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Dedicated to Professor Liviu Literat
On the occasion of his 85th birthday

ELECTROCHEMICAL BEHAVIOR OF SOME CATECHOLAMINES INVESTIGATED BY CYCLIC AND SQUARE-WAVE VOLTAMMETRY

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ABSTRACT. Electrochemical measurements of the oxidation of organic compounds can be helpful to understand how these compounds are metabolized by living organisms. Cyclic and square-wave voltammetry were used in view to estimate the electrochemical parameters of redox processes involving catecholamines compounds (isoprenaline, epinephrine, levonordefrin, norepinephrine, dopamine, d-DOPA and carbidopa). Redox processes related to catecholamines are quasi-reversible diffusion controlled reactions at a graphite electrode. According to the decreasing of the oxidation potential, the capacity to act as antioxidant of different catecholamines increases in the order: norepinephrine < levonordefrin < epinephrine < isoprenaline or carbidopa < d-DOPA < dopamine. Both series are influenced by the position of –OH and –NH_2 in *para*-alkyl substituents.

Keywords: *catecholamine, cyclic voltammetry, square wave voltammetry, antioxidant capacity.*

INTRODUCTION

Free radicals are highly reactive molecules generated by redox reactions in food, chemicals and even in living systems. Highly reactive free radicals and reactive oxygen species (ROS) present in biological systems can (i) oxidize nucleic acids, proteins and lipids, as a part of normal cell metabolism or (ii)

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act in the course of free-radical-mediated diseases such as: *Diabetes mellitus*, cancer, rheumatoid arthritis, renal, cardiovascular, inflammatory, infectious and neurological diseases [1]. Biologically important ROS (including superoxide anion ($O_2^{\bullet -}$), singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hydroxyl radical ($OH^\bullet$), peroxynitrite ($ONOO^-$), peroxyl ($ROO^\bullet$) and alkoxyl ($RO^\bullet$)) represent the most important class of free radicals generated in living system. These compounds are eliminated from the body by their interaction with antioxidants. Antioxidants significantly delay or avoid the oxidation of easy oxidable substrates.

The antioxidants are classified in (i) low-molecular weight compounds (e.g. vitamins E and C, β-carotene, glutathione, uric acid, bilirubin etc.) and (ii) high-molecular weight compounds (e.g. protein albumin, ceruloplasmin, ferritin, superoxide dismutase, glutathione peroxidase, catalase etc.).

The total antioxidant capacity (TAC) is the expression of the overall activity of antioxidants and antioxidant enzymes. The determination of antioxidant capacity has been widely investigated by traditional analytical techniques, such as: spectrophotometry, fluorescence or chromatography. In the last years, electrochemical methods have opened new possibilities to investigate the antioxidant capacity.

According to their polyphenolic nature, and due to the presence of conjugate benzene rings and hydroxyl groups, catecholamines are a group of compounds exhibiting good natural antioxidant properties, by reacting rapidly with ROS. [2]. Compounds having antioxidant capacity are helpful in reducing and preventing damage from free radical reactions because of their ability to donate electrons which neutralize the radical without forming another [3]. Thus, it is performed the decrease of ROS toxic action and is prevented the chain reactions involving free radicals in organism.

The aim of this work was to establish a relationship between chemical structure, electrochemical parameters (formal standard potential) and antioxidant activity of catecholamines (isoprenaline, epinephrine, levonordefrin, norepinephrine, dopamine, d-DOPA and carbidopa) using different electrochemical techniques (cyclic voltammetry and square-wave voltammetry). The influence of the experimental conditions (scan rate and frequency) on the electrochemical behavior of the investigated compounds on graphite electrode allowed to understand the kinetics of the involved redox process.

RESULTS AND DISCUSSION

Electrochemical behavior of catecholamines

The electrochemical redox reactions of catecholamines at the graphite electrode were studied by both cyclic voltammetry (CV) and square-wave voltammetry (SWV). Selected cyclic voltammograms recorded in the 10^{-3} M solution of catecholamines in the supporting electrolyte are presented in figures 1 A, B.

Within the potential range where the redox behavior of catecholamines is visible, the supporting electrolyte shows no characteristic peaks, besides charging the electrical double layer (thin lines in figures 1A, B).

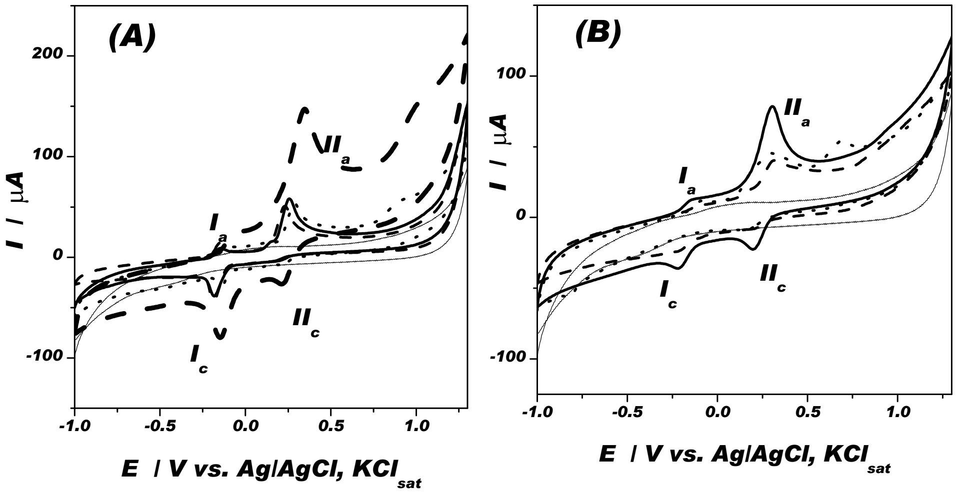
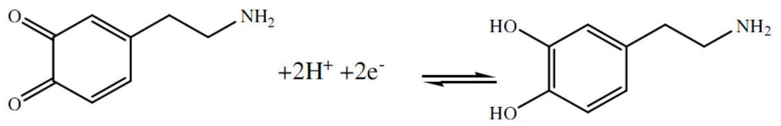


Figure 1. Cyclic voltammograms of 10^{-3} M isoprenaline (solid thick line), epinephrine (dash line), levonordeprine (dot line), norepinephrine (thick dash line) (**A**) and dopamine (solid thick line), d-DOPA (dash line), carbidopa (dot line) (**B**) at graphite electrode. Experimental conditions: electrolyte, 0.1 M phosphate buffer solution (pH 7) (solid thin lines); starting potential, -1 V *vs.* Ag/AgCl,KCl_{sat}; scan rate, 50 mV/s.

Cyclic voltammograms recorded in the catecholamines solutions (Figures 1A, B) show two pairs of peaks which charac-terize a global redox process of this compounds involving two protons, two-electron, as described by the typical reaction (1) for dopamine [4, 5].

It is worth to mention, that although, the current intensity of either the first, or the second anodic or cathodic peak is much smaller than the mirror corresponding one, this behavior prove a quasi-reversible redox behavior of the investi-gated catecholamine compounds.

The SWV investi-gation technique was chosen for the study of the catecholamines because it is the most advanced and most sophisticated method from the pulse voltammetric tech-niques and exhibits the advantages of a large speed and high sensitivity [6].



(1)

Figure 2 summarize the SW voltammograms obtained for the different catecholamines.

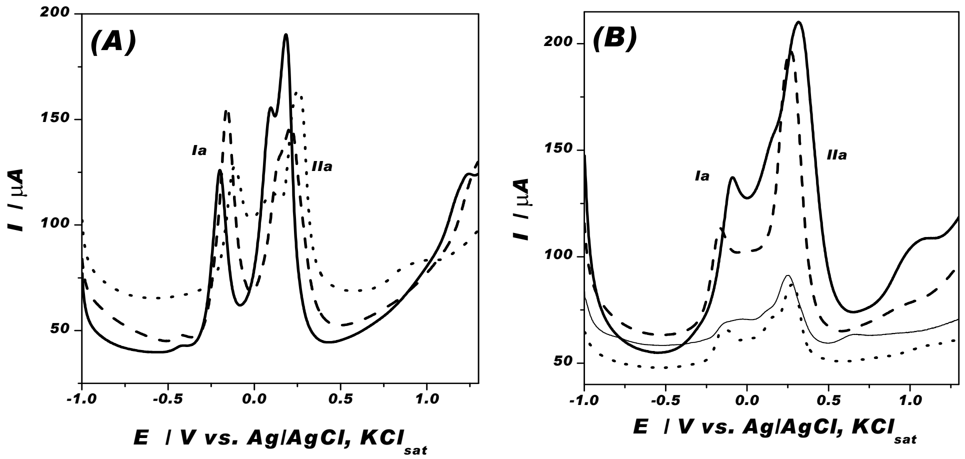


Figure 2. Square-wave voltammograms of 10^{-3} M isoprenaline (solid thick line), epinephrine (dash line), levonordefrine (dot line) (**A**) and norepinephrine (solid thick line), dopamine (dash line), d-DOPA (dot line) and carbidopa (solid thin line) (**B**) at graphite electrode. Experimental conditions: electrolyte, 0.1 M phosphate buffer (pH 7); initial potential, -1 V *vs.* Ag/AgCl,KCl_{sat}; step potential, 0.002 V; amplitude, 50 mV; frequency 10 s^{-1}.

The oxidation potentials (E_{pa}) and the estimated formal standard potential ($E^{0'}$) of the first and second anodic peaks for the studied catecholamines, by both SWV and CV techniques, are reported comparatively in table 1. The data of the table shows that in all cases, the peak potential for the anodic peak I or II is shifted to negative values with at least 50 mV in the SWV, than in the CV technique, because of the different experimental conditions.

Also, it is well-known that lower oxidation potentials correspond to a higher ability of a compound to donate an electron and therefore to act as an antioxidant. It is important to underline that the oxidation potential, because of its thermodynamic nature, does not give any information about the reaction rate. Although the oxidation process takes place at the two -OH groups linked to the benzene ring as described in the above reaction, it is probably that the presence of -OH, = O or $-CH_3$ groups on the ramification branch promotes or not this process.

Table 1. Electrochemical parameters for the studied catechoamines. Experimental conditions: see figures 1 and 2.

Catechoaminic compounds	**CV, v = 50 mV/s**				**SWV, f = 10 s^{-1}**	
	Peak Ia		**Peak IIa**		**Peak Ia**	**Peak IIa**
	E_{pa} /	**$E^{0\prime}$ /**	**E_{pa} /**	**$E^{0\prime}$ /**	**E_{pa} /**	**E_{pa} /**
	V *vs*. ER					
isoprenaline	-0.150	-0.168	0.255	0.220	-0.201	0.184
epinephrine	-0.146	-0.166	0.230	0.196	-0.159	0.212
levonordefrin	-0.097	-0.119	0.289	0.255	-0.117	0.254
norepinephrine	-0.067	-0.107	0.343	0.285	-0.098	0.315
dopamine	-0.141	-0.178	0.284	0.243	-0.171	0.266
d-DOPA	-0.107	-0.161	0.313	0.260	-0.140	0.275
carbidopa	0.289	0.231	0.685	-	-0.131	0.254

$E^{0\prime}$ is the mean value of anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}).

The catecholamine derivatives with electron-donating *para*-alkyl substituents were oxidized more easily depending on the position of the –OH and –NH_2 groups on the ramification branch. The effect of a conjugated double bond (=O) was, moreover, well evidenced, by the lowest oxidation potentials of d-DOPA and carbidopa comparing with dopamine [7].

Analyzing the influence of the intensity of the inductive/electromeric effect of substituents on the N atom in position 1 of the branches of the benzenic ring it can be concluded:

- For levonordefrin and norepinephrine, the substituent H does not induce any inductive effect and consequently the antioxidant behavior is similarly, confirmed by the quasi - identical values of the standard formal potential ($E^{\circ\prime}_{\text{levonordefrin}}$ = -0.119 V and $E^{\circ\prime}_{\text{norepinephrine}}$ = -0.107 V).
- For epinephrine and isoprenaline the -CH_3 (methyl) and -$CH(CH_3)_2$ (isopropyl) groups induce a positive inductive effect (+ I) increasing in the order methyl < isopropyl, which is neglected for antioxidant capacity because of the similitude of the standard formal potential values ($E^{\circ\prime}_{\text{epinephrine}}$ = -0.166 V and $E^{\circ\prime}_{\text{isoprenaline}}$ = -0.168 V), probably because is too far away from the oxidation site.
- Although the difference between dopamine and d-DOPA is the presence of a -COOH group which induces an electromeric effect overlapped on the inductive one (due to -NH_2 group), their antioxidant capacity were not significantly different (close $E^{\circ\prime}$ values, $E^{\circ\prime}_{\text{dopamine}}$ = - 0.178 V, $E^{\circ\prime}_{\text{d-dopa}}$ = - 0.161 V).
- Significant difference is observed between the antioxidant capacity of d-DOPA and carbidopa ($E^{\circ\prime}_{\text{d-dopa}}$ = -0.161 V and $E^{\circ\prime}_{\text{carbidopa}}$ = 0.231 V). The presence of C-NH-NH_2 having electromeric effect (+ E) lower than that of the -COOH group of carbidopa could be considered negligible in relation to the electromeric effect of -COOH group of d-DOPA.

Influence of scan rate and frequency

In order to establish the type of control corresponding to the studied redox reactions, cyclic voltammetry experiments were carried out at various scan rates. At pH 7, from cyclic voltammograms of differents catecholamines, the peak current intensities of peak **I** depend linearly on the square root of the scan rate (figure 3), how it was expected for diffusion controlled redox process.

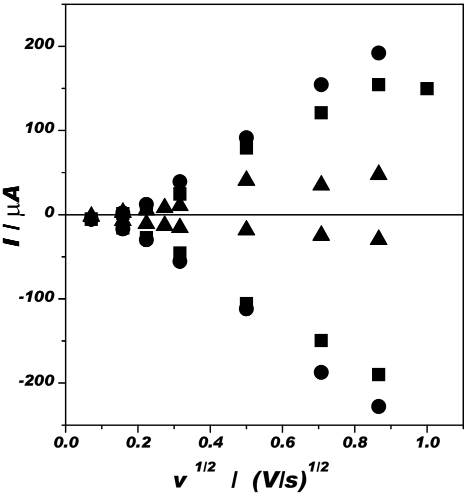


Figure 3. Influence of the scan rate on the peak current intensity of peak I for 10^{-3} M isoprenaline (■) epinephrine (●) and levonordefrin (▲). Experimental conditions: see figure 1.

This behavior is associated especially with a fast electron transfer in studied catecholamines. Also, from figure 3 could be seen, the ratio of both anodic and cathodic peak current is constant at different scan rates, confirmind the quasi-reversible redox electrode process [4].

Figure 4 shows the dependence of the ratio $I_pf^{-1/2}$ on $f^{1/2}$ for the oxidation of 10^{-3} M isoprenaline at graphite electrode. Note that the ratio $I_pf^{-1/2}$ corresponds to the dimensionless net-peak current Φ ($\Phi = I_p(nFS\,c^*_{ox})^{-1}(Df)^{-1/2}$) and the $f^{1/2}$ to the resistance parameter q ($q = R_\Omega(n^2F^2/RT)S\,c^*_{ox}(Df)^{1/2}$, (where n is the number of electrons, F is the Faraday constant, S is the electrode surface area, c^*_{ox} is the bulk concentration of the reactant Ox, R_Ω is the resistance of a thin film, f is the SW frequency and D is the diffusion coefficient).

For both peaks, the dependence $I_pf^{-1/2}$ *vs.* $f^{1/2}$ is associated with a well-developed maximum corresponding to reversible electrode reactions [8, 9].

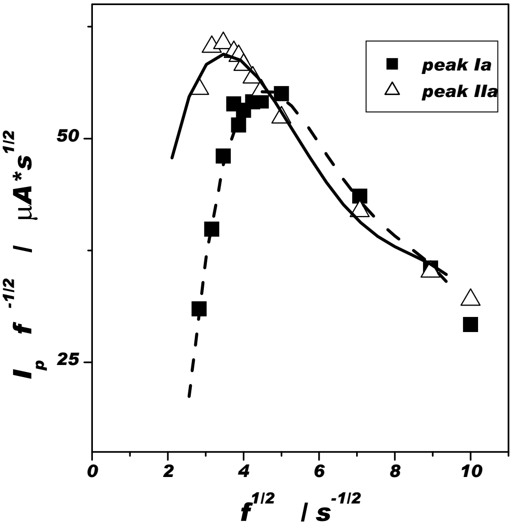


Figure 4. The influence of the SW frequency on the ratio of the SW peak current and the square root of the frequency for the oxidation of 10^{-3} M isoprenaline at graphite electrode. Experimental conditions: see figure 2.

CONCLUSIONS

Although based on different approaches, the use of both cyclic and square-wave voltammetry investigation techniques showed the effect of the -OH and $-NH_2$ groups of *para*-alkyl substituents on the antioxidant capacity of the studied catecholamine compounds. It is well-known that the antioxidant capacity increases with the decrease of the oxidation potential. Thus, for the studied catecholamaines the antioxidant capacity increase in the following order: norepinephrine < levonordefrin < epinephrine < isoprenaline or carbidopa < d-DOPA < dopamine.

Based on the importance of the intermediate oxidation products and the ability to control the redox process by electrochemical and selective methods (e.g., under potentiostatic conditions), further study of these compounds is required to fully achieve their potential applicability in both ageing and therapeutic processes.

EXPERIMENTAL SECTION

Reagents

The following compounds, with different number of –OH and $-NH_2$ groups, were used:

- 4-[1-hidroxi-2-(izopropilamino)etil]benzen-1,2-diol (isoprenaline),
- *(R)*-4-(1-hidroxi-2-(metilamino)etil)benzen-1,2-diol (epinephrine),
- 4-[(1*R*,2*S*)-2-amino-1-hydroxypropyl]benzene-1,2-diol (levonordefrin),
- 4-[(1*R*)-2-amino-1-hydroxyethyl]benzene-1,2-diol (norepinephrine),
- 4-(2-aminoetil)benzen-1,2-diol) (dopamine) and
- (*R*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid (d-DOPA)
- (2*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropanoic acid (carbidopa)

These p.a. quality compounds were supplied by Sigma–Aldrich GmbH and the corresponding 10^{-3}M solutions were prepared in 0.1M phosphate buffer solution (pH 7). The structures of the studied compounds are shown in figure 5.

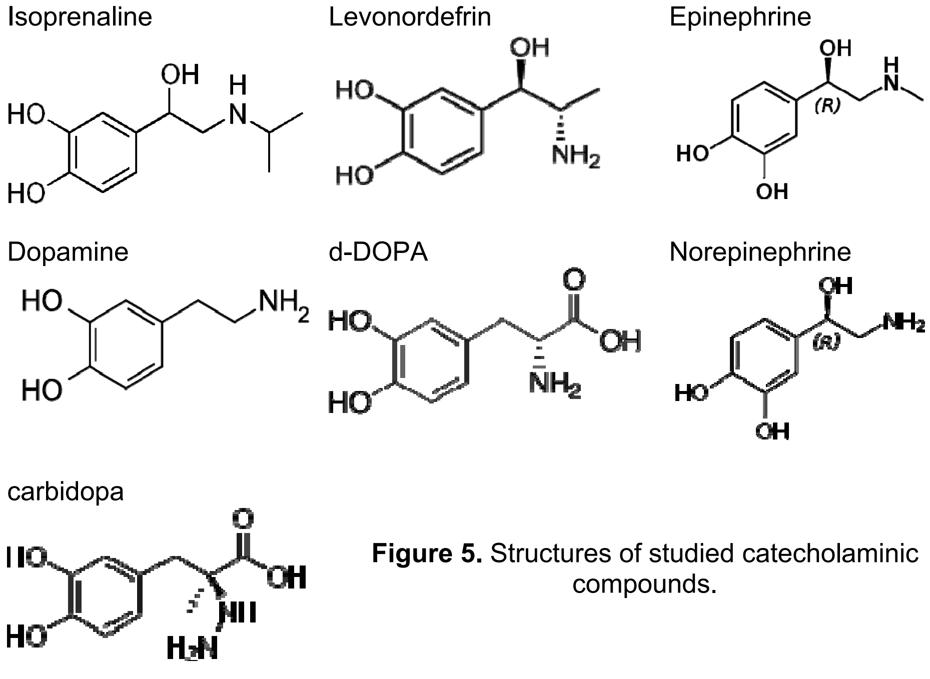


Figure 5. Structures of studied catecholaminic compounds.

The appropriate amounts of Na_2HPO_4, NaH_2PO_4, supplied also by Sigma, were used for preparing the 0.1M phosphate buffer solution.

Equipments

In order to assess the electrochemical behavior of the investigated compounds cyclic voltammetry (CV) and square-wave voltammetry (SWV) methods were used, employing a computer controlled - Autolab analytical unit (PGStat10, EcoChemie, Holland). For measurements was used an undivided cell equipped with following three-electrodes: a graphite working electrode (Ringsdorff, Germany) (diameter 0.3 cm), an Ag/AgCl, KCl_{sat} reference electrode (Radiometer, France) and a Pt wire counter electrode. The working electrode was mirror-polished with graded alumina powder prior to each experiment.

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