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Construction and analytical application of a biosensor based on stearic acid-graphite powder modified with sweet potato tissue in organic solvents

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Abstract A biosensor based on stearic acid-graphite powder modified with sweet potato (*Ipomoea batatas (L.) Lam.*) tissue as peroxidase source was constructed and applied in organic solvents. Several parameters were studied to evaluate the performance of this biosensor such as stearic acid-graphite powder and tissue composition, type and concentration of supporting electrolyte, organic solvents, water/organic solvent ratio (% v/v) and hydrogen peroxide concentration. After selection of the best conditions, the biosensor was applied for the determination of hydroquinone in cosmetic creams in methanol. At the peroxidase electrode hydroquinone is oxidized in the presence of hydrogen peroxide and the radical formed was reduced back electrochemically at –180 mV *vs* Ag/AgCl (3.0 mol L^{-1} KCl). The reduction current obtained was proportional to the concentration of hydroquinone from 6.2×10^{-5} to 1.5×10^{-3} mol L^{-1} (r = 0.9990) with a detection limit of 8.5×10^{-6} mol L^{-1}. The recovery of hydroquinone from two samples ranged from 98.8 to 104.1% and an RSD lower than 1.0% for a solution containing 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.10 mol L^{-1} tetrabutylammonium bromide methanol-phosphate buffer solution (95:5% v/v) (n = 10) was obtained.

Introduction

Since the invention of the first carbon paste electrode (CPE) by Adams in 1958 [1] several composite electrodes were developed [2, 3]. It generally consists of electrically conducting graphite powder and an organic liquid which is immiscible with the contacting aqueous solution. CPEs gain increasing popularity after modification of the composite by immobilization of both active mediators and enzymes [3]. These electrodes exhibit rather low background currents over a large range of potential when compared with other solid electrodes, offering an easy renewability of their surface as well as a high versatility and simplicity of modification [2–5].

The real chemical modification of a CPE was done by Cheek and Nelson [6] by introducing complexing functional groups to the carbon surface for the preconcentration of Ag(I). Enzymes have been used in conjunction with carbon paste electrodes ever since the late 1970s, when Yao and Musha used alcohol and L-lactate dehydrogenases [7]. Since then, an exponentially increasing number of publications followed, mainly dealing with modified carbon paste electrodes with crude extract [8, 9] and tissue [3, 10].

The first report of a biosensor in which the biocatalyst was in direct contact with the organic phase appeared in 1988, when Hall et al. described an amperometric enzyme electrode based on polyphenol oxidase for the measurement of phenols in chloroform [11, 12]. The ability of the enzyme to operate in organic solvents offers unique opportunities to the field of biosensors. Organic phase enzyme electrodes (OPEEs) have been employed in the detection of compounds of analytical and environmental interest. These include phenols [13] and peroxides [14–16]. The advantages of using OPEEs are well discussed in several articles [17–23].

The stearic acid-modified carbon paste electrode (StCPE) developed by Blaha and Lane [24] was extensively applied to in vivo monitoring of neurochemicals and one of the advantage of this electrode in voltammetric measurements is that it repels ascorbic acid and other negatively charged species and attracts positively charged neurotransmitters such as dopamine. The differences between StCPE performances after exposure to surfactant and brain tissues were recently presented by Petit et al. [25].

In this work, a biosensor based on stearic acid-graphite powder modified with sweet potato (*Ipomoea batatas (L.) Lam.*) tissue as peroxidase source was constructed and ap-

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plied to the determination of hydroquinone in methanol. The results obtained with this biosensor will be compared with those obtained using a paraffin-graphite powder electrode modified with the same potato tissue and also in terms of physical properties of the organic solvents used.

Experimental

Apparatus. All electrochemical experiments were carried out in a 15 mL thermostated glass cell at 25 °C. A three-electrode assembly incorporating stearic acid-graphite powder modified with sweet potato (*Ipomoea batatas (L.) Lam.*) tissue (StCPET) as working electrode, an Ag/AgCl (3.0 mol L^{-1} KCl) reference 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.

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.

StCPE and StCPET were prepared using stearic acid (Sigma) and graphite powder (grade # 38) from Fisher.

The following organic solvents obtained from Aldrich (Milwaukee, WI, USA) were used: acetone, acetonitrile, 1-butanol, chloroform, ethanol, methanol, 1-propanol, 2-propanol and tetrahydrofuran. Molecular sieves were used to remove trace amounts of water from all the solvents. The water content was less than 0.05% in weight which was determined using a Karl-Fischer procedure.

In the supporting electrolyte study tetraethylammonium bromide (TEAB), tetramethylammonium bromide (TMAB), tetrabutylammonium bromide (TBAB), tetrabutylammonium perchlorate (TBPAP) and tetrabutylammonium tetrafluoroborate (TBATFB) from Aldrich were used as conducting salts.

Hydroquinone was purchased from Sigma (St. Louis, MO, USA) and a 2.5×10^{-2} mol L^{-1} stock solution was prepared daily in 0.10 mol L^{-1} TBAB methanol-phosphate buffer solution (0.1 mol L^{-1} and pH 7.0) (95:5% v/v).

Solutions of citric acid, magnesium stearate, methylparaben, octylmethoxycinnamate, poly(ethylene glycol), propylparaben, and starch (Sigma) were prepared by dissolving the respective compounds in methanol and used for the interference study.

Hydrogen peroxide (Sigma) (10^{-2} mol L^{-1}) standard solutions were prepared daily in 0.10 mol L^{-1} TBAB methanol-phosphate buffer solution (0.1 mol L^{-1} and pH 7.0) (95:5% v/v).

Two Brazilian cosmetic creams such as Clariderm (Feliste, Guarulhos/SP, Brazil) and Claripel (Stiefel, Guarulhos/SP, Brazil) were obtained from a local store and analyzed using the proposed biosensor and United States Pharmacopoeia procedures [26].

Preparation of sweet potato tissue powder. Healthy sweet potato (*Ipomoea batatas (L.) Lam.*), purchased from a local producer were selected, washed, hand-peeled, chopped and lyophilizated for 3 h at 4 °C. A domestic liquefier was used to obtain fine powders and the particle size was selected by passing them through sieves with known mesh size (<100 μm), stored in a desiccator at 25 °C and used as a source of *peroxidase* (donor; hydrogen peroxide oxidoreductase, POD; EC.1.11.1.7).

StCPE and StCPET electrodes construction. The solid carbon paste electrodes were prepared manually by melting 0.140 g of stearic acid (28% w/w) in a mortar thermostatically controlled in a water bath at a temperature close to its melting point (67–69 °C). Subsequently, a mass of 0.285 g of graphite powder (57% w/w) was added and mixed for 5 min with a glass spatula to obtain a homogeneous paste. Thus, 0.075 g of solid bovine serum albumin (BSA) (15% w/w) was added for the StCPE preparation and mixed with a glass spatula for 2–3 min. A mass of 0.075 g of sweet potato powder (15% w/w), containing 1500 units of peroxidase, was used in another mixture of stearic acid-graphite powder, as prepared before, for StCPET preparation. A portion of each mixture (about 0.167 g) was packed into the tip of a 1 mL insuline plastic syringe and a silver wire was inserted to obtain the external electric contact as described elsewhere [8, 9].

These electrodes were immersed in 10 mL of organic solvent containing 0.1 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v) for 5 min before each determination and the StCPET was stored at 4 °C in a refrigerator when not in use.

Procedure. All measurements were made at room temperature (25 ± 1 °C) in 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.10 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v).

An accurate amount ranging from 1.5–3.0 g of each cosmetic cream sample was dissolved in 40 mL of methanol containing 0.1 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v) with stirring until sample dissolution and the volume was adjusted with this solvent in a 50 mL calibrated flask. Aliquots of 400 μL of this sample solution were added to a thermostated glass cell containing 10 mL of 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.1 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v). The determination of the hydroquinone content in the samples was performed after successive addition of reference hydroquinone solutions in the same organic-water mixture. After each aliquot addition, cyclic voltammograms were recorded by cycling the potential between +400 and –400 mV at a scan rate of 100 mV s^{-1}. The amperometric measurements were performed at –180 mV and the resulting cathodic current was displayed on the x-t recorder.

Results and discussion

Peroxidase catalytic cycle and voltammetric studies. The catalytic cycle involving native peroxidase (Fe^{3+}), HRP, hydrogen peroxide and hydroquinone, S, can be explained by the three following steps [27–33]:

$$HRP + H_2O_2 \rightarrow Cpd\ I + H_2O \quad (1)$$

$$Cpd\ I + S \rightarrow Cpd\ II + S^{\bullet} \quad (2)$$

$$Cpd\ II + S \rightarrow HRP + S^{\bullet} + H_2O \quad (3)$$

The first step (1) comprises a two-electron oxidation of the ferriheme prosthetic group of the native peroxidase, HRP, by hydrogen peroxide with formation of an unstable intermediate, two oxidizing equivalents above ferric state, consisting of ferryl iron and a porphyrin π cation radical (Cpd I) and water. In the next step (2), Cpd I loses one oxidizing equivalent by one-electron reduction of the first electron donor S forming Cpd II (iron with an oxidation state +4) and $S^{\bullet}$ free radical. Then, in the step (3), Cpd II receives an additional electron from S, whereby the enzyme is returned to its native state (Fe^{3+}). Also, the peroxidase immobilized in this electrode [33] can be oxidized by hydrogen peroxide according to reaction (1) and then subsequently reduced by electrons provided by the electrode, according to reaction (4):

$$Cpd\ I + 2\ e^{-} + 2\ H^{+} \rightarrow HRP + H_2O \quad (4)$$

As pointed out by Gorton et co-workers [31–33], this process is usually referred to a direct electron transfer (DET), i. e., when an electrode substitutes the electron donor substrates in a common peroxidase reaction cycle

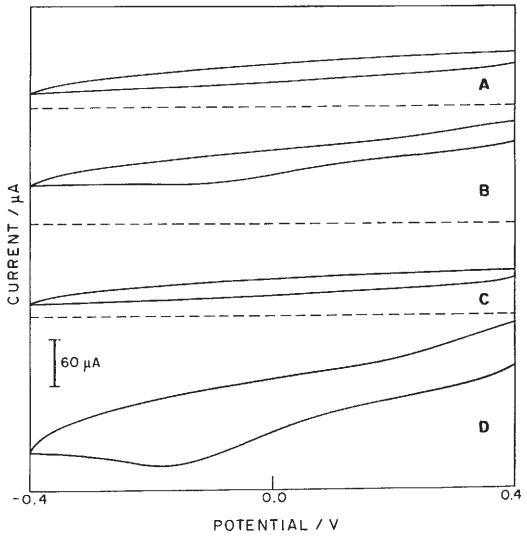

Fig. 1 Cyclic voltammographs obtained with StCPE electrode (1A and 1B) and StCPET (1C and 1D) in an unstirred 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.1 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v) without (1A and 1C) and with 7.3×10^{-4} mol L^{-1} hydroquinone solution (1B and 1D). Scan rate of 100 mV s^{-1} and Ecp of –180 mV, at 25 °C

(1)–(3). When an electron donor (S), such as hydroquinone, is present in a peroxidase-electrode system, both processes can occur simultaneously and the oxidized donor $S^{\bullet}$ is reduced eletrochemically by the electrode as shown in reaction (5):

$$S^{\bullet} + e^{-} + H^{+} \rightarrow S \quad (5)$$

The reaction sequence [33] (reactions (1), (2), (3) and (5)) is known as mediated electron transfer (ET), which is usually more efficient compared to DET.

In this study, cyclic voltammetric measurements were performed by scanning the potential between +400 and –400 mV vs Ag/AgCl at a scan rate of 100 mV s^{-1} using both electrodes StCPE and StCPET, as working electrodes.

Figure 1 shows cyclic voltammograms obtained with StCPE electrode (1 A and 1 B) and StCPET (1 C and 1 D) in an unstirred 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.1 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v) without (1 A and 1 C) and with 7.3×10^{-4} mol L^{-1} hydroquinone solution (1 B and 1 D). As can be seen, the StCPE (1 B) presented a cathodic current at a peak potential of *ca.* –124 mV. When the StCPET was used, there is an increase of electrode response as predicted by the above catalytic cycle at a peak potential of –180 mV.

Effect StCPET composition. The effect of the stearic acid varying from 13 to 33% (w/w) and graphite powder from 72 to 52% (w/w) at a fixed amount of sweet potato tissue of 15% (w/w) on the StCPET (biosensor) response for 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.1 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v) was initially investigated. The best stearic acid-graphite composition (*i.e.* electrode showed the best response (best S/N)) was found to be 28% (w/w) stearic acid with 57% (w/w) graphite powder. Therefore, this composition were then selected. An additional study was then conducted aiming to select the best sweet potato tissue (powder) amount. So, the effect of tissue amount from 5 to 20% (w/w), at a fixed graphite powder composition of 57% (w/w), on the StCPET response was studied. Biosensors containing 15% (w/w) of the tissue powder showed the best response (best signal/noise response). Thus, this composition was used in the construction of the StCPET.

In addition, biosensors constructed with a mixture of stearic acid + paraffin in the ratio of 28:0; 26:2, 24:4, 21:7 and 14:14% (w/w), respectively, at 57% (w/w) graphite powder and 15% (w/w) tissue powder amounts were also investigated. The biosensor response decreases (42.0; 32.6; 20.2; 12.7 and 12.3 μA, respectively) as the paraffin amount increases from 0 to 14% (w/w) in the composite, confirming the good performance of the StCPET obtained with stearic acid used as binding agent.

Effect of type and concentration of supporting electrolyte. The effect of supporting electrolytes generally used for the organic phase enzyme electrode such as TEAB, TMAB, TBAB, TBPAP and TBATFB at a fixed concentration each one of 0.05 mol L^{-1} for 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide solution in methanol-phosphate buffer solution (95:5% v/v) on the biosensor response was exploited. The StCPET response (cathodic peak current in μA) increased in the following order: TMAB (8.6), TEAB (25.7), TBPAP (34.8), TBATFB (37.2) and TBAB (39.8). Consequently, in all further studies TBAB was used as supporting electrolyte.

The effect of TBAB concentration varying from 0.025 to 0.30 mol L^{-1} in methanol-phosphate buffer solution (95:5% v/v) on the biosensor response was also investigated. The cathodic peak current signal increased with the increase of the electrolyte concentration up to 0.10 mol L^{-1} TBAB, then levels off in higher concentrations, indicating that in solutions with 0.10 mol L^{-1} concentration the migration of ions reached a constant value. Therefore, a concentration of 0.1 mol L^{-1} was selected.

Effect of solvents and physical properties. Klibanov [20] has shown that enzymes may be more stable in organic solvent than in water and that horseradish peroxidase from 10 to 100% is as active in optimal organic media as is in water. Hence, organic solvents can induce extensive changes in enzyme activity and specificity. This is because the enzyme structure and reactivity depends on several non-covalent interactions in the biocatalyst, such as hydrogen bonding, ionic, van der Waals and hydrophobic interactions [17, 18]. In the study of the effect of solvents on the biosensor response several solvent parameters have been considered like log P, solubility parameter (Hildebrand parameter), dielectric constant (ε), dipole moment (μ), hydrogen bonding (γ), and polarizability (α). Unfortunately, insufficient data of γ and α were available in the

Table 1 Response of the StCPET for hydroquinone in different organic solvents

log P values are as given by Laane *et al.* [30]
Solubility parameter values are as given by Morrison and Freiser [35]
Dielectric constant and dipole moment values are as given by Marcus and Kertes [36]
[a] These organic solvents dissolve solid stearic acid degrading the electrode carbon composite surface

Solvents	Current (μA)	Relative response (%)	log P	Solubility parameter	Dielectric constant	Dipole moment
Methanol	42.0	100	–0.76	14.5	32.6	1.67
Acetone	39.7	94.5	–0.23	10.0	20.7	2.8
Ethanol	26.7	63.6	–0.24	12.7	24.3	1.7
Acetonitrile	23.4	55.7	–0.33	–	36.2	3.4
1-Propanol	23.0	54.8	0.28	11.9	22.2	1.66
2-Propanol	21.8	51.9	–	11.5	13.8	1.65
1-Butanol	10.5	25.0	0.80	11.4	16.1	1.68
Tetrahydrofuran[a]	–	–	0.49	–	6.4	1.71
Chloroform[a]	–	–	2.00	9.3	4.8	1.20

literature to justify a comparison between biosensor response and these polarity parameters.

The parameter P (the partition coefficient of the solvent between octanol and water) is widely accepted as a quantitative measure of solvent polarity [23, 30]. The solubility parameter of Hildebrand is fundamentally based on the solution theory and describes the intrinsic thermodynamic state properties of a solvent and denotes a measure of the overall cohesive energy density of a solution [34, 35]. The dielectric constant is a measure of the relative permittivity, and governs the ability of solvents to weaken the electrostatic forces around the charged and polar active site of the enzyme [18, 36]. Finally, the dipole moment expresses the electrical polarity of a molecule. The dipole moment is a vector quantity since it has a definite direction as well as a magnitude [36].

The effect of different organic solvents containing 1% (v/v) water (buffer solution), such as acetone, acetonitrile, 1-butanol, chloroform, ethanol, methanol, 1-propanol, 2-propanol and tetrahydrofuran on the StCPET performance was studied. Table 1 presents the StCPET responses for 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide solution in 0.10 mol L^{-1} TBAB as a function of solvent parameters such as log P values, solubility parameter, dielectric constant and dipole moment. As can be observed from this table, there is a trend that the StCPET response decreases with the increase of the log P, indicating that the electrode response is directly proportional to the solvent polarity. This behavior is not expected, once polar solvents generally affect enzymatic activity by interacting directly with the essential water around the enzyme molecule. Although this interaction may not affect the enzyme itself, this electrode was active in both hydrophilic and hydrophobic organic solvents. One possible explanation for this is that peroxidase onto the stearic acid/graphite composite is capable of retaining its hydration shell very tightly even in polar organic solvents [23]. With increasing of the solubility parameter (Hildebrand parameter) there is a tendency of increasing response of the StCPET. The same tendency was observed for the dielectric constant. Regarding the dipole moment the response profile is not well defined.

A comparison of the StCPET used with the response of a paraffin/graphite electrode [37] in each of the solvents studied shows that the analytical signals of StCPET were much higher than those obtained with the paraffin/graphite electrode applying the same electrode compositions. For 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide in 0.10 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v), the response of paraffin/graphite electrode was 7.2 μA while StCPET was 42.0 μA. A possible explanation for this could be the strong hydrogen bonding between the carboxylate anion of stearic acid with the hydroxyl groups of hydroquinone that can approximate the analyte and the electrode surface with more efficacy than that of the biosensor based on paraffin. Another possibility is the distinct interaction between the active site of enzyme and stearic acid or paraffin in both electrodes, leading to a discriminated response for each of these biosensor.

Effect of water and hydrogen peroxide concentration. A minimum amount of water in the organic solvent is usually required for the catalytic enzyme activity. This is because water participates, directly or indirectly, in all noncovalent bonding and to the hydrogen bonds of the protein structure. Nevertheless, the appropriate amount of water necessary for enzyme activity in organic solvent has been discussed in several papers [17–22].

The amount of water required for the biosensor to respond in such media depends on solvent and enzyme. Thus, the effect of methanol or acetone in 0.1 mol L^{-1} TBAB phosphate buffer solution (pH 7.0) ranging from 0 to 50% (v/v) on the StCPET response for 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide solution is shown in Fig. 2. The addition of water in both solvents increased significantly the StCPET response up to 5% (v/v) water (buffer solution). For the methanol-water system the electrode response was constant for water contents higher than 5% (v/v) and in the case of the acetone-water system, the electrode response decreases about 35% for water contents from 5 to 50% (v/v). In this work, a composition of 95:5% (v/v) methanol-buffer solution was selected.

The effect of hydrogen peroxide concentration from 5.0×10^{-4} to 1.0×10^{-2} mol L^{-1} on the StCPET response was also exploited. The optimum hydrogen peroxide concentration found was 1.0×10^{-3} mol L^{-1}. This concentra-

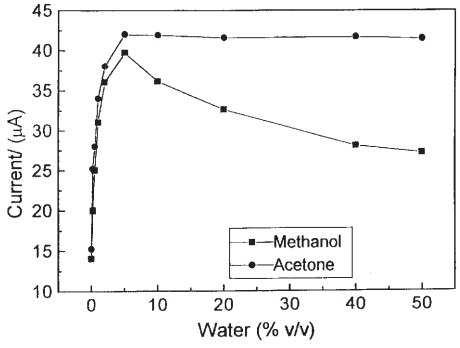

Fig. 2 Effect of methanol or acetone in 0.1 mol L^{-1} TBAB phosphate buffer solution (pH 7.0) ranging from 0 to 50% (v/v) on the StCPET response for 7.3×10^{-4} mol L^{-1} hydroquinone and 1.0×10^{-3} mol L^{-1} hydrogen peroxide solution

Table 3 Determination of hydroquinone in cosmetic creams using the Pharmacopeia and biosensor

Sample	Hydroquinone (mg g^{-1})		Relative error (%)
	Pharmacopoeia	StCPET	
Clariderm			
A	23.2 ± 0.1	23.0 ± 0.2	– 0.9
B	22.9 ± 0.1	22.5 ± 0.1	–1.7
C	22.6 ± 0.1	23.1 ± 0.1	+2.2
Claripel			
D	41.5 ± 0.1	41.9 ± 0.1	+1.0
E	40.8 ± 0.1	40.2 ± 0.1	–1.5
F	42.1 ± 0.2	41.3 ± 0.1	–1.9

n = 6, confidence level, 95%

Table 2 Optimization of biosensor (StCPET) parameters

Biosensor parameter	Range studied	Optimal value
Stearic acid	13 –33 (% w/w)	28 (% w/w)
Graphite powder	52 –75 (% w/w)	57 (% w/w)
Tissue composition	5.0–20 (% w/w)	15 (% w/w)
Electrolyte	TEAB, TMAB, TBAB, TBAP and TBATF	TBAB
TBAB	0.025–0.2 mol L^{-1}	0.1 mol L^{-1}
Solvent	Acetone, acetonitrile, 1-butanol, chloroform, ethanol, methanol, 1-propanol, 2-propanol, tetrahydrofuran	Methanol
Water	0.0–50.0 (% v/v)	5.0 (% v/v)
Hydrogen peroxide	5.0×10^{-4}–1.2×10^{-2} mol L^{-1}	1.0×10^{-3} mol L^{-1}
Scan rate	20–200 mV s^{-1}	100 mV s^{-1}

tion was then selected. Table 2 summarizes the range over which each variable was investigated and the optimal value found.

Study of interference, repeatability, reproducibility and lifetime. The effect of excipient substances frequently found with hydroquinone in cosmetic creams, such as citric acid, magnesium stearate, methylparaben, octylmethoxycinnamate, poly(ethylene glycol), propylparaben and starch were evaluated using the proposed StCPET. The ratios of the concentration of hydroquinone to those of the excipient substances were fixed at 0.1, 1.0 and 10.0. None of these substances interfered with the electrode response.

The relative standard deviation was <1.0% for solution containing 7.3×10^{-4} mol L^{-1} hydroquinone and 1×10^{-3} mol L^{-1} hydrogen peroxide solution (n = 10) in 0.10 mol L^{-1} TBAB methanol-phosphate buffer solution (95:5% v/v). The reproducibility of five biosensors shows only a slight variation of *ca.* 3.2% of the analytical curve slope.

During 7 months of intermittent use, the sensitivity of the electrode decreased to 50% of its initial value and at least 900 assays could be performed with a single biosensor, confirming the high stability of StCPET. The only two exception were observed in chloroform and tetrahydrofuran, because these organic solvents dissolve solid stearic acid degrading the surface of the carbon composite electrode.

Analytical characteristics and applications. Under the optimum conditions, *i.e.*, stearic acid/graphite/tissue sweet potato, 57:28:15% (w/w) 0.1 mol L^{-1} TBAB in methanol-phosphate buffer solution (95:5% v/v) and reference hydroquinone solution concentration from 6.2×10^{-5} to 2.7×10^{-3} mol L^{-1}, the analytical curve was established. Hydroquinone in cosmetic creams was determined amperometrically at a potential of –180 mV.

The analytical curve was rectilinear in the hydroquinone concentration range from 6.2×10^{-5} to 1.5×10^{-3} mol L^{-1} ($I_{cp} = 0.81 + 5.47 \times 10^4$ [H_2A]; r = 0.9990), where I_{cp} is the cathodic peak current in μA and [H_2A] is the hydroquinone concentration in mol L^{-1} with a detection limit (three times the signal blank/slope) of 8.5×10^{-6} mol L^{-1}.

Recoveries varying from 98.8 to 104.1% of hydroquinone from two commercial products (Claripel and Clariderm) were obtained using the StCPET. In this study, 27.0; 54.0; 80.4 and 105.7 mg L^{-1} of hydroquinone solutions were added to each sample and the cathodic current peak was obtained. The recovery results obtained did not show any matrix effect.

The procedure proposed was validated by applying it to the determination of hydroquinone in cosmetic creams. Table 3 shows the results obtained for six samples using a Pharmacopoeia [26] method in comparison to those determined by the proposed StCPET. Applying a paired-t test in the results obtained, it was found that all results are in agreement at the 95% confidence level and within an acceptable range of error.

Conclusions

Stearic acid/graphite or paraffin/graphite biosensors are a very good alternative to other carbon based matrices, because of their excellent characteristics such as compatibility with non-aqueous solvents and long lifetime. Additional advantages are renewability, simplicity, rapidity to prepare and low cost. The determination of hydroquinone in cosmetic creams directly in methanol without time-consuming sample treatment provides results comparable to the Pharmacopoeia method.

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References

1. Adams RN (1958) Anal Chem 30:1576
2. Kalcher K, Kauffmann JM, Wang J, Svancara I, Vytras K, Neuhold C, Yang Z (1995) Electroanalysis 7:5
3. Gorton L (1995) Electroanalysis 7:23
4. Chin Q, Göpel W, Ruzgas T, Gorton L, Heiduschka P (1997) Electroanalysis 9:357
5. Rice ME, Galus Z, Adams RN (1983) J Electroanal Chem 143:89
6. Cheek GT, Nelson RF (1978) Anal Lett 11:393
7. Yao T, Musha S (1979) Anal Chim Acta 110:203
8. Caruso CS, Vieira IC, Fatibello-Filho O (1999) Anal Lett 32:39
9. Vieira IC (1997) Development and application of enzymatic methods for the determination of phenol, sulfite and catecholamines using crude extract of sweet potato (*Ipomoea batatas (L.) Lam*.), Ph. D Thesis, PPGQ. Federal University of São Carlos/SP-Brazil, p 223
10. Oungpipat W, Alexander PW, Southwell-Keely P (1995) Anal Chim Acta 309:35
11. Hall GF, Best DJ, Turner APF (1988) Enzyme Microb Technol 10:543
12. Hall GF, Best DJ, Turner APF (1988) Anal Chim Acta 213:113
13. Wang J, Reviejo AJ, Mannino S (1992) Anal Lett 25:1399
14. Mannino S, Cosio MS, Wang J (1994) Anal Lett 27:299
15. Wang J, Lin Y, Chen L (1993) Analyst 118:277
16. Dong S, Guo Y (1994) Anal Chem 66:3895
17. Saini S, Hall GF, Downs ME, Turner APF (1991) Anal Chim Acta 249:1
18. Iwuoha EI, Smyth MR, Lyons, MEG (1997) Biosensors & Bioelectronics 12:53
19. Iwuoha EI, Smyth MR (1996) Anal Communications 33:23H
20. Klibanov AM (1986) Chemtech, june:354
21. Kazandjian RZ, Dordick JS, Klibanov AM (1986) Biosensors & Bioelectonics, 28:417
22. Klibanov AM (1997) TIBTECH 15:97
23. Díaz-Garcia MG, Valencia-González MJ (1995) Talanta 42: 1763
24. Blaha CD, Lane RF (1983) Brain Res Bull 10:861
25. Petit C, Gonzalez-Cortes A, Kauffmann JM (1996) Bioelectrochem and Bioenerg 41:101
26. United States Pharmacopeia (1995) National Formulary XXXIII US Pharmacopeial Convention, Rockville, MD, p 769
27. Campa A, Biological Roles of Plant Peroxidases: Known and Potential Function, In: Everse K, Grisham MB (eds), Peroxidase on Chemistry and Biology. Boston, CRC Press, vol 2, 1991, p 25
28. Ruzgas T, Csöregi E, Emnéus J, Gorton L, Marko-Varga G (1996) Anal Chim Acta 330:123
29. Whitaker JR, Principles of Enzymology for the Food Sciences (1972) Marcel Dekker Inc., New York, p 596
30. Lanne C, Boeren S, Vos K, Veeger C (1987) Biotechnol and Bioengin 30:81
31. Lindgren A, Emnéus J, Ruzgas T, Gorton L, Marko-Varga G (1997) Anal Chim Acta 347:51
32. Marko-Varga G, Emnéus J, Ruzgas T, Gorton L (1995) Trends Anal Chem 14:319
33. Gorton L, Lindgren A, Larsson T, Munteanu F D, Ruzgas T, Gazaryan I (1999) 400:91
34. Schneider LV (1991) Biotechnol and Bioengin 37:627
35. Morrison GH, Freiser H, Solvent Extraction in Analytical Chemistry (1957) John Wiley & Sons, New York, p 250
36. Marcus Y, Kertes AS, Ion Exchange and Solvent Extraction of Metal Complexes (1969) John Wiley & Sons, New York, p 927
37. Vieira IC, Fatibello-Filho O (2000) Talanta (in press)