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SENSORS

Use of a Melanin-Type Polymer to Improve the Selectivity of Glucose Biosensors

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ABSTRACT

This paper reports the advantages of using a melanin-type polymer to improve the selectivity of glucose biosensors based on carbon paste electrodes containing Pt and glucose oxidase (GO_x). The resulting bioelectrodes combine the high sensitivity of metallized electrodes with the selectivity given by the polymeric layer. They exhibit excellent performance for glucose with a rapid response (around 10 sec per sample), a wide linear range (up to 3.0×10^{-2} M), low detection limits (60 μM) and a highly reproducible response (R.S.D. of 4.9%). The bioelectrodes are stable with prolonged use and almost free from the interference of easily oxidizable compounds found in

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biological fluids, such as ascorbic acid (AA), uric acid (UA) and acetaminophen.

Key Words: Glucose biosensor; Glucose oxidase; Metallized electrode; Melanic polymer; Carbon paste; Platinum.

INTRODUCTION

One of the hardest challenges facing amperometric glucose biosensors is the development of biorecognition layers that allow the selective quantification of glucose. Glucose oxidase (GO_x) has been the most widely used biorecognition element in the preparation of glucose biosensors.[1,2] This enzyme catalyzes the oxidation of glucose to gluconolactone in the presence of oxygen, which, in turn, is converted to hydrogen peroxide during the enzymatic regeneration step.

Glucose amperometric biosensors are, in general, based on the detection of hydrogen peroxide obtained from the enzymatic reaction. Since high overpotentials are required for its oxidation, the transduction process suffers the interference of easily oxidizable compounds present in blood such as the endogenous ascorbic acid (AA) and uric acid (UA) as well as the exogenous acetaminophen (acet).[1,2] One of the strategies proposed to overcome this problem is the incorporation of metals into the biorecognition layer.[1,3,4] Rh,[5] Ru,[6] Ir,[7] Au,[8] Cu[3] or mixture of metals[9,10] incorporated in different carbon electrodes, have been successfully used as preferential catalysts of the redox behavior of hydrogen peroxide. However, in the case of platinum[11] and palladium,[12] although effectively catalyze the electrooxidation of hydrogen peroxide, they present the inconvenience of catalyzing the oxidation of AA and UA.

Another strategy frequently and successfully used to achieve the selective determination of hydrogen peroxide is the electropolymerization which can be used not only to reject usual interferents but also to immobilize GO_x at electrodes.[13–16] The electrodeposition of polymeric layers over electrodes allows the precise control of the charge passed during the polymer deposition and consequently, the reproducible deposition of the polymeric layer. In the literature there exist several examples of the electropolymerization of different monomers as a way to entrap enzymes.[13–16]

This paper describes the advantages of using a melanin-type polymer to effectively reject usual interferents in the glucose determination, as a way to improve the selectivity of the glucose determination. The melanin-type polymer obtained by electropolymerization of L-dopa, has been

demonstrated to possess excellent barrier properties for negatively charged compounds such as dopac, ascorbic acid and potassium ferrycyanide.[17,18] Thus, as it will be demonstrated in the following sections, the combination of the excellent catalytic properties of platinum towards hydrogen peroxide oxidation and of the permselective properties of a melanin-type polymer has allowed us to obtain a highly sensitive and selective glucose biosensor.

EXPERIMENTAL

Reagents

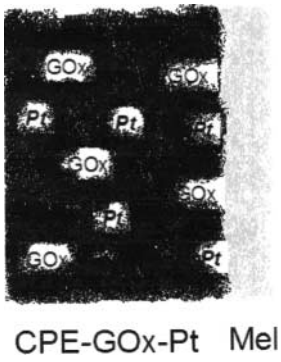
Hydrogen peroxide (30% V/V aqueous solution) was purchased from Carlo Erba. Uric acid and glucose were from Merck while ascorbic acid was from Fluka. L-dopa, glucose oxidase ((GO_x) (Type X-S, Aspergillus niger, (EC 1.1.3.4), 210,000 Units per gram of solid, Catalog number G-7141), and acetaminophen were from SIGMA. Other chemicals were reagent grade and used without further purification.

Ultrapure water ($\rho = 18\,M\Omega$) from a Millipore-MilliQ system was used for preparing all the solutions. A 0.050 M phosphate buffer solution pH 7.40 was used as supporting electrolyte.

Apparatus

Some amperometric experiments were performed with a BAS CV-37 potentiostat connected to a Linseis LY 1800 recorder while others were performed by using an Epsilon potentiostat (BAS).

The electrodes were inserted into the cell (BAS, Model VC-2) through its Teflon cover. A Ag/AgCl, 3M NaCl (BAS, Model RE-5B) and a platinum wire were used as reference and counter electrode, respectively. All the potentials are referred to that reference. Enzymatic metallized electrodes (CPE-Pt-GO_x) were prepared in the following way: the desired amount of enzyme (usually 15.0 mg/g) was mixed with mineral oil (Aldrich) in an agata mortar for 3 min followed by the incorporation of graphite powder containing 5.0 and 10.0% w/w platinum on Vulcan XC-72 (E-TEK, Inc) and mixing for 15 additional minutes. Carbon paste electrodes (CPE) containing GO_x (CPE-GO_x) were prepared in a similar way by using graphite powder (Fisher # 38) instead of the metallized carbon. The melanin-type polymer was obtained by applying 1.00 V for 120 min using a stirred air saturated 0.050 M phosphate buffer solution



Scheme 1. Schematic representation of the carbon paste electrode containing platinum microparticles and GO_x dispersed and covered by a melanin-type polymer, (CPE-Pt-GO_x-mel).

pH 7.40 containing 3.0×10^{-3} M L-dopa (Sch. 1). Once the polymer was obtained on the surface of the enzymatic electrode, it was washed with water and cycled in supporting electrolyte between −0.40 V and 0.80 V at 0.100 V/s (1 cycle). Enzymatic electrodes containing the melanin-type polymer (mel) were defined as CPE-GO_x-mel or CPE-Pt-GO_x-mel.

A portion of the resulting paste was packed firmly into the cavity (3 mm diameter) of a Teflon tube. The electric contact was established through a stainless steel screw. A new surface was obtained by smoothing the electrode onto a weighing paper.

A magnetic stirrer and a stirring bar were used for the convective transport when necessary. All the experiments were performed at room temperature. The amperometric ones were carried out by applying the desired potential and allowing the transient current to decay prior to the current monitoring.

RESULTS AND DISCUSSION

Figure 1 shows the effect of AA and UA on the amperometric response at 0.700 V of the enzymatically generated hydrogen peroxide obtained after the addition of glucose (glu) to a supporting electrolyte solution using different bioelectrodes, CPE-GO_x (15.0% w/w) (A), CPE-Pt (5.0% w/w)-GO_x (15.0% w/w) (B) and CPE-Pt (5.0% w/w)-GO_x (15.0% w/w)-mel (C). Since the electro-oxidation of hydrogen peroxide at carbon electrodes requires high overpotentials,[1] the amperometric

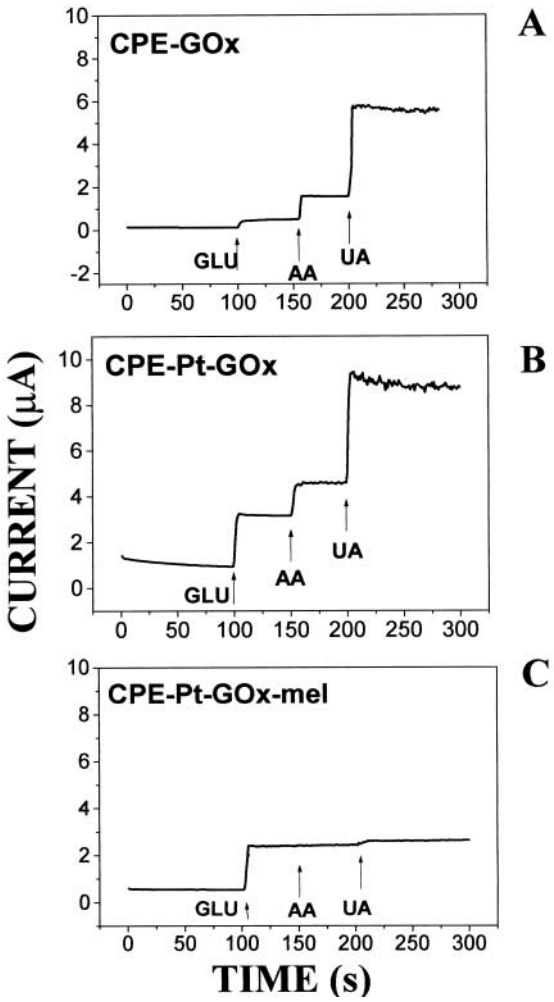


Figure 1. Current-time profiles at CPE-GO_x (15.0% w/w) (A), CPE-Pt (5.0% w/w)-GO_x (15.0% w/w) (B) and CPE-Pt (5.0% w/w)-GO_x (15.0% w/w)-mel (C) for an addition of 5.0×10^{-3} M glucose (GLU) followed by additions of 1.0×10^{-4} M ascorbic acid (AA) and 2.5×10^{-4} M uric acid (UA). Operating potential: 0.700 V. In the case of the electrode covered by the melanic polymer, it was obtained by applying 1.00 V for 120 min using a stirred air saturated 0.050 M phosphate buffer solution pH 7.40 containing 3.0×10^{-3} M L-dopa.

response at CPE-GO_x after the addition of 5.0×10^{-3} M glucose is very poor. When the addition of glucose is followed by the addition of 1.0×10^{-4} M AA and 2.5×10^{-4} M UA, a large interference (260 and 1050% for AA and UA, respectively) arises due to the easy oxidation of these compounds at CPE (Fig. 1A). A similar experiment was performed at CPE-Pt-GO_x (Fig. 1B) and, as expected,[11] due to the known electrocatalytic effect of the metal on the redox behavior of hydrogen peroxide, the incorporation of platinum to CPE-GO_x allows an important enhancement (almost 6 times) of the electro-oxidation current for the hydrogen peroxide enzymatically generated after the addition of 5.0×10^{-3} M glucose. Therefore, the interference of AA and UA is much smaller than that obtained at CPE-GO_x, it being 61 and 185% for AA and UA, respectively. Thus, the large improvement on the amperometric response of hydrogen peroxide after the incorporation of platinum in the biorecognition layer allows us to dramatically decrease the interference of AA and UA, although these interference values are still very large and not compatible with practical applications.

Figure 1C displays the effect of the melanin-type polymer covering the CPE-Pt-GO_x on the interference of AA and UA. Since AA and UA are negatively charged at the working pH (pK 4.1 and 5.4 for AA and UA, respectively), they are rejected by the melanic polymer.[17,18] Consequently, at CPE-Pt-GO_x-mel, the oxidation currents for AA and UA largely decrease, allowing the interference percentage goes down to 1.0 and 10.0% for AA and UA, respectively.

Similar experiments were performed with acetaminophen, another usual interferent in the glucose amperometric determination. The interference percentage for 4.0×10^{-5} M acetaminophen obtained at CPE-Pt-GO_x was 41% decreasing to 9.0% after covering the electrode with the melanic polymer.

The influence of different experimental parameters on the performance of the polymer-metallized enzymatic electrode, such as the electropolymerization conditions and platinum and GO_x content was evaluated. The effect of the polymeric layer on the response of the bioelectrode towards glucose and usual interferents is depicted in Fig. 2. Figure 2A shows calibration plots obtained from amperometric experiments at 0.700 V for successive additions of 5.0×10^{-3} M glucose at different electrodes: CPE-Pt(5.0% w/w)-GO_x(15.0% w/w) (a) and CPE-Pt(5.0% w/w)-GO_x(15.0% w/w) covered by melanic polymers obtained after different electropolymerization times (b–e). L-dopa was electropolymerized on the CPEs from a 3.0×10^{-3} M L-dopa solution by applying 1.0 V for 60 (b), 90 (c), 120 (d) and 150 (e) min. As it can be seen and in agreement with Fig. 1C, the hydrogen peroxide oxidation signal decreases when the electrodes are

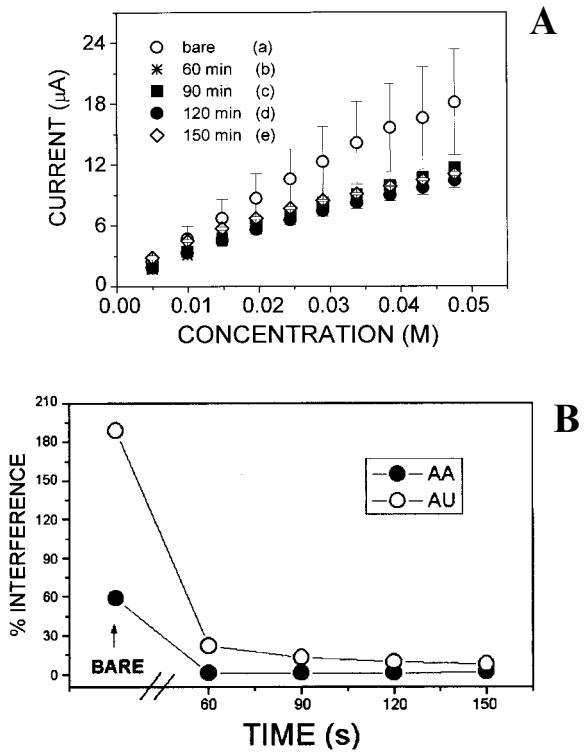


Figure 2. (A) Calibration plots obtained from the amperometric response to glucose using CPE-Pt (5.0% w/w)-GO_x (15.0% w/w) (a) and CPE-Pt (5.0% w/w)-GO_x (15.0% w/w) covered by the melanin-type polymer grown at different times: 60 (b), 90 (c), 120 (d) and 150 (e) min. (B) Percentage of interference for 1.0×10^{-4} M AA and 2.5×10^{-4} M UA in the response of the bioelectrode towards 5.0×10^{-3} M glucose (taken as ($i_{interferent}/i_{glucose} \times 100$)) at CPE-Pt (5.0% w/w)-$GO_x$ (15.0% w/w) covered by a melanin-type polymer grown at different times. Other conditions as in Fig. 1C.

covered by the polymer due to the effect of the polymer on the diffusion of the substrate towards the electrode (compare a vs b–e). Another interesting fact is that even when the sensitivities decrease for electrodes covered by the polymer, they remain almost constant independently of the polymerization time (b–e). This indicates that the thickness of the polymeric layer would not change drastically with the polymerization time. The sensitivities are $(417 \pm 7)\,\mu A\,M^{-1}$, $r = 0.9993$ for CPE-Pt-GO_x and $(248 \pm 7)\,\mu A\,M^{-1}$, $r = 0.998$; $(262 \pm 9)\,\mu A\,M^{-1}$, $r = 0.998$; $(2.5 \pm 0.1) \times$

$10^2\,\mu A\,M^{-1}$, $r = 0.997$ and $(2.5 \pm 0.2) \times 10^2\,\mu A\,M^{-1}$, $r = 0.993$ for CPE-Pt-GO_x covered by a polymer obtained after applying 1.0 V for 60, 90, 120 and 150 min, respectively.

Figure 2B shows the effect of polymeric layers grown at different times on the interference produced by 1.0×10^{-4} M AA and 2.5×10^{-4} M UA in the response of the biosensor to glucose. The barrier properties of the polymer are clear. At CPE-Pt-GO_x the interference percentages of AA and UA are 59 and 198%, respectively (taken as $((i_{interferent}/i_{glucose}) \times 100)$ for 5.0×10^{-3} M glucose and the given concentration of AA or UA), while for CPE-Pt-GO_x covered by the melanic polymer, this interference percentage notoriously decreases. When the polymer is grown for 60 min this percentage sharply decreases down to 1.0 and 22.0% for AA and UA, respectively while it decreases more slowly for electrodes covered by polymeric layers obtained for longer polymerization times. The best compromise between lower interference and higher sensitivity was obtained for a polymerization time of 120 min (interference percentages of 1.0% and 10.0% for AA and UA, respectively). Bioelectrodes covered by polymers obtained after longer electropolymerization times gave a slower and noisier response, without significant improvements in sensitivity and selectivity. Thus, a polymerization time of 120 min was selected for the subsequent work.

The effect of platinum on the amperometric response of the CPE-GO_x-mel at 0.700 V towards glucose, AA and UA is shown in Table 1. In all cases L-dopa was electropolymerized from a 3.0×10^{-3} M L-dopa solution by applying 1.0 V for 120 min. As expected from Fig. 1, a poor response for the enzymatically generated hydrogen peroxide after the addition of glucose is obtained at the electrode without platinum

Table 1. Oxidation currents for 5.0×10^{-3} M glucose and interference percentages for 1.0×10^{-4} M ascorbic acid (AA) and 2.5×10^{-4} M uric acid (UA) obtained from amperometric experiments at 0.700 V at CPE-GO_x containing different % w/w platinum and covered by the melanin-type polymer. Other conditions as in Fig. 1C.

% Platinum (w/w)	Oxidation current (μA)	% Interference	
	Glucose	AA	UA
0.0	0.13 ± 0.01	64.0	287.0
2.5	1.19 ± 0.02	8.0	21.0
5.0	1.8 ± 0.3	1.0	10.0

(CPE-GO_x(15.0% w/w)-mel). Consequently, in the absence of platinum, even when the electrode is covered with the melanic polymer, the interference of AA and UA at 0.700 V is very large. When 2.5% w/w platinum is incorporated into the bioelectrode covered by the melanin-type polymer, since platinum catalyzes the oxidation of hydrogen peroxide, there is a dramatic diminution in the interference percentage, which decreases even more when 5.0% w/w platinum is incorporated into the biorecognition layer. Thus, a 5.0% w/w platinum was selected for the subsequent work as the smaller interference occurs under these conditions.

We also evaluated the amount of GO_x in the electrode (not shown). The optimum amount was 15.0% w/w GO_x since under these conditions it was possible to obtain the best compromise between sensitivity and interference percentage.

Figure 3A shows a typical amperometric recording at 0.700 V obtained at CPE-Pt(5.0% w/w)-GO_x(15.0% w/w)-mel after successive additions of 5.0×10^{-3} M glucose (up to 8.5×10^{-2} M glucose). The response of the bioelectrode is very fast. The steady-state currents for the oxidation of the enzymatically generated hydrogen peroxide were obtained (6 ± 1) and (11 ± 2) seconds after the addition of 5.0×10^{-3} and 2.0×10^{-2} M glucose, respectively.

As Fig. 3B demonstrates, the enzymatic electrode presents a linear range up to 3.0×10^{-2} M (5.40 g/L), with a sensitivity of $(2.5 \pm 0.1) \times 10^2\ \mu A M^{-1}$, a correlation coefficient of 0.996 and a detection limit of 60 μM. This wide linear range makes the proposed electrode very promising for practical applications because one of the usual problems underwent by glucose biosensors is the oxygen dependence at glucose levels higher than 1.0×10^{-2} M. In this case the response is linear even for such a high glucose concentration value of 3.0×10^{-2} M without using any additional reagent. Figure 3C shows the corresponding Eadie-Hofstee plot (stationary current (I_{ss}) versus the ratio between I_{ss} and glucose concentration (I_{ss}/C). Two regions can be distinguished, one with negative deviations due to diffusional problems for glucose concentrations smaller than 2.5×10^{-2} M and a linear one at higher glucose concentrations. From this linear portion, the K_m^{app} and I_{max} were obtained, being $(3.8 \pm 0.1) \times 10^{-2}$ M and (17.2 ± 0.3) μA, respectively.

The reproducibility intra-electrodes, taken as the R.S.D. of the sensitivities of ten calibration plots, was 4.4%. Sixteen calibration plots obtained using seven electrodes prepared with two different enzymatic pastes gave a reproducibility of 3.9%.

The stability of enzyme-based sensors has been another important challenge in the development of devices that can be commercialized.

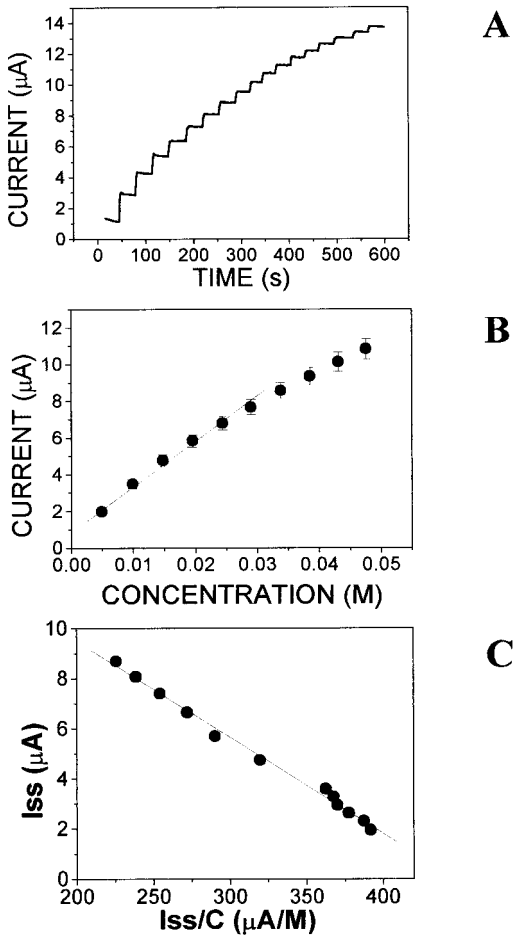


Figure 3. (A) Amperometric response for CPE-Pt (5.0% w/w)-GO_x (15.0% w/w)-mel to successive additions of 5.0×10^{-3} M glucose at 0.700 V. (B) Average calibration plot obtained from ten amperometric recordings performed with the same electrode. (C) Eadie-Hofstee plot obtained from the results shown in (A). Other conditions as in Fig. 1C.

The long-term stability of the biosensor was assessed with regard to storage dry at 4°C in connection with the response to glucose, AA and UA. After 24 h the signal for 5.0×10^{-3} M glucose increases as a consequence of an enzyme rearrangement into the composite matrix, to remain constant up to 6 days. Concerning the response for AA and UA

after six days, it increases 6.0% for 1.0×10^{-4} M AA and 6.0% for 1.0×10^{-4} M UA.

CONCLUSIONS

In conclusion, the excellent permselective properties of the melanin-type polymer incorporated at CPE containing GOX and Pt, has allowed us to obtain a dramatic improvement in the selectivity of the glucose biosensor. The combination of the advantages of this polymeric layer with the excellent catalytic properties of Pt towards hydrogen peroxide electro-oxidation, results in a highly selective and sensitive glucose biosensor.

The success of the use of this new polymer as a barrier to reject interferents provides a simple method to prepare enzymatic electrodes in a very reproducible way, turning it into a promising method for the fabrication of highly sensitive, selective and stable glucose biosensors. The methodology is also well suited for miniaturization since electro-polymerization can also be done on screen printed electrodes containing Pt and GO_x.

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