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Glucose biosensors based on the immobilization of copper oxide and glucose oxidase within a carbon paste matrix

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Abstract

The performance of amperometric glucose biosensors based on the dispersion of glucose oxidase (GOx) and copper oxide within a classical carbon (graphite) paste composite is reported in this work. Copper oxide promotes an excellent electrocatalytic activity towards the oxidation and reduction of hydrogen peroxide, allowing a large decrease in the oxidation and reduction overpotentials, as well as an important enhancement of the corresponding currents. Therefore, it is possible to perform the glucose biosensing at low potentials where there is no interference even in large excess of ascorbic acid, uric acid or acetaminophen. The influence of the copper oxide and glucose oxidase content in the paste on the analytical performance of the bioelectrode is discussed. The resulting biosensor shows a fast response, a linear relationship between current and glucose concentration up to 1.35×10^{-2} M ($2.43\,g\,L^{-1}$) and a detection limit of 2.0×10^{-5} M. The effect of the presence of the enzyme in the composite material on the dispersion of the copper oxide particles is also discussed.

Keywords: Glucose biosensor; Glucose oxidase; Copper oxide; Catalysis; Carbon paste; Composite

1. Introduction

Amperometric glucose biosensors based on the use of glucose oxidase (GOx) have received considerable interest [1,2]. Electrochemical biosensors are widely used for glucose monitoring due to their high accuracy, low cost, fast response, simplicity and better detection limits. In general, the transduction is performed from the hydrogen peroxide enzymatically generated during the enzyme recycling [1,2].

As it is widely known, the oxidation and reduction of hydrogen peroxide at carbon electrodes need elevated overvoltages [1,2]. Therefore, many efforts have been done in order to make the electrochemical detection of hydrogen peroxide more selective [3–16]. Among them, metallized carbon electrodes have allowed to obtain a very selective glucose determination based on the reduction of the enzymatically generated hydrogen peroxide. Platinum [10], palladium [11], rhodium [12], iridium [13] and gold [14] incorporated in carbon electrodes have been successfully used. More recently, we proposed the use of carbon electrodes modified with copper for glucose biosensing, based on the excellent electrocatalytic activity of copper towards the reduction of hydrogen peroxide [15].

In the present work, we report the characteristics of a bioelectrode based on the modification of carbon paste electrode with copper oxide and glucose oxidase, as well as its analytical performance for glucose biosensing. The influence of the content of copper oxide and enzyme in the biocomposite is also evaluated and discussed.

2. Experimental

2.1. Reagents

Hydrogen peroxide (30%, v/v aqueous solution) was purchased from Carlo Erba. Glucose, uric acid and

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acetaminophen were from Merck, while ascorbic acid was from Fluka. Glucose oxidase (Type X-S, *Aspergillus niger*, EC 1.1.3.4, 157,500 units per gram of solid, catalog number G-7141) was purchased from Sigma. Standatrol S-E-2 was obtained from Wiener Lab (Lote 301538). Mineral oil was purchased from Aldrich while graphite powder (grade 38) was from Fischer Scientific. Copper oxide was obtained from Aldrich.

Ultrapure water (ρ = 18 MΩ) 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.

2.2. Apparatus

The measurements were performed with an EPSILON potentiostat (BAS). The electrodes were inserted into the cell (BAS, Model VC-2) through its Teflon cover. Ag/AgCl, 3 M NaCl and a platinum wire were used as reference and counter electrodes, respectively. A magnetic stirrer and a stirring bar provided the desired convective transport, while the Cell-Stand C3 (BAS) was used during the amperometric determinations.

2.3. Electrode preparation and procedure

Carbon paste electrodes containing GOx and CuO (CuO–GOx–CPE) were prepared in the following way: the desired amount of enzyme (10.0%, w/w) and CuO (1.5%, w/w) was mixed with oil (26.6%, w/w) in an agate mortar for 5 min, followed by incorporation of graphite powder (61.9%) and mixing for additional 20 min. Carbon paste electrodes containing copper oxide (CuO–CPE) and copper (Cu–CPE) were prepared in a similar way. A portion of the resulting paste was packed firmly into a Teflon tube cavity (3 mm diameter). The electric contact was established through a stainless steel screw. A new surface was obtained by smoothing the electrode onto a weighing paper. Between experiments, the bioelectrode was stored at 4 °C.

All experiments were performed at room temperature. The desired potential was applied to the electrode, and the transient signal was allowed to reach its steady-state value prior to the substrate addition and current monitoring. When the bioelectrode was challenged with standard control serum, the corresponding aliquots of sample were added to 2.00-mL supporting electrolyte solution to obtain a final glucose concentration included in the linear portion of the calibration curve.

3. Results and discussion

Fig. 1 A displays cyclic voltammograms for 0.100 M hydrogen peroxide at CPE (a) and at CPE containing 3.0% (w/w) CuO (CuO–CPE) (b) at 0.050 V s^{-1}. As it is already known [1], at bare CPE, there is a poor electrochemical response for hydrogen peroxide, the oxidation starting at potentials higher than 0.70 V. On the contrary, at CuO–CPE, the oxidation starts at potentials more positive than 0.300 V, while the reduction starts at potentials more negative than 0.300 V.

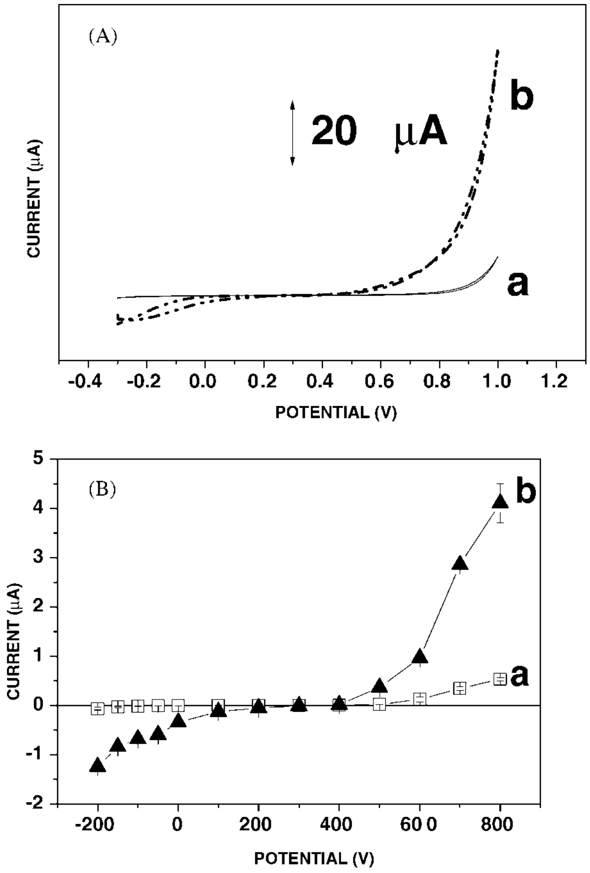

Fig. 1. (A) Cyclic voltammograms for 0.100 M hydrogen peroxide at bare graphite carbon paste electrode (CPE) (a) and at graphite carbon paste electrode modified with 3.0% (w/w) copper oxide (CuO–CPE) (b); scan rate: 0.050 V s^{-1}, potential range: −0.30 to 1.00 V. (B) Hydrodynamic voltammograms for 2.0 × 10^{-2} M hydrogen peroxide at CPE (a) and at CuO (1.5%, w/w)–CPE (b); supporting electrolyte: 0.050 M phosphate buffer solution (pH 7.40).

Fig. 1B shows the corresponding hydrodynamic voltammograms for 2.0 × 10^{-2} M hydrogen peroxide at CPE (a) and at CuO(1.5%, w/w)–CPE (b). As expected from Fig. 1A, the reduction of hydrogen peroxide starts at potentials more negative than 0.300 V, whereas the oxidation currents appear at more positive potentials. The ratios between the currents obtained at CuO–CPE, and those obtained at bare CPE at −0.200 V and 0.800 V are 18.9 and 7.8, respectively. At variance with Cu–CPE [15], a significant catalytic effect on the electrooxidation of hydrogen peroxide is also observed. Therefore, the important catalytic effect of copper oxide towards the reduction of hydrogen peroxide makes it possible the determination of glucose (from the hydrogen peroxide enzymatically generated) at lower potentials, where the interference of easily oxidizable compounds is negligible.

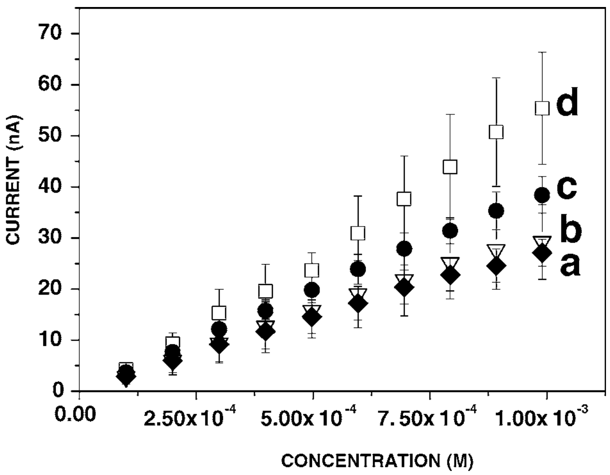

Fig. 2. Effect of copper oxide content in the paste on the response of the electrode towards hydrogen peroxide. Calibration plots obtained from amperometric recordings for successive additions of 1.0 × 10^{-4} M hydrogen peroxide using CPE containing 0.5% (a), 1.0% (b), 1.5% (c) and 3.0% (d) (w/w) CuO; working potential: −0.100 V, supporting electrolyte: 0.050 M phosphate buffer solution (pH 7.40).

Regarding the electrochemical behavior of usual interferents like ascorbic acid, uric acid and acetaminophen, there is almost no electrocatalytic effect of copper oxide towards these compounds (not shown).

The content of copper oxide in the paste proved to be a very critical variable for the performance of the bioelectrode. Fig. 2 shows the influence of the amount of copper oxide in the paste on the sensitivity of the modified carbon paste electrode. The calibration plots were obtained from amperometric experiments at −0.100 V for successive additions of 1.0 × 10^{-4} M hydrogen peroxide. As expected, the response of the electrode increases with the amount of copper oxide in the paste, with sensitivities of (2.70 ± 0.05) × 10^4 (a), (3.10 ± 0.05) × 10^4 (b), (4.00 ± 0.03) × 10^4 (c) and (5.4 ± 0.1) × 10^4 (d) nA M^{-1}, for electrodes containing 0.5, 1.0, 1.5 and 3.0% (w/w) of copper oxide, respectively. It is important to mention that at the same time that while the sensitivity increases with the amount of copper oxide, the response becomes less reproducible.

The selectivity is another important challenge in the development of a biosensor. In order to obtain the best compromise between sensitivity and selectivity, we evaluated the response of the electrode towards ascorbic and uric acids, and acetaminophen. Fig. 3 displays the effect of the amount of copper oxide in the paste on the selectivity of the bioelectrode. Additions of 4.7 × 10^{-4} M uric acid, 4.5 × 10^{-4} M ascorbic acid and 5.0 × 10^{-6} M acetaminophen were performed after an initial addition of 5.0 × 10^{-4} M hydrogen peroxide. The interference was evaluated at −0.100 V using electrodes prepared with 0.5, 1.0, 1.5 and 3.0% (w/w) of copper oxide. As it is shown in Fig. 3, for pastes with 3.0% (w/w) CuO, there is a very small interference of ascorbic acid (1.4%), whereas a strong interference is obtained for uric acid (21.3%). As the amount of copper oxide in the paste decreases, the interference also diminishes, and for pastes containing 0.5, 1.0 and 1.5% (w/w) CuO, there is no interference for any of the easily oxidizable compounds. Therefore, although the sensitivity is smaller than the paste containing 3.0% w/w (1.4-folds smaller, Fig. 3), the selected content of copper oxide was 1.5% (w/w) because the selectivity largely improves under these conditions.

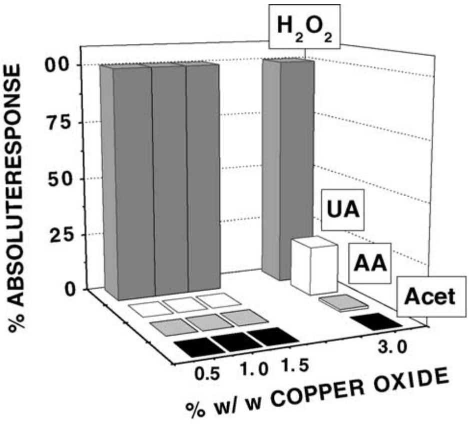

Fig. 3. Normalized response of CuO–CPE to 5.0 × 10^{-4} M hydrogen peroxide, 4.7 × 10^{-4} M uric acid (UA), 4.5 × 10^{-4} M ascorbic acid (AA) and 5.0 × 10^{-6} M acetaminophen (Acet) for different percentages of copper oxide in the graphite carbon paste (0.5, 1.0, 1.5, 3.0%, w/w). The 100% response corresponds to the signal of hydrogen peroxide in the absence of interferents. The percentage of absolute response obtained in the presence of UA, AA and Acet was obtained from the ratio between the current obtained in the presence and in the absence of the interferents multiplied by 100. Other conditions are as in Fig. 2.

An interesting effect on the response to hydrogen peroxide is observed when GOx is incorporated within the paste. Fig. 4A shows calibration plots obtained from amperometric determinations of hydrogen peroxide at −0.100 V at CuO–CPE without (a) and with (b) GOx. In the presence of the protein, there is an important enhancement in the sensitivity towards hydrogen peroxide (48 μAM^{-1} (a) versus 2790 μAM^{-1} (b)). Since hydrogen peroxide is not a substrate of GOx, this effect is clearly connected with a better dispersion of the copper oxide particles within the composite when the protein is present. This effect was also observed for Cu–GOx–CPE, where an important increase in the sensitivity was observed, although it was less pronounced than in the case of the composite containing copper oxide (not shown). Similar behavior was observed for CuO–CPE and Cu–CPE containing 10.0% (w/w) albumin instead of glucose oxidase, demonstrating the beneficial effect of the proteins in the dispersion of the metallic particles (not shown).

Fig. 4B shows the influence of the amount of GOx in the paste on the sensitivity of the amperometric determination of hydrogen peroxide at −0.100 V. The protein clearly improves the response of the electrode. The sensitivity increases as the content of GOx increases up to 10.0% (w/w) to become almost constant thereafter.

To select the optimum amount of the biorecognition element on the performance of the bioelectrode, calibration

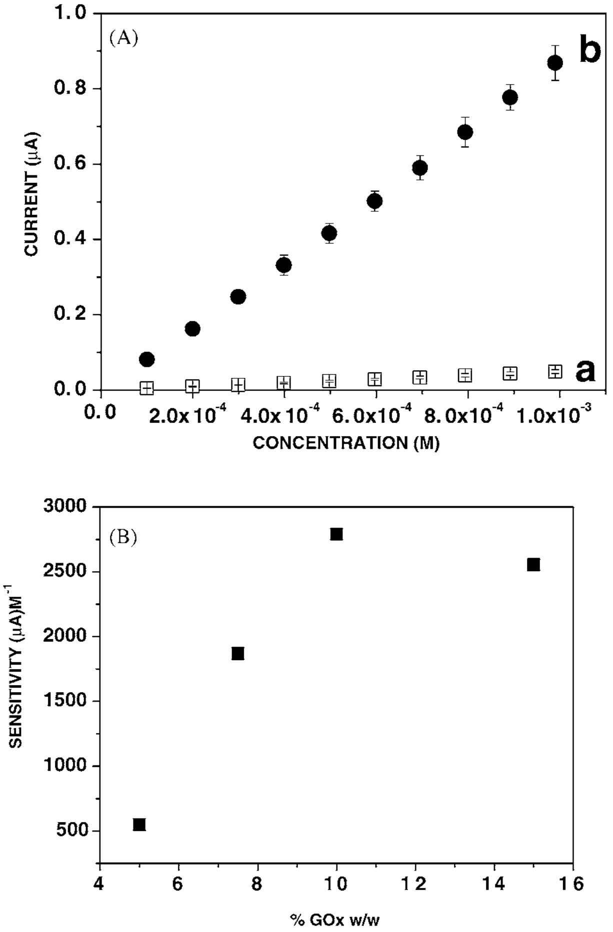

Fig. 4. (A) Calibration plots obtained from amperometric recordings at −0.100 V for successive additions of 1.0×10^{-4} M hydrogen peroxide at CuO–CPE (a) and at CuO–GOx (10.0%, w/w)–CPE (b). (B) Sensitivities of CuO–GOx–CPE as a function of the GOx content for amperometric experiments at −0.100 V performed for successive additions of hydrogen peroxide.

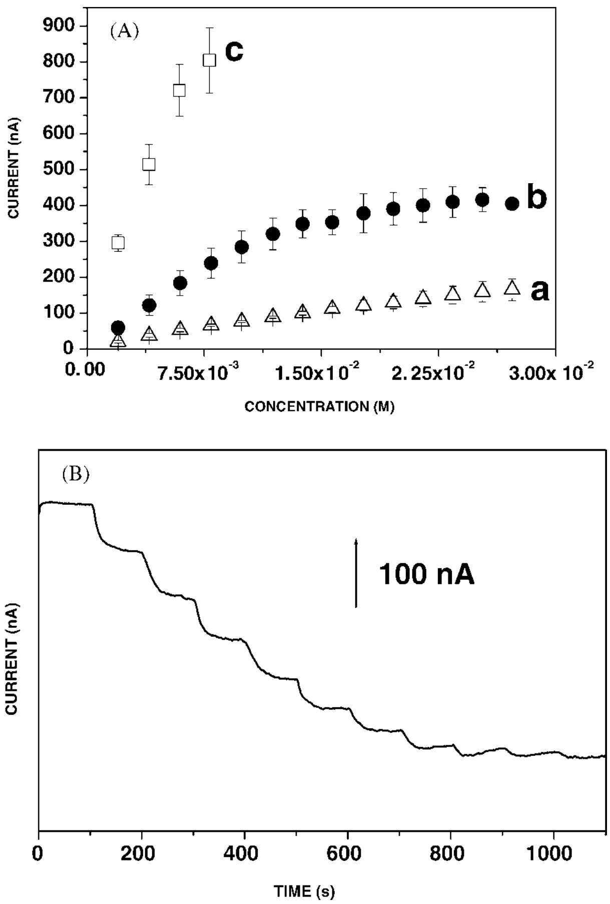

Fig. 5. (A) Calibration plots obtained from amperometric recordings at −0.100 V for successive additions of 2.0×10^{-3} M glucose. Working electrode: CPE containing 1.5% (w/w) CuO and 5.0% (a), 10.0% (b), 15.0% (c) (w/w) GOx. The plot is the average of the data obtained from four amperometric recordings performed with different surfaces. (B) Amperometric recordings for additions of 2.0×10^{-3} M glucose using a CPE containing 1.5% (w/w) CuO and 10.0% (w/w) GOx. Working potential: −0.100 V, supporting electrolyte: 0.050 M phosphate buffer solution (pH 7.40).

curves for glucose were performed using CuO(1.5% w/w)–CPE modified with different percentages of GOx. Fig. 5A shows calibration plots for glucose employing bioelectrodes modified with 5.0 (a), 10.0 (b) and 15.0 (c) % (w/w) of GOx. It is important to notice that the sensitivity towards glucose increases with the amount of GOx in the bioelectrode due to the biocatalytic effect, as well as to the better dispersion of the copper oxide particles (in agreement with Fig. 4). However, despite the fact that with 15.0% (w/w) GOx the sensitivity largely increases, there is like a saturation effect probably due to the oxygen consumption in the presence of such a high amount of GOx. The results obtained here demonstrate that the best compromise between sensitivity and dynamic linear range was reached with the bioelectrode containing 10.0% (w/w) of enzyme. Thus, the bioelectrode selected as optimum was CPE modified with 1.5% (w/w) CuO and 10.0% (w/w) GOx. Fig. 5B shows the current–time recordings for successive additions of 2.0×10^{-3} M glucose (corresponding to the calibration plot shown in Fig. 5 A-b). A fast response to glucose is observed after the addition of 2.0×10^{-3} M glucose (steady state currents were achieved at around 15 s). A linear relationship between current and glucose concentration was obtained up to 1.35×10^{-2} M glucose (2.43 g L^{-1}), thereafter the current increases non-linearly with the substrate concentration, as expected for biocatalytic reactions. Thus, the dynamic range covers not only physiological values but also pathological ones (normal range: 0.70–1.10 g L^{-1}). The average sensitivity measured at −0.100 V was $(2.94 \pm 0.09) \times 10^4$ nA M^{-1} and the detection limit was 2.0×10^{-5} M (3.60 mg L^{-1} taken as signal-to-noise ratio = 3).

The selectivity of the bioelectrode was also evaluated. Fig. 6 displays amperometric recordings at −0.100 V obtained after one addition of 5.0×10^{-3} M glucose, followed by additions of acetaminophen 9.8×10^{-6} M (up to a final concentration of 9.1×10^{-5} M), 3.0×10^{-5} M ascorbic acid (up to a final concentration of 1.5×10^{-4} M) and 9.7×10^{-5} M uric acid (up to a final concentration of 8.3×10^{-4} M (14.0 mg dL^{-1})). No interference was observed even for these concentrations larger than the maximum

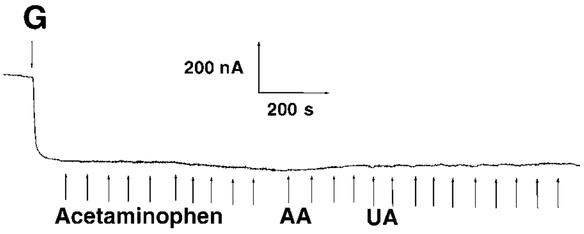

Fig. 6. Current–time profiles performed at CuO (1.5%, w/w)–GOx (10.0%, w/w)–CPE for one addition of 5.0×10^{-3} M glucose and successive additions of acetaminophen 9.8×10^{-6} M (up to a final concentration of 9.1×10^{-5} M), 3.0×10^{-5} M ascorbic acid (up to a final concentration of 1.5×10^{-4} M) and 9.7×10^{-5} M uric acid (up to final a concentration of 8.3×10^{-4} M (14.0 mg dL^{-1})). Other conditions are as in Fig. 5.

physiological levels found in human serum blood (AA level: 3.0 to 11.0×10^{-5} M, UA level: 3.5 to 6.0 mg dL^{-1}).

Under the working conditions, Eadie-Hofstee plots presented a linear response at glucose concentrations higher than 1.7×10^{-2} M. At lower substrate concentrations, a negative deviation was obtained due to a diffusional control (not shown). The kinetics parameters obtained from this plot were $K_M^{app} = (7.8 \pm 0.2) \times 10^{-3}$ M and $I_{max} = (549 \pm 5)$ nA.

The bioelectrode was challenged with a control serum (Standatrol S-E-2, Wiener Lab.) as real sample in order to evaluate its usefulness in practical applications. The range of concentration determinated for the corresponding additions of serum was (0.79–1.08) g L^{-1}, which was in agreement with the acceptable range reported by Wiener lab (0.75–1.01) g L^{-1}.

The long-term stability of the bioelectrode was analyzed by measuring the sensitivity of calibrations plots at -0.100 V obtained after storage of the bioelectrode at 4 °C (dried). After 120 days, the response remained almost constant (within experimental error), indicating that GOx keeps its biocatalytic activity within the CuO–CPE matrix (data not shown).

4. Conclusions

The excellent electrocatalytic activity shown by CuO towards the hydrogen peroxide enzimatically generated has allowed us to quantify glucose at potentials negative enough to avoid the interference of easily oxidizable compounds. The selection of the amount of catalyst in the composite bioelectrode was a very important factor to obtain a highly selective glucose biosensor, even in the presence of large excess of easily oxidizable compounds or complex matrices due to the preferential catalytic action on the reduction of hydrogen peroxide. The presence of the enzyme facilitated the dispersion of the copper oxide particles improving, thus, the catalytic effect. The resulting bioelectrode demonstrated a very good sensitivity, reproducibility and selectivity comparable and even better than those observed for other metallized glucose biosensors.

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