Flow-Injection Amperometric Determination of Ascorbic Acid Using a Graphite-Epoxy Composite Electrode Modified with Cobalt Phthalocyanine☆

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ABSTRACT: *A flow injection method is reported for the determination of ascorbic acid based on amperometric detection, using a cobalt(II) phthalocyanine (CoPc) modified graphite-epoxy electrode. The amperometric response was evaluated with regard to pH, ionic strength of the electrolyte, flow rate of the carrier solution, injected sample volume and conditioning time of the electrode. The limit of detection was 0.013 mM (50 μL injected), and the calibration plot was linear in the ranges of 0.025-1.0 mM (correlation coefficient, r = 0.9989, n = 6) and 1.0-10 mM (r = 0.9997, n= 5). The relative standard deviation for the measurement of ascorbic acid at several concentrations was ≤ 1%. The modified electrode was found to retain its full response in flowing streams for several days of operating time. The results of ascorbic acid quantification in fruit juices and vitamin tablets are reported.*

KEY WORDS: *Ascorbic acid, Cobalt phthalocyanine, Modified electrodes, Amperometry, Flow-injection analysis*

INTRODUCTION

Ascorbic acid (vitamin C) is an essential nutritional factor with many biochemical functions, such as being an enzyme cofactor, a reducing agent and involvement in neurotransmitter related enzymes[1,2]. This compound is found in numerous natural resources and is widely used as antioxidant agent in foods and drinks for the stabilization of color and aroma with subsequent extension of the storage time of the products [3]. Since the interest in harmful oxidative changes in food products has increased in recent years, the quantification of this ingredient in foods and beverages has received increasing attention [4]. Ascorbic acid is commonly used in various forms and for-

☆ *Dedicated to Professor Mahdi Golabi on the occasion of his 67th birthday.*

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mulations, such as tablets and multivitamin preparations in order to supplement its inadequate dietary intake, especially during pregnancy and lactation. Ascorbic acid measurement also has been used as a marker for product quality and food deterioration [5]. Therefore, it is of prime importance to determine ascorbic acid during production, transformation and storage. Quantification of ascorbic acid is generally based on its reducing properties or its capacity to produce colored substances. A survey of the literature reveals that ascorbic acid may be determined by various methods such as titrimetric, spectrophotometric, enzymatic and chromatographic [6–11]. Spectrophotometric methods, which are being the most used, suffer from tedious pretreatment, such as coupling with diazonium compounds to produce colored complexes [7], and have the drawback that both ascorbic acid and dehydroascorbic acid, which is the oxidation product of ascorbic acid, are determined simultaneously. Chromatographic techniques suffer from relatively long analysis time and expensive instrumentation [12-15]. Enzymatic methods, although highly selective, are difficult to manipulate and require specific conditions to prevent enzyme denaturation.

Ascorbic acid has been the subject of many electrochemical investigations and, on the basis of such studies several electrochemical methods have been developed for its determination [3, 16-24]. Electrochemical methods for ascorbic acid present many advantages as they do not require prior extraction, enjoy relatively high sensitivity and can be used with success in a colored matrix. Although ascorbic acid is electroactive, its oxidation at carbon and metallic electrodes typically requires undesirably high working potentials [19, 25-27] and as a result is prone to interference from other readily oxidizable compounds. This problem may be overcome by the use of modified electrodes, which favor electrocatalysis and anticipate the oxidation potential of the analyte. The design and application of the chemically modified electrodes for electroanalysis has been the subject of much research in recent years [28-31]. One of the most attractive modifiers is cobalt(II) phthalocyanine (CoPc), which is known for its effective electrocatalytic action towards a wide range of redox systems. Much research on the application of CoPc-modified electrodes has been reported. Wring and Hart [32], in their review on the application of chemically modified electrodes as electrochemical sensors for the analysis of biologically important compounds, have paid a special attention to the use of CoPc. The electrodes modified with this compound have been applied as amperometric sensors for the determination of glutathione [33,34], pesticides [35], hydrazine, oxalic acid and cysteine [36], and ascorbic acid [19,37]. They have also been used as detectors for liquid chromatographic detection of a number of biologically important compounds [38].

This paper reports a flow-injection amperometric system based on a graphite-epoxy composite electrode, modified with CoPc, for determination of ascorbic acid in pharmaceutical preparations and fruit juices. Owing to its versatility and flexibility, high sample throughput and ease of operation, flow-injection analysis (FIA) is now a well-known analytical technique [39]. Amperometric method is also known for its high sensitivity, fast response and relatively simple devices. Therefore, a combination of FIA with amperometric detection system allows very fast and sensitive methods of analysis to be developed. The graphite-epoxy composite electrodes offer desireable properties such as renewability (by polishing), ease of preparation, economy and rigidity [40]. The latter makes these electrodes applicable as electrochemical detectors in flow systems such as flow injection and chromatography. This is a marked advantage of composite graphite-epoxy over the carbon-paste electrodes that lack stability in flow systems due to swelling phenomena of the carbon-paste [41].

EXPERIMENTAL

Chemicals and reagents

Cobalt(II) phthalocyanine was obtained from Aldrich (Milwaukee, WI, U.S.A) and used as received. Epoxy resin was from (Applied Plastics Co., California). Ascorbic acid, graphite powder and all other chemicals (all from Merck, Darmstadt, Germany) were of highest purity available and used without any further purification. Doubly distilled, deionized water was used for preparing all solutions and throughout the experiments.

Ascorbic acid solution, 0.01 M, was prepared daily in phosphate buffer at the required pH and protected from light during all investigations. Solutions of 0.1 M of the sodium carbonate, potassium oxalate, sodium acetate, iron(III) chloride, copper(II) sulfate, tartaric acid, citric acid and benzoic acid were prepared for studying the effect of interferences. Unless otherwise specified, the supporting electrolyte and carrier solution were 0.05 M phosphate buffer prepared from potassium dihydrogen phosphate, phosphoric acid and potassium hydroxide solutions. These were mixed to give solutions of the required pH, using a pH meter, and diluted with water to give the final concentration. Voltammetric experiments were carried out in 0.05 M phosphate buffer solutions, deoxygenated by pure nitrogen.

Apparatus

Cyclic voltammetry was performed with a Model 746 VA Trace Analyzer in conjunction with a 747 VA stand (Metrohm, CH-9101, Switzerland). A conventional three-electrode cell was employed incorporating unmodified or modified electrodes, a saturated Ag/AgCl reference electrode and a Pt-wire counter electrode. Constant potential amperometric measurements in flowing streams were carried out on a Yanaco Model P8 detector (Yanagimoto, Kyoto, Japan) with a laboratory made flow-through electrochemical cell consisting of a CoPc modified as working electrode, an Ag/AgCl reference electrode and a platinum counter electrode. The thickness and width of the cell were 0.8 and 4 mm, respectively, and the cell volume was about 50 μL. Schematic diagram of the thin-layer electrochemical cell is shown in Fig. 1. The present cell design is simple and inexpensive and the reference electrode is close to the working electrode so as to minimize the uncompensated resistance [42].

The FIA system was constructed according to the conventional design (Fig. 2). Flow of the electrolyte solution was maintained by a syringe pump (Mettler Model DV11, Toledo Greifensee, Switzerland) using a 100 mL cartridge volume. A Rheodyne Model 7125(Rheodyne, L. P., Rohnert Park, U.S.A) injection valve with various fixed volume loops were used for sample introduction. PTFE tubing of 1 mm id was used for the connections.

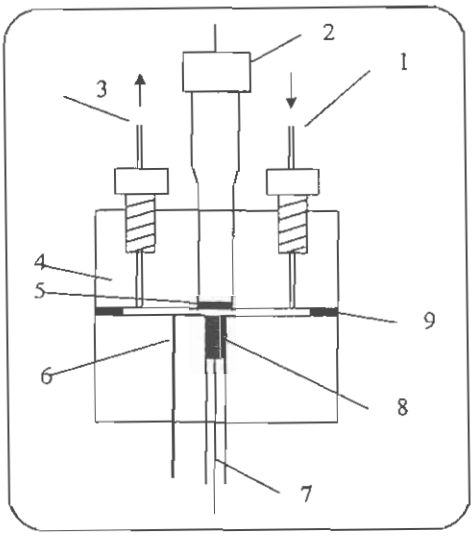

Fig.1: Schematic diagram of the thin-layer electrochemical cell. (1) Inlet; (2) Ag/AgCl reference electrode; (3) outlet; (4) Teflon body; (5) sintered glass; (6) auxiliary electrode; (7) copper wire; (8) graphite-epoxy composite electrode modified with CoPc as the working electrode; (9) Teflon spacer

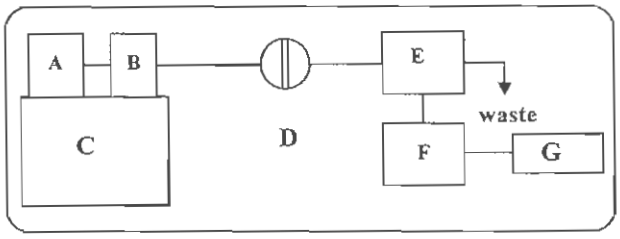

Fig. 2: Schematic diagram of the FIA system; (A) Carrier solution reservoir; (B) syringe; (C) pump drive; (D) injection valve; (E) thin-layer electrochemical cell; (F) current measuring system; (G) strip chart recorder

Modified electrode preparation

The modified electrode was prepared by mixing graphite powder with CoPc (5% w/w) and then adding epoxy resin (15% w/w); after thorough hand mixing in a mortar and pestle, a portion of the composite mixture was packed into the end of a Pyrex glass tubing (ca. 3 mm id. and 10 cm long). Electrical contact was made by forcing a copper wire down the glass tube and into the back of the mixture before hardening and left over night. The epoxy resin was cured according to instructions recommended by the manufacturer The working surface of the electrode was polished using a polishing paper and then rinsed several times with distilled water.

Sample preparation

Fresh juices were obtained by squeezing each of fruit samples into a glass beaker and then centrifuged as necessary to obtain a clear solution. The juices were then diluted with the supporting electrolyte (5 mL juice to a final volume of 25 mL) to give an ascorbic acid concentration between 10^{-3}–10^{-4} M.

For determination of ascorbic acid in vitamin tablets, each tablet was crushed with a mortar and pestle, and an appropriate amount of the powder was dissolved in about 20 mL of distilled water. The resulting solution was filtered and then appropriately diluted with phosphate buffer to reach the linear range of the calibration curve.

Each sample was injected at least three times at intervals of ~1 min. The peak height obtained after each injection was measured and the concentration of ascorbic acid was obtained through the calibration curve or by the standard addition method.

Analysis by the AOAC method [6]

Prepare fresh juice by pressing the fruit and filter. Add an appropriate aliquot to an equal volume of HPO_3-CH_3COOH solution (obtained by dissolving 1.5 g HPO_3 in 4 mL CH_3COOH and 20 mL water and then diluting to about 50 mL). Mix the solution and titrate with 2,6-dichloroindophenol until a light but distinct rose-pink color persists for ≥5 s. 2,6-dichloroindophenol solution is obtained by dissolving 50 mg of its sodium salt in 50 mL water to which has been added 42 mg $NaHCO_3$. Shake the solution to dissolve and then dilute to 200 mL with water. Filter the solution into an amber glass bottle. Standardize the solution against ascorbic acid solution as described above.

RESULTS AND DISCUSSION

The catalytic function of CoPc as a mediator in carbon-paste and other matrices has been observed towards a variety of compounds [32-38]. In an effort to develop a potentiometric sensor for ascorbic acid determination, we have recently studied voltammetric and potentiometric behavior of this mediator using a modified carbon-paste electrode [43]. We showed that the CoPc modified electrode not only reduced the overvoltage necessary for the oxidation of ascorbic acid, but also considerably increased the sensitivity of the current signal. Due to mechanical instability of the carbon-paste in flowing streams, in the present study we examined a graphite-epoxy composite electrode modified with CoPc as an electrochemical detector for flow-injection amperometric detection of ascorbic acid. Preliminary experiments, carried out by cyclic voltammetry, revealed that this electrode also behaves electrocatalytically towards ascorbic acid and shows a remarkable current enhancement and additionally, a marked decrease in the oxidation potential of ascorbic acid, similar to the modified carbon-paste electrode [43]. The catalytic function of the modified electrode is demonstrated in Fig. 3A by cyclic voltammogram of 1.0×10^{-2} M ascorbic acid solution at pH 5.0. The voltammogram of the modified electrode in supporting electrolyte is also shown in Fig. 3B. For amperometric detection of ascorbic acid, +0.4 V was found the most favorable potential to be applied to the modified electrode. Higher potentials resulted in a considerable increase in the residual current and lower reproducibility, but at lower potentials, the sensitivity of the measuring system was considerably decreased. In fact, 0.4 V was chosen as a compromise between sensitivity and reproducibility of the measuring system.

Since it has been reported that the stability of ascorbic acid is highly dependent on the pH [4], the effect of pH on the peak current of the FIA-amperometric detection system was studied in 0.1 M phosphate buffer in the pH

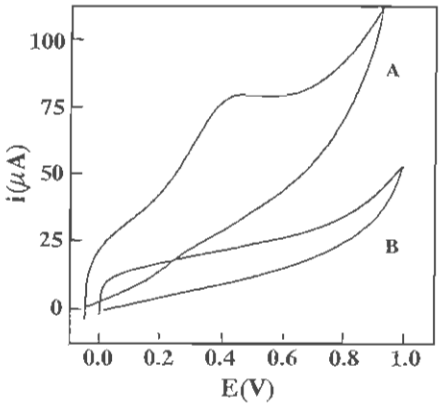

Fig. 3: Cyclic voltammogram of the CoPc-modified graphite-epoxy electrode in: (A) 1×10^{-2} M ascorbic acid solution, (B) in the background electrolyte, both at pH 5.0

range of 3–7. At each pH, at least three 50 μL of 1 mM ascorbic acid were injected at a carrier flow rate of 0.9 mL min^{-1}. The results of average responses are shown in Fig. 4. It was observed that the peak current is strongly affected by the pH of the solution; the largest sensitivity was obtained at pH 5. In fact it has been suggested that for maximum stability, ascorbic acid solutions should be buffered to pH ≤ 5.4 [44]. Ascorbic acid is unstable at higher pH values and can be easily oxidized by dissolved oxygen present as impurity in the solution [45]. This effect is intensified under alkaline conditions. so that a very small current was observed above the pH 7. It seems that the loss of a proton from ascorbic acid, giving a monoanion, results in an increase in its reducing properties. This may be the reason for the decrease of current amplitude at pH values lower than 5.

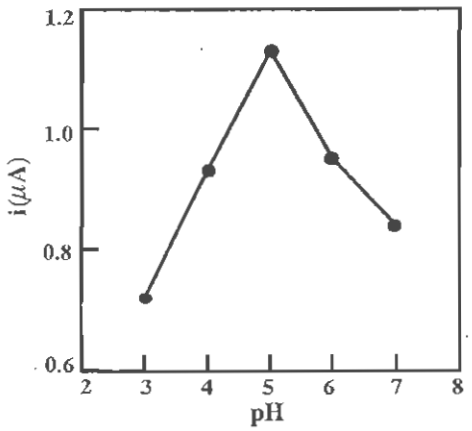

Fig. 4: Effect of pH on the peak height of the FIA recording for injection of 50 μL of 1 mM ascorbic acid. Each point is the average of at least three injections

The effect of concentration of the phosphate buffer, used as the carrier solution, on the amperometric signal was investigated in the range of 0.025 to 0.1 M by injecting several 50 μL volumes of 1 mM ascorbic acid solution at a flow rate of 0.9 mL min^{-1}. The magnitude of the measured current signal was found to increase by increasing the concentration of the phosphate buffer up to 0.05 M; beyond that the variation in current was not considerable. Based on the results of pH and ionic strength studies, 0.05 M phosphate buffer of pH 5 was used for further investigations.

Optimization of flow-injection variables

Before starting the measurement of ascorbic acid, it was necessary to condition the modified electrode for obtaining a stable base line. Conditioning was performed by passing the carrier solution through the flow cell while maintaining a constant potential of 0.4 V between the modified and reference electrodes. Maximum current, reproducible results and a flat base line were observed after conditioning the electrode for about 30 min. After this period, no cleaning or pretreatment of the indicator electrode was necessary after each injection.

Two operating parameters, the flow-rate of solution and the injection volume were optimized in this study. An increase in the peak height as well as a decrease in peak widths was observed when the flow-rate of the carrier solution (0.05 M phosphate buffer of pH 5) was increased from 0.5 to 1.4 mL min^{-1}, beyond which the sensitivity was considerably decreased. However, the reproducibility of the current deteriorated at flow rates higher than 0.9 mL min^{-1}. Hence, 0.9 mL min^{-1} was chosen as the optimum flow-rate. With this flow-rate, about 60 samples h^{-1} can be analyzed with good resolution. The effect of the sample volume injected was tested with several injection loops. An increase in peak heights and widths was observed by increasing the injected volume. A sampling loop of 50 μL was chosen as a compromise between sensitivity and reproducibility of the system.

Calibration curves and analytical characteristics

Fig. 5 shows the response of the modified electrode to injection of 50 μL of 0.25, 0.5 and 1 mM ascorbic acid. The proportionality between the amperometric current and the ascorbic acid concentration was proven from the calibration plot obtained by injecting ascorbic acid standard solutions. Under the optimized conditions, i.e. 0.05 M phosphate buffer of pH 5 at a flow rate of 0.9 mL min^{-1} and an injection volume of 50 μL, linear calibration curves were observed over the concentration ranges of 0.025-1.0 mM (r = 0.9989) and 1.0-10 mM (r = 0.9997), as shown in Fig. 6.

The limit of detection, calculated according to the $3S_b/m$ criteria, where m is the slope of the lower range of linearity and S_b is the standard deviation (n = 10) of the

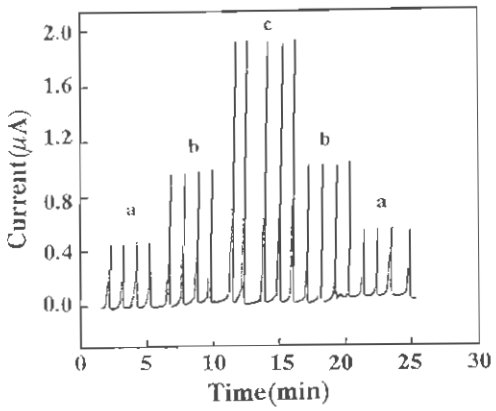

Fig. 5: Repetitive injections of 50 μL of samples containing 0.25, 0.50 and 1.0 mM of ascorbic acid (a-c) in 0.05 M phosphate buffer of pH 5. Applied potential: +0.4 V. Flow-rate: 0.9 ml min^{-1}

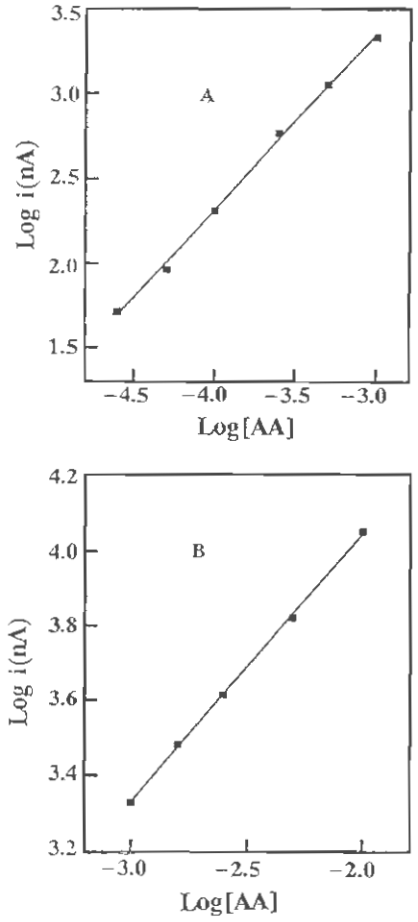

Fig. 6: Calibration plots, peak current vs. ascorbic acid concentration at two different concentration ranges (A and B) under optimized conditions. Each data point is the average of 5 measurements

signals from dilute ascorbic acid solution was 0.013 mM. This is a bout two orders of magnitude lower than the concentration generally found in fresh fruits. Fig. 7 shows FIA signals from 15 repeated injections of 2 mM ascorbic acid, which showed a RSD value of 0.96%. Good reproducibility was also observed in the case of orange juice sample. Fig. 8 shows FIA signals from orange juice and the same sample after spiking with various concentrations of ascorbic acid.

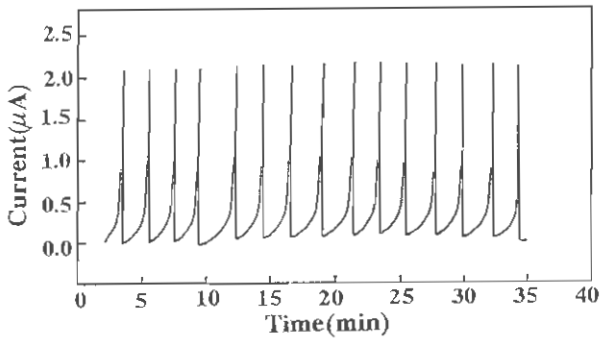

Fig. 7: FIA signals obtained with successive injections of 2 mM ascorbic acid under optimized conditions

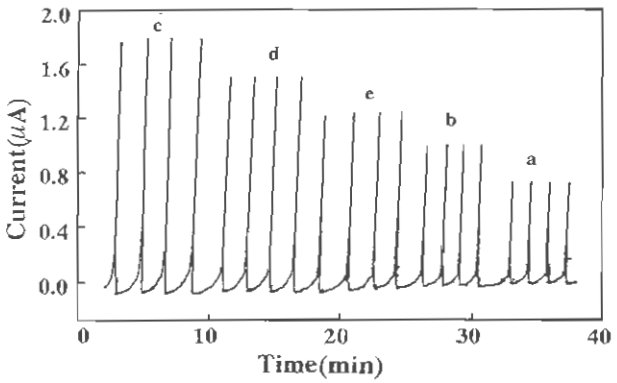

Fig. 8: FIA signals obtained with successive injections of orange juice; (a), 5 mL sample diluted to 25 mL with phosphate buffer; (b-e), orange juice sample spiked with various concentrations of ascorbic acid

The ease of electrode surface regeneration and its mechanical stability (rigidity of the electrode surface) are two of the very important aspects from a practical point of view. In fact, one graphite-epoxy electrode can be used for several days without a considerable deterioration of the signal. This constitutes a great advantage with respect to the modified carbon-paste matrix. To revive an elec-

trode that shows low sensitivity or non-reproducible results, it is enough to polish the electrode surface briefly using a polishing paper.

Interferences

The effect of several species including citric acid, benzoic acid, potassium oxalate, sodium acetate, tartaric acid, sodium carbonate, iron(III) chloride and copper(II) sulfate that may either naturally be present in fruit juices, as preservative, or contaminate them during processing and storage, was investigated for their possible interference with ascorbic acid determination. The presence of a species was tolerable if its effect on the signal is $\leq$ 3RSD of the ascorbic acid signal. Most of these species were found to cause no significant interference at the normal concentrations found in juices. The species and their concentrations not causing interference with 1 mM ascorbic acid were as follows: 100 mM benzoic acid; 50 mM tartaric acid, citric acid, acetate; and 25 mM oxalate and carbonate. The tolerance limit for most of the common anions and cations were high, but Fe(III) and Cu(II) ions severely interfered in the determination of ascorbic acid. The interference effect of these species is most probably due to oxidation of ascorbic acid. However, as it is shown in the next section, the existence of a good correlation between the results of fruit juice analysis by this method and a standard method may be taken as an indication that no other sample components significantly interacted with the CoPc modified electrode.

Determination of ascorbic acid in fruit juices and vitamin tablets

The flow injection amperometric assay was applied to the determination of ascorbic acid concentrations in freshly squeezed orange juice and vitamin-C tablets. The method of US Association of Official Analytical Chemists (AOAC) [6] based on using 2,6-dichlorophenolindophenol was applied as the reference method. The results for analysis of several samples obtained from different types of citrus fruits including orange, sour orange and sweet lemon, and of vitamin C tablets, using the proposed method, were in close agreement with the standard method ($r = 0.995$) (Table 1). Moreover, using the t-test and the matched pair methods for comparison of the results from the two methods, no difference in the results was obtained at the 95% significance level. Further validation of the method was done by analyzing solutions of an orange juice sample spiked with known amounts of ascorbic acid. The results of this experiment, given in Table 2, indicate recoveries in the range of 99.2-101.2%.

CONCLUSIONS

The results of this study demonstrate the suitability of graphite-epoxy electrode modified with CoPc for amperometric detection of ascorbic acid in flowing streams. The electrocatalytic behavior of the modified electrode and its mechanical stability coupled with FIA provides a sensitive, fast and economic method for determination of ascorbic acid in fruit juice samples and pharmaceutical preparations.

Table 1: Determination of ascorbic acid in fruit juices and vitamin tablets

	Amount of ascorbic acid found (mmol L^{-1})		
Sample	This method	Reference method	RSD (%)[a]
Orange juice	3.62	3.63	2.0
sweet lemon juice	3.03	2.86	1.8
sour orange juice	1.71	1.82	2.2
Vitamin C tablet [b]	1.42	1.46	3.0

(a) For the proposed method
(b) Concentration in mmole/tablet

Table 2: Recovery of ascorbic acid from spiked solutions of orange juice

Concentration of ascorbic acid (mg L^{-1})		
Added	Found	Recovery(%)
40.8	41.2	101.0
81.6	82.6	101.2
122.4	121.6	99.3
163.2	162.0	99.2

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