Determination of Ascorbic Acid by Flow Injection With Chemiluminescence Detection*

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Flow injection was used in combination with chemiluminescence detection to determine ≥30 amol of ascorbic acid based on a luminol–Fe^{3+}–hydrogen peroxide system. The method was applied to the determination of ascorbic acid in pure form, pharmaceutical preparations and fruit juices. The method is sensitive, rapid (sampling rate 2–56 samples h^{-1}) and tolerates the presence of common ingredients usually found in fruit juices and drug formulations.

Keywords: *Ascorbic acid determination; flow injection; chemiluminescence detection; pharmaceutical preparation; fruit juice*

The determination of ascorbic acid in vitamins and natural products is of great importance. Ascorbic acid occurs naturally in most fruit juices and vegetables. Often, it is added during the manufacture of juices or soft drinks to improve their nutritional value, for attracting consumers or to act as an antioxidant to prolong the shelf-life of the commercial product. However, its concentration in fruit juice requires careful regulation, as too low a concentration will affect its antioxidant properties but too high a concentration can cause certain disadvantages such as accelerated colour fading.[1,2]

Owing to the wide use of ascorbic acid in canned fruits, vegetables, animal foods and drugs, and because of its significance in human physiology, there are numerous conventional methods for and reviews on the determination of ascorbic acid. Reported methods include titrimetric,[3–5] fluorimetric,[6,7] enzymic,[8,9] chromatographic,[10,11] electroanalytical[12,13] and spectrophotometric[14–17] methods, each with their advantages and disadvantages.

The development of extremely sensitive and reliable instrumentation has generated much interest in reactions that produce chemiluminescence (CL) and bioluminescence. Several applications[18–21] have shown the high sensitivity and selectivity of CL detection compared with established methods such as ultraviolet–visible and fluorescence spectrometry and electroanalytical detection.

The sensitivity of CL methods for the determination of trace metals is well known.[22,23] Most such methods are based on the catalysis or inhibition of reactions involving the oxidation of reagents such as luminol, lucigenin, lophine and gallic acid. Iron(II) ions react with luminol in alkaline hydrogen peroxide and this reaction is sensitive and selective. The detection limit for iron(II) is of the order of 0.1 pmol.[21] This reaction was used as the basis for the CL determination of ascorbic acid. As CL measurements require that the analyte be mixed reproducibly with the reagents necessary for light production, flow injection (FI) is considered to be very suitable for CL methods of analysis, as it readily achieves rapid and reproducible mixing.[24,25]

This paper describes a procedure based on the reducing effect of ascorbic acid on iron(III) ion and measuring the iron(II)-catalysed light emission from luminol oxidation by hydrogen peroxide. The procedure is simple, precise, sensitive and selective for the accurate determination of ascorbic acid in pure solutions, pharmaceutical preparations and fruit juices. Other compounds normally present along with ascorbic acid do not interfere in the determination.

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Experimental

Reagents

Ascorbic acid solution. Prepared daily by direct weighing of ascorbic acid (Merck) in 1% m/v metaphosphoric acid.

Luminol stock solution, 0.1 mol l^{-1}. Prepared by dissolving 0.1772 g of luminol (general-purpose reagent, Sigma) in 0.1 mol l^{-1} carbonate buffer.

Iron(II) chloride hexahydrate stock solution, 0.1 mol l^{-1}. Prepared by dissolving 2.703 g of the compound (BDH, AnalaR) in 100 ml of distilled water and adding a few millilitres of 0.1 mol l^{-1} sulfuric acid for stabilization.

Hydrogen peroxide solution. Prepared daily by diluting a measured amount of 30% m/v H_2O_2 (BDH) with distilled water.

Metaphosphoric acid solution, 1% *m/v*. Obtained by dissolving 1 g of the crystals (Merck) in 100 ml of distilled water.

Sodium carbonate buffer solution, 0.1 mol l^{-1}. Prepared by dissolving 10.6 g of sodium carbonate (BDH, AnalaR) in water and diluting to 1 l.

Procedure for the Determination of Ascorbic Acid in Tablets, Capsules, Syrup and Fruit Juices

Tablets and capsules

Weigh a known amount of the powdered tablets or capsules and dissolve in and dilute to the required volume with 1% m/v metaphosphoric acid solution. Use the final solution for determining the ascorbic acid content as shown in Fig. 1.

Syrup

Weigh several drops (equal to approximately 100 mg of ascorbic acid) of the drug into a 100 ml calibrated flask and dissolve in and dilute to volume with 1% m/v metaphosphoric acid solution. Use the final solution for determining the ascorbic acid content as shown in Fig. 1.

Fruit juices

Natural fruits were bought freshly from the local market. Samples were prepared by extracting the juices, the extracts were centrifuged until a clear liquid was obtained and then weighed prior to analysis. Weighed amounts of these liquids were diluted to a known volume with 1% m/v metaphosphoric acid solution. The final solutions were used for determining the ascorbic acid content as shown in Fig. 1.

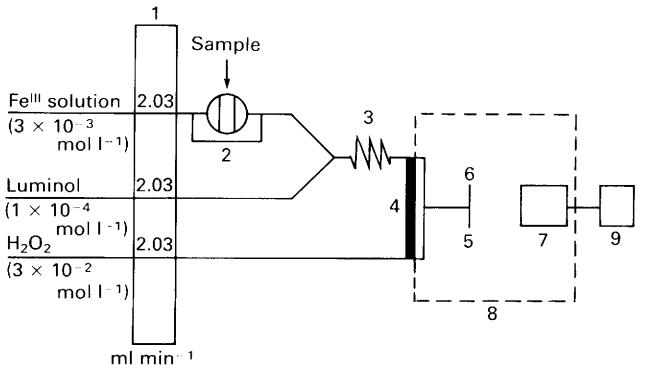

Fig. 1 Flow injection manifold for ascorbic acid determination: 1, peristaltic pump; 2, sample injection (30 μl); 3, reaction coil; 4, Perspex T-piece; 5, waste; 6, coiled flow cell; 7, PMT; 8, housing; and 9, recorder

Instrumentation for the Flow-through CL Detector

The flow cell was a coil made of 1.3 mm i.d. glass tubing spiralled to a diameter of 35 mm with five turns, enabling the flowing, emitting solution to remain in view of the detector for up to 30 s. The coiled glass flow cell, as used previously,[26] was backed by a mirror for maximum light collection and a sensitive photomultiplier tube (PMT) (Thorn EMI, 9789QB) for measurement of the emitted light intensity. The PMT was operated at 400 V, provided by a stable high-voltage power supply (Thorn EMI, Model PM 28BN). No wavelength selection was involved.

Flow System for the Determination of Ascorbic Acid by Chemiluminescence

A schematic diagram of the flow manifold is shown in Fig. 1. The acidified iron(III) solution (3 × 10^{-3} mol l^{-1}) was used as the carrier stream for the sample, which was usually acidified with 1% m/v metaphosphoric acid solution, and the luminol solution (1 × 10^{-4} mol l^{-1}) was mixed with the carrier stream at the reaction coil. Each solution was pumped at 2.03 ml min^{-1} by a peristaltic pump (Gilson Minipuls 3MP4). Hydrogen peroxide (3 × 10^{-2} mol l^{-1}) was pumped at 2.03 ml min^{-1} by a peristaltic pump (Ismatec Mini-S-820) and mixed with the other two solutions at a Perspex T-piece. Poly(tetrafluoroethylene) tubing (0.8 mm i.d.) was used to connect the reagent streams to the T-piece. Sample injection was effected with a Rheodyne Type RH 5020 rotary valve, fitted with a 30 μl sample loop. The CL emission was recorded on a chart recorder (Yokogawa Model 3021) and the peak heights were measured.

Results and Discussion

Optimization of Experimental Variables

A series of experiments were conducted to establish optimum analytical variables. The parameters optimized included flow rate, pH, reagent concentrations and reactor length.

Effect of luminol concentration

The effect of luminol concentration was studied in the range 1 × 10^{-3}–1 × 10^{-6} mol l^{-1}. It was observed that at concentrations higher and lower than 1 × 10^{-4} mol l^{-1} there was a sharp decrease in the CL. Therefore, 1 × 10^{-4} mol l^{-1} was selected for the analysis.

Effect of iron(III) concentration

The effect of the iron(III) concentration on the CL intensity from the ascorbic acid signal was studied in the range 1 × 10^{-2}–1 × 10^{-5} mol l^{-1}. The results obtained are shown in Fig. 2. A concentration of 3 × 10^{-3} mol l^{-1} was found to be optimum; higher concentrations (up to 1 × 10^{-2} mol l^{-1}) gave a significant increase in the signal, but with poorer reproducibility.

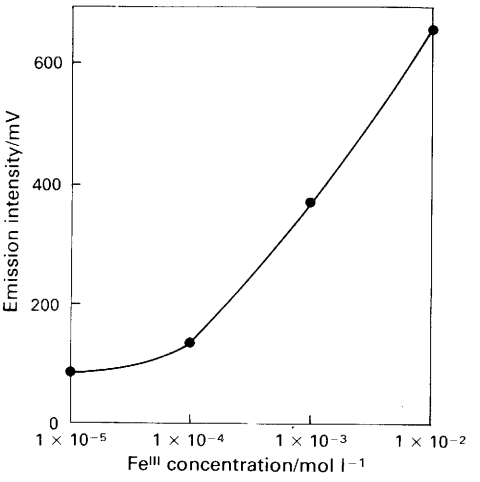

Fig. 2 Effect of Fe^{III} concentration on CL intensity of ascorbic acid (1 × 10^{-6} mol l^{-1}) in the presence of 1 × 10^{-4} mol l^{-1} luminol and 0.025 mol l^{-1} H_2O_2

Effect of hydrogen peroxide concentration

The optimum hydrogen peroxide concentration was 0.03 mol l^{-1}. Higher concentrations (up to 0.1 mol l^{-1}) gave higher emission, but the signal was noisy and irreproducible. The decrease in intensity at lower concentrations (≤0.01 mol l^{-1}) might be attributed to an inadequate amount of H_2O_2 available for reaction with the luminol.

Effect of ascorbic acid pH

The pH of the sample solution was found to be very critical. The results of a detailed study of the stability of ascorbic acid at different pH values are shown in Fig. 3. The maximum response was obtained at pH 1.5, indicating the best medium for the reducing activity of a solution of ascorbic acid. At pH >1.5, ascorbic acid became less stable and the destruction rate increased rapidly with increasing pH, causing the reducing activity to decrease.

Effect of flow rate

The flow rate of the solutions is very important and should be regulated. At flow rates that are too low or too high, CL is not emitted in the flow cell and hence the emitter is not detected. Fig. 4 shows the effect of total flow rate on CL intensity. The intensity increased with increasing flow rate. However, a total flow rate of 6.1 ml min^{-1} (2.03 ml min^{-1} for each channel) is recommended because of the greater precision and economy in the use of reagents.

Effect of reactor length

The proposed method for the determination of ascorbic acid is based on the reducing effect of the acid on iron(III) ion, and the chelating ability of luminol with iron(II) ion (in the presence of H_2O_2 as an oxidant) to provide a chemiluminescent product. Therefore, the optimum time for the reaction of ascorbic acid with iron(III) ion, in order to produce sufficient iron(II), was studied by varying the reactor length from 50 to 400 cm. Maximum emission intensity was obtained when the reactor length was 200 cm (the signal takes about 35 s to reach its maximum). Increasing the reactor length further caused a decrease in the emission intensity, probably owing to sample dispersion. Shortening the reactor length below 200 cm gave a ≥50% reduction in the emission intensity; probably

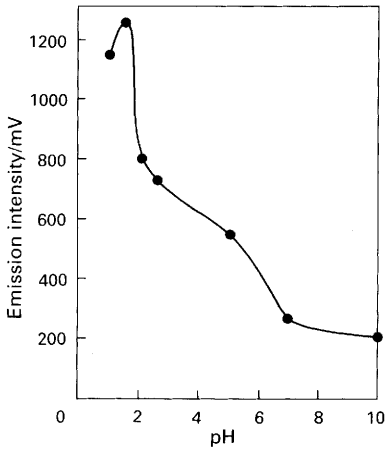

Fig. 3 Effect of pH on CL intensity of ascorbic acid (1×10^{-6} mol l^{-1}) in the presence of 3×10^{-3} mol l^{-1} Fe^{3+} and 0.03 mol l^{-1} H_2O_2

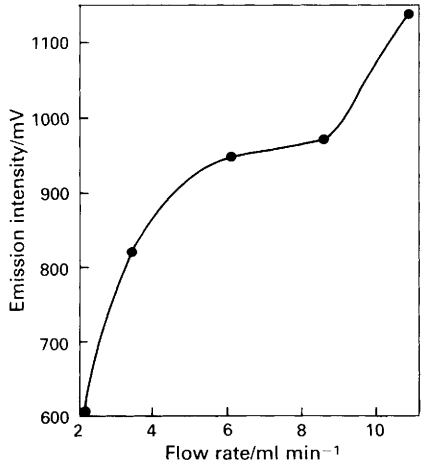

Fig. 4 Effect of total flow rate on CL intensity. Conditions: 1×10^{-6} mol l^{-1} ascorbic acid, 3×10^{-3} mol l^{-1} Fe^{3+}, 1×10^{-4} mol l^{-1} luminol and 0.03 mol l^{-1} H_2O_2

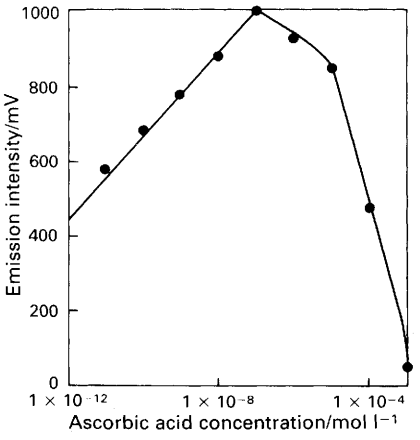

Fig. 5 Calibration graph for ascorbic acid (each point is the mean of four measurements under the recommended conditions)

Table 1 Effect of foreign ions (all 1×10^{-5} mol l^{-1}) on the signal for 1×10^{-6} mol l^{-1} ascorbic acid

	Signal height	
Interferent	mV*	%†
—	1060	100
Chloride	1090	102.83
Nitrate	1120	105.66
Nitrite	1100	103.77
Oxalate	1200	113.21
Sulfide	1400	132.08
Sulfate	1450	136.79
Tartrate	1060	100
Fructose	1060	100
Glucose	1060	100
Nicotinamide	1110	104.72
Riboflavin	1070	100.94
Starch	1080	101.89

* Results based on three injections per sample.
† Relative to ascorbic acid = 100%.

the iron(III) ion was in excess and the amount of iron(II) ion produced was not proportional to the amount of ascorbic acid in the sample.

Determination of Ascorbic Acid

The optimum values for the CL–FI variables are: flow rate per channel = 2.03 ml min^{-1}, reactor length = 200 cm, pH = 1.5, $[H_2O_2]$ = 0.03 mol l^{-1}, [luminol] = 1×10^{-4} mol l^{-1}, $[Fe^{3+}]$ = 3×10^{-3} mol l^{-1} and voltage supply to the PMT = 400 V. The optimization of the variables ensured the maximum emission intensity from the chemiluminescent product as the sample plug passed through the detector. The applicability studies involved the determination of the practical range, the repeatability in application, the effect of interferents and the reliability of the method for analysing a range of natural samples.

Practical Range and Repeatability in Application

An aqueous stock standard solution of 0.1 mol l^{-1} ascorbic acid was prepared, from which working standard solutions were prepared by serial dilution with 1% m/v metaphosphoric acid solution. A linear calibration graph for ascorbic acid was obtained over the range 1×10^{-11}–1×10^{-7} mol l^{-1} using the flow system described in Fig. 1, as shown in Fig. 5. The slope is 0.52. Above 1×10^{-7} mol l^{-1}, the slope decreased because the concentration of the reagents became a limiting factor (*i.e.*, there was not enough sample to react with the luminol). Four replicate injections of ascorbic acid were made per sample. The relative standard deviation for ten replicate sample injections of 1×10^{-6} mol l^{-1} ascorbic acid was 1.4%. There was no detectable blank signal. The ascorbic acid concentration that gave a signal-to-noise ratio of 2 was 1×10^{-12} mol l^{-1} (30 amol or 5 fg per injection), compared with 1×10^{-13} mol l^{-1} iron(II)[21] using a luminol–alkaline hydrogen peroxide system.

Interference Studies

The specificity of the method for ascorbic acid in the presence of frequently encountered excipients, common reducing agents and inorganic ions was studied, in addition to the effect of other vitamins that are likely to be present along with ascorbic acid in multivitamin formulations. The results obtained are summarized in Table 1.

Among the inorganic anions, oxalate, sulfide and sulfate interfered severely in the determination by enhancing the emission intensity. Nitrate, chloride and nitrite also affected the results but their effects were mild compared with those of oxalate, sulfide and sulfate. Interference by these ions was expected because the proposed method is based directly on the ability of ascorbic acid to reduce iron(III). Hence any species that can bring about this reduction would be expected to interfere. However, species such as tartrate, fructose, glucose, riboflavin and starch showed no interference. Nic-

Table 2 Determination of ascorbic acid in pharmaceutical preparations by the proposed method and the official method[27]

Preparation*	Amount of ascorbic acid/mg: Claimed†	Amount of ascorbic acid/mg: Proposed method‡	Recovery (%)
Multivitaplex tablets	30	28.24	94.13
Ascoplex capsules	175	164.44	93.95
Redoxon tablets	1000	908.20	90.82
Cebion tablets	1000	968.80	96.88
Multivitaplex syrup	90	91.94	101.15

* Multivitaplex tablets, Ascoplex capsules and Multivitaplex syrup were obtained from Dumex, Denmark; Redoxon tablets from La Roche, Switzerland; and Cebion tablets from Merck, Germany.
† According to the official method.[27]
‡ Average of six injections per sample.

Table 3 Determination of ascorbic acid in fruit juices

Juice	Amount of ascorbic acid/mg: Claimed*	Amount of ascorbic acid/mg: Found†	Recovery (%)
Tomato	980	1035.89	105.70
Orange	1050	1117.89	106.44
Lemon	1033	986.24	95.47
Banana	950	970.52	102.16

* According to the official method.[27]
† Average of six injections per sample.

otinamide gave a slight enhancement to the emission intensity but can be tolerated as an interferent in 10-fold excess over ascorbic acid.

Analysis of Pharmaceutical Preparations

The proposed method was applied to the analysis of several pharmaceutical dosage forms containing ascorbic acid either in tablets, capsules or a syrup. The results are summarized in Table 2. Most of the results agree with the reported values. However, non-significant differences were found in the results, which can be attributed to the quenching effect of the coloured solutions of the samples, to the heterogeneity of the samples or the effect of other reducing species in the preparations.

Analysis of Some Fresh Fruit Juices

Table 3 gives the ascorbic acid content of some fruit juices analysed by the proposed method. The sample matrix is very complex, because a large number of additives are now added to foods for different purposes, and such additives may affect the proposed method either by quenching or enhancing the emission intensity. However, the good recovery of ascorbic acid shown in Table 3 indicates that no significant interference occurred.

In conclusion, the determination of ascorbic acid by CL–FI has advantages over conventional methods in that it is faster and simpler, there is minimum manipulation and intervention of the operator, it is suitable for routine control, it can be carried out directly without any pre-treatment and it is extremely sensitive.

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