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Voltammetric Behavior and Determination of Isoniazid Using PEDOT Electrode in Presence Of Surface Active Agents

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An electrochemical sensor was developed by using poly(3,4-ethylenedioxy thiophene) electrode in presence of different types of surfactants. Voltammetric behavior of isoniazid was studied at this electrode in the presence and absence of SDS and CTAB and interesting electrocatalytic effects were found. The presence of surfactant in the medium plays a key role in the electrostatic attraction and repulsion of isoniazid towards the polymeric surface in different pH values. The electrochemical behavior of isoniazid was investigated by cyclic voltammetry, linear sweep voltammetry (LSV), and electrochemical impedance spectroscopy (EIS). The linear response obtained for isoniazid was in the range of 0.1 to 8 μmol L^{-1} and 10 to 100 μmol L^{-1} with correlation coefficients of 0.999 and 0.998 and detection limits 32 nmol L^{-1} and 45 nmol L^{-1}, respectively. The utility of this modified electrode was demonstrated for the determination of INH in human urine.

Keywords: Isoniazid; PEDOT; Surfactants; CV; EIS

1. INTRODUCTION

Isoniazid (pyridine-4-carboxilic acid hydrazide or isonicotinic acid hydrazide) is a widely used drug alone in the prophylaxis and in combination with other antituberculars in the treatment of all forms of tuberculosis [1]. Several methods for the analysis of isoniazid are available in the literature such as titrimetry [2,3], spectrophotometry [4–6], chemiluminescence [7–9], fluorimetry [10], high performance liquid chromatography (HPLC) [11–13], capillary electrophoresis [14– 16], and electroanalytical [17–19]. Electroanalytical techniques can be advantageous because they provide good sensitivity, precision and accuracy, simplicity and rapidity. In spite of this, there are only a few papers that report the analysis of isoniazid employing electroanalytical methods.

On the other hand, the use of mercury electrodes is not recommended due to the toxicity of this

metal. In addition, the voltammetric techniques require previous accumulation steps, precluding a high sampling frequency. A number of researchers have employed many kinds of polymeric film modified electrodes due to their features of good electrocatalysis and stability [20-22]. The chemically modified electrodes by poly(aniline) [23], poly (thiophene) [24, 25] or poly(pyrrole) [26] have been used to detect electroactive materials, such as dopamine, ascorbic acid, nitrate, norepinephrine and hemoglobin, and so on. Among various types of conducting polymers, Poly(3,4-ethylenedioxythiophene) (PEDOT) is a widely investigated electronically conducting polymer, which can be easily electrodeposited onto surface by electrooxidation of its monomer [27-30]. PEDOT modified electrodes have been extensively reported and showed excellent electrocatalytic effect on phenolic compounds [31], detection of DA [32], AA [33], and morphine [34]. Surfactants have been widely used in chemistry and in particular affecting several electrochemical processes [35]. Several applications of surfactants in electrochemistry are in electroplating [36], corrosion [37], fuel cells [38], electrocatalysis [39], and electroanalysis [40]. The area of surface modified electrodes is of particular interest because of its application in sensors. Rusling et al. [41] indicated the influence of surfactant aggregates at the electrode/electrolyte interface in micelle solutions. In his study [41], it was shown that the entry of an electrochemical reactant into this dynamic surface film is a key preceding electron transfer step. On the other hand, surfactants have proven effective in the electroanalysis of biological compounds and drugs. For example, it was recently shown that surfactants are highly effective in stabilizing the voltammetric response of serotonin by protecting the electrode surface from fouling [42, 25]. In another study, it was shown that anionic surfactants could also be used to improve the accumulation of some electroactive organic molecules such as ethopropazine at gold electrodes [43]. Recently, the influence of micelles in the simultaneous determination of two components was also demonstrated, as in the case of ascorbic acid and dopamine [44] and catechol and hydroquinone [45]. It was not clear whether the micelle interaction with the analyte in the solution phase contributes to the selective response. It is well established that interaction between aggregates and solutes in the solution phase is controlled by diffusion and takes place in the microsecond time scale [46].

In this work, PEDOT/Pt electrode is modified by hydrophobic adsorption of different types of surfactants on the surface of the electrode. This electrode is simple phase surface with a high density of anionic or cationic charges covered on the electrode surface according to the type of surfactant. Selectivity and sensitivity of the sensor is examined for the determination of isoniazid in presence of interfering molecules such as uric acid and ascorbic acid. Also estimation of the diffusion coefficient of isoniazid is calculated. Moreover, the sensor is utilized to determine isoniazid in human urine.

2. EXPERIMENTAL

2.1. Materials and reagents

All chemicals were used as received without further purification 3,4-(ethylene dioxy) thiophene EDOT, lithium per chlorate (LiClO4) , acetonitrile (HPLC) grade, isoniazid, ascorbic acid, uric acid, sodium dodecyl sulphate and cetyl trimethyl ammonium bromide (CTAB) were supplied by Aldrich

Chem. Co. (Milwaukee, WI. USA). Aqueous solutions were prepared using double distilled water. B-R buffer of pH 2-9 are prepared from (0.12 mol L^{-1} boric acid, 0.12 mol L^{-1} acetic acid and 0.12 mol L^{-1} ortho-phosphoric acid) and adjusted by 0.2 mol L^{-1} NaOH.

2.1.1. Preparation of PEDOT modified Pt-electrode

Electrochemical polymerization and characterization were carried out with a three-electrode/one-compartment glass cell. The working electrode was platinum disc (diameter: 1.5 mm). The auxiliary electrode was (10 cm long/2.0 mm diameter), platinum wire. All the potentials in the electrochemical studies were referenced to Ag/AgCl (3.0 mol L^{-1} NaCl) electrode. The Pt-electrode was polished by a BAS-polishing kit with 0.3 and 0.05 μm alumina slurry, rinsed and then sonicated in double-distilled water before starting each experiment. The electrochemical polymerization of the EDOT was carried out by the cyclic voltammetric method in non aqueous solution containing 0.01 mol L^{-1} EDOT, and 0.1 mol L^{-1} $LiClO_4$ in acetonitrile.

2.2. Instrumental and experimental set-up

2.2.1. Electrochemical measurements

The electrosynthesis of the polymer and its electrochemical characterization were performed using an Epsilon electrochemical analyzer (Bioanalytical systems, BAS, West Lafayette, USA). Linear scan voltammetry (LSV), cyclic voltammetry (CV) were used for the determination of the compounds using modified PEDOT/Pt electrode.

2.2.2. Spectroscopic measurements

Electrochemical impedance spectroscopy was performed using a Gamry-750 system and a lock-in-amplifier that are connected to a personal computer. The parameters in electrochemical impedance experiment were as follows: different potential values 0.65 V for INH at pH 2.3, and 0.25 V at pH 7.4 were studied at frequency range of 0.1– 10000 Hz with amplitude of 5 mV were applied on PEDOT/Pt electrode and tested in 0.5 mmol L^{-1} INH in presence and absence of each of 0.1 mol L^{-1} 150 μL SDS and 0.1 mol L^{-1} 150 μL CTAB in buffer pH 2.3 and pH 7.4.

2.3. Analysis of urine

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of INH in human urine samples. INH was dissolved in urine to make a stock solution with 5 mmol L^{-1} concentration. Standard successive additions of 10 μL of 5 mmol L^{-1} isoniazid in urine were added to the buffer pH 2.3 containing 150 μL SDS.

3. RESULTS AND DISCUSSION

3.1. Electrochemistry of isoniazid in different types of surfactants

3.1.1. Electrochemistry of isoniazid in pH 2.3

Although INH itself showed a very poor electrochemical response at the platinum electrode, the response could be greatly enhanced by using PEDOT/Pt electrode in presence of anionic surfactant SDS, which enables a sensitive electrochemical determination of the substrate INH in pH 2.3. Figure 1**A** shows the cyclic voltammograms of successive additions of 150 μL 0.1 mol L^{-1} SDS in 0.5 mmol L^{-1} INH at PEDOT/PT electrode in 0.1 mol L^{-1} B-R (pH 2.3). Two well-defined irreversible anodic peaks of INH at +0.63 V and +0.82 V are observed at the PEDOT/Pt electrode with anodic peak current 30 μA and 24 μA, respectively.

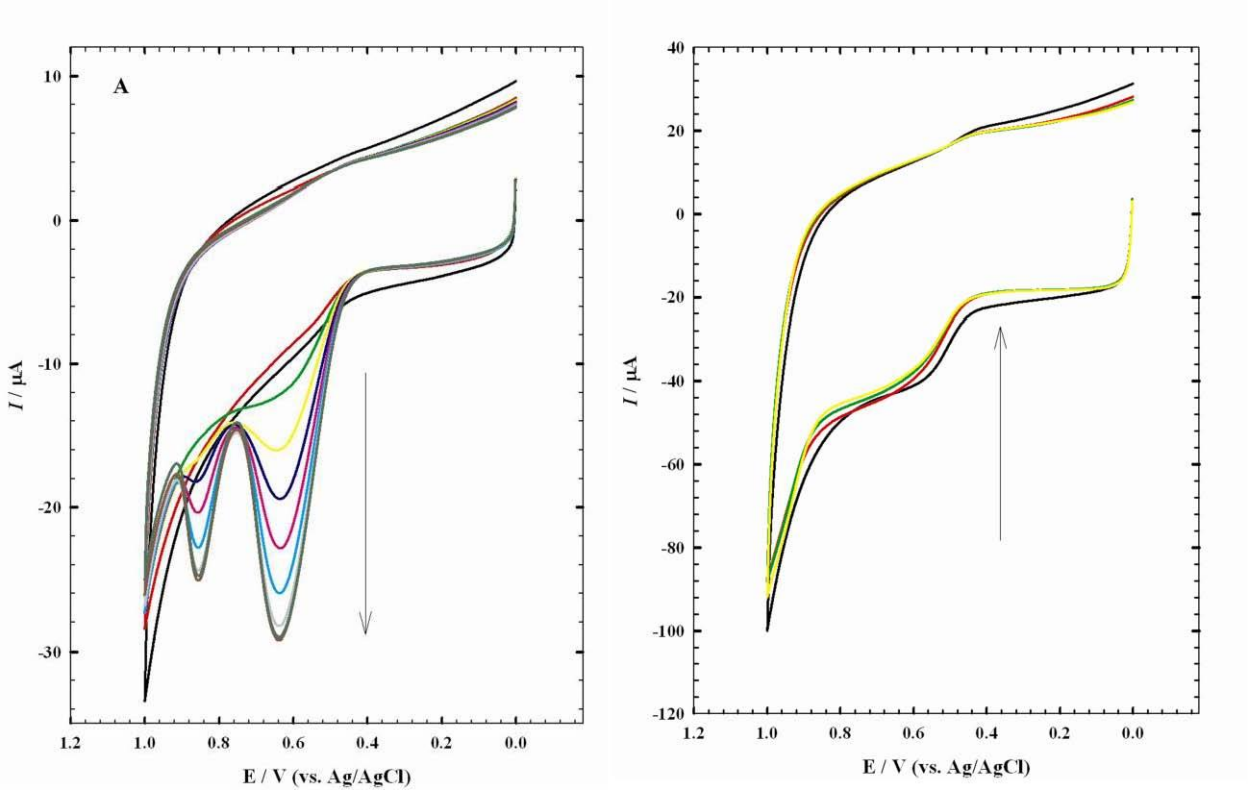

Figure 1. Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} INH / 0.1 mol L L^{-1} B-R, scan rate 50 mV s^{-1} at PEDOT/Pt electrode at pH 2.3 with successive additions (0 μL – 150 μL) of 0.1 mol L^{-1} (A) SDS, (B) CTAB.

These anodic peaks are attributed to the oxidation reaction of INH and the reaction is irreversible. Isoniazid has three p*Ka* values: 1.8 based on hydrazine nitrogen, 3.5 based on pyridine nitrogen and 10.8 based on acidic group. Since the pH of the solution is 2.3, therefore the oxidation peaks are attributed to protonated hydrazine nitrogen. The PEDOT/Pt electrode did not show any voltammetric peak for INH at this pH value (as the PEDOT has positive charge density it repels the

cationic molecules of INH from its surface and no peak is observed in the applied scale used). The anionic surfactant SDS enhances greatly the anodic peak current of INH which is attributed to the adsorption of the anionic surfactant SDS onto electrode surface forming a negatively charged hydrophilic film with the polar head group pointing to the bulk of the solution. This negatively charged hydrophilic layer facilitates reaching of INH to the electrode surface faster, and as a consequence, the reaction becomes easier. On the other hand, in Figure 1B the cyclic voltammograms of 0.5 mmol L^{-1} INH at PEDOT/PT electrode with successive additions of 150 μL 0.1 mol L^{-1} CTAB in 0.1 mol L^{-1} B-R (pH 2.3) shows the opposite behavior. Upon addition of the cationic surfactant CTAB, a weak broad peak at PEDOT/Pt electrode is observed which attributed to the oxidation of INH. The anodic current peak decreases by addition of CTAB, and this is attributed to the adsorption of the cationic surfactant CTAB onto electrode surface forming a positively charged hydrophilic film with the polar head group pointing to the bulk of the solution. This positively charged hydrophilic layer repels INH cationic molecules from the electrode surface, and as consequence, the diffusion becomes difficult. Therefore, in case of SDS the micellar effect on the oxidation of INH is basically an electrostatic interaction between the surfactant film adsorbed on the electrode and the protonated INH. The lower oxidation potential and higher current response clearly indicate that PEDOT/Pt electrode has excellent electrocatalytic activity towards isoniazid, which attributed to the presence of anionic SDS.

3.1.2. Electrochemistry of isoniazid in pH 7.4

At pH 7.4 the positive charge density of isoniazid decreases according to its pKa and becomes neutral, which affects its interaction with the polymeric layer and the surfactant molecules. The diffusion rate of INH towards the polymeric layer changed remarkably. (Figure 2 **A)** shows the cyclic voltammograms of successive additions of 150 μL 0.1 mol L^{-1} CTAB in 0.5 mmol L^{-1} INH at PEDOT/PT in 0.1 mol L^{-1} B-R (pH 7.4). At the beginning one anodic peak of INH at +0.26 V is observed at the PEDOT/Pt electrode with anodic peak current 38 μA which did not appear in pH 2.3, in this case INH is neutral and its diffusion towards the polymeric cationic film become easier. This indicates that PEDOT/Pt electrode in pH 7.4 have great response in INH, in its neutral form. By successive additions of 150 μL 0.1 mol L^{-1} CTAB, the anodic peak current increases to 55 μA then becomes almost stable. The cationic surfactant CTAB enhances greatly the anodic current peak of INH at pH 7.4 which is attributed to the adsorption of the cationic surfactant CTAB onto electrode surface forming a positively charged hydrophilic film with the polar head group pointing to the bulk of the solution [22]. The electrostatic force between CTAB and INH facilitates reaching of INH to the electrode surface faster, and as consequence, the reaction becomes easier. On the other hand, Figure 2B shows the CVs of successive additions of 150 μL 0.1 mol L^{-1} SDS in 0.5 mmol L^{-1} INH at PEDOT/PT in 0.1 mol L^{-1} B-R (pH 7.4). This figure indicates the opposite behavior, thus the anodic peak at 0.26 V at PEDOT/Pt electrode is observed which is attributed to the diffusion of INH towards the polymeric layer, this behavior did not occur in pH 2.3 in the previous section. By addition of the anionic surfactant SDS, the anodic current peak decreases which is attributed to the adsorption of the anionic surfactant SDS onto electrode surface forming a negatively charged hydrophilic film with the

polar head group points to the bulk of the solution. Due to the difference in the structure of the surfactants, the electrostatic force between SDS and INH is weaker in (pH 7.4) and as a consequence, the diffusion to the surface of the electrode becomes difficult and depends on the hydrophobic interaction. The adsorption of the surfactant molecules on the electrode surface that could be followed by the formation of micelle aggregates as the distance from the electrode surface increases [41, 47]. The possibility of aggregation of the drug with SDS in this pH can only be attributed to hydrophobic interactions and leads to reduced aggregation as compared to the SDS (in case of pH 2.3). The strength of interaction and binding between the drug and the surfactant should result in the observed distinct behavior and should also partially affect the transport of their corresponding aggregates in solution [48].

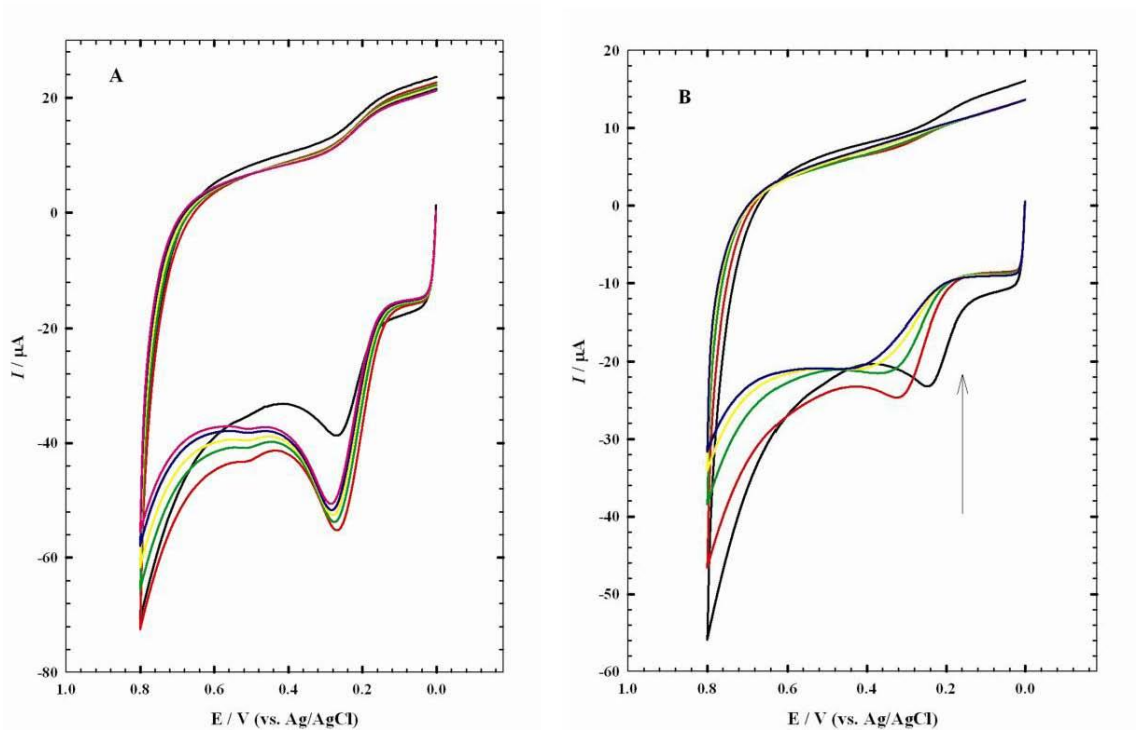

Figure 2. Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} INH / 0.1 mol L^{-1} B-R, scan rate 50 mV s^{-1} at PEDOT/Pt electrode at pH 7.4 with successive additions (0 µL – 150 µL) of 0.1 mol L^{-1} (A) CTAB, (B) SDS.

3.2 Effect of scan rate and apparent diffusion coefficient

As shown in figure 3, the oxidation peak currents (*i*p) at the PEDOT/Pt electrode in presence of 150 mL 0.1 mol L^{-1} SDS and 5 mmol L^{-1} INH solution (pH 2.3) varied linearly with change of square root of scan rate (ν). The dependence of the anodic peak current density on the scan rate has been used for the estimation of the "apparent" diffusion coefficient, Dapp, for the compounds studied. Dapp values were calculated from Randles Sevcik equation [49]

$$Ip = 2.69 \times 10^5 \, n^{3/2} \, A \, C_0 \, D^{1/2} \, \nu^{1/2}$$

Where *I*p is the peak current density (A cm^{-2}), n is the number of electrons transferred at T = 298 K, A is the geometrical electrode area (0.0176 cm^2), C_0 is the analyte concentration (5×10^{-6} mol cm^{-3}), and D is the diffusion coefficient of the electroactive species (cm^2 s^{-1}). Apparent surface area used in the calculations did not take into account the surface roughness, which is an inherent characteristic for all polymer films formed using the electrochemical techniques. D_{app} value at PEDOT/Pt electrode for INH is 4.2 x 10^{-4} cm^2 s^{-1} in presence of SDS, which is larger than their corresponding value at the PEDOT/Pt electrode 4.1 x 10^{-5} cm^2 s^{-1}. The anionic surfactant SDS affects remarkably the diffusion component of the charge transfer at the electrode surface as indicated by the D_{app} value. The diffusion coefficient can be considered as an average value of the diffusion process in the bulk, within the surfactant aggregates in solution and the surfactant layer adsorbed at the surface of the electrode. The size of the diffusion layer at the electrode surface proximity changes with the voltage scan used. At relatively slow voltage scans the diffusion layer grows much further towards the solution side and further from the electrode surface. Therefore, as the scan rate increases the flux to the electrode surface increases considerably. At relatively higher scan rates and in presence of SDS that mainly aggregates at the electrode surface and forms a pair with the drug in electrolyte, the diffusion layer grows less further from the vicinity of the electrode. The values indicated for D_{app} show that the diffusion is enhanced in presence of SDS than in absence of it.

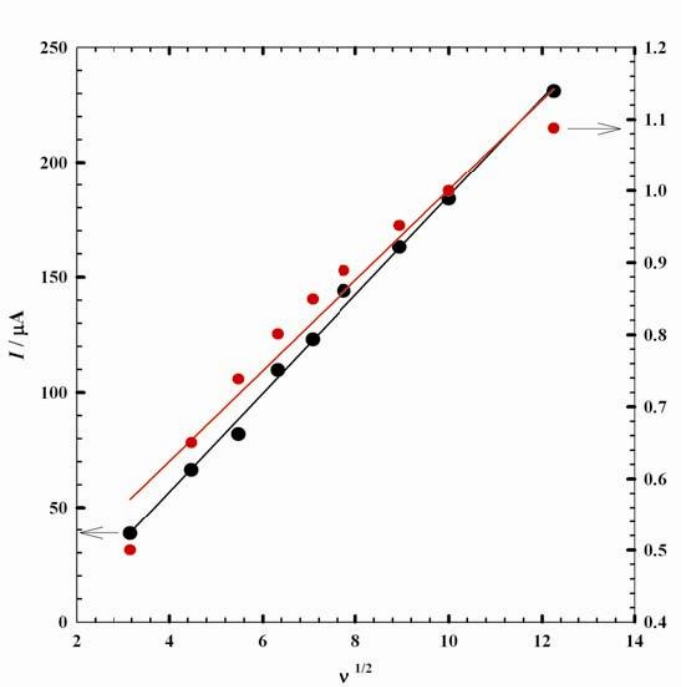

Figure 3. A plot of the anodic peak current values as a function of the square root of the scan rate at PEDOT/Pt electrode (a) in presence and (b) in absence of SDS.

3.3. Effect of pH

The electrochemical behavior of isoniazid was studied in acidic media of pH < 6 [50], and in neutral and basic media [51]. On the basis of polarographic and voltammetric measurements and by

regarding the tafel slopes and reaction orders, it was concluded that in a strongly acidic medium (pH < 2) the rate-determining step of the process was the loss of an ammonia molecule to yield isonicotinamide. Whereas at pH > 2 the process was controlled by the second one-electron irreversible transfer. Therefore, the influence of the solution pH on both catalytic peak currents and potentials was assessed through examining the electrode response in solution buffered in pH 2.3–9 in presence and absence of SDS and CTAB. The plot of Ip versus pH in absence and presence of CTAB, Figure 4A shows that the catalytic peak current decreases as the acidity of the solution is increased in absence and presence of CTAB curve a, b respectively. The plot of Ip versus pH in absence and presence of SDS Figure 4B shows that no peak current for INH in acidic medium in absence of SDS. Moreover, as the pH value increases the anodic peak current decreases in absence of SDS. In presence of SDS the maximum peak current is observed only in acidic medium as the structure of INH has high positively charged density and as the pH value increases the anodic peak current decreases.

(Supplementary 1A, B) shows the cyclic voltammograms of INH at different pH (2.3-9) in presence of SDS and CTAB. The peak potential shifted negatively with increase of pH in presence and absence of SDS and CTAB (inset), respectively. It could be explained by the consequence of deprotonation involved in the oxidation process which was facilitated at higher pH values. Our results showed that INH adsorbed readily on PEDOT/Pt electrode in presence of SDS in acidic medium and in presence of CTAB in neutral and alkaline medium.

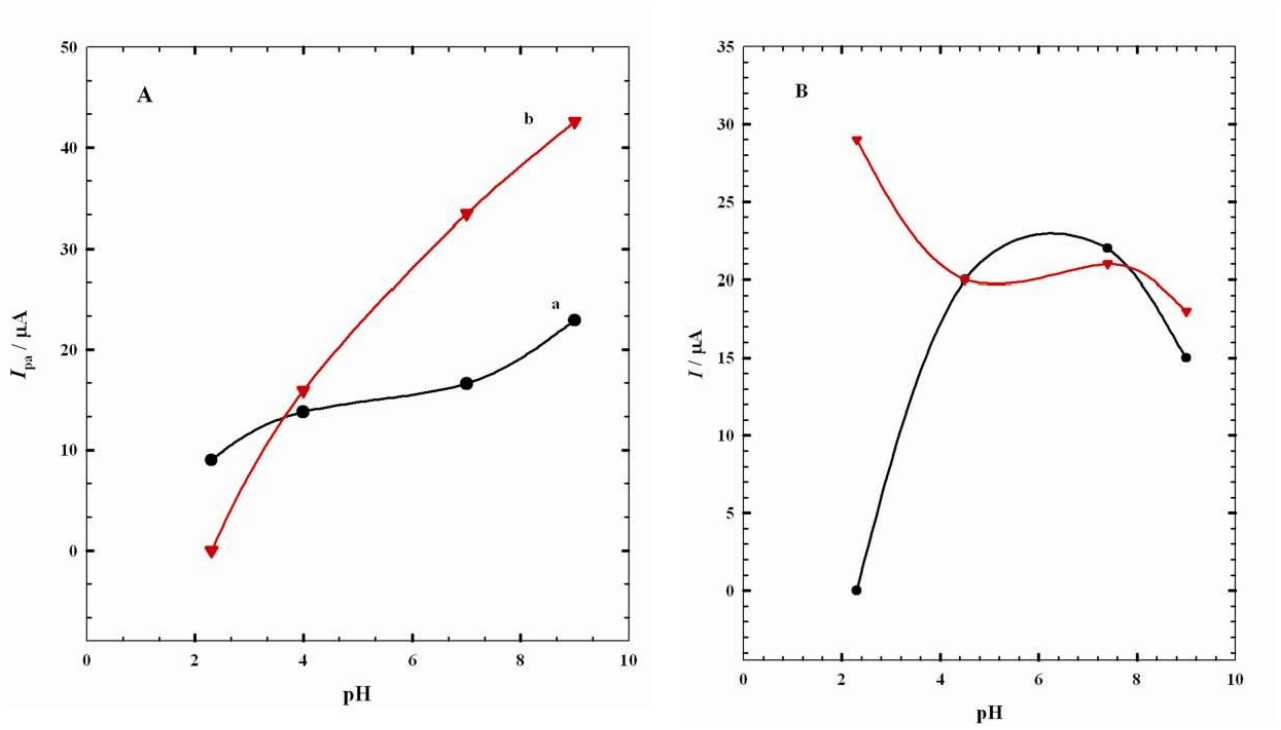

Figure 4. A plot of the anodic peak current values as a function of the pH value of the solution at PEDOT/Pt electrode (a) in absence and (b) in presence of (A) CTAB and (B) SDS.

On one hand, this is related to the differences in the surface properties of the electrode in absence and presence of SDS and CTAB and the adsorption interactions between INH and the

modified electrode surface according to its pKa value and the pH of the medium. On the other hand, the variation of electrostatic interaction between INH and the anionic SDS and cationic CTAB at different pH could also be responsible for this phenomenon. Based on above reasons, the current response increases with SDS in pH 2.3 and increases with CTAB in pH 7.4, pH 9.

3.4. Calibration graph

Figure 5A shows linear sweep voltammogram of the PEDOT/Pt electrode of different concentrations of INH (0.1 μmol L^{-1} -100 μmol L^{-1}) in presence of 150 μL 0.1 mol L^{-1} SDS in 0.1 mol L^{-1} B-R buffer solutions. Two anodic peaks current increases with increase of the INH concentration. Moreover, the calibration curve (figure 5B) shows linear behavior of peak current values versus different concentrations of INH ranging from of 0.1 to 8 μmol L^{-1} and from 10 μmol L^{-1} to 100 μmol L^{-1} with correlation coefficients of 0.999 and 0.998 and detection limits 32 nmol L^{-1} and 45 nmol L^{-1}, respectively.

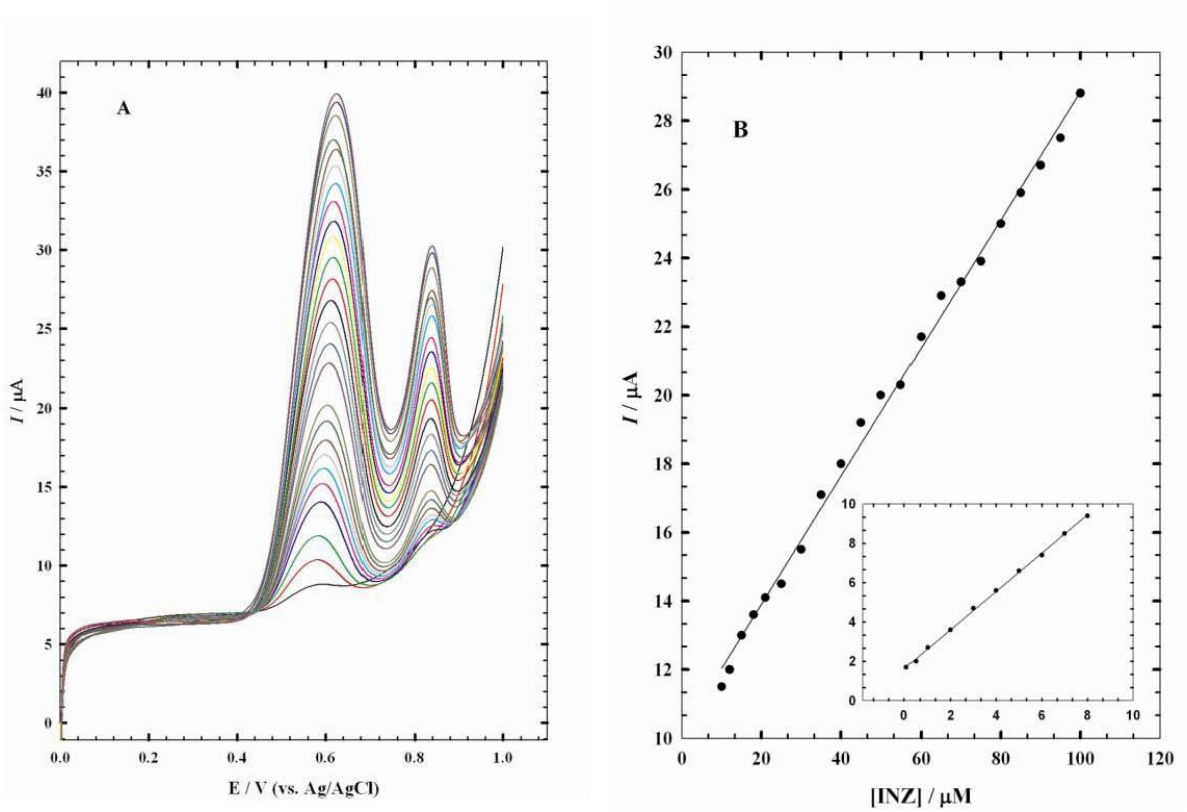

Figure 5 A. LSVs of different concentrations of INH (0.1 μmol L^{-1} -0.1 mmol L^{-1}) in 10 mL of 0.1 mol L^{-1} B-R pH 2.3 containing 150 μL 0.1 mol L^{-1} SDS at PEDOT/Pt electrode. **(B)** Calibration curve for INH of different concentrations from (10 μmol L^{-1} to 0.1 mmol L^{-1}) and from (0.1 μmol L^{-1} to 8 μmol L^{-1}) (inset).

3.5. Response stability of the modified electrode

Isoniazid 5.0×10^{-4} mol L^{-1} was determined repeatedly at the identical surface of PEDOT/Pt

electrode in 150 μL 0.1 mol L^{-1} SDS in 0.1 mol L^{-1} B-R buffer solutions pH 2.3 for 25 successive times, and the average current was 31 μA with the RSD of 3.2%, which showed an excellent reproducibility. The precision at renewed surface of PEDOT/Pt electrode in 150 μL 0.1 mol L^{-1} SDS was also investigated. The average peak current for 5.0×10^{-4} mol L^{-1} isoniazid was 30 μA with the RSD of 4.5% (n = 5). It indicated that this modified electrode has a remarkable reproducibility. The stability of PEDOT/Pt electrode in 150 μL 0.1 mol L^{-1} SDS was evaluated by measuring the anodic peak current response at a fixed 5.0×10^{-4} mol L^{-1} isoniazid over a period of 14 days. The PEDOT/Pt electrode was treated only once and stored in 0.1 mol L^{-1} B-R buffer solution (pH 2.3) after every determination. The RSD with 2.8% (n = 5) showed that the PEDOT/Pt electrode has a good stability.

3.6 Interference studies

3.6.1 Electrochemistry of INH in presence of UA and AA at pH 2.3.

In biological environments, the main interference of INH is the presence of high concentration of AA and UA. So it is important to investigate the electrochemical response of INH in the presence of AA, and UA. The LSV technique was used to investigate the interference study of successive additions of 150 μL 0.1 mol L^{-1} SDS, in a mixture of 50 mmol L^{-1} AA, 5 mmol L^{-1} UA and 0.5 mmol L^{-1} INH as shown in (Figure 6).

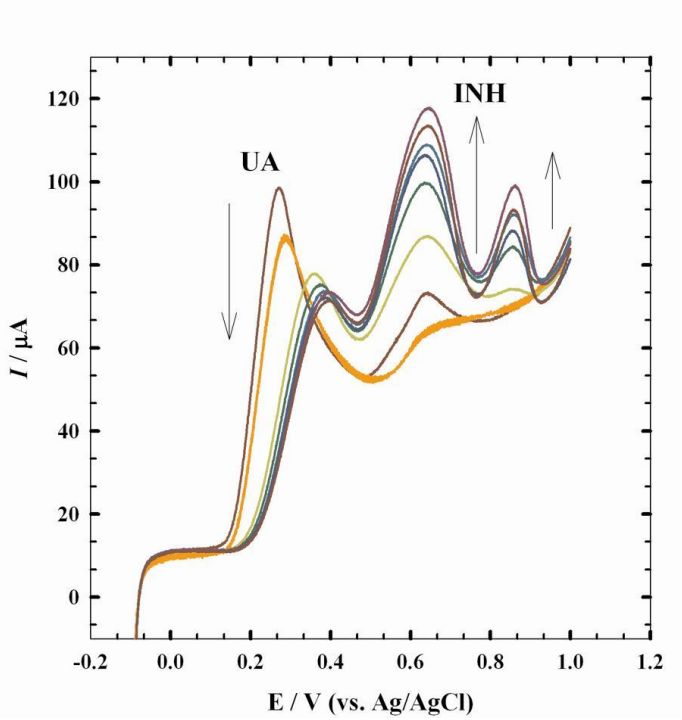

Figure 6. LSV for 50 mmol L^{-1} AA, 5 mmol L^{-1} UA and 0.5 mmol L^{-1} INH in B-R (0.1 mol L^{-1}), at PEDOT/Pt with successive additions of (0 -150 μL) 0.1 mol L^{-1} SDS , at pH 2.3

The oxidation peak of ascorbic acid was not identified at PEDOT/PT electrode tested in 150 μL

SDS 0.1 mol L^{-1} B-R (pH 2.3). The oxidation peak of UA at 0.27 V was observed. With the addition of SDS, the first anodic peak of INH is observed at +0.63 V which increases by increasing the concentration of SDS. The second anodic peak of INH was observed at + 0.8 V with the addition of SDS, while UA current response decreased. One possible explanation for this effect is that the adsorption of the anionic surfactant SDS onto electrode surface may result in a negatively charged hydrophilic film with the polar head group points to the bulk of the solution. This negatively charged hydrophilic layer facilitates reaching the INH to the electrode surface faster. This micellar effect on the oxidation of INH is basically an electrostatic interaction between the surfactant film adsorbed on the electrode and the protonated isoniazid. On the other hand, the adsorption of the anionic surfactant SDS may lead to electrostatic repulsion between the anionic film and anionic species (AA, UA) at electrode surface, thus, decreasing their electron transfer. This indicates the good selective determination of PEDOT/Pt electrode for INH in presence of high concentration of AA and UA at pH 2.3.

3.6.2. Simultaneous determination of INH, AA and UA at pH 7.4.

Figure 7 shows the LSVs of tertiary mixture of 0.5 mmol L^{-1} INH, 50 mmol L^{-1} AA and 5 mmol L^{-1} UA at PEDOT/Pt electrode with the successive additions of 10 µL of 0.1 mol L^{-1} CTAB in the mixture solution (pH = 7.4). A spontaneous adsorption of CTAB on the electrode surface causes electrostatic attraction force towards the anionic AA and UA. Two anodic peak currents are observed at + 0.1 V, + 0.486 V for AA and UA respectively. By successive additions of CTAB, anodic peak current for INH is observed at +0.7 V which increases by increasing the concentration of CTAB.

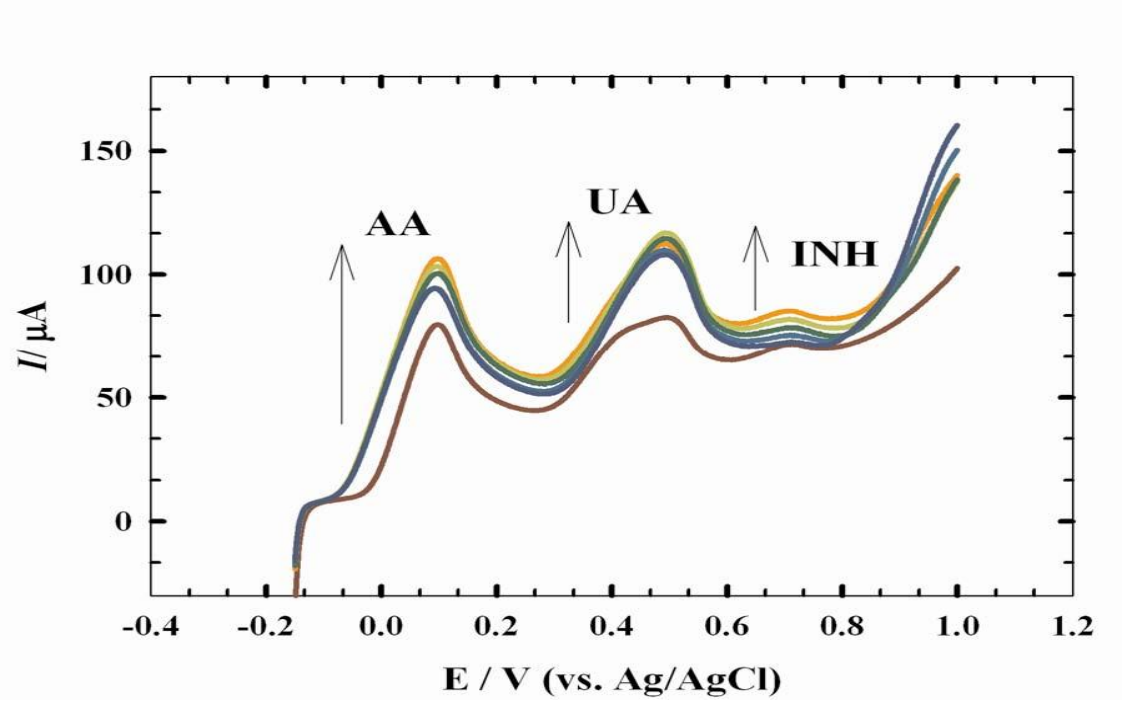

Figure 7. LSV for 50 mmol L^{-1} AA, 5 mmol L^{-1} UA and 0.5 mmol L^{-1} INH in B-R (0.1 mol L^{-1}), at PEDOT/Pt with successive additions of (0 -150 µL) 0.1 mol L^{-1} CTAB, at pH 7.3.

This is attributed to the neutral structure of INH which facilitates its diffusion by the cationic CTAB towards the polymeric layer. With stirring (5 minutes) and accumulation time (1 minute) after addition of the surfactant, the oxidation peak currents (Ipa) for AA, UA and INH increase correspondingly. This observation takes place in the case of pH 7.4 due to the anionic nature of molecules of AA, UA and neutral form of INH.

4. ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY (EIS) OF INH

Electrochemical impedance spectroscopy (EIS) is a simple and effective way to measure polarization resistance (Rp) and the capacitance of the double layer of the electrochemical reactions at the interface. The EIS measurements were performed in 5.0×10^{-4} mol L^{-1} of INH in B-R buffer solution pH 2.3-7.4 using the PEDOT/PT electrode in presence and absence of SDS and CTAB. (Figure. 8A) shows the complex plane diagram (Nyquist plot) of INH at PEDOT/Pt electrode in presence of SDS (a) presence of CTAB (b) and in absence of surfactants (c) at applied potential of 0.65 V in pH 2.3. It can be noticed that PEDOT/Pt in SDS, PEDOT/Pt in CTAB and PEDOT/Pt exhibit a semicircular behavior with a linear portion. The semicircle portion of the curve corresponds to the charge transfer process through the film. The diameter of the semicircle represents the magnitude of electron transfer resistance at the electrode surface. The measured EIS data were fitted with an equivalent circuit as shown in Figure 8 C. This equivalent circuit consists of R_u (the solution resistance), Rp (the polarization resistance), CPE (the predominant diffusion influence on the charge transfer process), and n is its corresponding exponent ($n < 1$). C_f represents the capacitance of the double layer. Diffusion can create impedance known as the Warburg impedance (W) resulting from the diffusion of ions from the bulk of the electrolyte to the interface. This equivalent circuit was used to fit the impedance spectra and calculate the values of CPE and Warburg impedance (W). Table 1A lists the best fitting values calculated from the equivalent circuit for the impedance data. EIS data were modeled using non-linear least-squares fit analysis (NLLS) software and electrical equivalent circuit. The error was 0.34 % represented as chi-square. It is clear that the charge transfer resistance of electrooxidation of INH decreases noticeably, and the charge transfer rate is enhanced by SDS and CTAB rather than that in case of PEDOT/Pt. This is attributed to the high resistance exerted within the polymeric film and repelled INH from its surface at pH 2.3, (section 3.1). The addition of SDS to the solution facilitates the diffusion of INH molecules towards the polymeric film. This is indicated from the low charge transfer resistant value for the PEDOT/Pt electrode in SDS, PEDOT/Pt electrode in CTAB which implies that the charge transfer process is relatively fast compared to the PEDOT/Pt electrode at pH 2.3. Comparing the data obtained, it can be concluded that the PEDOT/Pt electrode in SDS presented the lowest charge transfer resistance at the interface reflecting an improvement of the effective electron-transfer rate.

On the other hand, (Figure. 8B) shows the complex plane diagram (Nyquist plot) of INH at PEDOT/Pt electrode in presence of SDS (a) presence of CTAB (b) and in absence of surfactants (C), at oxidation potential 0.25 V in pH 7.4. It is clear that the charge transfer resistance of electro-oxidation of INH decreases greatly, and the charge transfer rate is enhanced by CTAB rather than that in case of

PEDOT/Pt in SDS. This is attributed to the neutral structure of INH at this pH, in which SDS as an anionic surfactant does not enhance the diffusion of the drug towards the polymeric layer. However, CTAB as a cationic surfactant enhance more the diffusion of INH and decreases its charge transfer resistance (section 3.1). The data shows that the behavior of INH at PEDOT/Pt electrode changes at pH 7.4 in presence of CTAB. In this case, its charge transfer resistance decreases in pH 7.4 than pH 2.3. This is attributed to INH pKa values (i.e. 1.8, 3.5, and 10.8); INH becomes neutral at this pH which facilitates its diffusion to the polymeric film. Table 1 B. Lists the best fitting values calculated from the equivalent circuit for the impedance data. Comparing the data obtained, it can be concluded that the PEDOT/Pt electrode in CTAB presented the lowest charge transfer resistance at the interface which reflects an improvement of the effective electron-transfer rate at pH 7.4.

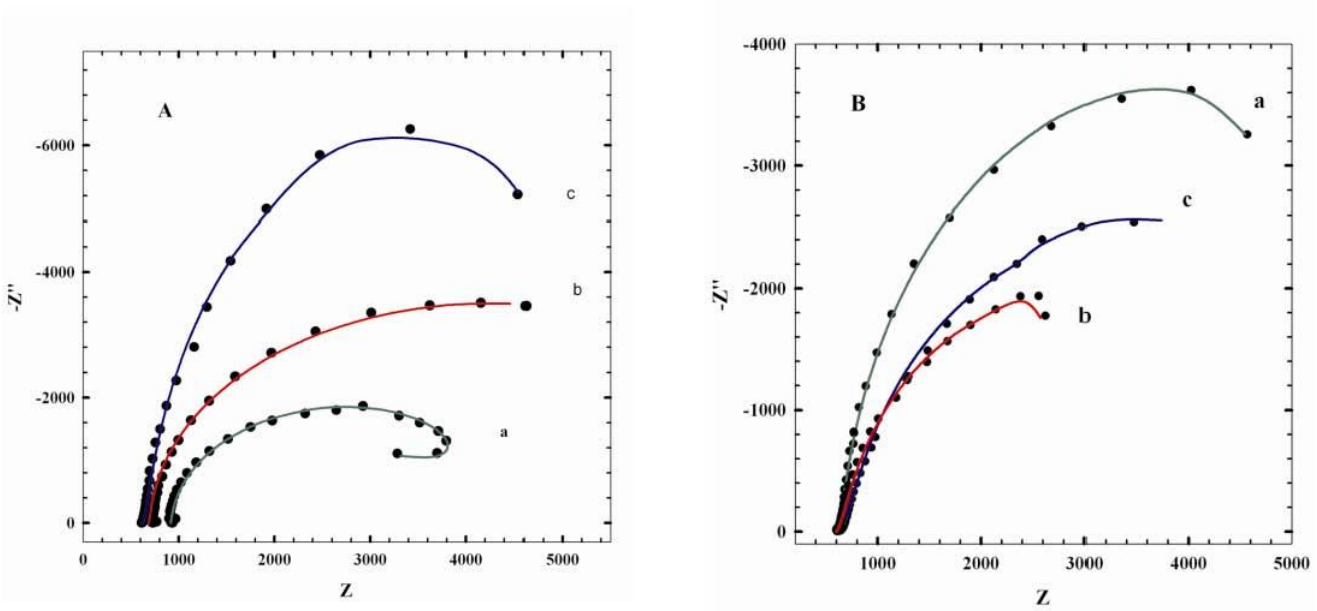

Figure 8. Nyquist diagrams (-Z″ vs. Z′) for the EIS measurements at PEDOT/ Pt (A) at potential 650 mV for INH at pH 2.3, (B) at potential 250 mV for INH at pH 7.4. (a) In presence of SDS, (b) in presence of CTAB and (c) in absence of surfactant. (5 mmol L^{-1} of INH, in 0.1 mol L^{-1} B-R. Amplitude: 5 mV, frequency range: 0.1–10000 Hz)**.**

Table 1. A) The data obtained from EIS in the determination of INH using PEDOT/Pt electrode in absence and presence of SDS and CTAB at different potentials, at pH 2.3

Electrode (PEDOT/Pt)	E (mV)	Rp (kΩ cm^2)	Ru (kΩ cm^2)	Cf (μF cm^{-2})	W (KΩ^{-1} cm^{-2})	CCPE (μFcm^{-2})	n
No surfactant	650	28.2	0.68	13	0.39	0.73	0.8
With SDS	650	3.8	0.51	82	0.18	30	0.6
With CTAB	650	7.1	0.61	38	0.28	9.2	0.9

Table 1. B) Same as in Table 1A but at pH 7.4

Electrode (PEDOT/Pt)	E (mV)	Rp ($k\Omega\ cm^2$)	Ru ($k\Omega\ cm^2$)	Cf ($\mu F\ cm^{-2}$)	W ($K\Omega^{-1}\ cm^{-2}$)	CCPE (μFcm^{-2})	n
No surfactant	250	5.6	0.62	27	0.42	2.0	0.3
With SDS	250	8.8	0.70	16	0.69	1.1	0.7
With CTAB	250	4.0	0.52	31	0.28	44	0.9

5. DETERMINATION OF INH IN HUMAN URINE SAMPLES

The utilization of the proposed method in real sample analysis is also investigated by direct analysis of INH in human urine. The same measurements were conducted successfully on urine samples. In this set of experiments, isoniazid was dissolved in urine to make a stock solution of 5 mmol L^{-1} concentration

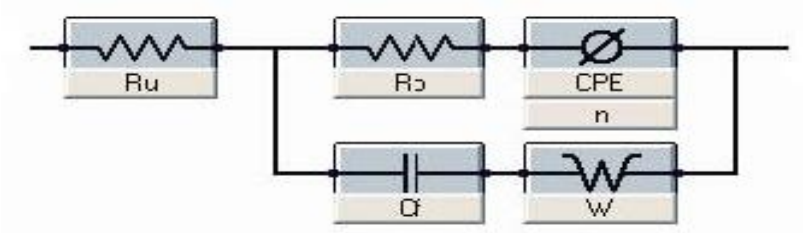

Figure 9. Equivalent circuit

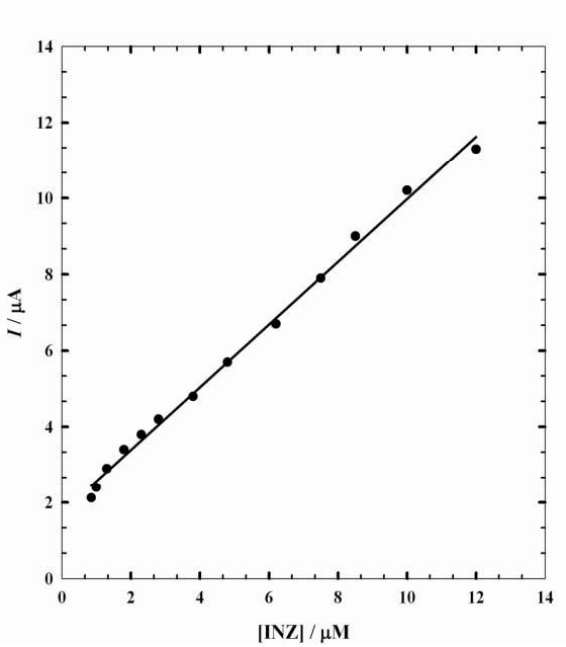

Figure 10. Calibration curve for successive additions of 10 µL of 5 mmol L^{-1} INH in urine to the buffer pH 2.3 containing 150 µL SDS.

Standard successive additions of 10 µL of 5 mmol L^{-1} isoniazid in urine were added to the buffer pH 2.3 containing 150 µL SDS, the corresponding LSV was then measured. Figure (9), shows the calibration curves of the anodic peak currents in the linear ranges of 0.8 to 12 µmol L^{-1} with correlation coefficients of 0.997 and detection limit 58 nmol L^{-1}.

The analytical results are summarized in Table 2. The recovery ranged from 92% to 100.1%, and the results are acceptable indicating that the present procedures are free from interferences of the urine sample matrix. The results strongly proved that INH can be selectively and sensitively determined at PEDOT/Pt electrode in urine sample in presence of SDS. In Table 3, response characteristics of the proposed method are compared with those obtained by some other reported methods. In comparison with some other modified electrodes for isoniazid determination, our method showed advantages in several aspects. The designed sensor is prepared in one simple step with cheap and simple reagents and no pretreatment needed before the measurements. This gives the sensor more advantages over other modified electrodes used in the literature. The designed sensor showed good reproducibility, high stability, sensitivity and anti-interference ability.

Table 2. Recovery data for synthesized biological solution spiked with various amounts of 0.5 mmol L^{-1} INH**,** in fresh urine sample from healthy volunteers

Urine sample	Spike (µmol L^{-1})	Found (µmol L^{-1})	Recovery (%)	R.S.D. (%)[a]
1	3.0	2.76	92	4.2
2	9.0	9.1	100.1	3.2
3	13.0	12.5	96.1	5.1

[a] Average of ten replicate measurements.

Table 3. Comparison of the proposed method with other reported methods for the determination of isoniazid in urine sample at different modified electrodes.

Type of electrode	Linear range	Detection limits
Au electrode [19]	$2.0x10^{-6}$-$2.3x10^{-4}$ M	$9.69x10^{-8}$ M
Mercury electrode [17]	$5.0x10^{-10}$-$2.0x10^{-6}$ M	$1.18x10^{-10}$ M
Polypyrrole modified GCE [52]	$3.99x10^{-6}$-$2.6x10^{-2}$ M	$3.15x10^{-6}$ M
PASA-GCE [53]	$5.0x10^{-8}$-$1.0x10^{-5}$ M	$1.0x10^{-8}$ M
This work	0.1-$8.0x10^{-6}$ M And 1-$10x10^{-5}$ M	$3.2x10^{-8}$ M $4.5x10^{-8}$ M

6. CONCLUSION

This work has shown that isoniazid can be determined using electrochemical method on the basis of electrostatic force of different types of surfactants at Poly(3, 4ethylene-dioxythiophene) electrode with wider linear range and lower detection limit. The proposed methods can be applied to

the detection of isoniazid in acid and neutral medium. The good properties of modified electrode, such as high sensitivity, easy fabrication, reproducibility, stability indicate that the proposed modified electrode will be promising for measurements of isoniazid in biological fluids without any interference. At the same time, it will expand the application of this modified polymer film in electrochemical field.

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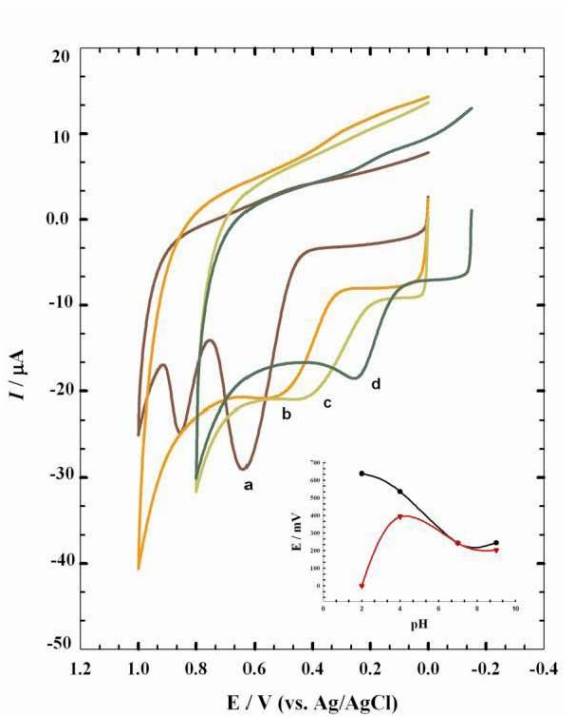
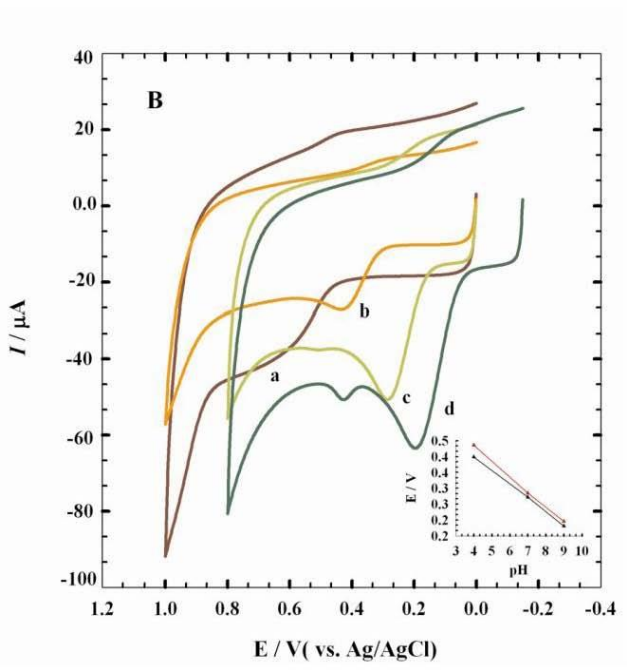


Supplementary 1A



Supplementary 1B