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ELECTROCHEMICAL DETERMINATION OF ANTICOCCIDIAL DRUG ROBENIDINE HYDROCHLORIDE

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The electrochemical oxidation of robenidine hydrochloride has been carried out in Britton–Robinson buffer at carbon paste and glassy carbon electrodes. Robenidine hydrochloride exhibits a well-defined irreversible oxidation peak over the entire pH range (2-8). Differential pulse voltammetry was used to determine robenidine hydrochloride in the pure form. The peak current varied linearly in the following ranges: 4.0 × 10^{-7} - 3.2 × 10^{-6} mol l^{-1} in the case of carbon paste electrode, and 4.0 × 10^{-7} - 2.8 ×10^{-6} mol l^{-1} with the glassy carbon electrode. In the case of carbon paste electrode the limits of detection and quantification were 7.33 × 10^{-8} mol l^{-1}, and 2.44 × 10^{-7} mol l^{-1}, respectively. For glassy carbon electrode, they were 7.93 × 10^{-8} mol l^{-1} and 2.64 × 10^{-7} mol l^{-1}, respectively. The percentage recoveries were found in the following ranges: 99.37-100.83 % and

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99.17-101 % for carbon paste and glassy carbon electrodes, respectively. The relative standard deviations were found in the following ranges: 0.376-0.973 % and 0.657-1.28 % in the case of carbon paste and glassy carbon electrodes, respectively. Differential pulse voltammetry method was successfully applied for the determination of robenidine hydrochloride in pharmaceutical form and chicken serum.

Introduction

Robenidine hydrochloride (1,3-bis[(4-chlorobenzylidene)amino]guanidinehydrochloride) is used as an aid in prevention of coccidiosis caused by *Eimeria tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunetti*, and *E. mivati* and as an aid in the control or prevention of necrotic enteritis caused or complicated by *Clostridium spp.* in chickens, turkeys and rabbits [1]. EC regulations set minimum and maximum content of robenidine hydrochloride in complete feeding stuffs at 30-36 and 50-66 mg kg^{-1} for poultry and rabbit feed, respectively [2]. Therefore, effective analytical methods are needed for determination of coccidiostats.

Carbon-based electrodes are currently in widespread use in electroanalytical chemistry, because of their broad potential window, low cost, rich surface chemistry, low background current, and chemical inertness. Carbon paste electrode (CPE) has found their place in modern electroanalysis due to its verstility, ease of preparation and composition modification, low cost ingredients and instrumentation, high sensitivity, reasonable reproducibility, broad potential range availability. The disadvantage of CPE is the tendency of the organic binder to dissolve in solutions containing an appreciable fraction of organic solvent. Glassy carbon electrode (GCE) is a class of nongraphitizing carbon that is widely used as an electrode material in electrochemistry. It is also known as vitreous carbon. Glassy carbon electrode is used very commonly because of its excellent mechanical and electrical properties, impermeability to gases, and extremely low porosity [3-4].

Literature search reveals that very few analytical procedures have been developed for the determination of robenidine hydrochloride (Roben). These procedures based on high performance liquid chromatography (HPLC) with UV spectrophotometric detection [5-6], normal phase HPLC with fluorescence detection after derivatization with dansyl chloride [7], high performance liquid chromatography (HPLC) with UV diode-array and mass spectrometer detectors after accelerated solvent extraction [8], and liquid chromatography-tandem mass spectrometry method were described for simultaneous detection of Roben and some other coccidiostats in eggs [9-11].

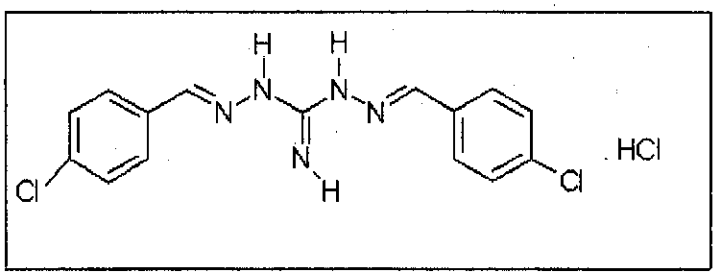

Fig. 1. The molecular structure of Robenidine hydrochloride

Electrochemical methods have proved to be fast, accurate, precise, simple, and sensitive for the determination of organic molecules that undergo oxidation or reduction reactions, including drugs and related molecules in pharmaceutical dosage forms and biological fluids [12-18].

In continuation to our previous work [19-25], the aim of this study was to establish and to optimize the experimental conditions for the determination of Roben in the pure form, pharmaceutical form and serum by using cyclic voltammetry and differential pulse voltammetry (DPV) techniques.

Experimental

Apparatus

The voltammetric measurements were carried out using a computer-driven AEW2 analytical electrochemical workstation with ECProg3 electrochemistry software (Sycopel, England) in combination with a C-2 stand with a three-electrode configuration: a glassy carbon disc electrode (BAS model MF-2012) or a carbon paste electrode (BAS model MF-2010) as working electrode, a Ag/AgCl/3 M NaCl (BAS model MF-2063) reference electrode, and a platinum wire (BAS model MW-1032) counter electrode. Origin 7.0 software was used for the transformation of the initial signal. A cyberscan 500 (EUTECH Instruments, USA) digital pH-meter with a glass combination electrode served to carry out the pH measurements.

Reagents

Robenidine hydrochloride (Roben) was supplied from El-Nasr Pharmaceutical Company, Egypt. Its pharmaceutical form (Cycostat powder) was manufactured by Efat Trading Company, Egypt. Stock 1.0×10^{-3} mol l^{-1} solution of Roben was

prepared by dissolving an appropriate amount of Roben in dimethylformamide (DMF) which was obtained from El-Nasr Pharmaceutical Company, Egypt. The stock solution was stored in a refrigerator. Britton–Robinson (BR) and, KCl-HCl buffers were prepared by using analytical grade reagents. Graphite powder and Nujol (which is a mineral oil) were supplied from Aldrich and Sigma, respectively. The serum sample obtained from healthy Chicken was collected and stored frozen until assay.

Preparation of the Working Electrodes

A paste was prepared by mixing 0.5 g graphite powder with 0.3 ml Nujol in a mortar with a pestle. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper until it had a shiny appearance.

To improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode (GCE) was polished manually with 0.5 μm alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

Assignment of the Optimum Conditions for the Determination of Robenidine Hydrochloride

To obtain the optimum pH, an appropriate amount of Roben working standard 1.0×10^{-3} mol l^{-1} solution was placed in the electrolytic cell, which contained 5 ml BR buffer solution of pH 2 and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values (3-11) and the optimum pH was obtained.

To study the effect of buffer type on the peak current (I_p) of Roben, the working electrode was immersed in buffer solution of the optimum pH containing an appropriate amount of the drug stock solution (1×10^{-3} M), and the cyclic voltammogram was recorded. The experiment was repeated by using a different buffer.

To study the effect of scan rate (v) on the peak current (I_p) of Roben, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Roben standard solution 1.0×10^{-3} mol l^{-1}, and the cyclic voltammograms were recorded at different scan rates over the scan range of 10-250 mV s^{-1}. A plot of log I_p vs log υ was constructed in order to recognize the nature of the process: whether a diffusion-controlled one or an adsorption-controlled one.

To study the effect of accumulation time, the working electrode was

immersed in the optimum buffer solution containing an appropriate amount of Roben standard 1.0×10^{-3} mol l^{-1} solution for selected times with stirring at 1200 rpm at open circuit condition. After accumulation, the cyclic voltammograms were recorded followed by plotting the peak current (I_p) vs time to obtain the optimum accumulation time.

The optimum instrumental conditions for the determination of Roben by using DPV method were chosen from a study of the variation of the peak current with pulse amplitude, pulse width and scan rate. During the study, each parameter was varied while the others were kept constant: pulse amplitude over the range of 25-100 mV, pulse width 30-90 ms, and scan rate 10-50 mV s^{-1}

General Procedure for the Determination of Robenidine Hydrochloride in the Pure Form

The voltammetric analyses were performed in 5 ml BR buffer. The solution was continuously stirred at 1200 rpm when accumulation potential (usually open circuit condition) was applied for a certain time to the working electrode. At the end of accumulation period, the stirring was stopped and a 5 sec rest period was allowed for the solution to become quiescent. The used drug was determined by using DPV method. Aliquots of the drug solution (1.0×10^{-3} mol l^{-1}) were introduced into the electrolytic cell and the procedure was repeated. The voltammograms were recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All measurements were carried out at room temperature.

Determination of Robenidine Hydrochloride in Cycostat Powder

The amount of the powder needed to obtain a 1×10^{-3} mol l^{-1} Roben was accurately weighed and transferred into 100-ml volumetric flask, 80 ml DMF was added; the flask was sonicated for about 15 min and the volume made up with the same solvent. The solution was then filtered to separate the insoluble excipients, rejecting the first portion of the filtrate. Aliquots of the drug solution were introduced into the electrolytic cell and the general procedure was carried out.

Determination of Robenidine Hydrochloride in Serum

After gentle thawing, 0.1 ml serum sample was transferred into 10-ml measuring flask, a suitable volume of the drug solution was added, and the volume was made up to the mark with methanol. After separation of proteins, the supernatant was

taken carefully, and an appropriate volume of supernatant liquor was transferred to 10-ml volumetric flask and diluted. The obtained solution was used for voltammetric determination by using DPV method as for the pure drug.

Result and Discussion

To elucidate the electrode reaction of Roben, the cyclic voltammograms at carbon paste and glassy carbon electrodes were recorded at different pH values and at different scan rates. As an example, Fig. 2 shows the cyclic voltammograms of 4.0 $\times 10^{-5}$ M Roben solution in BR buffer of pH 2 in the case of carbon paste and glassy carbon electrodes, at a scan rate of 100 mV s^{-1}. Each voltammogram exhibits one well-defined anodic peak, with no peak on the reverse scan, suggesting the irreversible nature of the electrode reaction.

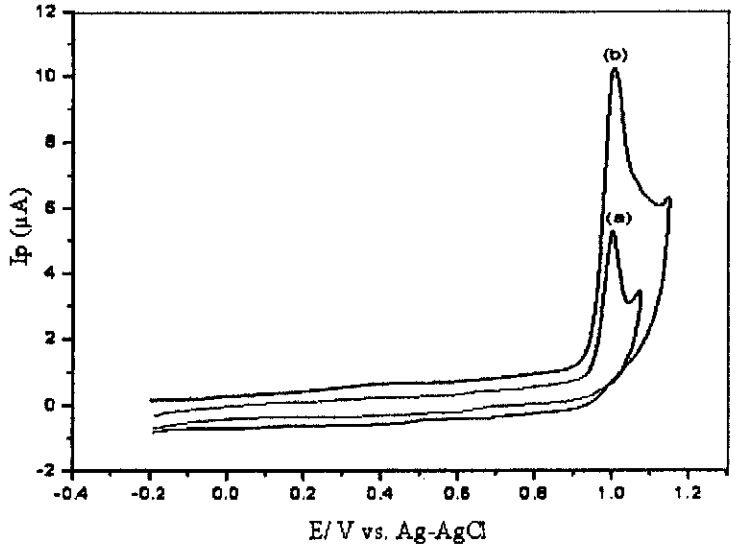

Fig. 2 Cyclic voltammograms of 4.0 $\times 10^{-5}$ mol l^{-1} Roben solution in BR buffer of pH 2 in the case of CPE (a) and GCE (b) at a scan rate of 100 mV s^{-1}

Effect of pH

The influence of pH on Roben at carbon paste and glassy carbon electrodes was studied. Figure 3 shows the plot of peak current (I_p) vs. pH. It is obvious from the figure that the peak current reaches its maximum value at pH 2 in the case of carbon paste and glassy carbon electrodes, i.e. acidic medium is suitable for the determination of Roben using DPV technique.

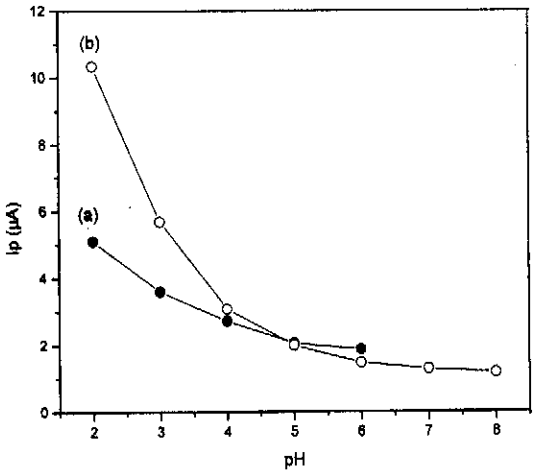

Fig. 3 Effect of pH on peak current of 4.0×10^{-5} mol l^{-1} Roben solution in BR buffer at CPE (a), and GCE (b) at a scan rate of 100 mV s^{-1}

Effect of Buffer Type

Cyclic voltammetry experiments of 4.0×10^{-5} mol l^{-1} Roben solution in BR buffer and HCl-KCl buffer at pH 2 in the case of carbon paste and glassy carbon electrodes were carried out. Figures 4 and 5 show the cyclic voltammograms of Roben using BR buffer and HCl-KCl buffer. It is obvious from the figures that BR buffer is suitable for electrochemical determination of Roben.

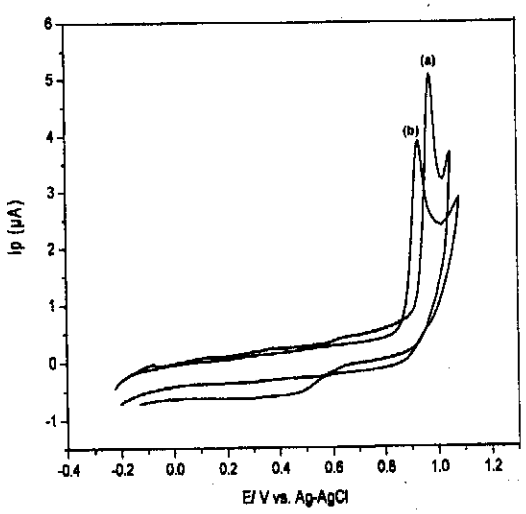

Fig. 4 Cyclic voltammograms of 4.0×10^{-5} mol l^{-1} Roben solution at CPE in BR buffer (a) and HCl-KCl buffer (b) at pH 2. Scan rate of 100 mV s^{-1}

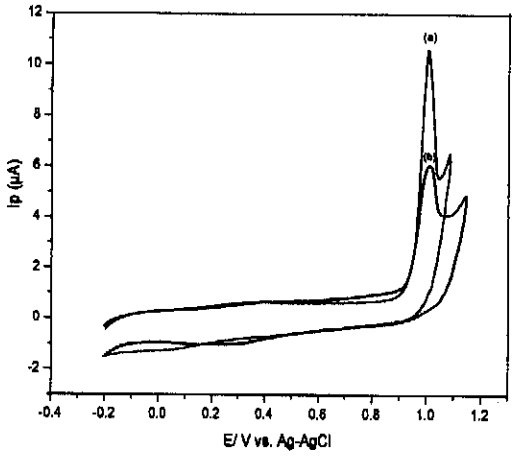

Fig. 5 Cyclic voltammograms of 4.0×10^{-5} mol l^{-1} Roben solution at GCE in BR buffer (a) and HCl-KCl buffer (b) at pH 2. Scan rate of 100 mV s^{-1}

Effect of Scan Rate

The effect of scan rate (v) on the peak current of Roben is shown in Fig. 6. Linear relationships were observed between log I_p and log v over the scan range of 10-250 mV s^{-1} and correspond to the following equations: $\log I_p = -0.44 + 0.61 \log v$, and $\log I_p = -0.25 + 0.43 \log v$ in the case of carbon paste electrode and glassy carbon electrode, respectively. The slope of 0.61 indicates a diffusion-controlled process with some adsorption character, and the slope of 0.43 is close to the theoretically expected value of 0.50 for a diffusion-controlled process [26].

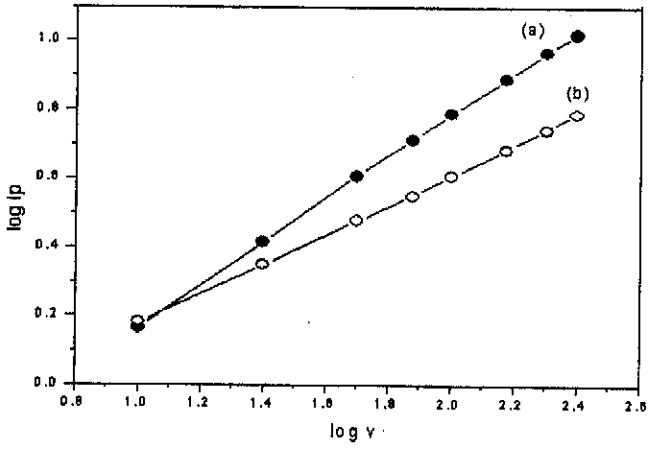

Fig. 6 Anodic peak current response of 4.0×10^{-5} mol l^{-1} Roben solution as a function of scan rate (v) in BR buffer of pH 2 at CPE (a) and GCE (b)

Effect of Accumulation Time

The effect of accumulation time on the anodic peak current of 4.0×10^{-5} mol l^{-1} Roben solution at pH 2 was studied for carbon paste electrode at open circuit condition and the results are shown in Fig. 7. It is concluded that increasing peak currents were obtained up to accumulation time of 90 sec. Hence 90 sec is chosen as the optimum accumulation time.

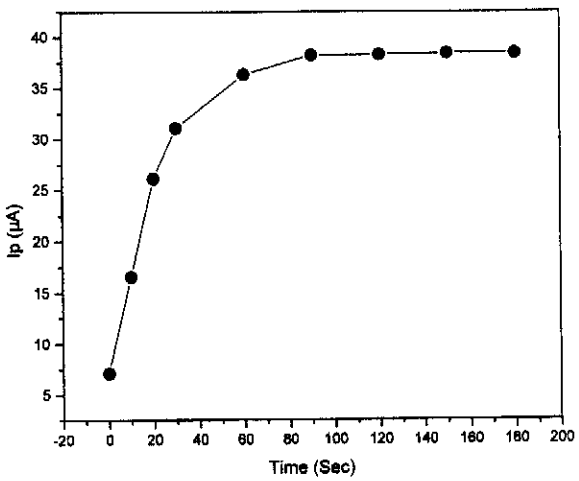

Fig. 7 Effect of accumulation time on the peak current of 4.0×10^{-5} mol l^{-1} Roben solution in BR buffer of pH 2 at CPE

Effect of Instrumental Parameters

It was found that the peak current was increased with increasing pulse amplitude and scan rate, while it decreased with the increasing pulse width. To obtain relatively high and narrow peaks, the values of 50 mV, 30 ms and 20 mV s^{-1} were finally chosen for pulse amplitude, pulse width and scan rate, respectively.

Determination of Robenidine Hydrochloride in the Pure Form

On the basis of the electrochemical oxidation of Roben at carbon paste and glassy carbon electrodes, analytical method was developed involving differential pulse voltammetry for the determination of the drug under investigation. Linear relations between the peak current (I_p) and Roben concentration (C) were found in the following range: 4.0×10^{-7} - 3.2×10^{-6} M for carbon paste electrode and 4.0×10^{-7} - 2.8×10^{-6} M in for glassy carbon electrode. The calibration plots were described

by the following equations:

1: I_p (μA) = 0.295C (μM) + 1.666 r = 0.9999 for carbon paste electrode
2: I_p (μA) = 0.509C (μM) + 0.514 r = 0.9999 for glassy carbon electrode

Three replicate calibration curves were obtained over the concentration ranges of 4.0×10^{-7} - 3.2×10^{-6} mol l^{-1} in the case of carbon paste electrode and 4.0×10^{-7} - 2.8×10^{-6} mol l^{-1} in the case of glassy carbon electrode. The limits of detection (LOD) and quantitation (LOQ) were calculated by using the following equations: $LOD = 3\ S.D./m$, and $LOQ = 10\ S.D./m$, where "$S.D.$" is the standard

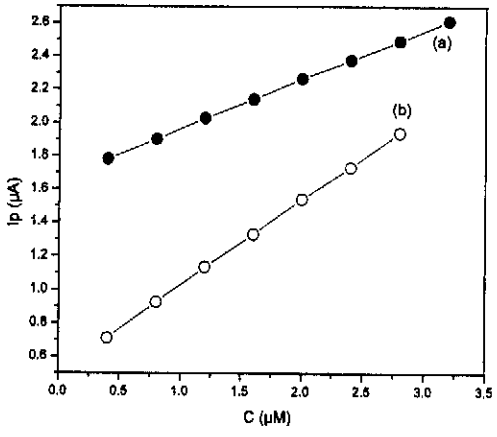

Fig. 8 Calibration curve of Roben at CPE (a) and GCE (b) by using DPV, the pulse amplitude of 50 mV at a scan rate of 20 mV s^{-1}

Table I Analytical parameters of robenidine hydrochloride

Parameter	Carbon paste electrode (CPE)	Glasy carbon electrode (GCE)
Linearity range, mol l^{-1}	4.0×10^{-7} - 3.2×10^{-6}	4.0×10^{-7} - 2.8×10^{-6}
Calibration curve equation	I_p (μA) = 0.295C (μM) + 1.666	I_p (μA) = 0.509C (μM) + 0.514
Correlation coefficient (r)	0.9999	0.9999
Limit of detection (LOD), mol l^{-1}	7.33×10^{-8}	7.93×10^{-8}
Limit of quantitation (LOQ), mol l^{-1}	2.44×10^{-7}	2.64×10^{-7}
Relative standard deviation (RSD), %	0.367-0.973	0.657-1.28
Recovery (R), %	99.37-100.83	99.17-101.00

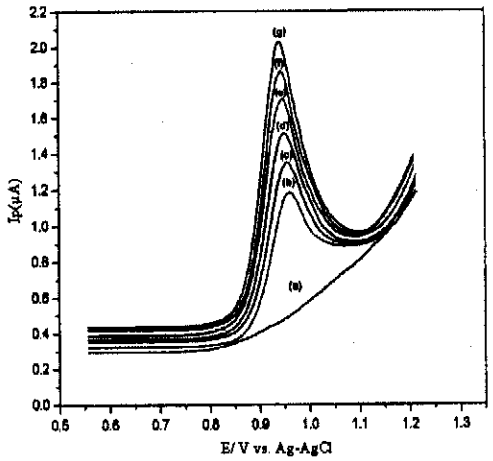

Fig. 9 Differential pulse voltammograms for different concentrations of Roben in serum samples at CPE, the pulse amplitude of 50 mV, t_{acc} = 90 sec, and at a scan rate of 20 mV s^{-1}. Blank (a), 0.8 (b), 1.2 (c), 1.6 (d), 2 (e), 2.4 (f), and 2.8 μM (g)

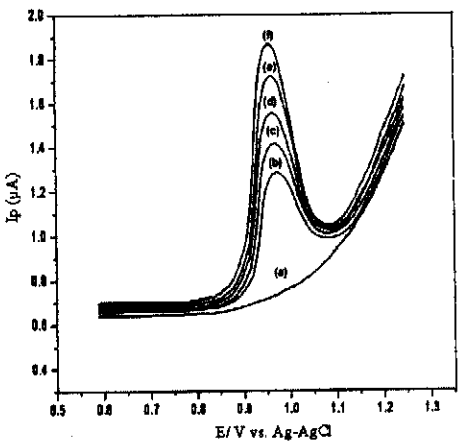

Fig. 10 Differential pulse voltammograms for different concentrations of Roben in serum samples at GCE, the pulse amplitude of 50 mV and at a scan rate of 20 mV s^{-1}. Blank (a), 0.8 (b), 1.2 (c), 1.6 (d), 2 (e) and 2.4 (f)

deviation of the intercept of the calibration curve and "*m*" is the slope of the calibration curve [27]. The limits of detection (*LOD*) and quantitation (*LOQ*) in the case of carbon paste electrode were 7.33×10^{-8} mol l^{-1} and 2.44×10^{-7} mol l^{-1}, respectively. For the glassy carbon electrode they were 7.93×10^{-8} mol l^{-1} and 2.64×10^{-7} mol l^{-1}, respectively.

Accuracy and precision of the proposed method were determined by replicate analyses of drug solutions, the results are shown in Table I. The recovery (*R*) was in the range of 99.37-100.83 % and the relative standard deviation (*RSD*)

was in the range of 0.367-0.973 % in case of carbon paste electrode.

For glassy carbon electrode, the recovery was in the range of 99.17-101 % and the relative standard deviation was in the range of 0.697-1.28 %. The recovery values of the proposed method and the relative standard deviations indicate adequate accuracy and precision.

The proposed method is more sensitive than that of high performance liquid chromatography (HPLC) with fluorescence detection [7] and HPLC with UV diode-array detector [8], both of them affording higher detection limits of 1.08×10^{-6} mol l^{-1} and 8.093×10^{-8} mol l^{-1}, respectively. Moreover, the proposed method is simple, rapid and inexpensive.

Determination of Robenidine Hydrochloride in Cycostat Powder

The proposed method was successfully applied to the direct determination of Roben in dosage form (Cycostat powder) without interference from some common excipients used in pharmaceutical preparations. The linearity range was 4.0×10^{-7} - 3.2×10^{-6} mol l^{-1} with the mean recovery of 100.30 % and the mean relative standard deviation of 0.726 % in the case of carbon paste electrode. For glassy carbon electrode the linearity range was 4.0×10^{-7} - 2.8×10^{-6} mol l^{-1} with the mean recovery of 100.27 % and the mean relative standard deviation of 0.677 %.

Determination of Robenidine Hydrochloride in Spiked Chicken Serum

The applicability of the proposed DPV method for the determination of Roben in spiked chicken serum was investigated. Figures 9 and 10 illustrate the differential pulse voltammograms for different concentrations of Roben in serum samples. The

Table II Determination of robenidine hydrochloride in spiked chicken serum

Parameter	Carbon paste electrode (CPE)	Glasy carbon electrode (GCE)
Linearity range, mol l^{-1}	8.0×10^{-7} - 2.8×10^{-6}	8.0×10^{-7} - 2.4×10^{-6}
Limit of detection (*LOD*), mol l^{-1}	1.32×10^{-7}	1.65×10^{-8}
Limit of quantitation (*LOQ*), mol l^{-1}	4.39×10^{-7}	5.49×10^{-7}
Relative standard deviation (*RSD*), %	0.589-0.976	0.619-0.903
Recovery (*R*), %	99.16-101.25	99.16-101.50

linearity range was 8.0×10^{-7} - 2.8×10^{-6} mol l^{-1} with the mean recovery of 100.28 % and the mean relative standard deviation of 0.768 % in the case of carbon paste electrode. For glassy carbon electrode the linearity range was 8.0×10^{-7} - 2.4×10^{-6} mol l^{-1} with the mean recovery of 100.32 % and the mean relative standard deviation of 0.752 %. The results are given in Table II.

Conclusion

The proposed differential pulse voltammetry method could be used successfully to determine robenidine hydrochloride in pure form, pharmaceutical forms and serum. It is a good alternative for the analytical determination of robenidine hydrochloride because it is simple, low cost, sensitive, accurate and precise. The proposed procedure showed clear advantages such as short period of real time of drug analysis and no pretreatment or time-consuming extraction steps were required prior to the analysis. Although CPE and GCE give acceptable results in the analysis of robenidine hydrochloride, we prefer GCE for biological analysis due to its high sensitivity.

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