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KINETIC DETERMINATION OF ASCORBIC ACID WITH SPECTROPHOTOMETRIC DETECTION

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Abstract

A simple and rapid method has been developed for the determination of ascorbic acid in fruit and pharmaceutical products. The method is based on the measurement of the reaction rate between ammonium molybdate and ascorbic acid in acidic media. The reaction was monitored spectrophotometrically at 800 nm by fixed-time and variable-time methods. The calibration graph was linear in the range of 50×10^{-6} - 6.0×10^{-3} M ascorbic acid when using a fixed-time method. The relative standard deviation for ten replicate analyses is 2.7% for 5.0×10^{-5} M ascorbic acid. The limit of detection is 2.0×10^{-6} M. The effect of various species was studied for this determination.

Introduction

Vitamin C, (l-ascorbic acid), is classified as a carbohydrate and has the chemical structure of l-keto-l-thero-hexono-γ-lactone-2,3-enediol. It is the enediol-group [-C(OH) = C(OH)-] which is responsible for the molecule acidic and reducing properties [1].

Ascorbic acid may reduce multivalent metal ions and their chelate to colored products. Methods of determination of ascorbic acid by spectrophotometry, chromatography, and electrochemistry have recently been reviewed [2-4]. Other methods such as adsorption potentiometric [5,6], coulometric [7,8], titrimetric [9], biosensor [10], cheluminescence [11] and photochemical [12] methods have also been used for the determination of ascorbic acid. Many of these methods suffer from a non-linear calibration [6-8] and/or have the drawback of requiring a reagent that is not commercially available [5,10,11], or needing complex instrumentation [12] or long reaction time to complete the determination [5,6,8].

Only a few kinetic methods for ascorbic acid determination are described in the literature [13]. A kinetic spectrophotometric method has been described [13] based on monitoring its reaction with toluidine blue. Recently, we reported a method for the determination of hydrazine based on its reaction with ammonium molybdate [14]. This paper describes the development of a new method for the determination of ascorbic acid based on its reaction with molybdate in hydrochloric acid media. This method is very rapid, simple, sensitive and relatively selective, and uses only readily available reagents.

Keywords: Ascorbic acid; Kinetic; Molybdate

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Experimental Section

Reagents

All the reagents were of analytical reagent grade and doubly distilled water was used throughout.

Mo(VI) standard solution (0.200 M) was prepared by dissolving 17.656 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (Merck) in about 300 ml of 0.01 M sodium hydroxide solution. Then the solution was neutralized by the addition of 0.1 M hydrochloric acid solution and diluted to 500 ml with

water in a 500-ml volumetric flask.

A stock solution of ascorbic acid (0.0100 M) was prepared from a pure 99.9% (BDH) compound, previously dried at 50°C in a vacuum oven magnesium perchlorate, by directly dissolving in water.

Solutions of hydrochloric and sulfuric acid were prepared from concentrated acid (Merck).

Apparatus

A spectrophotometer (Shimadzu, Model 256) was used for recording the absorbance spectra at various wavelengths. A spectrophotometer (Shimadzu, Model 120-01) with a 1.0 cm glass cell was used for absorbance measurements at a fixed wavelength (800 nm). A thermostat water bath (Gallenkamp Griffine) was used for maintaining the temperature of the solution.

Determination of Ascorbic Acid in Pharmaceutical Products

A vitamin C tablet solution was prepared by the following procedure: Ten tablets of the proprietary drug to be investigated were accurately weighed, crushed and powdered. An amount of this powder equivalent to 0.200 g was dissolved in 50 ml water. This was then left for 10 min for all gases to subside, filtered, washed and 2.0 mmoles of sulfuric acid added before finally being made up to volume in a 100-ml volumetric flask with water.

For the fruit juice, 10 ml of 10% trichloroacetic acid solution was added to a suitable aliquot of the fruit juice (for example 10 ml) to prevent oxidation of ascorbic acid. The solution was filtered and diluted to a suitable volume. Then its ascorbic acid content was determined by the proposed method with the standard addition method (due to the unknown ionic strength).

Recommended Procedure

The reaction between ascorbic acid and Mo(VI) in the presence of hydrochloric acid was followed spectrophotometrically by monitoring the absorbance change at 810 nm. Into a 10-ml volumetric flask, 1.0 ml of 0.40 M HCl solution and 4.0 ml of 0.20 M Mo(VI) solution were added. The solution was diluted to ca. 8 ml with water and was kept at 50°C in a thermostat bath for 10-15 min. Then 1.0 ml of ascorbic acid solution (>1 $\times 10^{-4}$ M) was added to initiate the reaction. The solution was diluted to the mark with water. After mixing the solution, an appropriate volume was transferred to the spectrophotometric cell to measure the absorbance increase. The time required for the absorbance to increase to 0.050 (or 0.500 for ascorbic acid concentrations greater than 0.005 M) was measured (variable-time method), or the increase in absorbance was measured against water in the 0.5-4.0 min from initiation of the reaction (fixed-time method). In both procedures, zero time was taken as the moment at which the last drop of ascorbic acid had been added and the solution was diluted to the mark with water.

Results and Discussion

In acidic solution, a solution of Mo(VI) in the presence of ascorbic acid (AA) undergoes a rapid reaction. This reaction, which produces blue soluble compounds, can be monitored spectrophotometrically by measuring the absorbance of the solution at 800 nm (Fig. 1). The variable-time and fixed-time methods were used for

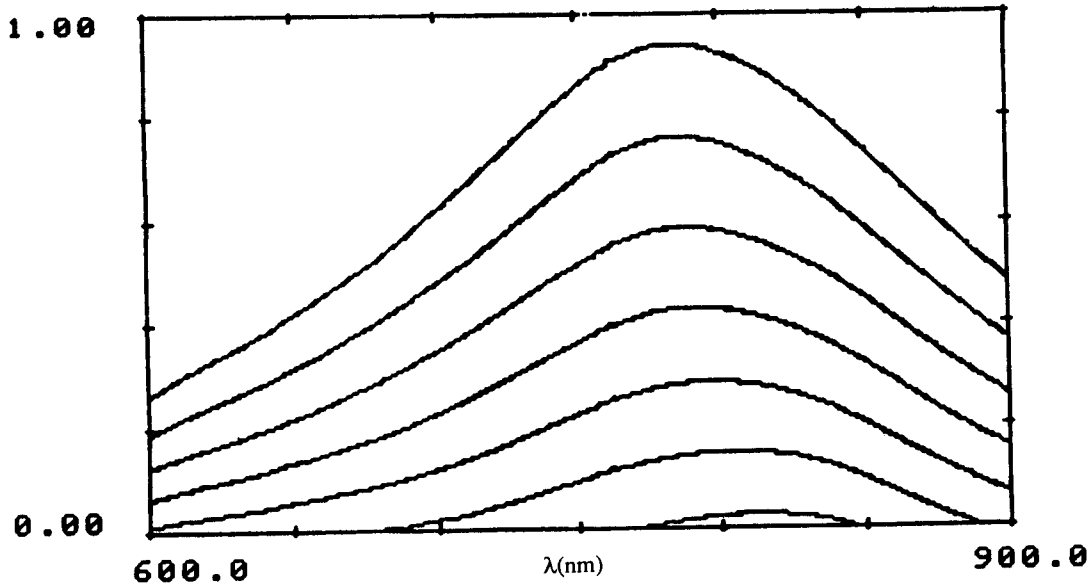

Figure 1. Variation of Mo(VI)-Ascorbic acid-HCl system with time; Conditions: HCl, 0.040 M; Mo(VI), 0.050 M; Ascorbic acid, 1.0×10^{-4} M at 33°C; a) 0.50, b) 2.0, c) 4.0, d) 6.0, e) 8.0, f) 10.0, g) 12.0 min from initiation of the reaction.

obtaining the calibration graph. For optimization of the conditions, the variable-time method was used.

The rate equation of the reaction is:

$$\text{Rate} = k[\text{Mo(VI)}]^a[H^+]^b[\text{AA}]^c \quad (1)$$

where k is the rate constant. Because $[H^+]$ and [Mo(VI)] >> [AA], $[H^+]$ and [Mo(VI)] can be considered to be constant, and c was found to be one. By integrating Equation (1) and incorporating Beer's law, we obtained the final expression for the fixed-time method:

$$\Delta A = (1/2.303)k'[\text{AA}]t \quad (2)$$

where t and ΔA are the reaction time and absorbance increase at the fixed time, respectively. For the variable time, with a fixed absorbance given, Equation (1) can be written as the final expression:

$$(1/\Delta t) = k'[\text{AA}] \quad (3)$$

where Δt is the interval time needed for a given fixed absorbance (net absorption of the solution).

Effect of Variables

Experimental results showed that the reaction between AA and Mo(VI) can proceed only in strongly acidic media (pH<3). Between sulfuric and hydrochloric acid, hydrochloric acid was the best at the same concentration. This is due to the fact that it has no effect on the blank

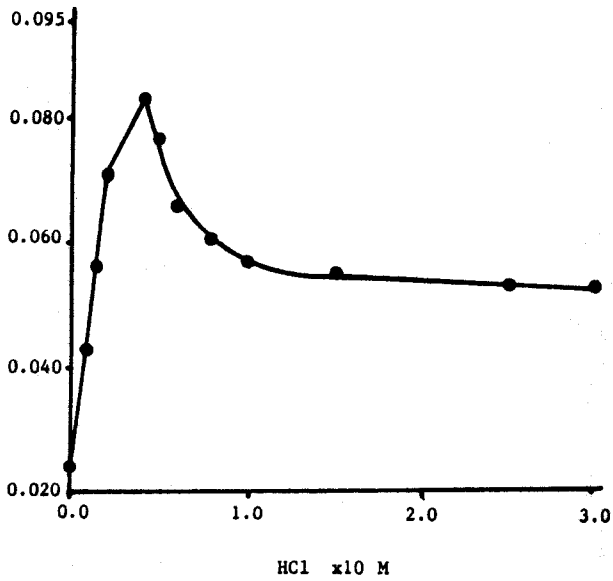

Figure 2. Effect of HCl concentration on the rate of reaction

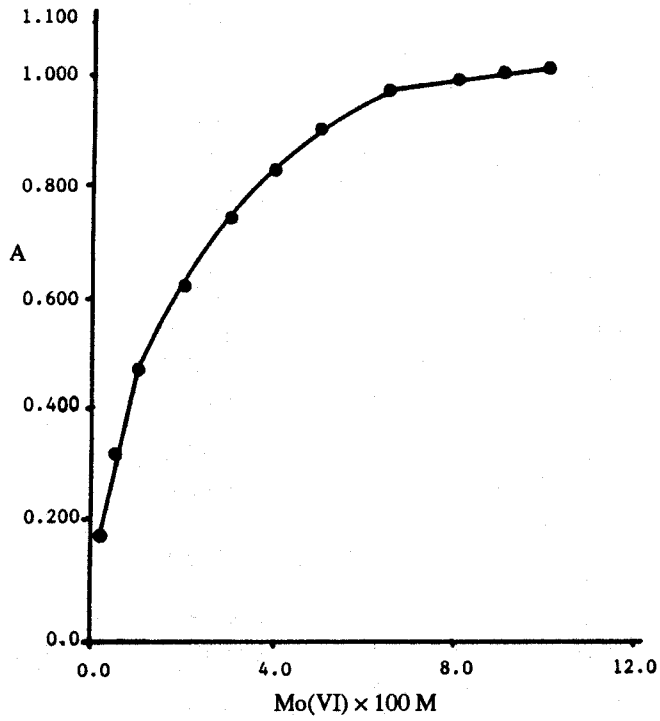

Figure 3. Effect of Mo(VI) concentration on the reaction rate

reaction. Thus, HCl solution was used for further study.

The effect of HCl concentration on obtaining a maximum reaction rate was investigated with 0.050 M Mo(VI) and 5.0×10^{-4} M ascorbic acid at 50°C (Fig. 2). The results show that the optimum concentration of HCl is 0.040 M in the final solution. This is due to the fact that in the diluted acid concentration, only one of the hydrogen groups of carbon No. 2 or No. 3 in AA is protonated, and thus produces an elimination group (H_2O^+) for oxidation of AA. On the other hand, however, and at the same time, in the presence of higher acid concentrations, two hydroxyl groups of the AA are protonated and thus the elimination reaction (oxidation reaction) is made more difficult. Consequently, 0.040 M HCl was selected for this study.

The effect of Mo(VI) concentration on the reaction rate was studied to achieve higher sensitivity. By increasing Mo(VI) concentration in the presence of 0.040 M HCl and 5.0×10^{-4} M ascorbic acid at 50°C, the reaction rate increased. Thus 0.080 M Mo(VI) concentration was selected for this study (Fig. 3).

The effect of reaction temperature was studied with optimum reagent concentration and 5.0×10^{-4} M ascorbic acid, in the range of 5-65°C. Figure 4 shows that by increasing temperature, the reaction rate was increased. At temperatures higher than 50°C, the reproducibility of the method becomes poor. Thus, 50°C was selected as the working temperature at which the study could easily be

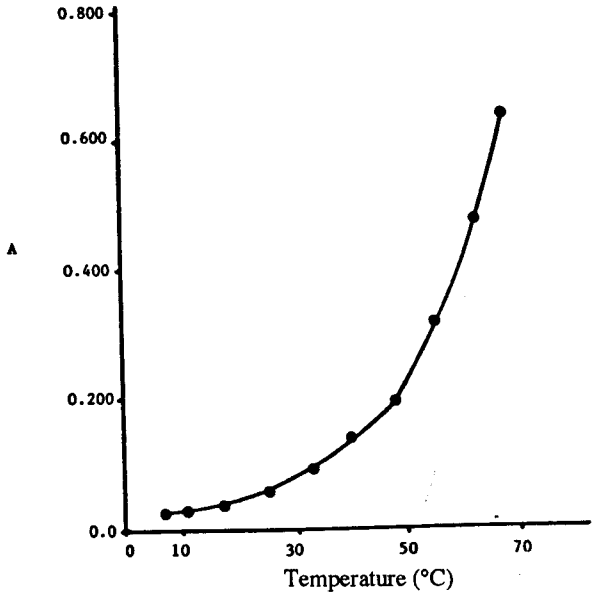

Figure 4. Effect of temperature on the rate of reaction

performed.

The effect of ionic strength on the reaction rate was studied with the optimum reagent concentration and 5.0×10^{-4} M AA concentration. Results show that by increasing ionic strength up to 1.5 M (using $NaNO_3$) a slight decrease in the reaction rate was caused.

Calibration Graph

Under the optimum conditions described above, calibration graphs were obtained by applying the variable-time and fixed-time methods. When the variable-time method was used, the calibration graph of (1/time) vs. ascorbic acid was linear for an ascorbic acid concentration of 5.0×10^{-6}-7.0×10^{-4} M (for a given absorbance of 0.040) with regression equation of: $(1/t)\ (min^{-1}) = 0.0114+571.5C_{AA}$ ($r = 0.9968$), and 7.0×10^{-4}-6.0×10^{-3} M (for a given absorbance of 0.500), the regression equation is: $(1/t) = 0.156+320.92C_{AA}$ ($r = 0.9988$), where C_{AA} is the molar concentration of AA.

For the fixed-time method, the calibration curve was linear for ascorbic acid concentration of 5.0×10^{-6}-5.0×10^{-3} M, with regression equation: $\Delta A = 1.292\times10^{-3}+311.05C_{AA}$ with $r = 0.9999$, where C_{AA} is the molar concentration of AA.

The 3s limit of detection [15] was 2.0×10^{-6} M and 4.0×10^{-6} M of ascorbic acid using the variable-time and fixed-time method, respectively.

The precision and accuracy of the analysis of ten replicate analyses of a series of samples containing various amounts of ascorbic acid by the variable-time method are shown in Table 1.

Table 2. Effect of foreign species on the determination of 5.0×10^{-5} M ascorbic acid

Species	Tolerance limit ($\mu g.ml^{-1}$)
Na^+, K^+, Ca(II), Mg(II), Co(II), Ni(II), Cr(II), Cd(II), Al(III), Mn(II), Hg(II), Sr(II), Ce(III), Ba(II), Li^+, SO_4^{2-}, HCO_3^-, $S_2O_3^{2-}$ acetate, benzoate, tartarate, citrate, Cl^-, Br^-, I^-, oxalate, glucose, fructose, saccharose	1000*
Fe(III), Cu(II), Zn(II), Pb(II)	100**

*Maximum amounts of substances studied
**In the presence of 5% EDTA solution

Interference Study

A study of interference was performed with sampling containing 5×10^{-5} M ascorbic acid and 1000 $\mu g.ml^{-1}$ of potential interferents. Table 2 shows the assayed

Table 1. Precision and accuracy of the method

Ascorbic acid present (M)	Ascorbic acid found (M)	RSD% (n= 10)	Relative error%
1.00×10^{-5}	1.01×10^{-5}	3.1	+1.0
5.00×10^{-5}	5.00×10^{-5}	2.7	0.0
1.00×10^{-4}	9.95×10^{-5}	2.4	-0.5
5.00×10^{-4}	4.98×10^{-4}	2.2	-2.0
1.00×10^{-3}	1.01×10^{-3}	2.2	+1.0

Table 3. Determination of ascorbic acid in Vitamin C tablets

Sample	AA contents (mg/tablet)	Ascorbic acid found (mg/tablet)	
		Proposed method (n = 10)	AOAC method (n = 10)
A	1000	992.2(±10.2)	998.1(±9.2)
B	500	502.1(±4.2)	499.3(±5.1)
C	500	490.5(±5.1)	491.2(±4.8)
D	500	493.9(±4.5)	497.5(±4.6)

Table 4. Determination of ascorbic acid in fruit juices

Sample	Ascorbic acid/μg.ml^{-1}	
	Proposed method	Standard method[16]
Grape	52.20±1.25	53.10±1.3
Orange	73.80±2.3	72.20±2.5
Pineapple	48.50±1.2	49.10±1.5

compounds. From these substances, only Cu(II), Fe(III), Pb(II), and Zn(II) are interfered. The tolerance limit of these ions can be increased in the presence of 5% EDTA solution. No interference is observed from organic compounds such as glucose, fructose, saccharose, tartarate, citrate, benzoate and maleic acid.

Analysis of Real Samples

Table 3 lists the results obtained on application of the proposed method to vitamin C tablets (Table 3) and fruit juice (Table 4). These results are compared with those obtained by the AOAC standard method [16], and indicate that the rapid and selective proposed method can be readily implemented on a very simple spectrophotometer.

References

1. Erdey, L. and Svehla, G. Ascorbinometric Titration, Academia Kiado, Budapest, (1973).
2. Sultan, S.M. *Talanta*, **40**, 593, (1993).
3. Sultan, S.M. and Bishop, E. *J. Pharm. and Biomed. Anal.*, **8**, 345, (1990).
4. Sultan, S.M., Abdennabi, A.M. and Suliman, F.E.O. *Talanta*, **41**, 125, (1994).
5. Pheto, G. *Acta Pharm. Hung.*, **52**, 228, (1982).
6. Nasser, T.A.K., Al-Rikabi, A.M. and Mansour, T.T. *Anal. Letters*, **20**, 627, (1987).
7. Strohl, A.N. and Curran, D.J. *Anal. Chem.*, **51**, 79, (1979).
8. Hernanez, J., Alonso, A., Almendral, M.J. and Garcia, C. *Anal. Chim. Acta*, **184**, 243, (1986).
9. Vermo, K.K., Jain, A. and Rawat, R. *J. Assoc. Off. Anal. Chem.*, **47**, 262, (1984).
10. Zhang, Z. and Qin, W. *Talanta*, **43**, 119, (1996).
11. Kim, J.M., Huang, Y. and Schmid, R.D. *Anal. Letters*, **23**, 2273, (1990).
12. Leon, L.E. and Catapan, J. *Anal. Letters*, **26**, 1741, (1993).
13. Safavi, A. and Fotouhi, L. *Talanta*, **41**, 1225, (1994).
14. Safavi, A. and Ensafi, A.A. *Anal. Chim. Acta*, **298**, 27, (1995).
15. Miller, J.C. and Miller, J.N. *Statistics for analytical chemistry*, Ellis Horwood, Chichester, (1986).
16. Herwitz, W. (ed). AOAC, *Official methods of analysis*, p. 476. AOAC, Washington D.C., USA, (1980).