Application of Glassy Carbon Electrode Modified with a Bilayer of Multiwalled Carbon Nanotube and Polypyrrole Doped with Nitrazine Yellow for Voltammetric Determination of Naltrexone

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Abstract

A new sensitive electrochemical sensor was fabricated based on a layer by layer process. In this process the glassy carbon electrode (GCE) is first coated by a thin film of multiwalled carbon nanotubes (MWCNTs). In the next step, the electropolymerization of pyrrole in the presence of Nitrazine Yellow (NY) as a dopant anion is performed on the surface of the MWCNTs precoated electrode. The electrochemical response characteristics of the modified electrode toward naltrexone (NTX) were studied by means of linear sweep voltammetry (LSV). A remarkable increase (~19 times) was observed in the anodic peak current of NTX on the surface of the modified electrode relative to the bare GCE. The effects of experimental parameters on the electrode response such as, drop size of the cast MWCNTs suspension, pH of the supporting electrolyte, accumulation conditions and the number of cycles in the electropolymerization process were investigated. Under the optimum conditions, the modified electrode showed a wide linear response to the concentration of NTX in the range of 4.0×10^{-8}–1×10^{-5} mol L^{-1} with a detection limit of 12 nmol L^{-1}. The prepared sensor exhibited high sensitivity, stability and good reproducibility for the determination of NTX. This sensor was successfully applied for the accurate determination of trace amounts of NTX in pharmaceutical and clinical preparations.

Keywords: Modified electrode, Multiwalled carbon nanotubes, Naltrexone, Nitrazine Yellow, Polypyrrole

1 Introduction

Naltrexone (NTX), a cyclopropyl derivative of oxymorphone opiate, is a potent, long acting and orally effective opiate antagonist with few side effects, which was first synthesized in 1965 [1–3]. NTX is used as a therapeutic agent for the treatment of opioid and alcohol dependence [4]. The blockade of opioid receptors is the basis behind its action in the management of opioid dependence. Its mechanism of action in alcohol dependence is not fully understood, but, as an opioid-receptor antagonist, it is likely to be due to the modulation of the dopaminergic mesolimbic pathway, which ethanol is believed to activate [5]. Many techniques have been developed for determination of NTX in pharmaceutical and clinical preparations such as high performance liquid chromatography (HPLC) [6–8], gas-liquid chromatography [9–11], gas chromatography-mass spectrometry (GC-MS) [12,13], LC-MS/MS [14], chemiluminescence [15], thin layer chromatography (TLC) [16], LC with electrochemical detection [17,18], attenuated total reflectance FT-IR spectrometry [5], LC-MS [19], spectrofluorimetry [4], LC-electrospray ionization (ESI) tandem mass spectrometry [20] and GC-nega-

tive ion chemical ionization-MS [21]. Spectrophotometric and chromatographic methods usually suffer from some disadvantages such as, expensive instrumentation, lengthy and tedious sample preparation, long analysis times and low sensitivity and selectivity. In comparison, electrochemical detection methods using electrochemical sensors and biosensors have shown to produce excellent results in the field of analysis of drugs of abuse and related molecules due to their remarkable advantages such as, high sensitivity, inexpensive equipment, relatively short analysis time and simple surface modification of electrodes [1].

The electrooxidation of NTX has been used for its determination in pharmaceuticals. In a previous paper [22], the electrochemical behavior of NTX on carbon paste electrode has been described using voltammetric techniques. It gives rise to an irreversible anodic process that can be ascribed to the oxidation of the aromatic hydroxy group. In addition, a second process with a peak potential more positive appears at pH values above 5, due to the oxidation of the tertiary amine group. In another work, flow injection analysis (FIA) with amperometric detection using a carbon paste electrode has been applied for the determination of NTX in pharmaceuticals [1]. A vol-

tammetric study of NTX has been carried out at the surface of a glassy carbon electrode (GCE) modified with Nafion-doped carbon nanoparticles [23]. This electrochemical sensor was developed for determination of NTX in pharmaceutical dosage form and human plasma. The electrochemical oxidation of NTX was investigated by cyclic and differential pulse voltammetric techniques.

Since their discovery by Iijima in 1991 [24], carbon nanotubes (CNTs) have been widely used in various research fields such as chemistry, physics, biology, medicine and engineering [25]. CNTs have unique structural, mechanical and electronic properties such as, high surface area, high electrical conductivity, good mechanical strength and outstanding thermal and chemical stability [26, 27–30]. As a consequence, CNTs have been recognized as attractive substrates for the fabrication of various sensors and biosensors. One promising application of CNTs involves their use as electrode modifiers for the preparation of electrochemical sensors, which promotes the kinetics of the electron transfer reaction, decreases surface fouling of the sensors and provides higher sensitivities and lower limits of detection [31, 32].

Recently, conducting polymers (CPs) have attracted much attention in the development of electrochemical sensors and biosensors, due to their electronic conducting properties and unique chemical and biochemical properties [31–34]. CPs are poly-conjugated polymers with electronic properties similar to metals, while retaining the properties of conventional organic polymers [35, 36]. Many reviews have been published about various applications of conducting polymers including analytical chemistry and biosensing devices [35–42]. Among various CPs, polypyrrole (PPY) is considered to be the most promising material for the development of sensor devices, because of its unique properties such as high conductivity, stability in ambient conditions, facile preparation and efficient polymerization at neutral pH, ease of formation from aqueous solutions, thickness controllability and good reversibility between its conducting and insulating states [43, 44].

In the recent years, many articles have been published in the field of CNTs/CPs composites. These composite materials have distinct, complementary properties of the individual components with a synergistic effect [45]. Combination of CPs and CNTs produces a new composite material with improved electrochemical and mechanical properties that were not observed in the individual components [46]. The desirable merging of properties of CPs such as reproducibility, good stability, strong adherence, high number of active sites and homogeneity in electrochemical deposition, together with high surface area and nanoporosity of CNT films, leads to a superior performance of the resulting sensing devices. According to the literature, two electrochemical approaches are commonly used for the preparation of composites of CNTs and CPs. One is the electropolymerization of a polymer film on electrode surfaces already modified by the adsorbed CNT coatings [31–33, 47]. The other is to

dissolve the monomer in a suspension of CNTs, and then co-deposit the polymer with the CNTs into a composite coating on the electrode surface [45, 48, 49].

In the present study, the GCE is modified with a nanostructured thin film using a layer by layer procedure. The modified electrode is prepared by electropolymerization of pyrrole in the presence of Nitrazine Yellow (NY) as a new dopant anion on the surface of a GCE precoated by a thin layer of MWCNTs. Scanning electron microscopy (SEM) was used to investigate the surface morphology of the modified electrode. The results of SEM images showed a porous thin film with high microscopic surface area on the electrode surface. This high microscopic surface area together with effective accumulation of NTX on the surface of the electrode resulted in a considerable enhancement in the oxidation peak current of NTX. The prepared modified electrode with advantages such as low detection limit, high sensitivity and relatively wide linear dynamic range was successfully applied for the voltammetric determination of NTX in pharmaceutical preparations and human blood serum samples.

2 Experimental

2.1 Materials

Multiwalled carbon nanotubes (MWCNTs, purity > 95 %) were prepared from Nanostructured & Amorphous Materials (USA). Naltrexone hydrochloride (NTX) in analytical grade was purchased from Sun Pharma, Pharmaceutical Industries Ltd (Scheme 1).

Pyrrole and dimethylformamide (DMF) were purchased from Merck. NY was obtained from Acros Organics. All other chemicals were of analytical reagent grade from Merck. All aqueous solutions were prepared with doubly distilled deionized water. Stock solutions of NTX were freshly prepared as required in appropriate buffer solution. In all electrochemical experiments, a stock Britton–Robinson (BR) buffer solution (containing $0.04 \, mol \, L^{-1}$ glacial acetic acid, orthophosphoric acid and boric acid) was used as the supporting electrolyte. Buffer solutions with different pHs were then prepared by the addition of $0.2 \, mol \, L^{-1}$ sodium hydroxide. All solutions were deoxygenated by purging with pure nitrogen gas (99.999 % from Roham Gas Company, Iran) prior to each experiment. NTX capsules (50 mg, Alhavi Pharmaceutical Co., Tehran, Iran) were purchased from the local pharmacies. Fresh frozen plasma was obtained from Iranian
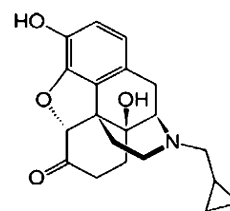
Scheme 1. Structure of NTX.

Blood Transfusion Organization. The serum samples were prepared by adding methanol (5 % (v/v)), hard mixing, and using to prepare the sample solutions by diluting 10 times with 0.04 mol L^{-1} BR buffer solutions of pH 6.

2.2 Apparatus

All electrochemical measurements were performed using an Autolab potentiostat/galvanostat model PGSTAT 101. An electrochemical cell with a three-electrode configuration was used with a glassy carbon working electrode (unmodified or modified), a saturated Ag/AgCl reference electrode and a Pt wire counter electrode.

A digital pH/mV/Ion meter (Metrohm, pH Lab 827) was utilized for the pH measurements and preparation of the buffer solutions, which were used as supporting electrolyte in the voltammetric experiments. The SEM experiments were performed on a HITACHI S4160 (Japan) scanning electron microscope.

2.3 Preparation of the Modified Electrode

Before the modification procedure, the GCE was polished with 0.05 µm alumina slurry on a polishing cloth, rinsed thoroughly with double-distilled water and sonicated in water for 5 min. An appropriate amount of pure MWCNTs was functionalized under concentrated nitric acid treatment process for 24 h in order to obtain more edge sites and better dispersion of nanotubes. The MWCNTs suspension was prepared by dispersing 1 mg of the functionalized MWCNTs (CNT-COOH) in 1 mL of DMF solvent under the ultrasonic agitation for 30 min, giving a black dispersion that is quite stable for 3–4 months. Then, a desired volume of the dispersed CNT was taken with a micro-syringe and after casting on the electrode surface, the electrode was heated in an oven at 50 °C for 30 min. This electrode is recognized as CNT/GCE. The electropolymerization of the PPY film on the surface of CNT/GCE was performed by potential cycling between −0.2 to +0.8 V (versus Ag/AgCl) at a scan rate of 50 mV s^{-1} for a total of 15 scans in an aqueous solution containing 5 mM pyrrole and 3 mM NY as dopant anion. This electrode, named as PPY/CNT/GCE, was used as the modified electrode in the electrochemical investigations of NTX.

3 Results and Discussion

3.1 Electrochemical Behavior of NTX on the Surface of Various Electrodes

Linear sweep voltammetry (LSV) was used to study the electrochemical response of NTX on the surfaces of various electrodes including bare GCE, CNT/GCE and PPY/CNT/GCE. Voltammetric responses of 1 µmol L^{-1} NTX in 0.04 mol L^{-1} BR buffer solution (pH 6.0), recorded at a scan rate of 100 mV s^{-1} after an accumulation time of 180 s at open circuit, are shown in Figure 1.
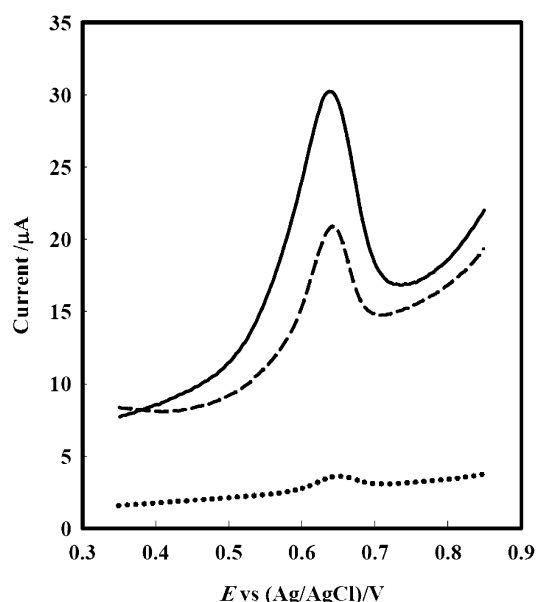

Fig. 1. LSVs of 1 µmol L^{-1} of NTX on the surface of various electrodes; bare GCE (dotted line), CNT/GCE (dashed line) and PPY/CNT/GCE (solid line). Potential sweep rate was 100 mV s^{-1} and supporting electrolyte was 0.04 mol L^{-1} BR buffer solution (pH 6.0).

As can be seen, NTX shows a very weak voltammetric response with an oxidation peak current of 0.85 µA on the surface of bare GCE. However, a remarkable increase in the peak current was obtained by a factor of 9.5 ($I_{p,a} = 8.1$ µA) and 18.8 ($I_{p,a} = 16.0$ µA) at the surface of CNT/GCE and PPY/CNT/GCE, respectively. The observed increase in voltammetric response at the PPY/CNT/GCE can be related to the increased active surface area of the electrode due to modification and strong adsorption of analyte on the surface of the modified electrode. During cyclic voltammetric studies of NTX, no cathodic peak was observed in the reverse scan, which suggests a totally irreversible behavior of the electrode process (data not shown).

3.2 SEM Characterization of the Electrode Surface

Surface morphology of the CNT/GCE and PPY/CNT/GCE was investigated by using SEM (Figure 2).

As can be seen in Figure 2A, the surface of GCE is coated with a homogeneous layer of MWCNTs with an average diameter of 70 nm. Figure 2B shows that by electrochemical polymerization of NY-doped PPY on the surface of CNT/GCE, the surface morphology of the electrode is obviously changed. It can be concluded from Figure 2 that PPY electropolymerization occurs on MWCNTs as a nanosized backbone and most MWCNTs are densely covered with a very thin, nanoporous and homogeneous layer of the polymer. As can be seen in Figure 2B, the diameter of nanotubes is further increased to around 105 nm after the electropolymerization process.
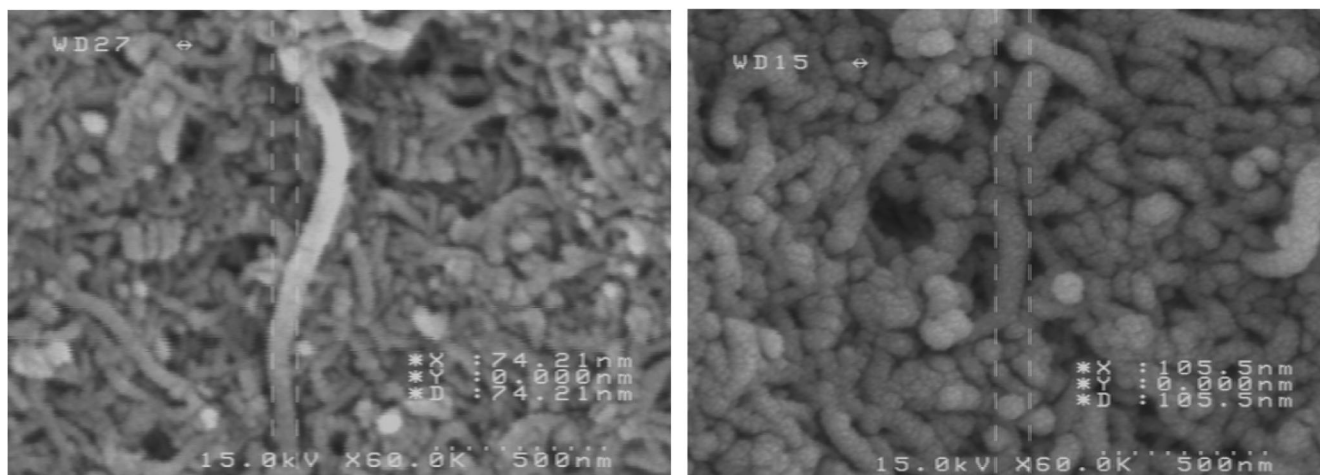
Fig. 2.   SEM images of (A) CNT coated GCE, and (B) 15 cycles deposition of NY-doped PPY film on the surface of GCE precoated with 8 μL of MWCNTs.

3.3 Electropolymerization of PPY Film

During the electropolymerization process, the presence of the counter anions (dopants) is necessary to balance out the positive charges produced by the oxidation process. Morphology, thickness, conductivity, stability and loading of the electrochemically generated polymer film are significantly influenced by the chemical nature and size of the dopant counter ions [44,50–52]. As we know stability is one of the important features of electrochemical sensors. PPY films doped with aromatic sulfonates are more stable than polymer films doped with inorganic anions [53–55].

In this work, NY (Scheme 2) was used as a new counter anion in the electropolymerization process.

NY is a yellow dye with two sulfonate groups, which dissolves easily in water. Cyclic voltammetry (CV) was used for the electrochemical polymerization of PPY film on the surface of GCE and CNT/GCE. The results of the electropolymerization process are shown in Figure 3. Increasing of the current on sequential scans indicates an efficient and stable polymer film deposition in the presence of NY (as dopant anion) on the electrode surface. NY with an aromatic structure and effective π–π interactions with the polymer and also MWCNTs backbone, can successfully serve as an anionic dopant in the electrodeposition process, which improves polymer loading on the surface of CNT/GCE and leads to formation of an adhesive polymer film on the surface of electrode. As can be
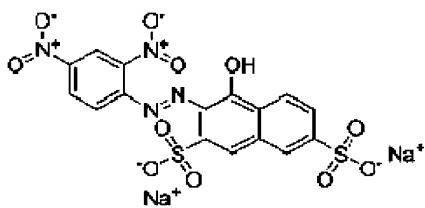
seen in Figure 3, on the surface of CNT/GCE, PPY film growth begins at the potential of about 0.5 V, while the beginning potential of PPY film growth on the bare GCE is about 0.6 V. Moreover, the growth current of PPY on CNT/GCE is much greater than on the bare GCE. The CNT/GCE has a very high real surface area, because of its porous structure and nanometric size, and is more active than the ordinary plane GCE. Therefore, lower nucleation energy is needed to initiate the polymerization.

The constructed electrode coated with an adhesive and porous film could be successfully used for the sensitive electrochemical determination of NTX. In this work, 15
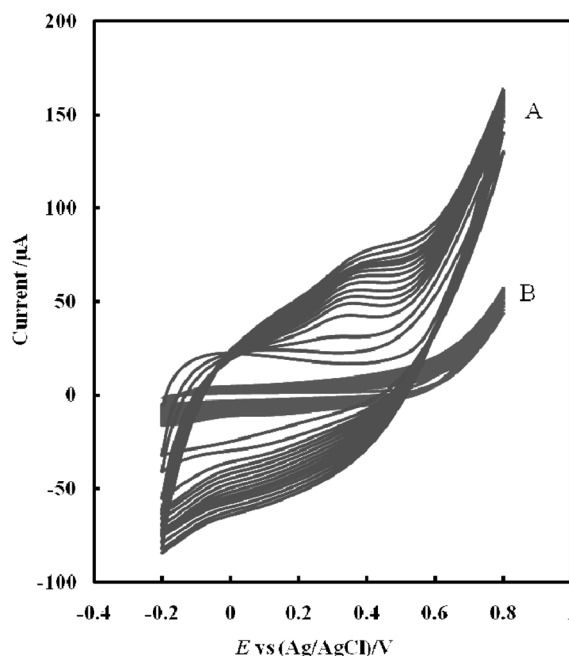

Scheme 2.   Structure of NY.

Fig. 3.   Repetitive cyclic voltammograms for the PPY polymerization reaction on (A) CNT/GCE, and (B) GCE in 5 mM pyrrole + 3 mM NY with 15 continuous cyclic scanning at 50 mV s^{-1}.
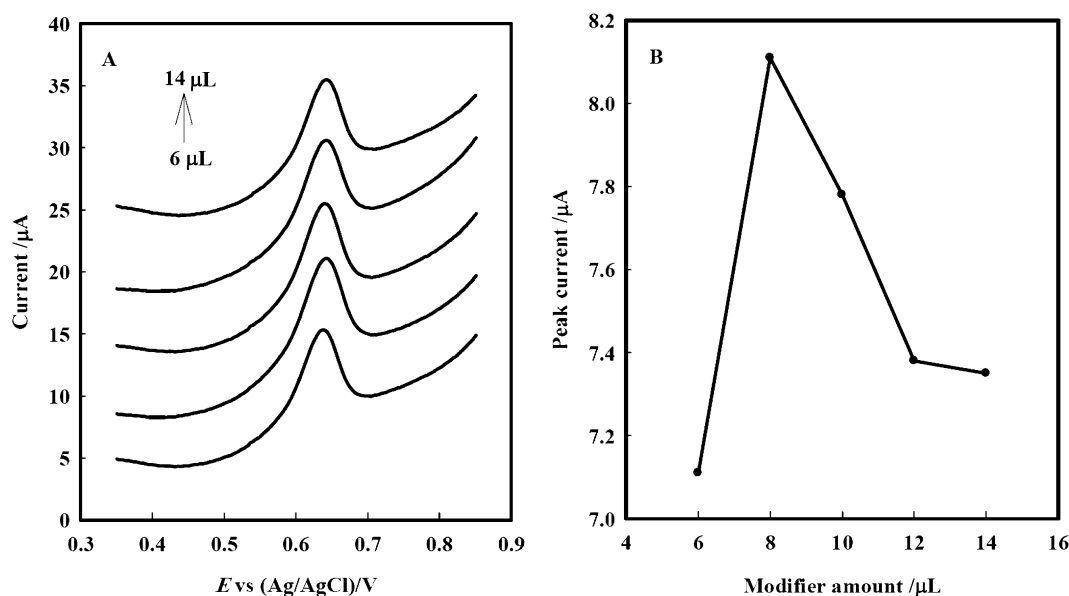
Fig. 4. (A) LSVs of 1 μmol L^{-1} of NTX on the surface of GCE coated with various amounts of 1 mg/mL of MWCNTs suspension. (B) The plot of $I_{p,a}$ versus amount of modifier. Supporting electrolyte was 0.04 mol L^{-1} BR buffer solution of pH 6.0 and sweep rate was 100 mV s^{-1}.

repetitive cycles in the range of -0.2 V to 0.8 V was selected for the preparation of PPY film. Higher scans more than 15 cycles resulted in increasing of the thickness of the polymer film and thus the capacitive background current is increased, which leads to decrease of the electrochemical response of NTX on the surface of PPY/ CNT/GCE. Moreover, increasing the thickness of the polymer film caused to increasing the resistance of the film modifier, leading to lowering the sensitivity of the electrochemical responses.

We have compared the electrochemical response of the modified electrode incorporating NY with the electrodes containing inorganic counter ions (such as Cl$^-$, ClO$_4^-$, NO$_3^-$, SO$_4^{2-}$). In all cases it was observed that the electrochemical response of NTX on the surface of the electrode containing NY is larger (~ 2 times) than polymer doped with inorganic ions. Also the sensor containing NY showed more long-time stability and reproducibility for the determination of NTX relative to other electrodes.

The pH range of NY (as a pH indicator), is 6.0 to 7.0, with a sharp neutral point at pH 6.6 [56]. Therefore, NY exists as azo form (scheme 2) in the experimental conditions (BR buffer solution pH 6.0). NTX pK_a values are 8.20 and 9.63 [57]. The first pK_a corresponds to the dissociation of the protonated aliphatic nitrogen proton and the second to the dissociation of the phenolic proton. Therefore, NTX exists as cationic form in the experimental conditions (BR buffer solution pH 6.0).

According to this information about pK_a values of NTX and NY we can conclude that there is an electrostatic interaction between NTX with positive charge and NY with two negative charges. Also there can be a hydrogen bond between these two molecules. These interactions improve the accumulation of NTX on the surface of

the modified electrode which leads to increasing sensitivity.

3.4 Effect of the Amount of Carbon Nanotubes on the Electrode Surface

In the case of thin film modifiers, the thickness of the modifier film on the surface of the electrode can affect the current responses for the analyte species. The mass transfer mechanism via diffusion through the porous film can be affected by the thickness of the modifier film [58–62]. In this work, the influence of the amount of MWCNTs suspension (cast on the electrode surface) on the response of the electrode toward NTX was investigated. For this mean, different volumes of a constant concentration of MWCNTs suspension (1 mg/mL) were cast on the GCE surface. Figure 4A shows voltammetric responses of 1 μmol L^{-1} NTX on the surface of GCE coated with different volumes of MWCNTs suspension.

As can be seen in Figure 4B, by increasing the volume from 6 to 8 μL, the oxidation peak current is increased. But, further increase in the volume of the CNT suspension from 8 to 14 μL caused a decrease in the oxidation peak current of NTX, while increasing the background current. This can be related to sluggish mass transfer process of NTX, because of large film thickness of the cast suspension on the GCE surface. Thus, a volume of 8 μL CNT was selected as optimum volume for preparation of the modified electrode. (The confidence interval (confidence level 95%) was calculated for the results and according to it, responses related to 8 and 10 μL of CNT are out of the experimental error range but the other responses are within the experimental error).
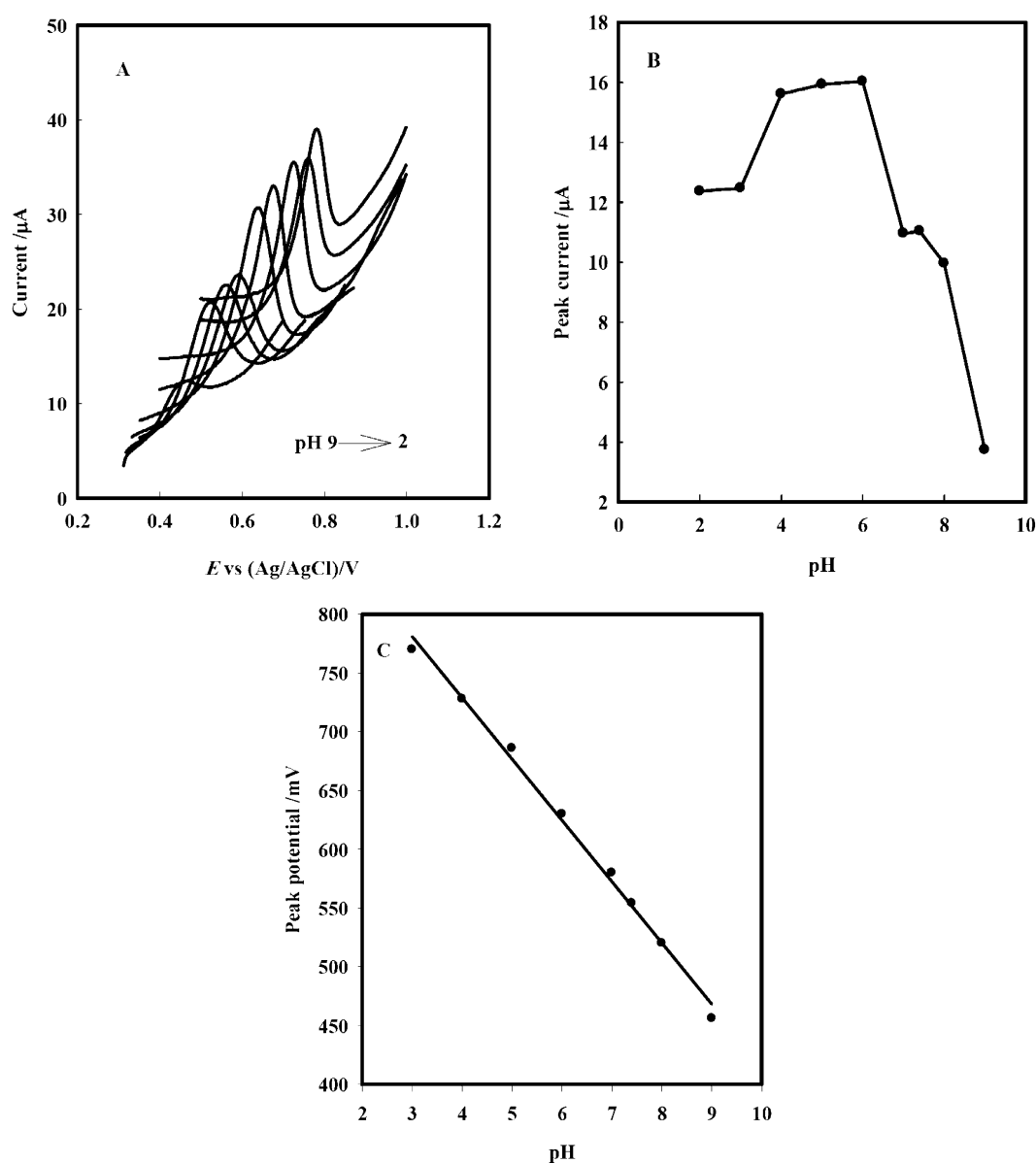
Fig. 5.   (A) LSVs of 1 μmol L^{-1} NTX at PPY/CNT/GCE in various pHs (from 2.0 to 9.0: 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 7.4, 8.0, 9.0) of buffer solution, dependence of (B) the oxidation peak current ($I_{p,a}$) and (C) the oxidation peak potential ($E_{p,a}$) with pH solution; scan rate 100 mV s^{-1}.

3.5 Effect of pH on the Voltammetric Responses

Voltammetric responses of 1 μmol L^{-1} NTX were recorded at different pH values in the range of 2.0–9.0 using 0.04 mol L^{-1} BR buffer as supporting electrolyte (Figure 5A).

As can be seen in Figure 5B, the anodic peak current increased with increasing pH from 2.0 to 4.0, then it was almost constant from pH 4.0 to 6.0. However, further increase in the pH of the buffer solution caused a decrease of the peak height. Therefore, BR buffer solution with pH 6.0 was used as supporting electrolyte for obtaining best sensitivity in all voltammetric determinations. It was observed that the oxidation peak potential of NTX shifted negatively with increasing pH, which indicates that H$^+$

participates in the oxidation process. Figure 5C shows a linear relationship between pH and $E_{p,a}$ in the pH range of 3.0–9.0, regarding the following equation:

$$E_{p,a} \text{ (mV)} = -52.16 \text{ pH} + 937.62 \qquad (R^2 = 0.9945) \quad (1)$$

A slope value of −52.16 mV per pH unit, which is close to the theoretical slope of −59 mV, indicates that equal number of electrons and protons are involved in the electrode reaction. The observed negative shift of the peak potential with increasing pH, confirms a one-electron and one-proton oxidation of phenolic (−OH) group in the anodic oxidation of NTX. A similar electrooxidation of NTX has been previously reported on the surface of

GCE coated with nafion-doped carbon nanoparticles [23].

3.6 Influence of the Potential Sweep Rate

The effect of the potential sweep rate (v) on the peak current was studied using LSV at different scan rates from 25 to 400 mV s^{-1}. It was found that the peak current has a linear relationship with the scan rate (Figure 6).

This relationship can be described by the following equation:

$$I_{p,a} = 0.057v + 1.079 \quad (R^2 = 0.9957; I_{p,a} : \mu A, v : mV\,s^{-1}) \quad (2)$$

This linear relationship confirms the adsorption-controlled process for the electrooxidation of NTX on the surface of PPY/CNT/GCE.

3.7 Effect of Accumulation Time and Potential

In the case of adsorption mechanisms, a simple and effective way to increase the sensitivity of the determinations is to use an accumulation step. The amount of the adsorbed NTX at the surface of the modified electrode can be affected by the accumulation time and corresponding potential, which are two important parameters in this step. The influence of these parameters on the peak current was studied in 1 μmol L^{-1} NTX solution using LSV (Figure 7A).

Before reaching the equilibrium adsorption, the amount of the adsorbed analyte and therefore the resulted peak current can be increased by longer accumulation times. As can be seen in Figure 7B, the oxidation peak current increased until 180 s and then became nearly con-
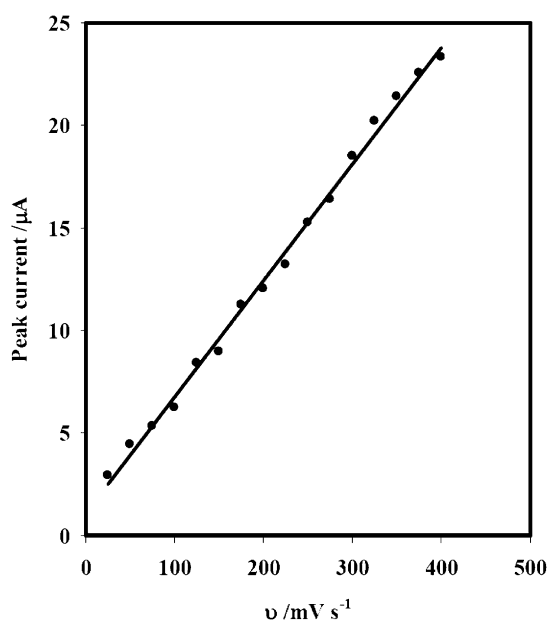
stant at higher accumulation times. Thus, 180 s was selected as the optimum accumulation time. The effect of the accumulation potential was examined from −0.5 V to 0.4 V at 180 s accumulation time. It was found that accumulation potential has no influence on the oxidation peak current. Also, in comparison to the open circuit conditions, it was observed that the accumulation potential has no obvious effect on the peak current. Therefore, accumulation of NTX was performed under the open circuit condition.

3.8 Voltammetric Determinations

The relationship between the oxidation peak current and the concentration of NTX was examined by LSV on the surface of PPY/CNT/GCE under the optimum experimental conditions. The results of the voltammetric studies are shown in Figure 8A.

Using the plot of the peak current versus the concentration of NTX, two linear ranges of 4.0×10^{-8}–1×10^{-6} mol L^{-1} and 1.0×10^{-6}–1.0×10^{-5} mol L^{-1} in 0.04 mol L^{-1} BR buffer solution (pH 6.0) were obtained (Figure 8B). Linear regression equations for these two regions are:

$$I_{p,a}(\mu A) = 14.953\,C\,(\mu mol\,L^{-1}) + 0.295 \quad (R^2 = 0.9907) \quad (3)$$

$$I_{p,a}(\mu A) = 4.493\,C\,(\mu mol\,L^{-1}) + 10.770 \quad (R^2 = 0.9975) \quad (4)$$

A detection limit ($S/N = 3$) of 12 nmol L^{-1} was obtained for NTX using the first range of the calibration plot.

As mentioned earlier, CV has been used for the determination of NTX on the surface of carbon paste electrode in highly acidic solutions (0.1 M HClO$_4$ as supporting electrolyte) [22]. In this work, a detection limit of 5 μmol L^{-1} and a calibration sensitivity of 9.13×10^3 μA mol^{-1} L are resulted for NTX. Also, a flow injection analysis with amperometric detection using a carbon paste electrode has been reported for detection of NTX in pharmaceuticals (in 0.1 M HClO$_4$ as supporting electrolyte) [1]. The method has shown a detection limit of 20 nmol L^{-1} and a calibration sensitivity of 1.16×10^5 μA mol^{-1} L. In another work, a GCE modified with nafion-doped carbon nanoparticles was used for the electrochemical determination of NTX [23]. A detection limit of 0.1 μmol L^{-1} with a calibration sensitivity of 0.177 μA μmol^{-1} L was obtained in this work. In comparison to the previous works [1,22,23], the present work exhibits lower limit of detection and higher sensitivity (14.95 μA μ mol^{-1} L) for the electrochemical determination of NTX. Thus, in this work we have presented a quite sensitive system with desirable electrochemical features by using an easy electrode modification method.

3.9 Reproducibility and Repeatability Evaluations

The repeatability of the response of the modified electrode was evaluated by recording the peak currents of 6
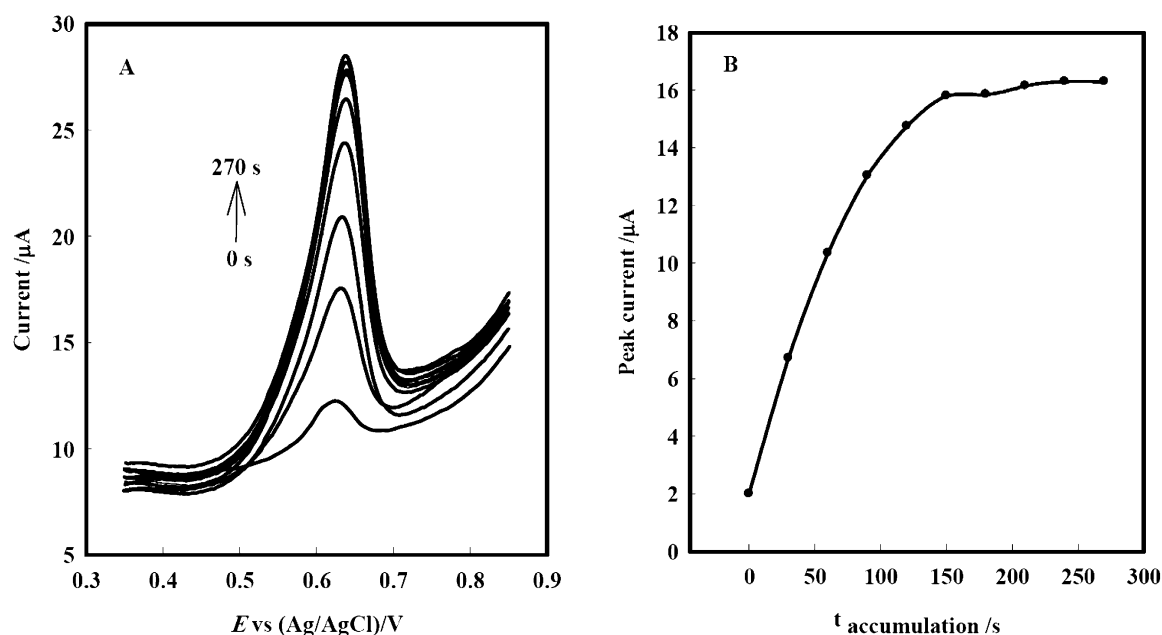
Fig. 6. Plot of $I_{p,a}$ vs. scan rate v.

Fig. 7. (A) LSVs of 1 μmol L^{-1} NTX in 0.04 mol L^{-1} BR buffer solution (pH 6.0) at PPY-CNT/GCE in various accumulation times; Scan rate was 100 mV s^{-1}. (B) Plot of $I_{p,a}$ vs. accumulation time.
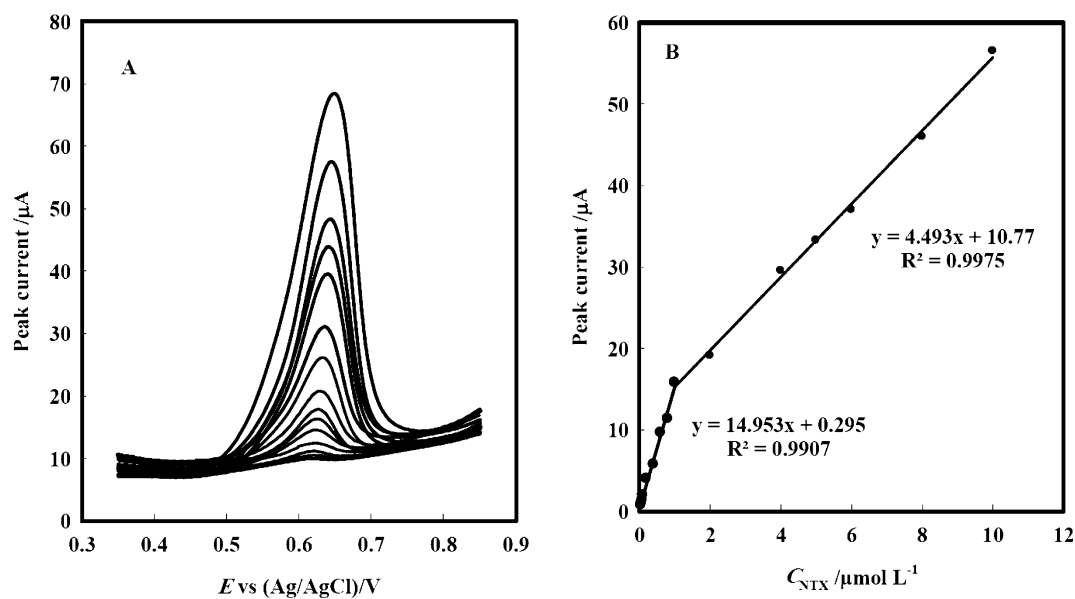

Fig. 8. (A) LSVs for various concentrations of NTX in the range of (down to up), 0.04–10 μmol L^{-1} in 0.04 mol L^{-1} BR buffer solution (pH 6.0) and (B) corresponding linear calibration curve of peak current vs. NTX concentration; scan rate 100 mV s^{-1}.

repetitive measurements at a fixed NTX concentration of 1 μmol L^{-1}. The relative standard deviation based on this replicates was 3.8%. In order to study the reproducibility of the method, 5 modified electrodes were prepared at different days, with the same fabrication procedure. Voltammetric responses of a 1 μmol L^{-1} NTX solution were determined under the optimized conditions using these electrodes. The relative standard deviation (RSD) for the between electrode peak currents was calculated to be 4.3%

Also, after storing the modified electrode under ambient conditions for two weeks, the peak current response of the electrode for a concentration of 1 μmol L^{-1} NTX retained 94.8% of its initial response. This shows the long term stability of the response of the modified electrode. Therefore, in this work we have fabricated a modified electrode with an excellent repeatability and reproducibility, which can be used for sensitive voltammetric determinations of NTX.

3.10 Analytical Applications

3.10.1 Determination of NTX in Pharmaceutical Preparations

In order to evaluate the applicability of the proposed method, the modified electrode was used for determination of the content of NTX in NTX capsules as a real pharmaceutical sample. Four capsules containing a labeled value of 50 mg NTX were accurately weighed and ground to a fine powder. A desired amount of the powdered sample was dissolved in 50 mL of BR buffer solution with pH 6.0 to get a nominal concentration of 1×10^{-4} mol L^{-1}. Then, a nominal concentration of 1×10^{-5} mol L^{-1} was achieved with further diluting with the same buffer solution. Then, 5.0 mL portions of the resulting solution was transferred to 50 mL volumetric flasks and spiked with standard NTX solutions in the range of 10^{-6}–10^{-5} M. The voltammetric responses and corresponding calibration plot of the peak current vs. the concentration are shown in Figure 9A and 9B.

The equation of the resulted standard addition-calibration plot was:

$$I_{p,a}(\mu A) = 4.330\, C\, (\mu mol\, L^{-1}) + 15.252 \quad (R^2 = 0.9968) \quad (5)$$

According to the obtained equation, it is concluded that the capsule matrix does not show any interference with the electrochemical analysis of NTX. An amount of 52.15 mg with good accuracy of 104.3% and a *RSD* of 4.6% was found for the analysis of capsule samples with a labeled value of 50 mg. Moreover, for accuracy studies, recoveries were evaluated in the lower, middle and higher level concentrations of the spiked NTX to the

pharmaceutical solutions. The recoveries were in the range from 94.3% to 102.1%.

3.10.2 Determination of NTX in Human Blood Serum

For preparation of serum samples, methanol was added to serum samples (5% (v/v)) and thoroughly mixed. Then, the serum samples were diluted 10 times with BR buffer solution (pH 6.0). The diluted serum samples were spiked with standard solutions of NTX in the range of 1×10^{-7}–1.0×10^{-6} mol L^{-1}. The modified electrode was applied for analysis of these samples. The results of the recovery evaluations showed an average recovery of 94.1% for NTX added to the serum samples.

4 Conclusions

In the present work, a new, highly sensitive and effective electrochemical sensor was constructed for the electrochemical measurement of NTX. Application of NY as a new kind of dopant led to efficient polymerization of pyrrole on the surface of CNT/GCE and formation of a highly stable and uniform film of the modifier on the electrode surface. The prepared modified electrode with important advantages such as, low detection limit, high sensitivity and relatively wide linear dynamic range was successfully applied for voltammetric determination of NTX in pharmaceutical and clinical preparations.

Acknowledgements
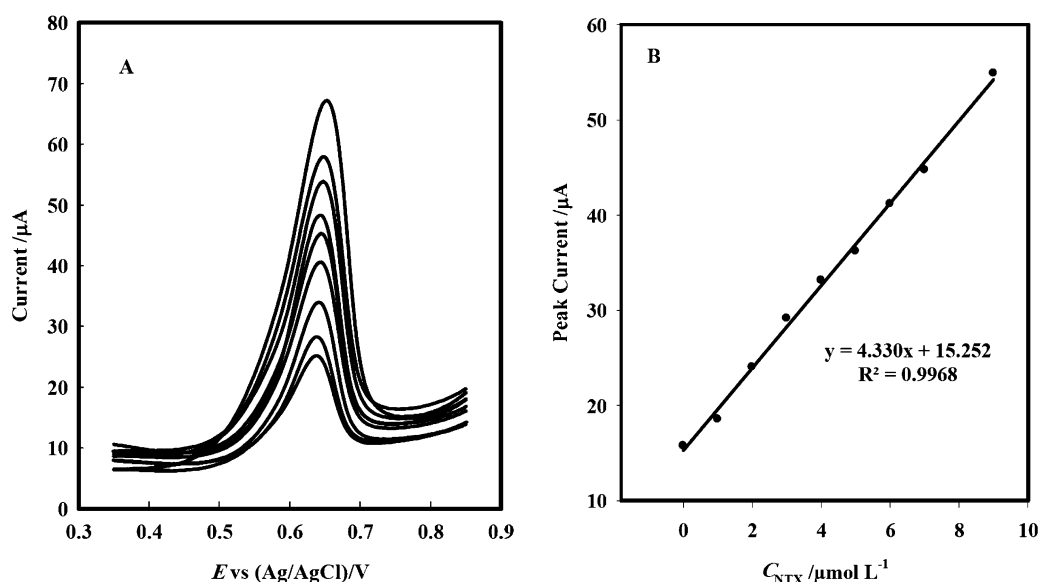
Fig. 9. (A) LSVs for the addition of different amounts of NTX (from down to up: 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0 µmol L^{-1}) in the real sample solution of NTX diluted with 0.04 mol L^{-1} BR buffer solution (pH 6.0) at PPY/CNT/GCE; scan rate 100 mV s^{-1} and (B) the plot of peak current vs. added concentration of NTX.

References

[1] M. T. Fernandez-Abedul, A. Costa-Garcia, *J. Pharm. Biomed. Anal.* **1997**, *16*, 15.

[2] F. S. Felix, L. Angnes, *J. Pharm. Sci.* **2010**, *99*, 4784.

[3] R. I. Shader, *J. Clin. Psychopharmacol.* **2003**, *23*, 321.

[4] J. A. M. Pulgarin, L. F. G. Bermejo, J. L. G. Lara, *Anal. Chim. Acta* **2003**, *495*, 249.

[5] M. Khanmohammadi, H. Mobedi, E. Mobedi, K. Kargosha, A. B. Garmarudi, K. Ghasemi, *Spectroscopy* **2009**, *23*, 113.

[6] P. Zuccaro, I. Altieri, P. Betto, R. Pacifici, G. Ricciarello, L. A. Pini, E. Sternieri, S. Pichini, *J. Chromatogr.* **1991**, *567*, 485.

[7] C. Kim, R. Cheng, W. A. Corrigall, *Chromatographia* **1988**, *25*, 91.

[8] K. Kambia, S. Bah, T. Dine, R. Azar, P. Odou, B. Gressier, M. Luyckx, C. Brunet, L. Ballester, M. Cazin, J. C. Cazin, *Biomed. Chromatogr.* **2000**, *14*, 151.

[9] G. L. Burce, H. B. Bhat, T. Sokoloski, *J. Chromatogr.* **1977**, *137*, 323.

[10] K. Verebely, S. J. Mule, D. Jukofsky, *J. Chromatogr.* **1975**, *111*, 141.

[11] K. Verebey, A. De Pace, D. Jukofsky, J. V. Volavka, S. J. Mule, *J. Anal. Toxicol.* **1980**, *4*, 33.

[12] M. Rezaei, K. Abdi, R. Dinarvand, S. Vosough-Ghanabri, M. Amini, *Chromatographia* **2009**, *70*, 1491.

[13] S. W. Toennes, G. F. Kauert, S. M. Grusser, W. Jakel, G. Partecke, *J. Pharm. Biomed. Anal.* **2004**, *35*, 169.

[14] C. Clavijo, J. Bendrick-Pearta, Y. L. Zhang, G. Johnson, A. Gasparic, U. Christians, *J. Chromatogr. B* **2008**, *874*, 33.

[15] A. Campiglio, *Analyst* **1998**, *123*, 1053.

[16] K. Verebey, J. Alarazi, M. Lehrer, S. J. Mule, *J. Chromatogr.* **1986**, *378*, 261.

[17] K. K. Peh, N. Billa, K. H. Yuen, *J. Chromatogr. B* **1997**, *701*, 140.

[18] A. F. Davidson, T. A. Emm, H. J. Pieniaszek, *J. Pharm. Biomed Anal.* **1996**, *14*, 1717.

[19] S. Valiveti, B. N. Nalluri, D. C. Hammell, K. S. Paudel, A. L. Stinchcomb, *J. Chromatogr. B* **2004**, *810*, 259.

[20] M. H. Slawson, M. Chen, D. Moody, S. D. Comer, E. S. Nuwayser, W. B. Fang, R. L. Foltz, *J. Anal. Toxicol.* **2007**, *31*, 453.

[21] W. Huang, D. E. Moody, R. L. Foltz, S. L. Walsh , *J. Anal. Toxicol.* **1997**, *21*, 252.

[22] M. T. Fernandez-Abedul, M. S. Velazquez-Rodriguez, J. R. Barreira-Rodriguez, A. Costa-Garcia, *Anal. Lett.* **1997**, *30*, 1491.

[23] F. Ghorbani-Bidkorbeh, S. Shahrokhian, A. Mohammadi, R. Dinarvand, *J. Electroanal. Chem.* **2010**, *638*, 212.

[24] S. Iijima, *Nature* **1991**, *354*, 56.

[25] H. Wu, X. Chang, L. Liu, F. Zhao, Y. Zhao, *J. Mater. Chem.* **2010**, *20*, 1036.

[26] B. Wu, Y. Kuang, X. Zhang, J. Chen, *Nano Today* **2011**, *6*, 75.

[27] L. T. Qu, L. M. Dai, *J. Am. Chem. Soc.* **2005**, *127*, 10806.

[28] H. C. Choi, M. Shim, S. Bangsaruntip, H. J. Dai, *J. Am. Chem. Soc.* **2002**, *124*, 9058.

[29] L. T. Qu, L. M. Dai, E. Osawa, *J. Am. Chem. Soc.* **2006**, *128*, 5523.

[30] K. P. Gong, F. Du, Z. H. Xia, M. Durstock, L. M. Dai, *Science* **2009**, *323*, 760.

[31] P. Manisankar, P. L. Abirama Sundari, R. Sasikumar, S. P. Palaniappan, *Talanta* **2008**, *76*, 1022.

[32] P. Manisankar, P. L. Abirama Sundari, R. Sasikumar, D. Jestin Roy, *Electroanalysis* **2008**, *20*, 2076.

[33] M. D. Shirsat, C. O. Too, G. G. Wallace, *Electroanalysis* **2008**, *20*, 150.

[34] Y. C. Tsai, S. C. Li, S. W. Liao, *Biosens. Bioelectron.* **2006**, *22*, 495.

[35] H. Peng, L. Zhang, C. Soeller, J. Travas-Sejdic, *Biomaterials* **2009**, *30*, 2132.

[36] J. C. Vidal, E. Garcia-Ruiz, J. R. Castillo, *Microchim. Acta* **2003**, *143*, 93.

[37] S. Nambiar, J. T. W. Yeow, *Biosens. Bioelectron.* **2011**, *26*, 1825.

[38] L. Xia, Z. Wei, M. Wanb, *J. Colloid Interf. Sci.* **2010**, *341*, 1.

[39] M. Ates, A. S. Sarac, *Prog. Org. Coat.* **2009**, *66*, 337.

[40] D. W. Hatchett, M. Josowicz, *Chem. Rev.* **2008**, *108*, 746.

[41] M. Trojanowicz, *Microchim. Acta* **2003**, *143*, 75.

[42] U. Lange, N. V. Roznyatovskaya, V. M. Mirsky, *Anal. Chim. Acta* **2008**, *614*, 1.

[43] J. Li, W. Wei, S. Luo, *Microchim. Acta* **2010**, *171*, 109.

[44] S. Sen, A. Gok, H. Gulce, *J. Appl. Polym. Sci.* **2007**, *106*, 3852.

[45] V. Branzoi, L. Pilan, F. Branzoi, *Electroanalysis* **2009**, *21*, 557.

[46] M. Hughes, G. Z. Chen, M. S. P. Shaffer, D. J. Fray, A. H. Windle, *Chem. Mater.* **2002**, *14*, 1610.

[47] S. Shahrokhian, E. Asadian, *J. Electroanal. Chem.* **2009**, *636*, 40.

[48] C. Peng, J. Jin, G. Z. Chen, *Electrochim. Acta* **2007**, *53*, 525.

[49] S. Shahrokhian, R. S. Saberi, *Electrochim. Acta* **2011**, doi:10.1016/j.electacta.2011.04.029.

[50] M. Gerard, A. Chaubey, B. D. Malhotra, *Biosens. Bioelectron.* **2002**, *17*, 345.

[51] F. R. R. Teles, L. P. Fonseca, *Mater. Sci. Eng. C* **2008**, *28*, 1530.

[52] A. Malinauskas, J. Malinauskiene, A. Ramanavicius, *Nanotechnology* **2005**, *16*, R51.

[53] A. Alumaa, A. Hallik, V. Sammelselg, J. Tamm, *Synth. Met.* **2007**, *157*, 485.

[54] P. Rapta, A. Neudeck, A. Bartl, L. Dunsch, *Electrochim. Acta* **1999**, *44*, 3483.

[55] M. Pyo, J. R. Reynolds, L. F. Warren, H. O. Marcy, *Synth. Met.* **1994**, *68*, 71.

[56] H. Wenker, *Ind. Eng. Chem.* **1934**, *26*, 350.

[57] M. Milewski, A. L. Stinchcomb, *Pharm. Res.* **2011**, *28*, 124.

[58] I. Streeter, G. G. Wildgoose, L. Shao, R. G. Compton, *Sens. Actuators B* **2008**, *133*, 462.

[59] G. P. Keeley, M. E. G. Lyons, *Int. J. Electrochem. Sci.* **2009**, *4*, 794.

[60] L. Xiao, G. G. Wildgoose, R. G. Compton, *Sens. Actuators B* **2009**, *138*, 524.

[61] C. E. Banks, T. J. Davies, G. G. Wildgoose, R. G. Compton, *Chem. Commun.* **2005**, *7*, 829.

[62] C. E. Banks, R. G. Compton, *Analyst* **2006**, *131*, 15.