Electroanalytical evaluation and determination of 5-(3′-indolyl)-2-thiohydantoin derivatives by voltammetric studies: possible relevance to *in vitro* metabolism

Síbel Süzen,*[a] B. Tolga Demírcígíl,[b] Erdem Buyukbingol[a] and Síbel A. Özkan[c]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Eczacilik), Ankara University, 06100, Tandoğan, Ankara, Turkey. E-mail: sibel@pharmacy.ankara.edu.tr*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330, Etiler, Ankara, Turkey*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy (Eczacilik), Ankara University, 06100, Tandoğan, Ankara, Turkey*

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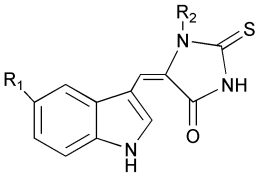
Some biologically important indolylthiohydantoin derivatives were investigated electroanalytically by voltammetric determination. Based on this study, a simple, rapid, sensitive and validated voltammetric method was developed for the determination of the indolylthiohydantoin derivatives that are readily oxidized at carbon-based electrodes. Due to the similarity between electrochemical and biological reactions it can be assumed that the oxidation mechanisms taking place at the electrode and in the body share similar principles. The oxidative behavior of the indole derivatives was studied as a function of pH at a glassy carbon electrode in different buffer media. The characteristics of the corresponding electrode reaction were discussed. The studied molecules are extensively metabolized *in vivo*, mainly through oxidative processes and we assume that the oxidation of the indolic compounds occurs on the nitrogen atom in the indole ring of the molecule. A linear response was obtained in the different media for all the compounds with a detection limit of 1.96×10^{-6} M, 2.32×10^{-6} M, 1.44×10^{-6} M and 7.10×10^{-7} M for compounds **1**, **2**, **3** and **4** respectively.

1. Introduction

The series of 5-(3′-indolyl)-2-thiohydantoin derivatives in Fig. 1 has been synthesized[1] and tested for the ability to inhibit the enzyme bovine lens aldose reductase which plays an important role in related diabetic disorders. Some of these derivatives also gave reasonable anti-cancer and anti-HIV activities.[2,3] Indole and hydantoin derivatives constitute an important class of therapeutic agents in pharmaceutical and medicinal chemistry.[4–8]

Indole and its derivatives are well known electroactive compounds that are readily oxidized at carbon-based electrodes, *e.g.* glassy carbon electrode.[9] Indole and its metabolites (*e.g.* tryptamine and serotonin) are of biochemical importance and analytical procedures have been developed for their determination in mixtures, based on liquid chromatography with electrochemical detection[10] and voltammetry.[11–13]



Compound	R_1	R_2
1	-H	-H
2	-H	$-COCH_3$
3	$-OCH_3$	$-COCH_3$
4	-CN	$-COCH_3$

Fig. 1 Voltammetrically analyzed derivatives of 5-(3′-indolyl)-2-thiohydantoin.

Electroanalytical techniques have also been shown to be excellent for the determination of pharmaceutical compounds in different matrices. Many of the active constituents of formulation, in contrast to excipients and endogenous substances of biological samples, can be readily oxidized or reduced. Voltammetric techniques in general and differential pulse voltammetry in particular are considered to be useful tools for the determination of indole derivatives.[11–15]

The use of electrochemical techniques for the determination of compounds of pharmaceutical interest is continually gaining in importance. The inherent sensitivity and high selectivity of the techniques allow very simple determinations, both in commercial samples and in body fluids in the presence of metabolites or impurities such as precursors used in the synthesis of these compounds.[16] The practical application of electrochemistry includes the determination of electrode oxidation mechanisms. Due to the known resemblance between electrochemical and biological reactions it can be assumed that the oxidation mechanisms taking place at the electrode and in the body share similar principles.

Cyclic voltammetry (CV) is perhaps the most effective and versatile electroanalytical technique available for the mechanistic study of redox systems. The obtained results from the redox properties of drugs and biomolecules might have profound effects on our understanding of their *in vivo* redox behavior or pharmaceutical activity.[17–19] Some metabolites can be differentiated from the parent compound since metabolism often proceeds through the addition or the modification of a substituent, and this will give rise to additional waves or to a shift of the main wave.

This paper reports a study of the electrochemical behavior of some indolylthiohydantoin derivatives. In order to understand the electrochemical process that occurs on the glassy carbon electrode, both pH and scan rate studies were carried out. The main purpose of this work was to establish experimental conditions for the electrochemical oxidation and determination of synthesized indole derivatives using a glassy carbon electrode. For the quantitative determination, differential pulse voltammetric methods were applied to the indole derivatives.

2. Experimental

The cyclic, linear sweep and differential pulse voltammetric (DPV) experiments at a stationary glassy carbon disc electrode were performed using a BAS 100 W Electrochemical Analyzer. A three-electrode cell system incorporating the glassy carbon disc electrode as working electrode (id = 3 mm, BAS): an Ag/AgCl (3 M KCl) reference electrode and a platinum-wire auxiliary electrode were also used. Before each measurements the glassy carbon electrode was polished manually with alumina (0.01 μm) in the presence of doubly distilled water on a smooth polishing cloth. DPV conditions were: pulse amplitude, 50 mV; pulse width 50 ms; sample width 17 ms; pulse period 200 ms; scan rate, 20 mV s^{-1}. A stock solution of all synthesized derivatives was prepared by direct dissolution in methanol. The working solutions of the indolylthiohydantoin derivatives were prepared by dilution of the stock solution and contained 20% methanol. Three different supporting electrolytes, namely sulfuric acid (0.1 M), phosphate buffer (as sodium salt) (0.2 M; pH 2.00–10.89) and acetate buffer (as sodium salt) (0.2 M; pH 3.50–5.80) were used. All the chemicals used were of reagent grade quality (Merck or Sigma and they were employed without further purification. All solutions were protected from light and were used within 24 h to avoid decomposition. All the studies were carried out at room temperature. The pH was measured using a pH meter Model 538 (WTW, Austria) using a glass electrode with an accuracy of ±0.05 pH.

3. Results and discussion

Differential pulse voltammetry is extremely useful for trace measurements of electroactive compounds in body fluids and tissues. Application of this technique allows discrimination of the unwanted capacity current from the required faradaic current. DPV curves are peak shaped and thus well suited to analytical purposes. In order to understand the electrochemical process occurring on the glassy carbon disc electrode, cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were carried out. All the compounds gave two or three anodic peaks or waves (Fig. 2) in all buffers and pH values. The CV, LSV and DPV behaviors of 1×10^{-4} M of all the compounds were examined with varying pH over a wide range of values from acidic (0.1 M H_2SO_4) to alkaline (pH 10.89). Different buffer solutions such as sulfuric acid, phosphate and acetate buffers were used.

Cyclic voltammetric measurements performed on different concentrations of all the compounds in the presence of 20% methanol show the irreversible nature of the peaks at the glassy carbon disc electrode in the range of scan rates comprised of between 10 and 150 mV s^{-1}. No cathodic peak or wave was observed (Fig. 2). The second peak of the indole ring was easily measurable. Hence, all subsequent work was based on measurement of the magnitude of this step. The possible mechanism of the first peak is also discussed at the end of this section.

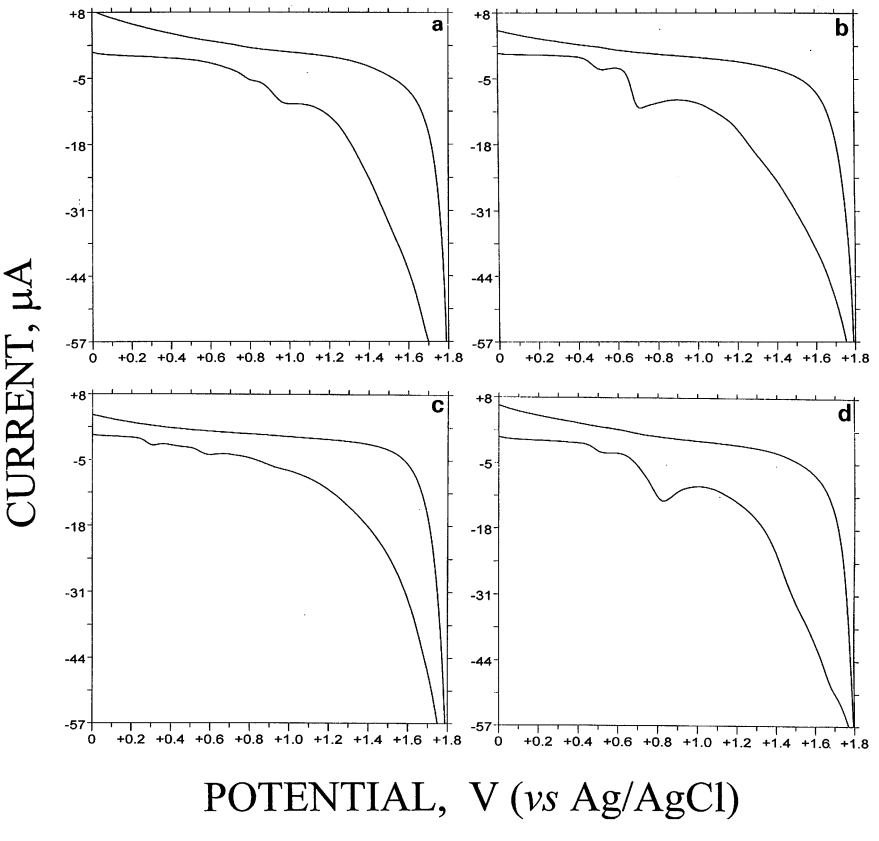


Fig. 2 Cyclic voltammograms of 1×10^{-4} M compound **1** (a) in 0.1 M H_2SO_4; compound **2** (b) in pH 2 phosphate buffer; compound **3** (c) in pH 4.71 phosphate buffer and compound **4** (d) in 0.1 M H_2SO_4in the presence of 20% methanol. Scan rate 100 mV s^{-1}.

Table 1 The results of the scan rates experiments on synthesized derivatives

Compound	Medium	Concentration/M	ΔE_p/ mV	Equation of V_v *vs.* I_p	Correl. coeff. (r)	Equation of log$V$ *vs.* logI_p	Correl. coeff. (r)
1	0.1 M H_2SO_4, 20% MeOH	4×10^{-5}	81	$y = 0.636x + 0.256$	0.995	$y = 0.504x + 0.187$	0.995
2	pH 2 phosphate buffer, 20% MeOH	4×10^{-5}	58	$y = 0.306x + 0.196$	0.995	$y = 0.575x + 0.690$	0.997
3	pH 4.71 phosphate buffer, 20% MeOH	6×10^{-5}	66	$y = 0.229x + 0.261$	0.994	$y = 0.586x + 0.871$	0.994
4	0.1 M H_2SO_4, 20% MeOH	4×10^{-5}	58	$y = 0.379x + 0.147$	0.995	$y = 0.579x + 0.591$	0.997

Table 2 Equations relating the logarithm of concentration *versus* logarithm of current on synthesized derivatives

Compound	Medium	Concentration range/M	Measured potential/V	Equation of log$c$ *vs.* log$I$	Correl. coeff. (r)
1	0.1 M H_2SO_4, 20% MeOH	1×10^{-5} to 1×10^{-4}	0.900	$\log I = 0.79\log C + 3.60$	0.989
2	pH 2 phosphate buffer, 20% MeOH	1×10^{-5} to 6×10^{-5}	0.850	$\log I = 0.76\log C + 3.44$	0.995
3	pH 4.71 phosphate buffer, 20% MeOH	1×10^{-5} to 6×10^{-5}	0.500	$\log I = 0.72\log C + 2.69$	0.976
4	0.1 M H_2SO_4, 20% MeOH	8×10^{-6} to 6×10^{-5}	0.750	$\log I = 0.81\log C + 3.87$	0.987

Different values of positive shifts in the peak potential were observed, which confirms the irreversibility of the process with a simultaneous increase in peak current. When the scan rate was increased, scan rate studies (Table 1) were then carried out to assess whether the process on glassy carbon electrodes occurred under diffusion or adsorption control. When the scan rate used was varied from 10 to 150 mV s^{-1}, a linear dependence of the peak intensity upon the square root of the scan rate was found, demonstrating diffusion behavior at all pH values and for all the compounds (Table 1).

The effect of scan rate on peak current was also examined under the above conditions with a plot of log$v$ *versus* log$I$, giving a straight line within the same scan rate range, which fitted the equations described in Table 1. The obtained slopes of log$v$ *versus* log$I$ equations are close to that theoretically expected (0.5) for an ideal reaction of solution species,[20] so in this case these processes had a diffusive component.

Tafel plots were obtained with a scan rate of 10 mV s^{-1} beginning from a steady-state potential as described for the scan rate studies and from the slope of the linear part $\alpha\eta$ was found to be 0.17, 0.40, 0.21 and 0.23 for the compounds **1**, **2**, **3**, **4** respectively. Tafel plots are frequently employed by physical electrochemists to determine exchange currents and transfer coefficients. The rate of the heterogeneous electron transfer relative to other controlling factors (*e.g.*, diffusion and coupled chemical reactions) is of critical importance to most experiments. These values together with the absence of cathodic voltammetry indicated the irreversibility of the oxidation reactions.

The peak potential of the oxidation process moves to less positive potentials with increasing investigated pH value for all the compounds. E_p–pH diagrams are convenient for summarizing equilibrium information about reactions that take place in solution. The variation of peak intensity and peak potential with pH at a concentration of 1×10^{-4} M for all the compounds were studied for the oxidation process by CV and DPV between pH 1.8 and 10.89. All obtained graphs were similar to each other. For this reason, only the E_p–pH graph obtained from the DPV results for compound **4** was given in Fig. 3. The plot of E_p *versus* pH showed one straight line, which can be expressed by the following equations between pH 1.8 and 10.89.

$E_p/V = 0.981 - 37.13pH$; $r = 0.994$ ($n = 12$) for compound **1**

$E_p/V = 0.774 - 56.49pH$; $r = 0.993$ ($n = 13$) for compound **2**

$E_p/V = 0.763 - 53.67pH$; $r = 0.991$ ($n = 13$) for compound **3**

$E_p/V = 0.845 - 57.46pH$; $r = 0.994$ ($n = 13$) for compound **4**

The peak potential was found to be pH dependent at all the pH values and in all the buffer solutions investigated. The linearity was observed over the pH range investigated giving a value less than 59 mV per pH unit for all the compounds. The pH effect on the oxidation mechanisms suggests a one electron–one proton for compounds **2**–**4**, but not for compound **1**. This might be explained in terms of a substituent effect in the hydantoin unit. These pH curves and values are in agreement with our previous study on the indole derivatives.[9]

The effect of pH on the peak current shows maxima in different supporting electrolytes and at different pH values. 0.1 M H_2SO_4 was chosen for compound **1** and **4**, phosphate buffer was chosen for compound **2** and pH 4.71 phosphate buffer was chosen for compound **3** to carry out the electroanalytical study. These media were selected for further study because they not only gave the highest peak current but also gave the best peak shape for the determination of these compounds using DPV techniques. The logarithm of current at different potentials for these compounds from the curves obtained in the selected medium having the concentration ranges described in Table 2 was plotted against the logarithm of concentration, and the lines are also shown in Table 2. The slope of these equations gives the reaction order. These kinetic parameters and reaction orders showed that there is a mechanism related to surface events for all the compounds, and reaction orders are close to first order kinetics (Table 2).

In order to develop a voltammetric methodology for determination of the drug, the differential pulse mode was selected

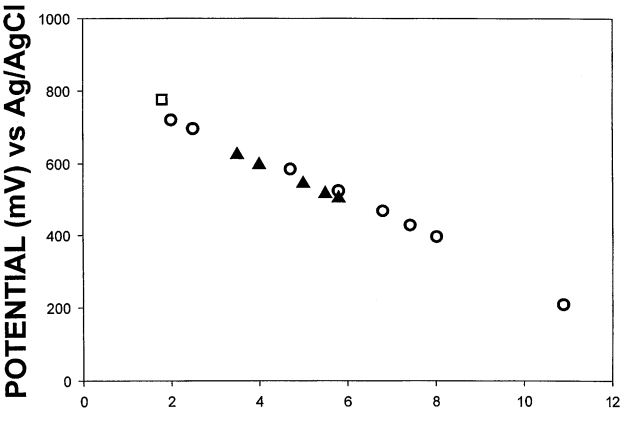


Fig. 3 Effects of pH on compound **4** peak potential. Sample concentration 1×10^{-4} M. 0.1 M H_2SO_4 (□), phosphate (○), acetate (▲) buffers

Table 3 Calibration parameters of synthesized derivatives

	Compound **1**	Compound **2**	Compound **3**	Compound **4**
Medium	0.1 M H_2SO_4 + 20% MeOH	pH = 2 phosphate buffer + 20% MeOH	pH = 4.71 phosphate buffer + 20% MeOH	0.1 M H_2SO_4 + 20% MeOH
Potential/V	0.904	0.456	0.556	0.812
Linearity range/M	1×10^{-5} to 1×10^{-4}	1×10^{-5} to 6×10^{-5}	1×10^{-5} to 6×10^{-5}	8×10^{-6} to 6×10^{-5}
Slope	16 514	64 286	5649,3	97 983
Intercept	0.4237	1.0286	0.3385	0.3169
Correlation coefficient	0.994	0.997	0.996	0.998
r.s.d. % of slope	0.624	1.052	1.609	1.036
r.s.d. % of intercept	1.768	0.683	1.698	1.492
Limit of detection/M	1.96×10^{-6}	2.32×10^{-6}	1.44×10^{-6}	7.10×10^{-7}

under the described conditions, as specified in the Experimental section. For quantitation the calibration graph method, with varying concentration range, was used for the DPV technique for all the compounds (Table 3). The calibration characteristics and related validation parameters are given in Table 3. Fig. 4 shows typical DPV curves obtained by analysis of compound **4**. This figure is given as an example of the DPV curve for all the compounds.

3.1 Mechanism

Voltammetric techniques are most suitable for investigating the redox properties of a new drug; this can give insights into its metabolic fate.[21] In some cases, it has been suggested by the research workers that the electrode mechanisms might mimic enzyme reactions; therefore, electrochemistry may be of value in the study of enzyme reactions in biological systems.[19] Cyclic voltammetric results from the redox properties of active compounds and biomolecules might have profound effects on our understanding of the redox mechanism related to the activity of the indolylthiohydantoin derivatives used in this study. Considering the voltammograms of these compounds, we may assume that the oxidation steps occur for all the compounds on the nitrogen atom in the indole ring, which is electroactive in both acidic and basic media, leading finally to hydroxylation of the benzene ring.[9,12,13,22] The second anodic oxidative behavior of compound **3** is like aromatic methoxy group oxidation, which was reported in the previous studies.[22–25] Our results revealed a good agreement with the redox mechanism postulated for similar compounds such as etodolac,[12] fluvastatin,[13] indole-3-propionamide derivatives,[9] and indolylthiohydantoin derivatives can be oxidized electrochemically by oxidation on the nitrogen atom on the indole ring. Studies of electrochemical oxidation of indole and some derivatives showed that the indole ring is most likely form dimers and trimers.[22,26,27] These are oxidized further to polymers in some cases. This is a possible mechanism for the indolylthiohydantoin derivatives also, since the data that we obtained are similar to the previous electrochemical studies.[22,26,27]

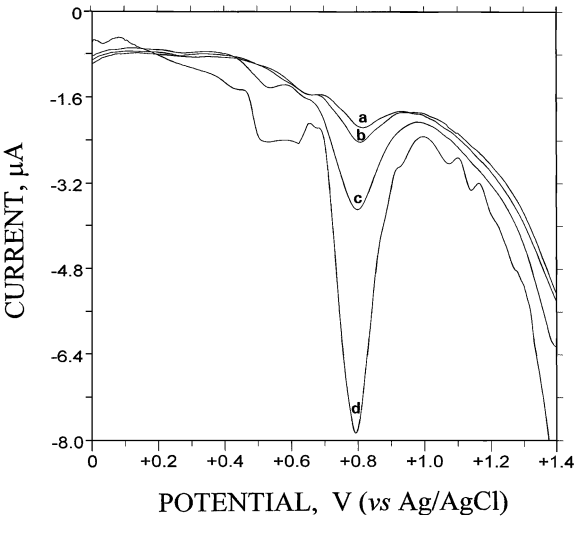


Fig. 4 Differential pulse voltammograms obtained for the determination of compound **4** in 0.1 M H_2SO_4 at different concentrations. (a) 8×10^{-6} M; (b) 1×10^{-5} M; (c) 2×10^{-5} M; (d) 6×10^{-5} M.

Additionally our results from compound **3** showed good agreement with the redox mechanism postulated for similar compounds *e.g.* formoterol fumarate, mefexamide, amisulpride, sulpride, and suggested that the anodic step investigated for compound **3** could be due to electrochemical oxidation of the methoxy group on the phenyl ring. Anodic oxidation of methoxybenzenes in aqueous acidic medium also leads to loss of the methoxy substituent, this time through *ipso*-substitution on the radical-cation by water. The results showed that, for compound **3**, because of the aromatic substitution of the indole ring, the peak height is lower than for the other compounds. Furthermore, the peak potential is found to be less positive, as Jennings *et al.*[26] found in their study. In these indole derivatives, the oxidation process was found to be a one electron transfer process.[28] This result can be seen from the E_p *versus* pH equations. A comparative study on 5-hydroxyindole and indole-3-acetic acid, regarding the indole oxidation step for all the compounds, and on anisole (phenyl methyl ether) regarding the methoxy group on the phenyl ring for compound **3**, was performed using CV at a glassy carbon electrode, as a function of pH, in order to identify the oxidation processes for all the compounds. Taking into account all studies performed, we suggest that the oxidation processes may be occurring on the nitrogen atom on the indole ring for all the investigated compounds and additionally, for the first oxidation process, it was suggested that this step may be occurring on the methoxy group. For the explanation of the redox mechanism the study was carried out with thiohydantoin and acetylthiohydantoin. There was no oxidation step detected for this compound. It is possible to say that the oxidation steps that were detected for all the compounds are not related to the thiohydantoin ring.

Voltammetric techniques in general and differential pulse voltammetry in particular are considered to be useful tools for the determination of indole derivatives.[12,14,15] In this study the measurements are two-dimensional, with the potential being related to qualitative properties and current related properties. Thus compounds may be selectively detected by these methods, namely cyclic and linear sweep and also differential pulse voltammetry. This is a rapid technique, which has been successfully applied to trace measurements of important pharmaceutical compounds such as indole.[9]

Acknowledgements

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