Poly(3,4-ethylene-dioxythiophene) electrode for the selective determination of dopamine in presence of sodium dodecyl sulfate

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ABSTRACT

A novel biosensor using poly(3,4-ethylene dioxythiophene) (PEDOT) modified Pt electrode was developed for selective determination of dopamine (DA) in presence of high concentrations of ascorbic acid (AA) and uric acid (UA) with a maximum molar ratio of 1/1000, and 1/100 in the presence of sodium dodecyl sulfate (SDS). SDS forms a monolayer on PEDOT surface with a high density of negatively charged end directed outside the electrode. The electrochemical response of dopamine was improved by SDS due to the enhanced accumulation of protonated dopamine via electrostatic interactions. The common overlapped oxidation peaks of AA, UA and DA can be resolved by using SDS as the DA current signal increases while the corresponding signals for AA and UA are quenched. The use of SDS in the electrochemical determination of dopamine using linear sweep voltammetry at modified electrode PEDOT/Pt resulted in detecting dopamine at relatively lower concentrations. The DA concentration could be measured in the linear range of 0.5 to 25 $\mu mol\ L^{-1}$ and 30 $\mu mol\ L^{-1}$ to 0.1 $mmol\ L^{-1}$ with correlation coefficients of 0.998 and 0.993 and detection limits 61 $nmol\ L^{-1}$ and 86 $nmol\ L^{-1}$, respectively. The validity of using this method in the determination of dopamine in human urine was also demonstrated.

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1. Introduction

Dopamine (DA) is an important neurotransmitter in the mammalian central nervous system. Low level of DA may result in neurological disorder such as Parkinson's disease and schizophrenia [1]. Uric acid (UA) is the primary end product of urine metabolism. It has been shown that extreme abnormalities of UA levels are symptoms of several diseases, such as gout and hyperuricemia [2]. Ascorbic acid (AA) is very popular for its antioxidant properties. Moreover, a number of studies have investigated the function of AA in gene expression and as a co-substrate of many important dioxygenases [3]. DA, UA and AA usually coexist in biological samples. Since the basal DA concentration is very low (0.01–1 μM) while the concentration of AA and UA is generally much higher than that of DA (100–1000 times) [1,4], it is essential to develop sensitive and selective methods for their determination in routine analysis. However, a major problem encountered is that UA, DA, and AA are oxidized at nearly the same potential with poor sensitivity at bare (unmodified) electrode. The overlap of their voltammetric responses makes their simultaneous determination highly difficult [5]. To overcome this problem, various modified electrodes have been constructed such as organic redox mediators modified electrodes [6], polymers modified electrodes [7–11], nanoparticles modified electrodes [12–14], boron-doped diamond electrode (BDD) [15], carbon ceramic

electrode [16], pyrolytic graphite electrode [17,18], screen-printed carbon electrode [19], carbon ionic liquid electrode [20], electrochemically oxidized GCE [21], and carbon nanotube microelectrode [22]. The successful route to overcome the problems of selectivity is to modify the electrode surface, because the modified electrode could decrease the overpotential, improve the mass transfer velocity and effectively enrich the substance [23,24]. Many sensors were fabricated with negatively charged polymer films, self-assembled monolayer or cation exchange. The major consideration is based on the different ion forms of DA, AA and UA at the physiological pH of 7.40. AA, UA exist in the anionic form ($pK_a = 4.10$), ($pK_a = 5.4$) respectively. While DA is in the cationic form ($pK_a = 8.87$) [25]. Surfactants, a kind of amphiphilic molecules with a hydrophilic head on one side and a long hydrophobic tail on the other, have been widely applied in electrochemistry to improve the property of the electrode/solution interface [26,27]. The surfactant-modified electrodes have been reported previously, Krishnananda et al. [28] have carried out the direct electrochemical studies on horse heart myoglobin and horseradish peroxidase at neutral surfactant-modified glassy carbon electrode. Improved electron transfer rate was found between these proteins and the modified electrode. Lenys and Hermes [29] have constructed a chemically modified glassy carbon electrodes by using surfactant/clay films containing ferrocenecarboxylic or ferrocenedicarboxylic acid. The results show that the surfactant molecules incorporated into the clay could increase the permeability and the positive surface of the film. Svancara et al. [30] also reported a carbon paste electrode modified with cationic surfactants, which was used to determine chromate based on synergistic pre-concentration of the

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chromate anion at modified electrode. Wen et al. [31] have investigated the micellar effect on the electrochemistry of dopamine and found that the anodic peak current of dopamine is enhanced in sodium dodecyl sulfate micelle, but the interference coming from ascorbic acid cannot be eliminated as selective sensor for dopamine in the presence of high concentration of AA and UA in biological fluids.

In this work an SDS modified PEDOT electrode is introduced by the hydrophobic adsorption of SDS on the surface of PEDOT/Pt electrode. This electrode is a simple phase surface with a high density of negative charges covered on electrode surface. The voltammetric response of SDS/PEDOT is highly selective toward DA compared to PEDOT. After an open circuit accumulation, a further enhancement of the oxidation peak current was observed, and a high selectivity for DA in presence of AA, UA is demonstrated for SDS/PEDOT. Furthermore, the electrochemical characterization of SDS/PEDOT was investigated by EIS technique. The determination of DA using this approach has the following advantages: easy to prepare, regeneration, low cost, high selectivity, and low detection limit.

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 (LiClO$_4$), acetonitrile (HPLC grade), dopamine, uric acid, ascorbic acid, and sodium dodecyl sulfate were supplied by Aldrich Chem. Co. (Milwaukee, WI. USA). B-R buffer (pH 2–9) was prepared from 0.12 M CH$_3$COOH, 0.12 M H$_3$BO$_3$ and 0.12 M H$_3$PO$_4$. Aqueous solutions were prepared using double distilled water.

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 M NaCl) electrode. The Pt electrode was polished by a BAS-polishing kit with 0.3 and 0.05 μl 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 M EDOT, and 0.1 M 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). Philips XL 30 instrument was used to obtain the scanning electron micrographs of the different films.

LSV and CV were used for the determination of DA using modified PEDOT/Pt electrode. From potential −150 to 800 mV, with scan rate 50 mV s^{-1}.

2.2.2. Impedance spectroscopy 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 0.125 V, 0.250 V, were studied at frequency range of 0.1 - 10,000 Hz with amplitude of 5 mV were applied on PEDOT/Pt electrode and tested in dopamine 0.5 mM in presence and absence of 0.1 M 150 μM SDS in buffer 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 DA in human urine samples. Dopamine 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} dopamine in urine were added to the buffer 7.4 containing 150 μl SDS.

3. Results and discussion

3.1. Electrochemistry of dopamine at PEDOT modified Pt electrode

The voltammetric behavior of DA was examined using cyclic voltammetry. Fig. 1(A,B) compares typical cyclic voltammograms of 0.5×10^{-3} mol L^{-1} DA in B-R buffer pH 2.3 and 7.4 at scan rate 50 mV s^{-1} using PEDOT/Pt electrode in presence of SDS. The SDS was added from stock 0.1 M with successive additions from 0 to 150 μl (a–q) and after each addition stirring takes place for 5 min and then holds for one minute before running the experiment. The CV in pH 2.3 is characterized by the appearance of distinct anodic peak at 0.5 V and a cathodic peak at 0.41 V. Furthermore, for DA an increase in anodic and cathodic peak current values were observed upon the successive additions of 150 μL SDS until the anodic and cathodic peak currents reached 6×10^{-5} A and 4.3×10^{-5} A, respectively. The same trend was observed in pH 7.4. In pH 7.4, anodic peak appeared at 0.223 V and the cathodic peak at 0.116 V upon addition 150 μl successive additions of SDS, the anodic and cathodic peak current values reached to 4.6×10^{-5}, 3.3×10^{-5}, respectively with shift in oxidation potential to less positive value. The effect of changing the pH of B-R buffer on the voltammetric response of dopamine was observed. Thus, the oxidation peak potential shifts to a more positive value and the oxidation current response increases as pH decreases. The suggested mechanisms for the aggregation of surfactants on the modified electrode surface in the form of bilayers, cylinder, or surface micelles (in the case of relatively higher concentrations added of SDS) could explain the increase in the current in the presence of surfactants [32]. The electron transfer process will take place when the electroactive species approaches the vicinity of the electrode surface. The facilitation of the transfer of the charge is due to the space of one to two head groups of adsorbed surfactant moieties that is extended from the electrode surface [33]. Furthermore, a possible mechanism suggests the formation of ion-pair that anchor onto the surface of the modified electrode that should posses some hydrophilic character [34]. The resulting ion-pair of the charged surfactant and dopamine tend to adhere to the modified surface through the lipophilic parts in both moieties.

3.2. Effect of solution pH in the presence and absence of SDS

The effect of changing the pH on the electrochemical response of dopamine at the modified PEDOT electrode was examined in the presence and absence of SDS. In Supplementary 1(A,B), the effect of changing the pH of B-R buffer on the voltammetric response of 0.5 mol L^{-1} dopamine in the presence and absence of 150 μl 0.1 mol L^{-1} SDS are shown, respectively. In general, the oxidation peak potential shifts to more positive values as the pH decreases in the absence and presence of SDS. Maximum oxidation current signal was obtained in pH = 2.3 and minimum current signal was observed in pH = 9, in the SDS containing solution Fig. 2. Therefore, all subsequent electrochemical measurements will be conducted in either pH = 2.3 or 7.4 (biological fluids). The pH dependency of the oxidation peak potential indicates that protonation/deprotonation is taking part in the charge transfer process.

3.3. Effect of scan rate on the voltammetric response of dopamine

The dependence of the anodic peak current density on the scan rate has been used for the estimation of the "apparent" diffusion coefficient,
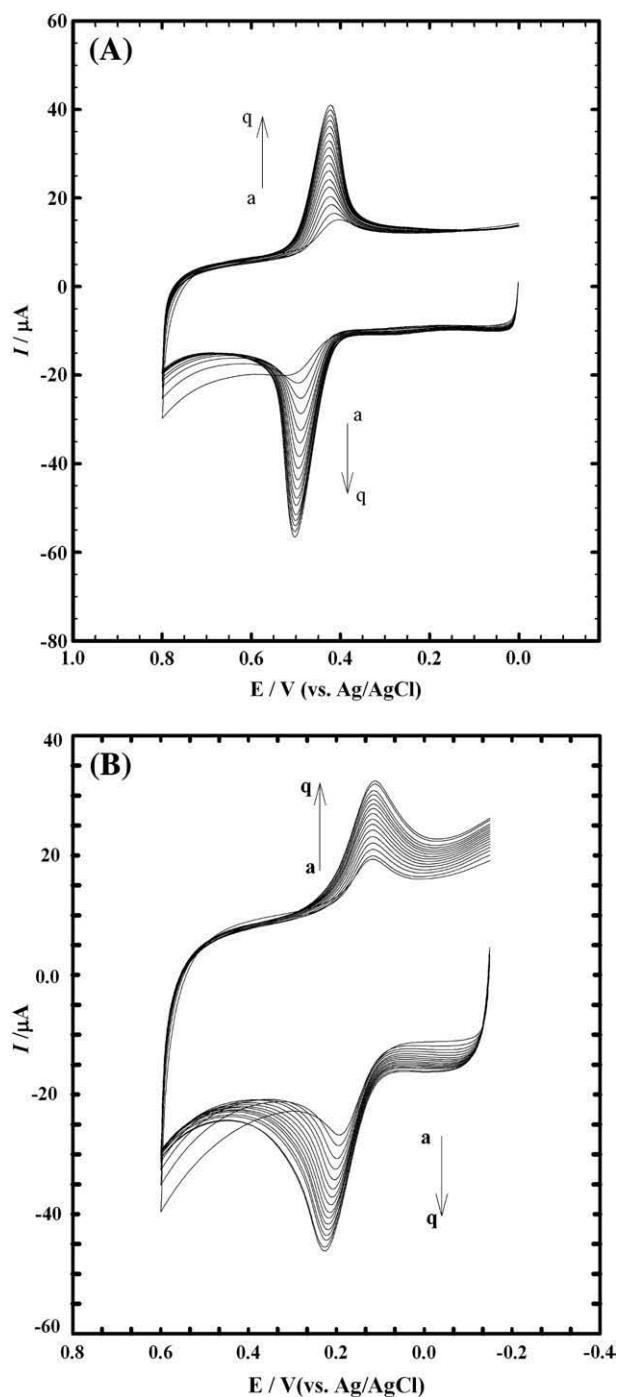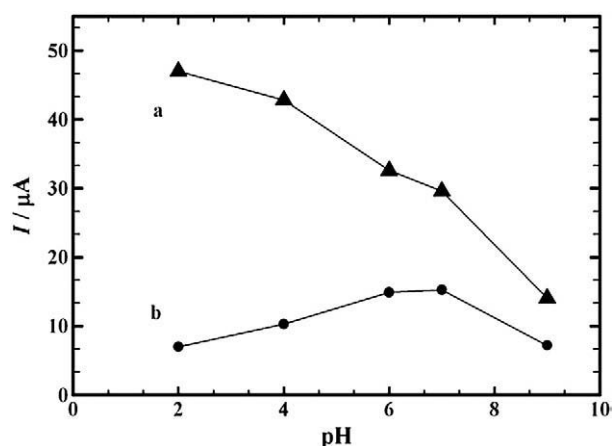
Fig. 2. Dependence of the anodic peak current of dopamine on pH value of the solution at PEDOT/Pt electrode (a) in presence and (b) in absence of SDS.

Where the constant has units (i.e. 2.687×10^5 C mol^{-1} V$^{-1/2}$).

In these equations: i_p is the peak current density (A cm^{-2}), n is the number of electrons appearing in half-reaction for the redox couple, v is the rate at which the potential is swept (V s^{-1}), F is Faraday's constant (96,485 C mol^{-1}), C_O is the analyte concentration (5×10^{-4} mol cm^{-3}), A is the electrode area (0.0176 cm^2), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K), and D is the electroactive species diffusion coefficient (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 is 1.401×10^{-3} cm^2 s^{-1} in the presence of SDS, and in absence of SDS D_{app} value is 5.160×10^{-4} 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} values. Careful inspection of data on the effect of scan rate (Supplement 2 (A,B)) reveals that the linearity of the relationship is realized up to a scan rate of 100 mV s^{-1} that is followed by a deviation from linearity at higher scan rates. This indicates that the charge transfer is under a partially diffusion control process and that adsorption of aggregates at the electrode surface is also possible. The relation between the oxidation peak potential E_{pa}, and the scan rate v, shows that deviation also begins at a scan rate more than 100 mV s^{-1}. 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 show that the diffusion is enhanced in presence of SDS than in absence of it.

3.4. Determination of dopamine using PEDOT/Pt in presence of SDS

The voltammetric behavior of DA was examined using linear scan voltammetry (LSV) with scan rate 50 mV s^{-1}. Fig. 3(A), shows typical LSV of successive additions of 10 µl 5 mmolL^{-1} of dopamine to 150 µL SDS in 0.1 mol L^{-1} B-R (pH 7.4). Fig. 3(A), shows that by increasing the concentration of dopamine the anodic peak current increases which indicate that the electrochemical response of dopamine is
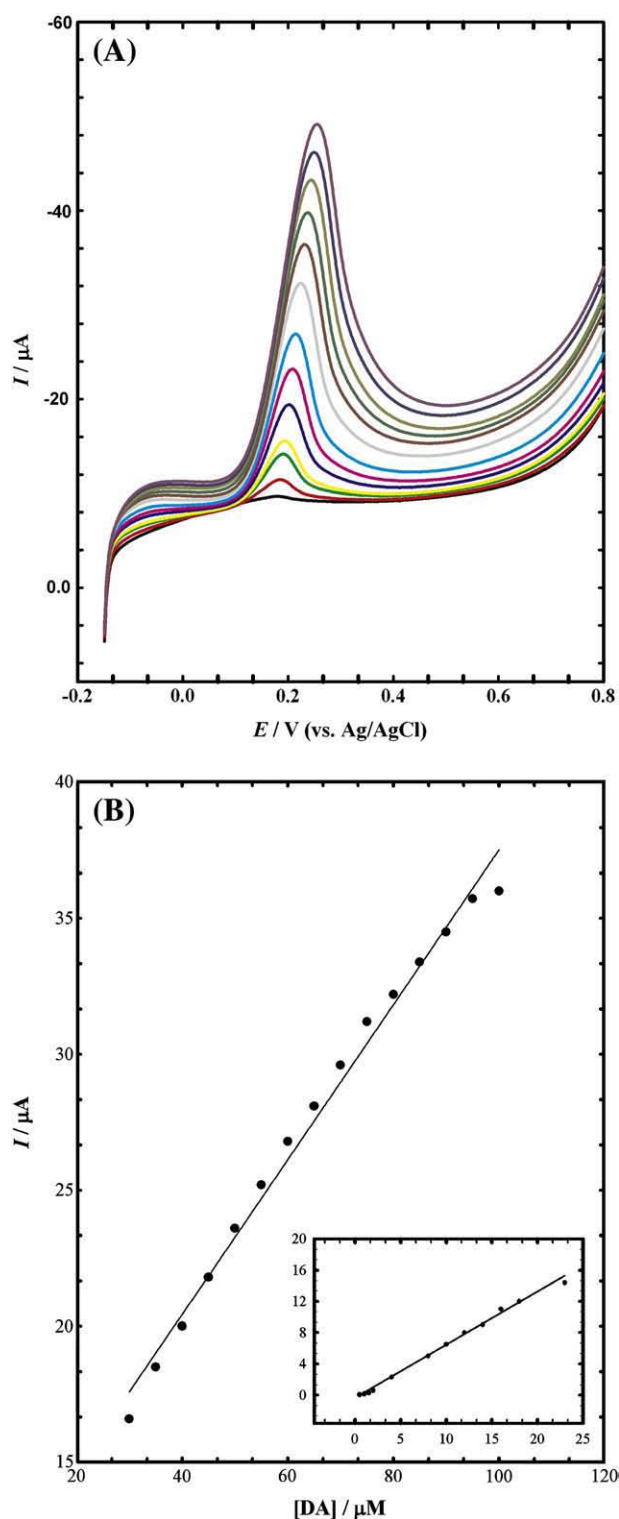
Fig. 1. Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} dopamine/0.1 mol L^{-1} B-R, scan rate 50 mV s^{-1} at PEDOT/Pt electrode with successive additions (10 µl–150 µl) of 0.1 mol L^{-1} SDS with stirring the solution for 5 min every addition and 1 min for occupation (a–q) at (A) pH 2.3 and (B) pH 7.4 .

D_{app}, for the compounds studied. D_{app} values were calculated from Randles Sevcik equation [35], and for the oxidized species [O]:

$$I_p = 0.4463 \left(F^3 / RT \right)^{1/2} n^{3/2} v^{1/2} D_O^{1/2} A\, C_O \qquad (1)$$

For T = 298 K (at which temperature the experiments were conducted), the equality holds true:

$$I_p = (2.687 \times 10^5)\, n^{3/2} v^{1/2} D_O^{1/2} A\, C_O \qquad (2)$$

Fig. 3. (A) LSVs of 10 ml of 0.1 mol L^{-1} B-R PH 7.4 at PEDOT/Pt electrode in 0.1 mol L^{-1} 150 μl SDS in different concentrations of DA (0.5 μmol L^{-1}–0.1 mmol L^{-1}) (B) calibration curve for DA for concentrations from (30 μmol L^{-1} to 0.1 mmol L^{-1}) and from (0.5 μmol L^{-1} to 25 μmol L^{-1}, the inset).

apparently improved by SDS due to the enhanced accumulation of protonated dopamine via electrostatic interaction with negatively charged SDS at the polymeric electrode surface. Fig. 3(B) and the inset show the calibration curves of the anodic peak current values in the linear range of 30 μmol L^{-1} to 0.1 mmol L^{-1} and 0.5 to 23 μmol L^{-1} with correlation coefficients of 0.993 and 0.998 and detection limits

86 nmol L^{-1} and 61 nmol L^{-1}, respectively. The detection limit was calculated from the equation: DL = 3 s/m, where s is the standard deviation and m is the slope [33]. Table 1(A, B) (Supplementary materials #3) summarize the data obtained from the calibration curves in the determination of dopamine using PEDOT/Pt electrode in absence and presence of SDS.

3.5. Interference studies

3.5.1. Electrocatalytic oxidation of UA, DA, and AA in their separate solutions

Fig. 4(A) shows typical cyclic voltammograms (CVs) of 0.5 mmol L^{-1} of DA, UA and AA at bare Pt in pH = 7.4 with scan rate 50 mV s^{-1}. AA and UA show broad oxidation peaks. For DA, the anodic and cathodic peaks appear at 315 mV and 70 mV, with potential peak separation of about 245 mV. It is clear that the reversibility of the electrochemical reactions of DA, UA at the bare Pt is slow, indicating the sluggish electron transfer kinetics of these compounds at the bare Pt, which may be related to the electrode fouling caused by the deposition of these compounds and their oxidation products on the electrode surface [16]. Fig. 4(B) shows the CVs of DA, UA and AA at the surface of PEDOT/Pt electrode. The anodic and cathodic peak potentials of DA appear at 223 mV and 146 mV, respectively, with potential peak separation 77 mV which indicates that the reversibility of DA at PEDOT/Pt is considerably improved. The peak current is also enhanced using PEDOT/Pt electrode. The peak potential for the oxidation of AA occurs at −25 mV and the peak current is enhanced and more pronounced at the PEDOT/Pt electrode. In the case of UA, a sharp oxidation peak at 355 mV and a small reduction peak at 284 mV were obtained at PEDOT/Pt electrode, and the peak current is enhanced in case of using PEDOT/Pt electrode. These results demonstrate that PEDOT/Pt electrode not only catalyses the oxidation of UA, DA, and AA, but also dramatically enlarge the peak separation among UA, DA, and AA in their mixture. The increase in separation of the anodic peak potentials, and the enhanced sensitivity, allows simultaneous determination of UA, DA, and AA. Fig. 4(C) shows CVs of DA, UA and AA at PEDOT/Pt electrode in presence of 150 μl SDS 0.1 mol L^{-1}, The electron transfer for the oxidation of DA, in the presence of anionic surfactant SDS, is faster due to the electrostatic attraction of positively charged DA (pK$_a$ = 8.92) with the anionic surfactant SDS Fig. 5(C) [31,36–38]. By adding SDS to the solution anodic peak potential of DA, Ep$_a$, at 248 mV and cathodic peak potential, Epc, at 126 mV were observed. In addition, an increase in both peak current values, Ipa and Ipc was noticed in presence of SDS. However, AA, UA have pK$_a$ = 4.10 and pK$_a$ = 5.4, respectively. In the experiment conditions the pH = 7.4 where AA and UA are in the anionic form and in micellar medium they established an electrostatic repulsion with anionic surfactant SDS, which provokes a large decrease in the peak current value, Ip$_a$. The change of the peak current values for AA, UA and DA after adding SDS can be assigned to the adsorption of the surfactant onto electrode surface, which may change the overpotential of the electrode and influence the electron transfer rate. The formation of micellar aggregates may also influence the mass transport of electroactive species to the electrode [31,39,40].

3.5.2. Electrochemistry of DA in the presence of ascorbic acid

It is well known that AA widely coexists with DA in real biological matrices. Therefore, eliminating AA interference is an important target for any dopamine analytical method. The interference of AA to DA detection arises from two aspects: the close oxidation potential values of AA and DA at ordinary electrode and the other is the electrocatalytic oxidation of dopamine by ascorbic acid [41]. Namely, oxidized dopamine, i.e., dopamine-o-quinone, is chemically reduced by ascorbic acid. There are different possible interactions that involved in the oxidation of ascorbate anion and dopamine at the PEDOT/Pt-modified electrode in the B-R buffer pH = 7.4 [42]. The voltammetric response of ascorbate anion at the PEDOT film has been due to the electrostatic interaction between the ascorbate anions and the cationic-fixed sites of
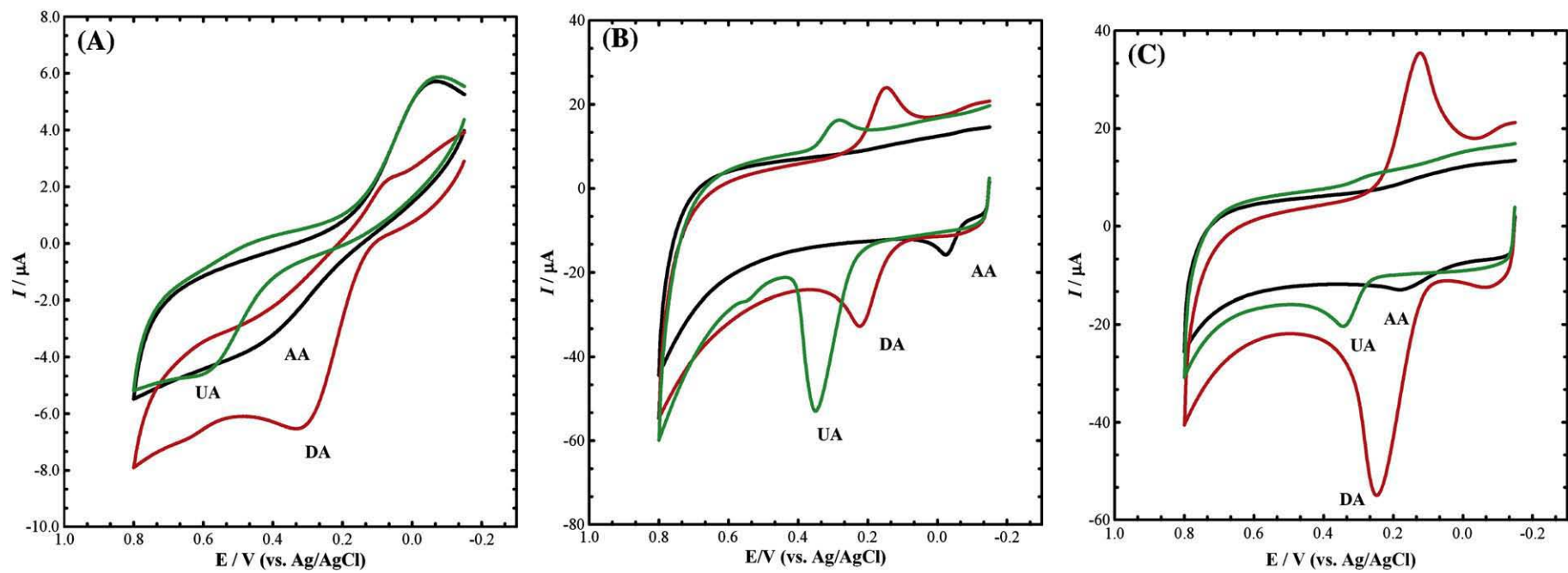
Fig. 4. (A) Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} DA, AA, UA/0.1 mol L^{-1} B-R, scan rate 50 mV s^{-1} at bare Pt electrode. (B) Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} DA, AA, UA/0.1 mol L^{-1} B-R, scan rate 50 mV s^{-1} at PEDOT/Pt electrode. (C) Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} DA, AA , UA/0.1 mol L^{-1} B-R, scan rate 50 mV s^{-1} at PEDOT/Pt electrode in the presence of 150 μl of 0.1 M SDS.
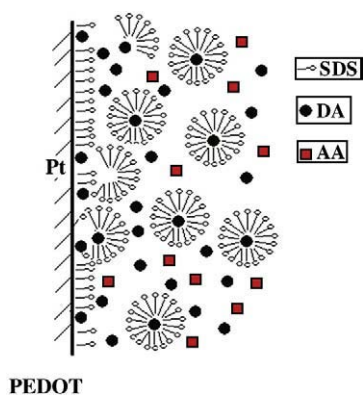
Fig. 5. Schematic drawing of electrostatic interaction between DA cation and the PEDOT in presence of SDS and AA.

polymer film. The amino group of dopamine is expected to be charged positively at pH = 7.4. The slow catalytic effect of polymer towards the dopamine molecules is attributed to the electrostatic repulsion between the polymer film and the positively-charged dopamine in B-R buffer solution. However in the present study, PEDOT films did not affect the electrochemical oxidation of DA that resulted in a relatively higher current signal compared to the "bare" Pt surface. One possible explanation for this effect is that, the effect generated due to the electrostatic repulsion might have been compensated by the hydrophobic interaction between the aromatic part of the dopamine and polymer film which was observed by Roy et al. [42]. Thus, the aromatic part of dopamine was involved in hydrophobic interaction with polymer film with its cationic part protruded outward. The authors also observed that incorporation of dopamine increased the net positive charge of the film and explained the shift of oxidation peak of ascorbic acid to more negative potential in the presence of dopamine by this hydrophobic interaction effect. In the present study, Fig. 5 shows that a possible 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 of the film allows the dopamine to reach the electrode vicinity faster. This micellar effect on the oxidation of DA is basically an electrostatic interaction between the surfactant film adsorbed on the electrode and the protonated dopamine [39,40,43,44]. On the other hand, the adsorption of the anionic surfactant SDS may lead to electrostatic repulsion between the anionic film and anionic species (AA and UA) at electrode surface, thus, decreasing their electron transfer. The cyclic voltammograms were recorded with DA at PEDOT/Pt electrode in binary mixture of 0.5 mmol L^{-1} of DA, and AA in B-R buffer pH = 7.4 with scan rate 50 mV s^{-1}, by successive additions of 150 µl, 0.1 mol L^{-1} SDS. All oxidation peak potentials of AA and DA shifted toward more negative values by increasing the solution pH from 2.3 to 7.4. This behavior is due to the deprotonation step involved in all oxidation process that is faster at higher pH value [45]. Both anodic peaks for AA and DA shift towards less positive values by increasing the pH of solution. However, as observed (figure not shown), the measurements performed using SDS allow a remarkable selective determination of DA in presence of AA. It is important to stress that according to the literature, the oxidation of AA involves two protons and two electrons in acid medium [39]. However, in the experimental condition (pH = 7.4), in which the pK_a of AA is (4.17); the oxidation process involves the loss of single proton and two electrons [38,46]. As the ascorbic acid is in the mono-protonated form, in micellar medium an electrostatic repulsion with anionic surfactant SDS takes place, which shifts the anodic peak potential, Ep_a, to less positive values and resulted in noticeable decrease in the peak current, Ip_a.

3.5.4. Electrochemistry of DA in presence of UA and AA

DA, UA and AA coexist in the extracellular fluid of the central nervous system and serum. The ability to selectively determine these species has been a major goal of electroanalysis research. Therefore, the electrochemical behaviors of DA, UA and AA in a mixture solution were studied. LSV mode was used for the oxidation of a solution containing equimolar mixture of DA, UA and AA at the bare Pt, PEDOT/ Pt and PEDOT/Pt in presence of SDS. At the working pH = 7.4 at the bare Pt as shown in Fig. 6, the oxidation peak potentials of DA, UA and AA are indistinguishable (Fig. 6, curve a). On the other hand, the oxidation peaks are resolved at PEDOT/Pt electrode with the peak potentials at 390 mV, 245 mV, and 4 mV for UA, DA, and AA, respectively (Fig. 6, curve b). The large separation of the peak potentials allows selective and simultaneous determination of UA, DA, or AA in their mixture. Using PEDOT/Pt in presence of SDS (Fig. 6, curve c), a sharp well defined oxidation peak of DA appeared at 272 mV and a smaller oxidation peak for UA appeared at 399 mV. The oxidation peak current for DA increased one and half times in presence of SDS. Moreover, there was a noticeable decrease in the oxidation peak current for UA and no oxidation peak for AA was observed. Therefore, the high response for dopamine was observed due to the electrostatic interaction of the anionic surfactant with the protonated DA in pH 7.4, but in case of AA and UA repulsion takes place (Fig. 6, curve c). Therefore, we can determine DA selectively in the presence of high concentration of AA, and UA.

3.5.5. Determination of DA in presence of high concentration of AA and UA

To verify the feasibility of the selective determination of DA at PEDOT/Pt electrode in presence of 150 µl of 0.1 M SDS, the effect of changing the concentration of DA in the presence of high concentration of AA and UA in B-R buffer pH 7.4 using LSV mode will be presented. The peak current of DA increases linearly with the increase in DA concentration from 0.5 µmol L^{-1} to 0.1 mmol L^{-1} (Supplement 4). The high concentrations of AA 500 µmol L^{-1} (1000 times relative to DA) and UA 50 µmol L^{-1} (100 times relative to DA) did not result in any
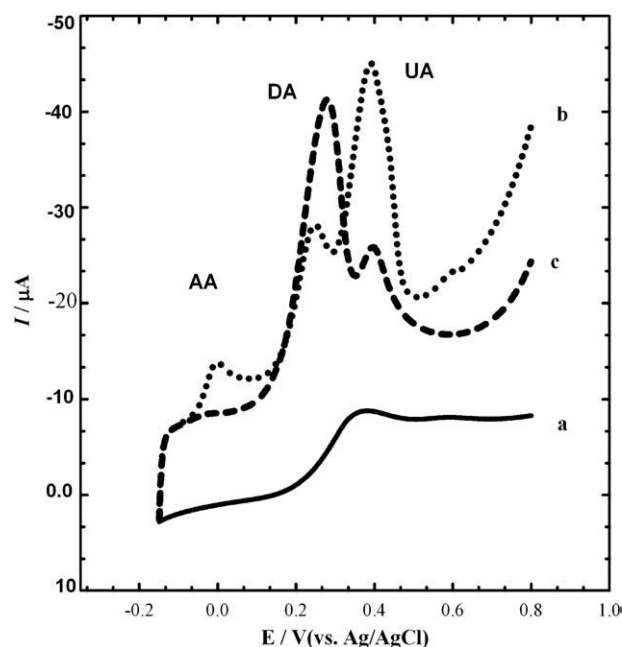

Fig. 6. LSVs for equimolar solution of 0.5 mmol L^{-1} AA, DA and UA in B-R (0.1 mol L^{-1}) at pH 7.4, scan rate: 50 mV s^{-1}. at (a) bare Pt, (b) PEDOT/Pt and (c) PEDOT/Pt in presence of 150 µl of 0.1 mol L^{-1} SDS.

noticeable interference for the detection of DA. The DA concentration could be measured in the linear range of 0.5 to 25 µmol L^{-1} and from 30 µmol L^{-1} to 0.1 mmol L^{-1} with correlation coefficients of 0.997 and 0.995 and detection limits 81 nmol L^{-1} and 89 nmol L^{-1}, respectively. From the data (Supplement 4 and the inset) the results strongly proved that DA can be selectively determined at the modified PEDOT/Pt electrode using SDS in presence of high concentrations of AA and UA.

3.5.6. Cyclic voltammetric study of dopamine in tertiary mixture in presence of SDS

Fig. 7 shows the CVs of 0.1 mmol L^{-1} DA in tertiary mixture with 0.2 mmol L^{-1} AA and 0.1 mmol L^{-1} UA at PEDOT/Pt electrode with the successive additions of 10 µl of 0.1 mol L^{-1} SDS in the mixture solution (pH = 7.4). A spontaneous adsorption of SDS on the electrode surface, electrostatic attraction force exerted towards the cationic DA and a repulsion force exerted towards the anionic species AA and UA take place. This indicates that the accumulation of SDS on the modified electrode forms a negative layer on the electrode surface. With stirring (5 min) and accumulation time (1 min), the reduction peak current (Ip$_c$) and the oxidation peak current (Ip$_a$) for DA increase correspondingly. Moreover, reduction peak current (Ip$_c$) and the oxidation peak current (Ip$_a$) for both AA and UA are suppressed. More sensitive response achieved in acidic solution and can be well explained by the proton participating in the oxidation process of DA, and in acidic solution SDS molecule is ionized easily (figure is not included).

3.6. Stability of the modified electrode

In order to investigate the stability of the SDS/PEDOT modified electrode, the CV for 0.5 mM L^{-1} DA in 150 µL 0.1 M L^{-1} SDS, 0.1 M L^{-1} B-R (pH = 7.4) solution were recorded every 10 min intervals. A total of 20 runs were performed without any noticeable decrease in the film response. It was found that both anodic and cathodic peak currents remained relatively stable. Repetitive measurements indicate that this electrode has a good reproducibility and does not suffer from surface
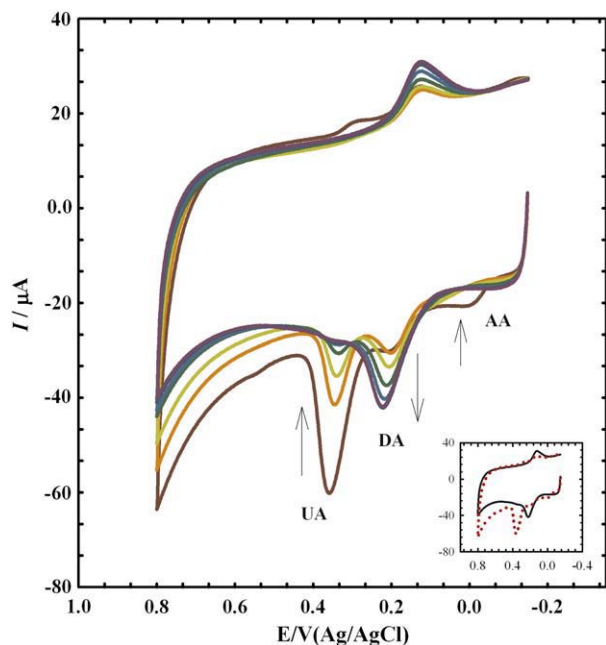
fouling during the voltammetric measurements. After measurements the electrode was kept in pH = 7.4 B-R buffer at room temperature. The current response decreased about 2% after 1 week and 6.1% after 2 weeks of storage.

3.7. Electrochemical impedance spectroscopy (EIS) of dopamine

It is well known that electrochemical alternating current imped-ance technique is a useful tool for studying the interface properties of surface-modified electrodes [47–50]. Therefore, EIS was used to investigate the nature of DA interaction at SDS/PEDOT surface. EIS data were obtained for the modified electrode at AC frequency varying between 0.1 Hz and 100 kHZ with an applied potential in the region corresponding to the electrolytic oxidation of DA in B-R buffer (pH = 7.4). Fig. 8A shows a typical impedance spectrum presented in the form of Nyquist plot of DA at PEDOT/Pt electrode in absence (a) and presence (b) of SDS. From this comparison, it is clear that the impedance responses of dopamine show great difference after addition of SDS. On the other hand, in the absence of SDS, the impedance spectra include a semicircle with a larger diameter (larger electron transfer resistance). However, after addition of SDS, the diameter of semicircle diminishes markedly. Thus, the charge transfer
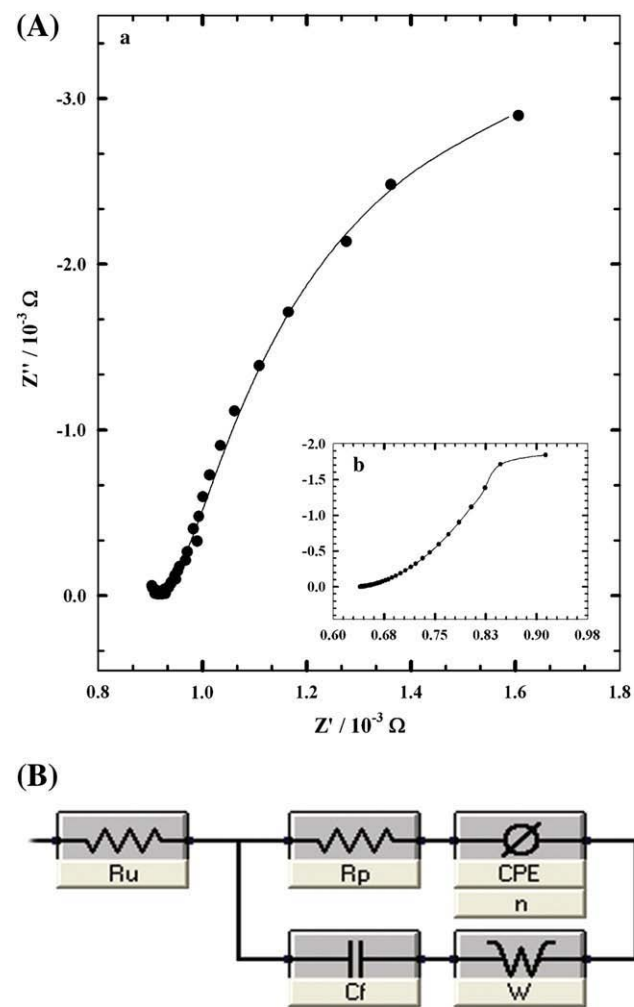
Fig. 8. Nyquist diagrams (−Z″ vs. Z′) for the EIS measurements at PEDOT / Pt (A) at potential 250 mV. (a) In absence of SDS and (b) in presence of SDS (inset). In 5 mmol L^{-1} DA in 0.1 mol L^{-1} B-R pH = 7.4. Amplitude: 5 mV, frequency range: 0.1–100,000 Hz (B) the equivalent circuit.

Fig. 7. Cyclic voltammogram for 0.2 mmol L^{-1} AA, 0.1 mmol L^{-1} UA and 0.1 mmol L^{-1} DA 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 7.4 , inset represents the initial and final CVs.

Table 1
The data obtained from EIS for the determination of DA using PEDOT/Pt electrode in absence and presence of SDS at oxidation potential (pH = 7.4).

PEDOT/Pt	E (mV)	R_p (kΩ cm^2)	R_u (kΩ cm^2)	C_f (μFcm^{-2})	W (k Ω^{-1} cm^{-2})	C_{CPE} (μFcm^{-2})	n
In absence of SDS	250	28.2	0.91	4.7	0.35	1600	0.57
In presence of SDS	250	15.8	0.64	7.8	0.32	2700	0.59

resistance of electro—oxidation of dopamine decreases greatly, and the charge transfer rate is enhanced by SDS.

To analyze the data obtained from EIS spectra, a simple equivalent circuit model in Fig. 8B was used to fit the results. In this circuit, R_u is the solution resistance, R_p is the polarization resistance, CPE represents the predominant diffusion influence on the charge transfer process (where n is its corresponding exponent), C_f is the capacitance of the double layer and W is the Warburg impedance due to diffusion.

Table 1 lists the best fitting values calculated from the equivalent circuit for the impedance data. The capacitive component of the charge at the PEDOT/Pt electrode in presence of SDS is relatively higher compared to that in absence of SDS. This is explained in terms of the increase in the ionic adsorption at the electrode/electrolyte interface. Moreover, the decrease in the interfacial electron transfer resistance is attributed to the selective interaction between SDS and DA that resulted in the observed current signal increase for the electro-oxidation process.

3.8. Morphology of PEDOT films

The ease of electron transfer is crucial to compare the performance of the PEDOT film, especially in biosensor related applications. In
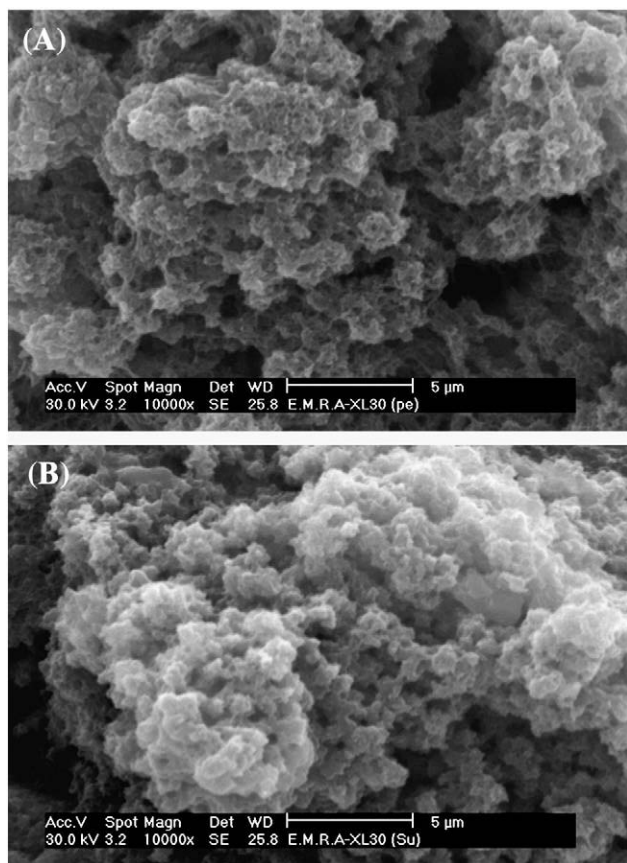

Fig. 9. SEM image of the PEDOT layers deposited on Pt wire from 0.01 mol L^{-1} EDOT in 0.1 mol L^{-1} LiClO$_4$ in acetonitrile (10 cycles) (magnification 10,000×, 30 kV), (A) in absence of SDS, (B) in presence of 150 μL 0.1 mol L^{-1} SDS.

addition to the backbone structure, the properties of conducting polymers are strongly dependent upon their morphologies [51]. Therefore, the surface morphologies of PEDOT films were characterized by SEM (Fig. 9). In Fig. 9, a significant difference in morphology was observed for film prepared by electrochemical deposition of PEDOT on Pt, and PEDOT on Pt in presence of surfactant (SDS). The morphology of PEDOT film without SDS (Fig. 9A) has globular morphology and the surface looks rough due to the Pt substrate. In case of SDS (Fig. 9B) the film becomes spongy and cotton-like due to the presence of anionic tailing structure. Thus, the aggregates of SDS that accumulates onto the surface of the polymer shown in the SEM picture influences the conductivity level of the film and helps the attraction of DA (selectively) to the surface of the electrode.

3.9. Determination of DA in human urine samples

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of DA in human urine. The same measurements were conducted successfully on urine samples. In this set of experiment, dopamine was dissolved in urine to make a stock solution with 5 mmol L^{-1} concentration. Standard additions of 10 μl of 5 mmol L^{-1} dopamine in urine were added to the buffer = 7.4 containing 150 μl SDS, the corresponding LSV were measured. The analytical results are summarized in Table 2. The recovery ranged from 99.7% to 100.0%, and the results are acceptable indicating that the present procedures are free from interferences of the urine sample matrix. The calibration curve of dopamine in urine with the detection limit of 67 nmol L^{-1} is given in Supplement 5. The results strongly proved that DA can be selectively and sensitively determined at PEDOT/Pt electrode in urine sample in presence of SDS.

4. Conclusion

In this work we demonstrated the selective and sensitive determination of DA in the presence of AA and UA in 0.1 M B-R (pH = 7.4) using PEDOT/Pt electrode in presence of SDS. A novel approach for the utilization of anionic surfactants in electroanalytical applications is described in this work. The negatively charged SDS adsorbed onto the electrode surface control the electrode reactions of AA, UA and DA that differ in their net charge. The oxidation of DA is facilitated at the negatively charged surfactant SDS on the polymeric film by electrostatic interaction of cationic DA with the SDS, increasing the anodic peak current by one and half folds. On the other hand, AA, UA, being negatively charged, is repelled from the negatively charged SDS in the polymeric film, causing quenching of both the anodic and cathodic peaks current. PEDOT/Pt electrode in presence of SDS was used to determine the dopamine in urine and simulated sample in presence of high concentration of AA and UA, and a satisfying result was achieved. The present modified electrode showed exceptional stability and relatively lower detection limit for the determination of DA compared to several surfaces mentioned in the literature [52,53].

Acknowledgments

Table 2

A. Determination of DA at PEDOT/Pt electrode in urine in the linear range 0.5×10^{-6} mol L^{-1} – 1×10^{-3} mol L^{-1}. B. Recovery data for synthesized biological solution spiked with various amounts of 0.5 mmol L^{-1} DA, in fresh urine sample from the healthy volunteers.

Dopamine pH = 7.4	E_{ox} (mV)	Linear range (mol L^{-1})	r	Slope	Intercept	S.E (slope)	S.E (intercept)	L.O.D	L.O.Q
PEDOT/Pt with SDS in urine	246	0.5×10^{-6} – 1.0×10^{-3}	0.994	0.0221	0.244	0.0005	0.267	6.7×10^{-8}	2.2×10^{-7}

Sample	Added DA (μmol L^{-1})	Found DA (μmol L^{-1})	Recovery (%)
1	4.00	3.99	99.7
2	6.00	5.89	98.1
3	10.0	10.01	100.0
4	15.0	14.98	99.8

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioelechem.2010.07.002.

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