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Original scientific paper

Voltammetric determination of dopamine using modified glassy carbon electrode by electrografting of catechol

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Abstract: By means of cyclic voltammetry as a diagnostic technique, the present study tried to describe the behaviour of dopamine (DP) at the bare and catechol electrografted glassy carbon electrode (CA/GCE). The results indicated that the CA/GCE exhibits high electrocatalytic activity toward DP. The DP cycling of the CA/GCE in DP showed two linear calibration ranges between 5.0–100 and 100–750 μM with a detection limit of 0.86 μM. For 6 DP replicate measurements (50 μM); the relative standard deviation (*RSD*) was 2.7 %. This method was successfully used for the determination of DP in serum samples.

Keywords: electrografted glassy carbon electrode; dopamine; catechol; cyclic voltammetry; electrochemical sensor.

INTRODUCTION

A type of catecholamine, dopamine (DP), acts as a significant neurotransmitter in mammalian brain fluids and tissues. It is responsible for the functions of the central nervous, hormonal, renal, and cardiovascular systems.[1,2] Thus, true and accurate measurement of DP for detection, monitoring, prevention and treatments of neurological disorders such as schizophrenia, Alzheimer's disease, Parkinson's disease and HIV infection is important.[3] Different methods are used for measuring DP concentration in biological samples such as capillary electrophoresis,[4] mass spectrometry,[5] high-performance liquid chromatography[6] and electrochemical sensors.[7,8]

Chemically modified electrodes are attractive tools for the detection of pharmaceutical compounds due to the high sensitivity, fast response, and low cost. Some of the used electrochemical techniques for measuring of DP concentration include the conducting polymer matrix/gold nanoparticles,[9] gold electrodes/*N*-

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acetylcysteine,[10] zinc oxide composite/glassy carbon electrode,[11] nanoparticles,[12] carbon nanotubes,[13] homogeneous mediators[14] and heterogeneous mediators.[15] Heterogeneous mediator is of particular interest in comparison with homogenous mediator because of high selectivity, environmental advantages, green chemistry and the reusability of these mediators.[16]

In this work, a heterogeneous catalyst based on catechol grafted glassy carbon electrode (CA/GCE) was used as a modified electrode for determination of DP. By means of catechol nitration by the nitrous acid and electroreduction of generating nitrocatechol, one can modify the electrode, which leads to catechol grafting.[17] We also showed the glassy carbon electrode (GCE) modified with electroactive catechol groups has the ability of dopamine determination in human blood.

EXPERIMENTAL

Apparatus

For electrochemical experiments, a potentiostat /galvanostat μAutolab model PGSTAT 204 (Eco Chemie, Utrecht, Netherlands) with NOVA 1.9 software NOVA was used. A 3-electrode cell was used with an Ag/AgCl electrode (KCl, 3 M) as the reference electrode. All potentials in the paper are expressed versus this reference electrode. For the working electrode, a modified GCE was used, and for the counter electrode, a Pt wire was used. The Ag/AgCl electrode and Pt wire were obtained from Metrohm, and the glassy carbon electrode was obtained from the AZAR Electrode. Moreover, the Metrohm model 827 pH/mV meter was used, for pH measurements. SEM (the scanning electron microscopy) was used for morphological specify the modified electrode with the electrografting.

Chemicals

Catechol (ReagentPlus®, ≥99%), sodium nitrite (ACS reagent, ≥97.0%) was obtained from Sigma-Aldrich. Other chemicals, however, were of analytical reagent grade. The DP was of analytical grade (Merck). Through the dissolving of an optimally precise amount of DP, a 1.0×10^{-2} mol L^{-1} stock solution was freshly prepared.

The optimal stock solution was sequentially diluted with distilled water for the preparation of other fresh solutions. For all the experiments, 0.1 M phosphate buffer solution was used with different pH values. All the electrochemical studies were conducted at 25±1 °C.

Electrografting of electrode surface

The published paper[15] was used as a guiding model for electrografting of the glassy carbon electrode. By means of a piece of cloth, the glassy carbon electrode was polished with an alumina fine powder (0.05 μm) in water slurry. Then, it was ultrasonically washed in ethanol and water. Then, the GCE was modified by the potential cycling ranging from 0.0 to –0.9 V in an aqueous solution which had HCl (pH 1.0), catechol and $NaNO_2$.

In all cases, the room temperature was used for the modification of GCE electrode with catechol (CA/GCE) in an electrolytic solution. The cyclic voltammetry in aqueous buffered solutions was used for the quantification of the catechol modified GCE redox activity.

Analytical procedure

In order to obtain a steady cyclic voltammogram in phosphate buffer (pH 8.0), the activation of CA/GCE by cycling between –0.3 and 0.4 V was used. Then, the electrodes were

dipped into a DP and buffer (pH 8.0) solution in a usual experiment. At a scanning rate of 20 mV s^{-1}, the potential was swept from –0.3 to 0.4 V. This was repeated while DP was presented as the sample solution.

Preparation of real samples

DP (2 ml, 0.01 mol L^{-1}) spiked in the blood serum sample was centrifuged, and the supernatant was diluted 50 times with water. Using the standard addition method, finally, 3.0 ml of the solution plus 7.0 ml of the phosphate buffer (pH 8.0) were used for the analysis. By means of the calibration curve method obtained from relevant calibration equations, quantitations were performed.

RESULTS AND DISCUSSION

Characterization and electrochemical behavior of the CA/GCE

Electrografting of glassy carbon electrode with catechol was performed as described in the experimental section. The cyclic voltammograms of CA/GCE in aqueous phosphate buffer (pH 8.0) at different scan rates are shown in Fig. 1. They show an anodic peak (A_1) in the positive-going scan and its corresponding cathodic peak (C_1) in the negative-going scan. These peaks belong to the oxidation/reduction of grafted catechol/*ortho*-benzoquinone redox couple[17] (Scheme 1). The plot of log I_{A1} *versus* log v in the range of 25 to 1000 mV s^{-1} is found to be linear with a slope of about one (Fig. 1, inset), which is a characteristic for the adsorption-controlled electrode process.[18]

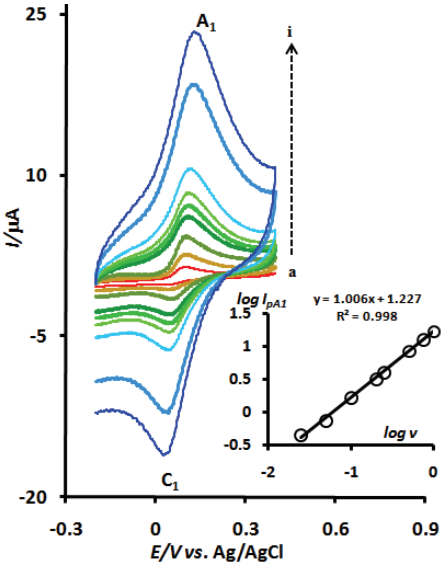
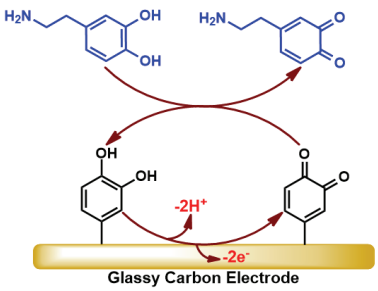


Fig. 1. Cyclic voltammograms of CA/GCE in a phosphate buffer solution (c = 0.2 M, pH 8.0) at different potential scan rates: a) 20, b) 50, c) 100, d) 200, e) 250, f) 400, g) 500, h) 750 and i) 1000 mV s^{-1}. Inset: corresponding plot of log I_{pA1} *vs.* log v. t = 25±1 °C.

The effect of the solution pH on the CA/GCE was studied in the next step (Fig. 2). The oxidation as well as the reduction peak potentials were found to shift to the negative potentials in the pH range 3–9 under study, with the depend-

ence being at –58 mV/pH unit. This is expected because of the participation of two electrons and two protons in the oxidation of catechol to *ortho*-benzoquinone.[17]



Scheme 1. The mechanism of electrocatalytic oxidation of Dp on CA/GCE.

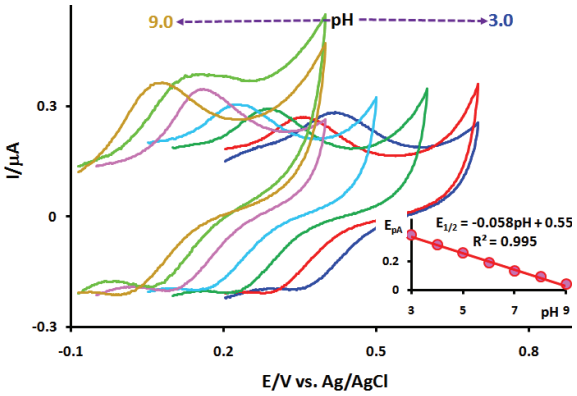


Fig. 2. Cyclic voltammograms of CA/GCE immersed in phosphate buffer solution at different pH values in the range of 3–9. Scan rate 20 mV/s. Inset: variation of anodic peak potential *vs*. pH; t = 25±1 °C.

Cyclic voltammograms of the bare GCE and CA/GCE in presence of DP at pH 8.0 and at a scan rate of 20 mV s^{-1} are shown in Fig. 3, curves b and c, respectively. Comparison of the behaviour of bare GCE and of the electrografted glassy carbon electrode (CA/GCE) shows that the anodic peak current (I_{pA1}) is significantly greater at the latter. On the other hand, with increasing the potential scan rate, the peak current ratio (I_{pC1}/I_{pA1}) increases because the time required for the reaction of DP with grafted *ortho*-benzoquinone is not enough (Fig. 4).[17] Scheme 1 shows how a catalytic reaction occurs after the process of the electron--transfer.

The influence of pH on the current response of a DP (0.1 mM) at CA/GCE in a phosphate buffered solution of was investigated in the pH range from 3.0 to 9.0 by the linear sweep voltammetry (Fig. 5). As can be seen, the oxidation peak current (I_{pA1}) increases gradually with the increasing pH up to pH 8.0 and then decreases. By increasing pH of the solution the anodic peak potential (E_{pA1}) also shifted to the negative potentials. This is expected as discussed earlier.[17]

Analytical measurements

Fig. 6 shows the cyclic voltammograms of CA/GCE in the presence of different concentrations of DP. The results showed that, by increasing the concentration of DP, the anodic peak current (I_{pA1}) increases. Under these circumstances, this dependence is linear, but in two segments: one in concentration range

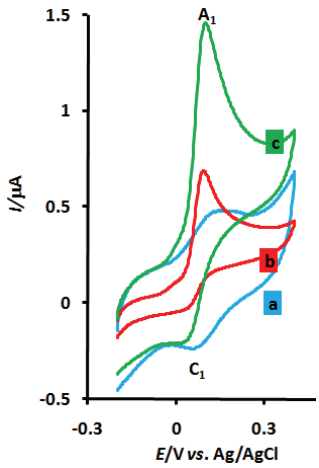


Fig. 3. Cyclic voltammograms of: CA/GCE in the absence (a) and presence of 0.1 mM DP (b) GCE; c) as b), but in phosphate buffer solution, pH 8; scan rate 20 mV/s. t = 25±1 °C.

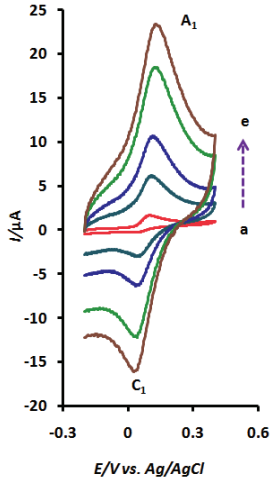


Fig. 4. Typical cyclic voltammograms of CA/GCE in the presence of DP (0.1 mM), in phosphate buffer solution (pH 8.0). Scan rates are: 25 (a), 200, 400, 750 and 1000 (e) mV/s.

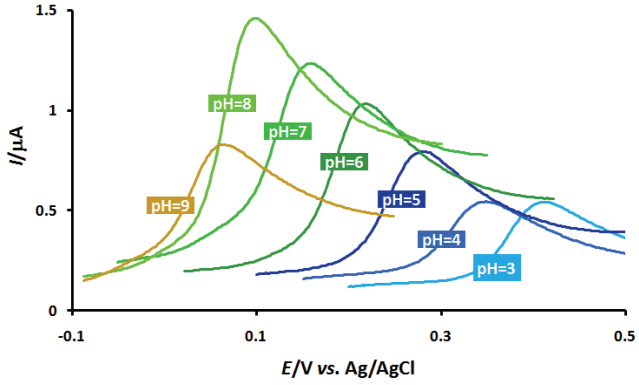


Fig. 5. Linear sweep voltammograms of CA/GCE in the presence of DP (0.1 mM) in phosphate buffer solutions with different pH values. Scan rate 20 mV/s. t = 25±1 °C.

5–100 μM (I_{pA1}/μA = 0.008[DP]/μM + 0.703) and second in the range 100–750 μM (I_{pA1}/μA = 0.003[DP]/μM + 1.248) with R^2 of 0.992 and 0.997, respectively. (Fig. 6B). The detection limit (LOD) for determination of DP was calculated according to the definition of $LOD = 3S_b/m$ (where, m and S_b are standard deviation of the blank (n = 6) and slope of the calibration graph, respectively). LOD was 0.86 μM in the lower range region. The reproducibility of CA/GCE was examined for 6 replicate measurements of 50 μM of DP. The relative standard deviation (RSD) of 2.7 % was obtained.

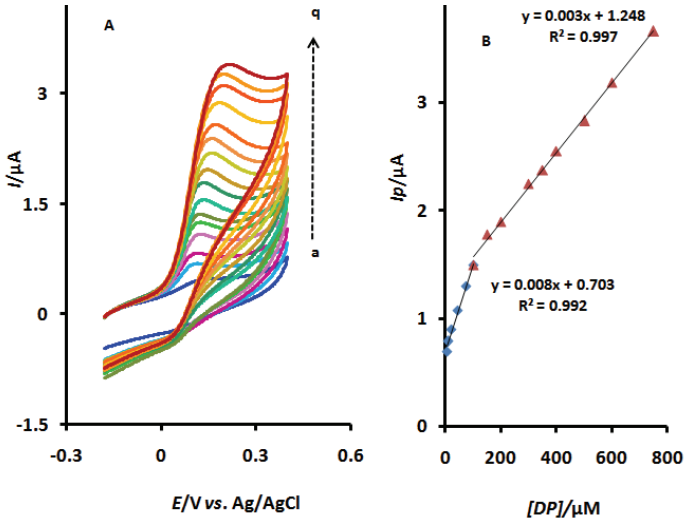


Fig. 6. A) Cyclic voltammograms of CA/GCE in the presence of different concentration of DP. Concentration of DP from a to q are: 0.0, 5.0,10.0, 20.0, 45.0,75.0,100.0,130.0,150.0, 200.0, 300.0, 350.0,400.0, 500.0, 600.0,700.0 and 750.0 µM. Scan rate: 20 mV/s. B) Plot of the anodic peak current *versus* concentration of DP; t = 25±1 °C.

Table I shows the comparison of this study and previously reported voltammetric methods for the determination of DP. As it is seen, the analytical parameters are comparable or better than the results reported for DP determination at the surface of other modified electrodes.

TABLE I. Comparison of the results of the proposed method with similar reports

Method	Electrode	Linear range, µM	Detection limit, µM	Reference
CV[a]	Cu_2O/Graphene/GCE	0.1–10	0.13	19
CV	Methionine/CPE	100–450	1	20
DPV [a]	RGO-AuNPs-CSHMs	1–200	0.3	21
DPV	TiO2/ grapheme /GCE	5–200	2	22
DPV	[c] SZP/MB composite electrode	6–100	1.7	23
DPV	Graphene /GCE	4–100	2.64	24
CV	CA/GCE	5–750	0.86	This work

[a]CV: Cyclic voltammetry; [b]differential pulse voltammetry; [c]SiO_2/ZrO_2/phosphate /methylene blue/graphite

The CA/GCE applicability was evaluated by measuring the DP concentration in human blood serum sample after the protein precipitation process. The recovery of DP from human blood serum was measured by the injection of a drug with a known amount of DP. Table II shows the results obtained by analysis of the DP content of the human blood serum samples. As Table II shows, the proposed method provides a potential tool for the determination of DP in real samples with good recoveries and good reproducibility.

TABLE II. Determination of DP in human serum samples under the optimum conditions (n = 6); Concentration sample: plasma

DP added, μM	DP found, μM	Recovery, %
50	55.0±0.3	110
10	11.5±0.4	115
5	5.6±0.7	112

The main problem in the DP determination is the difference between the DP and the simultaneous presence of such species as the ascorbic acid (AA) and the uric acid (UA). To determine the selectivity of the modified electrode, the electrocatalytic response of AA, UA and various substances (Table III) were evaluated under optimized experimental conditions in CA/GCE (Fig. 7). As can be seen, I_{pA1} in the absence (curve a) and in the presence of ascorbic acid (curve b), is nearly the same, but curve c shows the noticeable catalytic current when DP was added to the previous solution (curve c). Also, it is shown that the increasing UA to the previous solution does not influence on the I_{pA1} (curve d). The effects of various substances on DP determination (50 μM) were evaluated under optimal conditions. The tolerance limit was taken as the maximum concentration of the interfering substance that caused an error less than 5 % for determination of DP. We observed no significant interference up to 100 μM (Table III).

TABLE III. Interference of some foreign species on the determination of 50.0 μM DP under the optimized conditions.

Foreign spacies	Tolerant limits ($W_{substance}/W_{DP}$)
Ascorbic acid, sucrose, glucose, sodium benzoate, fructose	100
Uric acid	1000

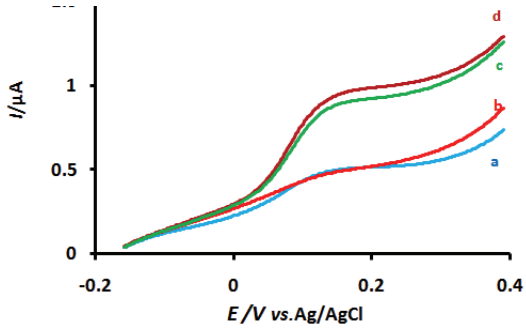


Fig. 7. Linear sweep voltammograms of CA/GCE: in the absence of AA (curve a) and in the presence of AA (0.1 mM) (curve b). c) Cyclic voltammograms of CA/GCE: in the presence AA (0.1 mM) and DP (0.05 mM). d) Cyclic voltammograms of CA/GCE: in the presence AA (0.1 mM), DP (0.05 mM) and UA (1 mM) aqueous solution containing phosphate buffer (c = 0.2 M, pH 8.0). Scan rate 20 mV/s.

CONCLUSIONS

In this study, we have prepared a new modified CA/GCE for DP determination. The modification of the glassy carbon working electrode with catechol has significantly improved the electrochemical response for DP determination.

Under the optimum conditions, the cyclic voltammetry of DP at the CA/GCE exhibited two different linear of calibration curves in the concentration range of DP of 5–100 μM and 100–750 μM with a detection limit of 0.86 μM in the lower range region. The modified electrode has been successfully applied as a new sensitive and selective sensor for detection of DP in real samples such as blood serum samples, spiked with low concentrations of the drug.

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ИЗВОД

ВОЛТАМЕТРИЈСКО ОДРЕЂИВАЊЕ ДОПАМИНА НА ЕЛЕКТРОДИ ОД СТАКЛАСТОГ УГЉЕНИКА ЕЛЕКТРОХЕМИЈСКИ МОДИФИКОВАНОЈ КАТЕХОЛОМ

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У раду је коришћењем цикличне волтаметрије као дијагностичке технике испитано понашање допамина на електроди од стакластог угљеника, чистој и електрохемијски модификованој катехолом. Резултати показују да стакласти угљеник модификован катехолом има високу активност за допамин. Волтаметријски пикови допамина на овој електроди показују две линеарне области, прву између 5,0 и 100 μM и другу између 100 и 750 μM, уз границу детекције од 0.86 μM. У шест поновљених мерења са раствором допамина концентрације 50 μM релативна стандардна девијација је износила 2,7%. Ова метода је успешно коришћена за одређивање допамина у узорцима серума.

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