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Spectrochimica Acta Part A 58 (2002) 2589–2594

SPECTROCHIMICA ACTA PART A

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Kinetic–spectrophotometric determination of ascorbic acid by inhibition of the hydrochloric acid–bromate reaction

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Received 26 November 2001; accepted 16 December 2001

Abstract

A new analytical method was developed for the determination of ascorbic acid in fruit juice and pharmaceuticals. The method is based on its inhibition effect on the reaction between hydrochloric acid and bromate. The decolourisation of Methyl Orange by the reaction products was used to monitor the reaction spectrophotometrically at 510 nm. The linearity range of the calibration graph depends on bromate concentration. The variable affecting the rate of the reaction was investigated. The method is simple, rapid, relatively sensitive and precise. The limit of detection is 7.6×10^{-6} M and calibration rang is 8×10^{-6}–1.2×10^{-3} M ascorbic acid. The relative standard deviation of seven replication determinations of 8×10^{-6} and 2×10^{-5} M ascorbic acid was 2.8 and 1.7%, respectively. The influence of potential interfering substance was studied. The method was successfully applied for the determination of ascorbic acid in pharmaceuticals.

Keywords: Ascorbic acid; Inhibition; Bromate; Methyl orange; Spectrophotometry

1. Introduction

Ascorbic acid is an essential vitamin with a recommended of daily intake of about 70 mg. Continuing interest in the benefits of a well-balanced vitamin intake has resulted in the fortification of many food products with a variety of vitamins. With insufficient uptake, the symptoms of scurvy appear [1].

Ascorbic acid occurs naturally in most fresh fruits and fruit juices, and is often added during the manufacture of juices or soft drinks. Consequently, there has been considerable interest in alternative methods of determining the ascorbic acid content of food products. Nowadays quantification of ascorbic acid is mainly performed by high-performance liquid chromatography or capillary electrophoresis [2–5]. As spectrophotometric methods are the instrumental methods commonly used in industrial laboratories; a great number of colorimetric methods have been proposed for the determination of ascorbic acid [6–15]. The majority of these methods are based on their oxidation–reduction properties or their ability to couple with diazotized aniline derivatives. Some of these methods are time consuming and

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1386-1425/02/$ - see front matter
PII: S1386-1425(02)00024-0

suffer from lack of selectivity or good sensitivity and/or have short linear dynamic range or have higher limit of detection and/or used reagents which are not commercially available. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis.

In the present work, a sensitive, facile, and relatively selective method was developed for the determination of ascorbic acid based on its inhibiting effect on the reaction of bromate with hydrochloric acid. The decolourisation of Methyl Orange by the reaction products was used to monitor the reaction spectrophotometrically at 510 nm.

2. Experimental

2.1. Reagents and chemicals

Doubly distilled water and analytical reagent grade chemicals were used. Ascorbic acid stock solution 0.010 M was prepared immediately before use by dissolving 0.1762 g ascorbic acid (Merck) in cold water. This solution was diluted quantitatively with water in a 100-ml volumetric flask.

A 0.010 M sodium bromate solution was prepared by dissolving 1.510 g $NaBrO_3$ (Merck) in water and diluting to 100 ml in a volumetric flask.

A solution of Methyl Orange (100 mg/l) was prepared by dissolving 0.0250 g Methyl Orange (Merck) in water and diluting to 250 ml with water. Hydrochloric acid was prepared by appropriate dilution of conc. hydrochloric acid (Merck).

2.2. Apparatus

A Spectronic 20 (Genesys) spectrophotometer with two matched 1-cm quartz cells was used to get an absorbance graph at various wavelengths. A Spectronic 20 (Genesys, Model 4001/4) spectrophotometer with a 1-cm glass cell was used to get absorption-time graphs at a fixed wavelength. A thermostat water bath (Memmert, Model KG 8540, Schwabachw, Germany) was used to keep the temperature of solutions at 35 ± 0.1 °C.

2.3. Recommended procedure

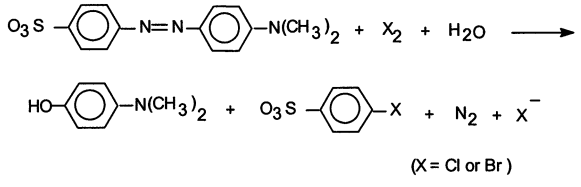
The inhibited reaction was followed spectrophotometrically by monitoring the change in absorbance of the mixed reagents solution at 510 nm. An aliquot of sample solution containing 8×10^{-5}–1.2×10^{-2} mmol ascorbic acid was transferred into a 10-ml volumetric flask, and then 1.4 ml of 2.33 M hydrochloric acid was added, followed by 1.0 ml 100 mg/l Methyl Orange solution. The solution was diluted to ca. 8 ml with water. Then 1.0 ml 1.44×10^{-3} M bromate was added to the solution and the result solution was diluted to the mark with water. The solution was mixed and a portion of the solution was transferred to the spectrophotometric cell. The change in absorbance with time was measured with time for 10–240 s from initiation of the addition of last drop of bromate solution. All the solution was kept at 35 °C.

3. Results and discussion

Bromate can be reduced by hydrochloric acid as follows:

$$10Cl^- + 2BrO_3^- + 12H^+ \rightleftharpoons 5Cl_2 + Br_2 + 6H_2O$$

The produced bromine and chlorine react with Methyl Orange and this reaction causes decolourisation of Methyl Orange [16] as in the following reaction:



This system has been used for determination of hydrazine [17] and arsenic [18]. This reaction can be monitored spectrophotometrically by measuring the decrease in absorbance of the reaction mixture at 510 nm at 35 °C. Ascorbic acid can react with the product of the reaction (bromine and chlorine); therefore, the induction period increases with increasing ascorbic acid concentration (Fig. 1). This inhibitory effect on the reaction

system depends on the concentration of ascorbic acid. The induction period can be measured mathematically from the regression equations of the linear part of the absorption-time graph. The regression equation for the first linear part of the graph is:

$$A = a_1 + b_1 t$$

and for the second linear part is:

$$A = a_2 + b_2 t$$

By equating these equations the induction period can be calculated as:

$$T_{ip} = a_1 - a_2/b_2 - b_1$$

Therefore, the calibration graph can be prepared by plotting t_{ip} versus ascorbic acid concentration.

3.1. Influence of variables

The effect of reagents concentration and temperature on the reaction system was studied to get the best sensitivity and find optimum conditions.

The influence of hydrochloric acid concentration on the sensitivity was studied over the range of 0.20–0.40 M with and without ascorbic acid at 30 °C. Fig. 2 shows the change in absorbance with time as a function of hydrochloric acid. In addition, Fig. 3 shows that by increasing HCl concentration up to 0.35 M, the difference in absorbance change for uninhibited reaction and inhibited reaction increases, whereas greater amounts of the acid concentration decrease this difference. Therefore, 0.35 M HCl was selected for study.

Fig. 4 shows the effect of bromate concentration on the induction period time. The results show that by increasing bromate concentration, the induction period time decreased and the slope of the absorbance change increases for uninhibited reaction (after initiation of the reaction). In

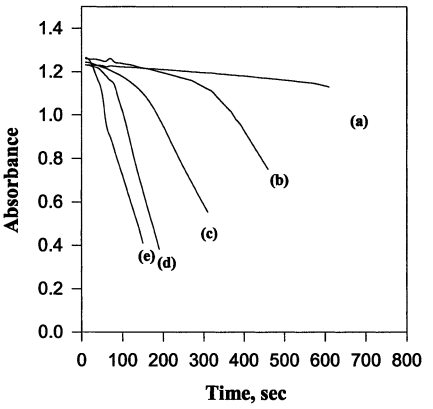


Fig. 2. Variation of absorbance with time as a function of hydrochloric acid; (a) 0.20 M; (b) 0.25 M; (c) 0.30 M; (d) 0.35 M; and (e) 0.40 M HCl; Conditions: 2.0×10^{-5} M ascorbic acid, 1.44×10^{-4} M bromate, and 10.0 mg/l Methyl Orange.

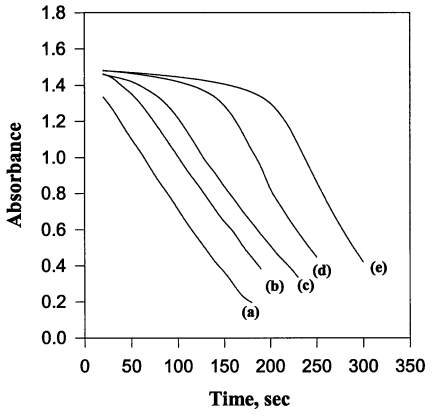


Fig. 1. Absorbance change of Methyl Orange–bromate–HCl system, (a) 0.00; (b) 1.0×10^{-5} M; (c) 2.0×10^{-5} M; (d) 4.0×10^{-5} M and (e) 6.0×10^{-5} M ascorbic acid. Conditions: 10.0 mg/l Methyl Orange, 1.44×10^{-4} M bromate, and 0.35 M HCl.

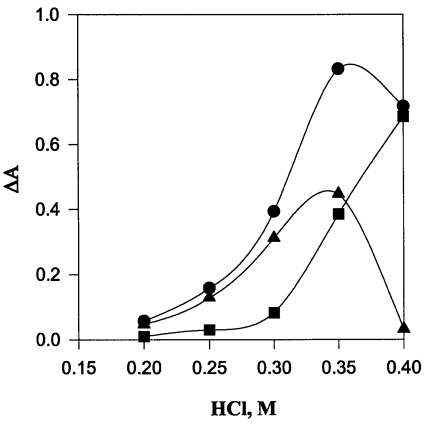


Fig. 3. Absorbance change for inhibited reaction (■), uninhibited reaction (●), and for their difference (▲) as a function of HCl concentration. Conditions: 2.0×10^{-5} M ascorbic acid, 1.44×10^{-4} M bromate, and 10.0 mg/l Methyl Orange.

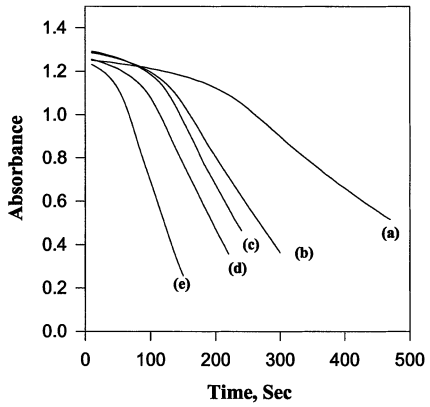


Fig. 4. Effect of bromate concentration on the induction period time; (a) 7.2×10^{-5} M, (b) 1.15×10^{-4} M, (c) 1.44×10^{-4} M, (d) 1.72×10^{-4} M, and (e) 2.16×10^{-4} M bromate. Conditions: 2.0×10^{-5} M ascorbic acid, 0.35 M HCl, and 10.0 mg/l Methyl Orange.

order to find the optimum concentration of bromate, the change in absorbance-time was plotted for the system with and without addition of ascorbic acid (Fig. 5), for the first 10–240 s from initiation of the reaction. The results show that the best sensitivity can be achieved in the presence of 1.44×10^{-4} M bromate concentration. Therefore, this concentration was selected for the study.

The influence of Methyl Orange concentration on the sensitivity was studied in the presence of

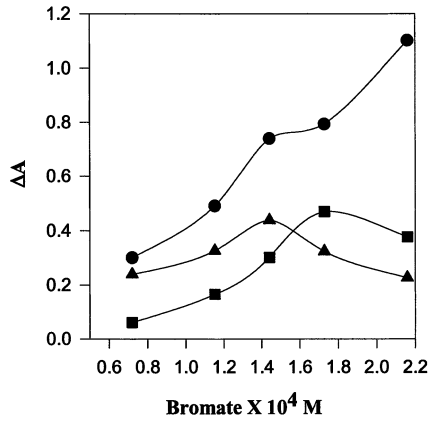


Fig. 5. Absorbance change for inhibited reaction (■), uninhibited reaction (●), and for their difference (▲) as a function of bromate concentration. Conditions: 2.0×10^{-5} M ascorbic acid, 0.35 M HCl, and 10.0 mg/l Methyl Orange at 35 °C.

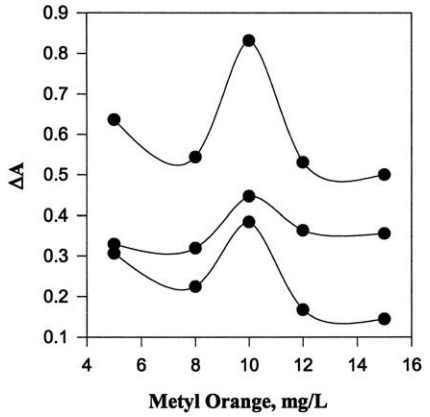


Fig. 6. Absorbance change for inhibited reaction (■), uninhibited reaction (●), and for their difference (▲) as a function of Methyl Orange concentration. Conditions: 2.0×10^{-5} M ascorbic acid, 0.35 M HCl, and 1.44×10^{-4} M bromate at 35 °C.

0.35 M HCl and 1.44×10^{-4} M bromate concentration at 30 °C with and without addition of ascorbic acid (Fig. 6). The results show that by increasing Methyl Orange concentration up to 10 mg/l, the sensitivity increases, whereas greater amount of the dye decreased the sensitivity. Therefore, a 10 mg/ml Methyl Orange was selected for the study.

The sensitivity of the system dose not depend on the ionic strength up to 1.0 M ionic strength (using 3.0 M K_2SO_4).

The influence of temperature on the sensitivity was studied over the temperature range of 10–40 °C in the presence of optimum reagent concentration. The results showed by increasing temperature up to 35 °C that the sensitivity increased, and after that the sensitivity decreased. This may be due to the fact that, at higher temperature, decomposition of ascorbic acid occurs and thus, the inhibitory effect decreases. Therefore, 35 °C was selected for the study.

3.2. Calibration graph, precision and limit of detection

Under the optimum conditions a linear correlation was found between the induction period and ascorbic acid concentration. The linear dynamic

Table 1
Linear regression parameters of calibration data for different concentration of bromate

BrO_3^- (M)	Slope (s/M)	Intercept (s)	r ($n = 13$)	Detection limit (M)	Calibration range (M)
1.44×10^{-4}	30.79×10^5	37.99	0.999	7.6×10^{-6}	8×10^{-6}–2.0×10^{-4}
4.32×10^{-4}	37.90×10^4	63.58	0.999	5.5×10^{-5}	4×10^{-5}–1.2×10^{-3}

Table 2
Tolerance limit for diverse ions on the determination of 14.0 μg/ml ascorbic acid

Substance	Tolerance limit ratio ($mole_{Substance}/mole_{ascorbic\ acid}$)
$C_2O_4^{2-}$, ClO_3^-, K^+, Na^+, NO_3^-, SO_3^{2-}, SO_4^{2-}, MoO_4^{2-}, PO_4^{3-}, ethanol, sucrose, glucose, fructose, galactose	500[a]
Fe^{3+}, Al^{3+}, Zn^{2+}, CN^-, CO_3^{2-}, Cl^-, salicyl aldehyde, acetate	100
Pb^{2+}, Ni^{3+}, Cd^{2+}, citrate, benzoic acid	50
Ca^{2+}, Mn^{2+}, Mg^{2+}	25
Co^{2+}, Cr^{3+}	5
Br^-, I^-, Cu^{2+}, SCN^-, Ag^+	<1

[a] Maximum concentration of substances tested.

range depends on the bromate concentration and the results are presented in Table 1. The limit of detection ($3s_b/m$, three of the standard deviation blank divided by slope of the calibration curve) was 7.6×10^{-6} M, ascorbic acid. The relation standard deviation for seven-replication determination of 8×10^{-6} M and 4.0×10^{-5} M ascorbic acid was 2.8 and 1.7%, respectively.

3.3. Effect of interfering substances

The effect of various substances on the determination of 14 μg/ml ascorbic acid was studied and the results are shown in Table 2. The tolerance was defined as the concentration of added substance causing a relative error less than 3%. Many substances did not interfere, even when present in 500-fold excess over ascorbic acid. Positive interference where observed from Fe(III) and benzoic acid.

3.4. Applications

In order to evaluate the potential of the proposed method to analysis of real sample, the method was applied to pharmaceutical samples for the determination of ascorbic acid. Pharmaceutical tablet solutions were prepared by dissolving each tablet in the water. Several pharmaceutical dosage forms containing ascorbic acid in tablets were diluted with water prior to spectrophotometric determination. The results ob-

Table 3
Determination of ascorbic acid in pharmaceutical compounds

Tablets (mg/tablet)	Ascorbic acid found (mg/tablet)	Standard method (mg/tablet)	Manufacture certified (mg/tablet)
Vitamin C[a] (Osvah-Iran Co.)	998 ± 10	985 ± 15	1000
Vitamin C[a] (Osvah-Iran Co.)	490.8 ± 5	497 ± 8	500
Vitamin C[a] (Modava Pharma Co.)	251 ± 5	246 ± 8	250
Multivitamin[b] (Osveh-Iran Co.)	63 ± 2	58 ± 5	60

[a] Containing 12 mg sodium saccharine.
[b] Containing vitamin C, vitamin B_1, B_2, B_3, B_6, B_{12}, folic acid, potassium chloride and calcium pantothenate.

tained by the proposed method and the 2,6-dichlorophenolindophenl [19] method are given in Table 3.

4. Conclusion

With the proposed kinetic method it is possible to determine ascorbic acid at trace levels. Because the detection limit is dependent on the concentration of bromate, it is expected that the limit could be lowered further if lower concentration of bromate is selected. The method is very simple, rapid, sensitive and relatively selective for determination of ascorbic acid. The procedure is suitable for the analysis of various pharmaceutical samples, with satisfactory results.

Acknowledgements

The authors are thankful to the Center of Excellency in Chemistry Research (IUT) and to the Research Council of Isfahan University of Technology for the support of this work.

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