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Repetitive determination of ascorbic acid using iron(III)-1.10-phenanthroline–peroxodisulfate system in a circulatory flow injection method

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Abstract

Ascorbic acid (AA) could be determined in large quantities of a co-existing oxidant. The incorporation of an on-line reagent regeneration step based on redox reaction eliminates the baseline drift in the procedure. This makes it possible to adopt a circulatory flow injection method (cyclic FIA) and to determine AA repetitively. The method is based on the reduction of iron(III) to iron(II) by the analyte, the reaction of the produced iron(II) with 1,10-phenanthroline (phen) in a weak acidic medium to form a colored complex, and the subsequent oxidation reaction of iron(II) to iron(III) by the co-existing peroxodisulfate. A solution (50 ml) of 3.0×10^{-4} mol l^{-1} ferric chloride, 9.0×10^{-4} mol l^{-1} phen and 5.0×10^{-2} mol l^{-1} ammonium peroxodisulfate in acetate buffer (0.2 mol l^{-1}, pH 4.5) is continuously circulated at a constant flow rate of 1.0 ml min^{-1}. Into this stream, an aliquot (20 μl) of the sample solution containing AA is quickly injected by means of a six-way valve. The complex formed is monitored spectrophotometrically (at 510 nm) in the flow system. The stream then returns to the reservoir after passing through a time-delay coil (50 m). The iron(II)–$(phen)_3$ complex is oxidized to iron(III)–$(phen)_3$ complex by peroxodisulfate which exists excessively in the circulating reagent solution. The proposed method allows as many as 300 repetitive determinations of 15 mg l^{-1} AA with only 50 ml reservoir solution. The contents of AA in commercial pharmaceutical products were analyzed to demonstrate the capability of the developed system.

Keywords: Ascorbic acid; Repetitive determination; Cyclic FIA; Regeneration of reagent; Reuse of reagents

1. Introduction

Ascorbic acid (AA) is one of the most important water-soluble vitamins and widely used as food additives and antioxidants. In the pharmaceutical and food industries, the determining or monitoring of AA content is particularly important. There is a need for a simple, fast, selective and automated method for its determination, particularly in routine analyses. Compared to conventional batch methods and chromatography, flow injection analysis (FIA) offers many significant advantages when the concentration of one analyte has to be determined or monitored. Although several determination methods have been developed for this purpose [1–5], spectrophotometry has become the most widely used detection technique for FIA [1,4,6–10]. Spectrophotometric methods are based on the reduction of iron(III) to iron(II) with AA, followed by complexation of reduced iron(II) with different reagents, such as 1,10-phenanthroline (phen) [4,11–15] and 2,2′-bipyridine derivatives [16,17]. If the reverse reaction, that is, the oxidation of iron(II) to iron(III) is integrated into the following reaction, a circulatory flow injection method (cyclic FIA) [18–26] may be designed to determine AA repetitively. We found that the reduction of iron(III) to iron(II) with AA occurred in the existence of a large amount of co-existing peroxodisulfate if the FIA system was adopted, and absorption based on the complexation of iron(II) with phen was detectable with signals obtained corresponding to the AA contents. The oxidation of iron(II) to iron(III) by a co-existing oxidant occurred completely after passing through the flow-through cell and a time-delay coil. Consequently, the main reagents were regenerated to its original

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concentration level and could be reused repeatedly. This is a striking characteristic of FIA, and can never be done by batch methods. The use of a cyclic FIA results in decrease of operating cost, shortening of determination time, providing a better utilization of reagents and minimizing waste.

The aim of this study was to develop a cyclic FIA for the repetitive determination of AA. Considering the simplicity of the flow system and the adoption of a well-known chemical reaction, spectrophotometry with AA-iron(III)–phen–peroxodisulfate system has been employed as a model. The proposed cyclic FIA is based upon a three stage chemical process. First iron(III) is reduced to iron(II) by AA, the second iron(II) reacts with phen in a weak acidic medium to form a colored complex which is monitored spectrophotometrically, and the third iron(II) is oxidized to iron(III) again by peroxodisulfate which exists excessively in a circulating reagent solution. The proposed method was satisfactorily applied to the determination of AA in commercial pharmaceutical products.

2. Experimental

2.1. Chemicals

All chemicals were of analytical-reagent grade. Water used for the preparation of solutions was obtained by a Milli-Q water purification system (Millipore). L(+)-Ascorbic acid, ammonium peroxodisulfate, iron(III) chloride hexahydrate (99.9%) and 1,10-phenanthroline monohydrate were purchased from Wako Pure Chemical (Osaka, Japan).

AA stock solution (500 mg l^{-1}) was prepared daily by dissolving 0.050 g of AA in 100 ml of 0.1 mol l^{-1} acetic acid solution. In this work, 0.1 mol l^{-1} acetic acid has been used in preparing the working solutions of AA, because acetic acid works as an effective stabilizer and AA oxidation is slower in it. Iron(III) stock solution (0.01 mol l^{-1}) was prepared by dissolving 1.352 g of iron(III) chloride hexahydrate in 500 ml of 1 mol l^{-1} HCl solution. Phen stock solution (0.03 mol l^{-1}) was prepared by dissolving 2.973 g of phen with 5 ml of 6 mol l^{-1} HCl and made up to 500 ml with water. Peroxodisulfate stock solution was prepared by dissolving 2.282 g of ammonium peroxodisulfate with 100 ml of water (0.1 mol l^{-1}).

Three solutions of pharmaceutical products were prepared by dissolving suitable amounts of the commercial samples in 0.1 mol l^{-1} acetic acid solution and diluted the resulting solution to adjust the concentration to that required by the experimental conditions adopted.

2.2. Apparatus

A single line manifold was assembled as described previously [24–26]. The FIA apparatus, consisted of a Sanuki DMX-2300T double plunger pump (Tokyo, Japan), a Rheodyne 9752 rotary injection valve (20 μl loop), a Soma Kougaku S-3250 visible spectrophotometer (Tokyo, Japan) equipped with a flow through cell (1 cm light pass, 8 μl inner volume), and a Nippon Denshi U-228 multi-range recorder (Tokyo, Japan). Flow lines were made of PTFE tubing (0.5 mm i.d.) and connectors.

2.3. Procedure

A typical circulating reagent solution was made up of 50-ml containing 3.0×10^{-4} mol l^{-1} iron(III) chloride, 9.0×10^{-4} mol l^{-1} phen and 5.0×10^{-2} M ammonium peroxodisulfate in 0.2 mol l^{-1} acetate buffer (pH 4.5). This solution in the reservoir was constantly stirred with the help of a magnetic stirrer. In the determinations, 20 μl of AA solutions were injected into the reagent stream. AA solution injected into the stream was allowed to form the colored iron(II)–$(phen)_3$ complex. In the flow-through cell, the complex was monitored spectrophotometrically at 510 nm and the response was fed to a strip chart recorder. Then the stream was carried back to the reservoir after passing through a delay coil (50 m long). A flow rate of circulating solution was 1.0 ml min^{-1}. Using a cycle time of 1 min, 60 injections h^{-1} were made. To evaluate the proposed method, the standard 15 mg l^{-1} AA solution was injected 100 times in sequence for regeneration and repeatability test.

3. Results and discussion

3.1. Co-existing oxidant

In this work, small quantities of AA is directly injected into the circulating reagent solution which contains iron(III)–$(phen)_3$ complex and peroxodisulfate. Because peroxodisulfate is a fairly strong oxidant and exists in excess, it can be presumed that AA injected is predominantly oxidized by peroxodisulfate instead of by iron(III), therefore, the color development based on the formation of iron(II)–$(phen)_3$ complex does not occur. In practice, however, it happens that the color development really occurred and its color intensity corresponded to the AA content. This is a striking characteristic of FIA, and can never be done by batch methods. This concept is very surprising and important; namely, the reaction is carried out in large quantities of an “inhibitor”. It can be easily extended so as to include the cases of the complex formation [24,25] and neutralization [26] as well as the redox reaction.

Preliminary tests have revealed that peroxodisulfate is preferable as a co-existing oxidant compared with hydrogen peroxide, because of its strong oxidizing power and no evolution of bubbles. The effect of peroxodisulfate concentration on the absorbance (peak height) was investigated. Peroxodisulfate concentration was found to play a critical role that determined the sensitivity of the method and the

number of samples as well as the regeneration of the main reagent. As shown in Fig. 1, the absorbance decreased with increasing the peroxodisulte concentration. It is obvious that AA reduces two oxidants, iron(III) and peroxodisulfate, simultaneously, when AA is injected into the reagent stream. A loss of sensitivity to some extent is unavoidable in the adoption of a cyclic FIA. Baseline absorbance increased with increasing the number of AA solution injected, because of the accumulation of the colored iron(II)–$(phen)_3$ complex in the system. Increase of baseline absorbance decreased with increasing the peroxodisulfate concentration (A)–(C) in Fig. 1. In contrast to those, no drift of baseline was observed and fairly constant flow signals were obtained (D). It is clear that the oxidation of iron(II)–$(phen)_3$ by peroxodisulfate took place completely, resulting in the re-formation of iron(III)–$(phen)_3$ complex which was nearly colorless and could be reused in the cyclic FIA system. The optimum concentration of peroxodisulfate was chosen as 5.0×10^{-2} mol l^{-1}, which is 167-fold higher than Fe(III) molar concentration, as a compromise between the sensitivity and the number of samples.

Similarly, the formation of iron(III)–$(phen)_3$ complex was affected by the length of a reaction (mixing) coil which was introduced between the sample injector and the flow-through cell. The signals decreased with increasing the reaction coil length, because the reduction occurred at the boundaries of the sample zone which was injected into the reagent solution. The mixing of AA was enlarged so as to react with peroxodisulfate, resulting in the decrease of the sensitivity. Therefore, the outlet of the injection valve and the inlet of the flow cell were connected directly (the effective coil length was 80 cm).

3.2. Cyclic FIA variables

Naturally, the reservoir volume in a cyclic FIA should be as small as possible to effectively reuse the reagents and to minimize the waste. At the same time, the selection of the reservoir volume is dictated mainly by the number of AA samples to be determined and the approximate content. In this work, the reservoir volume was decided to be only 50 ml, taking into account the stringent requirements.

Iron(II) reacts with phen to form the red iron(II)–$(phen)_3$ complex in the pH range 2.0–9.0 [27]. The effect of pH on the peak height was examined in the pH range 2.0–6.0. Maximum complex formation is achieved for pH values varying between 3.0 and 5.5. A sharp decrease in absorbance is noted for a pH smaller than 3.0. A buffer solution with a pH of 4.5 was adopted for the determination.

The volume of the sample injection should be as small as possible in a cyclic FIA, because a large quantity of sample solution causes dilution of the reagent concentration, resulting in lowering of sensitivity. Contrarily, the increase of the volume of sample injection increases the sensitivity of the AA determination. The injection loop volume varied from 10 to 200 μl, by changing the length of the sample loop in the injection valve. The absorbance increased with the increase of the sample volume, showing non-linearity above 100 μl. Taking into account these two opposite effects, 20 μl was chosen as the compromise between the dilution and the sensitivity.

In the initial studies, we used a short back-pressure coil (1 m long). After less than one hour of measurements, a gradual increment in the baseline was observed. Because the oxidation reaction of iron(II)–$(phen)_3$ to iron(III)–$(phen)_3$ by peroxodisulfate was relatively slow [28,29] and the time that allowed to proceed was too short, un-oxidized iron(II)–$(phen)_3$ complex remained in the reservoir, therefore, the background absorbance increased gradually. As a result, a time-delay coil was adopted to improve this defect. The dispersion and residence time of the reagent stream were controlled by the length of the tubing after the flow-through cell. The length of the time-delay coil was examined over the range 1–50 m using a flow rate of

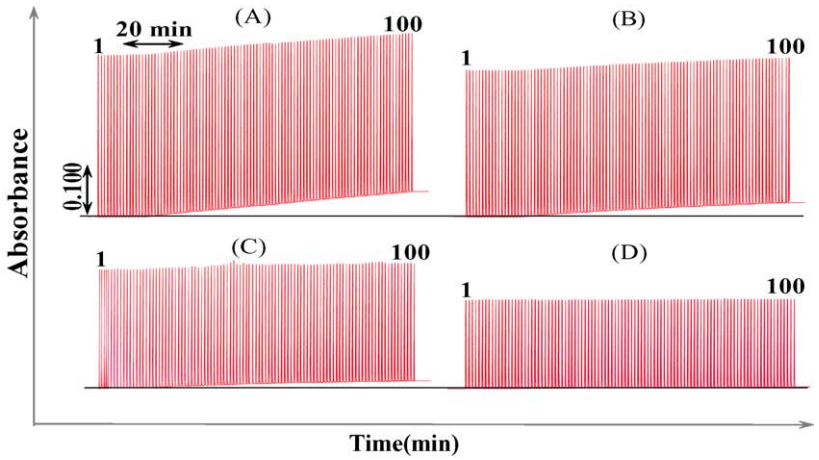

Fig. 1. Flow signals for 100 repetitive determinations of ascorbic acid in the presence of peroxodisulfate. Ammonium peroxodisulfate: (A) 1.0×10^{-3}; (B) 5.0×10^{-3}; (C) 1.0×10^{-2}; and (D) 5.0×10^{-2} mol l^{-1}. Ascorbic acid: 25 mg l^{-1}.

Table 1
Optimum conditions found for the cyclic FIA system

Variables	Optimal value	Range studied
Reservoir volume (ml)	50	–
Ferric chloride (mol l^{-1})	3.0×10^{-4}	$0.5–4.0 \times 10^{-4}$
1,10-Phenanthroline (mol l^{-1})	9.0×10^{-4}	$3.0–15.0 \times 10^{-4}$
Ammonium peroxodisulfate (mol l^{-1})	5.0×10^{-2}	$0.1–5.0 \times 10^{-2}$
pH	4.5	2.0–6.0
Buffer (acetate) (mol l^{-1})	0.2	0.1–1.0
Injection volume (μl)	20	10–200
Reaction coil length (cm)	80	80–180
Time-delay coil length (m)	50	1–50
Flow rate (ml min^{-1})	1.0	0.5–2.0
Wavelength (nm)	510	480–530

1.0 ml min^{-1}. The time-delay coil length finally chosen was 50 m.

Other FIA variables were optimized by maximizing the peak heights obtained. The variables optimized, the range studied and the optimal values are shown in Table 1.

3.3. Calibration, precision and repeatability

Under the optimal conditions given in Table 1, the calibration curve was linear in the concentration range from 0.2 to 30.0 mg l^{-1} of AA with a correlation coefficient of 0.9998. The detection limit of this method was 0.04 mg l^{-1}. The precision of the proposed method was checked by periodic injection of the same standard AA solutions. The relative standard deviations (R.S.D.) of 100 injections of each solution containing 5, 10, 15, 20, and 25 mg l^{-1} of AA were 1.2, 0.8, 0.3, 0.4, and 0.5%, respectively, on the sampling rate was 60 injections per hour with a reservoir volume of 50 ml. Furthermore, the repeatability of peak heights was determined by the same standard 15 mg l^{-1} AA samples in more than 300 injections. A slow decrease in peak height was noted; the total loss in height was 3% after 300 repetitive determinations. It was mainly due to the dilution of the reagent solution because 20 μl of reagent solution was placed by the same volume of sample solution for each injection. If the reservoir volume of 200 ml was adopted, no loss in peak height was observed after the same 300 determinations. It should be mentioned that the effects arising from the decrease of reagent concentration during continuous analysis could always be made negligible by employing a larger initial volume.

3.4. Robustness

To check the quality of products, for example, continuous monitoring or determining is required in every industry, where samples are periodically collected at constant intervals and analysis is carried out immediately. The robustness of the proposed method was evaluated by two kinds of long-term operations: (1) the system, except for the strip-chart recorder, ran continuously for 9 h, and at hourly intervals the standard AA solutions (0–25 mg l^{-1}) were injected and calibrations of AA were carried out. (2) All equipment (pump, spectrophotometer and recorder) were switch off before/after measuring. At hourly intervals and five minutes of warm up time for the equipment, the standard solutions were injected and calibrations of AA were carried out. In both cases, the precise and reproducible calibration signals were obtained and that of (1) is shown in Fig. 2, where the calibration peaks of AA were extracted at three hour intervals. It is clear that this method is applicable whenever an analysis is needed, and is suitable for such an urgent determination or monitoring. Another important feature of the proposed method is that after continuous use of the FIA system for more than 9 h, no disturbances of the baseline has been observed. This fact shows that the redox reaction of iron(II)–$(phen)_3$ to iron(III)–$(phen)_3$, vice versa, took place completely and there is no potential adsorption of iron(III)–$(phen)_3$ complex on the wall of the PTFE tubing and flow-through cell.

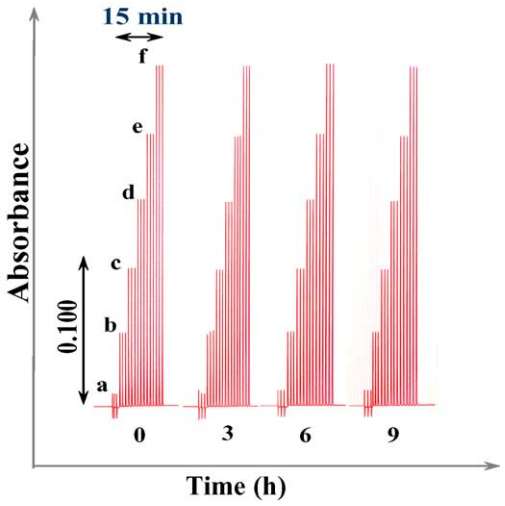

Fig. 2. Calibration peaks of ascorbic acid extracted at 3 h intervals. Ascorbic acid: (a) 0; (b) 5; (c) 10; (d) 15; (e) 20; and (f) 25 mg l^{-1}.

Table 2
Percent flow signal intensity for some interfering additives

Additives	Additive/ascorbic acid (%)			
	1/1	1/10	1/100	1/1000
Citric acid	108	90	63	37
Oxalic acid	108	105	102	72
Malic acid	101	105	108	82
L-Cysteine	99	101	135	264
Thiourea	104	107	110	111

L(+)-ascorbic acid: $10\,mg\,l^{-1}$.

3.5. Effect of additives

The effects of a number of additives on the determination of AA were examined by applying the method to a fixed concentration of $10\,mg\,l^{-1}$ AA solution and varying the concentration of each substance being studied. The pharmaceutical excipients, such as glucose, galactose, sucrose, lactose, fructose, maltose, sorbitol, glycine, serine, saccharine and urea did not interfere when presented in 1000-fold excess. Many amino acids did not interfered in concentrations up to at least 500-fold excess. Table 2 shows the effects of additives that interfered this method. Citric and oxalic acids caused positive errors when present in the same amount because of reducing agents. However, in concentration at 100- or 1000-fold excess, these acids caused serious negative errors because they acted as the masking agent for iron(II). L-Cysteine was tolerable in the presence of at least 10-fold excess.

3.6. Application

The applicability of the proposed method was checked by analyzing commercially available pharmaceutical products. A tablet or powder was dissolved in $0.1\,mol\,l^{-1}$ acetate, and insoluble residue was removed by filtration (Advantic 5C). After suitable dilution to fit the concentration of the analyte within the linear calibration range, the samples were injected five times. The results obtained are shown in Table 3. In all cases, the results obtained by the proposed method were in good agreement with the labeled amounts. The accuracy of the proposed method was further checked by adding known amounts of AA (5.0 and $10.0\,mg\,l^{-1}$) to the previously analyzed samples. The average recoveries obtained by the proposed method were between 96 and 104%, and the higher standard deviation was 1%, indicating good accuracy and precision.

Table 3
Determination of ascorbic acid in pharmaceutical products using circulatory flow injection method

Sample	Ascorbic acid		
	Labeled value ($mg\,g^{-1}$)	Found ($mg\,g^{-1}$)[a]	Error (%)
Takeda-1000	1000	984 ± 16	−1.6
Hicee granules	250	246 ± 2	−1.6
Cinal	200	198 ± 2	−1.0
Viscorin powder[b]	100	105 ± 1	+5.0

[a] Mean standard deviation ($n = 5$).
[b] The solution obtained was filtered through a Advantic 5C filter paper.

4. Conclusions

To determine ascorbic acid, an iron(III)–1,10-phenanthroline–peroxodisulfate system in a circulatory flow injection method was developed. The main reagent was successfully regenerated allowing the stable baseline and repetitive determination of ascorbic acid. The system developed permits a drastic reduction of reagent consumption and minimization of waste. This procedure is particularly appropriate when a rapid analysis is needed, or when a large number of samples have to be analyzed.

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