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Full Paper

Comparison of the Electroanalytical Oxidation of Ritodrine Hydrochloride at Carbon Paste, ZrO_2 Nano Particles Modified, Graphite Pencil and Glassy Carbon Electrodes

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Abstract- Electrochemical oxidation behavior of ritodrine hydrochloride (RT.HCl) was studied in Britton Robinson buffer at different pH range from 2 to 10 using carbon paste (CPE), modified zirconium oxide carbon paste (ZrO_2-MCPE), graphite pencil (GPE) and glassy carbon electrodes (GCE). Cyclic voltammetry (CV) was showed one well-defined, irreversible, diffusion-controlled anodic peak at pH 9 by using CPE and GPE electrodes and pH 8 and 7 at ZrO_2-MCPE and GCE electrodes, respectively. The linear response was obtained in the concentration range of 3.33×10^{-6}-4.33×10^{-5}, 6.67×10^{-8}-7.33×10^{-7}, 4.0×10^{-6}-6×10^{-5} and 5.0×10^{-6}-4×10^{-5} M with detection limit (LOD) of 1.57×10^{-6}, 6.18×10^{-8}, 1.71×10^{-6} and 3.32×10^{-6} mol L^{-1} by using differential pulse voltammetric method (DPV) at CPE, ZrO_2-MCPE, GPE and GCE, respectively. The linear concentration response was obtained in the concentration range of 1.33×10^{-6}-1.47×10^{-5}, 3.33×10^{-8}-3.67×10^{-7}, 4.0×10^{-6}-5.5×10^{-5} and 5.00×10^{-6}-4.5×10^{-5} M with detection limit (LOD) 5.78×10^{-7}, 1.11×10^{-8}, 1.74×10^{-6} and 1.84×10^{-6} mol L^{-1} by using square wave voltammetric method (SWV) at CPE, ZrO_2-MCPE, GPE and GCE, respectively. The repeatability and reproducibility of the method were 0.41-1.27, 0.25-1.11, 0.49-1.86 and 0.73-2.13% relative standard deviations (RSD) for anodic peak current by using square wave voltammetric method (SWV) at CPE, ZrO_2-MCPE, GPE and

GCE electrodes. The mechanism successfully confirmed by molecular orbital calculation (MOC). These calculations give the bond order, bond length and charge distribution. This helps the successful choice of the weakest bond which changed during oxidation or reduction. Therefore, the best pathway for the oxidation of this drug is correctly selected for proposed mechanism.

Keywords- Carbon paste electrode, Zirconium oxide nanoparticles, Graphite pencil electrode, Glassy carbon electrode, Differential pulse, Square wave voltammetry, Ritodrine hydrochloride

1. INTRODUCTION

Ritodrine hydrochloride is (Figure 1) a tocolytic drug which used to stop premature labor [1]. Ritodrine HCl has the IUPAC name 4-(2-((1R,2S)-1-hydroxy-1-(4-hydroxyphenyl)propan-2-ylamino)ethyl)phenol. It is a β-2 adrenergic receptor agonist that is a class of medication used for smooth muscle relaxation. Since ritodrine has a bulky N-substituent, it has high β 2 selectivity. Also, the 4-hydroxy groups on the benzene ring are important for their reactivity as it is needed to forming hydrogen bonds. However, the 4-hydroxy groups make it susceptible to metabolism [2]. A number of methods were used to determine RT.HCl including high performance liquid chromatography (HPLC) [3,11], gas chromatography [12,13], mass chromatography [14,16], capillary electrophoresis [17], spectrofluorimetric method [18,20], a colorimetric method [21], spectrophotometry [22-38], capillary zone electrophoresis [39] and ion-selective PVC membrane electrode [40]. Most of the previous methods involved intensive solvent usage, some extraction, and expensive device and also time consuming. To the best of our knowledge there are no voltammetric methods were reported for the direct determination of RT.HCl. Hence, the current electroanalytical research aims to study the CV, DPV and SWV behavior of RT.HCl in its pure form, pharmaceutical preparation and human urine. The proposed method was characterized by its simplicity, high sensitivity, relatively short time of analysis and low cost without any extraction.

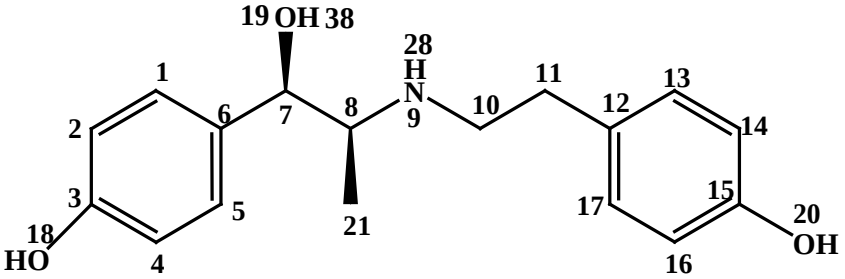

Fig. 1. Ritodrine neutral molecule numerical distribution

2. EXPERIMENTAL

2.1. Chemicals and reagents

Ritodrine hydrochloride and its pharmaceutical dosage form (Yutobar) were kindly provided by Pharco Company, Cairo, Egypt. All chemicals for preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma-Aldrich). Stock solution of RT.HCl (1×10^{-3} mol L^{-1}) was prepared by dissolving 16.19 mg in 50 mL deionized water and kept in the refrigerator (4 ^{o}C). Britton-Robinson buffer (0.04 mol L^{-1}) was prepared by mixing the acid mixture containing 0.04 M of phosphoric acid, acetic acid and boric acid [41]. The pH series was prepared in the range from 3 to 10 by using 0.2 M sodium hydroxide.

2.2. Apparatus

Voltammetric measurement was obtained using the electrochemical analyzer computrance system with 797 VA computrance software (1.0) from Metrohm, Switzerland. A three-electrode cell was employed. The working electrodes were a carbon paste (CPE), modified carbon paste with ZrO_2 nano particles (ZrO_2-MCPE), graphite pencil (GPE) and glassy carbon (GCE) electrodes. Ag/AgCl (3 mol L^{-1} KCl) was used as a reference electrode and platinum wire as a counter electrode. The pH measurements were performed using Jenway 3330 Research pH meter, U.K. Ultrasonic Cleaner, United Jeveiry Tool Supplies, model UTA-60, 6L capacity, Italy was used. Deionized water used throughout the present study was supplied from burette still plus deionized connected to a Hamilton-Aqua-Metric deionized water system, U.K. All the experiments were performed at an ambient temperature of 25 °C.

2.3. Molecular orbital calculations (MOC)

The MOCs were computed using for semi-empirical molecular orbital calculation of RT.HCl drug. The method used in these computations is the parametric method (PM7) described by Stewart [42]. The program is running under the molecular orbital calculation package MOPAC2012 by Stewart for microcomputers which depends on bond length, bond order and charge distribution to elucidate the proposed mechanism.

2.4. Working electrodes

a. The carbon paste electrode (CPE)

The carbon paste electrode was prepared by mixing of 0.5 g analytical grade graphite (particle dimension 20 µm) with 0.3 mL of paraffin oil in mortar with a pestle. A portion of composite carbon paste was packed into the hole of the insulin syringe body with diameter

3.0 mm which contains copper wire contacted the apparatus and the tip of the electrode was polished with a weighing paper till the surface become planed and shiny [43].

b. Nano modified carbon paste electrode (ZrO_2-MCPE)

Nano modified carbon paste electrode (ZrO_2- MCPE) was prepared as follows, The carbon paste was prepared by mixing of 20% (0.4 g graphite+0.1g nano ZrO_2), 30% (0.35 g graphite+0.15 g nano ZrO_2) and 40% (0.3g graphite+0.2 g nano ZrO_2) analytical grade graphite (particle dimension 20 µm, Sigma-Aldrich) and nano zirconium oxide (average crystal diameter 7 nm) which prepared by sol-gel method [44], respectively, for each percent batch with 0.3 mL of paraffin oil in mortar with a pestle. A portion of modified composite carbon pastee was packed into the hole of the insulin syringe body with diameter 3.0 mm which contain copper wire contacted the apparatus and the tip of the electrode was polished with a weighing paper until it had a shiny appearance.

c. Graphite pencil electrode (GPE)

Prepared by using rotring HB pencil leads with length of 60 mm and a diameter of 0.7 mm was employed. Electrical contact with the lead was achieved by soldering a metallic wire to the metallic part fixing the lead inside the pencil. The electrode was polished using a cloth felt pad with 0.05 m alumina slurry [45].

d. Glassy carbon electrode (GCE)

Mini glassy carbon disk electrode of the active zone: 2.8 mm, for ELCD 641/656. To improve the sensitivity and resolution of the voltammetric peaks, the GCE was polished manually with 0.5 mm alumina slurry on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and deionized water.

2.5. Area of the electrode

The active surface area of the CPE, ZrO_2-MCPE, GPE and GCE electrodes was obtained by the cyclic voltammetry method using solution of 1.0 mM $K_4Fe(CN)_6$ as an electrolyte at a different scan rates (20-200 mVs^{-1}). For a reversible process, the following Randles – Sevcik formula was used.

$$I_{pa} = (2.69 \times 10^5) n^{3/2} A_0 D_0^{1/2} C_0 * v^{1/2} \quad (1)$$

Where I_{pa} refers to the anodic peak current, n is the number of electrons transferred, A_0 is the surface area of the electrode, D_0 is the diffusion coefficient, v is the scan rate and C_0* is the concentration of $K_4Fe(CN)_6$. For 1.0 mM $K_4Fe(CN)_6$ in 0.1 M KCl electrolyte, n=1, Do=7.6×10^{-6} cm^2 s^{-1} then from the slope of the plot of I_{pa} vs. $v^{1/2}$, the electro-active surface area of working electrodes was calculated for carbon paste, modified carbon paste 30% ZrO_2 nano particles, graphite pencil and glassy carbon electrodes were found to be 0.202 0.269, 0.216 and 0.096 cm^2, respectively. Electroactive area of modified ZrO_2 nano particles carbon paste has greater response on peak current of RT.HCl drug in comparison with CPE, GPE and GCE.

2.6. Experimental procedure

In the electrochemical measurements, working electrodes (CPE, ZrO_2-MCPE, GPE or GCE electrodes) were electrochemically pretreated by scanning the potential between 0.02 and 0.24 V with a scan rate of 100 mVs^{-1} for 20 cycles in 0.04 mol L^{-1} Britton Robinson buffer (B-R). Voltammetric analyses were performed in 15 mL of B-R buffer solution at pH 9 and 8 for CPE and ZrO_2-MCPE electrodes, respectively, and in 10 and 20 mL of B-R buffer solution at pH 9 and 7 for GPE and GCE electrodes, respectively. Aliquots of the drug solution containing 8.095 mg mL^{-1} which equivalent to 10^{-3} mol L^{-1} of RT.HCl were introduced into the electrolytic cell and then a CPE, ZrO_2-MCPE, CPE or GCE electrodes was immersed into the supporting electrolyte. The cyclic voltammograms were recorded at a scan rate of 100 mVs^{-1}. The calibration curves of RT.HCl using DPV and SWV were determined by plotting the anodic peak current I_p (µA) against drug concentration (mol L^{-1}). The statistical parameters were calculated which indicates the accuracy and precision for the proposed method.

2.6.1 Analysis of pharmaceutical dosage form

The content of five tables of yutopar was transferred into a mortar to grind. A portion of the resulted fine powder accurately weighed equivalent to a solution of a concentration 1.0×10^{-3} M of RT.HCl then transferred into a 50 mL calibrated measuring flask and then diluted to the mark with deionized water. The measuring flask was sonicated for 20 min to complete the dissolution of the solution. The clear supernatant liquid was transferred into a 100 mL calibrated measuring flask and then diluted to the mark with deionized water. The solution was analyzed by using square wave voltammetry method (SWV). The square wave voltammograms were recorded in range from 0.2 to 1.2 V at frequency 20 Hz after 10 sec accumulation time with stirring at CPE, ZrO_2-MCPE, GPE or GCE electrodes. The slender content of RT.HCl was calculated using the linear regression equation.

2.6.2. Application to human urine

The method depends on the uses of human urine which was supplied from healthy volunteers. The urine was allowed to settle for about 15 minutes and then sonicated for 30 min and clear supernatant liquid was used for carrying out the experiments. An aliquot of RT.HCl was dissolved in deionized water to achieve a final concentration of 20 mg mL^{1}. Appropriate volumes of this sample were transferred into the voltammetric cell and diluted up to the volume with Britton-Robinson buffer at selected pH for CPE, ZrO_2-MCPE, GPE or GCE electrodes and subsequently analyzed according to the recommendation in the general analytical procedure.

3. RESULTS AND DISCUSSION

3.1. Effect of ZrO_2 nano particles content

In order to determine the best composition of modified electrode towards RT.HCl drug, three electrodes containing 20, 30 and 40% of ZrO_2 nano particles were prepared and tested for their use in the determination of RT.HCl at pH 8 applying DPV and SWV techniques. It was found that electrode containing 30% ZrO_2 nano particles was selected for further studies as it gave the highest density peak than other two electrodes (Figure 2).

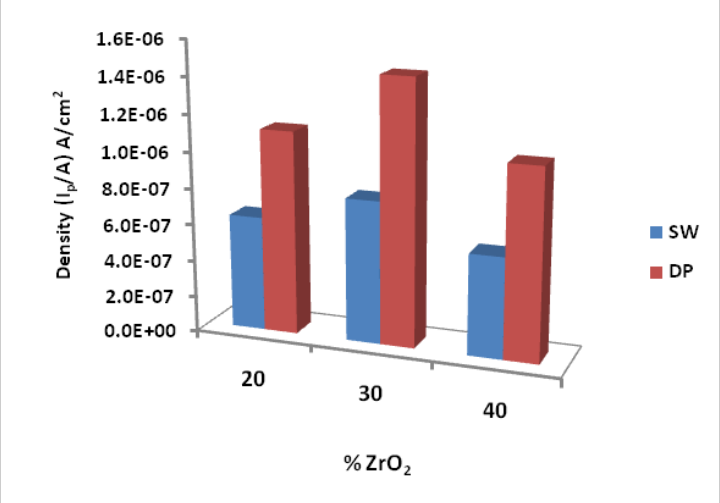

Fig. 2. Efficiency of ZrO_2 nano particles percent on anodic peak current

3.2. Electro-oxidation of RT.HCl

The oxidation behavior of RT.HCl drug was studied over pH range from 3.0 to 11.0 in B-R supporting electrolytes. The cyclic voltammetric method was employed to study oxidation behavior of RT.HCl in B-R buffer at different pH and the voltammogram showed the selected

pH of highest anodic peak current was obtained at pH 9.0 in case of CPE and GPE electrodes and pH 8 and 7 for ZrO_2-MCPE and GCE electrodes, respectively.

The oxidation peaks of the RT.HCl drug at the selected pH give one main irreversible anodic peak at 520 and 567 mV using CPE and GPE, respectively. While modified ZrO_2-MCPE and GCE electrodes have one irreversible anodic peak at 479 and 633 mV, respectively. RT.HCl has no reduction behavior on the reverse scan indicating that, the oxidation process of RT.HCl at CPE, ZrO_2-MCPE, GPE and GCE electrodes was an irreversible oxidation process.

3.3. Effect of pH

The experiment at a series of different pH range from 3 to 11 using B-R buffer solution has an influence on the electro oxidation peak of 1×10^{-3} mol L^{-1} RT.HCl was estimated by using cyclic voltammetry technique to examine the effect of pH on the CPE, ZrO_2-MCPE, GPE and GCE electrodes reaction. The pH affected on the peak current and peak potential of RT.HCl drug (Figures 3,4) frequently with the increase in pH of the solution where voltammetric peak potential was shifted to less positive values and expressed in the following equations:

E_P (mV)=1099.7-64.57 pH (r=0.9969) for CPE

E_P (mV)=936.11-57.13 pH (r=0.9993) for ZrO_2-MCPE

E_P (mV)=1107.5-60.93 pH (r=0.9993) for PGE

E_P (mV)=992.19-51.22 pH (r=0.9991) for GCE

The slope per pH unit from these equations at the selected pH was found to be 64.57, 57.13, 60.93 and 51.22 mV/pH for CPE, ZrO_2-MCPE, GPF and GCE, respectively. The data given in Figure (3) confirmed the closeness of the slope of Nernst equation to the expected theoretical value of 59 mV/pH [46]. This suggests that the number of the electrons (one electron) participate in the oxidation process of RT.HCl drug at CPE, ZrO_2-MCPE, GPF and GCE electrodes, respectively, is equal to the number of hydrogen ions (one hydrogen ion) participating in the oxidation reaction.

From (Figure 4) the relation between current (I_p) and pH values showed that the intensity of anodic peak current was reached highest value at pH 9 for CPE and GPE and pH 8 and 7 for ZrO_2-MCPE and GCE electrodes, respectively. The peak intensity decreases before and after the selected pH values (pH 7,8 and 9) because the best result of anodic peak accompanied with sharper response was obtained at selected pH values at the four electrodes.

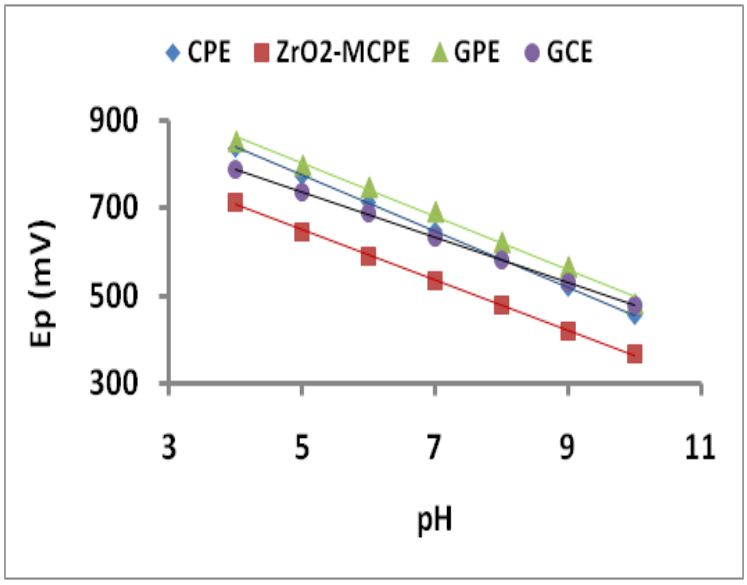

Fig. 3. The effect of pH on the peak potential of RT.HCl at CPE, ZrO_2-MCPE, GPE and GCE electrodes using CV

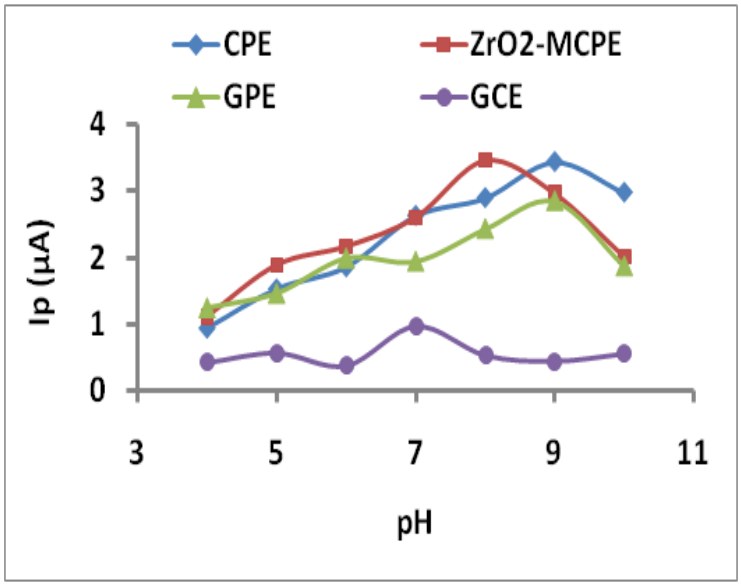

Fig. 4. The effect of pH on the current peak (µA) of RT.HCl at CPE, ZrO_2-MCPE, GPE and GCE electrodes using CV

3.4. Effect of scan rate

The scan rate studies were illustrated in (Figure 5) which describe the oxidation behavior of drug at electrodes surface asses adsorption or diffusion process. The results was accentuated that the linear relation between I_p and square root of scan rate was studied using CV over the scan range 20–240 mV/s with high correlation coefficient r^2=0.9992, 0.9995,

0.9993 and 0.9993 at CPE, ZrO_2-MCPE, GPE and GCE electrodes, respectively, which illustrated that the process is controlled diffusion process in the selected scan rate range [47]. The whole scan rate range studied which was represented in the following equations:

I (μA)=3.65 v_2^1+0.68 (r^2=0.9992) for CPE

I (μA)=4.93 v_2^1-0.69 (r^2=0.9995) for ZrO_2-MCPE

I (μA)=9.09 v_2^1+0.91 (r^2=0.9993) for GPE

I (μA)=3 v_2^1+0.3 (r^2=0.9993) for GCE

Furthermore, the date obtained from the linear relation between I_p versus log υ with slope less than 0.5 theoretical value were 0.328, 0.333, 0.496 and 0.342 at CPE, ZrO_2-MCPE, GPE and GCE electrodes, respectively, which sureness the process at the four electrodes surface were controlled diffusion reaction.

From the CV voltammogram there is appearance of another peak at 120 mV/s at the ZrO_2-MCPE electrode surface, so it was chosen for scan rate at ZrO_2-MCPE electrode because its sensitivity to detect another peak.

For an irreversible electrode process, according to relation between anodic potential current (E_p) and log scan rate Laviron [48], E_p is expressed by the following equation:

$$E_p = E^o + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RKK^o}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log v \quad (2)$$

Where E^o is the thermal standard redox potential, α is the transfer coefficient, n is the number of electrons transferred, K^o is the standard heterogeneous rate constant of the reaction, R is the universal gas constant (8.314 $Jmol^{-1}K^{-1}$), T is the temperature (298 K) and F is the Faraday Constant (96 485 $Cmol^{-1}$).

For an irreversible oxidation reaction, the value of αn can be easily calculated from the slope of E_p vs. log υ plot (Figure 6). In this system, the slope was 0.0626, 0.05617, 0.0767 and 0.0697 for CPE, ZrO_2-MCPE, GPE and GCE electrodes, respectively. Then αn value was calculated to be 0.9441, 1.0516, 0.7705 and 0.8479 for CPE, ZrO_2-MCPE, GPE and GCE electrodes, respectively, according to Bard and Faulkner [49], where α can be given as:

$$\alpha = \frac{47.7}{Ep - Ep/2} \text{ mV} \quad (3)$$

Where $E_{p/2}$ was the potential when the current was at half the peak value. From this, the value of α was calculated to be 0.53, 0.51, 0.40 and 0.49 for CPE, ZrO_2-MCPE, GPE and GCE electrodes, respectively. Further, the number of electrons (n) transferred in the electro-oxidation of RT.HCl was calculated to be 1.78, 2.07, 1.94 and 1.72 for CPE, ZrO_2-MCPE, GPE and GCE electrodes, respectively, which almost equal 2 for the four electrodes.

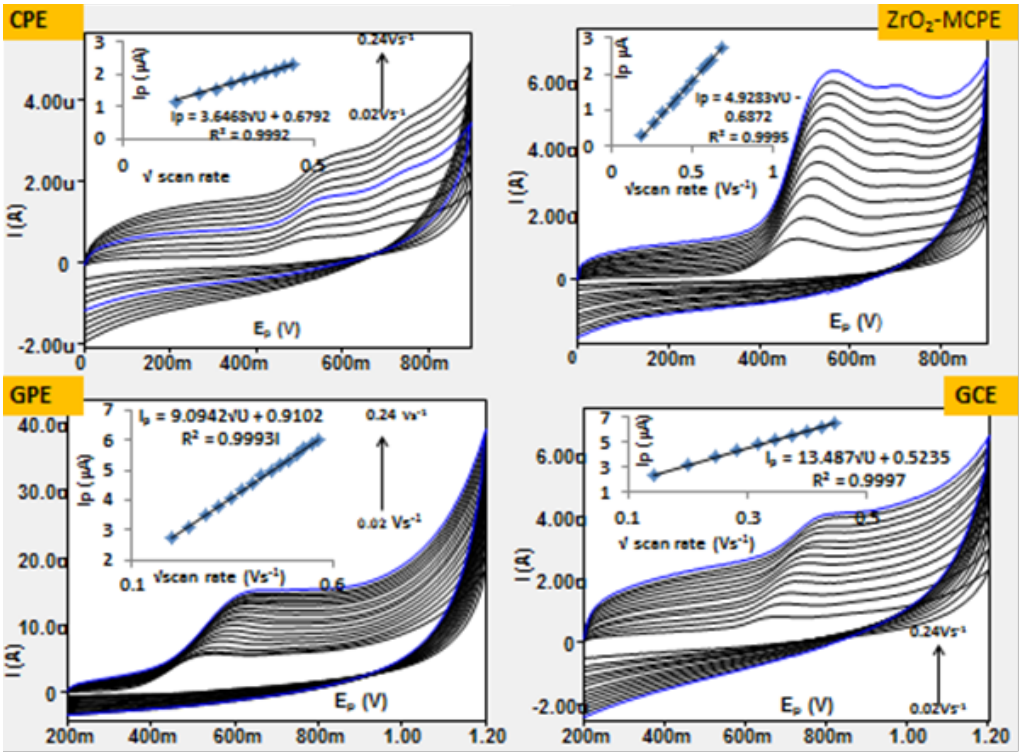

Fig. 5. Relation between current and scan rate for oxidation of RT.HCl drug at CPE, ZrO_2-MCPE, GPE and GCE electrodes

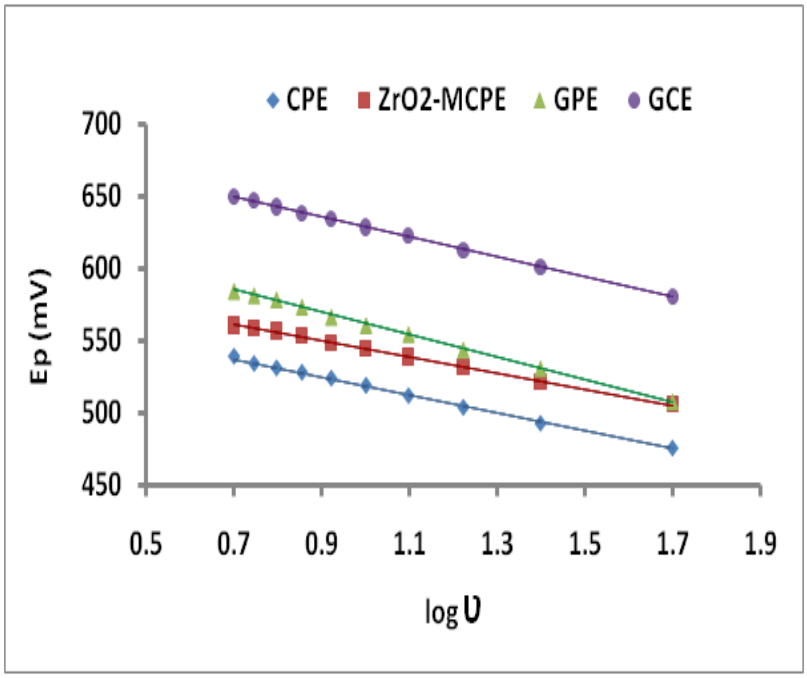

Fig. 6. Relation between peak potential (mV) and log scan rate (Vs^{-1}) for oxidation of RT.HCl drug at CPE, ZrO_2-MCPE, GPE and GCE electrodes

3.5. Molecular orbital calculations (MOC)

The MOC were performed using semi-emperical molecular orbital procedure [42], RT.HCl neutral molecule numerical distribution was shown in Figure (1).

Table 1. MOC properties of selected bond length and atomic bond order for both neutral and positive ritodrine molecule

Ritodrine	Bond Length (Å)		Bond order	
	Neutral	Cation	Neutral	Cation
C1C2	1.388	1.387	1.466	1.469
C1C6	1.400	1.398	1.396	1.402
C1H22	1.095	1.090	0.951	0.960
C2C3	1.399	1.401	1.365	1.359
C2H23	1.088	1.088	0.952	0.953
C3C4	1.395	1.397	1.388	1.372
C3O18	1.358	1.353	1.036	1.059
C4C5	1.393	1.391	1.426	1.446
C4H24	1.084	1.087	0.959	0.954
C5C6	1.395	1.397	1.439	1.422
C5H25	1.090	1.092	0.962	0.960
C6C7	1.502	1.500	0.997	0.994
N7C8	1.569	1.562	0.947	0.957
C7O19	1.421	1.422	0.938	0.928
C7H26	1.115	1.116	0.958	0.957
C8N9	1.473	1.476	0.984	0.961
C8C21	1.536	1.532	0.994	0.995
C8H27	1.123	1.124	0.957	0.957
N9C10	1.483	1.476	0.959	0.986
N9H28	1.020	1.022	0.900	0.897
C10C11	1.542	1.545	0.988	0.972
C10H29	1.112	1.114	0.966	0.960
C10H30	1.114	1.111	0.962	0.962
C11C12	1.495	1.460	1.006	1.073
C11H31	1.109	1.45	0.962	0.906
C11H32	1.108	1.131	0.962	0.889
C12C13	1.400	1.433	1.401	1.213
C12C17	1.395	1.433	1.443	1.204
C13C14	1.388	1.364	1.468	1.646
C13H33	1.092	1.100	0.959	0.945
C14C15	1.399	1.436	1.368	1.156
C14H34	1.087	1.099	0.955	0.939
C15C16	1.395	1.427	1.395	1.181
C15O20	1.361	1.312	1.025	1.252
C16C17	1.394	1.367	1.424	1.631
C16H35	1.083	1.095	0.960	0.943
C17H36	1.089	1.102	0.964	0.941
O18H37	0.987	0.992	0.875	0.869
O19H38	0.986	1.016	0.876	0.844
O20H39	0.975	0.979	0.883	0.872
C21H40	1.101	1.100	0.961	0.961
C21H41	1.093	1.096	0.970	0.964
C21H42	1.094	1.095	0.969	0.965

Table 2. MOC properties of selected partial atomic charge for both neutral and positive ritodrine molecule

Ritodrine	Atomic Charge	
	Neutral	**Cation**
C1	-0.059041	-0.099596
C2	-0.255154	-0.251869
C3	0.291918	0.306839
C4	-0.312186	-0.296041
C5	-0.105392	-0.072945
C6	-0.106335	-0.167169
C7	0.091415	0.10140
C8	0.030058	0.054956
N9	-0.552160	-0.550698
C10	-0.103109	-0.084381
C11	-0.297481	-0.364333
C12	-0.055343	0.175876
C13	-0.104426	-0.137019
C14	-0.228827	-0.147219
C15	0.277500	0.346318
C16	-0.303206	-0.235844
C17	-0.095870	-0.083225
O18	-0.468753	-0.450408
O19	-0.481081	-0.297235
O20	-0.563192	-0.595803
C21	-0.467316	-0.464154
H22	0.181336	0.147889
H23	0.181860	0.181607
H24	0.161326	0.175559
H25	0.150063	0.160107
H26	0.127291	0.139265
H27	0.144666	0.152884
H28	0.259791	0.271162
H29	0.140700	0.159012
H30	0.144861	0.132596
H31	0.150280	0.212280
H32	0.157355	0.231003
H33	0.160007	0.203163
H34	0.174642	0.215260
H35	0.155807	0.201629
H36	0.150933	0.207736
H37	0.326099	0.334773
H38	0.325488	0.365395
H39	0.316692	0.332973
H40	0.169110	0.169396
H41	0.169110	0.159333
H42	0.145696	0.159525

From the MOC it is found that the (H-O) bonds of RT.HCl drug appear as O19 H38 of bond order=0.8, N9 H28, O18 H37 and O20 H39 have bond order=0.9 that was shown in Table (1). These values mean that O19 H38 has low bond order so it was easily to lose the proton in comparative with N9 H28, O18 H37 and O20 H39. Also this was elucidated by change of atomic charge from neutral to cation during oxidation process that was shown in Table (2), where O19 changed from -0.48 to -0.29 which is the highest difference in comparatison with O20 (-0.56 to -0.59), O18 (-0.46 to -0.45) and N9 (-0.55 to -0.55). So O19 H38 bond was ruptured in the oxidation which supported the suggested mechanism. The proposed mechanism of the electro-oxidation of RT.HCl can be given as shown in Figure (7).

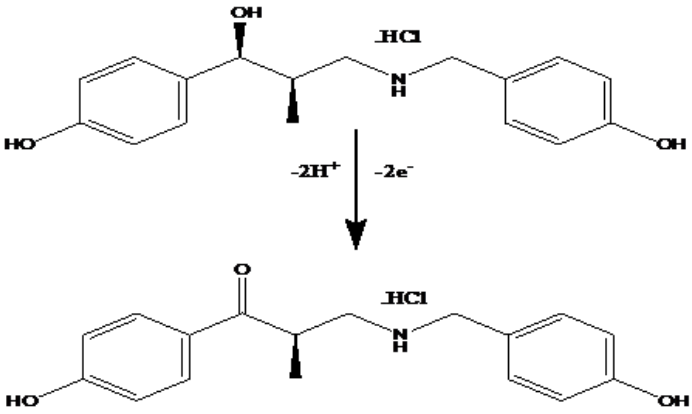

Fig. 7. Mechanism of the electro-oxidation of RT.HCl

3.6. Analytical applications

For amelioration a voltammetric methodology for determination of the RT.HCl drug, the DPV and SWV methods were utilized and the resulted voltammograms were given in Figures (8-11). Since the relation between current density (Ip/A) and concentration of the drug were sharper and better at lower concentration of RT.HCl than those obtained by cyclic sweep voltammetry with a lower back ground current leads to improvement in resolution.

For these studies, 1×10^{-3} mol L^{-1} RT.HCl drug using was used for CPE, GPE and GCE electrodes while 1×10^{-4} mol L^{-1} RT.HCl using was used for ZrO_2-MCPE electrode, and the results were given as shown in Tables (3-6). In order to provide DPV and SWV quantitative procedure, the dependence of the current density on the drug concentration was investigated. Linear calibration curves were obtained for RT.HCl concentration in the range from 3.33×10^{-6} to 4.33×10^{-5}, 6.67×10^{-8} to 7.33×10^{-7}, 4×10^{-6} to 5.2×10^{-5} and 5×10^{-6} to 3×10^{-5} mol L^{-1} for CPE, ZrO_2-MCPE, CPE and GCE electrodes, respectively, (Figures 8-11). The characterization of these graphs, the limit of detection (LOD) and the limit of quantification (LOQ) were shown in Tables (3-6).

LOD and LOQ were calculated on the peak current using the following equations:

LOD=3.3 SD/S

LOQ=10 SD/S

Where SD is the standard deviation of the peak current five runs and S is the slope of the calibration curve DPV or SWV [50].

To confirm the accuracy and precision of the proposed method, the four suggested electrodes were investigated for the determination of RT.HCl in a pharmaceutical tablet (Yutopar; 10 mg per tablet) and the results obtained were compared with the reference one [51]. The results of determination of RT.HCl in tablet dosage forms were shown in Table (7). A recovery of 98.00, 99.80, 99.28 and 99.44% with relative standard deviation of 0.41, 0.25, 0.49 and 0.73% using CPE, ZrO_2-MCPE, CPE and GCE electrodes were obtained, respectively.

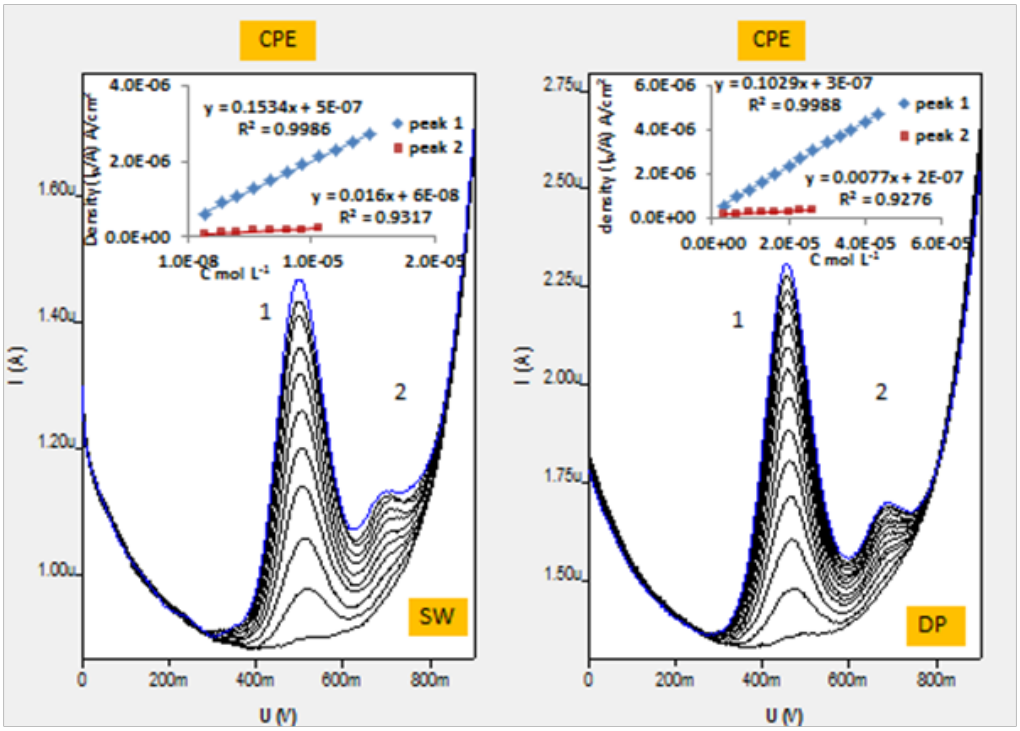

Fig. 8. Calibration graphs of RT.HCl drug by using (a) DPV and (b) SWV methods using CPE electrode

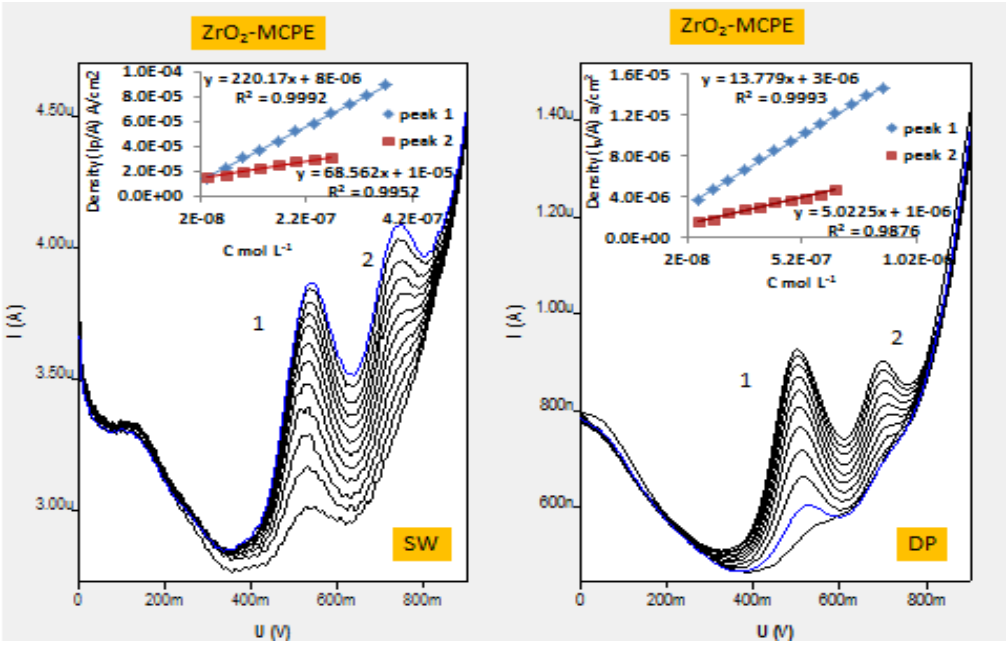

Fig. 9. Calibration graphs of RT.HCl drug by using (a) DPV and (b) SWV methods at ZrO_2-MCPE electrode

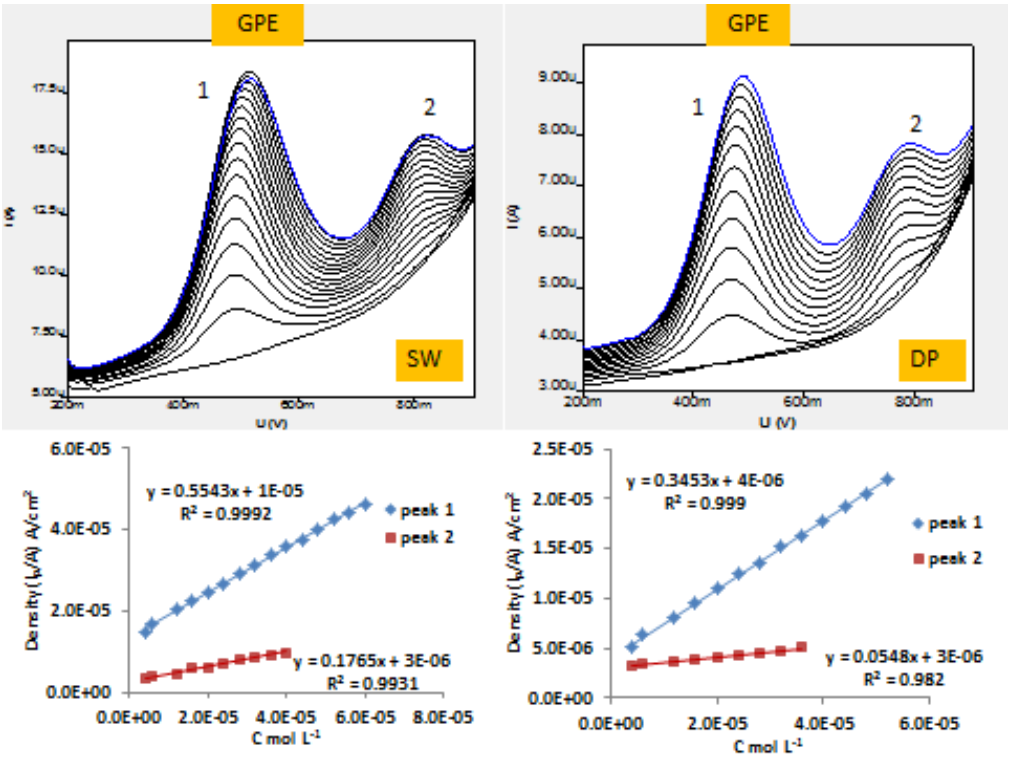

Fig. 10. Calibration graphs of RT.HCl drug by using (a) DPV and (b) SWV methods at GPE electrode

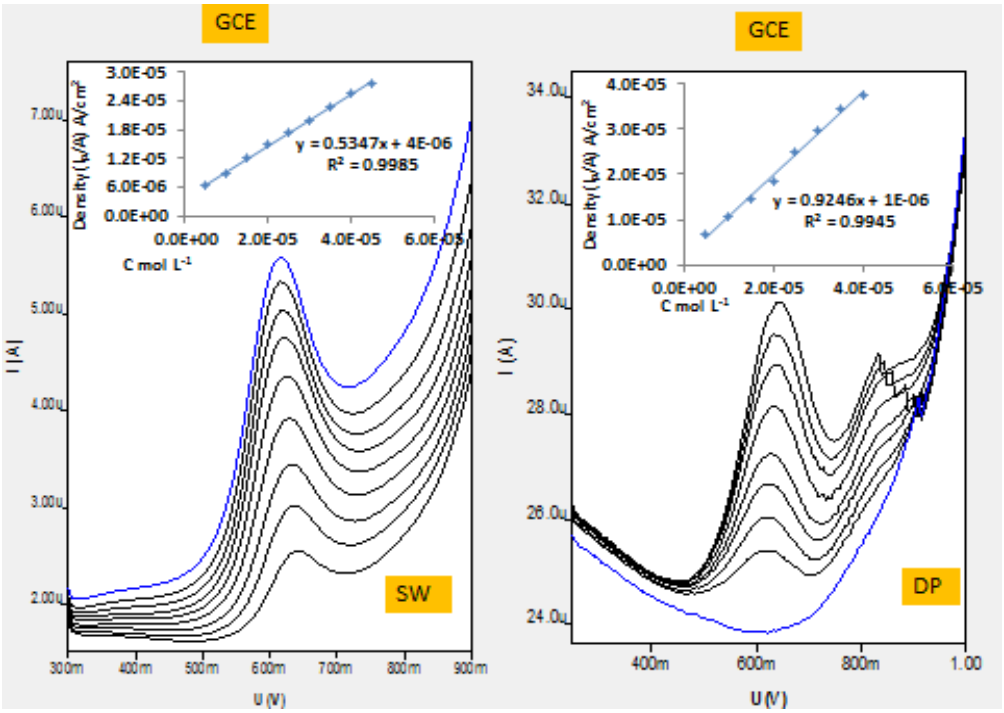

Fig. 11. Calibration graphs of RT.HCl drug by using (a) DPV and (b) SWV methods at GCE electrode.

Table 3. Analytical parameters of ritodrine .HCl at CPE electrode by using DPV and SWV methods

Parameters	**Differential pulse voltammetry**		**Square wave voltammetry (SWV)**	
	Peak 1	Peak 2	Peak 1	Peak 2
pH	9	9	9	9
concentration (mol L^{-1})	3.33×10^{-6} - 4.33×10^{-5}	2.0×10^{-5} - 4.33×10^{-5}	1.33×10^{-6} - 1.47×10^{-5}	5.33×10^{-6} - 1.47×10^{-5}
SD	4.88×10^{-8}	1.91×10^{-8}	2.69×10^{-8}	1.56×10^{-8}
RSD %	0.587	0.570	0.235	0.658
Slope of regression line (a)	0.1029	0.0077	0.1534	0.016
Intercept of regression line (b)	3.0×10^{-7}	2.0×10^{-7}	5.0×10^{-7}	6.0×10^{-8}
Correlation coefficient (r)	0.9988	0.9276	0.9986	0.9317
SEE	2.87×10^{-8}	2.88×10^{-8}	1.74×10^{-8}	1.88×10^{-8}
LOD (mol L^{-1})	1.57×10^{-6}	8.14×10^{-6}	5.78×10^{-7}	3.23×10^{-6}
LOQ (mol L^{-1})	4.74×10^{-6}	2.47×10^{-5}	1.75×10^{-6}	9.79×10^{-6}

Table 4. Analytical parameters of ritodrine .HCl at ZrO_2-MCPE electrode by using DPV and SWV methods

Parameters	**Differential pulse voltammetry**		**Square wave voltammetry (SWV)**	
	Peak 1	Peak 2	Peak 1	Peak 2
pH	8	8	8	8
concentration (mol L^{-1})	6.67×10^{-8} - 8.68×10^{-7}	2.67×10^{-7} - 8.68×10^{-7}	3.33×10^{-8} – 3.67×10^{-7}	1.30×10^{-7}- 3.67×10^{-7}
SD	2.60×10^{-7}	2.02×10^{-7}	7.41×10^{-7}	2.52×10^{-6}
RSD %	0.587	0.570	0.326	0.718
Slope of regression line (a)	13.779	5.0225	220.17	68.562
Intercept of regression line (b)	3.0×10^{-6}	1.0×10^{-6}	8.0×10^{-6}	1.0×10^{-5}
Correlation coefficient (r)	0.9993	0.9876	0.9992	0.9952
SEE	5.21×10^{-8}	1.22×10^{-7}	4.78×10^{-7}	1.88×10^{-7}
LOD (mol L^{-1})	6.18×10^{-8}	1.33×10^{-7}	1.11×10^{-8}	1.21×10^{-7}
LOQ (mol L^{-1})	1.87×10^{-7}	4.02×10^{-7}	3.36×10^{-8}	1.76×10^{-7}

Table 5. Analytical parameters of ritodrine .HCl at GPE electrode by using DPV and SWV methods

Parameters	**Square wave voltammetry (SWV)**		**Differential pulse voltammetry (DPV)**	
	Peak 1	**Peak 2**	**Peak 1**	**Peak 2**
pH	9	9	9	9
Concentration range (mol L^{-1})	4.0×10^{-6}-6×10^{-5}	2.4×10^{-6}–6.0×10^{-5}	4.0×10^{-6}-5.2×10^{-5}	2.0×10^{-5}– 5.2×10^{-5}
SD	2.92×10^{-7}	2.17×10^{-7}	1.78×10^{-7}	1.11×10^{-7}
RSD %	0.368	0.393	0.581	0.194
Slope of regression line (a)	0.5543	0.1765	0.3453	0.0548
Intercept of regression line (b)	1.0×10^{-5}	3.0×10^{-6}	4.0×10^{-6}	3.0×10^{-6}
Correlation coefficient (r)	0.9992	0.9931	0.999	0.982
SEE	1.57×10^{-7}	1.81×10^{-7}	1.03×10^{-7}	9.21×10^{-8}
LOD (mol L^{-1})	1.74×10^{-6}	9.88×10^{-7}	1.71×10^{-6}	6.54×10^{-6}
LOQ (mol L^{-1})	5.27×10^{-6}	2.99×10^{-6}	5.17×10^{-6}	1.98×10^{-5}

Table 6. Analytical parameters of ritodrine .HCl at GCE electrode by using DPV and SWV methods

Parameters	**Differential pulse voltammetry (DPV)**	**Square wave voltammetry (SWV)**
pH	7	7
concentration (mol L^{-1})	5.0×10^{-6}–4.0×10^{-5}	5.0×10^{-6}-4.5×10^{-5}
SD	9.06×10^{-7}	2.98×10^{-7}
RSD %	1.281	0.605
Slope of regression line (a)	0.9246	0.5347
Intercept of regression line (b)	1.0×10^{-6}	4.0×10^{-7}
Correlation coefficient (r)	0.9945	0.9985
SEE	7.06×10^{-7}	2.17×10^{-7}
LOD (mol L^{-1})	3.23×10^{-6}	1.84×10^{-6}
LOQ (mol L^{-1})	9.79×10^{-6}	5.58×10^{-6}

Table 7. Statistical parameters of pharmaceutical dosage form assay of the investigated RT.HCl drug

Electrode	**Drug**	**[RT.HCl] taken µg mL^{-1}**	**Proposed method ± RSD%, (n = 5)**	**Official method ± RSD%, (n = 5)**	**F-test**	**t-test**
CPE	Yutopar (10 mg/tablet)	20	99.98±1.11	100.2±1.32	1.78	2.45
		30	98.00±0.41	99.11±0.98	3.62	2.27
		40	100.3±1.27	99.19±0.89	2.51	1.71
ZrOCPE	Yutopar (10 mg/tablet)	20	99.74±1.08	99.04±2.44	3.87	1.34
		30	99.80±0.25	99.21±1.83	2.93	0.64
		40	98.22±1.11	99.12±1.06	1.94	0.77
CPE	Yutopar (10 mg/tablet)	20	99.28±0.49	99.41±1.41	2.65	1.37
		40	99.77±1.86	99.23±1.32	1.86	2.11
		30	100.1±0.82	98.74±1.00	2.81	1.69
GCE	Yutopar (10 mg/tablet)	20	99.98±0.94	99.74±1.00	1.85	2.23
		60	100.3±2.13	99.41±2.00	1.57	2.25
		60	99.44±0.73	100.3±1.39	2.57	2.12

3.7. Determination of RT.HCl in spiked human urine

The enforcement of the proposed technique to human urine was investigated. The obtained regression equation and related validation parameters are shown in Table (8). For the determination of RT.HCl drug in urine samples neither time consuming extraction and evaporation steps nor sample pretreatment were required. The proposed method gave reproducible results, easy to perform and sensitive enough for the quantitative determination of RT.HCl drug in human urine sample. The urine sample was tested by performing five consecutive analyses of the samples over a period of approximately 3 h, the recovery was in the range from 98.33 to 100.6%.

Table 8. Determination of RT. HCl in urine samples using CPE, ZrO_2-MCPE, GPE and GCE electrodes

Electrode	Urine spiked (µM)	Detected (µM)	Recovery%
CPE	6	5.90	98.33
	8	7.86	98.25
	11	10.86	98.72
	Mean±SD		**98.43±0.2**
ZrO_2-MCPE	8	8.03	100.4
	12	12.00	100.0
	15	14.96	99.73
	Mean±SD		**100.0±0.34**
GPE	5	4.99	99.80
	7	6.98	99.71
	10	10.06	100.6
	Mean±SD		**100.0±0.45**
GCE	5	4.93	98.60
	8	7.88	98.50
	12	11.92	99.33
	Mean±SD		**98.81±0.45**

4. CONCLUSION

The electro-oxidative behavior of RT.HCl drug was studied by using CV method and showed one well-defined irreversible anodic peak. Different parameters were tried and tested to optimize the determination conditions. The proposed method for determination of RT.HCl in pure form, pharmaceutical preparation and human urine sample was simple, accurate and sensitive by applying CV, DPV and SWV techniques. A comparison was made between the behavior of the RT.HCl drug at the CPE, ZrO_2-MCPE, GPE and GCE electrodes where

modified electrode (ZrO_2-MCPE) provide the highest sensitivity in terms of statistical parameters such as SD, RSD%, LOD and LOQ followed by GPE, CPE and GCE, respectively. The proposed method was also compared with the reported method [51] and was confirmed to be a satisfactory alternative for the fast, safe, clean and simple quantitative method for determination of RT.HCl.

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