

Full Length Research Paper

Electrochemical studies of Tram and DA in real pharmaceutical and human serum samples

Nefertiti Morsi^{1*}, Mohammed Rufi², Ibrahim O. Mubarak¹ and Hassan V. Hamama

¹ Electrochemistry Laboratory, Chemistry Department, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt.

² Faculty of Science, Ain Shams University, Cairo, Egypt.

Accepted 17 March, 2025

Modified platinum electrode based on the poly 8-(3-acetylmino-6-methyl-2,4-dioxopyran)-1-aminonaphthalene (PAMDAN) film was prepared by electrochemical polymerization technique. The modified electrode showed electrocatalytic activity toward tramadol (Tram) oxidation in acidic aqueous solution. It was demonstrated that Pt electrode modified by PAMDAN film (PAMDAN/Pt modified electrode) could be used for Tram detection using cyclic voltammetry (CV) and square wave voltammetry (SWV) techniques. This modified electrode was quite effective not only for detecting Tram, but also for simultaneous determination of Tram and dopamine (DA) in a mixture with satisfactory results.

Key words: Voltammetry, electrocatalysis, electro analysis, tramadol, dopamine, simultaneous determination.

INTRODUCTION

Tramadol is a centrally acting analgesic that was first introduced in Germany in 1977. Today, it has become the most prescribed opioid worldwide. It is generally said to be devoid of many of the serious adverse effects of traditional opioid receptor agonists such as the risk for respiratory depression and drug dependence.

Recently, reported results of post-marketing surveillance and case reports have shown that Tram abuse and Tram related fatalities have been noted. Its overall analgesic efficacy is comparable to that achieved using equianalgesic doses of morphine or alfentanil (National Corporation of Pharmacies, Sweden (Apoteket AB) Sale statistics, 2006; Scott and Perry, 2000; Karen and Lesley, 2003; Babaei et al., 2011). On the other hand, 3,4 -Dihydroxyphenyl ethylamine, also commonly known as dopamine (DA), is an important neuron transmitter compound widely distributed in the brain for message transfer in the mammalian central nervous system. Low levels of DA are related to neurological disorders (such as schizophrenia), Parkinson's disease and HIV infection (Wightman et al., 1988; Mo and Ogorevc, 2001). So DA is currently the subject of intense research focus by neuroscientists and chemists and it is

essential to develop rapid and simple methods for the determination of its concentration. Electrochemical methods can be used for determining DA because it is an electro active compound.

The electrochemical method is one of the most favorable techniques for the determination of most of the biological compounds in recent years, because of its fast response, high sensitivity and easy operation. However, the major drawbacks associated with conventional electrode materials for simultaneous electrochemical sensing of these compounds are poor selectivity arising from overlapping oxidation peaks and low reproducibility due to electrode fouling. Chemically modified electrodes have proved very useful to solve these problems (Ensafi et al., 2012; Hadi and Rouhollahi, 2012; Gong et al., 2012). The chemical modifications of bare electrodes with redox active films offer significant advantages in the design and development of the electrochemical sensors. Recently, our laboratory has developed several

*Corresponding author. E-mail: Nefertiti02@yahoo.com.

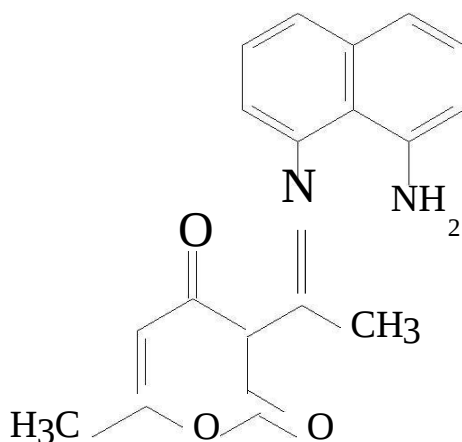


Figure 1. 8-(3-acetylimino-6-methyl-2,4-dioxopyran)-1-aminonaphthalene (AMDAN).

chemically modified electrodes (Hathoot, 2000; Abd El-Rahman et al., 2000; Diab et al., 2000; Yousef et al., 2001; Hathoot, 2003; Atia et al., 2008; Hathoot et al., 2009; Hathoot et al., 2010; Ismail et al., 2002; AbdelAzzem et al., 1998; Yousef et al., 2010; Ramiz et al., 2010).

In this work, poly 8-(3-acetylimino-6-methyl-2,4-dioxopyran)-1-aminonaphthalene (PAMDAN) film previously prepared by electrochemical polymerization technique (Abd El-Rahman et al., 2000; Yousef et al., 2001) was used for the detection of Tram in the presence of DA. Up to now and to the best of our knowledge, there are no reports in the literature of simultaneous electrochemical studies of Tram and DA in real pharmaceutical and human serum samples.

EXPERIMENTAL

Materials

Tramadol-HCl (Tram) was obtained from AMRIYA pharm. Ind. Co. - Egypt, whereas Dopamine (DA) was brought from EIPICO pharmaceutical co., Egypt. The chemicals 1,8-diaminonaphthalene, dehydroacetic acid analytical grade (Aldrich), lithium perchlorate (LiClO_4), acetonitrile (CH_3CN), sodium sulfate (Na_2SO_4) and sulfuric acid (H_2SO_4) were of analytical grade (Aldrich). The aqueous solutions were prepared from bi-distilled water.

Pharmaceutical dosage forms

For this study, Tram ampoule 100 mg/2 ml (CID pharmaceutical co. - Egypt) was used.

Human serum samples

These samples were supplied by the local Hospital Blood Bank and from the investigator. Human serum samples

were obtained by centrifugation of human blood after two hours of intravenous injection at 6000 rpm for 15 min and immediately frozen at -20°C until assay. Frozen human serum samples were left on the bench to thaw naturally and were vortex prior to their use.

Instruments

Electrochemical measurements were recorded using a potentiostat Model PST 006 from Voltalab-Radiometer analytical with software Model voltaMaster 4. All voltammograms were obtained in a three-electrode electrolytic cell from Bioanalytical system; model C-1A which contains a platinum disc electrode (Pt) of 3.0 mm diameter as working electrode, a platinum coil as counter electrode and Ag/AgCl as a reference one. All experiments were conducted at room temperature ($25^\circ\text{C} \pm 1$).

Preparations

Preparation of AMDAN Schiff base

AMDAN Schiff base (80% yield) was prepared by a condensation reaction between 1 mole of 1,8-diaminonaphthalene and 1 mole of dehydroacetic. The dehydroacetic acid in ethanol was added drop wise with stirring to hot 1,8-diaminonaphthalene in ethanol. The resulting Schiff base (Figure 1) was recrystallized twice from ethanol as faint brown granules (Abd El-Rahman et al., 2000).

Preparation of poly AMDAN

In preparing this sample, 1×10^{-3} M AMDAN Schiff base was dissolved in acetonitrile solution containing 0.1M LiClO_4 . The electrochemical polymerization of AMDAN at Pt electrode was carried out using repeated CV method between -0.2V and +0.8V for 20 cycles, at a scan rate of 0.1 V/s (Abd El-Rahman et al., 2000; Yousef et al., 2001).

Preparation of the drugs solutions

In preparing this solution, 1×10^{-2} M Stock solutions of Tram were prepared by dissolving an accurate weight in the appropriate volume of 0.1 M H_2SO_4 . The required concentrations were obtained by accurate dilution.

Preparation of the H_2SO_4 aqueous solution with different pH

The desired pH value was prepared by adjustment and addition of 1.0M NaOH solution to 0.1M H_2SO_4 , and the ionic strength was kept constant by using Na_2SO_4 .

RESULTS AND DISCUSSION

Electrocatalytic oxidation of Tram

Figure 2 shows the cyclic voltammograms of 1×10^{-4} M of

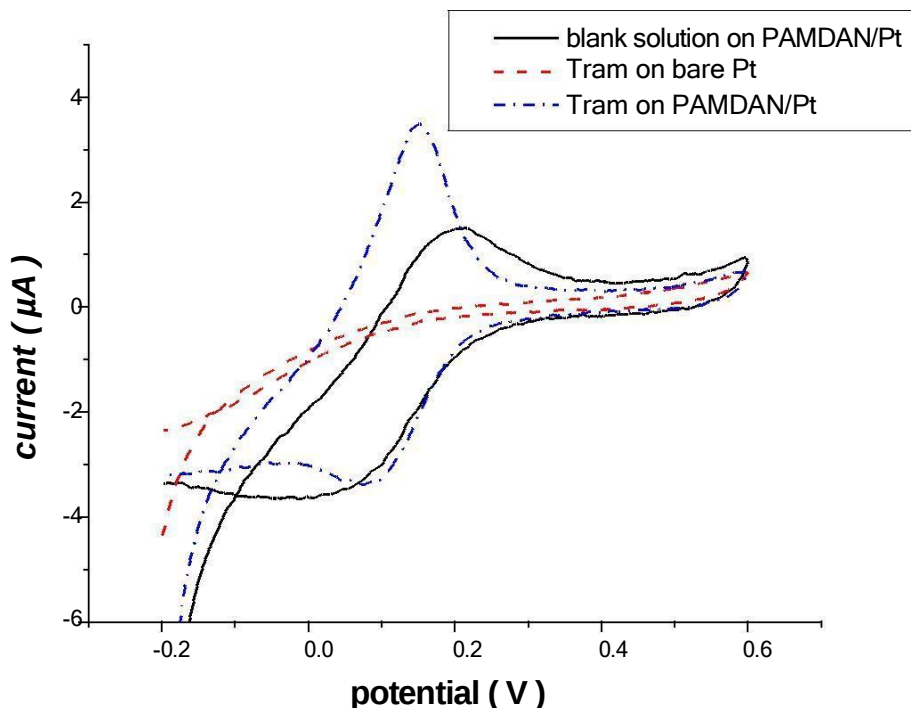


Figure 2. Cyclic voltammograms of 1×10^{-4} M of Tram at PAMDAN/Pt electrode, bare Pt electrode in 0.1 M H_2SO_4 and PAMDAN/Pt in blank solution (0.1 M H_2SO_4 , Tram free solution) scan rate of 0.1 V/s.

Tram obtained at PAMDAN/Pt and bare Pt electrodes in the 0.1 M H_2SO_4 aqueous solution, where the dashed line shows the CV obtained for Tram at bare electrode, dashed dotted line for CV of Tram at PAMDAN /Pt, and solid line illustrates the redox response of PAMDAN/Pt modified electrode in absence of Tram (blank solution). The voltammograms illustrate that the electrooxidation of Tram can be catalyzed by the modified electrode as a mediator, and it could be seen that the peak current was enhanced, which means that the polymer layer could accelerate electron transfer between the solution and Pt. The obtained results showed that Tram oxidation at bare electrode gave no peak, while its oxidation at PAMDAN/Pt modified electrode made the peak to appear at 0.18 V and the corresponding cathodic peak to appear at 0.1 V.

This behavior is typical of that expected for electrocatalysis at chemically modified electrodes (Gong et al., 2012; Hathoot, 2000; Abd El-Rahman et al., 2000; Diab et al., 2000).

For investigating the reaction mechanism, the effect of scan rate versus the peak current of Tram at PAMDAN/Pt modified electrode was investigated as shown in Figure 3.

The results showed that the anodic peak current (I_{pa}) of Tram was linearly proportional to the square root of scan rate ($v^{1/2}$) within the range of 0.01 - 0.2 V/s, with a regression equation:

$$I_{pa} = (-0.49) + (5.82) v^{1/2}$$

These results show that the Tram redox reaction process was a diffusion controlled kinetic process (Abd El-Rahman et al., 2000; Diab et al., 2000; Yousef et al., 2001; Hathoot, 2003; Atia et al., 2008; Hathoot et al., 2009; Hathoot et al., 2010; Ismail et al., 2002; AbdelAzzem et al., 1998; Yousef et al., 2010; Ramiz et al., 2010). Also, Figure 3 illustrates that the oxidation peak potential shifts towards more positive potential with increasing scan rates, confirming the kinetic limitation of the electrochemical reaction (Lu et al., 2012). The redox system should be affected by changes in pH values because of the involvement of protons in the overall electrode reaction. It was observed that the pH value obviously influenced the redox peak current where the maximum current response was obtained at lower pH (1 to 3), which confirmed our previously reported results that the polyaniline and their derivatives are electroactive at $\text{pH} \leq 4$ (Hathoot, 2000; Abd El-Rahman et al., 2000; Diab et al., 2000; Yousef et al., 2001; Ismail et al., 2002). The proposed oxidation mechanism of Tram is given as shown in Scheme 1.

Linear calibration curves, LOD and LOQ

CV and SWV were performed to investigate the

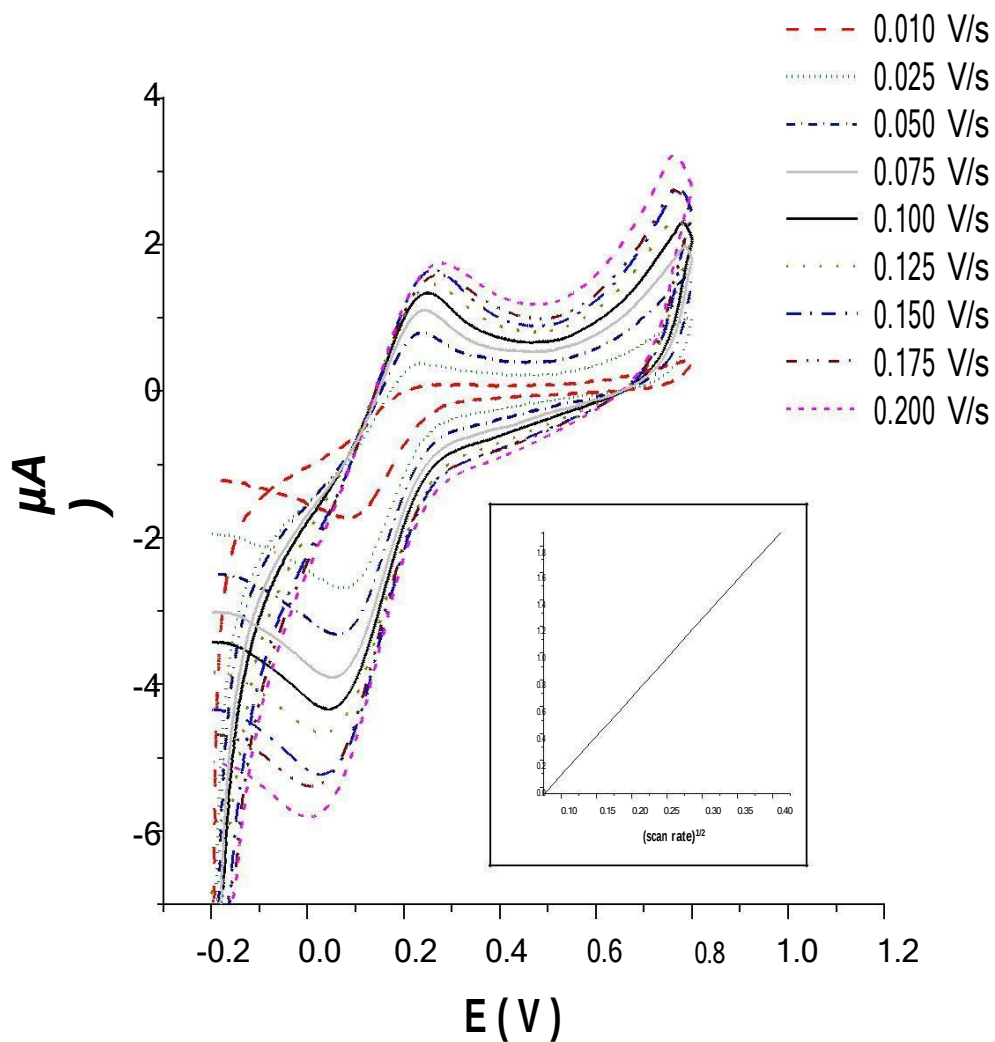
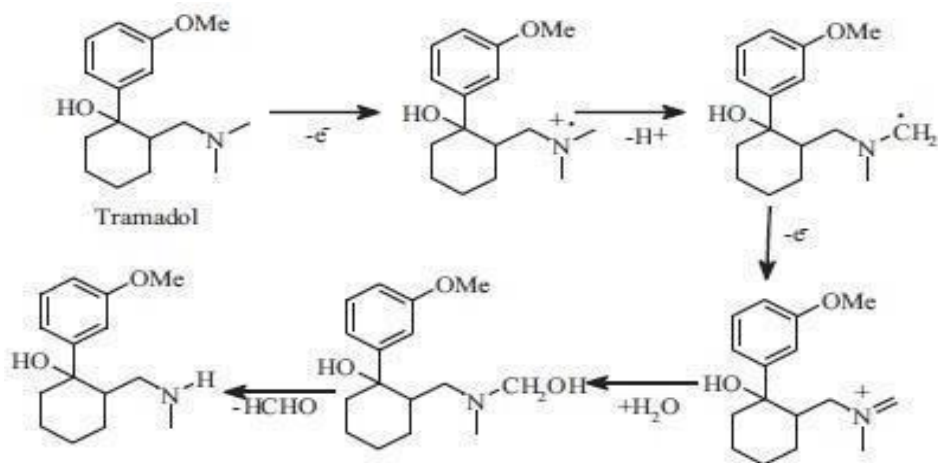


Figure 3. Cyclic voltammograms of 1×10^{-4} M of Tram in 0.1 M H_2SO_4 at PAMDAN/Pt modified electrode at different scan rates. The inner diagram represents the plot of I_{pa} vs. square root of scan rate ($u^{1/2}$) for Tram at PAMDAN/Pt modified electrode. The regression equation is $I_{pa} = (-0.49) + (5.82) u^{1/2}$.



Scheme 1. Probable oxidation mechanism for Tram.

Scheme1. Probable oxidation mechanism for Tram

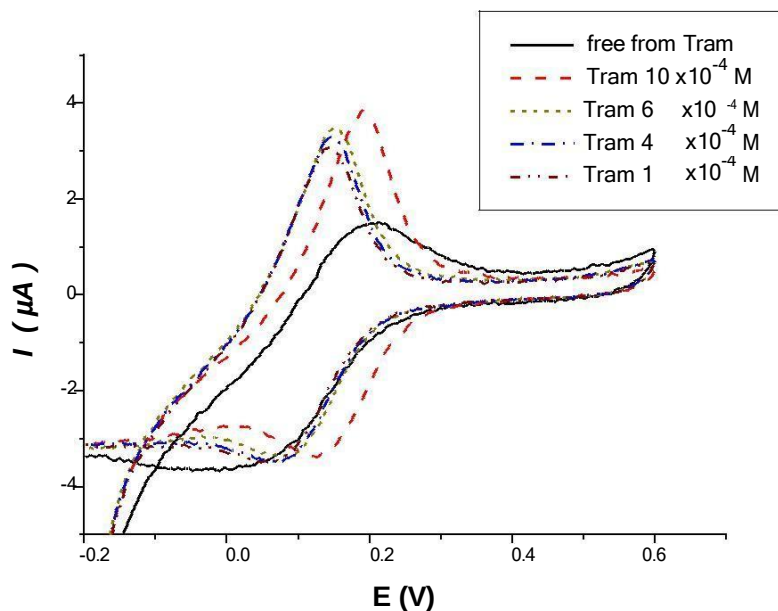


Figure 4. Cyclic voltammograms of different Tram concentrations in 0.1 M H_2SO_4 aqueous solution and blank solution (Tram free solution) at PAMDAN/Pt modified electrode at scan rate of 0.1 V/s.

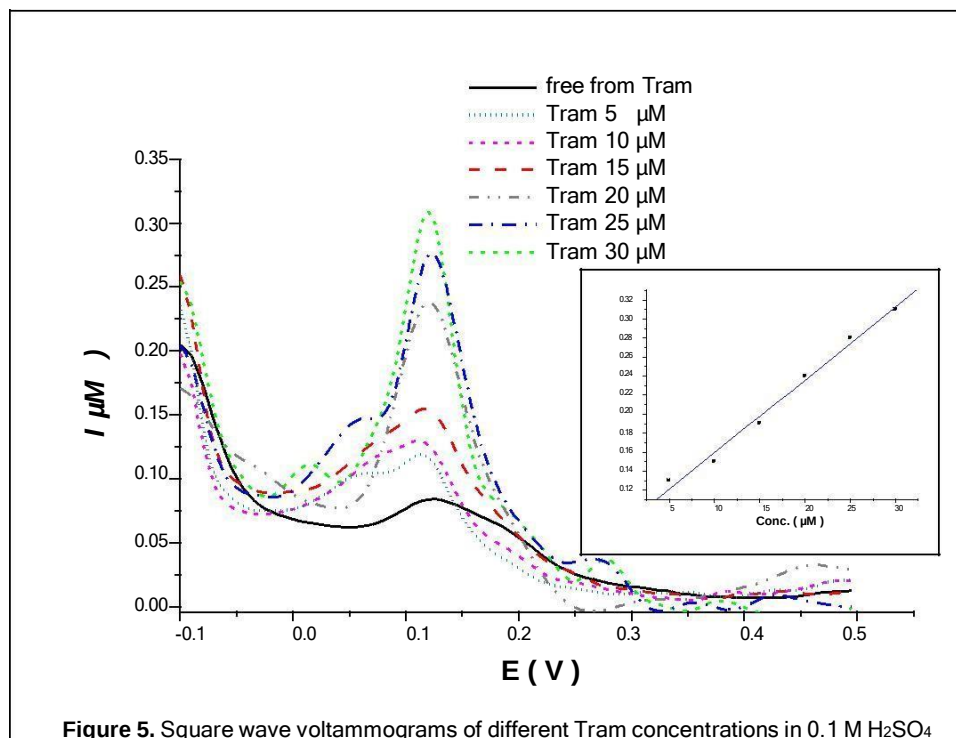


Figure 5. Square wave voltammograms of different Tram concentrations in 0.1 M H_2SO_4 aqueous solution and blank solution (Tram free solution) at PAMDAN/Pt modified electrode at scan rate 5 mV/s. Inset: Plot of peak currents as a function of Tram concentration.

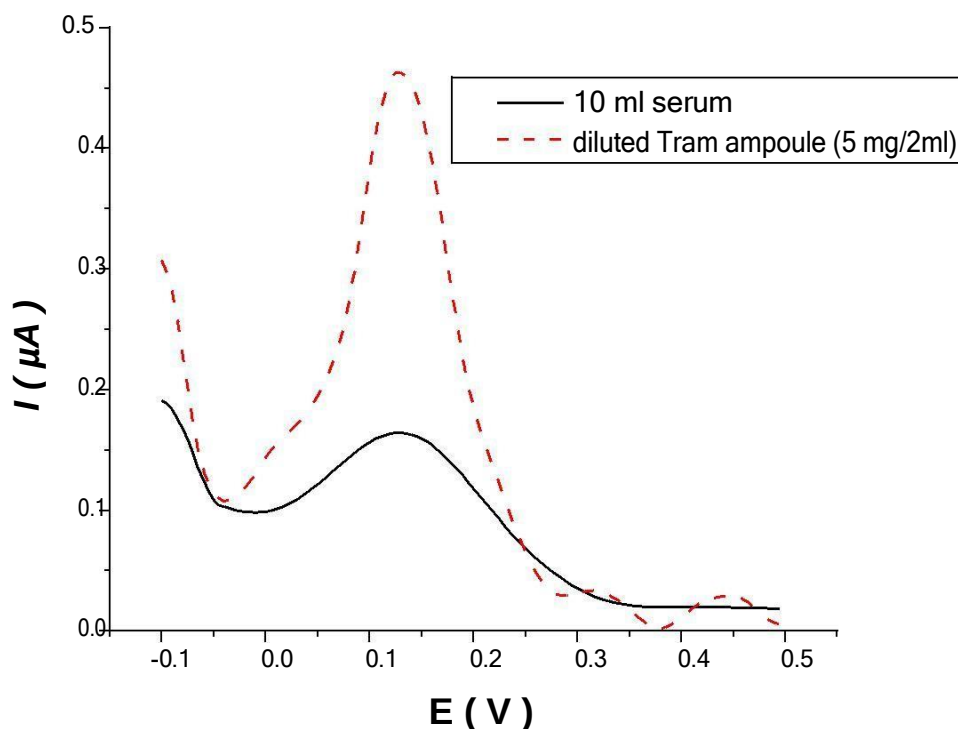
:Plot of peak currents as a function of Tram concentration.

relationship between the peak current height and concentration of Tram (Figures 4 and 5). It was found that the anodic peak currents were proportional to the Tram concentration (Zhao et al., 2009; Huang et al., 2008;

Ardakani et al., 2011; Qiu et al., 2008). The calibration curves were plotted and their parameters are shown in Table 1. Limit of detection (LOD) and quantification (LOQ) were determined according to the IUPAC protocol

Table 1. Calibration parameters of Tram at Pt/PAMADAN.

Technique	Regression equation	R ²	SD	S	LDR (μM)	LOD (μM)	LOQ (μM)
CV	$I_{pa}=2.989 + 0.083V$	0.998	0.018	0.083	100-1000	0.650	2.166
SWV	$I_{pa}=0.0083 + 0.0077V$	0.989	0.00084	0.0077	5-30	0.327	1.09

**Figure 6.** Square wave voltammograms of 5 mg/2 ml Tram in its pharmaceutical forms (Tram ampoule) and the human blood serum sample in 0.1 M H₂SO₄ at PAMDAN/Pt at a scan rate of 5 mV/s.**Table 2.** Determination of Tram in ampoule at Pt/PAMADAN.

Technique	Declared (mg/ml)	Found (mg/LI)	Recovery %
CV	50	46	92
SWV	50	52.1	104.2
HPLC	50	51.5	103

(Currie, 1995; Falciola et al., 2012):

$$\text{LOD} = 3 (\sigma_{\text{blank}}) / S$$

$$\text{LOQ} = 10 (\sigma_{\text{blank}}) / S$$

where S (indicating the method's calibration sensitivity) is the slope of the linear calibration plot and σ_{blank} is the blank standard deviation. LOD, LOQ and calibration sensitivity (S) are explained in Table 1, which shows that SWV technique possesses good LOD and LOQ as

compared to CV technique.

Detection of Tram in real sample

In order to test the practical application of the proposed methods, the PAMDAN/Pt modified electrode was applied to the analysis of Tram ampoule. The sample was determined in triplicate under the same conditions. Figure 6 illustrates the SWV of the diluted sample (dashed line). As illustrated in Table 2, the content of the Tram in the

Table 3. Determination of Tram in human serum at Pt/PAMADAN.

Technique	Spiked (mg/mL)	Found (mg/mL)	Recovery %
CV	0.16	0.16	100
SWV	0.16	0.167	104.3

Table 4. Comparisons of linear dynamic range (LDR) and detection limits (LOD) of various electrodes used for detection of Tram.

Electrode	LDR/ μ M	LOD/ μ M	Reference
GC	15-75	2.2	33
CNP-GC	10-100	5	34
	100-1000		
GC/MWCNTs	2-300	0.361	4
PAMDAN/Pt (SWV)	5-30	0.327	This work
PAMDAN/Pt (CV)	100-1000	0.650	This work

ampoule was calculated using CV and SWV as an electroanalytical method and HPLC as an analytical method. The good recoveries of the applied methods, for determination of Tram are presented in Table 2, which indicates that the determination of Tram using the modified electrode is effective and can be applied for the detection in real samples (Zhao et al., 2009; Huang et al., 2008; Ardakani et al., 2011; Qiu et al., 2008; Currie, 1995; Falciola et al., 2012; Noroozifar et al., 2010; Ensafi et al., 2009; Behera and RetnaRaj, 2007; Sivanesan and John, 2008).

In addition, the applicability of the proposed methods was also tested by measuring the concentration of Tram in the human serum sample. The analyses were performed in triplicate. Figure 6 (solid line) illustrates that SWV of the blood serum sample and the results obtained in Table 3 show that the recovery rates are acceptable and indicate that both the accuracy and repeatability of this study's proposed methods are very good. Thus, the modified electrode can be successfully used for the determination of Tram in serum samples.

The comparison on the calibration curve parameters (LOD, LOQ) of Tram at various modified electrodes (Babaei et al., 2011; Garrido et al., 2003; Bidkorbeh et al., 2010) are listed in Table 4. It was concluded that our proposed method has a satisfactory LOD compared to the listed values. Moreover, this electrochemical response has good stability as the peaks remain unchanged after 40 consecutive cyclic voltammetric scans, which means that the PAMDAN/Pt modified electrode showed good stability for the determination of Tram.

Simultaneous determination of Tram and DA

The electrooxidation processes of Tram and DA at PAMDAN/Pt in the mixture at different concentrations (in

a range from 0.01 to 0.001 M for both) was also investigated in aqueous H_2SO_4 solution, in the potential range of -0.2 to 0.8 V by SWV method, due to its higher current sensitivity and better resolution than CV (Huang et al., 2008; Ardakani et al., 2011; Qiu et al., 2008; Currie, 1995; Falciola et al., 2012; Noroozifar et al., 2010; Ensafi et al., 2009; Behera and RetnaRaj, 2007; Sivanesan and John, 2008; Badawy et al., 2010; Hathoot et al., 2012). Examination of Figure 7 shows that this modified electrode exhibits potent and persistent electron-mediating behavior followed by well-separated oxidation peaks towards Tram and DA. The peak current of Tram and DA increased with an increase in their concentrations with two well-distinguished anodic peaks at potentials of 0.18 and 0.60 V, corresponding to the oxidation Tram and DA respectively, whose separation was large enough to allow for the simultaneous determination of Tram and DA in one mixture. The calibration curves for both Tram and DA were plotted and it gave linear relation with correlation coefficient of 0.992 and 0.947 for Tram and DA respectively. The characteristics of the calibration curves of Tram individually and in mixture with DA in the solutions are presented in Table 5. The investigations showed the high efficiency of the prepared modified electrode for the simultaneous determinations in mixture.

Conclusion

In this paper, it was shown that the PAMDAN/Pt modified electrode exhibits electrocatalytic activity to oxidation of Tram. It was found that the catalytic currents increase with increasing Tram concentration in the examined range, with correlation coefficient of 0.992 in case of SWV method. The modified electrode displays good selectivity in the voltammetric measurements of Tram and DA in their mixture solutions. The peak potential separation for the oxidation of Tram and DA by SWV is

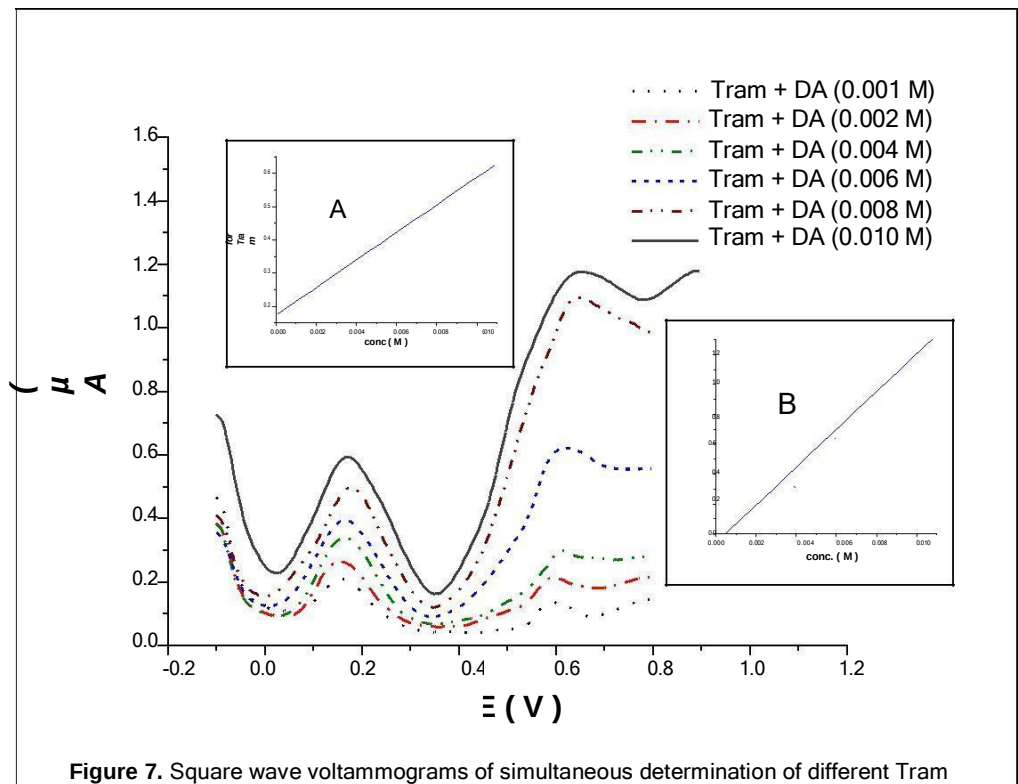


Figure 7. Square wave voltammograms of simultaneous determination of different Tram and DA concentrations at PAMDAN/Pt modified electrode at scan rate of 5 mV/s. Insets: (A) Plot of peak currents as a function of Tram concentration. (B) Plot of peak currents as a function of DA concentration.

Table 5. The characteristics of the calibration curves of Tram individually and in mixture with DA in solution at P/PAMADAN.

Drug	Tram individually	Tram in mixture
Regression equation	$I_{pa}=0.0083+0.0077C$	$I_{pa}=0.1739+41.411C$
R^2	0.989	0.992
SD	0.00084	0.1960
S	0.0077	41.411
LOD/ μ M	0.327	0.0142
LOQ/ μ M	1.09	0.04733

about 420 mV, so it is suitable for simultaneous determination. Moreover, the results obtained from the application of the proposed method for determining Tram in pharmaceutical real samples confirmed the good accuracy and precision of this study's proposed method. The proposed modified electrode is rapid, simple and low cost when compared with other reported methods. The results indicate that the modified electrode was successfully applied for determination of Tram in the presence of DA and can be used as a sensor in the biological systems.

REFERENCES

Abd El-Rahman HA, Hathoot AA, El-Bagoury M, Abdel-Azzem M (2000). Electroactive Polymer Films Formed from the Schiff Base Product of 1, 8-

Diaminonaphthalene and Dehydroacetic Acid. I. Preparation and Characterization. J. Electrochem. Soc., 147(1): 242-247.

AbdelAzzem M, Yousef US, Pierre G (1998). A cyclic voltammetric and coulometric study of a modified electrode prepared by electrooxidative polymerization of 1, 5-diaminonaphthalene in aqueous acidic medium, Eur. Polym. J., 34: 819- 26.

Ardakani MM, Beiollahi H, Amini MK, Mirjlili BF, Mirkhalaf F (2011). Simultaneous determination of epinephrine and uric acid at a gold electrode modified by a 2-(2, 3-dihydroxy phenyl)-1, 3-dithiane self-assembled monolayer. J. Electroanal. Chem., 651: 243-249.

Atia A, Hathoot AA, Abdel-Azzem M (2008). Studies of the Electrochemical Behavior of Poly 8-(3-acetylmino-6-methyl-2,4-dioxopyran)-1-aminonaphthalene Films Doped with an Anionic Surfactant. Int. J. Electrochem.

- Sci., 3: 1512-1522.
- Babaei A, Taheri AR, Afrasiabi M (2011). A multi-walled carbon nanotube-modified glassy carbon electrode as a new sensor for the sensitive simultaneous determination of paracetamol and tramadol in pharmaceutical preparations and biological fluids. *J. Braz. Chem. Soc.*, 22(8): 1549-1558.
- Badawy WA, El-Ries MA, Mahdi IM (2010). Electrochemical determination of some antidiabetic drugs for type 2 diabetic patients. *Talanta*. 82 (1) 106-112.
- Behera S, RetnaRaj C (2007). Self-assembled monolayers of thio-substituted nucleobases on gold electrode for the electroanalysis of NADH, ethanol and uric acid. *Sensors Actuat. B*, 128: 31-38.
- Bidkorbeh FG, Shahrokhian S, Mohammadi A, Dinarvand R (2010). Simultaneous voltammetric determination of tramadol and acetaminophen using carbon nanoparticles modified glassy carbon electrode. *Electrochim. Acta*. 55 2752-2759.
- Currie LA (1995). Nomenclature in evaluation of analytical methods including detection and quantification capabilities. *Pure Appl. Chem.*, 67(10): 1699-1723.
- Diab AS, Hathoot AA, Abdel-Azzem M, Merz A (2000). Preparation of a novel conducting polymer by electropolymerization of thiophenylidene 8-naphthylamine Schiff-base, *Eur. Pol. J.*, 36: 1959-1965.
- Ensafi AA, Rezaei B, Amini M, Bafrooei EH (2012). A novel sensitive DNA-biosensor for detection of a carcinogen, Sudan II, using electrochemically treated pencil graphite electrode by voltammetric methods. *Talanta*, 88: 244-251.
- Ensafi AA, Taei M, Khayamian T (2009). A differential pulse voltammetric method for simultaneous determination of ascorbic acid, dopamine, and uric acid using poly (3-(5-chloro-2-hydroxyphenylazo)-4, 5-dihydroxynaphthalene-2,7-disulfonic acid) film modified glassy carbon electrode. *J. Electroanal. Chem.*, 633: 212-220.
- Falciola L, Pifferi V, Possenti ML, Carrara V (2012). Square Wave Voltammetric detection of furan on platinum and platinum-based Screen Printed Electrodes, *J. Electroanal. Chem.*, 664: 100-104.
- Garrido EMPJ, Garrido JMPJ, Borges F (2003). Development of electrochemical methods for determination of tramadol-analytical application to pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.*, 32: 975-981.
- Gong W, Dou ZY, Liu P, Cai XY, He XQ (2012). Simultaneous determination of dopamine, ascorbic acid by polyethylene oxide (PEO) covalently modified glassy carbon electrode. *J. Electroanal. Chem.*, 666: 62-66.
- Hadi M, Rouhollahi A (2012). Simultaneous electrochemical sensing of ascorbic acid, dopamine and uric acid at anodized nanocrystalline graphite-like pyrolytic carbon film electrode. *Anal. Chim. Acta.*, 721: 55-60.
- Hathoot AA (2000). Electro-oxidative polymerization of Schiff-base of 1, 8-diaminonaphthalene and 3-acetylthiophene. I. Preparation and study the redox behaviour of the resulting polymer. *Eur. Pol. J.*, 36: 1063-1071.
- Hathoot AA, AbdelKader M, AbdelAzzem M (2009). Platinum Modified Poly 4-Aminoquinoline Film Electrodes and Their Electrocatalytic Application in Oxidation of Small Organic Molecules. *Int. J. Electrochem. Sci.*, 4: 208-222.
- Hathoot AA, Sherif D, AbdelAzzem M (2010). Composite Films of Metal Hexacyanoferrate and Poly (1, 8-diaminonaphthalene) Derivatives: Electrosynthesis and Properties. *Croatia Chem. Acta.*, 83(2): 143-149.
- Hathoot AA (2003). Electrochemical Behavior of Poly 8-(3-Acetylmino-6-methyl 2, 4-dioxopyran)-1-aminonaphthalene in Aqueous and Non Aqueous Media. *J. Bull. Korean Chem. Soc.*, 24(11): 1609-1612.
- Hathoot AA, Youssef US, Shatla AS, AbdelAzzem M (2012). Voltammetric simultaneous determination of glucose, ascorbic acid and dopamine on glassy carbon electrode modified by NiNPs@poly 1, 5-diaminonaphthalene. *Electrochim. Acta*. 85 531-537.
- Huang X, Li Y, Wang P, Wang L (2008). Sensitive Determination of Dopamine and Uric Acid by the Use of a Glassy Carbon Electrode Modified with Poly (3-methylthiophene)/Gold Nanoparticle Composites. *Anal. Sci.*, 24(12): 1563-1568.
- Ismail KM, Khalifa ZM, AbdelAzzem M, Badawy WA (2002). Electrochemical preparation and characterization of poly (1-amino-9, 10-anthraquinone) films, *Electrochim. Acta.*, 47(12): 1867-1873.
- Karen M, Lesley JS (2003). Tramadol/Paracetamol. *Drugs*, 63(11): 1079-1086.
- Lu D, Zhang Y, Wang L, Lin S, Wang C, Chen X (2012). Sensitive detection of acetaminophen based on Fe₃O₄ nanoparticles-coated poly (diallyldimethylammonium chloride)-functionalized graphene nano composite film. *Talanta*, 88: 181-186.
- Mo JW, Ogorevc B (2001). Simultaneous Measurement of Dopamine and Ascorbate at Their Physiological Levels Using Voltammetric Microprobe Based on Overoxidized Poly (1, 2-phenylenediamine)-Coated Carbon Fiber. *Anal. Chem.*, 73(6): 1196-202.
- National Corporation of Pharmacies (2006). Sweden (Apoteket AB) Sale statistics 2006.
- Noroozifar M, Motlagh MK, Taheri A (2010). Preparation of silver hexacyanoferrate nanoparticles and its application for the simultaneous determination of ascorbic acid, dopamine and uric acid *Talanta*, 80: 1657-1664.
- Qiu P, Chen X, Chen X, Lin L, Ai C (2008). Simultaneous determination of five toxic alkaloids in body fluids by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Chromatography B.*, 875(1): 471-477.

- Ramiz MM, Hassan KM, Khudaish EA, Deronzier A, Chardon-Noblat S, AbdelAzzem M (2010). Anodic polymerization of 1-amino-2-methyl-9, 10-anthraquinone in mixed solvent. *J. Electroanal. Chem.*, 647: 35-42.
- Scott LJ, Perry CM (2000). Tramadol: A Review of its Use in Perioperative Pain. *Drugs*, 60(1): 139-176.
- Sivanesan A, John SA (2008). Selective Electrochemical Epinephrine Sensor Using Self-Assembled Monomolecular Film of 1, 8, 15, 22-Tetraaminophthalocyanatonickel (II) on Gold Electrode. *Electroanal.*, 20: 2340-2346.
- Wightman RM, May LJ, Michael AC (1988). Detection of Dopamine Dynamics in the Brain, *Anal. Chem.*, 60(13): 769A-793A.
- Yousef US, Hathoot AA, Abdel-Azzem M (2001). Electrochemical synthesis and characterization of poly [8-(3-acetylimino-6-methyl-2, 4-dioxopyran)-1-aminonaphthalene] film electrodes in acidic aqueous medium. *Eur. Pol. J.*, 37: 1267-1276.
- Yousef US, Ragab AZ, AbdelAzzem M (2010). A new modified electrode based on electrochemical oxidation of 1-amino-5, 6, 7, 8-tetrahydronaphthalene in acetonitrile, *Electrochem. Acta*, 55: 1509-1518.
- Zhao C, Wan L, Wang Q, Liu S, Jiao K (2009). Highly Sensitive and Selective Uric Acid Biosensor Based on Direct Electron Transfer of Hemoglobin-encapsulated Chitosan-modified Glassy Carbon Electrode. *Analytical Sci.*, 25(8): 1013-1017.