Sensors and Actuators B 139 (2009) 570–575

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Carbon paste electrode modified with pine kernel peroxidase immobilized on pegylated polyurethane nanoparticles

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ARTICLE INFO

Article history:
Received 27 December 2008
Received in revised form 12 March 2009
Accepted 17 March 2009
Available online 27 March 2009

Keywords:
Pine kernel
Peroxidase
Nanoparticles
Polyurethane
Modified carbon paste electrode

ABSTRACT

In the present work, a carbon paste electrode based on pine kernel, a seed from Paraná pine (*Araucaria angustifolia*), for the determination of dopamine in pharmaceutical products was developed. The biodevice was constructed by the immobilization of peroxidase extracted from the pine kernel homogenate on pegylated polyurethane nanoparticles. Square-wave voltammetry (SWV) experiments were performed to investigate the performance of the modified carbon paste electrode. The best analytical response was obtained for a 75:15:10% (w/w/w) composition of graphite powder:mineral oil:polyurethane nanoparticles containing 2.5 units of peroxidase mg^{-1} of carbon paste, 0.1 mol L^{-1} phosphate buffer solution (pH 6.5), 2.0×10^{-3} mol L^{-1} hydrogen peroxide, 80 mV pulse height and 60 Hz frequency. The analytical curve was linear for dopamine concentrations from 9.9×10^{-5} to 1.6×10^{-3} mol L^{-1} ($r = 0.9995$) and the regression equation was found to be $\Delta I = 2.1 + 7.4 \times 10^{3}$ [dopamine] with a limit detection of 9.0×10^{-6} mol L^{-1}. The recovery of dopamine from pharmaceutical samples ranged from 97% to 103% and the results obtained using the proposed modified carbon paste electrode and those obtained by the official method are in agreement at the 95% confidence level.

1. Introduction

The immobilization of enzymes onto support materials has been proposed in the last years as a form to decrease mass-transfer limitations in analytical devices such as biosensors [1]. Support material, which plays an important role in the utility of an immobilized enzyme, should be readily available and non-toxic, and also should provide a large surface area suitable for enzyme reaction, and substrate and product transport with the least diffusion restriction. As support matrices, polymeric materials have attracted much attention because they can be produced easily in a wide variety of compositions [2]. A range of nanoparticles, nanotubes and nanowires, prepared from metals, semiconductor, carbon or polymeric species, have been widely investigated for their ability to enhance the response of biosensors. Nanoparticles can be used to modify the electrode surfaces, or to modify biological receptor molecules such as enzymes, antibodies and oligonucleotides [3].

Micro and nanoparticles of polyurethane (PU) are widely used in many industrial applications, as for the development of biomedical devices or drug delivery systems [4]. They have excellent physical properties, biocompatibility, and when functionalized with poly(ethylene glycol) (PEG), their surface become hydrophilic [5]. Many attempts have been done to construct biosensors and modified electrodes with immobilized enzyme into polyurethane materials. Veselova and Shekhovtsova [6] studied different esters, ethers and their mixtures with PU foam as support for the immobilization of horseradish peroxidase. Phadtare et al. [7] constructed a biosensor based on immobilized pepsin on the nano-gold-PU microparticles conjugates (templates), and Budriene et al. [8] have synthesized a series of PU microspheres to immobilize maltogenase.

Peroxidase constitutes a class of enzyme extensively distributed in the plant kingdom and can be easily extracted from almost all plant cells, animals and microorganisms. The use of homogenate and plant-tissue material, rather than isolated enzymes, represents not only an attractive alternative for a biodevice, but also simplicity, stability, longer lifetime, low cost and reduced co-factor requirements [9,10]. The pine kernel is a seed from Paraná pine (*Araucaria angustifolia*) and has constituted an important source of carbohydrates and its tissue homogenate can be used as a

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doi:10.1016/j.snb.2009.03.039

source of peroxidase. The Paraná pine is an endemic conifer of southern and southeastern of Brazil, which composes the Araucaria forest, an important biome endangered due the extensive loggings.

Dopamine is a very important catecholamine neurotransmitter in the mammalian central nervous system. The change in the level of dopamine has been proved to be a very effective route toward brain functions, and the loss of dopamine-containing neurons may result in serious disease such as Parkinson's disease [9]. Selective and sensitive determination of dopamine has been a long-standing goal, and electrochemical techniques have been proved to be one of the most advantageous ways in the determination of dopamine [11]. However, for this purpose other methods have also been applied such as chromatography [12,13], spectrophotometry [14,15] and spectrofluorometry [16].

In the present work, a new carbon paste electrode modified with peroxidase extracted from pine kernel was constructed by the immobilization of this enzyme on pegylated polyurethane nanoparticles. The characteristics and merits of the carbon paste electrode are described in the following sections. After optimization, the modified electrode was used for the square-wave voltammetric determination of dopamine in pharmaceutical products.

2. Experimental

2.1. Reagents, solutions and other supplies

All used chemicals were of analytical grade and prepared using water from a Milli-Q system (Millipore) with resistivity not less than 18 MΩ cm. The homogenate of several vegetables was used as a source of peroxidase. These vegetables were purchased from a local market of Florianópolis, Brazil, washed, hand-peeled, chopped and cooled in a refrigerator at 4 °C. A 0.05 mol L^{-1} Guaiacol (Aldrich) stock solution was prepared freshly in 0.1 mol L^{-1} phosphate buffer (pH 6.5) and a 0.12 mol L^{-1} hydrogen peroxide (Merck) stock solution was prepared in the same phosphate buffer. Reference solutions of dopamine from 9.9×10^{-5} to 1.6×10^{-3} mol L^{-1} were prepared by appropriate dilution of a 0.05 mol L^{-1} dopamine stock solution in 0.1 mol L^{-1} phosphate buffer (pH 6.5). The carbon paste electrodes were prepared using an Acheson 38 graphite powder (Fisher) and mineral oil (Aldrich). The pegylated polyurethane nanoparticles used as support for peroxidase immobilization were synthesized by miniemulsion technique using the following chemical products: poly(ethylene oxide)20-sorbitane monooleate (Tween 80, Beraca), isophorone diisocyanate (IPDI, Aldrich), natural triol (Kehl), poly(ethylene glycol) M_w = 400 g mol L^{-1} (PEG 400, Vetec), and olive oil (Yola).

2.2. Apparatus

A high-speed brushless centrifuge (model MPW-350R) was used in the preparation of the vegetables homogenate. An UV–VIS spectrophotometer (Pharmacia LKB-Ultrospec III) with a quartz cell (optical path of 1.00 cm) was used for peroxidase activity and total protein determinations. The polyurethane nanoparticles were synthesized using an Ultra-Turrax® T-18 (IKA®). The particles size was determined by dynamic light scattering technique using a Zetasizer equipment (Malvern) and transmission electron microscopy (JEOL-JEM). Cyclic (CV) and square-wave voltammetry (SWV) experiments were performed using an Autolab PGSTAT30 potentiostat/galvanostat (Eco Chemie) coupled to a computer with GPES 4.9 software. Measurements were carried out at room temperature using an electrochemical cell with three electrodes: Ag/AgCl (3.0 mol L^{-1} KCl) as the reference, a platinum wire as the auxiliary and the modified carbon paste electrodes as working electrodes.

2.3. Extraction of peroxidase from different vegetables

Vegetables such as persimmon (*Diospyros kaki* L.), star fruit (*Averrhoa carambola* L.), yam (*Dioscorea* sp.), saffron (*Crocus sativus* L.), yacón (*Smallanthus sonchifolius*), pea beans (*Pisum sativum* L.) and pine kernel from Paraná pine (*A. angustifolia*) were investigated for activity, total protein and specific activity determinations.

Each vegetable was homogenized using 25 g of peeled tissue in a blender with 100 mL of 0.1 mol L^{-1} phosphate buffer solution (pH 7.0) for 5 min at 4 °C. The homogenate was rapidly filtered and centrifuged at 15,000 rpm for 15 min at 4 °C. The resulting supernatant was stored at this temperature in a refrigerator and used as peroxidase source [9,17].

2.4. Peroxidase activity

The peroxidase activity present in the homogenate of the selected vegetables was determined in triplicate monitoring the formation of tetraguaicol at 470 nm. The reaction medium contained 0.2 mL of homogenate, 2.7 mL of 0.05 mol L^{-1} guaiacol and 0.1 mL of 10 mmol L^{-1} hydrogen peroxide in phosphate buffer (pH 7.0) at 25 °C [9,17]. The protein concentration was determined using the Biuret method [18].

2.5. Preparation of pegylated polyurethane nanoparticles

Nanoparticles of polyurethane were prepared by the miniemulsion technique as described elsewhere [5]. Briefly, a monomer mixture containing the diisocyanate (isophorone diisocyanate), the natural polyol, poly(ethylene glycol) (PEG) and the hydrophobe agent (olive oil) was added to an aqueous solutions containing the surfactant at room temperature. Nanodroplets of monomers were obtained using an Ultra-Turrax® T18 homogenizer at 18,000 rpm for 15 min. The resulting dispersions were maintained under mechanical stirring (800 rpm) at 60 °C for 4 h to allow the complete polyurethane nanoparticle formation. The whole monomer concentration in the polymerization medium was 5 wt %. Tween 80 was employed as surfactant at concentration of 10 wt% (versus monomer). The PEG 400 was added as a co-monomer in substitution to 50 mol% of natural triol.

2.6. Immobilization of peroxidase on pegylated polyurethane nanoparticles

Peroxidase immobilization was performed by adding aliquots from 30 to 630 μL of the pine kernel homogenate containing 100–2000 units mL^{-1} of peroxidase to 0.1 g of the pegylated polyurethane nanoparticles for 24 h at 25 °C. The polyurethane nanoparticles containing immobilized enzyme were dried in a desiccator at room temperature. The enzyme activity was not determined after immobilized into PEG–polyurethane nanoparticles, instead the performance of the modified carbon paste electrodes containing different amounts of immobilized enzyme was evaluated. The effect of enzyme concentration from 0.5 to 10 units of peroxidase mg^{-1} of carbon paste [75:15:10 (% w/w) of graphite:mineral oil:pegylated polyurethane] was studied by measuring the electrode response using square-wave voltammetry in the conditions described in the Section 2.8.

2.7. Electrodes preparation and optimization

Different electrodes were prepared to evaluate the performance of the carbon paste electrode modified with pine kernel peroxidase immobilized on pegylated polyurethane nanoparticles. The procedures for the electrodes preparation were as follows:

(a) *Carbon paste electrode containing pegylated polyurethane nanoparticles:* it was prepared mixing in a mortar 150 mg of graphite powder (75%, w/w) with 20.0 mg of pegylated polyurethane nanoparticles (10%, w/w) all together for 20 min. Subsequently, 30 mg of mineral oil (15%, w/w) was added and mixed for 20 min more.
(b) *Carbon paste electrode containing peroxidase extracted from pine kernel:* it was prepared by adding different amounts of enzyme (100–2000 units) to 150 mg of graphite powder. These materials were allowed to dry overnight at room temperature and before use they were mixed for 20 min. After that, 30 mg of mineral oil was added and mixed for 20 min more.
(c) *Carbon paste electrode:* it was prepared with 150 mg of graphite powder mixed with 30 mg of mineral oil for 20 min.
(d) *Carbon paste containing peroxidase immobilized on pegylated nanoparticles:* it was prepared mixing 150 mg of graphite powder (75%, w/w) with 20.0 mg of pegylated polyurethane nanoparticles containing immobilized peroxidase (100–2000 units of enzyme, 10%, w/w) all together for 20 min. Subsequently, 30 mg of mineral oil (15%, w/w) was added and mixed for 20 min more.

All the pastes described on the items (a), (b), (c) and (d) were transferred into a plastic syringe (3.0 mm internal diameter) and a copper wire was inserted for the electric contact. The working electrodes were stored at 4 °C when not in use [9,17].

To evaluate the performance of the modified carbon paste electrodes, parameters such as enzyme concentration, pH, frequency and pulse amplitude were optimized and the best values obtained are shown in Table 1. The concentration of hydrogen peroxide used in all experiments was determined in a previous work and it was 2×10^{-3} mol L^{-1} [19]. The carbon paste electrode containing 500 units of peroxidase immobilized on pegylated polyurethane nanoparticles (2.5 units mg^{-1} carbon paste) showed the best performance (see Table 1) and it was used for the determination of dopamine on pharmaceutical products.

2.8. Electrochemical measurements and analysis of pharmaceutical samples

Cyclic and square-wave voltammetric measurements were performed in an unstirred and not de-aerated 0.1 mol L^{-1} phosphate buffer (pH 6.5) containing 2.0×10^{-3} mol L^{-1} hydrogen peroxide at 25 °C. All potentials were measured and reported versus Ag/AgCl (3.0 mol L^{-1} KCl), after a suitable initial stirring time of 60 s. The cyclic voltammograms were recorded by cycling the potential between −0.2 and +0.5 V at a scan rate of 100 mV/s. The square-wave voltammograms were obtained in the potential interval from −0.4 to +0.5 V at a frequency of 60 Hz, pulse amplitude of 80 mV and step potential of 2.0 mV after successive additions of the dopamine stock solutions. The dopamine standard curve was constructed by plotting the magnitude of the resultant current peak against the corresponding dopamine concentration. The determination of dopamine in sample of pharmaceutical formulation followed the method of standard addition: an accurate volume of 140 μL of sample in 15.0 mL of 0.1 mol L^{-1} phosphate buffer (pH 6.5) containing 2.0×10^{-3} mol L^{-1} hydrogen peroxide was analyzed after successive additions of 3.3×10^{-4}, 6.6×10^{-4} and 9.8×10^{-4} mol L^{-1} of reference dopamine solution. After each addition, square-wave voltammograms were recorded. All measurements were performed in triplicate.

The results obtained from the proposed electrode will be compared using the Pharmacopeia standard method [20]. Briefly, the standard method consists of dissolving 30 mg of the dopamine sample in 7 mL of acetic acid and 1 mL of mercury acetate. The solution was mixed and potentiometrically titrated with 0.1N of perchloric acid for the final point determination. Each millilitre of the perchloric acid 0.1N solution is equivalent to 18.96 mg of dopamine.

Table 1
Optimization of experimental variables.

Parameters	Range studied	Optimal value
Peroxidase concentration[a]	100–2000	500
Peroxidase concentration[b]	0.5–10	2.5
pH	6.0–8.0	6.5
Frequency (Hz)	10–100	60
Pulse amplitude (mV)	10–100	80

[a] units mL^{-1}.
[b] units mg^{-1} carbon paste.

Table 2
Activity, total protein and specific activity obtained from various vegetables.

Vegetables	Activity (units mL^{-1})	Total protein (mg mL^{-1})	Specific activity (units mg^{-1} protein)
Persimmon	24.05	0.187	128
Star fruit	72.15	0.138	522
Yam	794	0.475	1671
Saffron	1010	1.033	977
Yacón	1766	0.391	4516
Pea beans	2428	0.345	7037
Pine kernel	3246	0.350	9274

3. Results and discussion

3.1. Source of peroxidase and stability

The peroxidase presented in several vegetables has been investigated successfully and employed to develop different types of electrodes [9,10,17]. In this work, different vegetable extracts were studied and the activity (units mL^{-1}), total protein (mg mL^{-1}) and specific activity (units mg^{-1} protein) were obtained using the homogenates, as shown in Table 2. As can be seen in this table, the persimmon (*D. kaki* L.) homogenate showed the lowest specific activity while the pine kernel homogenate showed the highest. Pine kernel from Paraná pine (*A. angustifolia*) is rich in peroxidase activity and, the enzyme from this plant is not yet commercially available. Therefore, the homogenate of this vegetable was used as a source of peroxidase and immobilized on pegylated polyurethane nanoparticles. The peroxidase activity values obtained from pine kernel were similar to the activity values obtained from other vegetables used for biosensor construction [9,10,17]. The stability of pine kernel peroxidase homogenate was also investigated. The specific activity of the free peroxidase remained constant at 4 °C during 21 days and after 60-day period, the enzyme activity decreased by 30%.

3.2. Characterization of pegylated polyurethane nanoparticles

The particles size was determined by dynamic light scattering (DLS) and transmission electron microscopy (TEM), as described in a previous work [5]. The average hydrodynamic radius of the particles was determined using Stokes–Einstein relation and it is equal to 131 nm. Fig. 1 shows the TEM image of pegylated polyurethane nanoparticle. As can be observed in the image, a fraction of PEG (hydrophilic diol) can migrate at the interface of the polyurethane particle–water, forming a protecting layer around the particle.

The zeta potential is a function of the particle surface charge, the layer adsorbed in the interface and the nature/composition of the surrounding medium in which the particle is suspended. The magnitude of the measured zeta potential is an indication of the repulsive force that is present and can be used to predict the long-term stability of the product. The value of zeta potential,

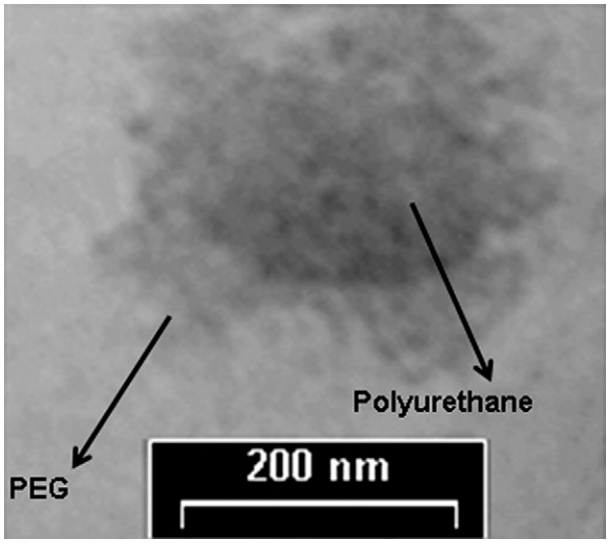

Fig. 1. Transmission electron microscopy (TEM) image of pegylated polyurethane nanoparticle.

already described in a previous work, obtained for the pegylated polyurethane nanoparticles was -22.1 ± 0.1 mV at pH equal to 6.9 [21].

3.3. Immobilization of peroxidase on pegylated polyurethane nanoparticles and enzymatic process of dopamine by the modified carbon paste electrode

The catalytic activity is dependent of the reagents and support material used for enzyme immobilization. In this work, the enzyme immobilization was performed at pH 6.5, which is the optimum pH of peroxidase [22]. Under such condition, the pegylated polyurethane nanoparticles are negatively charged, as evidenced by zeta potential value described on a previous work [21]. The isoelectric point of peroxidase is 7.2 [23], and therefore molecules of this enzyme carry a net positive charge in a pH 6.5. Hence, the enzyme can be physically adsorbed on the surface of pegylated polyurethane nanoparticles (negatively charged).

Furthermore, the PEG chains confer hydrophilic surfaces for the polyurethane nanoparticles. High hydration level on the surface seems to play a very important role, preserving enzyme conformation upon adsorption. This behavior was observed for immobilized pectinase on colloidal particles of block copolymer poly(styrene-b-acrylic acid), PS-b-PAA. The carboxyl groups on the particles surface provided an improvement in thermal and storage stabilities of immobilized pectinase [24]. Similarly, HRP molecules did not reduce the enzymatic activity when they were entrapped in mesoporous silica particles, which are very hydrophilic surfaces [25].

The schematic representation of the peroxidase immobilized in pegylated polyurethane nanoparticles and the electrochemical processes of the modified carbon paste electrode proposed are shown in Fig. 2. The peroxidase catalyses the oxidation of dopamine to the correspondent *o*-quinone in the presence of hydrogen peroxide and, the *o*-quinone produced is electrochemically reduced back to dopamine on the surface of the electrode, generating currents that are directly proportional to dopamine concentrations. This process is observed in the cyclic and square-wave voltammograms showed in the next sections.

3.4. Modified carbon paste electrode performance by cyclic voltammetry

The efficiency of peroxidase immobilization on pegylated polyurethane nanoparticles was evaluated using four different constructed electrodes: (a) carbon paste containing pegylated polyurethane nanoparticles; (b) carbon paste containing 500 units of peroxidase; (c) carbon paste and (d) carbon paste containing 500 units of peroxidase immobilized on pegylated nanoparticles (2.5 units mg^{-1} carbon paste). Fig. 3 shows the cyclic voltammograms obtained for these four electrodes. As can be seen, the electrode (a) showed the smallest analytical signal (cathodic current). The highest response was observed for the electrode (d), which resulted in a good performance of this biosensor. Furthermore, the electrode (b) containing only peroxidase showed a lower response than electrode (d). These results were expected since the enzyme immobilization on a polymeric support improves its activity and stability [22,24].

3.5. Reproducibility, stability and repeatability of the modified carbon paste electrode

The electrode-to-electrode reproducibility was determined for three modified carbon paste electrodes using a 6.6×10^{-4} mol L^{-1} dopamine solution. In this study a relative standard deviation (RSD) of 2% was obtained.

The stability study was investigated during a 200-day period (over 400 determinations). When the biosensor was stored at 4 °C and measured every 1–2 days, no obvious change was found in the response for eight successive assays for 6.6×10^{-4} mol L^{-1} dopamine in 0.1 mol L^{-1} phosphate buffer (pH

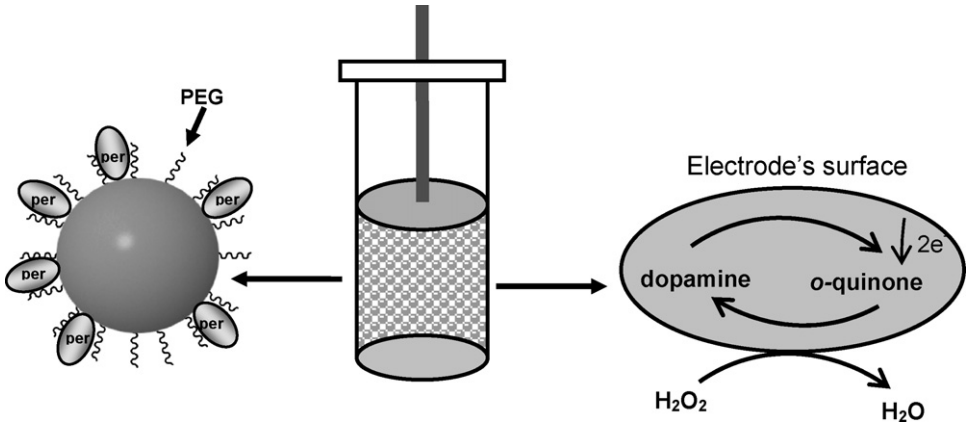

Fig. 2. Schematic representation of the enzymatic process among dopamine in the presence of hydrogen peroxide and peroxidase (per) immobilization on pegylated polyurethane nanoparticles.

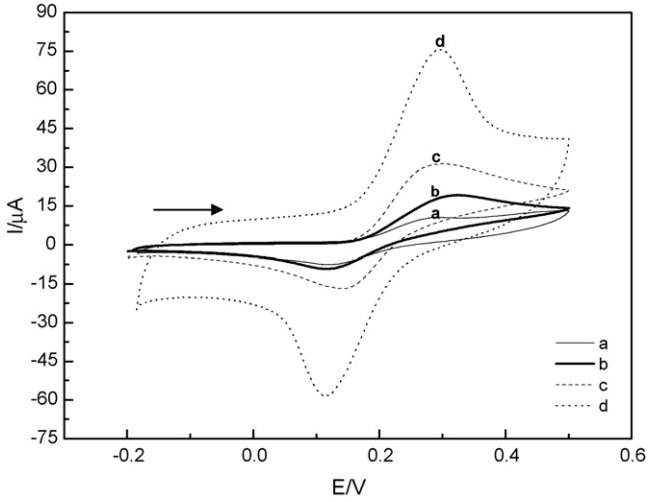

Fig. 3. Cyclic voltammograms obtained using the four different electrodes: (a) carbon paste + pegylated polyurethane nanoparticles; (b) carbon paste + peroxidase; (c) carbon paste and (d) carbon paste + peroxidase immobilized on pegylated polyurethane nanoparticles (2.5 units mg^{-1} carbon paste). The measurements were performed in an unstirred 0.1 mol L^{-1} phosphate buffer (pH 6.5) containing 2.0×10^{-3} mol L^{-1} hydrogen peroxide and 1.9×10^{-3} mol L^{-1} dopamine at 100 mV/s scan rate.

6.5) and 2.0×10^{-3} mol L^{-1} hydrogen peroxide solution. The relative standard deviation (RSD) of 5% was obtained.

The study of the repeatability was accomplished using ten successive measurements for a 6.6×10^{-4} mol L^{-1} dopamine in 0.1 mol L^{-1} phosphate buffer (pH 6.5). The values of the resulting currents were obtained and a RSD of 0.5% was found, showing thus a very good repeatability.

The good performance of the modified carbon paste electrode proposed in terms of reproducibility, stability and repeatability may be related to the efficiency of the immobilization of the peroxidase on pegylated polyurethane nanoparticles, which confer a favorable environment for the enzyme. Similar results were obtained for carbon paste electrodes modified with polymers containing enzymes extracted from vegetables [10].

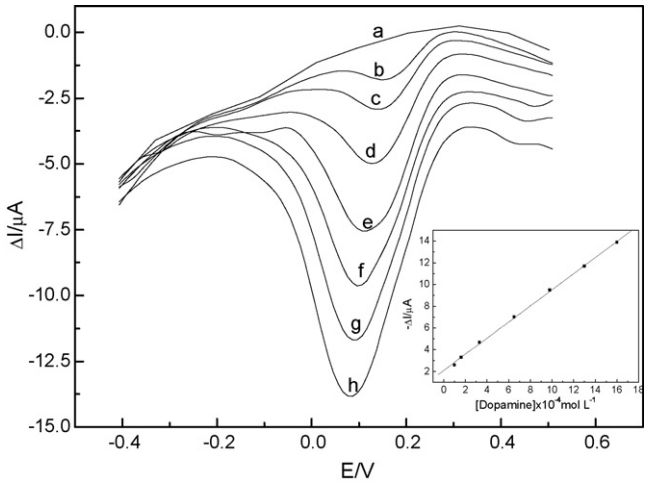

Fig. 4. Square-wave voltammograms obtained using the modified carbon paste electrode for (a) blank in 0.1 mol L^{-1} phosphate buffer solution (pH 6.5) containing 2.0×10^{-3} mol L^{-1} hydrogen peroxide, and dopamine solutions at the following concentrations: (b) 9.9×10^{-5}; (c) 1.6×10^{-4}; (d) 3.3×10^{-4}; (e) 6.5×10^{-4}; (f) 9.8×10^{-4}; (g) 1.3×10^{-3}; (h) 1.6×10^{-3} mol L^{-1} at pulse height 80 mV, frequency 60 Hz and increment 2.0 mV. Inset: the analytical curve of the modified carbon paste electrode proposed.

Table 3
Recoveries of dopamine standard solution in pharmaceutical products using the modified carbon paste electrode proposed.

Sample	Dopamine (mol L^{-1}) $\times 10^{-4}$		
	Added	Found	Recovery (%)
A	3.30	3.40 ± 0.04	103
	6.60	6.50 ± 0.05	98.5
	9.80	9.70 ± 0.03	99.0
B	3.30	3.20 ± 0.02	97.0
	6.60	6.70 ± 0.01	102
	9.80	9.90 ± 0.02	101

Table 4
Determination of dopamine (mg mL^{-1}) in pharmaceutical formulations using the official method and the modified carbon paste electrode proposed.

Sample	Label value	Official method	Biosensor	RE_1 (%)[a]	RE_2 (%)[b]
A	5.00	5.08 ± 0.01	4.80 ± 0.01	−4.0	−5.5
B	5.00	5.12 ± 0.02	5.10 ± 0.01	+2.0	−0.4

[a] RE_1 = biosensor versus label value.
[b] RE_2 = biosensor versus official method value.

3.6. Analytical curve, recovery study and analytical application

The square-wave voltammograms of the carbon paste electrode modified with pine kernel peroxidase immobilized on pegylated polyurethane nanoparticles (Fig. 4) were obtained with successive dopamine additions and in the conditions previously optimized. The analytical curve, Fig. 4 (inset), showed linear range from 9.9×10^{-5} to 1.6×10^{-3} mol L^{-1} ($r = 0.9995$) and the regression equation obtained was $\Delta I = 2.1 + 7.4 \times 10^3$ [dopamine], where ΔI is the resultant peak current in μA and [dopamine] is the dopamine concentration in mol L^{-1}. The detection limit was calculated using the formula DL = 3.3 s/S, where s refers to the estimated standard deviation of the blank signal and S to the angular coefficient of the analytical curve. A limit detection was found to be 9.0×10^{-6} mol L^{-1} dopamine, which is a good value if compared with some biosensors for dopamine determination [9,26].

The recovery study using the modified carbon paste electrode proposed was performed adding three different concentration of dopamine (3.3×10^{-4}, 6.6×10^{-4} and 9.8×10^{-4} mol L^{-1}) to each sample and the resultant current peak of square-wave voltammograms was obtained. The results of the recovery studies ranged from 97 to 103% of dopamine (Table 3) for two commercial samples (A and B). The obtained results confirmed the absence of matrix influence in these samples.

The modified carbon paste electrode proposed was validated by determinations of dopamine in pharmaceutical products. Table 4 gives the label value of dopamine in the samples. The results obtained from the proposed electrode were compared using the Pharmacopeia standard method [20]. Applying a paired t-test to the results obtained by each procedure, it was found that all results are in agreement at the 95% confidence level and within an acceptable range of error. Based on these results it may conclude that the method is suitable for the application tested.

4. Conclusions

In this work, a new carbon paste electrode modified with peroxidase from pine kernel immobilized on pegylated polyurethane nanoparticles was proposed. The pine kernel, a seed from Paraná pine (*A. angustifolia*), has been shown to be an alternative biocatalyst, not yet used as a source of peroxidase. In addition, the pegylated polyurethane nanoparticles showed to be an adequate support for enzyme immobilization, improving its stability. The modified electrode shows a simple design, low cost, lower limit of

detection and applicability in the determination in pharmaceutical products. We believe that this system is a significant contribution toward the fabrication of a sensor.

Acknowledgements

The authors acknowledge CNPQ, CNPq/PDJ, CAPES/PRODOC, FINEP and FAPESC for financial support.

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