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Glucose Biosensor Based on the Use of a Carbon Nanotube Paste Electrode Modified with Metallic Particles

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Abstract. This work reports on the performance of new glucose biosensors based on the combination of the electrocatalytic properties of metals and carbon nanotubes towards the reduction of hydrogen peroxide with the biocatalytic activity of glucose oxidase (GOx). The bioelectrodes were obtained by dispersing the metal particles, enzyme and multi-wall carbon nanotubes within a mineral oil binder. The strong electrocatalytic activity of copper and iridium towards the reduction of hydrogen peroxide has made possible an important improvement in the sensitivity for the determination of glucose compared to the carbon nanotube composite without metals. A highly sensitive and selective amperometric detection of glucose becomes possible at very low potentials (−0.100 V). The presence of the protein enables a better dispersion of the metals within the composite matrix, thus allowing an additional enhancement in the response to hydrogen peroxide. The influence of the amount of copper in the composite on the analytical performance of the bioelectrode is discussed. A biosensor containing 0.77% w/w Cu and 10.0% w/w GOx gave a fast response (10.0 s), a linear relationship between current and glucose concentration up to 1.20×10^{-2} M, and a detection limit of 2.0×10^{-5} M. A similar behavior was found for a carbon nanotube-composite electrode containing iridium.

Key words: Carbon nanotubes; composite; glucose biosensor; glucose oxidase; copper; iridium; hydrogen peroxide.

Carbon nanotubes (CNTs), discovered in 1991 [1], are a new type of carbon material obtained by folding graphene layers into carbon cylinders. They present a closed topology and tubular structure and possess diameters of several nanometers and lengths of many microns [2, 3]. Basically, there are two types of carbon nanotubes, multi-wall carbon nanotubes (MWCNTs) and single-wall carbon nanotubes (SWCNTs) [2]. They can behave as metals or semiconductors depending on their structure, mainly on their diameter and helicity [2–4].

Carbon nanotubes have received enormous attention for the preparation of electrochemical sensors due to their unique properties, as it was reviewed by Zhao et al. [5], Wang [6] and Gooding [7]. Several strategies have been proposed for the immobilization of carbon nanotubes on electrochemical transducers. Wang et al. reported a marked decrease in the overvoltage for the oxidation of NADH using composites obtained by dispersing CNTs in a Teflon binder [8, 9]. Other interesting protocol for immobilizing CNTs have been reported by Li et al. [10] who proposed the electrocatalytic oxidation of norepinephrine at a GCE modified with SWCNTs. Compton et al. [11]

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proposed the use of MWCNTs abrasively attached to the basal plane pyrolytic graphite (bppg). A decrease of 300 mV in the oxidation overvoltage for epinephrine and a significant increase in the associated peak current was obtained with the CNT-bppg in comparison with the bppg electrode. Compton et al. [12, 13] have reported interesting analogies in the electroactivity of basal plane pyrolytic graphite modified with carbon nanotubes and edge-plane pyrolitic graphite electrode, suggesting that the important electrocatalytic activity of carbon nanotubes can be attributed to edge-plane like sites present at the open ends of nanotubes. However, the global behavior would involve different contributions that include the edge-plane like reactivity, the special morphology and size of carbon nanotubes, and the properties of the system under investigation.

Glucose oxidase was immobilized at a GCE modified with CNTs dispersed in Nafion [14]. A very sensitive and selective glucose quantification was obtained due to the important electrocatalytic activity of CNTs towards the oxidation and reduction of hydrogen peroxide. Compton et al. [15] have proposed a glucose biosensor fabricated by immobilizing glucose oxidase encapsulated in a sol-gel matrix on basal plane pyrolytic graphite modified with MWCNTs. The resulting electrode made it possible an excellent glucose biosensing due to the important catalytic effect of MWCNTs on the reduction and oxidation of hydrogen peroxide enzymatically generated. GOx was also immobilized on Pt modified with chemically oxidized SWCNTs through covalent attachment using carbodiimide showing an excellent stability [16]. Wang et al. [17] reported the direct dispersion of GOx within the CNTs for continuous measurement of glucose with excellent performance. The use of aligned carbon nanotubes as a platform for the production of a conducting polymer-glucose oxidase based biosensor was also reported [18, 19].

Recently, we have proposed a new alternative for developing sensors based on the use of CNTs obtained by dispersion of MWCNTs within mineral oil [20]. The resulting electrode, so-called carbon nanotubes paste electrode (CNTPE), combines the ability of carbon nanotubes to promote electron-transfer reactions with the attractive advantages of composite materials. We have demonstrated the excellent catalytic properties of CNTs on the electrochemical behavior of different redox systems like dopamine, ascorbic acid, dopac, uric acid and hydrogen peroxide [20, 21]; guanine, adenine and nucleic acids [22]; phenol, catechol, NADH and hydroquinone [23]; amitrole [24]; epinephrine and norepinephrine [25]. The usefulness of CNTPE as a matrix for the immobilization of redox enzymes has been also demonstrated. In fact, glucose oxidase [20]; lactate oxidase, alcohol dehydrogenase and polyphenol oxidase [23] were immobilized to obtain sensitive sensors for glucose, lactate, ethanol, phenols and catechols. CNTPE prepared with short (1–5 microns length) and long MWCNTs (5–20 microns length) of 20–50 nm diameter were also successfully used as detectors in flow systems [25].

Wang et al. [26] proposed the use of CNTPE for the detection of homocysteine. Magno et al. [27] reported an amperometric biosensor for fructose determination prepared by mixing MWCNTs with mineral oil (60/40% w/w) and covered with a polymer obtained by electropolymerization of 1.0 mM 3,4-dihydroxybenzaldehyde (3,4-DHB). Palleschi et al. [28] presented a Prussian Blue (PB) modified with SWCNTs as an efficient platform for developing enzymatic electrodes. The PB was obtained in the presence of SWCNTs, starting from $K_3Fe(CN)_6$ and $FeCl_3$. Wang and coworkers [29] reported on the use of CNTPE containing Cu as a detector for Capillary Electrophoresis for the determination of carbohydrates. The electrode was prepared by hand mixing mineral oil, MWCNTs and copper powder in a weight ratio 1:1:2 (carbon/oil/Cu).

Here we are reporting on the effect of copper and iridium microparticles incorporated within the CNTPE containing GOx on the response to glucose based on the electrocatalytic activity of these metals towards hydrogen peroxide. The influence of the electrode composition on the sensitivity and selectivity of the resulting biosensor is discussed.

Experimental

Apparatus

Electrochemical measurements were performed with a TEQ_02 potentiostat. The electrodes were inserted into the cell (BAS, Model MF-1084) through holes in its Teflon cover. A platinum wire and Ag/AgCl, 3M KCl (CH Instruments) were used as counter and reference electrodes, respectively. All potentials are referred to the latter. A magnetic stirrer provided the convective transport during the amperometric measurements.

Scanning Electronic Microscopy (SEM) pictures were obtained with a Hitachi S3000N Microscope.

The carbon nanotubes paste electrode (CNTPE) was prepared by mixing in an agate mortar MWCNTs powder (60.0% w/w)

and mineral oil (40.0% w/w) for 30 min. CNTPE containing copper (Cu-CNTPE) or iridium (Ir-CNTPE) were prepared in a similar way, mixing first the metal microparticles with the mineral oil for 1 min, followed by the incorporation of the carbon nanotubes powder and additional mixing for 30 min. CNTPE containing GOx and Cu (Cu–GOx-CNTPE) or Ir (Ir–GOx-CNTPE) were prepared in the following way: the desired amount of enzyme and metal was mixed with the mineral oil in an agate mortar for 5 min, followed by incorporation of carbon nanotubes powder and mixing for additional 30 min. A portion of the resulting pastes was packed firmly into a Teflon tube cavity (3 mm diameter). The electric contact was established through a stainless steel screw. The paste was packed before performing every new experiment and the surface was smoothed onto a weighing paper. When not in use the bioelectrodes were stored at 4 °C.

Reagents

Hydrogen peroxide (30% V/V aqueous solution) was purchased from Baker and the concentration of the stock solutions was checked by redox titration with standardized $KMnO_4$. Ascorbic acid was obtained from Fluka. Acetaminophen and glucose oxidase (GOx) (Type X-S, Aspergillus niger, EC 1.1.3.4, 157,500 Units per gram of solid, Catalog number G-7141) were purchased from Sigma. Uric acid and glucose were obtained from Merck. Iridium (99.9% purity) and copper (99% purity) microparticles were acquired from Alfa Aesar. MWCNTs powder (diameter 15–45 nm, length 1–5 μm, 95% purity) was purchased from NanoLab, U.S.A. The mineral oil was from Aldrich. 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 5.0×10^{-2} M phosphate buffer solution pH 7.40 was employed as supporting electrolyte.

Procedure

Amperometric measurements were conducted in a stirred 5.0×10^{-2} M phosphate buffer solution pH 7.40 by applying the desired working potential and allowing the transient currents to decay to a steady-state value prior to the addition of the analyte and subsequent current monitoring. All measurements were performed at room temperature.

Results and Discussion

Figure 1A shows a SEM picture of the MWCNTs powder before dispersing it in the mineral oil. Figure 1B displays an image of the carbon nanotube composite obtained by dispersion of MWCNTs within the mineral oil. There is a distribution almost homogeneous of the tubules into the organic matrix, demonstrating that the carbon nanostructures are embedded within the binder. No changes were distinguished after the incorporation of 1.5 w/w% Cu microparticles, Fig. 1C.

Figure 2 depicts hydrodynamic voltammograms for 1.7×10^{-2} M hydrogen peroxide at different electrodes, CNTPE (a), CNTPE containing 1.50% w/w Cu (b), and CNTPE containing 1.50% w/w copper

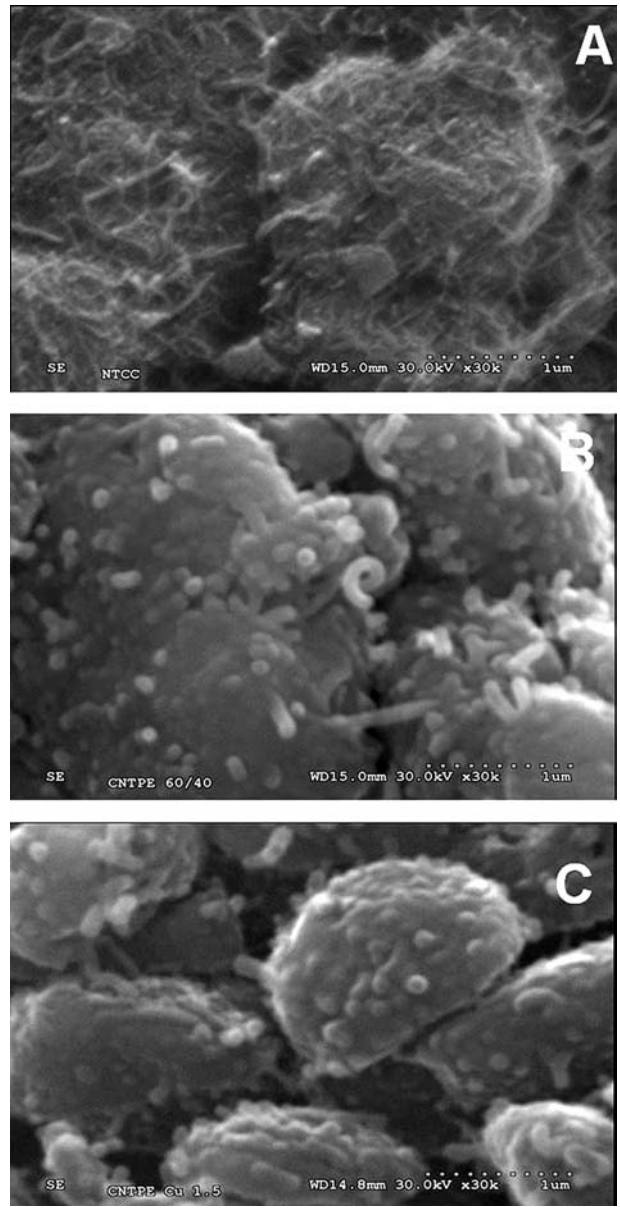

Fig. 1. SEM pictures of MWCNTs powder (A); Composite material containing 60.0% w/w MWCNTs and 40.0% w/w mineral oil (B); Composite material containing 1.50% w/w Cu, 59.25% w/w MWCNTs and 39.25% w/w mineral oil (C)

and 10.0% w/w GOx (c). In the presence of copper microparticles, due to the electrocatalytic effect of copper on the reduction of hydrogen peroxide [30], there is an important enhancement in the reduction current of this compound. At −0.100 V these currents increased almost in a factor of three compared to CNTPE ($-1.6\,\mu A\,cm^{-2}$ at CNTPE vs. $-4.5\,\mu A\,cm^{-2}$ at Cu-CNTPE).

An interesting effect on the response to hydrogen peroxide was observed at Cu-CNTPE containing GOx

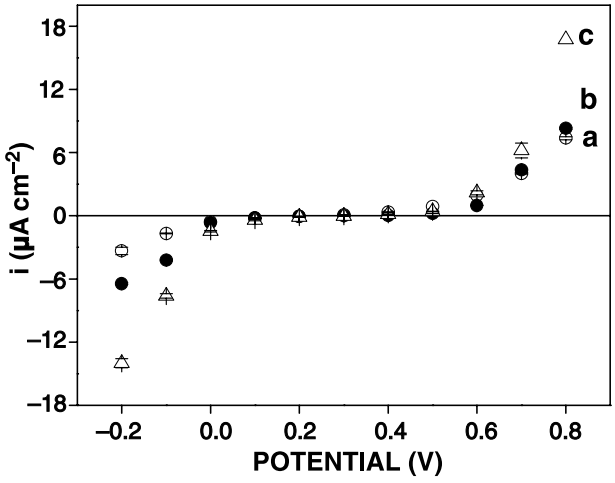

Fig. 2. Hydrodynamic voltammograms for 1.7×10^{-2} M hydrogen peroxide at CNTPE (*a*); at CNTPE containing 1.50% w/w copper microparticles (*b*) and at CNTPE containing 1.50% w/w copper microparticles and 10.0% GOx (*c*) Supporting electrolyte: 5.0×10^{-2} M phosphate buffer solution pH 7.40. Electrode composition: (*a*) 60.0% w/w MWCNT and 40.0% w/w oil; (*b*) 59.25% w/w MWCNT, 39.25% w/w oil and 1.50% w/w copper microparticles: (*c*) CNT (53.54% w/w), mineral oil (35.69% w/w), copper (0.77% w/w) and GOx (10.0% w/w)

(10.0% w/w) (Fig. 2c). As it is shown, in the presence of the protein there is an important enhancement in the oxidation and reduction currents. For instance, at −0.100 V, the current for the reduction of hydrogen peroxide is almost 2 and 5 times higher than those obtained at Cu-CNTPE (b) and at CNTPE (a), respectively. Since hydrogen peroxide is not a substrate of GOx, this effect is clearly connected with a better dispersion of the copper microparticles within the composite matrix containing the protein and/or changes in the permeability of the resulting composite. Therefore, the preferential catalytic activity of copper on the reduction of hydrogen peroxide makes the incorporation of this metal into the nanotubes composite, a very good alternative to improve the sensitivity of enzymatic biosensors based on the detection of hydrogen peroxide.

The influence of copper on the voltammetric behavior of common interferents in the glucose determination like ascorbic acid, uric acid and acetaminophen was also evaluated. Small decrease in the oxidation peak potentials in comparison with the response obtained at CNTPE was observed in the voltammetric behavior of ascorbic acid, uric acid and acetaminophen at Cu-CNTPE (not shown). Hence, there is also a catalytic effect of copper on the electrochemical behavior of these redox systems, although it is less pronounced than in the case of hydrogen peroxide.

Table 1. Sensitivities for the reduction of hydrogen peroxide at CNTPE containing different amounts of copper and 10.0% w/w GOx. Working potential: −0.100 V

% w/w of copper	Sensitivity ($\mu A\, M^{-1}$)
0	$(2.8 \pm 0.6) \times 10^2$
0.5	$(3.0 \pm 0.7) \times 10^2$
0.77	$(4.4 \pm 0.1) \times 10^2$
1.5	$(7.6 \pm 0.2) \times 10^2$
2.5	$(9.3 \pm 0.3) \times 10^2$

Therefore, since copper catalyzes not only the redox behavior of hydrogen peroxide, but also, although in lesser extension, the electrooxidation of uric acid, ascorbic acid and acetaminophen, the amount of copper present in the bioelectrode is an important parameter not only for the sensitivity of the bioelectrode but also for its selectivity.

The effect of copper on the electrocatalytic activity of the resulting modified CNTPE was evaluated. Table 1 shows the influence of the amount of copper in the bioelectrode on the sensitivity for hydrogen peroxide determination. The sensitivities were obtained from amperometric measurements at −0.100 V using electrodes containing 10.0% w/w GOx and different amounts of copper microparticles. As expected, an important increase in sensitivity is observed as the amount of copper in the composite increases.

The effect of the copper content on the selectivity of the biosensor was also evaluated from amperometric recordings at −0.100 V after additions of glucose and the common interferents using a CNTPE containing 10.0% w/w GOx and different amounts of copper. Figure 3 depicts the effect of 6.87×10^{-4} M uric acid, 4.11×10^{-4} M ascorbic acid and 3.16×10^{-4} M acetaminophen on the response of the bioelectrode to 5.0×10^{-3} M glucose. As expected, the interference for ascorbic acid and uric acid increases with the amount of copper in the composite. For CNTPE containing 2.5% of copper the interference for uric acid and ascorbic acid increased up to 28.0% and 20.0%, respectively, while no interference was observed for acetaminophen. Therefore, the selected value that has allowed to obtain the best compromise between sensitivity and selectivity was 0.77% w/w.

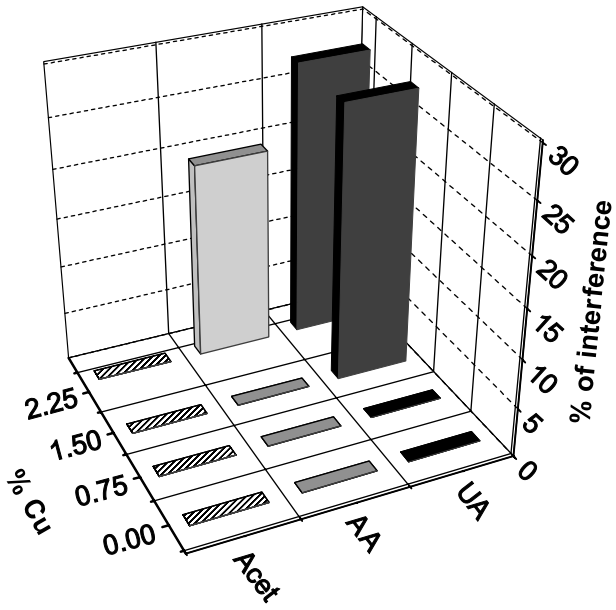

Fig. 3. Percentage of interference of ascorbic acid (4.11×10^{-4} M), uric acid (6.87×10^{-4} M) and acetaminophen (3.16×10^{-4} M) on the amperometric signal obtained after addition of 5.0×10^{-3} M of glucose as a function of the amount of copper in the electrode. It was taken as the percentage of the ratio between the oxidation current of the given interferent and the reduction current for the hydrogen peroxide enzymatically generated from glucose. Working potential: −0.100 V

Hence, 0.77% w/w-copper was the selected value for further work.

The catalytic action of copper facilitates a sensitive amperometric detection of glucose. Figure 4A shows amperometric recordings for glucose at −0.100 V for successive additions of 2.0×10^{-3} M glucose. The Cu–GOx-CNTPE responds rapidly to the changes in the glucose concentration. Figure 4B shows a calibration plot obtained as an average of three separated amperometric measurements like the one shown in Fig. 4A. A linear range was observed between 1.8×10^{-3} and 1.2×10^{-2} M glucose, with a sensitivity of $(2.97 \pm 0.05) \times 10^4$ nA M^{-1} cm^{-2} (r = 0.9991). Figure 4C shows the Eadie-Hofstee plot obtained from the data of Fig. 4B. A non-linear portion is obtained for low glucose concentrations as a consequence of the diffusional control of the overall process. The kinetic parameters were calculated from the linear portion at glucose concentrations higher than 6.0×10^{-3} M, being Imax = $(5.9 \pm 0.2) \times 10^2$ nA and $K_M^{app} = (70 \pm 3) \times 10^{-3}$ M.

The reproducibility in the sensitivities using five different electrodes was 12.0%. High stability is another

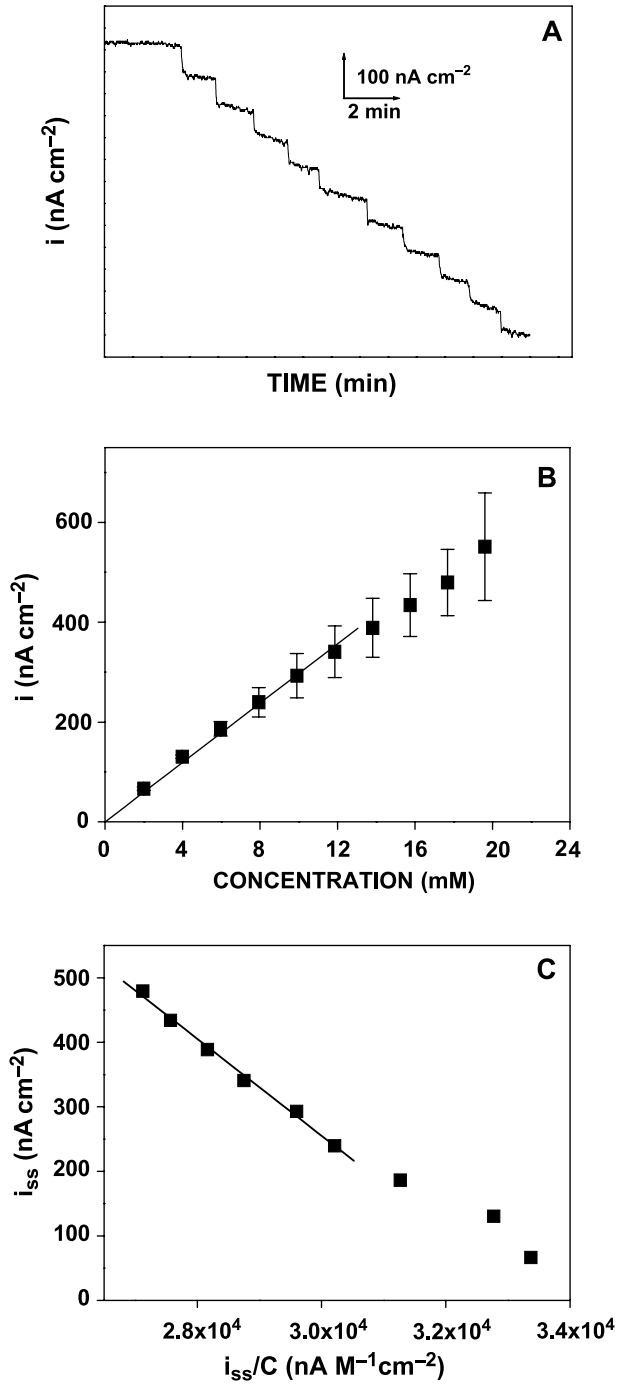

Fig. 4. (A) Amperometric recordings for successive additions of 2.0×10^{-3} M glucose. (B) Calibration plot for glucose obtained from the amperometric experiment shown in Fig. 4A. (C) Eadie-Hofstee plot obtained from (B). Electrode composition: CNT (53.54% w/w), mineral oil (35.69% w/w), copper (0.77% w/w) and GOx (10.0% w/w). Working potential: −0.100 V

attractive and important feature of Cu–GOx-CNTPE. In fact, the electrode demonstrated to be highly stable since after 2 months at 4 °C the response was almost

the same as the first day, decreasing a 24% after 6 months.

CNTPE Modified with Iridium Microparticles

The effect of iridium on the amperometric response of the biosensor was also evaluated. Hydrodynamic voltammograms for 1.7×10^{-2} M hydrogen peroxide at CNTPE (a) and at CNTPE containing 1.0% Ir (b) are shown in Fig. 5. In the presence of iridium there is an important improvement in the electrochemical behavior of hydrogen peroxide due to the strong electrocatalytic effect of iridium towards hydrogen peroxide [31]. An increase in the response to hydrogen peroxide was also observed in the presence of GOx.

Figure 6A displays the amperometric recordings at -0.100 V for successive additions of 2.0×10^{-3} M glucose. A well-defined and fast response (12 sec) was obtained after each addition of glucose. The analytical parameters obtained from the calibration plot shown in Fig. 6B are the following: linear range up to 2.0×10^{-2} M glucose and sensitivity of $(2.544 \pm 0.008) \times 10^4$ nA M^{-1} cm^{-2} ($r = 0.99990$). No interference was observed even in the presence of large excess of ascorbic acid, uric acid and acetaminophen.

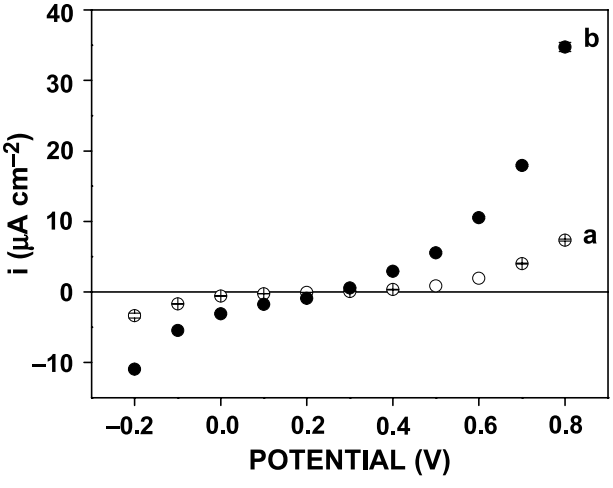

Fig. 5. Hydrodynamic voltammograms for 1.7×10^{-2} M hydrogen peroxide at CNTPE (*a*) and at CNTPE containing 1.00% w/w iridium microparticles (*b*). Supporting electrolyte: 5.0×10^{-2} M phosphate buffer solution pH 7.40. Electrode composition: CNT (59.40% w/w), mineral oil (39.60% w/w), iridium (1.00% w/w)

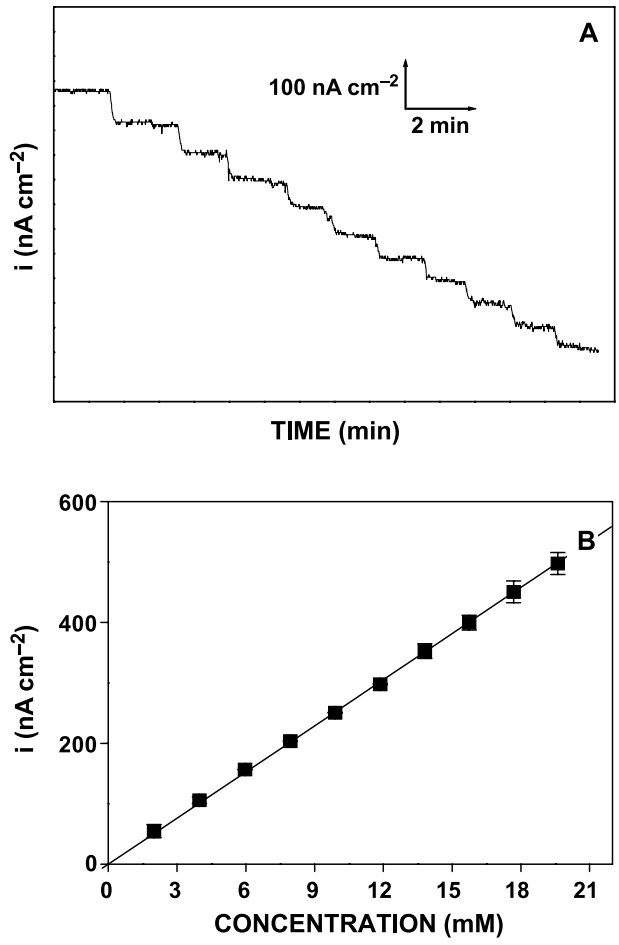

Fig. 6. (A) Amperometric recordings for successive additions of 2.0×10^{-3} M glucose at GOx–Ir-CNTPE. (B) Calibration plot for glucose obtained from the amperometric experiment shown in Fig. 6A. Electrode composition: CNT (53.40% w/w), mineral oil (35.60% w/w), iridium (1.00% w/w) and GOx (10.0% w/w). Working potential: -0.100 V

Conclusions

In summary, we have demonstrated that CNTPE can be successfully modified with metallic microparticles providing an useful avenue for developing highly sensitive and selective enzymatic biosensors. Cu–GOx-CNTPE and Ir–GOx-CNTPE offers a sensitive and stable constant-potential amperometric detection of glucose due to the combination of the electroactivity of CNTs with the electrocatalytic properties of metal microparticles towards the hydrogen peroxide enzymatically generated.

Future efforts aim at expanding the analytical applications of metallized CNTPEs to the sensitive detection of other biomolecules like aminoacids, peptides

and proteins. Our group is currently working in this direction.

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