Cheap and easy modification of glassy carbon electrode for voltammetric determination of dopamine in the presence of ascorbic acid

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ABSTRACT

Background and the purpose of the study: Different methods have been proposed to modify glassy carbon electrode in order to determine dopamine (DA), as one of the most important neurotransmitters in central nervous systems of mammalian. These methods are time comsuming and in some cases expensive. In this work, a very simple and cheap pretreatment method is developed for the bare glassy carbon electrode (GCE) to determine DA in the presence of Ascorbic acid (AA).

Methods: Cyclic voltammetry as an electrochemical activation procedure was used for activation of glassy carbon electrode in order to separate diffrential pulse peaks of DA and AA. The effect of different parameters such as pH for supporting electrolyte, range of potential and the number of cycles were investigated. Finally, differential pulse voltammetry was used to determine DA in the presence of AA.

Results: On the activated electrode under optimum condition, anodic peak of AA shifted to negative potentials and peak current decreased, but the peak current of DA increased. The peak current was linearly proportional to the bulk concentration of DA in the range of 6.5×10^{-7}-1.8×10^{-5} mol l^{-1}. The limit of detection was 6.2×10^{-7} mol l^{-1}.

Conclusion: A simple and cheap method was developed for the activation of glassy carbon electrode. It was possible to determine DA in the presence of AA on the treated electrode. The proposed method was used to determine DA in pharmacutical samples.

Keywords: Electrochemical determination, Activated electrode, Differential pulse voltammetry

INTRODUCTION

Dopamine (DA) is one of the most important neurotransmitters in the central nervous systems. Since like other catecholamines, dopamine is oxidized easily, its detection is possible by electrochemical methods (1-3). However, a major problem for electrochemical detection of DA in real biological matrices is the coexistence of ascorbic acid (AA). Since DA and AA are oxidized at nearly the same potential, as a result of overlapped voltammetric response (4, 5) the selectivity of the determination of DA decreases. Several methods have been applied to overcome the problems with selectivity. One of these methods is to cover the electrode surface with an appropriate permselective coating such as nafion (6) which is known to incorporate positively charged molecules and repels anionic ones due to its ion-exchange properties. Another method is to cover the electrode surface with two layers. The first layer is an electroactive material exhibiting catalytic activity toward dopamine and the second is nafion. This combination increases selectivity and sensitivity

of DA's detection (7). Another strategy is based on the modification of electrode by electrosynthesized polymeric film (8). The use of a specific procedure such as UV/ozone for electrode pretreatment (9) and electrochemical preanodization that leads to the differentiation of oxidation signals has been also successful. This approach is especially suited for carbon-based electrodes (10, 11). It has been shown that this type of treatment leads to a disruption of normal surface in which the oxygen proportion increases formatin of a graphitic oxide film. It is usually assumed that the surface functional groups generated during the activation process are phenolic, quinonic, carbonylic and epoxidic-like, which are immobilized and covalently bonded to the surface (12). Some of these groups are anionic groups and have high density of electron (13). Therefore the anionic analytes are repelled by the electrode while cationic analytes are attracted. At biological pH (pH 7) while AA exists in anionic form and is repelled from the electrode (14), DA exists in its protonated form and as a result there is an attraction between DA and the electrode. Thus

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the peak current of AA decreases while DA's peak current increases.

It has been shown that electron transfer kinetics of several reactions are affected by electrochemical activation at glassy carbon electrodes (15). Electrochemical activationn has been applied for differentiation voltammetric signals of ascorbic acid and dopamine at a graphite/ epoxy electrode (16). Also this method has been applied for detection of catechol derivative (10).

In this work, a new electrochemically activation procedure based on the modification of the sureface of GCE is described. The proposed electrode is very easy to fabricate and eco-friendly and re-usable for multiple time analysis. Also, it is novel for differentiation of oxidation signals of AA and DA and is employed for determination of DA in the presence of AA.

MATERIAL AND METHODS

All reagents were analytical grades (Merk) and used without further purification. Deionized water was used to prepare all solutions. All experiments were performed at room temperature and dissolved oxygen was not removed. Dopamine hydrochloride solutions were prepared daily and stored in the refrigerator. The electrochemical experiments were carried using an Autolab electrochemical analyzer (PGSTAT 20, Ecochemie, Netherlands) that connected to a PC for control and data storage. A glassy carbon electrode (2.00 mm in diameter) was used as a working electrode. The counter electrode was a platinum bar electrode. All potentials reported in this paper are referenced to a calomel electrode (SCE).

For the preparation of activated GCE, A glassy carbon electrode was successively polished with 0.5 μm alumina slurry and rinsed with deionized water prior to its activation. The electrode was placed in phosphate buffers (0.1M, pH 6) and activated by cyclic voltammetry. The potential range was 1.5-2 V in cyclic voltammetry and the number of cycles was 10 scans. All measurements were carried out in phosphate buffer (0.1M, pH 6) using differential pulse voltammetry (DPV). Differential pulse voltammograms were recorded in potential range of 0.0-0.40 V. The pulse amplitude was 10 mV.

About 0.1 ml of injection sample (Dopamine hydrochloride, Pharmacutical development Co., Rasht, Iran) was accurately pipeted in 100.0 ml volumetric flask and diluted to mark. Then 2.0 ml of diluted sample and 8.0 ml portion of buffer (phosphate, pH 6) were transferred to electrochemical cell and differential pulse voltamogram was recorded in the potential range of 0.0-0.4 V. Following of the addition of different amounts of standard solution of dopamine, differential pulse voltamograms were recorded. Finally, standard addition calibration curve was plotted.

RESULTS AND DISCUSSION

Electrochemical behavior

Differential pulse voltammogram (DPV) of 3×10^{-3} mol l^{-1} of AA and 3×10^{-5} mol l^{-1} of DA on the glassy carbon electrode in phosphate buffer of pH 6 is demonstrated in figure 1. As it can be found, the anodic peak current of DA and AA overlapped at the bare GCE. It is commonly difficult to obtain separate voltammetric waves for AA and DA at bare GCE because both of them are oxidized at very closed potentials. After activation, the oxidation potential for AA shifted to negative potentials and two well-resolved anodic peak were present at the activated GCE. The oxidation of ascorbic acid may be electocatalyzed at activated electrode so the first oxidation peak moved toward negative potentials. On the other hand, the electrocatalysing oxidation of dopamine may be negligible so after activation the second oxidation peak remained the same as before. The other drastic effect of the electrode activation was an increase in the relative sensitivity of DA/AA, judged by peak currents. The peak current of AA decreased while DA's peak current increased greatly. To determine the optimum conditions (high peak current for DA and enough peak separation between DA and AA) of the activation of GCE, different parameters must be investigated and optimized. As far as the electrode conditions was concerned, the scanning range and the number of scan were considered. In order to ensure about the oxidation of any impurity in electrode surface, positive potential must be selected in activation step and the scanning range was selected between 1.5-2V. Another important parameter is the number of scans that is used for activation. As can be seen in figure 2 after 10 scans, voltamogram of two compounds are separated. As shown in figure 3 DA's peak current increased by increase in the scan number until 10 cycles, but AA's peak current decreased by increase in the scan number. As a result 10 cycles was selected as optimum numbers. Also the effect of pH and composition of supporting electrolyte was investigated. The optimum peak separation was obtained by using phosphate buffer (pH 6) as supporting electrolyte during the activation process. Cyclic voltammograms of dopamine on activated GCE under optimum conditions with different scan rate are shown in figure 4A. A redox couple was observed for this system. The peak potential shifted to the more positive values as the sweep rate increased, suggesting involvment of a kinetic limitation in the reaction between activated GCE and DA (anodic peak potential increased linearly with logarithm of the scan rate, Fig. 4B). In order to check the mechanism of the reaction, the peak current was plotted against the square root of the scan rate (Fig. 4C). The linear ralationship between these parameteres indicate that the sufficient overpotential
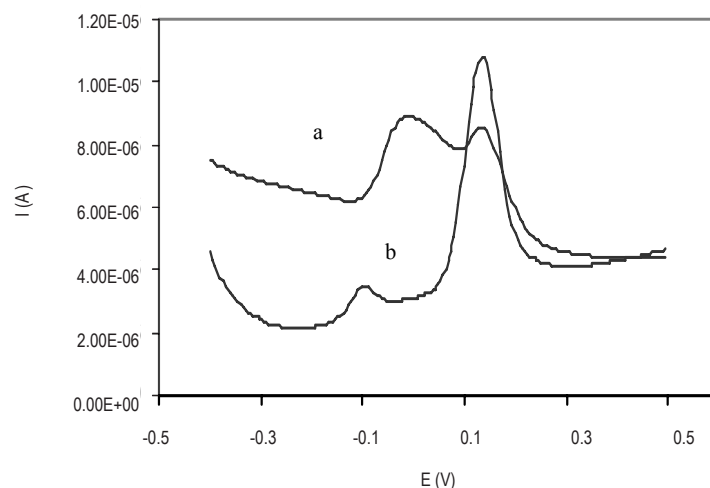
Figure 1. Differential pulse voltammograms of 3×10^{-3} mol l^{-1} of Ascorbic Acid (AA) and 3×10^{-5} mol l^{-1} of Dopamine (DA) obtained at, a: bare and b: actived Glassy Carbon Electrode (GCE).

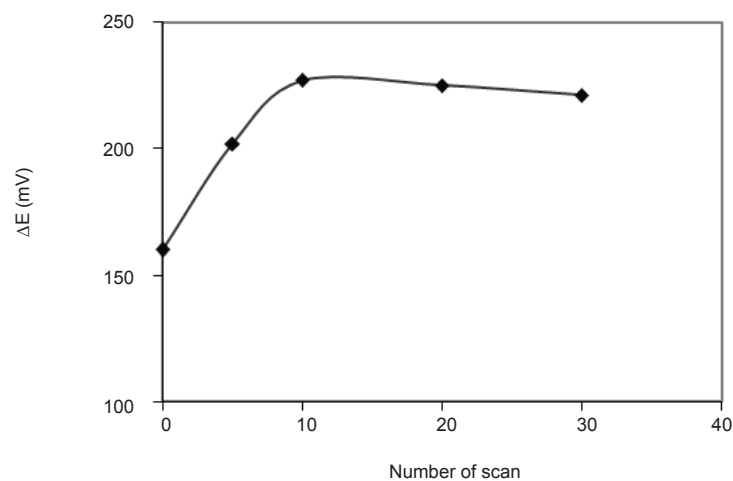**Figure 2.** Peak Separation potential ($\Delta E_p = E_{DA}-E_{AA}$) of 3×10^{-5} mol l^{-1} of Dopamine (DA) and 3×10^{-3} mol l^{-1} of Ascorbic Acid (AA) versus scan number of activation step.
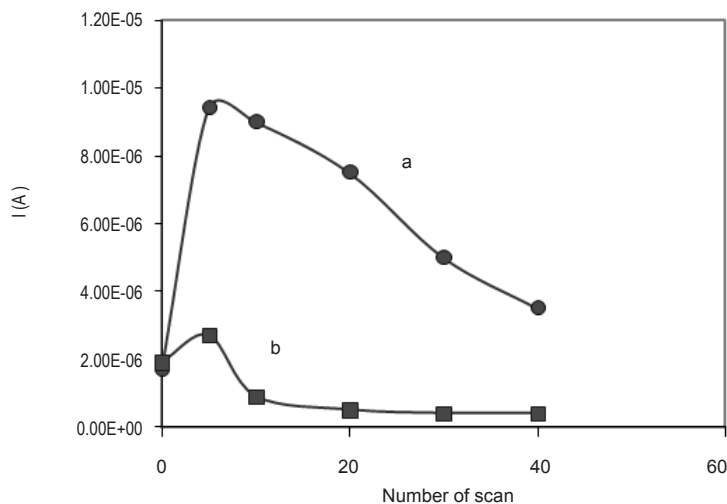
Figure 3. a: Peak current of 5×10^{-6} mol l^{-1} of Dopamine (DA), b: 3×10^{-3} mol l^{-1} of Ascorbic Acid (AA) versus scan number of activation step.
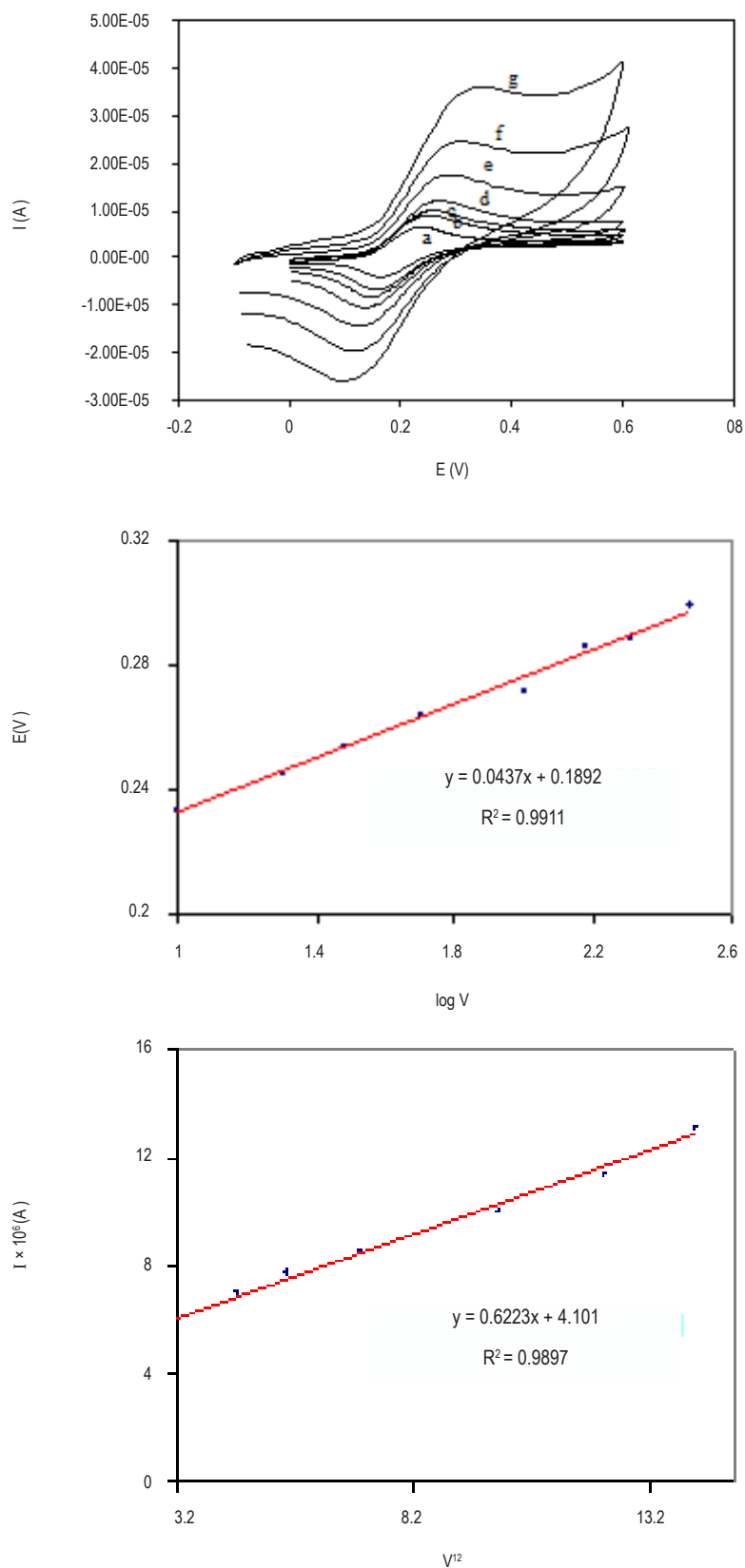
Figure 4. A : Cyclic voltammograms of 1×10 $^{-3}$ M mol l^{-1} of Dopamine (DA) at activate electrode with different scan rates a: 10, b: 20, c:30, d:50, e:100, f:150 and g:300 mV/S) B: anodic peak position of Dopamine (DA) vs. logharitm of scan rate C: anodic peak current of Dopamine (DA) vs. square root of scan rate.
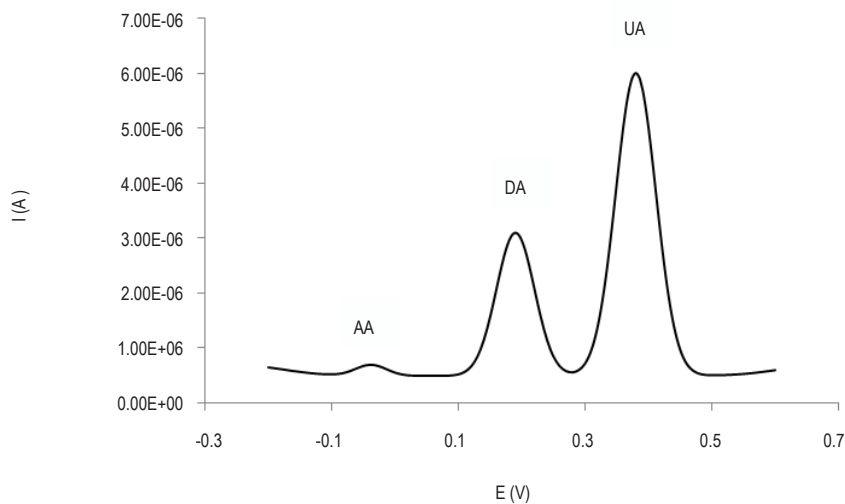
Figure 5. Differential pulse voltammograms of solution contained 1×10^{-4} mol l^{-1} of Uric Acid (UA), 5×10^{-6} mol l^{-1} Dopamine (DA) and 1×10^{-3} mol l^{-1} Ascorbic Acid (AA) obtained at actived Glassy Carbon Electrode (GCE).
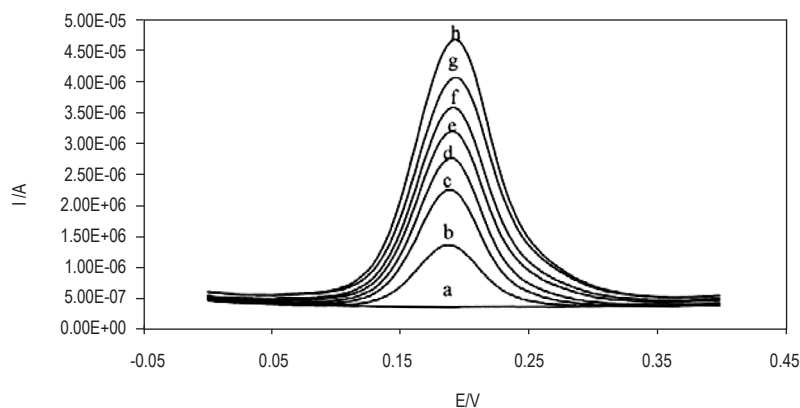

Figure 6. Differential pulse voltammograms a: supporting electrolyte, b: 5000 times diluted injection sample and c-h: after addition different amount of standard of Dopamine (DA) at activated Glassy Carbon Electrode (GCE).
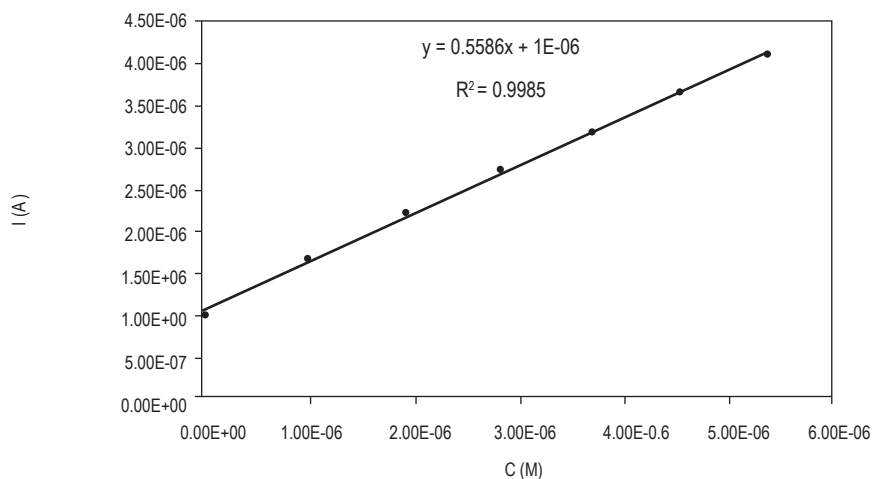

Figure 7. Standard addition calibration curve for diluted injection sample.

Table 1. Analytical performance data of the present and some previously reported methods for determination of dopamine.

Method	Linear range	Limit of detection	Ref.
Present work	$1.8 \times 10^{-5} - 6.5 \times 10^{-7}$M	6.2×10^{-7}M	-
Amperometric on over-oxidized polypyrrole-plant tissue composite	$1.1 \times 10^{-3} - 9.9 \times 10^{-6}$ M	3×10^{-6}M	(2)
Voltammetry at nanobiocomposite modified carbon-ceramic electrode	$1.2 \times 10^{-3} - 5 \times 10^{-6}$ M	3.41×10^{-6}M	(3)
Voltammetry on graphite nanosheet-nafion composite film modified electrode	$7 \times 10^{-5} - 5 \times 10^{-7}$ M	2×10^{-8} M	(6)
Voltammetry on poly(orthanilic acid)-multiwalled carbon nanotubes composite film-modified glassy carbon electrode	$9.0 \times 10^{-6} - 4.8 \times 10^{-7}$M	2.1×10^{-7}M	(17)

of the reaction is limited by diffusion.

Differential pulse voltammetry (DPV) which has been used as sensitive electrochemical method of the analytes (16-18) was employed in this study. Under optimum conditions, the catalytic peak current was linearly related to concentration over the range 6.5×10^{-7} - 1.8×10^{-5} mol l^{-1} with correlation coefficient of 0.9995. The detection limit calculated from the calibration curves for DA was 6.2×10^{-7} mol l^{-1}. To characterize the reproducibility of the method, repetitive measurement- regeneration cycle were carried out at concentration of 5×10^{-6} mol l^{-1} of DA. The results of 8 successive measurements showed a RSD <3.4%.

A comparison of the anlalytical charactristics of the proposed method with other reports (Table 1) showed that they are comparable but the proposed method has the advantage of simplicity of fubricating of electrode.

Uric acid is one of the main interferents in determination of dopamine using electrochemical methods where exhibits overlapping oxidation peaks at solid electrodes. Using the proposed method, oxidation peaks of these compounds are separated (Fig. 5) so it is possible to determine dopamine in the presence of uric acid without any interfering.

This method was applied for determination of DA in dopamine hydrochloride injection sample. In order to fit into linear range, the sample used for analysis was diluted 5000 times. To ascertain the correctness of the results, the samples were spiked with certain amount of DA at about the same concentration which was found in the sample. To remove the effect of matrix in determination of analyte, standard addition method was used. Figure 6 shows the voltammogram of solutions after addition of standards and Figure 7 shows standard addition calibration currve. The mean recovery and relative standard deviation for spiked samples were 95.55 and 1.8% , respectively.

CONCLUSION

In this work, a new voltammetric method was developed to pretreat the surface of glassy carbon electrode. The proposed method has many advantages such simplicity, cheapness and rapidness. The activated electrode showed very good selectivity to determine dopamine in the presence of ascorbic acid and uric acid.

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