ELSEVIER

www.elsevier.com/locate/farmac

Il Farmaco 56 (2001) 835–840

IL FARMACO

Synthesis and analytical evaluation by voltammetric studies of some new indole-3-propionamide derivatives

Sibel Süzen [a,*], Zeynep Ateş-Alagöz [a], B. Tolga Demircigil [b], Sibel A. Özkan [c]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Eczacılık), Ankara University, 06100 Tandoğan, Ankara, Turkey*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara, Turkey*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy (Eczacılık), Ankara University, 06100 Tandoğan, Ankara, Turkey*

Received 20 January 2001; accepted 12 June 2001

Abstract

Some biologically important and melatonin-related indole-3-propionamide derivatives were synthesized. The compounds synthesized were analyzed and characterized first by NMR and mass spectrometry and then investigated by analytical voltammetric techniques. Based on this study a simple, rapid and sensitive voltammetric method was developed for the determination of the indole derivatives that are readily oxidized at the carbon-based electrodes. The oxidative behavior of the indole derivatives was studied as a function of pH at a glassy carbon electrode. The characteristics of the corresponding electrode reaction were discussed. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: Indole-3-propionic derivatives; Synthesis; Voltammetry; Analysis

1. Introduction

Indole derivatives such as melatonin, melatonin-related indole structures, and indole-3-propionic (IPA) derivatives constitute an important class of antioxidant agents [1–7] potentially useful against Alzheimer's disease. Indole and its derivatives are the well-known electro active compounds that are readily oxidized at carbon based electrodes, i.e. glassy carbon electrode and therefore analytical procedures, based on liquid chromatography with electrochemical detection [8] and voltammetry [9,10] have been developed for the determination of important indoles such as tryptamine and serotonin in complex mixtures.

Practical application of electrochemistry includes the determination of electrode oxidation mechanisms. Owing to the existing 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.

Voltammetric techniques in general, and differential pulse voltammetry in particular, are considered to be useful tools for the determination of indole derivatives [8,9,11].

This paper reports the electrochemical behavior of synthesized indole derivatives. In order to understand the electrochemical process that occurs on 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 method was applied to the indole derivatives.

2. Experimental

2.1. Synthesis and voltammetric measurements of indole-3-propionamide derivatives

2.1.1. Apparatus and chemicals

Uncorrected melting points (m.p. (dec.)) were determined with a Büchi SMP-20 apparatus. The ^{1}H NMR

* Corresponding author.
E-mail address: sibel@pharmacy.ankara.edu.tr (S. Süzen).

PII: S0014-827X(01)01150-8

spectra were determined with a Bruker GmbH DPX-400, 400 MHz using $CDCl_3$ as solvent and TMS as an internal standard (chemical shifts in δ, ppm). Mass spectra were determined on a VG Platform II spectrometer by using Electron Ionization (Ionization Energy 70 eV). Chromatography was carried out using the flash method and Merck silica gel 60 (230–400 mesh ASTM). All the amines and indole-3-propionic acid were purchased from Aldrich. Petroleum spirit 60–80 °C was used.

The electrochemical measurements were made with BAS 100 W electrochemical analyzer (Bioanalytical System, USA). Voltammetric measurements utilized a glassy carbon working electrode (ϕ 3 mm, BAS), a platinum wire auxiliary electrode and Ag/AgCl (NaCl 3M, BAS) reference electrode. Before each experiment, the glassy carbon electrode was polished manually with alumina (ϕ 0.01 μm,) in the presence of double distilled water on a smooth polishing cloth.

Stock solutions under voltammetric investigation were prepared daily by direct dissolution in methanol. The working solutions were prepared by the dilution of the stock solution with selected supporting electrolytes. Three different supporting electrolytes, namely sulfuric acid (0.5 M), phosphate buffer (0.2 M, pH 5–7) and Britton–Robinson buffer (0.04 M, pH 2–11) were prepared in double distilled water.

2.1.2. General procedure for the synthesis of indole-3-propionamide derivatives

Compound **2** (m.p. (dec.) 79 °C) was prepared by the esterification of compound **1** in dry methanol using gaseous HCl and refluxing at 70 °C for 15 h. The methanol was evaporated to dryness on a rotary evaporator and then the crude product was extracted by ethyl acetate and purified by column chromatography ($CHCl_3$–hexan). The yield was 65%. All the amide derivatives (**3a**–**3g**) (Fig. 1) were synthesized by using the appropriate amine and compound **2**.

Compound **2** (1 mol) was dissolved in the appropriate amine (5 ml) and the mixture was refluxed by heating under nitrogen. The reaction was followed by TLC until no starting material could be detected. The excess amine was evaporated to dryness on a rotary evaporator using 40 °C water bath. The crude product was then purified by column chromatography and characterized by NMR and mass analyses.

Compounds **3a** and **3b** were prepared as described in Ref. [10].

*2.1.2.1. Synthesis of 3-(N-pentyl-propanamide-3-yl)-indole (**3c**).* Compound **2** was treated with pentyl amine according the general procedure. ^{1}H NMR: δ 0.79 (3H, t, CH_3), 1.19 (2H, sext, CH_2–CH_3), 1.26 (2H, m, CH_2–CH_2–CH_3), 1.30 (2H, m, NH–CH_2–CH_2), 2.49 (2H, t, NH–CH_2), 3.05 (2H, t, CH_2–CO), 3.10 (2H, t, CH_2–CH_2–CO), 5.25 (1H, brs, NH–CO), 6.95 (1H, d, indol-H_2), 7.05 (1H, t, indol-H_6), 7.13 (1H, t, indol-H_7), 7.28 (1H, d, indol-H_5), 7.53 (1H, d, indol-H_8), 7.74 (1H, brs, indol-NH). EI MS; m/z (%): 259 (12.3) $[M+1]^{+\bullet}$, 258 (56.4) $[M]^{+\bullet}$, 144 (77.2), 142 (100), 130 (100), 114 (29.4), 101 (13.1), 90 (5.3), 88 (15.9), 84 (20.6), 82 (28.2), 71 (38.8), 58 (47.8), 54 (40.3), 43 (53.3), 41 (60.7), 27 (36.8).

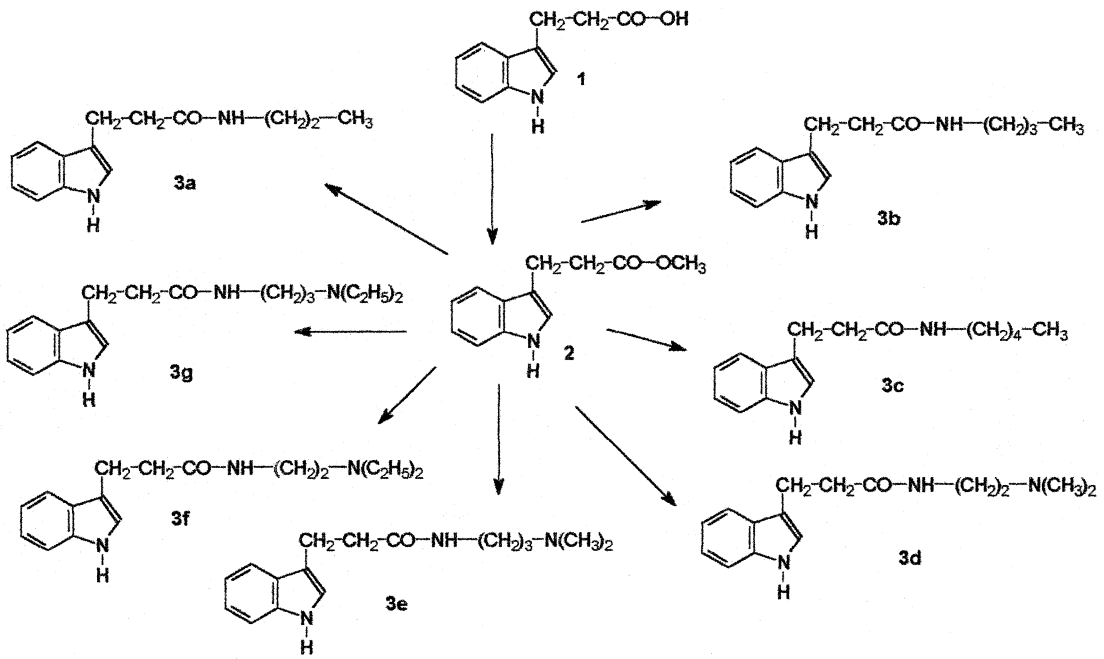
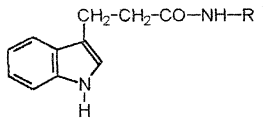


Fig. 1. Synthesis of the indol-3-propionamide derivatives.

Table 1
Indole 3-propionamide derivatives



No	R	Time (day)	Yield (%)	Eluent	m.p (°C)	Recryst. Solvent	Formula
3a	-$(CH_2)_2$-CH_3	3	16	$CHCl_3$	86	$CHCl_3$/Hx*	$C_{14}H_{18}N_2O$
3b	-$(CH_2)_3$-CH_3	7	53	Pet.sp/EtAc	65	$CHCl_3$/Hx	$C_{15}H_{20}N_2O$
3c	-$(CH_2)_4$-CH_3	6	45	$CHCl_3$	62	$CHCl_3$/Hx	$C_{16}H_{22}N_2O$
3d	-$(CH_2)_2$-$N(CH_3)_2$	5	18	$CHCl_3$/isopr	76	$CHCl_3$/isopr	$C_{15}H_{21}N_3O$
3e	-$(CH_2)_3$-$N(CH_3)_2$	5	19	$CHCl_3$/isopr:/NH_3	114	$CHCl_3$/isopr/NH_3	$C_{16}H_{23}N_3O$
3f	-$(CH_2)_2$-$N(CH_2$-$CH_3)_2$	5	54	$CHCl_3$/isopr	Oily	$CHCl_3$/isopr	$C_{17}H_{25}N_3O$
3g	-$(CH_2)_3$-$N(CH_2$-$CH_3)_2$	5	43	$CHCl_3$/isopr/NH_3	Oily	$CHCl_3$/isopr/NH_3	$C_{18}H_{27}N_3O$

* *n*-Hexane.

*2.1.2.2. Synthesis of 3-[N′-(N,N-dimethylaminoethyl)-propanamide-3-yl]-indole (**3d**).* Compound **2** was treated with 2-dimethylamino-1-ethyl amine according the general procedure. ^{1}H NMR: δ 2.06 (6H, s, $2CH_3$), 2.48 (2H, t, CH_2–N), 2.51 (2H, t, NH–CH_2), 3.05 (2H, t, CH_2–CO), 3.10 (2H, q, CH_2–CH_2–CO), 5.97 (1H, brs, NH–CO), 6.94 (1H, d, indol-H_2), 7.04 (1H, t, indol-H_6), 7.11 (1H, t, indol-H_7), 7.26 (1H, d, indol-H_5), 7.53 (1H, d, indol-H_8), 8.0 (1H, brs, indol-NH). EI MS; m/z (%): 260 (5.45) $[M+1]^{+\bullet}$, 259 (6.5) $[M]^{+\bullet}$, 188 (3.1), 144 (3.0), 143 (6.0), 130 (17.3), 115 (4.7), 105 (3.6), 103 (2.4), 89 (2.1), 84 (4.2), 82 (3.2), 77 (5.2), 72 (19.5), 71 (53.1), 58 (100), 42 (9.8), 32 (27.8).

*2.1.2.3. Synthesis of 3-[N′-(N,N-dimethylaminopropyl)-propanamide-3-yl]-indole (**3e**).* Compound **2** was treated with 3-dimethylamino-1-propylamine according the general procedure. ^{1}H NMR: δ 1.19 (2H, m, CH_2–CH_2–N), 2.16 (6H, s, $2CH_3$), 2.48 (2H, t, CH_2–N), 2.50 (2H, t, NH–CH_2), 3.04 (2H, t, CH_2–CO), 3.20 (2H, q, CH_2–CH_2–CO), 6.65 (1H, brs, NH–CO), 6.94 (1H, d, indol-H_2), 7.04 (1H, t, indol-H_6), 7.11 (1H, t, indol-H_7), 7.27 (1H, d, indol-H_5), 7.52 (1H, d, indol-H_8), 8.13 (1H, brs, indol-NH). EI MS; m/z (%): 273 (14.5) $[M+1]^{+\bullet}$, 272 (32.4) $[M]^{+\bullet}$, 142 (39.1), 130 (18.3), 128 (52.1), 114 (23.2), 101 (19.1), 97 (6.0), 89 (12.3), 83 (71.3), 75 (8.2), 72 (13.1), 69 (32.5), 57 (100), 42 (100), 34 (10.0), 30 (18.8), 27 (32.8).

*2.1.2.4. Synthesis of 3-[N′-(N,N-diethylaminoethyl)-propanamide-3-yl]-indole (**3f**).* Compound **2** was treated with 2-diethylamino-1-ethyl amine according the general procedure. ^{1}H NMR: δ 0.85 (6H, t, $2CH_3$), 2.33–2.39 (6H, m, CH_2–$N(CH_2)_2$), 2.50 (2H, t, NH–CH_2), 3.04 (2H, t, CH_2–CO), 3.18 (2H, q, CH_2–CH_2–CO), 6.02 (1H, brs, NH–CO), 6.92 (1H, d, indol-H_2), 7.03 (1H, t, indol-H_6), 7.10 (1H, t, indol-H_7), 7.25 (1H, d, indol-H_5), 7.52 (1H, d, indol-H_8), 8.23 (1H, brs, indol-NH). EI MS; m/z (%): 288 (13.0) $[M+1]^{+\bullet}$, 287 (5.5) $[M]^{+\bullet}$, 144 (1.5), 143 (2.9), 130 (9.7), 115 (2.0), 100 (5.6), 99 (13.0), 86 (100), 72 (1.5), 71 (1.5), 58 (8.9), 42 (2.8), 34 (10.0), 30 (18.8), 27 (32.8).

*2.1.2.5. Synthesis of 3-[N′-(N,N-diethylaminopropyl)-propanamide-3-yl]-indole (**3g**).* Compound **2** was treated with 3-diethylamino-1-propyl amine according the general procedure. ^{1}H NMR: δ 0.86 (6H, t, $2CH_3$), 1.46 (2H, m, CH_2–CH_2–N), 2.30–2.36 (6H, m, CH_2–$N(CH_2)_2$), 2.45 (2H, t, NH–CH_2), 3.04 (2H, t, CH_2–CO), 3.22 (2H, q, CH_2–CH_2–CO), 6.91 (1H, brs, indol-H_2), 7.02 (1H, t, indol-H_6), 7.09 (1H, t, indol-H_7), 7.25 (1H, d, indol-H_5), 7.51 (1H, d, indol-H_8), 8.41 (1H, brs, indol-NH). CO–N*H* not observed. EI MS; m/z (%): 302 (7.1) $[M+1]^{+\bullet}$, 301 (8.0) $[M]^{+\bullet}$, 272 (6.7), 229 (6.6), 144 (3.8), 142 (17.9), 130 (18.3), 129 (33.4), 114 (12.0), 111 (8.4), 99 (12.1), 85 (100), 71 (48.6), 57 (34.0), 43 (13.9), 41 (33.5), 30 (21.0), 27 (29.2).

3. Results and discussion

All synthesized compounds were analytically characterized and are represented by the general formula in Table 1.

The voltammetric oxidation of the synthesized indole derivatives at the glassy carbon electrode was investi-

gated in the pH range of 1.5–11.0 and different supporting electrolytes. Current–voltage curves of all the investigated indole derivatives showed one well-defined oxidation peak at pH = or > 4. It was observed that this single peak split into two overlapping peaks as the acidity was increased (Fig. 2).

The effects of potential scan rate between 10 and 1000 mV s^{-1} on the peak potential and the peak current of all indole derivatives (**3a**–**3g**) were evaluated. The linear increase in the oxidation peak current with the square root of the scan rate with similar slopes and correlation coefficients, showed the diffusion control process (Table 2). Positive shift in the peak potential was observed, which also confirms the irreversibility of the process, with a simultaneous increase in diffusion current when the scan rate was increased. A plot of

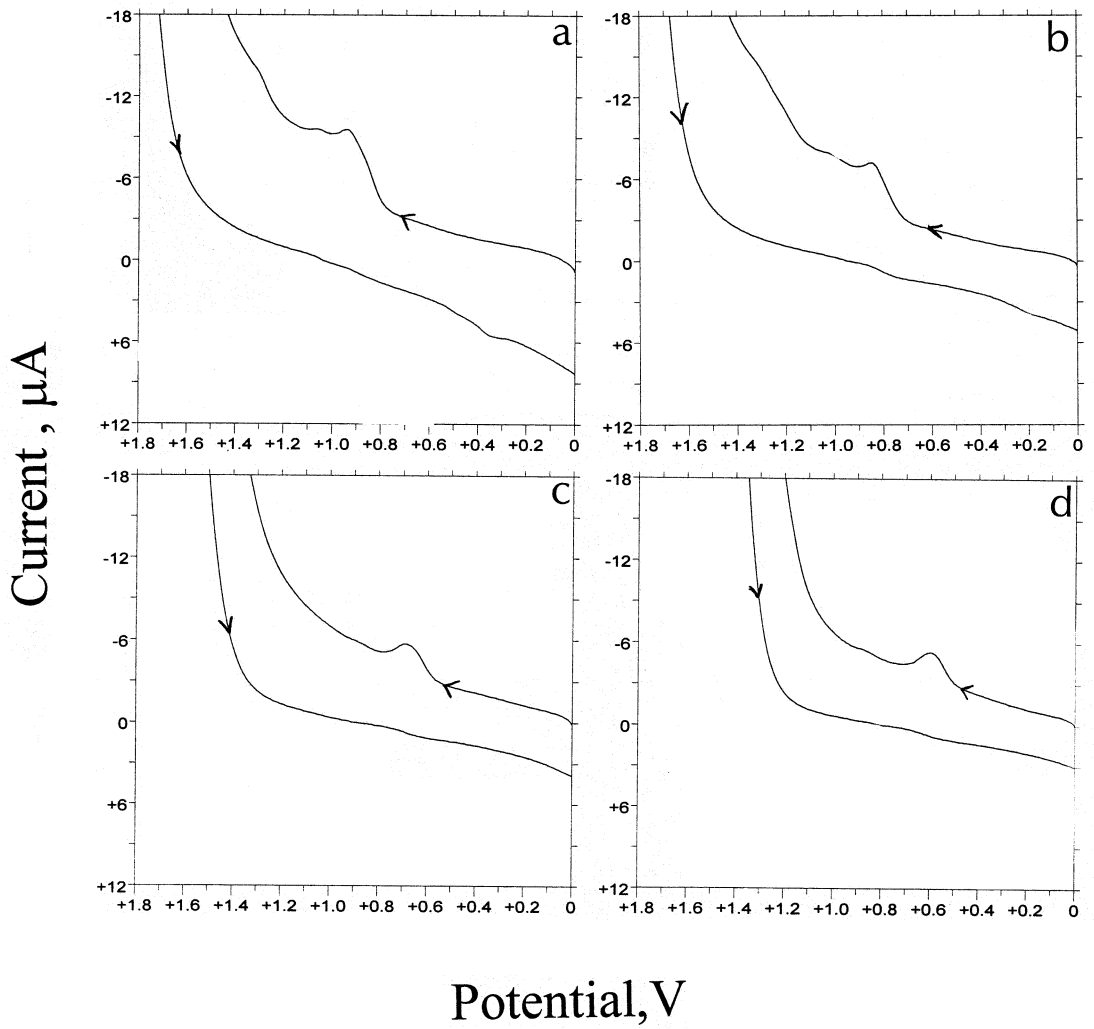


Fig. 2. Cyclic voltammogram of 1×10^{-4} M compound **3a** in Britton–Robinson buffer (20% MeOH); pH values $a = 2$, $b = 4$, $c = 7$, $d = 9$; scan rate 100 mV s^{-1}.

Table 2
Square root of the scan rate–peak current and logarithm of peak current–logarithm of scan rate results of synthesized indole derivatives

Samples	Equation of $\sqrt{v}-I_p$	Correlation coefficient	Equation of $\log v - \log I_p$	Correlation coefficient
3a	$y = 0.368x - 0.339$	$r = 0.993$	$y = 0.513x - 0.504$	$r = 0.995$
3b	$y = 0.322x - 0.476$	$r = 0.995$	$y = 0.510x - 0.536$	$r = 0.997$
3c	$y = 0.431x - 0.470$	$r = 0.994$	$y = 0.552x - 0.530$	$r = 0.998$
3d	$y = 0.352x + 0.165$	$r = 0.999$	$y = 0.483x - 0.396$	$r = 0.999$
3e	$y = 0.305x + 0.374$	$r = 0.995$	$y = 0.425x - 0.308$	$r = 0.994$
3f	$y = 0.793x - 1.180$	$r = 0.997$	$y = 0.608x - 0.404$	$r = 0.997$
3g	$y = 1.218x - 3.962$	$r = 0.991$	$y = 0.699x - 0.530$	$r = 0.999$

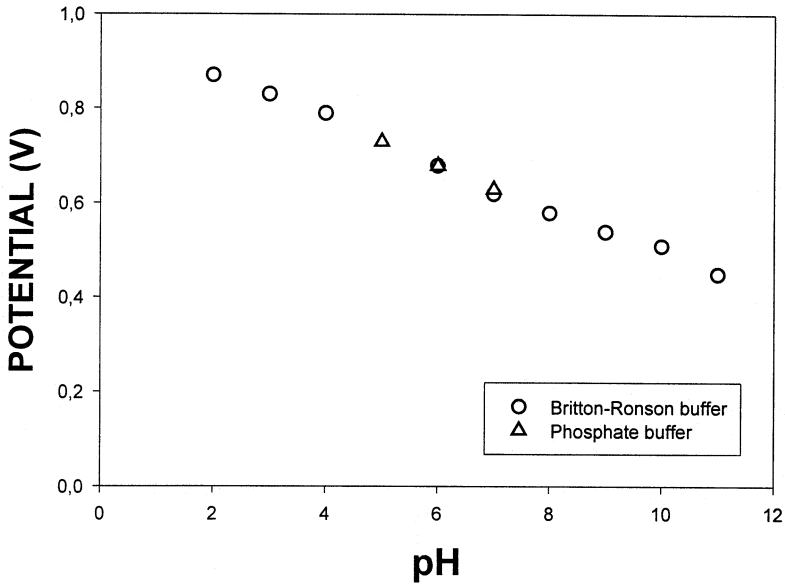


Fig. 3. Effects of the pH on compound **3a** peak potential.

Table 3
Calibration characteristics of synthesized indole derivatives

Sample	Medium	Linearity range (M)	Equation	Correlation coefficient	Measured potential (V)
3a	pH 2 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–8×10^{-5}	$y=26333x-0.1246$	$r=0.998$	0.87
3b	PH 5 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–1×10^{-4}	$y=19322x-0.1019$	$r=0.998$	0.73
3c	pH 3 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–1×10^{-4}	$y=24811x-0.1119$	$r=0.997$	0.83
3d	pH 10 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–1×10^{-4}	$y=21139x+0.0411$	$r=0.998$	0.51
3e	pH 2 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–8×10^{-5}	$y=21054x-0.056$	$r=0.996$	0.87
3f	pH 5 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–8×10^{-5}	$y=36842x+0.287$	$r=0.987$	0.73
3g	pH 10 Britton–Robinson buffer (%20 MeOH)	1×10^{-5}–1×10^{-4}	$y=31968x+0.197$	$r=0.998$	0.51

logarithm of peak current versus the logarithm of the scan rate gave a straight line with similar slopes and correlation coefficients for all the indole derivatives. Slopes of 0.50 and 1.0 are expected for ideal reactions of solution and surface species, respectively [12].

The peak potential shifted towards negative potentials with an increase in pH. Similar peak potential–pH curves were obtained for all the indole derivatives. Thus, compound **3a** is given in Fig. 3 as an example.

The quantitative evaluation is based on the dependence of the peak current on indole derivatives concentrations. The application of the differential pulse waveform (pulse amplitude: 50 mV) yielded voltammograms in which the peak currents were greater than those obtained by cyclic voltammetry. A pulse interval of 0.25 s gave rise to the sharpest and symmetrical peak shape. The optimum scan rate was found to be 20 $mV\,s^{-1}$ for all samples. For analytical purposes, best response (with regard to peak current sensitivity and morphology) was obtained with Britton–Robinson buffer at different pH (Table 3). Different linear calibration curves were obtained for indole derivatives. The characteristics of the calibration plots are listed in Table 3.

Fig. 4 shows a typical differential pulse voltammogram obtained follow by analysis of **3a** derivative. This figure is given as example of DPV curve for all the synthesized indole derivatives.

Voltammetric techniques are most suitable to investigate the redox properties of a new drug, this can give insights into its metabolic fate [13]. Some metabolites can be differentiated from the parent drug since metabolization often proceeds through the addition or the modification of a substituent, this will give rise to additional waves or to a shift of the main wave. Such situation may allow the simultaneous determination of the initial compound and the metabolized drug. Cyclic voltammetry has been used in studying the redox mechanism [14] that is related to antioxidant activity of the indole derivatives synthesized in this study. The results

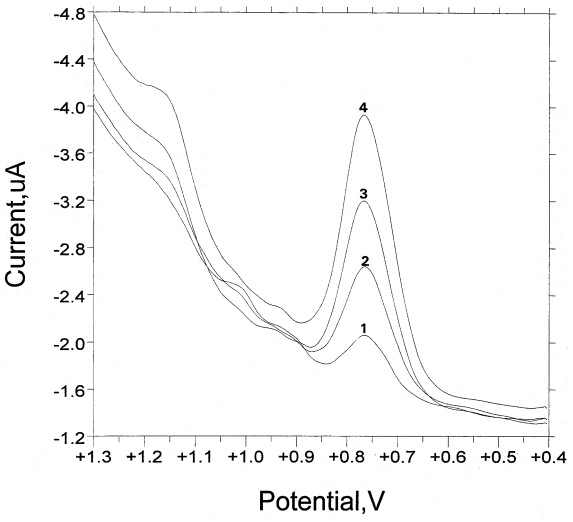


Fig. 4. Differential pulse voltammograms of different concentrations of compound **3a** in Britton–Robinson buffer pH 2: (1) 1×10^{-5} M; (2) 4×10^{-5}; (3) 6×10^{-5}; (4) 8×10^{-5} M.

showed that the compounds might have profound effects on our understanding of their in vivo redox processes and pharmaceutical activity. Indole-3-propionamide derivatives like melatonin have a heterocyclic aromatic ring structure with high resonance stability, which led us to suspect antioxidant activity in our compounds. Compound **3a**–**g** are extensively metabolized in vivo, mainly through oxidative processes we assume that the oxidation step of indolic compounds is located on the nitrogen atom in the indole ring of the molecule, which is electro active in both acidic and basic media leading finally to hydroxylation of benzene ring.

The electrochemical findings of the compounds show that it would be worth to synthesizing more indole-3-propionamide derivatives to investigate their free radical scavenger activities and analyze them with further tests. Biological evaluation of the synthesized compounds is under investigation.

Acknowledgements

This work was supported by the Ankara University Research and Development Grant 99-03-00-03.

References

[1] P.K. Li, P.A. Witt-Enderby, Melatonin receptors as potential targets for drug discovery, Drugs Future 25 (2000) 945–957.
[2] S. Süzen, E. Buyukbingol, Farmaco 54 (2000) 246–248.
[3] Z. Ateş-Alagöz, S. Süzen, Oxidative damage in the central nervous system and protection by melatonin. J. Fac. Pharm. Ank. Univ. (2001) in press.
[4] D. Avery, M. Lenz, C. Landis, Guidelines for prescribing melatonin, Ann. Med. 30 (1998) 122–130.
[5] Y.I. Chyan, B. Poeggeler, R.A. Omar, D.G. Chain, B. Frangione, M.A. Ghisc, Potent neuroprotective properties against the Alzheimer beta-amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid, J. Biol. Chem. 274 (1999) 21937–21942.
[6] A. Bast, G.R. Haenen, Regulation of lipid peroxidation by glutathione and lipoic acid: involvement of liver microsomal vitamin E free radical reductase, Adv. Exp. Med. Biol. 264 (1990) 111–116.
[7] R.J. Reiter, The role of the Neuro hormone melatonin as a buffer against macromolecular oxidative damage, Neurochem. Int. 27 (1995) 453–460.
[8] S. Yılmaz, B. Uslu, S.A. Özkan, Anodic oxidation of etodolac and its square wave and differential pulse voltammetric determination in pharmaceuticals and human serum, Talanta 54 (2001) 351–360.
[9] J.M.P. Carrazon, A.J.R. Garcia, L.M.P. Diez, Voltammetric determination of indole in micellar and emulsified media, J. Electroanal. Chem. 234 (1987) 175–183.
[10] J.M.P. Carrazon, A.J.R. Garcia, L.M.P. Diez, Determination of indole in shrimps by differential-pulse voltammetry in an emulsified medium, Analyst 115 (1990) 869–871.
[11] N.E. Zoulis, D.P. Nikoleis, C.E. Efstathiou, Pre-concentration of indolic compounds ay a carbon paste electrode and indirect determination of L-tryptophan in serum by adsorptive stripping voltammetry, Analyst 115 (1990) 291–295.
[12] Z. Şentürk, S.A. Özkan, Y. Özkan, H.Y. Aboul-Enein, Voltammetric investigation of oxidation of zuclopenthiol and application to its determination in dosage forms and in drug dissolution studies, J. Pharm. Biomed. Anal. 22 (2000) 315–323.
[13] J.C. Vire, J.M. Kauffmann, Trends in electrochemistry in drug analysis, Curr. Top. Electrochem. 3 (1994) 493–515.
[14] J. Wang, Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine, VCH, New York, 1988, pp. 20.