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Square Wave Voltammetric Determination of Ropinirole HCl in Bulk, Dosage Forms and Biological Samples on Carbon Paste Electrode

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Authors' contributions

This work was carried out in collaboration between all authors. Author MR designed the study. Author IHIH wrote the protocol, the first draft of the manuscript and managed the experimental process. Author RTEE managed the literature searches, performed the voltammetric study and the statistical analysis. Authors MR, DM and SM managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Voltammetric determination for of Ropinirole HCl.
Study: We will study the behaviour of Ropinirole HCl on the carbon paste electrode. So several factors such as pH, type of pasting oil, pulse amplitude and scan rate were studied to optimize the condition for voltammetric determination of drug.

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Place and Duration of Study: Microanalytical Chemistry Laboratory, Applied Organic Chemistry Department, National Research Centre, Dokki, Giza, Egypt, between August 2015 and December 2015.
Methodology: Square wave voltammetry (SWV) was employed in order to determine Ropinirole in bulk powder and plasma in a voltammetric cell containing 10 mL of 0.1 mole L^{-1} sulphuric acid as supporting electrolyte. After every aliquot addition, the solution was stirred for 30 s at 2000 rpm, rested for 10 s then SWV mode was ramped from +300 to +1600 mV with scan rate 100 mV s^{-1} and pulse amplitude 50 mV. The experiment was triplicated for every standard solution addition.
Results: A good linearity was obtained over a range of ($4.96x10^{-6}$ to $3.90x10^{-5}$ mol L^{-1}) with mean recovery and relative standard deviation (RSD) values of 99.15% and 3.7%, respectively. The lower detection (LOD) and quantification (LOQ) limits were found to be ($1.48x10^{-6}$ and $4.96x10^{-6}$ mol L^{-1}) respectively at Square wave (SWV) mode. The accuracy and precision of the method were presented with inter and intra days determinations which were within acceptable limits. The method was applied successfully for determining the active ingredients in pharmaceutical preparations with mean percentage recoveries ± RSD of 98.38±3.1 and in spiked human plasma with the mean of recoveries ± RSD, 99.56±3.63%.
Conclusion: An economic, accurate and precise electrochemical method has been developed and validated for the determination of Ropinirole HCl in bulk, dosage form and human plasma.

Keywords: Ropinirole; voltammetry; carbon paste electrode.

1. INTRODUCTION

Ropinirole Hydrochloride (ROP) is the hydrochloride salt of 4-[2-(dipropyl amino)ethyl]-1,3-dihydro-2H-indol-2-one monohydrochloride ,pale cream to yellow powder, soluble in water and has an empirical formula of $C_{16}H_{24}N_2O$•HCl. (Fig.1).of mol.wt 296.84 and it is official in USP [1]. It is a non-ergoline dopamine agonist which has been proven to be effective in both, monotherapy and combination therapy of idiopathic Parkinson's disease. In addition to ameliorating bradykinesia, rigor, and tremor, Ropinirole facilitates the daily life and improves depressive moods of patients with Parkinson's disease [2]. Dopamine agonists such as Ropinirole may be used to begin treatment of parkinsonism in attempt to delay therapy with levodopa ,particularly in younger patients. they also have an adjunctive use when levodopa is no longer effective alone or cannot be tolerated, and may be useful in reducing 'off' periods with levodopa and in ameliorating other fluctuations of mobility in the later stage of the disease [3]. It is rapidly absorbed from the gastrointestinal tract and mean peak plasma concentrations have occurred 1.5 hours after oral doses; the rate of absorption, but not the extent may be reduced if taken with food. Bioavailability is reported to be about 50%. It is widely distributed throughout the body and plasma protein binding is low (10 to 40%) [3]. Its mean maximum plasma concentrations is 1.16 µg/ml after a 1 mg dose and 5.11 µg/ml after 5mg have been observed. These concentrations are reached within 1.5 hours after oral administration [4].

Literature Survey reveals that few different analytical techniques have been applied including, the official HPLC method [1] and other HPLC methods with UV or Diode-array detection which were used for drug impurity profiling and stability-indicating assays [5-13], HPLC-MS detection [14-18], planer chromatography methods [19,20], spectrophotometric and spectrofluorimetric Methods [21-24], capillary electrophoresis [25] and electrochemical methods on glassy carbon electrode (GCE) [26] and at the surface of carbon nanotubes [27].

Because of time-consuming surface cleaning of glassy carbon electrode before each experiment as a result of strongly adsorbed oxidation product of drug on the GCE surface [26], it almost drastically restores to its original surface activity. Alternatively, the deteriorated or consumed GCE (due to unremovable sticky redox products on its surface) can be capped by carbon paste electrode as demonstrated later. The objective of the work in this paper is the determination of ROP in tablet and human serum through electrochemical oxidation on carbon paste electrode which is more economic and easily fabricated using square wave (SWV).

2. EXPERIMENTAL

2.1 Materials and Reagents

2.1.1 Pure materials

Pure Ropinirole HCl *B.N. RPN/WS/001/12* of purity 99.0% supplied by (RA CHEM Pharm Ltd.)

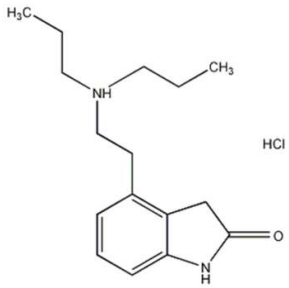


Fig. 1. Chemical structure of Ropinirole HCl

2.1.2 Pharmaceutical dosage form

TREMODECT tablets *B. N* 207128 provided by (EVA PHARMA –Cairo, Egypt) contains 4.56 mg of Ropinirole HCl equivalent to Ropinirole 4 mg.

2.1.3 Reagents

0.04 M Britton-Robinson (BR) buffers were: Prepared by mixing 10 mL of each 0.4 mol L^{-1} of boric acid, acetic acid and phosphoric acid in a beaker, adjusted to the desired pH (2-9) by drop wise of 1 mol L^{-1} sodium hydroxide solution, then transferred into a 100 mL measuring flask and completed to the volume with distilled water. All are of analytical grade. Paraffin oil (Fluka, Germany), silicon oil (of analytical grade), and Castor oil supplied from the pharmacy, synthetic carbon powder 1-2 micron (Aldrich, German) .Double distilled water was used in all work. Fresh human plasma was obtained from the blood bank (VACSERA, Cairo, Egypt).

2.1.4 Standard solutions

A stock solution of s ROP ($1x10^{-3}$ mol L^{-1}) was prepared by dissolving an appropriate amount of ROP HCl in double-distilled water and stored in the refrigerator at 4℃ for 72 hours.

2.2 Apparatus

The Metrohm model of VA processor 693 and VA stand 694 equipped with three electrodes is employed. The electrodes are a reference electrode of Ag/AgCl- 3 mol L^{-1} KCl and a platinum counter electrode. The carbon paste electrode CPE is used as a working electrode for electrochemical measurements and is prepared as described below.The Metrohm model 654 pH meter is also used.

2.3 Procedures

2.3.1 Preparation of the working electrode

The carbon paste is prepared by mixing 200 µL paraffin oil and 250 mg synthetic carbon powder 1 - 2 micron in an agate mortar. The sharp end of 1 mL-micropipette tip is cut off with a cutter to make like a cap with dimension 1 cm length and 0.75 cm internal diameter permitting to couple with and contact with the surface of the glassy carbon electrode. The cap is then packed with the carbon paste, compressed with the GCE and smoothed on a wetted Whatman filter paper. The CPE is activated by polishing every week (Fig. 2).

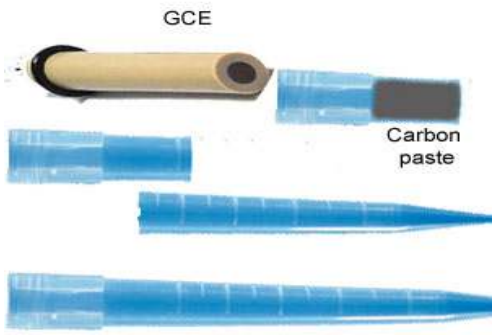


Fig. 2. Modified carbon paste electrode

2.3.2 Construction of calibration curve of ROP

Square wave voltammetry (SWV) was employed in order to determine ROP in bulk powder. Aliquots of ROP solution (1×10^{-3} mole L^{-1}), to give finally a concentration range of (4.96×10^{-6} to 3.90×10^{-5} mol L^{-1}), were transferred into a voltammetric cell containing 10 mL of 0.1 mol L^{-1} sulphuric acid. After every aliquot addition, the solution was stirred for 30 s at 2000 rpm, rested for 10 s then SWV mode was ramped from +300 to +1600 mV with scan rate 0.1 V/ s and pulse amplitude 50 mV, measurement time 6 ms. The experiment was triplicated for every standard solution addition. The anodic peak current was plotted versus final concentration in (µg mL^{-1}) to get the calibration curve and so the

corresponding regression equations were derived.

2.3.3 Application to pharmaceutical formulation

Five tablets were thoroughly grounded to a fine powder mixed well and an average weight of one tablet was accurately weighed to prepare a stock solution of $1.53x10^{-4}$ mole L^{-1} ROP HCl, transferred to a 100 mL of measuring flask, sonicated for 60 min in 50 mL of 0.1 mole L^{-1} sulphuric acid then completed to the final volume with the same solvent and mixed well. An aliquot from the clear supernatant was then analyzed according to the proposed voltammetric procedure based on standard addition method.

2.3.4 Application to spiked human plasma

0.5 ml of drug-free serum samples were transferred into10 ml centrifuge tube, fortified with 0.135 mL of 6.45 x 10^{-3} mol L^{-1} ROP solution (equivalent to 5.12 x 10^{-6} mol L^{-1} in final dilution). The solution mixture was treated with 1.5 ml acetonitrile, centrifuged for 20 min at 6000 RPM, followed by filtration of acetonitrile extract on 0.22 micro filter. Then required volume of the filtered acetonitrile extract was transferred into the voltammetric vessel and diluted to 10 mL with 0.1 mole L^{-1} sulphuric acid and analysed according to the proposed procedure using the standard addition method. The experiment is repeated with new activation of electrode surface by polishing and sweep activation as described before.

3. RESULTS AND DISCUSSION

3.1 Optimization of Experimental Conditions

3.1.1 Effect of pH

The variation of pH value, from 2 to 9, with oxidation potential and current, was studied using differential pulse (DP) sweep and scan rate 0.06 V/s. A well developed anodic peak current was observed over the pH range from 2 to 9 as shown in (Fig. 3) but an ill developed second peak current started to show which increased gradually with increasing the pH values. The first peak potential of oxidation process was shifted to the less-positive potential value with increasing the pH values up to 9 indicating the irreversible nature of the oxidation process which was confirmed by cyclic voltammetry (CV), (Fig. 4). CV showed that the corresponding peak current was decreased due to the hindrance of the oxidation process by decreasing the concentration of protons and strong adsorption of oxidation products of ROP on the electrode surface. This implies that H^+ ions were involved in the oxidation of ROP molecule and the deprotonation step proceeds before the electron transfer step.

The plot of the first peak potential versus pH showed one straight line between 2.0 and 9.0, which can be expressed by the following equation in Britton–Robinson buffer: (Fig. 5).

$$Ep = 1.45 - 0.5592\ pH$$
(r=0.997)

This slope is close to the Nernst theoretical value of 59 mV/pH [28] and according to the following equation

$$Ep = E° - \frac{RT}{nF}\ ln\frac{[Ox]}{[Red]} \pm \frac{2.303\ \partial RT}{nF} pH$$

Here, E° is standard peak potential in volt (V); [Ox] and [Red] are the equilibrium concentrations of oxidized and reduced species, respectively, and ∂ is the number of protons participated in mechanism and *n* is the number of electrons transferred. As demonstrated from the above equation, the ratio of proton to electron participated in the oxidation process was calculated as -0.9458 nearly equal 1 indicating the participation of equal number of protons and electrons in the oxidation of ROP [29].

3.1.2 Effect of supporting electrolyte

On using 0.1 mol L^{-1} sulphuric acid solution as alternative supporting electrolyte of BR-buffer solution at pH 2, it is found that 0.1 mol L^{-1} sulphuric acid (pH≈1) gave better peak than the buffer and was selected for the next study as demonstrated in (Fig. 6).

3.1.3 Effect of different oils and different composition of oil in the carbon electrode

The water-immiscible organic binder (pasting liquid), *viz.* Paraffin, silicon and castor oils, were studied in preparing carbon paste electrode and found that paraffin oil gave a slightly higher sensitivity with excellent correlation coefficient 0.9999 than castor oil did with r=0.9986 .On the other hand, studying the different percentage of

paraffin oil; it was found that 200 µl paraffin oil emerged high linear responses towards the Ropinirole HCl concentrations.

3.1.4 Effect of scan rate

The peak potential was shifted to more positive potential by increasing the scan rate, (Fig. 7) confirming the irreversibility of the oxidation electrode reaction of ROP and this was confirmed by no peak was noticed in the reverse direction in cyclic voltammetry indicating the irreversible oxidation behaviour of the adsorbed species at CPE as shown in (Fig. 4). As the peak currents decrease with successive potential scans suggesting an adsorbed oxidation product on the electrode surface.

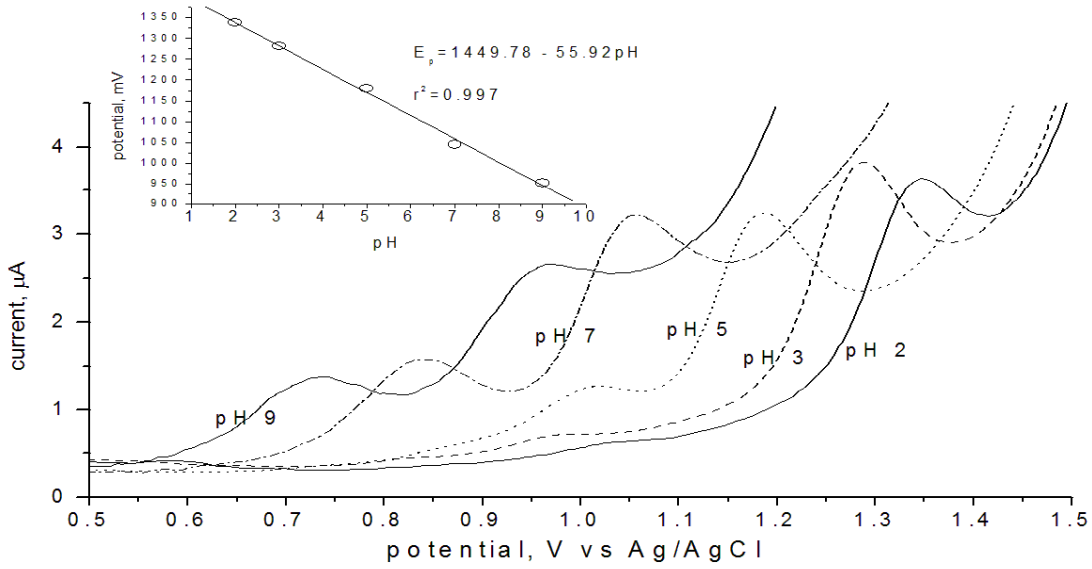


Fig. 3. Effect of pH on DP voltammetric peak potentials for 2.5 µg mL^{-1} Ropinirole HCl in Britton–Robinson buffer at CPE obtained with anodic peak Ox1 and anodic peak Ox2, stirring for 30 s at 2000 rpm, stop stirring for 10 s, scan rate 0.06 V/s and pulse amplitude 50 mV

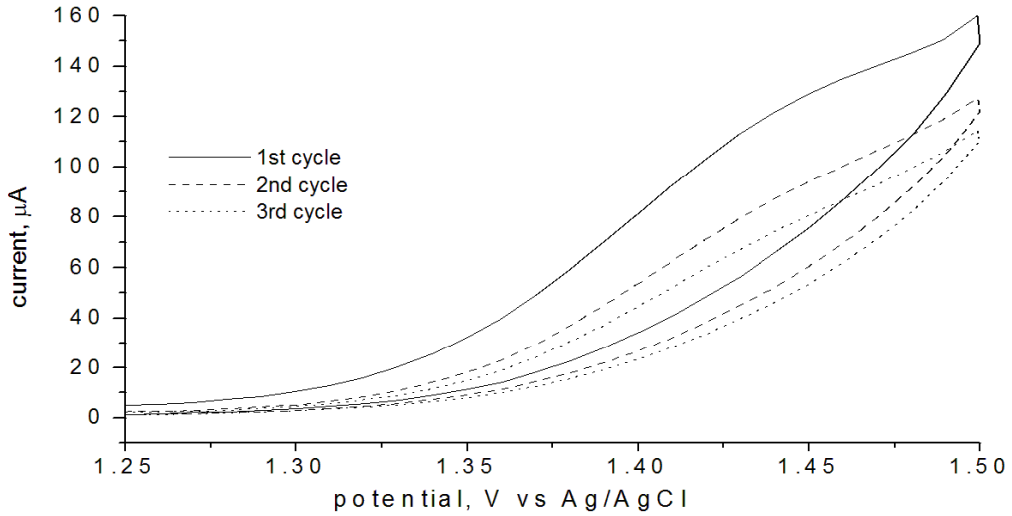


Fig. 4. Cyclic voltammogram for 2.5 µg g mL^{-1} Ropinirole HCl on the CPE in 0.1 mol L^{-1} sulphuric acid as supporting electrolyte, stirring for 30 s at 2000 rpm, stop stirring for 10 s, scan rate 0.06 V/s and pulse amplitude 50 mV

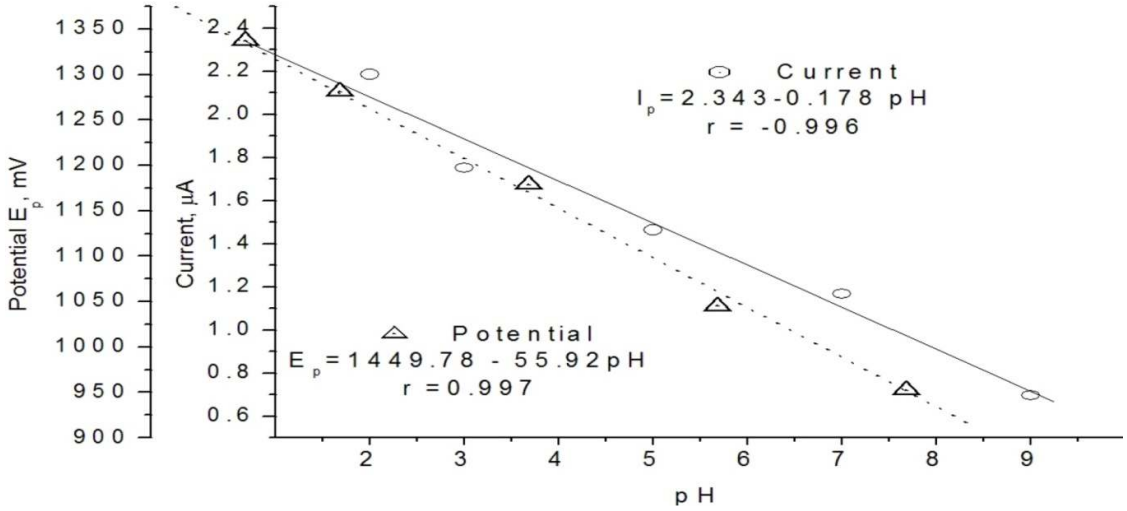


Fig. 5. Plotting of pH versus voltammetric peak potential and current of 2.5 µg g mL^{-1} Ropinirole HCl, on DP voltammetry, stirring for 30 s at 2000 rpm, stop stirring for 10 s scan rate 0.06 V/s and pulse amplitude 50 mV

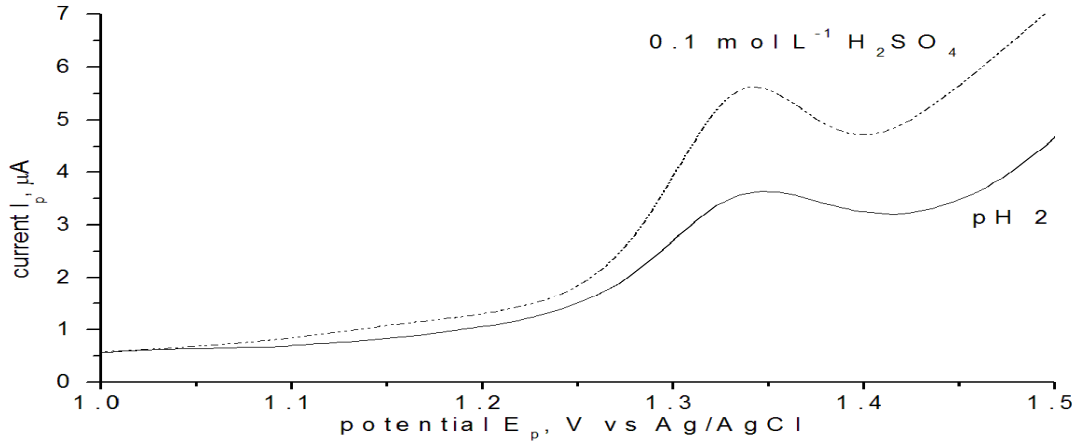


Fig. 6. The effect of pH 2 and 0.1 mil L^{-1} sulphuric acid as supporting electrolyte on voltammetric peak potentials for 2.5 µg g mL^{-1} Ropinirole HCl, on DP voltammetry, stirring for 30 s at 2000 rpm, stop stirring for 10 s, scan rate 0.06 V/s and pulse amplitude 50 mV

For this kind of mechanism, the relationship between the peak potential E_p and scan rate u was expressed and the $E°$ value at CPE can be deduced from the intercept of (E_p, *V)* versus scan rate (*u, V/s)* which equal to E°=1. 3533 *V*.

α*n* value can then be calculated from the equation [30]

$$E_{P_a} - E_{P_{a/2}} = \frac{1.857RT}{\alpha nF}$$

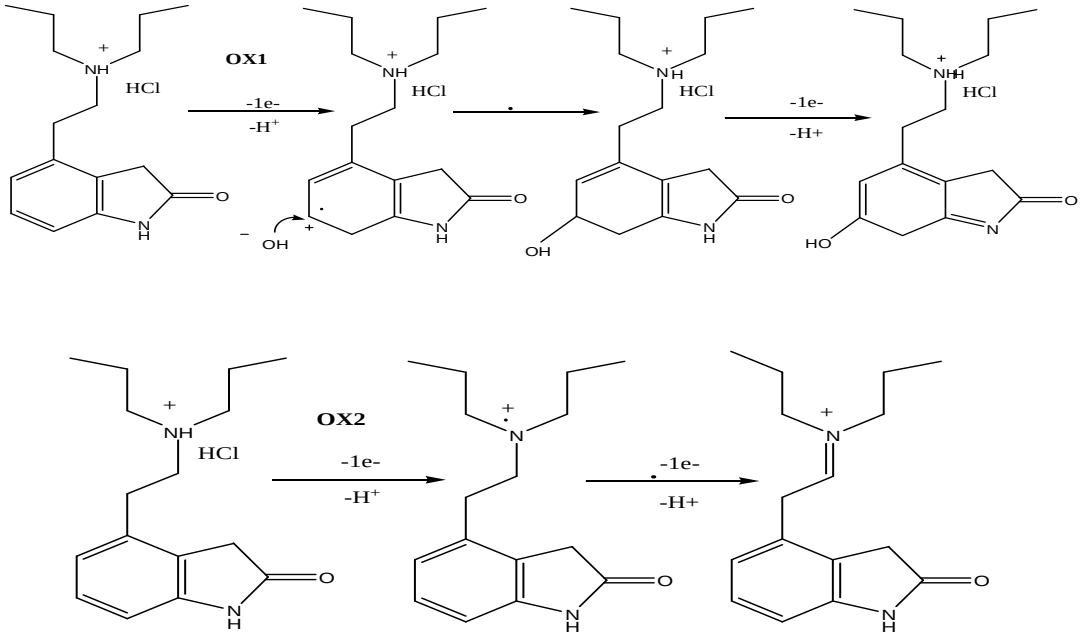
And at 25ºC, then

$$E_{P_a} - E_{P_{a/2}} = \frac{47.7}{\alpha n}$$

αn = 0.711 and by α =*0.35* (range from 0.30 to 0.70), thus the number of electrons transfer *n*= 2.034 for oxidation step which is most ≈ 2 indicating that two electrons was involved in the oxidation of ROP on the CPE. And as shown before that the total numbers of electrons and protons taking part in the charge transfer was the same so the electrochemical reaction process for ROP oxidation at CPE can be summarized as in (scheme 1).

Considering the molecular structure of ROP, the anodic peak is probably due to the oxidation in the indol-2-one ring such as isatin and ziprasidone [31,32] as well as the possibility of obtaining an oxidation signal in strong acidic media, the oxidation process can be attributed to the oxidation of indol-2-one ring moiety which is electroactive in both acidic and basic media, leading to probably hydroxylation of the benzene ring.

In supporting electrolytes of pH values 5.0 and above, ROP gave two separate oxidation steps as shown in (Fig. 3). Taking into account the break point of E_p vs. pH plot for Ox2 and anodic voltammetric behavior of some drugs which have tertiary amine group as only electroactive site on the molecule like doxepin, [33] the second oxidation step (Ox2) appeared in neutral and alkaline conditions could be located on the aliphatic nitrogen [34]. After deprotonation, ROP lost an electron to form a cation radical which in the subsequent step formed a quaternary Schiff base by losing a proton and an electron. Analysing the evolution of the SWV peak currents, it is possible to observe that this parameter shows dependence on the pH of the medium and supporting electrolyte composition. The slow decrease observed was accompanied by an appearance of second oxidation step Ox2 at less positive potentials. The second oxidation process became more pronounced as the pH decreased due to deprotonation of the amine group, however, the voltammetric signals Ox1 and Ox2 were not well-resolved. On the other hand, the peak current of Ox1 was best developed in the form of a sharp peak and was easily measurable as a single response in strongly acidic media. The anodic peak Ox1 reached the highest value in 0.1 mol L^{-1} sulphuric acid, which consequently was selected as the optimum supporting electrolytes for electroanalytical studies. The peak Ox1 decreased gradually by increasing the pH value.
By plotting the current *(I_P)* against scan rate (*υ)* it has been shown that the current was increased by increasing the scan rate (Fig. 8, left) and scan rate 0.1 V/s was selected, and the electrochemical process is diffusion controlled process as indicated by a value of the slope of (0.229) deduced from plotting logarithm current *(log I_P)* versus logarithm scan rate (log *υ)* as given in (Fig 8, right) which is less than 0.5. [28], [35-41].



Scheme 1. The proposed oxidation mechanism of ROP on CPE in 0.1 mol L^{-1} sulphuric acid as supporting electrolyte, stirring for 30 s at 2000 rpm, stop stirring for 10 s, scan rate 0.1 V/s and pulse amplitude 50 mV

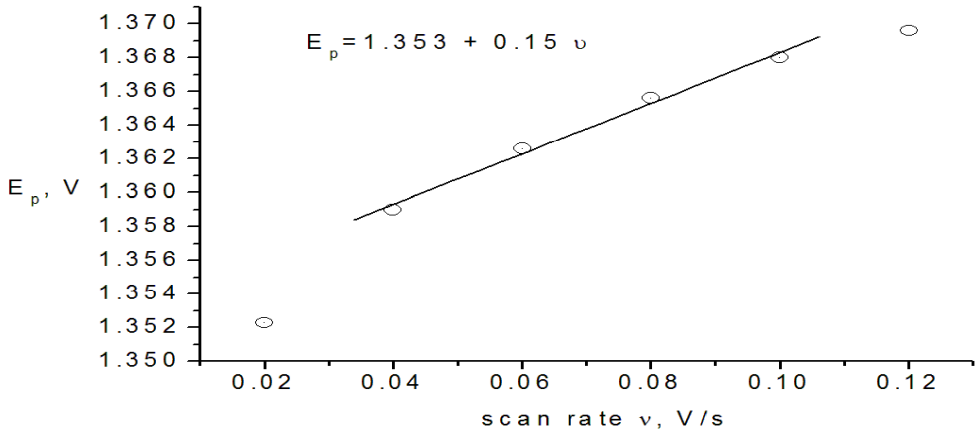


Fig. 7. Plotting of potential (Ep,V) against scan rate (u,V/s) of 2.5 µg mL^{-1} Ropinirole HCl in 0.1 mol L^{-1} sulphuric acid as supporting electrolyte, stirring for 30 s at 2000 rpm, stop stirring for 10 s and pulse amplitude 50 mV

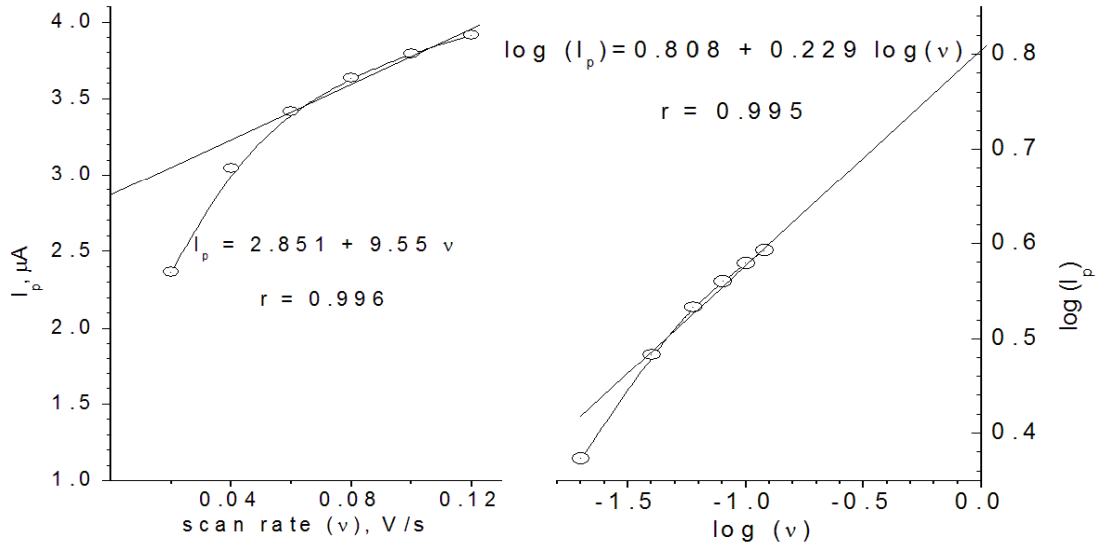


Fig. 8. Plot of current *(I_p,µA)* against scan rate (*u,V/s)* on left and log current *(log I_p)* against (log *u*) on right on 2.5 µg mL^{-1} Ropinirole HCl in 0.1 mol L^{-1} sulphuric as supporting electrolyte, stirring for 30 s at 2000 rpm, stop stirring for 10 s and pulse amplitude 50 mV

3.1.5 Effect of pulse amplitude

By studying the effect of pulse amplitude (ΔE,*mV*) against the current (IP, *µA*) and potential (E,*mV*) it was found to be 50 is the best.

3.1.6 Effect of stir

Stirring is necessary to prevent the depletion region around the surface of the electrode after each sweep, so it was found that rotating CPE gave higher current when compared to static form. And by studying the time of rotation, it was found the best time is the 30 s stirring without accumulation time as it leads to noise.

3.2 Linearity and validation parameters

3.2.1 Linearity and range

Voltammogram of four different modes of sweep, viz., direct current (DCT), differential pulse (DP), square wave (SWV) and first harmonic alternating current (AC1), are compared over a potential range from +300 to +1600 mV in the presence of 0.1 mol L^{-1} sulphuric acid with

stirring for 30 s, stop stirring for 10 s, scan rate of 0.1 V/s, and pulse amplitude of 50 mV (Fig. 9).

The SWV mode is selected due to its highest response and good linearity (Fig. 10) and applied successfully to determining the active ingredients in pharmaceutical preparations and in spiked serum. Calibration curves were constructed as a function of the concentrations of standard Ropinirole HCl in µg mL^{-1} (*X*) versus their peak currents, µA (*Y*) and the performance data of the proposed SWV method was presented in (Table 1).

3.2.2 Detection and quantification limits

The limits of detection and quantification (LOD) and (LOQ) were found to be 0.0.44 (µg mL^{-1}) and 1.47 (µg mL^{-1}) respectively, which indicates the sensitivity of the method in determination of ROP as presented in (Table 1).

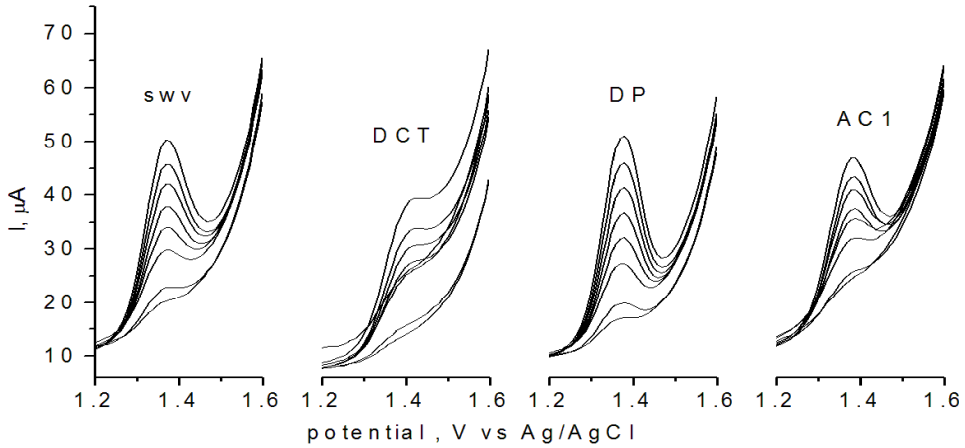


Fig. 9. Voltammogram of different modes on different concentrations of Ropinirole HCl in 0.1 mol L^{-1} sulphuric acid as supporting electrolyte, stirring for 30 s at 2000 rpm, stop stirring for 10 s and pulse amplitude 50 mV, scan rate 0.1 V/s on rotating CPE

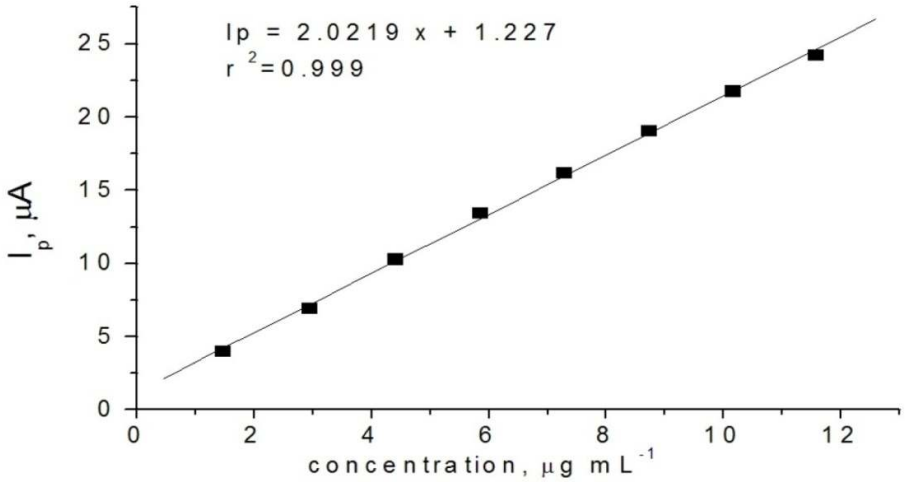


Fig. 10. The calibration curve of Ropinirole HCl on SWV mode in 0.1 mol L^{-1} sulphuric acid as supporting electrolyte, stirring for 30 s at 2000 rpm, stop stirring for 10 s, scan rate 0.1 V/s and pulse amplitude 50 mV on rotating CPE

Table 1. Performance data on the proposed SWV mode for determination of ROP in pure form

Mode	SWV
Concentration range (µg mL^{-1})	1.47-11.58
Slope	2.022
Intercept	1.22
Correlation (r)	0.999
$SD_{Y/X}$*	0.29
Average, %	99.15
RSD, %	3.7
RE, %	0.85
LOD (µg mL^{-1})	0.44
LOQ (µg mL^{-1})	1.47

**standard deviation y/x*

3.2.3 Accuracy and precision

The accuracy and precision data have been presented in (Table 2).The intra-day and inter-day data which were evaluated by replicate analysis of three different concentrations (4.40, 7.29 and 10.16 µg mL^{-1}) of authentic drug three times a day for three constitutive days with the same standard and without polishing of the electrode.

3.3 Applications

3.3.1 Determination of ROP in tablets

ROP was analyzed in commercial film-coated tablets (Tremodict). Well-defined SWV peaks were obtained and no interferences were observed (Table 3). It is clear from statistical data that no significance difference between the proposed method and official HPLC method [1].

3.3.2 Application on spiked plasma

The recovery studies of the drug in serum samples were performed using the standard addition and calculated by intercept method. The mean recoveries for ROP were achieved after protein precipitation and filtration of acetonitrile extract on 0.22 micro filter then direct determination without evaporation of acetonitrile. It shows mean of % recovery ± % RSD of (99.56±3.63) (Table 4).

Table 2. Precision data of the proposed SWV method for determination of ROP in pure form

Parameter	Intra-day precision (Repeatability)			Inter-day precision (intermediate precision)		
Concentration, µg mL^{-1}	4.40	7.29	10.16	4.40	7.29	10.16
	101.18	101.47	99.83	101.70	101.55	99.96
	101.37	101.77	100.00	99.67	100.22	100.04
	102.76	101.93	99.76	101.04	100.38	99.80
Average, %	101.77	101.73	99.87	100.80	100.72	99.93
RSD, %	0.85	0.23	0.12	1.03	0.72	0.12
ER, %	0.06	0.18	0.09	0.89	0.82	0.02

Table 3. Analysis of ROP in film-coated tablets by the proposed SWV, and official HPLC method [1]

Parameter	SWV method	Official HPLC method [1]
	Recovery, %	Recovery, %
	99.83	103.06
	96.67	105.75
	97.90	105.55
	96.04	
	103.70	
Average,%	98.83	104.79
RSD, %	3.12	1.43
F test	4.23 (19.25)	
Student t-test	0.54 (2.45)	

**Tabulated F and t values at* (*P* = .05) *confidence level*

Table 4. Determination of ROP in spiked plasma

Spiked plasma	Recovery found, %*
1	97.45
2	97.50
3	103.74
Average, %	99.56
RSD, %	3.63

**numbers of replicates are three for each determination*

4. CONCLUSION

The oxidation of ROP is a pH dependent, irreversible process in 0.1 mol L^{-1} sulfuric , ROP oxidation involves transference of two electrons and two protons in in indol- 2-one ring. This voltammetric response is used for electroanalytical measurements of the drug molecule. Possible oxidation mechanism of ROP was discussed. New SWV method for the electroanalytical determination of ROP on CPE which is economic was developed and validated. The voltammetric method proposed was applied to direct quantification of ROP in film-coated tablets and spiked human plasma.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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