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Electrochemical Determination of Tramadol in Ampoule Dosage Forms by Cyclic Voltammetry

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A voltammetric study was performed to examine the electroactive behaviour of tramadol hydrochloride. Tramadol was analyzed with cyclic voltammetry (CV) method in the concentration range 10-100 µg/mL (0.0336-0.33 mM) with a detection limit 5 µg/mL (0.0168 mM). The developed and validated method was successfully applied to the analysis of commercial ampoules. Recovery values were between 89 and 92 %. Developed electroanalytical method in this study was accurate, sensitive, precise and reproducible and could be directly and easily applied to pharmaceutical forms. Besides, the statistical results were compared with that of the data obtained from previous UV study. The reaction mechanism of this compound using cyclic voltammetric measurements at different scan rates was also tried to be explained by the electrochemical behaviour of tramadol.

Key Words: Tramadol HCl, Cyclic voltammetry, Electrochemical detection, Validation.

INTRODUCTION

Tramadol [tramadol hydrochloride: (1RS,2RS)-2-(dimethylamine)methyl-1-(3-methoxyphenyl)-cyclohexanol HCl] is among the typical central-acting opioids and is a synthetic 4-phenyl-piperidine analogue of codeine[1]. Tramadol, a synthetic opioid of the aminocyclohexanol group (Fig. 1), is an analgesic agent widely used in the treatment of chronic pain[2]. Tramadol was developed by the German pharmaceutical company Grünenthal GmbH and marketed under the trade name Tramal. Tramadol which is the efficient agent of the drug of which is a central acting analgesic is usually marketed as the hydrochloride salt (tramadol hydrochloride). Tramadol which is injected into vessel in the form of 100 mg ampoules and used to relieve the pain after the surgery has a strong pain-killer effect between codeine and morphine[1,2]. The marketed formulations of tramadol contain a racemic mixture of two enantiomers

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(50:50), each one displaying differing affinities for various receptors[1]. The drug is available in formulations suitable for oral, rectal and parenteral (IM and IV) administration[3,4].

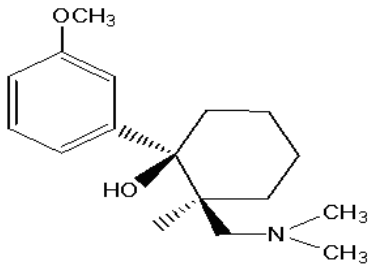

Fig. 1. Chemical structure of tramadol ($C_{16}H_{25}O_2N$·HCl)

Several Methods for determination of tramadol content in pharmaceutical preparations and plasma have been reported. These include GC[5-10], spectrophotometric[11,12], HPLC[13-22], chiral liquid chromatographic[23,24], capillary electrophoresis[25,26], LC-MS-MS[27] and GC-MS[28] methods in literature.

Although electrochemical methods have already proved to be very useful in the determination of pharmaceuticals due to their simplicity, low cost and relatively short analysis time when compared with other methods. It has been reported an electrochemical method in the literature. In this study, it had been investigated on the electrochemical behaviour of tramadol at a glassy carbon electrode and also a square-wave voltammetric (SWV) method and flow injection analysis system with amperometric detection had been developed for the determination of tramadol hydrochloride in pharmaceutical dosage forms[29].

Voltammetric methods are distinctly limited to easily reducible or oxidizable compounds. Cyclic voltammetry (CV) has become increasingly popular in all fields of chemistry as a means of studying redox states. The CV method enables a wide potential range to be scanned rapidly for reducible or oxidizable species. Oxidation of the electroactive species occurs at the electrode. This capability together with its variable time scale and good sensitivity make this the most versatile electro analytical technique[30]. The functional groups which show excellent voltammetric properties include the nitro, nitroso, quinine, azo, azoxy, azomethine, activated carbonyls and activated double bonds[31].

We are aimed to develop a voltammetric method which is sensitive, easy to apply and rapid and also make possible to determination of tramadol in pharmaceutical dosage forms. In this paper, we have achieved a direct electrochemical method for the determination of tramadol, its application to pharmaceutical forms and the comparison with the previous UV study[12]. At the same time, the voltammograms which has obtained at different scan rates and the reaction mechanism of tramadol has been tried to be found out by comparing the current functions.

The validation of method was carried out by establishing specifity, linearity, recovery values, the detection limit (LOD), the quantification limit (LOQ), intra-day and inter-day precision and accuracy according to International Conference on Harmonization guidelines for validation of analytical procedures[32]. The results obtained from these assay were statistically compared with t test.

EXPERIMENTAL

Tramadol standard was kindly provided by Grünenthal (Aachen, Germany). The dosage forms containing tramadol (ampoules containing 100 mg tramadol hydrochloride in 2 mL) were usually available the trade name of contramal on the market and this drug were obtained from Department of Anaesthesia (Faculty of Medicine, Ataturk University, Erzurum-Turkey).

The purity control of the certified tramadol standard was tested with the melting point (182.5 °C), UV, ^{1}H NMR and ^{13}C NMR spectra and it was decided that the results were appropriate according to the literature.

All chemicals used were analytical reagent grade. Acetonitrile was purified by drying with calcium hydride, followed by distillation from phosphorus pentoxide and then it was kept under molecular sieves in order to eliminate its water content as much as possible. 0.1 M lithium perchlorate ($LiClO_4$) used as supporting electrolyte was prepared in anhydrous acetonitrile.

Electrochemical: The voltammetric studies were carried out on BAS 100 B/W Electrochemical Analyser system equipped with a low current module (BAS PA-1). Cyclic voltammetric measurements were made at room temperature in an undivided cell (BAS model C3-cell stand). A three-electrode system consisting of a working electrode (0.068 cm^2) and a platinum wire counter electrode were used. All potentials were reported with respect to Ag/AgCl/KCl (3.0 M) reference electrode (BAS MF 2063). The working electrode was polished sweeping with an alumina paste (10 μm), rinsing by piranha solution (H_2O_2:H_2SO_4, 1:3) and then by double distilled water and acetonitrile for several times and then it was dried. The reference electrode was only cleaned by double distilled water. This procedure was always repeated before each experiment. Then, the electrodes were dipped into 10 mL supporting electrolyte solution containing tramadol in the voltammetric cell and the experiments were conducted. Voltammograms obtained for all the concentrations in calibration graphs were taken at the scan rate of 100 mV s^{-1}.

Preparation of standard solutions: A stock solution of tramadol to 200 μg/mL concentration was prepared in 0.1 M $LiClO_4/CH_3CN$. The standard solutions in 10, 25, 35, 50, 65, 75, 85 and 100 μg/mL concentrations were daily prepared by diluting the stock solution to a constant volume with same solutions. The most appropriate study range has been determined as 10-100 μg/mL, as no current has been observed in the chosen concentration study range of 1-10 μg/mL. The solutions were deoxygenated by passing dry nitrogen through the solution for 5 min prior to the experiment and during the experiments the flow was maintained over the solution. The quality

control samples (QC) were prepared at 25, 50 and 75 µg/mL concentrations from stock solution of tramadol. These samples were used in analysis of standard samples as quality controls for the purpose of checking recovery of analyte in the daily analyses of standard samples.

Sample preparation: Drug samples from 2 mL contramal ampoules containing 100 mg tramadol were prepared by diluting with the supporting electrolyte solution in 25 and 50 µg/mL concentrations. All solutions were filtered and then stirred while purging with oxygen-free nitrogen.

RESULTS AND DISCUSSION

Voltammetric analysis of tramadol: The oxidation behaviour of the tramadol was investigated in acetonitrile solutions containing 0.1 M $LiClO_4$ at Pt electrode. When the potential range is scanned starting at the initial potential, E_i = 500 mV and ending at the final potential, E_f = 1500 mV, the typical cyclic voltammogram for tramadol of 100 µg/mL in acetonitrile is showed only one oxidation peak (A) for all scan rates as shown in Fig. 2. During the return scan, no cathodic peak corresponding to the reduction of the first wave is appeared at 10 mV s^{-1} scan rate. However, as scan rate is increased up to 100 mV s^{-1}, the oxidation of the tramadol becomes partially reversible. An analysis of the scan rate dependence of the CV suggested that the reaction product at low scan rates is not completely stable within the time scale of experiment.

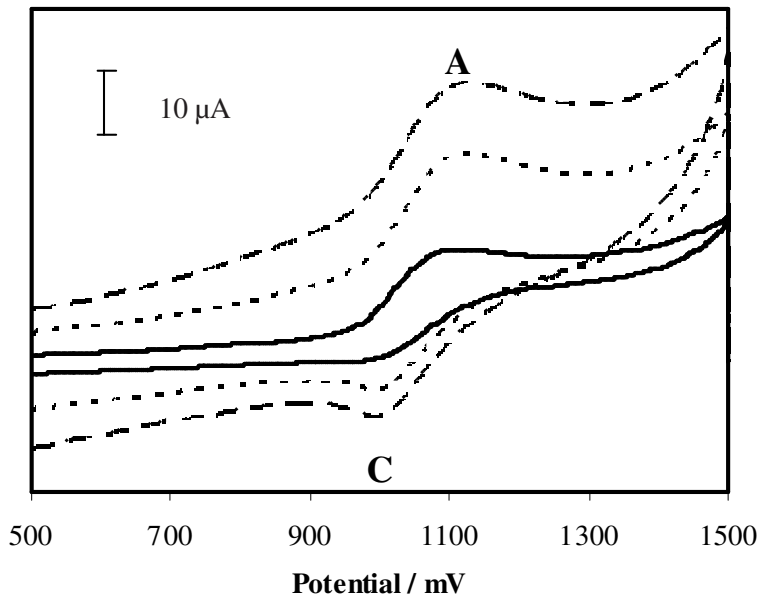

Fig. 2. Cyclic voltammograms obtained for tramadol of 100 µg/mL in 0.1 M $LiClO_4$ + acetonitrile at 10 (—), 50 (·····) and 100 (----) mV s^{-1} scan rates

The amplitude of the anodic peak current (I_{pi}) for the peak A increased linearly with the square root of the scan rate ($\nu^{1/2}$) indicating that the redox species was freely diffusing in solution (Fig. 3). Peak current (A) is linearly related to concentration in the range of 10-100 μg/mL. It was pointed out that the anodic peak current was rather big compared to cathodic peak current and the peak A could be used for analytical measurements.

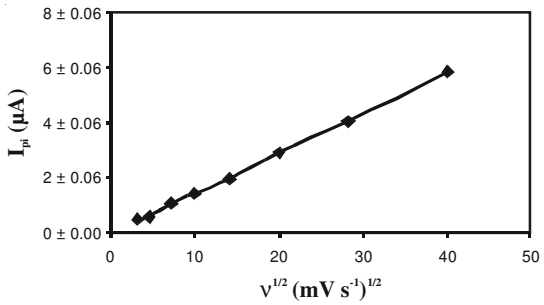

Fig. 3. Linear dependence of the peak current on the square root of the scan rate for 50 μg/mL concentration of tramadol

In addition, for such a system the current function ($I_{pi}/\nu^{1/2}$.C) decrease with $\nu^{1/2}$ (Fig. 4) and the peak potential of the primary oxidation wave (E_{pA}) shifted toward more positive potentials as scan rate (ν) increased as shown in Fig. 2. On the basis of the experimental results, it can be suggested that first an electron transfer then an irreversible chemical reaction, in other words, an EC type mechanism.

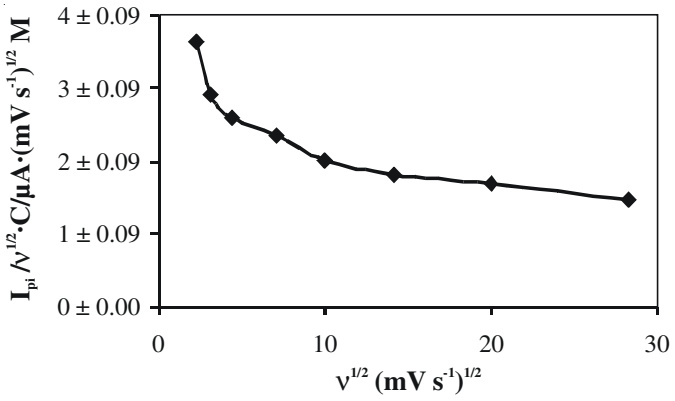

Fig. 4. Plot of $I_{pi}/\nu^{1/2}$ C function *versus* $\nu^{1/2}$ for 50 μg/mL concentration of tramadol

Linearity of calibration curves: The diffusion peak currents obtained have been recorded the concentration ranges between 10 with 100 μg/mL and the diagram of them has been drawn *versus* the concentrations of tramadol (Fig. 5). The peak current increased with increasing drug concentration from 10 to 100 μg/mL. The

calibration curve were established by plotting the peak currents *versus* tramadol concentrations (n = 6). The regression equation and the correlation coefficient, R was y = 1.891 x - 19.251 (y: peak currents, x: the concentration of tramadol) and 0.9912, respectively. The precision of calibration curve was expressed as RSD and the RSD values were found to be 3.44-9.20 % (except from LOQ value). The RSD values for intra-day and inter-day measurements have also been monitored ≤ 9.20 %.

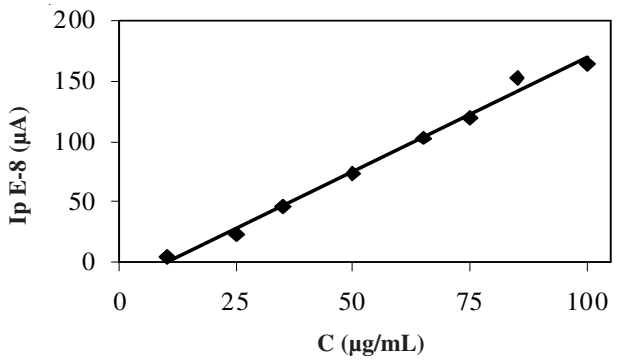

Fig. 5. Plot of the variation of the peak currents *versus* concentrations

Precision and accuracy: Repeatability was evaluated by assaying samples, at same concentration and during the same day. Assay precision and accuracy were assessed by assaying three quality control samples (25, 50 and 75 µg/mL) in six replicate on one day for intra-day precision and once daily for 6 days for inter-day precision. Concentrations of tramadol in quality control samples were determined by application of the appropriate standard curve obtained on that occasion. The intra-day relative standard deviation (RSD) were < 8.0 % (n = 6) and the inter-day relative standard deviation (RSD) were < 8.5 % (n = 6). Precision studies of CV method showed acceptable RSD values. The accuracy of this analytical method for assay determination was checked in same three concentration levels and relative errors for accuracy were < 5.0 %. These results also indicated a good reproducibility of this method (Table-1).

TABLE-1
RESULTS OF THE INTRA- AND INTER-DAY PRECISION ASSAYS OBTAINED FROM TRAMADOL THE CV METHOD (n = 6)

Precision	Intra-day			Inter-day		
Concentration (µg/mL)	Peak current, mean value ($\overline{X}$, Amper)	Standard deviation (SD)	RSD (%)	Peak current, mean value ($\overline{X}$, Amper)	Standard deviation (SD)	RSD (%)
25	4.50×10^{-7}	3.5×10^{-8}	7,77	3.37×10^{-7}	2.70×10^{-8}	8.18
50	1.08×10^{-6}	7.5×10^{-8}	6.97	7.88×10^{-7}	3.07×10^{-8}	3.90
75	2.00×10^{-6}	5.1×10^{-8}	2.59	1.47×10^{-6}	1.03×10^{-7}	7.02

Limit of detection (LOD) and limit of quantification (LOQ): The LOQ was defined as the lowest concentration on the calibration curve that presented a RSD that did not exceed 10 % and the LOD was defined as the lowest concentration that presented a RSD that did not exceed 20 %. LOD and LOQ values of method were found to be 10 and 5 μg/mL, respectively. In other words, the LOD was found to be 0.0168 mM with a minimum detectability.

Recovery: The accuracy was determined by recovery of known amounts of tramadol reference standard added the tablet samples at the beginning of the process. For recovery study, the tablet solutions according to the procedure described at in sample preparation were prepared. The tablet solutions to 25 and 50 μg/mL concentrations were transferred in tramadol standard solution to 10 μg/mL concentration. The final concentrations of these solutions were 25 and 50 μg/mL. The peak currents formed the straight equation from the voltammograms of solutions prepared was found. The per cent recovery of tramadol was calculated by comparing the found and added concentrations ($C_{found}/C_{added} \times 100$) in each case. The mean recoveries were found to be 89.0 and 92.0 %.

Statistical analysis of the results: The suggested cyclic voltammetric method was applied to the quantitative determination of tramadol in commercial ampoules. The results obtained from proposed method was evaluated statistically by the student's t tests (Tables 2 and 3) and also these results were statistically compared with reference method in literature[12]. The validity of the methods was tested. According to the results of the student's t-tests of 95 % confidence level were observed no significant differences between the performance of these two methods with regards to accuracy and precision of UV and CV methods as shown in Tables 2 and 3 ($p < 0.05$).

TABLE-2
APPLICATION OF THE PROPOSED CV METHOD FOR THE DETERMINATION OF TRAMADOL IN AMPOULES

Statistical values	25 μg/mL	50 μg/mL	t-test
Number of determination (n)	10	10	
Mean recovery* ($\overline{X}$, %)	89.30	92.14	t_c : 3.264
Standard deviation* (SD, %)	7.767	8.219	t_t : 2.556
Standard error (%)	2.518	2.049	

*Mean and standard deviations of ten determinations for samples, t_c; calculated t-value, t_t; tabulated t-value.

TABLE-3
APPLICATION OF THE PROPOSED UV METHOD FOR THE DETERMINATION OF TRAMADOL IN AMPOULES

Statistical values	Methanol	Water	t-test
Number of determination (n)	10	10	
Mean recovery* ($\overline{X}$, %)	100.931	89.120	t_c : 2.776
Standard deviation* (SD, %)	9.928	9.083	t_t : 1.734
Standard error (%)	3.140	2.872	

*Mean and standard deviations of ten determinations for samples, t_c; calculated t-value, t_t; tabulated t-value.

Conclusion

In this study, the reaction mechanism of compound has been studied and it has come out that there is a completely diffusion-controlled current process which isn't affected by adsorption phenomenon[4,5]. It has been also concluded that the peaks which have been observed in voltammogram can be used for the purpose of quantitative analysis. As a conclusion, the proposed method is rapid, simple, accurate, reproducibility and convenient since it do not require any special working conditions. The advantage of this alternative voltammetric method for analytical purposes lies in the simple and rapid determination of tramadol that is said to be electrochemically active. For this reasons, it can be used for determination from pharmaceutical preparations of tramadol in routine quality control measurement, where economy and time are essential.

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