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Short communication

Assay of the anti-psychotic drug haloperidol in bulk form, pharmaceutical formulation and biological fluids using square-wave adsorptive stripping voltammetry at a mercury electrode

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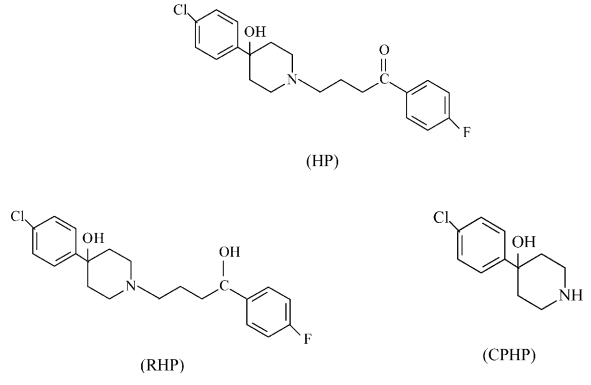
Abstract

The cyclic voltammetric behavior of haloperidol at a hanging mercury drop electrode was studied in Britton–Robinson buffer series of pH 2.5–11 containing 40% (v/v) ethanol. A single two-electron irreversible cathodic peak was obtained which attributed to reduction of the >C=O double bond. In addition, a small enhanced adsorptive pre-wave was observed at less negative potentials over the pH range 3.5–11. Controlled adsorptive accumulation of haloperidol onto the hanging mercury drop electrode provided the basis for its direct trace assay in bulk form, pharmaceutical formulation and human biological fluids using square-wave adsorptive cathodic stripping voltammetry. Following preconcentration of bulk haloperidol onto the HMDE a well-developed square-wave cathodic peak was generated in Britton–Robinson buffer especially at pH values 9–10; its peak current showed a linear dependence on the concentration of haloperidol over the range 1×10^{-9} M to 1.5×10^{-6} M depending on the preconcentration duration. The procedural parameters for assay of haloperidol were studied. The achieved limits of detection (LOD) and quantitation (LOQ) were 3.83×10^{-10} M and 1.28×10^{-9} M bulk haloperidol, respectively. The procedure was successfully applied to assay haloperidol in tablets (Safinace®) and in spiked human serum and urine. LOD of 3.3×10^{-9} M and 5.46×10^{-9} M, and LOQ of 1.10×10^{-8} and 1.82×10^{-8} M haloperidol were achieved in spiked human serum and urine samples, respectively.

Keywords: Haloperidol; Cyclic voltammetry; Square-wave adsorptive cathodic stripping voltammetry; Safinace® tablets; Human urine and serum

1. Introduction

Haloperidol (HP), a major tranquilizer, is a neuroleptic used in clinical medicine mainly for treatment of schizophrenia, mania and neurological disorders with hyperkinesias [1]. Its therapeutic plasma concentrations are in the range of 4–20 ng ml^{-1} [2]. Metabolites of haloperidol are mainly the reduced haloperidol (RHP) and the 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) [3,4].



Structures of haloperidol (HP) and its metabolites (RHP) and (CPHP).

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Several analytical methods have been reported for assay of haloperidol including capillary electrophoresis [4], acidimetric titration in non-aqueous medium [5], UV-spectrophotometry [6–8], derivative spectrophotometry [9], conductometry [6], fluorimetry [10], colorimetry [11–15], H-nuclear magnetic resonance [16], densitometry [17], gas chromatography (GC) with electron-capture detection [18,19], GC with surface ionization detection [20], liquid chromatography (LC) with electrochemical detection [21], LC with UV detection [22], gas–LC with nitrogen–phosphors detection [23,24], LC with mass spectrometric (MS) detection [25,26], high-performance liquid chromatography (HPLC) with diode array detection [27], HPLC with UV–vis detection [28-30], HPLC with MS detection [3], micellar electrokinetic chromatography [31] and radioreceptor assay [32]. However, most of the reported methods did not allow the rapid quantification and identification of haloperidol in a one single run since most of these methods necessitate samples pretreatment and/or time-consuming extraction steps prior to analysis of the drug. Besides, most of these methods require expensive equipment and considerable skill is necessary to operate them successfully.

On the other side, haloperidol has been determined in bulk form and urine samples by differential-pulse stripping voltammetry at a glassy carbon electrode [33] and in bulk form by differential-pulse polarography [34]. The electrochemical behavior of haloperidol at the dropping and hanging mercury drop electrodes has been studied in different supporting electrolytes (phosphate, acetate and borate buffers) containing a mixture of 20% propylene carbonate −30% methanol at pH <9 or 40% dimethylformamide at higher pH values [34]. To date no square-wave adsorptive cathodic stripping voltammetric procedure for quantification of haloperidol is reported in literature.

The aim of this study was to identify the characteristics of the electrode reaction of haloperidol at a hanging mercury drop electrode in Britton–Robinson (B–R) buffer containing 40% ethanol (pH 2.5–11), and consequently to develop a direct, simple, rapid, sensitive, precise and inexpensive square-wave adsorptive stripping voltammetric procedure for its quantification in bulk form, pharmaceutical formulation and human biological fluids.

2. Experimental

2.1. Materials

Haloperidol bulk substance, {4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone}, was supplied from Sigma (St. Louis, MO, USA). The pharmaceutical formulation Safinace® tablets was supplied from Kahira Pharm. & Chem. Ind. Co. (Cairo, Egypt) as labeling to contain 1.5 mg haloperidol per tablet as an individual drug (the occupants were cellulose, magnesium stearate, glucose, lactose, gelatin, and starch).

2.2. Reagents and solutions

2.2.1. Bulk haloperidol solutions

A standard stock solution (1×10^{-3} M) of haloperidol (molecular weight = 375.87 g) was prepared by accurately weighing of 18.8 mg of bulk haloperidol substance, then transferred into a 50-ml volume calibrated flask containing 30 ml ethanol (Merck). The content of the flask was sonicated for few minutes, then made up to the volume with ethanol and stored at 4 °C. More dilute solutions (10^{-6} M to 10^{-4} M) were prepared daily by accurate dilution. Haloperidol solutions were stable and their concentrations did not change with time.

2.2.2. Tablet solutions

Five Safinace® tablets were weighed and powdered. Accurately weighed portion of the powder was placed into a 25-ml volume calibrated flask containing 15 ml ethanol (Merck). The content of the flask was sonicated for about 10 min and then made up to the volume with ethanol. The solution was filtered through a 0.45 μm Milli-pore filter (Gelman, Germany). The desired concentrations of haloperidol were obtained by accurate dilutions with ethanol. The solution was directly analyzed, according to the proposed stripping voltammetric procedure.

2.2.3. Spiked serum samples

Serum samples were obtained from healthy volunteers and stored frozen until the assay. Aliquots of human serum sample were fortified with different concentrations of bulk haloperidol. A 100 μl aliquot of each of these solutions was diluted to a 1.0 ml volume with ethanol in a 2-ml volume centrifuge tube. The precipitated proteins were separated by centrifugation for 3 min at 14,000 rpm. The clear supernatant layer of each solution was filtered through a 0.45 μm Milli-pore filter to produce protein-free human serum solutions spiked with different concentrations of haloperidol. Then the protein-free spiked serum solutions were directly analyzed, according to the proposed stripping voltammetric procedure.

2.2.4. Spiked urine samples

Urine samples, 1 ml each, spiked with 10–100 μl aliquots of haloperidol working solution were made up to 10 ml with Britton–Robinson buffer to obtain concentrations of 10–100 μg ml^{-1} urine. Then the solution was directly analyzed according to the proposed stripping voltammetric procedure.

2.2.5. Supporting electrolyte

Britton–Robinson buffer of pH 2–11 (a mixture of an equal volumes of 0.04 M of boric, acetic and phosphoric acids, the pH was adjusted by 0.2 M sodium hydroxide solution) was prepared in de-ionized water [35]. The pH values were measured using a digital pH-meter (Crison, Bercelona, Spain). All the chemicals used were of analytical-reagent grade and were used without further purification.

2.3. Instrumentation

The voltammetric measurements were carried out employing the Electrochemical Analyzers models 263A and 394-PAR (Princeton Applied Research PAR, Princeton, NJ, USA). The electrode assembly (303A-PAR) incorporated with a micro-electrolysis cell comprising of a hanging mercury drop electrode (HMDE) as a working electrode (surface area = 0.026 cm^2), an Ag/AgCl/3 M KCl reference electrode and a platinum wire auxiliary electrode, was used. A magnetic stirrer (305-PAR) and a stirring bar were used to provide the convective transport during the preconcentration step.

A centrifuge (Eppendorf model 5417C, Hamburg, Germany) was used for separation of the precipitated proteins from the human serum samples before assay of the drug. A digital micropipetter (Volac, John Poulten Ltd., Essex, UK) was used for solution transfer during the present electrochemical measurements. A Mettler balance (Toledo-AB104, Switzerland) was used for weighing the solid materials. Deionized water was obtained from a Purite Still Plus Hp deionizer attached to an AquaMatic bidistillation water system (Hamilton, UK).

2.4. General analytical procedure

For stripping voltammetric analysis, the analyte solution was introduced into a 10-ml volume calibrated flask, and then completed to the volume with the B–R buffer of a selected pH value. The solution was transferred into the voltammetric cell, and a pure nitrogen stream was passed for 5 min before analysis. The analyte was preconcentrated by accumulation onto the HMDE under the optimized accumulation parameters (t_{acc} = 60–100 s for analysis of tablets and t_{acc} = 180 s for analysis of bulk haloperidol solutions and human biological fluids, and E_{acc} = −1.3 V) while stirring the solution. At the end of the preconcentration duration the stirring was stopped and 5 s were allowed for the solution to become quiescent. Then the voltammograms were recorded by scanning the potential towards the negative direction under the optimized instrumental parameters (frequency = 40 Hz, scan increment = 6 mV and pulse-height = 25 mV). Recovery measurements were carried out by means of both the calibration curve and standard addition methods.

3. Results and discussion

3.1. Cyclic voltammetric study

Voltammograms of 1.25×10^{-4} M haloperidol at the HMDE in B–R buffers of pH 2.5–11 exhibited a single two-electron cathodic peak which may be attributed to the reduction of >C=O double bond of haloperidol molecule, in addition, a small enhanced adsorptive pre-peak was observed at less negative potential over the pH range >3. No oxidation peak was observed in the positive scanning half-cycle, indicating the irreversible nature of the electrode process [36]. On increasing the pH of the medium the peak potential E_p shifted to more negative values indicating the involvement of protons in the electrode reaction and the proton-transfer reaction precedes the electrode process proper [37]. A linear E_p − pH plot of slope = 52 mV was obtained; from which values of αn_a = 1.13 and α = 0.57 were estimated. The irreversible nature of the reduction process was confirmed by the shift of the peak potential E_p to more negative values with the increase of scan rate ν [38], Fig. 1. The plots of E_p versus ln ν at different pH values were straight lines of slope values of 62–66 mV (slope, mV = 59 /αn_a), from which values of αn_a (0.89–0.95) and α (0.45–0.48) at the different pH values were estimated.

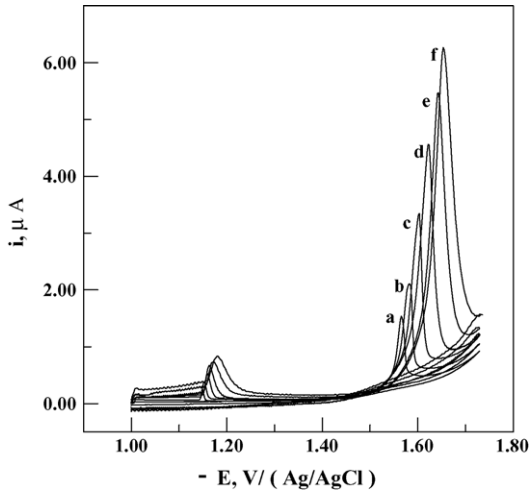

Fig. 1. Cyclic voltammograms for 1×10^{-4} M haloperidol in a B–R buffer of pH 10 at different scan rates: (a) 25 mV/s; (b) 50 mV/s; (c) 100 mV/s; (d) 200 mV/s; (e) 300 mV/s; and (f) 400 mV/s.

The adsorptive character of haloperidol onto the mercury surface was identified from the cyclic voltammograms of 5×10^{-7} M bulk haloperidol in B–R buffer of different pH values recorded without or following preconcentration of the compound onto the HMDE for 30 s at a potential E_{acc} of −1.1 V. A much-developed peak current was achieved following preconcentration of haloperidol onto the HMDE especially over the pH range 9–10. Fig. 2 shows the cyclic voltammograms of 5×10^{-7} M haloperidol in a B–R buffer of pH 9 (a) accumulated initially at open circuit potential; (b) following preconcentration by accumulation onto the HMDE for 30 s at E_{acc} = −1.1 V; and (c) repetitive cycle at the same mercury drop. A well-defined cathodic peak (E_p = −1.55 V) of a much-developed peak current was observed following preconcentration of the analyte by accumulation onto the HMDE (curve b) that completely disappeared in the repetitive cycle (curve c) at the same mercury drop. This behavior indicated the interfacial adsorptive character of haloperidol onto the mercury surface during the preconcentration step.

The electrode surface coverage (Γ_0 mol cm^2) with haloperidol species was determined from the amount of charge (Q) consumed by the surface process as calculated

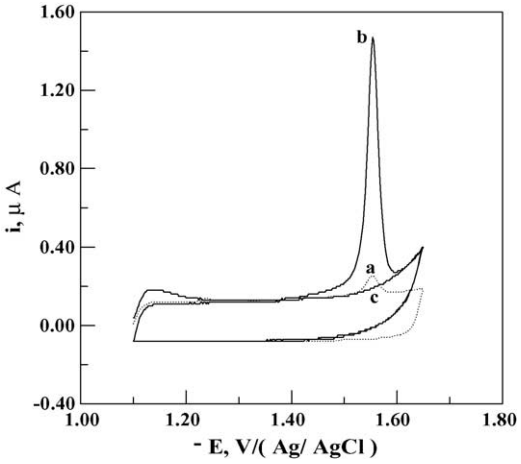

Fig. 2. Cyclic voltammograms for 5×10^{-7} M haloperidol in a B–R buffer of pH 9: (a) accumulated initially at open circuit potential; (b) following preconcentration for 30 s onto a HMDE at $E_{acc} = -1.1$ V; and (c) repetitive cycle at the same mercury drop; scan rate = 200 mV/s.

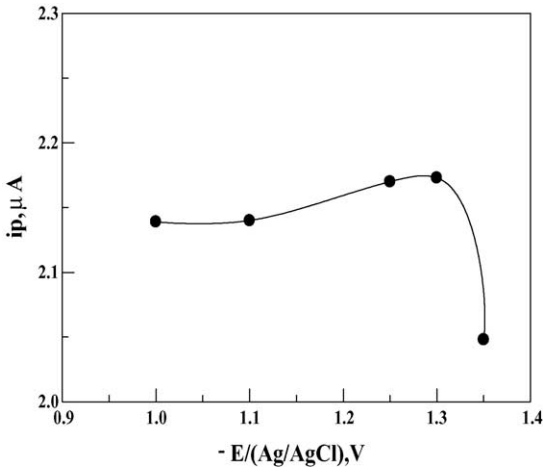

Fig. 3. Effect of accumulation potential (E_{acc}) on the square-wave adsorptive cathodic stripping (SWAdCS) voltammetric peak current (i_p) for 5×10^{-7} M bulk haloperidol in a B–R buffer of pH 9; $t_{acc} = 30$ s; $f = 80$ Hz, $\Delta E_s = 10$ mV and $a = 25$ mV.

by the integration of the area under the cyclic voltammetric peak of haloperidol in a B–R buffer of pH 9 using the equation $\Gamma_0 = Q/nFA$, where n is the number of electrons consumed in the reduction process ($n = 2$), F is the Faraday's constant (96,487 C) and A is the electrode surface area (0.026 cm^2). On dividing the number of coulombs transferred 795.4 nC by the conversion factor nFA (5017.3×10^9 nC), a monolayer surface coverage of 1.58×10^{-10} mol cm^{-2} was obtained. Thus, each adsorbed haloperidol molecule occupies an area of 1.05 nm^2.

The cyclic voltammetric peak current (i_p) of 5×10^{-7} M haloperidol measured following preconcentration of haloperidol onto the HMDE for 30 s, increased linearly with the increase of scan rate ν (25–500 mV s^{-1}) according to the equation $\log i_p = 0.89 \log \nu - 1.88$, $r = 0.998$. The slope value of 0.89 is very close to the expected theoretical value 1.0 for an ideal reaction of surface species [39], indicating the interfacial adsorptive character of haloperidol onto the surface of mercury electrode.

3.2. Adsorptive stripping voltammetric study

3.2.1. pH of the medium

The square-wave adsorptive cathodic stripping voltammetric response for 5×10^{-7} M haloperidol in B–R buffers of pH 2.5–11 without preconcentration and following preconcentration onto the HMDE for 30 s at −1.0 V (frequency $f = 80$ Hz, scan increment $\Delta E_s = 10$ mV and pulse-height $a = 25$ mV) was studied. The response preceded by preconcentration step increased extensively, and the peak current was much developed over the pH range 9–10, therefore a B–R buffer of pH 9 as a supporting electrolyte was considered for the rest of the present analytical study.

3.2.2. Potential and duration of accumulation

Effect of accumulation potential E_{acc} on the square-wave adsorptive cathodic stripping (SWAdCS) voltammetric peak current (i_p) of 5×10^{-7} M haloperidol was examined over the range −1.0 V to −1.4 V following preconcentration onto the HMDE for 30 s. A much-developed peak current was achieved over the potential range of −1.0 V to −1.3 V; at more negative potentials a gradual decrease of the peak current was observed (Fig. 3). Therefore, an accumulation potential of −1.3 V was chosen in the rest of analytical study.

The dependence of the SWAdCS voltammetric peak current of 5×10^{-7} M; 1×10^{-7} M; and 5×10^{-8} M haloperidol in a B–R buffer of pH 9 on the preconcentration duration onto the HMDE at $E_{acc} = -1.3$ V was studied. As shown in Fig. 4 for a 5×10^{-7} M haloperidol the response was linear up to 180 s, then leveled off and decreased (curve a); this behavior may be attributed to the complete coverage of the

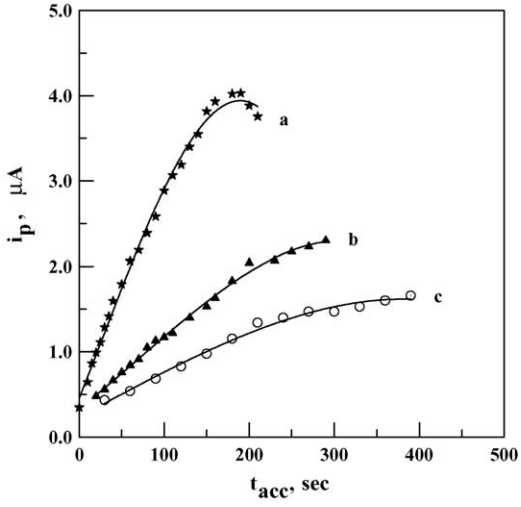

Fig. 4. Effect of preconcentration duration (t_{acc}), on the SWAdCS voltammetric peak current (i_p) for: (a) 5×10^{-7} M; (b) 1×10^{-7} M; and (c) 5×10^{-8} M bulk haloperidol in a B–R buffer of pH 9; $E_{acc} = -1.3$ V. The other operational conditions are as those illustrated in Fig. 3.

mercury electrode surface with the analyte species. While for 5×10^{-8} M haloperidol a preconcentration duration (t_{acc}) of 200 s was required (curve c). The lower the concentration of the analyte, the longer of the preconcentration duration is. Thus, the accumulation duration of choice will be dictated by the sensitivity needed.

3.2.3. Square-wave operational parameters

Since the square-wave parameters are strongly dependant on those of the excitation signal, optimization of frequency (f), pulse-height (a) and scan increment (ΔE_s) were attempted for 5×10^{-7} M haloperidol in a B–R buffer of pH 9 following preconcentration for 30 s at $E_{acc} = -1.3$ V. The SWAdCS voltammetric peak current was linearly dependent on the frequency 10–90 Hz while a well-defined peak was achieved at 40 Hz. Also, the peak potential (E_p) shifted to more negative values with the increase of frequency. The plot of E_p versus $\log f$ [40] was a straight line following the relation E_p, $V = 0.067 \log f + 1.48$. So, value of αn_a for the electrode reaction was calculated and found to equal 0.88. Since $n_a = 2$, a value of transfer coefficient $\alpha = 0.44$ was obtained. On the other hand, the peak current was practically dependent on the pulse-height up to 30 mV. In the present study, a pulse-height, a, of 25 mV was used. Although, the peak current increased with the increase of scan increment ΔE_s (2–10 mV), however a well-defined peak current was observed at scan increment of 6 mV. On the other hand, the square-wave signal was found to increase as the mercury electrode area was increased (0.01–0.026 cm^2). Therefore, the present study was carried out at a large HMDE area (0.026 cm^2). The influence of the rest time was also considered and a time period of 5 s was chosen. Accordingly, the optimal experimental and instrumental operational conditions of the proposed SWAdCS voltammetric procedure are: $E_{acc} = -1.3$ V, $t_{acc} = 60$–200 s, $f = 40$ Hz, $\Delta E_s = 6$ mV, $a = 25$ mV and a B–R buffer of pH 9 as a supporting electrolyte.

3.2.4. Validation of the analytical procedure

Linear calibration graphs over different concentration ranges between 1×10^{-9} M and 1.5×10^{-6} M bulk haloperidol, depending on the preconcentration duration, were obtained. Characteristics of these calibration graphs at different preconcentration duration are reported in Table 1. Validation of the proposed procedure for assay of bulk haloperidol was examined via evaluation of limit of detection (LOD), limit of quantitation (LOQ), repeatability, reproducibility, precision, selectivity, robustness and ruggedness. The LOD and LOQ were calculated from the calibration graphs obtained following preconcentration of haloperidol onto the HMDE for different durations, using the relations: LOD = 3 S.D./b and LOQ = 10 S.D./b [41], where S.D. is the standard deviation of the intercept of the calibration curve and b is its slope. The results reported in Table 1 indicated the reliability of the proposed SWAdCS voltammetric procedure for the trace assay of haloperidol.

The repeatability, reproducibility, precision and accuracy [42] of analysis of haloperidol by means of the proposed procedure were identified by performing five replicate measurements for each of 1×10^{-8} M, 5×10^{-8} M and 5×10^{-7} M bulk haloperidol ($t_{acc} = 180$ s and $E_{acc} = -1.3$ V) over 1 day (intra-day assay) and for 3 days over a period of 1 week (inter-day assay). The results obtained during 3 days measurements for repeatability (intra-assay precision), the intermediate (inter-day) precision and accuracy are summarised in Table 2. The relative standard deviation (R.S.D.%) was used as a measure of precision and the relative difference between found and taken concentrations (relative error RE%) was a measure of the accuracy. The intra-day precision (R.S.D.%) ranged from 1.0% to 2.46% and the accuracy (RE%) was measured as −1.15% to 1.24% ($n = 5$). Satisfactory mean intermediate recoveries and relative standard deviations of $98.9 \pm 2.43\%$, $99.7 \pm 1.15\%$ and $100.3 \pm 1.34\%$ for 1×10^{-8} M, 5×10^{-8} M and 5×10^{-7} M haloperidol ($n = 15$), respectively, were achieved and no significant differences were observed between the amount of haloperidol taken and the amount found indicating the reproducibility, precision and accuracy of the proposed procedure for assay of haloperidol.

The selectivity [42] of the optimized procedure for assaying haloperidol was tested by study the effect of occupants

Table 1
Characteristics of the calibration plots of haloperidol in bulk form and biological fluids at different preconcentration durations; B–R buffer of pH 9, $E_{acc} = -1.3$ V, $f = 40$ Hz, $\Delta E_s = 6$ mV and $a = 25$ mV, at 25 °C

t_{acc} (s)	Regression equation: i_p (μA) = bC (μM) + $a \pm$ S.D.	Linearity range (M)	r	LOD (M)	LOQ (M)
Bulk form					
60	$i_p = 3.59C + 0.44 \pm 2.12 \times 10^{-3}$	5×10^{-9} to 1.5×10^{-6}	0.998	1.77×10^{-9}	5.90×10^{-9}
100	$i_p = 5.41C + 0.50 \pm 2.05 \times 10^{-3}$	2×10^{-9} to 1×10^{-6}	0.995	1.14×10^{-9}	3.79×10^{-9}
180	$i_p = 9.23C + 0.60 \pm 1.18 \times 10^{-3}$	1×10^{-9} to 5×10^{-7}	0.987	3.83×10^{-10}	1.28×10^{-9}
Human urine					
60	$i_p = 3.29C + 0.59 \pm 2.42 \times 10^{-2}$	7×10^{-8} to 1×10^{-6}	0.998	2.21×10^{-8}	7.35×10^{-8}
100	$i_p = 5.08C + 0.62 \pm 2.51 \times 10^{-2}$	5×10^{-8} to 1×10^{-6}	0.998	1.48×10^{-8}	4.94×10^{-8}
180	$i_p = 6.04C + 0.79 \pm 1.10 \times 10^{-2}$	1×10^{-8} to 8×10^{-7}	0.991	5.46×10^{-9}	1.82×10^{-8}
Human serum					
60	$i_p = 2.21C + 0.60 \pm 1.48 \times 10^{-2}$	6×10^{-8} to 9×10^{-7}	0.993	2.00×10^{-8}	6.70×10^{-8}
100	$i_p = 3.30C + 0.68 \pm 1.27 \times 10^{-2}$	4×10^{-8} to 7×10^{-7}	0.996	1.15×10^{-8}	3.85×10^{-8}
180	$i_p = 5.22C + 0.82 \pm 5.74 \times 10^{-3}$	1×10^{-8} to 5×10^{-7}	0.999	3.30×10^{-9}	1.10×10^{-8}

Table 2
Analytical precision and accuracy of haloperidol determination by the proposed SWAdCS voltammetric procedure ($E_{acc} = -1.3$ V and $t_{acc} = 180$ s)

Concentration (M)	Day	Intra-day				Inter-day	
		Concentration found (M) (mean ± S.D.)	Mean *R*%	Precision (R.S.D.%)	Accuracy (RE%)	Mean *R*%	Precision (R.S.D.%)
Lab (1)							
1×10^{-8}	1	$0.9885 \times 10^{-8} \pm 0.0227$	98.85	2.30	−1.15	98.90	2.43
	2	$0.9890 \times 10^{-8} \pm 0.0241$	98.90	2.44	−1.10		
	3	$0.9895 \times 10^{-8} \pm 0.0243$	98.95	2.46	−1.05		
5×10^{-8}	1	$4.9855 \times 10^{-8} \pm 0.0543$	99.71	1.09	−0.29	99.70	1.15
	2	$4.9875 \times 10^{-8} \pm 0.0603$	99.75	1.21	−0.25		
	3	$4.9820 \times 10^{-8} \pm 0.0498$	99.64	1.00	−0.36		
5×10^{-7}	1	$5.0170 \times 10^{-7} \pm 0.0627$	100.34	1.25	0.34	100.30	1.34
	2	$4.9650 \times 10^{-7} \pm 0.0769$	99.30	1.55	−0.70		
	3	$5.0620 \times 10^{-7} \pm 0.0557$	101.24	1.10	1.24		
Lab (2)							
5×10^{-7}	1	$5.0970 \times 10^{-7} \pm 0.0861$	101.94	1.69	1.94	101.8	1.83
	2	$5.0810 \times 10^{-7} \pm 0.0904$	101.62	1.78	1.62		
	3	$5.0925 \times 10^{-7} \pm 0.0988$	101.85	1.94	1.85		

that often accompany the drug in various pharmaceutical preparations. An attractive feature of an analytical procedure is its relative freedom from interference of the occupants. Samples of 5×10^{-7} M bulk haloperidol solution in absence and presence of 5×10^{-7} M haloperidol of standard Safinace® tablet solution (containing the occupants) were analyzed by means of the proposed procedure following preconcentration onto the HMDE for 60–100 s in both cases. No significant differences in the recoveries or the standard deviation achieved in the absence (100.34 ± 1.25%) and presence (101.7 ± 2.4%) of occupants. On the other hand, the haloperidol metabolites RHP and CPHP were found electro-inactive at the HMDE under the experimental conditions of the proposed procedure. Accordingly, no significant interference from occupants and also from metabolites was observed in the assay of haloperidol. Thus, the procedure can be considered selective.

The robustness [42] of the measurements by means of the proposed SWAdCS voltammetric procedure was examined by study the effect of variation of some of the procedural conditions such as pH (9–10), preconcentration potential (−1.2 V to −1.3 V) and preconcentration duration (170–180 s) on recovery and standard deviation of 5×10^{-7} M bulk haloperidol. The obtained mean percentage recoveries and standard deviations (98.2 ± 2.1 to 100.6 ± 1.4) were not significantly affected within the studied range of variation of the procedural conditions, and consequently the proposed procedure can be considered robust.

The ruggedness [42] of the measurements is defined as the degree of reproducibility of results obtained by analysis of the same sample under variety of normal test conditions such as different laboratories, different analysts, different instruments and different lots of reagents. This was examined by SWAdCS voltammetric assay of 5×10^{-7} M haloperidol using two Potentiostats (PAR) models 263 A, lab (1) and 394, lab (2) under the same operational conditions at different elapsed time by two different analysts (Table 2). The mean percentage recoveries obtained due to lab-to-lab and even day-to-day were found reproducible (100.3 ± 1.34–101.8 ± 1.83).

3.3. Application

3.3.1. Assay of haloperidol in Safinace® tablets

The proposed SWAdCS voltammetric procedure was successfully applied to direct determination of haloperidol in the commercial Safinace® tablets (as labeling to contain 1.5 mg haloperidol per tablet as an individual drug) without the necessity for samples pretreatment and/or time-consuming extraction steps prior to the analysis. The mean percentage recovery of haloperidol, based on the average of five replicate measurements was found to be 101.7 ± 2.4 using the calibration curve method. The results were statistically compared with those obtained (100.4 ± 1.8) by a reported spectrophotometric method [9]. The calculated *F*-value and *t*-test were 1.77 and 0.97, respectively. The theoretical values of *F*- and *t*-test at the 95% confidence limit (for $n_1 = 5$ and $n_2 = 5$) are 6.39 and 2.30, respectively. Since the calculated *F*-value did not exceed the theoretical one, there was no significant difference between the proposed and reported methods with respect to reproducibility [43]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by *t*-test [43]. The accuracy of the proposed procedure was also judged by applying the standard addition method [44] for three standard concentrations of haloperidol added to pre-analyzed tablet solutions. The analysis exhibited satisfactory results as a good percentage recovery (100.4 ± 1.2) of added haloperidol was achieved.

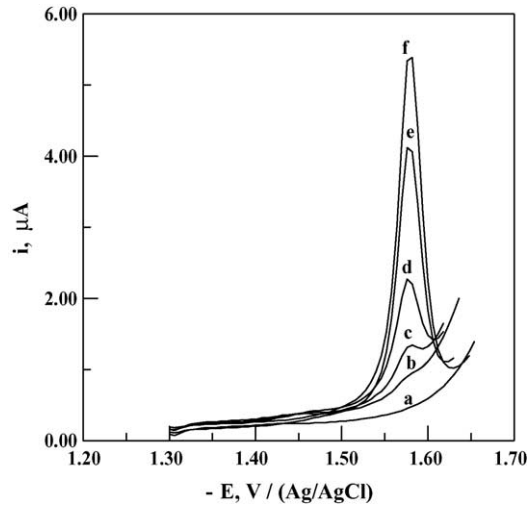

Fig. 5. SWAdCS voltammograms for different concentrations of haloperidol in spiked human urine: (a) background; (b) 2.5×10^{-8}; (c) 1×10^{-7}; (d) 2.5×10^{-7}; (e) 5×10^{-7}; and (f) 7.5×10^{-7} M; $E_{acc} = -1.3$ V $t_{acc} = 180$ s; $f = 40$ Hz, $\Delta E_s = 6$ mV, $a = 25$ mV and a B–R buffer of pH 9.

3.3.2. Assay of haloperidol in spiked human biological fluids

The applicability of the proposed procedure for assay of haloperidol in spiked human biological fluids was accessed without sample pretreatment or time-consuming extraction or evaporation steps prior to analysis of the drug. Fig. 5 illustrates the response of successive concentrations of haloperidol in spiked human urine, following its preconcentration onto the HMDE for 180 s. Calibration plots for the haloperidol in spiked human serum and urine samples were carried out by means of the proposed analytical procedure; their characteristics are reported in Table 1. Mean percentage recoveries of 99.3 ± 1.4 and 98.4 ± 1.6 were achieved for the drug in human serum and urine, respectively. Limits of detection of 3.3×10^{-9} M and 5.46×10^{-9} M and limits of quantitation of 1.1×10^{-8} M and 1.82×10^{-8} M haloperidol in spiked human serum and urine samples were achieved, following preconcentration of the drug onto the HMDE for 180 s. The assay results of haloperidol in spiked human urine and serum samples are favorably compared to that obtained by means of a reported HPLC method [28].

4. Conclusion

The electrochemical behavior of haloperidol at the HMDE in B–R buffers of pH 2–11 is investigated and discussed. Also a fully validated square-wave adsorptive cathodic stripping voltammetric procedure is described for trace assay of haloperidol in bulk substance, pharmaceutical formulation and human biological fluids, without the necessity for samples pretreatment and/or time-consuming extraction or evaporation steps prior to analysis of the drug. The proposed procedure could be recommended for pharmacokinetic studies and analysis of haloperidol in quality control and clinical laboratories.

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