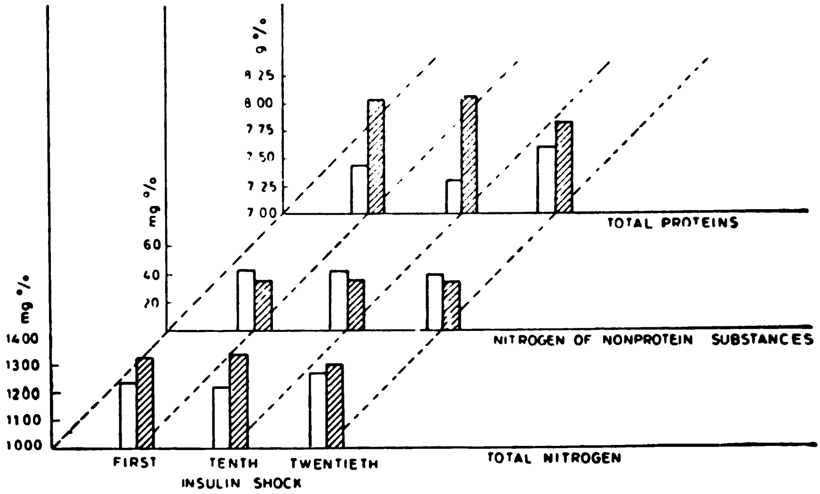

Figure 1

CONCENTRATIONS OF TOTAL AND FREE α-AMINO ACIDS AND POLYPEPTIDES IN BLOOD SERUM OF SCHIZOPHRENIC PATIENTS (WOMEN) IN THE FIRST, TENTH AND TWENTIETH INSULIN SHOCK

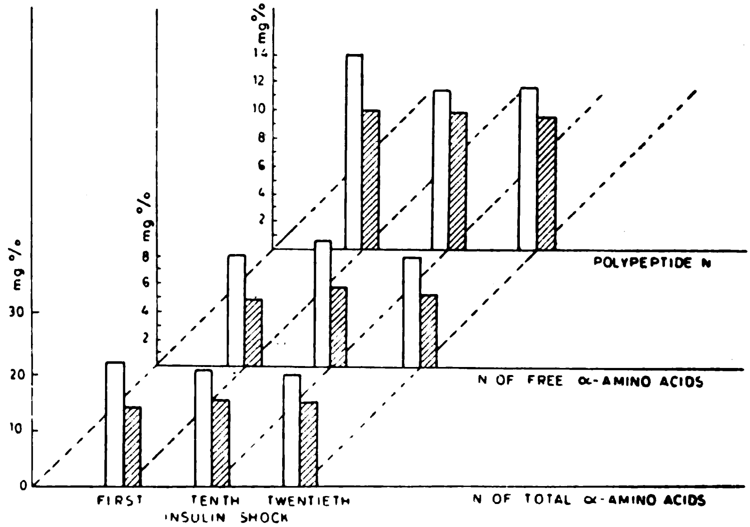

Figure 2

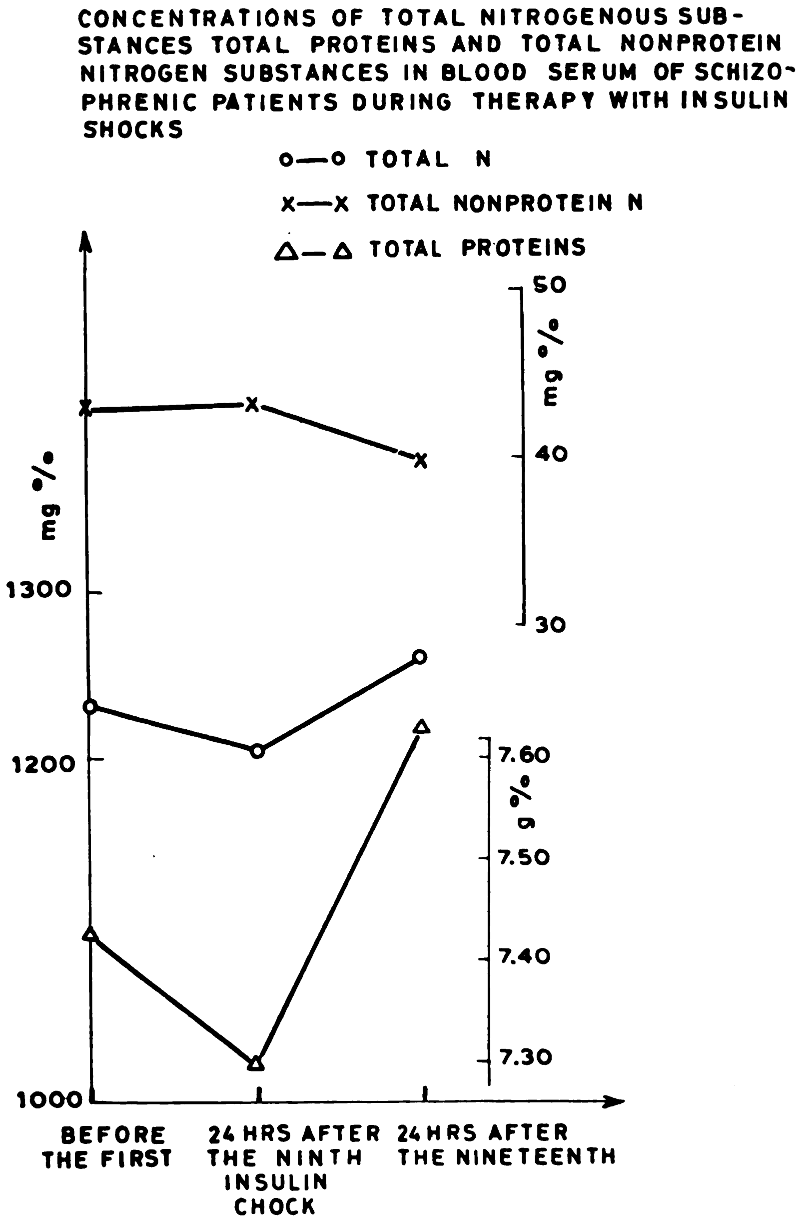

Figure 3

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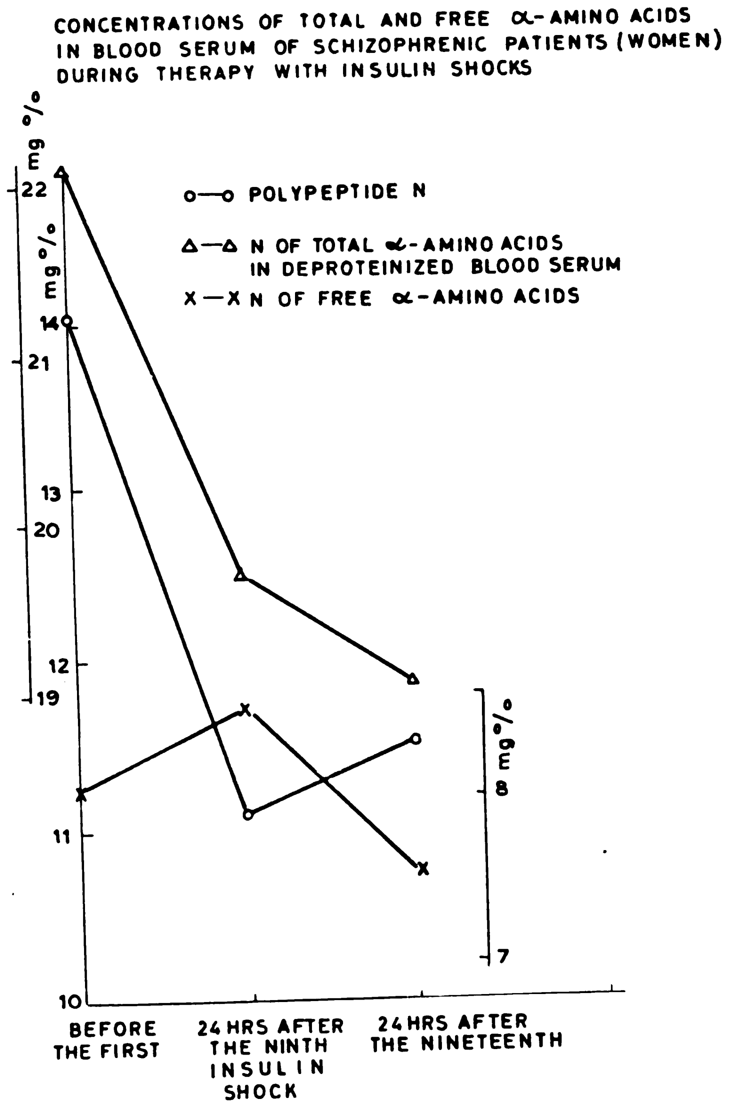

Figure 4

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IMPROVEMENT OF THE ADSORBENT PROPERTIES OF CERTAIN YUGOSLAV SEMI-COKES, AND THE POSSIBILITY OF THEIR USE FOR THE DEPHENOLIZATION OF INDUSTRIAL WASTE WATERS

(Part II)

by

S. JANKOVIĆ, S. DAKOVIĆ and I. KOSTIĆ

INTRODUCTION

This work is a continuation of an earlier study of the changes of adsorbent properties of some Yugoslav semi-cokes by means of oxidation(1). It is based on the same hypotheses as the earlier work, and the same apparatus was used. The results are arranged in the same manner, and phenol was determined by the same modification of the Koppeschar method.

THEORY

Like graphite, oxidized semi-coke forms interstitial lamellate compounds. The aim of this study was to determine the activity of individual substances during the formation of these compounds.

The carbonization temperature of semi-coke influences the molecules of reacting substances which are distributed in the free space between two graphite layers. Thus two semi-cokes whose thermal-treatment conditions are almost the same were chosen.

Pure nitric acid is the best means for oxidation of semi-cokes carbonized at 600°C. Nitric acid was mixed with $Na_2Cr_2O_7$ or $KMnO_4$. Better results were obtained with $HNO_3 + KMnO_4$ than with $HNO_3 + Na_2Cr_2O_7$. This can be explained by the influence of the diameter of the particles of the reacting substancas when the interstitial compounds are created.

In the literature there is no data on the diameter of ions of $Cr_2O_7^{-2}$ and ions of MnO_4^{-1}, but from the data(2) for

O^{-2} —1.40 Å
Cr^{+6} —0.52 Å
Mn^{+7}—0.46 Å

it can nevertheless be seen that $Cr_2O_7^{-2}$ must be bigger than MnO_4^{-1}, taking into account that in aqueous solutions the ions have different diameters because of hydration(3).

Thus it can be expected that it will be difficult for the bichromate ion to penetrate inio the free space between two carbon layers in semi-coke, so that lamellate metastable compounds will form to a smaller extent.

Both seme-cokes showed great improvement under the effect of the mixture of nitric acid and permanganate, whereas the mixture of nitric acid and dichromate did not cause such improvement.

The improvement of the adsorbent properties of chemically treated semi-cokes is also influenced by the redox process between the semi-coke carbons and the means of oxidation. Some of the carbon oxidizes and new free spaces are created in the semi-coke.

The effect of hydrogen peroxide cannot be studied the same way. It was not mixed with nitric acid. The diameter of its particles probably does not prevent the creation of lamellate compounds.

Acid probably increases the porosity of the treated material by dissolving the inorganic admixtures in the coke. The absence of acid in the mixture in this case is evidenced by the decreased capacity of the semi-coke to form interstitial compounds in its free spaces. The weak effect of hydrogen peroxide can partly be explained by the fact that in water and hydrogen peroxide mixtures the mobility of hydrogen ions is significantly decreased.(4)

EXPERIMENTAL PART

Two kinds of semi-cokes were chosen for the experimental analysis of the adsorbent properties of semi-coke towards phenolic materials, "Bogovina" and "Lubnica". Both were carbonized at 600°C. The initial material for the first was brown coal and for the second lignite.

Both semi-cokes were crushed and sifted, and the 1—4 mm fraction was used.

Both semi-cokes were treated in the following way:

Treatment with the mixture of nitric acid and potassium permanganate: 200 g of semi-coke is placed in a porcelain dish, on to which is poured 800 ml of 3% $KMnO_4$ solution in dilute HNO_3 (300 ml H_2O and 500 ml HNO_3 conc.) After standing for 24 hours the semi-coke is profiltered and rinsed with distilled water to a neutral reaction. It is then dried at room temperature and kept in a closed vessel until needed.

Treatment with the mixture of nitric acid and sodium dichromate: 800 ml of 3% $Na_2Cr_2O_7$ in dilute nitric acid (300 ml H_2O and 500 ml HNO_3 conc.) is poured on 200 g of semi-coke. The rest of the treatment is the same as with the HNO_3—$KMnO_4$ mixture.

Treatment with the solution of hydrogen peroxide: 3% aqueous solution of hydrogen peroxide is poured on 200 g of semi-coke.

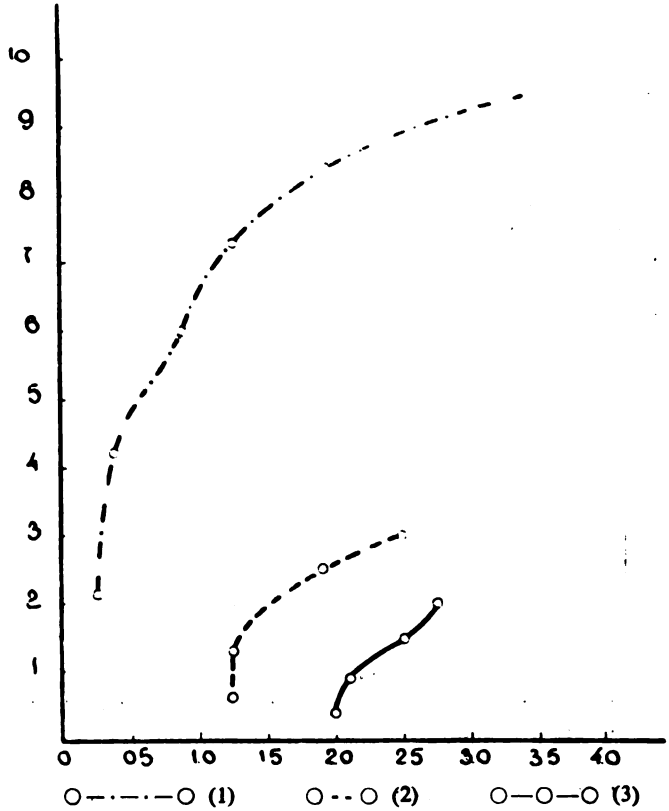

Figure 1

Comparison of dephenolization of treated and untreated semi-cokes.
Abscissa: Concentration of phenol in the effluent, g/lit
Ordinate: Adsorbed phenol, g/kg semi-coke
1. Bogovina semi-coke treated with HNO_3—$KMnO_4$
2. Bogovina semi-coke treated with HNO_3—$Na_2Cr_2O_7$
3. Bogovina semi-coke untreated

The semi-coke stands for thee hours, and is then washed with distilled water until the last traces of H_2O_2 are eliminated.

Six types of semi-cokes emerged from these treatments of the initial materials.

1. Bogovina semi-coke treated with HNO_3 $KMnO_4$, 1—3 mm. fraction.

2. Bogovina semi-coke treated with HNO_3—$Na_2Cr_2O_7$, 1—3 mm fraction.

3. Untreated Bogovina semi-coke, 1—3 mm fraction.

4. Lubnica semi-coke treated with HNO_3 and $KMnO_4$ 0.5——02. mm. fraction.

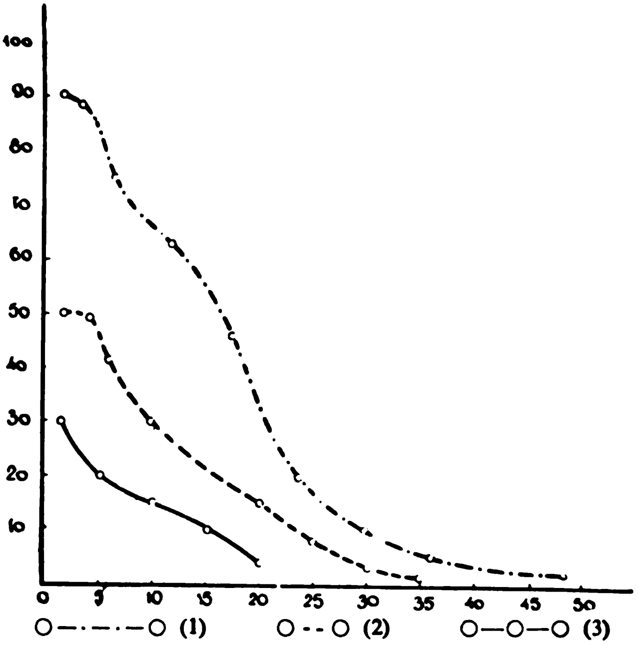

Figure 2
Comparison of dephenolization of treated and untreated semi-cokes.
Abscissa: Effluent discharge time, min.
Ordinate: % adsorbed phenol
1. Bogovina semi-coke treated with HNO_3—$KMnO_4$
2. Bogovina semi-coke treated with HNO_3—$Na_2Cr_2O_7$
3. Bogovina semi-coke untreated

5. Lubnica semi-coke treated with H_2O_2, 2.0—3.5 mm fraction.
6. Untreated Lubnica semi-coke 1—3 mm fraction.

Adsorption was done on these semi-cokes vith the same phenol concentration of 2.5—3.0 g phenol per liter and X industrial waste water with phenol concentration of 9—10 g/lit. Since the industrial water contained a large quantity of suspended materials it was strained through a layer of glass wool before its phenol was determined.

After each adsorption the column of semi-coke was rinsed with tap water for 24 hours to eliminate the adsorbed phenol and prepare the semi-coke for further work.

The results of this experiment confirm most of our hypotheses.

The chemical treatments gave varying results, but adsorption on the treated semi-coke was in general better than that on the untreated. The improvement of the adsorbent properties was proportional to the properties of the untreated semi-coke. Since both the semi-cokes had similar properties in untreated form, the results of chemical treatment were similar.

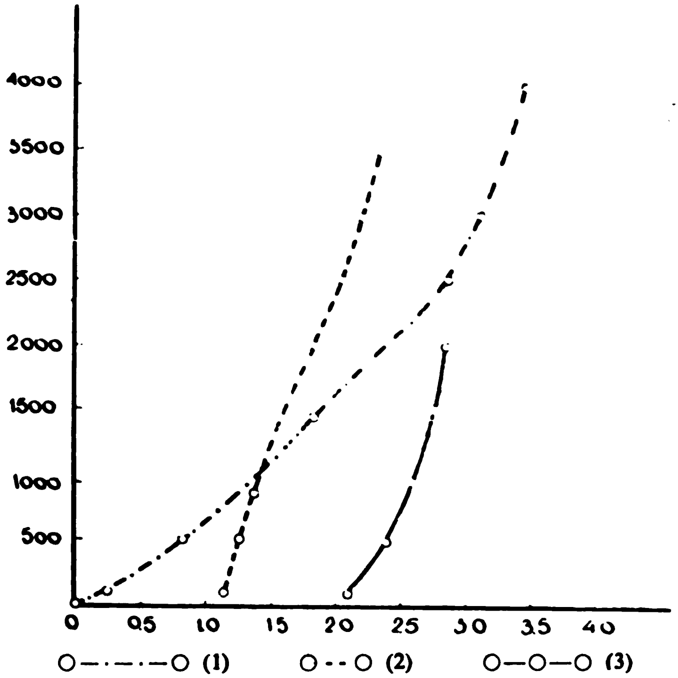

Figure 3

Comparison of dephenolization of treated and untreated semi-cokes.

Abscissa: Concentration of phenol in the effluent, g/lit

Ordinate: Volume of effluent sample, ml.

1. Bogovina semi-coke treated with HNO_3—$KMnO_4$
2. Bogovina semi-coke treated with HNO_3—$Na_2Cr_2O_7$
3. Bogovina semi-coke untreated.

Untreated Bogovina semi-coke adsorbed about 2 g of phenol per kg, and after treatment with a mixture of nitric acid and potassium permanganate it adsorbed 9 g PhOH per kg. After treatment with a mixture of nitric acid and sodium bichromate the amount of adsorbed phenol was only 3 g per kg.

When an untreated sample of Lubnica semi-coke which adsorbed 2.5 g phenol was treated with a mixture of nitric acid and potassium permanganate it adsorbed 7 g/kg; when treated with H_2O_2 it adsorbed 4 g/kg.

During the work with industrial waste water the percentage of adsorption remained almost the same, although the absolute adsorption increased because of the greater concentration of phenol.

The interaction between the semi-coke and the oxidation materials can partly be explained by the lowering of pH values in the collected effluents and by their colors which can be interpreted in terms of the interaction between the metastable compounds of the semi-coke and the phenol materials adsorbed in it.

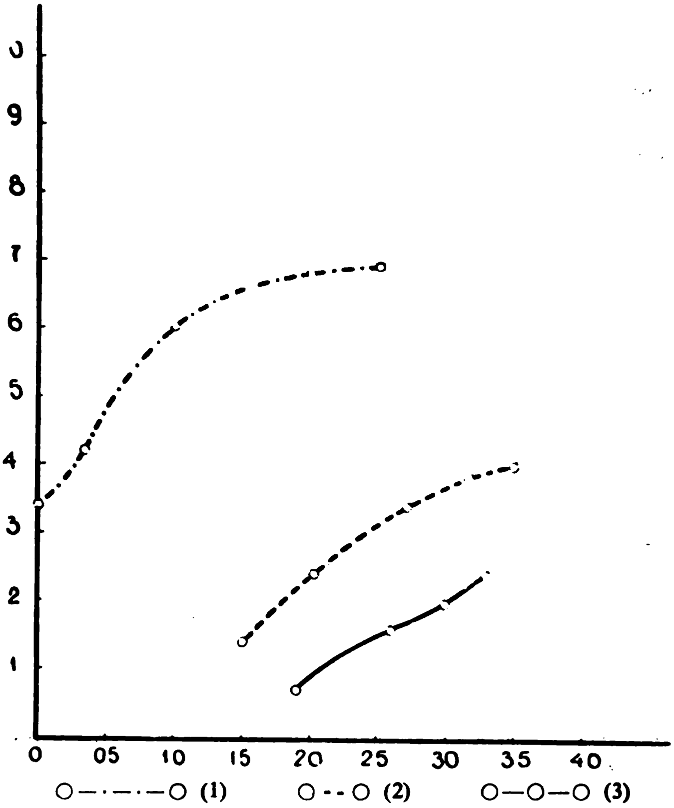

Figure 4
Comparison of dephenolization of treated and untreated semi-cokes.
Abscissa: Concentration of phenol in the effluent, g/lit
Ordinate: Adsorbed phenol, g/kg semi-coke
1. Lubnica semi-coke treated with HNO_3—$KMnO_4$
2. Lubnica semi-coke treated with H_2O_2
3. Lubnica semi-coke untreated.

During work with a pure phenol dilution, semi-coke treated with a mixture of nitric acid and sodium dichromate yielded a yellow effluent, whereas semi-coke treated with nitric acid and potassium permanganate yielded a light rose effluent.

During experiments with industrial waste water the affected effluent's color was not different from the color of water which was not passed through treated semi-coke. When the effluent was collected, foam formed.

This means that both treated and untreated semi-cokes have a discoloring effect on industrial waters, which are intense dark red. As the phenol concentration in the effluent increases, the foam decreases.

The results are shown in Tables I and II, and in graphs 1 to 5.

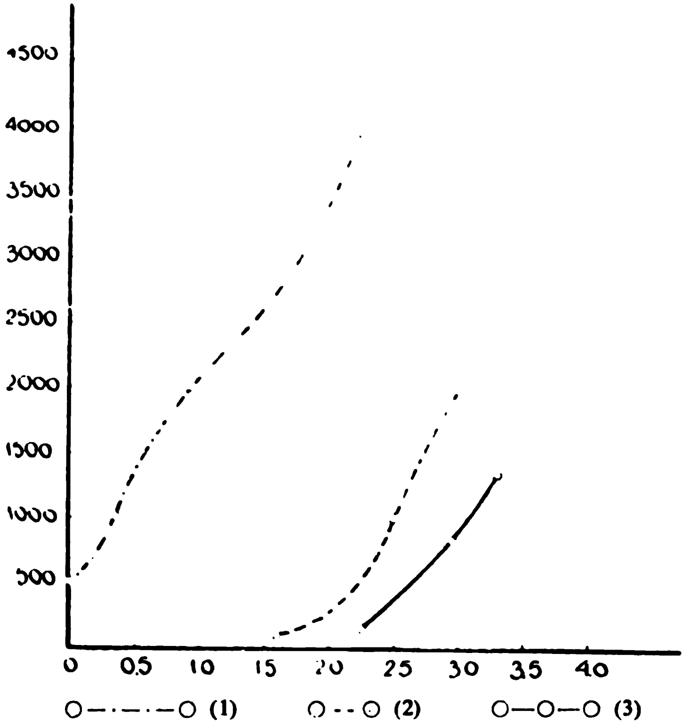

Figure 5

Comparison of dephenolization of treated and untreated semi-cokes.

Abscissa: Concentration of phenol in the effluent, g/lit

Ordinate: Volume of effluent sample, ml.

1. Lubnica semi-coke treated with HNO_3—$KMnO_4$
2. Lubnica semi-coke treated with H_2O_2
3. Lubnica semi-coke untreated.

TABLE I

Results obtained with X industrial waste water, whose concentration in the case of Lubnica semi-coke was 7 g PhOH/lit, and in the case of Bogovina 10 g PhOH/lit.

Time of effluent's outflow in min.	% of adsorbed phenol		Quantity of adsorbed PhOH g/kg	
	Bogovina semi-coke treated with $HNO_3 + KMnO_4$	Lubnica semi-coke treated with $HNO_3 + KMnO_4$	Bogovina semi-coke treated with $HNO_3 + KMnN_4$	Lubnica semi-coke treated with $HNO_3 + KMnO_4$
1.2	57	100	3.8	3.5
2.4	50	100	7.1	7.0
2.4	47	95	10.23	10.9
6.0	44	92	13 20	13.68
6.0	39	64	15.84	15.92
6.0	—	24	18.14	16.77
6.0	28	23	20.01	17.57
6.0	13	15	20.92	17.62
6.0	8	14	21.47	18.12
6.0	3	11	21.67	18.52
12.0	—	3	22.0	19.0

Although the total quantity of adsorbed phenol was about the same for both semi-cokes, it can be seen from the table that the percentage of adsorption was different. This is assumed to be a consequence of the difference in the materials from which the semi-coke was produced.

TABLE II

Results obtained with industrial waste water X, whose phenol concentration in the case of semi-coke Lubnica was 10 g PhOH/lit, and of Bogovina 9 g PhOH/lit.

Time of effluent's outflow in min.	% of adsorbed phenol		Quantity of adsorbed PhOH g/kg	
	Bogovina semi-coke treated with $HNO_3 + KMnO_4$	Lubnica semi-coke treated with $HNO_3 + KMnO_4$	Bogovina semi-coke treated with $HNO_3 + KMnO_4$	Lubnica semi-coke treated with $HNO_3 + KMnO_4$
1.2	36	50	1.28	4.98
2 4	32	35	2.40	8.48
2.4	32	29	3.51	11.38
6 0	28	11.5	4.51	12.53
6.0	27	8.0	5.46	13.33
6.0	12	5.0	5.86	13.83
6.0	2	3.0	5.90	14.20
6.0		2.0	6.0	14.27
				14.3

CONCLUSION

The following conclusions can be drawn from the experiments which were carried out:

a) The explanation of mechanism of adsorption in certain semi-cokes based on analogy with graphite(1) has been confirmed.

b) The authors' hypothesis on the influence of the temperature of carbonization on the size of free spaces where interstitial compounds are formed has been confirmed through the treatment of semi-cokes Bogovina and Lubnica with the same means of oxidation.

c) Means of oxidation which significantly improve the adsorption characteristics of materials at relatively low cost of chemical treatment would be of interest to industry. Acids which are means of oxidation play an important role in improving the adsorption characteristics of semi-coke. Thus these acids should be used in the chemical treatment of semi-coke.

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MEASUREMENT OF LOCAL COEFFICIENTS OF THE TRANSFER OF MASS TO CYLINDRICAL SURFACES UNDER DETERMINED HYDRODYNAMIC CONDITIONS

by

GORDANA POPOVIĆ and S. KONČAR-DJURDJEVIĆ

The rate of mass and heat transfer to cylindrical surfaces is not uniform around the perimeter, but depends on the nature of fluid flow over such surfaces.

Well-defined and proved laws for this form of mass transfer do not yet exist. Experimental results, and their generalisations on the basis of dimensional analysis, give the perimeter-mean values of this phenomenon.

In order to understand this event on the surface of a cylindrical object it is necessary to know local coefficients of mass transfer. This knowledge can serve as a starting point for the accurate interpretation of experimental data.

With respect to the difficulty of determining local coefficients of the transition of mass, V. T. Levich said, "Unfortunately data is not available which would allow us to make quantitave confirmation"(1). The author was thinking of the expression, based on known theories in this field, for calculating the qnantity of material transferred from a fluid current in motion to the cylinder. Levich's work stimulated us to try to solve the problem of the mass transfer to a cylinder in a flowing fluid. The method of adsorption was used to study the fluid flow and the transfer of mass and heat, and the transfer of momentum. This method was elaborated in the Institute for Chemical and Metallurgical Engineering of the Technological Faculty in Belgrade.(2, 3, 4)

METHODS

Previous methods, measured change of weight of the geometrical object or the concentration of the solution which flows around it, giving average values for the total quantity of material transferred to an object of known geometrical form. The method of adsorption uses color solutions and objects covered with white adsorbents. In this way the adsorbed quantity of color can very easily be determined and measured on any part of the covered surface.

The local coefficient of mass transfer k_c was measured on a cylinder placed vertically and normal to the direction of flow of the liquid.

The cylinders were copper or aluminum tubes with diameters of 27 and 25 mm, respectively. They were fixed to the bottom of the channel where the liquid color solution flowed at a constant speed, measured by a special apparatus. The fluid was a water solution of methylene blue, concentration 0.00025%, in the amount of 800 liters. A centrifugal pump circulated the fluid continuously.

The experiments were carried out at the following speeds:

	Material	Speed
1.	Cu	0.636 m/sec
2.	Al	0.612 m/sec
3.	Cu	0.557 m/sec
4.	Cu	0.445 m/sec
5.	Cu and Al	0.308 m/sec
6.	Cu	0.237 m/sec
7.	Al	0.210 m/sec
8.	Al	0.127 m/sec
9.	Cu	0.120 m/sec
10·	Cu	0.087 m/sec

The mass transfer was measured at 10 different speeds in the interval from 0.087 to 0.636 m/sec.

Silica gel, in an acetone solution of nitrocelulose, was used as and adsorbent, with a suspension of which the cylinders were covered.

Adsorption is basically an unsteady process. For an approximately steady adsorption, the experiments lasted 2.5 min(5).

The dried objects were examined with a colorimeter designed for solid surfaces. Made at the Technological Institute, the colorimeter was provided with an apparatus for the colorimetry of cylindrical surfaces of different diameters(6, 7). A 15 mm wide zone 52 mm from the bottom was measured on all the cylinders. Colorimetry was carried out at 29 or 26 positions around the perimeter, every 0.222 or 0.245, i.e. every 12°43′ or 14° of arc. A known quantity of adsorbed material corresponds to every measured color intensity. Thus it was possible to calculate the local coefficients of mass transfer. The results were not expressed in terms of these coefficients. Sherwood's non-dimensional coefficient, a similarity criterion of mass transfer, was used:

$$Sh = k_c \frac{d}{D}$$

where k_c represents the coefficient of mass transfer in $\left(\frac{\text{cm}}{h}\right)$

d — diameter of the cylinder in (cm)

D — coefficient of color diffusion in $\left(\frac{\text{cm}^2}{h}\right)$

Sherwood's similarity criterion of mass transfer corresponds to Nusselt's similarity criterion in heat transfer.

With increasing color intensity, i.e. with darker color, the quantity of material transferred by adsorption is greater, and Sherwood's number increases.

DISCUSSION OF THE RESULTS

Logarithms of the Sherwood numbers in a polar coordinate system are shown in Figs. 1, 2 and 3. The Sherwood numbers are a function of the angle the radius of the cylinder makes with the direction of the liquid flow. The flow has different speeds: $w_1 = 0.612$ m/sec; $w_2 = 0.308$ m/sec; $w_3 = 0.210$ m/sec.

Sherwood's number changes around the perimeter of the cylinder. The left-right symmetry of the mass transfer to the cylinder can easily be seen. The asymmetries which can be seen in the graph probably result from the fact that the cylinder was not always centrally placed in the channel and that the layers of adsorbents were not always adequately homogeneous. Because of the great sensitivity of the method, the least deviation in the quantity of material transferred is registered.

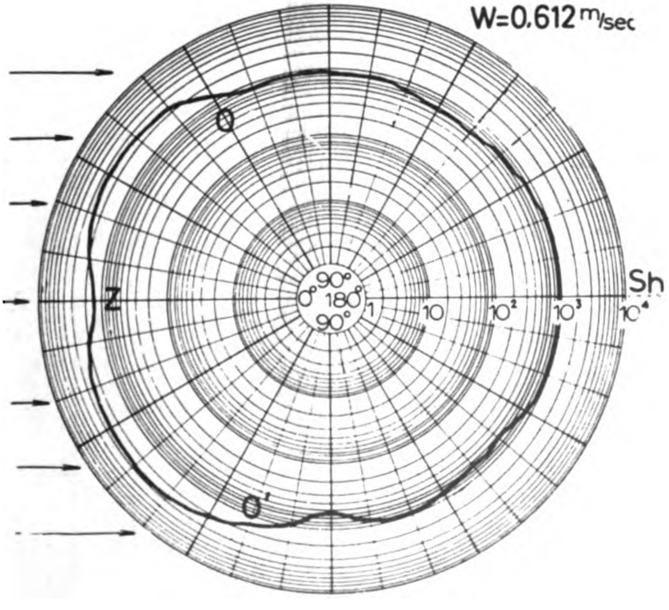

Figure 1.

Dependence of Sherwood's Number on the Position of the Surface, Shown in a Polar Coordinate System. Speed of Solution 0.610 m/sec.

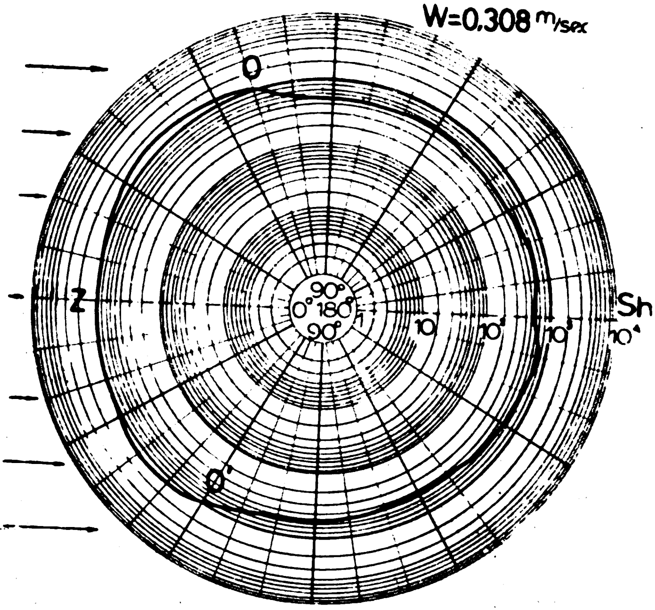

Figure 2.

Dependence of Sherwood's Number on the Position of the Surface, Shown in a Polar Coordinate System. Speed of Solution 0.308 m/sec.

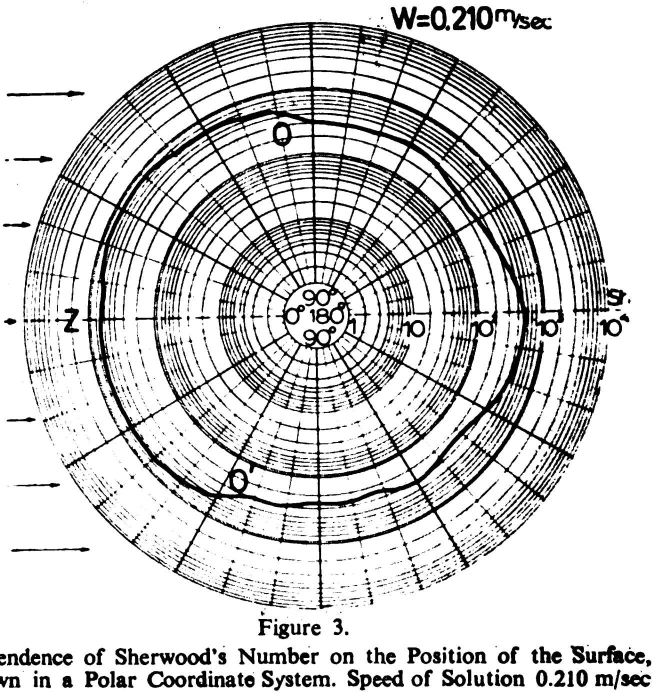

Figure 3.

Dependence of Sherwood's Number on the Position of the Surface, Shown in a Polar Coordinate System. Speed of Solution 0.210 m/sec

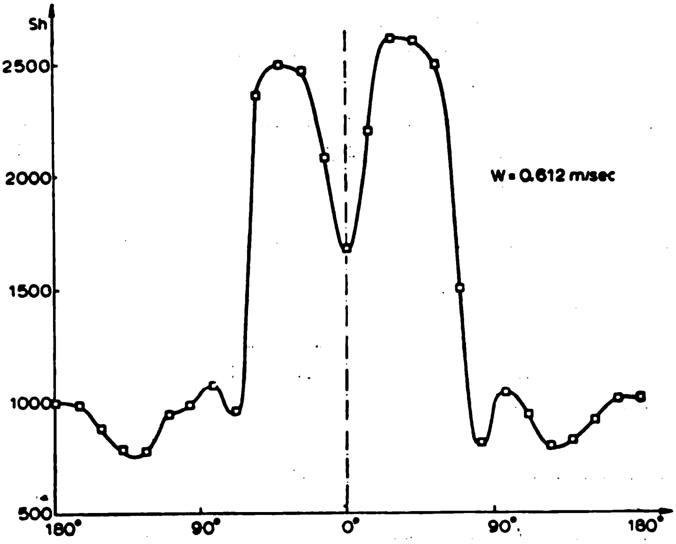

Figure 4
Dependence of Sherwood's Number on the Position of the Surface, Shown in a Rectangular Coordinate System. Speed of Solution 0.610 m/sec.

On the front side of the cylinder, in the immediate surroundings of the stagnation point, there is a zone of low transfer of material, with small Sherwood numbers. Further along the perimeter of the cylinder, in the direction of the flow, there is a zone of intensive transfer with large Sherwood numbers which extends to the Line where the boundary layer separates from the surface of the cylinder

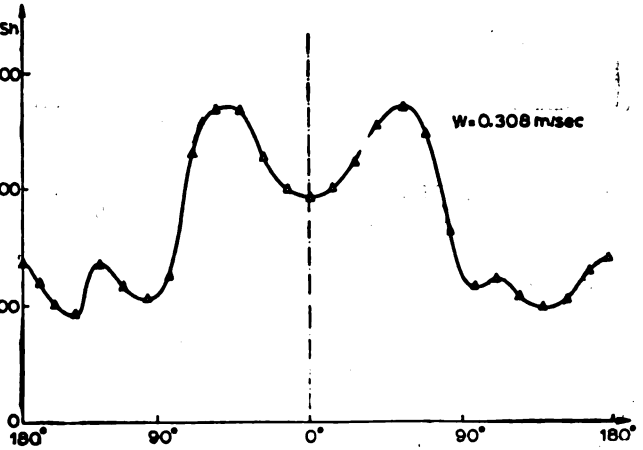

Figure 5
Dependence of Sherwood's Number on the Position of the Surface, Shown in a Rectangular Coordinate System. Speed of Solution 0.308 m/sec.

(0,0′). Behind the line of separation Sherwood's number falls suddenly. However, because of very complex flow at the rear of the cylinder, the number increases somewhat, then falls again, and in the central part of the rear side rises again.

Since the other side of the graph has the same zones, six zones of mass transfer can clearly be distinguished on one side. Along the entire perimeter there are 10 zones. Only two zones, the front and rear sides of the cylinder, are mentioned in the literature on the transfer of mass to cylinders. However, the actual situation is much more complex. The work of Trüb is an exception to the view usually held.(8)

When the speed decreases, the zone of low transfer around the stagnation point Z, gradually disappears and it merges with the zones on its left and right.

However, the seven zones on the back side clearly differ from each other at low speeds. While the three front zones merge into one with a decrease in speed, it seems that the rear zones become less clearly distinguished with increase in speed.

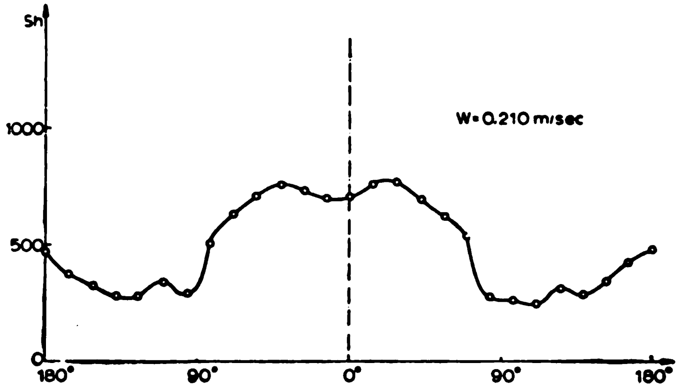

Figure 6
Dependence of Sherwood's Number on the Position of the Surface, Shown in a Rectangular Coordinate System. Speed of Solution 0.210 m/sec.

The dependence of Sherwood's numbers on the position on the circumference of the cylinder is shown more clearly in a rectangular coordinate system where the length along the circumference is on the abscissa and Sherwood's number on the ordinate (Figs. 4, 5, 6).

Here the symmetry of the curves for the left and right sides of the cylinder is clearer, as is the difference in the mass transfer coefficients and in the Sherwood numbers.

The graphs are analogous to graphs of the distribution of pressure around the cylinder and the distribution of local transfer of momentum coefficients.(9)

The results, which are reproducible, have yielded more reliable data on the transfer of mass to cylinders under given hydrodynamic conditions than that found by other researchers in this field, as far as can be seen from the literature available to us. We have also demonstrated the applicability of the adsorption method.

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INVESTIGATION OF THE POLARITY OF ELECTRIC CHARGE CAUSED BY ELECTRIZATION OF PARTICLES DURING FLUIDIZATION AND EXPLANATION OF THE SHAPE OF SEPARATED MATERIAL

by

SLOBODAN K. KONČAR-DJURDJEVIĆ, DEJAN M. BAJIĆ,
and BOJAN D. DJORDJEVIĆ

This work is a continuation of our earlier works treating the phenomenon of electrization of particles during fluidization.(1, 2) These showed the particular complexity of this problem with many factors which can be directly observed, and without doubt some not observed which implicitly influence the phenomenon.

The phenomenon of electrization during fluidization is very complex because it involves both electrical and hydrodynamic phenomena. The system in which electrization takes place is also very complex because it consists of particles of solid material of different size and shape. The different rates of movement of particles with different dielectric permittivities and surface properties, and other materials whose presence is hard to avoid (electrode, net, inside surface of the fluidization column, etc.) make this phenomenon still more complex. To all this we must add the influence of the relative moisture content and temperature of the air and the material, and the shape of the electric field in which fluidization and separation take place. Change of the shape of the electrode surface due to the separation of particles onto it causes a change in the intensity of the electric field, thus constantly changing the experimental conditions.

It is obvious that the complex nature of this phenomenon requires special study in connection with the electrization procedure, i.e. with the particle polarity of the system.

The purpose of the work reported here was to qualitatively study the charging phenomenon and the particle polarity if a monolithic mass of one dielectric or a mixture of two or three dielectrics is used. We hoped to use these results to explain some phenomena which we only registered phenomenologically before(1).

In our first paper(1) we pointed out the existence of electric charging during the fluidization of monolithic powder mass and studied the change of potential difference between the net and the electrode. In another paper(2) we showed that separation of material by electric charging during fluidization is possible and that with respect to dielectric permittivity the electrization of particles is in agreement with Cohen's rule. The possibility of applying this phenomenon in industry was also pointed out.

EXPERIMENTAL

Apparatus

During the fluidization of a solid material in an air stream, under certain conditions electrical charging of the particles in the fluidized bed by friction occurs. Then separation of two or more component materials is possible if a metal rod, an electrode is submerged in the fluidized bed. The complex nature of this phenomenon suggests a more detailed study of it from the electrical aspect.

In our experiments the electrode was insulated or short-circuited to the net or to ground, and a d—c generator was used to investigate the polarity of the separated material or to change the electric field of the fluidized bed.

In these electrical measurements the problem of insulation is of particular importance because the voltages are relatively high and the quantities of electricity relatively small (voltage 1000—5000 V quantity of electricity of the order of 10^{-9} C, and particle charge 10^{-13} C).

The electrical measurements are also considerably influenced by the moisture content of the material(1, 2, 3). Therefore dry material was used (dried at 105° to constant weight) and the air humidity was maintained below 20%. Thus satisfactory reproducibility of measurements was obtained.

Figure 1 shows a schematic view of the fluidization apparatus.

The blower (1) provides an air current of suitable pressure and flow rate. From the blower the air flows into the silica gel drying column (2). From there it passes through a rotameter (3) for flow rate measurement. Thence it enters the fluidization column, passing through the wire net (4) which holds the material to be fluidized. The vertical fluidization column (5), 50 mm in diameter and 1360 mm long, is of metal up to the net and of glass above, with a spherical top end of 130 mm diameter. This spherical extension of the column is designed to reduce the transport of material out of the column. The air from the column flows into a tube (7) containing thermometers for measuring the temperature and moisture content.

In the column there is an electrode (6) whose lower part is a thin cylindrical metal net 25 mm in diameter and 100 mm long. Switch (9) in position *a* shorts the electrode to the net (mass). When the switch is in position *b* the electrode is electrically insulated. In position *c* the switch connects in an electrostatic voltmeter which measures the potential difference between the electrode and the mass. Switch (10) switches on and off the d—c generator (11) (0—5 kV). This generator is connected between the net and the upper end of the electrode. The polarity of the terminals is changed as required.

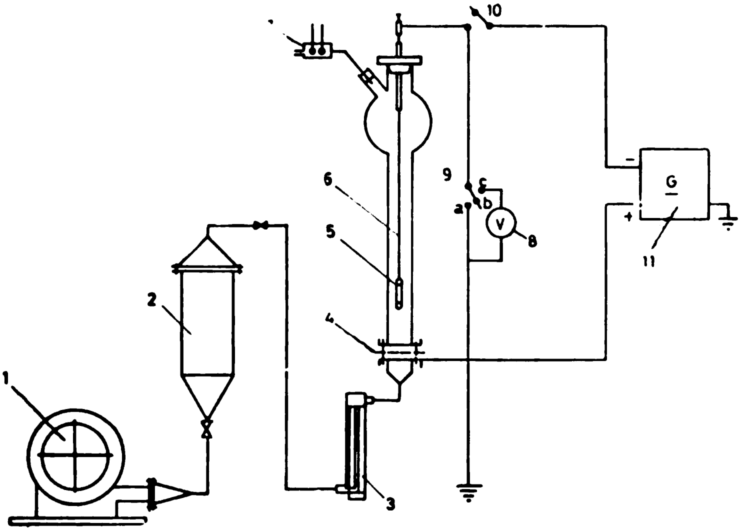

Figure 1
Fluidization apparatus — 1. Blower: 2. Silica-gel drying column; 3. Rotameter; 4. Wire net; 5. Fluidization column; 6. Metal electrode; 7. Apparatus for temperature and humidity measurements; 8. Voltmeter; 9. and 10. Switches; 11. D—C generator.

a) *Electric charging of a one-component mass during fluidization*

In this part of the work we wanted to study the nature of particle electrization during fluidization of a one-component material. In this case we had to bear in mind that the net, metal electrode and fluidization column were made of materials different from that of the particles, which may have a considerable effect on the electrization.

To reduce this effect to a minimum we made a series of measurements with granulated plexiglass fluidized in a plexiglass column. In this case the particles mostly collided with each other, but they also hit the metal electrode too. The particles separated on the metal electrode in large quantities, which indicates that even in a one-component powder particles get electrified by friction. If the electrization of the electrode was changed, either positively or negatively, by means of the d—c generator particles were always separated onto the electrode. This indicates that there were positively and negatively charged particles. Similar experiments were made in a glass column, as shown in Table 1(4).

TABLE 1

Separation and electrization of one-component material during fluidization

Component	Short-circuited electrode (g)	electrode polarized +5 kV	electrode polarized −5 kV
NaCl 20 g	g = 2.5 g t = 1 min q = 140 l/min	g = 5.1 g t = 10 sec q = 140 l/min	g = 3.9 g t = 10 sec q = 140 l/min
$MgCO_3$ 20 g	g = 0.1 g t = 3 min q = 120 l/min	g = 0.9 g t = 30 sec q = 130 l/min	g = 0.45 g t = 10 sec q = 130 l/min
$BaSO_4$ 30 g	g = 3.2 g t = 2 min q = 150 l/min	g = 1.0 g t = 20 sec q = 160 l/min	g = 1.5 g t = 15 sec q = 150 l/min
Coal 6 g	— t = 2 min q = 55 l/min	g = 0.5 g t = 10 sec q = 60 l/min	g = 0.55 g t = 10 sec q = 60 l/min
KNO_3 20 g	g = 1 g t = 1.5 min q = 140 l/min	g = 4 g t = 15 sec q = 140 l/min	g = 3.0 g t = 15 sec q = 140 l/min

In Table 1 g is the quantity of material separated, t the fluidization time and q the flow rate. It can be seen that the investigated materials separated onto the electrode both when it is short-circuited to the net and when a d—c voltage is applied between it and the net. The particles separated whether the particles were positively or negatively charged. Hence it follows that some of the particles were positively and some negatively polarized by collision.

The phenomenon of different polarities of charged particles of a one-component material, apart from being interesting in itself, may be useful in explaining certain phenomena which occur in the separation of two and many-component mixtures and some other phenomena in technology.

b) *Electrization of two-component systems during fluidization*

Electric charging is intense in fluidized heterogeneous systems, i.e. systems with components of different dielectric permittivity. In this case one component separates onto the electrode short-circuited to the net, while the other remains in the fluidized bed. Because of its structure and shape the material separated onto the electrode is called a "grape"(1,2).

To study the phenomena of electrization it was necesary to determine the polarity of the separated particles and the polarity of the electrode or the part of it onto which particles separated. Since particles get electrized during fluidization it is to be excpected that under the effect of an additional electric field, electrostatic forces will act on them and affect their movement. This could be used to determine their polarity and to separate differently charged particles.

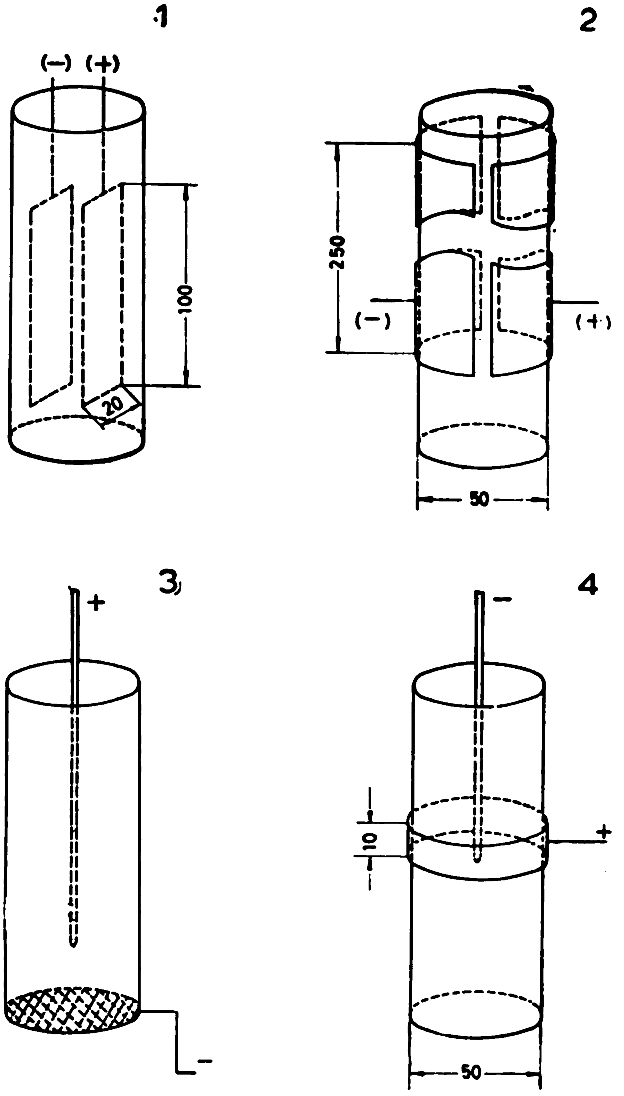

Fig. 2. — Shapes of the electrodes

The electric field produced by an external source can, by induction, affect the polarization of particles making it stronger or weaker depending on whether the additional field assists or opposes the field already existing.

The external generator gave 1—5 kV d—c and was connected to electrodes of different shapes mounted in the column or around it (Fig 2).

The electrodes were: 1. — Two parallel copper plates, 100 mm long, 20 mm wide and 0.5 mm thick submerged in the fluidized bed (Fig. 2—1); 2. — Two semi-cylindrical copper plates 250 mm long, 25 mm wide and 0.5 mm thick, against the outside wall of the glass fluidization column, opposite each other (Fig. 2—2); 3. — A metal electrode and wire net (Fig. 2—3); 4. — An electrode and a copper ~~ring~~ around the fluidization column at the height of the lower end of the electrode (Fig 2—4).

With these electrodes in various positions we got electric fields of various shapes and intensity, allowing us to investigate the separation of two-component systems with respect to different polarities.

In all these cases one component of a two-component system always separated on the positive and the other on the negative electrode. Hence the component separated on the negative electrode was positively charged and the one separated on the positive electrode negatively charged. The polarity of the components did not change with change of polarity of the electrodes by means of the external generator. This implies that the polarity of the components of a given two-component system is always the same.

The polarity was determined using the external generator applied to two parallel electrodes submerged in the fluidized bed. This gave the charged particles the same mechanical possibility to separate the only decisive factor being electrization.

By such measurements we determined the polarity of particles of both components. However, it is not definite whether the particles of one component are unipolar or bipolar, with a larger quantity of positive or negative electricity, the larger charge determining the overall polarity. Also some particles of one component might get charged positively during fluidization, some negatively, and some bipolar. The results shown in Table 1 help explain this.

Table 2 shows polarities of charging of the components of two-component systems during fluidization.

In the table the dielectric permittivity increases from left to right and from top to bottom. Each rectangle has a sign indicating the polarity of the component in that horizontal row obtained by fluidization with the component in that vertical column.

It can be seen from the table that most of the components obey Cohen's rule(5, 6). There are deviations in all systems with KNO_3 and in the systems $MgCO_3 + NaCl$ and $Pb(NO_3)_2 + BaSO_4$. For the last system it was found that the component that weighs more in

TABLE 2.

Polarities of components during fluidization of two-component systems

	KNO_3	SiO_2	$NaCl$	$MgCO_3$	$CaCO_3$	$BaSO_4$	$Pb(NO_3)_2$	coal
ε	2.56	4.8	6.12 - 6.29	8.1	8.5	10.2 - 11.4	16	
KNO_3	▨	+	+	+	+	+		+
SiO_2	–	▨	–	–	–	–	–	–
$NaCl$	–	+	▨	+	–	–	–	
$MgCO_3$	–	+	–	▨	–	–		
$CaCO_3$	–	+	+	+	▨	–		–
$BaSO_4$	-	+	+	+	+	▨	+ –	
$Pb(NO_3)_2$		+	+			– +	▨	
coal	–	+			+			▨

the mixture is positively charged. The table also shows that deviations appear in those systems whose components have rather similar dielectric permittivies, as can be seen around the diagonally shaded regions in the table.

c) *Electrization of three-component systems during fluidization*

Electric charging of three-component systems during fluidization was studied on mixtures in which one of the components was always quartz sand. Sand was used for several reasons: in two-component mixtures it always got negatively charged; it is easily determined analytically; it frequently accompanies natural mixtures.

The following systems were investigated: Coal + $CaCO_3 + SiO_2$; $MgCO_3 + CaCO_3 + SiO_2$; $BaSO_4 + CaCO_3 + SiO_2$; $MgCO_3 + BaSO_4 + SiO_2$; $BaSO_4 + NaCl + SiO_2$; $KNO_3 + MgCO_3 + SiO_2$;

In these experiments two parallel electrodes (Fig. 2—1), were used, one connected to the positive and the other to the negative pole of the d—c generator. Sand always separated onto the positive and the other two components onto the negative electrode. This indicates that in this case too sand was always negatively charged and the other two components positively. Hence in these three component systems the important factor is how the other component are charged relative to sand, and not their mutual relative charge, as is the case in the fluidization of a two-component system.

In experiments on these three-component systems with only one electrode, short-circuited to the net, without additional electric field, sand was not separated on the electrode, while the other two components formed a "grape". An exception was the system $BaSO_4 + NaCl + SiO_2$, where sand was separated on the electrode short-circuited to the mass. This is not surprising since sand was also separated on the electrode in the two-component system $BaSO_4$ + sand(4).

The two components thus separated from the three-component mixture can be separated by a subsequent separation.

d) *Explanation of the phenomenon of various shapes of material separated onto the electrode.*

It is interesting to note that during fluidization, under given conditions, the shape of grapes of the material separated onto the electrode is specific. In case the electrode is short-circuited to the net, during the fluidisation of a two-component system one of the components separates in the shape of a grape on the bottom end of the electrode submerged in the fluidized bed (Fig. 3a). In case the electrode is not short-circuited, i.e. when the electric circuit is not closed, two grapes are formed during the fluidization of both two- and three-component systems (Fig. 3 b). The two grapes are separated, one at the top of the electrode and the other at

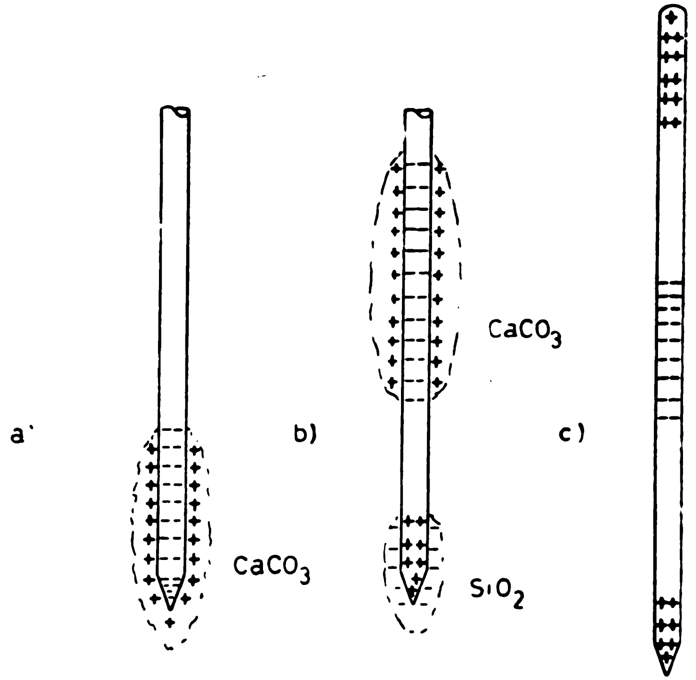

Figure 3

a) *"Grape" on short-circuited electrode* b) *Two "grapes" on insulated electrode*
c) *Distribution of charge on the insulated electrode for two-grapes phenomenon.*

some distance from it. In this case it seems that the higher grape is always that component which is separated when the electrode is short-circuited. The size of the grapes and their separation greatly depend on the flow rate, the quantity of charge, composition of the mixture, fluidization time, etc.

The appearance of one grape may be due to the action of the field of the fluidized bed upon the lower end of the metal electrode submerged in it. This field has a certain direction between the fluidized bed and the electrode, depending on the shape of the electrode. The thinner the electrode and the sharper its tip, the stronger the field immediately around it. The field is strongest immediately beside the electrode. Its direction depends on the composition of the mixture. Under the action of this field the electrode gets charged. Electricity of one polarity remains on the lower part of the electrode while that of the opposite polarity is short circuited to the net, i.e. to the mass.

Since this field is induced by friction between the particles it is clear that particles with the highest charge determine the direction of the field and their inductive action upon the electrode, and its corresponding polarization, dominate. This is why particles of this material separate onto the electrode. It was found that relatively better separation is obtained of that material of which there is less in the mixture. This implies that the particles of this material have more chance to collide with particles of another material, and thus of getting charged with one polarity.

Two grapes are formed in the following way: first, at a certain flow-rate on the lower end of the electrode a grape is formed of the material which would separate onto an electrode short-circuited to the net. Shortly afterwards this grape begins to move up the electrode and is replaced by material of the other component. Thus two grapes, one above the other, are quickly formed. If during fluidization the electrode is short-circuited to the net the lower grape falls off and the upper grape takes its place, so only one component is separated. Likewise, if during fluidization with a short-circuited electrode the electrode and the net are disconnected, two grapes are formed again. The grape which was until then on the lower end of the electrode begins to move upwards and is replaced by material of the other component.

The formation of two grapes is interesting from the theoretical point of view. It can be explained by the fact that the resultant electric field in the electrode is zero, since the electrode is isolated.

At the beginning of under the effect of an externally applied field the charge on the upper part of the electrode has a certain polarity, e. g. positive if it is negative at the lower end. Consequently, positively charged particles begin to separate onto the lower end, and in this case the separation is similar to that when the electrode is short-circuited. Since the positively charged separated material is held on by the negative electricity on the lower end of the electrode the excess of positive electricity tends to spread over the whole electrode to establish equilibrium between the electric fields in the electrode. Of course, most of it accumulates at the lower end of the electrode. A dynamic process develops in which positive electricity accumulates at the lower end of the electrode. Due to the electric field of the fluidized bed there is a further tendency to produce positive electricity on the upper end of the electrode. Thus, to maintain equilibrium between the electric fields in the electrode negative electricity is accumulated in between the lower and the upper ends (Fig. 3c). At the same time negatively charged material is separated on the lower end and positively charged a little further up along the electrode. (Fig. 3b).

When this phenomenon stabilizes the separated positive material (upper grape) neutralizes the negative electricity on the electrode. The positive electricity on the lower part is partly held on by the negatively charged separated material and partly by the electricity at the upper end of the electrode. In this way in the electrode itself the resultant field is zero, i.e. the quantities of electricity on the electrode do not move.

When the upper end of the electrode is short-circuited to the mass, the positive electricity leaves the electrode, thus disturbing the stable positive field at the top of the electrode. The separated negative material falls off and only the negative electricity of the electrode remains which under the effect of the electric field of the fluidized bed occupies the lower part of the electrode, on which opositely charged material begins to separate.

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OPTIMAL CONDITIONS FOR THE SEPARATION OF MATERIALS BY ELECTRIZATION DURING FLUIDIZATION

by

SLOBODAN K. KONČAR-DJURDJEVIĆ, DEJAN M. BAJIĆ
and BOJAN D. DJORDJEVIĆ

INTRODUCTION

In our earlier works we mentioned the possibility of separating materials by electrization during fluidization and we also pointed out that certain conditions must be satisfied to obtain the highest weight and purity of the separated material(1, 2, 3).

The purpose of this work was to find out, by systematic measurements, factors essential for the separation of materials by electrization during fluidization for several two-component systems. In addition to general factors such as the humidity and temperature of the material and the air, the shape of the column and the material of which it is made, etc., we studied several other factors that have considerable influence on the separation of materials. The most important of these were found to be the shape of the electrode, the distance between the electrode and the net, the weight ratio of the components, the weight of the mixture, the particle-size distribution, the flow-rate of the mixture, and the fluidization time.

For all these factors we found the optimum values for quantity of material separated with satisfactory purity (93—99%).

EXPERIMENTAL PROCEDURE

All measurementes were made on the apparatus described in our earlier papers. The temperature was the ambient temperature and was about 20°C. The moisture content of the material was about 20%. This amount of moisture does not influence the reproducibility of measurements. The material was first heated at 105° C to constant weight. The experiments were always performed in the same column of plexiglass with dimensions 45 × 6 × 6.

Since the purpose of experiments was to separate only one component, the electrode was always short-circuited to the net, i.e. to the mass.

During the experiments one of the above factors was varied while the others were kept constant. The electrode was always the same. It was the one on which we found most material separated in all cases.

The following systems were studied:

$CaCO_3 + SiO_2$; $KNO_3 + SiO_2$; $Pb(NO_3)_2 + SiO_2$; $MgCO_3 + SiO_2$; $NaCl + SiO_2$; $KNO_3 + NaCl$; $MgCO_3 + BaSO_4$; $CaCO_3 + KNO_3$; $NaCl + CaCO_3$; $NaCl + BaSO_4$.

This paper presents the results for the $CaCO_3 + SiO_2$ system in which $CaCO_3$ was separated on the electrode.

The shape of the curves for all the systems was studied and the corresponding optimus for these factors were found.

Since material separates on it, the electrode is one of the essential elements. For this reason we made a series of experiments to find an electrode which would on the one hand give high field intensity on its surface, and on the other, would be able to hold the largest possible quantity of separated material.

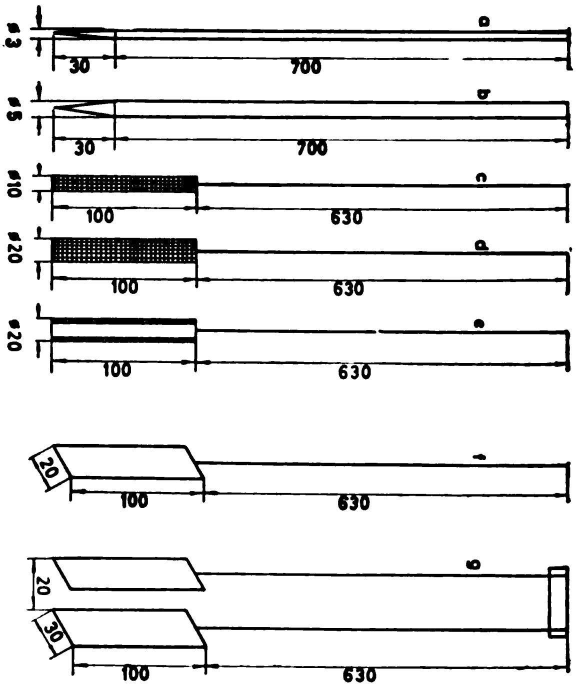

Figure 1. — Electrodes of different shapes

Figure 1 shows some of the electrodes used. We noticed that large-diameter solid cylinders (10—20 mm) (Fig. 1, a and b) or hollow tubes (e) were not suitable because the field on their surface is relatively weak. On them the material separated only on the sharp edges, i. e. on the top. On very thin electrodes, 1—3 mm in diameter, the field was considerably stronger and more material separated. However, the quantity of material separated was not satisfactory because a considerable fraction of it fell off under its own weight and the influence of the air flow.

This indicates that it is essential for the electrode to have a very large electric field on its surface so that it can hold the largest possible quantity of material. An electrode made of thin wire mesh bent in the form of a cylinder satisfies these conditions best (Fig. 1, c and d). The mesh wire diameter was 0.1 mm, thus providing an intense electric field along the whole electrode. All the wires of the electrode are carefully connected at the bottom and top end so the electrode is a good conductor. The upper end of the electrode is connected to a metal rod which is connected to the net by a wire. With this kind of electrode, 100 mm long and 20 mm in diameter, we got the maximum separation of materials in all the investigated systems. This would be expected since such an electrode has the greatest electrical capacitance relative to the net of the fluidization column.

TABLE 1

Influence of the shape and dimensions of the electrode on the separation of materials. System: $SiO_2 + MgCO_3$; particle size distribution +40, DIN 1 171; distance between the lower edge of the electrode and the net of the column $H_0 = 68$ mm; air flow rate, $q = 110$ l/min; weight ratio of components 55:5.

Electrode	Time (sec)	Capacitance (pF)	Separated (g)	Purity of grape %	Total separated
Solid metal rod Ø = 1.5 mm	30	30	2.21	97.1	44.2
Solid metal rod Ø = 3.5 mm	30	32.5	2.30	96.8	45.8
Cylindrical net Ø = 20 mm	30	33.8	4.01	95.5	80.3

Table 1 shows the influence of the shape and dimensions of the electrode on the separation of material and its purity. It can be seen that the cylindrical net electrode gives the best results, so we used it in all further experiments.

To study the effect of the level of sumbergence of the electrode in the fluidization bed upon the quantity of material separated we varied the depth of the submergence, with other conditions held constant. The submergence means the distance between the lower edge of the electrode and the net of the column.

The results of these measurements are shown in Fig. 2.

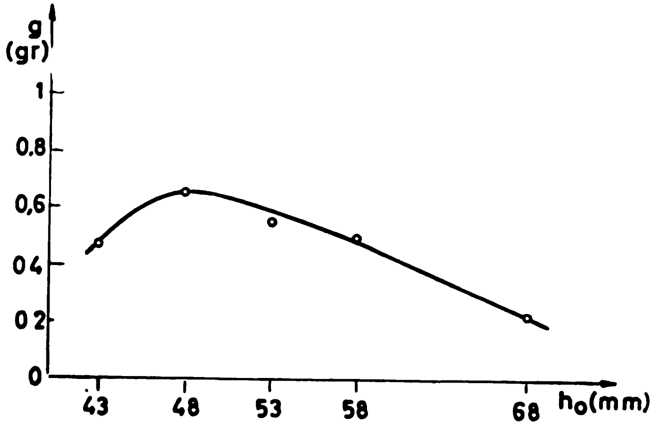

Figure 2.

Quantity of material separated as a function of the electrode submergence. System: $CaCO_3 + SiO_2$; ratio: 1:3; q = 160 l/min; fluidization time t = 15 sec; weight of mixture G = 40 g; particle size + 30.

The graph shows that at too great a submergence the quantity of material separated is small. This may be explained as follows: in a deeply submerged position the electrode interrupts the formation of a fluidized bed; the particles do not get charged enough and the air flow takes off a lot of already separated material from the electrode. If the electrode is far from the net of the column, i.e. if it is only partly submerged in the fluidized bed, then again a small quantity of material is separated and the quantity of material separated is decreased the less the electrode is submerged in the fluidized bed. This is because there is less electrode in the fluidized bed and thus less material can separate onto it.

We found that there is an optimum between these two extreme values, when the quantity of material is separated. For the given system, other conditions constant, the optimum submergence was found to be 48 mm.

By a series of measurements we found that the quantity of material separated on the electrode varies with the weight ratio of the components.

Figure 3 shows the quantity separated as a function of the weight ratio of the components (curve a). It may be seen that for a weight ratio 1:1 the amount separated is slightly less than for a ratio 10:1, which was found to give the maximum quantity of separated material. Further increase of the ratio, i.e. more sand and less $CaCO_3$, steadily decreased the quantity of material separated on the electrode. The graph also shows the percentage ratio of the separated $CaCO_3$ to the total $CaCO_3$ in the mixture (curve b). This curve rises sharply up to a certain ratio of the components in the mixture (in Fig. 3 up to 20:1), and beyond this it increases only slightly.

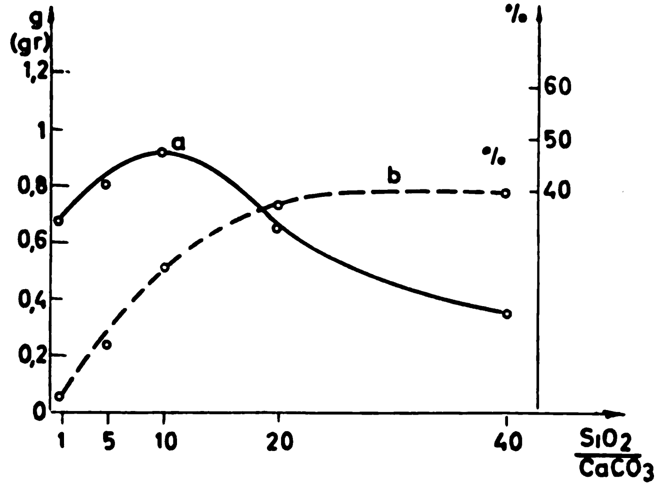

Figure 3.

a) Quantity of material separated as a function of the weight ratio of the components of the mixture; b) Weight ratio of separated $CaCO_3$ to total $CaCO_3$ in the mixture. System: $CaCO_3 + SiO_2$; q – 140 l/min; t – 15 sec; G – 40 g; $H_0 = 48$ mm; particle size +30.

The sharp increase of the relative separation indicates a positive aspect of increased friction between the particles of the two components. However, excessive increase of the relative ratio, i.e., great decrease of one component, leads to saturation, and the relative separation is more or less constant. The percentage relative separation is very high, about 40% of the component whose fraction in the mixture is only 2.5%.

The effect of the air flow rate used for fluidization of the mixture on the quantity of material separated is shown in Fig. 4.

This graph shows that the flow rate has a strong influence, At low flow rates the separation is practically negligible. It increases sharply with increasing flow rate, reaching a maximum at an optimum flow rate, other conditions being constant. In our case this flow rate was 140 l/min. With further increase of the flow rate the quantity of material separated sharply decreases and if the increase is too excessive the material is carried out right out of the fluidization column. The air flow rate is one of the essential factors in fluidization. At low flow rates particle collision is relatively weak, causing only weak electric charging of particles; the height of the fluidized bed is very shallow and the electrode is only partly submerged in it. Increase of the flow rate produces more intense collision, the fluidized bed is raised and covers more of the electrode, and the quantity of material separated increases too. At an optimum flow rate these factors are such that maximum separation is obtained. With further increase of flow rate the mechanical force tending to carry off material becomes stronger than the electrostatic force attracting particles to the electrode, so the quantity of material seperated suddenly decreases.

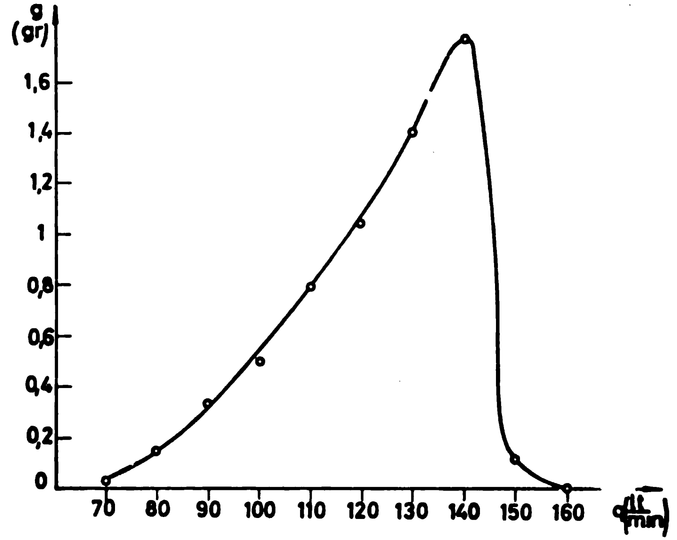

Figure 4.

Quantity of material separated as a function of the air flow rate. System: $CaCO_3 + SiO_2$; ratio 1:3; t = 15 sec; G = 40 g; H_0 = 48 mm; particle size +40.

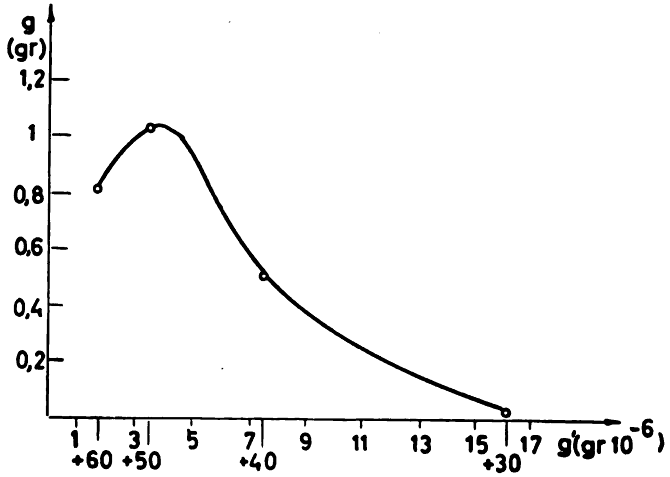

Figure 5.

Quantity of material separated as a function of the particle size distribution; System: $CaCO_3 + SiO_2$; ratio: 1:3; q = 100 l/min; t = 15 sec; G = 40 g; H_0 = 48 mm·

To determine the influence of particle size on separation we investigated mixtures with different particle size distribution, with other conditions held constant. The curve in Fig 5 shows the influence of particle size on the quantity of material separated. It can be seen that for a mixture with large particles (particle size +30 per DIN 1171) at a given flow rate the quantity of material separated is very small. With decreasing particle size at the same flow rate the quantity of material separated increases. We found maximum separation with particle size +50. With further reduction of particle size the quantity of the material separated falls and eventually all the material is carried off. The phenomenon which occurs on variation of the particle size at a constant flow rate (Fig. 3) is closely connected with that occurring on variation of the flow rate at constant particle size (Fig. 4), and these two phenomena can be explained in a similar way.

At a given flow rate, if the particles are too large, they are hardly movable and the friction and particle charging are therefore insufficient. The electrode is insufficienty submerged in the fluidized bed. If the particles are smaller their mobility is greater and the electrode gets more submerged.

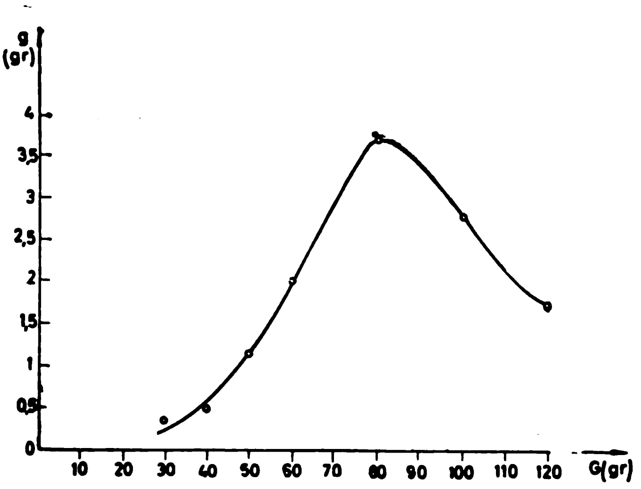

Figure 6.

Quantity of material separated as a function of the weight of the mixture; System: $CaCO_3+SiO_2$; ratio: 1:3; q - 140 l/min; t - 20 sec; $H_0=48$ mm; particle size +30.

The total weight of the mixture for a given column and other constant conditions also has a great influence on the quantity of material separated. Figure 6 shows the quantity separated as a function of the weight of the mixture, in our case 80 g for maximum separation. The size and material of the column primarily influence the optimum amount of mixture. It also depends on the distance of the electrode from the net and the capacitance of the electrode. In case of very small quanities of mixture the electrode is far from the fluidized bed so only a small number of particles reach it and get captured. In case of very large quantities of material, the electrode is too deeply submerged in the fluidized bed, and even into the mixture, so separation is reduced.

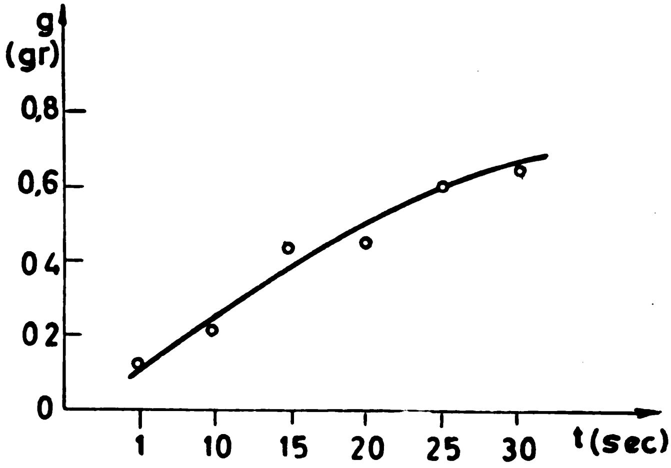

Figure 7

Quantity of material separated as a function of the fluidization time
System: $CaCO_3 + SiO_2$; ratio: 1:3; $q = 140$ l/min;
$G = 40$ g; $H_0 = 48$ mm; particle size +30

We found that the fluidization time is an import factor. Figure 7 shows the quantity of material separated as a function of the fluidization time. As can be seen, if the fluidization time is too short the quantity of material separated is small. If it is long, in our case longer than 15 seconds, the separation does not increase proportionally with it. The increase is smaller and the quantity of impurities increases. Hence, there is an optimum fluidization time for a given column and mixture, with other conditions kept constant.

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THE AMMONIA-DIMETHYL ETHER LIQUID-VAPOR EQUILIBRIUM

by

JOVAN D. PUPEZIN, ŽIVOJIN V. KNEŽEVIĆ and SLOBODAN V. RIBNIKAR

Dimethyl ether forms unstable addition compounds with various substances. Some of them are liquid and distillable, such as boron trifluoride and hydrogen chloride compounds (1,2). Such compounds are applicable in stable isotope separation, in a process called exchange distillation (3,4).

The distillabilities of some low temperature systeme involving dimethyl ether have been investigated. The first to be reported is the ammonia-dimethyl ether system.

From thermal analysis it is known that below —89°C a one-to-one compound is formed in the solid phase (5), but there are no data on liquid and gaseous mixtures.

1. EXPERIMENTAL PROCEDURE

The procedure used in detecting a possible distillable compound or mixture was analysis of the distillate of a condensed mixture.

A low temperature distillation column of the Podbielniak type, with 5 to 8 theoretical plates, was loaded with the condensed mixture. The distillate composition was analysed by means of a Rayleigh interference refractometer, which allowed rapid and quite precise analyses. One of its gas cells (100 cm long) was filled with pure ammonia. Some of the distillate mixture was let into the other.

The instrument was first calibrated with mixtures of known compositions. The zero setting of the instrument corresponded to pure amonia, and full deflection to pure ether. To obtain this, a pressure of 100 mm Hg in both cells was maintained, because of the relatively large differences in the refractive indices.

Dimethyl ether was obtained by heating mixtures of methyl alcohol and sulfuric acid to 140°C. The gas was washed with concentrated sulfuric acid, and dried with phosphorus pentoxide. Final purification was by rectification. Technical grade ammonia gas was purified by rectification.

2. DISTILLATION OF THE MIXTURE

A sample distilation run of a one-to-one ammonia-dimethyl ether mixture showed irregular behavior at the end of distillation. The first two thirds of the distillate was a constant boiling mixture which was gradually enriched with ether towards the end of distillation.

Rectification experiments, using the column, confirmed these results. Two runs with mixtures having an excess of either ammonia or dimethyl ether are shown in Fig. 1. In both cases the first portion of the distillate at atmospheric pressure consisted of a mixture with approximately 33 mol percent dimethyl ether. The excesses of ether or ammonia appeared at the end of the rectification. Rectification of the first fractions did not show any further separation.

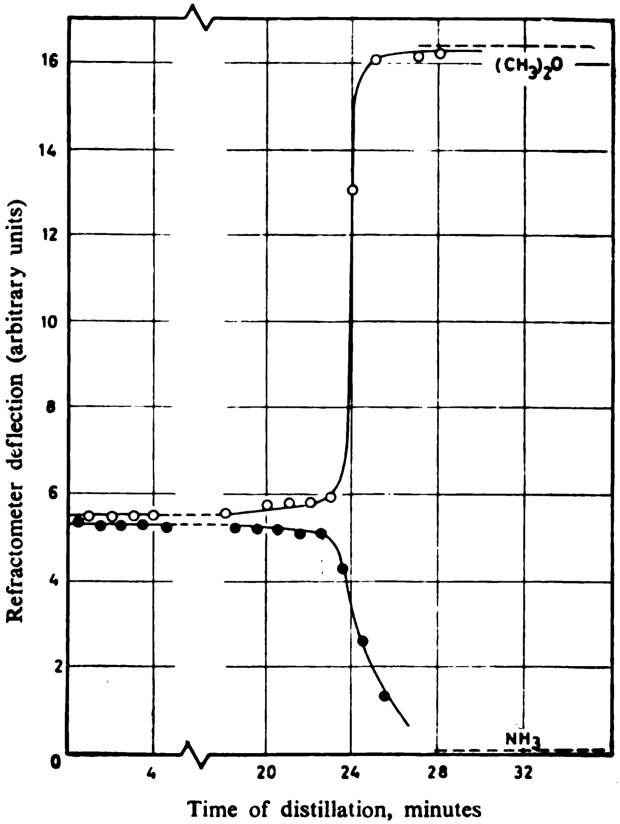

Fig. 1. Distillation diagram for dimethyl ether-ammonia mixtures.

Sample with an excess of ether — ○, excess ammonia— ●

3. THE LIQUID-VAPOR EQUILIBRIUM

To complete the picture of the liquid-vapor equilibrium, vapor pressures at various initial concentrations were measured. Simultaneously, compositions of the vapor phase were recorded.

Mixtures of pure gases of known compositions were condensed into an equilibrating bulb. The capacity of the bulb was 74 cc. Quantities of 2 liters of gas mixture were usually employed. The bulb was placed in a large Dewar vessel filled with alcohol. With the addition of the necessary amounts of dry ice, desired constant temperatures could be maintained. The alcohol bath was mixed with a stirrer and the bulb connected to a vibrating device to shorten the equilibrium time. An electric heater was immersed in the bath so that its temperature could be raised when necessary. The measurements were made in the range —65° to —40°C. The temperature was measured on an ammonia vapor pressure thermometer, using the data of Gragoe *et al* (6).

When equilibrium was reached, the gas phase in the bulb was allowed to expand slowly into the measuring cell of the refractometer. At the same time, pressure data were taken on a mercury manometer. Readings were taken at intervals of 3 or 4 degrees, so that interpolation to rounded-off temperatures could be made.

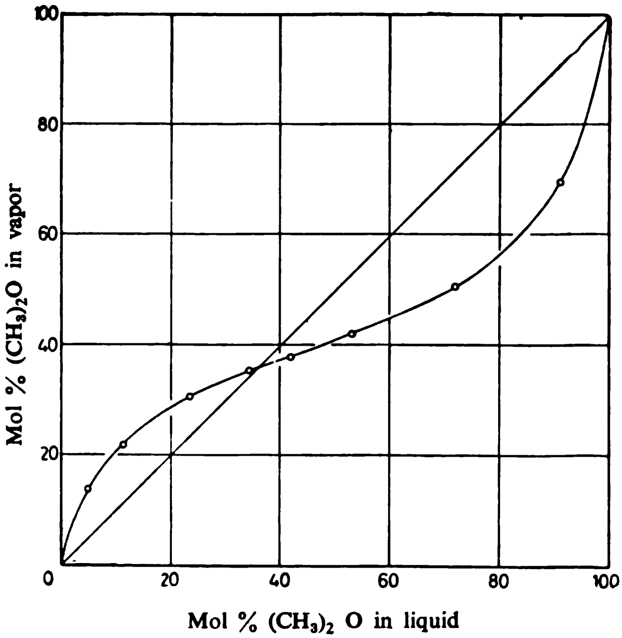

Fig. 2. The liquid-vapor equilibrium at —50°C.

Table 1 shows data on the vapor pressures of various ammonia-dimethyl ether mixtures at —60°, —50°, and —40°C. The liquid-vapor composition equilibria at – 50° are given in Table 2 and Fig. 2. Assuming ideal behavior of the vapor phase, absolute and partial pressures of the components at —50° are shown graphically in Fig. 3.

TABLE 1

Vapor pressures of condensed ammonia — dimethyl ether mixtures at various temperatures

—40°C		—50°C		—60°C	
% $(CH_3)_2O$	p (mm Hg)	% $(CH_3)_2O$	p (mm Hg)	% $(CH_3)_2O$	p (mm Hg)
0.0	538.7	0.0	307.1	0.0	164.7
4.4	592.1	4.7	341.9	4.8	187.5
9.6	620.9	9.9	359.4	10.0	197.6
17.7	646.7	18.0	373.5	18.0	204.9
25.7	657.4	25.8	379.2	25.9	207.8
34.4	659.6	34.4	381.0	34.3	209.0
42.6	653.3	42.3	378.8	41.9	208.7
54.7	639.6	53.9	373.4	53.4	207.3
74.0	597.7	73.0	351.7	72.4	197.0
92.4	494.7	91.7	292.9	91.3	165.0
98.0	408.2	97.7	242.6	97.5	137.3
100.0	370.0	100.0	218.0	100.0	122.0

TABLE 2

Equillibrium composition of the liquid and vapor phases of the ammonia-dimethyl ether system at—50°C.

—50°C	
% $(CH_3)_2O_{liq}$	% $(CH_3)_2O_{vap}$
4.9	13.9
11.3	21.8
23.7	30.8
34.5	35.3
42.0	38.0
53.3	42.0
72.1	50.7
91.2	69.7
97.4	88.7

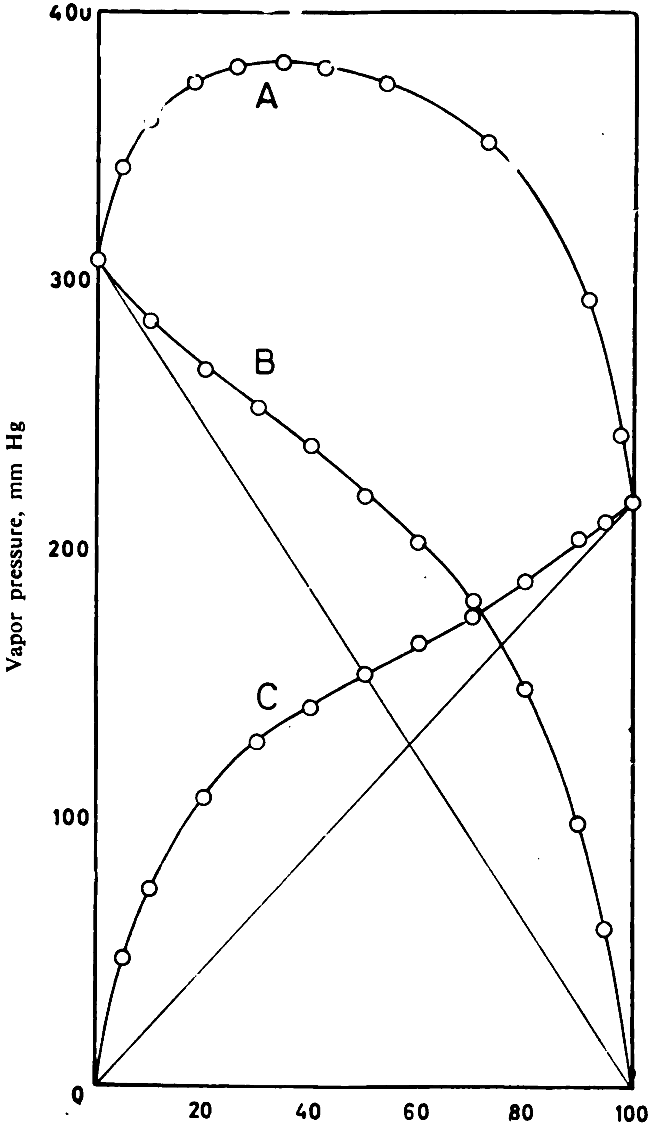

Fig. 3. Pressure-composition diagram *A* — total pressure, *B* — partial pressure of ammonia, *C* — partial pressure of dimethyl ether.

The reliability of the experimental results was checked by the two-constant Margules equation (7). The values of the constants at —50° and a concentration of 40 percent ether are $_{3} = 1.645$ and $_{2} = 2.015$.

In another column experiment the azeotrope composition at various temperatures was determined. It was found that an increase in temperature favors an increase of the ammonia content of the azeotrope. This fact is in accordance with the second rule of Wrewsky (8), since ammonia has a higher heat of vaporization than dimethyl ether. In the interval from 205° to 235°K, the temperature dependence can be fitted by the equation

$$x = 0.730 - 0.00169\,\mathrm{T},$$

where x represents the mol fraction of dimethyl ether in the azeotropic mixture at an absolute temperature T. The slope obtained agrees well with that calculated from the equation of Storonkin and Morachevskii (9). The heats of vaporization taken for this calculation were 5.77 and 5.31 k cal/mol at —50°C for ammonia and dimethyl ether, respectively*. The value obtained for the slope was 0.00167.

Assuming that ammonia and dimethyl ether form a regular solution, it is possible to give a value for the heat of mixing of these two substances. From the vapor pressure of the pure components and the azeotrope composition at —50°C, a maximum heat of mixing (for a 1 : 1 mixture) of —130 cal/mol is obtained. The order of magnitude of this effect, and the fact that the system shows a positive deviation from Raoult's law, suggests a weakening of the hydrogen bonds in liquid ammonia when it is mixed with dimethyl ether.

4. DISSOCIATION OF THE VAPOR PHASE

Closer consideration of the measured refractive indices of different mixtures of known compositions shows a remarkable proportionality between them and the composition. The relationship

$$\log n_{\mathrm{mix}} = x \log n_1 + y \log n_2$$

where the n's are the indices of refraction and x and y the mol fractions of the constituents, is fully satisfied. In case of molecular association this would not be possible. The conclusion there is no association at room temperature therefore seems justified.

Gas density measurements at lower temperatures were made using the Regnault technique. Two experiments were made, at —37.0° and —46.°C, at pressures below the saturation pressure at the given temperature, i.e. at 399 and 385 mm Hg, respectively. The gas had the composition of the normal pressure azeotrope. The measured mean molecular weight in both cases was 26.67. This is the exact value calculated for a 33.2 percent ether mixture, assuming complete dissociation.

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* There are two values available for the heat of vaporization of dimethyl ether at its boiling point: 5.14 (10) and 4.48 kcal/mol (11). The value of 5.31 kcal/mol used is the mean of these two, extrapolated to —50°C using Watson's equation.

5. CONCLUSION

Contrary to the dimethyl ether *compounds* with boron trifluoride or hydrogen chloride, dimethyl ether and ammonia form typical low boiling azeotropes in the investigated temperature range of —37° to —65°C. The azeotrope composition varies with temperature fram 33.2 mol percent ether at its normal boiling point of —37.5°, to 37.0 mol percent at —60°C. There is no association in the vapor phase of the mixtures. The pure azeotrope can be stably distilled for long periods of time.

Azeotrope formation suggests depolimerization of the ammonia molecules in liquid ammonia when it is mixed with dimethyl ether, a finding which is similar, for example, to the recently published results of Clusius *et al.* obtained in the condensed nitric oxide-methane system (12).

ABSTRACT:

Condensed mixtures of ammonia and dimethyl ether show maxima in their vapor pressure-composition curves. The azeotropic mixture boiling at —37.5°C and 760 mm Hg contains of 33.2 mol percent ether. The azeotrope at —60°C contains 37.0% dimethyl ether. Complete dissociation is found in the gas phase within the range 20° to —46°C.

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THE CHELATE COMPOUND OF NICKEL WITH 2,3 QUINOXALINEDITHIOL AND ITS pH INDICATOR PROPERTIES

by

D. B. STEVANČEVIĆ

The synthesis of 2,3 quinoxalinedithiol was first carried out in 1956 by Morrisson and Furst(1). They found this compound gives chelates with a number of transition elements. On the basis of this work, Skoog(2) and co-workers suggested a highly sensitive method for the spectrophotometric determination of nickel in ammoniacal solutions.

In an earlier work(3), the absorption spectra of quinoxalinedithiol and its chelate compounds with nickel and cobalt, were studied. By the molar ratio continuous variation methods it was found that in an alkaline medium the reagent: metal ratio is 2 and the charge of the Ni-complex ion is negative. In a later work(4) complex compounds with transition metals were investigated polarographically and by current measurement.

More recently, Burke and Yoe(5) suggested a method for the simultaneous spectrophotometric determination of cobalt and nickel with this compound. Ayres and Annand examined the reactions of these metals in 80% dimethylformamide slightly acidified with formic acid.

The nickel complex is red in alkaline and blue in acid media. This work comprises research necessary to evaluate the properties of this compound as an indicator. The structure of the complex in acid media was studied and its absorption spectra in various media were measured.

EXPERIMENTS AND RESULTS

Reagents and Apparatus

2,3 quinoxalinedithiol (QDT) was prepared by the method of Morrison and Furst: 2,3 dichloroquinoxaline was treated with thiourea and hydrolizing the isothiouronium salt. The product was purified in the following way: it was dissolved in 0.1 N NaOH, passed through a Jena G 4 sintered glass crucible and reprecipitated with acetic acid. After three precipitations a product whose structure approximated the theoretical structure was gotten. A standard reagent solution was prepared by dissolving the QDT in an equivalent quantity of 0.1 N NaOH and diluting to the desired concentration. Fresh solutions were made every day.

A standard nickel solution was gotten by dissolving nickel chloride and by gravimetric standardization with dimethylglyoxime.

All other chemicals were of p. a. quality. Triple-distilled water was used for the solutions.

A Beckman Model DU spectrophotometer was used to measure the absorption, and a Beckman Model GS pH-meter to measure the pH values.

The Structure of the Complex in an Acid Medium

The mole ratio method was used to determine the structure of the complex in an acid medium. In an acetate buffer. (pH-4), the QDT concentration was kept constant at 3.10^{-5} M and different quantities of standard nickel solution were added. Precipitation of the compound was prevented by adding 0.001% gelatin. The absorptance was measured at 620 mμ. A value of 1:2 was gotten for the metal:reagent ratio.—The same ratio as had been gotten for the complex in an alkaline medium(4.) Burke and Yoe(5) and Ayres and Annand(6) found the same ratio for the complex in an acid medium and in mixed solvents. Ayres and Annand also got a ratio 1:2.4 in experiments where the nickel concentration was kept constant and different quantities of QDT were added. When in some of our experiments the QDT was not completely fresh, similar behavior was found.

Absorption Spectra

Figure 1 shows the spectra of the nickel complex in different buffered media. In very acid media HCl solutions were used. Acetate buffers was used in the pH range 1.8—5.8 and a phosphate buffer in the neutral and slightly alkaline region. The ion strength was kept at approximately $\mu = 0.1$. A gelatin solution was added to stabilize the color. The nickel complex was added as a weak amoniacal solution (red form) and its final concentration was $1.7 \cdot 10^{-5}$ M. The absorption was measured 30 minutes after the complex was added.

In the alkaline region the complex absorbs strongly, at 515 mμ (red form) with molar absorptivity $\varepsilon_{515} = 22{,}000$. In a slightly acid medium the absorption maximum is at 620 mμ (blue form). There is also a third, green form, in very acid media with absorption maximum at 660 mμ.

Dependence of the Color Change on the pH

Figure 2 shows the dependence of the absorption on the pH for wavelengths of 515 and 620 mμ. The change from red to blue takes place in a very narrow pH interval, between 6 and 7. From the midpoint of the curves in Figure 2 it was found that the in-

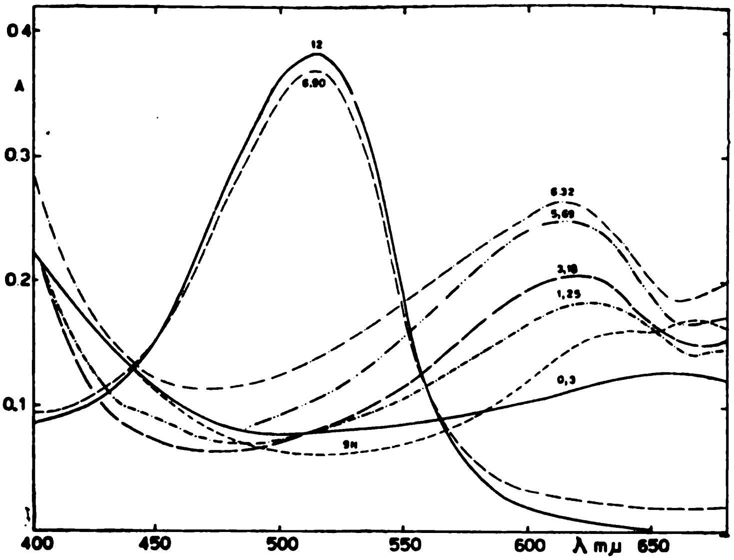

Fig. 1. Absorption spectra of nickel complexes with 2,3 quinoxalinedithiol. Concetration $1.7 \cdot 10^{-5}$ M.

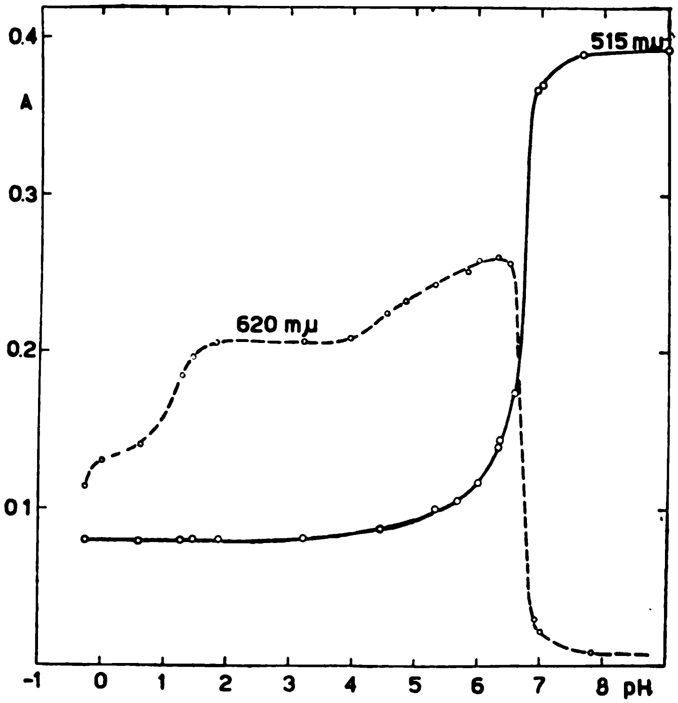

Fig. 2. Dependence of the absorption on the pH at wavelengths of 515 and 620 mμ.

dicator ratio equals unity when the pH is 6.7. The second transition, from the blue to the green form, was found in the acid region at pH$\approx$1.

An attempt was made to determine how many dissociable hydrogen atoms take part in the reaction from the plot of pH against log (I)/(H_xI). However, a uniform slope could not be gotten. In pH region 5.4—6.1 the slope was 2, and above this region it increased to 4. This behavior can be explained by the assumption that true equilibrium was not reached in the solution during measurement of the absorption.

Ion Exchange and Extaction Experiments.

These experiments were made to determine the ionic charge of the complex species.

In an alkaline medium the red form complex was not adsorbed on cation exchange resin. However, the adsorption was marked on Dowex – 1 anion resin. This shows that the red complex is an anion.

The blue complex was not adsorbed either on the cation or the anion resin, which shows it behaves as an uncharged molecule. Similar results were obtained by extraction. The blue complex was extracted with organic solvents even though its solubility, in them is relatively low. The red complex was not extracted by non-polar solvents. However it was extracted with alcohols, probably because of solvation reaction. This reaction increased significantly when amines were added to the alcohols, for example tributyl amine in butyl alcohol. This also shows the anionic nature of the red complex.

Structure of the Complexes.

2,3 quinoxalinedithiol may exist in solution in several tautomeric forms. It was earlier assumed that the thiol form is the reactive species in an alkaline medium and it was suggested that the red complex has a square planar configuration with two negative charges(3). Burke and Yoe suggested a similar structure for the blue complex, taking that the semiacid form is the reactive species. Both of these structures are consistent with our ion-exchange and extraction experiments. However, it would be difficult to reconcile the dipolar ion structure suggested by Burke and Yoe as one of the possibilities with the present experiments. The green complex is probably a protonized form of the blue complex.

The color change of the complexes induced by dissociation results from some intermolecular structural changes. To identify these changes further research (infrared spectra, magnetic moments) is necessary.

The Possibility of Using the Nickel Complex as a pH Indicator

Many acid-base titrations were carried out with the nickel complex as an indicators. These titrations were compared with identical experiments with phenolphthalein and bromothymol blue as indicators. No differences in the amount of titrant used in the two series of titrations were observed.

The nickel complex was added as a 0.05% solution in an ethanol-butanol mixture. The complex was made by reaction of QDT with an excess of nickel in a slightly ammoniacal environment. The precipitate filtered, washed and dried.

Because of its two color changes the complex is convenient for estimating the pH values of well-buffered solutions by color matching with reference buffer solutions.

For different pH values the complex has the following colors:
$pH \leqslant 1$ green
1.8—5.2 blue
5.2—7.3 different violet tones
$pH \geqslant 7.3$ red

The complex easily adsorbs on paper and thus it can be used as a pH indicator paper. Strips of Whatman filter paper were soaked for 10 minutes in a weakly ($8 \cdot 10^{-4}$M) ammoniacal solution of the complex and dried. When immersed various buffers these strips assumed colors as previously described. Artificial aging for 20 days at 130°C, or the actions of ultraviolet light for 8 hours, did not cause the loss of the indicator properties. The complex is adsorbed on the paper, so that the tested solution is not colored when the strips are soaked in it.

The attempt to use the complex as a metalochromic indicator for complexometric titration did not succeed because the stability constant of the nickel-QDT complex is the same order of magnitude as for the Ni-EDTA complex.

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INFRARED SPECTROSCOPIC DETERMINATION OF THE DEGREE OF ESTERIFICATION OF CELLULOSE MOLECULES

by

MILENA NOVOGRADSKI

INTRODUCTION

The application of infrared spectroscopy in chemical analysis is very widespread nowadays. Absolute identification of compounds, quantitative analysis and qualitative identification of atomic groups in molecules by infrared spectroscopy, wich is a nondestructive analysis, is very popular. Sometimes infrared spectroscopy is the only method which can be used for the investigation of some compounds. It is very useful if combined with other methods because it is quick, specific and precise.

The spectrum of a compound is specific for the compound, like a fingerprint in a card index, and it allows positive identification of the compound by comparison with the spectrogram of a known compound.

Infrared spectroscopy was first applied in petroleum industry, but today its application has been extended to all branches of organic chemistry, even to the chemistry of modern explosives(1,2).

Using differential analysis(3,4) in this work we observed the degree of esterification of the celulose molecule by infrared spectroscopy and quantitatively measured the band intensity of the NO_2 group.

EXPERIMENTAL PROCEDURE

A Perkin-Elmer Model 13 U infrared spectrophotomer with two beams and an NaCl prism was used. Two cells 40 microns thick, slit width 0.153 mm, optical density 0.300 were used. Concentration of nitrocellulose 2 g/100 ml of an ether alcohol mixture. The degree of esterification of a celullose molecule was determined by differential analysis, which is very suitable for quantitative work.

The sample, in a suitable solvent, is put in one beam, and only solvent is put in the other, the reference beam. The sample must not absorb more than 50%. If high precision is required the conditions are best if the absorption is about 37%. The spectrum to be observed should not be absorbed by the solvent at all, or only

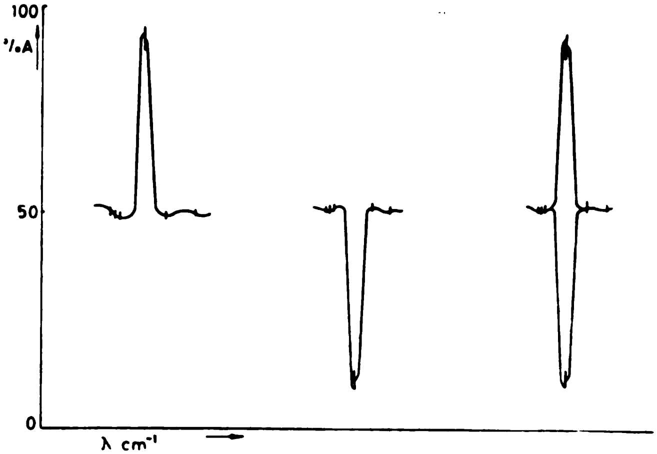

Fig. 1. Band intensity at 6.1 microns

slightly. The advantage of this method is that it graphically increases small differences between the two solutions(5). The effect of any difference in cell thickness is eliminated by changing the cells over.

Each sample of nitrocellulose with a different percentage nitrogen content is recorded three times and the mean value of the surface, as measured by a planimeter in square millimeters, is taken. If cell A with the sample on the beam is recorded, a contour is obtained of the absorption band whose upper end is directed upwards, and when the cells are changed over the same band is obtained with its upper end directed downwards so that the two bands form a closed surface, as shown in Fig. 1.

DISCUSSION AND RESULTS

Cellulose nitroester, like other nitro compounds, has a characteristic NO_2 group which, by absorbing infrared radiation is excited to certain kinds of oscillation. Depending on the energy absorbed by the molecule, each oscillation has a definite place in the spectrum, a definite form and intensity. The NO_2 group has two characteristic bands, one of very high intensity and the other a little lower, at 6.06—6.67 and 7.4—8.0 microns respectively, or in reciprocal centimeters from 1650—1500 and from 1350—1250 sm^{-1}.

In the nitrocellulose molecule (Fig. 2) a characteristic band, called an asymmetrical valence oscillation, appears at 6.1 microns or 1939 sm^{-1}. The other bands which appear in the spectrum are connected with the oscillations of other atoms or atomic groups.

Treating the cellulose molecule with a sulphonitric mixture changes the degree of esterification, and the changes occurring in

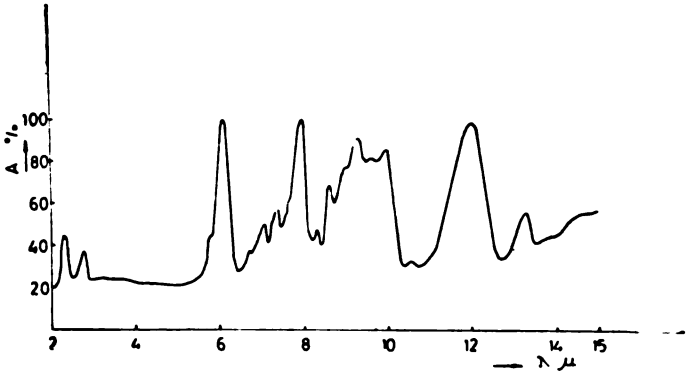

Fig. 2. Nitrocelullose spectrum

the molecule will also be reflected in changes of the oscillation of the NO_2 group.

The nitro group will oscillate at the same frequency, with the same band shape at 6.1 microns, only the intensity of the band will change because of changes of the dipole moment. For slightly nitrated cellulose the band at 6.1 microns will have a lower intensity and it will enclose a smaller surface. Therefore, the problem consists only in observing the intensity changes of the 6.1 micron band.

For quantitative work it is more conveninet to work with liquids, so a solvent for nitrocellulose must be chosen which does not absorb where there are bands of the NO_2 group. In the present case we used a 2:1 ether-alcohol mixture ratio.

In the section describing the experimental procedure it was stated how data can be obtained for plotting the working curve. The curve graphically shows the band area against the percentage of nitrogen, from which the degree of esterification of any nitrocellulose whose nitrogen percentage does not exceed 12.5 can be obtained. More strongly nitrated cellulose does not dissolve in an alcohol-ether mixture, only in acetone. In this case acetone cannot be used as a solvent because it has bands at places in the spectrum where there is a characteristic band of the NO_2 group.

The results of the measurements are shown in Table 1.

TABLE 1

Sample	Area in mm^2	Nitrogen percentage
1	1.068.78	12.03
2	1.027.42	11.93
3	1.021.73	11.91
4	958.88	11.87
5	798.25	11.52
6	746.74	11.35
7	534.90	11.02

Like all other analytical methods, differential analysis has sources of errors. It is important to take care that the entry slit always has a constant width, that the zero line is always the same, and that the thickness of the cell does not vary.

Results showing the precision of the differential analysis method are shown in Table 2.

TABLE 2

Sample	Weighed % N	Found % N	Difference
I	11.82	11.67	−0.15
II	11.66	11.69	+0.03
III	11.63	11.52	−0.11
IV	11.57	11.52	−0.05
V	11.48	11.36	−0.12
VI	11.37	11.30	−0.07
VII	11.09	11.22	+0.13

The infrared spectroscopy method of determining the nitrogen percentage is fast, elegant and reliable. It requires small amounts of the sample, 5 microliters of solution, and the complete analysis takes only a few minutes. The work is done in a pure atmosphere without the presence of nitrous gases as in the case of the nitrometric method. The precision of the differential analysis method is 1.2% and of the nitrometric method 0.5%, which is not much difference.

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NATURE OF OIL-SHALE KEROGENS. OXIDATIONS WITH POTASSIUM PERMANGANATE IN ACETONE SOLUTION. II. OXIDATION OF ALEKSINAC OIL-SHALE KEROGEN

by

DRAGOMIR K. VITOROVIĆ and MILICA DJURIČIĆ

In the study of the chemical nature of kerogens of various oil-shales, investigations have been made on the action of potassium permanganate in acetone solution on Aleksinac oil-shale kerogen. The aim of this works was to study the effect of the amount of potassium permanganate and the time of heating on the yields and properties of the degradation products, and to determine the exact balance of organic carbon.

In the literature there are some data on the oxidation of Aleksinac oil-shale kerogen with potassium permanganate in acetone and alkaline solutions, stating the following:

a) Oxidation with acetone solution gives much more ether-soluble degradation products that oxidation with an alkaline solution. From 600 g of oil-shale (with an organic carbon content of 57.1 percent), after 16 hours of heating with 3 kg of permanganate in 5 l of acetone, 38.5% of acid and 2.0% of neutral products were obtained. From these data it is evident that most of the oxidation products are acid. However, these products were only partly investigated(1).

In the systematic study of the action of alkaline potassium permanganate solution on Aleksinac oil-shale, the experimental conditions were varied according to the scheme applied in the study with acetone solution: the same portions of enriched oil-shale were oxidized with 100, 200 and 300 g of potassium permanganate for periods of 16, 32, 48 and 64 hours. The results showed that the first oxidation steps involved the formation of products which on prolonged heating with excess permanganate were further oxidized to carbon dioxide and water soluble products. Under the most drastic conditions, in alkaline solution the organic carbon is completely oxidized to carbon dioxide(2).

In the present communication we give the results obtained in the oxidation of Aleksinac oil-shale kerogen with potassium permanganate in acetone solution. The experiments were performed under various conditions according to the usual scheme: portions of 20 g of enriched oil-shale (organic carbon content: 8.54 g) in 400 ml of acetone were oxidized with 100, 200 and 300 g of $KMnO_4$ for periods of 16, 32, 48 and 64 hours (time of the addition of $KMnO_4$ included). The oxidation and the separation of acid and neutral products were performed as described in an earlier paper(3). Acid and neutral products and the unoxidized part were analyzed and the balance of organic carbon was determined.

Comparison of the results obtained here with those of the oxidation of the same oil-shale with alkaline solution, reveals that, like Colorado oil-shale, Aleksinac oil-shale is considerably more resistant to the acetone solution than to the alkaline solution of potassium permanganate(3). As already mentioned, in oxidation with alkaline solution the kerogen of this oil-shale is completely degraded. On the other hand, in acetone solution a considerable amount of ether-soluble products is obtained and most of the carbon is unoxidized.

The results have also shown that the changes caused by different oxidation conditions are very similar to those found in the study of Colorado oil-shale(3).

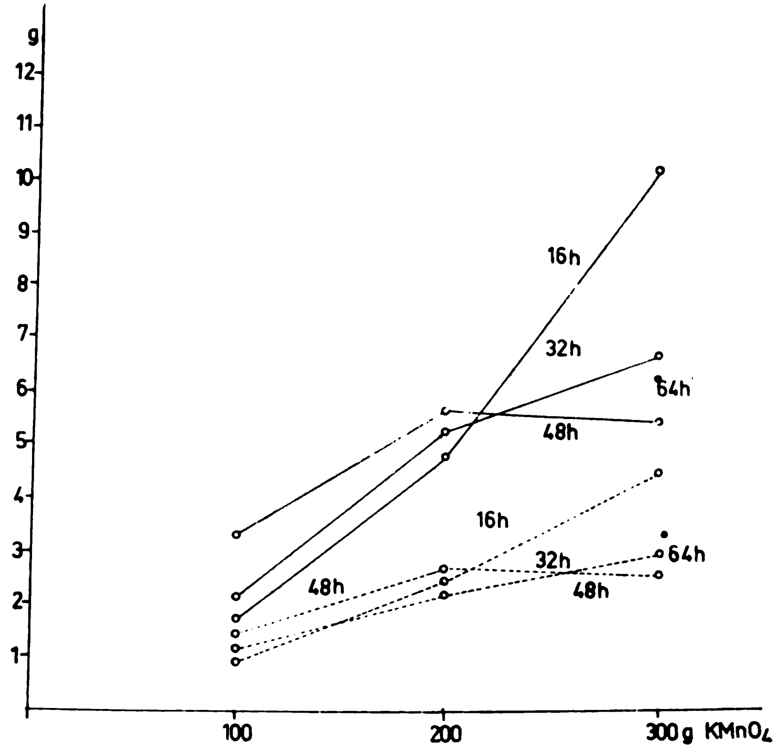

Fig. 1. Yields and carbon contents of acid oxidation products

----- Carbon content
_____ Yield of acids

REACTION PRODUCTS

a) *Acid products.* With increase of the permanganate quantity (100, 200, 300 g) the yields of acid products rapidly increase (1.59, 4.69, 10.11 g) in experiments in which the heating period is 16 hours. When the period is 32 hours, the increase of the yields is less pronounced, as shown in Fig. 1. Under more drastic conditions, with heating of the reaction mixture for 48 hours with various amounts of potassium permanganate, the yields, of acid products first increase and then slightly decrease.

The diagram in Fig. 1 shows the carbon contents of the acid products calculated from the elementary analyses.

On the other hand if we take the quantity of permanganate as constant, and observe the yields of acid oxidation products in dependence on the duration of heating (Fig. 2), we may observe the following:

1. In oxidation with 100 g of permanganate the yield increases (1.59, 2.07, 3.28) with increasing heating periods (16, 32, 48). The organic carbon contents also increase regularly (0.84, 1.07 and 1.40, i.e. 9.84%, 12.53% and 16.39% respectively, of the initial organic carbon content).

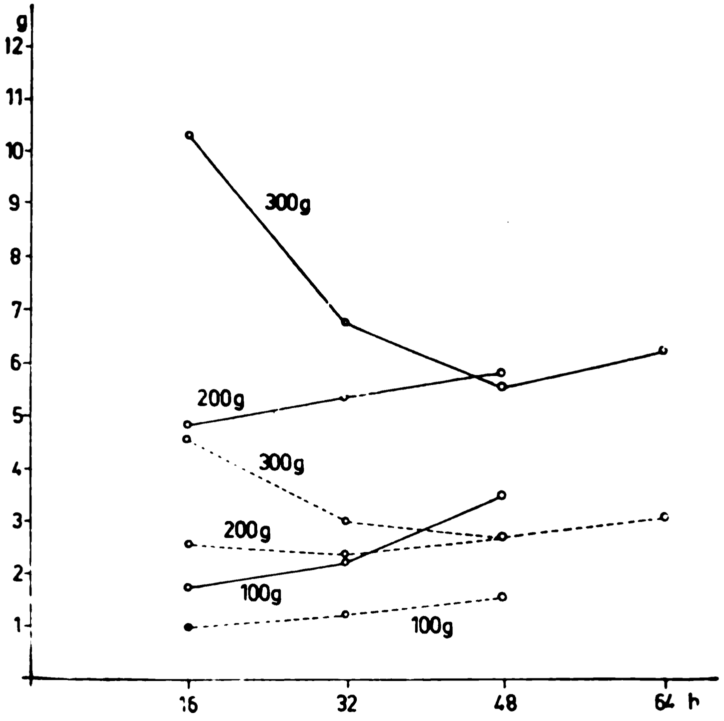

Fig. 2. Yield and carbon content of acid oxidation products.

- - - - Carbon content
______ Yield of acids

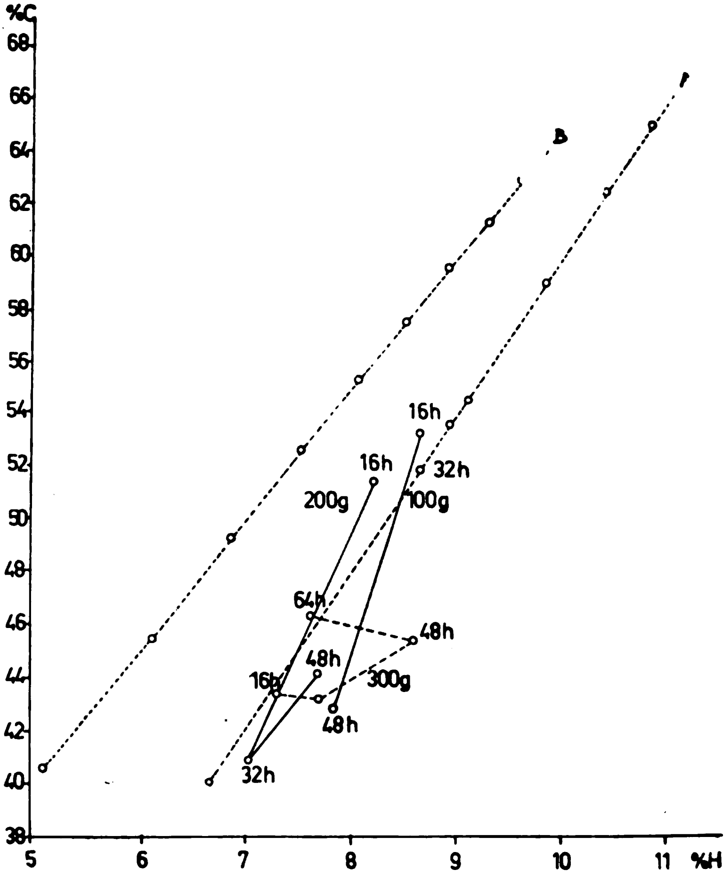

Fig. 3. C,H—content of acid oxidation products

C,H—content of: A = monocarboxylic acids B = dicarboxylic acids

2. In oxidations with 200 g of permanganate the yield of acid gradually increases (4.69, 5.19 and 5.5 g) with increasing periods of heating, but the carbon content is not essentially changed (2.41, 2.12 and 2.45 g i.e. 28.22%, 24.82% and 28.69% respectively, of the initial organic carbon content).

3. In oxidations with 300 g of permanganate, with increasing periods of heating the yield first diminishes rapidly (10.11 g, 16 hrs: 6.54 g, 32 hrs), then less rapidly and finally slightly increases (5.41g, 48 hrs; 6.12g, 64 hrs). The carbon content changed in the same manner (4.38, 2.82, 2.45 and 2.84 g i.e. 51.29%, 33.02%, 28.69% and 33.25%, respectively, of the initial organic carbon content). These results show that on prolonged heating, initially formed products are further degraded.

The C,H— content of acid products obtained under various experimental conditions did not show a regular change, as may be seen from the graph in Fig. 3. The graph shows the results of the elementary analyses of the acid products and the C,H—values of mono—and dicarboxylic fatty acids. The contents of carbon and hydrogen in products of oxidations with 100g of permanganate for various heating periods decrease. The changes in products of oxidations with 200 and 300g of permanganate are not regular.

The sulphur content is low (about 1%) but it is present in all fractions. The most of sulphur is present in the product of oxidation with 100g of permanganate for 16 hours.

The variations of acid numbers and equivalents are regular for products of oxidations with 100 and 200g of permanganate for various heating periods: the acid numbers increase with increasing heating time (graph in Fig. 4). In oxidations with 300g of permanganate the changes are not regular; the irregularity is especially pronounced in the acid products of 16 hours reaction, when the equivalent is very high and the carbon content very low. These results may be explained by gradual degradation of various parts of kerogen. A better insight will be obtained by further investigation of the degradation products.

Slightly higher iodine numbers are found only in products of 16 hours heating. The values of other products are in general low and within the limits of experimental error.

b) *Neutral products.* The yields of neutral products are not greatly or regularly changed by variations of experimental conditions. From 20g of enriched oil-shale (organic carbon content: 8.54g) about 0.4—0.7g of neutral products is obtained.

The carbon contents are high and range from 70 to 80%. The hydrogen contents lie between 10 and 12 percent.

The organic carbon in neutral products is small compared with that in acid products (about 3—6% of the carbon originally present).

All the neutral products contain about the same amount of sulphur: 2—2.5%. Less sulphur is obtained the more drastic conditions.

Unoxidized parts. From the analyses of the unoxidized parts it may be stated that with acetone potassium permanganate solution, under the most drastic conditions employed, a great part of the organic substance is not degraded to ether-soluble products. When the quantity of permanganate is increased and the heating period prolonged, the organic carbon of oil-shale is further oxidized. However, there are exceptions: after treatment with 300 g of permanganate for 64 hours, 68.5% of organic carbon remained in the unoxidized part.

The balance of organic carbon shows that most carbon appears in products of oxidation with 300 g of permanganate for 16 hours (51.29%). The residues of oxidations with 100 g of permanganate for various heating periods contain the greatest fraction of unoxidized organic carbon (90.05%, 92.30% and 81.50%).

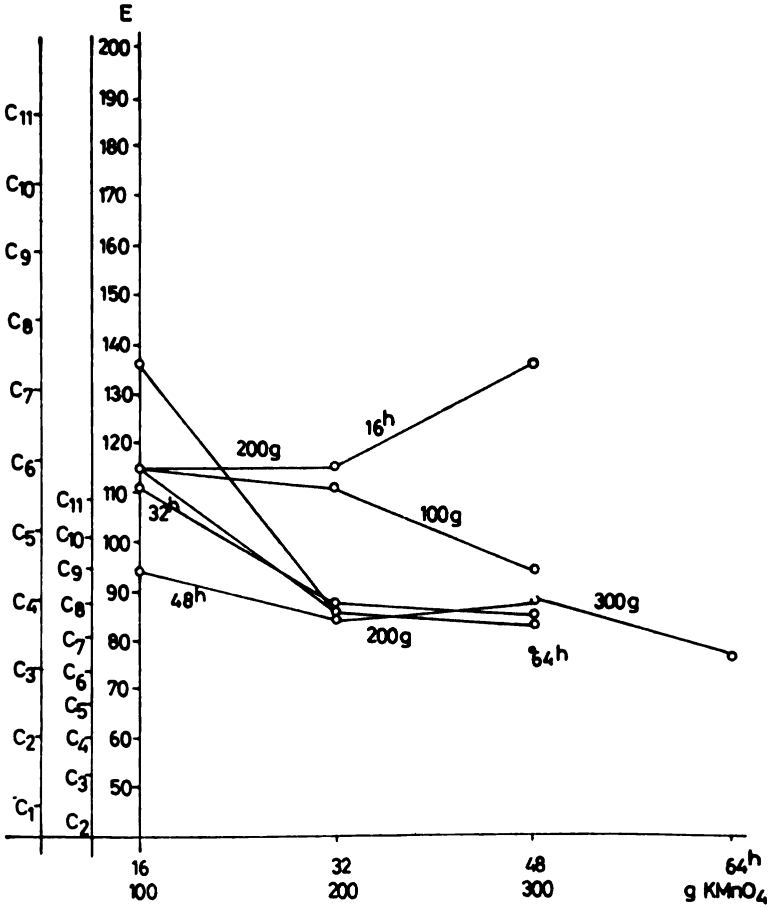

Fig. 4. Equivalents of acid oxidation products

EXPERIMENTAL

Aleksinac oil-shale was oxidized, which on distillation up to 540°C gives the following products (weight percentages): 27.8% oil, 5% water, 59.5% of semi-coke, 7.7% gases, with losses.

Shale was ground in a ball-mill (100 mesh, Tyler 0.149), then treated with hydrochloric acid (1:1), washed with water until negative chloride ion test and dried at 60°C to constant weight; 1000 g of crude oil-shale gave 795 g of enriched concentrate. The composition of the concentrate was: C—43.72%, H—6.15%, or C—71.94%, H—10.12% for the combustible part. The same oil-shale was used earlier in the study of the action of potassium permanganate in alkaline solution.

Potassium permanganate was ground into a fine powder. Acetone was purified by boiling with $KMnO_4$, then dried over anhydrous calcium chloride and distilled.

The experiments were always carried out with the same amount of enriched shale (20 g of shale with carbon content of 8.54 g) The experimental conditions were varied according to the scheme described in an earlier paper(3).

A total of ten experiments were performed under various conditions. Blank tests were also performed since it was establish that acetone gave rise to some acid product, mainly acetic acid.

The oxidations were carried out in the usual way: solid permanganate was added to the mixture of oil-shale and acetone under reflux, taking care that the reaction was not too violent. The time of the addition was 7 hours when the reaction mixture was to be heated for 16 hours, and 9 hours when the heating period was 32, 48 of 64 hours respectively. After oxidation, acetone was removed by distillation and the cooled reaction mixture was diluted with water. The excess of permanganate and manganese dioxide was reduced with sulphur dioxide and the oxidation products were extracted with ether. Acid products were separated from the neutral products by extraction with 5 potassium hydroxide.

a) *Acid products.* Yields of acid products are shown in Table 1.

TABLE 1

Yields (in g) of acid oxidation products (20g of enriched shale with 8.54g of organic carbon).

Heating period hrs	Amount of permanganate g		
	100	200	300
16	1.59	4.69	10.11
32	2.07	5.19	6.54
48	3.28	5.55	5.41
64	—	—	6.12

C,H-contents of acid oxidation products are shown in Table 2.

TABLE 2

C,H-contents of acid oxidation products

Heating period hrs	Amount of permanganate g		
	100	200	300
16	57.7	51.41	43.35
	8.68	8.23	7.28
32	51.81	40.74	43.13
	8.63	7.00	7.69
48	42.56	44.05	45.27
	7.78	7.70	8.58
64	—	—	46.29
			7.57

Carbon contents of acid oxidation products are expressed in percentages of the initial carbon content calculated from the analyses and given in Table 7.

Acid numbers were determined by titration with 0.1 N potassium hydroxide solution. The results are shown in Table 3.

TABLE 3

Acid numbers of acid oxidation products

Heating period hrs	Amount of permanganate g		
	200	200	300
16	488.3	488.2	412.0
32	506.0	651.8	667.7
48	599.4	670.1	635.1
64	—	—	729.1

b) *Neutral products.* Neutral products were isolated from ethereal solution after extraction of acids with potassium hydroxide solution. The yields of these products are given in Table 4.

TABLE 4

Yields (in g) of neutral oxidation products (20g of enriched shale with 8.54g of organic carbon)

Heating period hrs	Amount of permanganate g		
	100	200	300
16	0.5532	0.6952	0.6476
32	0.4270	0.6947	0.3150
48	0.5630	0.4610	0.3837
64	—	—	0.5280

C,H-contents of neutral products are given in Table 5; carbon contents are expressed in percentages of initial carbon content given in Table 7.

TABLE 5

C,H-contents of neutral oxidation products

Heating period hrs	Amount of permanganate g		
	100	200	300
16	72.13	73.49	70.56
	11.30	10.93	10.99
32	76.51	78.26	71.68
	11.28	11.03	10.82
48	70.97	76.90	77.96
	10.75	11.83	12.05
64			68.48
			9.99

c) *Unoxidized parts.* Organic carbon content in unoxidized parts was determined on the basis of C,H-analyses. The results are given in Table 6.

TABLE 6

Carbon contents of unoxidized parts

Heating period hrs	Amount of permanganate g		
	100	200	300
16	7.69	4.83	4.78
32	7.86	6.52	6.16
48	6.96	6.18	5.83
64	—	—	5.85

The balance of organic carbon was calculated on the basis of the analyses of acid and neutral oxidation products and the unoxidized parts. The balance is given in Table 7.

TABLE 7

Balance of organic carbon
(k – acid products; n – neutral products; o – unoxidized parts; z – total)

Heating period hrs		Amount of permanganate g		
		100	200	300
16	k	9.84	28.22	51.22
	n	4.67	5.98	5.35
	o	90.05	56.61	55.96
	z	104.56	90.71	112.96
32	k	12.53	24.82	
	n	3.83	6.37	
	o	92.30	76.34	
		108.66	107.53	
48	k			
	n			
	o			
	z	102 57		
64				

From the above balance it may be seen that the total carbon in most experiments is above 100% This is due to the presence of acid products produced by the solvent.

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CHEMICAL INVESTIGATION OF WHEAT

6*

GRAIN LIPIDS OF SOME HIGH YIELD WHEAT VARIETIES

by

GEORGE ALLAN GARTON, MIHAILO LJ. MIHAILOVIĆ, MIHAILO ANTIĆ and DIMITRIJE HADŽIJEV

INTRODUCTION

As yet there has been relatively little research on the fatty acids of wheat oil(1) and wheat phospholipids(2). However, the first reports on this subject immediately showed the difficulties of separating lipids from wheat grain. This concerns especially phospholipids, whose isolation in the pure state was hampered by the presence of other phosphorus compounds in the wheat grain, such as phytin, nucleoproteins and inorganic phosphorus salts, and by the fact that phospholipids are usually present as carbohydrate and protein complexes(3, 4).

Because of these difficulties and unsatisfactory methods of analysis, varying results have been obtained, which are mostly questionable and uncertain.

Intensive and detailed study of wheat lipids was begun only recently. Phosphatidyl-choline and phosphatidyl-ethanolamine have been studied by Antener and Högl(5) and by Friese *et al.*(6). Schulte and Krause(7) have isolated the serine component of wheat phospholipids. Faure and Morelec-Coulon(8) have demonstrated the presence of phosphatidyl-inositol. Law(9) has separated Carter's phytoglycolipides containing phytosphingosine as the base, and glucosamine, hexuronic acid, arabinose, galactose and mannose bound to the inositol part of the molecule(9). Carter *et al.*(10) isolated from wheat flour cerebrosides with phytosphingosine, dehydrophytosphingosine and dihydrosphingosine as bases, and with glucose and mannose as sugar components. The presence of glycolipids, such as mono- and digalactosyl-glycerol lipids was also revealed(11).

Recent investigations show that more than half the lipids in wheat flour are firmly bound to the proteins in the form of lipoprotein complexes(13,14). These complexes are extremely stable and cannot be disrupted by rinsing the gluten with water, nor by extract-

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* See paper 5.(18)

ing it with diethyl ether or petroleum ether. The role of these lipoprotein complexes in wheat flour is now also being extensively studied(15).

Previous investigations of wheat quality and especially of baking properties were mainly concerned with wheat grain proteins, *i.e.* the quality of gluten(12). The recent study of wheat flour by the X-ray diffraction technique proved the existence of an oriented bimolecular leaflet structure of phospholipids, acting as "lubricant" between protein platelets in gluten(16). The elastoplastic properties of dough disappear if the phospholipids are removed. These and other findings(17) led to the conclusion that the study of the baking quality and other varietal properties of wheat must include, besides gluten, the lipids of wheat grain.

From this point of view our present study was conducted along the following lines:

1. To establish, if possible, differences existing in the grain lipid fractions of some high yield Italian wheat varieties of fairly good and poor baking quality.

2. If any correlation is found between the baking quality and lipid fractions of wheat grain, to verify the possibility of increasing the accumulation of this lipid fraction by crop production techniques, *e. g.* by applying increasing amounts of nitrogen, phosphorus and potassium fertilizers. Since nitrogen and phosphorus are constituents of phospholipids, application of increasing levels of fertilizer containing these elements might be expected to influence the extent of phospholipid biosynthesis.

This report restricts itself to the investigation of the content of total lipids, free fatty acids, neutral lipids and phospholipids in the grain of some Italian high yield wheat varieties of fairly good and poor baking quality.

MATERIALS AND METHODS

The preparation of wheat samples, the extraction of lipids and their fractionation on a column of silicic acid and hyflo super cel (3 : 1), the preparation of fatty acid methyl esters and their separation by gas-liquid chromatography were described in our previous report(18).

The separation of the methyl ester of the metastable form of linoleic acid from other methyl esters of fatty acids, such as lauric, myristic, pentadecanoic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic and linolenic acids, was carried out by thin layer chromatography on silicic, acid, activated according to Stahl (Merck). The thickness of the silicic acid layers applied on glass plates 20 × 20 cm was 250—275 microns. The amount of the ester mixture used was 100 micrograms. The plates were developed with a 0.5 per cent solution of diethyl ether in petroleum ether (BDH; b. p. 40—60°). The spots were detected in UV light, after spraying the plates with a 0.2% solution of 2,3-dichlorofluoresceine. The elution of the metastable form of linoleic acid methyl ester (Rf value 0.00—0.06) was performed with ethanol, and its UV absorption spectrum, in the same solvent, was obtained by using a Hilger-Uvispek spectro-photometer.

RESULTS AND DISCUSSION

The results obtained for the contents of neutral lipids, free fatty acids, ether-soluble non-saponifiable products, phospholipids and total lipids in the grain of some high yield wheat varieties are given in Table 1.

TABLE 1

*The contents of neutral lipids, free fatty acids, non-saponifiable products, phospholipids and total lipds in the grain of wheat of high yield varieties**

Wheat variety	Moisture content (in %)	In *mg* per 100 *g* of dry grain				
		Neutral lipids	Free fatty acids	Ether-soluble non-saponifiable products	Phospholipids	Total lipids
Abbondanza	10.97	624.3	893.2	109.0	577.5	2204.0
Autonomia	11.40	1551.4	191.9	127.6	606.1	2477.0
Fortunato	11.12	602.5	496.2	106.0	479.3	1684.0
Leonardo	11.55	725.2	599.7	134.0	485.1	1844.0
Mara	10.68	1134.7	510.5	91.3	529.5	2266.0
Produttore	11.91	528.7	499.6	141.0	492.7	1662.0
San Pastore	10.82	707.5	552.0	142.0	543.1	1945.0

* In this and the following tables all results are for dry material.

As shown in Table 1, the total lipid content in wheat grain varied from 1662 (variety *Produttore*) to 2477 mg per 100 g (variety *Autonomia*). Of the separated fractions, the neutral lipid fraction was the most abundant. The highest content of this fraction was found in the variety *Autonomia* (1551.4 mg per 100 g) and the lowest content in the variety *Produttore* (528.7 mg per 100 g).

The amounts of free fatty acids also varied considerably with the variety investigated. However, these differences are probably due to the veriable intensity of the enzymatic action of lipase in ground wheat samples.

The investigated wheat varieties had different phospholipid contents. The highest amount was found in the variety *Autonomai* (606.1 mg per 100 g) and it decreased in the following order: *Abbondanza* (577.5 mg%) *San Pastore* (543.1 mg%) and *Mara* (529.2 mg per 100 g). The varieties *Fortunato, Leonardo* and *Produttore* had similar phospholipid contents which amounted, on the average, to 485.7 mg per 100 g.

With a view to investigating more closely the possible relationship between the baking quality and the lipid content in the grain of high yield wheat varieties, our attention was concentrated on the problem of the qualitative composition of some individual lipid fractions. Table 2 lists some usual data for isolated phospholipids.

TABLE 2

Nitrogen and phosphorus contents of wheat grain phospholipids and whole wheat grain.

Wheat variety	Phospholipid-N (as % of grain phospholipids)	Phospholipid-P (as % of grain phospholipids)	Phospholipid ratio N/P	Phospholipid-N (in *mg* per 100 *g* of grain)	Whole grain total N (in %)	Phospholipid-N (as % of grain total N)	Phospholipid-P (in *mg* per 100 *g* of grain)	Whole grain total P (in %)	Phospholipid-P (as % of grain total P)
Abbondanza	1.23	1.84	1.49	7.10	2.09	0.34	10.63	364.6	2.91
Autonomia	1.25	1.83	1.51	7.58	2.28	0.33	11.10	423.7	2.62
Fortunato	1.22	1.83	1.48	5.85	2.46	0.24	8.77	378.6	2.32
Leonardo	0.99	1.78	1.23	4.80	2.58	0.19	8.63	367.3	2.35
Mara	0.99	1.71	1.28	5.24	2.22	0.24	9.05	351.4	2.57
Produttore	0.96	1.71	1.24	4.73	2.05	0.23	8.43	318.0	2.65
San Pastore	0.90	1.66	1.20	4.89	1.97	0.25	9.02	387.2	2.33

Table 2 shows that the atomic rations N/P of isolated phospholipids varied with the variety. The highest ratios were found for *Autonomia* (1.51), *Abbondanza* (1.48) and *Fortunato* (1.48), which nearly corresponds to 3N : 2P. For the variety *Mara* this ratio is 1.28; for other varieties it is slightly lower, with an average of 1.22. Earlier investigations of some Yugoslav wheat varieties of good baking quality gave a mean value of 1.49 for this ratio(18).

Nitrogen bound to phospholipids, expressed as a percentage of total grain nitrogen, amounted to 0.34% in the variety *Abbondanza* (Table 2). In *Fortunato, Mara, Produttore* and *San Pastore* this percentage was practically constant, with a mean value of 0.24%. Although the amount of nitrogen bound to phospholipids corresponded to that of other varieties, the percentage value in the variety *Leonardo* (based on total N of grain) was only 0.19 per cent, owing to the higher anount of proteins present in wheat grain.

Phospholipid phosphorus in the investigated wheat varieties, expressed as a percentage of total phosphorus present in grain, had a mean value of 2.54 (Table 2). Some data cited in earlier literature reported higher values, *i.e.* 5—6%. The same is true of phospholipid-*P*, expressed in mg per 100 g of grain.

To explain this discrepancy, also observed in Yugoslav wheat varieties⁽¹⁸⁾ phospholipid phosphorus content in wheat grain of the *San Pastore* variety was determined using also the methods of other authors⁽¹⁹⁾. The results were as follows:

1. If the phospholipids are determined in an extract of chloroform-methanol (2:1 v/v) or directly in methanol solution, *i.e.* in the "crude" lipid extract, the content of phospholipid phosphorus amounts to 15.5 mg per 100 g or 0.035% P_2O_5. This result is identical with that of Kurmies⁽¹⁹⁾.

2. If the "crude" lipid extract is washed with distilled water, the resulting water extract contains orthophosphate phosphorus in an amount of 4.2 mg P per 100 g (0.0097% P_2O_5). The average content of orthophosphate phosphorus in ripened grain of high yielding wheat varieties was previously found to be 0.0448% P_2O_5⁽²⁰⁾. According to this result, it can be concluded that methanol extraction involves the orthophosphate present in wheat grain up to 21.6%.

3. If the "crude" lipid extract, from which the orthophosphate was previously removed. is washed according to Folch *et al.*⁽²¹⁾, the phospholipid-*P* content amounts to 7.2 mg P per 100 g* or 0.0165 P_2O_5. This result indicates that before applying the washing proce dure of Folch *et al*⁽²¹⁾, about 4.0 mg% P (0.0092% P_2O_5) of non-lipid phosphorus [phytin and phosphoproteide phosphorus(?)] was present in the crude extract containing the phospholipids. This finding agrees with the results we obtained by the direct purification of the crude lipid extract, according to *Folch et al.*⁽²¹⁾.

The qualitative and quantitative composition of the free fatty acid fraction isolated from the grain of the investigated wheat varieties is listed in Table 3.

As previously mentioned⁽¹⁸⁾, the enzymatic lipase action on the lipids present is intensified in ground wheat grain samples, thus considerably increasing the amount of free fatty acids within a few days. Since all the high-yield Italian varieties were milled at the same time and stored until the moment of analysis under the same conditions, the amount of accumulated fatty acids might represent a measure of the enzymatic activity of lipases in the investigated varieties. Being particularly characteristic, the variety *Abbondanza* may be cited as an example. Accumulation of free fatty acid content in this variety amounted to 57.7% of the total fatty acids present in the grain. But as can be seen from Tables 3, 4 and 5, the observed enzymatic activities were not particularly selective. This explains why the contents of fatty acids, expressed as percentages of total acids present, are very similar in all the investigated varieties.

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* This result (7.2 mg per 100 g) for phospholipid phosphorus was obtained for the variety *San Pastore*, provenience Novi Sad, while the value 9.2 mg% P, cited in Table 2, corresponds to the variety *San Pastore*, provenience Zrenjanin.

TABLE 3

Fatty acid composition of the fraction of free fatty acids

Wheat variety	The amount of free fatty acids (in *mg*% per 100 *g* of grain	Percentage of total acids *									
		Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Abbondanza	893.2	tr *	tr	tr	15.3	0.3	tr	1.5	19.3	58.1	5.3
Autonomia	191.9	tr	0,7	tr	22.2	tr	tr	2.8	13.3	57.0	4.0
Fortunato	496.2	0.3	0.2	tr	20.9	0.3	tr	1.2	12.0	60.4	4.8
Leonardo	499.7	tr	tr	tr	19.0	0.4	tr	1.4	13.6	61.4	4.2
Mara	510.5	0.3	0.2	tr	19.6	0.3	tr	1.1	13.5	60.6	4.4
Produttore	499.6	0.5	0.3	tr	16.3	0.6	tr	1.7	14.7	60.6	5.3
San Pastore	552.0	0.3	0.3	tr	19.9	tr	tr	1.2	13.8	58.7	5.8

* tr = traces (in this and the following tables).

TABLE 4

Fatty acid composition of the fraction of neutral lipids

Wheat variety	The amount of fatty acids bound to neutral lipids (in mg per 100 g of grain)	As Percentage of total acids									
		Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Abbondanza	303,3	0.2	tr	tr	15,8	0.5	0.2	0.9	13.8	63.6	5.2
Autonomia	1392.0	tr	tr	tr	17,8	0.3	tr	0.7	14.8	61.7	4.7
Fortunato	443,3	0.2	0.2	tr	15.9	0.7	0.3	0.9	13.5	62.8	5.5
Leonardo	592.4	0.2	tr	tr	15.6	tr	tr	2.8	16.5	60.2	4.7
Mara	565.3	0.2	tr	0.2	19.0	0.8	0.2	1.0	17.6	57.4	3.5
Produttore	467.6	tr	tr	tr	16.1	0.5	tr	1.1	14.7	62.8	4.8
San Pastore	538.9	0.2	tr	tr	15.0	0.6	tr	0.7	14.5	62.9	6,1

Linoleic acid predominates in the fraction of free fatty acids (Table 3). While its amount in some Yugoslav wheat varieties(18) averaged 54.3%, in the high yield varieties linoleic acid was found in amounts of 60% (average). The next most abundant unsaturated acid is oleic acid, with an average of 14.3%; this percentage is slightly lower than the amount (15.2%) found in Yugoslav wheat varieties of good baking quality(18). The linolenic acid content averages 5% *i.e.* 1 per cent more than in Yugoslav wheat varieties.

Of saturated fatty acids in the fraction of free fatty acids, palmitic acid predominates. Other saturated acids, with the exception of stearic acid (average content 1.6%), are present only in small amounts or traces (Table 3).

The fatty acid content in the neutral lipid and phospholipid fractions, and the per cent distribution of individual fatty acids within these fractions are listed in Tables 4 and 5.

TABLE 5

Fatty acid composition of the fraction of phospholipids

Wheat variety	The amount of fatty acids bound to phospholipids (in mg per 100 g of grain)	As Percentage of total acisd									
		Lauric acid	Myristic acid	Pentadecanoic acid	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Abbondanza	350.5	tr	tr	tr	18.3	tr	tr	1.6	9.3	67.4	3.5
Autonomia	395.0	tr	tr	tr	23.9	tr	tr	1.4	10.3	61.1	3.3
Fortunato	316.1	tr	tr	tr	18.0	tr	tr	1.2	9.4	67.8	3.6
Leonardo	226.1	tr	tr	tr	18.1	tr	tr	2.0	9.1	67.4	3.4
Mara	409.8	tr	tr	tr	20.1	tr	tr	1.4	10.6	64.7	3.2
Produttore	290.6	tr	tr	tr	20.1	tr	tr	1.3	10.4	64.2	4.1
San Pastore	371.0	0.4	0.3	tr	20.8	0.7	tr	0.9	10.6	63.8	2.5

As seen from these Tables, linoleic and palmitic acids predominate in both fractions. As in Yugoslav varieties(18), their amounts as percentages of total acids are slightly higher in the fraction of phospholipids while the contents of other acids are very similar.

In comparison with some Yugoslav varieties of good baking quality, the per cent distribution of fatty acids within both fractions of the high yield varieties does not show a significant difference.

Finally, as with Yugoslav varieties(18), the presence of fatty acids with an odd number of carbon atoms was also confirmed in the Italian high yield wheat varieties. As evident from gas-liquid chromatograms shown in Figs. 1, 2 and 3, the biosynthesis of these acids in the high yield varieties is favored to the same extent as the biosynthesis of some acids with an even number of carbon atoms, such as lauric, myristic and palmitoleic acid. This finding agrees with the results obtained with the Yugoslav varieties[18].

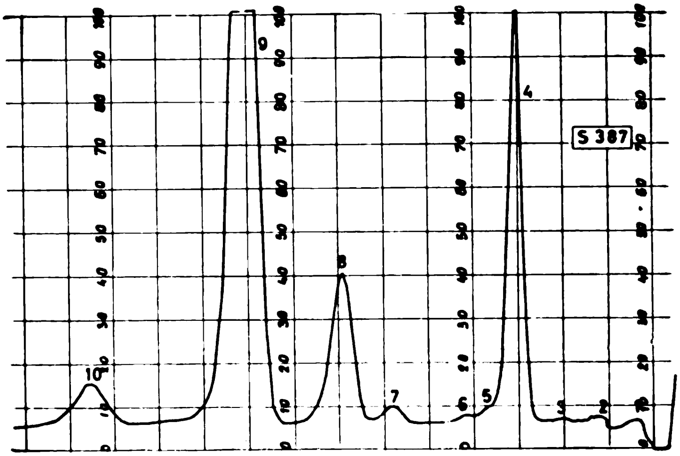

Fig. *1.* — GLC of fatty acids of the fraction of free fatty acids from the grain of wheat variety *Fortunato*

Column—acid washed Embacel /EGS, 8 : 2; column temperature 170°; argon flow rate 50 cc/min; detector voltage 1250 V; recorder sensitivity x 3; chart speed 30″ /hr; volume of esters applied 0.1 microliter.

1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Heptadecanoic acid; 7. Stearic acid; 8. Oleic acid; 9. Linoleic acid; 10. Linolenic acid

The recent detection of the C-13 fatty acid in some commercial gluten samples of Italian wheat varieties[22,23] and our own investigations of some Yugoslav and high yield wheat varieties[18] have shown that the biosynthesis of tri-, penta-, and heptadecanoic acids in wheat grain was a general phenomenon, regardless of the variety investigated. The presence of these acids in wheat is not restricted to any particular lipid fraction, *i.e.* they are present in the fraction of free fatty acids, of neutral lipids and in the phospholipid fraction.

As our previous gas-liquid chromatography investigations of some Yugoslav low-yield varieties have shown, after the position of arachidonic acid in the phospholipid fraction a double peak appears. As is evident from the gas-liquid chromatogram given for the variety *Abbondanza* (Fig. 3, peak 11) the shape and position of this doublet as well as of other peaks are identical with those obtained for other high-yield varieties and some Yugoslav low-yield varieties[18].

Simultaneously and independently, the same doublets were detected by Coppock and Daniels[24] in the course of investigating the action of iodate on the lipids of wheat flour.

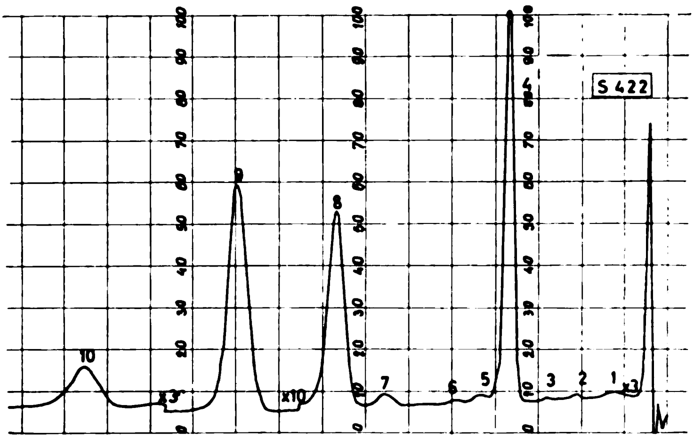

Fig 2. GLC of fatty acids of the fraction of neutral lipids from the grain of wheat variety *Abbondanza*.

Experimental data for the separation were the same as for the variety *Fortunato* (Fig 1.), except the recorder sensitivity was x 10 for linoleic acid.

1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Heptadecanoic acid; 7. Stearic acid; 8. Oleic acid; 9. Linoleic acid; 10, Linolenic acid.

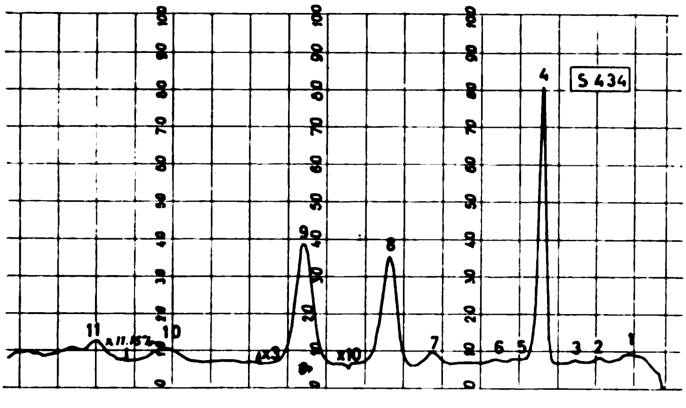

Fig. 3. GLC of fatty acids of the fraction of phospholipids from the grain of wheat variety *Abbondanza*.

Experimental data for the separation were the same as for the fraction of neutral lipids (Fig. 2.), except the volume of esters applied was 0.05 microliter.

1. Lauric acid; 2. Myristic acid; 3. Pentadecanoic acid; 4. Palmitic acid; 5. Palmitoleic acid; 6. Heptadecanoic acid; 7. Stearic acid; 8.Oleic acid; 9. Linoleic acid; 10. Linolenic acid; 11. Double peak of „metastable" linoleic acid.

The fact that the content of unsaturated fatty acids decreased when dough was treated with iodate, but was regenerated after the process of baking, led these authors to conclude that the detected doublet was a transient form of these acids, which during baking might act as an oxygen transfer agent in the oxidation of the sulphur bonds in cystein, glutathione and thioglycolic acid. The same authors showed that after baking the transient form disappears and the individual acids are regenerated almost quantitatively.

Our investigations have shown that the detected doublet was present in all ground wheat samples, regardless of the variety, when these samples were stored at room temperature for at least a few days. This indicates that the appearance of the doublet on the chromatogram is not restricted to the action of oxidizing agents such as iodates, bromates and persulphates, but even air oxygen is sufficient for its formation.

In addition, our present study has demonstrated that the transient form of linoleic acid is found only in the phospholipid fraction and does not appear in other lipid fractions, not even in traces. This finding undoubtedly supports the important role attributed to phospholipids in the process of baking.

The detection of the transient form of unsaturated fatty acids in the phospholipid fraction prompted us to examine its nature more closely. Thus the transient form was isolated in measurable amounts by thin-layer chromatography on silicic acid. From its ultraviolet absorption spectrum (absorption maxima at 232 and 268 mμ) the presence of conjugated chromophores, characteristic of partially polymerized linolelc acid(25), was confirmed.

It is of interest to note the stability of the transient form of linoleic acid under the conditions of our extraction procedure and gas-liquid chromatography analysis (temperature 170°; Embacel/EGS, 8:2). The stability of this form was also confirmed by Coppock and Daniels who carried out the gas-liquid chromatography separation at an even higher temperature (190°) and used polyethylene glycol adipate as the polar stationary phase (24).

In order to facilitate study of the relationship of the lipid content of wheat grain to the varietal-quality characteristics of the wheats investigated, some results of farinological analyses are listed in Table 6.

According to Grosskreutz (16), the protein platelets in gluten are bound to the present phospholipids (or more exactly to the lipoprotein complexes consisting of phospholipid and protein molecules) by hydrogen or salt-like linkages, the amount of phosphatides (*i.e.* lipoprotein complexes) determining the elastoplastic properties (including baking characteristics) of flour dough. By comparing the phospholipid content in wheat grain varieties of different quality (B-1 to C-2) (Table 1) with the elastoplastic properties of their dough (Table 6), it can be seen that the accumulation of phospholipids in the high yield varieties, contrary to Yugoslav low yield varieties(18), does not follow without exception the above-mentioned assumption. However, with a few exceptions, a certain regularity was observed in the distribution of linoleic and palmitic acids

TABLE 6

*Mean farinological values of flour prepared from the investigated wheat varieties**

Farinological characteristics	Wheat variety						
	Abbondanza	*Autonomia*	*Fortunato*	*Leonardo*	*Mara*	*Produttore*	*San Pastore*
Year of crop	1959—1961	1958—1961	1961	1961	1958—1961	1958—1961	1961
Wet gluten (%)	30.2	32.6	28.6	25.1	29.7	30.5	30.9
Dry gluten (%)	10.3	10.5	9.7	—	10.3	10.0	10.1
Gluten extensibility **	normal	—	—	normal	strong	very strong	very strong
Water absorption (%)	56.8	57.5	57.3	59.1	58.4	61.7	58
Dough development in mins	1.75	1.50	1.50	1.25	1.25	1.25	1.50
Dough stability in mins	0.50	1.50	0.75	0.75	0.50	0.50	0.50
Softening grade in F. u.	80	105	110	100	120	165	165
Quality index	56.2	51.3	48.9	51.4	41.2	21.1	28
Quality grade ***	B—1	B—2	B—2	B—2	C—2	C—2	C—2
Energy in cm^2	63.1	61.4	50.5	53.5	30.6	20.8	31
Resistance to extension in C units	315	245	185	340	125	105	160
Extensibility in mm	134	154	157	112	122	97	141
Resistance/extensibility	2.58	1.62	1.15	3.04	0.87	0.77	1.0

* Analyses correspond to flour of 60-70 % extraction

** Estimated by Kranzkosmin method

*** Graded in decreasing order of baking quality, *viz.* A-1, A-2, B-1, B-2, C-1 and C-2.

in the hydrophobic ends of the phospholipid molecules. An increased percentage of linoleic acid in most cases improves the baking quality of flour, while an increase of palmitic acid (*i.e.* partial substitution of linoleic acid by palmitic acid) lowers this quality. This finding is in agreement with the identification of the polymeric ("metastable") form of linoleic acid and its participation during baking in the oxidative processes, which with other factors correlates directly with the baking quality.

By comparing the lipid date given in Table 1 with farinological analyses in Table 6 (according to which the varieties are classified into corresponding quality grades), it is difficult, for the time being, to establish a definite relationship which would permit a better understanding of the varietal-quality characteristics of the high yield wheat varieties investigated. Undoubtedly these results only confirm the complex role of lipids in regulating the elastoplastic properties of dough, and the baking quality in general.

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THE IMPROVEMENT OF THE ADSORPTIVE PROPERTIES OF SOME YUGOSLAV SEMI-COKES BY MEANS OF CHEMICAL TREATMENT AND THE POSSIBILITY OF THEIR USE FOR THE DEPHENOLIZATION OF WASTE INDUSTRIAL WATERS

by

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1. INTRODUCTION

Adsorption on different materials has become one of the most important processes suggested for the dephenolization of waste industrial waters. This subject has been treated in the following works: ion exchangers and different active coals were studied by the Polish authors Jurkjevicz and Janczur(1); these problems were also studied by the Soviet authors Ivanov, Sharanov and Shullman(2); anion exchangers were studied by Saharnov, Bogatirev and Shkuderovich(3); coke and chemically treated wood shavings were studied by Maidanovskaya and Glumov(4); the mixture of wood shavings and magnesium carbonate and active coal covered with thin layers of silver and copper were studied by Maidanovskaya and Naidenova(5); coal ashes were studied by Ivanov and Kozak(6); slag was investigated by the Czech authors Šolin, Jungwirt, Kustka and Palaty(7); wood shavings activated by sulphuric acid and mercerized in sodium hydroxide were studied by Kotkowski and Wojtkiewicz-Matenko(8); lignite ashes were studied by the Roumanian authors Blum, Bolchi and Mihail(9); different types of charcoal were investigated by Nicolini in Peru(10); activated charcoal treated by various means of oxidation was studied by Mukherjee and Bhattacharya in India(11); fuller's earth was studied by Rios Carlos(12); and varios coals were investigated by the American authors Eveson, Ward and Worthington(13).

The adsorptive properties of Yugoslav materials and the possibility of their use for the dephenolization of waste industrial waters have been studied in Yugoslavia. However, the adsorptive properties of Yugoslav semi-cokes have not yet been studied, and the possibilities of their improvement by means of chemical treatment have not been investigated. The possibility of using this relatively new Yugoslav product for the dephenolization of certain waste industrial waters is the subject of this paper.

Two types of Yugoslav semi-coke ("Kosovo" and "Bogovina"), and their adsorptive properties toward phenol materials, were examined. Untreated semi-cokes were used, as well as samples which had previously undergone chemical treatment with sulphuric or nitric acid. The aim was to explain the adsorption mechanism in the light of new knowledge in this field.

2. THEORY

The influence of acids on the adsorptive properties of semi-cokes has not been explained. However, since coking is partly a graphitization of coal material, which depends on temperature, semi-coke has a partially graphite structure.

The carbonization temperatures of the two semi-cokes used in this experiment different by abount 200°C. This was expressed in their different structures and their different behavior with respect to their adsorptive properties. Thus the explanation of the effect of acid on semi-coke was based on the following interpretations of the behavior of graphite(14):

Under given conditions graphite interacts with certain substances building interstitial compounds with a characteristic initial graphite structure, leading to modifications depending on the nature of the substance with which the graphite interacted. There are three kinds of interstitial compounds, but this study was concerned with lamellate interstitial compounds where a layer of reacting substance is located between two carbon layers. The number of individual layers of reacting substance depends on their concentration and activity. Lamellate interstitial compounds of sulphuric and nitric acid with graphite are well known. Electric measurement has established them to be partly ionized. When these compounds are formed, the distribution and orientation of the molecules of the reacting substance depends on the free space inside the graphite and the diameter of the particles of the reacting substance. By X—ray diffraction it has been established that molecules of sulphuric acid require a free space of 4.63Å between two carbon layers, whereas nitric acid molecules are distributed in a space of 4.49Å, due to a different orientation and size. These compounds have a metastable nature and in certain circumstances decompose, with an electron exchange. Even the presence of water can sometimes cause such changes. But these changes are much greater in the presence of means of oxidation or reduction.

It was taken as logical that the size of the free space in the examined semi-cokes differs because of the different carbonization temperatures, and that the increase in space would be parallel with the increase of carbonization temperature. It was assumed that the semi-cokes would behave differently when treated with sulphuric acid than when treated with nitric acid. These assumptions were confirmed during the experimental work. Semi-coke carbonized at 800°C showed a greater tendency to build compounds with sulphuric acid, whereas semi-coke carbonized at 600°C showed a tendency to carbonize with citric acid. This was concluded when examining the dephenolization, i.e. the increase of adsorption potential of the treated semi-coke compared to that of the untreated semi-coke.

The interaction between the phenol materials in the solution which is passed through the semi-coke column as an adsorbent and the interstitial compounds in the mass of the adsorbent was established by measuring the pH of the effluent from the column with treated semi-coke and comparing it with those for the columns with untreated semi-coke. In the first case the effluent retained the properties of the phenol solution, whereas in the second case it showed definitely base pH values. The presence of SO_4 ions in the effluent was qualitatively determined. This also plays a role in the interaction between the phenol materials and the interstitial compounds.

This gives a probable explanation of the mechanism of dephenolization on treated semi-cokes.

3. EXPERIMENTAL PART

3.1 For the comparative study of adsorptive properties in relation to phenol materials in solution, two semi-cokes were used: Kosovo semi-coke from Kosovo coal carbonized at 800°C and Bogovina semi-coke from brown coal carbonized at 600°C.

Both semi-cokes were granulated and sifted, and the 1—2 mm fraction was used. Then the semi-cokes were treated with nitric and sulphuric acid under identical conditions.

Treatment with nitric acid: 800 ml of nitric acid, in an approximate concentration of 54%, was poured on 200 g of semi-coke in a porcelain container. During the first 3 — 4 hours, the mixture was occasionally stirred. It then stood for 20 hours. after which it was separated from the mixture by means of Büchner's funnel, across which a layer of fiberglass was placed. The semi-coke was then rinsed with distilled water to a neutral reaction. It was dried in air two to three days at room temperature, after which it was placed in a closed grass container in a dry place until needed.

Treatment with sulphuric acid has been applied to various types of coals(15) and the data show that temperatures higher than 100°C are not advisable. 800 ml of industrial sulphuric acid was poured on 200 g of semi-coke in a porcelain container. This was heated during four hours to a temperature of 50 to 100°C, with occasional stirring. The cooled mixture stood overnight, and then the semi-coke was separated in the same way as with the nitric acid treatment. Both treatments were carried out in a fume cupboard because of the intensive giving off of NO_2 or SO_3 vapors. The long process of rinsing to get the remaning acid out of the treated material can be shortened if the semi-coke is placed in distilled water for 24 hours after the first rough rinsing.

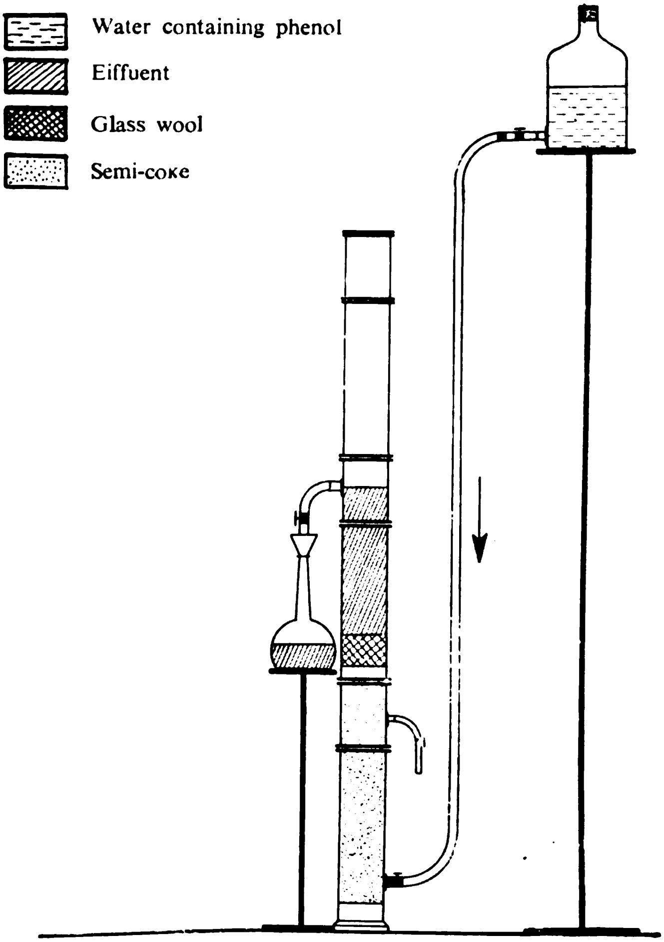

Fig. 1. Sketch of dephenolization column

After this treatment 6 different materials were available for adsorption:

1. Kosovo semi-coke treated with HNO_3, 1 — 2 mm fraction
2. Kosovo semi-coke treated with H_2SO_4 ,, ,,
3. Untreated Kosovo semi-coke ,, ,,
4. Bogovina semi-coke treated with HNO_3 ,, ,,
5. Bogovina semi-coke treated with H_2SO_4 ,, ,,
6. Untreated Bogovina semi-coke ,, ,,

3.2 Pure phenol solution in a concentration of 2.5 — 3 g phenol per liter was adsorbed on all these materials. Adsorption was also carried out with waste industrial water A, phenol content 5.6 g/lit, and waste industrial water B, phenol content 8.0 g/lit. Before being poured through the semi-coke, the waters were filtered through a sheet of fiberglass to remove suspended materials which would decrease the adsorptive area of the semi-coke.

The phenol content was determined by means of a modification of Koppeschar's method(16). A certain amount of n/10 bromide-bromate solution, which was judged to be adequate, was added to the water under investigation. The solution was acidified with 10 ml 1 : 3 H_2SO_4 and allowed to brominate in the dark for 15 to 20 minutes. Then 25 ml of 10% KI solution was added, and it was again placed in the dark for 3 to 5 minutes. The liberated iodine, which corresponds to the surplus bromide-bromate which did not react, was titrated with a solution of n/10 $Na_2S_2O_3$ with starch as an indicator. To prevent the creation of sediments of bromine derivates in the phenol, which would confuse the indication of the end of the reaction, chloroform is added during the reaction to dissolve the sediment. The phenol concentration was calculated from the amount of thiosulphate used up according to the following formula:

$$c = \frac{(\text{ml } KBr/KBrO_3 - \text{ml } Na_2S_2O_3)\ 1.57}{\text{ml for the test}} \cdot 1000\ (\text{mg/lit})$$

One ml of used up n/10 bromide-bromate solution corresponds to 1.5685 mg phenol.

3.3 Using the adsorbents listed above, the process of adsorption was studied by the dynamic method(17). A column of plexiglass (see diagram) was filled with semi-coke, and a sheet of fiberglass was placed on the bottom of the column and the top of the semi-coke. The phenol water was allowed to flaw out of a four liter container. The pressure under which the liquid flowed out was kept approximately constant. The liquid moved from the bottom up, and after 30 — 35 minutes the first drop of effluent appeared. Two kg of semi-coke were always placed in the column, which made it 50 cm high, and there was about another 20 cm of unfilled space to the effluent outlet. In all the experiments the liquid flowed through with a speed of 100 ml/min to 100 ml/1.2 min. The phenol concentration in the effluents was determined by a modification of Koppeschar's method. The data needed for the graph were derived from the data on the phenol concentration in the effluent:

— amount of adsorbed phenol in g/kg of semi-coke:

$$\frac{\textit{basic phenol concentration} - \textit{phenol concentration in effluent}}{\textit{kg semi-coke in column}}$$

— % of adsorbed phenol:

$$\frac{\textit{basic phenol concentration} - \textit{phenol concentration in effluent}}{\textit{basic phenol concentration}} \cdot 100$$

— the time of flowing through was not constantly measured but was calculated from the known volume of the effluent and the average speed of flow.

After each experiment the column was rinsed for 48 hours with tap water. This period sufficed to get rid of the last traces of phenol. It must be mentioned that the aim of this desorption was not to regenerate the adsorbing substances but only to prepare them for further work. When Kosovo semi-coke treated with nitric acid was regenerated, and the adsorption was repeated under the same conditions, exactly the same results were obtained. This demonstrated the adequacy of the desorption and at the same time showed satisfactory adsorptive properties of the semi-coke.

3.4 The results of the series of experiments carried out in the columns with 6 adsorbents and three phenolic waters showed that chemically treated semi-coke has better adsorptive properties than untreated. This improvement of the adsorptive properties is proportional to the properties of the untreated semi-coke. Untreated Kosovo semi-coke adsorbed 7.75 g of phenol per kg, and after chemical treatment about 14 g of phenol per kg. Untreated Bogovina semi-coke adsorbed less than 2 g of phenol per kg, and after treatment with nitric acid the adsorption was increased by more than three times.

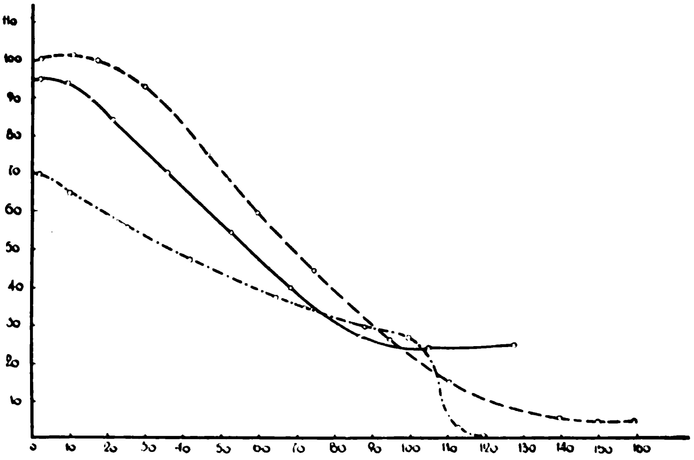

Fig. 2

——————— Kosovo semi-coke treated with nitric acid, 1—2 mm fraction.

— — — — — Kosovo semi-coke treated with sulphuric acid, 1—2 mm fraction.

—·—·—· Kosovo semi-coke, untreated, 1—2 mm fraction.

Abscissa: time in mins.

Ordinate: percentage of phenol adsorbed.

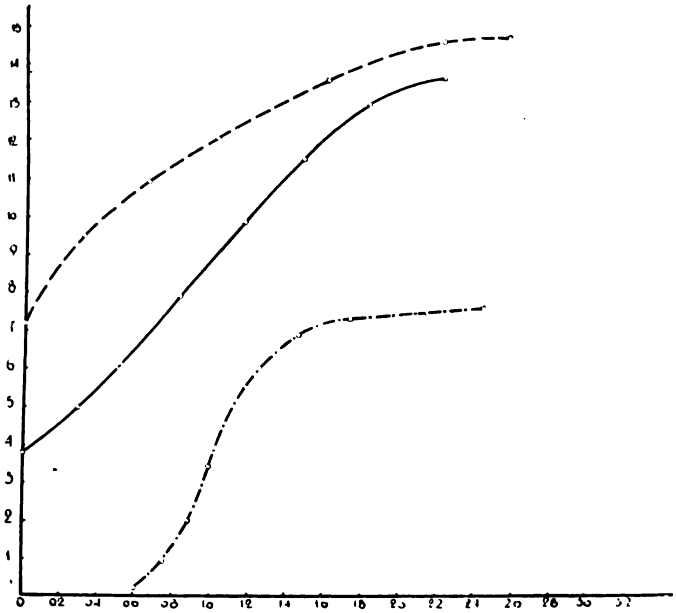

Fig. 3

———— Kosovo semi-coke treated with nitric acid, 1—2 mm fraction.

— — — — Kosovo semi-coke treated with sulphuric acid, 1—2 mm fraction.

—·—·—· Kosovo semi-coke, untreated, 1—2 mm fraction.

Abscissa: phenol concentration in effluent, g/lit.

Ordinate; quantity of phenol adsorbed, g/kg of semi-coke.

However, even though the Bogovina reached a value of 8 g of phenol per kg, its adsorption was much poorer than that of Kosovo semi-coke.

The two semi-cokes were also different in external appearance. Bogovina particles had a smooth surface, which in itself shows weaker adsorptive properties, whereas Kosovo had a significantly rougher surface.

The improvement of the adsorption was not manifested only in the increased quantity of adsorbed phenol but also in the degree of dephenolization of phenol water and in the time needed for saturation of the adsorbent. The maximum dephenolization of Kosovo semi-coke in the untreated state was only 70% and lasted 2 — 3 minutes. After treatment with sulphuric acid the maximum dephenolization in 10 minutes was 100%. The time needed to saturate the adsorbent was 120 minutes for the untreated semi-coke, and 160 minutes for that treated with sulphuric acid.

This data, for both semi-cokes, is shown in graphs 1 — 6.

The fact that Kosovo semi-coke improved more on treatment with sulphuric than with nitric acid, and that Bogovina improved more on treatment with nitric acid, tends to support the earlier assumption that the action of nitric or sulphuric acid depends on the structure of the semi-coke.

In adsorption from phenol industrial waste water, the efficacy of the semi-cokes was the same, but the amount of phenol adsorbed was much greater. This increase is in complete agreement with Freundlich's equation:

$$a = \alpha \cdot c^{1/n}$$

which shows that the quantity of material adsorbed (a) increases with increasing concentration (c) in the solution which is passed through. Since phenolic industrial waste waters A and B had much larger phenol concentrations then the pure phenol solution, the reason for the increased quantity of adsorbed phenol is obvious.

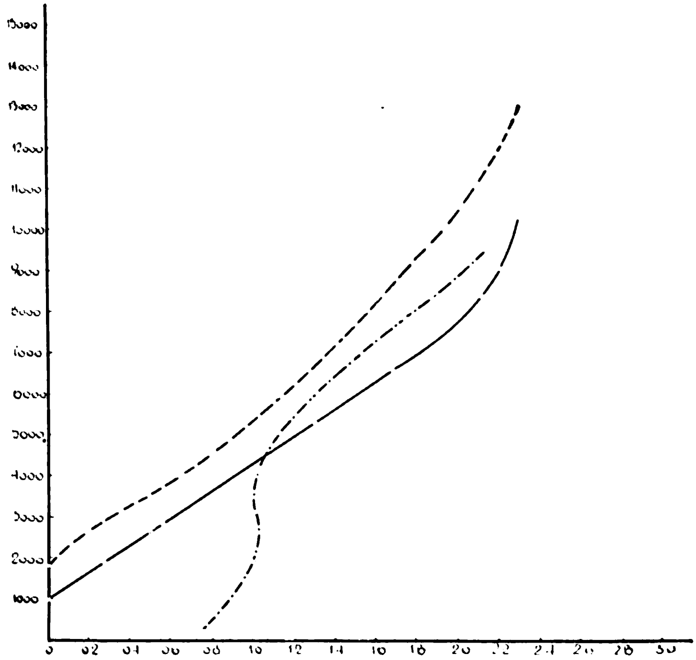

Fig. 4

———— Kosovo semi-coke, treated with nitric acid, 1—2 mm fraction.
— — — — Kosovo semi-coke treated with sulphuric acid, 1—2 mm fraction.
—·—·—· Kosovo semi-coke untreated, 1—2 mm fraction.
Abscissa: phenol concentration in effluent, g/lit.
Orbinate: effluent volume, ml.

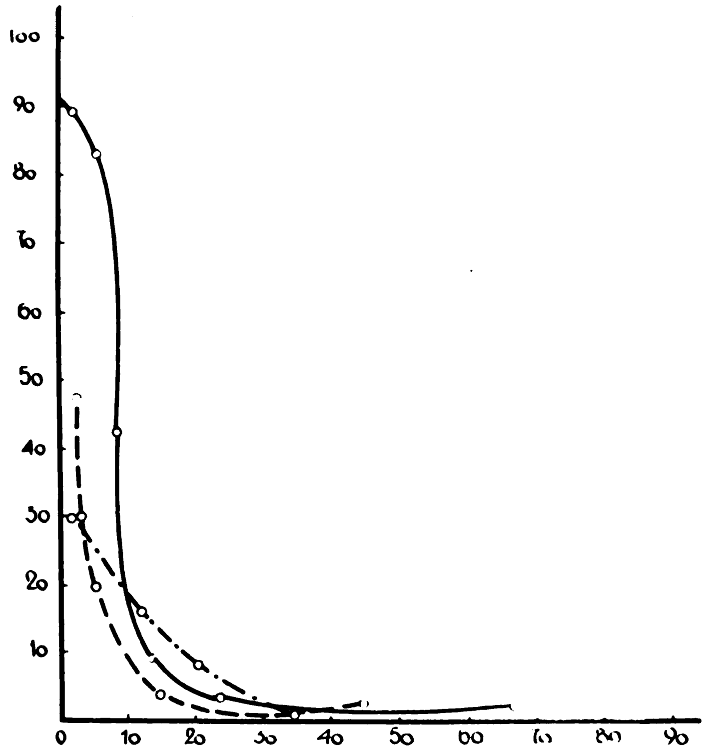

Fig. 5

——— Bogovina semi-coke treated with nitric acid, 1—2 mm fraction

— — — — Bogovina semi-coke treated with sulphuric acid, 1—2 mm fraction

—·—·—· Bogovina semi-coke, untreated, 1—2 mm fraction

Abscissa: time im mins.

Ordinate: percentage of phenol adsorbed

The possible influence of other substances in the waste waters, such as sulphides, cyanides and others, must not be excluded, since these substances may influence the adsorption process. The effect of these compounds on adsorption was not considered in this work.

Tables 1 and 2 show the results obtained with industrial waste waters A and B in the case of untreated Kosovo semi-coke, Kosovo treated with sulphuric acid, untreated Bogovina, and Bogovina treated with nitric acid.

Table 1 shows the results obtained with industrial waste water B, phenol concentration of 8 g/lit.

Table 2 shows results obtained with industrial waste water A, phenol concentration 5.6 g/lit.

TABLE 1

Time effluent takes to flow through (in minutes)	% of adsorbed phenol		Amount of adsorbed phenol g/kg	
	Bogovina semi-coke treated with nitric acid	Untreated Bogovina semi-coke	Bogovina semi-coke treated with nitric acid	Untreated Bogovina semi-coke
1	73	50	2.93	2.00
2	70	48	5.63	3.95
2	—	—	—	—
5	40	46	9.00	7.50
5	—	—	—	—
5	30	15	11.40	8.70
5	19	0	12.40	8.70
5	20	—	13.35	—
10	1.5	—	13.95	—
10	0.6	—	14.00	—

TABLE 2

Time effluent takes to flow through (in minutes)	% of adsorbed phenol		Amount of adsorbed phenol g/kg	
	Kosovo semi-coke treated with sulphuric acid	Untreated Kosovo semi-coke	Kosovo semi-coke treated with sulphuric acid	Untreated Kosovo semi-coke
1	100	100	2.50	2.90
2	100	100	5.08	5.80
2	100	—	7.62	—
5	96	80	10.06	10.30
5	86	60	12.32	12.06
5	—	—	—	—
5	74	45	14.38	14.30
5	—	44	—	15.40
5	53	—	16.28	—
5	—	22	—	16.63
10	23	17	17.90	17.03
10	20	—	19.25	—
10	16	9	20.22	17.53
10	—	5	—	17.67
10	7	—	21.74	—
10	4	1	22.24	17.70
10	2	—	22.37	—
10	0	—	22.40	—

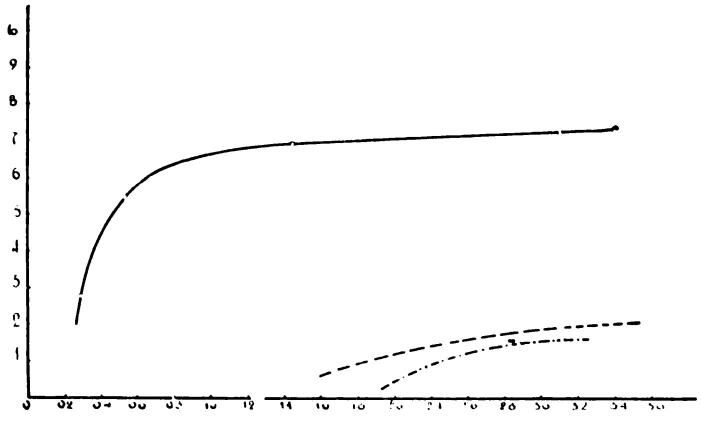

Fig. 6

——— Bogovina semi-coke treated with nitric acid, 1—2 mm fraction

— — — — Bogovina semi-coke treated with sulphuric acid, 1—2 mm fraction

—·—·—· Bogovina semi-coke, untreated, 1—2 mm fraction

Abscissa: phenol concentration in effluent, g/lit.

Ordinate: quantity of phenol adsorbed, g/kg of semi-coke

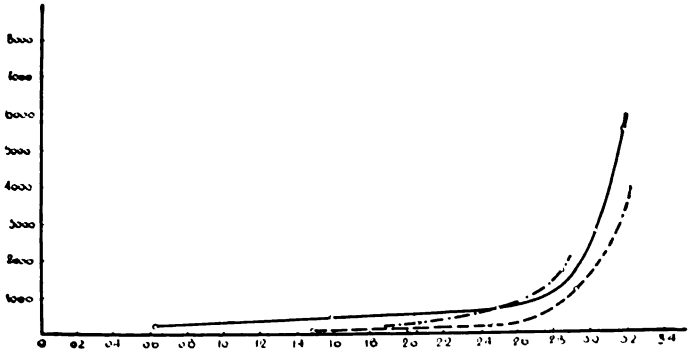

Fig. 7

——— Bogovina semi-coke, treated with nitric acid, 1—2 mm fraction

— — — — Bogovina semi-coke treated with sulphuric acid, 1—2 mm fraction

—·—·—· Bogovina semi-coke, untreated, 1—2 mm fraction

Abscissa: phenol concentration in effluent, g/lit.

Ordinate: effluent volume, ml.

With pure phenol dilutions and untreated semi-cokes, the treated effluents remained completety colorless. With treated semi-cokes all treated effluents were more or less yellow. Only Bogovina semi-coke treated with sulphuric acid did not give a yellow effluent. This is another proof that sulphuric acid was not able to significantly improve the adsorptive properties of this semi-coke. Because of spacial obstacles, the sulphuric acid hardly even formed interstitial compounds.

The experiment was carried out in conditions wich correspond to industrial practice:

— the liquid passed from the bottom up through the stationary adsorbent,

— the desorption of the adsorbent was possible without extraction of the material from the column,

— the examined industrial water even though it contained other substances, din not decrease the adsorptive power of the semi-coke toward phenol materials,

— the effluent which came out of the column was hard to capture because it formed a foam,

— the industrial waters did not discolor, not did they lose the characteristic smell of tar.

Economic handling industrial waste water with a phenol content of 0.5 to 1 g/lit thus appears practical. This limiting value is determined by the capacity of the adsorbent and the quantity of waste water which it can dephenolize.

CONCLUSIONS

a) Chemical treatment of Yugoslav semi-cokes significantly improved their adsorptive properties toward phenol. Heated Kosovo semi-coke improved when treated with sulphuric acid, and Bogovina cold semi-coke improved when treated with nitric acid. Compared with the untreated semi-cokes, the adsorptive properties of the Kosovo were two times greater, and those of the Bogovina 4 times.

b) Due to different structures of the two semi-cokes, in one case sulphuric acid was more effective in improving the adsorptive properties, whereas in the other case nitric acid was more effective. The different structures result from the difference in the temperature at which the coals are carbonized in the production of semi-coke.

c) The mechanical process which takes place during chemical treatment of semi-coke is interpreted by an analogy with graphite. It is assumed that certain interstitial compounds are formed which during dephenolization interact with phenol materials.

d) It is possible to use these materials in industry, depending on their capacity and the quantity of phenolic industrial waste water.

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