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Short communication

Single Walled Carbon Nanotubes/polypyrrole–GOx composite films to modify gold microelectrodes for glucose biosensors: Study of the extended linearity

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ABSTRACT

A glucose biosensor was assembled using gold microelectrodes (diameter of 250 μm) coated by Single-Walled Carbon Nanotubes (SWCNTs), via the Electrophoresis Deposition Process (EPD). This nanostructured platform was successfully used to deposit the poly(pyrrole)/glucose oxidase film (PPy/GOx). The most important result of this biosensors was the wide linear range of concentration, ranging from 4 to100 mM (covering the hypo- and hyper-glycemia range, useful in diabetes). This extended linearity offered the possibility to measure glucose from 0.560 to 12.0 mM, with a detection limit of 50 μM (useful for hypo-glycemia disease).

1. Introduction

The glucose detection in blood and urine has played a crucial role in the diagnosis of diabetes, and it represents one of the most usual determinations performed in clinical analysis (Jaffari and Turner, 1995). Since the first amperometric biosensor was fabricated for continuous monitoring of glucose in cardiovascular surgery in 1962 (Clark and Lyons, 1962), a large number of methodologies focusing on improvements of the amperometric signal and immobilization strategies of the GOx have been extensively developed. A wide range of glucose biosensor prototypes have been developed during these years (2012) but the real challenge is to obtain a biosensor capable of measuring the glucose concentration in the normal range (i.e, representative of healthy patients) and also in the range of hypo- and hyper-glycemia values (especially for diabetes patients, for clinical diagnosis of this widespread pathology). Only in one case of study, Wang et al. (2005) proposed a new glucose biosensor prototype based on over-oxidized polypyrrole/Multi-Wall Carbon Nanotubes (MWCNTs)/GOx nanocomposite film, to modify electrodes. In this way GOx entrapment was easier to perform, resulting in glucose biosensors with improved analytical performances, especially in terms of an extended linearity (still 50 mM ≅ 901 mg/dl, useful for the iper-glycemia pathology values), high selectivity toward the most common interferents and an improved stability (Wang et al., 2005). In this last paper, the LOD resulted of 0.2 mM, for the assembled biosensors, using MWCNTs, which just represented the lower limit of the normal clinical range of glucose, present in human blood. Consequently, such biosensor prototype, presented by Wang et al. (2005) would not be able to measure glucose in patients with hypo-glycemia disease. According to this last consideration, our biosensor prototype resulted very innovative under several interesting point of view, listed below: (1) we can be able to cover all the entire clinical useful range of glucose analyte: normal (in the case of healthy patients), ipo-, and iper-glycemia range (which are representatives of diabetes diseases); (2) we used SWCNTs, instead of MWCNTs (used before by Wang et al. (2005)) because their electronic, electrochemical features, and their mechanical properties are better than those exhibited by the more defective Multi-Wall Carbon Nanotubes, according to the literature (Saito et al., 1998). In addition, the topography of MWCNTs makes more difficult the polymers entrapment because they exhibit more concentric walls (Scheme 1 in Supporting information); (3) we assembled a new prototype of glucose biosensors based on a new Electrophoresis Deposition Process (EPD), which was able to guarantee a oriented an aligned coating of the electrochemical transducer surfaces; (4) we used Au microelectrodes (diameter of 250 μm) instead of a bare Glassy Carbon electrode (GC, $\phi = 3$ mm,

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by Wang et al. (2005)), which could be very useful as implantable and not-invasive miniaturized devices, for possible future applications. The analytical performances of these resulting biosensors were significantly improved, especially in terms of a wider linear range of concentration, if compared to the PPy/MWCNT/GOx based GC biosensors described in the literature (Wang et al., 2005), and other biosensor systems reported previously in references Mascini et al. (1989), Moscone et al. (1993). In this case, glucose could be detected in a range which covered the hypo-, and hyper-glicaemic values (ranging from 0.560 to 100 mM, according to the clinical values for diabetes: 10–1802 mg/dl; Weast et al., 1968; Pesce and Kaplan, 1987). This extended linearity seems to be related to the size-exclusion effects exhibited by the thickness and mesoporosity properties of the resulting nanocomposite-based barrier, toward the glucose diffusion. This results had been confirmed by the FE-SEM/EDX and BET studies, performed on this modified micro-electrode surface, for the first time. In addition the operational and long term stability, for this new EPD-SWCNT-modified biosensors, resulted better than those obtained in the case of PPy/MWCNT/GOx based GC biosensors, described in the literature (Wang et al., 2005) for an extended linearity of glucose, only in the hyper-glycemia range of values.

2. Experimental

The assembly and testing of the SWCNTs modified Au micro-electrodes was the final step of a long analytical procedure. Details on the sample preparation and materials are given in the Supporting information (Figs. 1S–6S; Tables 1S–9S).

3. Results

Several polymeric films having different thicknesses were electrochemically synthesized, using different monomer concentrations (ranging from 0.5 M to 0.05 M). Working with the lowest pyrrole concentration, 0.05 M, the film resulted in a typical network structure (see Fig. 1(a)) maintaining the typical SWCNT's morphology, which demonstrated the presence of a thinner polymeric film. In particular, SEM image (Fig. 1(b)) of the same sample displayed the SWCNT's bundles surrounded by the PPy film. These results demonstrated that carbon nanotubes could act also as nucleation centers for the subsequent poly(pyrrole) growth, and not only as dopant agent (as reported by (Wang et al., 2005). Increasing the monomer concentration, using 0.1 M pyrrole (Fig. 1(c)), the resulting polymeric films appeared homogeneous and uniform on the electrode surface, having smaller polymeric globules, resulting in a larger electrochemical area. This means that higher GOx amount could be entrapped in the polymeric matrix (with a higher biocatalyst loading). For the biosensors assembled with 0.3 M pyrrole concentration, a homogeneous and continuous layer on the Au microelectrode surfaces was also observed, which resulted very similar to that obtained in Fig. 1(d), where 0.5 M pyrrole was employed during the synthesis. Fig. 1(d) showed a homogeneous, uniform, and dense packed coating on the entire Au microelectrode surfaces (Fig. 1(d)). In this case, the polymeric layer appeared as large and compact globular structures, ranging from 15 to 60 μm (Fig. 1(e), Scheme 1). According to the literature (Matsumoto et al., 1993) the glucose diffusion in the polymeric matrix (glucose permeability) was proportionally delayed when the polymeric film thickness was increased (Matsumoto et al., 1993). According to this, in Table 4S some interesting parameters, such as PPy–GOx film thickness, and the surface coverage (Γ mol cm^{-2}) of the modified Au electrodes were reported. These data showed how decreasing the pyrrole concentration during the electrodeposition (0.3 M, 0.1 M, and 0.05 M, respectively) thinner polymeric films were grown. Table 4S reports different estimates of PPy film thickness using Fortier et al. (1990) approach based on the electrical charge required for the poly(pyrrole) growth. These results showed how using higher pyrrole concentration (i.e. 0.5 M) during the electrodeposition, more electrical charge (mC cm^{-2}) was required for the film growth, obtaining thicker polymeric films. Contrarily, using lower pyrrole concentrations, thinner poly(pyrrole) films were synthesized, requiring lower amount of the electrical charge (mC cm^{-2}). The results were also confirmed by SEM study, and these agreed very well with the literature (Reyes et al., 2004), concerning the over-oxidized poly(pyrrole) film permeability toward H_2O_2 and glucose substrate. In addition, mesoporosity and surface nominal area parameters (reported in Supporting information, Table 9S) confirmed these results because for higher monomer concentration, lower value of mesoporosity was observed. In particular, the mesoporosity is strictly related to the interstitial spaces of the carbon nanomaterials used, therefore a decrease of mesoporosity would result in a more restricted availability of interstitial spaces and thus in a reduced mobility of glucose, toward the surface of the chemically modified micro-transducers. The resulting polymeric films showed different morphologies (which influenced the electro-analytical properties) depending on the kinetic of the electropolymerization, which was strongly related to the monomer concentration. Some authors reported that at lower monomer concentrations, as monomer concentration increases, the rate of polymerization increases by mass action (Reyes et al., 2004). At this level, as the polymer grows the monomer is consumed faster than it diffuses from the bulk resulting in a more permeable structure (with higher permeability and diffusion coefficients for glucose, Table 5S). At relatively high monomer concentrations, while the polymer grows faster, monomer is abundant enough so that it is always available to react with growing polymer resulting on a more compact film (Reyes et al., 2004), with lower permeability toward glucose (Table 5S). Instead, when lower pyrrole concentrations were used (i.e. 0.1 and 0.05 M monomer) less dense packed polymeric films were grown on the electrode surfaces (Fig. 1). According to these results, a quantification of the surface area coverage Γ (mol cm^{-2}), obtained using Laviron's approach (Laviron, 1979), gives a more accurate determination of permeability allowing correlation of permeability with film thickness and monomer concentration. In Table 4S the highest Γ (mol cm^{-2}) values were observed using 0.5 M and 0.3 M pyrrole, and these corresponded to those reported in literature for a typical monolayer film.

These assembled biosensors were characterized by amperometry, and the analytical parameters were reported in Table 6S. The best analytical performances, in terms of the lower detectable glucose concentration, were obtained when 0.05 M monomer was used to synthesized the PPy–GOx film to modify SWCNT/Au microelectrodes. In fact, in this case, the LOQ resulted of 560 μM ≈ 10 mg/dl glucose, especially useful for diabetes applications, covering the hypo-glicaemic values. As displayed in Table 6S, it was evident that for the highest sensitivity, more restricted response linearity was obtained (ranging from 0.560 to 12 mM glucose, working with 0.05 M monomer, in aerobic conditions). This result could be explained considering that in the presence of thinner polypyrrole films, the glucose biosensor mechanism was controlled by a typical Michaelis–Menten kinetic (Table 7S), where the enzyme electrode affinity resulted very high toward its substrate producing a saturation effect up to 12 mM glucose. When thicker polymeric films were used (i.e. 0.5 M pyrrole) the biosensors mechanism was controlled by glucose diffusion. The presence of the thicker polymeric matrix produced a diffusion delay of glucose (Matsumoto et al., 1993), yielding an extended linear range of concentrations (ranging from 4 to