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Electrosynthesis of Clozapine Drug Derivative *via* an EC Electrochemical Mechanism

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The fact that oxidation, as one of the main routes of phase I metabolism of drugs, follows by conjugation reactions, and also formation of nitrenium ion as one of the clozapine oxidation products, directed us to investigate the oxidation of clozapine (CLZ) in the presence of nucleophile. The oxidation of clozapine (CLZ) has been studied on a glassy carbon electrode in the absence and presence of 2-thiobarbituric acid (TBA) as nucleophile in aqueous medium by means of cyclic voltammetry and on the graphite rods in controlled-potential coulometry. Cyclic voltammetry studies were realized for CLZ in the pHs 1.0 to 8.0. Results indicate that the electrochemical behavior of CLZ depends on the pH. Based on the obtained electrochemical results, an ECE mechanism was proposed to explain the electrochemical oxidation of CLZ. The results revealed that oxidized CLZ participates in Michael type addition reaction with TBA and via an EC mechanism converts to the corresponding new dibenzodiazepin derivatives. The product has been characterized by IR, ¹H NMR, ¹³C NMR and MS.

Keywords: Clozapine (CLZ), Electrosynthesis, Dibenzodiazepin, Cyclic voltammetry, 2-Thiobarbituric acid (TBA)

INTRODUCTION

Clozapine, 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e]-[1,4]diazepine (CLZ), is a dibenzodiazepine derivative with a piperazinyle side chain. Clozapine is an effective antipsychotic drug that has been shown to be effective in the treatment of refractory schizophrenia [1,2]. Electrochemistry as a useful tool for electrosynthesis, mechanistic and kinetics studies, permits the generation and identification of intermediates, as well as elucidation of redox mechanisms [3-9]. Both electrochemical and chemical synthesis were applied in the syntheses of organic molecules [10,11]. However electrochemical synthesis is a green methodology and toxic and dangerous oxidizing or reducing reagents can be replaced and the electrons serve the role of reagents. Furthermore, unstable or hazardous reagents can be produced *in situ* and reactions carried out

under milder conditions [12-14]. On the other hand, electro-synthesis was introduced as an environmental friendly processes for green and simple synthesis of new organic compounds under mild condition [15,16].

Among the electrochemical methods, voltammetric techniques, are well established in pharmaceutical and biomedical analysis. Electrooxidation of amines were reported by many workers at different conditions such as aqueous and nonaqueous solvents or at various pH values [17,18]. There are some literatures about electrochemical behavior of clozapine. Kauffmann and co-workers studied clozapine electrochemically. Their investigation results revealed at low acidity condition and the electrooxidation process of clozapine is proposed to be an ECE mechanism [19,20]. The type of electrochemical mechanism is depended on some parameters such as; nature of the electroactive species, nature of nucleophile (electron withdrawing or donating) and the electrolysis conditions like solvent and pH [17,21-23].

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We have previously shown that clozapine can be oxidized electrochemically to nitrenium ion. The nitrenium ion formed are quiet reactive and can be attacked by a variety of nucleophiles such as arylsulfonic acid. These nucleophiles undergo Michael reactions according to EC mechanism, with the consumption of two electrons per molecule of clozapine [24]. Following our experience in electrosynthesis of organic compounds based on oxidation and *in situ* generation of Michael acceptors, we performed the synthesis of new dibenzodiazepine derivative based on the oxidation of clozapine in the presence of barbituric acid derivatives. The barbituric acid and its derivatives are of particular interest, they are used in the preparation of barbiturates, long active on central nervous system and polymerization catalysts [6,25]. This method represents a facial one-pot electrochemical method for the synthesis of a new dibenzodiazepine derivative. This reaction is carried out in a single step with high atom economy under ambient conditions at a carbon electrode in an undivided cell.

EXPERIMENTAL

Apparatus and Reagents

Cyclic voltammetry (CV), controlled potential coulometry, and preparative electrolysis were performed using an Autolab model PGSTAT 12 potentiostat/galvanostat. The working electrode used in the voltammetry experiments was a glassy carbon electrode (1.8 mm diameter) and a platinum wire was used as the counter electrode. The working electrode used in controlled-potential coulometry was an assembly of three carbon rods (8 mm diameter and 8 cm length) and a large platinum gauze was used as counter electrode. The working electrode potentials were measured *versus* the SCE reference. An undivided cell was used for coulometry. Melting points of synthesized compounds were determined in open capillary tubes and are uncorrected. The IR spectra were recorded on a Shimadzu model IR Prestige 21 FTIR spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker spectrometer operating at 400 and 100 MHz, respectively. The mass spectra were obtained using Agilent Technologies (HP) 5973 Network mass selective detector.

Clozapine was pharmaceutical grade material (purity > 99.8%) and is received from Sobhan pharmaceutical

company from Iran. Phosphate salts, 2-thiobarbituric acid were of pro-analysis grade from E. Merck. All other chemicals used in this investigation were of analytical grade. Solutions were prepared in distilled water. The total concentrations of prepared buffers are 0.2 M. All experiments were conducted at room temperature.

Electro-organic Synthesis of (3)

For the synthesis of 3, 0.3 mmol of CLZ (1) and 0.3 mmol TBA (2) was subjected to electrolyze in phosphate buffer solution (80 ml, 0.2 M, pH 7.2) at 0.48 V versus SCE, in an undivided cell. The process was interrupted during the electrolysis and the carbon anode was washed in acetone in order to reactivate it. At the end of electrolysis, the precipitated solid was collected by filtration and washed several times with water. After purification and re-crystallization in ethanol tested by thin layer chromatography. the dark brown product (yield 50%) was characterized by IR, ^1H NMR, ^{13}C NMR, and MS. M.p = 201-203 °C. IR_(KBr) = 3480, 3310, 2812, 1620, 1550, 1480, 1360 cm^{-1} . MS: m/z (%relative intensity); 469 (M^+ , 5), 457 (2), 444 (5), 421 (9), 363 (5), 334 (17.2), 302 (3), 232 (7), 221 (11), 174 (25), 162 (8), 121 (100), 89 (31), 77 (17), 63 (10). ^1H NMR (400 MHz DMSO- d_6): δ_{H} (ppm) 2.19 (3H, s, NCH₃), 2.37 (4H, m, 2CH₂), 3.30 (4H, m, 2CH₂), 6.83-7.35 (7H, m, 6H-Ar and NH), 10.03 (1H, s, CO-CH-CO), 10.98 (2H, broad, 2NH). ^{13}C NMR (100 MHz, DMSO- d_6): δ_{C} (ppm) 21.03 (NCH₃), 45.48 (COCHCO), 46.68 (CH₂), 54.17 (CH₂), 120.30, 120.61, 122.36, 122.47, 122.90, 125.44, 126.66, 129.74, 131.96, 132.05, 141.82, 142.14, 153.92, 162.72, 172.12 (NHCSNH).

RESULTS AND DISCUSSIONS

The Effect of pH

Cyclic voltammograms of 1.0 mM solution of CLZ in aqueous solutions at various pHs are shown in Fig. 1. All voltammograms indicate one oxidation peak (A_1) which is corresponding to at wo-electron electrooxidation of clozapine in the positive-going scan. Two cathodic peak (C_1 and C_2) in the negative-going scan can be seen at pHs ≥ 4 . Peak C_1 is the counterpart of anodic peak (A_1), due to the reduction of the oxidation product while the C_2 peak can be related to the occurrence of side chemical reaction. We

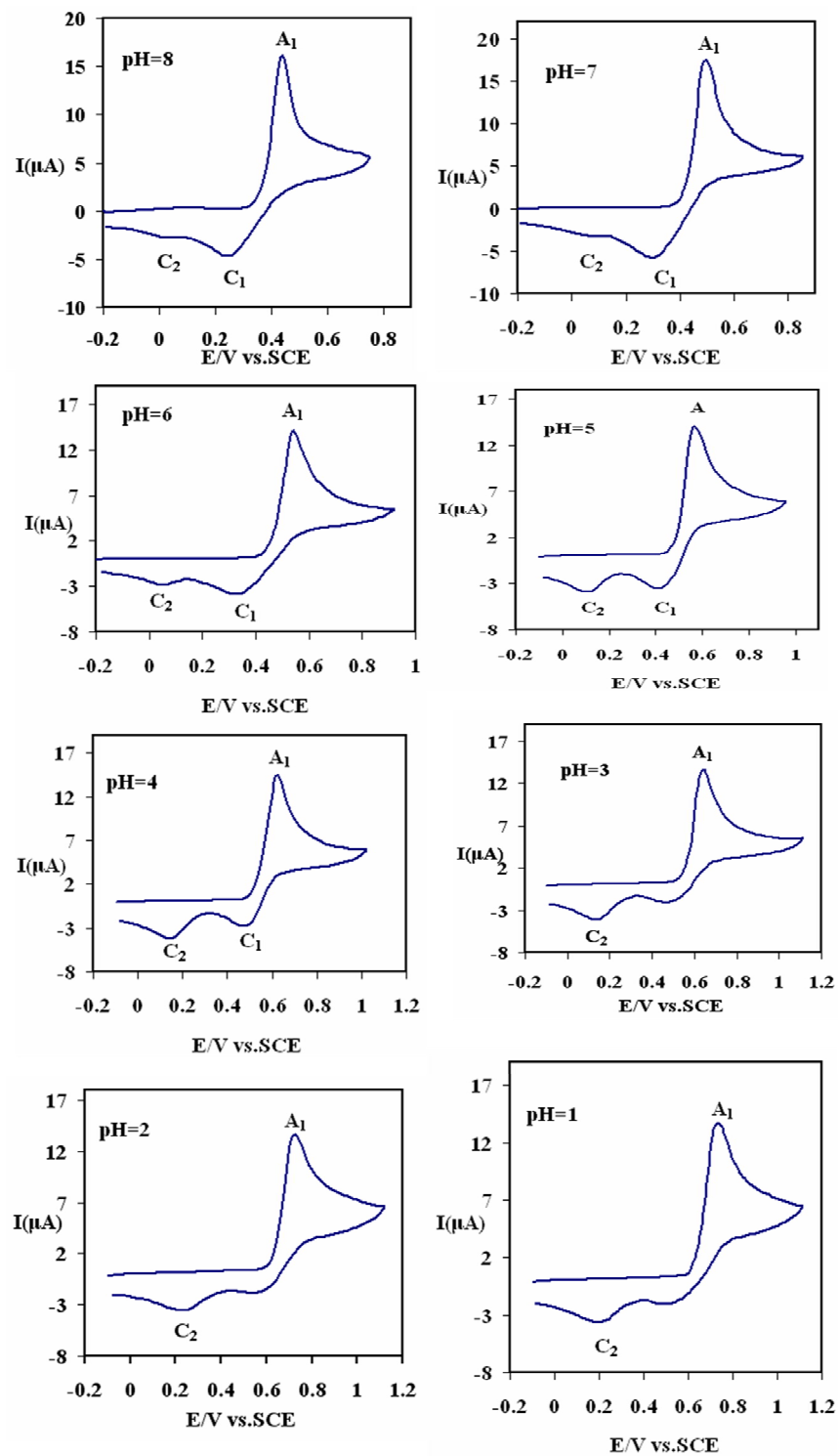
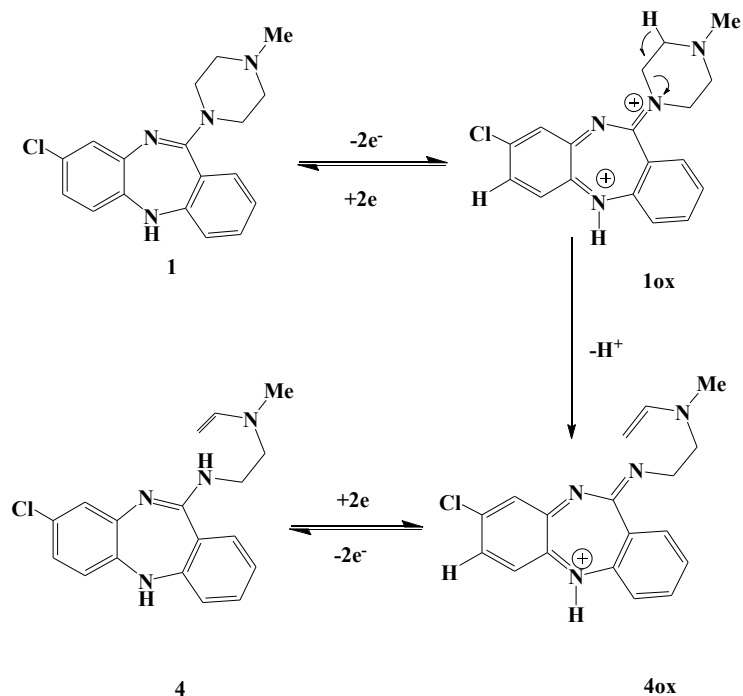
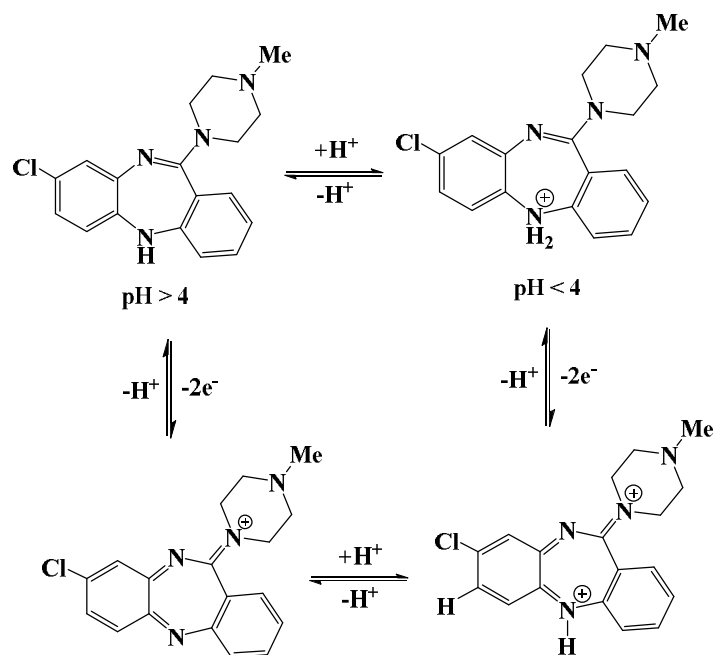


Fig. 1. Cyclic voltammograms of CLZ (1.0 mM) in buffer solution with various pH values at a glassy carbon electrode. pHs from are 1-8. Scan rate 100 mV s^{-1} .



Scheme 1. Occurrence of a side chemical reaction such as piperazine ring breaking reaction subsequent to the clozapine oxidation under the experimental conditions



Scheme 2. A foursquare diagram for CLZ, Protonated CLZ and their oxidized forms

suggest two alternative plan for justification of this observation. The first hypothesis is that the C_2 peak can be related to the occurrence of a side chemical reaction such as piperazine ring breaking reaction, subsequent to the clozapine oxidation under the experimental conditions (Scheme 1) [29]. The second hypothesis is, as the voltammograms show, the occurrence of side reaction is pH dependent process and apparently passes from an acid catalyzed pathway, and then its inhibition with increasing of pH is reasonable. With decreasing of pH the peak current of C_1 decreases and reaches to about zero at $\text{pH} \leq 3$ (Fig. 1). These changes can be related to the increasing of protonated portion of the secondary amine group on oxidized CLZ parallel to decreasing of pH. The ΔE_p between the A_1 and C_1 peaks reveals a quasi-reversible redox process. By decreasing the pH (8.0 to 1.0), the peak potentials for A_1 , C_1 and C_2 shifted to more positive values. This originates from the participation of proton(s) in the oxidation of CLZ (Scheme 2).

The plot of E_p versus pH (Fig. 2) shows that the peak potential is pH dependent. potential-pH equation [26] a potential-pH diagram for CLZ, includes two lines with different slopes. The second region at higher pH values may correspond to the loss of one hydrogen ion by the radical cation to give a radical. These results agree with Kauffman reports [19]. These results indicate that protons were involved in the electrochemical reaction of CLZ. Therefore, physiologic pH (7.2) was selected as a suitable medium for the electrochemical investigation and the synthesis of new dibenzodiazepine derivatives.

Figure 3 represents the first and second cycles of cyclic voltammograms of CLZ at pH 7.2 and 100 mV s^{-1} . In the first cycle, one quasi-reversible oxidation peak (A_1) is obtained at +0.48 V which is due to the electro-oxidation of CLZ. The electrochemical oxidation of tricyclic antidepressants (TCAs) occurs at the nitrogen atom in heterocyclic ring resulting in the formation of a radical that is similar to that for the oxidation of methyliminobiphenyl [27]. On the reverse sweep, two reduction peaks (C_1 and C_2) at 0.29 and 0.027 V are observed. The cathodic peak (C_1) is the counterpart of oxidation peak (A_1) and (C_2) represents the reduction of new compound which gets subsequently oxidized (A_2) in the second anodic cycle (Fig. 3, curve b). This corresponds to an ECE mechanism in which the initial

oxidation product is chemically transformed into a compound that is more readily oxidized than the parent compound [18].

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of CLZ at different scan rates from 10 to 1000 mVs^{-1} at pH 7.2 was also studied. Figure 4 part a, shows the effect of potential sweep rate on the normalized cyclic voltammograms of CLZ. Normalized cyclic voltammograms are obtained by dividing the current of cyclic voltammograms by the square root of the scan rate [24]. In lower scan rates, the peak current ratio (I_{pC1}/I_{pA1}) is less than one and increases with increasing scan rate, which is indicative of the following chemical reaction after the electron transfer step. Figure 4 part b, shows the plot of increasing peak current ratio (I_{pC1}/I_{pA1}) versus scan rate for CLZ. At scan rates higher than 300 mVs^{-1} , the current ratio (I_{pC1}/I_{pA1}) becomes constant, but at slower potential sweep rates, it increases as a result of decreasing of chemical reaction. The current of the cathodic peaks C_1 and C_2 strongly depends on the potential scan rate. It is seen that when the scan rate is increased, the current ratio of (I_{C1}/I_{C2}) increased with increasing of the potential sweep rate (Fig. 4. part c). This corresponds confirms an ECE mechanism for the electrochemical oxidation of CLZ in an aqueous medium. The same pathway for the oxidation of CLZ has been reported by other researchers [28-30].

For more data, we plotted $\log I_p$ for the peak A_1 versus $\log v$ (Fig. 4. part d). It was reported that when the slope is 0.5, the electrochemical reaction is a diffusion controlled process [31]. The plot of $\log I_{pA1}$ versus the $\log v$ is found to be linear with a slope of 0.446 (This value is close to the theoretically expected value) and correlation coefficient, $R = 0.9921$, so the electrochemical process is governed by diffusion control.

Voltammetric Studies CLZ in the Presence of TBA

Cyclic voltammogram of a 3 mM solution of CLZ in aqueous solution containing 0.2 M phosphate buffer (pH 7.2) is shown in Fig. 5 curve a. As can be seen, one anodic (A_1) and two cathodic peaks C_1 and C_2 were obtained. The oxidation of CLZ in the presence of TBA as a nucleophile was studied in some details. Figure 5 curve c, shows the

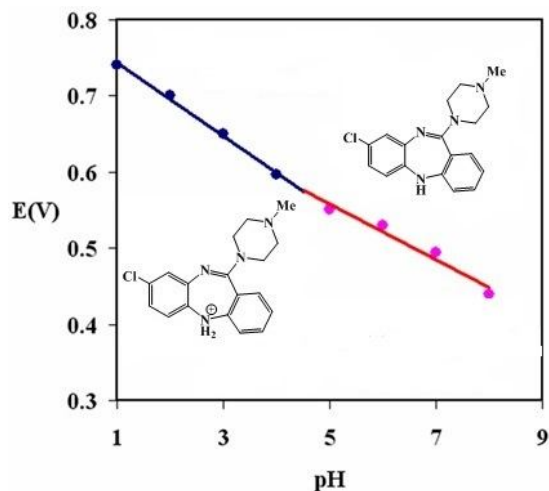


Fig. 2. Potential-pH diagram of CLZ at various pH values for Fig. 1.

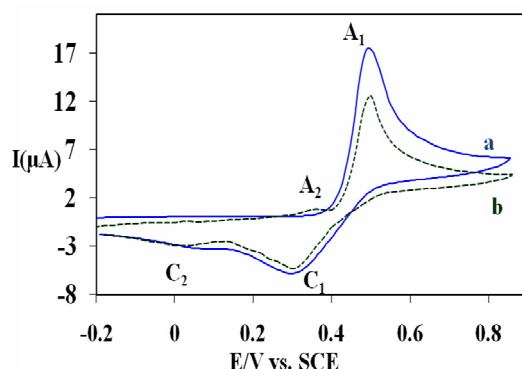


Fig. 3. Two repeated cyclic voltammograms scans of 1 mM CLZ:(a) the first and (b) the second cycle in phosphate buffer solution ($c = 0.2$ M, $\text{pH} = 7.2$), at a glassy carbon electrode, scan rate: 100 mV s^{-1} .

cyclic voltammogram obtained for a 3 mM solution of CLZ in the presence of 3 mM 2-thiobarbituric acid. Comparison of this voltammogram with the cyclic voltammogram of CLZ in the absence of TBA shows that in the reverse scan, the cathodic peak C_1 and C_2 disappears and the current of anodic peak A_1 decreases. The occurrence of a chemical reaction after electron transfer process is supported by the disappearing of peak C_1 and C_2 during the reverse scan, which could indicate that CLZ oxidized, formed at the surface of the electrode is consumed by a chemical reaction with TBA. In Fig. 5, curve b is related to by the cyclic voltammogram of TBA.

More studies were performed by varying the potential scan rate in a solution of CLZ in the presence of TBA. The results indicate that the peak current ratio (I_{pC_1}/I_{pA_1}) is dependent on the potential scan rate and increases with increasing it. The same results were obtained by decreasing the concentration of TBA (data not shown).

Controlled potential coulometry was performed in aqueous solution (phosphate buffer 0.2 M, $\text{pH} 7.2$) containing 0.3 mmol of CLZ and 0.3 mmol of 2-thiobarbituric acid at 0.5 V versus SCE. The electrolysis progress was monitored using cyclic voltammetry (Fig. 6). It is shown that, proportionally to the advancement of

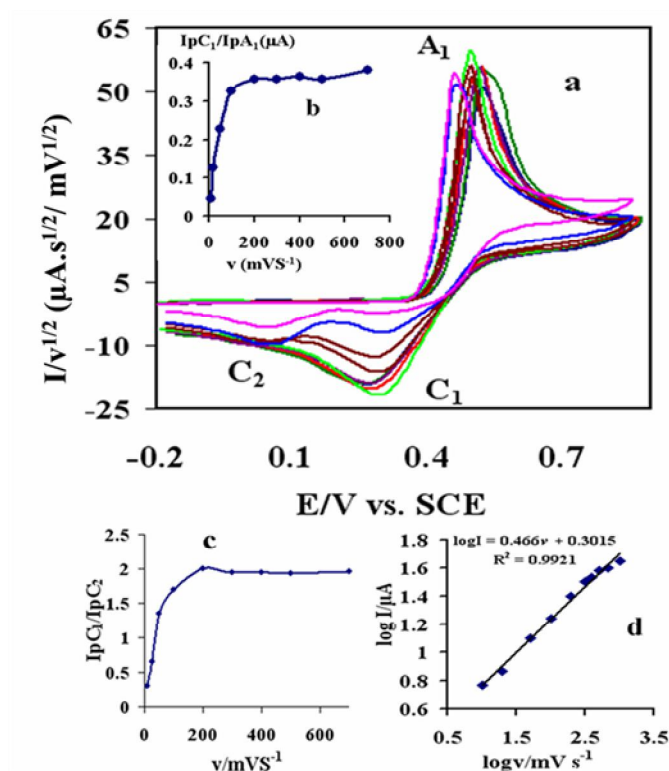


Fig. 4. Part a: Normalized cyclic voltammograms of 1.0 mM CLZ at various scan rates. Scan rates are: 10, 25, 50, 100, 200, 300, 400, 500, 700 and 1000 mV s^{-1} , respectively. Part b: Variation of peak current ratio (C_1/A_1) vs. Scan rate. Part c: The plots of current ratio of (C_1/C_2) vs. Scan rate and Part d: Variation of $\log I_{pA_1}$ vs. $\log v$. At glassy carbon electrode, in phosphate buffer solution ($c = 0.2 \text{ M}$, $\text{pH} = 7.2$).

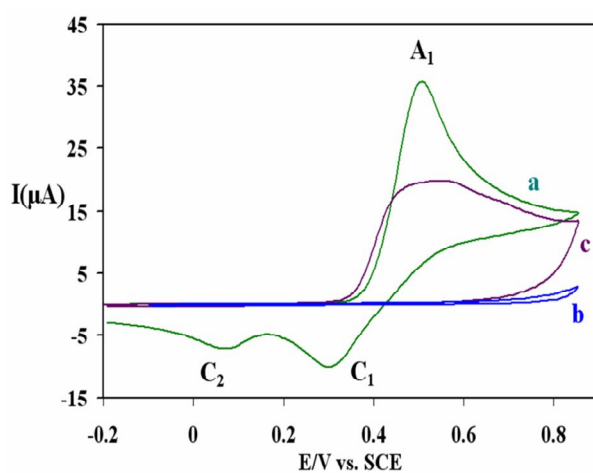


Fig. 5. Cyclic voltammograms of 0.3 mM CLZ: (a) in the absence of TBA; (b) 0.3 mM TBA in the absence of CLZ, and (c) in the presence of 0.3 mM TBA, at a glassy carbon electrode, in phosphate buffer solution ($C = 0.2 \text{ M}$, $\text{pH} = 7.2$); scan rate: 100 mV s^{-1} .

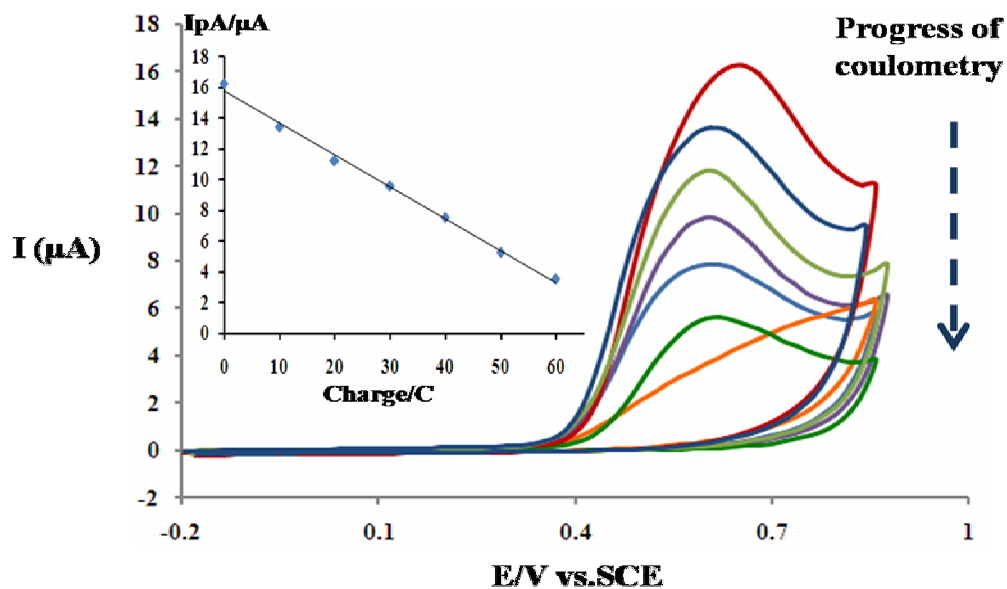
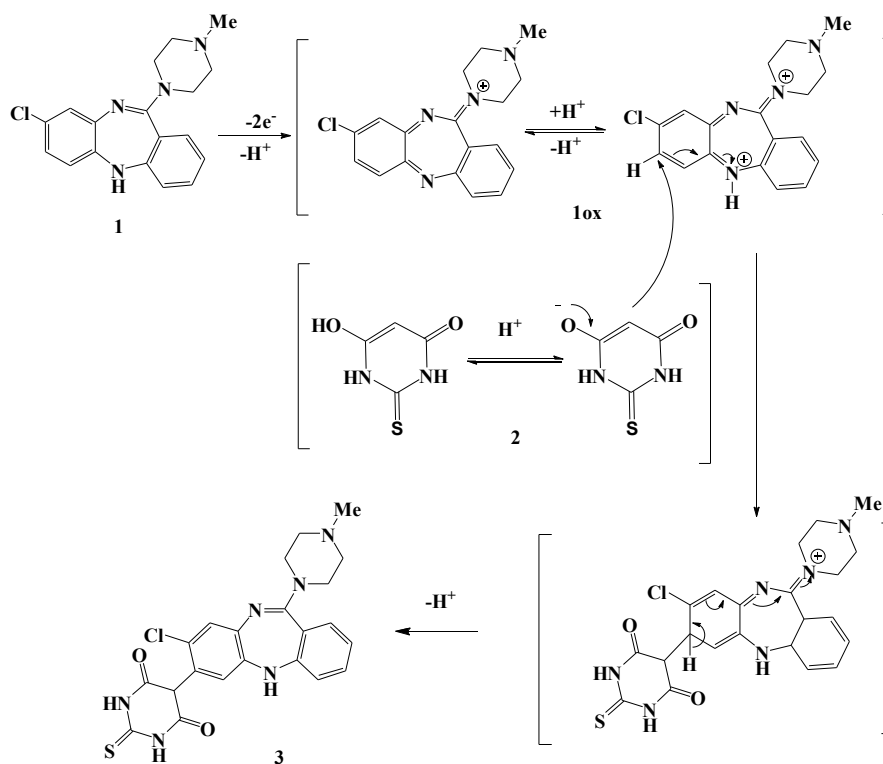


Fig. 6. Cyclic voltammograms of 0.3 mmol clozapine in the presence of 0.3 mmol TBA during controlled potential coulometry at 0.5 V vs. SCE. Inset: variation of peak current ($I_p A$) vs. charge consumed.



Scheme 3. Electrochemical oxidation mechanism of CLZ in the presence of 2-thiobarbituric acid (TBA) in aqueous medium.

coulometry, the anodic peak A_1 decreases and disappearance after consumption of about $2e^-$ per molecule of CLZ. After formation of nitrenium ion *via* Michael addition reaction it can be attacked by the TBA to yield of product. However, thin layer chromatography (TLC) indication of formation of one component in electrooxidation of CLZ in the presence of TBA.

The existence of an EC electrochemical mechanism of electrooxidation of CLZ in the presence of nucleophile [21] is supported by coulometry and voltammetry results accompanied by the ^1H NMR and ^{13}C NMR data and molecular mass. Scheme 3 can be proposed as a possible reaction mechanism to explain the electrochemical oxidation of CLZ in the presence of TBA.

CONCLUSIONS

In this article, cyclic voltammetry and controlled potential coulometry of CLZ has been investigated in the presence of TBA as a nucleophile in aqueous solutions in comparison of that in the absence of nucleophile. The electrochemical data indicate that the mechanism of the electrochemical oxidation of CLZ in an aqueous medium is ECE. According to previous reports on the products of chemical reactions following the oxidation reaction [29], and our cyclic voltammetry data, an acid catalyzed reaction is suggested to produce the final product (4/4ox). The redox potential of the suggested product could be obviously easier than CLZ that is in agree with C2/A2 peaks in related cyclic voltammogram data. Also, the electrochemical oxidation of CLZ in the presence of TBA as a nucleophile, show that the oxidized CLZ, after the consumption of $2e^-$ per molecule of CLZ is attacked by the nucleophile, *via* an EC electrochemical mechanism, to give the final product 3.

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