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# Carbon nanotubes paste electrode

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**Abstract**

The performance of carbon nanotubes paste electrodes (CNTPE) prepared by dispersion of multi-wall carbon nanotubes (MWNT) within mineral oil is described. The resulting electrode shows an excellent electrocatalytic activity toward ascorbic acid, uric acid, dopamine, 3,4-dihydroxyphenylacetic acid (dopac) and hydrogen peroxide. These properties permit an important decrease in the overvoltage for the oxidation of ascorbic acid (230 mV), uric acid (160 mV) and hydrogen peroxide (300 mV) as well as a dramatic improvement in the reversibility of the redox behavior of dopamine and dopac, in comparison with the classical carbon (graphite) paste electrodes (CPE). The substantial decrease in the overvoltage of the hydrogen peroxide reduction (400 mV) associated with a successful incorporation of glucose oxidase (GOx) into the composite material, allow the development of a highly selective and sensitive glucose biosensor without using any metal, redox mediator or anti-interference membrane. No interference was observed at −0.100 V even for large excess of ascorbic acid, uric acid and acetaminophen. A linear response up to 30 mM (5.40 g $l^{-1}$) glucose with a detection limit of 0.6 mM (0.11 g $l^{-1}$) were obtained with the CNTPE modified with 10% w/w GOx. Such an excellent performance of CNTPE toward hydrogen peroxide, represents a very good alternative for developing other enzymatic biosensors.


*Keywords:* Carbon nanotubes; Composite; Biosensor; Glucose oxidase; Glucose; Catalysis

## 1. Introduction

Since the discovery of carbon nanotubes in 1991 [1], they have been the target of numerous investigations due to their unique properties [1–4]. Carbon nanotubes are built from $sp^2$ carbon units and they present a seamless structure with hexagonal honeycomb lattices, being several nanometers in diameter and many microns in length [2,3]. There are two groups of carbon nanotubes, multi-wall carbon nanotubes (MWNTs) and single-wall carbon nanotubes (SWNTs) [2]. MWNTs can be visualized as concentric and closed graphite tubules with multiple layers of graphite sheet defining a hole typically from 2 to 25 nm separated by a distance of approximately 0.34 nm [1–3]. A SWNTs consist of a single graphite sheet rolled seamlessly, defining a cylinder of 1–2 nm diameter. Carbon nanotubes can behave as metals or semiconductors depending on the structure, mainly on the diameter and helicity [2,3].

Several authors [5–12] have reported the excellent electrocatalytic properties of nanotubes in the redox behavior of different biomolecules. Britto et al. [5] have reported a dramatic improvement in the electrochemical behavior of dopamine with $\Delta E_p$ of 30 mV at nonactivated carbon nanotube electrodes constructed by using bromoform as binder. Li et al. [6] have reported the advantages of using glassy carbon modified with single wall carbon nanotubes on the voltammetric behavior of norepinephrine, dopamine and ascorbic acid. The ability of a SWNT-glassy carbon modified electrode for the highly sensitive and selective detection of dopac in the presence of 5-hydroxytryptamine was also demonstrated [7]. Li et al. [8] have described the catalytic properties of activated SWNT film-modified glassy carbon electrodes toward the reduction/oxidation of cytochrome *c*. Wang et al. [9] demonstrated that carbon-nanotubes-modified

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glassy carbon electrodes show a very significant electrocatalytic activity toward NADH with an important decrease in the oxidation overpotential. Recent studies demonstrated improved electrochemical behavior of hydrogen peroxide and catecholamine neurotransmitters on glassy carbon electrodes modified with SWNTs and MWNTs solubilized in nafion [10]. Hill et al. [11] have reported a preliminary work demonstrating the feasibility to use carbon nanotubes as an electrode material, packing the tubes into a glass capillary in mineral oil, deionised water, nujol or bromoform. They evaluated the electrochemical properties of the resulting electrode by using cytochrome *c* and azurin and found that these proteins can be immobilized on and within opened nanotubes without denaturation. Recently, Wang and Musameh [12] described the attractive performance of a composite material prepared by dispersion of MWNTs within Teflon. The resulting electrode keeps the excellent electroactivity of MWNTs even in presence of the hydrophobic material and allows the incorporation of enzymes like alcohol dehydrogenase and glucose oxidase for the development of enzymatic biosensors.

This article reports on the advantages of carbon nanotubes paste electrodes (CNTPE) prepared in an easy, fast and very effective way by using mineral oil as binder. The resulting CNTPE retains the properties of the classical carbon paste electrode (CPE) such as the feasibility to incorporate different substances, the low background currents, the easy renewal and composite nature. Therefore, this new composite electrode combines the ability of carbon nanotubes to promote electron-transfer reactions with the attractive advantages of composite materials. The electrochemical behavior of different biomolecules such as dopamine, ascorbic acid, dopac and uric acid as well as hydrogen peroxide, compound widely involved in enzymatic reactions of interest at this new composite material is described. The suitability of CNTPE for developing highly sensitive glucose enzymatic biosensors by incorporation of glucose oxidase (GOx) within the composite matrix is also illustrated in the following sections.

## 2. Experimental

### 2.1. Apparatus

The measurements were performed with an EPSILON potentiostat (BAS). The electrodes were inserted into the cell (BAS, Model MF-1084) through holes in its Teflon cover. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrode, respectively. All potentials are referred to the latter. A magnetic stirrer provided the convective transport during the amperometric and stripping measurements.

The carbon nanotubes paste electrode (CNTPE) was prepared by mixing in an agata mortar multiwalled carbon nanotubes powder (diameter 20–50 nm, length 1–5 μm, NanoLab, USA) and mineral oil (Aldrich) in a ratio 60.0% w/w nanotubes powder and 40.0% w/w mineral oil. The classical carbon (graphite) paste electrode (CPE) was prepared in a similar way by mixing graphite powder (Fisher Grade No. 38) with mineral oil. CPE and CNTPE containing GOx were prepared in the following way: the desired amount of enzyme (10.0% w/w) was mixed with mineral oil (usually 30.0% w/w) in an agata mortar for 3 min followed by the incorporation of graphite or carbon nanotubes powder and mixing for additional 30 min. A portion of the resulting paste was packed firmly into the cavity (3.0 mm diameter) of a Teflon tube. The electric contact was established via a stainless steel screw. A new surface was obtained by smoothing the electrode onto a weighing paper.

### 2.2. Reagents

Hydrogen peroxide (30% V/V aqueous solution) was purchased from Baker. Ascorbic acid was obtained from Fluka. Dopamine, 3,4-dihydroxyphenylacetic acid (dopac) and glucose oxidase (GOx) (Type X-S, *Aspergillus niger* (EC 1.1.3.4), 157,500 U per gram of solid, Catalog No. G-7141), and atomic absorption copper standard solution (985 ppm Cu in 1% $HNO_3$) were purchased from Sigma. Uric acid and glucose were obtained from Merck. Other chemicals were reagent grade and used without further purification.

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

### 2.3. Procedure

The amperometric experiments were carried out in a phosphate buffer solution (0.050 M, pH 7.40) by applying the desired potential and allowing the transient current to decay to a steady-state value prior to the addition of the analyte and the subsequent current monitoring. The cyclic voltammetric experiments were performed using a 0.050 M phosphate buffer solution, pH 7.40. Linear-sweep voltammograms were recorded in different solutions (0.050 M phosphate buffer, pH 7.40, 0.10 M sulfuric acid and 0.10 M NaOH).

Constant-current chronopotentiometric experiments were performed with a TraceLab Potentiometric Stripping Unit PSU 22 (Radiometer, France) connected to a PC. According to the TraceLab protocol, the potentials were sampled at a frequency of 30 kHz and the derivative signals ($dT/dE$) versus potential ($E$) were recorded following baseline fitting. The protocol for metals de-

termination consisted of two steps: metal preconcentration (accumulation or deposition) in a stirred copper solution prepared in 0.100 M nitric acid at −0.50 V during 3 min followed by the chronopotentiometric transduction in a 0.100 M nitric acid solution by applying a constant current of 8.0 μA with an initial potential of −0.50 V. All the experiments were conducted at room temperature.

## 3. Results and discussion

### 3.1. Electrochemical behavior

Since the work reported by Adams in 1958 [13], carbon composite electrodes have received enormous attention. Among them, the composites made of graphite powder and mineral oil are the most widely known [14]. As its analogous CPE, carbon nanotubes paste electrodes display a broad potential window and a low background current. Fig. 1 shows linear-sweep voltammograms obtained at 0.100 V $s^{-1}$ in different deoxygenated solutions: 0.10 M sulfuric acid (a), 0.050 M phosphate buffer, pH 7.40 (b) and 0.10 M NaOH (c) at CNTPE. In sulfuric acid solution the oxygen evolution starts at potentials close to 1.0 V, while the reduction at potentials more negative than −0.50 V. In phosphate buffer solution the oxidation of the solvent starts at 0.8 V and the oxygen reduction at potentials more negative than −0.30 V. In 0.10 M NaOH, the solvent starts to oxidize at 0.30 V and the oxygen reduces at −0.25 V. Therefore, CNTPE shows a potential window comparable to that of CPE and better than that of glassy carbon paste electrodes [15].

Fig. 2 displays cyclic voltammograms obtained at 0.100 V $s^{-1}$ for $1.0 \times 10^{-3}$ M ascorbic acid (A), uric acid (B), dopamine (C) and dopac (D) at CPE, at CPE containing 10% w/w carbon nanotubes and at CNTPE (in all cases the content of mineral oil was 40.0% w/w). The CNTPE shows sharper and larger voltammetric peaks as well as lower overvoltages for the different redox systems, indicating the improved electron-transfer kinetics. In general, the charge transfer is facilitated even modifying the CPE with a 10% w/w of carbon nanotubes.

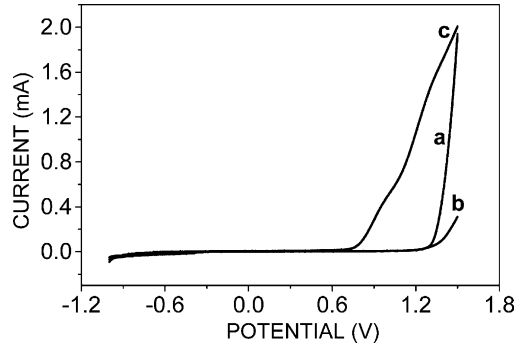


Fig. 1. Linear scan voltammograms of CNTPE in different electrolytes solutions: (a) 0.10 M sulfuric acid, (b) 0.050 M phosphate buffer solution, pH 7.40, and (c) 0.10 M sodium hydroxide. Scan rate: 0.100 V $s^{-1}$.

Tables 1 and 2 summarize the voltammetric parameters for these compounds obtained at the different electrodes. As it can be seen from the comparison between CPE and CNTPE, a decrease in the oxidation peak potential of 230 and 160 mV was obtained for ascorbic acid and uric acid, respectively, at CNTPE (Table 1). In the case of dopamine and dopac, compounds that display a quasi-reversible behavior at CPE, a dramatic improvement in the reversibility was found at the CNTPE (Table 2). The $\Delta E_p$ for dopamine and dopac decreased 133 and 313 mV, respectively, while the anodic-to-cathodic ratio decreased from 5.75 to 2.83 for dopamine and from 3.09 to 1.33 for dopac. These facts demonstrate that even in the presence of the pasting liquid the nanotubes keep an excellent electrochemical reactivity.

As it was already demonstrated for other carbon materials, the state of the surface of CNTPE plays an important role on the electrode kinetics. Various activation procedures were evaluated, being the one performed by cycling the potential between −1.00 and 1.50 V (18 cycles) at 1.0 V $s^{-1}$ in a 0.050 M phosphate buffer solution, pH 7.40, the one that allows to obtain the best response. The $\Delta E_p$ for $1.0 \times 10^{-3}$ M dopamine at activated CNTPE decreased 30 mV while the anodic oxidation peak currents increased from 34.3 to 64.9 μA (not shown).

The effect of the amount of mineral oil on the performance of the CNTPE was also evaluated (not shown). Electrodes with oil content smaller than 40.0% w/w were difficult to pack into the Teflon tube. Pastes containing 50.0% w/w of mineral oil showed a behavior similar to the one observed with CNTPE containing 40.0% w/w. For instance, for $1.0 \times 10^{-3}$ M dopamine, the $\Delta E_p$ was 0.140 V and the cathodic-to-anodic currents ratio was 2.89. This $\Delta E_p$ is 40 mV higher than that at CNTPE containing 40.0% w/w mineral oil, while the currents ratio is almost the same (see Table 2). However, the consistence of the CNTPE containing 50.0% w/w mineral oil was difficult to handle for preparing the electrode. Therefore, the selected composition was 60.0% w/w carbon nanotubes and 40.0% w/w mineral oil.

### 3.2. Electroanalytical performance

#### 3.2.1. Glucose biosensor based on CNTPE modified with GOx

Hydrogen peroxide is of considerable interest for developing oxidases-based biosensors. Fig. 3(A) shows the hydrodynamic voltammograms for $1.0 \times 10^{-2}$ M

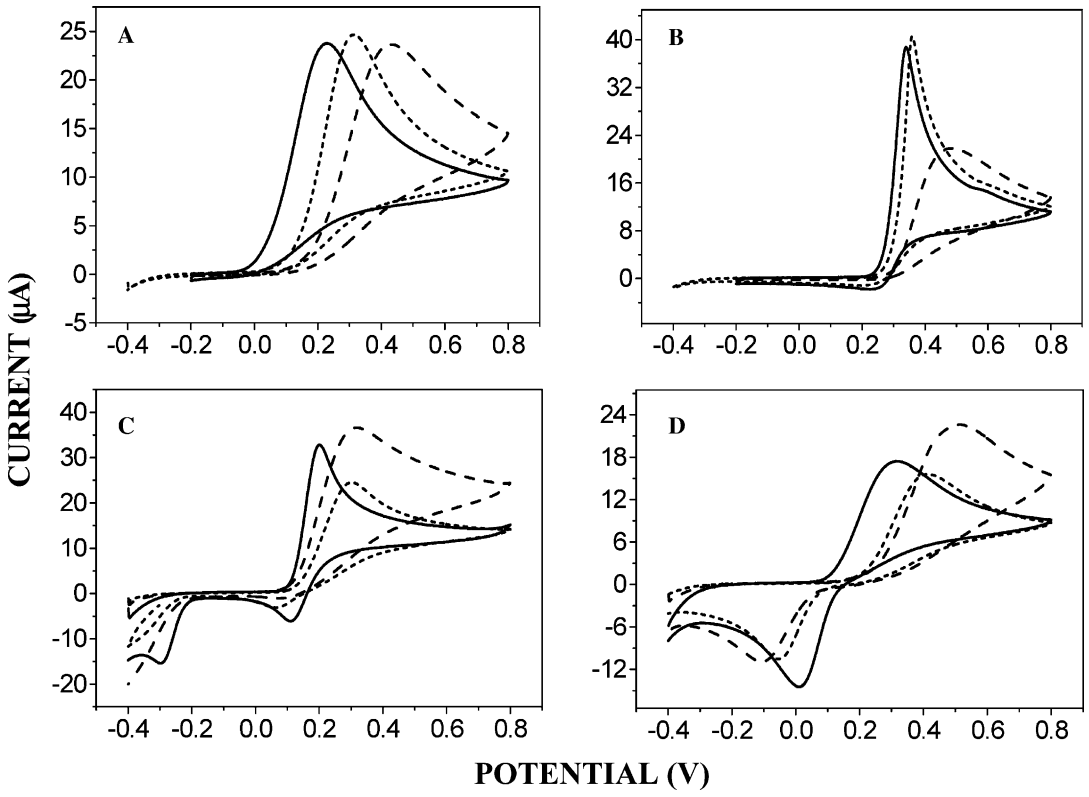


Fig. 2. Cyclic voltamograms for $1.0 \times 10^{-3}$ M ascorbic acid (A), uric acid (B), dopamine (C) and dopac (D) at different electrodes: (- - -) CPE; (. . .) CPE containing 10% w/w carbon nanotubes; (———) CNTPE. Supporting electrolyte: 0.050 M phosphate buffer solution, pH 7.40. Scan rate: 0.100 V $s^{-1}$.

Table 1
Voltammetric parameters for $1.0 \times 10^{-3}$ M ascorbic acid and uric acid at CPE, CPE containing 10% w/w carbon nanotubes and CNTPE

| Compound | CPE | | CPE containing 10% w/w nanotubes | | CNTPE | |
|---|---|---|---|---|---|---|
| | Current (μA) | Potential (V) | Current (μA) | Potential (V) | Current (μA) | Potential (V) |
| Ascorbic acid | 23.3 | 0.440 | 24.2 | 0.320 | 23.3 | 0.210 |
| Uric acid | 21.6 | 0.490 | 40.2 | 0.360 | 51.2 | 0.330 |

Table 2
Voltammetric parameters for $1.0 \times 10^{-3}$ M dopamine and dopac at CPE, CPE containing 10% w/w carbon nanotubes and CNTPE

| Compound | CPE | | CPE containing 10% w/w nanotubes | | CNTPE | |
|---|---|---|---|---|---|---|
| | $\Delta E_p$ (V) | $i_{pa}/i_{pc}$ | $\Delta E_p$ (V) | $i_{pa}/i_{pc}$ | $\Delta E_p$ (V) | $i_{pa}/i_{pc}$ |
| Dopamine | 0.233 | 5.75 | 0.242 | 3.21 | 0.100 | 2.83 |
| Dopac | 0.610 | 3.09 | 0.46 | 2.08 | 0.297 | 1.33 |

hydrogen peroxide at CPE (a) and at CNTPE (b). At CNTPE the oxidation starts at around 300 mV and the reduction at 200 mV. Therefore, decreases of 300 mV in the oxidation overpotential and 400 mV in the reduction overpotential are obtained for hydrogen peroxide. Fig. 3(B) shows calibration plots for hydrogen peroxide performed at −0.100 V at CPE (a) and CNTPE (b). The corresponding sensitivities are 0.33 ($r = 0.9990$) and 16.8 ($r = 0.996$) μA $M^{-1}$, respectively, indicating that at CNTPE there is an enhancement of 50 times in sensitivity. Therefore, in agreement with Fig. 3(A), an excellent electrocatalytic effect for the reduction of hydrogen peroxide is obtained at CNTPE, comparable to that obtained with metallized carbon electrodes [16]. This fact is very promising due to the possibility of using CNTPE for developing enzymatic biosensors.

Based on the electrocatalytic properties of carbon nanotubes towards the oxidation and reduction of hydrogen peroxide, the electrode was also used for the preparation of glucose biosensors by incorporation of GOx into the composite matrix. It is widely known that GOx catalyzes the oxidation of glucose to gluconolactone while oxygen, its natural mediator, is converted into hydrogen peroxide. Fig. 4(A) shows the ampero-

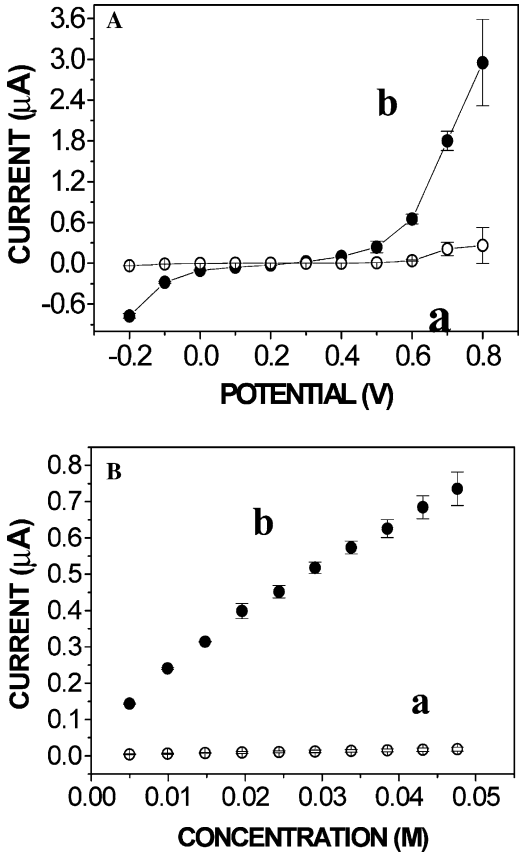


Fig. 3. (A) Hydrodynamic voltammogram for $1.0 \times 10^{-2}$ M hydrogen peroxide at CPE (a) and CNTPE (b). (B) Calibration plot for hydrogen peroxide obtained at CNTPE. Working potential: −0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution, pH 7.40.

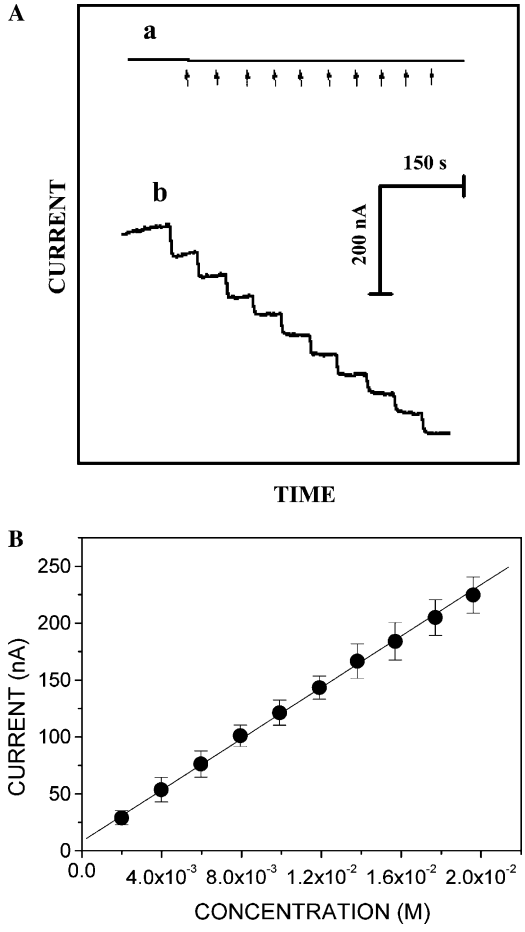


Fig. 4. (A) Amperometric recording obtained at CPE–GOx (a) and at CNTPE–GOx (b) for successive additions of 5 mM glucose. The content of GOx was 10.0% w/w in both electrodes. (B) Calibration plot obtained from amperometric recording for successive additions of 2 mM glucose. Working potential: −0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution, pH 7.40.

metric response obtained at −0.100 V for a CPE–GOx (a) and for a CNTPE–GOx (b) (10.0% w/w GOx in both cases) to successive additions of 5 mM glucose. As expected, a small response is observed at the enzymatic CPE, while a well defined, fast (10 s) and 43 times more sensitive response is obtained at the CNTPE–GOx. Fig. 4(B) shows the calibration plot for additions of 2.0 mM glucose at CNTPE–GOx obtained as an average of four calibrations. A linear relationship is observed in the whole range. The response was linear even up to 25 mM glucose (4.5 g $l^{-1}$), covering, thus, not only the physiological range, but also pathological values, avoiding the problem of oxygen consumption for high levels of glucose. The corresponding sensitivity is $(1.13 \pm 0.01) \times 10^4$ nA $M^{-1}$, with a correlation coefficient of 0.9994 and a detection limit of 0.6 mM (0.11 g $l^{-1}$).

One of the most important problems of first generation glucose biosensors based on the determination of hydrogen peroxide, is the interference of easily oxidizable compounds such as ascorbic acid and uric acid. Since the response for hydrogen peroxide is very important, the interference of uric acid and ascorbic acid oxidation is negligible even despite the catalytic effect of CNTPE toward these compounds, in special ascorbic

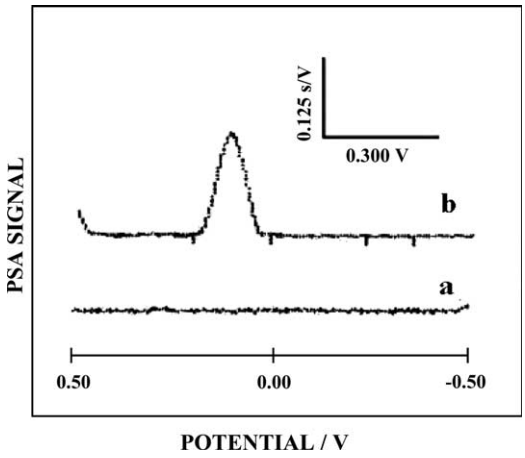


Fig. 5. Chronopotentiograms for 0.100 M nitric acid (a) and 100 ppb copper (b) solutions obtained at CNTPE activated by cycling between −1.00 and 1.50 V at 1.0 V $s^{-1}$ (18 cycles in a 0.050 M phosphate buffer solution, pH 7.40). Deposition: 3 min at −0.50 V. Stripping conditions: electrolyte, 0.100 M nitric acid solution; initial potential, −0.50 V; current, 8.0 μA.

acid. In fact, the interference percentage at −0.100 V related to 5 mM glucose (G), is zero even for $5.0 \times 10^{-4}$ M uric acid (UA) and $2.5 \times 10^{-4}$ M acetaminophen (A). At variance with Teflon-nanotubes composite electrode [12], no interference was observed for $5.0 \times 10^{-4}$ M ascorbic acid, concentration five times higher than the maximum physiological level. Therefore, the CNTPE–GOx allows the highly selective quantification of glucose without adding any membrane or metals particles.

*3.2.2. Quantification of trace metals*

Carbon nanotubes paste electrode also offers an attractive chronopotentiometric stripping operation. Fig. 5 displays a typical chronopotentiogram for the blank (a, 0.100 M $HNO_3$) and 100 ppb copper (b) solutions. Well-defined peaks were obtained following a short preconcentration period (180 s) at −0.500 V onto an activated CNTPE (18 cycles between −1.00 and 1.50 V at 1.0 V $s^{-1}$ in 0.050 M phosphate buffer solution). A detection limit of 1 ppb copper was attained even for such a short accumulation time (not shown). Therefore, the association of the low background current with the efficient electron transfer of carbon nanotubes, allowed convenient quantitation of ppb concentrations of copper, expanding the analytical application of this composite electrode.

## 4. Conclusions

The properties of CNTPEs based on the dispersion of carbon nanotubes within mineral oil have been demonstrated. The new material combines the advantages of composite materials with the electrochemical properties of carbon nanotubes. CNTPEs offer a dramatic improvement in the electrochemical behavior of dopamine, ascorbic acid, uric acid, dopac and hydrogen peroxide. The feasibility of incorporating GOx into the matrix was also illustrated. The important decrease in the hydrogen peroxide reduction overpotential (400 mV) allowed us to develop a highly selective and sensitive glucose biosensor without incorporating metals, redox mediators or membranes. The attractive properties of this new composite material open the doors to new analytical applications.

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