Glassy Carbon Electrodes Modified with Multiwall Carbon Nanotubes Dispersed in Polylysine

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Abstract
We report the analytical performance of glassy carbon electrodes (GCE) modified with a dispersion of multiwall carbon nanotubes (MWCNT) in polylysine (Plys) (GCE/MWCNT-Plys). The resulting electrodes show an excellent electrocatalytic activity towards different bioanalytes like ascorbic acid, uric acid and hydrogen peroxide, with important decrease in their oxidation overvoltages. The dispersion of 1.0 mg/mL MWCNT in 1.0 mg/mL polylysine is highly stable, since after 2 weeks the sensitivity for hydrogen peroxide at GCE modified with this dispersion remained in a 90% of the original value. The MWCNT-Plys layer immobilized on glassy carbon electrodes has been also used as a platform to build supramolecular architectures by self-assembling of polyelectrolytes based on the polycationic nature of the polylysine used to disperse the nanotubes. The self-assembling of glucose oxidase has allowed us to obtain a supramolecular multistructure for glucose biosensing. The influence of glucose oxidase concentration and adsorption time as well as the effect of using polylysine or MWCNT-Plys as polycationic layers for further adsorption of GOx is also evaluated.

Keywords: Carbon nanotubes, Glassy carbon, Enzymatic biosensor, Glucose oxidase, Glucose biosensor, Catalysis, Polylysine, Carbon nanotubes dispersion, Electrochemical sensor

1. Introduction

Carbon nanotubes (CNTs) can be described as a sheet of carbon atoms rolled up into a cylinder held together by van der Waals interactions [1–5]. Multiwall carbon nanotubes (MWCNT) consist of concentric cylindrical shells of graphene sheets arranged around a central hollow area. Since their discovering [1], they have gained considerable interest due to their unique structural, mechanical and chemical properties [1–5].

The first attempt to use CNTs for developing an electrochemical sensor based on CNTs was performed by Britto et al. [6] who demonstrated the excellent electrocatalytic properties of single wall carbon nanotubes (SWCNTs) dispersed within bromoform. Since then, the interest of using CNTs for developing modified electrodes has largely increased due to their known advantages connected with the high surface area, favorable electronic properties and electrocatalytic effects [7–11]. Compton et al. [12–14] have demonstrated that the interesting electrocatalytic properties of CNTs are mostly due to the presence of defects like the edge planes of pyrolytic graphite located mainly at the end of the tubes.

A major problem to manipulate CNTs is their insolubility or poor solubility in common solvents. Therefore, the selection of the conditions for a proper solubility or dispersion of CNTs is a major challenge for the development of electrochemical sensors based on CNT. In such a sense, the dispersion in solvents [15], surfactants [16, 17] polymers [18, 25], and mineral oil [26–29] have demonstrated to be very efficient.

Here, we report for the first time the design and characterization of electrochemical transducers based on the modification of glassy carbon electrodes (GCE) with multi-wall carbon nanotubes (MWCNT) dispersed in polylysine (Plys). The advantages of the new transducer are demonstrated in connection with the detection of electrochemical bioanalytes like hydrogen peroxide, ascorbic acid and uric acid. The feasibility to use the MWCNT-Plys layer immobilized at the GCE as a platform to build supramolecular multistructures by self-assembling of polyelectrolytes is also discussed, in this case using glucose oxidase (GOx).

2. Experimental

2.1. Reagents

Ascorbic acid (AA) was obtained from Fluka and uric acid (UA) was from Merck. Polylysine (Plys), average MW > 30 000, Catalog number P9494) was purchased from Sigma.

Ferrocenemethanol (FcOH) and potassium ferricyanide (FCN) were from Aldrich. Multiwalled carbon nanotubes powder (MWCNT, (30 ± 15) nm diameter, $(1-5)$ microns length) was obtained from NanoLab (USA). Hydrogen peroxide and ethanol were from Baker. Other chemicals were reagent grade and used without further purification. Ultrapure water ($\rho = 18$ MΩ cm) 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.

2.2. Apparatus

Scanning Electronic Microscopy (SEM) images were obtained with a Hitachi S3000N Microscope equipped with secondary and backscattered electron detectors.

IR experiments were performed with a Nicolet 5-SXC FT-IR spectrometer. Samples for IR were prepared in ethanol/water 50/50 V/V and sonicated for 15 min.

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, 3 M NaCl (BAS, Model RE-5B) 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 Electrochemical Microscopy (SECM) measurements were performed with a CHI 900 (CH Instruments Inc., USA). $A \approx 10$ μm diameter homemade carbon fiber electrode served as SECM tip while a glassy carbon electrode (GCE) of 3 mm diameter (Model CHI104, CH Instruments) modified with MWCNT-Plys was used as SECM substrate. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. The experiments were carried out in a 0.10 M phosphate buffer solution pH 7.40, using FCN and FcOH as redox mediators. Cyclic voltammograms were recorded for each redox mediator at 0.100 V s^{-1}. The tip potential was held at 0.000 V to produce the reduction of FCN or 0.500 V to produce the oxidation of FcOH, while the substrate potential was held at 0.500 V or 0.000 V to permit the feedback between the electrodes, since FCN$_{red}$ or FcOH$_{ox}$ generated at the tip can be regenerated to their parent compounds. An approach curve was conducted on the CNT film at a tip scan rate of 0.5 μm/s. The tip was stopped when i_T reached ca. 1.25 times the value of $i_{T,\infty}$. ($i_{T,\infty} = 4\ nFDCa$, where F is the Faraday constant, n is the number of electrons transferred in the tip reaction, D is the diffusion coefficient of the electroactive species, C is the bulk concentration of the species and a is the tip radius). According to the theoretical curve that describes the dependence of i_T with the distance tip-substrate (d), 1.25 times of $i_{T,\infty}$ corresponds to a $d \approx 10$ μm, when using a 5 μm tip radius [30]. After the approach curve a series of constant height 100 μm × 100 μm area SECM images were recorded at a tip scan rate of 1 μm/s. The results are presented in the

dimensionless form of I_T, by normalizing the experimental feedback current (i_T) to the steady-state current obtained when the tip was far from the substrate ($i_{T,\infty}$), i.e., $I_T = i_T/i_{T,\infty}$.

2.3. Preparation of the Working Electrodes

2.3.1. Preparation of MWCNT-Plys Dispersion

It was obtained by dispersing 1.0 mg of MWCNTs within 1.0 mL of 1.0 mg/mL Plys solution (prepared in 50:50 v/v ethanol/water) followed by sonication for 15 min.

2.3.2. Preparation of Glassy Carbon Electrodes Modified with MWCNT-Plys Dispersion (GCE/MWCNT-Plys)

The glassy carbon electrode (GCE) was polished with alumina slurries of 1.0, 0.30, and 0.05 μm for 2 min each. Then, it was modified with the MWCNT-Plys dispersion in the following way: an aliquot of 20 μL was dropped on top of a polished GCE and then the solvent was allowed to evaporate at room temperature.

2.3.3. Preparation of GCE/MWCNT-Plys/GOx/(Plys/GOx)$_n$

The biosensor was prepared in the following way: the GCE was cast with 20 μL of the MWCNT-Plys dispersion, and after drying, it was immersed in a 2.5 mg/mL GOx solution (prepared in 0.050 M phosphate buffer solution pH 7.40) for 20 min. For building the multilayers system, the electrode was alternately immersed in 1.0 mg/mL Plys and 2.5 mg/mL GOx solutions for 20 min. In some experiments, the layer of Plys was obtained by dropping 20 μL of Plys solution on the GCE/MWCNT-Plys/GOx, continuing with GOx immobilization once it was dry.

2.3.4. Preparation of GCE/MWCNT-Plys/GOx/ (MWCNT-Plys/GOx)$_n$

The biosensor was prepared by casting the GCE with 20 μL of the MWCNT-Plys dispersion and after drying, it was immersed in a 2.5 mg/mL GOx solution (prepared in 0.050 M phosphate buffer solution pH 7.40) for 20 min. To build the multilayers system, 20 μL of MWCNT-Plys were dropped on the top of the electrode and allowed to dry. The next step was the immersion of the resulting electrode in a 2.5 mg/mL GOx solution for 20 min. The following layers were obtained by alternating the last two steps.

3. Results and Discussion

3.1. Characterization of MWCNT-Plys Dispersion

Figure 1 displays a SEM picture of GCE/MWCNT-Plys obtained at $20\,000 \times$. The image reveals that CNTs are well entrapped within the polymeric net and homogeneously distributed covering the whole glassy carbon surface.

Figure 2 displays IR spectra for MWCNT (a), MWCNT dispersed in Plys (b) and Plys (c). The spectrum for unmodified MWCNT shows a weak peak at 1648 cm^{-1} assigned to C=C stretching of phenyl ring vibration. IR spectrum for Plys shows the typical profile with a peak at 3260 cm^{-1} due to the asymmetrical and symmetrical N−H stretching vibrations, two peaks at 2860 y 2940 cm^{-1} due to the stretching of CH y CH$_2$, and two peaks at 1660 and 1530 cm^{-1} due to the stretching of amide I (C=O) and the bending of amide II (C−N), respectively. The spectrum of MWCNT-Plys dispersion is very similar to that of the Plys alone. No shifting of the typical bands of the polycation is observed, indicating that there are not changes in the functional groups of Plys and
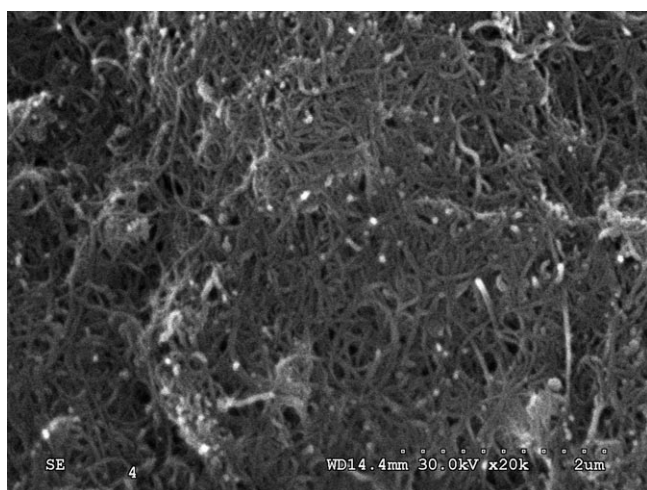

Fig. 1. SEM pictures obtained for a GCE modified with the dispersion of MWCNTs in Plys. Magnification: 20 000 × .

that the interaction with MWCNT is weak. If the MWCNT-Plys dispersion is filtered before obtaining the spectrum, weak bands due to the stretching of amide I (C=O) and the bending of amide II (C−N) of polylysine are obtained (not shown). These results confirm that the interaction of MWCNT with Plys is weak and non-covalent and that Plys is mainly wrapped on the surface of MWCNT.

The glassy carbon surface modified with MWCNT dispersed in Plys was also studied by SECM. Figure 3A displays the images obtained when the tip is scanned in close proximity (ca. 10 μm) to the substrate electrode (GCE/MWCNT-Plys) using FCN (a) and FcOH (b) as redox mediators. An irregular topography, compared to the one obtained for bare GCE [21], is observed at GCE/MWCNT-Plys. These results indicate that CNTs are electrically connected with GCE and that there are regions of different electroactivity according to the distribution of CNTs on the electrode surface. No high conductivity spots were found, suggesting that CNT were homogeneously dispersed before modifying the electrode. One interesting aspect is that, at variance with the expected behavior, the normalized current (I_T) of the images varies depending on the redox mediator. When using FCN the I_T was higher than the one obtained when using FcOH (1.44 vs. 1.28 μA, respectively), indicating that some other process, in addition to the electron transfer, is taking place. Cyclic voltammograms of FCN at GCE/MWCNT-Plys (Figure 3B, c) show a $\Delta E_p = 36$ mV and i_{pa}/i_{pc} ratio greater than one for the Fe(CN)$_6^{3-}$/Fe(CN)$_6^{4-}$ couple, suggesting that the increase in the charge density after reduction of FCN would facilitate the adsorption of the reduced compound (Fe(CN)$_6^{4-}$) at the electrode surface, producing an enhancement in the associated current. On the other hand, the CV for FcOH (Fig. 3B, d) shows a $\Delta E_p =$
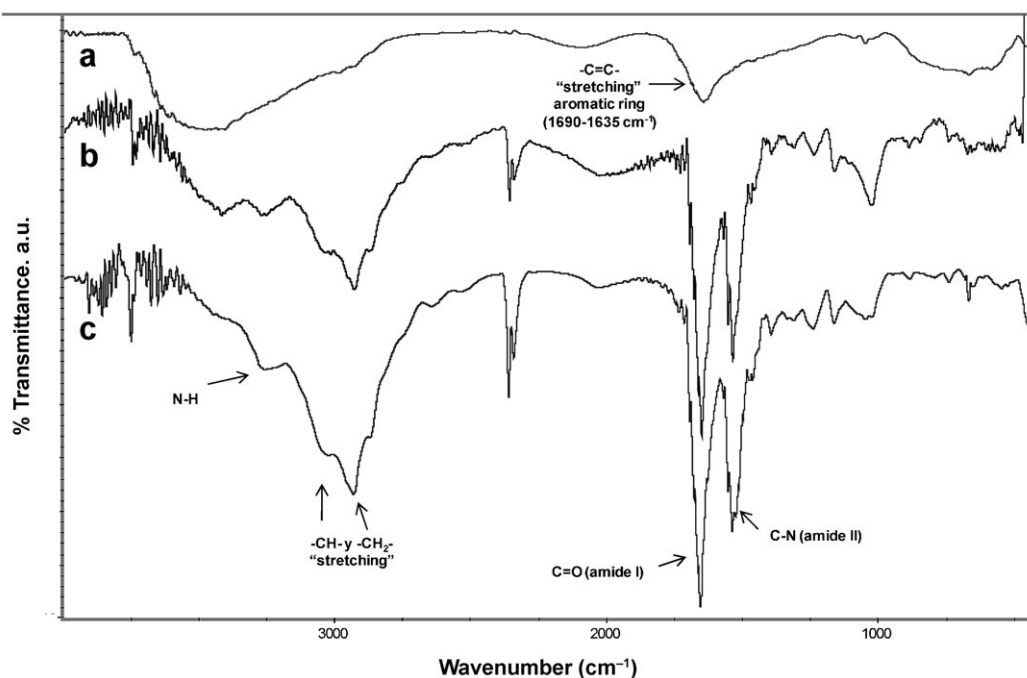

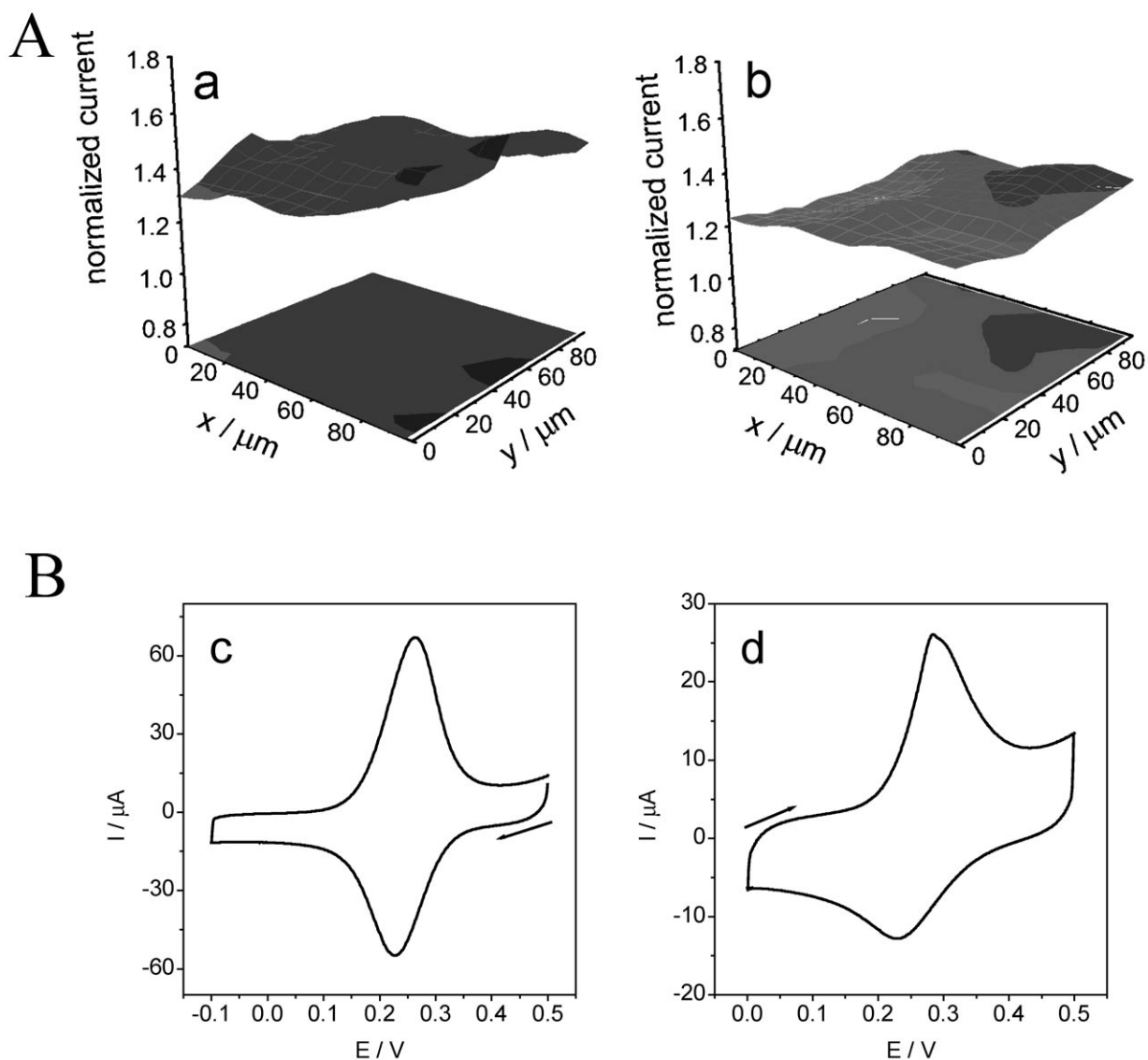Fig. 2. FTIR spectrum of a) raw MWCNT, b) MWCNT-Plys and c) Plys.

Fig. 3. A) SECM surface-plot images of GCE/MWCNT-Plys recorded using the feedback mode in solution of 1.0 mM FCN (a) and 1.0 mM FcOH (b). Tip carbon fiber of 5 μm radius; image parameters: 100×100 μm at 1 μm/s tip scan. B) Cyclic voltammograms for 1.0 mM FCN (c) and 1.0 mM FcOH (d) at GCE/MWCNT-Plys. Scan rate: 0.100 V/s. Supporting electrolyte 0.100 M phosphate buffer pH 7.40.

58 mV, indicating that the one-electron process is not complicated with the adsorption of the redox mediator in the film. Furthermore, a peak current ratio close to 0.8 was observed, probably due to a repulsion between the oxidation product of FcOH, the ferricinium ion, and the positively charged film producing a lower feedback current in SECM images.

Cyclic voltammetry can also help in the characterization of the behavior of GCE/MWCNT-Plys. Figure 4A shows cyclic voltammograms for 2.0×10^{-2} M hydrogen peroxide at GCE (a and inset) and at GCE/MWCNT-Plys (b). At variance with the behavior observed at GCE, at GCE/ MWCNT-Plys there is a very significant decrease in the overvoltages for the oxidation and reduction of hydrogen peroxide, as well as an important increase in the associated currents. The oxidation starts at 0.300 V, while the reduction

currents appear at potentials more negative than 0.050 V, clearly evidencing the advantages of CNTs on the analytical performance of the electrode. Figure 4B shows the calibration plots for hydrogen peroxide obtained from amperometric experiments at bare GCE (a) and GCE/MWCNT-Plys (b) at 0.700 V. In agreement with Figure 4A, the response at the bare electrode (a) is very poor (sensitivity = (42 ± 2) μA M^{-1}), while huge signals are obtained at the electrode modified with CNTs (sensitivity = $(13.0 \pm 0.8) \times 10^3$ μA M^{-1}), demonstrating once more the significant enhancement in sensitivity for hydrogen peroxide oxidation in the presence of CNT. These results indicate that CNTs keep their electrocatalytic activity even when dispersing within the Plys matrix, and that the successful dispersion of CNTs produces a noticeable increase in the active area once immobilized on the glassy carbon surface. The stability of

GCE/MWCNT-Plys is a very important parameter, especially considering further applications for the development of biosensors. No changes in the cyclic voltammetric profiles of the supporting electrolyte at a given GCE/MWCNT-Plys were observed after 60 min use. Moreover, ten successive calibration plots for hydrogen peroxide (obtained from amperometric experiments at 0.700 V), gave an *RSD* of 3.8% for the average sensitivity, clearly demonstrating the stability of the MWCNT-Plys layer.

Geckeler et al. [31] established that Plys can be irreversibly adsorbed on CNTs. TEM images showed that SWCNTs are fully covered or wrapped with Plys after high-speed vibration milling. Molecular models suggested that the free amino groups of Plys are adsorbed or strongly interact on the surface of SWCNTs. Chen et al. [32] demonstrated that the mechanism of Plys-CNTs interaction in aqueous medium could be due to hydrophobic interactions between the hydrocarbon backbone of the polymer and the sidewalls of CNTs, or cation-π interaction between
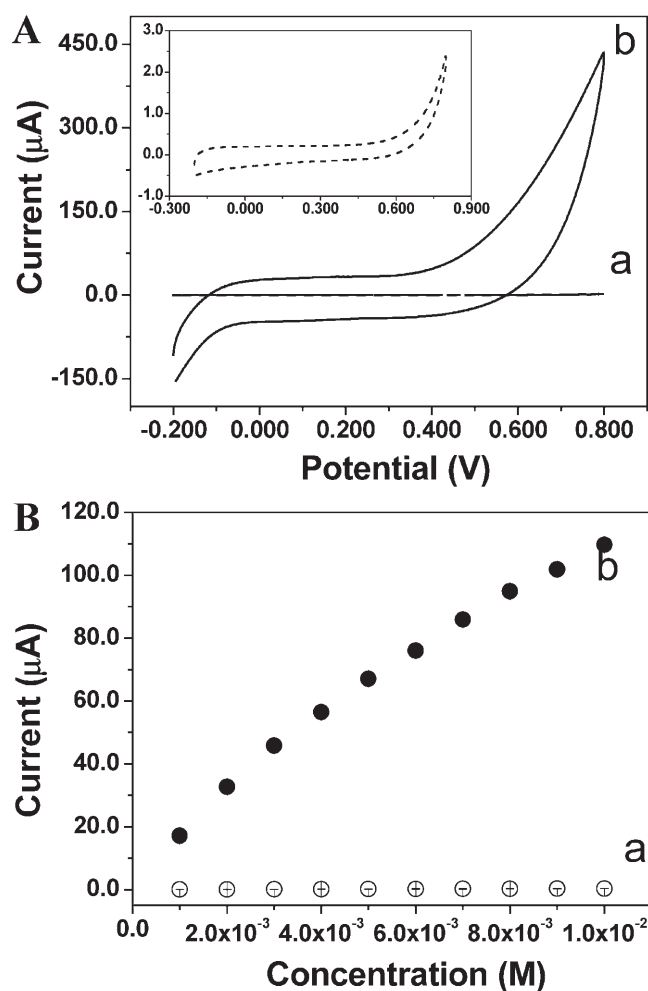
Table 1. Effect of the amount of MWCNT dispersed within 1.0 mg/mL polylysine on the sensitivity for amperometric determinations of hydrogen peroxide at 0.500 V.

MWNTC amount (mg mL^{-1})	Sensitivity (μA M^{-1})
0.5	$(42 \pm 3) \times 10^2$
1.0	$(59 \pm 5) \times 10^2$
2.0	$(104 \pm 3) \times 10^2$
3.0	$(116 \pm 3) \times 10^2$

protonated amino groups and the π-electron system of the SWCNTs. These interactions would be responsible for obtaining stable dispersions that allow a highly efficient response once modifying the glassy carbon surfaces.

The effect of the amount of CNTs dispersed in polylysine solution (0.5; 1.0; 2.0 and 3.0 mg/mL MWCNT) once immobilized on the top of the glassy carbon surface was also evaluated from amperometric experiments for hydrogen peroxide at 0.500 V (Table 1). The sensitivity increases almost linearly with the amount of CNTs in the Plys solution from 0.5 to 3.0 mg/mL MWCNT. However, even when higher sensitivities are obtained for dispersions containing 2.0 and 3.0 mg/mL MWCNT, they were not stable. In both cases, after 24 hours preparation, two clearly distinguished phases were observed, making them useless. Therefore, 1.0 mg/mL dispersion was selected as the best compromise between sensitivity and stability.

The MWCNT-Plys immobilized at GCE demonstrated to be highly stable. The sensitivity obtained from amperometric experiments for hydrogen peroxide at 0.500 V decreased 10.0% after two weeks of the preparation day, while after 20 days the sensitivity decreases almost 20% (not shown).

The advantages of MWCNTs present at GCE/MWCNT-Plys were also demonstrated using two other interesting bioanalytes, AA and UA. Figure 5 shows cyclic voltammograms for 1.0×10^{-3} M AA (A) and UA (B) obtained at GCE (a) and GCE/MWCNT-Plys (b). At GCE/MWCNT-Plys there is a shifting of 176 mV and 95 mV in the peak potential oxidation for AA and UA, respectively, as well as a noticeable enhancement in the oxidation peak currents (5 and 15-folds enhancement for AA and UA, respectively), mainly due to the significant increase in the electroactive area of the resulting GCE/MWCNT-Plys.

Fig. 4. A) Cyclic voltammograms for 2.0×10^{-2} M hydrogen peroxide at GCE (a) and at GCE/MWCNT-Plys (b) (The inset shows the current-time profiles for hydrogen peroxide at GCE in an expanded scale). B) Calibration plots obtained from amperometric experiments performed at 0.700 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Scan rate: 0.100 V/s.

3.2. Use of MWCNT-Plys as Platform for (Bio)Sensor Design

Layer-by-layer (LbL) self-assembling of multilayers has demonstrated to be a very successful alternative to prepare (bio)sensors due to the versatility that the technique offers regarding the rational design of biosensors at molecular level [33–36]. Li et al. [37–39] have proposed several enzymatic biosensors obtained by LbL self-assembling of poly(diallyldimethylammonium) and glucose oxidase [37], choline oxidase and horseradish peroxidase [38] or acetylcholinesterase [39] on a glassy carbon surface modified with
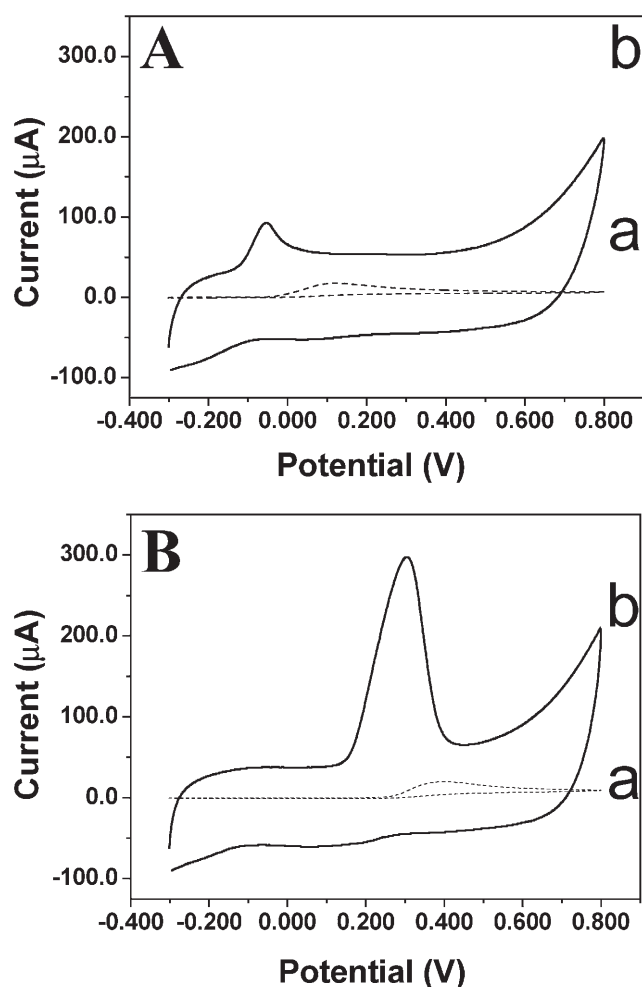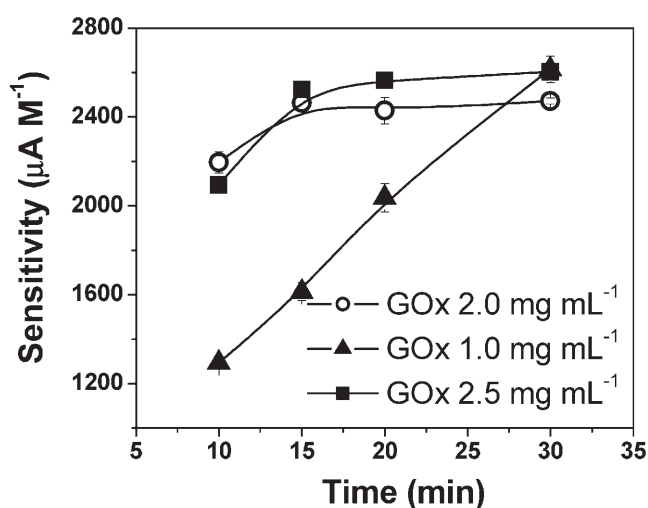
Fig. 6. Sensitivity of GCE/MWCNT-Plys/GOx as a function of the deposition time of GOx for different concentrations of GOx: 1.0; 2.0; and 2.5 mg/mL. The sensitivities were obtained from amperometric recordings at 0.700 V for successive additions of glucose. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

Fig. 5. Cyclic voltammograms for 1.0×10^{-3} M of ascorbic acid (A) and uric acid (B) at GCE (a) and at GCE/MWCNT-Plys (b). Other conditions as in Figure 4.

carboxylated MWCNT. In this work, we took advantage of the polycationic nature of Plys to evaluate the use of the MWCNT-Plys layer immobilized at GCE as a platform for building supramolecular multistructures by layer-by-layer (LbL) self-assembling of polyelectrolytes. We demonstrate this concept by studying the immobilization of glucose oxidase (GOx) at GCE/MWCNT-Plys.

GOx adsorption conditions demonstrated to be critical for the development of an efficient supramolecular multi-structure devoted to glucose quantification. Figure 6 displays the effect of GOx adsorption time for different concentrations of GOx (1.0; 2.0; and 2.5 mg/mL) on the sensitivity obtained from amperometric determinations at 0.700 V after successive additions of glucose. The analytical signal is due to the oxidation of the hydrogen peroxide enzymatically generated. In general, the sensitivity increases for longer adsorption times. In the case of 1.0 mg/mL GOx solution, even after 30 min adsorption there is a linear relationship between sensitivity and adsorption time. As the GOx concentration increases, the deposition times for obtaining the maximum sensitivity become shorter, due to

a faster screening of the MWCNT-Plys charges, being 15 min for 2.0 and 2.5 mg/mL GOx. The GOx concentration and adsorption time selected as optimum were 2.5 mg/mL and 20 min, respectively.

Figure 7 displays amperometric recordings obtained at 0.700 V at GCE/MWCNT-Plys/GOx after successive additions of 0.20 mM glucose solution (A) as well as the corresponding calibration plot (B). This plot represents the average of calibration plots obtained for 7 electrodes prepared with the same dispersion. Under these conditions, the average sensitivity is $(28 \pm 3) \times 10^2 \, \mu A \, M^{-1}$ ($r = 0.9993$; RSD of 11%) and the detection limit is 32 µM. The linear range goes up to 1.4 mM glucose. The RSD for sensitivities obtained using four different dispersions was 9.4%. These results are clear evidence that the MWCNT-Plys layer behaves as a "precursor" film allowing the efficient self-assembling of GOx. The average sensitivity for glucose after ten calibration plots obtained from successive amperometric experiments at 0.700 V using the same GCE/MWCNT-Plys/GOx was $(27.7 \pm 0.8) \times 10^2 \, \mu A \, M^{-1}$, $r = 0.9997$; RSD of 2.9%. Therefore, even after 2.5 hours continuous use, the sensitivity remains highly constant, evidencing the great stability of the biorecognition layer.

The interference of easily oxidizable compounds usually present in biological fluids on the amperometric response of glucose (considered as 100%) was also evaluated. The effect of 1.0×10^{-4} M AA and 4.0×10^{-4} M UA (maximum physiological concentrations) on the amperometric response of 0.50 mM glucose at 0.700 V was studied at GCE/MWCNT-Plys/GOx and at GCE/MWCNT-Plys/GOx/Nafion. An important interference (57% for AA and 275% for UA) is observed when using GCE/MWCNT-Plys/GOx, while it drastically decreases (to 0.0%) when the electrode is covered with a layer of Nafion (not shown).
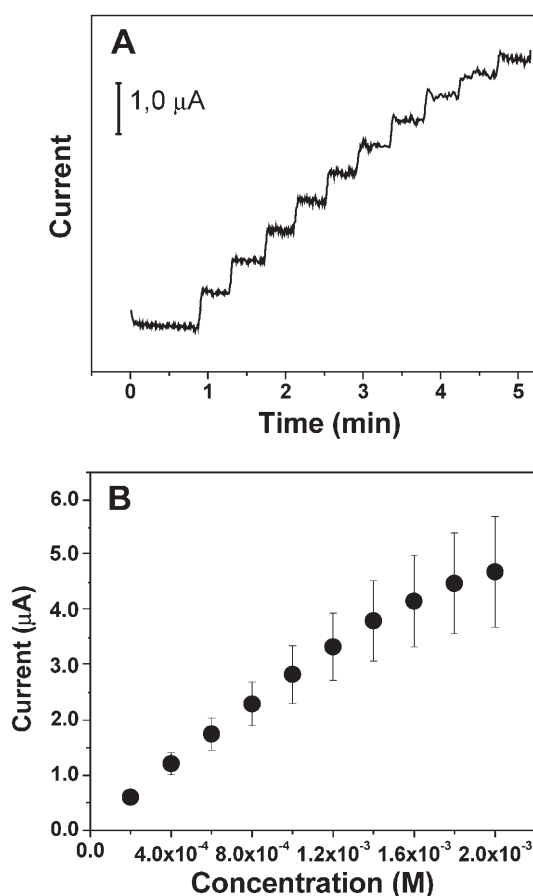
GOx layers, suggesting a most efficient immobilization of the biocatalytic layer (Fig. 8A, b).

In order to evaluate the contribution of CNTs in the improvement of the response observed when using MWCNT-Plys, additional electrodes were prepared in a similar way to that of (GCE/MWCNT-Plys/GOx/(MWCNT-Plys/GOx)$_n$), but using a Plys layer without MWCNT (until dryness). As Figure 8B shows, the sensitivity to glucose is almost the same either if the Plys layer is immobilized by immersion or by dropping/drying. One interesting aspect to remark is that when the last layer of the architecture is either Plys or MWCNT-Plys, a small decrease in sensitivity to glucose is observed, probably due to a more difficult accessibility of GOx (not shown).

We also studied the response of GCE/MWCNT-Plys/GOx/(Plys/GOx)$_n$ (using 20 min immersion in Plys solution) and GCE/MWCNT-Plys/GOx/(MWCNT-Plys/GOx)$_n$ to hydrogen peroxide directly added to the cell instead of evaluating the response to the enzymatically generated hydrogen peroxide after the addition of glucose. Figure 8C shows the variation in sensitivity towards hydrogen peroxide for each step during the construction of the multilayers system obtained from amperometric experiments at 0.700 V. Starting with GCE, when a layer of MWCNT-Plys is added, as expected, there is an important increase in sensitivity due to the presence of MWCNT ($(10 \pm 1) \times 10^2$ µA M^{-1} vs. $(20 \pm 1) \times 10^3$ µA M^{-1} for GCE and GCE/MWCNT-Plys, respectively). When adsorbing the first layer of GOx, the sensitivity decreases to $(125 \pm 4) \times 10^2$ µA M^{-1}, probably as a consequence of some destabilization of the MWCNT-Plys layer due to the compromise of Plys that supports the MWCNTs in the interaction with GOx and/or some diffusional problems for hydrogen peroxide. When adsorbing the following layers, the behavior is different depending if using Plys alone or MWCNT-Plys. In the case of GCE/MWCNT-Plys/GOx/(Plys/GOx)$_n$, the sensitivity decreases either if adsorbing Plys or GOx, suggesting important barrier effects. On the contrary, when using MWCNT-Plys instead of Plys, after the decrease in sensitivity observed when immobilizing the first layer of GOx, it increases if adsorbing a new layer of MWCNT-Plys (GCE/MWCNT-Plys/GOx/MWCNT-Plys). Once a second layer of GOx is immobilized, a new decrease in sensitivity is observed, to reach the original value after the adsorption of another layer of MWCNT-Plys. No further changes in sensitivity are observed after immobilizing the following layers of MWCNT-Plys and GOx.

From the analysis of Figure 8, it is possible to conclude that the presence of MWCNT is necessary to obtain a more efficient arrange of the multilayers system and to decrease the barrier effects observed when using Plys alone. Therefore, the increase in sensitivity to glucose observed when incorporating additional layers of MWCNT-Plys/GOx in the supramolecular architecture GCE/MWCNT-Plys/(GOx/MWCNT-Plys)$_n$ can be attributed to a more efficient immobilization of GOx. Equivalent experiments using electrodes prepared by dropping 20 µL Plys/drying instead of dipping in a Plys solution for 20 min, gave similar results (not shown).

Fig. 7. A) Amperometric recording for successive additions of 2.0×10^{-4} M glucose. B) Calibration plot obtained from experiments like the one shown in (A) using different electrodes. Working potential: 0.700 V. GOx adsorption: 20 min from a 2.5 mg/mL GOx solution; Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

The number of biocatalytic layers is a very important aspect to be considered when designing supramolecular architectures based on the self-assembling of multilayers. Figure 8A and B show the variation of sensitivity towards glucose as a function of the number of GOx layers employing two different "polycationic layers" to reverse the negative charge of GOx. In one case, the enzymatic electrode was obtained by alternate immersions of GCE/MWCNT-Plys in GOx (2.5 mg/mL) and in Plys (1.0 mg/mL) solutions for 20 min each (GCE/MWCNT-Plys/GOx/(Plys/GOx)$_n$). In the other case, GCE/MWCNT-Plys was first immersed for 20 min in the 2.5 mg/mL GOx solution, and then, the resulting electrode was dropped with 20 µL MWCNT-Plys (1.0 mg/mL MWCNT in 1.0 mg/mL Plys). After dryness, the building of the multilayers system continued by repeating this procedure alternately (GCE/MWCNT-Plys/GOx/(MWCNT-Plys/GOx)$_n$). When Plys is the polycationic layer, the sensitivity to glucose remains almost constant even for the increasing number of GOx layers (Fig. 8A, a). On the contrary, when using MWCNT-Plys, the sensitivity increases linearly with the number of
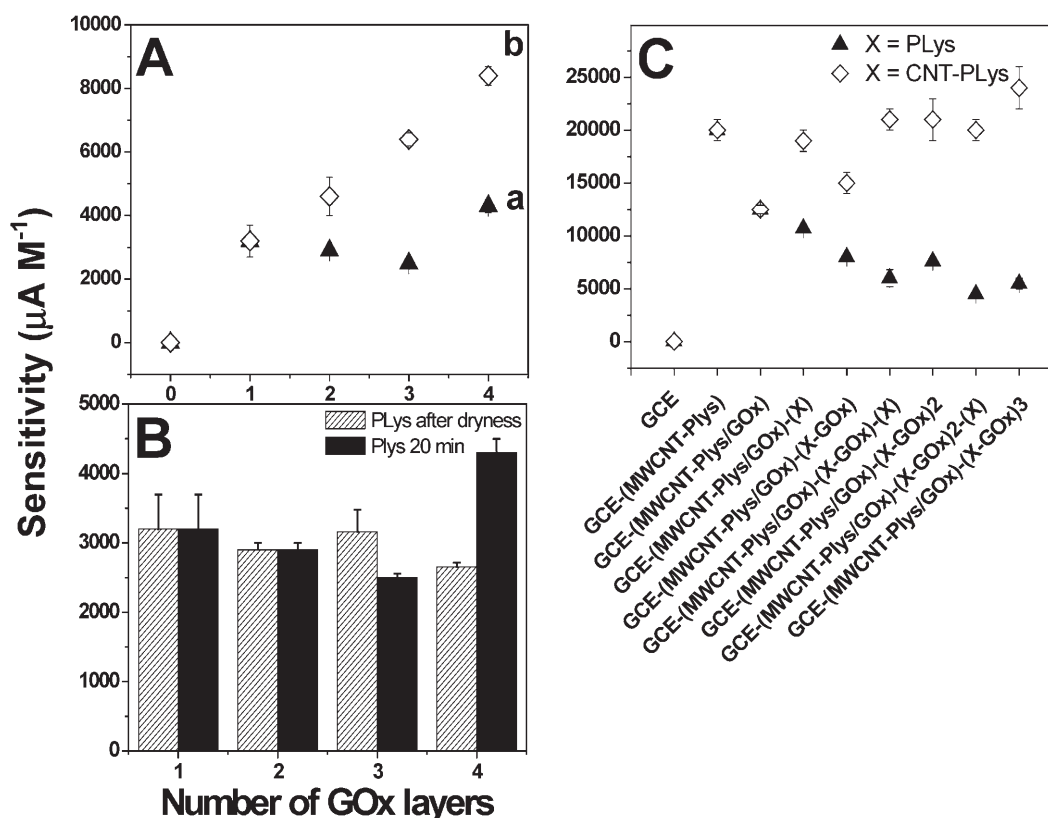
Fig. 8. A) Dependence of the sensitivity obtained from amperometric recordings after successive additions of glucose on the number of GOx layers immobilized on the electrode: a) Plys adsorption time: 20 min; Plys concentration: 1.0 mg/mL. b) MWCNT-Plys adsorption conditions: 20 μL 1.0 mg/mL MWCNT/Plys until dryness. a), b) GOx adsorption time: 20 min; GOx concentration: 2.5 mg/mL. B) Comparison between the sensitivities obtained using two different ways of Plys immobilization: (black) 20 min adsorption from a 1.0 mg/mL Plys solution; (striped) 20 μL 1.0 mg/mL Plys until dryness; (black and striped) GOx adsorption: 20 min from a 2.5 mg/mL GOx solution. C) Dependence of the sensitivity obtained from amperometric recordings after successive additions of hydrogen peroxide on the different layers during the building of the supramolecular architecture: (▲) X = Plys; Plys adsorption time: 20 min; Plys concentration: 1.0 mg/mL; (◇) X = MWCNT-Plys; MWCNT-Plys adsorption conditions: 20 μL 1.0 mg/mL MWCNT/Plys until dryness. a), b) GOx adsorption conditions: the same as in (B). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Working potential: 0.700 V.

4. Conclusions

In summary, we are proposing for the first time the successful construction of highly sensitive electrochemical sensors based on the modification of GCE with a dispersion of MWCNTs in Plys. The ability of the MWCNT-Plys layer as a new platform for self-assembling of polyelectrolytes/biomolecules has been also demonstrated, in this case in connection with GOx. The combination of the unique properties of MWCNTs with the versatility of LbL self-assembling of polyelectrolytes, represent a very interesting strategy for further developments of (bio)sensors using different biorecogniton layers.

5. Acknowledgements

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