Kinetic determination of rutin

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A kinetic method is described for the determination of rutin based on its inhibitory effect on the Fe(II)-AA catalysis of the oxidation of C_6H_5COONa with hydrogen peroxide. Detection limit of this method is 0.16 ng cm^{-3}. The relative error ranges between 0.9 to 9.8 % for the concentration interval 0.82 ng cm^{-3} to 8.2 ng cm^{-3}. Kinetic equations are proposed for the investigated process. The effects of certain foreign ions upon the reaction rate were determined for the assessment of the selectivity of the method.

Keywords: kinetic method, rutin, determination.

INTRODUCTION

Rutin and the related flavonoids have attracted much interest owing to their action in decreasing the fragility of blood capillaries in guinea-pigs and these substances were once trough to possess vitamin like activity in humans.[1]

The determination of flavonoids at the 10 M level has generally been carried out by spectrofluorometric methods measuring the intrinsic fluorescence of these compounds.[2] Sensitive methods have been used to determine lower concentrations of flavonoids (mainly rutin) based on the formation of fluorescent complex with ethonium[3] or by spectrofluorometrically following the oxidation of flavonoids in alkaline pH[4] with determination ranges of 2.5–25 mol dm^{-3} for both rutin and kaempferol.

The capabilities of spectrophotometric and electrochemical detection techniques were investigated for the high-performance liquid chromatographic determination of flavonoids. The method developed was applied to the analysis of flavanones and flavonols in a real sample, such as an extract of orange juice. Even though quercetin glucoside is mostly present in orange juice as rutin, other different glycosides of this flavonol could be present; on this basis, the hydrolysis of all glycosides to aglycone allows one to obtain more accurate data on the flavonol concentration in orange juice.[5]

A new kinetic method for the determination of rutin (R), with sensitivity of 0.82 ng cm^{-3}, is described in this paper. The oxidation of C_6H_5COONa with hydrogen per-

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\# Serbian Chemical Society active member.

oxide in an acetic acid solution gives a colored product. The reaction is catalyzed by traces of complex Fe(II)-AA.[6] We have observed that small amounts of rutin strongly inhibits the catalysis of this reaction by complex Fe(II)-AA. The rate of the reaction decreases proportionally with increasing concentration of rutin. This fact was used as the basis of a kinetic method for determining ultra micro amounts of rutin.

EXPERIMENTAL

Apparatus

A spectrophotometric method was used for following the reaction rate investigated. The dependence of the absorbance (A) on time (t) was measured by a Perkin-Elmer Lamda 15 spectrophotometer, connected to a thermocirculaing bath. The pH was measured by means of a Radiometer PHM 29b pH meter and a combined glass-calomel electrode, GK 2311C. The solutions were thermostated at 25 0.1 ºC before the beginning of the reaction.

Reagents

The stock $FeCl_2$ solution (2×10^{-3} mol dm^{-3}) was prepared by dissolving $FeCl_2$ in water. The ascorbic acid solution (2×10^{-3} mol dm^{-3}) was prepared by dissolving ascorbic acid in water. The complex Fe(II)-AA solution (1×10^{-3} mol dm^{-3}) was prepared by mixing $FeCl_2$ solution (2×10^{-3} mol dm^{-3}) and ascorbic acid solution (2×10^{-3} mol dm^{-3}) 1:1 v:v. The C_6H_5COONa solution (1×10^{-3} mol dm^{-3}) was prepared from dissolving C_6H_5COONa in water. The acetic acid solution (1 mol dm^{-3}) was prepared from the 99.9 % glacial acetic acid. The hydrogen peroxide solution (1 mol dm^{-3}) was prepared from the 34 % reagent. The rutin (1×10^{-3} mol dm^{-3}) was prepared by dissolving rutin in methanol.

All chemicals were of analytical reagent grade, and were provided by Merck unless indicated otherwise. The solutions were made using deionized water. All the stock solutions were stored in polyethylene containers. The working solutions of Fe(II) and H_2O_2 were prepared immediately before use.

All the polyethylene containers and the glassware used were cleaned in aqueous HCl (1:1) and then thoroughly rinsed with deionised water.

Procedure

Measured amounts of hydrogen peroxide were stored in one compartment of a special vessel (Budarin's vessel), Fe(II)-AA solution was placed in the second compartment, C_6H_5COONa in the third compartment and acetic acid (acetic acid and rutin) and water (up to total volume of 15 cm^3) in the fourth compartment. The spectrophotometer cell was rinsed well and filled with the solution. The absorbance at 540 nm, was measured every 30 s over a period of 5–8 min after mixing. Instead of the reaction rate (dc/dt), the quantity dA/dt was used.

The measurement were made at 25 0.1 ºC.

RESULTS AND DISCUSSION

Kinetic studies

A differential variant of the tangent method[6] was used for the processing of the kinetic data, because a linear correlation exists between the absorbance and time during the first 5 to 8 min after mixing. The reaction rate was followed by the change in the values of the tangent of the angle (tan) of the slope of the linear part of the kinetic curve to the abscissa in the coordinates A–t, since tan   = dA/dt.
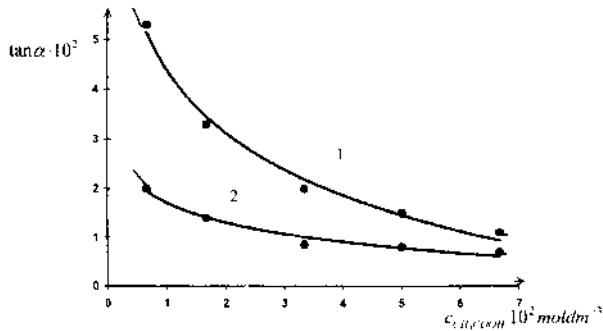
Fig. 1. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the acetic acid concentration. Initial concentrations: $c_{C_6H_5COONa} = 2 \cdot 10^{-4}$ mol dm^{-3}; $c_{H_2O_2} = 0.133$ mol dm^{-3}; $c_{Fe(II)-AA} = 2 \cdot 10^{-4}$ mol dm^{-3}; R = 8.2 ng cm^{-3}; $T = 293$ K.

In order to determine the lowest possible determinable concentration of rutin, the conditions needed to be optimized. Therefore, the dependencies of the rates of both the catalytic and the inhibited reactions on the concentration of each of the reactants were determined.
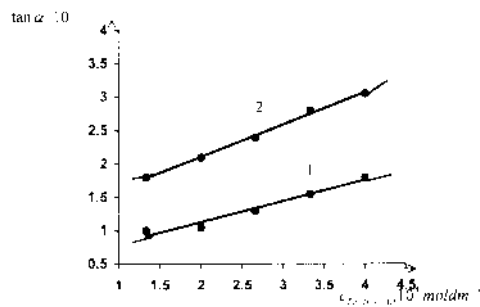

Fig. 2. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the Fe(II)-AA concentration. Initial concentrations: $c_{C_6H_5COOH} = 0.033$ mol dm^{-3}; $c_{H_2O_2} = 0.133$ mol dm^{-3}; $c_{Fe(II)-AA} = 2 \cdot 10^{-4}$ mol dm^{-3}; R = 8.2 ng cm^{-3}; $T = 293$ K.
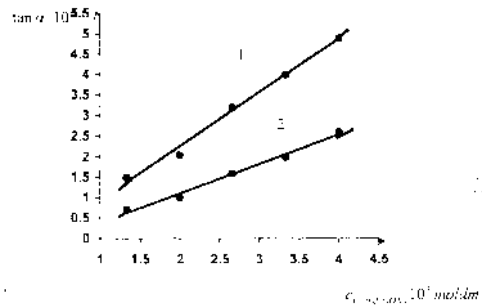
Fig. 3. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the C$_6$H$_5$COONa concentration. Initial concentrations: $c_{Fe(II)-AA} = 2 \cdot 10^{-4}$ mol dm^{-3}; $c_{CH_3COOH} = 0.033$ mol dm^{-3}; $c_{H_2O_2} = 0.133$ mol dm^{-3}; R = 8.2 ng cm^{-3}; $T = 293$ K.

Figure 1 shows the influence of pH on the rate of both reactions. It can be seen that the greatest difference between the reaction rates occurs at $c_{CH_3COOH} = 3.33 \cdot 10^{-2}$ mol dm^{-3}, when rutin maximally decreases the catalytic reaction rate. For further work an acetic acid concentration of $3.33 \cdot 10^{-2}$ mol dm^{-3} was selected. From Fig. 1 it appears that there is a complicated relationships between the c_{CH_3COOH} and the catalytic and inhibited reactions rate in the range of concentrations studied. The linear relationship between the logarithm of the tan and logarithm c_{CH_3COOH} was found for catalyzed and inhibited reactions. The order of catalyzed reaction is – 0.68 with respect to the c_{CH_3COOH} in the all investigate interval. The order of inhibited reaction is – 0.50 with respect to the c_{CH_3COOH} 10^{-2} mol dm^{-3}. The correlation between tan and the Fe(II)-AA concentration is shown in Fig. 2. Both reactions are first order with respect to

the Fe(II)-AA concentration. A Fe(II)-AA concentration of 2 $\quad$ $^{-4}$ mol dm^{-3} was selected, for further work, because at higher concentrations the linear part of the kinetic curve (*A–t*) is rather short.

The dependence of tan $\quad$ on the C$_6$H$_5$COONa concentration is shown in Fig. 3, which shows that the difference in the rates of the ihibition and catalytic reactions increases with increasing C$_6$H$_5$COONa concentration. For further work a C$_6$H$_5$COONa concentration of 3.33 $\quad$ 10^{-4} mol dm^{-3} was selected, with respect to the $c_{CH3COOH}$.

The dependence of the reaction rates on the concentration of H$_2$O$_2$ is shown in Fig. 4, from which it can be seen that the inhibited and catalytic reactions are first order with respect to the H$_2$O$_2$ concentration. A H$_2$O$_2$ concentration of 0.133 mol dm^{-3} was selected, for further work.
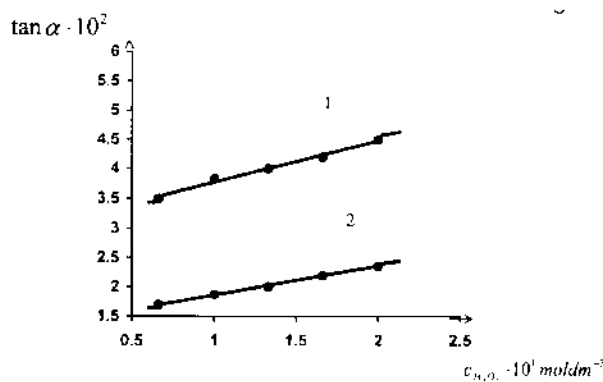

Fig. 4. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the H$_2$O$_2$ concentration. Initial concentrations: $c_{CH3COOH}$ = 0.033 mol dm^{-3}; $c_{Fe(II)-AA}$ = 2 $\quad$ $^{-4}$ mol dm^{-3}; $c_{C6H5COONa}$ = 3.33 10^{-4} mol dm^{-3}; $_R$ = 8.2 ng cm^{-3}; *T* = 293 K.
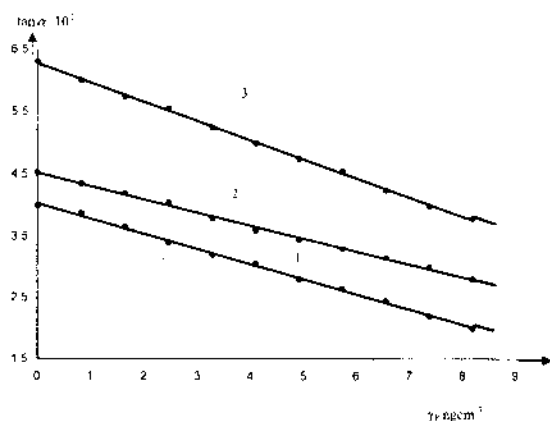

Fig. 5. Dependence of the rate of the reaction on the rutin concentration. Initial concentrations: $c_{CH3COOH}$ = 0.033 mol dm^{-3}; $c_{Fe(II)-AA}$ = 2 10^{-4} mol dm^{-3}; $c_{C6H5COONa}$ = 3.33 10^{-4} mol dm^{-3}; c_{H2O2} = 0.133 mol dm^{-3}; 1) *T* = 293 K; 2) *T* = 295 K; 3) *T* = 298 K.

Under the optimal reaction conditions:

$c_{CH3COOH}$ = 3.33 $\quad$ $^{-2}$ mol dm^{-3}, $\qquad$ $c_{C6H5COONa}$ = 3.33 $\quad$ 10^{-4} mol dm^{-3},

$\quad$ $c_{Fe(II)-AA}$ = 2 $\quad$ 10^{-4} mol dm^{-3}, $\qquad$ c_{H2O2} = 0.133 mol dm^{-3}

the rutin concentration was varied from 0.82 ng cm^{-3} to 8.2 ng cm^{-3}.

Figure 5 shows the three calibration lines, at three different temperatures which can be used for the determination of the rutin concnetration in the interval mentioned.

The following kinetic equations were deduced on the basis of the graphic correlations obtained for the investigated process.

For the catalyzed reaction:

$$\frac{dc}{dt} = k_1 \ c_{CH_3COOH}^{0.68} \ c_{C_6H_5COONa} \ c_{Fe(II)-AA} \ c_{H_2O_2} \tag{1}$$

were k_1 is constant proportional to the rate constant of the catalyzed reaction.

For the inhibited reaction:

$$\frac{dc}{dt} = k_2 \ c_{CH_3COOH}^{-0.5} \ c_{C_6H_5COONa} \ c_{Fe(II)-AA} \ c_R^{-1} \tag{2}$$

for $c_{CH_3COOH} \geq 3.33 \times 10^{-2}$ mol dm^{-3}, were k_2 is a constant proportional to the rate constant of the inhibited reaction. On the basis of these equations, the rate constants for the inhibited and catalyzed reactions were calculated (Table I).

TABLE I. Rate constants for the catalysed (k_1) and inhibited (k_2) reactions at three temperatures

T/K	$k_1 \times 10^4$	$k_2 \times 10^{-3}$
293	4.44	1.04
296	5.0	1.19
298	6.94	1.64

The linear relationship between the logarithm of the rate constant and the reciprocal of the absolute temperature (Table II) was found for the inhibited as well as catalyzed reaction. The activation energies were found to be 5.45 kJ mol^{-1} for the catalyzed reaction and 15.0 kJ mol^{-1} for the inhibited reaction, at 293 K.

TABLE II. Logarithm of rate constants for the catalyzed ($\log k_1$) and inhibited ($\log k_2$) reactions at three different temperatures

$1/T(10^{-2} K^{-1})$	$\log k_1$	$\log k_2$
3.413	4.64	−2.983
3.378	4.699	−2.924
3.356	4.809	−2.785

The accuracy and precision[7] of the measurements are presented in Table III. The relative error ranges from 0.9 to 9.7 % for rutin concentrations from 0.82 ng to 8.2 ng cm^{-3}.

The minimum concentration of rutin, which could be determined by this method, may be calculated by the method given by Perez-Bendito and Silva.[8] The detection limit is 0.48 ng cm^{-3}.

TABLE III. Accuracy and precision of rutin determination

Taken/ng cm^{-3}	Found ($\bar{x}$)/ng cm^{-3}	n	S 10^{10}/g cm^{-3}	G/%	$(x-)$ 100 / ; %
0.82	0.88	5	0.16	9.70	+7.4
4.10	4.09	5	1.22	3.70	−0.27
8.20	8.22	5	0.27	0.90	+1.22

x – Mean value; – true value; n – number of determinations; S – standard deviation; G – relative error (= 100 t s/x $\sqrt{n}$, where n = 5 and t is Student's for 95 % confidence)

TABLE IV. Tolerance levels for foreign ions in the kinetic determination of 4.1 ng cm^{-3} rutin

Tolerance level (c_{ION}/c_R)	Ion added
10^4	Na$^+$; Cl$^-$
10^3	NO$_3^-$; CH$_3$COO$^-$; PO$_4^{3-}$
10^2	Ca(II); Mg(II); K(I); SO$_4^{2-}$
10	CO$_3^{2-}$; Citric acid; gallic acid; Se(IV); Zn(II); Cd(II); Co(II); Pb(II)
1	Al(III); C$_2$O$_4^{2-}$; SCN$^-$; Br$^-$; rutin sulphat
1	Quercetin; morin inhibited

To assess the selectivity of the method, the influence of several foreign ions on the rate of the inhibited reaction rates was studied at a constant rutin concentration of 4.1 ng cm^{-3} (Table IV). Regarding selectivity as determined by 2-s criterium,[9] for a rutin concentation of 4.1 ng cm^{-3} it may be seen that quercetin and morin in a 1:1 ratio to rutin interfere with the reaction. The other ions investigated have practically no influence on the determination of rutin by this method.

ИЗВОД

КИНЕТИЧКО ОДРЕЂИВАЊЕ РУТИНА

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У овом раду предложена је нова кинетичка метода за одређивање рутина која се базира на његовом инхибиционом ефекту у реакцији оксидације C$_6$H$_5$COONa водоник-пероксидом, која је катализована комплексом Fe(II) са аскорбинском киселином. Граница детекције је 0,16 ng cm^{-3}. Релативна грешка методе се креће од 0,9–9,7 % за концентрациони интервал од 0,82 ng cm^{-3} – 8,2 ng cm^{-3}. Предложене су кинетичке једначине за предложене процесе. Испитан је утицај већег броја страних јона на брзину реакције и утврђено је да је селективност методе задовољавајућа. Метода ће бити примењена за квантитативно одређивање рутина изолованог из различитих биљних врста.

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