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Short communication

Adsorptive stripping voltammetric quantification of the antipsychotic drug clozapine in bulk form, pharmaceutical formulation and human serum at a mercury electrode

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Abstract

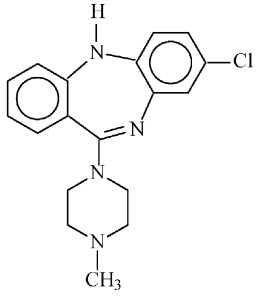
Using the cyclic voltammetry technique and the Britton–Robinson buffer (pH 2–10) as a supporting electrolyte, clozapine was found to reduce at the hanging mercury drop electrode in a single two-electron irreversible step corresponding to reduction of the azomethine group of the seven-member heterocyclic ring. Based on the interfacial adsorptive character of clozapine onto the hanging mercury drop electrode, a validated square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure was described for the quantification of bulk clozapine with limits of detection and quantitation of 4.5×10^{-10} and 1.5×10^{-9} M, respectively. The proposed procedure was successfully applied to the quantification of the drug in pharmaceutical formulation (Leponex®) and human serum without the necessity for samples' pretreatment or any time-consuming extraction or evaporation steps prior to the analysis. The limits of detection and quantitation of clozapine in spiked human serum were found to be 1×10^{-9} and 3.3×10^{-9} M, respectively. The proposed procedure for quantification of clozapine in bulk form, tablets and human serum has the advantage of being simple, rapid, sensitive, precise and inexpensive compared to most of the reported methods.

Keywords: Clozapine; Leponex® tablets; Human serum; Cyclic voltammetry; Square-wave adsorptive stripping voltammetry

1. Introduction

Clozapine: 8-chloro-11-(4-methylpiperazine-1-yl)-5H-dibenzo [b, e] [1,4] diazepine is the first atypical antipsychotic drug to demonstrate superior efficacy to the typical antipsychotic drugs in treatment-resistant schizophrenia. The drug is an effective antipsychotic with a low incidence of extrapyramidal symptoms and good antipsychotic efficacy in schizophrenic patients [1].

Clozapine has been effectively used in treating schizophrenic patients [2]. The beneficial and adverse effect of clozapine in both therapeutic and overdose situations can be largely explained by its receptor pharmacology. The therapeutic effects of antipsychotic agents seem to be related to competitive inhibition of dopamine D_2 receptors [3].



(Structure of clozapine)

Several analytical methods have been reported for the determination of clozapine in formulations and plasma samples including spectrophotometry [4–6], colorimetry [7], high-performance capillary electrophoresis [8], capillary zone electrophoresis with amperometric detection [9], capillary zone electrophoresis with UV detection [10], high-performance capillary electrophoresis-frontal analysis [11], capillary gas chromatography [12], gas

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chromatography–mass spectrometry with single-ion detection [13], liquid chromatography–electrospray tandem mass spectrometry [14], high-performance liquid chromatography (HPLC) with diode array detection [15], HPLC with UV detection [16–35], HPLC with electrospray detection [36] and HPLC with amperometric detection [37,38]. In addition, several electrochemical methods have been reported in the literature for determination of clozapine in bulk form and tablets including dc- and differential-pulse polarography [39], differential-pulse adsorptive cathodic stripping voltammetry at a hanging mercury drop electrode [40], linear sweep anodic stripping voltammetry at an activated glassy carbon electrode [41], anodic voltammetry at rotating platinum, glassy carbon disk and stationary carbon paste electrodes [42] and a wax-impregnated graphite electrode [43]. The behavior of clozapine was studied by adsorptive anodic voltammetry in order to carry out its direct determination in biological fluids [44]. Up to date, no square-wave adsorptive cathodic stripping voltammetric procedure was described for the quantification of clozapine in bulk, formulations and biological fluids. Most of the above reported methods for quantification of the drug required samples' pretreatment or time-consuming extraction steps prior to analysis of the drug and/or used different kinds of solid working electrodes. The solid electrodes required several preparation, pretreatment and activation steps prior to the application for analysis of the drug.

Therefore, the aim of this work was to develop a simple, fast, sensitive, precise and inexpensive square-wave adsorptive cathodic stripping voltammetric procedure for the trace quantification of clozapine in bulk form, pharmaceutical formulation and human serum.

2. Experimental

2.1. Reagents

A stock solution of 1×10^{-3} M bulk clozapine substance (Sigma, St. Louis, MO, USA) was prepared in methanol (Merck), and then stored at 4 °C. Working solutions (10^{-6} to 10^{-4} M) were prepared daily by appropriate dilution with methanol. Clazopine solutions were stable, and their concentrations did not change by time. Britton–Robinson (B–R) universal buffer of pH 2–11 [45] was used as a supporting electrolyte in the present study. All the chemicals used were of analytical reagent grade. The de-ionized water was supplied from a Purite-Still Plus de-ionizer connected to an AquaMatic double-distillation water system (Hamilton Laboratory Glass LTD, Kent, UK).

2.2. Apparatus

The voltammetric measurements were carried out using the Princeton Applied Research (PAR) Electrochemical Analyzers Models 394 and 263A (Princeton, NJ, USA), interfaced with a personal computer loaded with the software package 270/250 (from PAR). The PAR-303A electrode assembly incorporated with a micro-electrolysis cell and a three-electrode configuration system comprising of a hanging mercury drop electrode (HMDE) as a working electrode (area = 0.026 cm^2), an $Ag/AgCl/KCl_s$ reference electrode and a platinum wire auxiliary electrode was used. Stirring of the solution in the electrochemical cell was performed using a magnetic stirrer and a stirring bar to provide the convective transport during the preconcentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus. A polarograph Model 4001 Sargent Welch (Skokie, Illinois, USA) was used for study of the polarographic behavior of clozapine in B–R buffer of different pH values. The characteristics of the dropping mercury electrode were: m=1.03 mg s^{-1} and t=3.3 s, at a 60 cm mercury height.

A Mettler balance (Toledo-AB104, Switzerland) was used for weighing the solid materials. A pH meter incorporated with combined glass and saturated calomel electrodes (Crison, Barcelona, Spain) was used for the pH measurements. A centrifuge (Epindorf 5417C, Germany) was used for separation of the precipitated proteins from human serum samples.

2.3. General analytical procedure

A known volume of standard solution of clozapine was pipetted into a 10-ml volume calibrating flask and then made up to the volume with the B–R buffer. Then, the solution was introduced into the electrolysis cell and de-oxygenated with pure nitrogen for 10 min in the first cycle and 30 s for each successive cycle; the nitrogen was then kept over the solution during measurements. The preconcentration of clozapine onto the HMDE was performed at −0.7 V for a selected duration while stirring the solution at 400 rpm with a magnetic stirrer. After an equilibrium time of 5 s was allowed for the solution to become quiescent, the voltammograms were recorded by scanning the potential toward the negative direction.

2.3.1. Analysis of Leponex® tablets

Ten tablets of Leponex® (Novartis Pharma AG, Basel, Switzerland) labeled as containing 25-mg clozapine per tablet were crushed. Adequate amounts of the homogenous powder corresponding to stock solutions of 1×10^{-5} to 1×10^{-3} M clozapine were individually weighed and each transferred into a 25-ml volume calibrated flask, and then dissolved in methanol by sonication for 10 min, followed by mechanical shaking for 10 min and lastly centrifuged for 5 min at 14,000 rpm. A portion of the centrifuged clear solution was diluted with the supporting electrolyte to achieve the desired concentration. Then, voltammograms of different concentrations were recorded as explained in the general analytical procedure.

2.3.2. Analysis of spiked human serum

Serum samples were obtained from healthy volunteers and stored frozen until the assay. Into each of 10 centrifugation tubes containing a certain concentration of clozapine, a 0.1 ml of the human serum sample was transferred, and then mixed well with 1.0 ml of methanol to precipitate the blood proteins. The precipitated proteins were separated by centrifugation for 5 min at 14,000 rpm. The clear supernatant layer was filtered through a 0.45-μm milli-pore filter to produce protein-free human serum samples spiked with different concentrations of clozapine (10^{-6} to 10^{-4} M). The spiked human serum sample was transferred into a 10-ml calibrated flask, completed to the volume with B–R buffer of pH 7, and then introduced to the voltammetric cell. After de-aeration for 10 min with pure nitrogen, the voltammograms were recorded as explained in the general analytical procedure.

Recovery and quantification of clozapine in the different matrixes were performed by means of both the calibration curve and standard addition methods.

3. Results and discussion

3.1. Cyclic voltammetric studies

The cyclic voltammograms of 1.2×10^{-4} M clozapine in B–R buffer of pH 2–11 exhibited a single irreversible cathodic peak. The peak current was practically pH-independent over the pH range 2–10. The absence of any anodic peak on the reverse scan indicated the irreversible nature of the electrode reaction of clozapine. A displacement of the peak potential (E_p) to more negative values with the increase of pH indicated the involvement of protons in the electrode reaction and that the proton transfer reaction preceded the electrode process proper [46]. The number of protons (Z_{H^+}) involved in the electrode reaction of clozapine was determined from its dc-plarographic data in B–R buffers (Table 1) as $Z_{H^+}=S_2/S_1$ [47,48],where S_1 is the slope (mV) of the applied potential E_{de} versus $\log(i/i_1-i)$ plots at different pH values and S_2 is the slope (mV) of the half-wave potential $E_{1/2}$ versus pH plot. The cyclic voltammetric peak potential E_p versus pH plot was a straight line with a slope (S_3) of 73 mV, which agrees well with that of the polarographic $E_{1/2}$–pH plot (Table 1). Also the achieved transfer coefficient α values of the electrode process by means of both dc-polarography and cyclic voltammetry techniques agree well. The shift of the peak potential (E_p) of clozapine to more negative values on the increase of scan rate ν confirmed the irreversible nature of its electrode reaction. The plots of E_p versus log ν at different pH values were straight lines, with slope values (S_4) of 39–46 mV (Table 1) proportional to αn_a.

The interfacial adsorptive character of clozapine onto surface of the HMDE was identified by recording the cyclic voltammograms in B–R buffer at pH 7–8 without and following preconcentration for 30 s at −0.7 V (Fig. 1). Following preconcentration of the clozapine onto the HMDE even for a short duration, a substantial enhancement of the peak current was obtained during the first scan (curve b) compared with that of the subsequent scan at the same mercury drop (curve c) or with that measured without preconcentration at a new mercury drop (curve a). This behavior indicated that clozapine has an interfacial adsorptive character onto surface of the HMDE.

The adsorptive character of the drug was also identified by plotting $\log(i_p)$ versus log ν at different pH values for scan rates 25–500 mV s^{-1} following preconcentration for 30 s; straight lines were obtained with slope values 0.85−0.96 mV, which are very close to the theoretical value of 1.0 mV that was expected for the electrode reaction of surface-confined species [49]. The cyclic voltammetric peak potentials (E_p) of the adsorbed species at different pH values shift to more negative values with the increase of scan rate ν. The E_p–log ν plots ($t_{acc} = 30$ s) at different pH values were linear with slope values of 39–47 mV (slope $= 59/\alpha n_a$, mV). Values of αn_a (1.26–1.5) and α (0.63–0.75) were obtained.

3.2. Electrode surface coverage

The electrode surface coverage (Γ_0 mol/cm^2) was calculated using the equation $\Gamma_0 = Q/nFA$, where Q is the charge consumed by the surface process as estimated by the integration of the area under the peak of the cyclic voltammogram corrected to residual current [50], 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). Dividing the number of coulombs

Table 1
Data of dc polarographic and cyclic voltammetric studies for 1.2×10^{-4} M clozapine at different pH values; 25 °C

Dc polarography								Cyclic voltammetry					
pH	S_1 (mV)	αn_a	α ($n_a = 2$)	S_2 (mV)	Z_{H^+} (S_2/S_1)	αn_a	α ($n_a = 2$)	S_3 (mV)	αn_a	α ($n_a = 2$)	S_4 (mV)	αn_a	α ($n_a = 2$)
1.0	42	1.40	0.70	75	1.8	1.57	0.78	73	1.6	0.80	43	1.37	0.68
1.8	42	1.40	0.70		1.8						42	1.40	0.70
7.0	44	1.34	0.67		1.7						39	1.50	0.75
8.0	45	1.31	0.65		1.7						45	1.31	0.65
9.0	46	1.28	0.64		1.6 $Z_{H+} \cong 2$						46	1.28	0.64

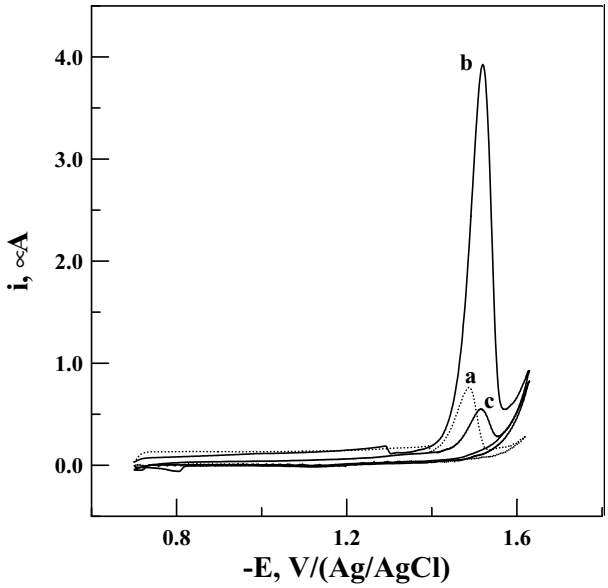

Fig. 1. Cyclic voltammograms of 2×10^{-6} M clozapine in a B–R buffer at pH 7: (a) without preconcentration, $t_{acc} = 0$ s; (b) following preconcentration for 30 s at $E_{acc} = -0.7$ V; and (c) the repetitive cycle at the same mercury drop, scan rate = 200 mV s^{-1}.

transferred, 2.10 μC, by the conversion factor *nFA* yielded a monolayer surface coverage of 4.19×10^{-10} mol/cm^2. Each adsorbed clozapine molecule therefore occupies an area of 0.397 nm^2.

From chronocoulometry experiments for 10 ml B–R buffer (at pH 7–9) alone (blank) and in the presence of 1×10^{-6} M clozapine, the total charge (Q_{total}) versus the square root of time ($t^{1/2}$) plots were obtained for the blank and after preconcentration of clozapine onto the HMDE for 30 s. The amount of clozapine adsorbed onto the mercury electrode surface Γ_0 (mol/cm^2) was estimated on applying the following relation [51]:

$$Q_{total} = Q_{diff} + Q_{ads} + Q_{dl}$$

$$Q_{total} = \int_0^t i\,dt = \frac{2nFACD^{1/2}t^{1/2}}{\pi^{1/2}} + nFA\Gamma_0 + Q_{dl}$$

where Q_{total} is the total charge, Q_{dl} is the double layer charge and Q_{ads} is the amount of charge required to reduce the adsorbed reactant species. The plot of total charge (Q_{total}) versus $t^{1/2}$ has an intercept equal to ($nFA\Gamma_0 + Q_{dl}$). Since Q_{dl} was measured in a separate experiment for the supporting electrolyte alone, the contribution of the adsorbed species can be determined. The deference between the intercepts of Q_{total} versus $t^{1/2}$and Q_{dl} versus $t^{1/2}$ plots is the amount of charge required to reduce the adsorbed reactant species (Q_{ads}). So, the surface coverage Γ_0 was found to equal 4.23×10^{-10} mol/cm^2, and each adsorbed clozapine molecule therefore occupies an area of 0.393 nm^2, which agrees well with the value estimated from cyclic voltammetry measurements.

3.3. Square-wave voltammetric studies

The square-wave voltammograms of 2×10^{-7} M clozapine following preconcentration onto the HMDE for 30 s at −0.7 V exhibited a single two-electron irreversible cathodic peak over the pH range 2–10 (Fig. 2). The response preceded by preconcentration step increased extensively, and a more developed peak current intensity was achieved at pH 7, which was chosen for the rest of the present analytical study. The peak potential (E_p) shifted toward more negative

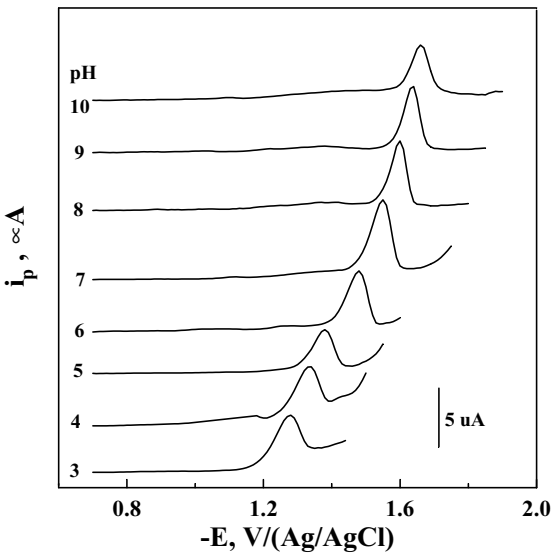

Fig. 2. SWAdCS voltammograms of 2×10^{-7} M clozapine in B–R buffer at different pH values; $t_{acc} = 30$ s, $E_{acc} = -0.7$ V, frequency $f = 120$ Hz, scan increment $\Delta E_s = 10$ mV, pulse height $a = 25$ mV, stirring rate = 400 rpm, and rest period = 5 s.

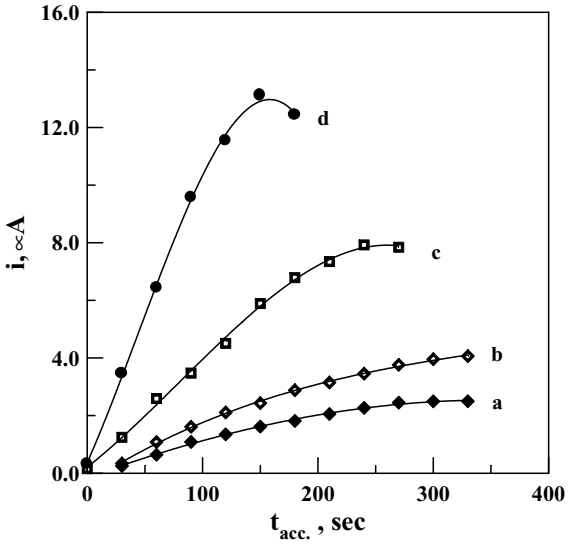

Fig. 3. Effect of accumulation duration (t_{acc}) on SWAdCS voltammetric peak current of four different concentrations of clozapine: (a) 5×10^{-9}; (b) 2×10^{-8}; (c) 5×10^{-8}; and (d) 1×10^{-7} M in B–R buffer of pH 7 at $E_{acc} = -0.7$ V, $a = 50$ mV. Other operational parameters were as those indicated in Fig. 2.

values with the increase of pH. This behavior also clearly indicated the involvement of protons in the electrode reaction of clozapine.

The dependence of the stripping peak current of 1×10^{-7} M clozapine in B–R buffer of pH 7 on the preconcentration potential (E_{acc}) was identified over the range -0.1 to -1.2 V following preconcentration of clozapine onto the HMDE for 30 s. A potential of -0.7 V (versus $Ag/AgCl/KCl_s$) was chosen as the accumulation potential, which has given a more developed peak current. Also, the effect of preconcentration duration was studied for different concentrations of clozapine (5×10^{-9}, 2×10^{-8}, 5×10^{-8} and 1×10^{-7} M). The plots of peak current (i_p) versus the preconcentration duration (t_{acc}) for 5×10^{-9} to 5×10^{-8} M were linear up to approximately 200 s, while for 1×10^{-7} M clozapine, the response was linear up to 120 s (Fig. 3). Thus, a preconcentration duration of choice will be dictated by the sensitivity needed.

The peak current of 1×10^{-7} M clazopine in B–R buffer of pH 7, following preconcentration for 30 s at $E_{acc} = -0.7$ V, was optimized by changing the pulse amplitude (a), scan increment (ΔE_s) and frequency (f) over the range of 10–100 mV, 2–10 mV and 10–120 Hz, respectively. The dependence of the peak current (i_p) on the square-wave frequency (f) was linear according to the equation: i_p (μA) $= 0.031f$ (Hz) $- 0.17$ ($r = 0.995$). The peak potential (E_p) was linearly depending on the logarithm of frequency (f), with a slope ($59/\alpha n_a$, mV) [52] of 43 mV. Accordingly, values of $\alpha n_a = 1.37$ and $\alpha = 0.68$ were obtained, which agree well with those obtained by cyclic voltammetry technique. Although the peak current intensity increased with the increase of pulse amplitude up to 100 mV, however pulse amplitude of 50 mV was chosen in the rest of this study to achieve a more developed and sharp peak. With increasing the scan increment (2–10 mV), the peak intensity also increased linearly; thus a 10-mV scan increment was used over the rest of study. As expected, an increase of the HMDE area yielded an increase in the peak height, so a large electrode area of 0.026 cm^2 was considered suitable. The influence of rest time on the peak current was also considered, and a value of 5 s was chosen.

Accordingly, the established optimal operational parameters of the proposed square-wave adsorptive cathodic stripping voltammetric procedure were: $E_{acc} = -0.7$ V, $t_{acc} =$ 60–200 s, $\Delta E_s = 10$ mV, $a = 50$ mV and $f = 120$ Hz, area of the HMDE $= 0.026$ cm^2, rest period $= 5$ s and a B–R buffer of pH 7 as a supporting electrolyte.

3.3.1. Validation of the proposed analysis procedure

This was examined via evaluation of limit of detection LOD, limit of quantitation LOQ, repeatability, recovery, selectivity, robustness and ruggedness. The SWAdCS voltammograms of different concentrations of bulk clozapine were recorded following preconcentration onto the HMDE for different durations (60–200 s); four replicate measurements were carried out for each concentration. The corresponding calibration graphs were linear over the concentration ranges reported in Table 2. The LOD and LOQ were estimated as LOD $= 3\ S_B/b$ and LOQ $= 10\ S_B/b$ [53], where S_B is the standard deviation of the blank and b is the slope of the calibration curve. The estimated values of LOD and LOQ (Table 2) show an improvement in sensitivity of the proposed SWAdCS voltammetric procedure for quantification of clozapine in bulk and tablets compared to that of the reported [39–44] electrochemical methods (LOQ $= 1 \times 10^{-6}$ to 8×10^{-9} M).

The selectivity [54] of the proposed procedure for the assay of clozapine was identified by studying the effect of excipients that often accompany clozapine in pharmaceutical preparations. An attractive feature of an analytical procedure is its relative freedom from interference by the excipients. Samples containing 1×10^{-8} M bulk clozapine in the absence and presence of excipients were analyzed by means of the proposed procedure. The obtained mean percentage recovery (R%) and the relative standard deviations (% R.S.D.), based on three replicate measurements (98.7 ± 1.4% to 97.9 ± 1.8), showed no significant interference from excipients; thus the procedure was able to assay clozapine in the presence of excipients, and hence it can be considered selective.

The intra- and inter-day precision of the measurements by means of the proposed procedure was determined by analyzing different concentrations of clozapine for three times in 1 day and for 7 days over a period of 1 week. The intra- and inter-day recoveries of clozapine (Table 3) indicated repeatability, accuracy and high precision of measurements by means of the proposed procedure.

The robustness [54] of the proposed procedure for analysis of 5×10^{-8} M clozapine was also examined by evaluating the

Table 2
Characteristics of the calibration plots of clozapine in B–R buffer of pH 7 by means of the proposed procedure

t_{acc} (s)	Regression equation i_p (μA) = bC (nM) ± a		r	Linearity range	S_B (blank)	LOD (M)	LOQ (M)
	Slope (b)	Intercept (a)					
Bulk							
60	$0.054 \pm 4.4 \times 10^{-3}$	$0.16 \pm 1.80 \times 10^{-2}$	0.997	1×10^{-8} to 2×10^{-7}	0.0190	1.1×10^{-9}	3.5×10^{-9}
120	$0.093 \pm 3.0 \times 10^{-3}$	$2.10 \pm 1.87 \times 10^{-2}$	0.998	8×10^{-9} to 1×10^{-7}	0.0198	6.4×10^{-10}	2.1×10^{-9}
200	$0.134 \pm 2.1 \times 10^{-3}$	$0.70 \pm 1.91 \times 10^{-2}$	0.997	2×10^{-9} to 6×10^{-8}	0.02010	4.5×10^{-10}	1.5×10^{-9}
Serum							
60	$0.020 \pm 3.5 \times 10^{-3}$	$0.6 \pm 7.8 \times 10^{-3}$	0.995	2×10^{-8} to 1×10^{-7}	0.009	1.4×10^{-9}	4.5×10^{-9}
120	$0.024 \pm 2.5 \times 10^{-3}$	$1.3 \pm 8.5 \times 10^{-3}$	0.996	6×10^{-9} to 8×10^{-8}	0.008	1.0×10^{-9}	3.3×10^{-9}

S_B is the standard deviation of the blank.

Table 3
Precision and accuracy of determination of clozapine in bulk and spiked human serum samples by the proposed procedure at $t_{acc} = 120$ s ($n = 5$)

Concentration (nM) (taken)	Intra-day			Inter-day		
	R (%)	Accuracy (bias %)	Precision (% R.S.D.)	R (%)	Accuracy (bias %)	Precision (% R.S.D.)
Bulk						
5	101.4	1.4	1.5	100.6	0.6	1.1
10	98.9	−1.1	1.0	99.1	−0.9	0.9
20	100.5	0.5	0.6	100.1	+0.1	0.6
Serum						
20	97.9	−2.1	1.2	96.9	−3.1	1.4
40	98.6	−1.4	1.1	98.1	−1.9	1.8
60	98.8	−1.2	0.9	99.3	−0.7	1.1

influence of small variation in some of the most important procedural conditions including pH (7–8), preconcentration duration (120–125 s) and preconcentration potential (−0.7 to −0.8 V) on the recovery of clozapine. The obtained mean recoveries (98.7 ± 1.8 to 97.4 ± 2.1%) were not significantly affected within the studied range of variations of some of the procedural conditions, and consequently, the proposed procedure was reliable for assay of clozapine and it could be considered robust.

3.3.2. Applications

3.3.2.1. Analysis of Leponex® tablets. The content of clozapine in Leponex® tablets was determined directly using the proposed procedure, without the necessity for samples' pretreatment or any time-consuming extraction steps prior to the analysis. The recovery obtained (97.9 ± 1.8%) showed a good agreement with that obtained by a reported spectrophotometric method [4]. The results showed that the calculated F-value (4.6) and t-test (0.45) did not exceed the theoretical values (95% confidence limits for five degree of freedom), from which one can conclude that the proposed procedure does not differ significantly from the reported method.

3.3.2.2. Analysis of spiked human serum. A quantitative assay of clozapine spiked in human serum was carried out by the proposed SWAdCS voltammetric procedure, t_{acc} = 120 s and $E_{acc} = -0.7$ V without the necessity for samples' pretreatment or any time-consuming extraction or evaporation steps prior to the analysis other than the centrifugal separation of the precipitated proteins by methanol. Representative voltammograms of spiked human serum are shown in Fig. 4. The linear dependence that existed between the stripping peak current and concentration of clozapine gave

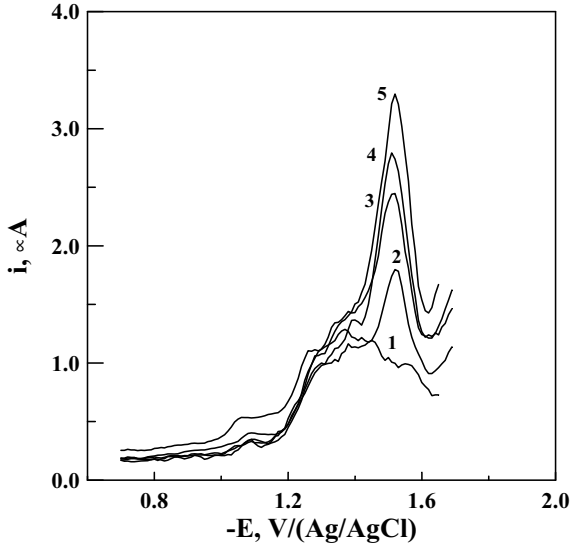

Fig. 4. Representative SWAdCS voltammograms in B–R buffer of pH 7 for different concentrations of clozapine spiked in human serum: (1) background; (2) 2×10^{-8}; (3) 4×10^{-8}; (4) 6×10^{-8}; and (5) 9×10^{-8} M; at $t_{acc} = 120$ s, $E_{acc} = -0.7$ V, $f = 120$ Hz, $\Delta E_s = 10$ mV and $a = 50$ mV.

a calibration curve that extended over the range 6×10^{-9} to 8×10^{-8} M ($n = 10$). The LOD and LOQ were found to be 1×10^{-9} and 3.3×10^{-9} M, respectively. The recovery of clozapine spiked in human serum was determined in triplicate for the concentrations of 20, 40 and 60 nM clozapine/ml of human serum (Table 3). The obtained mean recoveries and standard deviation values indicated good accuracy and precision of measurements by means of the proposed procedure. The results are favorably compared with those obtained by means of a reported method [4].

4. Conclusion

A fully validated, highly sensitive, simple and precise square-wave adsorptive cathodic stripping voltammetric procedure was described for quantitation of clozapine in bulk form, pharmaceutical formulation and human serum without the necessity for samples' pretreatment or any time-consuming extraction or evaporation steps prior to the analysis. The estimated limits of detection and quantitaton of clozapine show an improvement in sensitivity of the proposed procedure to quantification of clozapine compared at least to that reported in the literature [36–41] using the different electrochemical techniques (LOQ $= 1 \times 10^{-6}$ to 8×10^{-9} M).

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