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

ELECTROCHEMICAL STUDY OF ISOPRENALINE AND EPINEPHRINE AT PLATINUM-NANOPARTICLES-CHITOSAN MODIFIED GRAPHITE ELECTRODE

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ABSTRACT. Two catecholamines (epinephrine and isoprenaline) were studied by cyclic (CV) and square-wave voltammetry (SWV) at bare graphite (G) and Pt nanoparticles (Pt-NP) - chitosan modified graphite electrodes (G/Pt-NP-Chitosan). The obtained results shown similarly redox behavior for both investigated compounds by mentioned electrochemical techniques at the investigated electrodes, consisting in two pairs of peaks (peak I and II) corresponding to a quasi-reversible processes. The difference between the structures of compounds doesn't influence the electrochemical parameters values. As expected, the presence of the Pt nanoparticles acts as a diffusion barrier on the electrode surface leading to a decrease of the current intensities values, when electrodes were investigated by CV and SWV. The G/Pt-NP-Chitosan nanocomposites matrix can be satisfactorily used for detecting catecholamines.

Keywords: *catecholamines, Pt nanoparticles, chitosan, cyclic voltammetry, square wave voltammetry.*

INTRODUCTION

Catecholamines are produced by sympathetic nervous system activation and act as hormones and neurotransmitters to monitor heart rate, brain muscles activity, glycogenolysis, fatty acid mobilization and body temperature [1]. Epinephrine and isoprenaline are two important catecholamines having similar structures.

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Epinephrine (often called adrenaline) is an important catecholamine neurotransmitter in mammalian central nervous system and biological body fluids. It has been used for the treatment of myocardial infarction, hypertension, bronchial asthma, cardiac arrest, and cardiac surgery in clinics. Studies show that many physiological phenomena are correlated to the changes of its concentration in the body fluids [2-3].

Isoprenaline (or isoproterenol) is a catecholamine drug that affects the heart by increasing inotropic and chronotropic activity. This sympathomimetic beta-receptor stimulant was used for the treatment of bradycardia (slow heart rate), heart block and rarely for asthma. The cardiovascular effects of isoprenaline are compared with the epinephrine, which can relax almost every kind of the smooth musculature that receives adrenergic nervous, but this effect is pronounced in the musculature of bronchus and also in the gastrointestinal tract [4-5].

Therefore, quantitative determination of these compounds in biological fluids and/or pharmaceutical preparations is very important. In the literature, a great number of methods have been presented, such as: chromatography (high performance liquid- or gas-), capillary electrophoresis, flow injection, chemiluminescence, fluorimetry, spectrophotometry and electrochemical methods [4, 6].

Among these methods, the electrochemical techniques and mainly the applications of chemical modified electrodes have increased enormously, because of their simplicity, rapidity, high selectivity and sensitivity. Thus, modified electrodes with various nanomaterials having unique electronic and catalytic properties were used in electrochemical studies of some catecholamine compounds [5].

The main purpose of the paper is to investigate the electrochemical behaviour of two catecholamines (epinephrine and isoprenaline) having similar structures, at a new prepared Pt nanoparticles-chitosan modified graphite electrodes, by cyclic and square-wave voltammetry.

RESULTS AND DISCUSSION

Electrochemical behavior of catecholamines by cyclic voltammetry

Figure 1 presents the cyclic voltammogramms of the studied catecholamines recorded on G and G/Pt-NP-Chitosan electrodes. Within the studied potential window, the supporting electrolyte shows no characteristic peaks, besides the charging of the electrical double layer (thin lines in figures 1A, B), while the studied catecholamines shown two pairs of peaks (abbreviated I, II) on both G and G/Pt-NP-chitosan investigated electrodes.

It is worth to mention, that the whole mechanism reaction consisting by chemical (C) and electrochemical (E) steps, involves different forms of epinephrine (scheme 1) and was detailed in literature. Briefly, it consists by the succession of ECCCEE steps, where the first E is assigned to IIa peak, followed by deprotonation (first C), cyclization (second C), and disproportionation (third C), and then again E (for Ic peak), and finally E (for Ia peak) [1, 7-9].

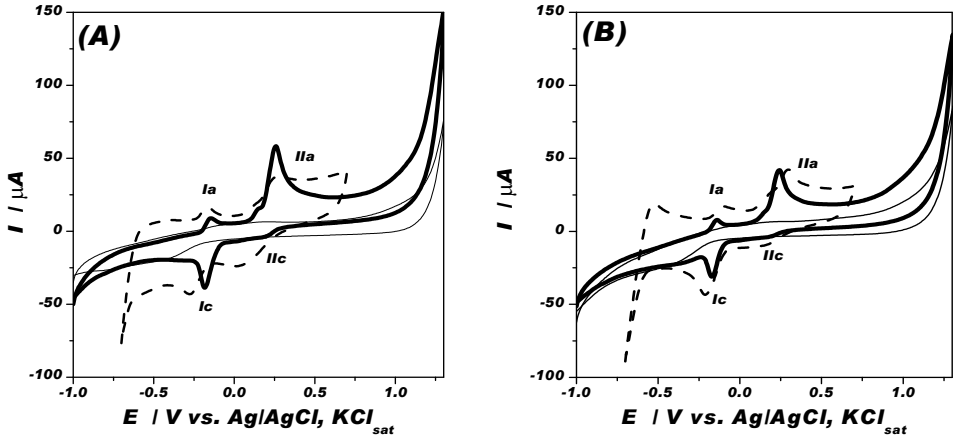
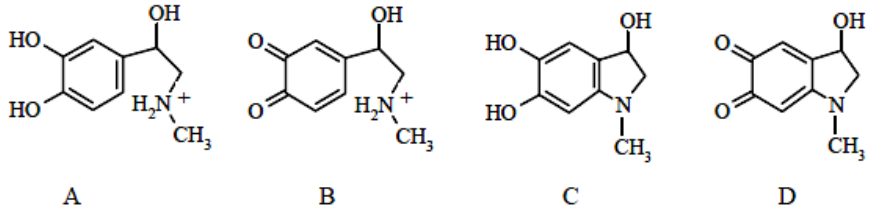
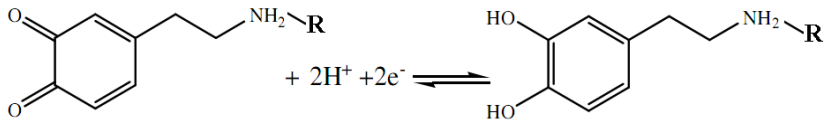


Figure 1. Cyclic voltamograms of 10^{-3} M isoprenaline (**A**) and 10^{-3} M epinephrine (**B**) at G (thick solid line) and G/Pt-NP-chitosan (dash thick line) electrode. Experimental conditions: electrolyte, 0.1 M phosphate buffer, pH 7 (thin solid line); scan rate, 50 mV/s; starting potential, -1 or 0.75 V *vs.* Ag/AgCl, $KCl_{sat.}$



Scheme 1. Structures of differents forms of protonated epinephrine (A), protonated epinephrinequinone (B), leucoadrenochrome (C) and adrenochrome (D).

At pH 7, regardless the type of used working electrode, the total redox process consists in the oxidation (two protons, two-electron) of epinephrine or isoprenaline to its corresponding open chain quinone epinephrinequinone or isoprenalinequinone following the reaction depicted in scheme 2:



Scheme 2. Redox process at pH 7 for epinephrine (R =-CH_3) or isoprenaline (R=–$CH(CH_3)_2$)

The electrochemical parameters describing the redox behavior of the investigated compounds are synthesized in table 1.

Table 1. Electrochemical parameters of 10^{-3} M epinephrine and 10^{-3} M isoprenaline investigated by cyclic voltammetry. Experimental conditions: see figure 1.

Parameters		E_{pa}/	I_{pa}/	E_{pc}/	I_{pc}/	ΔE/	$E^{0'}$/	I_{pa}/I_{pc}
Electrode	Peak	V/ER	A	V/ER	A	V	V/ER	
epinephrine								
G	**I**	-0.146	$1.22\ 10^{-5}$	-0.185	$-2.99\ 10^{-5}$	0.039	-0.166	0.41
	II	0.230	$4.05\ 10^{-5}$	0.162	$-2.63\ 10^{-6}$	0.068	0.196	15.4
G/NP-Pt-	**I**	-0.104	$6{,}25\ 10^{-6}$	-0.212	$-2.68\ 10^{-5}$	0.108	-0.158	0.23
chitosan	**II**	0.296	$2.07\ 10^{-5}$	0.164	$-4{,}27\ 10^{-6}$	0.132	0.230	4.84
isoprenaline								
G	**I**	-0.150	$8.10\ 10^{-6}$	-0.185	$-2.74\ 10^{-5}$	0.035	-0.168	0.29
	II	0.255	$4.65\ 10^{-5}$	0.186	$-2.93\ 10^{-6}$	0.069	0.220	15.9
G/NP-Pt-	**I**	-0.168	$7.48\ 10^{-6}$	-0.270	$-1.47\ 10^{-5}$	0.102	-0.219	0.51
chitosan	**II**	0.257	$1.46\ 10^{-5}$	0.062	$-1.04\ 10^{-5}$	0.195	0.160	1.4

where: ER is the reference electrode; $\Delta E_p = E_{p,a} – E_{p,c}$ is the peak potentials separation; $E^{0'} = (E_{pa} + E_{pc})/2$ is the formal standard potential; E_{pa} and E_{pc} are the anodic and cathodic peak potentials, respectively.

As it can be seen, the electrochemical behavior of isoprenaline is similar with the redox behavior of epinephrine due to their analogous structure. A shift towards positive (peak II at G) or negative (peak I and II at G/Pt-NP-chitosan) directions of peak potentials of isoprenaline comparing to epinephrine is visible. If the difference between the $E^{0'}$ of isoprenaline and epinephrine is of + 24 mV (peak II) at G electrode, the presence of Pt-NP lead to a difference of - 61 mV (peak I) and -70 mV (peak II), the two compounds becoming better separated.

Also, the variation of the current intensities of either the Ia, or the IIc peaks which are much smaller than the mirror corresponding one (I_{pa}/I_{pc} much greater/smaller than 1), and additionally with the variation of the peak potentials separation values ($\Delta E_p = E_{p,a} – E_{p,c}$ = 0.035 ÷0.195 V, at 50 mV/s), confirms that the redox behaviour of isoprenaline and epinephrine correspond to a quasi-reversible process at both type of investigated electrodes. However, because peak IIc is hardly visible, it can be considered that the process II corresponds to an irreversible oxidation of epinephrine or isoprenaline, at pH 7.

With increasing of the scan rate, the peak current intensity is increasing and the corresponding peak potentials of Ia, IIa and Ic are shifting towards positive and negative values, respectively (results not shown). The plots of log(Ip)- log(v) for each peaks currents (I and II) are linear with a slope close to 0.5 (see Table 2), proving that the adsorption of studied compounds is very weak at G/Pt-NP-chitosan and bare G electrodes, at pH 7.

Table 2. Parameters of the linear regression for the $\log(I_p/\mu A)$ - $\log(v/(V*s^{-1}))$ dependence. Experimental conditions: see figure 2.

Electrode	Slope R/n			
	Peak Ia	Peak Ic	Peak IIa	Peak IIc
		isoprenaline		
G	-	0.732 ± 0.019 0.9983/7	0.306 ± 0.024 0.9882/6	0.257 ± 0.055 0.9015/7
G/NP-Pt-chitosan	0.599 ± 0.116 0.9025/8	0.411 ± 0.055 0.9654/6	0.269 ± 0.027 0.9800/6	0.263 ± 0.032 0.9711/6
		epinephrine		
G	-	0.760 ± 0.015 0.9989/7	0.476 ± 0.040 0.9892/5	-
G/NP-Pt-chitosan	0.473 ± 0.059 0.9551/8	0.434 ± 0.058 0.9497/8	0.342 ± 0.076 0.9142/6	0.291 ± 0.025 0.9852/6

As consequence the epinephrine or isoprenaline redox electrode process can be considered to take place under diffusion control [10]. Also, the plot I *versus* $v^{1/2}$ is linear (see Figure 2), as expected the current intensities at G/Pt-NP-chitosan electrode being smaller than at bare G electrode.

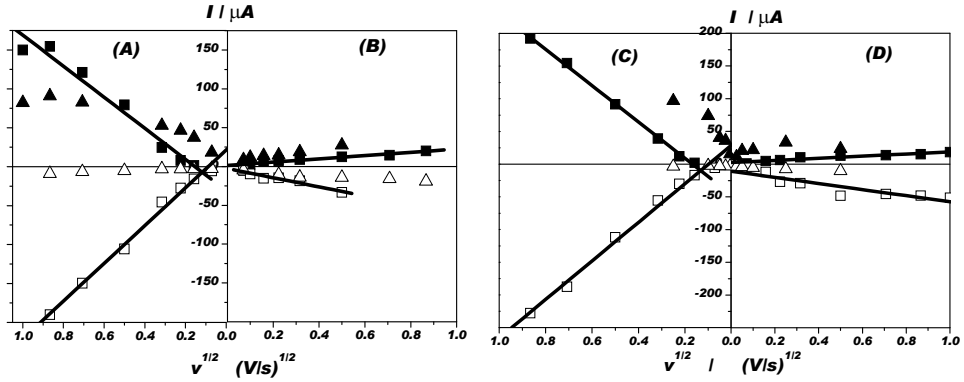


Figure 2. Influence of the scan rate on the current intensity of peak I (■, □) and peak II (▲, △) of 10^{-3} M isoprenaline (**A, B**) and 10^{-3} M epinephrine (**C, D**) at G (**A, C**) and G/Pt-NP-chitosan (**B, D**) electrode. Experimental conditions: see figure 1.

For a reversible process, using the Randles-Sevcik equation (1) the diffusion coefficients of catecholamines could be calculated.

$$I_{p,a} = 2.69*10^{-5}\, n^{3/2}\, A\, D_0^{1/2}\, v^{1/2}\, c_{ox}^{*} \quad (1)$$

where: $I_{p,a}$ (A) refers to the anodic peak current, n is the number of transferred electron, A (cm^2) is the area of the electrode, D_0 ($cm^2\ s^{-1}$) is the diffusion coefficient, c_{ox}^{*} (mol cm^{-3}) is the catecholamine concentration and v ($V\ s^{-1}$) is the scan rate.

From the slope of I_{pa} *versus* $v^{1/2}$ plots, and presuming the surface area of graphite electrode being 0.071 cm^2, for a 10^{-3} M catecholamine concentration, the diffusion coefficients were $D_0 = 5.8\ 10^{-5}$ $cm^2\ s^{-1}$ for epinephrine and $D_0 = 0.3\ 10^{-5}$ $cm^2\ s^{-1}$ for isoprenaline at G electrodes, values which are in accordance with literature data (i.e., for epinephrine $D = 4.2\ 10^{-5}$ $cm^2\ s^{-1}$ at CPE [11] or $1.4\ 10^{-4}$ $cm^2\ s^{-1}$ at poly(*p*-xylenolsulfonephthalein) glassy carbon modified electrode [3]).

Electrochemical behavior of catecholamines by square-wave voltammetry

The square-wave voltammetry investigation technique was chosen for the study of the catecholamines because it is the most advanced and most sophisticated method from the pulse voltammetric techniques and exhibits the advantages of a large speed and high sensitivity [1, 12].

In Figure 3A are shown the SW voltammograms obtained for the epinephrine and isoprenaline at different electrodes. As expected, the current intensities obtained at G/Pt-NP-chitosan electrodes comparing with those at bare G are smaller, confirm that the Pt-NP-chitosan matrix act as diffusion barrier for epinephrine or isoprenaline towards electrode interface. At both investigated electrodes, the peak potentials have close values ($E_{a,I}$ = -0.160 V; -0.200 V and $E_{a,II}$ = 0.210 V; 0.185 V for epinephrine and isoprenaline at G electrode, respectively; $E_{a,I}$ = -0.150 V; -0.200 V and $E_{a,II}$ = 0.240 V; 0.200 V for epinephrine and isoprenaline at G/Pt-NP-chitosan electrode, respectively) than in CV technique.

According to the theoretical model proposed by Mirceski and coworkers for SWV, a dependence of $I_p f^{-1/2}$ *vs.* $f^{1/2}$ associated with a well-developed maximum corresponds to a quasi-reversible electrode reactions [13] and an exponential dependence indicate a diffusion-controlled electrode process [14]. Note that the ratio $I_p f^{-1/2}$ corresponds to the dimensionless net-peak current (Φ) and the $f^{1/2}$ to the resistance parameter (q), where: $\Phi = I_p(nFS\, c_{ox}^{*})^{-1}(Df)^{-1/2}$, $q = R_\Omega(n^2F^2/RT)S\, c_{ox}^{*}\, (Df)^{1/2}$, n is the number of electrons, F is the Faraday

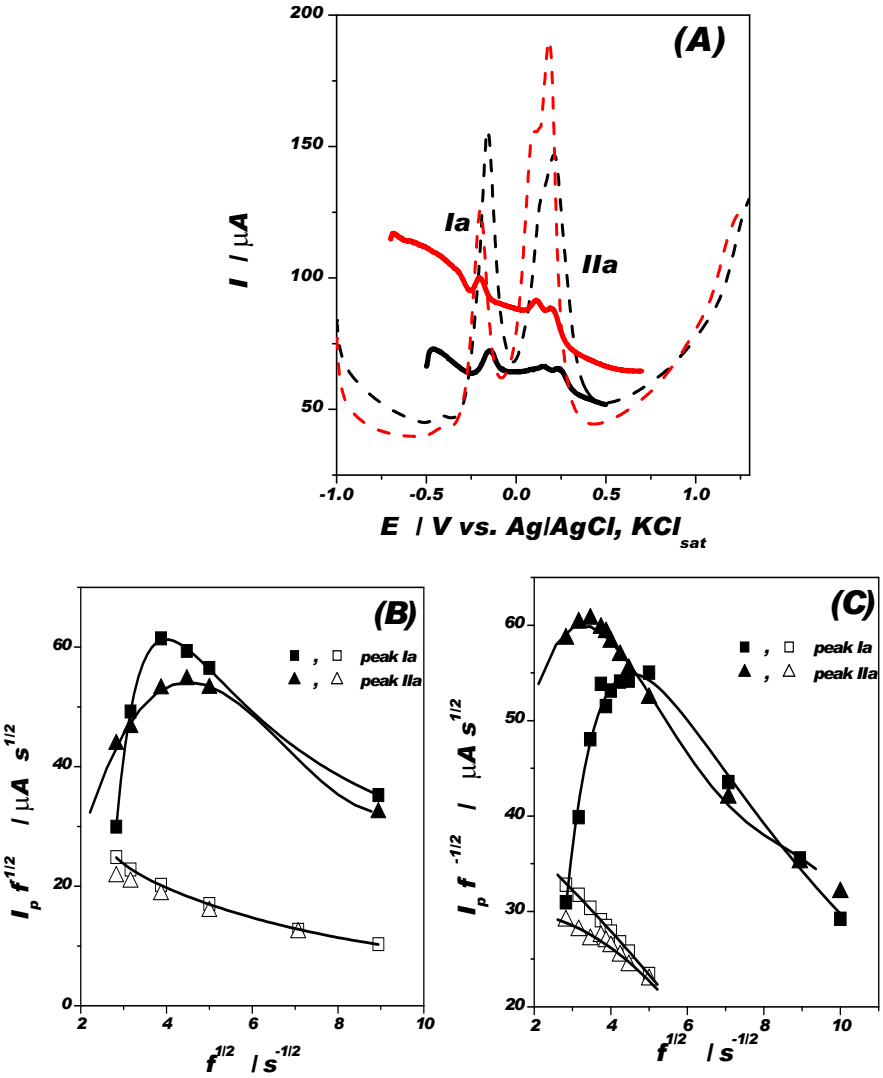


Figure 3. (A) Square-wave voltamograms of 10^{-3} M isoprenaline (red solid or dash line) and 10^{-3} M epinephrine (black solid or dash line) at G electrode (dash lines) and G/Pt-NP-chitosan (solid lines). Influence of SW frequency on the ratio of the SW peak current and the square root of the frequency for the oxidation of 10^{-3} M epinephrine (**B**) and 10^{-3} M isoprenaline (**C**) at graphite electrode (■, ▲) and G/NP-Pt-chitosan (□, △) electrode. Experimental conditions: electrolyte, 0.1 M phosphate buffer (pH 7); interval time, 0.1 s; initial potential, -1 V *vs.* Ag/AgCl,KCl_{sat}; step potential, 0.00195 V; amplitude, 50 mV; frequency, 10 s^{-1}.

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.

Figures 3B and 3C show the dependence of the ratio $I_p f^{-1/2}$ on $f^{1/2}$ for the oxidation of 10^{-3} M isoprenaline and epinephrine, respectively. For both peak pairs, the maximum of the mentioned dependence at G electrode proves the existence of a quasi-reversible electrode process and the exponential decrease at G/Pt-NP-chitosan prove the diffusion-controlled oxidation process, as observed in the CV experiments. Also, the results are in good qualitative agreement with the theoretical variation predicted for an EC type mechanism [15].

CONCLUSIONS

A new modified electrode consisting by Pt nanoparticles immobilized in a biocompatible chitosan matrix on graphite surface was prepared employing a simple and general methodology.

At G and G/Pt-NP-Chitosan electrodes, epinephrine and isoprenaline having similar structures, cyclic voltammograms showed two pairs of peaks (peaks I and II), corresponding to a quasi-reversible redox processes. As expected, the presence of the Pt nanoparticles acts as a diffusion barrier on the electrode surface leading (i) to a decrease of the peak current intensities values, when electrodes were investigated by CV or SWV and (ii) to a negative shift of the redox formal potential of isoprenaline comparing to the epinephrine, when CV was used as investigation method.

The easy and low cost modified electrode containing Pt nanoparticles seems to be of great utility for making a voltammetric sensor for the detection of epinephrine and isoprenaline using cyclic or square wave voltammetry.

EXPERIMENTAL SECTION

Reagents

The p.a. quality of 4-[1-hidroxi-2-(izopropilamino)etil]benzen-1,2-diol (isoprenaline), *(R)*-4-(1-hidroxi-2-(metilamino)etil)benzen-1,2-diol (epine-phrine) and chitosan were supplied by Sigma–Aldrich GmbH. The corresponding 10^{-3}M solutions of catecholamines were prepared in 0.1 M phosphate buffer solution (pH 7). The appropriate amounts of Na_2HPO_4, NaH_2PO_4, supplied also by Sigma, were used for preparing the 0.1 M phosphate buffer solution. Nanoparticles of Pt stabilised on graphite powder (Pt-NP) were a kindly gift from dr. Dan Goia (Clarkson University, Potsdam, USA) and is greatly acknowledged here. Acetic acid was purchased from Reactivul-Bucuresti.

Equipments

In order to investigate the electrochemical behavior of the compounds cyclic voltammetry (CV) and square-wave voltammetry (SWV) methods were used, employing a computer controlled - Autolab analytical unit (PGStat10, EcoChemie, Holland). For electrochemical measurements an undivided cell was used, equipped with the following three-electrodes: a modified graphite working electrode (Ringsdorff-Werke Gmbh, Bonn-Bad Godesberg, Germany) (diameter 0.3 cm), an Ag/AgCl, KCl_{sat} reference electrode (Radiometer, France) and a Pt wire counter electrode. For comparison, all experiments were, also carried out on unmodified graphite. Before each experiment, the bare graphite was mirror-polished with different grit emery papers.

G/Pt-NP-Chitosan electrode modification

Chitosan (10 mg) was dissolved in 10 ml acetic acid 0.1 M. A suspension of 1 mg of Pt-nanoparticles in 500 μL chitosan solution was strongly mixed by sonication, for 10 min. A volume of 5 μL of the suspension was deposited on the bare freshly polished graphite electrode. The obtained G/Pt-NP-Chitosan electrodes were used after solvent evaporation.

ACKNOWLEDGEMENTS

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