HIGHLY SELECTIVE AND STABLE GLUCOSE BIOSENSORS BASED ON POLYANILINE / CARBON NANOTUBES COMPOSITES

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In this paper, a new selective and stable glucose biosensor based on polyaniline (PANI) / functionalized single-walled carbon nanotubes (SWCNTs) / Prussian Blue (PB) composite films was investigated. For biosensor synthesis we combined two widely used techniques to produce modified electrodes that are the electroreduction of diazonium salts and the electropolymerization. Thus, in a first step, SWCNTs deposited at a PB modified glassy carbon (GC) electrode were functionalized with p-nitrophenyl group by electrochemical reduction of p-nitrobenzenediazonium salt in nonaqueous media. Then, the nitro group was reduced electrochemically to amine functionality. The enzyme doped PANI film can easily be grafted onto the surface of such obtained aminophenyl-modified SWCNTs electrodes. The marked synergistic electrocatalytic activity of SWCNTs and PB film toward H_2O_2 produced during enzymatic reaction of glucose oxidation permitted effective low-potential amperometric measurement of glucose.

Keywords: diazonium salt, amperometric biosensor, carbon nanotubes, conductive polymers, nanocomposite, selectivity

1. Introduction

During recent years, enzymatic biosensors have received much attention by many researchers due to their rapid response, high sensitivity and intrinsic selectivity [1,2]. Research in this field is still focused on the improvement of sensor properties by new sensing approaches.

The biosensor based on glucose oxidase (GOx) for glucose sensing continues to be the primary model system in the development of new sensing materials and methods [3,4]. The glucose concentration can be determined by amperometric detection of the oxidation current of hydrogen peroxide (H_2O_2), a side product during the enzymatic reaction. A great drawback for this model is represented by high overpotential (usually 0.6V *vs.* Ag/AgCl) [5] required for H_2O_2 oxidation. In clinical application the high positive working electrode

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potential required leads to interference from reducing species such as ascorbic acid and uric acid.

Carbon nanotubes (CNTs) have attracted considerable attention in the last decades in fabricating electrochemical sensors because of their remarkable properties such as high-chemical stability, unique electronic properties, high-mechanical strength, and high-surface area [6-8]. The dramatic decrease in the overpotential of H_2O_2 oxidation or reduction as well as the direct electron transfer of glucose oxidase (GOx) observed at CNT-modified electrodes indicate great promise for the biosensing of glucose [9].

The polyaniline (PANI) in the form of conductive polymer (CP) has attracted much attention due to its interesting electrical, electrochemical and optical properties [10]. Among the most promising applications of PANI is electrochemical biosensing [11], as it can provide a suitable environment for the immobilization of biomolecules, and the PANI-modified electrodes have several advantages such as impressive signal amplification and elimination of electrode fouling.

As both PANI and CNTs are excellent materials for the construction of electrochemical sensors and biosensors, the combination of these two materials is also expected to be an excellent platform for electrochemical sensing applications [12]. Conductive polymers/carbon nanotubes composites can be obtained using three major synthesis methods: electrodeposition of the CP onto a preformed CNT-modified electrode [13], chemical synthesis [14], and electro-co-deposition of the CNTs and CP onto a bare electrode [15]. In this work, for composite films synthesis the first procedure has been applied.

One of the problems that can arise when using conductive polymer films electrodeposited on the surface of carbon nanotubes to obtain biosensors is the stability of detection layer adsorbed at the electrode. It is well known that the nanotube surface, like the basal plane of graphite, is a low energy surface that interacts only weakly with many of the known organic materials. This weak interaction can result in poor contact, also known as poor wetting, between the organic material and the nanotubes. Various strategies have been reported in the literature to enhance the interaction between the CP matrix and the nanotubes [16–18]. In this context, CNTs functionalization represents an effective way to improve interfacial bonding. We report here the functionalization of single-walled carbon nanotubes (SWCNTs) through the electrochemical reduction of aryl diazonium salts. The electrochemical functionalization based on the electrogeneration of reactive radicals near the nanotube surface shows several advantages, compared with some other grafting methods such as clean and nondestructive chemical functionalization or selective electrochemical modification of individual material [19].

In this work, we report a simple and efficient approach for electrochemical preparation of an electrochemical biosensor for glucose having improved stability and selectivity. For this, we combine two widely used techniques to produce modified electrodes, that are the electroreduction of diazonium salts at SWCNTs based electrodes and then, the electropolymerization of a conductive polymer at the functionalized SWCNTs electrode surface. Thus, in a first step, a SWCNTs electrode was functionalized with 4-nitrophenyl group by electrochemical reduction of 4-nitrobenzenediazonium salt in nonaqueous media. Then, the nitro group was reduced electrochemically to amine functionality [20]. The PANI film can easily be grafted onto the surface of such obtained aminophenyl-modified SWCNTs electrodes. In our previous studies we have demonstrated that these PANI/SWCNTs films exhibit significantly improved electrochemical stability [21].

It is well known that Prussian Blue (PB) is an excellent catalyst for H_2O_2 reduction at low potentials. Moreover, PB is a relatively cheap and stable electrocatalyst compared to some enzymes such as peroxidase. As a result, it is an attractive material for possible mass production of oxidases based biosensors [22]. With the introduction of PB, the PANI/SWCNTs/PB hybrid composite show synergistic enhancement of the response current for H_2O_2 detection. Therefore, a glucose biosensor was further constructed by immobilization glucose oxidase on the PANI/SWCNTs/PB hybrid composite film during the polymeric film electrogeneration. The resulted biosensor exhibits high sensitivity, long-term stability and freedom of interference from other co-existing electroactive species.

2. Experimental

2.1. Reagents, Solutions, and Instrumentation

Single-walled carbon nanotubes, 0.7-1.11 nm diameter produced by CoMoCAT® using Catalytic Chemical Vapor Deposition (CVD) method were purchased from Aldrich. Anhydrous acetonitrile (99.8%, noted ACN) and tetra-n-butylammonium tetrafluoroborate (99%, noted TBATFB) were purchased from Aldrich and were used as received. Aniline (99.5%, Fluka) was used as supplied. Bidistilled water was used for all aqueous solutions preparation. p-Nitrophenyl diazonium tetrafluoroborate (PNBDTFB) was prepared by standard diazotation of the corresponding amine with $NaNO_2$ in acidic medium as described in our previous studies [21]. Glucoxidase from Aspergillus Niger 200U/mg (GOx) was purchased from Aldrich.

All electrochemical measurements were performed with a 128N Autolab potentiostat. A three-electrode configuration of cell consisting of bare or modified glassy carbon (GC, Metrohm, ***disks, diameter*** 2 ***mm***) as working electrodes, Hg/Hg_2Cl_2 (3M KCl) and Ag/10 mM $AgNO_3$, 0.1 M TBATFB as reference electrodes for electrochemical experiments in aqueous and organic solvents, respectively, and a Pt wire as counter electrode was used.

All acetonitrile solutions were deoxygenated by bubbling high purity Ar for 15 min prior to experiments.

2.2. Preparation of modified electrodes

Prior to electrode modification, the GC electrode surface was polished in 0.05μm alumina slurry on a microcloth pad. After polishing the electrode was thoroughly rinsed with water and sonicated in ACN for 5 min. A PB film was then electrodeposited on GC electrode by using cyclic voltammetry in the potential range (-0.1, 1)V, at a scan rate of $0.1Vs^{-1}$, from an aqueous solution containing 2mM $K_3Fe(CN)_6$, 2mM $FeCl_3 \cdot 6H_2O$, 0.1M KCl and 1mM HCl.

The SWCNTs electrodes were obtained by depositing a drop of a SWCNTs-dispersion (obtained after a few minutes by ultrasonication of 5 mg of SWCNTs in 5mL ACN) on the PB/GC electrode surface and evaporating the solvent after the drop addition.

The functionalization procedure of SWCNTs deposited at PB/GC electrode with 4-nitrophenyl group was carried out in ACN solutions containing different concentrations of the PNBDTFB salt and 0.1M TBATFB. The SWCNTs/PB/GC modified electrode potential was scanned in the potential range of (+0.5, -1) V, at a scan rate of 0.1 Vs^{-1}. The electrode was then removed from the synthesis solution and rinsed with large volumes of ACN. Electrochemically functionalized SWCNTs electrodes were denoted *NB*SWCNTs.

The carbon nanotubes / polyaniline composite films were fabricated by two different ways. One was to prepare these composite structures simply by electrogeneration of the conductive polymer at the SWCNTs/PB/GC electrode surface and the other one consisted in the electropolymerization of the monomer at the already functionalized *NB*SWCNTs. In the second approach, prior the aniline electropolymerization, the nitrophenyl groups grafted on SWCNTs surface were electrochemically reduced in 1M H_2SO_4 solutions to amine functionality (Fig. 3). Such functionalized SWCNTs were denoted *NBred*SWCNTs. The resulting electrodes were washed with water before use and stored in water at room temperature when were not in measurements.

The PANI-GOx films were then synthesized at the previously modified electrodes by electropolymerization from an aqueous solution consisting of 0.1M monomer, 1mg/mL GOx in 0.1M acetate buffer pH = 5.2. Conventional cyclic voltammetry electropolymerization was performed from −0.3 to 1V with a scan rate of $0.1Vs^{-1}$ for 20 cycles.

3. Results and Discussion

3.1. PANI-GOx/SWCNTs/PB/GC composite synthesis

PB thin films are generally formed by electrochemical reduction of solutions containing iron(III) and hexacyanoferrate(III) ions. Reduction of the brown-yellow soluble complex Prussian brown [PX, iron(III) hexacyanoferrate(III), present in equilibrium with the iron(III) and hexacyanoferrate(III) ions], is the main electron-transfer process in PB electrodeposition as shown in the equation below:

$$[Fe^{III}Fe^{III}(CN)_6]\ (PX) + e^- \rightarrow [Fe^{III}Fe^{II}(CN)_6]^-\ (PB) \qquad (1)$$

Charge compensating cations (initially Fe(III), then K(I) ions at potential cycling in potassium ion containing supporting electrolyte) are present in the PB film for electroneutrality [23]. The typical cyclic voltammogram recorded during PB/GC electrode electrosynthesis as described in the Experimental section, is shown in Fig. 1. Two sets of peaks can be observed in cyclic voltammetry recordings for PB/GC modified electrodes synthesis which correspond to the reduction and oxidation of PB to Prussian White ($E_{1/2}$ = 0.2 V) and to Berlin Green ($E_{1/2}$ = 0.9 V), respectively.

The SWCNTs electrodes were obtained afterwards by depositing a drop of a SWCNTs-dispersion (obtained after a few minutes by ultrasonication of 5 mg of SWCNTs in 5mL ACN) on the PB/GC electrode surface and evaporating the solvent after the drop addition. We observed a strong adherence of the sonicated SWCNTs on PB/GC surface that allowed us to carry out the electrochemical studies similarly to modified electrodes, without loss of the immobilized material.

The SWCNTs/PB/GC electrodes were then modified with 4-nitrophenyl group by electrochemical reduction of the diazonium salt from solutions containing 3mM PNBDTFB and 0.1M TBATFB in ACN. The potential of SWCNTs/PB/GC modified electrode was scanned in a range of (+0.5, -1)V at a scan rate of 0.1 Vs^{-1}. The reduction process of PNBDTFB using CV at SWCNTs electrodes shows one peaks in the first cathodic scan (Fig. 2). The electrode surface was passivated after the first scan as evidenced by the absence of cathodic peaks in the second cycle. The electrode was then removed from the grafting solution and rinsed with large volumes of ACN.

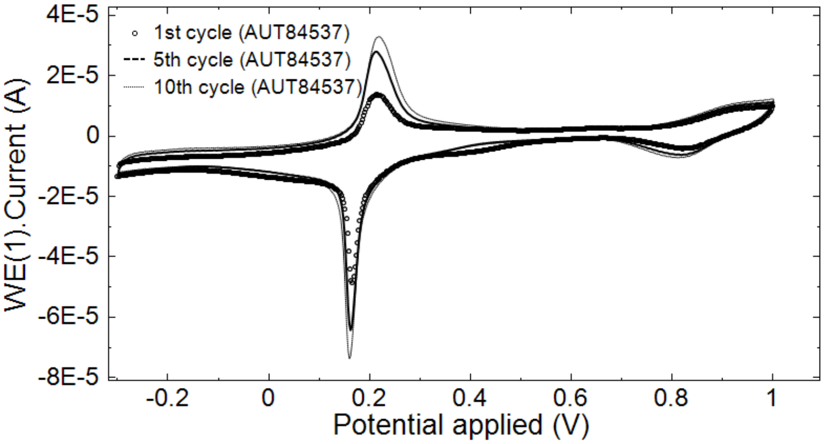

Fig. 1. Cyclic voltammograms of Prussian Blue film synthesis at GC electrodes. Scan rate: 0.1 Vs^{-1}

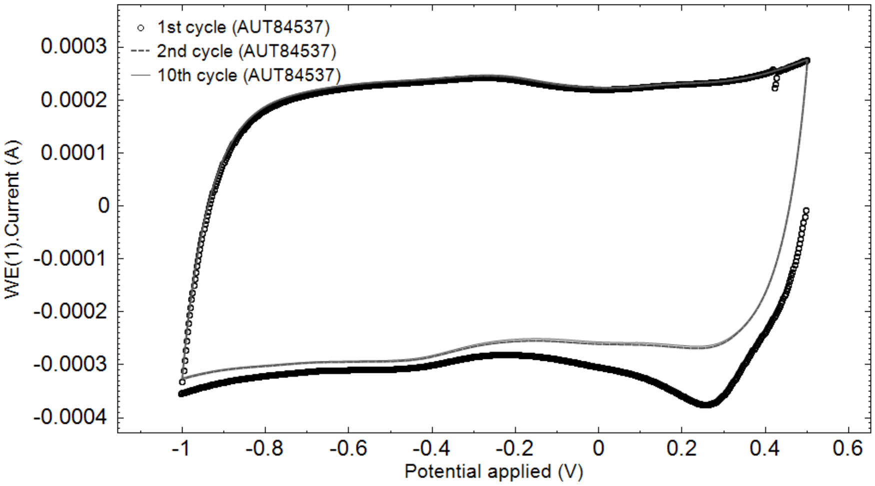

Fig. 2. Cyclic voltammograms of *NB*SWCNTs/PB/GC electrodes preparation by 4-nitrophenyl group grafting at SWCNTs surface in 3mM PNBDTFB +0.1M TBATFB solutions in ACN. Scan rate: 0.1 Vs^{-1}

After the formation of the 4-nitrophenyl layer, the nitro moieties were electrochemically reduced to amine functionality. As it is shown in Fig. 3, the CV of the *NB*SWCNTs in 1M H_2SO_4 shows no any peak in the first anodic scan. After performing the scan in the cathodic region up to a potential of -0.4V, in the subsequent scans, a pair of reversible redox peaks appears at a half wave potential of approximately 0.5 V. This reversible couple from electrochemical point of view can be attributed to the formation of the hydroxylamine intermediate according to the previous studies on p-nitrophenyl group reduction at GC and CNTs surface [21, 24]. These reduced electrodes were denoted *NBred*SWCNTs.

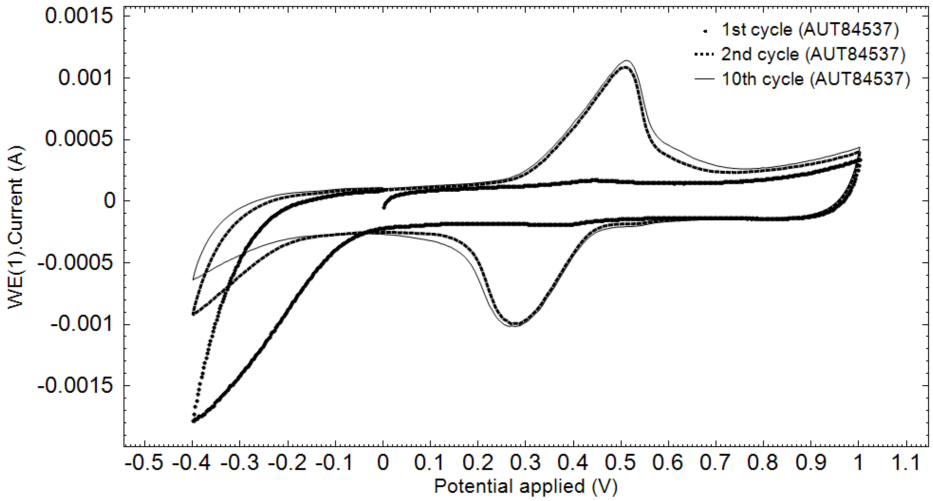

Fig. 3. Cyclic voltammograms of *NBred*SWCNTs/PB/GC electrodes preparation by the reduction of surface-grafted 4-nitrophenyl groups on the SWCNTs/PB/GC electrode in1M H_2SO_4 aqueous solution. Scan rate: 0.1 Vs^{-1}

The polyaniline films can be electrogenerated at SWCNTs electrodes in two different ways. One is to prepare these composite structures simply by electropolymerization of the monomer at the SWCNTs/ PB/GC electrode and the other one consists in the electrosynthesis of the polymer at the already functionalized SWCNTs surface; this structure is denoted as *NBred*SWCNTs/PB/GC electrode. Thus, the PANI-GOx films were obtained at the surface of the two types modified electrodes by electropolymerization from an aqueous solution consisting of 0.1M monomer, and 1mg/mL GOx in 0.1M acetate buffer pH = 5.2. To obtain a compact and relatively thin film, conventional cyclic voltammetry electropolymerization was performed from −0.3 to 1V with a scan rate of 0.1Vs^{-1} for 20 cycles (Fig. 4). From Fig. 4 the three pairs of redox peaks can be observed, indicating the presence of discrete electroactive regions in the film similar to those well-known from the literature.

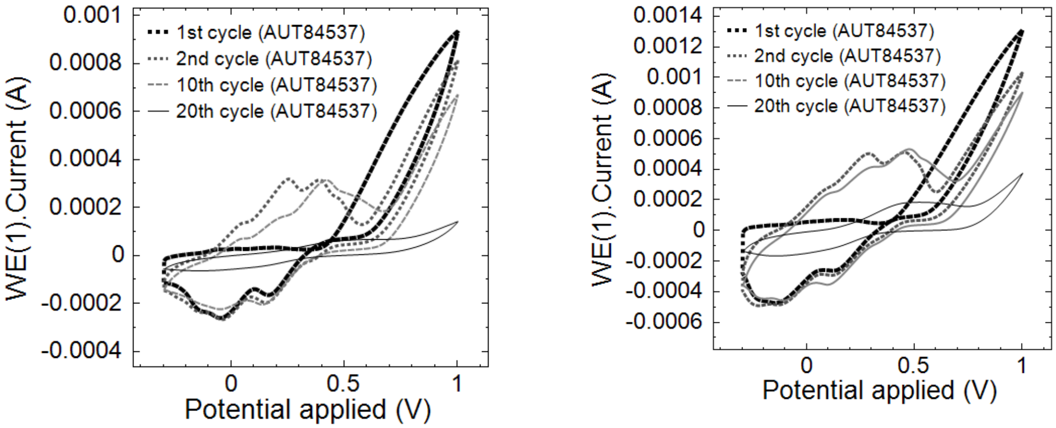

Fig. 4. Cyclic voltammograms of PANI-GOx film electrosynthesis at SWCNTs/PB/GC (a) and *NBred*SWCNTs/PB/GC (b) electrodes from aqueous solution containing 0.1M aniline, 1mg/mL glucose oxidase in 0.1M acetate buffer pH = 5.2, at a scan rate of $0.1Vs^{-1}$.

3.2. Biosensor testing

Analytical performances of the modified electrodes for glucose detection were investigated. Both types of modified electrodes display an expanded linear response range (from 10^{-5} M to 5×10^{-3}M) with a good correlation coefficient and high sensitivities. The high sensitivity may be attributed the superior transducing ability of PANI and the excellent performance of the composites of PANI/SWCNTs.

Fig. 5 shows amperometric responses of the PANI-GOx/*NBred*SWCNTs/PB/GC modified electrode in 0.1M phosphate buffer, pH 7.4, at different operating potentials. It can be observed that the electrode displays the highest selectivity when operating at the potential of 0 V. Similar results were obtained in the case of PANI-GOx/SWCNTs/PB/GC electrode.

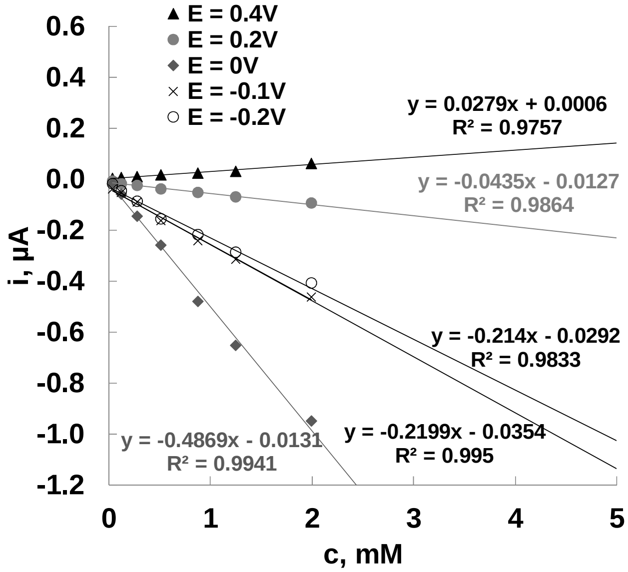

Fig. 5. Calibration curves for glucose detection in pH 7.4 0.1M phosphate buffer at PANI-GOx/*NBred*SWCNTs/PB/GC modified electrode at different operating potentials

Sensitivities of PANI-GOx/N*Bred*SWCNTs/PB/GC and PANI-GOx/SWCNTs/PB/GC modified electrodes toward glucose at different operating potentials are reported in Fig. 6. Both electrodes displayed the highest sensitivities at the potential of 0 V. When comparing the two types of electrodes, it can be seen that, regardless the operating potential, the sensitivities are slightly higher in the case of composite films based on non-functionalized SWCNTs than in the case of PANI-GOx/N*Bred*SWCNTs/PB/GC (sensitivities of 18.6 µAm$M^{-1}cm^{-2}$and 15.5 µA $mM^{-1}cm^{-2}$, respectively, at the potential of 0 V). This means that the sensitivity slowly decreases by adding the intermediated layer of p-nitrophenyl groups grafted at SWCNTs surface.

The operational stability of PANI-GOx/*NBred*SWCNTs/PB/GC and PANI-GOx/SWCNTs/PB/GC electrodes was investigated by successive measurements, at the potential of 0V, of the amperometric response of 2mM glucose in 0.1M phosphate buffer, pH 7.4. The PANI-GOx/*NBred*SWCNTs/PB/GC electrode response decreased by about 8% after 20 measurements, which indicated the electrode has a good operational stability.

The storage stability of the biosensors was also studied. The steady-state response current of 2mM glucose was determined every 3 - 4 days. When not in use the electrodes were stored in 0.1M phosphate buffer pH 7.4 at 4°C. The response current of PANI-GOx/*NBred*SWCNTs/PB/GC electrode only decreased by 10% after 60 days measurements which indicates that the electrode was considerably stable. In the case of PANI-GOx/SWCNTs/PB/GC electrode, the response current decreased after only 32 days measurements from 1.14 μA to 0.9 μA, that means a maintenance of 80% from the initial response. The above results confirm the fact that the PANI-GOx/*NBred*SWCNTs/PB/GC electrode has a considerable higher stability than the one obtained by the direct electrosynthesis of the enzymatic layer at the SWCNTs surface.

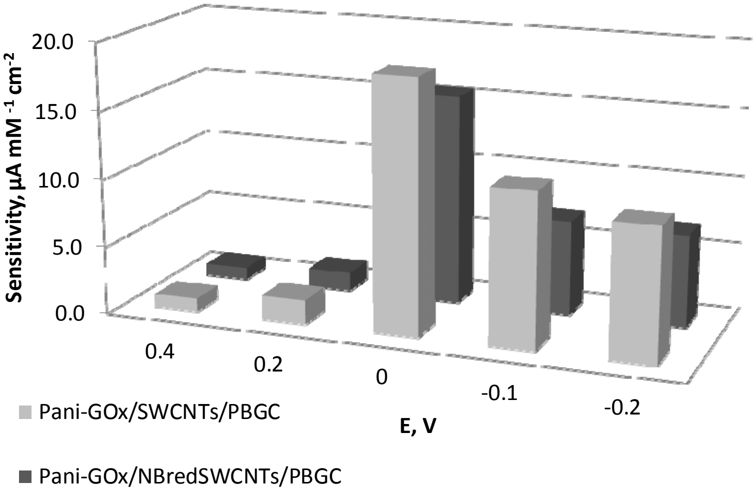

Fig. 6. Sensitivities of PANI-GOx/*NBred*SWCNTs/PB/GC and PANI-GOx/SWCNTs/PB/GC modified electrodes toward glucose at different operating potentials

A general problem in the electrochemical detection of glucose is the interferences from redox-active species that are usually present in physiological samples. The most common electrochemical interfering species for glucose such as acetaminophen, uric acid, lactate, and ascorbate were evaluated. As shown in Fig. 7, the addition of 0.1mM acetaminophen, 0.5mM uric acid, 1mM lactate, 0.1mM ascorbate to 0.5mM glucose did not produce observable interference in the PANI-GOx/N*Bred*SWCNTs/PB/GC biosensor response when operated at 0 V.

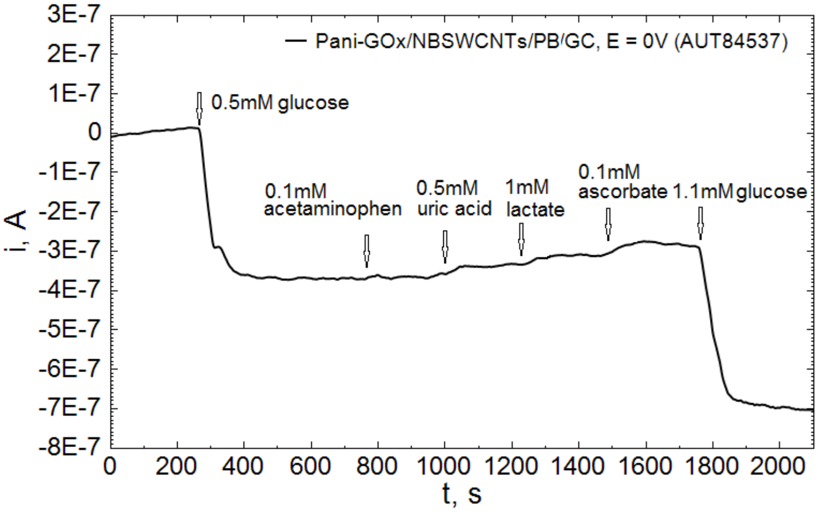

Fig. 7. Current–time recording at the PANI-GOx/*NBred*SWCNTs/PB/GC biosensor for an addition of 0.5mM glucose, followed by addition of 0.1mM acetaminophen, 0.5mM uric acid, 1mM lactate, 0.1mM ascorbate and, finally, 1.1mM glucose.

4. Conclusions

A novel route for fabrication PANI / SWCNTs composite material is proposed by electroreduction of p-nitrobenzenediazonium salts at SWCNTs surface and then, the electropolymerization of the conductive polymer at the functionalized SWCNTs. With the modification of the substrate electrode with a thin film of PB, a glucose biosensor based on PANI/*NBred*SWCNTs/PB hybrid composite was constructed. The biosensor exhibited high sensitivity and excellent stability and non-interferences. The good results can be attributed the synergistic effect between SWCNTs, PANI and PB and the process for fabricating the biosensor. The PANI/*NBred*SWCNTs/PB composite has the potential to provide the access to a large group of oxidase enzymes for designing a variety of bioelectrochemical devices.

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