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Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science 4(3) (2016) 250-256

Electrochemical Determination of Dopamine and its Simultaneous Resolution in the Presence of Uric Acid at Poly(Pyrocatechol Violet) Modified Glassy Carbon Electrode: A Voltammetric Study

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ABSTRACT

The present techniques describe sensitive determination of dopamine (DA) at the surface of newly established poly(pyrocatechol violet) modified glassy carbon electrode (poly(PCV)MGCE). The developed electrode was characterized using electrochemical impedance spectroscopy. The modified electrode showed a synergic and very remarkable electrocatalytic performance in sensing of DA at pH 5.0. Simultaneous determination of DA in the presence of uric acid (UA) is validated. The modified chemical sensor was easy to fabricate, very stable and reusable, and free of interference from UA. The practical analytical application of poly(PCV)MGCE toward the determination of DA was successfully demonstrated.

Key words: *Dopamine, Cyclic voltammetry, Dopamine, Differential pulse voltammetry, Electrochemical impedance spectroscopy, Pyrocatechol violet, Simultaneous determination.*

1. INTRODUCTION

Dopamine (DA) is an important neuromodulator that belongs to catecholamine group and plays a very important role in the central nervous, renal, hormonal, and cardiovascular systems. It has been disturbed in many neurological disorders such as schizophrenia and Parkinson's disease due to abnormal concentration levels [1,2]. The monitoring of concentration of DA has a great impact to examine the several neurological disorders; therefore, a robust research is needed to develop rapid and simple methods for the determination of DA. Uric acid (UA) (2, 6, 8-trihydroxypurine, UA) is the end metabolic product of purine metabolism and is existing in blood and urine. However, abnormal levels of UA caused many diseases such as gout, hyperuricemia, and Lesch-Nyhan [3]. In general, DA and UA are coexisting substances in real biological fluids and are oxidized at similar potential at the conventional electrodes. The coexisting of their voltammetric responses makes their simultaneous resolution highly difficult [4]. Therefore, their simultaneous determination is speciously in the diagnosis and treatment of diseases.

Over the past decade, several analytical methods have been employed for the determination of DA such as fluorescence [5], spectrophotometry [6,7], and chromatography [8-11]. However, these methods suffer from certain disadvantages such as high cost, over analysis time, in some cases poor selectivity and low sensitivity. Meanwhile, electrochemical methods have received great importance due to their good sensitivity, often associated with high selectivity, low cost, rapidity, and precision for quantification of important compounds in biological and clinical point of view [12-15]. In electroanalytical techniques, the redox behavior of the analyte occurs at high over potential owing to the slow electron transfer rate at conventional electrodes (such as glassy carbon electrode [GCE], Au, and Pt). Thus, one promising approach to overcome this problem through modification of electrodes using various materials. Especially, electropolymerization of organic dyes is one of interesting approach due to the polymeric film has good stability and reproducibility [16,17].

Recently, polymer modified electrodes have attained a great importance. In addition, the electropolymer film-coated electrodes with organic dyes exhibit high stability, more active sites, reproducibility and homogeneity in electrochemical deposition [18,19]. Numerous redox dyes are available as artificial

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electron donors [20]. Pyrocatechol violet (PCV) (4-[3-(3,4-dihydroxyphenyl)-1,1-dioxo-2,1λ6-benzoxathiol-3-yl]benzene-1,2-diol) is a complexometric indicator dye and is capable to undergo electropolymerization in aqueous solutions and produces redox stable active layers. Hence, it can be used as a modifier for sensing different biologically important compounds.

The present study describes the quantification of DA at poly(PCV) modified glassy carbon electrode (MGCE) by cyclic voltammetry (CV) technique. The modified electrode showed better sensitivity and selectivity toward the determination of DA at low-level concentration and in the presence of UA. Literature survey resulted that no examination has been done toward the determination of DA at poly(PCV)MGCE. The practical application of poly(PCV)MGCE toward the determination of DA was successfully demonstrated.

2. EXPERIMENTAL

2.1. Chemicals

DA, UA, and PCV were from Sigma-Aldrich. All of them were used without any further purification. The stock solution of 10 mM DA was prepared and stored in a refrigerator, and working solution was prepared by diluting the stock solution with buffer solution. 0.1 M phosphate buffer solution (PBS) was prepared from $NaH_2PO_4.2H_2O$ and Na_2HPO_4. All reagents are of analytical grade and used without purification.

2.2. Instrumentation

CHI 1200 electrochemical analyzer was used for the measurements of CV, and CHI 660D electrochemical workstation was used for the measurements of electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). A conventional three-electrode system was employed, which consists of a modified/unmodified GCE as working electrode; saturated Ag/AgCl electrode as reference electrode to measure cell potentials and platinum wire as an auxiliary electrode to measure current. Elico U 120 pH meter combined with pH CL 51 B was used to get pH values.

2.3. Preparation of Poly(PCV)MGCE

The GCE was polished on polishing leather pads with 0.3 and 0.05 μm of alumina suspension to get a mirror shine and cleaned thoroughly with distilled water successively. The poly(PCV) film was formed on the electrode surface by continuous potential cycling between +1.5 and −1.5 V by CV. The polymerized electrode was cleaned with water to remove physically absorbed material. The resulted electrode was abbreviated as poly(PCV)MGCE (Figure 1).

3. RESULT AND DISCUSSION

3.1. Electrochemical Impedance Study of Poly(PCV) MGCE

EIS is the most emerging analytical tool for the characterization of surface nature of the electrode, and it gives the specific information about the nature of the surface. At present, we have studied the electrochemical impedimetric behavior of bare and poly(PCV)MGCE at 1 mM $[Fe(CN)_6]^{3-}$ in 1 M KCl. Figure 2A represents bode plot for the impedimetric behavior of the two different electrodes which depicts that the poly(PCV)MGCE (indicated with blue color) has less value when compared to bare GCE (indicated with red color). Figure 2B shows the Nyquist plot of bare and poly(PCV)MGCE, which clearly gives the information about charge transfer resistance (R_{ct}). From Figure 2B, poly(PCV)MGCE has less R_{ct} value when compared to the bare GCE, indicating the good electron transfer nature at the surface of poly(PCV)MGCE. In the Nyquist plot, the diameter of the semicircle at high frequency corresponds to the magnitude of the charge transfer resistance (R_{ct}). This value significantly varies based on the modification of the electrode surfaces [21].

3.2. Electrochemical Investigation and Electrochemistry of DA

Figure 3 shows the cyclic voltammograms of 1 mM DA in 0.1 M PBS (pH 5.0) at a scan rate of 0.1 V at GCE and poly(PCV)MGCE. A pair of redox peaks was observed with the oxidation peak potential about 0.407 V and the reduction peak potential about −0.020 V at GCE. However, at poly(PCV)MGCE, a well-defined redox peaks of DA was observed with the anodic and cathodic peak potentials at 0.178 V and 0.141 V, with enhanced peak currents. It signifying that more reversible electron transfer process at oxidation of DA at poly(PCV)MGCE. The peak to peak separation potentials at poly(PCV)MGCE, (ΔEp)

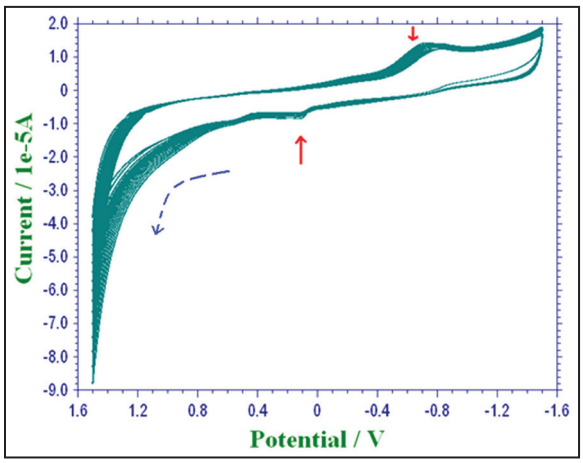


Figure 1: Cyclic voltammograms for the electrochemical polymerization of pyrocatechol violet at glassy carbon electrode.

was 37 mV, which was on accordance with a Nernst reversible behavior. The shifting of Ep_a toward less positive potential, Ep_c toward more positive potential, and enhancement in redox peak currents showed the electrocatalytically activity of poly(PCV) MGCE toward oxidation of DA. The overall redox mechanism of DA could be explained at poly(PCV)MGCE as illustrated in Scheme 1.

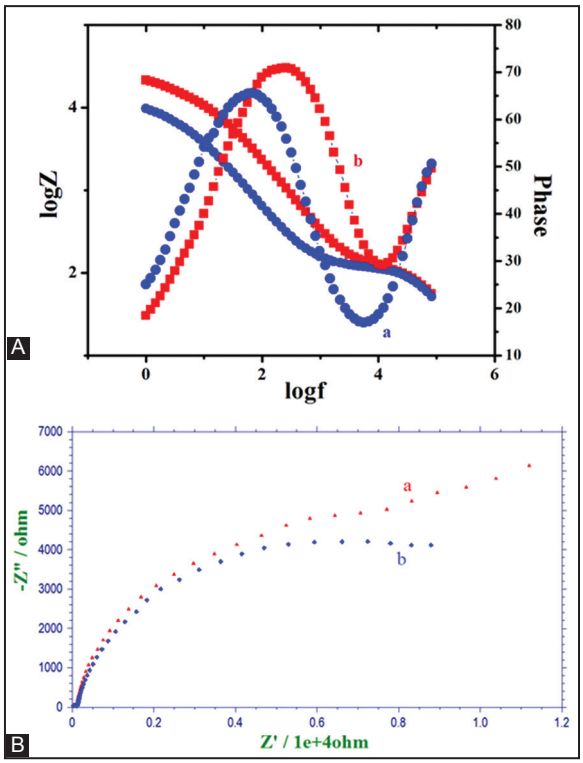


Figure 2: (A) Electrochemical impedance spectroscopy (EIS) spectrum bode plot. (B) EIS spectrum nyquist plot 1 M KCl solution containing 2.5 mM $[Fe(CN)_6]^{-3/-4}$ at (a) glassy carbon electrode, (b) poly(pyrocatechol violet) modified glassy carbon electrode.

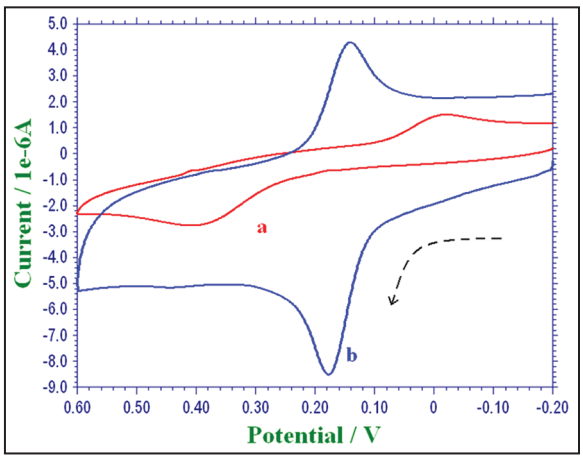
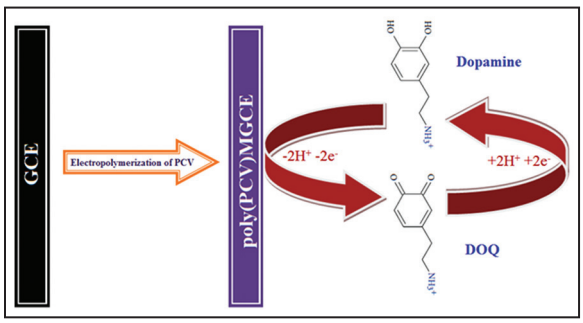


Figure 3: Cyclic voltammograms for the electrochemical response of 1 mM dopamine at (a) bare glassy carbon electrode, (b) poly(pyrocatechol violet) modified glassy carbon electrode in 0.1 M phosphate buffer solution of pH 5.0 at a scan rate of 0.1 V/s.

3.3. Effect of Solution pH

Figure 4A and B show the effect of solution pH on the electrochemical response of DA at poly(PCV)MGCE in 0.1 M PBS at different pH values (5.0-8.5). As the



Scheme 1: Schematic diagram of preparation of poly(pyrocatechol violet) modified glassy carbon electrode (poly(PCV)MGCE) and dopamine mechanism at poly(PCV)MGCE.

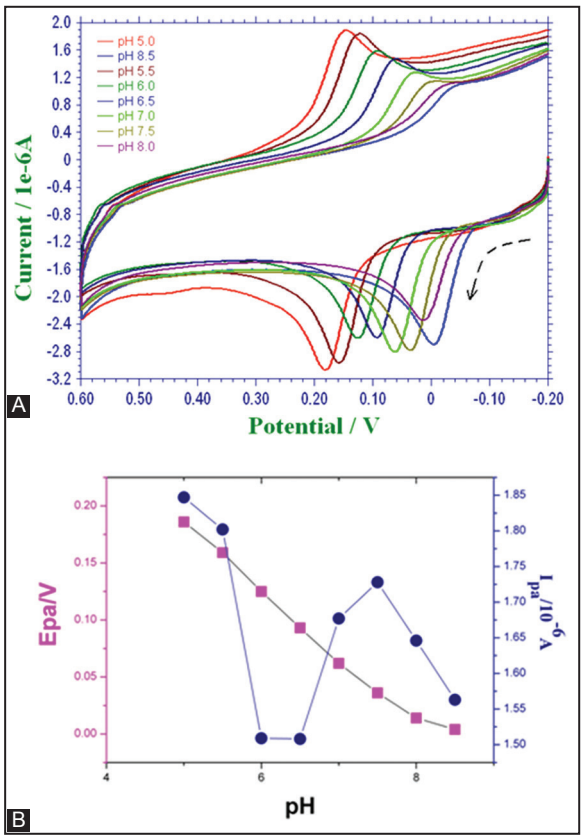


Figure 4: (A) Cyclic voltammograms obtained at poly(pyrocatechol violet) modified glassy carbon electrode in 0.1 M phosphate buffer solution containing 1 mM dopamine (DA) at different pH values. (B) A plot between DA oxidation peak potential versus pH of 0.1 M phosphate buffer solution and A plot of DA oxidation peak current versus pH.

pH of the analyte was increased, then the peak currents of redox system decreased and the maximum peak current was observed at pH 5.0. Therefore, PBS of pH 5.0 was selected for all subsequent electrochemical studies of DA. Furthermore, the anodic peak potential shifted linearly toward more negative side as the pH of the solution was increased from 5.0 to 8.5. This indicates that the participation of protons in the electrochemical oxidation of DA at poly(PCV)MGCE. A plot of pH of the solution versus anodic peak potential (Figure 4B) was constructed and was linearly connected with a linear regression equation of:

E_{pa} (V) = 0.45404-0.05469 pH (R = 0.99265).

The plot has linearity with a slope of 54 mV/pH; this value was nearly closer to the theoretical value (59 mV/pH) of the Nernstian equation for equal number of electrons and protons transfer reaction [22]. This indicates the involvement of equal number of protons and electrons in the oxidation of DA at poly(PCV)MGCE.

3.4. Effect of Potential Scan Rate

The effect of scan rate on the peak currents of DA at poly(PCV) MGCE was investigated at different scan rates at 0.1 M PBS pH (5.0) and was shown in Figure 5A. According to results, both anodic and cathodic peak currents were increased with increasing potential scan rate, and it can be seen in Figure 5B. The linear response of anodic peak current versus scan rate was Ipa (10^{-6} A) = −0.50837-0.00949 (V/s) with a correlation coefficient of 0.98823 which indicates that adsorption controlled process occurs at poly(PCV) MGCE. By increasing the scan rate of system, the anodic peak potentials of DA shifted to the more positive potential and the cathodic peak potentials to less positive potential.

3.5. Effect of DA Concentration

The dependence of anodic peak currents on the concentration of DA was carried out in PBS (pH 5.0) at poly(PCV)MGCE. Figure 6A shows that the redox peak current at modified electrode was proportional to the DA concentration which shows relationships between 15×10^{-6} to 2.0×10^{-4} M. A plot was drawn between peak currents and concentration of DA and it was shown in Figure 6B.

The detection limit and quantification limit were calculated using the formulas, LOD=3S/M and LOQ=10S/M [23-26], where S is the standard

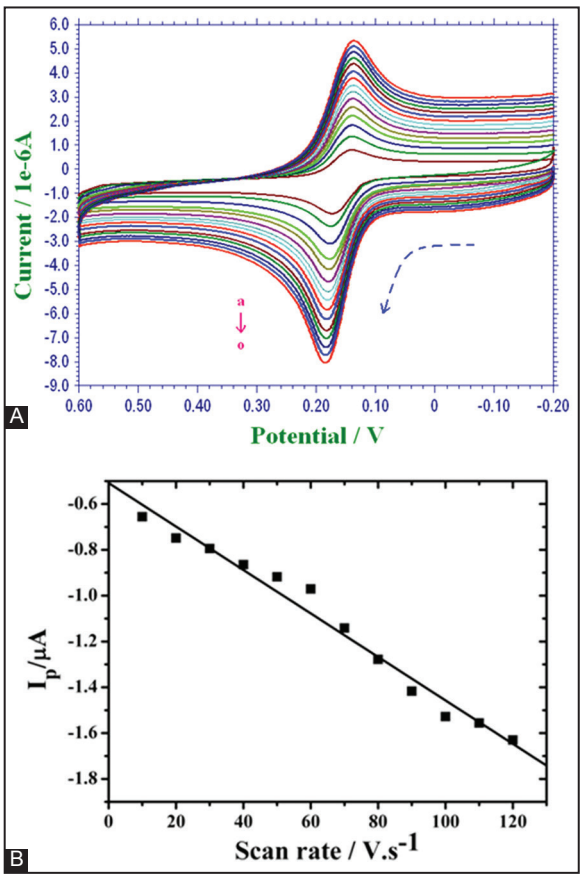


Figure 5: (A) Cyclic voltammograms of dopamine in 0.1 M phosphate buffer solution of pH 5.0 at different scan rates (a-o), 0.01-0.15 V. (B) Calibration plots for the redox peak currents versus scan rate.

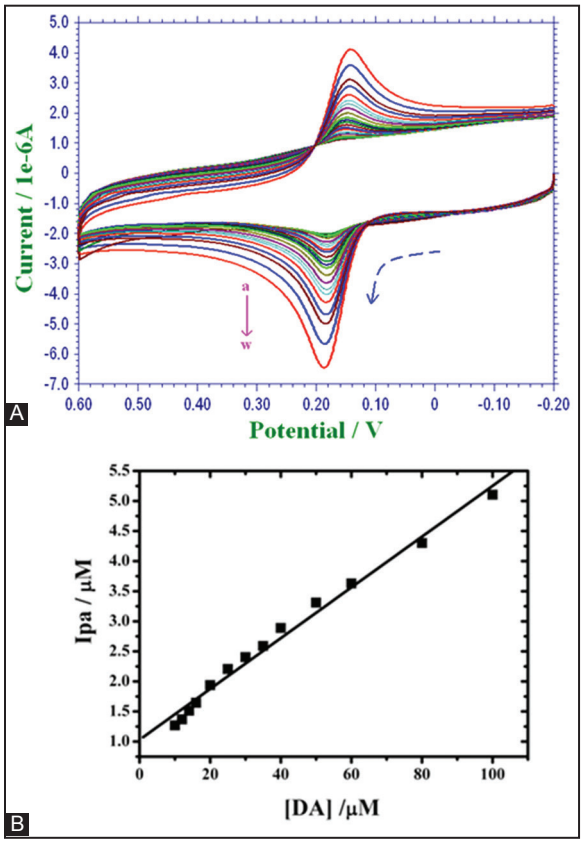


Figure 6: (A) Cyclic voltammograms of dopamine (DA) with the different concentrations in phosphate buffer solution at pH 5.0. (B) Calibration plot of DA concentration.

deviation and M is the slope obtained from five different calibration curves. The LOD and LOQ of DA were found to be 9.7×10^{-6} M and 3.2×10^{-5} M, respectively.

3.6. Stability, Repeatability, and Reproducibility of Poly(PCV)MGCE

Stability of the developed poly(PCV)MGCE was investigated for several times by examining the cyclic voltammograms of DA in PBS (pH 5.0). The CV expressed after 2nd cycle, the anodic and cathodic peak currents of DA remains constants. It was observed that activity of modified electrode was stable during the DA determination. A plot was drawn between number of multiple cycles versus I_p values; it can be seen that the measurements were almost parallel, confirming the stability of the poly(PCV)MGCE.

3.7. Simultaneous Resolution of DA and UA

The electroactive biomolecule UA has very similar oxidation potential as that of DA. Thus, there is a possibility of ascetic interference of UA with DA. Hence, it is important to explore this interference toward the determination of DA. Simultaneous determination of DA was carried out in the presence of UA using DPV technique. Figure 7 shows the voltammograms of two systems, corresponding to DA and UA and their individual anodic peak potentials were 0.034 V and 0.178 V, respectively. This corresponds to two distinct oxidation peaks due to the oxidation of DA and oxidation of UA. These results indicate that the present method can detect DA in the presence of an excess of UA.

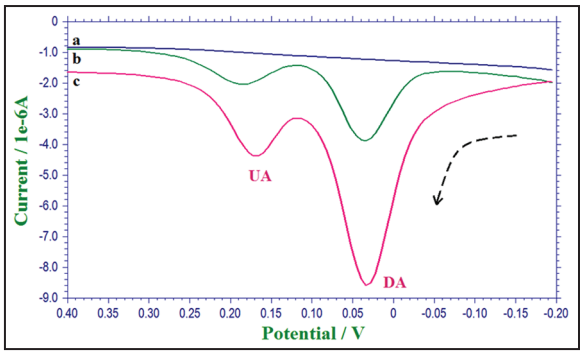


Figure 7: Simultaneous determination of dopamine and uric acid in 0.1 M phosphate buffer solution of pH 5.0 (a) blank (b) glassy carbon electrode (c) poly(pyrocatechol violet) modified glassy carbon electrode.

Table 1: Determination of DA in real samples.

Real sample	Added (µM)	Found (µM)	Recovery (%)
DA injection	30	29.0	96.6
DA injection	35	33.5	95.7
DA injection	40	39.6	99.0

DA: Dopamine

3.8. Determination of DA in Real Samples

The analytical application of the poly(PCV)MGCE was validated toward the determination of DA in an injection sample (DA hydrochloride injection solution). The DA injection sample was purchased from Sterile Specialties India (Pvt.) Ltd., with a specified content of DA 40.0 mg/mL. Injection sample was prepared approximately to get the concentration range of a standard analyte. The resultant injection solution was analyzed in 0.1 M PBS (pH 5.0) using DPV technique with a standard addition of the working analyte. The results were shown in Table 1. From the Table 1, it can be seen that the recovery and RSD values are reasonable, and thus, the modified electrode is suitable for the determination of DA in formulations.

4. CONCLUSIONS

In this study, the modification of the GCE with PCV metallochromic indicator has led to the fabrication of stable sensor, exhibit highly electrocatalytic activity to DA oxidation. The major difficulty from the interference of UA can be efficiently overcome using poly(PCV)MGCE in PBS 5.0. The proposed method can be applied for the detection of DA in pharmaceutical formulation samples.

5. ACKNOWLEDGMENT

The authors are very much thankful to the authorities of Sri Venkateswara University, Tirupati (India), for providing the necessary support toward this work.

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