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Direct and simple electrochemical determination of morphine at PEDOT modified Pt electrode

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Abstract

A simple and rapid method for morphine detection is described based on PEDOT electrode in the presence of SDS. The electrochemistry of morphine was investigated by CV, LSW and SWV. The effect of common interferences on the current response of morphine namely AA and UA is studied. The electrode is applied to the selective determination of morphine in urine samples in the linear ranges 0.3-8 $\mu molL^{-1}$ and 10-60 $\mu molL^{-1}$, with low detection limits of 50 and 68 $nmolL^{-1}$, respectively and recovery of 96.4%. The application of PEDOT is realized in determination of morphine in tablets successfully.

Keywords: Poly(3,4-ethylenedioxythiophene); narcotics; morphine; codeine; surfactants.

Introduction

As a major component in opium, morphine is often used to relieve severe pain in patients, especially those undergoing a surgical procedure. It is recommended by the World Health Organization (WHO) for the relief of moderate cancer-related pain (1). However, it is toxic in excess and when abused. Different methods have been used for the determination of morphine in plasma, urine, and opium samples, such as gas chromatography (GC) [2], liquid chromatography (LC) [3], high performance liquid chromatography (HPLC) [4], ultraviolet (UV) spectroscopy [5], GC–mass

spectroscopy (GC–MS) [6], fluorimetry [7], chemiluminescence [8], surface plasmon resonance (SPR) [9], and electrochemical methods [10]. The morphine molecules are usually purified using liquid–liquid extraction or solid phase extraction [11]. Even though chromatography and GC–MS are well-developed methods for morphine detection with a low detection limit, the bulky and expensive apparatus still hinder their practical applications. There still remains a great need for a fast and user-friendly device for morphine sensing. However, the range of morphine concentration in human beings changes greatly. For example, the concentration of morphine ranges between 8 and 80 ng/ml when curing [4] and the concentration of morphine in the urine would also change with time [12]. Thus the major shortcoming for these easy testing methods is their relatively high detection limits and low selectivity. Recently, some new electrochemical detection methods have been proposed for morphine detection. For example, an adsorptive differential pulse stripping method [13] and its conjugation with least-squares support vector machines [14] have been developed for trace morphine detection. Fast Fourier transformation with continuous cyclic voltammetry at Au microelectrode [15, 16] has been devised for morphine detection in a flow injection system. Furthermore, different modified electrodes have been developed for morphine detection. For example, Jin and co-workers prepared a cobalt hexacyanoferrate modified carbon paste electrode combined with HPLC and successfully detected morphine in vivo [17]. Ho et al. devised a Prussian blue-modified indium tin oxide (ITO) electrode [18] and molecularly imprinted electrodes for morphine determination [19, 20]. Multiwalled carbon nanotubes modified preheated glassy carbon electrode has also been used for the morphine detection [21]. Prussian blue film modified-palladized aluminum electrode [22] has recently been used for morphine detection.

A cheap, user-friendly, highly sensitive, highly selective method is still in great demand for morphine sensing. The conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT), was previously utilized to prepare the MIP–PEDOT thin film at the ITO electrode to enhance the selectivity of the modified electrode in detecting morphine [20].

In this work, it was found that morphine could be effectively adsorbed and accumulated on PEDOT /Pt electrode in the presence of anionic surfactant SDS. In the present work, a simple, rapid and sensitive voltammetric method for morphine detection is introduced. The method could readily discriminate morphine in presence of catecholamines. As a potential application, the electrochemical detection of morphine in tablets and spiked urine samples is demonstrated.

2. Experimental

2.1. Materials and reagents

All chemicals were used as received without further purification 3,4-ethylenedioxythiophene (EDOT), lithium perchlorate ($LiClO_4$), acetonitrile (high-performance liquid chromatography [HPLC] grade), dopamine hydrochloride (DA), ascorbic acid (AA), uric acid (UA), epinephrine, sodium dodecyl sulphate (SDS) were supplied by Aldrich Chem. Co. (Milwaukee, WI. USA). Morphine (MO) and codeine 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); the pH was 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 ml 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), linear scan voltammetry (LSV), and square wave voltammetry (SWV) were used for studying the electrochemical behavior of MO using modified PEDOT/Pt electrode in presence of SDS.

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: potential value for MO at 420 mV was studied at frequency range of 0.1–100000 Hz with AC amplitude of 5 mV applied on PEDOT/Pt electrode and tested in 0.5 mM Morphine in presence and absence of 0.1 mol L^{-1} 100 µmol L^{-1} SDS in B-R pH 7.4.

2.3. Analysis of urine and tablets

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of MO in human urine samples. MO was dissolved in urine to make

a stock solution with concentration of 5 mmol L^{-1}. Standard successive additions of 10 µL of 5 mmol L^{-1} MO in urine were added to the buffer 7.4 containing 100 µL SDS. Tablets of morphine sulphate 20 mg were used as received. Tablets were dissolved to form 2.6 mmol L^{-1} stock solution, standard successive additions of 10 µL of 2.6 mmol L^{-1} MO solution were added to 10 mL buffer 7.4 containing 100 µL SDS.

3. Results and discussion

3.1 Electrocatalytic oxidation of morphine at the PEDOT/Pt electrode

Historically, platinum [23] and glassy carbon electrodes [24, 25] have been used to perform MO electrocatalytic oxidation. In this study, we introduce the conductive PEDOT electrode in presence of SDS in order to oxidize morphine with excellent current response compated to conventional electrodes. Figure 1 shows the cyclic voltammograms of 0.5 mmol L^{-1} MO in 0.1M B-R (pH 7.4) at PEDOT/PT in presence (a) and absence (b) of 100 µL SDS and at and Pt (c). One well-defined anodic peak for the oxidation of MO is observed at +0.41 V at the PEDOT/Pt electrode in presence of SDS Figure 1 (a). The current signal obtained in this case is 2 and 12 -fold larger in magnitude compared to those at the PEDOT/Pt (b) and Pt electrode (c), respectively. This oxidation peak is attributed to the oxidation reaction of the phenolic group (–OH) at the 3-position which involves one-electron transfer and is responsible for the major peak. The oxidation of the phenolic group leads to the formation of pseudomorphine (PM) as the main product. Since the structure of pseudomorphine possesses two phenolic groups it makes its further oxidation possible. However, as shown in Figure 1, the oxidation occurs at the same potential as morphine [25]. Therefore, the peak at +0.41V in Figure 1 is ascribed to oxidation of the phenolic groups in morphine and pseudomorphine Scheme 1. The PEDOT/Pt electrode also showed a similar voltammetric peak at +0.41 V with lower current response (b). 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 MO 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. This negatively charged hydrophilic layer facilitates reaching of MO to the electrode surface faster, and as consequence, the reaction becomes easier [26]. This micellar effect on the oxidation of MO is basically an electrostatic interaction between the surfactant film adsorbed on the electrode and the protonated MO. The lower oxidation potential and higher current response clearly indicate that PEDOT/Pt electrode has excellent electrocatalytic activity towards morphine, which is attributed to the presence of anionic SDS.

$$\text{M-OH (morphine)} \leftrightarrows \text{M-O}^- + \text{H}^- \quad (1)$$

$$\text{M-O} \leftrightarrows \text{M-O}^* + \text{e}^- \quad (2)$$

$$2\text{M-O}^* \leftrightarrows \text{PM-OH} \quad (3)$$

$$\text{PM-OH} \leftrightarrows \text{PM-O}^- + \text{H}^+ \quad (4)$$

$$\text{PMO}^- \leftrightarrows \text{PM-O}^* + \text{e}^- \quad (5)$$

Scheme 1. The reaction scheme of morphine oxidation.

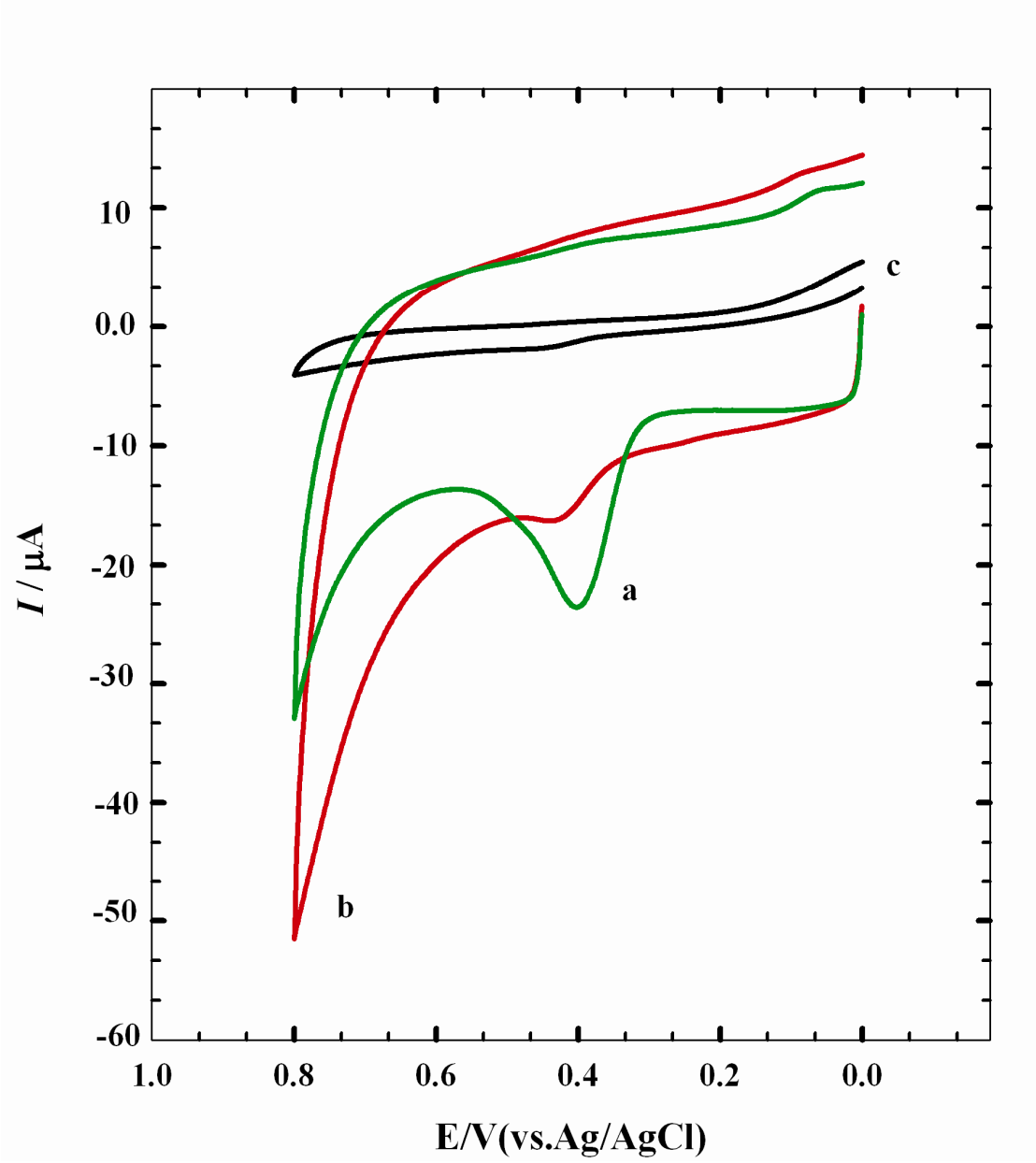


Figure 1

3.2 Effect of scan rate

As shown in supplementry 1, the oxidation peak currents (*i*p) of MO at the PEDOT/Pt electrode in presence of 100 μL 0.1 mol L^{-1} SDS and 5 mmol L^{-1} morphine solution (pH 7.4) varied with change of scan rate (v). In the range of 30–150 mV s^{-1}, the relation obeys the following equation:

$$\log ip = -0.40 + 0.69 \log \nu \quad (6)$$

(R = 0.996, where ν is in mV s^{-1} and ip is in μA),

This indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [27].

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 [28]

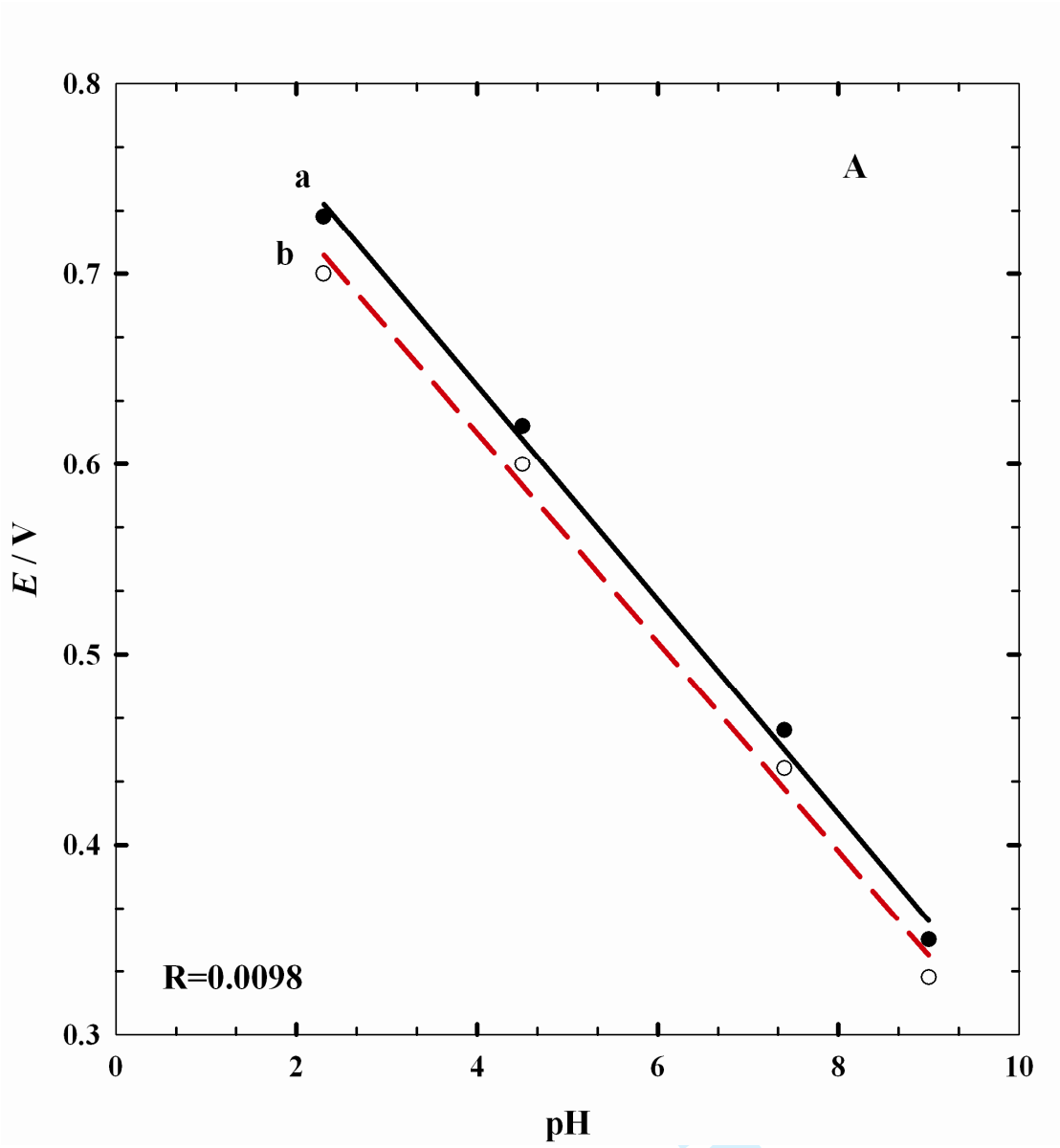
$$ip = 2.69 \times 10^5 n^{3/2} A C_0 D^{1/2} \nu^{1/2} \quad (7)$$

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 (5×10^{-7} mol cm^{-3}), and D is the diffusion coefficient of the electroactive species (cm^2 s^{-1}). Apparent surface area used in the calculations did not take into account the surface roughness, which is an inherent characteristic for all polymer films formed using the electrochemical techniques. D_{app} value at PEDOT/Pt electrode for morphine is 2.6×10^{-4} cm^2 s^{-1} in presence of SDS, which is larger than its corresponding value at the PEDOT/Pt electrode 1.1×10^{-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} value [29]. The diffusion coefficient can be considered as an average value of the diffusion process in the bulk, within the surfactant aggregates in solution and the surfactant layer adsorbed at the surface of the electrode. The size of the diffusion layer at the electrode surface proximity changes with the voltage scan used. At relatively slow voltage scans the diffusion layer grows much further towards the solution side and further from the electrode surface. Therefore, as the scan rate increases the flux to the electrode surface increases considerably. At relatively higher scan rates and in presence of SDS that mainly

aggregates at the electrode surface and forms a pair with the drug in electrolyte, the diffusion layer grows less further from the vicinity of the electrode. The values indicated for D_{app} show that the diffusion is enhanced in presence of SDS than in absence of it.

3.3. Effect of pH

Effect of pH (2.3, 4.0, 7.4, and 9.0) on the oxidation peak current and peak potential for the morphine oxidation were also investigated in presence and absence of SDS (supplementary 2 (A, B)). The peak potential shifted negatively with increase of pH in presence and absence of SDS. This is explained by the consequence of deprotonation involved in the oxidation process which was facilitated at higher pH values [30]. A plot of peak potential vs. pH values Figure 2A was found to be linear over the pH range of 2.3–9, corresponding to the mechanism involving the same number of electrons and protons. Also it was found that the current increased with the decrease of the pH in presence (a) and absence (b) of SDS Figure 2B. Our results showed that morphine adsorbed readily on PEDOT/Pt electrode in presence of SDS in acidic medium. First, this is related to the differences in the surface properties of the electrode in absence and presence of SDS and the adsorption interactions between morphine and the modified electrode surface. Second, the variation of electrostatic interaction between morphine and the anionic SDS at different pH could also be responsible for this phenomenon. Third, the decrease of the peak current in alkaline medium might be due to the decomposition of morphine. Based on the above points the current response decreases with the increase of the pH [31].


A
a
b
R=0.0098
E / V
pH
0.8
0.7
0.6
0.5
0.4
0.3
0
2
4
6
8
10

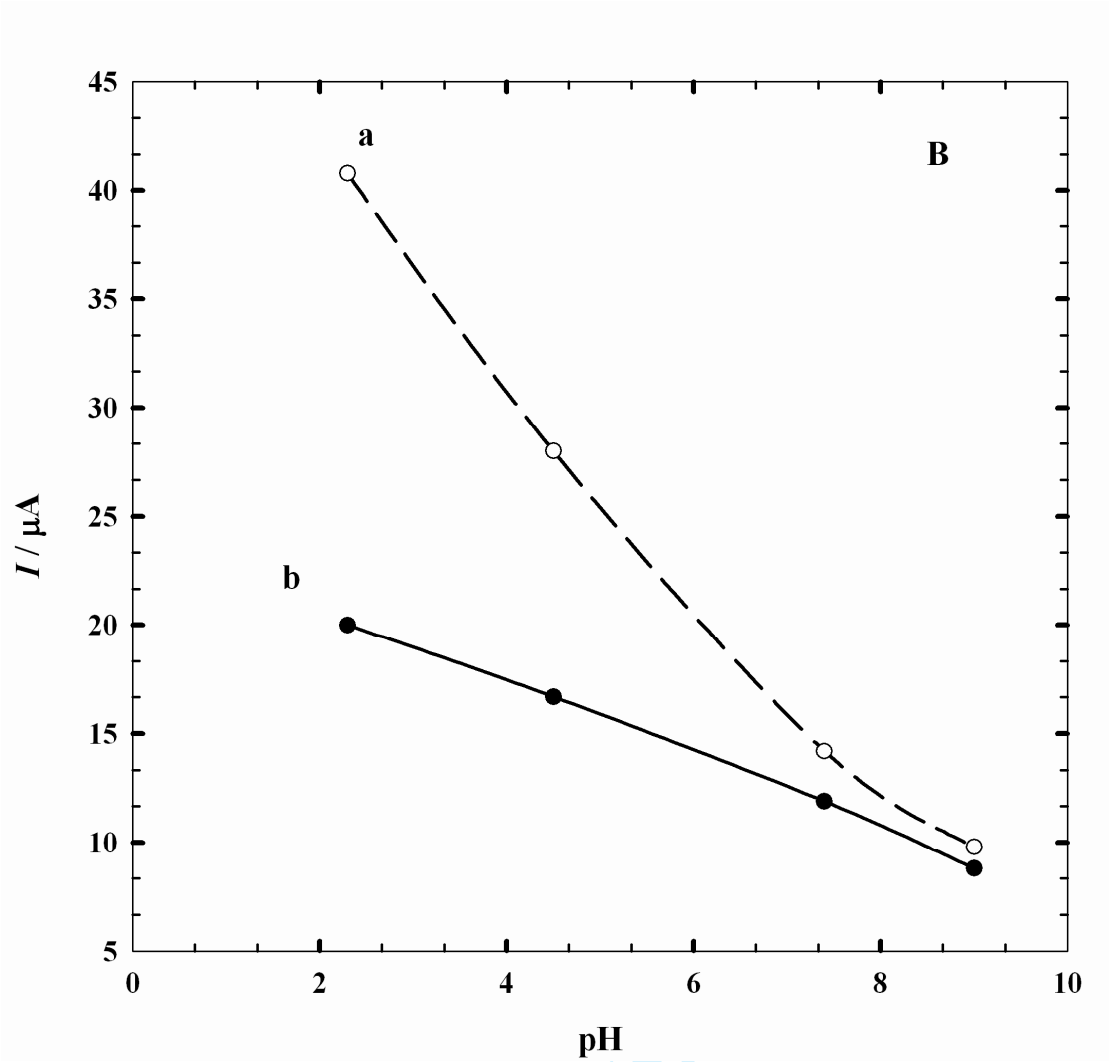


Figure 2 A, B

3.4. Calibration graph

Figure 3 shows LSVs of different concentrations of MO (0.3-16 µmol L^{-1}) in presence of 100 µL 0.1 mol L^{-1} SDS in 0.1 mol L^{-1} B-R buffer solutions (pH 7.4) at PEDOT/Pt electrode. Anodic peak current increases with increase of the morphine concentration. Moreover, the calibration curve (inset) shows linear behavior of peak current values versus different concentrations of morphine ranging from 0.3 µmol L^{-1} to 16 µmol L^{-1}, with correlation coefficients of 0.996 and detection limit of 46 nmol L^{-1}.

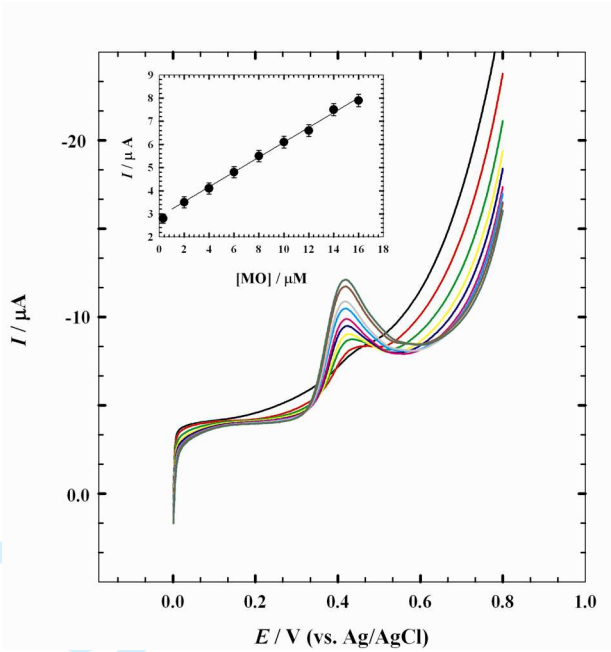


Figure 3

3.5. Response stability of the modified electrode

In order to investigate the stability of the PEDOT/Pt electrode in presence of SDS, the CV for 0.5 mmol L^{-1} MO in 100µL 0.1 mol L^{-1} SDS, 0.1 mol L^{-1} B-R (pH 7.4) solution were recorded for every 10 minutes interval and is stable for 20 runs without any noticeable change from the polymer film response. Thus, the anodic peak current remained almost without decrease in value. 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.

4. Action of morphine on biological compounds

4.1. Morphine and neurotransmitters

The increase of plasma catecholamines that occurs during surgery can be reduced by administration of morphine. This is due to the fact that morphine specifically blocks

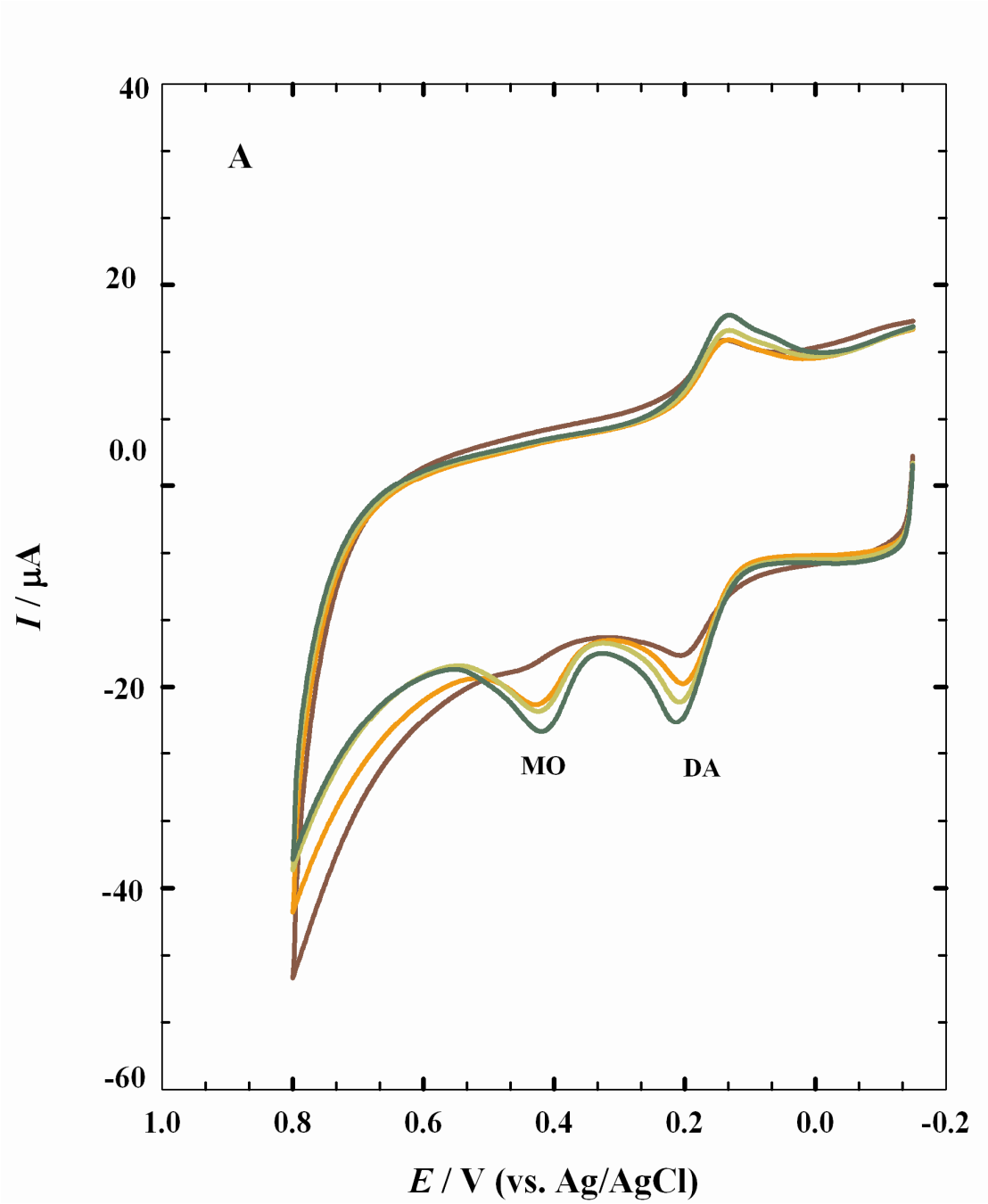
nociceptive stimulation during surgery. The mechanism of action of morphine may have its etiology in the concurrent modulation of more than one neurotransmitter. Moreover, in invertebrates, dopamine acts as the major molecule used in neural systems. In vertebrates, epinephrine emerges as the major end of the catecholamines. In this work, we illustrate the simultaneous determination for the morphine in the presence of some catecholamine compounds.

Figure (4A) shows the voltammetric response at the PEDOT/Pt electrode, in 0.5 mmol L^{-1} morphine solution containing 0.5 mmol L^{-1} DA in presence of three successive additions of 100 µL SDS in 0.1 mol L^{-1} B-R (pH 7.4). DA usually interferes with MO analysis in urine or blood. By using the PEDOT/Pt electrode in the presence of SDS, this electrode gives two well defined oxidation peaks at +0.22V and +0.41V for DA and MO, respectively. This illustrates that it is possible to discriminate morphine from dopamine with good separation in peak potential (ca. 20 mV) and with relatively high oxidation current values.

Another study was conducted using the LSV technique to investigate the effect of increasing the concentration of MO (1 µmol L^{-1}-110 µmol L^{-1}) in presence of 0.5 mmol L^{-1} and 100 µL SDS 0.1 mol L^{-1}, B-R (pH 7.4) using PEDOT/Pt electrode as shown in Figure 4B. Initially the peak current value of DA at 0.22 V was higher than MO at 0.41 V. Upon successive additions of MO, the current signal of MO oxidation increases while DA oxidation current response was almost constant. This confirms that this electrode can detect MO in presence of high concentration of DA.

Morphine withdrawal increases the turnover of adrenalin in the heart so studying both compounds in presence of each other is necessary. The LSV technique is used to investigate the effect of increasing the concentration of MO in presence of 0.5 mmol L^{-1} EP and 100 µL SDS, 0.1 mol L^{-1} B-R (pH 7.4) using PEDOT/Pt electrode (figure

is not shown). Initially the peak current value of EP at 0.26 V was higher than MO at 0.44 V, with successive additions of MO, the current signal of MO increases while EP current response almost became constant. So using the biosensor PEDOT/Pt electrode in presence of SDS for selective determination of morphine in presence of neurotransmitter compounds is possible with high sensitivity and reproducibility.



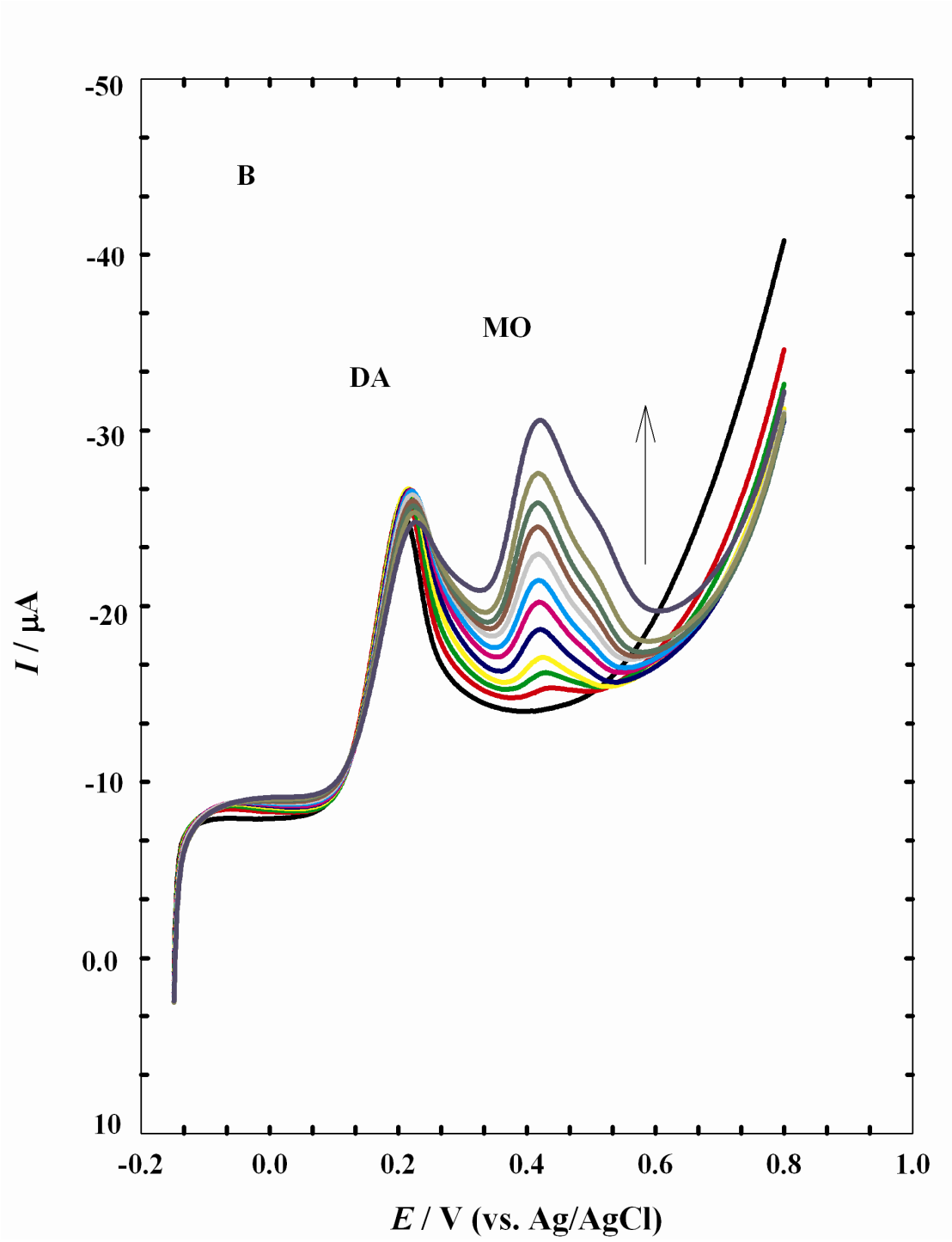


Figure 4 A, B

4.2. Morphine, Ascorbic acid and uric acid

Acute and chronic morphine administrations increase dopamine (DA), turnover [32] and release [33] in terminal fields of dopaminergic neurones. Increased dopaminergic activity in the limbic area and in the striatum is paralleled by increased locomotor

activity and stereotyped behavior [34]. The dopaminergic system is also involved in the reinforcing effects of abused drugs [35]. Experimental evidence suggests that ascorbic acid (AA) may modulate central dopaminergic transmission [36] as well as behavior [37]. AA is not synthesized in the brain. However, AA is found in high concentrations throughout the mammalian brain, and then diffuses at the blood-brain barrier site. AA is a very active component of the neuronal antioxidant pool, since it is rapidly oxidized by reactive oxygen species (ROS) [38]. AA is the main scavenger of ROS generated from catecholamine oxidation in vivo [39].

Large doses of AA have been reported to suppress withdrawal symptoms in opiate addicts and to prevent the development of tolerance to and physical dependence on morphine. Moreover, MO increases uric acid levels and AA oxidation. Therefore, the electrochemical behavior of MO, UA and AA in a mixture solution is extremely important to investigate. Cyclic voltammetry was used for the characterization of a solution containing mixture of 0.5 mmol L^{-1} MO, 5 mmol L^{-1} UA and 50 mmol L^{-1} AA, pH 7.4 at PEDOT/Pt electrode in presence of SDS. As depicted in Figure (5), the oxidation potential peaks appeared at potentials 0.44 V, 0.33 V, and 0.28 V for MO, UA, and AA, respectively. The large separation of the peak potentials allows simultaneous determination of MO, UA, and AA in their mixture. Second using PEDOT/ Pt in presence of four successive additions of 100 µL SDS in 0.1 mol L^{-1} B-R (pH 7.4); a sharp well defined oxidation peak of MO appeared at 0.42 V. Moreover, the oxidation peak current for MO increased in presence of SDS, while the oxidation peaks for UA and AA disappeared. Therefore, the high response for morphine was observed due to the electrostatic interaction of the anionic surfactant with the protonated MO in pH 7.4, but in case of AA and UA repulsion takes place. This is due to the anionic nature of the acids at this working physiological pH. Therefore, it is

possible to determine MO selectively in presence of high concentration of AA, and UA.

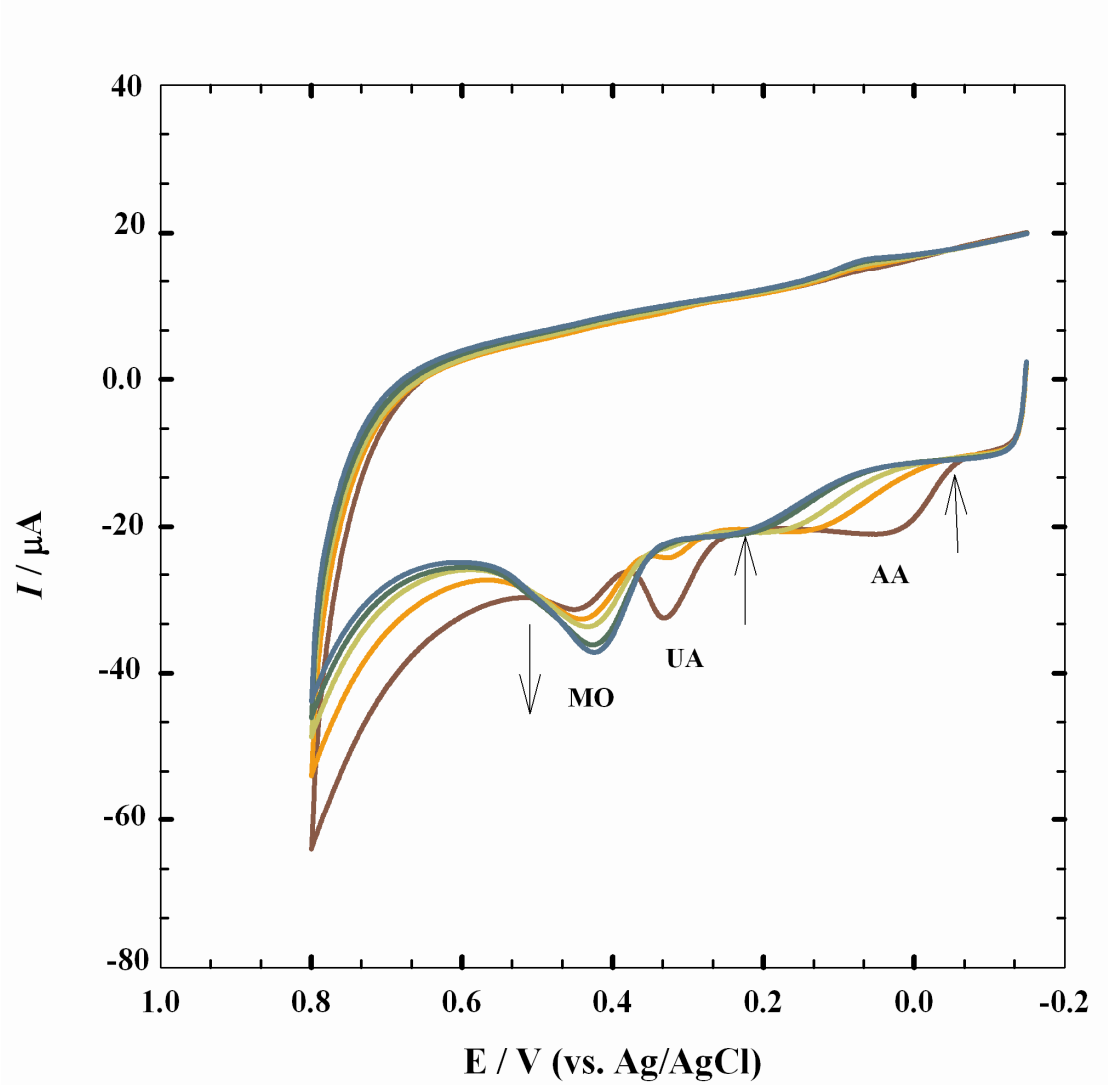
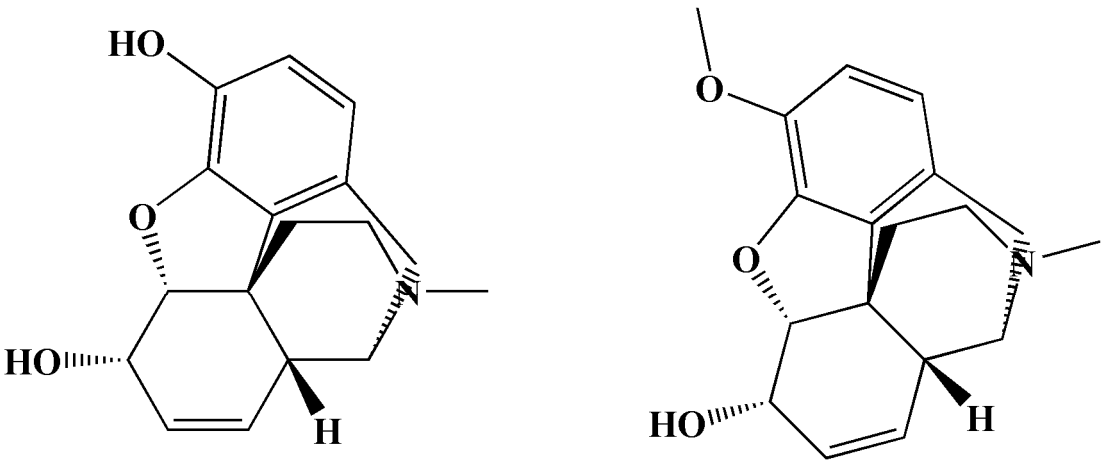


Figure 5

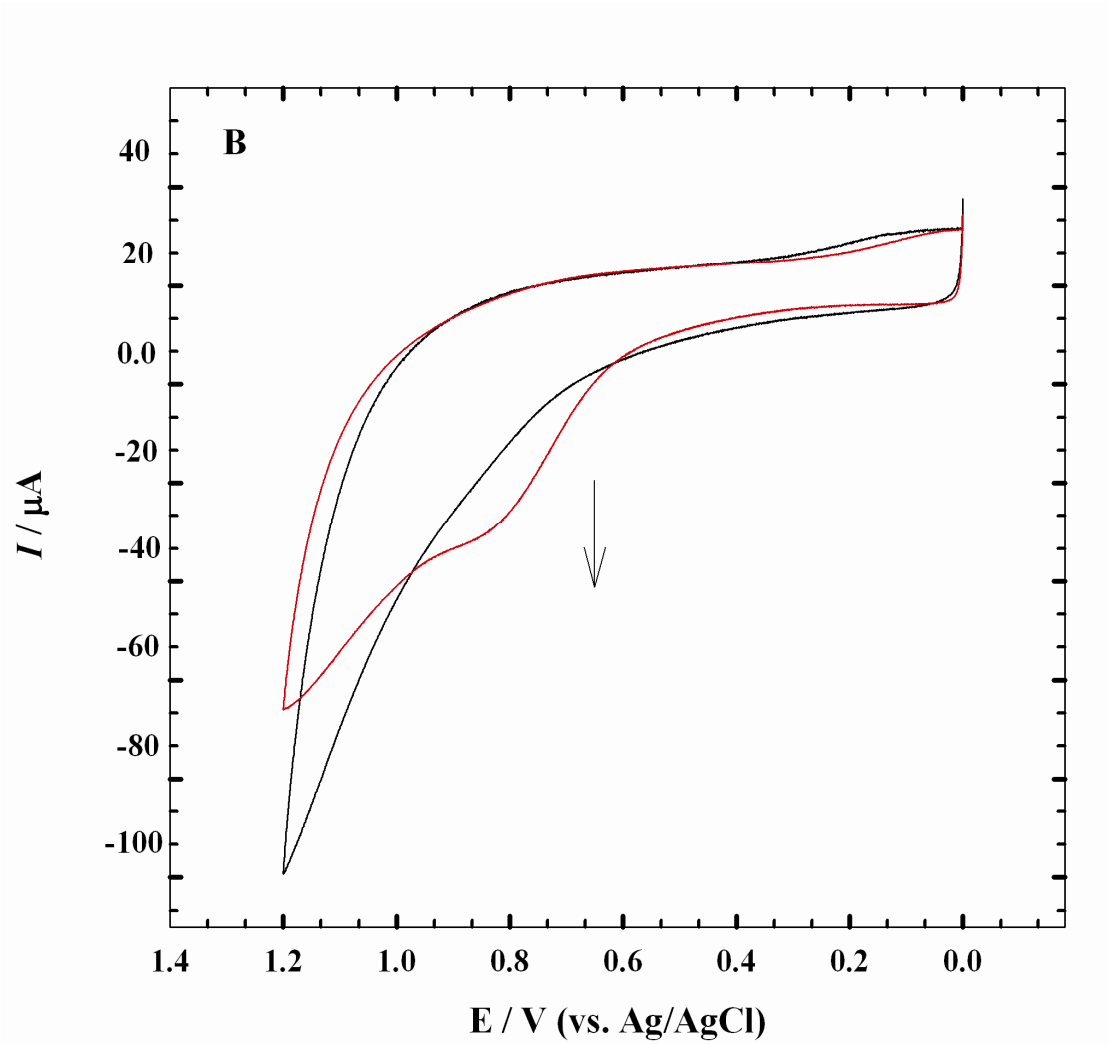
5. Discrimination of morphine from codeine

Codeine, with the methyl ether group (–OCH_3) substituted for the phenolic group (–OH) in the 3-position, is very similar to morphine in structure as shown in Figure 6A. It usually interferes with morphine analysis in urine or blood. The oxidation peak of codeine appeared at 0.86 V (Figure 6B) in presence of 100 μL SDS at PEDOT/Pt electrode in 0.1 mol L^{-1} B-R (pH 7.4) due to the absence of phenolic group at the 3-position [31]. Therefore, the modified electrode PEDOT/Pt electrode in presence of

SDS can easily discriminate morphine from codeine. The adsorption of morphine by the PEDOT/Pt electrode involved interactions between morphine molecules and the anionic surfactant. The presence of codeine may affect the detection of morphine due to its competitive adsorption. Voltammetric response at the PEDOT/Pt electrode in 0.5 mmol L^{-1} morphine solution containing 0.5 mmol L^{-1} codeine was also examined in presence of successive additions of 100 µL SDS in 0.1 mol L^{-1} B-R (pH 7.4). The results in Figure 6C did not show significant interference and an oxidation peak at 0.55 V for MO is observed. These results prove that PEDOT/Pt electrode in presence of SDS can detect MO with no interference from the coexisting codeine. Since MO was the major component in opium poppy, therefore SDS can be used for the determination of morphine in opium poppy in presence of codeine. It was possible to determine morphine in presence of 10 fold concentration of codeine.



morphine codeine



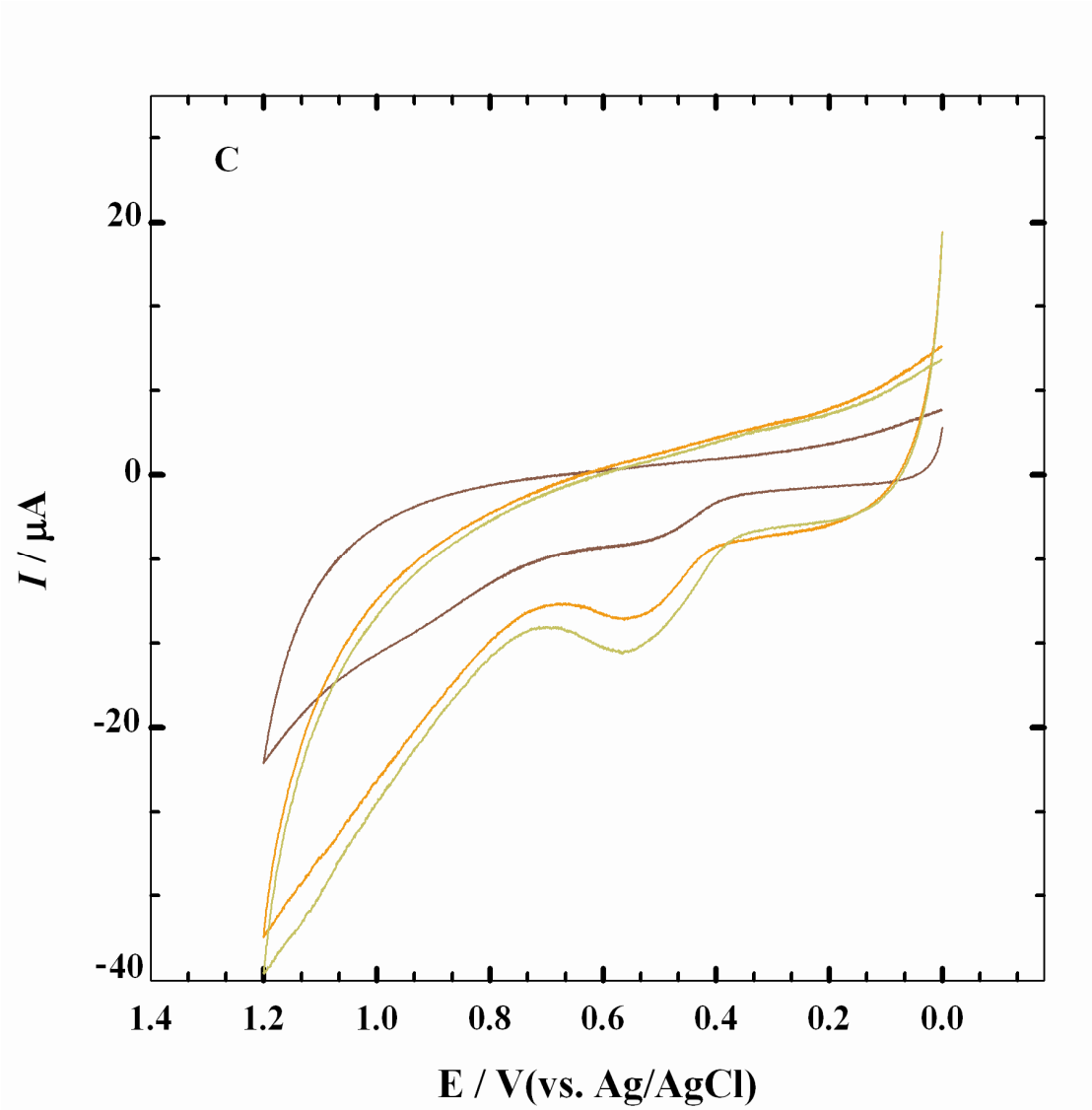


Figure 6 A, B, C

6. Determination of morphine in urine

The proposed method in real sample analysis was also examined in human urine samples. In this set of experiments, morphine was mixed in urine to make a stock solution of 5 mmol L^{-1} concentration. Standard additions of 10 µL of 5 mmol L^{-1} morphine in urine were added to B-R pH 7.4 containing 100 µL SDS and the corresponding LSV was measured. The results show that the oxidation peak current increases with addition of morphine. The calibration plots (supplementary 3) are linear in the concentration range of 0.3 to 8 µmol L^{-1} and 10 to 60 µmol L^{-1} with

correlation coefficients of 0.999, 0.995 and detection limits 50, 68 nmol L^{-1}, respectively.

Validation of the procedure for the quantitative assay of the morphine 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 94% and 100.1%. The R.S.D. (n = 5) was less than 6.0%.

In Table 2, the response characteristics of the proposed method are compared with those obtained by some reported methods. In comparison with some other voltammetric methods of morphine determination, our method showed advantages in several aspects. The designed sensor is prepared in one simple step with cheap and simple reagents and no pretreatment needed before the measurements. This gives the sensor more advantages over other modified electrodes used in the literature. This sensor showed good reproducibility, high stability, sensitivity and anti-interference ability. The sensor was further utilized to determine MO level in human urine and satisfactory results were obtained with low detection limit.

Table 1.

Results of determination of morphine in urine sample

Urine sample	Spike (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	R.S.D. (%)[a]
1	4.0	3.76	94	4.2
2	9.0	9.4	100.1	3.2
3	16.0	15.62	97.6	5.1

[a] Average of five replicate measurements.

Table 2
Comparison the proposed method with other reported methods

Detection method	Limit of detection	Sample	Recovery (%)	Reference
LPME[a]–HPLC[b]	0.05 mg L^{-1}	Urine	92.4–106.8	[40]
Amperometry	0.2 mmol L^{-1}	Not applied	-	[41]
Amperometry-MIP[c]	0.3 mmol L^{-1}	Not applied	-	[42]
SIA[d]	0.076 µgm L^{-1}	Urine	96.3	[43]
Voltammetry	0.2 µmol L^{-1}	Urine	95.1–106.6	[31]
Voltammetry	50-68 nmol L^{-1}	Urine	94–100.1	This work

[a] Liquid phase micro extraction.
[b] High-performance liquid chromatography.
[c] Molecularly imprinted polymer.
[d] Sequential injection analysis.

7. Applications on commercial tablets

The MO tablets were dissolved in buffer solution with a "start concentration" of 2.6 mmol L^{-1}. Standard successive additions of 10 µL of 2.6 mmol L^{-1} of morphine buffer solution were added to the buffer (pH 7.4) containing 100 µL SDS. The effect of changing the concentration of Morphine in the presence of 0.1 mol L^{-1} SDS in pH 7.4 was studied by square wave voltammograms, SWV, using a PEDOT/Pt working electrode Figure 7. The following are the parameters for the SWV experiments: Ei = 0.30 V, Ef = 0.60V, scan rate = 50 mV s^{-1}. The oxidation peak current for MO is linearly proportional to the concentration of the drug in the range of 8 µmol L^{-1} to 150 µmol L^{-1} with correlation coefficient, r = 0.996, and the detection limit, DL, 67 nmol L^{-1} calculated from the equation:

$$DL = 3S/m \quad (8)$$

Where s is the standard deviation ($s = 9.0 \times 10^{-4}$) and m is the slope.

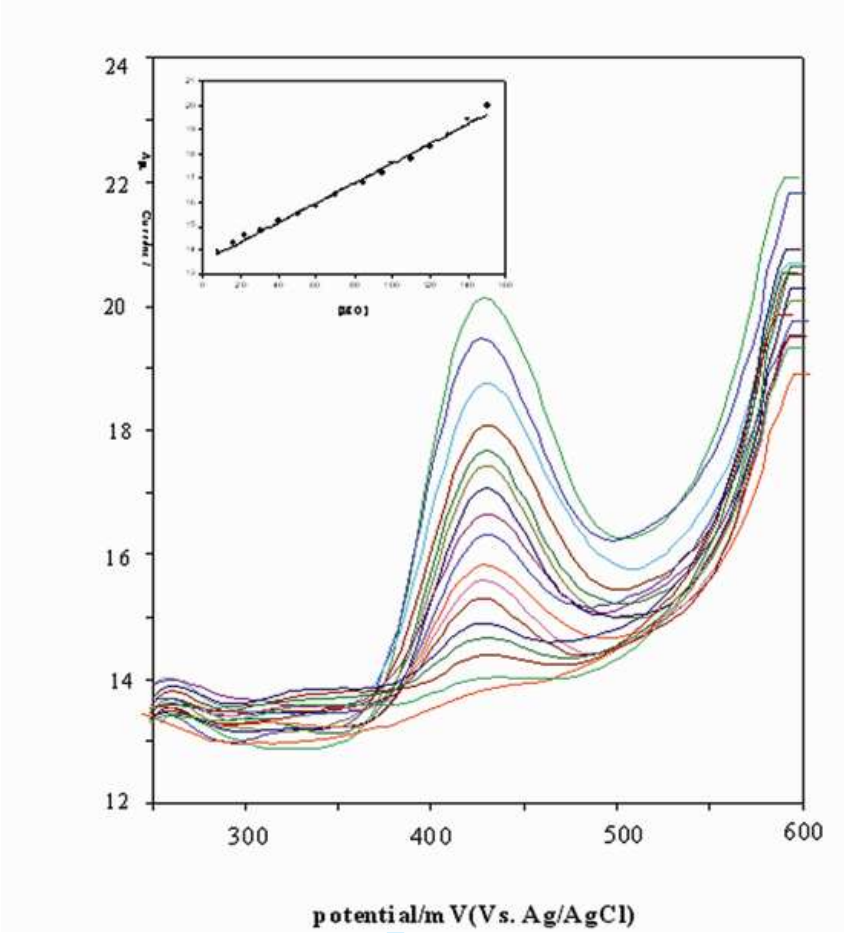


Figure 7

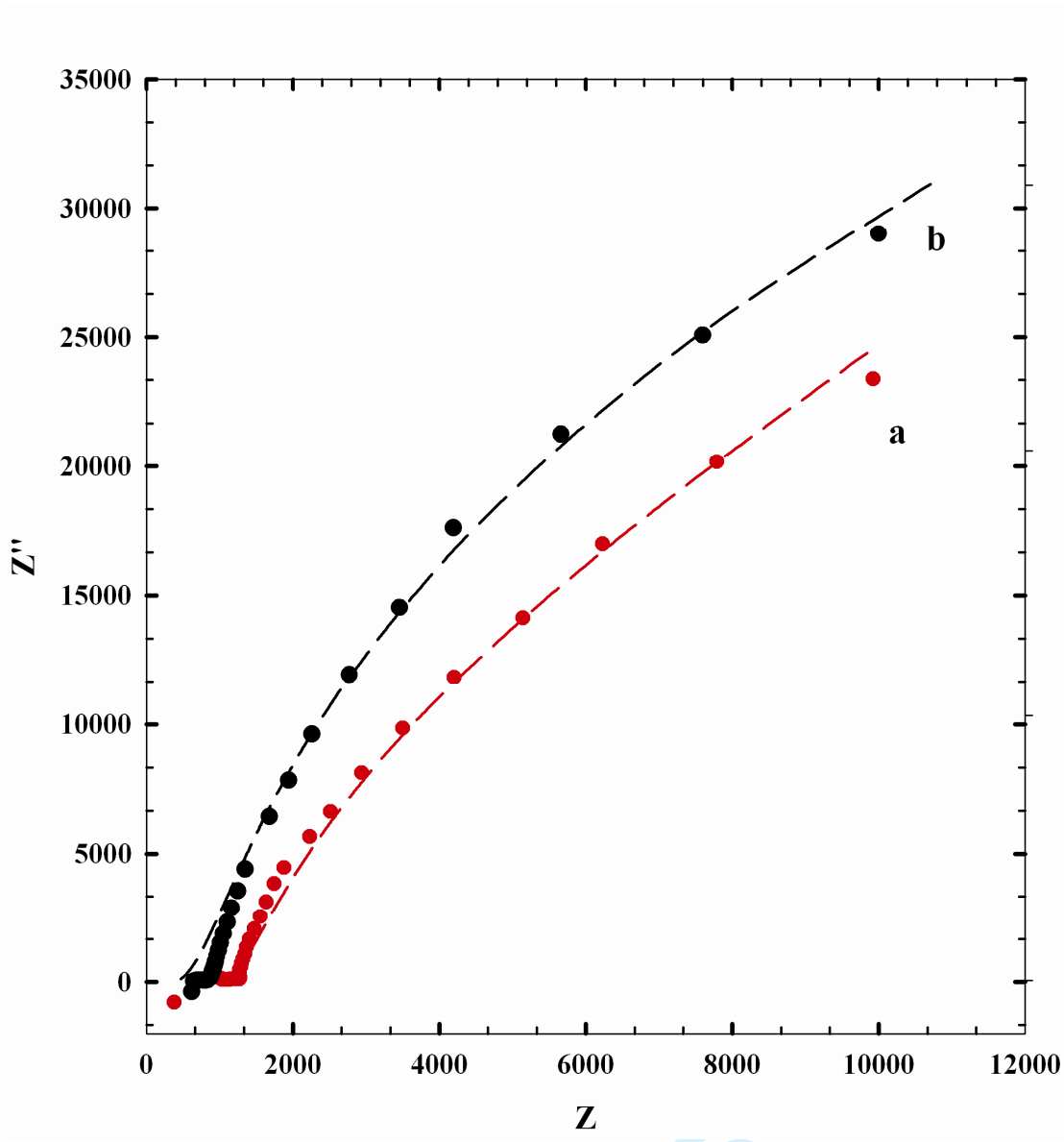
8. Electrochemical impedance spectroscopy (EIS) of MO

It is well known that EIS technique is a useful tool for studying the interface properties of surface-modified electrodes [44-45]. Therefore, EIS was used to investigate the nature of MO interaction at PEDOT/Pt surface in presence and absence of SDS. In EIS, the semicircle diameter in the Nyquist plot represents the electron transfer resistance. Figure 8 A, shows the complex plane diagram (Nyquist plot) of MO at PEDOT/Pt electrode in the presence (a) and absence of SDS (b) at oxidation potential 0.42 V. From this comparison, it is clear that the impedance responses of MO show great difference after addition of SDS. On the other hand, in absence of SDS, the impedance spectra include a semicircle with a larger diameter. However, after addition of SDS, the diameter of semicircle diminishes markedly. The charge

transfer resistance of electrooxidation of morphine decreases greatly, and the charge transfer rate is enhanced by SDS. The data proves that SDS facilitates the electron transfer between MO and electrode.

Figure 8 B, represents the circuit used, in the fitting where 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 impedance known as the Warburg impedance (W).

Table 3 lists the fitting values calculated from the equivalent circuit for the impedance data. The PEDOT/Pt electrode in presence of SDS shows increased values of the capacitive component compared to the case of absence of 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 MO which accelerate the electron transfer between the electrode and morphine.


35000
30000
25000
20000
15000
10000
5000
0
Z''
0
2000
4000
6000
8000
10000
12000
Z
b
a

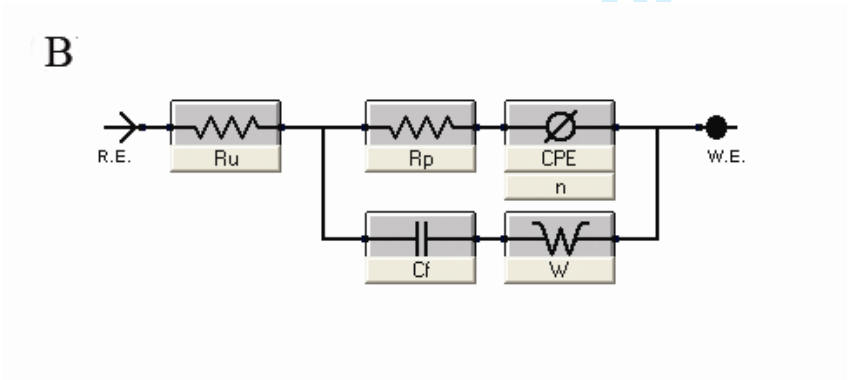

B
R.E.
Ru
Rp
CPE
n
W.E.
Cf
W

figure 8 A, B

Table 3
Summary of the data obtained from EIS in the determination of morphine using PEDOT/Pt electrode in absence and presence of SDS at the oxidation potential.

Electrode PEDOT/Pt	E mV	R_p ($k\Omega\ cm^2$)	R_u ($k\Omega\ cm^2$)	C_f ($\mu F cm^{-2}$)	W ($K\Omega^{-1} cm^{-2}$)	C_{CPE} ($\mu F\ cm^{-2}$)	n
In absence of SDS(ST)	420	122	0.39	45	2.49	75	0.88
In presence of SDS(ST)	420	52	0.5	50	2.38	279.8	0.7

9. Conclusion

Voltammetric determination of morphine has been successful at the PEDOT modified pt electrode in presence of SDS. The main advantage of the proposed method is simple, cheep and fast compared to other determination methods. Furthermore, a low detection limit and a wide range of concentrations are enough for usual analytical purpose.

The method has demonstrated that it is easily to discriminate morphine from AA and UA as common interferences in biological fluids. Moreover, it is possible to determine morphine in presence of codeine as they are having the same structure and coexist in opium poppy. The determination of morphine in real human urine without any sample pretreatment has been successful with consistent results compared to those of obtained when using other techniques such as HPLC and LPME. The proposed method provides the basis for designing portable morphine sensor due to its easy, fast preparation, and low cost.

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Figure 1. Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} morphine / 0.1 mol L^{-1} B-R, pH 7.4, at (a) PEDOT/Pt electrode in the presence of SDS, (b) PEDOT / Pt electrode (c) Pt electrode, at scan rates 50mVs^{-1}

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Figure 4B. LSVs for MO of different concentrations (1 µmol L^{-1} – 110 µmol L^{-1}) in 5 mmol L^{-1}, 100 µL 0.1 mol L^{-1} SDS, 0.1 mol L^{-1} B-R PH 7.4 at PEDOT /Pt electrode.

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Figure 6B. Cyclic voltammograms for 5 mmol L^{-1} codeine in 0.1 mol L^{-1} B-R pH 7.4 at PEDOT /Pt electrode (a) in 0.1 mol L^{-1} 100 µL SDS (b).

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Figure 7. SWV for the successive additions of 10 µL of 2 mmol L^{-1} morphine tablets solution to the buffer pH 7.4 containing 100 µL SDS.

Calibration curve of MO for concentrations from (8 µmol L^{-1} - 150 µ mol L^{-1}) (inset).

Figure 8A. Nyquist diagrams (–Z″ vs. Z′) for the EIS measurements at PEDOT/ Pt at potential 420 mV for 5 mmol L^{-1} MO in 0.1 mol L^{-1} B-R pH 7.4, (a) in presence of SDS and (b) in absence of SDS. Amplitude: 5 mV, frequency range: 0.1–100000 Hz**.**

Figure 8B. The equivalent circuit**.**

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Supplementary 1. Cyclic voltammograms for 0.5 mmolL^{-1} morphine 0.1 mol L^{-1} B-R pH 7.4 at PEDOT /Pt electrode in 0.1 mol L^{-1} 100 µL SDS at different scan rates 30,50,70,100,120,150 mV s^{-1}. Relation between log ip and log ν as function of scan rate (inset).

Supplementary 2A. Effect of solution pH on the behavior of PEDOT/Pt electrode in 5.0×10^{-4} mol L^{-1} morphine/0.1 mol L^{-1} B-R in presence of SDS: at different pH values (a-e) 2.3, 4.0, 7.4, 9, scan rate 50 mV s^{-1}.

Supplementary 2B. Effect of solution pH on the behavior of PEDOT/Pt electrode in 5.0×10^{-4} mol L^{-1} morphine/0.1 mol L^{-1} B-R in absence of SDS: at different pH values (a-e) 2.3, 4.0, 7.4, 9, scan rate 50 mV s^{-1}.

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