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Cathodic adsorptive stripping voltammetry of drotaverine hydrochloride and its determination in tablets and human urine by differential pulse voltammetry

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ABSTRACT

The stripping voltammetric behaviour of drotaverine hydrochloride (DvCl) was studied using a hanging mercury drop electrode (HMDE). The adsorptive stripping response has been evaluated with respect to pH, accumulation time, accumulation potential, scan rate and other variables. Differential pulse DP mode; over the potential range −400 to −1200 mV, is used in the presence of 0.04 M Britton–Robinson buffer pH 2. Cyclic voltammetric study indicates that the reduction process is irreversible and controlled by adsorption. The response of DP technique is linear over the concentration range 21.70–257.34 ng/ml. Limit of detection and limit of quantification were 3.15 and 10.50 ng/ml, respectively. The proposed method was successfully applied for the determination of the drug in commercial tablets and spiked human urine samples.

1. Introduction

Drotaverine, 1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline, [14009-24-6], is an antispasmodic drug, structurally related to papaverine. It is a selective inhibitor of phosphodiesterose 4 and has no anticholinergic effect [1,2]. It is used in treating renal colic [3] and has also been used to accelerate labor [4] (Scheme I).

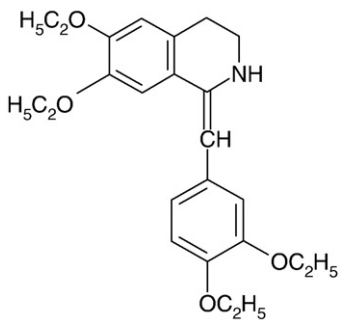
Few methods have been reported for the determination of drotaverine in dosage forms and in biological fluids including, high performance liquid chromatography (HPLC) [5–8] thin layer densitometric [9] spectrophotometric [9–12] differential spectrophotometric [13,14] computer-aided spectrophotometric [15] and potentiometric [16–19] methods. Also Fulop [20] proposed a polarographic method for determination of drotaverine in 1 M H_2SO_4 at −420 mV in the range 4–80 μg and recently Ziyatdinova [21] proposed voltammetric method for determination of the drug by oxidation at a graphite electrode in 0.1 M H_2SO_4 at 1.05 and 1.28 V, but up to now nothing has been published concerning the adsorptive cathodic stripping voltammetric determination of this drug using HMDE.

The aim of this study was to optimize and develop a sensitive, fast and accurate differential pulse adsorptive cathodic stripping voltammetric (DP/AdCSV) method for the determination of DvCl in pharmaceutical formulations and human urine samples.

2. Experimental

2.1. Apparatus

All voltammetric measurements were performed using Metrohm 757 VA Computrace (Herisau, Switzerland) equipped with a Metrohm VA 694 stand. Three electrodes assembly cell consisted of hanging mercury drop electrode (HMDE) as working electrode, an Ag/AgCl in 3 mol/l KCl (Metrohm 6.0728.000) as a reference electrode and platinium wire (Metrohm 6.0343.000) as an auxiliary electrode. The pH measurement were carried out with Jenway model 3305 pH meter.



Scheme I. Structural formula for drotaverine.

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2.2. Reagents and materials

Drotaverine hydrochloride was obtained from Eva Pharma. Co., Cairo, Egypt, and the pharmaceutical product Spasmocure® tablets (60 mg/tablet) was obtained from Alfa Chem. Advanced Pharmaceutical Industries Co. (ACAPI), Cairo, Egypt. A stock solution 1×10^{-3} M drotaverine hydrochloride was prepared by dissolving the required amount of this drug in bidistilled water. Dilute working standard solutions were then prepared daily with bidistilled water. As a supporting electrolyte, a series of 0.04 M Britton–Robinson (BR) buffer pH 2.0–11.5 (a mixture of each of acetic, orthophosphoric and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide was prepared. All reagents used were of analytical reagent grade.

2.3. Procedure

A known amount of the drug solution was pipetted into 10 ml measuring flask and completed to the mark by 0.04 M Britton–Robinson buffer pH 2. The solution was transferred into the voltammetric cell and deaerated with pure nitrogen for 3 min. to remove oxygen, the accumulation potential E_a at −400 mV was applied to a new mercury drop (drop area 0.3 mm^2) whilst still stirring the solution (2000 rpm); following the accumulation period (40 s): the stirring is stopped and allowed to equilibrium for 10 s. The differential pulse voltammogram is obtained by scanning from −400 to −1200 mV with scan rate of 60 mV/s and pulse amplitude of 50 mV.

2.4. Determination of drotaverine hydrochloride in Spasmocure® tablets

Twenty tablets (Spasmocure®, 60 mg/tablet) were accurately weighed and powdered in a mortar, the required amount from the crushed tablets powder was dissolved in about 30 ml bidistilled water and filtered in a 100 ml measuring flask. The residue was washed three times with bidistilled water and the volume was completed to the mark by the same solvent. A suitable volume of the above tablets solution is pipetted into 10 ml measuring flask, completed to the mark by 0.04 M Britton–Robinson buffer pH 2 and the procedure is repeated as described. The nominal content of the tablets is calculated using standard addition technique.

2.5. Determination of drotaverine hydrochloride in spiked human urine

1 ml of 10^{-3} M DvCl and 1 ml of urine of a healthy person were completed to 10 ml to prepare 10^{-4} M DvCl in spiked urine sample, different volumes of the above spiked urine sample (10-20 µl) are pipetted into a 10 ml measuring flask and completed to the mark by 0.04 M Britton–Robinson buffer pH 2 and the procedure is repeated as described. The amount of DvCl is calculated using standard addition technique.

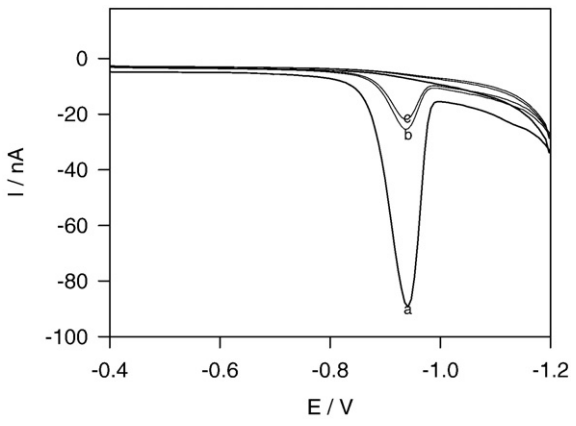

Fig. 1. Successive cyclic voltammograms of 5×10^{-6} M DvCl solution in 0.04 M BR buffer pH 2 and scan rate of 50 mV s^{-1} after an accumulation of 30 s.

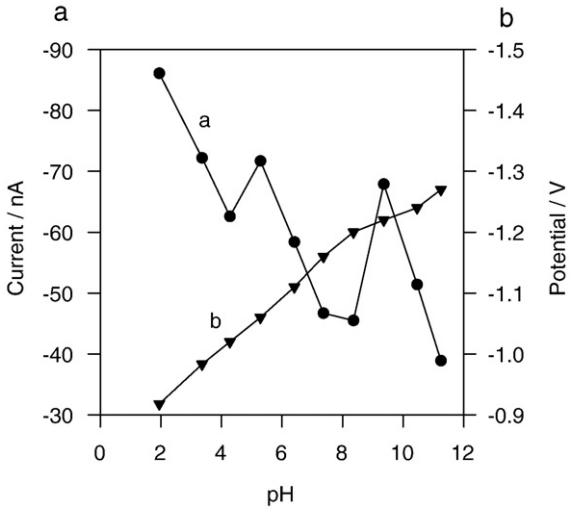

Fig. 2. Effect of pH on the DP adsorptive stripping peak current, a and peak potential, b of (10^{-6} M DvCl) in 0.04 M BR buffer, t_a**=30 s,** E_a**=−0.4 V,** ν**=50 mVs^{-1} and pulse amplitude 50 mV.**

3. Results and discussion

3.1. Cyclic voltammetric studies

Fig. 1 illustrates the repeatative cyclic voltammograms for 5×10^{-6} M drotaverine solution in 0.04 M Britton–Robinson buffer at pH 2.0, scan rate υ=50 mVs^{-1} and accumulation potential E_a of −400 mV. A well defined reduction peak appears at −0.941 V which was a result of five repetitions. This peak may be due to the reduction of olefinic bond of the drug and no oxidation peak is observed in the anodic branch which suggests that the process is irreversible. The repeatative cyclic voltammograms show that the peak current decreases sharply in the second and third cycles and this behavior gives an indication of an adsorption character. A plot of logarithm of peak current versus logarithm of the scan rate gave a straight line relation with a slope of 1.17 which is close to the theoretically expected 1.0 for an ideal

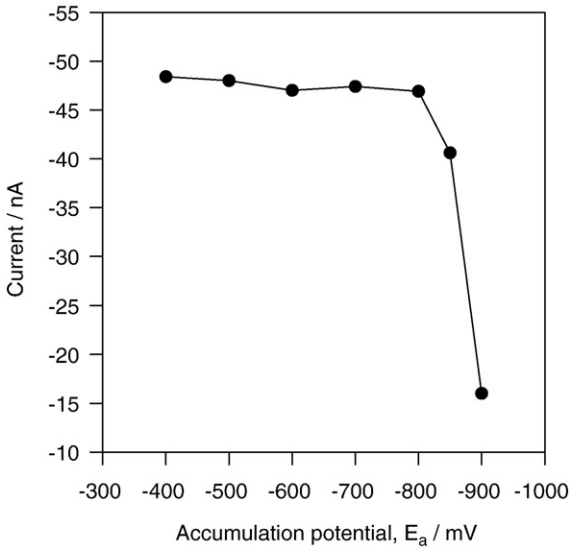

Fig. 3. Effect of accumulation potential on the peak current for 5×10^{-7} M DvCl, in 0.04 M BR buffer pH 2 at t_a **30 s,** ν**=50 mVs^{-1} and pulse amplitude 50 mV.**

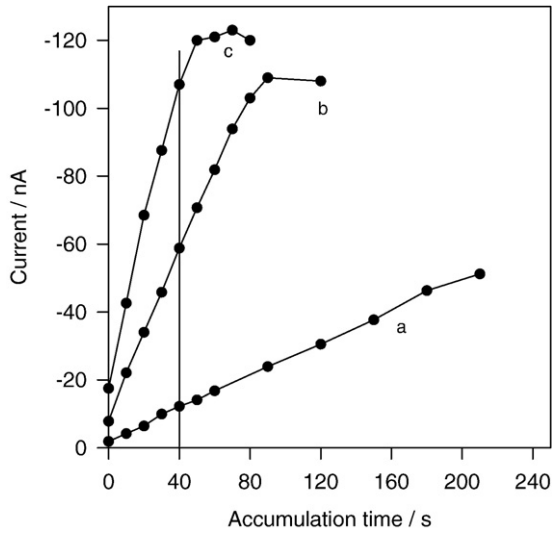

Fig. 4. Effect of accumulation time on the peak current for a, 1×10^{-7}; b, 5×10^{-7} and c, 1×10^{-6} M DvCl, in 0.04 M BR buffer pH 2 at E_a **−0.400 V, ν=50 mVs^{-1} and pulse amplitude 50 mV.**

reaction of surface species [22]. Also the peak potential shifts to more –ve values on increasing the scan rate which confirm the irreversibility of the reduction process.

3.2. DP voltammetric studies

The conditions affecting the enhancement of the peak associated with the preconcentration step was studied. Various electrolytes such as 1 M sulphuric acid, Britton–Robinson, acetate, phosphate and phthalate buffers were examined. Britton–Robinson buffer gave the highest peak current and the best peak shape than in case of 1 M sulphuric acid and the other mentioned buffers, so Britton–Robinson buffer was selected for further work. The effect of pH on the peak current and the reduction potential was studied over the range 2.0–11.25. Plots of pH versus peak current and peak potential are given in Fig. 2. The peak current has its maximum value at pH 2, the peak potential is varied by the linear relation E=−0.84–0.04 pH with correlation coefficient r=0.9953 and the potential is shifted to more negative values indicating the irreversible nature of the reduction process. The appearance of two maxima at pH values 5 and 9 may be attributed to the deprotonation of the positively charged ion formed in acid solution and ionization of the nitrogen-proton, leading to formation of anionic species. The study of the effect of the supporting electrolyte (BR buffer) concentrations (0.01, 0.04 and 0.1 M) indicated that the highest peak current was obtained at 0.04 M BR buffer.

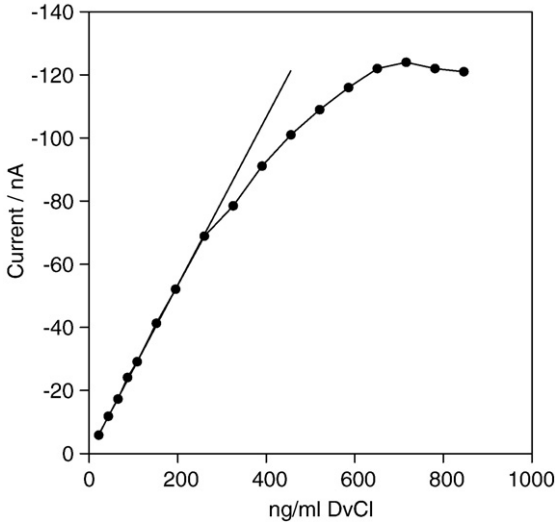

Fig. 5. Regression line for DP/AdCSV determination of DvCl with t_a**=40 s, E_a=−0.4 V, ν=60 mVs^{-1}, pulse amplitude 50 mV and pH=2.**

Table 1
Statistical comparison between the results of Spasmocure® tablets using the proposed DP/AdCSV method and the reference method

Parameter	Proposed method	Reference method [13]
Mean recovery, %	97.91	98.77
SD	0.946	0.994
RSD, %	0.966	1.006
F-ratio (5.409)[a]	1.104	
t-test (2.306)[b]	1.382	

Average of six determinations for the proposed method and four determinations for the reference method.
[a] Tabulated F-value at 95% confidence level.
[b] Tabulated t-value at 95% confidence level and 8 degrees of freedom.

The effect of accumulation potential on the adsorptive peak current was studied for 5×10^{-7} M DvCl at 30 s accumulation time (Fig. 3), the current peak was nearly constant on changing the accumulation potential (E_a) from −400 to −800 mV, but it decreased gradually by increasing accumulation potential more than −800 mV.

The effect of the accumulation time on the adsorptive peak current was studied at three concentration levels, 1×10^{-7}, 5×10^{-7} and 1×10^{-6} M DvCl (Fig. 4). The current increases linearly with increasing the accumulation time (t_a), indicating that the longer the accumulation time, the increase the drug concentration at the electrode surface, and the larger the peak current, then as the accumulation time increases the peak current tends to level off, 40 s accumulation time was generally used for subsequent studies. However ultimate choice of accumulation time should depend on the concentration range studied.

The optimum instrumental parameters were chosen from a study of the variation of the peak current of 5.0×10^{-7} M DvCl with change in scan rate and pulse amplitude. The current increases linearly with the increase in scan rate over the range 20–60 mVs^{-1} then remain nearly constant. The current also increased linearly with increasing the pulse amplitude over the range 10 to 60 mV and the reduction peak is displaced towards less negative potentials. Therefore the optimum instrumental parameters are established at scan rate υ=60 mVs^{-1} and pulse amplitude=50 mV, hence these values were used for further measurements.

3.3. Calibration graph, limit of detection and limit of quantitation

The dependence of the DP/AdCSV peak current on drotaverine hydrochloride concentration under the optimum conditions, shows a linear relationship from 21.70–257.34 ng/ml DvCl (Fig. 5). The linear regression equation was I (nA)=−0.442–0.2646C (ng/ml) with correlation coefficient of 0.9998. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the relation ($k(SD_a)/b$) [23] where k=3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept and b is the slope of the calibration curve, were found to be 7.26×10^{-9} M (3.15 ng/ml) and 2.42×10^{-8} M (10.50 ng/ml) for

Table 2
Determination of drotaverine hydrochloride in spiked urine samples using the proposed DP/AdCSV method

Taken (M)	Recovery, %	SD	RSD, %
1.0×10^{-7}	97.96	1.315	1.342
2.0×10^{-7}	97.43	1.679	1.723

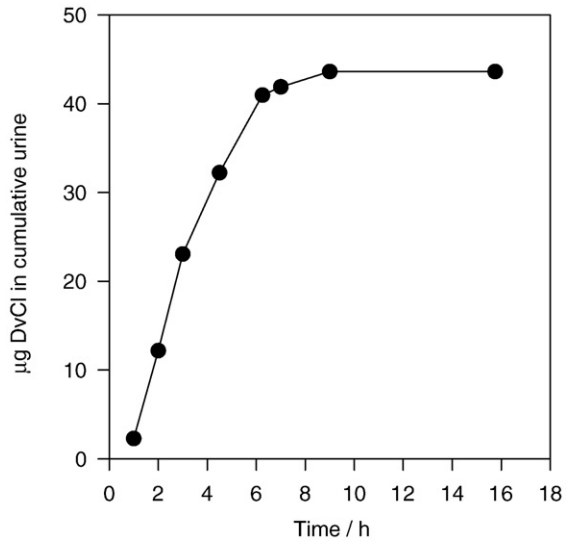

Fig. 6. Concentration of DvCl in cumulative urine vs time after administration of a 60 mg oral dose to a healthy volunteer.

LOD and LOQ, respectively. The precision expressed by relative standard deviation was 2.047% (n=8) for 2×10^{-7} M DvCl.

The robustness [24] of the proposed procedure was also examined for 2×10^{-7} M drug concentration by evaluating the effect of small change in some of the most important procedure parameters including pH (1.9–2.3) and accumulation potential (−0.4 to −0.6 V). The results showed that none of the changes significantly affect the recovery of the drug and provide an indication of the reliability of the proposed procedure and it could be considered robust.

3.4. Assay of drotaverine hydrochloride in Spasmocure® tablets and urine samples

The proposed DP/AdCSV method was applied to the determination of DvCl in Spasmocure tablets (60 mg DvCl/tablet) using standard addition technique. The mean recovery and the relative standard deviation values are summarized in Table 1. The results are in good agreement with those obtained from the spectrophotometric reference method [13]. Student's t- and F-tests (at 95% confidence level) were applied [23]; the results show that the caculated t- and F-values did not exceed the theoretical values.

The determination of DvCl in spiked urine samples was also carried out by the standard additions method at two different levels of concentrations (1×10^{-7} and 2×10^{-7} M). Four determinations were carried at each concentration level (Table 2). The mean recoveries for the two concentrations were 97.96 and 97.43% with relative standard deviations of 1.342% and 1.723%, respectively.

Urine samples were also collected from four healthy male volunteers up to 20 h after single dose, 60 mg drotaverine hydrochloride (Spasmocure® tablets) and the concentration of drotaverine hydrochloride was determined by applying the proposed technique. The results indicate that the drug appear in urine after the first hour and the concentration of the drug in urine increases till 3 h and then decreases and the excreted drug through urine was found to be 0.07–0.09% of the initial dose after 9 h (Fig. 6). This results is in agreement with previous studies that drotaverine is rapidly and extensively metabolized by the liver [5].

4. Conclusion

The reduction behaviour of drotaverine hydrochloride at HMDE after a preconcentration step at a cathode potential of −0.4 V was described and reduction peak close to −0.941 V was used for establishment of a sensitive, accurate, rapid and low cost DP/AdCSV procedure for the determination of drotaverine hydrochloride in pharmaceutical dosage form and human urine. The method gave a lower LOD (3.15 ng/ml) than the reported HPLC [5–7] methods (6, 10 and 50 ng/ml). The LOQ in the present method (2.42×0^{-8} M) is lower than that of glassy carbon oxidation method (2.16×10^{-5} M) reported by Ziyatdinova [21]. The proposed DP/AdCSV procedure can be considered as an alternative substitute to HPLC methods.

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