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Dedicated to Professor Luminița Silaghi-Dumitrescu on the occasion of her 65th anniversary

ELECTROCHEMICAL DETERMINATION OF DOPAMINE WITH GRAPHENE-MODIFIED GLASSY CARBON ELECTRODES

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ABSTRACT. Two new glassy carbon modified electrodes were prepared by drop casting of chemically reduced graphene oxide (rGO) or graphene oxide (GO) on glassy carbon (GC) and then protected with a polymeric layer of Nafion or chitosan (Chit) (GC/Chit/rGO/Chit and GC/GO/Nafion). Their investigation by cyclic- and square-wave voltammetry for dopamine (DA) oxidation was aiming to estimate the analytical parameters that have the best values for GC/Chit/rGO/Chit electrode (*i.e.*, the highest sensitivity of 1.002 ± 0.025 μA/ μM, a detection limit of 2.67 μM μM, for a linear domain from 4 to 18 μM DA). The modified GC/Chit/rGO/Chit electrode was also used for the detection of dopamine in injection vials using the standard addition method with a recovery of 99.42%.

Keywords: graphene oxide, reduced graphene oxide, dopamine, cyclic voltammetry, square wave voltammetry

INTRODUCTION

Dopamine (DA) is a neurotransmitter with important roles in the function of central nervous, cardiovascular, renal and hormonal systems [1]. It is associated in human behaviours such as: reward, cognition, motor function, motivation, learning and memory [2]. Deficiency of DA may cause serious diseases like schizophrenia and Parkinson's disease, so its detection becomes necessary and helpful for correct diagnosis [3-4].

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The detection of dopamine could be realized by capillary electrophoresis, liquid chromatography, calorimetric method, chemiluminescence, fluorescence etc. Because of its redox activity, dopamine is an interesting subject in electroanalysis, consequently electrochemical methods have gained more attention due to their advantages such as: selectivity, low-cost, portability, easy handling and less-time consuming [2].

However, in real body fluids, dopamine coexists with interfering substances (*e.g.*, ascorbic acid and uric acid), having similar oxidation potential. In this context, for selective and discriminative detection of DA, surface modification of the working electrodes with appropriate agents such as graphene [5] and carbon nanotubes is a promising method [6].

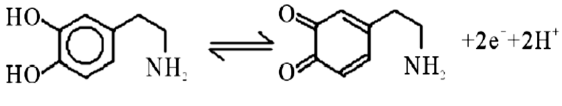
Graphenes, an atomically thin layer of sp^2-bonded carbon atoms, stacked in a two-dimensional (2D) honeycomb lattice, belong to a new class of carbon nanomaterials [7] that display versatile properties including thermal/electrical conductivity, good mechanical properties, large surface area etc. [8]. Due to these properties, graphenes can be successfully used in electrochemical sensing devices for selective detection of chemical species. Nevertheless, it should be mentioned that graphene is electrochemically active only in its reduced form, but electrode modification with graphene oxide can also be achieved by the introduction of a pretreatment step, which reduces the graphene oxide to graphene. The reduction of GO to graphene can be done either chemically, electrochemically or thermally [2].

The present work was focused on the electrochemical studies of chemically reduced graphene oxide (rGO) and electrochemically reduced graphene oxide (GO) immobilized *via* drop-casting method [9] on glassy carbon (GC) electrodes and protect by either Nafion [10] or Chitosan [11] polymers. The obtained modified electrodes were used for studying dopamine oxido-reduction process by cyclic- and square-wave voltammetry.

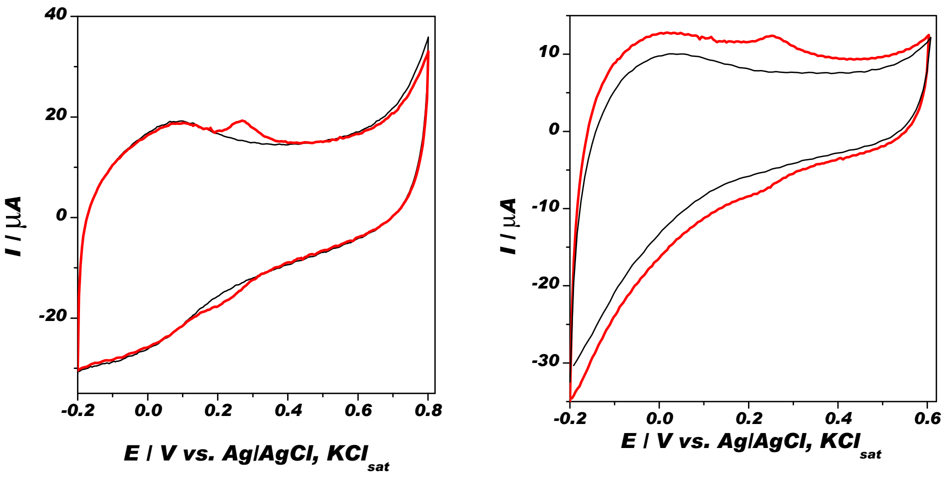
RESULTS AND DISCUSSION

Electrochemical behaviour of dopamine at different glassy carbon modified electrodes

The oxido-reduction of 10^{-3} M DA at various modified electrodes was investigated by cyclic voltammetry (CV). As shown in figure 1, at both modified electrode a pair of peaks appeared at around +0.250 V *vs.* Ag/AgCl, KCl_{sat} and was attributed to the oxidation of dopamine to form dopaminequinone with the release of two electrons as described by the following reaction (1):



(1)



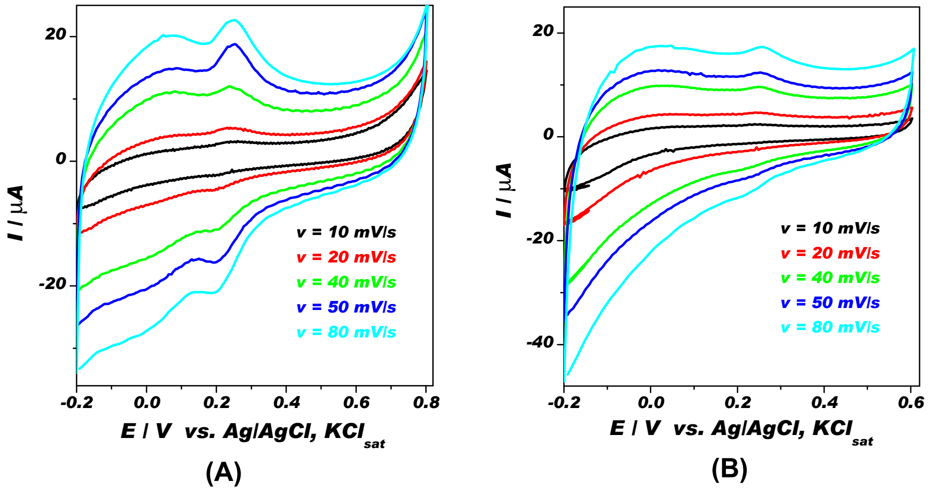
(A) (B)

Figure 1. Cyclic voltammograms of 15 μM dopamine (thick) at GCE/GO/Nafion/(red) (**A**) and GCE/Chit/rGO/Chit (**B**) modified electrode. Experimental conditions: electrolyte, 0.1 M phosphate buffer (pH 7) (thin layer); scan rate, 50 mV/s; starting potential, -0.2 V *vs.* Ag/AgCl, KCl_{sat}.

Dopamine being an easily oxidizable catecholamine, the separation of redox peaks (ΔE_p, calculated as the difference between E_{pa} and E_{pc}) was found 0.046 V for GCE/GO/Nafion/(red) and 0.023 V for GCE/Chit/rGO/Chit electrode, respectively. On the other side, the I_{pa}/I_{pc} ratio was found 1.68 (at GCE/GO/Nafion/(red) electrode) and 1.39 (at GCE/Chit/rGO/Chit electrode), suggesting a quasi-reversible reaction. Despite the fact that the peak potentials for GCE/Chit/rGO/Chit electrode are placed more closely one from another, the formal peak potential ($E^{0'}$, calculated as the midpoint of E_{pa} and E_{pc}) was estimated to be 0.242 V for both studied modified electrodes. The smaller value of ΔE_p and the I_{pa}/I_{pc} ratio value closer to 1 confirm that the redox process occurring at GCE/Chit/rGO/Chit electrode is more reversible than that occurring at GCE/GO/Nafion/(red) electrode. The difference between the two modified electrodes could be explained by the different porosity of the protective polymeric membrane used (*i.e.*, chitosan and Nafion).

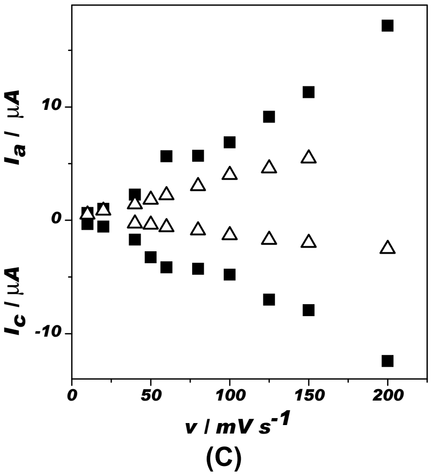
Influence of scan rate

The effect of scan rate on the peak current of dopamine was recorded in the range of 10 – 200 mV/s in order to investigate the electrode reaction kinetics and verify if diffusion is the only factor controlling the process (Fig 2A-B). As it can be seen in Fig 2A-B, for both studied electrodes the scan rate increasing leads to the increase of the peak current and the anodic and cathodic peak potentials are slightly shifted towards the positive and negative direction, respectively [2].



(A)

(B)



(C)

Figure 2. Influence of the scan rate on the cyclic voltammograms of 15 μM dopamine recorded at GCE/GO/Nafion/(red) (■) (**A**) and GCE/Chit/rGO/Chit (Δ) (**B**) modified electrode. Dependency I *vs.* scan rate (C). Experimental conditions: electrolyte, 0.1 M phosphate buffer (pH 7); starting potential, -0.2 V *vs.* Ag/AgCl, KCl_{sat}.

Fig 2C illustrates for both anodic and cathodic processes a linear relationship between log I_p and log v with a slope close to 1 (Table 1), proving a surface adsorption-controlled kinetics for oxidation of dopamine at the electrode surface during the experiment. Also, from Figure 2C it can be observed that the current intensities for GCE/Chit/rGO/Chit electrodes have lower values than those of GCE/GO/Nafion/(red) electrode. This behaviour, at the working pH 7, could be explained by an enhanced attractive interactions between the positively charged dopamine (pKa = 8.89 [10]) and the negatively charged groups

(SO_4^{2-}) of the Nafion protective polymers for GCE/GO/Nafion/(red) electrode. Such interactions could lead to an accumulation of DA on the surface of the modified electrode explaining the increase in peak current [4]. In the case of GCE/Chit/rGO/Chit electrode repulsive interactions between dopamine and the positively charged chitosan are noticed. Nevertheless, this effect is partially counteracted by the well-known phenomenon of swelling of Chit which increase the protective layer porosity [12].

Table 1: Influence of the potential scan rate on the voltammetric response of GCE/GO/Nafion/(red) and GCE/Chit/rGO/Chit modified electrodes. Experimental conditions: see Fig 2.

Electrode type	**Slope of the log I_p – log v dependencies**			
	oxidation	**R^2/n**	**reduction**	**R^2/n**
GCE/Chit/rGO/Chit	0.899 ± 0.034	0.9916 / 9	1.417 ± 0.056	0.9779 / 8
GCE/GO/Nafion/(red)	1.121 ± 0.07	0.9817 / 9	1.237 ± 0.092	0.9706 /10

where: R is the correlation coefficient and n is the no. of experimental points.

Analytical parameters for dopamine detection

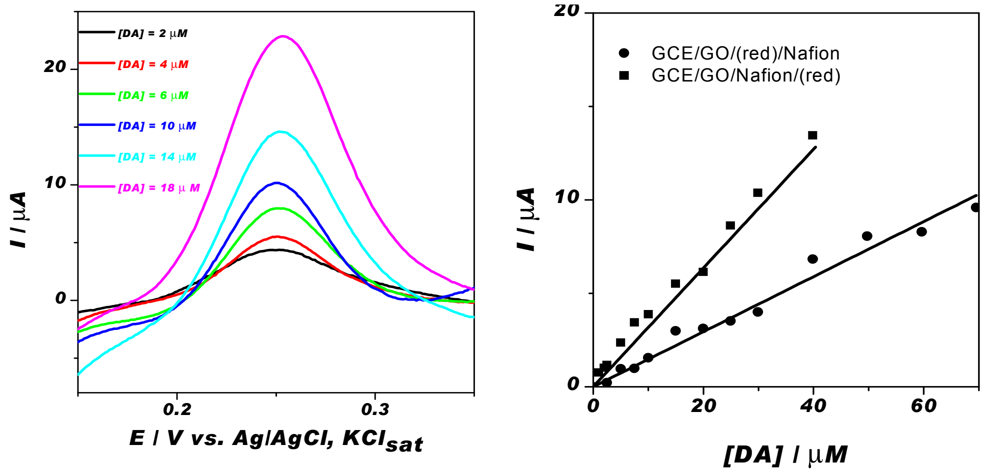
The analytical parameters were determined by using square-wave voltammetry (SQW).

Case of G/GO/Nafion electrode

The GCE/GO/Nafion electrodes were activated by electrochemical reduction of GO, consisting by cycling the electrode potential between -1 and 0 V *vs.* Ag/AgCl,KCl_{sat} for 10 cycles. This activation step was done before (*i.e.,* GCE/GO/(red)/Nafion) and after (*i.e.,* GCE/GO/Nafion/(red)) deposition of the protective layer of Nafion. Square-wave voltammetric measurements carried out at GCE/GO/Nafion electrodes in the absence and in the presence of dopamine (Figure 3A) allowed obtaining of calibration curves. Based on these curves (Figure 3B), the electroanalytical parameters of the modified electrodes were estimated (Table 2).

It is worth mentioning that the step of electrochemical reduction of GO before or after applying the protective layer of Nafion has a great influence on the electrode sensitivity. Comparing the slopes of the calibration curves we can observe an increase of the sensibility from 0.147 ± 0.004 μA/μM (for

GCE/GO/(red)/Nafion electrode) to 0.318 ± 0.007 µA/µM (for GCE/GO/Nafion/(red) electrode). For GCE/GO/(red)/Nafion electrode the linear domain was between 2.5 - 70 µM DA and more narrow for GCE/GO/Nafion/(red) (*i.e.*, 1 - 40 µM DA).



(A) **(B)**

Figure 3. SQW voltammograms of dopamine (after baseline corrections) at GCE/GO/(red)/Nafion electrodes (**A**) and the corresponding calibration curve for dopamine detection at GCE/GO/(red)/Nafion (●) and GCE/GO/Nafion/(red) (■) (**B**). Experimental conditions: phosphate buffer 0.1 M (pH 7); frequency, 10 Hz; amplitude, 25 mV; step potential, 10 mV; starting potential -1 V *vs*. Ag/AgCl, KCl$_{sat}$.

Case of GCE/Chit/rGO/Chit electrode

In the case of GCE/GO/(red)/Nafion electrode, after a short time storage of the electrode surface, either by immersion in buffer solution or on air, the protective layer cracked. This is the reason for which Nafion was replaced by chitosan. In view to determine the ideal concentration of the chitosan solution used for surface protection, a concentrated (1%) solution and a diluted (0.1%) solution was used.

It was determined that the GCE/Chit/rGO/Chit electrode obtained using diluted solution shows a much higher sensitivity for detecting DA (*e.g.*, 1.0 µA/µM) compared with those obtained with concentrated one (*e.g.*, 0.04 µA/µM). Such high sensitivity can be obtained with a much lower limit of detection (*i.e*, 2.67 µM compared to 6.6 µM DA) (results not shown).

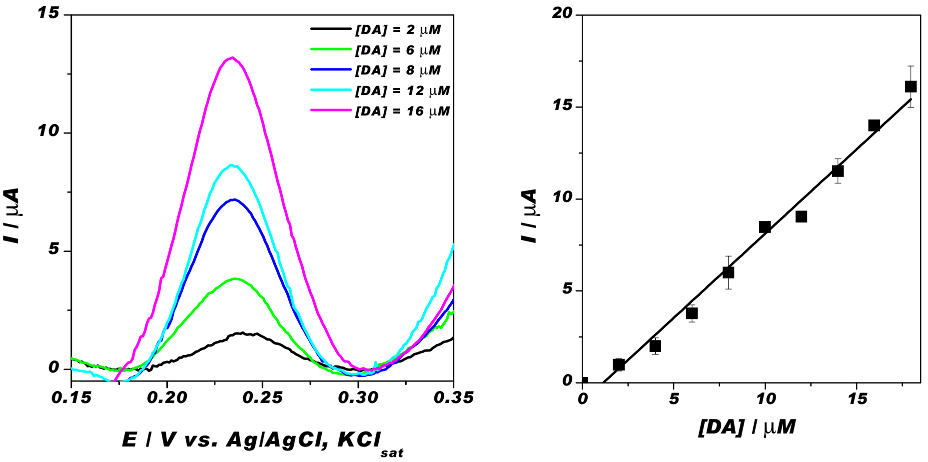


Figure 4. SQW voltammograms of dopamine (after baseline corrections) at GCE/Chit/rGO/Chit electrodes (A) and the corresponding calibration curve for dopamine. Experimental conditions: phosphate buffer 0.1 M (pH 7); frequency, 10 Hz; amplitude, 25 mV; step potential, 10 mV; starting potential -1 V *vs*. Ag/AgCl,KCl_{sat}; error bars: mean of measurements with 3 different electrodes.

The analytical parameters of the mean measurements with 3 different GCE/Chit/rGO/Chit electrodes are summarised in table 2.

In the case of three measurements with 3 different electrodes of 18 µM DA at GCE/Chit/rGO/Chit in 0.1 M phosphate buffer pH 7, the mean peak intensity is 16,11 ± 1.955 µA, with a RSD of 12.14% and the mean peak potential is 0.234 ± 0.006 V *vs.* Ag/AgCl, KCl_{sat}, with a RSD of 2.65%, respectively. The obtained RSD values indicate a good reproducibility of the obtained electrode, recommending the GCE/Chit/GO/Chit as a good sensor for DA detection.

Real sample analysis

To investigate the application potential of the GCE/Chit/GO/Chit electrodes for the detection of DA, the injections vials of DA (5 mg/ml) were analyzed by the standard addition method in order to overcome possible matrix effects.

Table 2. Electroanalytical parameters of the G/GO/Nafion and GCE/Chit/rGO/Chit modified electrodes. Experimental conditions: see fig. 3 and 4.

Electrode type	Sensitivity µA / µM	Linear range/ µM	LOD* /µM	R^2 / n
GCE/GO/(red)/Nafion)	0.147 ± 0.004	2.5 - 70	1.35 µM	0.9980/ 9
GCE/GO/Nafion/(red)	0.318 ± 0.007	1 - 40	0.69 µM	0.9980/10
GCE/Chit/GO/Chit **	1.002 ± 0.025	4 -18	2.67 µM	0.9969/ 7

* calculated using Miller approach and SMAC software based on the confidence bands of the calibration function; ** mean of 3 measurements obtained with 3 different electrodes.

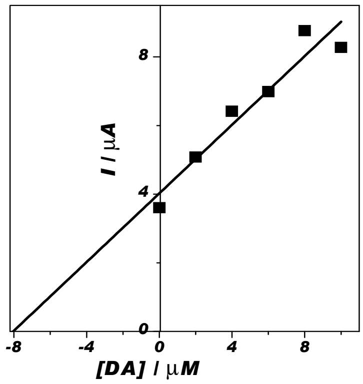


Figure 5. Standard addition method for determination of DA concentration from injection vials using GCE/Chit/GO/Chit electrode. Experimental conditions: see fig 4.

The linear equation for GCE/Chit/GO/Chit electrode obtained with standard addition method was I/µA = (4.023 ± 0.435) + (0.5 ± 0.072) [DA]/µM (R^2 = 0.9236 n = 6), and lead to calculate the unknown concentration of the DA, which was estimated as 8.046 µM DA, for an added concentration of 8 µM DA. The value is in good agreement with that indicated by the producer and confirms that the proposed method could be efficiently used for the determination of DA in injection vials with a recovery of 99.42%.

EXPERIMENTAL SECTION

Reagents

The graphene oxide (GO) were prepared by dr. C. Cotet at the Faculty of Chemistry and Chemical Engineering and was a kindly gift which is gratefully thanked here. The reduced graphene oxide (rGO) was provided by Chengdu Organic Chemicals Co. Ltd., Chinese, Academy of Sciences. Dopamine hydrochloride 99% and the glacial acetic acid were provided by Alfa Aesar and by Merck, respectively. The Na_2HPO_4 2 H_2O, NaH_2PO_4 12 H_2O and high viscosity chitosan were provided by Sigma Aldrich. The 5% Nafion in ethanol solution was supplied from Sigma Aldrich and ethanol 100% from Reactivul Bucuresti, respectively. All chemicals were of analytical grade and used without any purification step. For avoiding DA polymerization, stock solutions of dopamine were prepared fresh every day. The DA injection vials were produced by Zentiva with a specified content of DA of 5 mg/ml.

The graphene oxide (GO) 1 mg/mL solutions were prepared from flakes dissolved in distillated water during 30 minutes in a vortex mixer followed by 30 minutes sonication.

The 1 mg/mL dispersion of reduced graphene oxide (rGO) was obtained by mixing the appropriate quantity of powder for 30 minutes on a vortex mixer, followed by sonication (2 hours) and mixing (30 minutes on a vortex mixer). Before every usage, the stored dispersion was further sonicated, and then mixed for 30 minutes.

A 0.1 % chitosan solution was prepared by dissolving appropriate quantity of salt in 0.1 M acetic acid solution.

A 0.1 M phosphate buffer solution was prepared by using appropriate amounts of Na_2HPO_4 and NaH_2PO_4 and adjusting the pH values with 0.1 M H_3PO_4 or NaOH solutions, respectively.

Methods

Electrochemical measurements were performed with a PGStat 12 computer controlled potentiostat (Autolab, The Netherlands). An undivided electrochemical cell containing the modified glassy carbon electrode (GCE, 3 mm diameter) as the working electrode, a platinum wire as the counter and a Ag/AgCl,KCl_{sat} as reference electrode was connected to the potentiostat. All experiments were carried out at room temperature.

Electrode preparation

The cleaning of glassy carbon electrodes (GCE) surfaces consist in polishing with 1200 abrasive paper and Al_2O_3 powder, followed by ultrasonication for 3 minutes. Between each step the electrode surface was rinsed with distillated water and inspected *via* microscope. The modified electrodes were prepared by drop casting.

GCE/GO/Nafion was obtained by dropping 5 µL of 1 mg/mL of GO solution on the clean GC surface and dried in air for solvent evaporation. After that the electrode surface was covered with 5 µL of 1.25% Nafion solution. The active form of GO was obtained by cycling the potential between -1 and 0 V *vs.* Ag/AgCl/KCl_{sat} for 10 cycles either before (**GCE/GO/(red)/Nafion**) or after (**GCE/GO/Nafion/(red)**) protection with Nafion membrane.

GCE/Chit/rGO/Chit was prepared by successive deposition of 2 µL Chit, and a mixture of 5 µL rGO dispersion and 3 µL Chit solutions, respectively. Between each deposition steps, the solvent was evaporated in air drying. This alternation was needed to eliminate the significant differences in surface structure of glassy carbon electrodes, thus obtaining a “universal” procedure that would yield matching results on all used GC electrodes.

CONCLUSIONS

Modified GCE/GO/Nafion and GCE/Chit/rGO/Chit electrodes were prepared by drop casting method and investigated for studying the dopamine oxido-reduction process by cyclic voltammetry. The electrochemical parameters ($\triangle$Ep, $E^{o\prime}$, I_{pa}/I_{pc}, influence of scan rate) are in accordance with the literature data describing a $2e^-/2H^+$ reversible electrode process.

The prepared GCE/GO/Nafion and GCE/Chit/rGO/Chit electrodes, tested by square-wave voltammetry, exhibit greater sensitivity and selectivity for the determination of DA with a wide linear range and a low detection limit.

The GCE/Chit/rGO/Chit electrode was also used for detection of DA in real samples and satisfactory results were obtained.

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