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Flow electrochemical determination of ascorbic acid in real samples using a glassy carbon electrode modified with a cellulose acetate film bearing 2,6-dichlorophenolindophenol

Ageliki B. Florou, Mamas I. Prodromidis, Miltiades I. Karayannis, Stella M. Tzouwara-Karayanni *

Department of Chemistry, University of Ioannina, GR-45110 Ioannina, Greece

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Abstract

An ascorbate sensor based on a glassy carbon electrode modified with a cellulose acetate polymeric film bearing 2,6-dichlorophenolindophenol (CA/DCPI-CME) was constructed. The overall reaction obeys a catalytic regeneration mechanism (EC mechanism) and the electrochemical rate constant k_f for the electrocatalytic oxidation of ascorbic acid was evaluated. The modified electrodes were mounted in a flow injection (FI) manifold, poised at +100 mV versus Ag/AgCl/3 M KCl at pH 6.5 and utilized for the determination of ascorbic acid in beverages and pharmaceuticals. Good correlation with a reference method was attained. Interferents of various molecular sizes were tested. Calibration graphs were linear over the range 0.02–1 and 0.1–6 mM ascorbic acid for CA/DCPI sensors hydrolyzed in KOH and $ZnCl_2$ solution, respectively. The throughput was 25 samples per hour and the CV was for a 0.4 mM ascorbic acid solution 0.75 (n=14) and 1.2% (n=10) for CA/DCPI sensors hydrolyzed in KOH and $ZnCl_2$ solution, respectively. The recovery was 92–110%. The sensors showed very good repeatability and operational stability.

Keywords: Electrochemical determination of ascorbic acid; Controlled porosity cellulose acetate film; Beverages; Pharmaceuticals; Flow injection analysis; 2,6-Dichlorophenolindophenol

1. Introduction

Ascorbic acid (vitamin C) has an important role in body health as it is necessary for the formation of collagen, assists in the absorption of iron by promoting its reduction to the ferrous state and lack of it provokes increased susceptibility to many kinds of infections and slows down the rate at which wounds and fractures heal [1]. The amperometric determination of ascorbic acid is based on its electrochemical oxidation. At bare platinum or glassy carbon electrodes, the reaction proceeds at potentials above +500 mV. However, at chemically modified electrodes (CME), the necessary overpotential is lowered substantially [2]. CMEs based on polyvinylferrocene [3], or on 7,7,8,8-tetracyanoquinodimethane (TCNQ) in conjunction with tetrathiafulvalene or ferrocene [4] have been proposed. Screen printed electrodes based on

* Corresponding author. Tel.: +30-651-98346; fax: +30-651-98407.
E-mail address: mkaragia@cc.uoi.gr (S.M. Tzouwara-Karayanni).

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TCNQ [5] or cobalt phthalocyanine [6] have also been reported. Kissinger and his colleagues have proposed a method for the determination of ascorbic acid and dehydroascorbic acid using liquid chromatography with ultraviolet and electrochemical detection [7,8]. Recently, CMEs containing Fe(III)Y zeolite [9] or zeolite molecular sieves [10] were applied for the detection of ascorbic acid in aqueous solutions.

Problems associated with electrochemical detectors, such as fouling and selectivity, could be potentially faced combining the advantages of working at low redox potential of the mediator and the permselective properties of a polymeric membrane employed in the probe [11,12]. In membrane-based electrochemical devices, characteristics of great analytical interest, such as retention of mediators, co-factors or activators, sufficient permeability to analyte, extension of response linearity over the environmental concentration range, could be achieved [13].

The present work explores the construction of an analytical device with the required properties of interferents diffusion restriction through a controlled porosity cellulosic film and a highly reactive mediator towards ascorbic acid [14,15]. This approach provides the feasibility for a sensitive, reliable, easy to perform and fast method for the determination of ascorbic acid in beverages and pharmaceuticals. To our knowledge, this is the first sensor dealing with the electrochemical determination of ascorbic acid using 2,6-dichlorophenolindophenol (DCPI) as a mediator and based on the redox reaction between ascorbic acid and DCPI according to the reaction scheme presented in Fig. 1.

2. Experimental

2.1. Reagents

2,6-dichlorophenolindophenol (DCPI) sodium salt dihydrate ($C_{12}H_6Cl_2NNaO_2 \cdot H_2O$, Cat. No. 103028), aluminum oxide (particle size 0.3 μm) and potassium hydroxide (pro analysis) were obtained from Merck (Darmstadt, Germany) and were used without further purification. Cyclohexanone (99+%) and acetone (99+%) were supplied by Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Ascorbic acid (disodium salt) and cellulose acetate (CA) (approximately 40% acetyl) were purchased from Sigma (St. Louis, USA). Zinc chloride was obtained from Riedel-de Haen (Seelze, Germany). All other chemicals were of analytical grade from Merck and Sigma.

Working solutions of ascorbic acid (10 mM) were prepared daily by dissolving 0.0176 g of ascorbic acid in 10.0 ml of the working buffer solution (50 mM phosphate in 50 mM KCl, pH 6.5).

For the validation experiments, a DCPI solution (1 mM) was prepared by dissolving 0.0816 g of DCPI in 250 ml of distilled water. The concentration of the solution was checked before analysis by measuring its molar absorptivity at the isosbestic point ε_{522}=8600 l mol^{-1} cm^{-1} [16].

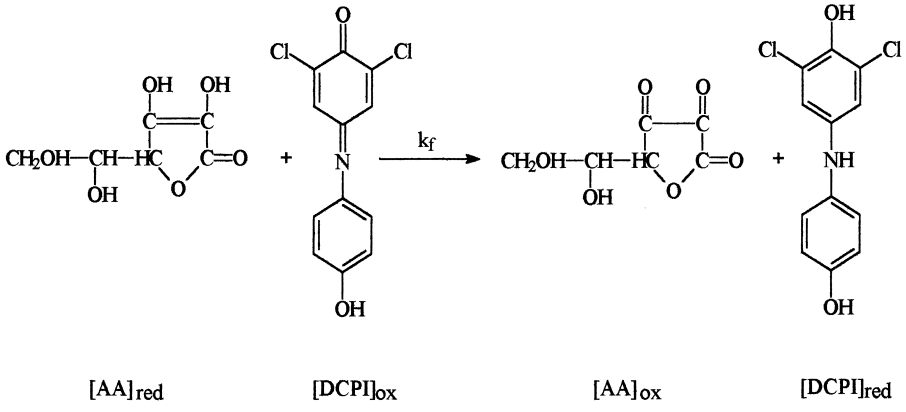

Fig. 1. The redox reaction scheme between ascorbic acid and DCPI.

2.2. Apparatus

All electrochemical experiments were conducted with a computer controlled potentiostat, the Autolab Electrochemical Analyzer (Eco Chemie, Utrecht, The Netherlands). The flow injection (FI) experiments were carried out using an in-house fully automated FI manifold. A detailed description of the FIA manifold and the resident program, which was employed for the full control of the FI components, is given elsewhere [17]. The working electrode was a glassy carbon electrode (MF-2070, 3 mm diameter, BAS, West Lafayette, IN, USA) used in a wall-jet type flow through detector (Metrohm 656, volume $<1\,\mu$l, Herisau, Switzerland).

Cyclic voltammetry experiments were performed with a voltammetry cell (VC2, BAS) using glassy carbon as the working electrode, an Ag/AgCl/3 M KCl reference electrode (BAS) and a Pt wire as auxiliary electrode with a gold connecting pin (BAS). The total effective electrode area was determined by chronocoulometric measurements in hexacyanoferrate(III) ($D^0=7.63\times10^{-6}\,cm^2\,s^{-1}$) and found equal to the geometrical one.

2.3. Procedure

The applied potential for the FI measurements was +100 mV (versus Ag/AgCl/3 M KCl). After the potential had been applied to the modified electrode, the background current was allowed to decay to a stable value under continuous flow (1–2 nA within 20–35 min). The carrier (0.05 M phosphate buffer in 0.05 M KCl, pH 6.5) stream was pumped continuously at a flow rate of $0.32\,ml\,min^{-1}$. Standard or sample solutions of ascorbic acid were introduced as short pulses of 130 μl via the loop injection valve. The peak height of the current response was taken as a measure of the ascorbic acid concentration.

2.4. Sample preparation

Juice and sport drink samples were directly diluted in 10 ml of working buffer, while carbonated samples were sonicated (15 min, 100 W) prior to use. Pharmaceutical tablet solutions were prepared by dissolving each tablet in the working buffer solution.

2.5. Membrane modification and electrode assembly

CA/DCPI membrane was prepared by dissolving 0.0326 g (1 mM) of DCPI to 100 ml of 1% CA solution. The latter was prepared by dissolving 1 g of CA in a mixture of 55 ml of acetone and 45 ml of cyclohexanone. 10 μl of the solution was pipetted onto the surface of the probe and left to air dry, leaving a thin polymeric film bearing DCPI. Membranes were then hydrolyzed in a 0.07 M KOH or in a 3.2 M $ZnCl_2$ solution [18].

Modified electrodes were immersed in a stirred base or inorganic salt solution for 20 and 16 min, respectively, washed thoroughly with double distilled water and then immersed in a stirred solution of the supporting electrolyte for 10 min to wash out the residual solvent. CA/DCPI-CME was then incorporated in the electrochemical cell of the FI manifold and left to equilibrate with the working buffer while applying the working potential.

3. Results and discussion

3.1. Permeability studies of the polymeric film

The permeability of the cellulose film increases upon increasing the hydrolysis period. The dependence of the catalytic current on the hydrolysis time is illustrated in Fig. 2. Hydrolysis in 0.07 M KOH or in 3.2 M $ZnCl_2$ takes place in a well-stirred solution of the hydrolysis agents and a sufficient permeability for ascorbic acid is achieved after 20 and 16 min, respectively. At this extent of hydrolysis, the catalytic current is almost 70% of the maximum catalytic current in the case of $ZnCl_2$ treatment and more than 80% in the case of alkaline treatment. As maximum catalytic currents were taken the responses obtained after 20 or 24 min of hydrolysis using $ZnCl_2$ or KOH, respectively. Furthermore, high selectivity towards compounds with higher molecular weight and superior retaining properties of DCPI in the polymeric film were obtained. It is important to clarify that any increase in the catalytic current is due to the easier permeation of the ascorbic acid through the film and has no relation with the electrocatalytic properties of the reaction studied. Permeability

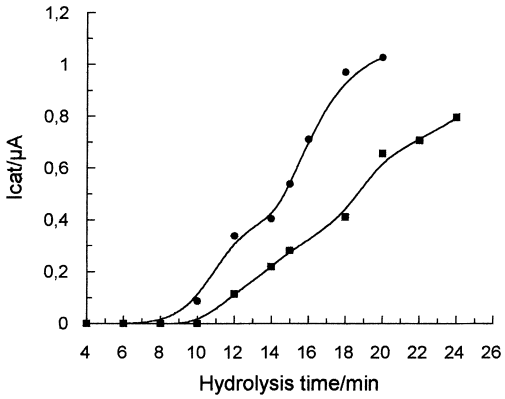

Fig. 2. Dependence of the catalytic current of ascorbic acid on the hydrolysis time in a (■) 0.07 M KOH and (●) a 3.2 M $ZnCl_2$ solution. Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5. The catalytic currents of ascorbic acid were obtained with a CA/DCPI-CME and corrected for the anodic currents observed in the absence of ascorbic acid.

studies have also been performed in the absence of DCPI (glassy carbon covered with CA) recording the oxidation current of different reductive species at their oxidation potential. Comparison of these results shows that DCPI may be involved in the hydrolysis mechanism since, in its absence, the same permeabilities were achieved after 22–23 min of hydrolysis [18].

3.2. Electrocatalytic oxidation of ascorbic acid

The reduction of DCPI by ascorbic acid has been studied kinetically by the stopped-flow technique. The reaction is first-order with respect to the reactants and reaction rate constant of $5.6\times10^4\,mol^{-1}\,s^{-1}$ has been calculated [14]. Fig. 3 shows cyclic voltammograms obtained with CA/DCPI modified electrodes treated with KOH for 15 min (I), 20 min (II) and with $ZnCl_2$ (III) in the presence of different concentrations of ascorbic acid at pH 6.5. There is a significant increase in the current at the potential where $[DCPI]_{ox}$ ($E^{0\prime}$=74–79 mV) is formed, compared to the current recorded at this potential in the absence of ascorbic acid. This behavior indicates a strong electrocatalytic effect and can be interpreted by the diffusion of ascorbic acid present in the solution through the CA film, thus reducing the electrochemically produced $[DCPI]_{ox}$. The anodic current increases proportionally with the concentration of the reductive species and a shift of the anodic potential is observed as the sweep rate is increased, indicating that a kinetic limited regime dominates in the reaction between the DCPI and ascorbic acid. However, plot (Fig. 4) of the catalytic peak current is increased linearly with the square root of the sweep rate, suggesting that, at sufficient overpotential, the reaction is transport-limited [19].

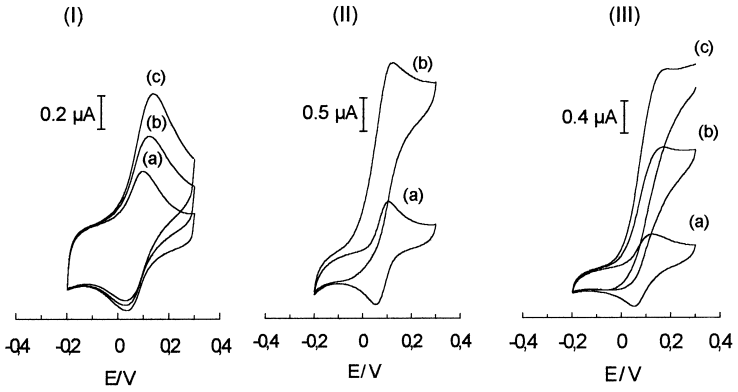

Fig. 3. Cyclic voltammograms illustrating the catalytic oxidation of ascorbic acid mediated by immobilized DCPI in a cellulosic membrane treated with (I) 0.07 M KOH for 15 min, (II) 0.07 M KOH for 20 min and (III) 3.2 M $ZnCl_2$ for 16 min. (a): CVs of CA/DCPI-CME in a buffer solution, (b) CVs of the same CA/DCPI-CME in a buffer solution containing 3 mM ascorbic acid and (c) 6 mM ascorbic acid. Scan rate: $50\,mV\,s^{-1}$. Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5. Surface coverage: (I) $0.19\,nmol\,cm^{-2}$, (II) $0.11\,nmol\,cm^{-2}$, (III) $0.18\,nmol\,cm^{-2}$.

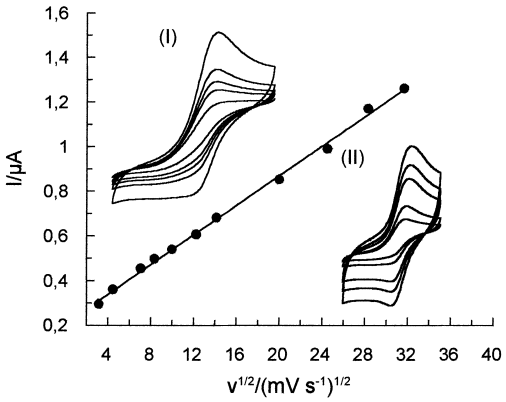

Fig. 4. Variation of I_{pa} with the square root of the scan rate for the DCPI/CA-CME hydrolyzed in 0.07 M KOH for 20 min in the presence of 3 mM ascorbic acid. Embedding cyclic voltammograms represent the experimental data for scan rates: (I) 10, 20, 50, 70, 100 mV s^{-1} and (II) 150, 200, 400, 600, 800 mV s^{-1}. Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5. Surface coverage: 0.05 nmol cm^{-2}.

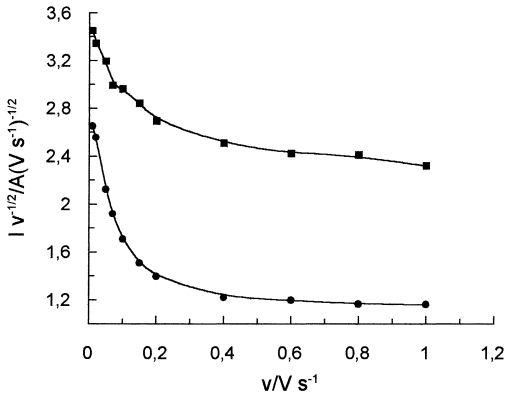

Fig. 6. Variation of the sweep rate-normalized current with the sweep rate for CA/DCPI modified electrodes in the presence of 3 mM ascorbic acid. Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5. Surface coverage: (●) 0.05 nmol cm^{-2} and (■) 0.12 nmol cm^{-2}.

A current measurement-based diagnostic test was utilized for the identification of the mechanism illustrated in Fig. 5 [20]. A catalytic regeneration mechanism (variation of the EC mechanism) was justified by the shape of the plot of the sweep rate-normalized current ($i/v^{1/2}$) versus sweep rate (Fig. 6).

3.3. Determination of k_f

Andrieux and Saveant [21] developed a theoretical model for such a mechanism and derived a relation between the peak current and the concentration of the substrate for the case of slow sweep rate (v) and large catalytic rate constant k_f according to the Eq. (1):

$$i_{cat} = 0.496nFAD^{1/2}v^{1/2}C^* \left(\frac{nF}{RT}\right)^{1/2} \quad (1)$$

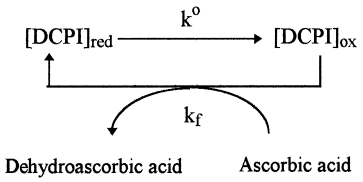

Fig. 5. Catalytic regenerated EC mechanism, where k^0 is the electrochemical rate constant of the DCPI redox couple within the film and k_f the electrochemical rate constant for the catalytic oxidation of ascorbic acid present in the solution.

where D and C^* are the diffusion coefficient and the concentration of ascorbic acid, respectively. The other symbols have their usual meaning. Low values of k_f result in values of the coefficient lower than 0.496. For low sweep rates (2–10 mV s^{-1}), an average value of this coefficient, of 0.120, was found for a CA/DCPI electrode with a coverage of $\Gamma = 1.66 \times 10^{-10}$ mol cm^{-2} in the presence of 3×10^{-3} mol l^{-1} ascorbic acid. The diffusion coefficient of ascorbic acid under the specific experimental conditions (through CA membrane) was calculated as $D^0 = 2.5 \times 10^{-9}$ cm^2 s^{-1} [18].

Value $i_{cat}/[nFAD^{1/2}v^{1/2}C^*(nF/RT)^{1/2}]$ was calculated from the corresponding cyclic voltammograms of the CA/DCPI electrode in the presence of ascorbic acid and then the value of $\log[k\Gamma/D^{1/2}v^{1/2}C^*(nF/RT)^{1/2}]$ was determined graphically from Fig. 1(b) in the article of Andrieux and Saveant [21]. A rate constant of 1.7×10^4 M^{-1} s^{-1} (pH<3) was calculated. Comparison with reported values shows that the reaction which takes place in the polymeric film, and exhibits a rate constant larger than the reaction in homogeneous solution at pH 6.5 [14,22]. Catalytic rate constant can be also evaluated by double-step chronocoulometry from the ratio of the total charge in the forward and the reverse step (Q_r/Q_f) [23] or with rotating disk voltammetry [24]. However, Andrieux and Saveant's approach was selected as it is convenient and accurate [19].

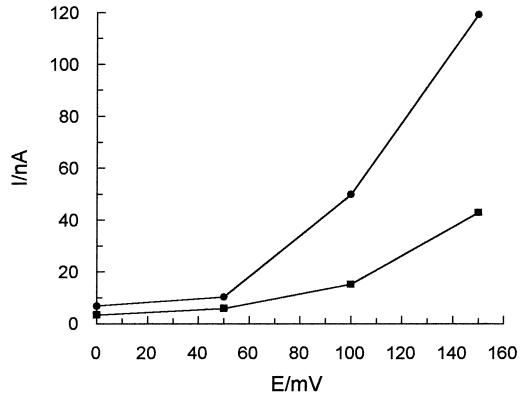

Fig. 7. Response of ascorbic acid at different applied voltages for (■) bare glassy carbon electrode and (●) CA/DCPI modified electrode hydrolyzed for 20 min in 0.07 M KOH. Buffer: 0.05 M phosphate in 0.05 M KCl, pH 5. Flow rate: 0.32 ml min^{-1}. Sample volume: 130 μl.

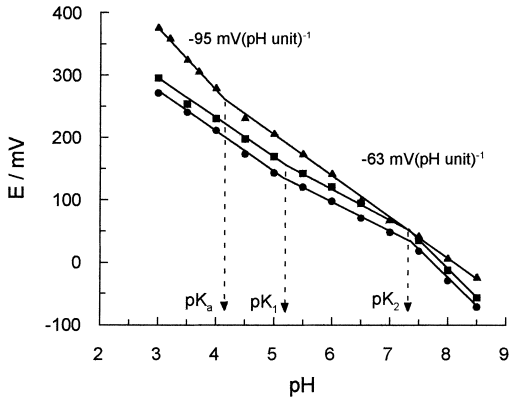

Fig. 8. Determination of p*K*. (▲) Plot of peak potentials vs. pH for ascorbic acid oxidation with CA/DCPI-CME treated in 0.07 M KOH for 20 min. Plots of (■) peak potentials and (●) formal potentials vs. pH for DCPI immobilized in the cellulose acetate membrane hydrolyzed in 0.07 M KOH for 20 min. Scan rate 20 mV s^{-1}. Buffer: 0.25 M phosphate in 0.5 M KCl. pK_1 and pK_2 are referring to the dissociation constants of DCPI and are found to be 5.2 and 7.4, respectively.

Fig. 7 illustrates FIA-peak currents recorded at different applied voltages for bare and CA/DCPI modified electrodes. These results (see also CV-grams in Fig. 3) are a strong evidence that a fast and almost quantitative oxidation of ascorbic acid can be achieved in the presence of the DCPI in the cellulosic film. The mediator achieved a more than 370 mV lowering of the overpotential for the oxidation of ascorbic acid in the film, indicating very efficient electrocatalysis. In all cases, the current intensities for the mediated electrodes are higher by a factor of 3–4 compared to these for the bare electrodes.

3.4. Determination of pK_a of ascorbic acid

The dependence of the oxidation peak potential of ascorbic acid on the pH was investigated by cyclic voltammetry. As the E_p is directly related to the formal potential of the redox couple, it is useful for qualitative analysis of electroactive species [25]. The variation E as a function of pH (Fig. 8) clearly indicates that the peak shifted to more negative potentials with increasing pH. The slope of the line is −95 mV (pH unit)$^{-1}$ between pH 3 and 4.25 in accordance with a $2e^-/3H^+$ and −63 mV (pH unit)$^{-1}$ between pH 4.25 and 8 in accordance with a $2e^-/2H^+$ electron reaction. pK_a was determined graphically by the intersection of the two straight lines at pH 4.25 and coincides with the literature value of 4.1 for the pK_a of ascorbic acid [26]. As can be seen from Fig. 8, the oxidation potential of ascorbic acid follows that of the formal (or the oxidation peak) potential of the DCPI [18]. The deprotonation of ascorbic acid, according to the experimental results, is independent, as expected, of that of DCPI.

3.5. Working conditions

The pH value of the working buffer was investigated covering the pH range 6–8. A narrow pH range was selected since the efficiency of the sensor with respect to pH is also affected by the value of the formal potential ($E^{0\prime}$) of the DCPI. In the selected range, we can assume that a bias has not occurred since $E^{0\prime}$ is ranking between 0 and 150 mV. At pH 6, the efficiency of the sensor decreases due to the non-quantitative oxidation of DCPI at the applied potential of 100 mV, while for values higher than 6.5, the efficiency of the sensor remains low due to the small chemical rate constant of the reaction in this pH range [14]. The optimum pH is 6.5 as shown in Fig. 9. The efficiency of the sensor was also investigated in dependence of the flow rate (reaction time) in the range 0.2–0.7 ml min^{-1}. An overall flow rate of 0.32 ml min^{-1} which reconciles fairly high peaks and satisfactory sample throughput (25 h^{-1}) was

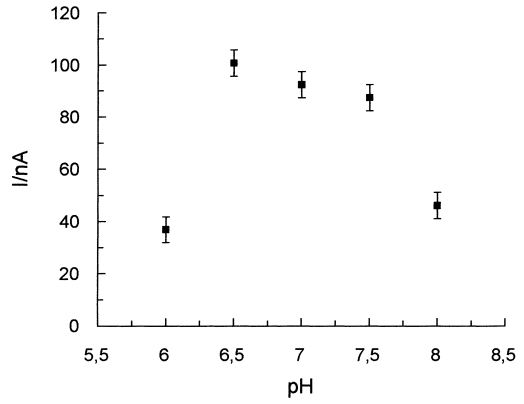

Fig. 9. pH profile of the ascorbate chemical sensor. Buffer: 0.25 M phosphate in 0.5 M KCl, Ascorbic acid concentration: 1 mM. Flow rate: 0.32 ml min^{-1}. Sample volume: 130 μl. Applied potential: +0.1 V.

Table 1
Interference effect of various compounds on the assay of ascorbic acid[a]

Interferent	Relative activity (%)
None	100
Paracetamol (4)	97
Uric acid (4)	99
Ampicillin (4)	99
Dopamine (0.4)	133
Cysteine (4)	102
Homocysteine (4)	98
Theophylline (4)	97
Sodium sulphide (4)	364
Sodium sulphite (4)	96
Glutathione (4)	96

[a] The values in parentheses are the concentrations of the compounds in mM. All solutions contained 0.4 mM ascorbic acid and were compared with the activity of pure 0.4 mM ascorbic acid taken as 100%.

finally selected. A sample volume of 130 μl was used as it prevented peak broadening (dispersion coefficient 1.19–1.23) and ensures high sensitivity.

3.6. Interferents

Interference from various reductive species present in real samples was investigated by applying the method of mixed solutions in the presence of 0.4 mM ascorbic acid. Interferents were added at concentrations much higher than those normally present in the real samples. Experiments were carried out immediately after the addition of ascorbic acid, preventing any loss due to its oxidation by the air. The relative responses are illustrated in Table 1. Only sodium sulphide exhibits significant positive interference (364% at a 10-fold concentration). Since sodium sulphide rarely co-exists with ascorbic acid in real samples, its interference is not important. Dopamine interference (133% at the same concentration with ascorbic acid) is also not important, as even in the extracellular fluid of the central nervous system, its concentration level is three orders of magnitude smaller than that of ascorbic acid [27].

3.7. Application to real samples

Under optimum conditions, calibration curves, current/nA=f([ascorbic acid/mM]), were constructed applying the least squares method. Using CA/DCPI membrane hydrolyzed with 0.07 M KOH for 20 min, a linear relation was obtained between the response and the ascorbic acid concentration in the range 0.02–1 mM, with a correlation coefficient r=0.9992 (Fig. 10(I)). Data fit the equation y=(3.40±0.67)+(177.93±2.62) [ascorbic acid/mM]. The detection limit was 0.01 mM ascorbic acid for a signal to noise ratio of 3 (S/N=3) and the coefficient of variation (CV) of the method was 0.75 (n=14) for 0.4 mM ascorbic acid (Fig. 10(II)).

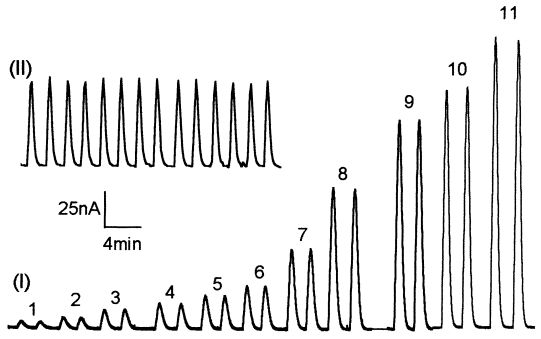

Fig. 10. (I) FIA-grams for ascorbic acid with a CA/DCPI-CME treated with 0.07 M KOH for 20 min. Peaks 2–11 correspond to concentrations within the linear range, while peak 1 represents a concentration of 0.01 mM ascorbic acid. (II) The reproducibility of the method for 0.4 mM ascorbic acid, n=14. Buffer: 0.05 M phosphate in 0.05 M KCl, pH 6.5. Flow rate: 0.32 ml min^{-1}. Sample volume: 130 μl. Applied potential: +0.1 V.

Table 2
Determination of ascorbic acid in various real samples[a]

Sample	Dilution ratio	Proposed method[b] (mg/100 ml)	Reference method[c] (mg/100 ml)	Relative error (%)
Orange juice (IVI)[d]	11	8.52	8.70	−2.1
Carrot & fruits juice (Amita)[d]	11	6.39	6.50	−1.7
Blackcurrant juice (Ribena)	11	21.31	21.20	+0.52
Sport drink (Refresh)	11	15.69	15.80	−0.7
Ice tea (Lipton)	11	3.29	3.35	−1.8
Orange juice (Kliafas)[d]	5	not detected	–	–
Lemon juice (IVI)[d]	11	2.70	2.68	+2.00
Orange juice (ISOTON)	11	2.70	2.75	−1.80
Supradin[e]	20	10.92	11	−0.70
Upsavit[e]	20	42.27	42.5	−0.54
Aspirin+Vit. C[e]	20	67.63	66.5	+1.67
Salospir+Vit. C[e]	20	84.54	85	−0.54
Cebion[e]	40	290	293	−1.00
Redoxon[e]	40	310	302	+2.60
Cal-C-Vita[e]	40	290	285	+1.70

[a] The standard deviation of the mean ranges from 0.02 to 0.08 mg/100 ml.
[b] Average of three runs.
[c] Titrimetric method with DCPI [28].
[d] Greek non-alcoholic beverages.
[e] Each tablet was diluted in 100 ml of the working buffer.

By using CA/DCPI membrane, hydrolyzed with 3.2 M $ZnCl_2$ for 16 min, a linear calibration curve for the concentration range 0.1–6 mM ascorbic acid was plotted. The equation for the straight line was $y=(0.61\pm0.34)+(27.16\pm0.11)$ [ascorbic acid/mM] with correlation coefficient $r=0.9995$. The detection limit ($S/N=3$) and the CV of the method were 0.04 mM ascorbic acid and 1.2 ($n=10$) for 0.4 mM ascorbic acid, respectively.

The proposed method was applied to pharmaceuticals and non-alcoholic beverages for the determination of ascorbic acid. The results for various samples are summarized in Table 2. Each sample of beverages required a minimum dilution of 1+10, while for

Table 3
Recovery of ascorbic acid added to real samples

Sample	Added ($\times10^{-4}$ M)	Found ($\times10^{-4}$ M)	Recovery (%)
Orange juice (IVI)	0.8	0.86	107
Carrot & fruits juice (Amita)	0.5	0.53	106
Blackcurrant juice (Ribena)	1.0	1.1	110
Sport drink (Refresh)	1.5	1.68	112
Ice tea (Lipton)	2	2.25	112
Orange juice (Kliafas)	2	2.18	109
Lemon juice (IVI)	2	1.83	92
Orange juice (ISOTON)	2	2.17	108
Supradin	1.5	1.4	94
Upsavit	3	2.9	97
Aspirin+Vit. C	2	2.2	110
Salospir+Vit. C	2.5	2.4	96
Cebion	1	1.01	101
Redoxon	1	1.06	106
Cal-C-Vita	1	1.03	103

pharmaceutical samples, a dilution of 1+39 is recommended. The results were compared with those obtained using the official titrimetric method with DCPI [28]. The mean relative errors were 1.52 and 1.25%, respectively. The accuracy of the method was also verified by recovery studies adding standard ascorbic acid solutions to samples. Recoveries of 92–110% were achieved, as shown in Table 3.

3.8. Stability of the sensor

The operational stability of the sensor was studied by continuous exposure to the flow stream. As a result, the final CA/DCPI-CME activity (current response versus initial current response × 100%) was higher than 92% after 10 h of continuous operation with standards and real samples. This minor decrease in activity is due to the leaching out effect of DCPI from the three-dimensional structures of the polymeric film. This phenomenon is due to the inhomogeneity of the polymeric film and was mainly observed during the first stage of operation. This improved stability of the sensor also illustrates the fouling protection achieved over prolonged use as desired in practical applications. The sensor displayed good storage stability if stored dry at 4°C. The CA/DCPI-CME retained 95 and 80% of its initial activity after 1 week and 2 weeks of storage, respectively.

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