J. Serb. Chem. Soc. 69 (6) 477–483 (2004)
JSCS – 3174

UDC 582.685:543.23
Original scientific paper

Kinetic determination of morin nanoamounts

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(Received 22. September, revised 10 December 2003)

Abstract: A kinetic-spectrophotometric method is proposed for the determination of morin. The method is based on the inhibition effect of morin on the oxidation of C_6H_5COONa by hydrogen peroxide in the presence of the complex Fe(II)-AA(ascorbic acid), which acts as a catalyst. The concentration range for the determination of morin is one of the lowest achieved so far (a linear calibration graph was obtained for morin from 2.255–22.55 ng cm^{-3}). The limit of detection of the method is 0.28 ng cm^{-3}. The relative error ranges between 1.42 to 5.10 % for the given concentration interval. Kinetic equations are proposed for the investigated process. The effects of certain foreign ions upon the reaction rate were determined in order to assess the selectivity of the method. The major advantages of this kinetic-spectrophotometric assay are its sensitivity, selectivity, reproducibility, speed and simplicity.

Keywords: kinetic method, morin, determination.

INTRODUCTION

Flavonoids are widely distributed dietary constituents derived from plants. They possess a wide spectrum of pharmacological properties, the mechanisms of which remain, to a great extent, unknown. Flavonoids are antioxidants and act as scavengers of oxygen radicals, such as the super oxide anion, singlet oxygen and hydroxyl radicals. A wealth of evidence in the literature strongly suggests the involvement of oxygen free radicals in celular processes that underline the mechanisms of the induction of certain diseases, such as arterioscelerosis, arthritis and cancer.[1]

Morin is a bioactive flavonoid found in yellow Brazil wood. It is an effective antioxidant, concentrations of 75 – 100 mM, inhibits the oxidation of low density lipoprotein (LDL) induced by free radicals or by Cu(II).[2,3]

The determination of flavonoids at the 10 μM level has generally been carried out by spectrofluorometric methods and measuring the intrinsic fluorescence of these compounds.[4] Sensitive methods have been used to determine lower concentrations of flavonoids (mainly morin) based on the formation of a fluorescent complex with

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ethonium[5] or spectrofluometrically following the oxidation of flavonoids at alkaline pH[6] (with determination ranges of 2.5–25 μmol dm^{-3} for both morin and kaempferol).

A new kinetic method for the determination of morin, with a sensitivity of 2.255 ng cm^{-3}, is described in this paper. The oxidation of C_6H_5COONa with hydrogen peroxide in acetic acid solution gives a colored product.[7] The reaction is catalyzed by traces of the complex of Fe(II) with ascorbic acid (AA). It was observed that small amounts of morin strongly inhibit the catalysis of this reaction by complex Fe(II)-AA. The rate of the reaction decreases proportionally with increasing concentration of morin. This fact was used as the basis of a kinetic method for determining ultra micro amounts of morin.

EXPERIMENTAL

Apparatus

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

Reagents

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

A morin solution (1×10^{-3} mol dm^{-3}) was prepared by dissolving morin in methanol.

All the 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 with four compartment), Fe(II)-AA solution was placed in the second compartment, C_6H_5COONa in the third compartment and acetic acid (acetic acid and morin) and water (up to a 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 change of dA/dt was followed.

The measurement were made at 25 ± 0.1 ºC.

RESULTS AND DISCUSSION

Kientic studies

A differential variant of the tangent method[8] was used for processing the kinetic data, because there is a linear correlation between the absorbance and time during the

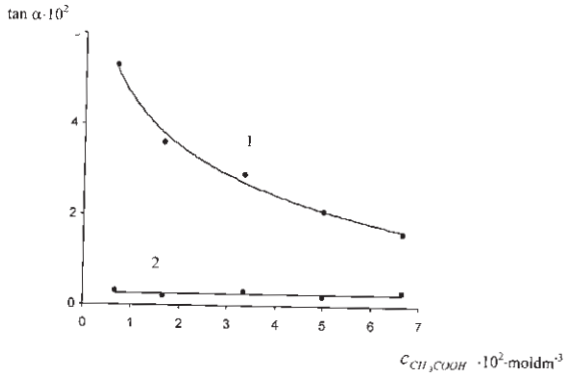

Fig. 1. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the acetic acid concentration. Initial concentrations: $c_{C_6H_5COONa} = 2 \times 10^{-4}$ mol dm^{-3}; $c_{Fe(II)\text{-}AA} = 2 \times 10^{-4}$ mol dm^{-3}; $c_{H_2O_2} = 0.133$ mol dm^{-3}; $c_{morin} = 0.226$ μg cm^{-3}; $t = 25 \pm 0.1$ °C

first 5 to 8 min after mixing. In order to determine the lowest possible determinable concentration of morin, the conditions needed to be optimised. Therefore, the dependencies of the rates of both the catalytic and the inhibited reactions on the concentration of each of the reactants were determined.

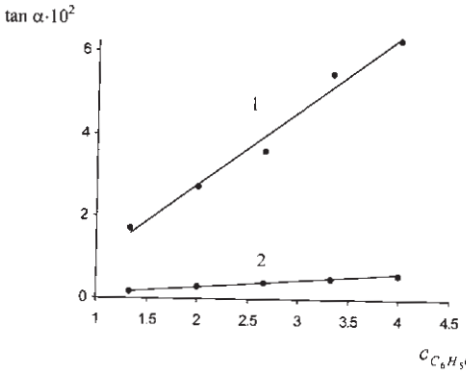

Fig. 2. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the C_6H_5COONa concentration. Initial concentrations: $c_{CH_3COOH} = 3.33 \times 10^{-2}$ mol dm^{-3}; $c_{Fe(II)\text{-}AA} = 2 \times 10^{-4}$ mol dm^{-3}; $c_{H_2O_2} = 0.133$ mol dm^{-3}; $c_{morin} = 0.226$ μg cm^{-3}; $t = 25 \pm 0.1$ °C

The dependencies of the reaction rate *s* on the acetic acid concentratios is shown in Fig. 1, which shows that the inhibited reaction is zero order with respect to the acetic acid concentration. From Fig. 1 appears that there is a complicated relationship between the acetic acid and the catalytic reaction rate in the range of concentration stud-

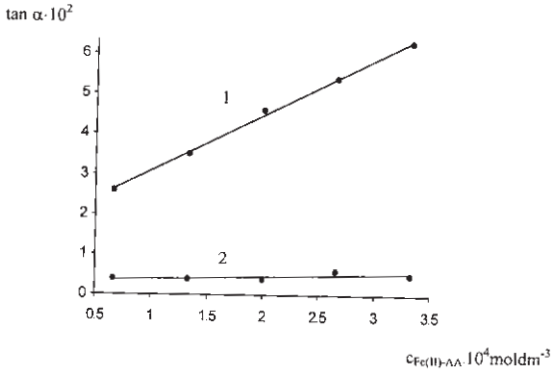

Fig. 3. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the complex Fe(II)-AA concentration. Initial concentrations: $c_{CH_3COOH} = 3.33 \times 10^{-2}$ mol dm^{-3}; $c_{C_6H_5COONa} = 2 \times 10^{-4}$ mol dm^{-3}; $c_{H_2O_2} = 0.133$ mol dm^{-3}; $c_{morin} = 0.226$ μg cm^{-3}; $t = 25 \pm 0.1$ °C

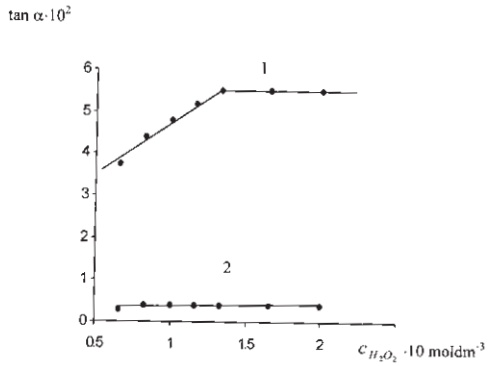

Fig. 4. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the H_2O_2 concentration. Initial concentrations: $c_{CH_3COOH} = 3.33 \times 10^{-2}$ mol dm^{-3}; $c_{C_6H_5COONa} = 2 \times 10^{-4}$ mol dm^{-3}; $c_{Fe(II)\text{-}AA} = 2 \times 10^{-4}$ mol dm^{-3}; $c_{morin} = 0.226$ μg cm^{-3}; $t = 25 \pm 0.1$ °C

ied. It can be seen that the greatest difference between the reaction rates occurs at $c_{CH_3COOH} = 3.33 \times 10^{-2}$ mol dm^{-3}, when morin maximally decreases the catalytic reaction rate. For further work, an acetic acid concentration of 3.33×10^{-2} mol dm^{-3} was selected. The pH of all solutions was constant 3.3.

The dependence of tan α on the C_6H_5COONa concentration is shown in Fig. 2, which shows that the difference in the rates of the inhibited and catalized reactions increases with increasing C_6H_5COONa concentration. Both reactions are first order with respect to the C_6H_5COONa concentration. For further work, a C_6H_5COONa concentration of 3.33×10^{-4} mol dm^{-3} was selected, because at higher concentrations the linear part of the kientic curves is rather short.

TABLE I. Summary of the kientic data for the oxidation of C_6H_5COONa by hydrogen peroxide in the presence of the complex Fe(II)-AA in acid medium

Variable and concentration range	Partial order for catalyzed reaction	Partial order for the inhibited reaction
0.666×10^{-2} mol dm^{-3} > c_{CH_3COOH} < 6.66×10^{-2} mol dm^{-3}	–0.5	0
1.33×10^{-4} mol dm^{-3} > $c_{C_6H_5COONa}$ < 4×10^{-4} mol dm^{-3}	+1	+1
0.667×10^{-4} mol dm^{-3} > $c_{Fe(II)\text{-}AA}$ < 3.33×10^{-4} mol dm^{-3}	+1	+1
$c_{H_2O_2} > 0.133$ mol dm^{-3}	0	0
$c_{H_2O_2} < 0.133$ mol dm^{-3}	+1	0

The correlation between tan α and the concentration of the Fe(II)-AA complex concentration is shown in Fig. 3, from which it appears that the inhibited reaction is zero order with respect to the concentration of the Fe(II)-AA complex, whereas the catalytic reaction is first order with respect to the concentration of the Fe(II)-AA complex. A Fe(II)-AA complex concentration of 2×10^{-4} mol dm^{-3} was selected for further work.

TABLE II. Accuracy and precision of the morin determination

Taken ng cm^3	Found ng cm^3	n	$S\times10^{10}$	$E\times10^{10}$	G %	× 100
2.255	2.195	5	0.73	0.9	5.1	1.7
11.275	11.22	5	2.23	0.997	2.4	1.5
22.55	22.43	5	1.15	0.5	1.42	0.53

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

TABLE III. Tolerance levels of interference in the kinetic determinatio of 11.25 ng cm^{-3} morin using the optimum conditions

Tolerance level c_{ION}/c_{morin}	Ion added
10^4	Na^+; Cl^-
10^3	NO_3^-; CH_3COO^-; PO_4^{-3}
10^2	K(I); Ca(II); Mg(II)
10	SO_4^{-2}; CO_3^{-2}; gallic acid; citric acid
	Fe(III); Cd(II); Co(II); Pt(II); Se(IV)
1	Al(III); SCN–; $C_2O_4^{2-}$; Br^-
1	rutin; rutin sulphate; quercetin(inhibited) interfere

The dependence of the reaction rates on the concentration of H_2O_2 is shown in Fig. 4. It can be seen that the catlaytic reaction is first order with respect to the H_2O_2 concentrfation up to 0.133 mol dm^{-3} and zero order for higher concentrations. The inhibited reaction is zero order with respect to the H_2O_2 concentrations. A H_2O_2 concentration of 0.133 mol dm^{-3} was selected for further work.

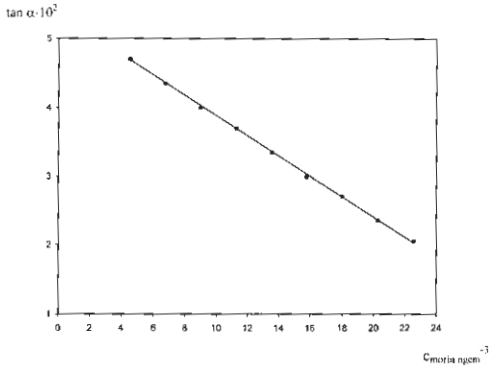

Fig. 5. Dependence of the rate of the reaction on the morin concentration. Initial concentrations: c_{CH_3COOH} = 3.33 × 10^{-2} mol dm^{-3}; $c_{C_6H_5COONa}$ = 2 × 10^{-4} mol dm^{-3}; $c_{Fe(II)\text{-}AA}$ = 2 × 10^{-4} mol dm^{-3}; $c_{H_2O_2}$ = 0.133 mol dm^{-3}; t = 25 ± 0.1 ºC

Under the optimal reaction conditions:

c_{CH_3COOH} = 3.33 × 10^{-2} mol dm^{-3}, $c_{C_6H_5COONa}$ = 3.33 × 10^{-4} mol dm^{-3}, $c_{Fe(II)\text{-}A\text{-}A}$ =

2×10^{-4} mol dm^{-3}, $c_{H_2O_2} = 0.133$ mol dm^{-3} the morin concentration was varied from 2.255 to 22.55 ng cm^{-3}.

Figure 5 shows the obtained calibration line, which can be used for the determination of the morin concentration in the mentioned concentration range. A linear dependece was established between tan α and the concentration of Se(IV):

$$\tan \alpha = 0.0535 - 0.0015\, c_{\text{morin}} \quad r = 0.998 \text{ at } 25 \pm 0.1\ ^{\circ}\text{C} \tag{1}$$

The partial orders in the different variables, for oxidation of C_6H_5COONa by hydrogen peroxide in the presence of the complex Fe(II)-A-A in acid medium are summarized Table I.

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

For the catalyzed reaction:

$$\frac{dc}{dt} = k_1 \cdot c_{CH_3COOH}^{-0.5}\, c_{C_6H_5COONa}\, c_{\text{Fe(II)-A-A}}\, c_{H_2O_2} \tag{2}$$

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

0.667×10^{-2} mol dm$^{-3} \geq c_{CH_3COOH} \leq 6.67 \times 10^{-2}$ mol dm^{-3}

$c_{H_2O_2} < 0.133$ mol dm^{-3}

For the inhibited reaction:

$$-\frac{dc}{dt} = k_2\ c_{C_6H_5COONa}\, c_{\text{Fe(II)-A-A}}\, c^{-1}_{\text{morin}} \tag{3}$$

were k_2 is a constant proportional to the conditional rate constant of the inhibited reaction.

On the basis of these equations, the conditional rate constants for the catalyzed and inhibited reactions were calculated.

$$k_1 = 1.11 \times 10^6 \text{ mol}^{2.5} \text{ dm}^{-7.5} \text{ s}^{-1}$$

$$k_2 = 2.07 \times 10^{-2} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

The minimum concentration of morin which could be determined by this method may be calculated by the method given by Perez–Bendito and Silva.[9]

The detection limit is 0.28 ng cm^{-3}.

The accuracy and precision of the measurements are presented in Table II. The relative error ranges from 1.42 to 5.10 % for morin concentrations from 2.255 to 22.55 ng cm^{-3}. To assess the selectivity of the method, the influence of several foreign ions on the rate of the activated reaction rates was studied at a constant morin concentration of 11.25 ng cm^{-3} (Table III). Regarding the selecitivty as determined by the 2s-kriterium.[10] It can be seen that for a morin concentration of 11.25 ng cm^{-3} quercetin and rutin and rutin sulphate in a 1:1 ratio to morin interfere with the reaction. The other investigated ions have practically no influence on the determination of morin by this method.

ИЗВОД

КИНЕТИЧКО ОДРЕЂИВАЊЕ НАНОКОЛИЧИНА МОРИНА

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Предложена је нова кинетичко-спектрофотометријска метода за одређивање морина. Метода се заснива на инхибиционом ефекту морина на оксидацију натријум бензоата водоник-пероксидом у киселој средини у присуству комплекса Fe(II)-AA(аскорбинска киселина), који има улогу катализатора. Граница детекције је 0,28 ng cm^{-3}. Релативна грешка методе се креће од 1,42–5,1 % за концентрациони интервал у опсегу од 2,255–22,55 ng cm^{-3}. Постављене су кинетичке једначине за испитиване процесе. Ради оцене селективности испитан је утицај већег броја страних јона и органских молекула на брзину реакције. Реакција је високо селективна према неорганским анјонима и катјонима. Главне карактеристике ове методе су висока осетљивост, селективност, репродуктивност, брзина извођења и једноставност.

(Примљено 22. септембра, ревидирано 10. децембра 2003)

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