



FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF ASCORBIC ACID USING IRON (III)-BATHOPHENANTHROLINE DISULFONIC ACID DISODIUM SALT

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A single step straightforward, indirect flow injection spectrophotometric determination of ascorbic acid is proposed. The solution of iron (III) and bathophenanthroline disulfonic acid salt in 1×10^{-4} M HCl media is used as an oxidative reagent. Reagent is reduced to tris-bathophenanthroline disulfonic acid-iron (II) chelate, by the ascorbic acid at room temperature. Spectrophotometric monitoring of absorbance signal of tris chelate at 535 nm is directly related to the concentration of ascorbic acid. The effect of various parameters e.g. pH, flow rate, sample volume, reaction coil length, etc. on the analytical signal were examined and optimized. Under optimized parameters such as sample volume (100 μ L), flow rate- (2.2 ml/min), reagent concentration (2×10^{-4} M ammonium iron(III) sulfate + 2×10^{-4} M bathophenanthroline disulfonic disodium salt.), pH (4.0), reaction coil length (50 cm) and wave length (535 nm), ascorbic acid can be determined in the range of 1-10 μ g/ml ($R=0.9924$) with sample throughput of 80 samples per hour. Single channel flow injection analysis (FIA) manifold raises the sample throughput as compared to other existing procedures. The validity of the proposed method is tested by analyzing citrus fruits using present and the standard addition method. Results of the two methods are in good agreement.

Keywords: FIA, Ascorbic acid, Indirect determination

1. Introduction

Ascorbic acid (vitamin C) plays an important role in the maintenance of good health of human being and in scurvy, cardiovascular and cancer disease. The determination of ascorbic acid is of great significance in pharmaceuticals, fruits and vegetables. [1, 2]. Methods for the determination of ascorbic acid by spectrophotometry, chromatography, spectrofluorometry and electrochemistry have been well reviewed. [3,4]. Spectrophotometry is commonly used for the indirect determination of ascorbic acid by the reagents which produces specific color reaction.

A common approach to the determination of ascorbic acid is based on the reduction of solvated Fe(III) to Fe(II) by ascorbic acid, followed by the determination of Fe(II) after masking the activity of excess of residual Fe(III). The Fe(II) can be conveniently measured by complexation with 1,10-phenanthroline, 2,2-bipyridine, 4,7-diphenyl-1,10-phenanthroline, 2,4,6-Tn.(2.pyridyl)-1,3,5-triazine (TPTZ), 3-(2-pyridyl)-5,6-bis (phenyl sulfonic acid) or with ferrocene [5].

The use of iron (III)-1,10-phenanthroline mixture

as an oxidant for the determination of ascorbic acid has attracted much attention over the past few years. [4,6-10]. The reduction of greenish brown 1,10-phenanthroline-iron(III) [7] required 7 minutes for the reduction reaction and formation of orange red tris- 1, 10-phenanthroline- iron (II), (ferroin). Excess of residual reagent iron (III), left after the completion of reduction reaction, has affected the ruggedness of the method. Another method using 1, 10-phenanthroline iron (III) as an oxidative reagent requires 30 minutes heating at 40°C to promote the reduction and formation of colored product tris-1,10-phenanthroline-iron(II) [8]. Slow reaction affected the sampling frequency, hence making it less attractive for routine analysis of large number of samples. 1,10-phenanthroline iron (III) has been used as an oxidant for the indirect determination of ascorbic acid, using flow injection thermal lens microscopy, with a sampling rate of 40 samples h^{-1} . The use of special detection system prohibits the use of this system for the routine analysis of ascorbic acid in commercial products [9, 10].

In this paper a simple, sensitive and rapid, flow injection spectrophotometric method is described

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Table 1. Amount of ascorbic acid determined in fruit juices.

Sample	Amount of ascorbic acid mg/L (Std.Dev)		
	Proposed method	Standard addition method	% Error
Orange juice(fresh)	4.28 ± 0.06	3.86 ± 0.04	+9.8
Tang (powder)	12.0 ± 0.005	12.0 ± 0.005	0
Energile (powder)	13.4 ± 0.003	13.1 ± 0.002	+2.2

for the indirect determination of ascorbic acid. Bathophenanthroline disulphonic acid – iron (III) is directly used as an oxidant at room temperature in a single line FIA manifold. The reagent is rapidly reduced to tris – (bathophenanthroline sulfonic acid) iron (II). On-line absorbance measurement of tris chelate at 535 nm is directly related to the concentration of ascorbic acid. The validity of method is judged by analyzing spiked and real samples of fruit juices.

2. Experimental

2.1 Reagents

All reagents used were of AnalaR grade. Double distilled water was used throughout the work. A stock solution of 1 mg/ml ascorbic acid was prepared from pure L-ascorbic acid (Merck) in 1×10^{-4} M HCl and stored in an amber bottle. Working solutions were prepared by appropriate dilution from stock solution with 1×10^{-4} M HCl. Ammonium iron(III) sulfate heptahydrate 1.67g (Merck), bathophenanthroline disulfonic acid disodium salt 3.2g (Merck) were dissolved and diluted to 1L with 1×10^{-4} HCl to give pale yellow mixture of iron (III) and bathophenanthroline disulphonic salt.

2.2 Instrumentation and procedure

A single channel manifold was used in this work. The reagent stream was pumped at a flow rate of 2.2 ml/min (except for the study of flow rate) via a peristaltic pump (Gilson Minipuls 3) equipped with a PVC pump tubing (Ana-Chem). The ascorbic acid sample (100 μ l) was introduced into the reagent stream via a rotary teflon valve (Rheodyne 5020). Teflon tubing of 0.5 mm was used throughout the system. The detector was a Spectronic 20 visible spectrophotometer equipped

with a flow through cell (10 mm, 80 μ l) set at 535 nm.

2.3 Determination of ascorbic acid in fruit juices

Equal volumes of fruit juice and 1×10^{-4} M HCl were immediately mixed after getting juice to prevent air oxidation of ascorbic acid and was further diluted to get measurable signal. Solid samples were simply weighed and diluted with 1×10^{-4} M HCl. Results obtained for these diluted solution of samples were compared to validate the method by standard addition method (Table 1). In order to stabilize vitamin C and to provide the pH required for the redox reaction, acidic media was used to dilute samples.

3. Results and Discussion

3.1 Effect of reaction coil length

The effect of reaction coil length on analytical signal is shown in Fig. 1. The reaction coil length was varied between 50 to 300 cm. Fig. 1 shows that the peak absorbance decrease between 50 to 200 cm and then remain constant. This shows that

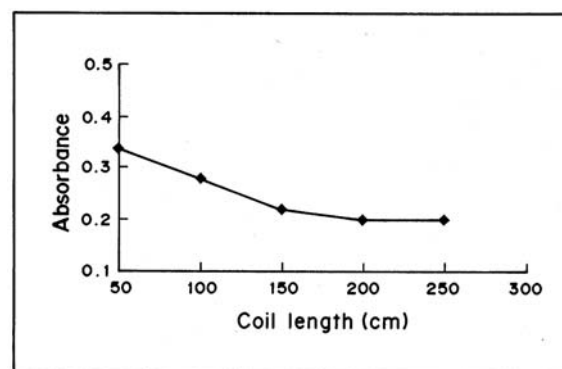


Figure 1. Effect of coil length on the absorbance.

the reaction is fast and reaches at its maximum in short time. The decrease in absorbance upto 200 cm is due to the longer residence time of sample in the reaction coil which increase the diffusion of the product. The method was optimized by using a reaction coil length of 50 cm which also increase sample throughput.

3.2. Influence of reagent flow rate

The flow rate was found to have pronounced effect on the sample throughput, reagent consumption and sensitivity of the procedure. Fig. 2 shows the effect of increasing flow rate on the absorbance. The absorbance was almost constant from 0.5 to 2.2 ml / min, however, a slight decrease was observed beyond this flow rate. This decrease in absorbance at higher flow rates is due to smaller residence time, whereas increase in absorbance at low flow rate is due to greater residence time. A flow rate 2.2 ml / min was thus chosen for assay in present case to match with the requirement of sensitivity and high sample throughput.

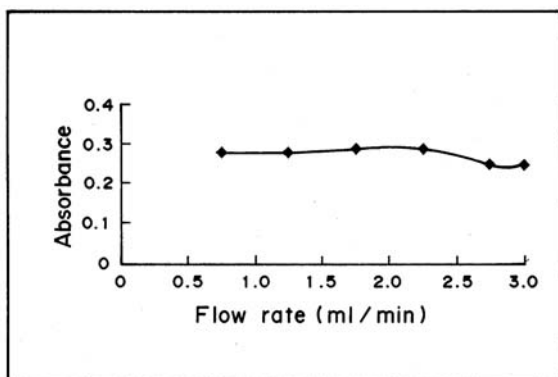


Figure 2. Effect of flow rate on the absorbance.

3.3 Effect of sample volume

The sample volume was found to have pronounced effect on the sensitivity of the analytical signal, shape of the peak and width of the peak. The peak height increases with an increase in sample volume. In present case the effect of sample volume from 30-150 μ L was investigated keeping the ascorbic acid concentration constant and results are shown in Fig. 3. There was gradual increase in the absorbance upto 100 μ L, further increase in the sample volume was producing a double peak showing the improper mixing of reagent and sample. A sample volume of 100 μ L was chosen as an optimized sample volume.

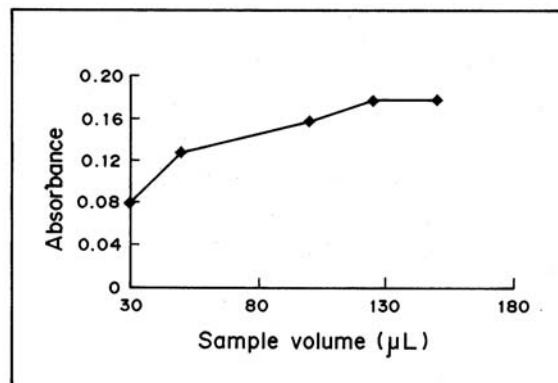


Figure.3. Effect of sample volume on the absorbance.

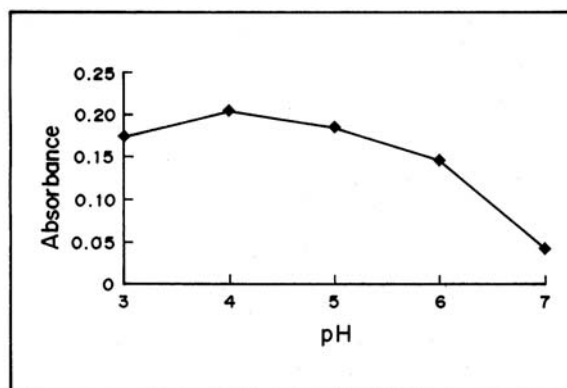


Figure 4. Effect of pH on absorbance

3.4. Effect of pH

The pH of the reagent solution was varied by adding 0.01 M HCl in the range pH 3.0-7.0 (Fig. 4). Absorbance was almost same from pH 3 – 5 and then it starts decreasing and is minimum or negligible at pH 7. The pH 4 was chosen, as an optimized pH for the determination of ascorbic acid using present method as this pH is also consistent with maximum reducing activity of ascorbic acid.

3.5 Calibration and reproducibility

Ascorbic acid standard covering the range 1-10 μ g/L were analyzed using the optimized FIA manifold (Fig. 5). A linear calibration graph represented by equation $y = 0.0459x + 0.004$ ($R^2 = 0.9924$) was obtained (Fig. 6). The relative standard deviation for $n = 10$ (10 μ g/L) was $\pm 4\%$ with sampling frequency 80 samples per hour.

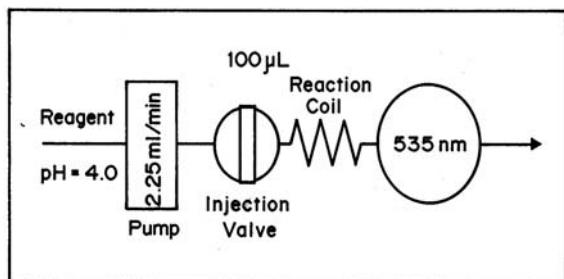


Figure 5. Optimized FIA set up.

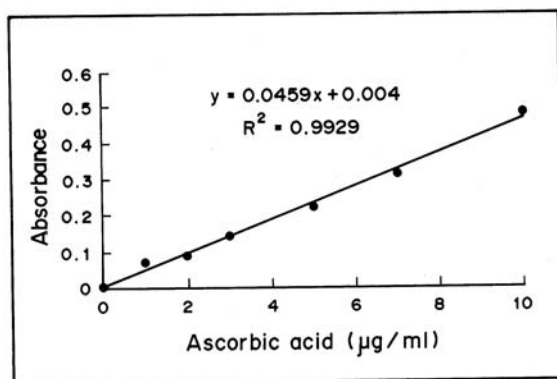


Figure 6. Sample volume (100µL), flow rate calibration plot (2.25 ml/min), reagent concentration (2×10^{-4} M ammonium iron(III) sulfate + 2×10^{-4} M bathophenanthroline disulfonic disodium salt), pH (4.0), reaction coil length (50 cm) and wave length (535 nm).

3.6. Validation of the method by standard addition technique

As the fruit juices contain additives, coloring agents, etc. and natural fruits contain reductones, enzymes, etc. that may interfere in the determination, calibration should approximate the composition of the samples to be analyzed in order to minimize the effects of these components of the sample on the measured absorbance. In present method some of these effects from various matrix

components on the absorbance are reduced by dilution due to appreciable sensitivity of the method. But in order to observe that at what extent these matrix effects influence in the determination of vitamin C. Results obtained for different samples are compared in Table 1, which shows that the interference is larger in case of fresh fruits but is in acceptable limits.

4. Conclusions

Ascorbic acid can be conveniently determined by an FIA method which overcomes the difficulties encountered in the normal spectrophotometric or FIA methods. The present method has advantages over existing methods in terms of simplicity and sample throughput and can be used for the routine analysis of ascorbic acid in fruits and vegetables.

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