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Adsorptive stripping voltammetric behaviour and quantification of the tricyclic antidepressant drug doxepin hydrochloride in bulk form, pharmaceutical preparations and biological medium

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ABSTRACT

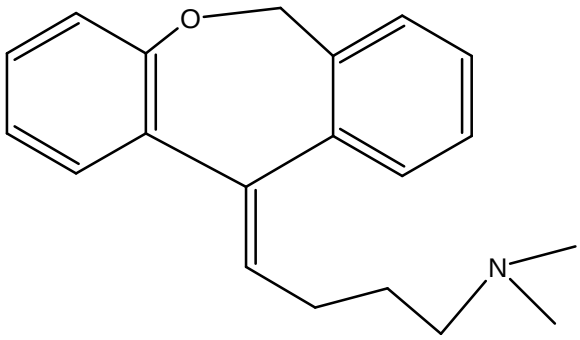
The electro reductive behaviour of doxepin has been investigated and one irreversible well-defined cathodic peak was observed at -0.62 V vs. Ag/AgCl (3M KCl). The electrochemical reduction and adsorption of doxepin hydrochloride was studied in phosphate buffer medium by cyclic (CV), differential pulse cathodic adsorptive stripping (DP-CAdSV) and square-wave cathodic adsorptive stripping (SW-CAdSV) voltammetric techniques at glassy carbon electrode. The voltammograms showed a single 2-electron irreversible cathodic peak, which may be attributed to reduction of the C=C double bond of the doxepin hydrochloride and the mechanism of reduction was postulated on the basis of controlled potential electrolysis. A fully validated, simple, sensitive and reproducible cathodic adsorptive stripping voltammetric procedure for the trace determination of the doxepin hydrochloride bulk drug in pharmaceutical formulation and in biological medium has been developed. The achieved LOD and LOQ were 4.83 x 10^{-6} mL^{-1} and 1.5 x 10^{-7} mL^{-1} by SWCAdSV and 8.9 x 10^{-6} mL^{-1} and 2.4 x 10^{-7} mL^{-1} by DPCAdSV respectively. The procedure was applied to the assay of the drug in tablets form with mean percentage recoveries of 100.17% with SWCAdSV and 100.16% with DPCAdSV. Applicability to assay the drug in spiked human urine and serum samples were illustrated and minimum detectability were found to be 2.1 × 10^{-7} mol L^{-1} and 3.71× 10^{-7} mol L^{-1} and 3.65 × 10^{-7} mol L^{-1} and 6.3 × 10^{-7} mol L^{-1} for DP–CAdSV and SW–CAdSV, respectively. respectively.

Key words: doxepin hydrochloride; cathodic adsorptive stripping voltammetry; biological sample.

INTRODUCTION

Doxepin hydrochloride is a psychotropic agent with tricyclic antidepressant [1] and anxiolytic properties. It is used primarily to treat depression and to treat the combination of symptoms of anxiety and depression and insomnia [2,3]. It has also been used to support smoking cessation programs. Doxepin hydrochloride is chemically known as 1-propanamine, 3-dibenz [b, e] oxepin-11(6H)-ylidene-N, N-dimethyl-, hydrochloride (Scheme-1).

It displays a potent central anticholinergic activity and can inhibit both nor epinephrine and serotonin (5-HT) reuptake in synapses in brain [4]. It is neither a central nervous stimulant nor a monoamine oxidase inhibitor. In general, lower dosages of doxepin hydrochloride are recommended. Where the presenting symptoms are mild in nature, it is advisable to initiate treatment at a dose of 10–50 mg daily. At high concentrations, severe adverse effects and toxicity can appear [5]. Therefore, the development of an analytical method sensitive and selective enough for determining doxepin hydrochloride in both pharmaceutical and biological samples are of great importance.



(Scheme-1)

Several analytical methods have been developed to determine the concentrations of doxepin hydrochloride in biological fluids and pharmaceutical preparations. Most of the reported methods are high-performance thin layer chromatography [6], high-performance liquid chromatography (HPLC) [7-8], liquid chromatography coupled with mass spectrometry [9], capillary electrophoresis [10], electro analytical method of analysis [11] spectrophotometry [12,13], extractive spectrophotometry [14-15] and spectrofluorimetry [16-19]. Although the selectivity and the detection limit have been improved in these methods, these are rather time-consuming methods and require large number of complicated steps to follow on for analysis. For this purpose, the desirable technique for the analysis of drugs should be rapid, simple, low cost, and of high sensitivity in analysis.

MATERIALS AND METHODS

Doxepin hydrochloride was obtained from Best Laboratories Pvt. Ltd. New Delhi, India, and was used as received. A standard stock solution ($1x10^{-3}$ mol L^{-1}) of bulk doxepin hydrochloride was prepared by dissolving an accurate mass of the drug in an appropriate volume of DMF, which was then stored in the dark at 4°C. More dilute solutions were prepared by accurate dilution just before use. phosphate buffers of pH 2–10 (mixtures of 0.04 mol L–1 acetic, orthophosphoric, and boric acids; adjusted to the required pH with 0.1M sodium hydroxide solution and 0.1M hydrochloric acid) were prepared and used as supporting electrolytes. All chemicals used were of analytical reagent grade quality and were employed without further purification.

RESULTS AND DISCUSSION

The electrochemical investigation of doxepin hydrochloride at the GCE was studied by using cyclic voltammetry (CV), differential pulse cathodic adsorptive stripping voltammetry (DPCAdSV) and square-wave cathodic adsorptive stripping voltammetry (SWCAdSV). In all electrochemical methods, doxepin hydrochloride gave one well-defined reduction peak in phosphate buffer (pH 3.2) at glassy carbon electrode.

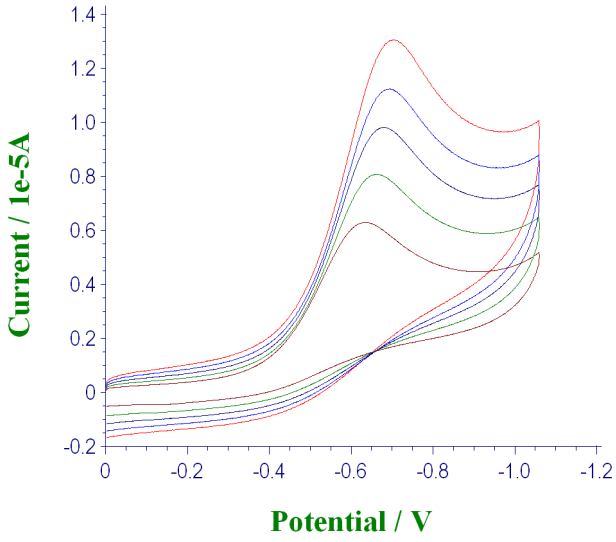


Figure1. Cyclic voltammograms in the presence of $1x10^{-4}$ M concentration of doxepin hydrochloride in pH 3.2 phosphate buffer solution on glassy carbon electrode at different scan rates, (1)–(5), 100, 200, 300, 400, 500 mVs^{-1}.

4.1 Cyclic Voltammetric Studies:

Typical cyclic voltammograms (Figure 1) for doxepin hydrochloride were recorded within the wide range (-200 to -1200 mV) of the potential at different pH, scan rate and concentration. The shape of cyclic voltammograms clearly indicated the irreversible nature of reduction.

The effect of pH value on the reduction peak current of doxepin hydrochloride was examined in the range of pH 2-10 phosphate buffers at a target concentration of $1x10^{-4}$ mol L^{-1} doxepin hydrochloride solution. With the rise in pH, the peak potential shifted towards more negative potential, which indicated the existence of a protonation reaction coupled with the doxepin hydrochloride reduction process [20-25].

The relation between E_p of the wave and pH of the medium over the range of 2-10 is expressed by the following equations:

For CV, pH 2-10: E_p (V) = 0.0396+0.962(V) pH, r^2 = 0.996

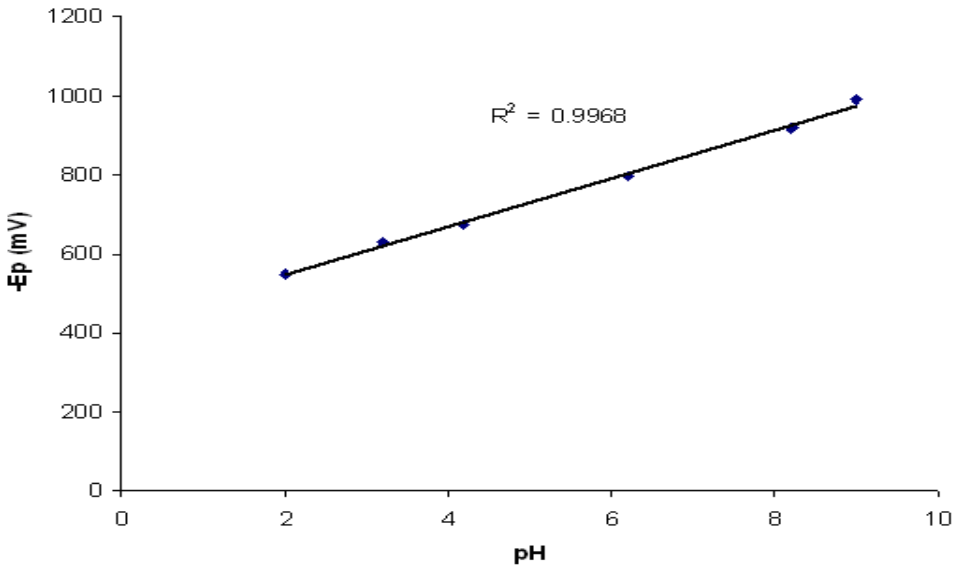


Figure 2. The linear relation between -Ep and pH

Linear pH dependence of the peak potential for reduction waves in the range of 2-10 shows that protonate participates directly in the reduction process. After pH 10, no significant displacement in peak potential was observed. The effect of scan rate (ν) on the cathodic peak current by using the solution of the concentration $1x10^{-4}$ mole L^{-1} and recording CV's at 100, 200, 300, 400 and 500 $mVsec^{-1}$ scan rate (Figure 1).

The relation between the cathodic peak current, i_{pc} (µA), the diffusion coefficient of the electro active species, D_0 (cm^2 s^{-1}), and the scan rate, ν (mV s^{-1}), is given by **Randles-Sevick** equation:

$i_{pc} = (2.99x10^{-5})n\ \alpha^{½}\ A\ C_o D_o^{½}\ \nu^{½}$

The **Randles–Servick** equation also indicates that i_{pc} is directly proportional to concentration. A plot of this equation (i_{pc}/concentration) for doxepin hydrochloride yields a straight line according to the equation:

i_{pc} (µA) = 0.10 C (mol L^{-1})+0.01, r^2 = 0.997, n=5

Table -I. Summarize voltammetric data for doxepin hydrochloride in the acidic medium at different scan rates

S.NO.	SR(ν) (mV/sec)	Ip/ν½ (mV/sec)	Log.SR (mV/sec)	Ep (mV)	Ep/2 (mV)	Ip (µA)	Log. Ip(µA)	α (mV)
1.	100	0.270	2	-634	-459	2.700	0.4313	0.0682
2.	200	0.2317	2.301	-662	-486	3.277	0.5154	0.0675
3.	300	0.2222	2.477	-680	-502	3.849	0.585	0.0670
4.	400	0.2109	2.602	-695	-516	4.219	0.625	0.0686
5.	500	0.2169	2.698	-704	-534	4.852	0.6859	0.0678

4.2 Stripping voltammetric studies

Stripping voltammetric methods were optimized for trace determination of doxepin hydrochloride by pulse and square wave potential-waveforms. Stripping voltammograms of bulk doxepin hydrochloride in the PHOSPHATE buffer (pH 2 to 10) recorded by differential pulse and square wave voltammetry following its preconcentration onto the GCE by adsorptive accumulation for 20 s exhibited a well-defined single irreversible cathodic peak with a better enhanced peak current magnitude at pH 3.2. Therefore, a PHOSPHATE buffer of pH 3.2 was chosen as a supporting electrolyte in the rest of study.

4.3 Validation of the Procedur:

Validation of the proposed procedure for assay of the drug at trace levels was examined via evaluation of the limit of detection (LOD), limit of quantization (LOQ), reproducibility, recovery, selectivity, robustness and ruggedness. The Limits of detection (LOD) and quantification (LOQ) of doxepin hydrochloride were calculated using the following equations: [26-29].

LOD= 3s/b
LOQ=10s/b

Where s is the standard deviation of the intercept and b is the slope of the calibration curve reproducibility, accuracy and precision [30] of results applying the described stripping voltammetric methods were examined by performing five replicate analysis of standard solutions of bulk doxepin hydrochloride.

The mean percentage recovery (%R) had been calculated for the found concentrations as a percent of the nominal concentrations in the standard solutions. Accuracy was expressed as relative error (RE %) while precision was assessed from the relative standard deviation in percentage (RSD %) of the mean recovery. The obtained results confirmed the reliability of the described stripping voltammetric methods for assay of doxepin hydrochloride.

4.4 Assay of doxepin hydrochloride in pharmaceutical form

Differential pulse cathodic adsorptive stripping voltammetry (DP-CAdSV) method. The optimum operational conditions of pulse-height scan rate and preconcentration parameters for determination of bulk doxepin hydrochloride applying differential pulse cathodic adsorptive stripping voltammetry (DP-CAdSV) at the GCE were identified. This was carried out by recording voltammograms of 5×10^{-6} mol L^{-1} bulk doxepin hydrochloride in the phosphate buffer of pH 3.2 under each of the following conditions: scan rate ν (10 mV s^{-1}), pulse height a (5 to 50 mV), preconcentration potential (E_{acc}) and preconcentration time tacc (0 to 20 sec.). DP-CAdS voltammograms of various concentrations of doxepin hydrochloride were recorded under the optimal operational conditions.

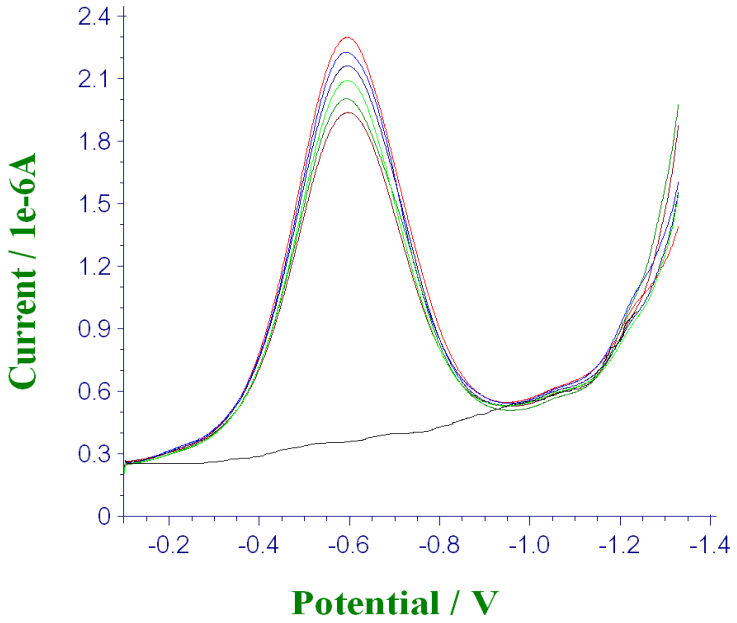


Figure 3. The DPCAdS voltammograms for increased concentrations of doxepin hydrochloride in pharmaceutical forms

A linear variation of the peak current (i_{pc}) with concentration (C) of bulk doxepin hydrochloride was obtained within the concentration range of 5×10^{-6} to 1×10^{-5} mol L^{-1}. LOD of 8.9×10^{-6} mol L^{-1} and a LOQ of 2.4×10^{-7} mol L^{-1} bulk doxepin hydrochloride were achieved (Table-3&4) applying the described DP-CAdSV method.

Table II: Application of the stripping voltammetric determination of Doxepin hydrochloride drug in bulk, pharmaceutical formulation using SWCAdSV and DPCAdSV Modes.

Techniques	SWCAdSV	DPCAdSV
Added (µg cm^{-3})	5 6 7 8 9 10	5 6 7 8 9 10
Found (µg cm^{-3})	5.02 6.03 6.98 7.99 9.01 10.1	5.01 5.99 7.02 8.03 9.02 10.01
N	6	6
Average recovery %	100.40 100.50 099.17 099.87 100.11 101.00	100.20 099.83 100.28 100.37 100.22 100.10
Mean S.D RSD %	100.17 0.623 0.622	100.16 0.187 0.187
Bias %	-0.17	-0.16

Square wave cathodic adsorptive stripping voltammetry (SW-CAdSV) method. Optimum operational conditions of both preconcentration and pulse-parameters for determination of bulk doxepin hydrochloride applying square wave cathodic adsorptive stripping voltammetry (SW-CAdSV) were identified. This was carried out by studying the effect of changing of each of preconcentration potential (E_{acc}), preconcentration time tacc (0 to 20 sec.), pulse-height a (5 to 50 mV), frequency f (40 Hz) and scan increment ΔEs (10 mV) on peak current magnitude of 2×10^{-6} mol L^{-1} bulk doxepin hydrochloride in the phosphate buffer of pH 3.2. SW-CAdS voltammograms of various concentrations of doxepin hydrochloride were recorded under the optimal operational conditions. A linear variation of the peak current (i_{pc}) with concentration (C) of bulk doxepin hydrochloride was obtained within the concentration range of 5×10^{-6} to 1×10^{-5} mol L^{-1}. A LOD of 4.8×10^{-6} mol L^{-1} and a LOQ of 1.5×10^{-7} mol L^{-1} .

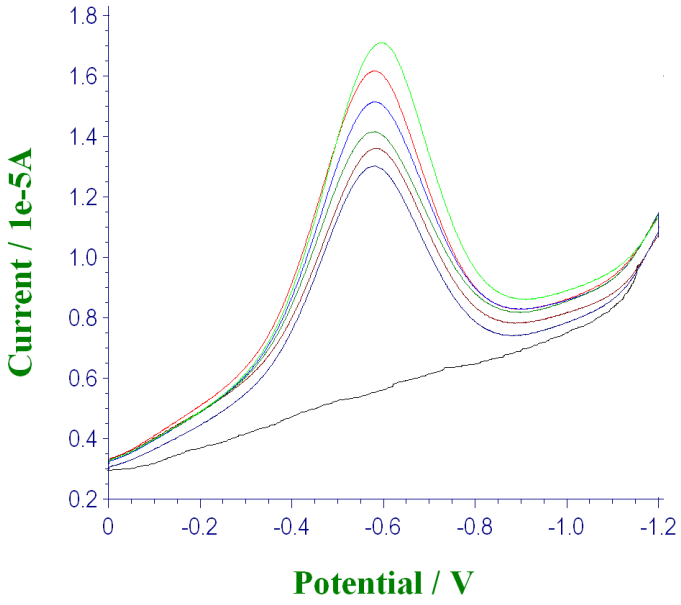


Figure 4: The SWCAdS voltammograms for increased concentrations of doxepin hydrochloride in pharmaceutical forms: (*1*) blank; (*2*) $5x10^{-6}$; (*3*) $6x10^{-6}$; (*4*) $7x10^{-6}$; (*5*) $8x10^{-6}$; (*6*) $9x10^{-6}$; (*7*) $1x10^{-5}$; mol L^{-1}; $E_{acc.}$=0.0 V, $t_{acc.}$=20 s, a=50 mV, f=40 Hz, ΔE=10 mV and phosphate buffer (9 mL) of pH 3.2.

4.5 Assay of doxepin hydrochloride in spiked human urine

Doxepin hydrochloride in spiked human urine was successfully analyzed by the described voltammetric methods (DP-CAdSV and SW-CAdSV) without the necessity for extraction of the drug prior to the analysis. No interfering peaks were observed in the blank human urine within the studied potential range. Linear variations of the peak current (i_{pc}) with concentration of doxepin hydrochloride in spiked human urine were obtained within the concentration ranges of 3×10^{-7} to 2.5×10^{-6} mol L^{-1} (DP-CAdSV) and 1×10^{-7} to 2.5×10^{-6} mol L^{-1} (SW-CAdSV) following the regression equations: (r = 0.999 and n = 6), and (r = 0.999 and n = 7), respectively. Detection limits of 2.1×10^{-7} and 3.65×10^{-7} mol L^{-1} and quantitation limits of 7.06×10^{-7} and 1.2×10^{-8} mol L^{-1} doxepin hydrochloride were achieved by the described DP-CAdSV and SW-CAdSV methods. Mean percentage recoveries and relative standard deviations of 100.40 ± 0.247 (DP-CAdSV) and 100.21 ± 0.452 (SW-CAdSV) were achieved based on replicate measurements of 5×10^{-6} mol L^{-1} doxepin hydrochloride in spiked human urine. These results confirmed the reliability of the described stripping voltammetric methods for assay of doxepin hydrochloride in human urine.

4.6 Assay of doxepin hydrochloride in spiked human serum

Doxepin hydrochloride in spiked human serum was successfully analyzed by the described voltammetric methods (DP-CAdSV and SW-CAdSV) without the necessity for extraction of the drug prior to the analysis. No interfering peaks were observed in the blank human serum within the studied potential range [30-34]. Linear variations of the peak current (i_{pc}) with concentration of doxepin hydrochloride in spiked human serum were obtained within the concentration ranges of 4×10^{-7} to 1.2×10^{-6} mol L^{-1} (DP-CAdSV) and 3×10^{-7} to 1.2×10^{-6} mol L^{-1} (SW-CAdSV) following the regression equations: (r = 0.998 and n = 7), and (r = 0.997 and n = 8), respectively. Detection limits of $3.71\times10^{-7}$and 6.3×10^{-7} mol L^{-1} and quantification limits of 1.2×10^{-8} and 2×10^{-8}mol L^{-1} doxepin hydrochloride were achieved by the described DP-CAdSV and SW-CAdSV methods. Mean percentage recoveries and relative standard deviations of 100.62 ± 0.287 (DP-CAdSV) and 100.12 ± 0.268 (SW-CAdSV) were achieved based on replicate measurements of 5×10^{-7} mol L^{-1} doxepin hydrochloride in spiked human serum [35]. These results confirmed the reliability of the described stripping voltammetric methods for assay of doxepin hydrochloride in human serum.

CONCLUSION

The electrochemical investigation of doxepin hydrochloride at the glassy carbon electrode and a Pt foil electrode in phosphate buffer solution, based on the adsorption behavior of doxepin hydrochloride onto the glassy carbon electrode surface. The Cyclic voltammetric behavior show well defined irreversible cathodic peak at -0.64 V, so (C=C) group is converted in to the (C-C) bond. A fully validated, simple, sensitive, selective, fast and low-cost differential pulse and square wave adsorptive cathodic stripping voltammetric methods were developed for determination of doxepin hydrochloride in bulk form, in spiked human urine and serum. Since the proposed procedure allowed the achievement of a detection limit of the drug at the trace level in spiked human urine and serum by means of the described stripping voltammetric methods are low as well as they offer good possibilities for determination of drug in low-dosage bulk pharmaceutical preparations and in real biological fluids. The described methods could be recommended for use in trace analysis, quality control and clinical laboratories.

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