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Investigation of Redox Mechanisms of Biologically Active Hydantoin Derivatives by Different Voltammetric Methods

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Abstract- The electrochemical behavior of four biologically active cycloalkane espirohydantoins with 5-, 6-, 7-, and 8-membered rings: 1,3-diazaspiro[4.4] nonane-2,4-dione (CSH(5)); 1,3-diazaspiro[4.5]decane-2,4-dione (CSH(6)); 1,3-diazaspiro[4.6]undecane-2,4-dione (CSH(7)); and 1,3-diazaspiro[4.7]dodecane-2,4-dione (CSH(8)) has been investigated at the pH range 1.81-12 by modern electrochemical techniques as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The electrochemical studies were carried out on a hanging mercury drop electrode (HMDE) at room temperature. CSH(5) and CSH(6) at pH 5.50 and 7.63 and CSH(7) at pH 5.50 showed a single quasireversible peaks. For CSH(7) at pH 7.63 and CSH(8) at two pH values the irreversible nature of the electrode process was proved. The effect of scan rate, pH and concentration on peak current and peak potential was investigated. Physical parameters like diffusion coefficient and heterogeneous electron transfer rate constant were determined from scan rate and concentration effects. The number of proton transfer in the electrochemical reaction was determined from the peak potential shift as a function of pH and a mechanism of cycloalkanespiro-5-hydantoins derivatives on the basis of CV, SWV and DPV results was proposed. The proposed mechanisms of the cited cycloalkane spiro-5-hydantoins could be essential in regard to the hidden pathways by which such compounds exert their biochemical actions.

Keywords- Hydantoin, Cycloalkanespiro-5-hydantoins, Voltammetry, Redox mechanism, Heterogeneous electron transfer rate constant, Electro-reduction

1. INTRODUCTION

Hydantoins have been found to have broad spectrum of applications in the field of medicine [1-13], industry and agriculture [10-13]. The derivatives of imidazolidine-2,4-dione (hydantoin) are promising candidates especially in the syntesis of new medicines on account of their wide spread use in the treatment of high blood pressure, cancer pain, attention deficit hyperactivity disorder. Phenytoin (5,5-diphenylhydantoin, Dilantin, Epilan) is from the first generation of antiepileptic drugs introduced in 1938 by Meritt and Putnam, which is employed in cases of generalized tonic-clonic seizures (so-called grand mal epilepsy) and focal motor seizures [14]. The anticonvulsant properties of Phenytoin are responsible for the synthesis of many hydantoin analogs [15-17]. It is known that derivatives of hydantoin have been demonstrated to exert various effects on nervous systems, most of which are compatible with an anticonvulsant and antinociceptive action. Although the anticonvulsant action of hydantoin derivatives is due to the selective block of the voltage-sensitive sodium channels this site on the sodium channel is still not completely defined [18,19]. Development of new compounds with increased selectivity for it may provide significant activity and fewer side effects. In order to improve efficiency and to overcome the side effects of the known in the medicine drugs automatically switched the attention of scientists to search more effective imidazolidine 2,4-dion-based compounds [20,21].

Cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings are the subject of extensive investigations due to their biological properties [22,23]. Some cycloalkanespiro-5-hydantoins also have modest anticonvulsive effect. It has been observed that the growth in the cycloalkane ring size increases the effect [22,24].

The structural characteristics of hydantoins as well hydantoin derivatives as the cycloalkanespirohydantoins are interesting in terms of development of novel drugs [25] and for a better understanding of the relationship between chemical structure and biologically activity. A number of biologically active compounds are included in oxidation-reduction reaction that is why many drugs can be determined thanks to their reduction or oxidation properties. As mentioned, the hydantoin derivatives have a broad range of applications but the understanding of the electrochemical redox mechanism of more of their derivatives is not clarified. It has been found that there is relationship between pharmaceutical properties of most of the hydantoins and their redox-pH behavior [26]. The results of sensitive electrochemical techniques have shown that some of hydantoin derivatives can be oxidized at the glassy carbon electrode involving the same number of electrons and protons [26]. Hydantoin derivatives used as sedatives can be also determined using their anodic waves at mercury drop electrode. [27]. In this paper we report the electrochemical behavior of 1,3-diazaspiro[4.4]nonane-2,4-dione (CSH(5)); 1,3-diazaspiro[4.5]decane-2,4-dione (CSH(6)); 1,3-diazaspiro[4.6]undecane-2,4-dione (CSH(7)); and 1,3-diazaspiro[4.7]dodecane-2,4-dione (CSH(8)) (Fig. 1) and propose the mechanism for their reduction at mercury drop electrode.

The proposed mechanisms of cited cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings could be essential in regard to the hidden pathways by which such compounds exert their biochemical properties. The cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings derivatives were prepared according to Bucherer-Lieb reaction and fully characterised by TLC, IR and NMR spectroscopy [23,28].

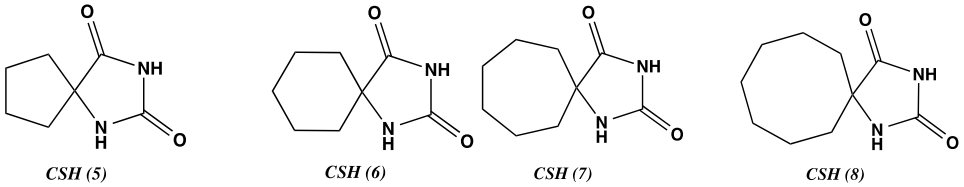


Fig. 1. Chemical structures of the cycloalkanespiro-5-hydantoins

2. MATERIAL AND METHODS

2.1. Apparatus

The voltammograms (cyclic, DPV and SW) were recorded on a Metrohm 797 VA trace analyzer and a 797 VA stand. The Ag/AgCl electrode in 3 mol L^{-1} aqueous solution of KCl was used as a reference electrode, the hanging mercury drop electrode (HMDE) as a working electrode, and a carbon electrode as an auxiliary electrode. All the voltammetric experiments were conducted in a high purity nitrogen atmosphere at room temperature (25±1 ^{o}C). The pH measurements were carried out with digital pH-meter (Jenway).

2.2. Material and reagents

All chemicals used were of analytical grade. Twice distilled water was used in all experiments. The stock solutions of 0.09435 mol L^{-1} CSH(5), 0.0999 mol L^{-1} CSH(6), 0.11028 mol L^{-1} CSH(7) and 0.05373 mol L^{-1} CSH(8) were prepared by dissolving the test substances in double-distilled water. Britton-Robinson (BR) buffer solutions (0.04 mol l^{-1}) over the range pH 1.81-12 were also prepared.

2.3. Methods for determination

2.3.1. Cyclic voltamperometry (CV)

An aliquot of stock solution of cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings (200 μL of the solutions of CSH(5), CSH(6), CSH(7) and 400 μL of the solution of CSH(8)) was placed in a 10 mL-electrode cell containing 10 mL Britton-Robinson buffer (0.04 mol L^{-1}, pH=1.81). Oxygen was removed by bubbling of pure nitrogen through the solution for 400 seconds. Deposition of the test substances was carried at -1.2 V for 60 s

with continuous stirring at speed of 2000 rpm. The stirrer was stopped and the solution was allowed to rest for 10 s, then the voltammograms were recorded using HMDE with a drop size of ≈0.15 mm^2, voltage step 5 mV and variation of scan rate "ν" from 10 to 100 mV s^{-1} over reduction potential range from -0.020 to -1.2 V. After that the pH of the solution was increased from 1.81 to 12.00, respectively and the voltammograms were recorded at the same conditions.

2.3.2. Differential pulse voltammetry (DPV)

The differential pulse polarogram of the (200 μL of the solutions of CSH(5), CSH(6), CSH(7) and 400 μL of the solution of CSH(8) in 10 ml Robinson buffer was recorded after oxygen removal by passing nitrogen gas for 400 s. The cathodic peaks for all of cycloalkanespiro-5-hydantoins were registered at a hanging mercury dropping electrode (HMDE), deposition: 60 s, pulse amplitude –50 mV, voltage step 5 mV, voltage step time 0.4 s at pH rank from 1.81 to 12.00.

2.3.3. Square wave voltammetric (SWV)

The square wave voltammetric (SWV) experiments of the 200 μL of the solutions of CSH(5), CSH(6), CSH(7) and 400 μL of the solution of CSH(8) in 10 ml Robinson buffer were performed at 50 Hz frequency and 2 mV potential increments corresponding to scan rate of 100 mV s^{-1}.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry

Cyclic voltammetry of CSH(5), CSH(6), CSH(7) and CSH(8) was carried out using 18.8, 20.0, 22.1 and 21.5 mM solutions at pH 5.50 and 7.63 using 0.04 mol l^{-1} Robinson buffer at a hanging drop mercury electrode between the limits from 0 to -1V (Fig. 2). on proceeding from 0 to -1 V of the all cycloalkanespiro-5-hydantoins, single reduction signals were observed as follows: at pH 5.5: -0.365 V, -0.366 V, -0.383 V and -0.401 V; at pH 7.63: -0.562 V, -0.609 V, -0.580 V and -0.627 V. The immediate reverse potential scan in a positive direction, oxidation peaks were observed at potentials corresponding to: -0.273 V, -0.308 V, -0.288 V and -0.252 V at pH 5.5 and -0.413 V, −0.437 V, 0.318 V and -0.389 V at pH 7.63, respectively. One can see that increasing the number of the cycloalkane ring shifted to more negative potentials the reduction peaks. It can be attributed to the stronger effect of the cycloalkane ring on carbonyl group from hydantoin ring and its easier reduction. The appearance of corresponding oxidation peaks in the reverse scan is often indication for reversible or quasi-reversible nature of the reduction process. The distance between both peaks, ΔE_p ($\Delta E_p=(E_{pc}-E_{pa})$) is between 60-200 mV and the ratio of peak heights: I_{pc}/I_{pa} is

greater than one, suggesting the quasi-reversible nature of the electrode process [29]. ΔE_p is higher than 200 mV for CSH (7) and CSH (8) at pH 7.63 and it indicated the reduction process to be irreversible. However, E_{pc}-$E_{pc/2}$ values between 30 and 80 mV peaks showed the quasi-reversible nature of the electrochemical process for all cycloalkanespiro-5-hydantoins. It is noticed that the distance between cathodic (E_{pc}) and anodic (E_{pa}) potentials is increased with increasing the alkalinity of the medium. The peak potentials were shifted toward more negative values also at pH>8 which may indicate that the electrode process could involve a strong adsorption phenomenon on HMDE at pH>8 [29].

The blank voltammogram of the Robinson buffer in the potential window (from 0 to -1 V) was done and confirm that the oxidation/reduction signals are of hydantoins only. We also observed that the initial potential of the blank voltammogram of the Robinson buffer takes place at positive than 0 V potential and gives the anodic current around 10 μA because anodic oxidation of the mercury from the mercury electrode. Therefore the cyclyc voltammograms showing on the figures were registered at initial potential around -0.020 V.

Cyclic voltamperograms of the selected cycloalkanespiro-5-hydantoins obtained in Robinson buffer of pH ranging from 2 to 12 at 100 mV s^{-1} scan rate are shown in Fig. 3.
The effect of pH of the media was used to establish the redox mechanism of the analytes. With increase in pH of the medium, both peaks (cathodic and anodic) were shifted cathodically indicating that protons participate directly in the reduction process and that proton-transfer reaction precedes the electrode process proper. Maximum sensitivity was observed at pH 7.63 (Fig. 2 and Fig. 3) for cycloalkanespiro-5-hydantoin all structures. Furthermore the cited cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings were studied in pH 5.50 and 7.63 where was observed the difference of nature of electrode processes and the current sensitivity. It has been reported by previous researchers that the phenytoin structure is polarographically inactive, and it lacks cathodic peak [30]. Other authors reported that same of hydantoins could be determined using anodic waves at mercury drop electrode [27]. We found that the electrochemical behavior of cited cycloalkanespiro-5-hydantoins is different than that for other reported hydantoins. The presence of the electron withdrawing groups such as cycloalkyl with 5-, 6-, 7-,and 8-membered rings leads to increase the lipophilicity and the steric bulk, consistent with the enlargement of the cycloalkane ring size in the molecule bonded with hydantoin ring and it could be stabilized the compound. This appears from the reversibility of the redox peaks and is possible because the electron-withdrawing caused by cycloalkane group makes a compound more positively charged, i.e., more easily reduced. It can be seen (Fig. 3) that the stability of the compounds increases by increasing the withdrawing character (CSH(5)>CSH(6)>CSH(7)>CSH(8)).

E_{pc} of cycloalkanespiro-5-hydantoins as a function of pH was present in Fig. 4 (A-D). A good linear relation was observed in the pH rang 2-10 with a slope ≈95 mV per pH unit (Fig. 4 (A-D)) which suggested that the number of proton taking part in the electrode reaction is

similar to the number of electrons. It can be concluded from the expression $\Delta E_p / \Delta pH = 0.059\, p / \alpha n$ that the ratio between protons *p* and fractional electrons transferred αn is 1.61. "αn" for quasi-reversible electrode reaction were calculated from the relation $(E_p - E_{p/2}) = 0.048 / \alpha n$, where $E_{p/2}$ is a potential at half peak current [31]. The values of $(E_p - E_{p/2})$ as well αn for CSH(5), CSH(6), CSH(7) and CSH(8) at pH 5.50 and 7.63 are given in Table 1-A and Table 1-B. It can be concluded, from these results and number of electrons, *n* (from DPV) that the numbers of protons participated *p* is 1 for all cycloalkanespiro-5-hydantoins and 2 only for CSH(7) at pH 7.63 and CSH(8) at pH 5.50 and 7.63. Moreover, the peak potentials were not changed at pH more than 10. This ruled out the proton involvement during reduction process of cycloalkanespiro-5-hydantoins in highly basic media. The pK_a values of CSH(5) (9.6), CSH(6) (9.5), CSH(7) (9.7) and CSH(8) (10.4) evaluated from intersection of the two linear segments of the function E_{pa}= f (pH) (Fig. 4 A-D) are given in Table 2. According to the other references the values of pK_a constants of structurally related species like phenytoin, thymine, *5-benzylideneimidazolidine-2,4-dione* and 5-(4-methoxy benzylidene)imidazolidine-2,4-dion are pK_a=8.31, pK_a=9.9, pK_a=9.7 and pK_a=10.1, respectively [26].

The influence of pH on the current of cathodic peaks has been also analyzed for 18.8, 20.0, 22.1 and 21.5 mM CSH(5), CSH(6), CSH(7) and CSH(8) (Fig. 4 A-D). From the plots of I_p *vs.* pH, it is seen that the peak current reaches maximum values in the pH 7.63. The ratio of cathodic and anodic peak heights is greater than one (Table 1-A), suggesting the quasi-reversible nature of the electrode process.

Cyclic voltammograms of 47.2 mM CSH(5), 50,0 mM CSH(6), 22.0 mM CSH(7) and 21.5 mM CSH(8) solutions were also obtained in pH 5.50 and pH 7.63 in the range from 10 to 100 mV sec^{-1}. A slight shift to more negative potentials was observed with increase in scan rate indicating the reduction process to be of quasi-reversible nature under these conditions. More negative peak potential shift of the catodic peak with increase in scan rate (ν) predicted the electrochemical process of CSH (8) at pH 7.63 to be of irreversible nature [32]. Furthermore, the peak current increased with increasing the scan rate, after that 70 $mVsec^{-1}$ the peak current is not change. For that reason, 100 mV s^{-1} was selected for further studies.
Scan rate studies were also carried out to establish whether the process at the hanging mercury drop electrode is diffusion or adsorption controlled (Fig. 5).

The cathodic peaks current of cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings increase linearly with the square root of scan rate (Fig. 5). The diffusion coefficient of all these compounds listed in Table 2 were determined at two different pH values using Randles-Secik equation and number of electrons, *n* (from DPV).

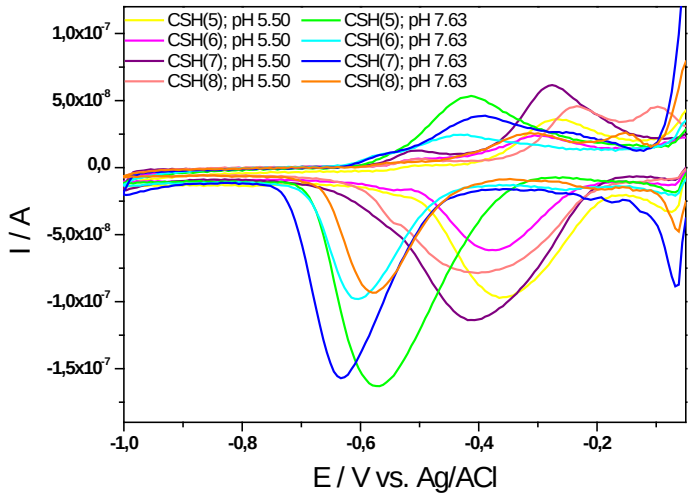


Fig. 2. Cyclic voltammograms of 18.8, 20.0, 22.1 and 21.5 mM cycloalkanespiro-5-hydantoins at HMDE in pH 5.50 and 7.63 at 100 mV s^{-1} scan rate

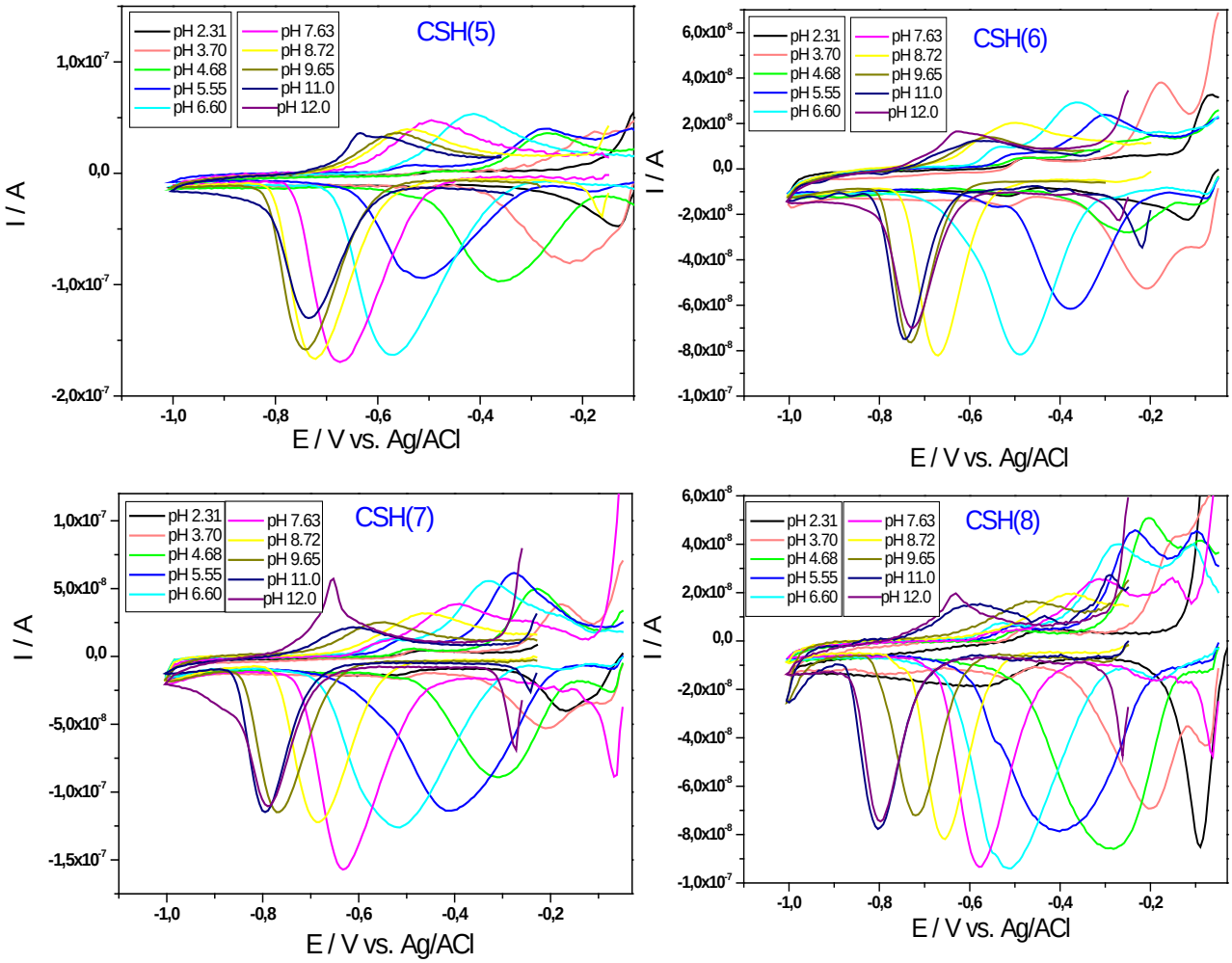


Fig. 3. Cyclic voltammograms of 18.8, 20.0, 22.1 and 21.5 mM CSH(5), CSH(6), CSH(7) and CSH(8) at HMDE in Robinson buffer at different pH and 100 mV s^{-1} scan rate

The equations of regression of a plot of logarithm of peak current versus logarithm of scan rate at pH 5.50 and 7.63 are given in Table 3. The slopes of the straight lines in the logarithm plots of peak currents against logarithm of scan rate at pH=5.50 and 7.63 (Table 3) are less than 1.0 (theoretical value), which shows the adsorption controlled electrode process and more than 0.5 (theorethical value), which is expressed for the diffusion controlled electrode process. This case indicates both diffusion and adsorption controlled electrode process [33,34].

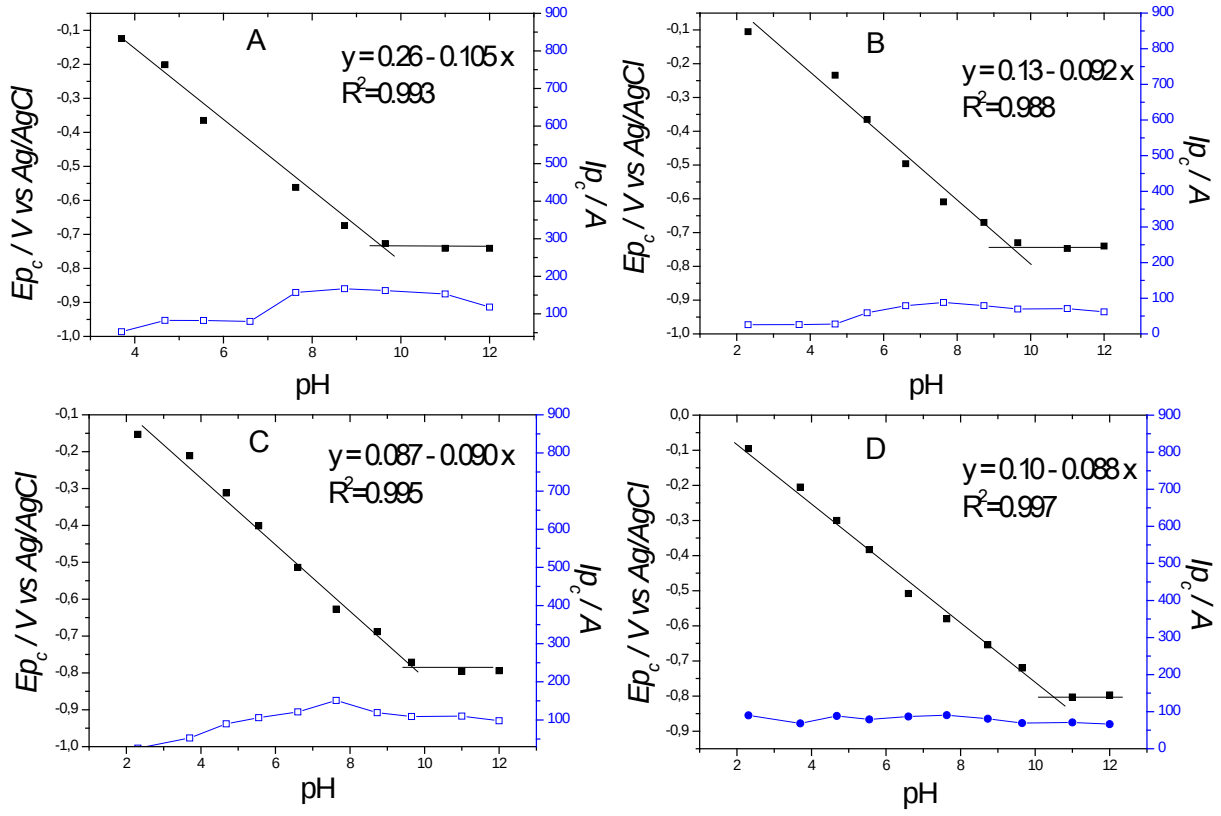


Fig. 4. (A-D) Plot of (□) Ip_c and (■) Ep_c *vs* pH for CSH (5) (18.8 mM), CSH(6) (20.0 mM), CSH(7) (22.1 mM) and CSH(8) (21.5 mM).

Therefore, it can be said that the analytical signal is displayed from the reduction process not only of cycloalkanes hydantoin molecules which were already adsorbed on the mercury electrode surface prior to the potential scan stage, but also of those which reach to the electrode by diffusion. Some of the sharp peaks obtained at pH 5.50 (Fig. 3) could also be indication about the adsorption controlled part of the mixed electrode process [31].

Peak current was plotted as a function of concentration and heterogeneous electron transfer rate constants for all cycloalkanespiro-5-hydantoins were evaluated using Reinmuth expression: *Ipc* = *nFACoKsh* [35]. We found that the peak current increased with increasing the concentrations of the compounds. The values of K_{sh} for all cycloalkanespiro-5-hydantoins

are also summarized in Table 2. The K_{sh} values with an order of 10^{-3} cm s^{-1} characterize the cycloalkanespiro-5-hydantoins reduction process to be quasi-reversible [36].

Table 1-A. Values of cathodic and anodic current, current ratio and fractional electrons transferred *αn* for CSH(5), CSH(6), CSH(7) and CSH(8) obtained by CV studies

CSH	***-I_{pc}, nA***		***I_{pa}***		***I_{pc}/I_{pa}***		***αn***	
	pH 5.5	pH 7.6	pH 5.5	pH 7.6	pH 5.5	pH 7.6	pH 5.5	pH 7.6
(5)	82.3	157	39.8	50.9	2.1	3.1	0.56	0.58
(6)	59.3	88.3	20.3	25.7	2.9	3.4	0.85	0.64
(7)	106	151	49.5	35	2.1	4.3	0.83	0.96
(8)	79.1	90.7	25.1	15.2	3.1	6.0	0.68	1.14

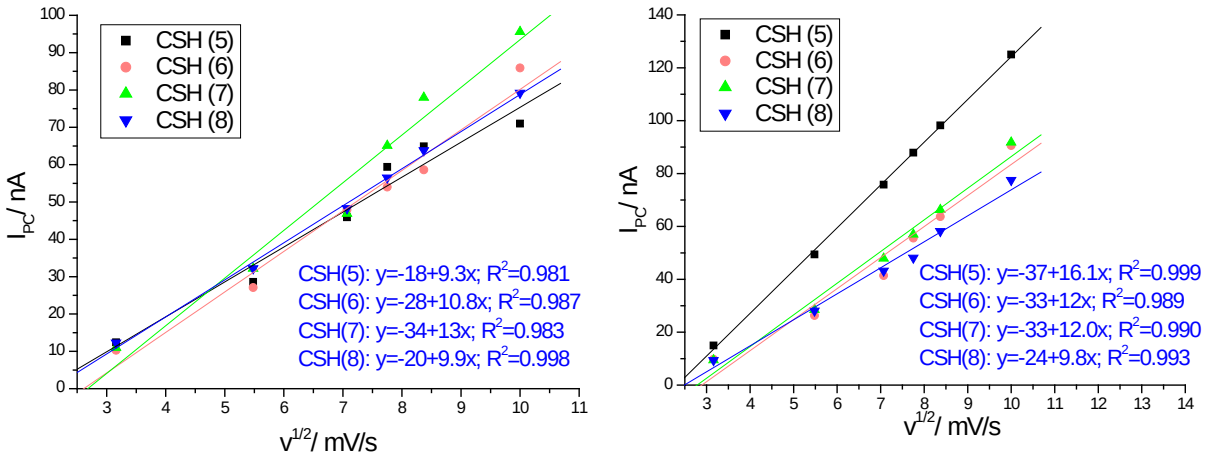


Fig. 5. (A) Plot of I_{pc} at pH 5.55 and (B) I_{pc} at pH 7.63 *vs.* $v^{1/2}$ (mV s^{-1})

Table 1-B. Values of cathodic and anodic peak potential and E_{pc}-$E_{pc/2}$ for CSH(5), CSH(6), CSH(7) and CSH(8) obtained by CV studies

CSH	***-E_{pc}, mV***		***-E_{pa} mV***		***E_{pc}-$E_{pc/2}$ mV***	
	pH 5.5	pH 7.6	pH 5.5	pH 7.6	pH 5.5	pH 7.6
(5)	365	562	273	413	85	82
(6)	366	609	308	437	56	75
(7)	383	580	288	318	58	42
(8)	401	527	252	389	70	50

Table 2. Values of diffusion coefficient (D), heterogeneous electron transfer rate constant (k_{sh}^{0}) and pK_a for CSH(5), CSH(6), CSH(7) and CSH(8) obtained by CV studies

CSH	***Dx10^{-7} cm^2 s^{-1}***		***k_{sh}^{0} x10^{-3} cm s^{-1}***		***pK_a***
	pH 5.5	pH 7.6	pH 5.5	pH 7.6	
(5)	2.93	10.7	2.66	2.11	9.6
(6)	1.41	3.12	4.08	4.35	9.5
(7)	3.70	3.29	3.78	0.406	9.7
(8)	0.893	1.17	2.23	0.830	10.4

Table 3. The equations of regresion of the function of log I_{PC}, nA= f(log (ν, mV s^{-1})) at pH 5.55 and 7.63 for CSH (5) (18.8 mM), CSH(6) (20.0 mM), CSH(7) (22.1 mM) and CSH(8) (21.5 mM)

Compound	pH	Equation of regresion	R^2
CSH (5)	5.50	log I_{PC} =0.28+0.81 log (ν)	0.991
	7.63	log I_{PC} =0.27+0.93 log (ν)	0.996
CSH (6)	5.50	log I_{PC} =0.08+0.92 log (ν)	0.998
	7.63	log I_{PC} =0.01+0.97 log (ν)	0.996
CSH(7)	5.50	log I_{PC} =0.08+0.96 log (ν)	0.996
	7.63	log I_{PC} =0.01+0.99 log (ν)	0.999
CSH (8)	5.50	log I_{PC} =0.28+0.82 log (ν)	0.998
	7.63	log I_{PC} =0.07+0.92 log (ν)	0.998

3.2. Differential pulse voltammetry

DPVs of 18.8 mM CSH(5), 20.0 mM CSH(6), 22.1 mM CSH(7) and 21.5 mM CSH(8) solutions were carried out using different pH values and have been depicted in Fig.6 and Fig. 7.

One can see that in DPV mode one cathodic peak from electrolyte buffer (Fig. 8 A-D, curves 1') at pH 2.31 and 3.70 is observed and it influence into the peaks of cycloalkanespiro-5-hydantoins. The experimental values of $W_{1/2}$ (width at half peak height) of ≈75 mV (Fig. 7 A-D, Table 3) for CSH(5), CSH(6) and CSH(7) at pH 5.50 and for CSH(5), CSH(6) at pH 7.63 is very closed to the theoretical one at pulse amplitude ΔE=50 mV and evidenced one electron reduction process [37]. The values of $W_{1/2}$ of ≈50 mV for CSH(8) at pH 5.50 and for CSH(7) and CSH(8) at pH 7.63 (Table 4) predict two electron reduction process. Moreover,

the experimental data for $W_{1/2}$ and the symmetrical form of the peaks at pH 5.50 and pH 7.63 shows the reversible nature of electrochemical process according criteria proposed in [37]. The shift of peak potential with rise in alkalinity of the medium showed similar tendencies as obtained from cyclic voltammetry. E_{pc} was plotted against pH and a slope of ≈80 mV (Fig. 8) per pH unit (close to the theoretical value of 59 mV per pH unit) authenticated the CV results of the same number of electron and proton during reduction process. pK_a values were found to have values very close to those obtained in cyclic voltammetry. All parameters obtaining by differential pulse voltammetry are summarized in Table 4.

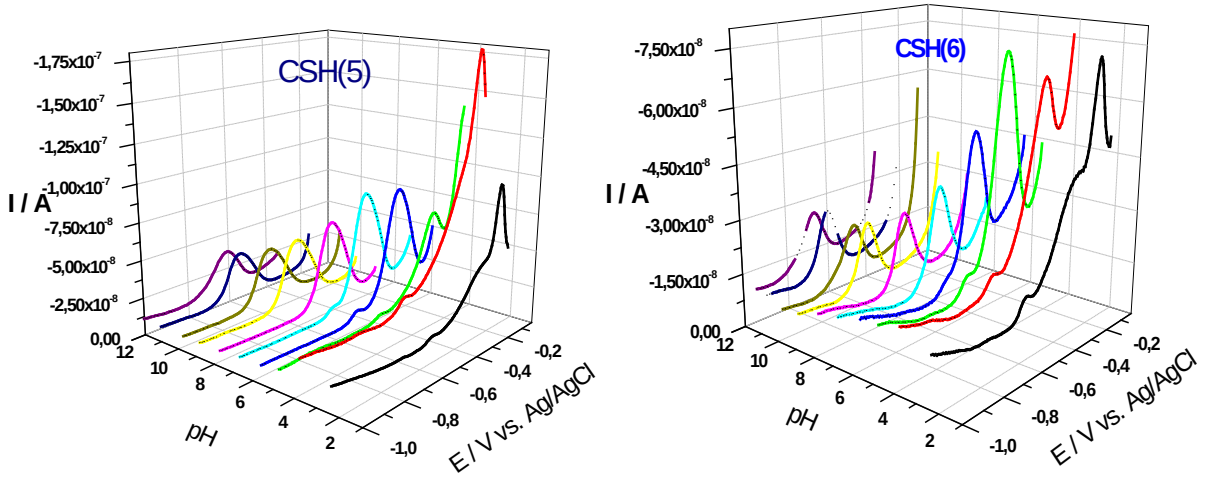


Fig. 6. DPVs of 18.8 mM CSH(5) and 20.0mM CSH(6) at HMDE in different pH at 100 mV s^{-1} scan rate

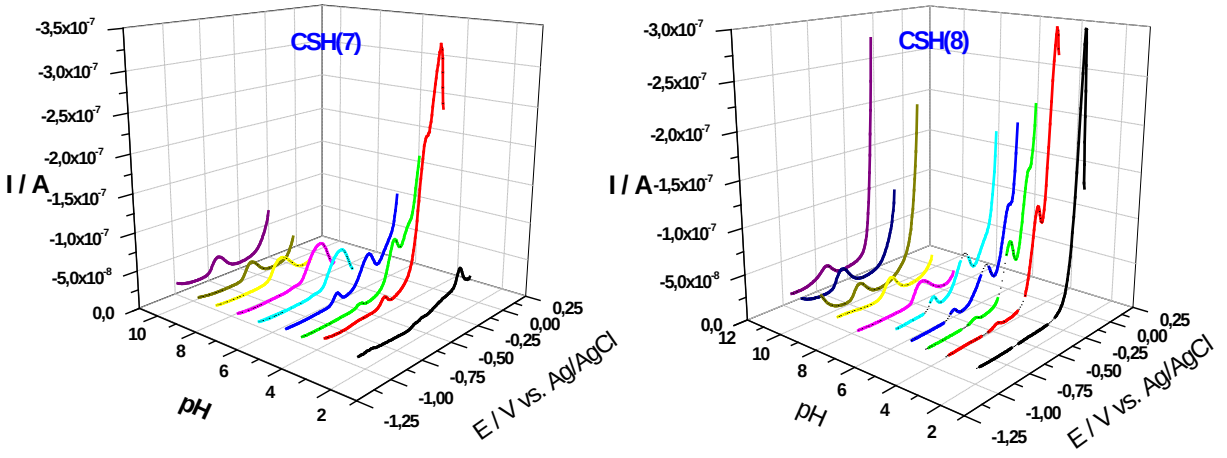


Fig. 7. DPVs of 22.1mM CSH(7) and 21.5 mM CSH(8) at HMDE in different pH at 100 mV s^{-1} scan rate.

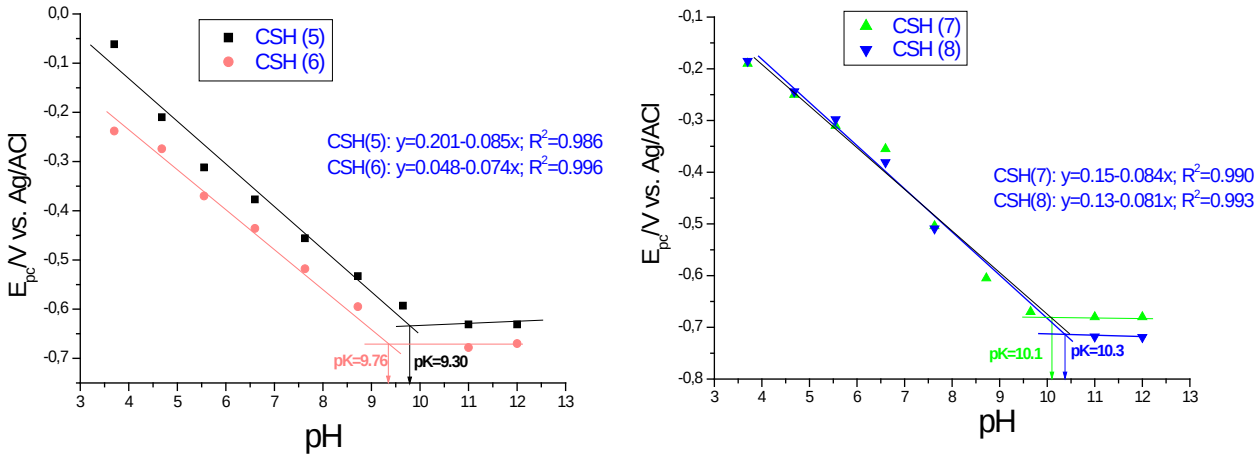


Fig. 8. Plot Ep_c *vs* pH for CSH(5) (18.8 mM), CSH(6) (20.0 mM), CSH(7) (22.1 mM) and CSH(8) (21.5 mM) using HMDE as working electrode.

Table 4. Summary of results for cathodic current and peak potential, value of width at half peak height ($W_{1/2}$) and pK_a constant for CSH (5) (18.8 mM), CSH(6) (20.0 mM), CSH(7) obtained from DPV studies

	$-I_{pc}$, nA		$-E_{pc}$, mV		$W_{1/2}$		pK_a
CSH	pH 5.5	pH 7.6	pH 5.5	pH 7.6	pH 5.5	pH 7.6	
(5)	64	53.6	0.312	0.456	79	78.5	9.3
(6)	49.6	23.8	0.370	0.518	70.1	68.2	9.7
(7)	27	26.1	0.310	0.504	68.4	52.6	10.1
(8)	33.5	29.6	0.298	0.509	42.1	47.4	10.3

3.3. Square-wave Voltammetry (SWV)

The main objective of SWV is to confirm the reversibility, irreversibility or quasi-reversibility of the redox process of the compound. At pH 5.55 and 7.63, the square-wave voltamograms of 18.8 mM CSH(5), 20.0 mM CSH(6), 22.1 mM CSH(7) and 21.5 mM CSH(8) solutions are presented in Figure 9 A-D. As can be seen in Fig. 9, all cycloalkanespiro-5-hydantoins are characterized with one cathodic and one anodic peaks. However, the cathodic peaks of CSH(8) at pH 5.50 and CSH(7) and CSH(8) at pH 7.63 were not accompanied by anodic peaks, which indicates that their redox reactions were totally irreversible. A different situation from CV was also encountered in the backward scan when oxidation peaks of CSH(5) at pH 5.50 was obtained at the same E_P values as the reduction

peaks one (Fig. 9, Table 4) and predict the reversible nature of reduction process. All parameters obtaining by SWV at pH 5.50 and 7.63 are summarized in Table 5.

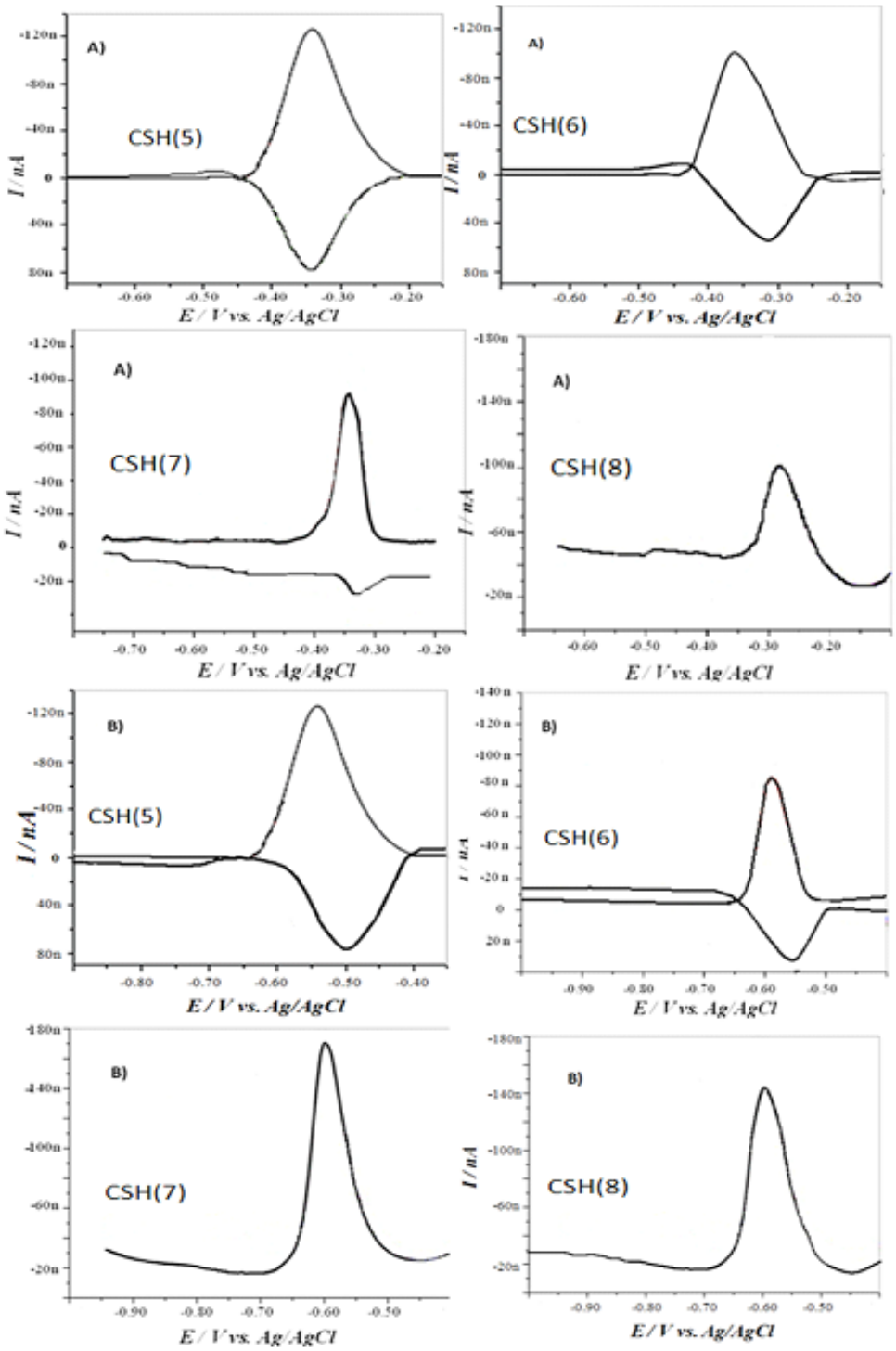


Fig. 9. Square-wave voltamograms at HMDE of 18.8 mM CSH(5), 20.0 mM CSH(6), 22.1 mM CSH(7) and 21.5 mM CSH(8) at pH 5.50 (A) and 7.63 (B). Experimental conditions: pulse height, *E*sw=20 mV; scan increment, d*E*=2 mV; frequency, *f* =50 Hz; scan rate, *v*=100 mVs^{-1}

Table 5. Data from SWV studies of CSH(5), CSH(6), CSH(7) and CSH(8) at pH 5.50 and 7.63.

CSH			***-I_{pc}, nA***	***I_{pa} nA***	***I_{pc}/I_{pa}***	***- E_{pc} mV***	***- E_{pa} mV***	***Process***
(5)	*pH*	5.5	119	75	1.58	350	348	Re[a]
		7.6	121	60	2.02	548	500	Qre[b]
(6)	*pH*	5.5	95	48	1.97	342	315	Qre[b]
		7.6	82	38	2.15	575	557	Qre[b]
(7)	*pH*	5.5	80	20	4.00	350	320	Qre[b]
		7.6	140	-	140	592	-	Irr[c]
(8)	*pH*	5.5	52	-	52	282	-	Irr[c]
		7.6	130	-	130	591	-	Irr[c]

[a]reversible process; [b]quasi-reversible process; [c]irreversible process

3.4. Redox Mechanism

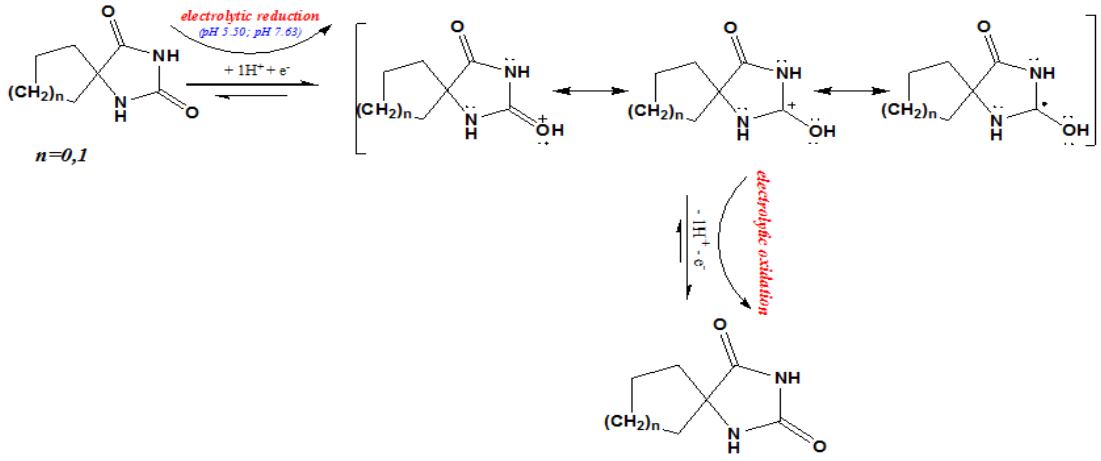
The results obtained from all three electrochemical experiments (CV, DPV and SW) are useful in suggesting the electrode reaction mechanisms of CSH(5), CSH(6), CSH(7) and CSH(8).

Proposed mechanism of CSH(5) and CSH(6) -From the results presented above is obvious that the electrochemical reduction of CSH(5) and CSH(6) at pH 5.50 and 7.63 is a process leading to cleavage of π-bond from C=O group. As one electrons can be required in the reduction of the carbonyl group and one proton is involved in the rate-determining step, a mechanism (Fig 10-A) can be proposed for the reduction of CSH(5) and CSH(6) in the pH range of 2.0–10.0. The proposed mechanism (Fig 10-A) has a support from the increase in cathodic potential with pH, as the protons are consumed in the reduction process.

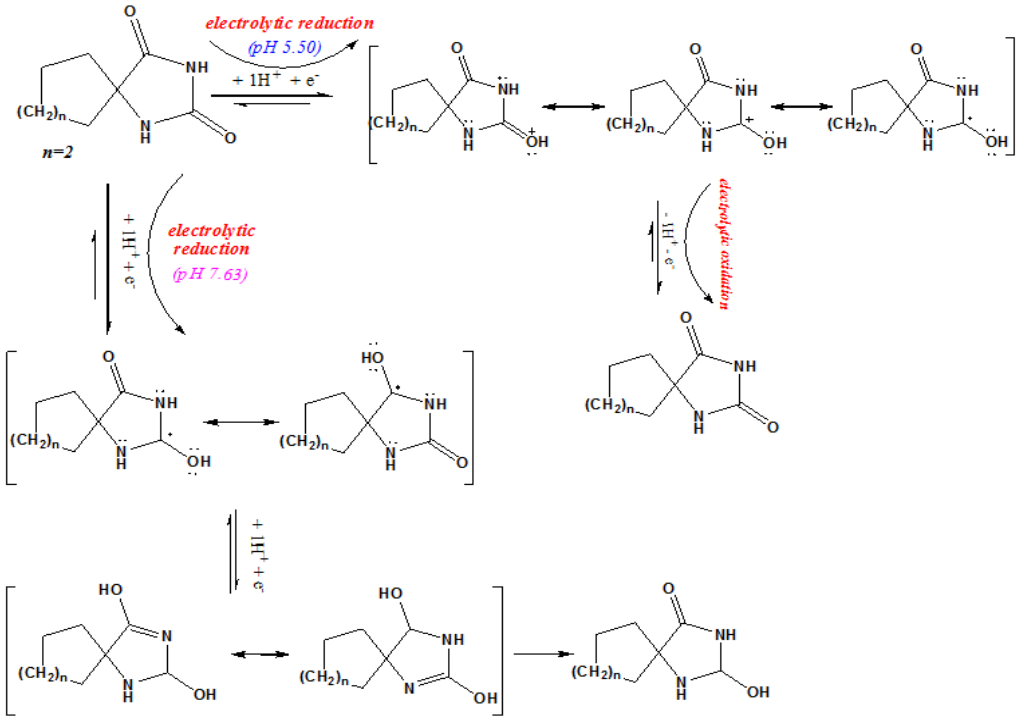
Proposed mechanism of CSH (7) – The reduction of CSH (7) at pH 5.50 was found to occur by the transfer of a single electron as determined from the average $W_{1/2}$ value of 68.4 mV. The plot of the cathode potential to the acidity of the medium with a slope of 80 mV pH^{-1} indicated the reduction to occur by one electron one proton process at pH 5.50 (Fig 10-B). The data obtained by SW voltammetry proved that the reduction of CSH(7) at pH 7.63 is irreversible. Based upon the data obtained from three electhrochemical techniques it was proposed that carbonyl group of CSH(7) after the loss of two electron and proton results in the formation of oxygen radical, which can exist in two resonance forms (Fig 10-B). The form in which electron is residing on carbon atom having attached carbonyl group gets

converted to 2-hydroxy-1,3-diazaspiro[4.6]undecan-4-one by the cleavage of π-bond from C=O group as shown in Fig 10-B.

Proposed mechanism of CSH (8) – One reduction peak was found to have $W_{1/2}$ of 42.1 mV with 84 mV potential shifts per pH unit at pH 5.50 and $W_{1/2}$ of 47.4 mV with 81 mV potential shifts per pH unit at pH 7.63, respectively. This suggests the conversion of ring carbonyl group (>C=O) to >CH-OH by the gain of $2e^-$ and $2H^+$ as shown in Fig 10-C.



(A)



(B)

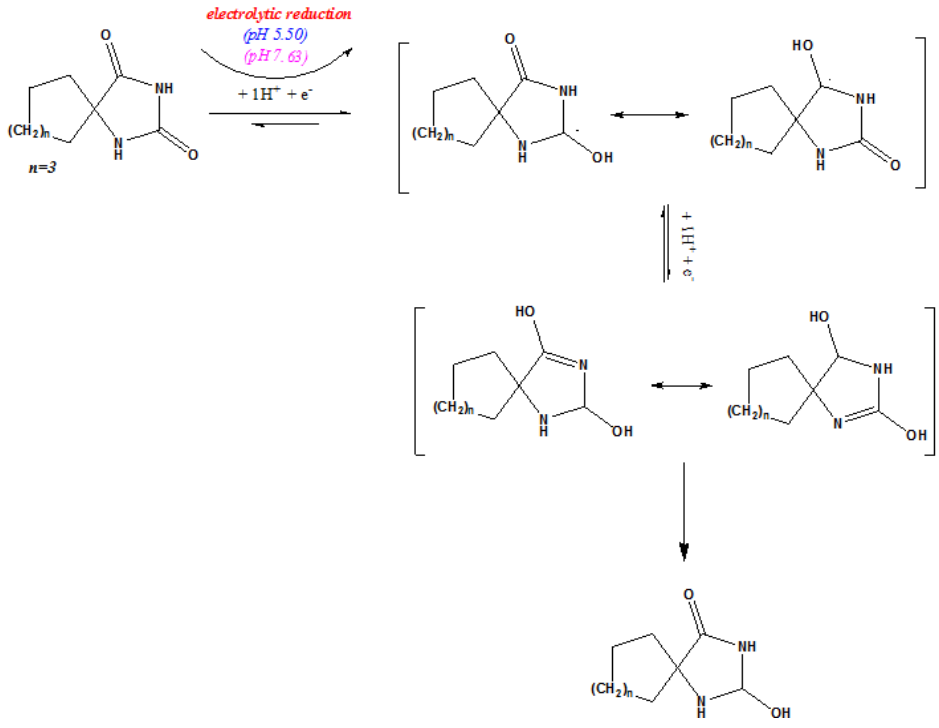


Fig 10. Proposed redox mechanism of cycloalkanespirohydantoins with A) 5- 6-; B) 7-, and C) 8-membered rings

4. CONCLUSION

This work has reported the electrochemical behavior of four biologically active cycloalkanespiro-5-hydantoins on hanging mercury drop electrode at pH 5.50 and 7.63. The electrochemical processes for CSH(5), CSH(6), CSH(7) at pH 5.50 are proved to be quasireversible and pH dependent. The reduction of CSH(8) at pH 5.50 and CSH(7) and CSH(8) at pH 7.63 was found to occur in a irreversible pH dependent manner from pH 4 to 10. The diffusion coefficient, pKa and heterogeneous electron transfer rate constant values of the cycloalkanespirohydantoins with 5-, 6-, 7-, and 8-membered rings were successfully evaluated from electrochemical data. A two-electron two proton mechanism for the redox reactions of CSH(7) at pH 7.63 and CSH(8) at pH 5.50 and 7.63 were observed. The proposed mechanisms of the cited cycloalkane spiro-5-hydantoins could be essential in regard to the hidden pathways by which such compounds exert their biochemical action. These investigations could be help in further understanding of the mechanism of interaction as required for the design of new anticancer drugs.

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