



Simultaneous Determination of Catecholamines and Serotonin on Poly(3,4-ethylene dioxythiophene) Modified Pt Electrode in Presence of Sodium Dodecyl Sulfate

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A promising electrochemical sensor was developed using poly(3,4-ethylene dioxythiophene) modified platinum electrode in the presence of sodium dodecyl sulphate (SDS). This sensor is sensitive for the determination of catecholamine compounds, namely dopamine, epinephrine, L-norepinephrine, and L-DOPA, as well as serotonin (ST) in the presence of interference molecules such as uric acid, ascorbic acid (AA), and glucose. The presence of SDS in the medium plays a key role in the electrostatic attraction of these compounds toward the polymeric surface and causes repulsion toward the interfering ones. Cyclic voltammetry, linear sweep voltammetry, ultraviolet-visible (UV-vis), nuclear magnetic resonance and electrochemical impedance spectroscopy were used to verify the behavior of the studied compounds in micellar media. In the presence of an anionic surfactant, the presence of large excess of AA and glucose did not interfere with the voltammetric responses of catecholamine and ST. The linear response was obtained for serotonin in the range of 0.05–10 $\mu\text{mol l}^{-1}$ and 20–100 $\mu\text{mol l}^{-1}$ with correlation coefficients of 0.997 and 0.998 and detection limits 48 and 71 nmol l^{-1} , respectively. The utility of this modified electrode was demonstrated for the determination of ST in human urine.

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There is considerable interest in developing electrochemical techniques for measurement of catecholamines such as epinephrine (E), norepinephrine (NE), dopamine (DA), and serotonin (ST), which are important neurotransmitters in mammalian species.¹ DA is widely distributed in the brain tissues for message transfer in mammalian central nervous system. The deficiency of DA can result in some neurological disorders such as schizophrenia and Parkinson's disease.^{2–4} ST is also distributed in the brain and plays a crucial role in emotional system together with other neurotransmitters. Uric acid (UA) is the primary end product of purine metabolism. Abnormal levels of UA are symptoms of several diseases such as hyperuricaemia, gout, and Lesch–Nyan disease.⁵ Thus, detecting and determining the concentrations of catecholamines in the presence of interfering species is an important goal in electrochemical analysis. Much attention has been given to the design and development of novel materials coated on electrode surfaces with improved molecular recognition capabilities.^{6–9} The determination of monoamine neurotransmitters have been carried out by using spectrophotometry,¹⁰ fluorescence,¹¹ chemical luminescence,¹² pseudopolarography,¹³ voltammetry,¹⁴ capillary electrophoresis,¹⁵ and sensors based on enzymatic amplification.¹⁶ High performance liquid chromatography (HPLC) with electrochemical detection is most often used for the analysis of catecholamines and their metabolites.^{1,16–18} The detection of neurotransmitters and their metabolites by electrochemical methods have attracted great interests because of their simplicity, rapidness, high sensitivity, and the ability of sensing neurotransmitters in living organisms and in vivo real time analysis.^{19,20} Electrochemical analysis on the unmodified electrodes such as glassy carbon (GC), Pt, and Au electrodes has limitations because of overlapping voltammetric peaks, and high concentrations of ascorbate and UA in typical biological matrices.^{21,22}

Acetaminophen or paracetamol (ACOP) is an antipyretic and analgesic drug widely used worldwide for the relief of mild to moderate pain associated with headache, backache, arthritis, and postoperative pain. It is also used for the reduction of fevers of bacterial or viral origin.²³

Simultaneous measurement of DA and ST levels is of greater value because of their coexistence in biological systems. The major problem found is that the voltammetric responses of DA and ST interfere with each other. Numerous reports demonstrated that DA and ST influence each other in their respective releasing.^{24,25}

Therefore, electrochemical techniques based on various approaches have been made to overcome these difficulties for the determination of DA and ST.^{26–34}

The simultaneous determination of DA, UA, and ascorbic acid (AA) was reported by using different modified electrodes.^{35–38} Choline and acetyl choline modified glassy carbon electrode³⁹ and poly(phenosafranin) modified electrode⁴⁰ were developed for the simultaneous determination of DA, AA, and serotonin. Among the above approaches, polymer film modified electrodes are widely used because they can provide more active sites than that fabricated through covalent bonding or adsorption.⁴¹

Among various types of conducting polymers, poly(3,4-ethylene-dioxythiophene) (PEDOT) is a widely investigated and can be easily electrodeposited on several surfaces by electro-oxidation of its monomer.^{42–45} PEDOT modified electrodes have been extensively reported and showed excellent electrocatalytic effect for phenolic compounds,⁴⁶ detection of DA,⁴⁷ AA,⁴⁸ and morphine.⁴⁹

Surfactants have been widely used in chemistry and in particular affecting several electrochemical processes.⁵⁰ Several applications of surfactants in electrochemistry are in electroplating,⁵¹ corrosion,⁵² fuel cells,⁵³ electrocatalysis,⁵⁴ and electroanalysis.⁵⁵ Rusling⁵⁶ indicated the influence of surfactant aggregates at the electrode/electrolyte interface in micelle solutions. In his study,⁵⁶ it was shown that the entry of an electrochemical reactant into this dynamic surface film is a key preceding electron transfer step.

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.⁵⁷ 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.⁵⁸ Recently, the influence of micelles in the simultaneous determination of two components was also demonstrated, as in the case of ascorbic acid and dopamine,⁵⁹ catechol and hydroquinone.⁶⁰ 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.⁶¹

In this work, the modification of PEDOT/Pt electrode is introduced by hydrophobic adsorption of sodium dodecyl sulphate (SDS) on the surface of PEDOT/Pt electrode. This electrode is a simple phase surface with a high density of negative charges covered on the electrode surface. Selectivity and sensitivity of the sensor is examined for the determination of catecholamine compounds and

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ST in the presence of interfering molecules such as uric acid, ascorbic acid, and glucose. Competitive adsorption between ST and DA at the modified sensor is investigated. Also estimation of the diffusion coefficients for the compounds studied is calculated. Moreover, the sensor is utilized to determine ST in human urine.

Experimental

Materials and reagents.— All chemicals were used as received without further purification. (3,4-ethylenedioxy)thiophene (EDOT), lithium perchlorate, acetonitrile (HPLC grade), dopamine hydrochloride, epinephrine, L-norepinephrine, L-DOPA, paracetamol, ascorbic acid, uric acid, tryptophan, serotonin hydrochloride, glucose, and sodium dodecyl sulphate were supplied by Aldrich Chem. Co. (Milwaukee, WI, USA). Aqueous solutions were prepared using double distilled water. B-R buffer of pH between 2 and 9 are prepared from 0.12 mol l⁻¹ boric acid, 0.12 mol l⁻¹ acetic acid, and 0.12 mol l⁻¹ orthophosphoric acid and adjusted by 0.2 mol l⁻¹ NaOH.

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 disk ($\phi = 1.5$ mm). The auxiliary electrode was a Pt wire (10 cm long/2.0 mm diameter). All the potentials in the electrochemical measurements 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 sonicated in double-distilled water before starting each experiment. The electrochemical polymerization of the EDOT was carried using cyclic voltammetric method in non aqueous solution containing 0.01 mol l⁻¹ EDOT and 0.1 mol l⁻¹ LiClO₄ in acetonitrile.

Instrumental and experimental setup.—**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) and cyclic voltammetry (CV) were used for the determination of the compounds using modified PEDOT/Pt electrode. Electrochemical impedance spectroscopy was performed using a Gamry-750 system and a lock-in-amplifier that was connected to a personal computer. The parameters in electrochemical impedance experiment were as follows: different potential values 0.350 V for ST and 0.02 V for AA were studied at frequency range of 0.1–10,000 Hz with an ac amplitude of 5 mV applied on PEDOT/Pt electrode and tested in 0.5 mmol l⁻¹ of each of serotonin and ascorbic acid in the presence and absence of 150 μl , 0.1 mol l⁻¹ SDS in buffer pH 7.4. Data analysis software was provided with the instrument and applied nonlinear least square fitting with Levenberg–Marquardt algorithm.

Spectroscopic measurements.—All UV measurements were performed using a Shimadzu 1601 spectrophotometer (Kyoto, Japan).

Nuclear magnetic resonance (NMR) measurements were performed using a 300 MHz Varian NMR instrument in DMSO (Dimethyl sulphoxide) and with TEMAC (triethylmethylammonium chloride) as internal standard.

Analysis of urine.— The utilization of the proposed method in real sample analysis was also investigated by direct analysis of ST in human urine samples. Serotonin 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 serotonin in urine were added to the buffer pH 7.4 containing 150 μl SDS.

Results and Discussion

Electrochemistry of catecholamine neurotransmitters, acetaminophen, uric acid, and ascorbic acid.— Initial studies of the voltammetric behavior of all compounds were performed using cyclic voltammetry. Table I summarizes the electrochemical data for the oxidation of some molecules of biological interest, namely, catecholamines (dopamine, epinephrine, L-norepinephrine, and L-DOPA), as well as serotonin (5-hydroxytryptamine), tryptophan, acetaminophen, and some interfering compounds such as uric acid and ascorbic acid. The data were collected from the cyclic voltammograms at PEDOT/Pt electrode in the absence and presence of 150 μl SDS. The scan rate is 50 mV s⁻¹, and the analyte concentration is 0.5 mmol l⁻¹ in 0.1 mol l⁻¹ universal buffer (B-R) pH 7.4. Figure 1a shows the cyclic voltammograms for one of the cationic catecholamines, L-norepinephrine using PEDOT/Pt modified electrode. The scan rate is 50 mV s⁻¹, and the analyte concentration is 0.5 mmol l⁻¹ in 0.1 mol l⁻¹ universal buffer (B-R) pH 7.4. The SDS was added from a stock containing 0.1 mol l⁻¹ with successive additions from 0 to 150 μl . After each addition, stirring takes place for 5 min before starting the experiment. An increase in anodic and cathodic peak current values was observed upon addition of SDS due to the electrostatic interaction between the surfactant film adsorbed on the electrode and the cationic catecholamine. For anionic compounds such as UA, shown in Fig. 1b, the oxidation current response decreases in the presence of SDS. Tryptophan, AA, and UA are in the anionic form (pH = 7.4), and in micellar medium, they establish an electrostatic repulsion with anionic surfactant SDS, which provokes a large decrease in the peak current value, I_{pa} . L-DOPA and ACOP are neutral species at physiological pH (i.e., pH = 7.4); hence, SDS would not affect the kinetic of these compounds as illustrated in Table I. The change of the peak current values for cationic and anionic compounds 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.²⁸

Electrocatalytic oxidation of ST.— Figure 2a shows the cyclic voltammetric curves (CVs) of 0.5 mmol l⁻¹ ST in pH 7.4 universal

Table I. Summary of CV results obtained at PEDOT/Pt electrode and PEDOT/Pt electrode in the presence of SDS, for 5.0×10^{-4} mol l⁻¹ of each compound in 0.1 mol l⁻¹ B-R pH = 7.4 scan rate 50 mV s⁻¹ with additions of 150 μl , 0.1 mol l⁻¹ SDS.

Compound	PEDOT/Pt			PEDOT/Pt SDS			Amount of current changed
	CV			CV			
	E_{pa} (mV)	I_{pa} (10^{-5} A)	D_{app}	E_{pa} (mV)	I_{pa} (10^{-5} A)	D_{app}	
Dopamine	223	3.3	5.1×10^{-4}	248	5.5	1.4×10^{-3}	1.6
Serotonin	358	1.2	6.8×10^{-5}	353	2.4	2.7×10^{-4}	2
Epinephrine	206	1.2	6.8×10^{-5}	212	1.7	1.3×10^{-4}	1.4
L-norepinephrine	306	3.1	4.5×10^{-4}	325	5.6	1.4×10^{-3}	1.8
L-DOPA	190	2.6	3.2×10^{-4}	212	2.2	2.3×10^{-4}	-0.84
Paracetamol	388	0.9	3.8×10^{-5}	394	0.8	3.0×10^{-5}	-0.88
Ascorbic acid	20	2.9	4×10^{-4}	—	—	—	0
Tryptophan	22	0.9	3.8×10^{-5}	—	—	—	0
Uric acid	336	1.1	5.7×10^{-5}	342	0.7	2.3×10^{-5}	-0.6

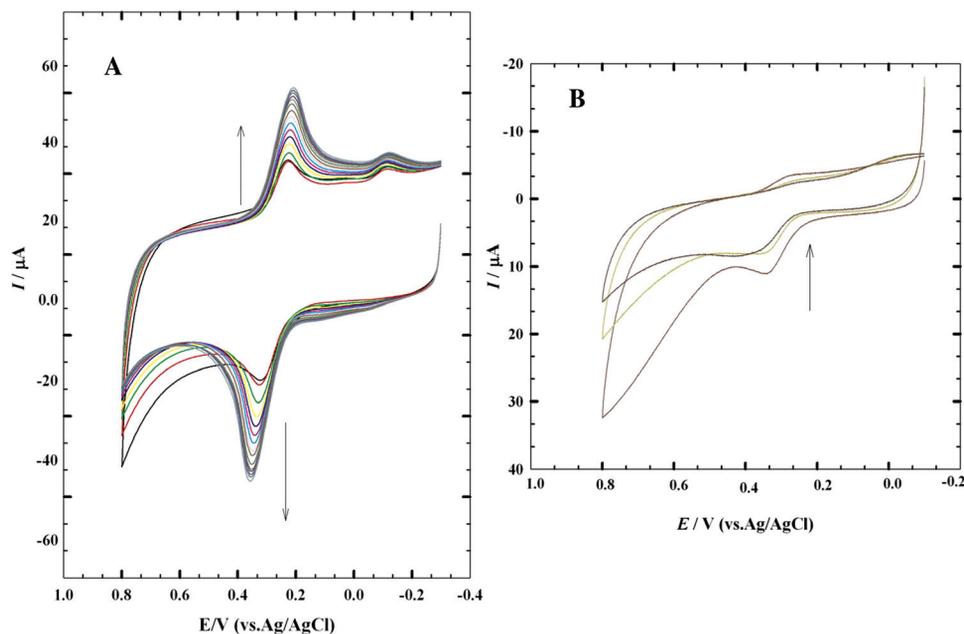


Figure 1. (Color online) Cyclic voltammograms of $5.0 \times 10^{-4} \text{ mol l}^{-1}$ (a) L-nor-epinephrine, (b) uric acid/ 0.1 mol l^{-1} B-R, scan rate 50 mV s^{-1} at PEDOT/Pt electrode with successive additions ($0\text{--}150 \mu\text{l}$) of 0.1 mol l^{-1} SDS at $\text{pH} = 7.4$.

buffer solution (B-R), scan rate: 50 mV s^{-1} at bare Pt electrode (curve a), PEDOT/Pt (curve b), and PEDOT/Pt electrode in the presence of $150 \mu\text{l}$ SDS (curve c), respectively. A relatively high anodic peak current $2.4 \times 10^{-5} \text{ A}$ at 0.35 V with a small cathodic peak at about -0.037 V was observed at PEDOT/Pt electrode in the presence of SDS (curve c), which was two- and sixfold in oxidation current response relative to that at the PEDOT/Pt (curve b) and Pt electrode (curve a), respectively. This was attributed to the quasireversible reaction of ST oxidation process. The PEDOT/Pt electrode (curve b) also showed small anodic peak current $1.25 \times 10^{-5} \text{ A}$ at 0.35 V . A very weak current response was observed at the bare Pt electrode (curve a). The anionic surfactant SDS enhances greatly

the anodic current peak of ST, which is due to the adsorption of the anionic surfactant SDS onto electrode surface which results in a negatively charged hydrophilic film with the polar head group points to the bulk of the solution. This negatively charged hydrophilic layer accelerates serotonin to reach the electrode surface faster, and as consequence, the reaction becomes easier. Two peaks in the reduction direction are characteristic for the polymer film of PEDOT and have been previously mentioned in the literature.⁶²

Another study was performed to illustrate the voltammetric behavior of ST at the PEDOT/Pt electrode in the presence of $150 \mu\text{l}$ SDS using LSV with scan rate 50 mV s^{-1} . Figure 2b shows typical LSVs for successive additions of $10 \mu\text{l}$, 5 mmol l^{-1} of serotonin to

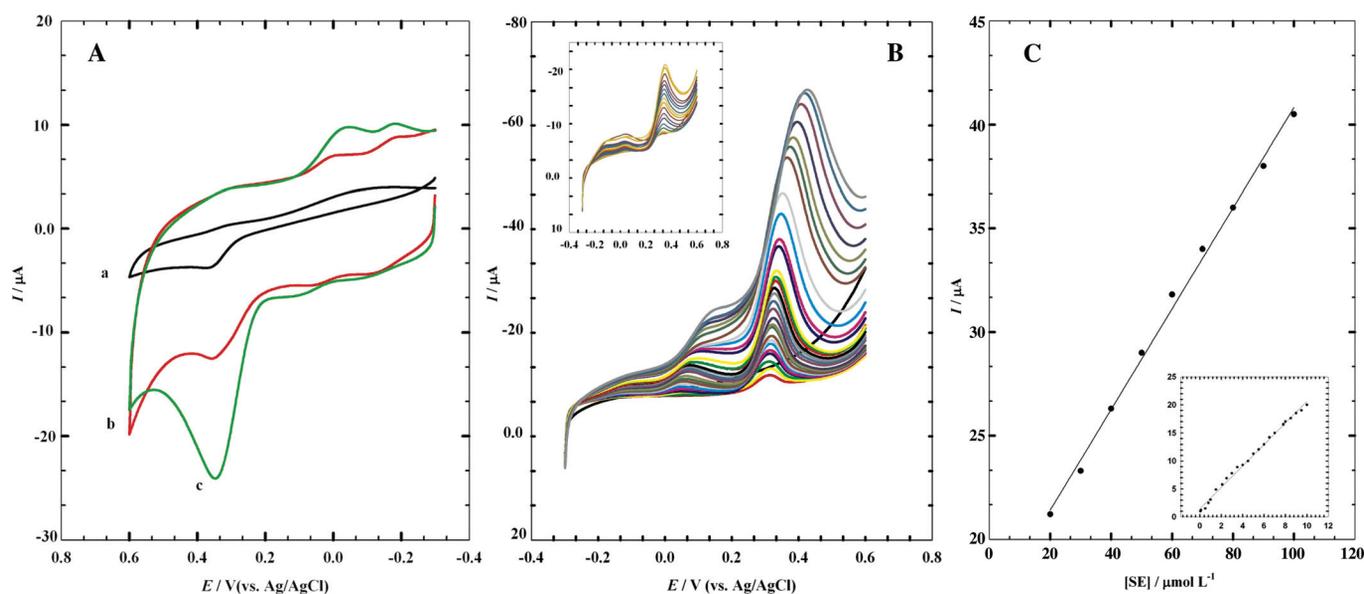


Figure 2. (Color online) (A) Cyclic voltammograms of $5.0 \times 10^{-4} \text{ mol l}^{-1}$ serotonin/ 0.1 mol l^{-1} B-R, $\text{pH} = 7.4$, at (a) Pt electrode, (b) PEDOT/Pt electrode, (c) PEDOT/Pt electrode in the presence of SDS, at scan rates 50 mV s^{-1} and (B) LSVs of different concentrations of ST ($0.05 \mu\text{mol l}^{-1}$ – 0.1 mmol l^{-1}) in 10 ml of 0.1 mol l^{-1} B-R, $\text{pH} = 7.4$, containing 0.1 mol l^{-1} $150 \mu\text{l}$ SDS at PEDOT/Pt electrode. The inset without SDS. (C) Calibration curve for ST of different concentrations from $20 \mu\text{mol l}^{-1}$ to 0.1 mmol l^{-1} and from 0.05 to $10 \mu\text{mol l}^{-1}$ (inset).

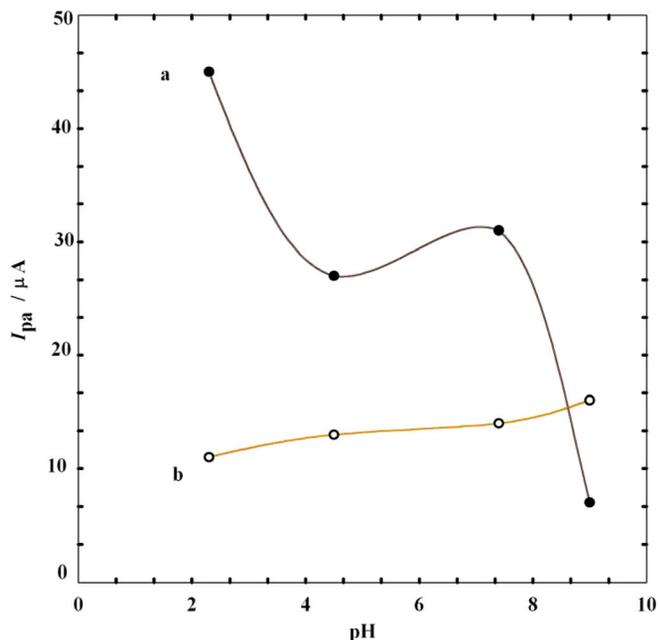


Figure 3. (Color online) Plot of the anodic peak current values as a function of the pH value of the solution at PEDOT/Pt electrode (a) in the presence and (b) in the absence of SDS.

150 μL SDS in 0.1 mol l^{-1} B-R (pH 7.4). The data shows that by increasing the concentration of serotonin the anodic peak current increases, which indicates that the electrochemical response of serotonin was apparently improved by SDS due to the enhanced accumulation of protonated serotonin via electrostatic interaction with negatively charged SDS at the polymeric electrode surface. The inset shows the same experiment in the absence of SDS with lower current response during successive additions of ST. Figure 2c shows the calibration curves of ST in the linear ranges of 0.05–10 $\mu\text{mol l}^{-1}$ (inset) and 20–100 $\mu\text{mol l}^{-1}$ with correlation coefficients of 0.997 and 0.998 and detection limits 48 and 71 nmol l^{-1} , respectively.

Response stability of the modified electrode.— In order to investigate the stability of the PEDOT/Pt electrode in the presence of SDS, the CV of 0.5 mmol l^{-1} ST in 150 μL (0.1 mol l^{-1}) SDS, 0.1 mol l^{-1} B-R (pH 7.4) solution was recorded for every 10 min intervals, and it shows excellent signal stability for 20 runs without any change from the polymer film response. Thus, the current signals for both anodic and cathodic peak currents remained the same. Repetitive measurements indicate that this electrode has a good reproducibility and does not undergo surface fouling during the voltammetric measurements. After measurements, the electrode was kept in pH 7.4 B-R solution at room temperature. Repeating the experiment after longer time, it was found that the current response decreased about 2% in 1 week and 5.1% in 2 weeks relative to the “original” signal recorded for freshly prepared surfaces.

The effect of pH .— The effect of changing the pH on the electrochemical response of ST at the modified PEDOT electrode was examined in different pH values 2.3, 4.5, 7.4, and 9.0 in the presence and absence of SDS. Supplementary 1(A and B) shows the effect of changing pH of B-R buffer on the current response of 0.5 mmol l^{-1} serotonin in the presence and absence of 150 μL (0.1 mol l^{-1}) SDS, respectively.⁶³ The electrochemical oxidation of ST is believed to take place at the phenol group of the molecule to form the corresponding ketone. The absence of the corresponding reduction peak indicates the instability of this oxidation product, which then undergoes chemical reaction to form a product that is easily oxidizable. This product is believed to be the reduced hydroquinone. In general, the oxidation peak potential shifts to more positive val-

ues as the pH decreases in the absence and presence of SDS. Also, we can evaluate the number of protons involved in the voltammetric oxidation of ST. ST oxidation varies linearly with increasing pH ($E_p = 0.67 - 0.025 pH$, $r = 0.99$) and is shifted to less positive potentials with a slope of 0.025 per pH unit, revealing that one proton is involved in the oxidation process. The peak current of ST increases with increasing pH in the absence of SDS, while in the presence of SDS, the peak current of ST decreases with increasing pH as shown in Fig. 3. Maximum oxidation current signal was obtained in pH 2.3, and minimum current signal was observed in pH 9, in the SDS containing solution. In the present study, all electrochemical measurements have been conducted in pH 7.4 (physiological media).

Effect of scan rate.— The effect of scan rate on the oxidation peak potential and peak current of ST was studied at the surface of PEDOT/Pt in the presence and absence of 150 μL (0.1 mol l^{-1}) SDS in 0.1 mol l^{-1} B-R buffer solution. Supplementary 2 shows the cyclic voltammetric curves of ST obtained at different scan rates 10–150 mV s^{-1} to investigate the kinetics of electrode reactions and verify whether SDS affects the diffusion of the mass transport process or not. A linear relation between oxidation peak current and square root of scan rate from 10 to 150 mV s^{-1} is observed for ST in the absence and presence of 150 μL (0.1 mol l^{-1}) SDS in 0.1 M B-R buffer solution as shown in Fig. 4. This linearity suggests that the electrochemical reaction of ST at the surface of PEDOT/Pt is a diffusion-controlled process. Moreover, in case of SDS, the current increases, which indicates that the anionic surfactant SDS accelerates the diffusion of the cationic ST. Also, the cyclic voltammetric results show that the oxidation peak potentials of ST in the absence of SDS are shifted slightly to more positive value with increasing scan rate from 10 to 150 mV s^{-1} . It is important to notice that no reverse peak is observed at low scan rates and relatively small reverse peaks are observed at high scan rates (0.1 V s^{-1} and higher),

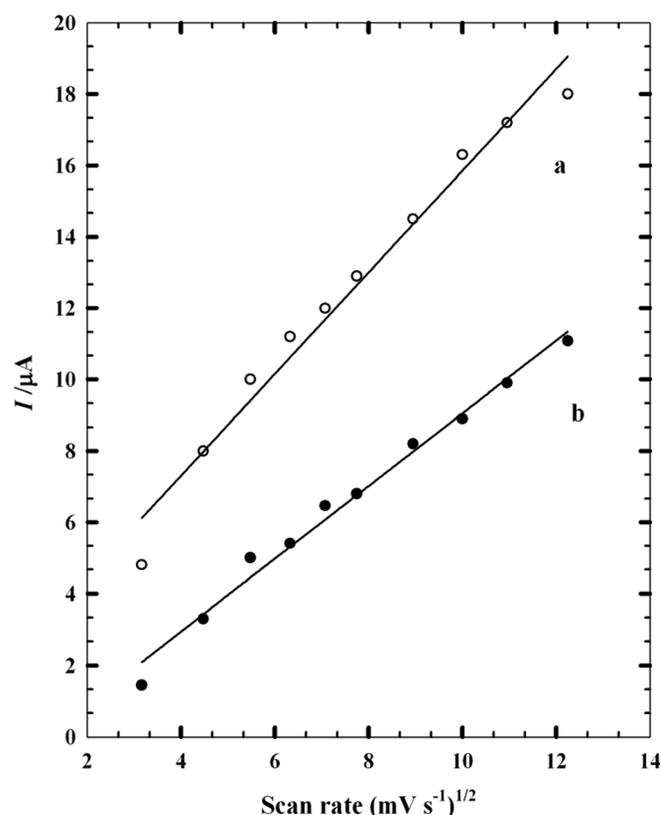


Figure 4. Plot of the anodic peak current values as a function of the square root of the scan rate at PEDOT/Pt electrode (a) in the presence and (b) in the absence of SDS.

which suggests irreversible or quasireversible electrode process for ST (figure not shown). On the other hand, in the presence of SDS, the oxidation peak potentials of ST are shifted to more positive values with increasing scan rate from 10 to 150 mV s⁻¹ with noticeable increase in the current values.

The dependence of the anodic peak current density on the scan rate has been used for the estimation of the "apparent" diffusion coefficient, D_{app} , for the compounds studied. D_{app} values were calculated from Randles Sevcik equation⁶⁴

$$I_p = 2.69 \times 10^5 n^{3/2} A C_0 D^{1/2} v^{1/2}$$

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 cm²), C_0 is the analyte concentration (5×10^{-7} mol l⁻¹), and D is the diffusion coefficient of the electroactive species (cm² 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} values at PEDOT/Pt electrode for cationic molecules are in the range of 10^{-5} – 10^{-4} cm² s⁻¹, and D_{app} values at the PEDOT/Pt electrode in the presence of SDS are in the range of 10^{-4} – 10^{-3} cm² s⁻¹, which are in good agreement with the previous values reported in the literature.^{65,66} The reverse was obtained for anionic molecules where the calculated D_{app} in the presence of SDS is lower than that calculated in the absence of SDS. The anionic surfactant SDS affects remarkably the diffusion component of the charge transfer at the electrode surface as indicated by the D_{app} values in Table I. 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

toward the solution side and far 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 the 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 the presence of SDS than in the absence of it.

Effect of interferences on the behavior of ST.— In biological environments, the main interference of catecholamines compounds is the presence of high concentration of AA and also the presence of

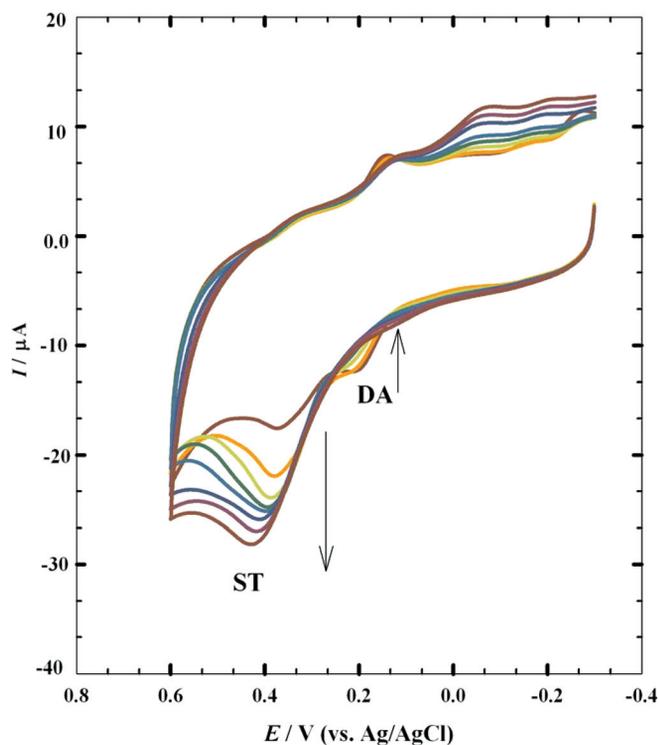
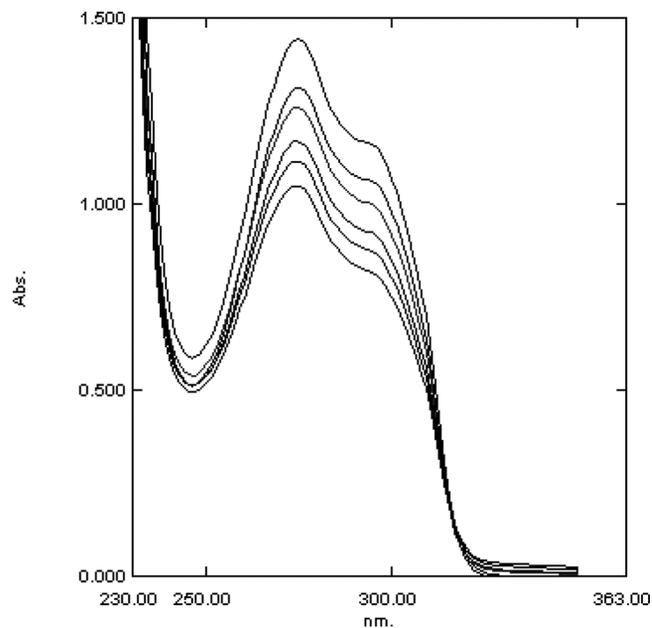
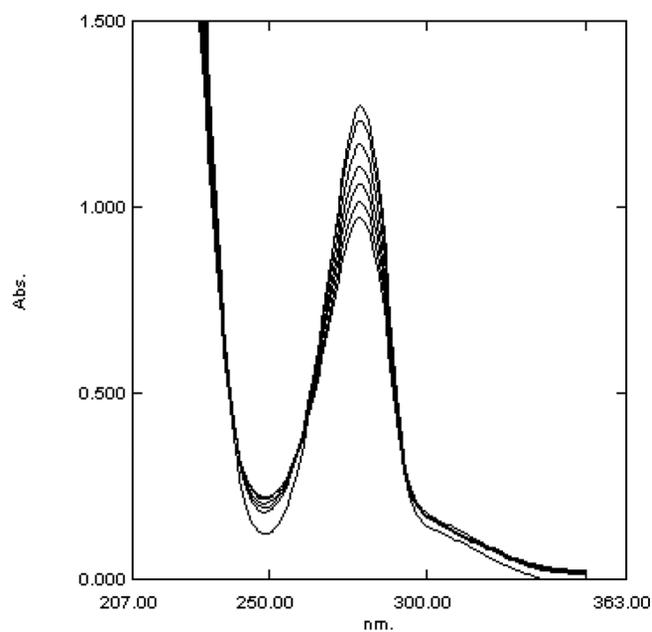


Figure 5. (Color online) Cyclic voltammograms of equimolar solution 0.5 mmol l⁻¹ of both DA and ST in B-R (0.1 mol l⁻¹), scan rate 50 mV s⁻¹ at PEDOT/Pt electrode pH = 7.4 with successive additions of 10 μl 0.1 mol l⁻¹ SDS (0–150 μl).



A



B

Figure 6. Absorption spectra of five successive aliquots of 0.2 ml of 0.01 mol l⁻¹ SDS added to 4.0 ml of (a) 2.5×10^{-4} mol l⁻¹ serotonin, (b) 5×10^{-4} mol l⁻¹ for DA.

glucose. So, it is important to investigate the electrochemical response of ST in the presence of AA and glucose. The LSV technique was used to investigate the interference study of successive additions ($0.05\text{--}0.1\ \mu\text{mol l}^{-1}$) of $5\ \text{mmol l}^{-1}$ ST, in a mixture of $5\ \text{mmol l}^{-1}$ AA, and $0.5\ \text{mmol l}^{-1}$ glucose as shown in Supplementary 3. The glucose signal was invisible at PEDOT/PT electrode tested in $150\ \mu\text{l}$ SDS $0.1\ \text{mol l}^{-1}$ B-R ($\text{pH} = 7.4$). Initially, the peak current value of AA at $0.13\ \text{V}$ was larger than ST at $0.32\ \text{V}$. However, with the addition of ST, the signal of ST increased while AA current response decreased. The presence of more than 1000-fold excess of AA and 100-fold excess of glucose did not interfere with the response of ST.

Competitive adsorption of DA and ST.— Determination of DA and ST is of great importance because levels of DA and ST in biological fluids such as serum may also provide further information of diagnostic value in the aforementioned disorders. Furthermore, both DA and ST coexist in a biological system and they influence each other in their respective releasing. So, it was interesting to study the interaction of both compounds with SDS. First, the voltammetric behavior of $0.5\ \text{mmol l}^{-1}$ DA and $0.5\ \text{mmol l}^{-1}$ ST mixtures were investigated by CVs. As shown in Fig. 5, DA and ST yielded two well-defined oxidation peaks at 0.20 and $0.35\ \text{V}$ at the PEDOT/Pt electrode, respectively. The current response of ST increased while DA response decreased with successive additions of $150\ \mu\text{l}$ SDS in $0.1\ \text{mol l}^{-1}$ B-R ($\text{pH} = 7.4$). This is due to the competitive interac-

tion of DA and ST with the PEDOT/Pt film that is more pronounced in the case of ST. This is presumably due to a large conjugated structure of ST, which have the possibility to intercalate into the interior of PEDOT/Pt film. Another reason is the presence of $-\text{NH}_2$ and $-\text{NH}$ groups in serotonin, which increase the positive charge density toward the anionic surfactant SDS, which in turn facilitates its diffusion to the polymeric film. The competition between ST and DA is indicative of similar interaction mechanism for both of these compounds.

Another study was done by using the CV technique to investigate the competitive adsorption of DA and ST at PEDOT/PT electrode surface by successive additions of ST ($0\text{--}70\ \mu\text{mol l}^{-1}$) in $0.5\ \text{mmol l}^{-1}$ DA and $150\ \mu\text{l}$ SDS ($0.1\ \text{mol l}^{-1}$) B-R ($\text{pH} = 7.4$) (Supplementary 4). At the beginning, the peak current value of DA at $0.22\ \text{V}$ was higher than ST at $0.35\ \text{V}$. With the successive additions of SDS, the current response of ST increases while DA response decreases then becomes constant. This confirms the increase of adsorption of ST at the surface of the modified electrode.

Spectroscopic Measurements

UV/visible studies.— Interaction of anionic surfactant (SDS) with serotonin and dopamine in aqueous B-R buffer solutions was followed by UV-vis spectroscopy. Figures 6a and 6b shows the effect of successive additions of SDS surfactant on the absorption spectrum of each of serotonin and dopamine. Basically, the anionic

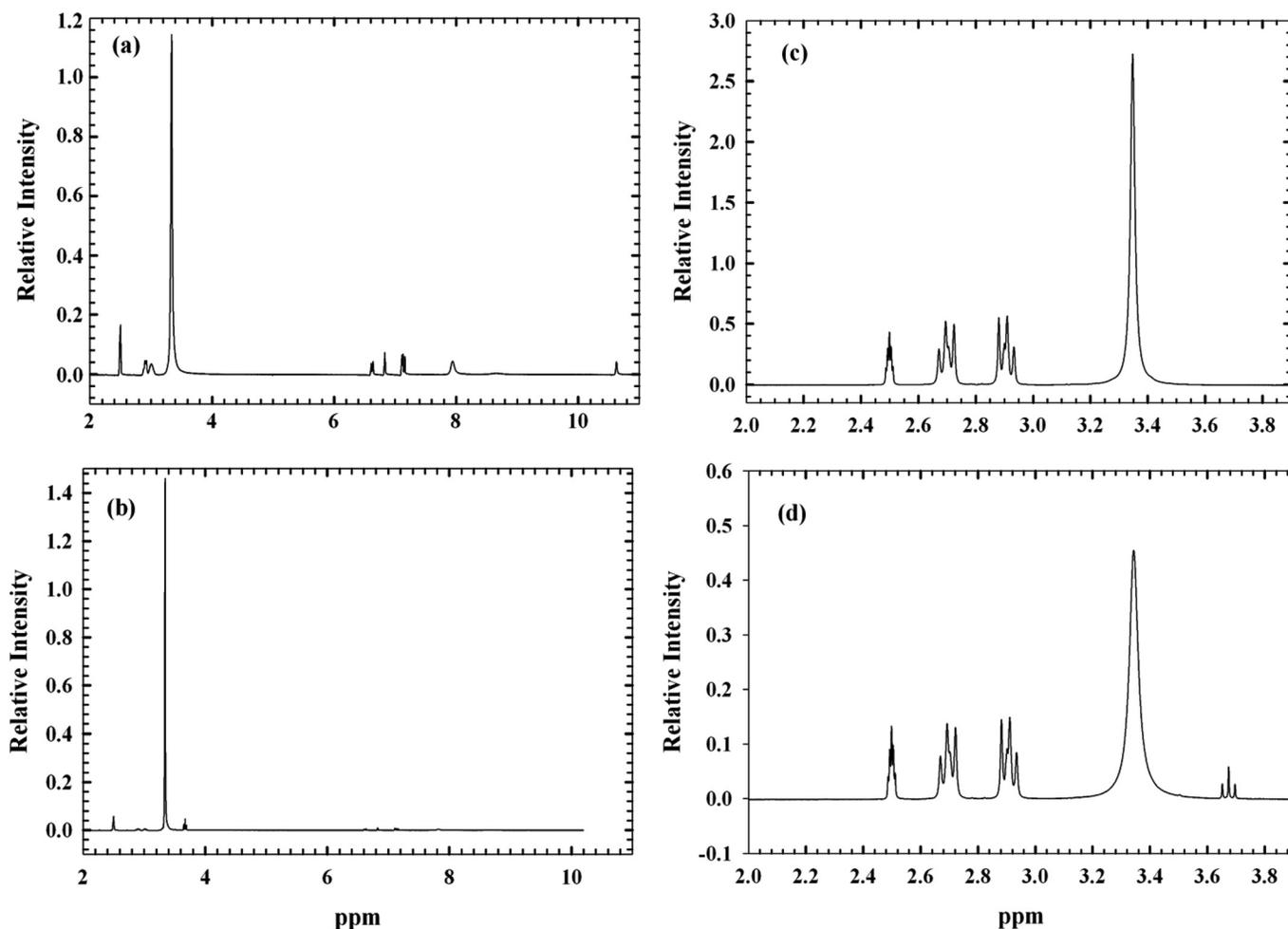


Figure 7. $^1\text{H-NMR}$ spectrum of (a) ST in the absence of SDS, (b) ST in the presence of SDS, (c) DA in the absence of SDS, and (d) DA in the presence of SDS.

surfactant SDS showed no absorption background. Successive aliquots of 0.2 ml (0.01 mol l^{-1}) SDS were added to each of the UV-vis cuvette containing 4.0 ml (0.25 mmol l^{-1}) serotonin and 0.5 mmol l^{-1} for DA ($\text{pH} = 7.4$).

In Fig. 6a, a band is identified at 274 nm and a shoulder is present at 296 nm for ST⁶⁷; these two peaks are due to the different possible sites of protonation in the case of indole nucleus having various constituent groups. It was assumed that protonation on nitrogen always occurs first and then takes place in other places.⁶⁸ These bands decrease its absorbance from 1.31 to 0.95 with five SDS additions. In Fig. 6b, a sharp band is formed at 279 nm for DA, which

decreased its absorbance from 1.27 to 0.97 with five SDS additions. The anionic character of SDS favors coulombic attraction forces with the compounds and should lead to the formation of aggregates in the solution phase. The aggregation in aromatic systems could be attributed to the formation of larger units (possibly due to the formation of longer repeat unit chains).⁶⁸ This "oligomerization" was due to the London–Margenau attractive forces between the π -electrons that are counter balanced by the coulombic and Lenard–Jones repulsive forces. This should be accompanied with a blueshift⁶⁸ or a redshift⁹ in the corresponding spectra that was not observed in the present case for ST and DA. This indicates that the charge interaction of the compound with SDS is the main contribution to the association that resulted in the decrease in the absorption spectra. Also, it was noticed from the results that the decrease in absorbance in the case of ST was larger than in the case of DA, which shows that there is more charge interaction of the ST with SDS than in case of DA. So, the spectrophotometry data are in good agreement with what we obtained from the voltammetry experiments.

NMR studies.— NMR measurements led us to similar conclusions and ascertain to a great extent the involvement of direct interaction between the drug and the SDS. The proton NMR spectra of ST are given in Fig. 7a. As noticed, NMR spectra of ST show characteristic signals for the 3-indole moiety at 7.93 and 6.63 ppm. The multiplets between 2.92 and 3.02 ppm are attributed to the protons of the $-\text{CH}_2$ methylene alpha and β -N moieties. Therefore, the two regions of interest in which the chemical shift and interactions are observed upon the addition of surfactant are for 3-indole and $-\text{CH}_2$ methylene alpha and β -N moieties, respectively. We believe that the most clearly influenced environment of ST protons is that of the $-\text{CH}_2$ methylene group of the molecule as shown in Fig. 7b. The protons are expected to be in close proximity to the interacting NH_3^+ group with the incoming polar end, in the particular case of SDS. α - CH_2 is shifted from 2.92 to 2.86 ppm, and β - CH_2 is shifted from 3.02 to 2.90 ppm in the presence of SDS. On the other hand, the protons of 3-indole moieties shifted from 7.93 to 7.83 and from 6.63 to 6.59 ppm, which is affected by the hydrophobic interaction of the surfactant's hydrocarbon chains for SDS.

Figure 7c shows the proton NMR spectra of DA. As could be noticed, NMR spectra of DA show characteristic signals of $-\text{CH}_2$ methylene group at 2.93 and 2.67 ppm. In the presence of SDS, these signals are almost unaffected with to appreciable chemical shift compared to ST and shifted to 2.93 and 2.66 ppm, respectively, as shown in Fig. 7d. In conclusion, SDS interacts more effectively with ST compared to DA.

Electrochemical impedance spectroscopy (EIS) of ST and AA.— It is well known that EIS technique is a useful tool for studying the interface properties of surface-modified electrodes.^{70,71} Therefore, EIS was used to investigate the nature of ST and AA interaction at PEDOT/Pt surface in the presence of SDS. In EIS, the semicircle diameter equals the electron transfer resistance. Figures 8a and 8b shows the complex plane diagram (Nyquist plot) of ST, AA at PEDOT/Pt electrode in the (a) presence and (b) absence of SDS at oxidation potentials 350 and 20 mV, respectively. From these

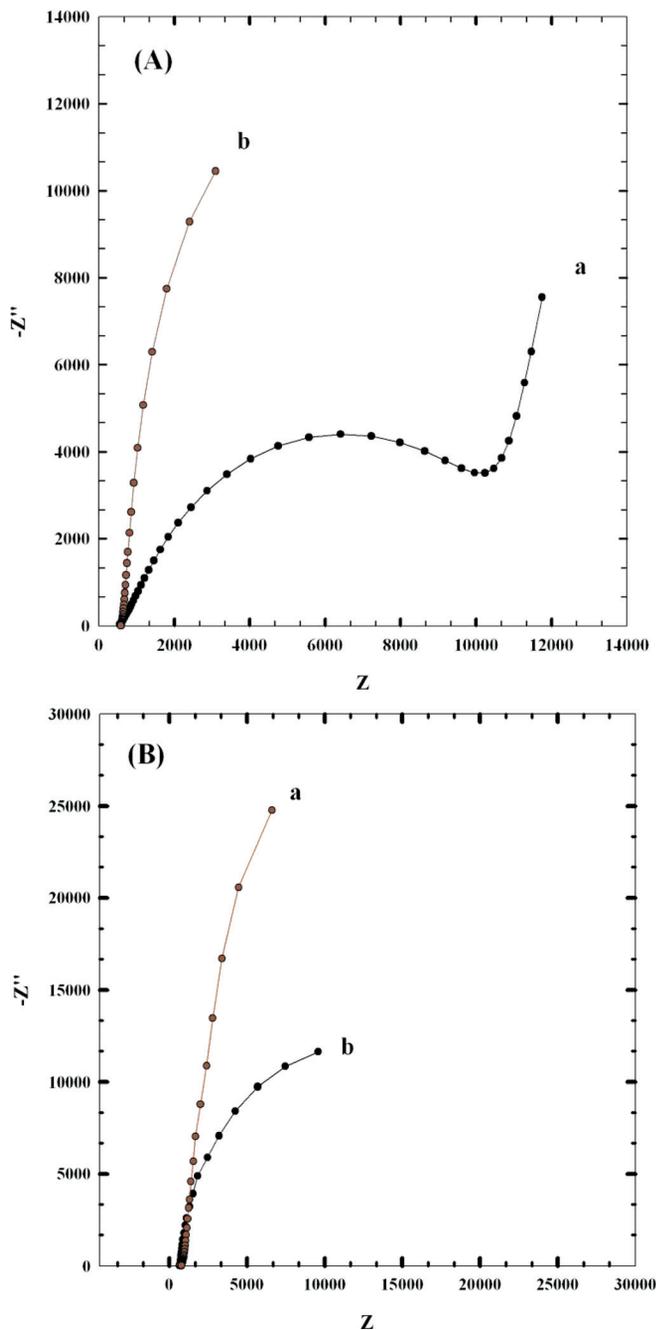


Figure 8. (Color online) Nyquist diagrams ($-Z''$ vs Z') for the EIS measurements at PEDOT/Pt (A) at $E_{\text{app}} = 350 \text{ mV}$ for ST and (B) at potential $E_{\text{app}} = 20 \text{ mV}$ for ascorbic acid, (a) in the presence of SDS and (b) in the absence of SDS. For 5 mmol l^{-1} of each ST and AA, in 0.1 mol l^{-1} B-R $\text{pH} = 7.4$. Amplitude: 5 mV , frequency range: 0.1 – 10000 Hz .

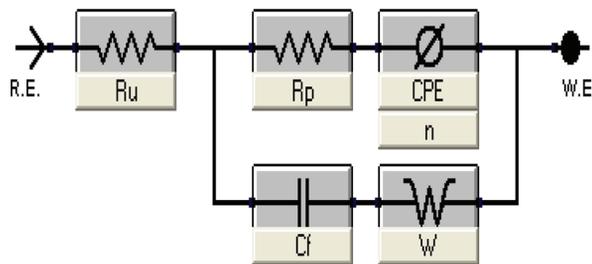


Figure 9. (Color online) The equivalent circuit used to model the data.

Table II. The data obtained from EIS in the determination of serotonin and ascorbic acid using PEDOT/Pt electrode in the absence and presence of SDS at oxidation potentials.

Electrode PEDOT/Pt	E/mV	R_p (k Ω cm 2)	R_u (k Ω cm 2)	C_f (μ F cm $^{-2}$)	W (K Ω^{-1} cm $^{-2}$)	C_{CPE} (μ F cm $^{-2}$)	n
In the absence of SDS(ST)	350	77	0.58	90	0.42	237	0.51
In the presence of SDS(ST)	350	10.5	0.52	120	0.3	2590	0.88
In the absence of SDS(AA)	20	23.9	0.60	77	0.6	210	0.57
In the presence of SDS(AA)	20	91.9	0.64	63	2.5	99	0.59

comparisons, it is clear that the impedance responses of serotonin and ascorbic acid show great difference after addition of SDS. In case of cationic molecule, in the absence of SDS, the impedance spectra include a semicircle with a larger diameter. However, after addition of SDS, the diameter of semicircle diminishes markedly. Thus, the charge transfer resistance of electro-oxidation of serotonin decreases greatly, and the charge transfer rate is enhanced by SDS. The data proves that SDS facilitates the electron transfer between ST and PEDOT/Pt electrode.

Figure 9 represents the circuit used; in this circuit, R_u is the solution resistance, R_p is the charge transfer resistance, CPE represents the predominant diffusion influence on the charge transfer process, n is its corresponding exponent ($n < 1$), and C_f represents the capacitance of the double layer. Diffusion can create impedance known as the Warburg impedance (W).

Table II lists the best fitting values calculated from the equivalent circuit for the impedance data. The PEDOT/Pt electrode in the presence of SDS shows increased values of the interfacial capacitance component than without SDS due to more conducting character of the surface regarding to ionic adsorption at the electrode surface and the charge transfer process. Also, the decrease in the interfacial electron transfer resistance is attributed to the selective interaction between SDS and ST, which accelerate the electron

transfer between the electrode and serotonin. The average error (χ^2) of the fits for the mean error of modulus was in the range: $\chi^2 = (2.4-3.6) \times 10^{-2}$.

On the other hand, in the case of ascorbic acid, reverse behavior was observed in the presence of SDS, increase in the charge transfer resistance, decrease in the capacitive component, and increase in the electron transfer resistance, which was attributed to the electrostatic repulsion force between SDS and AA that decreases electron transfer between the electrode and the electrolyte.

Determination of ST in Human Urine Samples

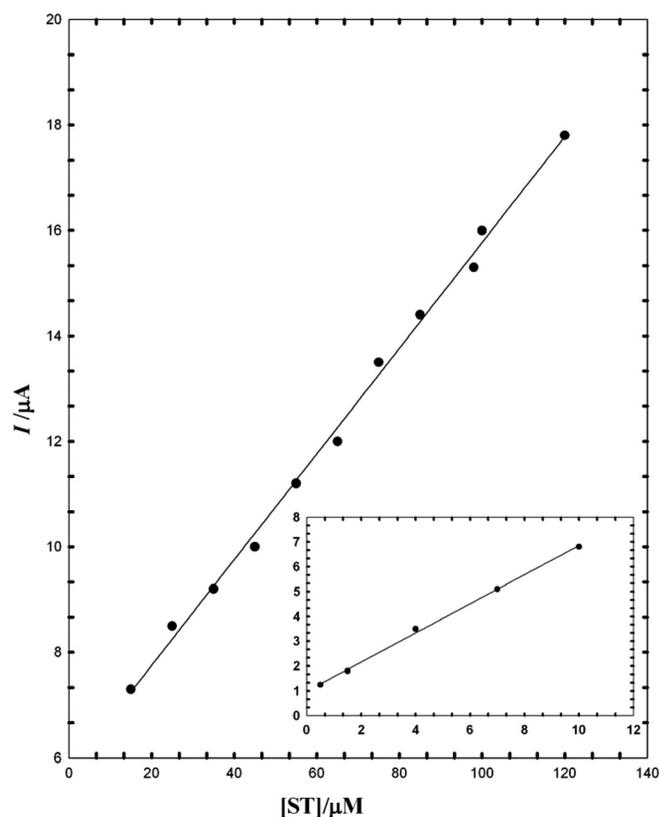
The utilization of the proposed method in real sample analysis is also investigated by direct analysis of ST in human urine. The same measurements were conducted successfully on urine samples. In this set of experiment, ST was dissolved in urine to make a stock solution of 5 mmol l $^{-1}$ concentration. Standard additions of 10 μ l of 5 mmol l $^{-1}$ ST in urine was added to the buffer pH 7.4 containing 150 μ l SDS, and the corresponding LSV was performed. The analytical results are summarized in Table III. 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. Figure 10 shows the calibration curves of the anodic peak current in the linear ranges of 0.5–10 μ mol l $^{-1}$ and 15–120 μ mol l $^{-1}$ with correlation coefficients of 0.996 and 0.993 and detection limits 60 and 78 nmol l $^{-1}$, respectively. Table III results strongly proved that ST can be selectively and sensitively determined at PEDOT/Pt electrode in urine sample in the presence of SDS.

Table III. Recovery data for synthesized biological solution spiked with various amounts of 5 mmol l $^{-1}$ ST in fresh urine sample from the healthy volunteers.

Sample	Added ST (μ mol l $^{-1}$)	Founded ^a ST (μ mol 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

Conclusion

This work demonstrated that PEDOT/Pt electrode in the presence of SDS exhibited remarkably electrocatalytic activity toward catecholamines and ST oxidation, improving the current response. In the anionic surfactant SDS, a remarkable electrostatic interaction is established with positively charged compounds, as a consequence, the peak current increases. On the other hand, the negatively charged AA and UA are repelled from the electrode surface, and their corresponding oxidation peak currents are quenched. While DA coexisted with ST, it was found that the PEDOT/Pt electrode showed a high affinity for ST rather than DA due to the difference of their molecular size, structure, and positive charge density especially on adding SDS. 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.⁷²⁻⁷⁴ The designed sensor showed good reproducibility, high stability,

**Figure 10.** Calibration curve for successive additions of 10 μ l of 5 mmol l $^{-1}$ serotonin in urine were added to the buffer pH = 7.4 containing 150 μ l SDS.

sensitivity, and anti-interference ability. The sensor was further utilized to determine ST level in human urine, and satisfactory results were obtained with low detection limit.

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