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Analytical Methods Section

Determination of ascorbic acid in fruits and vegetables by stripping voltammetry on a glassy carbon electrode

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A sensitive and selective method is described for the determination of ascorbic acid by stripping voltammetry on a glassy carbon electrode. It involves formation of ferroin when ascorbic acid reacts with a mixture of iron (III) and 1,10-phenanthroline. Ferroin is adsorbed on the glassy carbon electrode followed by stripping voltammetric measurement in the cathodic direction. A linear concentration range is obtained from $2{\cdot}0 \times 10^{-7}$–$2{\cdot}2 \times 10^{-6}$ g/ml for an accumulation period of 30 s. The method was successfully employed for the determination of ascorbic acid in fruit and vegetable juices. Comparison of the results obtained for the juices demonstrated reasonable agreement with those obtained by a spectrophotometric method.

INTRODUCTION

The most widely used procedures for the determination of reduced ascorbic acid depend upon its oxidation to dehydroascorbic acid. For example, titrimetric and spectrometric methods have been used for the determination of ascorbic acid (Freed, 1966; Skaltsa *et al.*, 1987; Amir, 1987; Barbas-Pardilid *et al.*, 1989; Wang & Chen, 1991; Huang *et al.*, 1993; Zhang & Hung, 1993), but the methods have certain limitations due to the presence of interfering substances. Polarographic methods can be used for ascorbic acid determinations (Lento *et al.*, 1963; Ohmon *et al.*, 1977; Ratzkowski & Korol, 1977; Sontage & Kainz, 1978; Branca, 1980; Amin, 1983; Lao *et al.*, 1985; Kozar *et al.*, 1988; Fernhunde, 1992; Wang *et al.*, 1992). The method involving oxidation of ascorbic acid at a dropping-mercury electrode, and the advantages of the method, have been described. The main limitations of polarography have been the relatively low sensitivity and the limited anodic voltage range of the mercury electrode. Stripping voltammetry can be used for ascorbic acid determinations (Linquist, 1975; Lechien *et al.*, 1982), but the selectivity is poor. In an attempt to develop a sensitive and accurate method for the assay of ascorbic acid, we have studied the application of stripping voltammetry (glassy carbon electrode used as working electrode). The present method is based upon oxidation of ascorbic acid with a mixture of iron (III) and 1,10-phenanthroline to form ferroin complex followed by stripping voltammetry measurement of ferroin. Following this, the ascorbic acid can be determined. It is used for the determination of ascorbic acid in fruit and vegetable juices with good results. This electrochemical method is simple, reliable and quite sensitive, and avoids the use of mercury in stripping voltammetry.

MATERIALS AND METHODS

Apparatus

A Model AD-3 polarograph (Jintan Analytical Instrumental Factory, China) was used. A three-electrodes system was used with a glassy carbon electrode as working electrode. The reference and counter electrodes were saturated calomel and platinum wire electrodes, respectively. A 751-spectrophotometer (Shanghai, China) with 1-cm cell and a pH meter were used.

Reagents

All reagents, unless otherwise stated, were of analytical grade and doubly-distilled water was always used.

Ascorbic acid standard solution, 100 μg/ml, was prepared daily. 1,10-Phenanthroline (phen) solution was $7{\cdot}6 \times 10^{-3}$ M. A 100 μg/ml iron (III) solution was prepared by weighing and dissolving 0·08634 g $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 2 ml of 1 M nitric acid and the solution was diluted to 100 ml with water ($1{\cdot}8 \times 10^{-3}$ M iron (III)). 0·14 M HAc–0·08 M NaAc buffer solution was prepared.

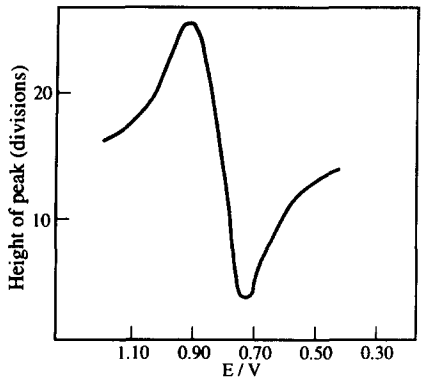

Fig. 1. Stripping voltammetric response of ferroin on glassy carbon electrodes. HAc–NaAc buffer solution (pH 4·5); iron (III), $7{\cdot}2 \times 10^{-5}$ M; phen, $4{\cdot}6 \times 10^{-4}$ M; ascorbic acid, 0·8 μg/ml.

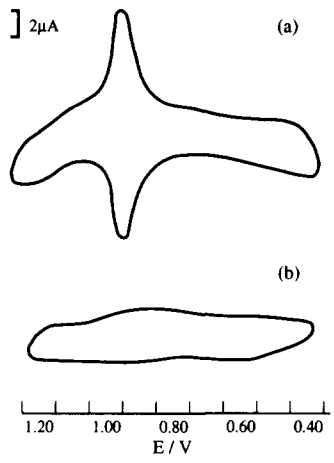

Fig. 2. Cyclic voltammetric response of ferroin on the glassy carbon electrodes. (b) HAc–NaAc buffer solution (pH 4·5); iron (III), $7{\cdot}2 \times 10^{-5}$ M; ascorbic acid, 1·0 μg/ml. (a) As in (b) + phen, $4{\cdot}6 \times 10^{-4}$ M.

Procedure

Standard ascorbic acid solution was mixed with 2·0 ml of $1{\cdot}8 \times 10^{-3}$M iron (III), 3·0 ml of $7{\cdot}6 \times 10^{-3}$M phen solution and diluted to 50 ml with HAc–NaAc buffer solution (pH 4·5). Then the solution was transferred to a cell. The measurements were carried out after a preconcentration step (open circuit accumulation), in which the solution was stirred for 30 s. After a rest period of 30 s, the response curve was recorded by scanning the potential in the negative direction (from 1·2 V to 0·3 (versus Saturated Calomel Electrode (SCE)), a well-defined stripping peak was obtained (Fig. 1), with a peak potential of 0·87 V (versus SCE). After each electrochemical determination, the surface of the electrode should be polished with Al_2O_3 (0·05 μm) on a pad (Worsted) for 30 s, and then washed with doubly-distilled water and blotted with filter paper. All of this is essential for a successful stripping peak.

RESULTS AND DISCUSSION

Formation of stripping peak

The method is based on oxidation of ascorbic acid to dehydroascorbic acid by iron (III) in the presence of phen, with consequent formation of ferroin (Tris-1,10-phenanthroline-ferrous ion). Figure 2(a) shows cyclic voltamperograms for 1·0 μg ascorbic acid, in a HAc–NaAc buffer solution containing $7{\cdot}2 \times 10^{-5}$M iron (III) and $4{\cdot}6 \times 10^{-4}$M phen, after 30 s accumulation in an open circuit with stirring. One cathodic peak is observed at 0·87 V (versus SCE) during the negative-going scan. Scanning in the reverse direction exhibits an anodic peak (Fig. 2(a)). No iron (II) peak was observed for a solution of iron (II) at the peak potential of 0·87 V in the absence of phen (Fig. 2(b)). When an amount of phen is added to the solution containing iron (II) and HAc–NaAc buffer solution, a ferroin complex peak appears at 0·87 V (versus SCE). Therefore, we can infer that the ferroin complex peak resulted from the oxidation of the surface-adsorbed iron (II)–phen (ferroin) complex and reduction of the oxidized form.

Effect of the preconcentration time

The effect of the preconcentration time was studied. It was found that the peak height increased to a preconcentration time of 30 s. Saturation of the electrode surface was obtained at greater times. The preconcentration time was chosen to be 30 s in all subsequent work.

Effect of pH on the peak height

Stoichiometric reaction and maximum peak height were obtained at pH 2·0–5·5. The decrease in peak height at pH values outside this range may be due to incomplete oxidation of ascorbic acid at low pH or atmospheric oxidation at high pH, or to partial decomposition of ferroin by protonation or hydrolysis. The optimum pH is about 4·5 in our experiments.

Interferences

Ascorbic acid is absorbed at the electrode in an open circuit, and no voltage was applied during the entire process; most of the metal ions will therefore not be deposited at the electrode so as to interfere with its determination. The tolerance for various foreign ions was studied for determination of 1·0 μg/ml ascorbic acid. The results showed that at least 20-fold amounts of Mg (II), Cu (II), 50-fold Zn (II), tartaric acid, 100-fold starch, citric acid, sucrose, glucose have little effect on the determination.

Calibration graph

Under the conditions described above, the peak height is directly proportional to the ascorbic acid concentration over the range $2{\cdot}0 \times 10^{-7}$–$2{\cdot}4 \times 10^{-6}$ g/ml (correlation coefficient = 0·998). The relative standard deviation was 5·6% (n = 8).

Table 1. Determination of ascorbic acid in orange and vegetable juices (n = 4)

Sample	Proposed method (mg/g)	Spectrophotometric method (mg/g)
Orange 1	42·5 ± 0·51[a]	41·5
Orange 2	43·3 ± 0·48	42·3
Orange 3	46·1 ± 0·39	47·4
Tomato	42·0 ± 0·43	41·0
Garlic bolt	18·1 ± 0·53	17·8
Green pepper	23·3 ± 0·46	22·6

[a] ± Standard deviation.

Sample analysis

The procedure described above was applied to determine ascorbic acid in samples of orange and vegetable. The samples were treated with 10% acetic acid to minimize the losses of ascorbic acid (Manuel *et al.*, 1989). Three different samples of orange juice were analysed. The results were reproducible, as indicated by the standard deviation values (Table 1). Three different samples of vegetable juice were analysed for ascorbic acid by the proposed method, reproducibility being of the same order as that found for orange juice. In order to evaluate the validity of the proposed method to determine ascorbic acid in orange juice, recovery studies were carried out on samples to which known amounts of ascorbic acid had been added. The recovery values were 102–105%.

This complex ferroin has a maximum absorbance at 510 nm; it may be used in spectrophotometric analysis (Amir, 1987). The results agree well with those obtained by the method developed by Amir.

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