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Article

L-Ascorbic Acid Determination in Pharmaceutical Formulations Using a Biosensor Based on Carbon Paste Modified with Crude Extract of Zucchini (*Cucurbita pepo*)

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Um biossensor baseado em pasta de carbono modificada com extrato bruto de abobrinha (*Cucurbita pepo*) como fonte de *peroxidase* é proposto para a determinação de ácido L-ascórbico em formulações farmacêuticas. Esta enzima na presença de peróxido de hidrogênio catalisa a oxidação de hidroquinona a p-quinona cuja redução eletroquímica a hidroquinona foi obtida em potencial de pico de -0,14V. Assim, quando ácido L-ascórbico é adicionado à solução, este ácido pode reduzir p-quinona quimicamente para hidroquinona e/ou reduzir peróxido de hidrogênio, decrescendo a corrente de pico proporcionalmente ao aumento de sua concentração. A recuperação do ácido L-ascórbico em cinco amostras variou de 98,1 a 102,1% e uma curva analítica linear no intervalo de concentração de ácido ascórbico de $2,0x10^{-4}$ a $5,5x10^{-3}$ mol L^{-1} (r=0.9992) foram obtidos. O limite de detecção foi $2,2x10^{-5}$ mol L^{-1} e o desvio padrão relativo foi <1,3% para solução de ácido ascórbico $4,0x10^{-3}$ mol L^{-1}, hidroquinona $7,0x10^{-3}$ mol L^{-1} e peróxido de hidrogênio $2,0x10^{-4}$ mol L^{-1}. Os resultados obtidos para o ácido L-ascórbico em formulações farmacêuticas usando o biossensor proposto e o procedimento da Farmacopéia estão em concordância a um nível de confiança de 95%.

A biosensor based on carbon paste modified with crude extract of zucchini (*Cucurbita pepo*) as a source of *peroxidase* is proposed for determining L-ascorbic acid in pharmaceutical formulations. This enzyme in the presence of hydrogen peroxide catalyses the oxidation of hydroquinone to p-quinone whose electrochemical reduction back to hydroquinone was obtained at peak potential of -0.14V. Thus, when L-ascorbic acid is added to the solution, this acid can reduce chemically p-quinone to hydroquinone and/or reduce hydrogen peroxide, decreasing the peak current obtained proportionally to the increase of its concentration. The recovery of L-ascorbic acid from five samples ranged from 98.1 to 102.1% and a rectilinear calibration curve for L-ascorbic acid concentration from $2.0x10^{-4}$ to $5.5x10^{-3}$ mol L^{-1} (r=0.9992) was obtained. The detection limit was $2.2x10^{-5}$ mol L^{-1} and relative standard deviation was < 1.3% for a solution containing $4.0x10^{-3}$ mol L^{-1} L-ascorbic acid, $7.0x10^{-3}$ mol L^{-1} hydroquinone and $2.0x10^{-4}$ mol L^{-1} hydrogen peroxide. The results obtained for L-ascorbic acid in pharmaceutical formulations using the proposed biosensor and those obtained using the Pharmacopeia method are in agreement at the 95 % confidence level.

Keywords: L-ascorbic acid, carbon paste biosensor, peroxidase, zucchini (*Cucurbita pepo*)

Introduction

L-ascorbic acid (vitamin C) is a γ-lactone that is synthesized by plants and almost all animals except primates and guinea pigs. Its prolonged deficiency in the diet of humans results in the disease know as scurvy which is caused by the impairment of collagen formation. Scurvy generally results from a lack of fresh food[1].

Plant tissue[2-5] and crude extract[6-12] have been found to be effective catalytic materials in the enzymatic method. This kind of biocatalytic material maintains the enzyme of interest in the natural habitat which can result in a considerable stabilization of enzyme activity (long lifetimes), high enzymatic activity, low cost, ease of preparation and in several materials the presence of the enzyme cofactor[3].

Many enzymatic procedures have been proposed in the literature[13-17] for determining L-ascorbic acid using *ascorbate oxidase* (E.C. 1.10.3.3.). This enzyme catalyses the oxidation of L-ascorbic acid to dehydroascorbic acid and the oxygen uptake was measured with a Clark oxygen type electrode.

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A slice of squash (*Cucurbita pepo*) or cucumber (*Cucumis sativus*) mesocarp (0.3 mm thick) supported in a polyamide net was placed over a PTFE membrane of an oxygen electrode for the monitoring of L-ascorbic acid oxidation by the plant-tissue *ascorbate oxidase*[13] An amperometric sensor for L-ascorbic acid has been made by immobilization of *ascorbate oxidase* in the reconstituted collagen membrane and mounting the enzyme-collagen membrane onto a Clark oxygen electrode. This enzyme was purified from the cucumber peel (*Cucumis sativas*) and the response of the electrode was linear from $5.0x10^{-5}$ to $5.0x10^{-4}$ mol L^{-1} L-ascorbic acid[14].

Cucumber (*Cucumis sativus L.*) juice has been used as the carrier solution in an amperometric flow-injection system for the determination of L-ascorbic acid[15]. The determination time was 1 min and the calibration graph was rectilinear in the L-ascorbic acid concentration range from $5.0x10^{-4}$ to $7.0x10^{-3}$ mol L^{-1}. L-ascorbic acid was determined by concentration-step amperometry using a thin layer of carbon felt impregnated with cucumber juice as an enzyme solution of *ascorbate oxidase*[16]. The dilute fruit juice was added on top of the carbon felt and the decreases of current peak caused by the enzymatic reaction was measured. The peak current was proportional to the concentration of L-ascorbic acid in the concentration range $2.5x10^{-4}$ - $1.6x10^{-3}$ mol L^{-1}.

A biosensor for L-ascorbic acid based on enzyme kinetics of *ascorbate oxidase* was developed. The enzyme was extracted from *Cucurbita maxima* and immobilized by covalent bonding, using glutaraldehyde as a bifuncional agent, on alkylamine glass beads, with and without enzyme active site protection. A low-cost, home-made oxygen electrode was applied as a transductor[17].

In this work, a biosensor is proposed for determining L-ascorbic acid in pharmaceutical formulations. *Peroxidase* in the presence of hydrogen peroxide catalyses the oxidation of hydroquinone to p-quinone whose electrochemical reduction back to hydroquinone was obtained at peak potential of -0.14V. Thus, when L-ascorbic acid is added to the solution, this acid can reduce chemically p-quinone to hydroquinone and/or reduces hydrogen peroxide, decreasing the peak current obtained proportionally to the increase of L-ascorbic acid concentration.

Experimental

Reagents and solutions

All reagents were of analytical-reagent grade and all solutions were prepared with water from a Millipore (Bedford, MA, USA) Milli-Q system, Model UV Plus Ultra-Low Organics Water.

Guaiacol, Nujol and hydrogen peroxide were purchased from Aldrich (Milwaukee, WI, USA). Acetylsalicylic acid, L-ascorbic acid, citric acid, glucose, lactose, magnesium stearate, oxalic acid, poly(ethylene glycol) 1500, sodium chloride, starch, sucrose, tartaric acid were purchased from Sigma Co (St. Louis, MO, USA) and graphite powder (grade # 38) were purchased from Fisher.

A $2.5x10^{-2}$ mol L^{-1} L-ascorbic acid stock solution was prepared daily in 0.1 mol L^{-1} phosphate buffer of pH 6.5 previously de-oxygenated with nitrogen and standardized by a conventional method[18]. Reference solutions from $1.9x10^{-4}$ to $5.4x10^{-3}$ mol L^{-1} were prepared from the stock solution in 0.1 mol L^{-1} phosphate buffer of pH 6.5.

Five Brazilian pharmaceutical formulations containing L-ascorbic acid such as Cebion (Merck, Rio de Janeiro, RJ), Redoxon (Roche, Rio de Janeiro, RJ), Energil C (Legrand, São Paulo, SP), Vitamina C (Schering-Plough, Rio de Janeiro, RJ) and Aspirina C (Bayer, São Paulo, SP) were obtained from a local drug store and analyzed using the proposed biosensor.

The Polyclar SB-100 used as a protective and/or stabilizer agent in the crude extract preparation was kindly donated by GAF (Wayne, NJ, USA).

Healthy zucchini (*Cucurbita pepo*), a variety of squash with a long, narrow shape and a greenish rind, purchased from a local producer, was selected, washed, hand-peeled, chopped, cooled in a refrigerator at 4ºC and used as a source of *peroxidase* (donor; hydrogen peroxide oxidoreductase, POD; E.C.1.11.1.7.).

Instrumentation

A DuPont Instruments (Newtown, CT, USA) Model RC-5B centrifuge, provided with a Model SS-34 rotor, was used in the preparation of the crude extract of the zucchini root.

A Hewlett-Packard (Boise, ID, USA) Model 8452A UV-visible spectrophotometer with a quartz cell (optical path of 1 cm) was used in *peroxidase* activity measurement and total protein determination.

All experiments were performed in a 15 mL electrochemical cell. A three-electrode assembly incorporating a crude extract-modified carbon paste electrode (biosensor), an Ag/AgCl reference electrode and a platinum auxiliary electrode was used in all measurements. Cyclic voltammetric and amperometric measurements were performed with an EG&G PAR, Model 264A Polarographic Analyzer/ Stripping voltammeter.

Zucchini crude extract preparation

A 25g amount of the frozen peeled zucchini was homogenized in a liquefier with 100 mL of 0.1 mol L^{-1}

phosphate buffer (pH 6.5) containing 2.5 g of Polyclar SB-100 for 2 min at 4-6 ºC. The homogenate was rapidly filtered through four layers of cheesecloth and centrifuged at 13,500 rpm for 15 min at 4ºC. The resulting supernatant was stored at this temperature in a refrigerator and used as the enzymatic source after the determination of the *peroxidase* activity and total protein.

Peroxidase activity and total protein determinations

Peroxidase (POD) activity present in the crude extract was determined in triplicate by measurement of the absorbance at 470 nm of tetraguaiacol[12,19] produced by the reaction between 0.2 mL of supernatant solution, 2.7 mL of 0.05 mol L^{-1} guaiacol solution and 0.1 mL of 10.0 mmol L^{-1} hydrogen peroxide solution in 0.1 mol L^{-1} phosphate buffer (pH 6.5) at 25ºC. The initial rate of guaiacol peroxidation reaction was a linear function of time for 1.5-2.0 min. One activity unit is defined as the amount of enzyme that causes an increase of 0.001 absorbance per minute under the experimental conditions described previously.

Total protein concentration was determined in triplicate using bovine serum albumin as standard[20].

Biosensor construction

A mixture of 1,400 units of *peroxidase* mg^{-1} protein of zucchini root crude extract with 375 mg of graphite powder (75 % m/m) was initially prepared in a mortar. This mixture was dried for 12 h under reduced pressure in a desiccator at 4ºC. Subsequently 125 mg of mineral oil (25% m/m) was added into this powder and mixed in a mortar for at least 20 min to produce the final paste. The modified carbon paste was packed into the tip of a 1 mL plastic syringe and a platinum soldered to a copper wire was used to provide the external electric contact[6]. Other enzyme concentrations varying from 700 to 3,500 units of *peroxidase* mg^{-1} protein and other carbon paste compositions containing graphite powder from 65 to 75 % m/m and mineral oil amounts ranging from 35 to 25 % m/m in that activity range of POD were also prepared.

Analysis of pharmaceutical samples

The contents of 10 tablets were well mixed; from the fine powder an accurately weighed portion was taken and dissolved in 0.1 mol L^{-1} phosphate buffer solution (pH 6.5) previously degased with nitrogen.

All experiments were performed at room temperature in a 0.1 mol L^{-1} phosphate buffer (pH 6.5) solution containing 2.0×10^{-2} mol L^{-1} hydrogen peroxide and 7.0×10^{-3} mol L^{-1} hydroquinone. Aliquots of pharmaceutical samples were added into a 15 mL thermostated glass cell, homogenized with the aid of a magnetic stirrer, degased with nitrogen for 1 min and the amperometric measurements were performed at -0.14V and the resulting cathodic current was displayed on the x-t recorder.

Results and Discussion

Study of the enzymatic process

Reactions catalyzed by enzymes have long been used for analytical purposes in the determination of different analytes, such as substrates, inhibitors and also the enzymes. Biosensors, which combine the selectivity of enzymes with the high sensitivity of electrochemical measurements, provide an excellent tool for analytical chemistry[21].

Figure 1 shows a scheme of the enzymatic process between hydroquinone (H_2Q), hydrogen peroxide (H_2O_2), L-ascorbic acid (AsA) and *peroxidase* (POD) of the crude extract incorporated into the carbon paste electrode. For the reduction wave, hydrogen peroxide oxidizes the native form of POD in a single two-electron process resulting in the oxidized form of POD (denoted compound I) and water[22] Thus, hydroquinone is enzymatically oxidized to p-quinone (Q) which at a potential of - 0.14V was electrochemically reduced to hydroquinone providing a peak current related to its concentration. Following this reduction wave scheme, when L-ascorbic acid solution is added to the solution, this acid can reduce chemically p-quinone to hydroquinone[23] and/or reduce hydrogen peroxide to water with dehydroascorbic acid (DAsA) formation[24] (not shown in this Figure) decreasing the peak current proportionally to the increase of L-ascorbic acid concentration. A scheme which explains the oxidation wave is also presented in this figure and will not be discussed once it was not used in the ascorbic acid detection.

Effect of paste composition

The effect of the graphite powder, containing 1,400 units of *peroxidase* mg^{-1} of protein varying from 65 to 75% (m/m) and Nujol from 35 to 25% (m/m) on the biosensor response using 4.0×10^{-3} mol L^{-1} hydroquinone was investigated. The best carbon paste composition was found using 0.375 g of graphite powder containing zucchini crude extract (75% (m/m)) and 0.125 g of Nujol (25% (m/m)).

The effect of enzyme concentration from 700 to 3,500 units *peroxidase* mg^{-1} of protein in the 75:25 % m/m of graphite powder plus crude extract/Nujol, respectively, on the biosensor response was also studied. The analytical signals (cathodic peak currents) for 4.0×10^{-3} mol L^{-1} hydroquinone increased with the increase of the enzyme

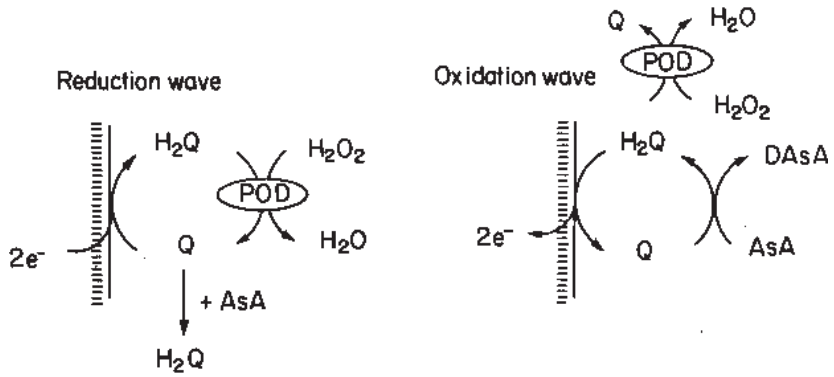

Figure 1. Schematic representations of the reduction and oxidation waves of the enzymatic process between hydroquinone (H_2Q), hydrogen peroxide (H_2O_2), L-ascorbic acid (AsA) and *peroxidase* (POD) of the crude extract incorporated into the carbon paste electrode

concentration used up to 1,400 units *peroxidase* mg^{-1} protein. Thus, a concentration of 1,400 units mg^{-1} protein POD was used in all biosensors.

Effect of pH and hydrogen peroxide concentration

The effect of pH in the range from 3.0 to 8.0 on the electrode response of a $4.0x10^{-3}$ mol L^{-1} hydroquinone solution was also investigated. A maximum current (maximum signal/noise ration) is obtained at a pH range of 6.0-7.0. Therefore, a pH of 6.5 was used in the other experiments, since L-ascorbic acid is more stable at this pH.

The effect of varying hydrogen peroxide concentration from $5.0x10^{-4}$ to $1.2x10^{-2}$ mol L^{-1}, for $4.0x10^{-3}$ mol L^{-1} hydroquinone and $4.0x10^{-3}$ mol L^{-1} ascorbic acid solution on the biosensor response was also evaluated. The optimum hydrogen peroxide concentration found was $2.0x10^{-3}$ mol L^{-1}. This concentration was then used.

Effect of dissolved oxygen

The effect of dissolved oxygen on the biosensor response for $4.0x10^{-3}$ mol L^{-1} hydroquinone solution, $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide solution and L-ascorbic acid ranged from $2.0x10^{-4}$ to $5.5x10^{-3}$ mol L^{-1} was evaluated in an air-saturated and degased conditions. From the results obtained, it was found that oxygen has very little effect on the biosensor response in the L-ascorbic acid concentration range studied for recently prepared L-ascorbic acid solutions. As it is known, solutions of L-ascorbic acid are highly susceptible to oxidation by dissolved oxygen and degrade in a short period of time in alkaline solution[25,26]. Thus, in order to obtain a good compromise between stability and performance of the biosensor, measurements were made in phosphate solution (pH 6.5), after efficient deoxygenation with nitrogen of all solutions used.

Cyclic voltammetry and analytical characteristics

Figure 2 shows the cyclic voltammograms obtained with plain carbon paste electrode (A, B, C and D) and zucchini crude extract modified carbon paste electrode (biosensor) (E, F, G and H) in the potential range from +0.7 to -0.4 V vs Ag/AgCl at a scan rate of 100 mV s^{-1} in 0.1 mol L^{-1} phosphate buffer solution (pH 6.5). Cyclic voltammograms A and E were obtained in 0.1 mol L^{-1} phosphate buffer (pH 6.5) with unmodified electrode and biosensor, respectively. Cyclic voltammograms B and F were obtained for $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide 0.1 mol L^{-1} in phosphate buffer (pH 6.5), C and G for $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide and $3.0x10^{-3}$ mol L^{-1} L-ascorbic acid in 0.1 mol L^{-1} phosphate buffer (pH 6.5) solution and finally cyclic voltammograms D and H were obtained for $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide, $3.0x10^{-3}$ mol L^{-1} L-ascorbic acid and $7.0x\ 10^{-3}$ mol L^{-1} hydroquinone in 0.1 mol L^{-1} phosphate buffer (pH 6.5) solution for these both electrode (plain and biosensor), respectively. It can be seen that L-ascorbic acid (AsA) was oxidized to dehydroascorbic acid (DAsA) at a potential of 0.4-0.5 V using the plain electrode (cyclic voltammogram C) and at a potential range of 0.2-0.4 V using this biosensor (cyclic voltammogram G). On the other hand, in the presence of hydroquinone (cyclic voltammograms D and H), two waves were observed: a reduction wave of reduction of quinone to hydroquinone (see also Figure 1) and an oxidation wave of hydroquinone to quinone and/or oxidation of AsA to DAsA. As it can be observed, the sensitivity of the biosensor was much better than that of the plain electrode (cyclic voltammogram H).

Figure 3 shows the cyclic voltammograms obtained with the modified carbon paste biosensor in the potential range from + 0.7 to - 0.4V vs Ag/AgCl at a scan rate of 100 mV s^{-1} in 0.1 mol L^{-1} phosphate buffer solution (pH 6.5). The baseline A was obtained using a $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide in phosphate buffer solution (pH 6.5). Cyclic voltammogram 3B

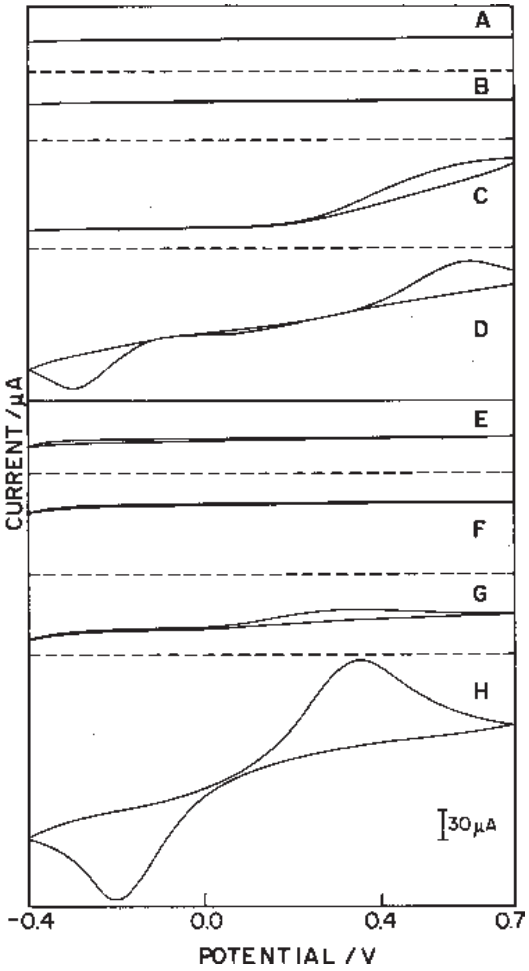

Figure 2. Cyclic voltammograms obtained with plain carbon paste electrode (A, B, C and D) and zucchini crude extract modified carbon paste (E, F, G and H) in the potential range from + 0.7 to - 0.4 vs Ag/AgCl at a scan rate of 100 mV s^{-1} in 0.1 mol L^{-1} phosphate buffer solution (pH 6.5), at 25ºC (see details in the text).

was obtained with $2.0x10^{-3}$ mol l^{-1} hydrogen peroxide and $3.0x10^{-3}$ mol L^{-1} L-ascorbic acid solutions in the same buffer solution described above in the experimental section. On the other hand, cyclic voltammograms presented in Figure 3C were obtained for $7.0x10^{-3}$ mol L^{-1} hydroquinone and $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide (cyclic voltammogram 1) and twenty other cyclic voltammograms (from 2 to 21) with the addition of L-ascorbic acid solution in the concentrations of (2) $2.0x10^{-4}$; (3) $3.9x10^{-4}$; (4) $7.7x10^{-4}$; (5) $1.1x10^{-3}$; (6) $1.5x10^{-3}$; (7) $1.8x10^{-3}$; (8) $2.1x10^{-3}$; (9) $2.5x10^{-3}$; (10) $2.8x10^{-3}$; (11) $3.1x10^{-3}$; (12) $3.3x10^{-3}$; (13) $3.6x10^{-3}$; (14) $3.9x10^{-3}$; (15) $4.1x10^{-3}$; (16) $4.4x10^{-3}$; (17) $4.6x10^{-3}$; (18) $4.9x10^{-3}$; (19) $5.1x10^{-3}$; (20) $5.3x10^{-3}$ and (21) $5.5x10^{-3}$ mol L^{-1}. The cathodic peak current obtained at a potential of -0.14 V (electrochemical reduction of p-quinone to hydroquinone) was thus related with the L-ascorbic acid concentration. The analytical curve obtained for L-ascorbic acid solution in the concentration range from $2.0x10^{-4}$ to $5.5x10^{-3}$ mol L^{-1} was I_{cp} =147.82 - $2.00x10^{5}$ [AsA], r= 0.9992, where I_{cp} is the cathodic peak current in mA and [AsA] is the L-ascorbic acid concentration in mol L^{-1}. A detection limit (three times the signal blank/slope) of $2.2x10^{-5}$ mol L^{-1} and a relative standard deviation <1.3% for eight consecutive determinations of a solution containing $4.0x10^{-3}$ mol L^{-1} L-ascorbic acid, $7.0x10^{-3}$ mol L^{-1} hydroquinone and $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide solution were obtained. This biosensor retained 90% of its initial enzyme activity for five months when stored at 4ºC and the lifetime of this biosensor was at least 7 months (over 900 samples were analyzed with the same carbon paste electrode), which is much higher than the lifetime of biosensors using pure *peroxidase* and/or *ascobate oxidase*[22,27].

Study of potential interferents

The effect of other substances on the determination of L-ascorbic acid in pharmaceutical formulations, such as acetylsalicylic acid, citric acid, glucose, lactose, magnesium stearate, oxalic acid, poly(ethylene glycol) 1500, sodium chloride, starch, sucrose, tartaric acid was investigated. The ratios of the concentrations of L-ascorbic acid to those of these excipient substances were fixed at 0.1, 1.0 and 10.0. None of these substances interfered in the proposed method.

Biosensor application

Using the above established conditions, *i.e.* 75:25 % m/m of graphite powder and Nujol, respectively and 1,400 units of *peroxidase* mg^{-1} of protein, pH 6.5, $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide, $7.0x10^{-3}$ mol L^{-1} hydroquinone, amperometric measurements at a fixed potential of – 0.14 V were carried out with the proposed biosensor in the recovery study and also in the determination of L-ascorbic acid in several pharmaceutical formulations.

The recovery studies of L-ascorbic acid in five pharmaceutical formulations, Cebion, Redoxon, Energil C, Vitamina C and Aspirina C (n=5) were undertaken using the carbon paste modified with the crude extract. In these studies, three different standard concentration solutions (0.207; 0.432 and 0.587 g L^{-1}) were added to each sample and recoveries varying from 98.1 to 102.1% of L-ascorbic acid were obtained (Table 1). This is a good evidence of the accuracy of proposed method and absence of matrix effects on those determinations.

The proposed method was validated by applying it to the determination of L-ascorbic acid in many pharmaceutical formulations (Table 2). The results obtained by the proposed procedure are in good agreement with those obtained by an official procedure[28] and also with the values claimed (calculated *t*-values did not exceed the theoretical value at 95% confidence level) and within an acceptable range of error.

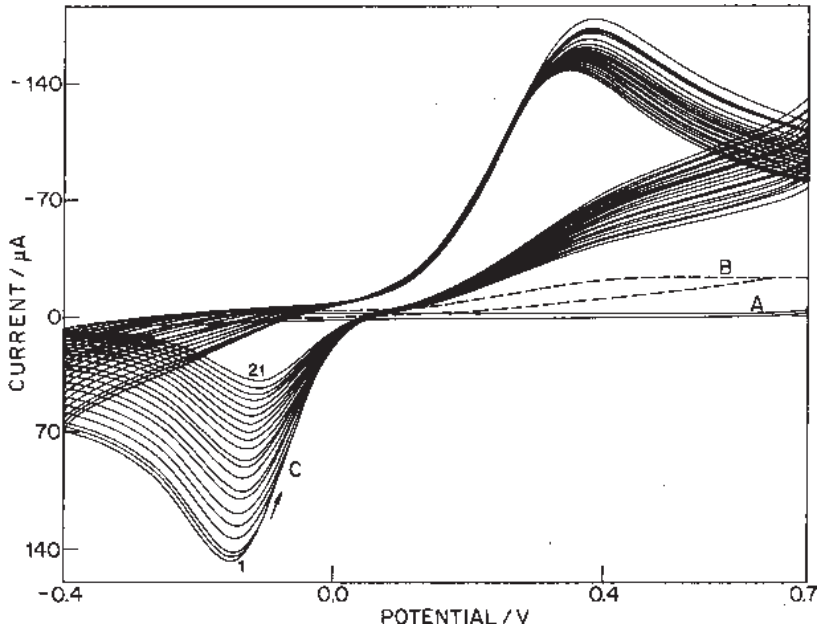

Figure 3. Cyclic voltammograms in 0.1 mol L^{-1} phosphate buffer solution (pH 6.5) for: (A) $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide solution; (B) $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide and $3.0x10^{-3}$ mol L^{-1} L-ascorbic acid solution and (C) $7.0x10^{-3}$ mol L^{-1} hydroquinone and $2.0x10^{-3}$ mol L^{-1} hydrogen peroxide (cyclic voltammogram 1) and twenty other cyclic voltammograms (from 2 to 21) with the addition of L-ascorbic acid solution in the concentration range from $2.0x10^{-4}$ to $5.5x10^{-3}$ mol L^{-1}. Scan rate of 100 mV s^{-1} and E_{cp} of -0.14V, at 25ºC.

Table 1 Results of the addition-recovery experiment using L-ascorbic acid of three standard concentrations

	L-ascorbic acid (g L^{-1})		
Sample	Added	Found	Recovery (%)
Cebion	0.207	0.208	100.5
	0.432	0.439	101.6
	0.587	0.585	99.7
Redoxon	0.207	0.211	101.9
	0.432	0.430	99.5
	0.587	0.580	98.8
Energil C	0.207	0.205	99.0
	0.432	0.441	102.1
	0.587	0.598	101.9
Vitamina C	0.207	0.204	98.6
	0.432	0.430	99.5
	0.587	0.594	101.2
Aspirina C	0.207	0.203	98.1
	0.432	0.439	101.6
	0.587	0.589	100.3

Table 2 Determination of L-ascorbic acid in pharmaceutical formulations using the official method[28] and the biosensor

	L-ascorbic acid (g/tablet)			
Sample	Label value	Official method	Biosensor	Relative error (%)
Cebion	2.0	2.08±0.08	2.10±0.04	+ 1.0
Redoxon	1.0	1.09±0.07	1.05±0.03	- 3.7
Energil C	1.0	0.98±0.07	1.01±0.03	+ 3.1
Vitamina C	0.50	0.52±0.06	0.51±0.02	- 1.9
Aspirina C	0.24	0.24±0.05	0.25±0.02	+ 4.1

Conclusions

The biosensor based on carbon paste modified with crude extract of zucchini (*Cucurbita pepo*) for determining L-ascorbic acid in pharmaceutical formulations is stable, selective, sensitive, simple and low cost. These characteristics make this biosensor an attractive alternative to those procedures that use pure enzymes (*ascorbate oxidase*) used for pharmaceutical, clinical and food applications.

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