Kinetic Spectrophotometric Method for the Determination of Trace Amounts of Oxalate by an Activation Effect

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A new, simple and inexpensive kinetic catalytic spectrophotometric method for the determination of oxalate is described. The method is based on an activation effect of oxalate on a catalytic effect of iron(II) on the oxidation of iodide by bromate. The reaction is monitored by measuring the absorbance of triiodide ion at $\lambda_{\text{max}} = 352$ nm. A calibration graph was obtained from 0.10–7.0 $\mu\text{g cm}^{-3}$ of oxalate with a detection limit of 0.080 $\mu\text{g cm}^{-3}$. The standard deviations for ten replicate determinations of 0.50, 1.0 and 5.0 $\mu\text{g cm}^{-3}$ of oxalate were 4.0, 2.6 and 1.8%, respectively. The applicability of the method was demonstrated by the determination of oxalate ion in real samples.

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Introduction

Oxalic acid is one of the most common nutrient chelaters in the human diet. Oxalates are found in a wide variety of foods, such as spinach, mushroom and beet leaves. Oxalic acid is a primary chelater of calcium, and its effect in the human diet is well known. Absorbed oxalic acid causes assimilated calcium to be precipitated as insoluble salts and the formation of renal calculi.¹ Thus, a sensitive, precise and rapid determination of oxalate is of interest. Various analytical methods were reviewed up to 1993^{2–4} for the determination of oxalic acid. Recently, oxalate (or oxalic acid) has been determined by a variety of methods, including high-performance liquid chromatography,^{5–7} ion chromatography,^{8,9} differential pulse voltammetry¹⁰ and capillary electrophoresis.^{11–14} Some of these methods provide high sensitivity and specificity, but also have drawbacks, such as having high costs for equipment and assays, being time-consuming and involving complicated operation. Oxalate biosensors have also been constructed.^{15–18} A disadvantage of these biosensors is that the lifespan is short due to an instability of the enzyme, and they are relatively expensive.

Kinetic catalytic spectrophotometric methods have been widely used because of significant advantages in the determination of many analytes at trace levels. A number of kinetic catalytic methods based on an inhibition or enhancing effect of oxalic acid on the rate of well-known reaction systems have been developed.^{19–25} Some of these methods have higher limits of detection^{20–23,25} and/or lower dynamic ranges^{19,21,22,24} compared to the proposed method.

In the present work a new kinetic catalytic spectrophotometric method is proposed for the determination of oxalate based on a catalytic effect of iron(II) on the oxidation of iodide by bromate. Oxalate acts as an activator on the catalytic effect of iron(II). The absorbance change of triiodide ion (I_3^-) at 352 nm is proportional to the oxalate concentration.

Experimental

Reagents and chemicals

Doubly distilled water and analytical reagent-grade chemicals were used. A stock iron solution containing 1000 $\mu\text{g cm}^{-3}$ Fe(II) was prepared by dissolving 0.7021 g of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (Merck) in 0.10 mol dm^{-3} sulfuric acid in a 100- cm^3 volumetric flask. A 0.120 mol dm^{-3} iodide solution was prepared by dissolving 2.0021 g of potassium iodide (Merck) in water and diluting to the mark in a 100- cm^3 volumetric flask. A 0.100 mol dm^{-3} bromate solution was prepared by dissolving 1.5090 g of sodium bromate (Merck) in water in a 100- cm^3 volumetric flask. An acetate buffer solution of pH = 5.0 was prepared by mixing appropriate amounts of a 0.20 mol dm^{-3} acetic acid solution with a 0.20 mol dm^{-3} sodium acetate solution and then adjusting the pH of the resulting solution. A standard oxalate solution as 1000 $\mu\text{g cm}^{-3}$ was prepared by dissolving 0.1523 g of $\text{Na}_2\text{C}_2\text{O}_4$ (Merck) in a 100- cm^3 volumetric flask.

Apparatus

For recording the UV-visible spectra and measuring the absorbance at a fixed wavelength, a Shimadzu UV-160 spectrophotometer with a 1.0-cm quartz cell was used. Measurements of the pH were made using a Metrohm 691 pH-meter equipped with a glass-saturated calomel combined electrode. A water-bath thermostat (Gallenkamp Griffin, BJ-240-V) was employed to control the reaction temperature. A stopwatch was used to record the reaction time.

Recommended procedure

All of the solutions were kept in a thermostated bath at 20.0°C for 30 min before initiation of the reaction. A sample solution was prepared by pouring a suitable amount of the standard oxalate solution (1.0–70.0 μg) into a 10- cm^3 standard flask; then, 2.0 cm^3 of the buffer solution (pH = 5.0), 1.0 cm^3 of 7.0 $\mu\text{g cm}^{-3}$ iron(II) and 1.0 cm^3 of a 0.120 mol dm^{-3} potassium iodide solution were added. After the mixture was diluted to ca. 8 cm^3 with water, 1.0 cm^3 of a 0.10 mol dm^{-3} sodium bromate solution

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Fig. 1 Effect of the pH. Conditions: bromate, 0.010 mol dm⁻³; iodide, 0.010 mol dm⁻³; iron(II), 0.50 μg cm⁻³; oxalate, 2.5 μg cm⁻³; temperature, 25.0°C.

Fig. 2 Effect of the iron(II) concentration on the reaction rate. Conditions: pH, 5.0; bromate, 0.010 mol dm⁻³; iodide, 0.010 mol dm⁻³; oxalate, 2.5 μg cm⁻³; temperature, 25.0°C.

Fig. 3 Effect of the potassium iodide concentration on the net reaction rate. pH, 5.0; bromate, 0.010 mol dm⁻³; iron(II), 0.70 μg cm⁻³; oxalate, 2.5 μg cm⁻³; temperature, 25.0°C.

Fig. 4 Effect of the sodium bromate concentration on the net reaction rate. pH, 5.0; iron(II), 0.70 μg cm⁻³; iodide, 0.012 mol dm⁻³; oxalate, 2.5 μg cm⁻³; temperature, 25.0°C.

was added and the solution was diluted to the mark. Then, an appropriate quantity of the reacting solution was transferred to the spectrophotometer cell, and the increase in absorbance at 352 nm was recorded against water for the first 0.5–4.0 min from initiation of the reaction (ΔA_s). The measurement in the absence of oxalate ion was repeated to obtain the absorbance change for the inactivated reaction; this blank value (ΔA_b) was subtracted from the previous value to yield the net analytical signal ($\Delta A_s - \Delta A_b$).

Procedure for preparation of real samples

In analyzing spinach and mushroom, an appropriate amount of each sample (2.5 g of spinach or 25 g of mushroom) was cut into small pieces and pounded into a paste in a mortar. After the obtained paste was mixed with water in a 250 cm³ reflux flask and boiled for 20 min, the mixture was cooled. The suspension was centrifuged and then filtered through filter paper (Whatman No. 1). The filtrate was diluted to 1000 cm³, and 2.0 cm³ of the sample solution was used for the determination of oxalate ion by the proposed method.

Results and Discussion

Bromate reacts with iodide in acidic media to produce triiodide according to the following reaction:

Reaction (1) was very slow at pH = 5.0. On the other hand,

the oxidation of iodide by bromate was catalyzed by iron(II) in the presence of ultra-trace amounts of oxalate ion as the activator. The mechanism of the catalytic effect of iron(II) and the activation effect of oxalate ion on reaction (1) is discussed in our previous report.²⁶ The reaction can be followed spectrophotometrically by monitoring the change in absorbance at 352 nm due to an increase in the triiodide (I_3^-) concentration.

Influence of variables

The effect of the pH on the net reaction rate was investigated by varying the pH in the range of 2.0–6.0 (Fig. 1). The results show that the net reaction rate increased up to pH = 5.0, whereas a high pH value caused a decrease in the net analytical signal. Thus, an acetate buffer solution with pH = 5.0 was selected for the study.

The effect of the iron(II) concentration on the reaction rate was studied at pH = 5.0 with an oxalate concentration of 2.5 μg cm⁻³ and 0.010 mol dm⁻³ bromate at 25°C. The results (Fig. 2) show that by increasing the iron(II) concentration up to 0.70 μg cm⁻³, the reaction rate increased, whereas greater iron(II) concentrations had no considerable effect on the sensitivity. Thus, a 0.70 μg cm⁻³ iron(II) concentration was selected for further studies.

Figure 3 shows the effect of the potassium iodide concentration on the net reaction rate with 2.5 and 0.70 μg cm⁻³ of oxalate and iron(II) concentration, respectively and pH = 5.0 at 25.0°C. The results show that an increase in the iodide concentration up to 0.012 mol dm⁻³ caused an increase in the net reaction rate and sensitivity, whereas at higher values of the iodide concentration the sensitivity remained nearly constant.

Table 1 Dynamic range and detection limit of some catalytic methods for oxalate determination

Reaction system	Oxalate effect	Dynamic range and DL ^b / $\mu\text{g cm}^{-3}$	Reference
$\text{I}^- + \text{H}_2\text{O}_2$ catalyzed by W	Inhibition	1 – 5	19
Methyl red + K_2CrO_4	Enhancing	0 – 140.8 (3.68)	20
Phenylfluorone + SnCl_2	Enhancing	0 – 1	21
Rhodamine B + $\text{K}_2\text{Cr}_2\text{O}_7$	Enhancing	0.06 – 4 (0.02)	22
Alizarin red S + Cr(III)	Enhancing	0.8 – 14	23
$\text{MnSO}_4 + \text{KIO}_4$	Enhancing	0.05 – 1.25 (0.027)	24
Victoria blue B + $\text{K}_2\text{Cr}_2\text{O}_7$	Enhancing	1.0 – 80.0 (0.80)	25
$\text{I}^- + \text{BrO}_3^-$ catalyzed by Fe^a	Enhancing	0.10 – 7.0 (0.080)	—

a. Proposed method.

b. Detection limit.

Table 2 Interferences for the determination of oxalate ($2.0 \mu\text{g cm}^{-3}$)

Species	Tolerance limit (weight ratio) (W species/W oxalate)
Cl^- , NO_3^- , Br^- , Cr^{3+} , K^+ , Na^+ , ClO_3^- , ClO_4^-	1000 ^a
Glucose, sucrose, fructose	500
Tartaric acid, benzoic acid, F^-	400
PO_4^{3-} , NO_2^-	200
Cd^{2+} , uric acid	40
Ba^{2+} , Co^{2+} , Ni^{2+} , Al^{3+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , Cu^{2+}	20 (200 ^b)
SO_3^{2-}	10

a. Maximum ratio tested.

b. After acidification and passing through a cation exchanger resin.

Thus, a $0.012 \text{ mol dm}^{-3}$ potassium iodide concentration was selected for the further studies.

The influence of the sodium bromate concentration on the sensitivity was studied in the range of $(0.10 - 1.2) \times 10^{-2} \text{ mol dm}^{-3}$ under other optimized conditions at 25.0°C . The results (Fig. 4) show that with increasing the bromate concentration up to $0.010 \text{ mol dm}^{-3}$ the sensitivity increased and then decreased slightly. Therefore, a $0.010 \text{ mol dm}^{-3}$ bromate concentration was used for further studies.

The influence of the temperature on the sensitivity ($\Delta A_s - \Delta A_b$) was studied at $\text{pH} = 5.0$, and optimized concentrations of other reagents in the range of $10.0 - 60.0^\circ\text{C}$. The results show that below 20.0°C , the net analytical signal ($\Delta A_s - \Delta A_b$) increased with temperature, but at temperatures higher than 20.0°C , it decreased due to an increase in ΔA_b . Thus, the temperatures of the reagent solutions and reaction mixture were fixed at 20.0°C .

The salt effect on the net reaction rate was studied using NaNO_3 in the concentration range of $0.05 - 0.20 \text{ mol dm}^{-3}$. The results show that the ionic strength of the reaction mixture had no considerable effect on the net reaction rate and the sensitivity.

Analytical parameters

Under the chosen experimental conditions, the difference in the absorbance change between blank and sample solutions varied linearly with the concentration of oxalate ion in the range from 0.10 to $7.0 \mu\text{g cm}^{-3}$ with the regression equation $\Delta A = 5.35 \times 10^{-3} + 0.049C_{\text{oxalate}}$ ($r = 0.9989$ for $n = 10$), where ΔA is the

Table 3 Analysis of spiked water samples by the proposed method

Sample	Oxalate added/ $\mu\text{g cm}^{-3}$	Oxalate found ^a / $\mu\text{g cm}^{-3}$	Recovery, %
Tap water	0.40	0.38 (± 0.02)	95.0
Tap water	3.00	2.92 (± 0.05)	97.3
Tap water	6.00	6.21 (± 0.11)	103
River water	0.80	0.76 (± 0.03)	95.0
River water	4.00	3.78 (± 0.07)	94.5

a. $\pm$ Standard deviation ($n = 5$).

Table 4 Results for the determination of oxalate in real samples

Sample	Added/ $\mu\text{g cm}^{-3}$	Found ^a / $\mu\text{g cm}^{-3}$	Recovery, %	Content in sample/ g kg^{-1}	
				This method	Reference method ³
Spinach	—	3.81 (± 0.08)	—	7.62	7.75
	2.00	5.70 (± 0.15)	94.5		
	3.00	6.74 (± 0.19)	97.7		
Mushroom	—	1.63 (± 0.05)	—	3.26	3.50
	2.00	3.54 (± 0.10)	95.5		
	4.00	5.71 (± 0.12)	102		

a. $\pm$ Standard deviation ($n = 5$).

difference in the absorbance change between a blank and the sample ($\Delta A_s - \Delta A_b$), and C_{oxalate} is the oxalate concentration in $\mu\text{g cm}^{-3}$.

The 3σ limit of detection was $0.080 \mu\text{g cm}^{-3}$. The relative standard deviations for ten replicate determinations of 0.50 , 1.0 and $5.0 \mu\text{g cm}^{-3}$ of oxalate were 4.0% , 2.6% and 1.8% , respectively. A comparison between the proposed method and other reported methods is shown in Table 1. This comparison shows that the proposed method has a better dynamic range and/or detection limit than other reported methods.

Interference study

The effect of various substances on the determination of oxalate in a sample solution containing $2.0 \mu\text{g cm}^{-3}$ oxalate was examined under the optimum conditions. The tolerance limit was defined as the concentration at which the species caused an error in the net signal less than three-times standard deviation of the sample solution. The obtained results are given in Table 2. The results show that the most of the anions could not interfere in the determination of oxalate ion. Some cations, such as Ba^{2+} , Co^{2+} , Ni^{2+} , Al^{3+} , Ca^{2+} , Zn^{2+} , Mn^{2+} and Cu^{2+} , form a complex or precipitate with oxalate ion and interfere at 20-fold. The interference of this cations can be reduced to 200-fold with acidification of the solution and passing it through a cation exchanger resin, and then undergoing neutralization of the passed solution by the droplet addition of a sodium hydroxide solution.

Application

To investigate the applicability of the method to real samples, the proposed method was applied directly to the determination of oxalate ion in spiked water samples and water extracted from spinach and mushroom. The food samples were prepared according to the recommended procedure for preparation of real samples; the standard-addition method was used for the

analysis. The results are summarized in Tables 3 and 4.

Conclusion

The presented results clearly demonstrate that the activation effect of oxalate on the catalytic oxidation of iodide by bromate can be used for the determination of oxalate. The reliability and simplicity of this method permits the analysis of a large number of samples with satisfactory precision and accuracy.

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