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Direct and Sensitive Determination of Atropine Sulfate at Polymer Electrode in Presence of Surface Active Agents

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A novel, selective, rapid and simple electrochemical method is demonstrated for the first time for the determination of atropine sulfate, using poly (3, 4-ethylene-dioxythiophene) (PEDOT) electrode film modified platinum electrode in presence of sodium dodecyl sulfate (SDS). The modified electrode displays an obvious increase in the peak current (8 times) compared to the bare platinum electrode (Pt). The results indicate that PEDOT/Pt electrode in presence of SDS remarkably enhances the electrocatalytic activity towards the oxidation of Atropine sulfate. Cyclic voltammetry (CV), linear sweep voltammetry (LSV), and Electrochemical impedance spectroscopy (EIS) are used to verify the voltammetric behavior of Atropine sulfate in micelles media. Simultaneous determination of Atropine and morphine, and selective determination of atropine sulfate in presence of codeine, ascorbic acid and uric acid are obtained with high sensitivity and good resolution. The PEDOT/Pt in presence of SDS has also been successfully applied to the determination of Atropine sulfate in atropine sulfate injection in the linear ranges of 0.1 to 0.8 μmol L^{-1} and 2.5 to 100 μmol L^{-1} with low detection limits of 27 and 64 nM, respectively. The results indicate that the PEDOT/Pt electrode can successfully be applied for the analysis of pharmaceutics in biological fluids.

Keywords: PEDOT; SDS; Atropine sulfate; Morphine; Codeine; Electrochemical Analysis.

1. INTRODUCTION

Atropine, the tropane alkaloid could be readily extracted from the seeds of Datura metel and it is commonly used as antispasmodic, anticholinergic and antidote [1]. Its structure contains an aromatic ring, carbonyl group and oxygen atom as shown in schematic diagram 1. Atropine sulfate as an active alkaloid lowers the parasympathetic activity of all muscles and glands regulated by the parasympathetic nervous system as it blocks acetylcholine receptor sites. This occurs because atropine

is a competitive antagonist of the muscarinic acetylcholine receptors (acetylcholine being the main neurotransmitter used by the parasympathetic nervous system). Therefore, it may cause swallowing difficulties and reduced secretions.

Atropine is used in the treatment of bradycardia (an extremely low heart rate), asystole and pulseless electrical activity (PEA) in cardiac arrest. This works because the main action of the vagus nerve of the parasympathetic system on the heart is to decrease heart rate. Atropine blocks this action and, therefore, may speed up the heart rate**.** Atropine can be used to reduce the effect of the poisoning by blocking muscarinic acetylcholine receptors, which would otherwise be over stimulated by excessive acetylcholine accumulation.

Atropine has wide medical applications, e.g. for dilating the pupils in the ophthalmic operations, as an antispasmodic and as an antidote for poisoning of opium, eserine and muscarine [2]. A variety of methods are employed for the determination of atropine such as spectrophotometry [3], ion-selective electrode (ISE) [4], liquid chromatography [5]. However, spectrophotometry suffers from the low sensitivity. The liquid chromatography is more sensitive but expensive apparatus, long procedures and a number of chemicals are involved. In ISE method the response is affected by electrical properties of the film and electric double-layer capacitance.

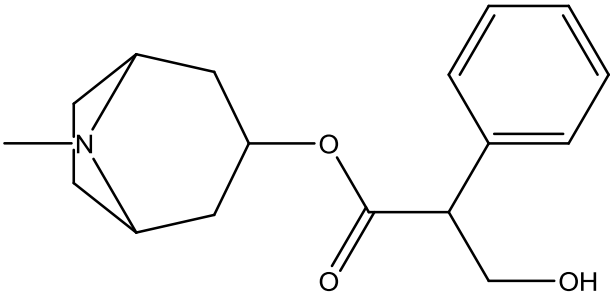
Cyclic voltammetry is often the first experiment performed in an electrochemical study of a compound, a biological material, or an electrode surface. It is effectively used in the fields of environmental electrochemistry, organic chemistry, inorganic chemistry, and biochemistry. The effectiveness of CV results from its capability for rapidly observing the redox behavior over a wide potential range.

Further, the electroanalytical techniques have been shown to be excellent for the determination of pharmaceutical compounds in different matrices. Many of the active constituents of formulations, in contrast to excipients, can be readily oxidized. The selectivity of this method is normally excellent because the analyte can be readily identified by its voltammetric peak potential. Advances in experimental electrochemical techniques in the field of analysis of drugs are because of their simplicity, low cost, and relatively short analysis time compared to other techniques [6, 7]. The use of various electrodes, viz. mercury [8–14], solids [15–21], and modified electrodes [22–26], for electroanalytical measurements has increased in recent years because of their applicability to the determination of active compounds that undergo oxidation reactions which is a matter of great importance in the field of clinical and pharmaceutical analysis. Other works as Molecular imprinted polymers [27, 28] are also used but this process is time consuming. polymerization of *o*-phenylenediamine (o-PD) with aniline (An) to imprint the template atropine was studied [29]. A bulk acoustic wave (BAW) device was used as a transducer to imprint atropine sulfate over *o*-phenylenediamine (o-PD). Some surfactants (such as cyclodextrin [30] and sodium dodecyl sulfate [31]) are typically used as additives in the electrophoretic buffers.

Moreover, selective assessing elements of sensors is important as a procedure of modification that is not easy and perhaps decreases the performance properties of sensor which greatly limits the application of this artificial material as sensing components of chemical sensor. Therefore, simple and convenient new and simple electrochemical methods for the determination of the important drug

atropine sulfate are increasingly needed. The alkaloid, atropine, is unstable in aqueous solution but atropine sulfate is stable [32]

In this work, we found that atropine sulfate is effectively adsorbed and accumulated on PEDOT /Pt electrode in the presence of anionic surfactant SDS. Therefore a novel, simple, rapid and sensitive voltammetric method for atropine determination is explored. Simultaneous determination of atropine in presence of other alkaloids namely, morphine and codeine is also demonstrated with high response and good resolution. As a potential application, the electrochemical detection of atropine in injection and spiked urine samples was performed.



atropine

Schematic diagram 1.

2. EXPERIMENTAL

2.1. Materials and reagents

All chemicals were used as received without further purification. 3, 4-ethylenedioxy) thiophene EDOT, lithium per chlorate ($LiClO_4$), acetonitrile (high-performance liquid chromatography [HPLC] grade), Atropine sulfate, ascorbic acid, uric acid, and sodium dodecyl sulfate were supplied by Aldrich Chem. Co. (Milwaukee, WI. USA). Codeine, morphine were supplied from Forensic chemistry Laboratory, Medico Legal Department, Ministry of Justice, Cairo, Egypt. 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} orthophosphoric 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).

Cyclic voltammetry (CV) and Linear scan voltammetry (LSV) were used for studying the electrochemical behavior of atropine sulfate using modified PEDOT/Pt electrode in presence of SDS with scan rate 50 mV s^{-1} in the designated potential range as indicated.

2.2.2. Impedance 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: applied potential value at 650 mV, frequency range of 0.1-100000 Hz with AC amplitude of 5 mV on PEDOT/Pt electrode and tested in 0.3 mmol L^{-1} atropine sulfate in presence and absence of 150 μmol L^{-1} 0.1 mol L^{-1} SDS in B-R pH 7.4. The data analysis software is provided with the instrument and applied a non-linear least-square fitting with the Levenberg-Marquadt algorithm.

2.3. Analysis of Atropine sulfate in urine

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of atropine sulfate in human urine samples. Atropine sulfate was dissolved in urine to make a stock solution with concentration of 3 mmol L^{-1}. Standard successive additions of 10 μL of 3 mmol L^{-1} atropine sulfate in urine were added to the 5 mL buffer pH 7.4 containing 150 μL SDS.

3. RESULTS AND DISCUSSION

3.1 Electrocatalytic oxidation of atropine at the PEDOT/Pt Electrode

Figure 1 shows the cyclic voltammograms of 0.3 mmol L^{-1} atropine sulfate at PEDOT/PT in SDS (a), PEDOT/Pt (b), and Pt (c) in 0.1 mol L^{-1} B-R (pH 7.4). One well-defined anodic peak of atropine sulfate at +0.67 V is observed at the PEDOT/Pt electrode in presence of SDS figure 1(a) which displays 4 and 8-fold increase in current response relative to that at the PEDOT/Pt (b), and Pt electrode (c), respectively. This oxidation peak is due to the oxidation of atropine sulfate in which the functional group involved in this oxidation is the tertiary substituted nitrogen atom of the atropine

molecule, an electrolytic N- dealkylation almost certainly occur to produce formaldehyde and a secondary amino compound, [32]. The PEDOT/Pt electrode (b) also showed a similar voltammetric peak at +0.62 V with lower current response. Only a very weak current response has been observed in case of using bare Pt electrode (c).

The anionic surfactant SDS enhances greatly the anodic current peak of atropine sulfate 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[33]. This negatively charged hydrophilic layer facilitates reaching atropine sulfate to the electrode surface faster, and as consequence, the reaction becomes easier. This micellar effect on the oxidation of atropine sulfate is basically an electrostatic interaction between the surfactant film adsorbed on the electrode and the protonated atropine sulphate. The lower oxidation potential and higher current response clearly indicate that PEDOT/Pt electrode has excellent electrocatalytic activity towards atropine, which is attributed to the presence of anionic SDS.

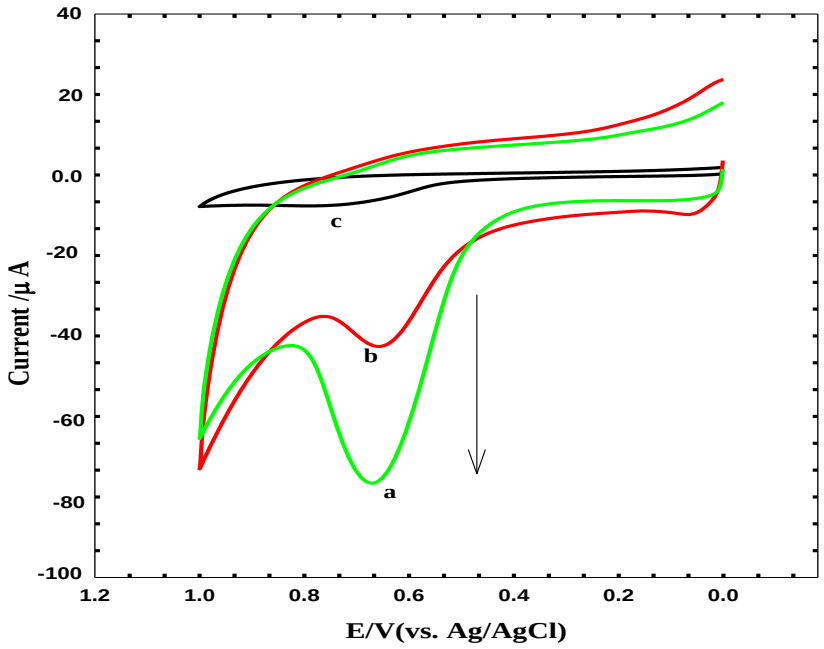


Figure 1. Cyclic voltammograms of 3.0×10^{-4} mol L^{-1} atropine sulfate/ 0.1 mol L^{-1} B-R, pH 7.4, at PEDOT/Pt electrode in the presence of SDS (a), PEDOT / Pt electrode (b), and Pt electrode (c), at scan rates 50 mVs^{-1}

3.2. Calibration graph

Figure 2A shows linear sweep voltammograms of different concentration of atropine sulfate at the PEDOT/Pt electrode in presence of 150 μL 0.1 mol L^{-1} SDS in 0.1 mol L^{-1} B-R buffer solutions. The results show that the anodic peak current increases with the increase of atropine sulfate concentration. The calibration curve Figure 2B corresponding to peak current is linear versus different

concentrations of atropine sulfate in the linear ranges 0.1 µmol L^{-1} to 0.8 µmol L^{-1} and 1.2 µmol L^{-1} to 100 µmol L^{-1}, with correlation coefficients of 0.999 and 0.995 and with low detection limits of 27 nmol L^{-1} and 64 nmol L^{-1} respectively.

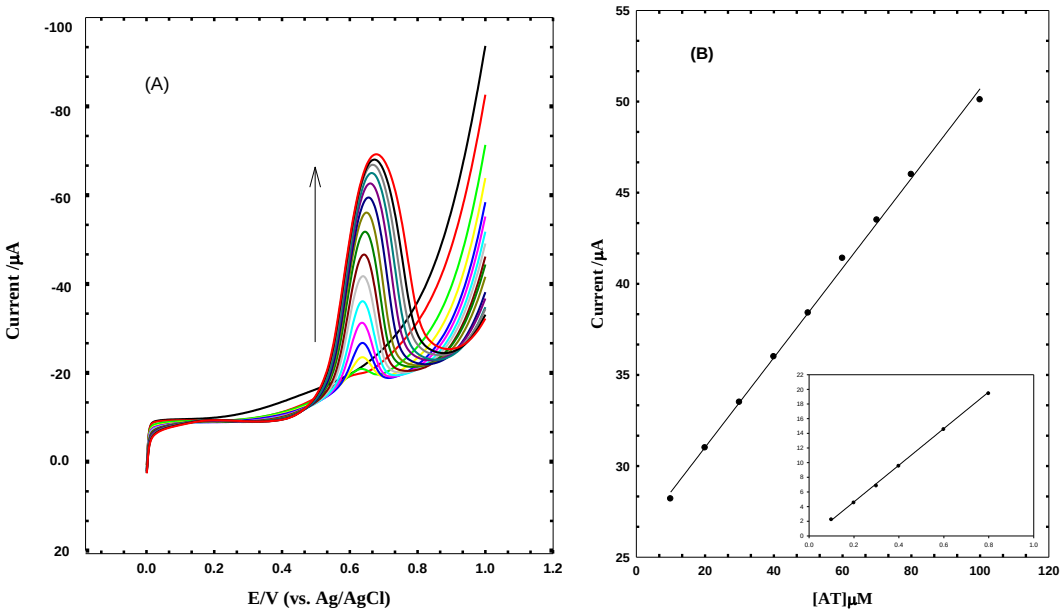


Figure 2. **(A)** LSVs of different concentrations of atropine sulfate (0.1 µmol L^{-1} - 0.1 mmol L^{-1}) in 10 ml of 0.1 mol L^{-1} B-R pH 7.4 containing 150 µL 0.1 mol L^{-1} SDS at PEDOT /Pt electrode. **(B)** Calibration curve for atropine sulfate of different concentrations from (1.2 to 100 µ molL^{-1}) and from (0.1 to 0.8 µmol L^{-1}) (inset).

3.3. Stability of response of the modified electrode

In order to investigate the stability of the PEDOT/Pt electrode in presence of SDS, the CV for 0.3 mmol L^{-1} atropine sulfate in 150 µL 0.1 mol L^{-1} SDS, 0.1 mol L^{-1} B-R (pH 7.4) solution were recorded for every 10 minutes intervals and over 20 successive runs without any deterioration of the polymer film. It is found that anodic peak current remains basically 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% after 1 week and 5.1% after 2 weeks of usage.

3.4 The effect of pH

Atropine sulfate absorption in human depends on the pH-values of the medium. Thus, when the pH values of the gastric juice are low, absorption of atropine sulfate is slower in the stomach and quicker in the intestines. Therefore, studying the effect of the pH is very important. Cyclic

voltammograms show the effect of changing the pH on the electrochemical response of atropine sulfate at the modified PEDOT electrode. The experiments were performed in different pH values, namely 2.3, 4.5, 7.4 and 9.0 in presence of SDS as shown in Figure 3A. Figure 3B shows that at pH 2.3 and 4.5 the oxidation peak potential is lower than that in pH 7.4. While at pH 9.0 no oxidation peak appears, this takes place in absence (a) and presence (b) of SDS. Figure 3C shows the effect of changing pH of B-R buffer on the current response of 0.3 mmol L^{-1} atropine sulfate in the absence (a) and in presence (b) of 150 µL 0.1 mol L^{-1} SDS. The peak current of atropine increases at pH 7.4 in absence and presence of SDS. No current signal was observed in pH 9. Thus, electrochemical measurements are conducted in pH 7.4, (physiological media).

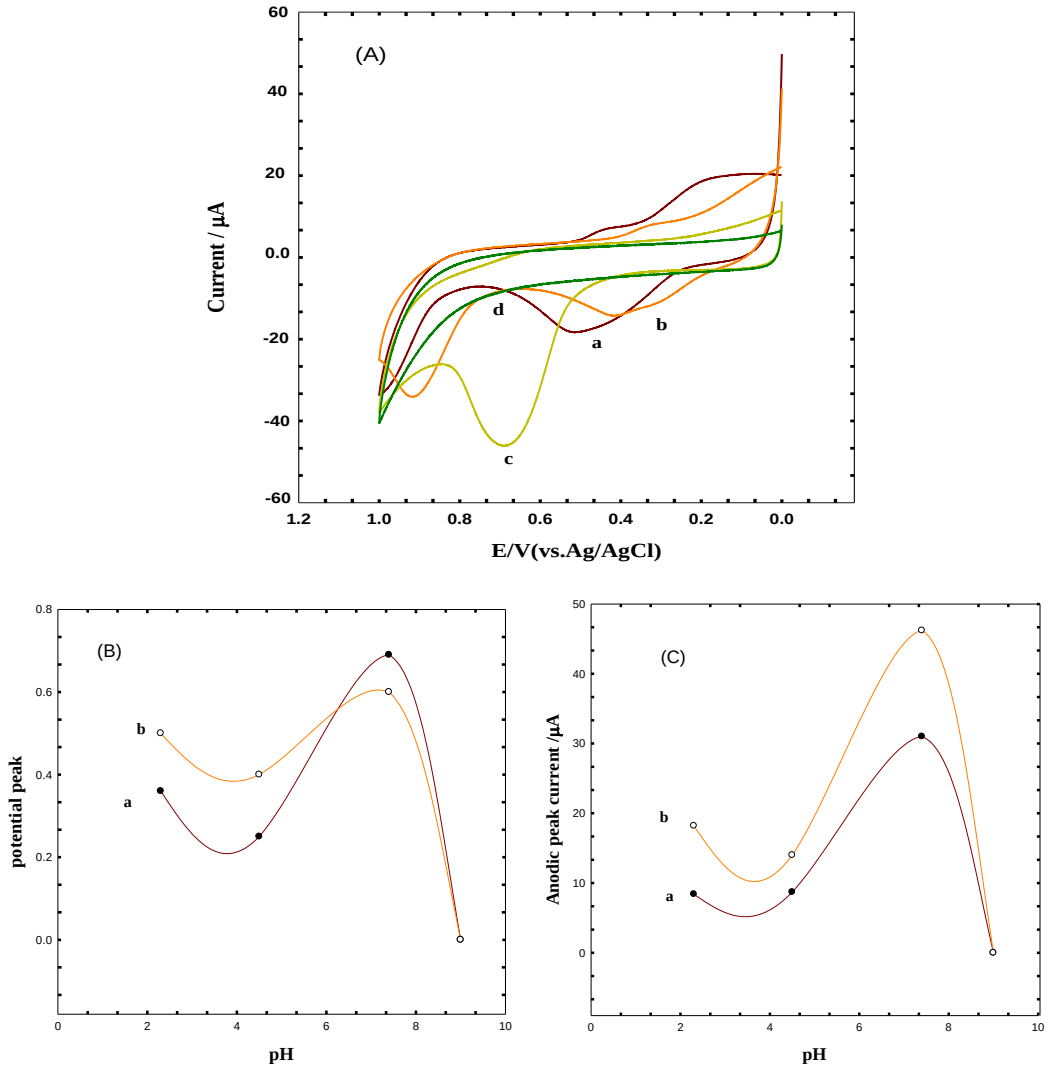


Figure 3. (A) Cyclic voltammograms of PEDOT / Pt electrode in 3.0×10^{-4} mol L^{-1} atropine sulfate / 0.1 mol L-1 B-R in presence (A) of SDS: at different pH values (a-d) pH 2.3, pH 4.0, pH 6, pH 7.4, pH 9 scan rate 50 $mV.s^{-1}$.

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

3.5. Effect of scan rate

The effect of scan rate on the oxidative peak potential and peak current of atropine sulfate at the surface of PEDOT/Pt in presence and absence of 150 μL 0.1 mol L^{-1} SDS in 0.1 mol L^{-1} B-R buffer solution was studied. Figure 4A, shows the cyclic voltammetric curves of atropine obtained at different scan rates 30-150 mVs^{-1} to investigate the kinetics of electrode reactions and verify whether the presence of SDS affects the diffusion process. Figure 4B shows a linear relation between oxidative peak current and square root of scan rate from 30 to 150 m V s^{-1} that is observed for atropine sulfate in absence (a) and presence (b) of 150 μL 0.1 mol L^{-1} SDS, 0.1 mol L^{-1} B-R buffer solutions. This linearity suggests that the electrochemical reaction of atropine at the surface of PEDOT/Pt is a diffusion process that is followed by an adsorption step.

Moreover, in case of SDS the current increases which indicates that the anionic surfactant SDS accelerates the diffusion of the cationic atropine sulfate. Moreover, the cyclic voltammetric results show that the oxidative peak potentials of atropine sulfate in absence of SDS are shifted slightly to more positive values with increasing scan rate from 30 to 150 m V s^{-1}. In presence of SDS the oxidative peak potentials of atropine are shifted to more positive values with increasing scan rate from 30 to 150 m V 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 equation [34]

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

Where i_p is the peak current density (Acm^{-2}), n is the number of electrons transferred at T=298K, A is the geometrical electrode area (0.0176 cm^2), C_0 is the analyte concentration (3×10^{-7} $molcm^{-3}$), 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} values at PEDOT/Pt electrode in absence and presence of SDS for atropine sulfate are 9.0×10^{-4} cm^2 s^{-1} and 3.0×10^{-3} cm^2 s^{-1}, respectively. The anionic surfactant SDS affects remarkably the diffusion component of the charge transfer at the electrode surface as indicated by the D_{app} values. 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[35]. The values indicated for D_{app} show that the diffusion is enhanced in presence of SDS.

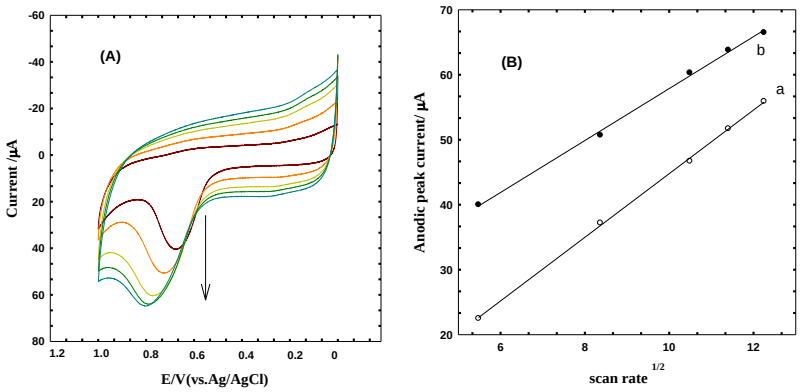


Figure 4. (A) Cyclic voltammograms of 3.0×10^{-4} mol L^{-1} atropine sulfate / 0.1 mol L^{-1} B-R, pH 7.4, at PEDOT / Pt electrode at different scan rates (30, 70, 110, 130 and 150 mV s^{-1}. in presence of 150 µL 0.1 mol L^{-1} SDS. (B) A plot of the anodic peak current values as a function of the square root of the scan rate at PEDOT/Pt electrode (a) in absence and (b) in presence of SDS.

3.6. Simultaneous determination of Morphine and Atropine

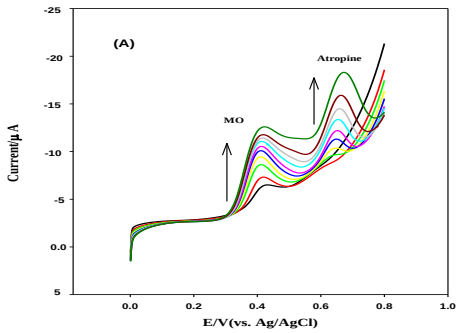


Figure 5. LSVs of different concentrations of a solution formed by successive injections of 10 µL from a solution containing a mixture of 0.6 mg atropine and 0.01 gm morphine / 1 mL to 10 mL of 0.1 mol L^{-1} B-R pH 7.4 containing 150 µL 0.1 mol L^{-1} SDS at PEDOT /Pt electrode.

Since Morphine and Atropine are alkaloids, a mixture of Morphine and Atropine are commonly used in premedication in anesthesia. Moreover, they both play an important role in respiratory system and alveoli during surgery.

Successive additions of 10 µL from solution containing a 1 mL mixture of 0.6 mg atropine and 0.01 gm morphine is tested in 10 mL of 0.1 mol L^{-1} B-R pH 7.4, and in presence of 150 µL 0.1 mol L^{-1} SDS. This experiment was performed in order to study the simultaneous determination of the two alkaloids [36, 37]. The LSVs in Figure 5 show a well-defined peak for the oxidation of MO at +422 mV; a second less-defined peak appears at + 648 mV for the oxidation of Atropine sulfate after successive additions . This indicates that in presence of relatively high concentration of morphine compared to atropine, it is possible to successfully determine morphine and atropine selectively. The two peaks appear for the oxidation of MO and Atropine with a separation of more than 226 mV. From

the calibration curve corresponding to the data of atropine in the linear range from 0.13 µmol L^{-1} to 96 µmol L^{-1}, with correlation coefficient of 0.999 the detection limit was 78 nmol L^{-1} and for MO in the linear range from 0.01 µmol L^{-1} to 2.6 µmol L^{-1} and from 5 µmol L^{-1} to 270 µmol L^{-1}, with correlation coefficients of 0.999 and 0.994 the detection limits were 50 nmol L^{-1} and 73 nmol L^{-1}(figures not shown).

3.7. Determination of Atropine sulfate in presence of Codeine

The combination of the spasmolytic action of atropine with the pain-relieving properties of codeine brings the drug near to morphine in the intensity of pain-relieving action. Figure 6A shows that the oxidation peak of codeine appears at +0.86 V in absence (curve a) and in presence (curve b) of 150 µL 0.1 mol L^{-1} SDS at PEDOT/Pt electrode in 0.1 mol L^{-1} B-R (pH 7.4).

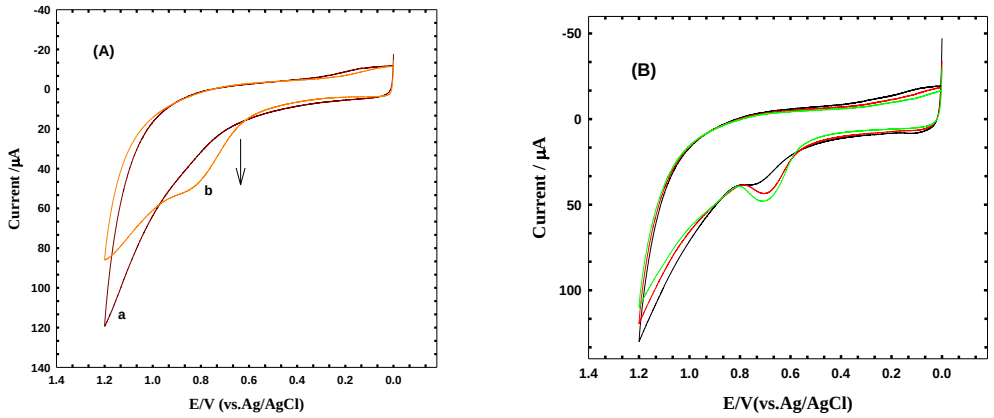


Figure 6. **(A)** Cyclic voltammograms for 3 mmol L^{-1} codeine in 0.1 mol L^{-1} B-R pH 7.4 at PEDOT /Pt electrode in absence (a) in presence of 100 µL SDS 0.1 mol L^{-1} (b). **(B)** Cyclic voltammograms for equimolar solution 0.3 mmol L^{-1} for each of atropine sulfate and codeine in 0.1 mol L^{-1} B-R pH 7.4, at PEDOT/Pt electrode with two successive additions of 100 µL 0.1 mol L^{-1} SDS, scan rate 50 $mV.s^{-1}$.

Figure 6B shows a voltammetric response of 0.3 mmol L^{-1} atropine sulfate solution containing 0.3 mmol L^{-1} codeine at the PEDOT/Pt electrode in presence of successive additions of 150 µL SDS in 0.1 mol L^{-1} B-R at pH 7.4. The results indicated that the PEDOT/Pt electrode in presence of SDS can easily discriminate atropine from codeine (CO) [37, 38]. The adsorption of atropine sulfate by the PEDOT/Pt electrode involved interactions between atropine molecules and the anionic surfactant. Codeine did not show significant interference and an oxidation peak at +0.69 V for atropine sulfate is observed. The proposed method is useful for toxicological analysis.

3.8. Determination of Atropine sulfate in presence of ascorbic and uric acids

To verify the feasibility of the selective determination of atropine at PEDOT/Pt electrode in presence of 150 µL of 0.1 mol L^{-1} SDS, the electrochemical behavior of a solution containing a

mixture of atropine sulfate, UA and AA is studied. LSV mode was used to study the oxidation of a solution containing a mixture of 0.3 mmol L^{-1} atropine sulfate, 3 mmol L^{-1} UA and 30 mmol L^{-1} AA at (a) PEDOT/Pt and (b) PEDOT/Pt in presence of SDS. As indicated in Figure 7, at the working pH 7.4 the oxidation peaks are resolved at the PEDOT/Pt electrode with the peak potentials at 87 mV, 496 mV and 800 mV for AA, UA, and AT, respectively (Figure 7, curve a).

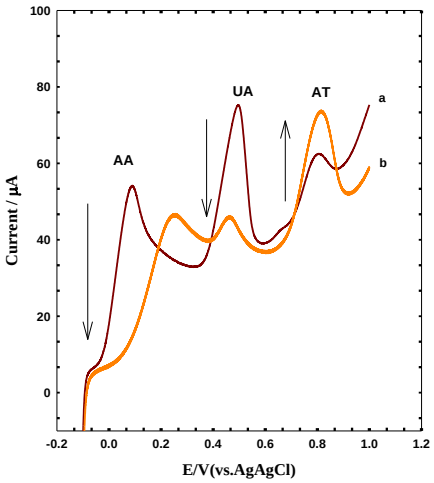


Figure 7. LSV for a mixture solution of 30 mmol L^{-1} AA, 3 mmol L^{-1} UA, 0.3 mmol L^{-1} AT sulfate, in 0.1 mol L^{-1} B-R pH 7.4 at PEDOT /Pt electrode in absence (a) in presence of 150 µL SDS 0.1 mol L^{-1} (b) scan rate 50 mV s^{-1}.

The large separation of the peak potentials allows selective and simultaneous determination of AA, UA, and AT in their mixture. Using PEDOT/ Pt in presence of SDS (Figure 7, curve b), a sharp well defined oxidation peak of AT appeared at 800 mV and a relatively smaller oxidation peaks for UA and AA appear at 466 mV and 244 mV, respectively. The oxidation peak current increases for AT in presence of SDS. Moreover, there is a noticeable decrease in the oxidation peak currents for UA and AA. Therefore, the high response for atropine was observed due to the electrostatic interaction of the anionic surfactant with the protonated AT in pH 7.4, but in case of AA and UA repulsion takes place[39] (Figure 7, curve b). Therefore, it is possible to determine atropine sulfate selectively in presence of relatively high concentrations of AA, and UA.

3.9. Determination of Atropine sulfate in urine

The proposed method in real sample analysis is also examined in human urine samples. In this set of experiments, Atropine sulfate is dissolved in urine to make a stock solution with 3 mmol L^{-1} concentration. Standard additions of 10 µL of 3 mmol L^{-1} atropine sulfate in urine are added to 5 mL B-R pH 7.4 containing 150 µL SDS, the corresponding LSV is then measured. The results showed that the oxidation peak current increases by increasing the atropine concentration. The calibration plot

(Figure 8) is found to be linear in the concentration range of 0.5 to 65 µmol L^{-1} with correlation coefficient of 0.994 and detection limit 82 nmol L^{-1}.

Validation of the procedure for the quantitative assay of the atropine sulfate by performance characteristics method was examined in B-R buffer pH 7.4. Three different concentrations on the calibration curve are chosen to be repeated for five times to evaluate the accuracy and precision of the proposed method, which is represented in table 1. The recovery of the spiked samples ranged between 95% and 102.5%. The R.S.D. (n = 5) was less than 5.2%.

In Table 2, response characteristics of the proposed method are compared with those obtained by some reported methods. In comparison with some other methods of atropine sulfate determination, the present 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. The sensor was further utilized to determine atropine level in human urine and satisfactory results are obtained with low detection limit.

Table 1. Results of determination of Atropine in urine sample.

Urine sample	Spike (µmol L^{-1})	Found (µmol L^{-1})	Recovery (%)	R.S.D. (%)[a]
1	2.0	1.9	95	5.2
2	8.0	8.2	102.5	3.7
3	15.0	14.62	97.4	4.5

[a] Average of five replicate measurements.

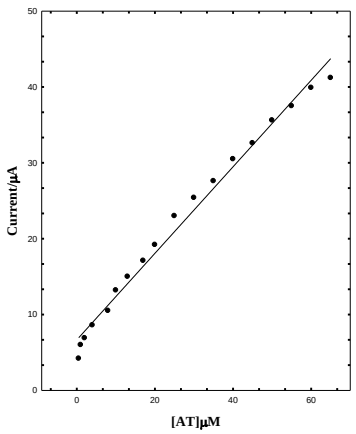


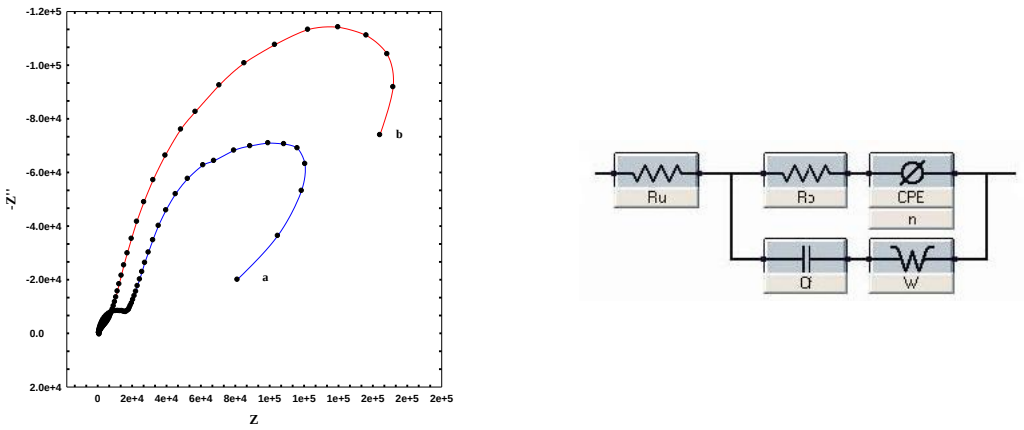
Figure 8. Calibration curve for successive additions of 10 µl of 3 mmol L^{-1} atropine sulfate in urine were added to the buffer pH 7.4 containing 150 µL SDS.

Table 2. Comparison of the proposed method with other methods for the determination of atropine sulfate

Method	Application	Calibration range (mM)	Detection limit (M)	Recovery (%)	References
ISE	Pharmaceutical	0.017–20		106.8	[40]
spectrophotometry	Pharmaceutical	2.9×10^{-3}–1.4×10^{-2}		99.0	[3]
FIA-ISPs	Pharmaceutical	0.02–100	2.0×10^{-6}	98–102	[41]
HPLC	Pharmaceutical Biological fluids	2.6×10^{-3}–0.13	2.6×10^{-6}	90.7–97.7	[5]
PQC	Pharmaceutical		0.01		[42]
This work	urine	0.5×10^{-3}-65×10^{-3}	82×10^{-9}	95-102	

3.9. Electrochemical impedance spectroscopy (EIS) of Atropine sulfate

It is well known that electrochemical alternating current impedance technique is a useful tool for studying the interface properties of surface-modified electrodes [43, 44]. Therefore, EIS was used to investigate the nature of atropine sulfate interaction at PEDOT/Pt surface in presence of SDS. In EIS, the semicircle diameter equals the electron transfer resistance. Figure 9A shows the complex plane diagram (Nyquist plot) of atropine sulfate at PEDOT/Pt electrode in the presence (a) and absence of SDS (b) at oxidation potential 650 mV. From this comparison, it is clear that the impedance responses of atropine sulfate show great difference after addition of SDS. On the other hand, in the absence of SDS, the impedance spectra display a semicircle with a larger diameter. However, after addition of 150 µL 0.1 M SDS, the diameter of semicircle diminishes markedly. Thus, the charge transfer resistance of electro–oxidation of atropine sulfate decreases greatly, and the charge transfer rate is enhanced by SDS. The data proves that SDS facilitates the electron transfer between atropine and electrode and indicates that adsorption is taking place at the electrode surface.



A **B**

Figure 9. (A) Nyquist diagrams (−Z″ vs. Z′) for the EIS measurements at PEDOT/ Pt electrode at potential 650 mV for atropine sulfate (a) in presence of SDS and (b) in absence of SDS in 0.1 mol L^{-1} B-R pH 7.4. Amplitude: 5 mV, frequency range: 0.1–10000 Hz**. (B)** The equivalent circuit**.**

Table 3. Fitting values calculated from the equivalent circuit for the PEDOT/PT electrode in presence and absence of SDS in atropine sulphate.

Electrode PEDOT/Pt	Pot. mv	R_p(kΩ cm^2)	R_u(kΩ cm^2)	C_f (μFcm^{-2})	W(K$\Omega^{-1}cm^{-2}$)	C_{CPE}(μFcm^{-2})	n
In absence of SDS	650	120	0.50	35	3.79	85	0.6
In presence of SDS	650	60	0.43	80	3.68	400.8	0.9

Figure 9B, represents the circuit used, 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, and n is its corresponding exponent (n < 1). C_f represents the capacitance of the double layer. Diffusion can create an impedance component known as the Warburg impedance (W).

Table 3. Lists the best fitting values calculated from the equivalent circuit for the impedance data (the average error (χ^2) of the fits is $\chi^2 = 3.5 \times 10^{-3}$). The PEDOT/Pt electrode in presence of SDS shows increased values of the capacitive 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 atropine sulfate which accelerate the electron transfer between the electrode and atropine sulfate.

4. CONCLUSION

A novel voltammetric method for determination of atropine has been applied at the PEDOT modified pt electrode in presence of SDS. Several advantages of using the proposed method are proved. Thus, this method is new, simple, selective, cheep and fast for the determination of atropine sulfate. Furthermore, a low detection limit and a wide dynamic range of concentrations are obtained at this surface that renders this method applicable for usual analytical purposes.

The method also demonstrates that it easily discriminates atropine sulfate from MO and CO. Moreover, the determination of atropine in real human urine without any sample pretreatment is successful with consistent results compared to those obtained using HPLC and Spectrophotometric methods. The proposed method seems useful for toxicological analysis of the three drugs (atropine sulfate, morphine and codeine), because of simplicity, selectivity and reproducibility.

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