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Electrooxidation and Determination of Tramadol at Mixed Ion Pair Chemically Modified Carbon Paste Electrode

Sayed S. Badawy, M. A. Sultan, Yomna M. Ahmed

Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt

Summary: A new chemically modified carbon paste electrode (CMCPE) based on mixed ion-exchangers tramadol-reineckate (TR-Rn) and tramadol-tetraphenyl borate (TR-TPB) was designed for the sensitive determination of tramadol hydrochloride (TR-HCl) in pure solutions and in pharmaceutical preparations. The electrooxidation of TR-HCl was achieved by cyclic voltammetry. The effect of buffer solution (pH), potential scan rate and TR-HCl concentration on the voltammetric performance were investigated. The sensor showed high selectivity towards TR-HCl over many common drug excipients. This modified electrode was successfully and effectively applied for TR-HCl determination in pharmaceutical dosage forms, without any sample pretreatment, utilizing cyclic voltammetry and potentiometric titration techniques with satisfactory results and excellent percent recovery values.

Several medicines are categorized as opioid analgesics drugs and may lead to addiction. One of the most misused as addiction drugs is tramadol. Tramadol hydrochloride (TR-HCl), trans-(±)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is commonly used to relieve postoperative pain, moderate surgical pain, cancer pain control, obstetric pain and chronic pain [(1)]. Its structure can be written as shown in Fig. (1).

The literature reveals various methods for the determination of Tramadol in biological fluids and pharmaceutical preparations. Among these methods are high performance liquid chromatography (HPLC) [(2)], liquid chromatography-mass spectrometry (LC-MS) [(3)], gas chromatography (GC) [(4)], capillary electrophoresis [(5)] and spectrophotometry [(6)]. These instrumental techniques are too expensive and require time consuming procedures and qualified personnel. They include derivatization procedures, extraction steps and are not applicable to colored or turbid sample solutions. Thus, there is an urgent need for the development of direct inexpensive method for drug analysis. Electrochemical methods [(7,8)], have proven to be very useful for drug analysis due to their simplicity, reliability, sensitivity and relatively short analysis time compared to other techniques.

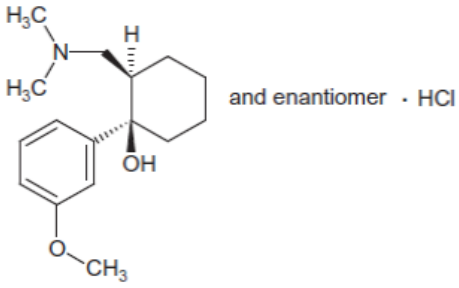


Fig. 1. The chemical structure of tramadol hydrochloride

Carbon paste, i.e., a mixture of carbon (graphite) powder and a binder (pasting liquid), has become one of the most popular electrode materials used for the laboratory preparation of various electrodes, sensor and detectors [9]. They are characterized by their ease of preparation, surface renewal capabilities, stability and fast response, reasonable selectivity, low background current, wide range of used potential, applicability to colored and turbid solutions and possible interface with automated and computerized systems.

In the present work, a chemically modified carbon paste electrode (CMCPE) incorporating 2.8% w/w tramadol-reineckate (TR-Rn) and 1.2% w/w tramadol-tetraphenyl borate (TR-TPB) as a mixed ion-exchanger, and dibutylphthalate (DBP) as a liquid binder was fabricated and has been successfully used for the determination of TR-HCl in both pure solutions and pharmaceutical preparations using cyclic voltammetric technique.

Experimental

Materials and reagents

All reagents used in the present investigation were of analytical grade. Double distilled water was used throughout all experiments. Tramadol hydrochloride (TR-HCl, M.wt = 299.8 g/mol) and its pharmaceutical preparations (Tramadol capsules, 50 mg/capsule and Tramadol tablets, 225 mg/tablet) were obtained from October pharma, Cairo, Egypt. The ion-exchangers (TR-Rn and TR-TPB) and carbon paste electrodes were prepared as described elsewhere [10,11].

Britton Robinson (BR) buffer solutions covering the pH range of 2.96 - 7.82, and phosphate buffer in the pH range of 5.29 – 8.04 were prepared as recommended elsewhere [12,13]. Working solutions were prepared from a stock concentrated solution (1×10^{-2} M) of pure TR-HCl by appropriate dilution. For the analysis of pharmaceutical preparations, the content of five tramadol capsules or tablets were well grinded and an average weight of this powder equivalent to one capsule or tablet was dissolved in a proper volume of the solvent. The insoluble residues were filtered out and removed.

Apparatus

Voltammetric measurements were carried out using a VoltaLab 06 (PST 006 and Voltamaster 4) Potentiostat. Ag|AgCl|3M KCl and platinum wire were used as the reference and the auxiliary electrodes, respectively. Chemically modified carbon paste electrode (CMCPE) was the working electrode. All electrochemical measurements were carried out in a one-compartment voltammetric cell. Potentiometric and pH measurements were performed using a JENWAY pH-millivoltmeter.

Results and discussion

Electrode composition

Electrode composition and paste ingredients are important parameters that affect the electroanalytical performance of the indicator electrodes. For this reason, several electrodes of different compositions were constructed and their emf against log [TR-HCl] were measured, in order to choose the optimum composition displaying Nernstian behavior. Electrodes incorporating pastes with and without ion-exchanger were constructed. The unmodified CPE (binary mixture of graphite powder and pasting liquid) displayed no measurable response towards TR-HCl. Thus, modification to the CPEs was necessary. Different CMCPEs were constructed using DBP as pasting liquid and varying percentages of the ion-exchanger (TR-Rn and / or TR-TPB). The results showed that response of the electrodes containing only one type of the previously mentioned ion-exchangers was not promising, but when mixing the two ion-exchangers together, there was an obvious improvement in the electrode response toward TR-HCl ions. The electrode showing the best Nernstian response (57 mV/decade) was the one containing (2.8 % TR-Rn + 1.2 % TR-TPB) mixed ion-exchanger.

Effect of electrode composition on the electrochemical behavior of TR-HCl

Cyclic voltammograms (CVs) of 10 mM TR-HCl solution in Britton Robinson buffer pH 6.98 were recorded at 100 mV/s scan rate using unmodified carbon paste electrode along with five other chemically modified ones. The compositions of the modified electrodes are shown in Table (1).

Table 1: Compositions of modified carbon paste electrodes used in the analysis of the voltammetric behavior of TR-HCl

Sensor	(Ion-exchanger % w/w)/CPE	Graphite % w/w	Liquid binder
I	(4% TR-Rn)/CPE	96	DBP
II	(4% TR-TPB)/CPE	96	DBP
III	(2% TR-Rn + 2% TR-TPB)/CPE	96	DBP
IV	(1.2% TR-Rn + 2.8% TR-TPB)/CPE	96	DBP
V	(2.8% TR-Rn + 1.2% TR-TPB)/CPE	96	DBP

As shown from the cyclic voltammograms in Fig. (2), the TR-HCl electrochemical behavior was greatly affected by the composition of working electrode. No peaks were detected using the unmodified carbon paste electrode, the same behavior was observed when the paste was modified with 4% w/w TR-Rn ion-exchanger (sensor I), but the current increased. One peak corresponding to TR-HCl anodic oxidation appeared at 974 mV using sensor II. Mixed ion-exchanger modified electrode represented by sensor IV showed an oxidation peak at 945 mV and its current was slightly decreased. When mixing the two types of ion-exchangers by equal weights (sensor III) the oxidation peak was shifted to the left at 910 mV with more reduced peak current. The peak appeared at lower potential of 844 mV with greatly increased current due to the enhanced electron transfer process on using the mixed ion-exchanger electrode in sensor V; indicating the synergetic effect of the two ion-exchangers at this percentage. This behavior agrees with potentiometric data where (2.8% TR-Rn + 1.2% TR-TPB)/CPE showed Nernstian slope of 57 mV/decade.

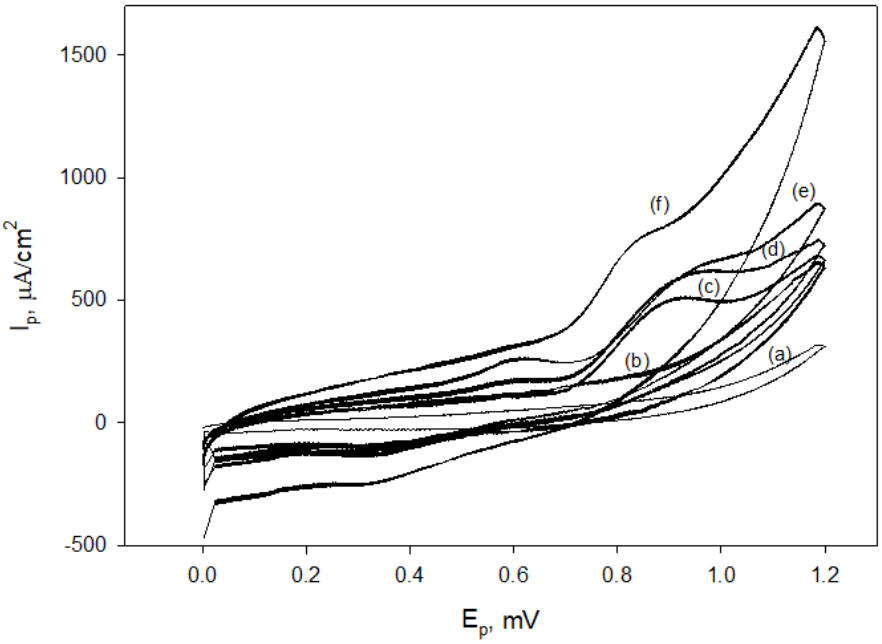
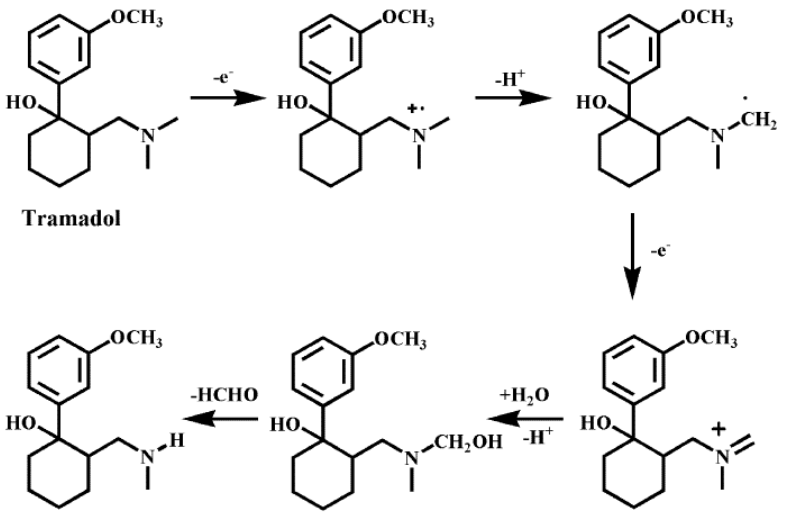


Fig. 2. CVs of 10 mM TR-HCl in pH 6.98 BR buffer and scan rate 100mV/s at unmodified CPE (a), 4% TR-Rn/CPE (b), 2% TR-Rn + 2% TR-TPB/CPE (c), 1.2% TR-Rn + 2.8% TR-TPB/CPE (d), 4% TR-TPB/CPE (e) and 2.8% TR-Rn + 1.2% TR-TPB/CPE (f)

From the obvious electrochemical behavior noticed in the cyclic voltammograms above and from previous studies [7,8], the oxidation mechanism of tramadol hydrochloride can be deduced.

Scheme 1. The probable mechanism of tramadol oxidation



Effect of support electrolyte type and pH

In order to optimize the anodic oxidation of TR-HCl, CVs were recorded in two different types of supporting electrolytes (BR buffer and phosphate buffer) with different pH values. The results are summarized in Table (2) which shows that, the peak current density values (I_p) in both buffers were increased with increasing the pH. On the other hand, the peak potential (E_p) was shifted to less positive values upon increasing the solution's pH with a slope -41.8 mV/pH in case of BR buffer and -43.6 mV/pH in case of phosphate buffer which is close to the expected value of −59 mV/pH for involving same number of electron and proton coupled electron transfer process [11].

Table 2. Supporting electrolytes' pHs and the corresponding anodic peak currents and potentials.

Britton Robinson buffer (BR)			**Phosphate buffer (PB)**		
pH	I_p (µA/cm^2)	E_p (mV)	pH	I_p (µA/cm^2)	E_p (mV)
2.96	440.8	1000	-	-	-
4.46	470.0	959	-	-	-
5.00	555.0	917	5.29	239	890
5.89	647.0	882	6.24	450	850
6.98*	759.0	844	7.17	825	810
7.82	710.0	799	8.04	910	770

*Optimum pH

Figs. (3 a and b) represent a graphical relation between pH and anodic peak potential (E_{pa}), and pH against peak current (I_{pa}), respectively. The linear regression equations in BR buffer and phosphate buffer are (E_{pa} = -41.8 pH + 1131) and (E_{pa} = -43.6 pH + 1121), respectively. It has been noticed that in the case of using phosphate buffer with pHs 7.17 and 8.04, the anodic peak current density appears to have a large value, but the problem is that the peak appears to merge with oxygen evolution region leading to a difficulty in locating the peak corresponding to TR-HCl oxidation. For this reason, it was found that Britton Robinson buffer of pH 6.98 is more suitable as a supporting electrolyte medium for TR-HCl catalytic oxidation; as the anodic peak in this case is more well defined and easy to locate, and it relatively has a large current density of 759 µA/cm^2.

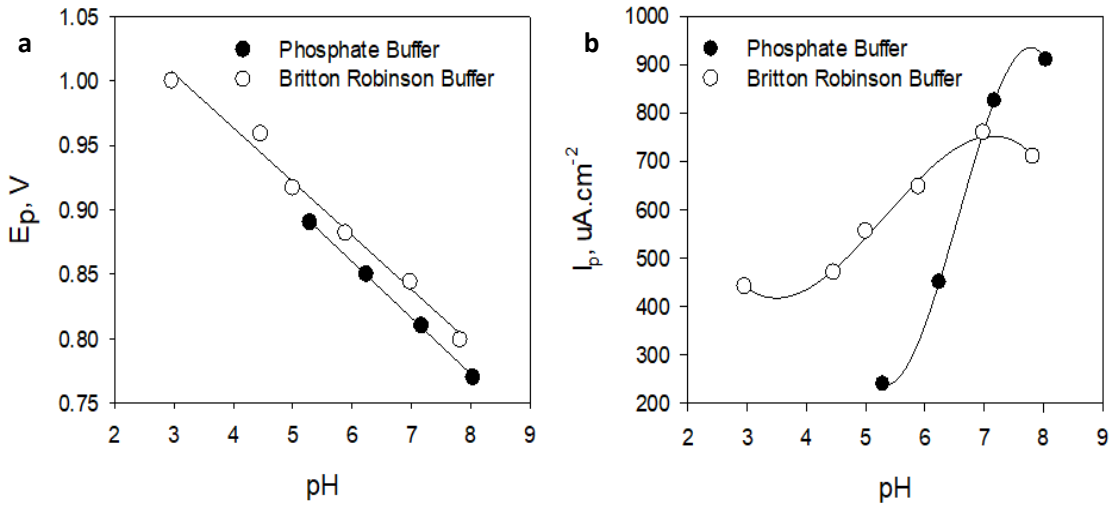


Fig. 3. Effect of pH of Britton Robinson buffer and phosphate buffer on, oxidation peak potential (a), and oxidation peak current (b)

Effect of scan rate (v)

The effect of potential scan rate on TR-HCl electrooxidation process at the modified carbon paste was studied. These experiments were performed in BR buffer with pH 6.98 containing 10 mM TR-HCl at scan rates range of 10-175 mV/s. It was found that, with increasing the scan rate, the height of I_p increases, according to the equation [$I_p = 2.99 \times 10^5$ n $(\alpha n_\alpha)^{1/2}$ A $D^{1/2}$ $v^{1/2}$ C], which states that for an irreversible electron transfer reaction, I_p is directly proportional to the square root of the scan rate $v^{1/2}$ [14]. In addition, E_p is slightly shifted to more positive values which may be attributed to the charge transfer kinetics arising from chemical interaction between the electrolyte ions and the modified electrode i.e., the IR drop effect generated at high current density values [15].

Variation of the TR-HCl oxidation peak current density and peak potential with the square root of scan rate are represented in Figs. (4 a and b), respectively. Both the TR-HCl oxidation peak current density and peak potential in Britton Robinson buffer with pH 6.98

containing 10 mM TR-HCl are found to increase linearly with the increase of the scan rate. This relation demonstrates that the electrooxidation process is a surface diffusion-controlled [16,17].

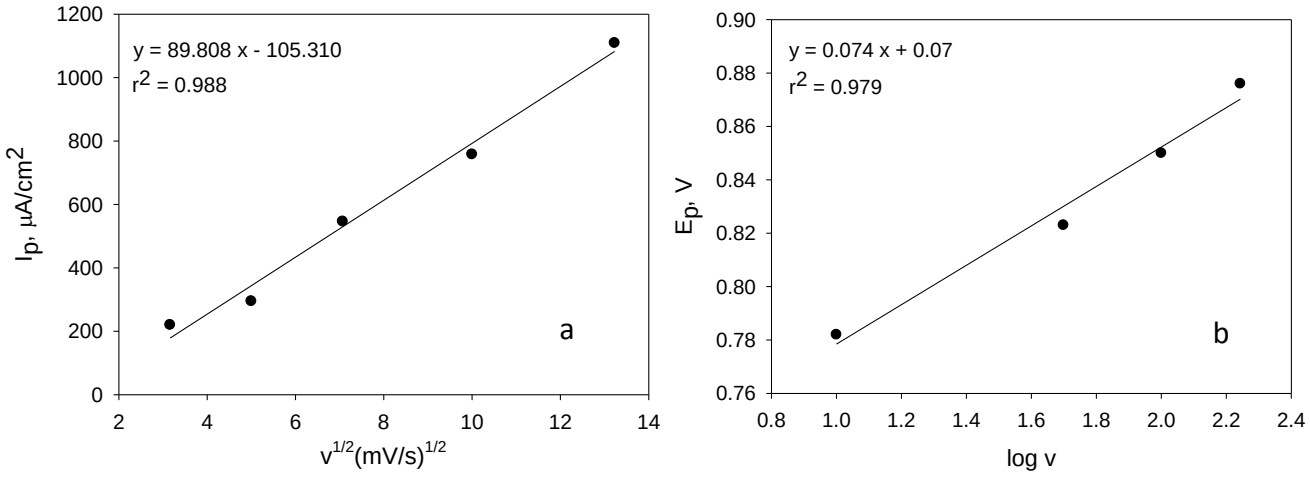


Fig. 7. Relationship between I_p and $v^{1/2}$ (a) and E_p and log v (b), at sensor V in 10 mM TR-HCl in BR buffer pH 6.98

Concentration range, limit of detection and reproducibility

The cyclic voltammetric behavior of sensor V in BR buffer pH 6.98w was studied at scan rate 100 mV/s using different concentrations of TR-HCl. It was found that the anodic peak current density due to TR-HCl oxidation increases linearly as the drugs concentration increase in the range of 1×10^{-5} - 1×10^{-2} mol/L. Fig. (5) represents a calibration graph of I_p against TR-HCl concentration, a straight line was obtained with a slope of 49.99 µA/cm^2.mM and linear regression (r^2) of 0.995. The limit of detection (LOD) was found to be 0.01 mM, and the limit of quantification (LOQ) can be easily calculated. The LOQ has the value of 0.033 mM.

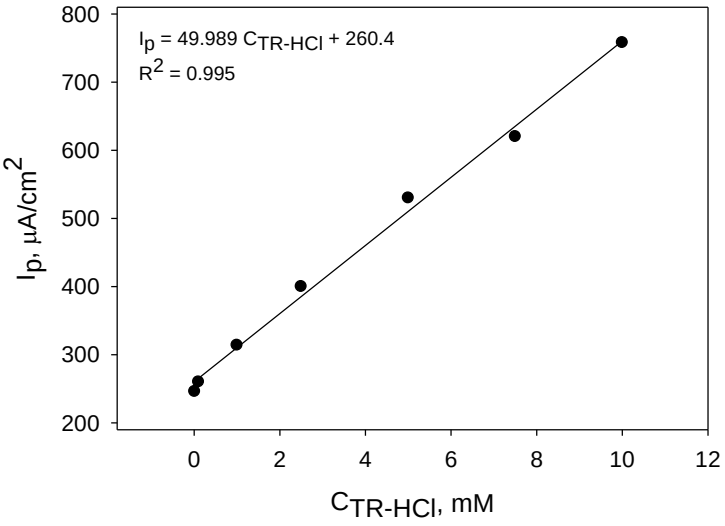


Fig. 5. Relation between I_p and TR-HCl concentration

The performance characteristics of the proposed electrode was investigated in the terms of its reproducibility, accuracy and precision, such results are represented in Table (3). In order to investigate the reproducibility of the CMCPE four replicate measurements of 6.0 mg TR-HCl solution in B-R buffer pH 6.98 were performed within the same day (intra-day precision) and yielded RSD of 0.88%. Similar procedures were carried out but between days (inter-day precision) and the RSD was found to be 1.08%. In addition to the high reproducibility of the proposed electrode, it also possessed excellent recovery values indicating the ability of the electrode to detect TR-HCl ions with high accuracy.

Table (3): Values of percent recovery and relative standard deviation of sensor V after four replicate measurements in 6.0 mg pure drug solution in BR buffer pH 6.98 within intra-day and inter-days

	Taken (mg)	Recovery %	RSD % *
Intra-day	6.0	101	0.88
Inter-day	6.0	102	1.08

*Four replicate measurements

Interference study

The aim of the present work is to detect TR-HCl ions in pharmaceutical preparations; and for this reason, the effect of common excipients on the electrochemical performance of TR-HCl was investigated. The analysis was made using the drug solution with concentration of $1x10^{-3}$ M and adding successive amount of the interferent with concentration of $1x10^{-1}$ M, in order to create excess interferent concentration, in Britton Robinson buffer pH 6.98 and scan rate 100mV/s using sensor V. The maximum tolerable concentration ratio was taken such that the foreign substance caused about ±5% relative error in the electrode response. As shown in Table (4), the tolerated ratio was 20-fold for glucose, 15-fold for sucrose and 10-fold for lactose and starch, while glycine, citric acid and ascorbic acid had apparent influence on the analytical signal of the electrode. It is important to mention that some excipients, such as magnesium stearate, are water insoluble and thus it can be concluded that such excipients will cause no interference with the present work.

Table 4. Effect of some foreign substances on the electrode response towards 1 mM TR-HCl solution in BR buffer of pH 6.98 at scan rate of 100 mV/s.

Foreign species	concentration (mM)	Signal Change (%)
Glucose	20	1.02
Sucrose	15	1.01
Lactose	10	0.95
Starch soluble	10	1.05
Glycine	10	1.17
Citric acid	10	1.59
Ascorbic Acid	10	3.80

Analytical application

In order to investigate the applicability of the present work on pharmaceutical preparations of TR-HCl, tramadol capsules and tablets were analyzed with two analytical techniques, cyclic voltammetry and potentiometric titration.

A- Cyclic voltammetry

Calibration curve method was applied to determine the concentration of TR-HCl in pure solution and both pharmaceutical preparations; capsules and tablets. The values of percent recovery ranged from 98.0-101.2% and relative standard deviation were in the range of 0.83-4.82% as tabulated below in Table (5).

Table 5. The values of % recovery and RSD of sensor V after four replicate measurements obtained from cyclic voltammetry for pure solution, capsules and tablets of TR-HCl with different weights of each.

Sample	Taken (mg)	Recovery %	RSD %*
Pure solution	0.60	98.0	1.57
	3.00	98.4	1.02
	6.00	101.2	0.88
Capsules (50 mg)	0.60	98.6	2.47
	3.00	99.0	3.19
	6.00	100.8	1.42
Tablets (225 mg)	0.60	100.2	4.82
	3.00	99.2	2.45
	6.00	99.9	0.83

*Four replicates

B- Potentiometric titration

A solution of sodium tetraphenyl borate was used to titrate tramadol hydrochloride in the pure solution and in its both pharmaceutical preparations, capsules and tablets. A 25 mL of the drug solution was transferred to 250 mL titration vessel and was titrated against 5 x 10^{-4} mol/L sodium tetraphenyl borate solution. The end point of such titration can be easily determined from the conventional S-shaped curve which is a plot of measured potential in mV against the volume of titrant added in mL. The first derivative curve also offers an easier method to determine the end point of the titration with high accuracy. The values of percent recovery ranged from 97-105% and relative standard deviation were in the range of 0.29-5.77% as tabulated below in Table (6).

Table (6): The values of % recovery and RSD of sensor V after four replicate measurements obtained from potentiometric titration for pure solution, capsules and tablets of TR-HCl with different weights of each.

Sample	Taken (mg)	Recovery %	RSD%*
Pure solution	0.75	90.0	3.12
	3.75	98.0	1.17
	7.5	99.5	0.29
Capsules (50 mg)	0.75	105.0	5.77
	3.75	101.0	4.76
	7.5	99.0	1.15
Tablets (225 mg)	0.75	90.0	5.12
	3.75	97.0	1.76
	7.5	99.0	0.58

*Four replicates

Conclusion

A new chemically modified carbon paste electrode was developed as a sensing platform for the determination of TR-HCl. Two types of ion-exchangers were used: TR-Rn and TR-TPB. However, the electrochemical response towards TR-HCl using mixed ion-exchangers was significantly enhanced over the unmodified CPE and single ion-exchanger MCPE; due to the synergistic electrocatalytic activity of the two ion-exchangers. The modified electrode showed a relatively low detection limit, wide linear range, high sensitivity, and rapid response towards TR-HCl concentration. The proposed electrode enables the detection of TR-HCl in presence of many common drug excipients. The sensor was successfully applied to TR-HCl determination in real pharmaceutical dosage forms with satisfactory recovery values. Compared to other instrumental methods used for the assay of TR-HCl, such as HPLC, GC, capillary electrophoresis and spectrophotometry, the proposed method is simple, coast effective and time saving.

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