Full Paper

Carbon Nanotubes Paste Electrodes. A New Alternative for the Development of Electrochemical Sensors

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Abstract
In this work we summarize the recent activities of our group regarding the analytical performance of a new composite material, the so-called carbon nanotubes paste electrode (CNTPE) obtained by dispersion of multiwall carbon nanotubes in mineral oil. The electrocatalytic properties towards different redox systems, especially those involved in important enzymatic reactions are discussed. Significant shifting in the overpotentials for the oxidation and/or reduction of hydrogen peroxide, NADH, phenol, catechol, dopamine, ascorbic acid, uric acid and hydroquinone are obtained at CNTPE in comparison with the analogous graphite paste electrode (CPE). The usefulness of the electrode as a matrix for immobilizing enzymes is also demonstrated. Highly sensitive and selective glucose quantification is accomplished even without using permselective films or redox mediators. Enzymatic biosensors obtained by incorporation of lactate oxidase, polyphenol oxidase and alcohol dehydrogenase/NAD^+ within the composite material have allowed the successful quantification of lactate, phenol, dopamine, catechin and ethanol. The sensitive quantification of traces of oligonucleotides and double stranded calf thymus DNA by adsorptive stripping is reported. The confined DNA layer demonstrated to be stable either in air, acetate or phosphate buffer. The advantages of incorporating copper particles for the quantification of amino acids and albumin is also discussed.

Keywords: Multiwall carbon nanotubes, Glucose oxidase, Lactate oxidase, Polyphenol oxidase, Alcohol dehydrogenase, NADH, Catechin, Iridium, Copper, Nucleic acids, Enzymatic biosensors, DNA, Amino acids, Albumin

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1. Introduction

Since 1991, carbon nanotubes (CNTs) have been the center of numerous investigations due to their outstanding structural, electronic and mechanical properties that make them a very unique material [1–6]. Carbon nanotubes are built from sp^2 carbon units and present a seamless structure with hexagonal honeycomb lattices, being several nanometers in diameter and many microns in length [1, 4]. Each end of the nanotube is capped with half of a fullerene molecule. The curvature in the planar hexagonal graphite lattice is obtained from topological defects like pentagons [4, 5].

There are two groups of carbon nanotubes, multiwall (MWCNTs) and singlewall (SWCNTs) carbon nanotubes [4, 5, 7]. MWCNTs can be visualized as concentric and closed graphite tubules with multiple layers of graphite sheet defining a hole typically from 2 to 25 nm and separated by approximately 0.34 nm [4, 5, 7]. SWCNTs consist of a single graphite sheet rolled seamlessly, defining a cylinder of 1–2 nm diameter. MWCNTs can be considered as a mesoscale graphite system while SWCNTs are real single large molecules [8].

The combination of size, structure and topology gives nanotubes important mechanical and surface properties. The electrical properties of CNTs depend sensitively on their diameter and chirality [2, 5, 8]. According to the structural parameters, SWCNTs can be either a metal, semiconductor or small-gap semiconductor [2, 5, 6–8]. Since the topological defects in nanotubes result in local perturbations of their electronic structure, the pentagonal defect of the caps make them more metallic than the cylinders. These defects enhance the chemical reactivity of the ends giving the possibility to functionalize them, to open the tubes and fill them with foreign substances [1, 9, 10].

Due to their properties, CNTs have received enormous attention for the preparation of electrochemical sensors, as it was widely reviewed [3, 11, 12]. Since they are not readily soluble in usual media, different strategies for immobilizing them on the surface of electrodes have been proposed. Dispersion of CNTs in acidic solutions [13, 14], *N*,*N*′-dimethylformamide [15], Nafion [16, 17] and chitosan [18] have been successfully used. The incorporation of CNTs in composite matrices using different binders like Teflon [19], bromoform [20], mineral oil [21–24] and inks [25] has been also proposed. The resulting electrodes modified with CNTs have been employed for the detection of several bioanalytes like glucose [19, 21], DNA [13, 22], homocystein [26], neurotransmitters and related compounds [20, 21, 24, 27], uric acid [28] and ethanol [19, 23], amitrol [29] among others.

In this work we present a review of the current activities of our group on the analytical applications of an electrode proposed by us some years ago, the carbon nanotubes paste electrode (CNTPE), a composite material obtained by dispersion of MWCNTs in mineral oil. The electrochemical behavior of different redox systems at CNTPE as well as the performance of enzymatic electrodes developed by incorporation of enzymes within the CNTPE matrix is reported in the following sections.

2. Experimental

2.1. Reagents

Hydrogen peroxide (30% V/V aqueous solution) was purchased from Baker. Ascorbic acid was obtained from Fluka. Dopamine, 3,4-dihydroxyphenylacetic acid (dopac), glucose oxidase (GOx) (Type X-S, *Aspergillus niger*, (EC 1.1.3.4), 157500 Units per gram of solid, Catalog number G-7141), lactate oxidase (LOx) (from *Pediococcus* species, 36 units/mg solid), alcohol dehydrogenase (ADH) (EC 1.1.1.1. from bakers yeast, 428 units/mg protein, 393 units/mg solid), NAD^+, NADH, lactate, polyphenol oxidase (PPO) (EC 1.14.18.1 from mushroom, 2870 units/mg solid), bovin seric albumin (A-4503) and all amino acids were purchased from Sigma. Uric acid, glucose, hydroquinone, and phenol, L-amino acids were obtained from Merck. Catechol was from Mallinckrodt. Ethanol, methanol and isopropanol were from Baker. Other chemicals were reagent grade and used without further purification. Copper (99% purity) microparticles (−325 mesh, 10% max +325 mesh, 99%) were acquired from Alfa Aesar.

MWCNTs powder (diameter 20–50 nm and lengths of 1–5 microns (short CNTs) and 5–20 microns (long CNTs), 95% purity, were obtained from NanoLab, (U.S.A). Glassy carbon spherical powder 0.4–12 μm, type 2 was purchased from Alfa AEsar. Graphite powder (grade # 38) was from Fisher.

$Oligo_X$ (5′-ATG TGG AAA ATC TCT AGC AGT-3′) was purchased from Life Technologies (Grand Island, New York, USA) as its ammonium salt. Double stranded calf thymus DNA (dsDNA) (activated and lyophilized, catalog number D-4522) was purchased from SIGMA (St. Louis, MO). All other reagents were of analytical grade. DNA stock solutions (nominally 1000 mg/L) were prepared with TE buffer (1 × concentrate, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Ultrapure water ($\rho = 18$ MΩ) from a Millipore-MilliQ system was used for preparing all the solutions.

2.2. Apparatus

The amperometric and voltammetric measurements were performed with EPSILON (BAS) and TEQ-02 potentiostats. 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 DNA accumulation experiments.

Constant-current chronopotentiometric experiments were performed with a TraceLab Potentiometric Stripping Unit PSU 22 (Radiometer, France) connected to a PC. According to the Trace Lab 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 working electrodes were graphite paste electrode (CPE), carbon nanotubes paste electrode (CNTPE) or glassy carbon paste electrode (GCPE). The CNTPE was prepared by mixing in an agata mortar MWCNTs powder and mineral oil (Aldrich) in a ratio 60.0% w/w nanotubes powder and 40.0% w/w mineral oil. CPE and GCPE were prepared in a similar way by mixing graphite powder or glassy carbon microparticles with mineral oil. CPE and CNTPE containing enzymes were prepared in analogous way by including the desired amount of enzyme. CNTPE containing copper (CNTPE-Cu) was prepared in a similar way, mixing first the copper microparticles with the mineral oil for 1 min, followed by the incorporation of the carbon nanotubes powder and additional mixing for 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. In case of DNA experiments, the mineral oil was DNAse, RNAse, protease free (Aldrich)

Scanning Electronic Microscopy (SEM) pictures were obtained with a Hitachi S3000N Microscope equipped with secondary and backscattered electron detectors, as well as with a X-ray detector.

2.3. Procedure

The amperometric and cyclic voltammetric experiments were carried out in a phosphate buffer solution (0.050 M, pH 7.40). The amperometric ones were performed 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.

2.3.1. Nucleic Acids Detection

Nucleic acids detection consisted of the following steps:

- Pretreatment: By applying 1.300 V for 20 s in a 0.200 M acetate buffer solution pH 5.00.
- Nucleic acid immobilization: performed at 0.200 V for a given time by immersion of the pretreated CNTPE in a stirred 0.200 M acetate buffer pH 5.00 containing DNA
- Washing of the DNA modified electrode with a 0.200 M acetate buffer solution pH 5.00 for 5 s.

– Chronopotentiometric transduction: it was performed after medium exchange in a 0.200 M acetate buffer solution pH 5.00 applying a constant current of 8.0 μA with an initial potential of 0.500 V. The anodic signal at around 1.0 V, corresponding to the guanine oxidation, was used as the analytical signal.

Repetitive measurements were carried out by polishing the surface of the electrode on a weighing paper and repeating the above assay. All experiments were performed at room temperature.

2.3.2. Amperometric Determination of Aminoacids

Measurements were conducted in a stirred 0.050 M phosphate buffer solution pH 7.40 by applying a potential of 0.000 V.

2.3.3. Albumin Determination

A square-wave voltammogram between −0.200 V and 1.000 V was obtained after holding the CNTPE-Cu at −0.100 V for 10 min in a stirred solution of albumin. The voltammetric parameters are the following: frequency of 25 Hz, pulse amplitude of 25 mV, staircase step 10 mV and scan rate of 0.250 Vs^{-1}

3. Results and Discussion

3.1. Electrochemical Behavior of Different Analytes at CNTPE

Our group proposed for the first time a new composite material based on the dispersion of MWCNTs within mineral oil, the called carbon nanotubes paste electrode (CNTPE) [21]. The results demonstrated that the presence of the nonconductive phase did not impair the excellent properties of CNTs for the electron transfer. A homogeneous distribution of CNTs within the mineral oil after incorporation into the electrode can be observed in the SEM pictures (Fig. 1).

A potential window comparable to that of CPE and better than that of the analogous glassy carbon composite was obtained in different supporting electrolyte solutions [30]. Figure 2 displays cyclic voltammograms obtained at 0.100 V/s for 1.0×10^{-3} M ascorbic acid (A), uric acid (B), dopamine (C) and hydroquinone (D) at CPE (dotted line) and at CNTPE (solid line). An important decrease in the overvoltages for the oxidation of these redox systems was observed at CNTPE, indicating that CNTs effectively improve the electron-transfer kinetics. It is important to remark that the excellent electrocatalytic properties of the carbon nanotubes composite material were observed even with electrodes containing just 10.0% w/w CNTs (not shown). A decrease in the oxidation peak potential of 230 and 160 mV was obtained at CNTPE for ascorbic acid and uric acid, respectively. A significant improvement in the

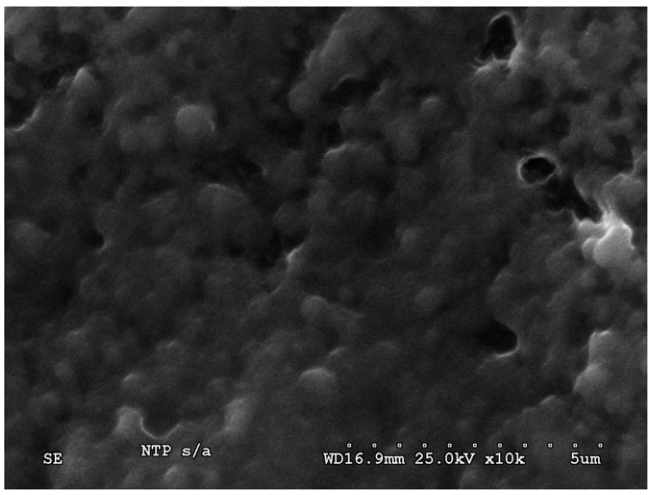

Fig. 1. SEM picture of CNTPE (60.0% w/w CNT and 40.0% w/w oil). Magnification 10 000 × .

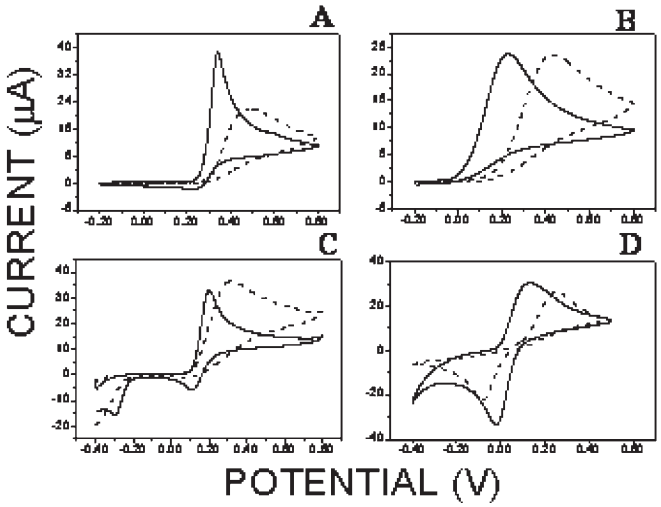

Fig. 2. Cyclic voltammograms for 1.0×10^{-3} M ascorbic acid (A), uric acid (B), dopamine (C) and hydroquinone (D) at different electrodes: CPE (dotted line) and CNTPE (solid line). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Scan rate: 0.100 V/s. CNTPE (60.0% w/w CNT and 40.0% w/w oil. Adapted from Figure 2, Electrochemistry Communications 5 (2003) 689–694.

reversibility was found at the CNTPE for dopamine and hydroquinone, compounds that display a quasireversible behavior at CPE, In fact, the ΔE_p for dopamine and hydroquinone decreases 141 mV and 181 mV, respectively, while the anodic-to-cathodic peak currents ratio decreases from 5.21 to 3.00 for dopamine and from 1.79 to 1.12 for hydroquinone. These results demonstrate that the improved electrocatalytic activity of CNTs due to the nanotubes dimensions, electronic structure and topological defects present on their surface [31, 32], are retained even in the presence of the pasting liquid.

Hydrogen peroxide is an important molecule involved in numerous enzymatic processes. Therefore, the development of new methodologies for the sensitive and selective hydro-

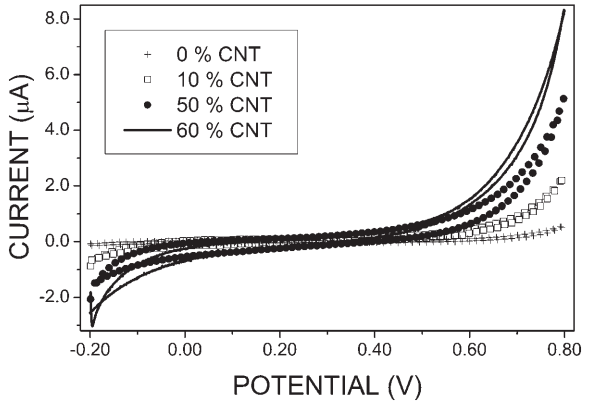

Fig. 3. Cyclic voltammograms for 2.0×10^{-2} M hydrogen peroxide obtained at graphite composite electrodes containing different amounts of carbon nanotubes: 0.0 (+), 10.0 (□), 50.0 (●) and 60.0 (—)% w/w. In all cases the percentage of mineral oil was 40.0% w/w. The 100% w/w was completed, when necessary, with graphite powder. Scan rate: 0.100 V/s. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

gen peroxide determination is receiving enormous attention. Figure 3 shows the cyclic voltammetric response of 2.0×10^{-2} M hydrogen peroxide at carbon composites electrodes containing different amounts of MWCNTs. Even for composites containing 10.0% w/w CNTs, the catalytic effect is clear. The overvoltages for the oxidation and reduction of hydrogen peroxide largely decrease in the presence of CNTs and the oxidation and reduction currents present a noticeable increase. For instance, at -0.100 V the reduction currents for hydrogen peroxide were 0.090, 0.360, 0.790 and 1.360 μA, while the oxidation currents at 0.700 V were 0.250, 0.950, 2.880 and 3.260 μA at carbon composites containing 0.0; 10.0; 50.0 and 60.0% w/w CNTs, respectively. The significant decrease in the overvoltage for the reduction of hydrogen peroxide has allowed working at potentials where the interference of easily oxidizable compounds can be eliminated, opening the doors to many analytical applications connected to enzymatic electrodes based on the use of hydrogen peroxide as analytical signal.

The effect of CNTs on the electrochemical behavior of NADH is also interesting. It is widely known that the oxidation of this compound requires elevated overvoltages at carbon materials and that the electrode is passivated after its oxidation [33]. In the case of CNTPE the oxidation starts at -0.100 V, that is, at potentials 400 mV less positive than at CPE, evidencing once more the unique electrocatalytic properties of CNTs (not shown) [23]. The amperometric response of 1.0×10^{-5} M NADH solution at CNTPE at 0.400 V decreased only 20.0% after 15 min, at variance with the behavior observed at CPE, where a decrease of 80.0% was obtained after the same period (not shown).

3.2. Effect of the Length of CNTs and Paste Composition

The influence of the nature of CNTs on the electrochemical response of the electrodes was also evaluated. Short carbon nanotubes (1 – 5 μm) demonstrated to be more effective for the oxidation of ascorbic acid, dopamine and dopac than the long ones (5 – 20 μm) (not shown). The effect of the content of mineral oil is another important aspect to consider when studying these materials. For instance, no significant differences were observed in the voltammetric parameters of ascorbic acid, dopac and dopamine when using composites containing 40.0 and 45.0% w/w mineral oil. Pastes with 50.0% w/w oil were difficult to handle and a shifting of the peak potentials in the positive direction was observed, suggesting a slower charge transfer. When long CNTs were used to prepare the composite, the situation was different. Pastes containing 40.0% w/w oil were difficult to handle due to the inherent characteristics of the long CNTs that require more oil to obtain a homogeneous dispersion. Pastes with 45.0 and 50.0% w/w long CNTs gave a similar response, demonstrating that with these CNTs is possible to obtain composites with more oil content.

3.3. Effect of Electrochemical Pretreatments

Different schemes for CNTs activation have been proposed. Many of them have been based on the chemical oxidation. In general, as a consequence of the pretreatment, there is an increase in the density of oxygenated functional groups on the surface of CNTs, mainly carboxylic ones [5]. Depending on the pretreatment conditions (nature of the acid, temperature, time and sonication), the tubules can be opened or even shortened [34, 35]. Electrochemical pretreatments have been also used, although, in general, they were used as a complement of a previous chemical oxidation scheme.

The state of the surface of CNTPE plays an important role on the electrode kinetics. For instance, the electrochemical behavior of dopamine was highly dependent on the surface pretreatment. 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 in a 0.050 M phosphate buffer solution pH 7.40 the one that has allowed us to obtain the best response. As it is shown in Figure 4, after this pretreatment the ΔE_p for 1.0×10^{-3} M dopamine decreases 30 mV while the anodic oxidation peak current increases from 34.3 to 64.9 μA and the currents peak ratio decreases from 3.00 to 2.80.

The influence of different potentiostatic and potentiodynamic pretreatments on the adsorption and electrooxidation of DNA was also evaluated. Potentiodynamic pretreatments performed either in a 0.200 M acetate buffer solution pH 5.00 or in a 0.050 M phosphate buffer solution pH 7.40 by cycling the potential between -1.000 and 1.500 V at 1.0 V/s were not effective because they produced a large increase in the background signal that makes the chronopotentiometric detection of guanine electrooxidation very difficult. On the contrary, potentiostatic pretreatments demonstrated to be effective for the adsorption and electrooxidation of oligonucleotides, being the selection of the applied potential and time a critical point. The pretreatments were performed by applying different potentials in a

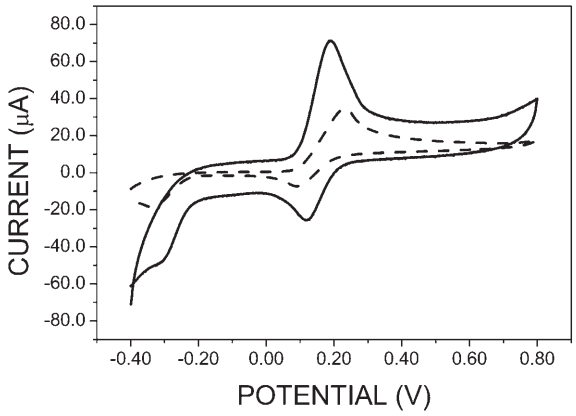

Fig. 4. Cyclic voltammograms for 1.0×10^{-3} M dopamine at untreated CNTPE (dotted line) and at electrochemically activated CNTPE (solid line). Electrochemical pretreatment: 18 cycles between -1.00 and 1.50 V at 1.0 Vs^{-1} in supporting electrolyte. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Scan rate: 0.100 V/s.

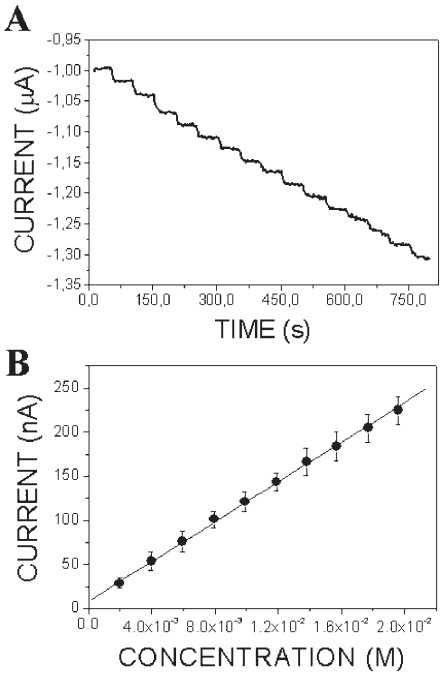

Fig. 5. A) Amperometric recordings obtained at CNTPE-GOx (10.0% w/w) for successive additions of 2.0 mM glucose. B) Average calibration plot obtained from (A). Working potential: -0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Adapted from Figure 4, Electrochemistry Communications 5 (2003) 689–694.

0.200 M acetate buffer solution pH 5.00 for 20 s. The guanine oxidation signal rose with the increase in the activation potential, mainly due to the increase in the density of oxygenated groups on CNTs. However, potentials more positive than 1.300 V produced a large background current as a consequence of the catalytic effect of CNTs on the solvent oxidation. This increase in the background current made the resolution of the guanine oxidation peak very complicated. A pretreatment of 20 s at 1.300 V was enough to improve the guanine oxidation signal. Under those conditions, a signal almost 13 times higher than that at CPE pretreated was obtained. Experiments performed at 1.300 V for different times showed that times longer than 20 s produce a larger increase in the background signal (not shown).

3.4. Enzymatic Biosensors Based on the Use of CNTPE

The dramatic improvement in the electrochemical behavior of NADH, hydroquinone and hydrogen peroxide, represents a very interesting starting point for the development of enzymatic biosensors that involve the detection of these compounds, like oxidases and dehydrogenases-NAD^+ dependent. The performance of the resulting biosensors involving CNTs is discussed below.

3.4.1. CNTPE Modified with Glucose Oxidase and Lactate Oxidase

Based on the excellent electrocatalytic properties of CNTs towards the oxidation and reduction of hydrogen peroxide, the CNTPE was used for the preparation of glucose biosensors by the incorporation of GOx into the composite matrix. As it is widely known, GOx catalyzes the oxidation of glucose to gluconolactone while oxygen, its natural mediator, is converted into hydrogen peroxide [36]. The selection of the working potential is very important to obtain the selective determination of the hydrogen peroxide enzymatically generated. Figure 5A shows the amperometric response at -0.100 V obtained at CNTPE-GOx (10.0% w/w) for successive additions of 2.0 mM glucose. A well defined, fast and very sensitive response was obtained at the CNTPE-GOx. Figure 5B shows the corresponding calibration plot obtained as an average of four independent experiments. The response was linear even up to 25 mM glucose (4.5 g/L), covering, thus, not only the physiological range, but also pathological values. The corresponding sensitivity and detection limit were $(1.13 \pm 0.01) \times 10^4$ nA M^{-1} ($r = 0.9994$) and 0.6 mM (0.11 g/L), respectively. Therefore, the sensitivity obtained with CNTPE-GOx was 43 larger than that with the analogous CPE-GOx.

Figure 6 shows the effect of common interferents on the amperometric response of 5.0×10^{-3} M glucose at -0.100 V using CNTPE after additions of 1.0×10^{-4} M ascorbic acid (AA), 5.0×10^{-5} M acetaminophen and 2.5×10^{-4} M uric acid (UA). No interference of these easily oxidizable compounds was found even at these levels, indicating that under these conditions it is possible to obtain not only a sensitive but also a highly selective glucose determination without adding any membrane or redox mediator.

The usefulness of a CNTPE containing lactate oxidase (LOx) for the detection of lactate was also studied [23]. As expected, due to the noticeable improvement in the electron transfer of hydrogen peroxide at CNTPE, a well defined and fast response to lactate was reported. A linear range was

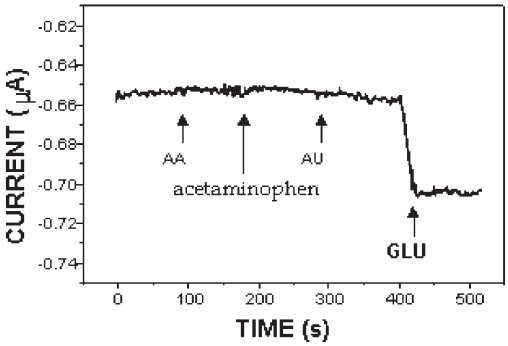

Fig. 6. Current-time profiles performed at CNTPE-GOx (10.0% w/w) for one addition of 1.0×10^{-4} M ascorbic acid (AA), 5.0×10^{-5} M acetaminophen, 2.5×10^{-4} M uric acid (UA) and 5.0×10^{-3} M glucose (GLU). Other conditions as in Figure 5.

observed up to 7.0×10^{-3} M lactate, with a sensitivity of (14.4 ± 0.4) μA M^{-1} and a detection limit of 3.0×10^{-4} M.

3.4.2. CNTPE Modified with Polyphenol Oxidase

Polyphenol oxidase is an enzyme that catalyzes the oxidation of phenols and catechols to the corresponding quinones in the presence of oxygen [23]. Figure 7A shows a calibration plot obtained from amperometric experiments at -0.050 V after successive additions of 1.0×10^{-6} M dopamine. The sensitivity was $(3.36 \pm 0.03) \times 10^3$ μA M^{-1}, that is, 12 times higher than that obtained with CPE-PPO, while the detection limit was 1.0×10^{-6} M.

The CNTPE-PPO was also used for the quantification of polyphenols, compounds that are receiving important attention in the pharmacological and food industry due to their interesting antioxidant properties. The bioelectrode demonstrated to be very useful for the detection of catechin, a polyphenol present in some products like tea and wines. Figure 7B shows the amperometric recordings obtained at -0.050 V for successive additions of 1.0×10^{-5} M catechin. The corresponding calibration plot is shown in Figure 7C. The sensitivity and detection limits were $(2.03 \pm 0.09) \times 10^3$ μA M^{-1} ($r = 0.996$) and 1.0×10^{-6} M, respectively. The electrode demonstrated to be highly stable, since after 60 days the sensitivity was the same as the original one.

3.4.3. CNTPE Modified with Alcohol Dehydrogenase

A fast response was obtained at 0.400 V after additions of 5.0×10^{-3} M ethanol at CNTPE modified with ADH (12.0% w/w) and NAD^+ (12.0% w/w) with a sensitivity of (44 ± 3) μA M^{-1} (not shown) [23]. No response was observed for methanol even for high concentrations. In the presence of isopropanol the sensitivity was one third of that obtained for ethanol.

The electrode demonstrated to be highly stable since after 2 months at 4 °C the response was still the same as the first day, while after 6 months it decreased just 24%. The bioelectrode was used to determine the content of ethanol in different alcoholic beverages.

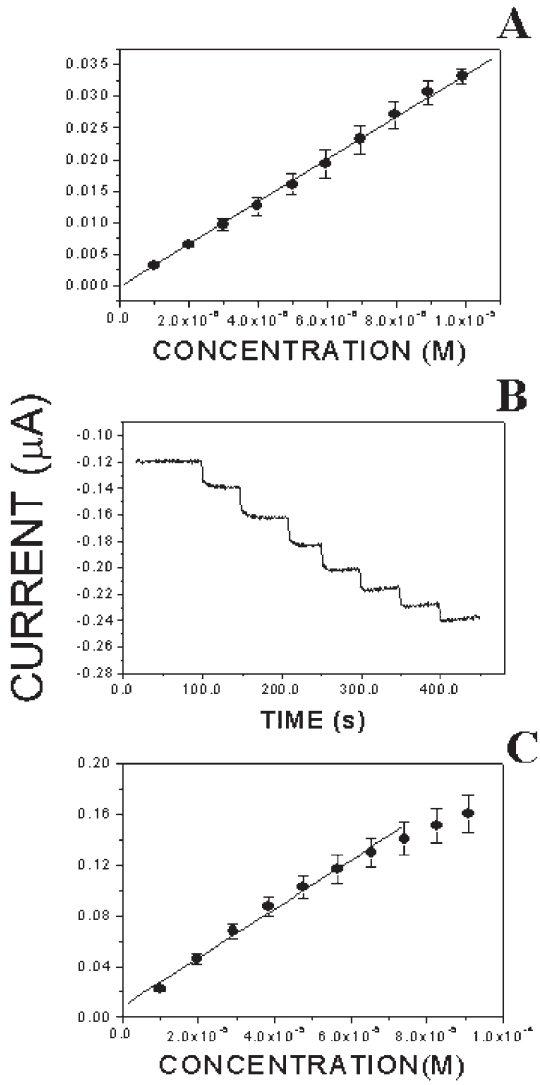

Fig. 7. A) Calibration plot for dopamine obtained from amperometric experiments at PPO-CNTPE. B) Amperometric recording for successive additions of 1.0×10^{-5} M catechine at PPO-CNTPE. C) Calibration plot for catechin obtained from (B). Electrode composition: 1.5% w/w PPO, 60.0% w/w carbon nanotubes, 38.5% w/w mineral oil. Working potential: -0.050 V.

3.5. Direct Quantification of Nucleic Acids at CNTPE

Figure 8 shows the calibration plot for calf thymus double stranded DNA after 5 min accumulation at 0.200 V. There is a linear relationship up to 15.0 mg L^{-1} and a sensitivity of (12.3 ± 0.9) ms L mg^{-1}, ($r = 0.995$). Detections limits of 2 and 170 μg L^{-1} (calculated as 3 times the standard deviation of the blank over the sensitivity), were obtained for oligox and dsDNA, respectively.

The inset of Figure 8 depicts chronopotentiograms for different concentrations of $oligo_X$: 0.30, 0.50, 0.80 and 1.00 mg L^{-1}. The calibration plot for this oligonucleotide gave a sensitivity of (163 ± 5) ms L mg^{-1}, ($r = 0.995$), and a linear range up to 1.00 mg L^{-1} after 1 min accumulation at 0.200 V (not shown).

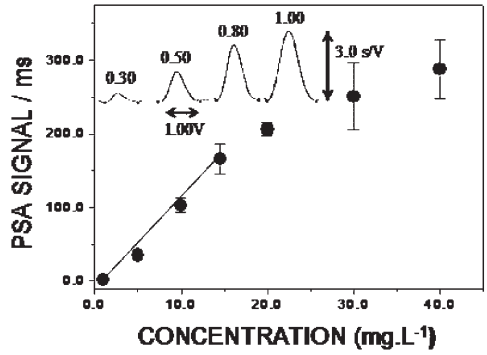

Fig. 8. Calibration plot for dsDNA at pretreated CNTPE. The inset shows chronopotentiograms for oligo $(d_X)_{21}$. Experimental conditions: Adsorption at 0.200 V for 1 min (A) or 5 min (B) in 0.200 M acetate buffer solution pH: 5.00, containing different concentrations of the corresponding nucleic acid. Stripping: in 0.200 M acetate buffer solution pH: 5.00; $i = 8.0\ \mu A$, initial potential: 0.500 V. Adapted from Figure 5, Electrochemistry Communications 6 (2004) 10–16.

The layer of DNA confined to the electrode demonstrated to be stable in air, in 0.200 M acetate buffer pH 5.00 and in 0.020 M phosphate buffer pH 7.40 + 0.50 M NaCl. The stability of the adsorbed nucleic-acid layer upon transfer to the blank solutions results promising for developing different biosensors based on the use of CNTPE and nucleic acids as biorecognition element.

3.6. Effect of the Presence of Copper Microparticles within the CNTPE

3.6.1. Glucose Biosensor

Since copper is an excellent catalyst for the reduction of hydrogen peroxide [37] it was incorporated into the CNTPE to improve the sensitivity of glucose determinations. The careful selection of the amount of copper has allowed an important improvement in sensitivity without compromising the selectivity. A value of 0.77% w/w was selected as the optimum to achieve the best compromise between sensitivity and selectivity [38].

The sensitivity of CNTPE-Cu-GOx was almost three times larger than in the absence of copper, although the linear range was more restricted. The electrode demonstrated to be highly stable since after 60 days at 4 °C the response was almost the same as the first day.

3.6.2. Aminoacids and Albumin Sensor

The incorporation of copper into the carbon nanotubes composite was also used to design amino acids and albumin sensors based on the facilitated copper oxidation due to the complex formation with the amino acid [39].

Figure 9 shows amperometric recordings at − 0.100 V for successive additions of 5.0×10^{-4} M L-histidine, L-lisine and L-proline at CNTPE (a) and CNTPE-Cu (b). Since the

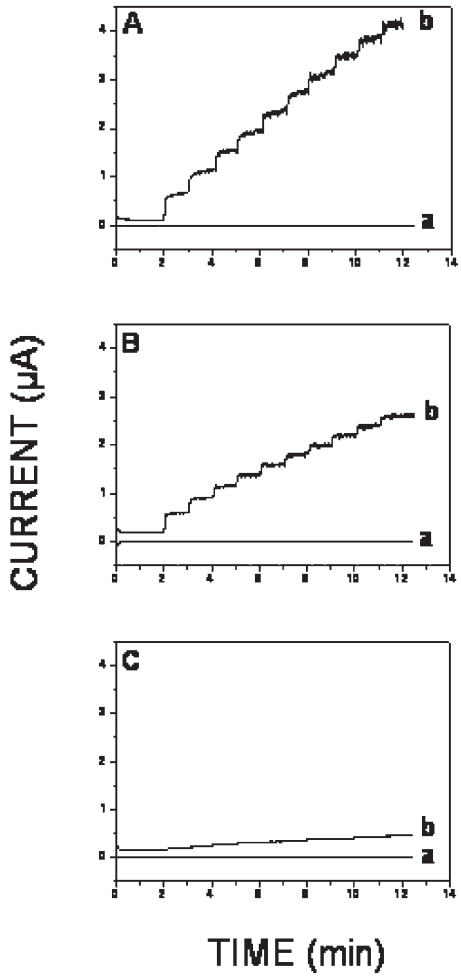

Fig. 9. Amperometric recordings for successive additions of 5×10^{-4} M L-amino acids at CNTPE 60/40 (a) and CNTPE + Cu 18% (b) for A) L-histidine, B) L-lisine, C) L-proline.

amino acids are not electroactive, no response is observed at CNTPE. On the contrary, at CNTPE/Cu, a fast and sensitive response is obtained after successive additions of the amino acids due to the facilitated oxidation of copper. It is important to remark that the sensitivity is highly dependent on the nature of the amino acid due to the strength of the complex formation between Cu (II) and each amino acid [40]. Therefore, the incorporation of copper within CNTPE have allowed us the detection of amino acids, electroactive or not, at very low potentials and at physiological pHs.

Based on these properties, the electrode was also used for the square-wave voltammetric-detection of albumin. Figure 10 shows square-wave voltammograms obtained for 10.0 mg/mL albumin at CNTPE (dotted line) and CNTPE-Cu (solid line). At the composite without metal, there is only the direct oxidation of tyrosine and triptophan residues at around 0.7 V. In the presence of copper, besides the direct amino acids oxidation, there is a huge peak at − 0.100 V due to the facilitated oxidation of copper in the presence of the amino acid residues. A linear relationship between peak current and concentration was obtained up to 25.0 mg/L albumin. The methodology was validated using the classical

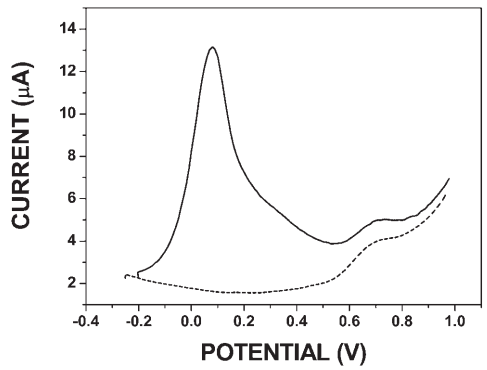

Fig. 10. Square-wave voltammograms for 10.0 mg/mL albumin at CNTPE (dotted line) and at CNTPE-Cu (18.0%, w/w) (solid line). Conditions: protein accumulation at −0.100 V for 10 min followed by square-wave voltammogram between −0.200 and 1.000 V. Frequency of 25 Hz, pulse amplitude of 25 mV, staircase step 10 mV and scan rate of 0.250 V s^{-1}.

spectrophotometric determination, with excellent correlation.

4. Conclusions

CNTPE combines the advantages of composite materials with the unique electrocatalytic properties of carbon nanotubes. The increased reactivity of carbon nanotubes has allowed the fast and highly sensitive detection of different analytes like hydrogen peroxide, ascorbic acid, uric acid, dopamine, dopac, NADH and hydroquinone. Trace levels of nucleic acids could be also detected by adsorptive stripping at CNTPE.

The feasibility to incorporate enzymes and cofactors into the composite matrix has paved the way for the construction of different enzymatic electrochemical biosensors. For instance, the important decrease in the reduction overpotential of hydrogen peroxide (400 mV) has allowed the highly sensitive and selective glucose quantification based on the incorporation of GOx into the composite matrix without need of redox mediators or anti-interferents membranes. The CNTPE has also offered an attractive chronopotentiometric detection of metals, like copper, associated with very short accumulation times [21], expanding, in this way, the analytical application of this composite electrode.

The incorporation of copper into the CNTPE has allowed the highly sensitive detection of electroactive and non electroactive amino acids and albumin at very low potentials and at physiological pH.

In summary, this new composite material represents a very good alternative for designing novel electrochemical sensors with a large number of analytical applications.

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6. References

[1] S. Iijima, *Nature* **1991**, *354*, 56.
[2] H. Dai, *Acc. Chem. Res.* **2002**, *35*, 1035.
[3] C. E. Banks, R. G. Compton, *Analyst* **2006**, *131*, 15.
[4] M. L. Cohen, *Mater. Sci. Eng. C* **2001**, *15*, 1.
[5] P. M. Ajayan, *Chem. Rev.* **1999**, *99*, 1787.
[6] P. M. Ajayan, T. W. Ebbesen, *Rep. Prog. Phys.* **1997**, *60*, 1025.
[7] H. Dai, *Surface Science* **2002**, *500*, 218.
[8] P. M. Ajayan, O. Z. Zhou, *Applications of Carbon nanotubes in Carbon nanotubes* (Eds: G. Dresselhaus, Ph. Avouris) Springer, Heidelberg, **2001**, pp. 391 – 425.
[9] P. M. Ajayan, T. W. Ebbesen, T. Ichihashi, S. Iijima, K. Tanigaki, H. Hiura, *Nature* **1993**, *362*, 522.
[10] S. C. Tsang, Y. K. Chen, P. J. F. Harris, M. L. H. Green, *Nature* **1994**, *372*, 159.
[11] J. Wang, *Electroanalysis* **2005**, *17*, 7.
[12] M. Valcarcel, B. M. Simonet, S. Cardenas, E. B. Suarez, *Anal. Bioanal. Chem.* **2005**, *382*, 1783.
[13] M. Musameh, J. Wang, A. Merkoci, Y. Lin, *Electrochem. Commun.* **2002**, *4*, 743.
[14] J. Wang, A.-N. Kawde, M. Musameh, *Analyst* **2003**, *128*, 912.
[15] X. X. Yan, D.-W. Pang, Z.-X. Lu, J.-Q. Li, H. Tong, *J. Electroanal. Chem.* **2004**, *1*, 47.
[16] J. Wang, M. Musameh, Y. Lin, *J. Am. Chem. Soc.* **2003**, *125*, 2408.
[17] G. A. Rivas, S. A. Miscoria, J. Desbrieres, G. Barrera, *Talanta* **2007**, *71,* 270.
[18] M. Zhang, A. Smith, W. Gorski, *Anal. Chem.* **2004**, *76,* 5045.
[19] J. Wang, M. Musameh, *Anal. Chem.* **2003**, *75,* 2075.
[20] P. J. Britto, K. S. V. Santhanam, P. M. Ajayan, *Bioelectrochem. Bioenerg.* **1996**, *41*, 121.
[21] M. D. Rubianes, G. A. Rivas, *Electrochem. Commun.* **2003**, *5*, 689.
[22] M. L. Pedano, G. A. Rivas, *Electrochem. Commun.* **2004**, *6*, 10.
[23] M. D. Rubianes, G. A. Rivas, *Electroanalysis* **2005**, *17*, 73.
[24] F. Valentini, A. Amine, S. Orlanducci, M. L. Terranova, G. Palleschi, *Anal. Chem.* **2003**, *75*, 5413.
[25] J. Wang, M. Musameh, *Analyst* **2004**, *129*, 1.
[26] N. S. Lawrence, R. P. Deo, J. Wang, *Talanta* **2004**, *63*, 443.
[27] M. Chicharro, A. Sánchez, E. Bermejo, A. Zapardiel, M. D. Rubianes, G. A. Rivas, *Anal. Chim. Acta* **2005**, *543*, 84.
[28] Z. Wang, Y. Wang, G. A. Luo, *Analyst* **2002**, *127*, 1353.
[29] M. Chicharro, E. Bermejo, M. Moreno, A. Sánchez, A. Zapardiel, G. A. Rivas, *Electroanalysis* **2005**, *17*, 476.
[30] M. C. Rodríguez, G. A. Rivas, *Anal. Chim. Acta* **2002**, *459*, 43.
[31] P. J. Britto, K. S. V. Santhanam, V. Alonso, A. Rubio, P. M. Ajayan, *Adv. Mater.* **1999**, *11*, 154.
[32] T. W. Odom, J.-L. Huang, P. Kim, C. M. Lieber, *J. Phys. Chem. B* **2000**, *104*, 2794.
[33] C. Hua, S. Walsh, M. Smyth, I. Švancara, K. Vytřas, *Electroanalysis* **1992**, *4*, 107.

[34] O. Zhou, H. Shimoda, B. Gao, S. Oh, L. Fleming, G. Yue, *Acc. Chem. Res.* **2002**, *35*, 1045.
[35] C. G. Hu, W. L. Wang, S. X. Wang, W. Zhu, Y. Li., *Diamond Rel. Mater.* **2003**, *12*, 1295.
[36] M*ethods in Enzymology,* Vol. XLIV (Ed: K. Mosbach), Academic Press, London **1976**.
[37] M. C. Rodríguez, G. A. Rivas, *Electroanalysis* **2001**, *13*, 1179.
[38] G. L. Luque, N. F. Ferreyra, G. A. Rivas, *Microchim. Acta* **2006**, *152*, 277.
[39] J.-M. Zen, C.-T. Hsu, A. S. Kumar, H.-J. Lyu, K.-Y. Lin, *Analyst* **2004**, *129*, 841.
[40] G. L. Luque, N. F. Ferreyra, G. A. Rivas, *Talanta* **2007**, *71,* 1282.

