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AMPEROMETRIC GLUCOSE BIOSENSOR BASED ON ELECTROPOLYMERIZED CARBON NANOTUBE/POLYPYRROLE COMPOSITE FILM

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In this paper we report a one-step preparation procedure of an amperometric biosensor for glucose based on incorporating single-walled carbon nanotubes (SWCNTs) that were covalently functionalized with a water soluble conducting polymer, poly (m-aminobenzene sulfonic acid (SWCNT-PABS) and the glucose oxidase (GOD) within an electropolymerized polypyrrole (PPY) film. The nanocomposite films were electrochemically synthesized by electrooxidation of pyrrole in an aqueous solution containing appropriate amounts of GOD and CNTs. The amperometric detection of glucose was assayed by potentiostating the enzyme electrode at a potential to oxidize the enzymatically produced H_2O_2 with minimal interference from the coexisting electroactive compounds. The results indicate that the PPY-CNTs-GOD nanobiocomposite film is highly sensitive and suitable for glucose biosensor function. The biosensor exhibits excellent response performance to glucose with a linear range from 0.5 to 50 mM. Furthermore, the biosensor shows rapid response, high sensitivity and long-term stability.

INTRODUCTION

An important work in the design and fabrication of biosensors is to find an effective immobilization method, an appropriate support material and a fast and simple procedure.

Electropolymerization is an attractive and well-controlled method for immobilizing enzymes onto electrodes. In this methodology, the enzyme is mixed with a monomer, which is electropolymerized at an inert electrode, whereupon the enzyme becomes embedded into the polymer matrix. The incorporation of the enzyme into the matrix is often promoted through electrostatic interactions. Advantages of the electropolymerization approach include the good control over the film thickness and the ability to selectively attach the biomolecules onto nanoscale electrode surfaces. Numerous enzymes have been incorporated into electropolymerized films.[1-2] In many cases conductive polypyrrole has been used as a polymer matrix. This choice relates to the fact that pyrrole is inexpensive, its polymerization is well known and it can be electropolymerized at neutral pH, which allows the entrapment of a wide range of biomolecules.

Direct electron transfer between biomacro-molecules and electrode surface is important in the development of biosensors. Unfortunately, it is difficult for enzymes to exchange electrons with electrode surface directly because their redox centers are deeply immersed in the insulated protein shells. A variety of attempts have been done to promote the electron transfer between redox enzymes and electrodes.[2–11] Recently, direct electrochemistry and catalytic activity of many biomolecules have been obtained at electrodes modified with CNTs.[2, 7-10]. Their unique geometrical structure and electrical, chemical, and mechanical properties make them attractive for constructing electrochemical biosensors through utilizing the high electron transfer ability of CNT between biomolecules and electrode surface acting as nanowiring of biomolecules. Many researches have demonstrated that the direct electrochemistry of biomolecules could be promoted by CNTs [2, 7-10].

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Glucose oxidase has been one of the most extensively researched enzymes for the construction of enzyme-based glucose biosensors. GOD contains two flavine adenine dinucleotide (FAD) cofactors as redox centers and the FAD redox centers are electrochemically insulated by a protective protein shell. In order to improve the electron transfer between the redox centers of GOD and the electrode, CNTs can be used. [11] The drawback is that the use of CNTs in applications as biosensors is difficult due to their poor solubility in most solvents.[12]

Of the several methods reported for the solubilization of CNTs, the most often used is the functionalization of CNTs by an oxidation process, which involves extensive ultrasonic treatment in a mixture of concentrated nitric and sulfuric acid.[12] By this procedure, the ends and sidewalls of the treated CNTs become decorated with carboxyl groups.

Polypyrrole has proven effective at electrically wiring the enzymes and CNTs bearing carboxylic groups due to their ability to function as an anionic dopant in the polymeric matrix. On this basis, glucose biosensors have been fabricated from pyrrole, GOD and oxidized multiwalled carbon nanotubes [2, 13]

In this study we used single-walled carbon nanotubes that were covalently functionalized with a water soluble conducting polymer, poly (m-aminobenzene sulfonic acid) (SWCNT-PABS) (Figure 1). The SWCNT-PABS graft copolymer has excellent solubilities in water.[14] The nanocomposite enzymatic films were electrochemically synthesized by electrooxidation of pyrrole in an aqueous solution containing appropriate amounts of GOD and functionalized CNTs.

The enzymatic nanocomposite films were prepared using electrochemical polymerization technique in which glucose oxidase, carbon nanotubes and polypyrrole were deposited simultaneously.

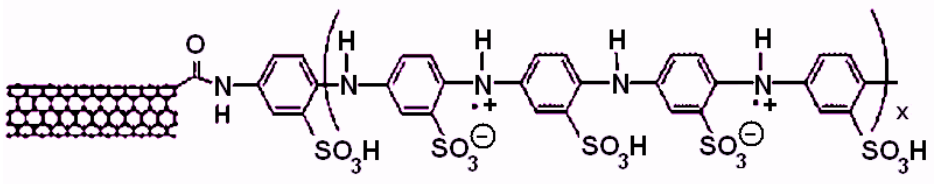

Fig. 1 – Chemical structure of SWCNTs functionalized with PABS.

EXPERIMENTAL

PABS functionalized SWCNTs were provided by Carbon Solutions, Inc (www.carbonsolution.com, Riverside, CA). Pyrrole (98%) was purchased from Aldrich. All solutions were prepared from double-distilled water. Glucose oxidase (GOD, Type X-S, *Aspergillus niger* (EC 1.1.3.4), 179 units/mg) and D(+) glucose were purchased from Sigma. Different amounts of the functionalized nanotubes (usually 100 mg/L) were dispersed in bidistilled water by sonication for 1 hour. The selected amount of GOD (usually 1 mg/mL) was then added to the CNTs solution. In the end, pyrrole was added (at a concentration of 0.5 M) to the GOD-CNTs mixture and the electropolymerization proceeded at a current density of 0.5 mA cm^{-2} for different times.

The electropolymerization was carried out at a pH of 7, above the isoelectic point of the glucose oxidase, in order to provide it an overall negative charge. After the electropolymerization, the PPY/CNTs/GOD film was subjected to overoxidation by cycling the potential from 0 to 0.9 V for 100 cycles at 50 mV/s in a phosphate buffer solution of pH = 7. It is well known that overoxidation of PPY/GOD electrodes leads to a loss of the PPY electroactivity and to an enhanced sensitivity and selectivity to glucose.[15-16] For comparison, a PPY/GOD film also was obtained, its preparation was done in a similar manner to that of PPY/CNTs/GOD film, but in absence of CNTs.

The amperometric detection of glucose was assayed by potentiostating the enzyme electrode at a potential to oxidize the enzymatically produced H_2O_2 with minimal interference from the coexisting electroactive compounds. In a typical measurement, 50mL of sample was transferred to the cell. Measurements of glucose were carried out in a 0.1M phosphate buffer solution (pH = 7) supporting electrolyte medium. Amperometric detection was proceeded under batch condition with stirring. Constant-potential amperometry required the preconditioning (~30 min) and operation of the electrode at a constant potential. When the current reached a baseline in the absence of substrate, substrate was added.

Electrochemical measurements were performed using a three-electrode cell with a platinum wire working electrode (1cm^2), a Pt gauze auxiliary electrode and a saturated calomel reference electrode (SCE).

Electrochemical characterization experiments were performed using a Gill AC potentiostat/galvanostat.

RESULTS AND DISCUSSION

As we presented in the Experimental section, the PPY/CNTs nanocomposites films were electrochemically synthesized by electrooxidation of pyrrole in an aqueous solution containing appropriate amounts functionalized CNTs. Because no electrolyte has been added into the deposition bath, functionalized CNTs were acting as both an electrolyte and a dopant during the

electropolymerization process. The PPY/CNTs nanocomposite films obtained at a current density of 0.5mA/cm^2, for a charge density of 1800 mC/cm^2 shows a typical cyclic voltammogram as presented in figure 2. For comparison, the cyclic voltammetric investigation of a PPY film doped with dodecylsulphate (DS) anion obtained in the same conditions was also carried out and the corresponding results are shown in the same figure. It can be observed that the CNTs counter ion has a profound effect upon the redox properties of the resulting PPY/CNTs film. The PPY/CNTs film displays improved redox characteristics when comparing with to those of the PPY/DS one, with a wide conductive region. Moreover, the background current of PPY/CNTs is apparently larger than that of PPY/DS, which indicates the PPY/CNTs modified electrode has larger effective surface area.

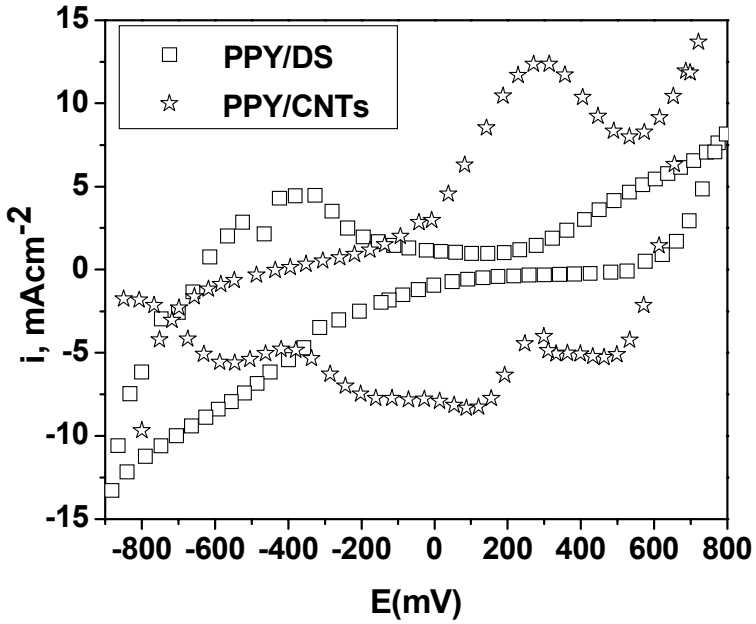

Fig. 2 – Cyclic voltammograms at the PPY/CNTs and PPY/DS electrodes in a 0.25M KCl solution, for a scan rate of 50 mV/s.

The growth of the PPY/CNTs/GOD films was accomplished also without an additional electrolyte. In this case, both the functionalized CNT and GOD are incorporated within the growing film and serves as the charge-balancing 'counter-ions'.

Scanning electron microscopy (SEM) was used for examining the morphology of the PPY/CNTs/GOD film. As one can see from figure 3, the surface morphology of the PPY films differed remarkably between the PPY/CNTs/GOD and PPY/DS films. SEM image of PPY/CNTs/GOD film reveals a very fibrous three-dimensional reticular structure with interlocking pores whereas the image of PPY/DS displays typical cauliflower morphology. Such morphology is in agreement with other studies of conducting-polymer/CNT composites.[2, 17]

The characteristics of the PPY/CNTs/GOD electrode towards glucose sensing have been then investigated using the amperometric method. The catalytic reaction of glucose biosensor is as follows:

$$\text{Glucose} + \text{GOD-FAD} \rightarrow \text{GOD-FADH}_2 + \text{gluconolactone}$$

The determination of the response current is based on the formation of H_2O_2:

$$\text{GOD-FAD} + 2e^- + 2H^+ \leftrightarrow \text{GOD-FADH}_2$$
$$\text{GOD-FADH}_2 + O_2 \rightarrow \text{GOD-FAD} + H_2O_2$$

During the enzyme-catalyzed reaction, the hydrogen peroxide is detected by the amperometric current method during oxidation at the enzyme electrode:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

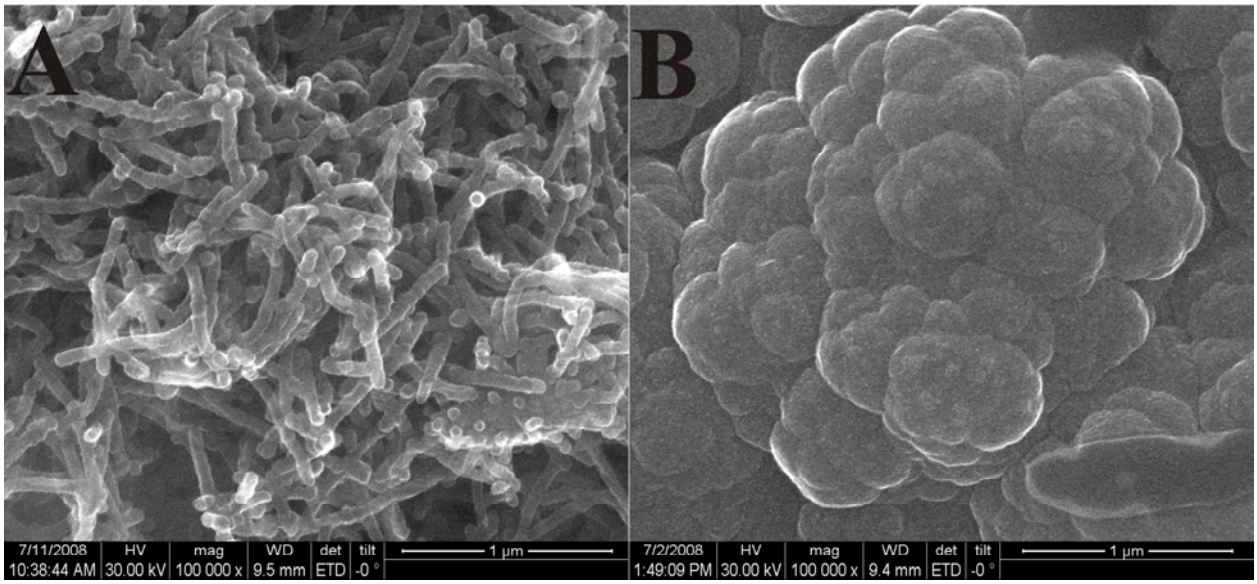

Fig. 3 – Scanning electron micrographs of PPY/CNTs/GOD (A) and PPY/DS (B) films.

The choice of the applied potential at the working electrode is fundamental to achieve the lowest detection limit and to avoid the electrochemical interfering species. In order to select an optimal applied potential to the working electrode for glucose detection, the effect of the applied potential on the current response of the PPY/CNTs/GOD electrode was examined. As shown in figure 4, the biosensor was operated at potentials in 0 – 800 mV range. The background current of the working electrode in the buffer without substrate was measured firstly and, when it reached steady values, the substrate was added. In figure 4 is presented the comparison in response in a 20mM glucose solution in 0.1M phosphate buffer of pH = 7 for the PPY/CNTs/GOD and PPY/GOD electrodes. From this experiment it results that the PPY/CNTs/GOD modified electrode exhibited stable electrocatalytic activity toward hydrogen peroxide oxidation in a wide potential range. As one can see, the anodic glucose response of the CNT-based biosensor increases rapidly above +600 mV. Also, a significant decrease in overvoltage for the oxidation of hydrogen peroxide was observed in comparison with conventional PPY/GOD biosensor.

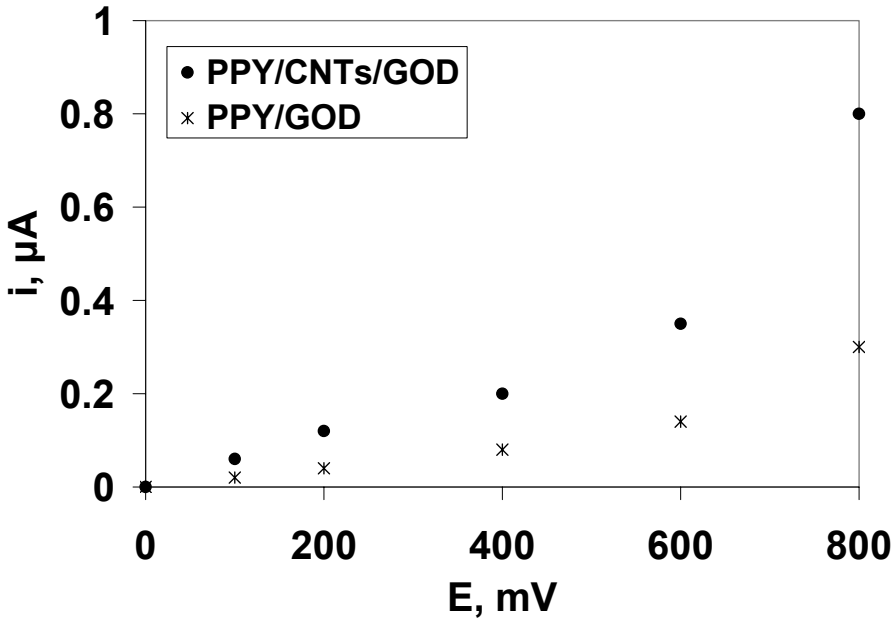

Fig. 4 – The anodic response in a 20mMglucose for the PPY/CNTs/GOD and PPY/GOD electrodes.

The diffusion properties of the substrate (glucose) within the PPY/CNTs/GOD film will have a significantly effect on the sensor properties. The thickness of the PPY/CNTs/GOD nanocomposite film can be controlled by the electropolymerization time, i.e. the total charge passed during film formation in the galvanostatic method. The PPY/CNTs/GOD biosensors performance for different film thickness was investigated by amperometric detection of glucose in 0.1M phosphate buffer (pH = 7) solution at +600 mV/SCE. The biosensor responses improved by increasing the total charge from 90 to 300 $mC \cdot cm^{-2}$, then the response decreased for the longer electrodeposition times (> 300 $mC \cdot cm^{-2}$). A thicker PPY/CNTs/GOD nanocomposite film obtained at a higher electropolymerization time allowed an increase of the entrapped GOD and, as a result, a higher activity, but a thicker film will lead to a slower substrate transport through the PPY/CNTs/GOD nanocomposite. It can be concluded that an ideal biosensor configuration should have the thinnest possible biocomponent layer with the highest possible biochemical activity.[18]

A series of experiments have been performed in order to investigate the sensor response and the typical amperometric responses at a potential of +600 mV/SCE for the PPY/CNTs/GOD nanocomposite modified electrode by the successive addition of glucose are shown in figure 5. The responds to the addition of glucose are quickly, sensitively and reach a steady state in approximatively 10 s.

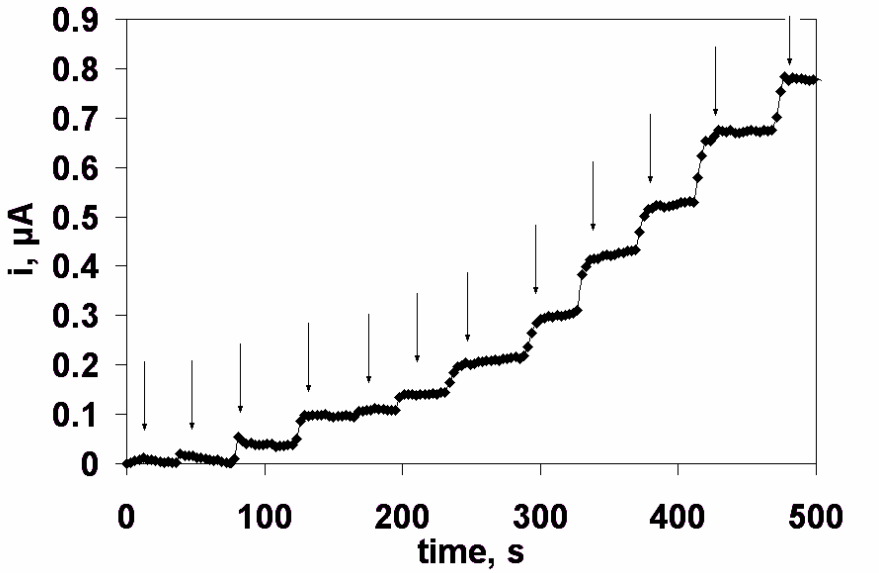

Fig. 5 – Current–time recordings for successive additions of glucose in 0.1 M phosphate buffer solution pH = 7, at the PPY/CNTs/GOD modified electrode measured at +600mV/SCE (after the injection of glucose at a certain interval).

The relationship between glucose concentration and the response current of the glucose biosensors PPY/CNTs/GOD and PPY/GOD, respectively, in the 0.1 M phosphate buffer of pH = 7 is shown in figure 6.

The PPY/CNTs/GOD nanocomposite demonstrated enhanced sensor performance when compared to PPY/GOD electrode. In the case of nanocomposite electrode, we observed that the response current is linear with glucose concentration in the range from 0.5 to 50 mM (figure 7).

The analytical parameters of PPY/CNTs/GOD modified electrode towards glucose sensing are presented in table 1.

Table 1

Analytical performance of PPY/CNTs/GOD modified electrode towards glucose sensing

Linear range (mM)	Regression equation	Sensitivity ($\mu A \cdot M^{-1}$)	Response time (s)	Detection limit (M)
0.5 - 50	y = 15.56x + 0.03	15.56	~ 10	$5 \cdot 10^{-4}$

The operational stability of the PPY/CNTs/GOD glucose biosensor was also studied. The PPY/CNTs/GOD electrode was incorporated in the electrochemical cell with stirred 10 mM glucose solution in order to study its operation stability under continuous use for 10 hours. The response decreased by about 10% within the first 2 hours, and about 40% within 10 hours, which indicated the modified electrode has a good operational stability.

Such an ability of conductive polymers/carbon nanotubes composites to promote the hydrogen peroxide electron transfer reaction with a short response time (~10 s) and long-term stability, a low detection limit, an extended linear concentration range and a high sensitivity suggests great promise for oxidases based amperometric biosensors.

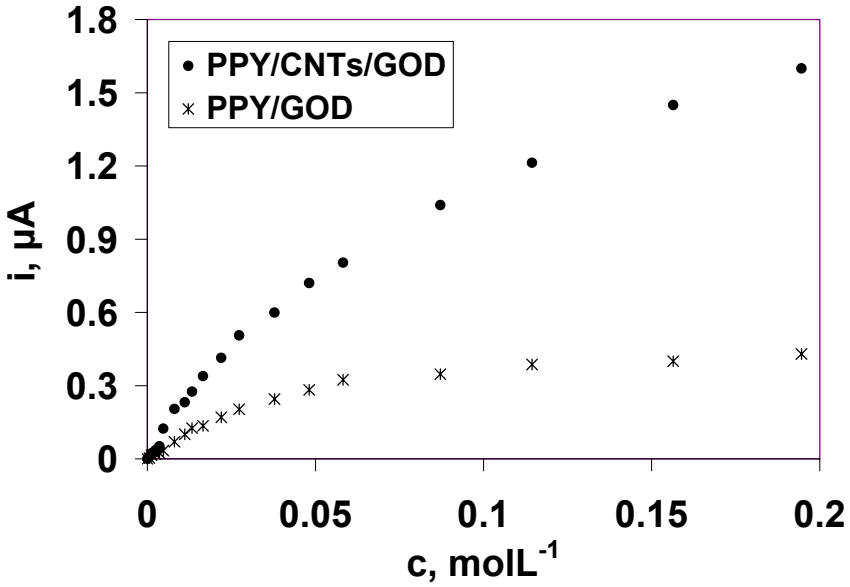

Fig. 6 – Calibration plot for the sensing of glucose using PPY/CNTs/GOD nanocomposite-modified electrode vs. PPY/GOD electrode.

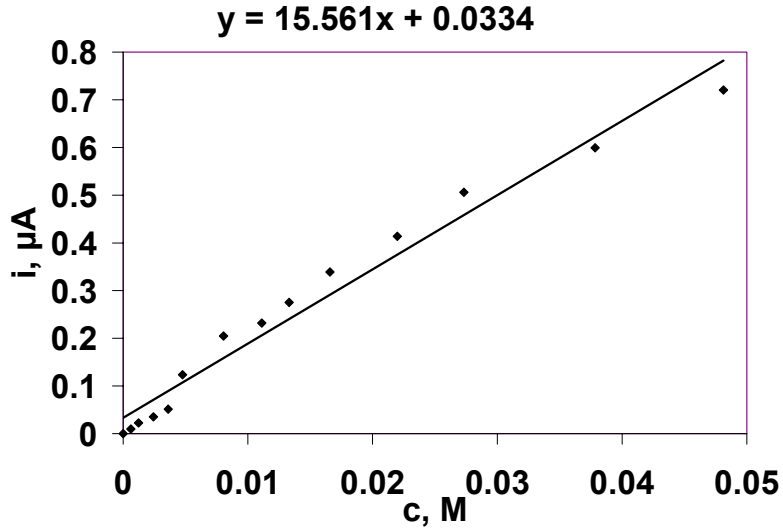

Fig. 7 – Linear range displayed by PPY/CNTs/GOD nanocomposite-modified electrode.

CONCLUSIONS

In this work, we have demonstrated a one-step preparation of enzyme electrodes based on the incorporation of functionalized CNTs dopants and the simultaneous entrapment of the GOD. The obtained electrode displays a high sensitivity and a wide linear range. The PPY/CNTs/GOD nanocomposite demonstrated enhanced sensor performance when compared to PPY/GOD

electrode. Such simultaneous incorporation of CNTs and GOD represents a simple and effective route for preparing enzyme electrodes.

The concept presented within the context of glucose sensing, it can be extended to other amperometric enzyme electrodes based on the judicious selection of the biocatalyst.

The biosensor exhibits excellent response performance to glucose with a linear range from 0.5 to 50 mM. Furthermore, the biosensor shows rapid response (~10 s), high sensitivity and long-term stability.

Long-term stability is crucial for practical application of biosensors. Future work will address parameters such as the storage stability as well as interferences.

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