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FLOW INJECTION COLORIMETRIC METHOD FOR THE ASSAY OF VITAMIN C IN DRUG FORMULATIONS USING TRIS,1-10-PHENANTHROLINE–IRON(III) COMPLEX AS AN OXIDANT IN SULFURIC ACID MEDIA

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Summary—A simple, fast and accurate colorimetric flow injection (FI) method suitable for the assay of vitamin C in drug formulations was proposed. In the method, vitamin C was injected into a flowing stream of iron(III) and then mixed with 1,10-phenanthroline in 0.05*M* sulphuric acid media. The mixture was allowed to react in a 45-cm long coil and the resulting solution of tris, 1-10-phenanthroline–iron(II) complex was monitored at 510 nm. The method was adopted by fully investigating the kinetics of the reaction and proposing a suitable mechanism. A throughput of 100 samples per hour was achieved with a relative standard deviation of 0.88% for vitamin C concentration range of 100–400 ppm.

Vitamin C (1-keto-1-threo-hexono-g-lactone-2,3-enediol) is commonly known as 1-ascorbic acid. Methods of determination of vitamin C by spectrophotometry, chromatography and electrochemistry have recently been reviewed.[1,2]

Flow injection analysis (FIA) has been applied to the determination of vitamin C with amperometric[3–6] and coulometric[3] detection suitable for determination in the nanogram range and with low sampling throughput. The FIA colorimetric methods[7,8] reported so far are complex involving the use of many reagents, subject to numerous interferences and limited to the determination of vitamin C in biological fluids. Very recently a photochemical FIA method[9] has been developed but with a maximum sampling frequency of 15 samples per hour. The recently published FIA colorimetric method[1] is suitable for the assay of vitamin C in drug formulations but it is a kinetic method and suffers low sampling frequency.

In the British Pharmacopoeia (BP) monograph[10] a titrimetric method involving the use of cerium(IV) as a titrant and ferroin sulphate as an indicator in sulphuric acid media, is the one described for determination of vitamin C in drug formulations.

In the present paper, a flow injection colorimetric method is described. The procedure involves the use of a stream of a solution of iron(III) in sulphuric acid generated in a flow system as a carrier and 1,10-phenanthroline in sulphuric acid media as a reagent. The method was validated by investigating the mechanism of vitamin C oxidation by tris,1-10-phenanthroline–iron(III) in sulphuric acid, thus reacting with ascorbate anion as the active reductant in the first rate-determining step followed by the reaction of the ascorbate anion radical producing dehydro ascorbic acid in the fast second step.

Reagents

1,10-phenanthroline. A 0.25% solution was prepared by dissolving the required amount in 0.05*M* sulphuric acid.

Iron(III). A 0.01*M* iron(III) solution was prepared by dissolving 5.1567 g of dried ammonium ferric sulphate [$NH_4Fe(SO_4)_2\ 12H_2O$] in about 400 ml of 0.05*M* sulphuric acid; left overnight and finally diluted to 1000 ml with the same acid in a calibrated flask.

Vitamin C generic standard. A 1000-ppm stock solution was prepared from a pure 99.9% (BDH) compound, previously dried at 50° *in vacuo* over magnesium perchlorate, by directly dissolving in water.

Vitamin C tablets. Ten tablets of the proprietary drug to be investigated were accurately weighed, crushed and powdered. An amount of this powder equivalent to 0.2 g of vitamin C was dissolved in 50 ml water, left for 10 minutes for all gases to subside, then filtered, washed and 5.0 mmoles of sulphuric acid were added; and

finally made up to volume in a 100-ml calibrated flask with water.

Apparatus

An Alitea USA/FIA Lab. (Medina, Washington, U.S.A.) apparatus described previously[11] was used for FIA measurements. The apparatus is comprised of a peristaltic pump, injector, reactor module and a Spectronic 20 spectrophotometer connected to a single-channel strip-chart recorder (Cole Parmer, Chicago, IL, U.S.A.).

A Varian Model 2300 UV/V/IR spectrophotometer was used for preliminary investigations and the kinetics of the reaction. This instrument was connected with a Varian DS-15 data station and an Epson LX-86 printer.

Manifold and procedure

A two-line FIA manifold configuration (Fig. 1) was used. A 110-μl vitamin C sample was injected into iron(III) solution already dissolved in 0.05*M* sulphuric acid as a carrier stream which was pumped through a PVC tubing of 1.3 mm i.d. after merging with 1,10-phenanthroline solution. The sample injector used was of a Rheodyne Model 5041 four-way poly(tetra fluoroethylene) (PTFE) rotary valve (Cotati, CA, U.S.A.) type. The reactants passed a 45-cm PTFE of 0.5 mm i.d. reaction coil and products were transported to waste through a Univic ultra micro flow cell (Plainsville, New York, U.S.A.) of 20-μl size and 1.0-mm path length. The absorbance was monitored at the wavelength of 510 nm and finally recorded at the rate of 0.5 cm/min.

RESULTS AND DISCUSSION

Kinetics and mechanism

The present FI method was based on the oxidation of vitamin C with iron(III) using 1,10-phenanthroline indicator in sulphuric acid media.

The kinetics of this reaction was thoroughly investigated by monitoring the increase of absorbance of tris-1,10-phenanthroline iron(II) red complex at the wavelength of maximum absorbance at 510 nm.

The mechanism could be described as a two-step reaction. In the first step a rise was observed which was followed by a gradual and almost horizontal increase in absorbance in the second step. It was also observed that reaction rate accelerates at low hydrogen ion concentrations and slows down at higher concentrations. This phenomenon suggests that the ascorbate anion (HA^-) is the active reductant for iron(III) and not the ascorbic acid (H_2A) itself. Ascorbic acid formation at higher acidic media retards the reaction and stops it at 0.10*M* sulphuric acid.

As a result of the kinetics data, the overall reaction rate expression could be represented as follows:

$$\text{Rate} = -[Fe^{3+}]dt = K[Fe^{3+}][H_2A][H^+]^{-3}$$

where K is the overall rate constant.

The inclusion in the rate law of a simple inverse dependence on the concentration of a species, *i.e.*, the negative reaction order, usually indicates that this reagent features as the product of a rapid step preceding the rate determining step.[12] The proton loss due to oxidation of ascorbic acid appeared at a later second step of the reaction following the rate

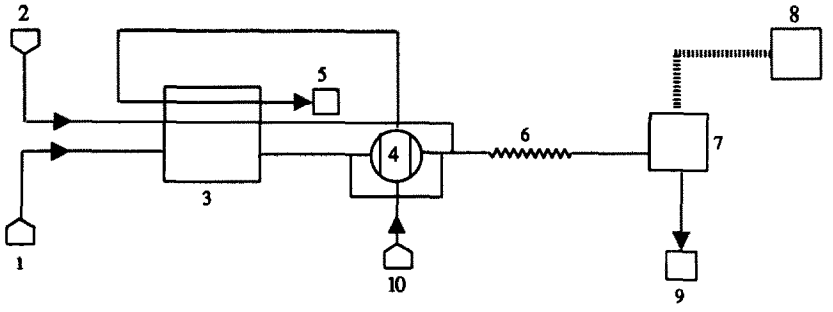

Fig. 1. Two-line FIA manifold comprised of: 1. 0.01*M* iron(III) solution carrier; 2. 0.25% 1,10-phenanthroline; 3. peristaltic pump; 4. injector of 157 mm^3 loop size; 5. drug sample waste; 6. 45-cm reaction coil; 7. spectronic Mini 20 spectrophotometer; 8. XY recorder; 9. products waste; and 10. drug samples injected.

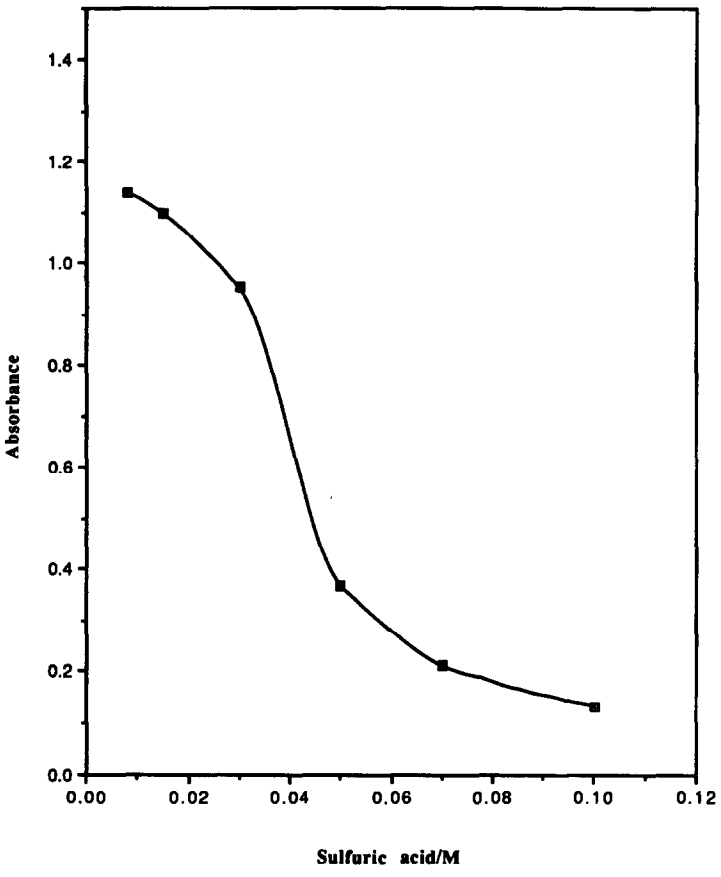

Fig. 2. Influence of variation of sulphuric acid on absorbance taking constant concentration of 300 ppm vitamin C, 0.01*M* iron(III), 3.2-ml/min flow rate and 45-cm reaction coil length.

determining step and was not expected to be included in the rate expression. Under the conditions of the experiments at higher acid concentrations in particular, there had been no evidence for the hydrolysis of the iron(III) in solution. Therefore, the inclusion of $[H^+]^{-3}$ term in the rate law, where -3 is a negative integer, can be attributed to three protons being a product of a pre-equilibrium step of a deprotonated species. These three protons are likely to be a result of the reaction of iron(III) with the protonated 1,10-phenanthroline as follows:

$$Fe^{3+} + 3H^+phen \Leftrightarrow [Fe(phen)_3]^{3+} + 3H^+$$

The above reaction is strongly supported by the fact that at higher acidities, no reaction will take place before the addition of 1,10-phenanthroline. This concludes that the reaction in fact occurs between the ascorbate anion and the tris 1,10-phenanthroline–iron(III) and not with solvated iron(II)–hydroxyl, sulphate or bisulphate complexes. In fact the solvated iron(II) has an oxidation potential of 0.68 V, therefore it is not by itself a strong oxidant. However, by the addition of 1,10-phenanthroline, iron(II) complex, it becomes a considerably stronger oxidant due to preferential stabilization of iron(II) by 1,10-phenanthroline, which raises the potential up to 1.06 V, thus increasing the reactivity rate by 10^6 fold.[13,14] For this reason, the FI configuration used, was constructed so that vitamin C was injected into the iron(III) carrier and immediately after; meets the 1,10-phenanthroline solution to be complexed before it is transported to the reaction coil.

The stoichiometry of the reaction was found to be 1:2 ascorbic acid to iron(III), respectively. Since iron(III) is a one-electron oxidant, the ascorbate anion reacts with the complex in the first reversible rate determining step thus forming the ascorbate anion radical ($HA^{\cdot}$) which is further oxidized to dehydrosascorbic acid (A^{2-}). The formation of the ascorbate and ascorbate anion radical have been reported earlier.[2,15–17]

The above reaction steps could be represented as follows:

$$H_2A \Leftrightarrow HA^- + H^+$$

$$HA^- + [Fe(phen)_3]^{3+} \Leftrightarrow [Fe(phen)_3]^{2+} + HA^{\cdot}$$

$$HA^{\cdot} + [Fe(phen)_3]^{3+} \Leftrightarrow [Fe(phen)_3]^{2+} + A^{2-} + H^+$$

$$H_2A + 2[Fe(phen)_3]^{3+} \Leftrightarrow [Fe(phen)_3]^{2+} + A^{2-} + 2H^+$$

Optimization

Because of the complexity of the chemical reaction and the fact that maximization of absorbance in the vicinity of the second step of the reaction would not result in correlating absorbance with reagent concentration, optimization was achieved with respect to throughput and Beer's Law validation range.

Sample volume size and iron(III) concentration were found to have no significant effect on throughput and concentration range, therefore, kept constant at 110 μl and 0.01M, respectively. Reaction coil length was kept at 45 cm because for longer coils residence time is higher and dispersion leads to lower throughputs.

Acidity and flow rate were found to be the most serious parameters that affect throughput and sample concentration range.

The effect of sulphuric acid concentration on absorbance is shown in Fig. 2. It is evident that at low acid concentrations, lower than 0.03M, the increase in absorbance becomes gradual and steady and the FI runs for different concentrations of vitamin C *vs* absorbance, resulted in a linearity of a very narrow limited range of about 10–50 ppm only. At this absorbance range, where the second step of the reaction was found to be dominant, the ascorbate anion radical is considered to be the active species. Below 0.1M acid the reaction rate was found to be very slow and the sensitivity was not favourable. It was therefore decided to conduct the FI experiment at an acid concentration of 0.05M.

The effect of variation in the flow rate with respect to the absorbance is shown in Fig. 3. It

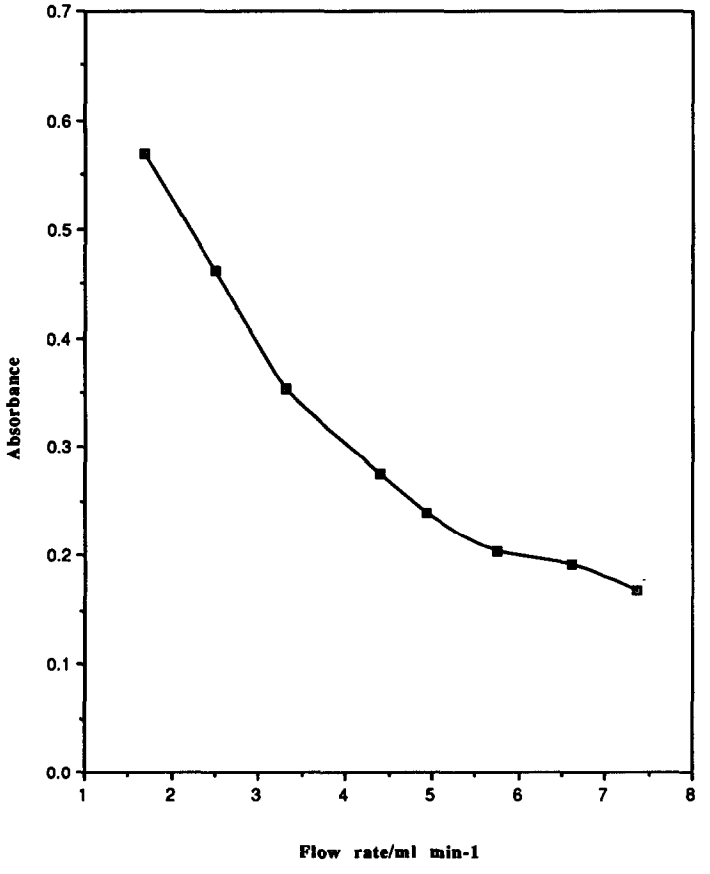

Fig. 3. Influence of variation of flow rate on absorbance taking constant concentration of 0.05M sulphuric acid, 0.01M iron(III) and 45-cm reaction coil length.

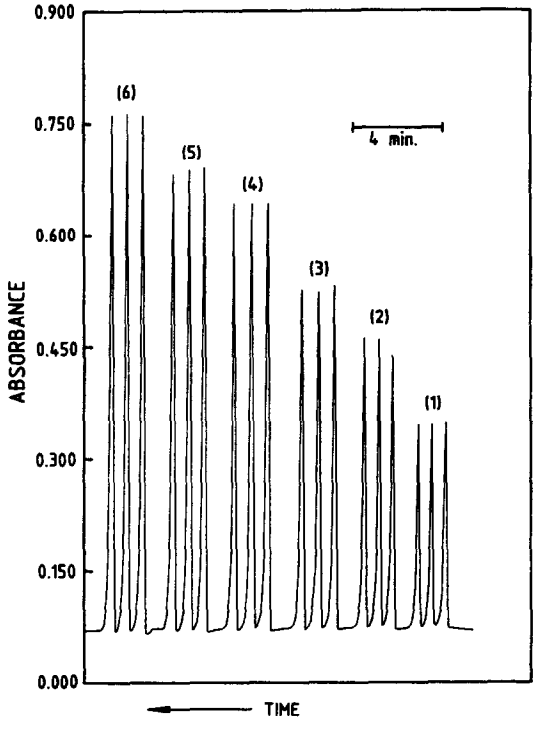

Fig. 4. Recorder tracing for FIA calibration plot for absorbance vs series of standard vitamin C solutions of: 1, 50; 2, 100; 3, 200; 4, 250; 5, 300; and 6, 350 ppm.

is clear that the absorbance decreases as the flow rate increases and this could be attributed to the fact that the residence time was not enough for a considerable formation of products; therefore the flow rate was kept at a value of 3.2 ml/min.

To summarize, optimum values for the FIA parameters are: 110-μl sample size, 45-cm reaction coil length, 0.01M iron(III), 0.05M sulphuric acid and 3.2-ml/min flow rate.

Calibration plot

Series of standard solutions of vitamin C injected resulted in the calibration plot shown in Fig. 4 for absorbance vs vitamin C concentrations. Linearity was found to be perfect when taking six data points in the range 50–400 ppm vitamin C with a correlation coefficient (r) of 0.997 and the following calibration equation was obtained:

$$A = 0.2134 + 1.343 \times 10^{-3}C$$

where A is the absorbance and C is the concentration in ppm.

Peak width at the baseline was measured to be 36 sec thus defining a sampling frequency of 100 samples per hour and a one and a half minute total analysis time per sample was possible. A relative standard deviation of 0.88% was obtained for five repeated runs of 200 ppm of vitamin C solutions thus proving excellent reproducibility.

The present FIA method was applied to the determination of vitamin C in the proprietary drugs; Cebion and Redoxon in tablet formulations. Results of the determination were statistically compared with the BP method[10] by calculating Student t-test values which are summarized in Table 1.

Interferences

Interferences were studied by applying the method to the determination of vitamin C in tablet formulations containing 1.0 g of glucose and 0.5 g of starch as in Cebion and Redoxon proprietary drugs. Results obtained in Table 1 showed no interferences of these common excipients. The presence of carbonates as much as 2.0 g also showed no interferences to the results.

CONCLUSION

The present method is faster with a higher throughput than the previous reported methods and could be applied for the determination of vitamin C in a wider analytical range. It is suitable for the assay of vitamin C in tablet formulations without interferences from excipients as glucose and sugar which are usually added in dosage form. The full information on the investigation of the reaction mechanism has validated the proper conditions of the reaction and hence the assay method.

Table 1. Results obtained by the FIA method and the BP method[10] for the analysis of proprietary drugs

Drug	Supplier	Mean recovery + SD(%)*		
		FIA method	BP method	t†
Cebion	Merck, Darmstadt, F. R. Germany	99.82 ± 0.8	99.77 ± 0.8	0.14
Redoxon	F. Hoffman-La Roche LTD, Basel, Switzerland	99.88 ± 0.5	99.94 ± 0.7	0.29

*$n = 5$.

†t theoretical value = 2.31, $n = 5$.

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