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Voltammetric Resolution of Dopamine in Presence of Ascorbic Acid and Uric Acid at Poly (Brilliant Blue) Modified Carbon Paste Electrode

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Abstract

Poly (brilliant blue) modified carbon paste electrode was fabricated for the detection of dopamine in the presence of large excess of ascorbic acid and uric acid in phosphate buffer solution of pH 7.4. The redox peaks obtained at poly (brilliant blue) modified carbon paste electrode shows good electrocatalytic activity towards the oxidation of dopamine. From the study of scan rate variation the electrode process was found to be adsorption controlled. The limit of detection of dopamine was found to be 6.7×10^{-7} M. and the simultaneous study shows the good result with peak to peak separation between dopamine and other two analytes ascorbic acid and uric acid by both cyclic voltammetry and differential pulse voltammetric techniques.

Keywords: Poly (brilliant blue); Dopamine; Ascorbic acid; Uric acid; Electrocatalytic oxidation; Cyclic voltammetry; Differential pulse voltammetry

Introduction

Dopamine (DA), Ascorbic acid (AA), and Uric acid (UA) are the compounds that play a very important role in the field of biomedicine and neurochemistry. DA is an important neurotransmitter molecule of catecholamine which is widely distributed in the mammalian central nervous system for message transfer. It plays a very important role in the function of central nervous, renal, hormonal and cardiovascular system and medicament to drug addiction and Parkinson's disease [1]. AA is a water soluble vitamin that takes part in many important life processes; it has been used in the prevention and treatment of common cold, mental illness and cancer [2]. It can be chemically or electrochemically oxidized to dehydroascorbic acid [3]. UA is primary product of purine metabolism [4] abnormal levels of UA are symptoms of several disease like gout, hyperuricamia [5] enhanced urate level also leads to phenumonia and leukaemia [6]. Therefore the monitoring the levels of these compounds are of great importance.

DA, AA and UA usually coexist in biological fluids, and also the direct electrochemical oxidation of DA and AA at bare electrode is reversible and requires high potential. Moreover the DA and AA get oxidized at the same potential [7-9]. Therefore the development of various techniques for detecting the DA, AA and UA of neurotransmitters in the central nervous system has attracted much attention of researchers during the past few decades. However the methods like electrophoresis [10], chromatography [11], and chemiluminescence [12] have been employed for the detection of AA, DA and UA. Among all these methods electrochemical methods have been found great applicability [13-17]. The electroanalytical methods using modified electrodes are more promising for the simultaneous detection of these electroactive species [18-21]. In recent days the modification of electrode by the electropolymerisation technique is of great importance because of its sensitivity, selectivity and more reproducible results. There were so many reports on the electropolymerisation of N, N-dimethylaniline [22] styrene sulphonic acid [23] aminobenzoic acid [19] sulfosalicylic acid [18] and glycine [24] to modify the electrode for the detection of DA in presence of AA.

The present work describes the fabrication of stable electrode by electropolymerising coomassie brilliant blue R-250(brilliant blue) on the surface of carbon paste electrode to achieve the challenge of simultaneous determination of DA in presence of high concentration of AA and UA at physiological pH. Brilliant blue is the name of triphenylmethane dye widely used for staining proteins in the analytical biochemistry [25,26] and also used as a food colourant. Although no examination of the detection of dopamine in presence of large excess of AA and UA in physiological pH at poly (brilliant blue) film coated carbon paste electrode has not been reported. The carbon paste was modified with the different quantity of brilliant blue was used for the electrochemical determination of DA in presence of AA [27] and this work mainly reports the electropolymerisation of brilliant blue on bare carbon paste electrode and used for the determination of DA in presence AA and UA. This modified carbon paste electrode shows very good enhancement when compared to brilliant blue modified carbon paste electrode. This work reports about sensitivity, selectivity, stability and reproducibility of neurotransmitter at poly (brilliant blue) film coated carbon paste electrode at physiological pH.

Experimental Section

Reagents

Dopamine hydrochloride (DA), Uric acid (UA), Ascorbic acid (AA), Coomassie Brilliant Blue R-250(Brilliant Blue) was purchased from Himedia. The stock solution 25×10^{-4} M DA, 25×10^{-3} M UA, 25×10^{-3} M AA was prepared in 0.1 M perchloric acid, 0.1 M NaOH, and double distilled water respectively. Buffer used was 0.2 M Phosphate buffer (PBS) solution of pH 7.4. Graphite powder of 50 µm particle size was purchased from Merck and silicone oil from Himedia was used to prepare Carbon Paste Electrode (CPE). All the chemicals mentioned

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were all of analytical grade used as received without any further purification.

Apparatus

The electrochemical experiments were carried out using a model CHI-660C (CH Instrument-660 electrochemical workstation). A conventional three electrode cell was used with a saturated calomel electrode (SCE) as a reference, a platinum counter electrode, and bare or poly (brilliant blue) modified electrode as working electrode.

Preparation of bare carbon paste electrode

The bare CPE was prepared by hand mixing of 70% graphite powder and 30% silicone oil in an agate mortar for about 45min until a homogeneous paste was obtained. The paste was then packed into a cavity of PVC tube of 3 mm internal Diameter and smoothened on a tissue paper. The electrical contact was provided by a copper wire connected to the end of the tube.

Preparation of poly (brilliant blue) modified carbon paste electrode

The paste packing procedure was same as that at bare carbon paste electrode (BCPE). Electrochemical polymerization of brilliant blue at the CPE was carried out using cyclic voltammetric method in aqueous solution containing 0.5mM brilliant blue in 0.1 M NaOH solution. The electropolymerisation was achieved by the formation of film that grew between -0.5 V to +1.5 V at the scan rate of 0.1 Vs^{-1} for 10 cycles. After that the electrode was rinsed thoroughly with double distilled water.

Result and Discussion

Electrochemical polymerization of brilliant blue on CPE

The poly (brilliant blue) modified carbon paste electrode (MCPE) was prepared by placing 0.5 mM brilliant blue with 0.1 M NaOH in an electrochemical cell. The potential window was maintained from -0.5 V to 1.5 V with scan rate 0.1 Vs^{-1} for 10 multiple cycles. Figure 1 shows during the process of multiple cycles the voltammogram has gradually descended with increase of cyclic time. This indicates that the poly (brilliant blue) film was formed and deposited on the surface of CPE [28,29] the structure of brilliant blue was shown in scheme 1.

Effect of multiple cycles on the preparation of poly (brilliant blue) MCPE

From the obtained experimental results the thickness of the film affects the electrocatalytic property of the carbon paste electrode. The coating was controlled by varying the multiple cycles on the CPE (from 5 to 20 multiple cycles) and corresponding electrocatalytic activity towards oxidation of 0.2mM DA in phosphate buffer solution (PBS) of pH 7.4 was investigated. The Figure 2 shows that at 10 multiple cycles the both anodic and cathodic peak currents was increased to almost 10 folds. Therefore ten cycles was chosen for the electropolymerisation of brilliant blue.

Electrochemical characterization of poly (brilliant blue) MCPE using standard potassium ferrocyanide system

The freshly prepared solution of 1 mM potassium ferrocyanide and 1 M KCl as supporting electrolyte was placed in the electrochemical cell. Figure 3 shows the cyclic voltammograms obtained for the 1 mM potassium ferrocyanide at both BCPE (dashed line) and poly(brilliant blue) MCPE (solid line) at the scan rate 0.1 Vs^{-1}. The low redox peak currents response was obtained at BCPE but at the poly (brilliant blue) MCPE exhibited stable enhancement of redox peak currents and also it shows the fast rate of electron transfer kinetics. The result obtained greatly improved the voltammetric response of potassium ferrocyanide at poly (brilliant blue) MCPE this suggests that the surface property of the modified electrode has been significantly changed and also the results proves that the electrocatalytic activity of our poly (brilliant blue) MCPE. The surface area available for reaction of species in solution can be estimated by the Randles-sevcik equation (1) [30,31].

$$Ip = 2.69\times10^{5} n^{3/2} A D^{1/2} C_0 \nu^{1/2} \quad (1)$$

where, Ip is the peak current in A. C_0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electrons exchanged, D is the diffusion coefficient in cm^2S^{-1}, and υ is the scan rate (Vs^{-1}), A is the electroactive area (cm^2). For poly (brilliant blue) MCPE the electroactive surface area is maximum (0.03658 cm^2) as compared with BCPE (0.02892 cm^2).

Electrochemical response of DA at poly (brilliant blue) MCPE

Figure 4 shows the cyclic voltammograms recorded for 0.2 mM DA at BCPE and poly (brilliant blue) MCPE in 0.2 M PBS solution of pH 7.4 with scan rate 0.1 Vs^{-1}. At BCPE (dashed line) the dopamine shows oxidation and reduction potentials in the low current signals and at poly (brilliant blue) MCPE (solid line) shows significant increase in current signals and also it shows electrocatalytic activity of poly (brilliant blue) MCPE towards the detection of DA. The overall oxidation mechanism of DA could be explained on the basis of the dissociation ability of the $-SO_3H$ functional group of the poly (brilliant blue) at physiological pH of 7.4. The $-SO_3H$ group of poly (brilliant blue) could dissociate favorably into a negatively charged SO_3^- group under this condition the $-NH_2$ group of DA could obtain a proton and form the positive ion of DA. Therefore the negative charge $-SO_3^-$ on the surface of poly (brilliant blue) MCPE has a well affinity to the DA positive ions and could catalyse and promote the oxidation of DA [32-34].

The effect of scan rate

The effect of scan rate for 0.2 mM DA in PBS of pH 7.4 at poly (brilliant blue) MCPE was studied by cyclic voltammetric technique. According to Randles-sevciks equation the peak current is directly proportional to scan rate. Figure 5 shows the increase of redox peak currents with increase in scan rate from 0.05 Vs^{-1} to 0.6 Vs^{-1}. The graph

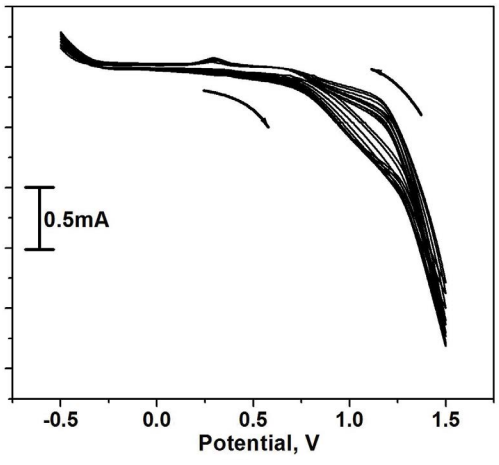

Figure 1: Cyclic voltammograms of preparation of poly (brilliant blue) MCPE. 0.5mM aqueous solution in 0.1 M NaOH at 10 cycles with scan rate 0.1 Vs^{-1}.

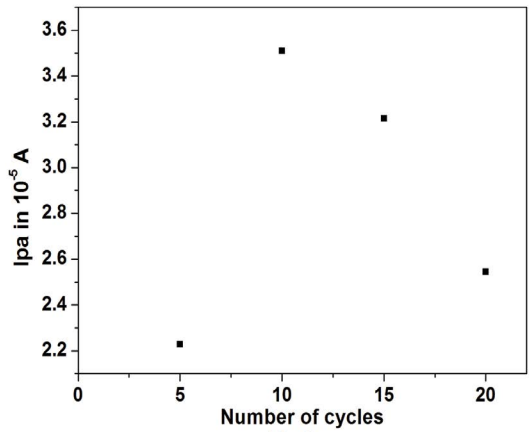

Figure 2: Graph of anodic peak current Vs number of polymerisation cycles.

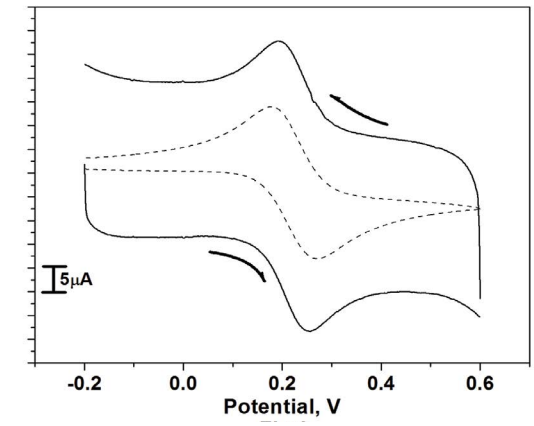

Figure 3: Cyclic voltammograms of 1 mM potassium ferrocyanide at BCPE (dashed line) and poly (brilliant blue) MCPE (solid line) at scan rate of 0.1 Vs^{-1}.

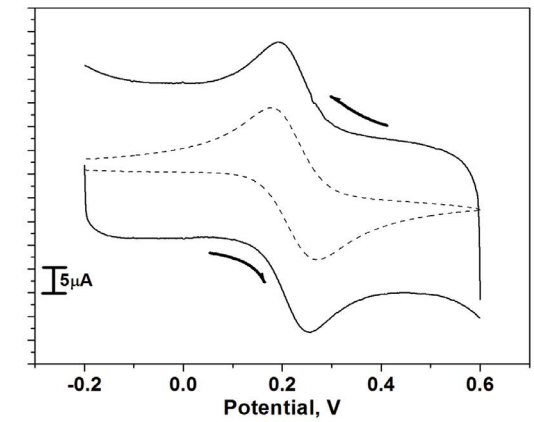

Figure 4: Cyclic voltammograms of 0.2 mM DA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (brilliant blue) MCPE (solid line) at scan rate of 0.1 Vs^{-1}.

of anodic peak current (Ipa) vs. scan rate (υ) was plotted as shown in Figure 5a, the graph obtained was good linearity between the scan rate and Ipa in the range from 0.05 Vs^{-1} to 0.6 Vs^{-1}. The correlation coefficient was r^2=0.9989 which indicates the electrode reaction was adsorption-controlled [35,36]. According to an equation previously reported [37,38] for determining the value of k° form experimental (ΔEp) values equation (2) was a valid approximation of such curves for ΔEp>10 mV. The values of k° for the DA were determined from the experimental ΔEp values, the data are in Table 1. The values of k° indicates that strong adsorption of reactants and products are involved, here the k° is the heterogeneous rate constant, and ΔEp is the potential difference between the anodic and cathodic peak potentials. The heterogeneous rate constant (k°) was estimated using equation (2). The values of k° obtained at a scan rate of 0.35 Vs^{-1} for the poly (brilliant blue) MCPE exhibits larger heterogeneous rate constant compared with those determined in other scan rate variation studies. The calculated data's are tabulate in Table 1.

$$\Delta Ep = 201.39 \log (\nu / k°) - 301.78 \qquad (2)$$

Effect of concentration of DA

The electrocatalytic oxidation of DA was carried out by varying its concentration at poly (brilliant blue) MCPE. Figure 6 shows increasing the concentration of DA, the electrochemical anodic and cathodic peak current goes on increasing with shifting Epa towards more positive and Epc towards negative side. The concentration of DA from 0.510×10^{-4} M to 4.310×10^{-4} M showed that Epa was increased from 0.155 V to 0.238 V. The graph of anodic peak current Vs concentration of DA was plotted as shown in Figure 6a. The anodic peak current was linearly increasing upto the concentration of 2.358×10^{-4} M and further excess of increase of DA concentration shows less increase in Ipa and remains almost same this may due to the kinetic limitation [39].

The limit of detection was calculated according to the previous reported literature [37] and the detection limit on the lower concentration range for DA was 6.7×10^{-7} M for the poly (brilliant blue) MCPE and limit of quantification was 22.3×10^{-7} M. The proposed electrode exhibited a relatively lower detection limit than those recently reported [40-43] as shown in Table 2.

Electrochemical oxidation of AA and UA at poly (brilliant blue) MCPE

Figure 7 shows that the oxidation of 2 mM AA at BCPE and poly (brilliant blue) MCPE in 0.2 M PBS of pH 7.4 at the scan rate 0.1 Vs^{-1}. From the Figure 7 it is observed that the oxidation potential of AA at

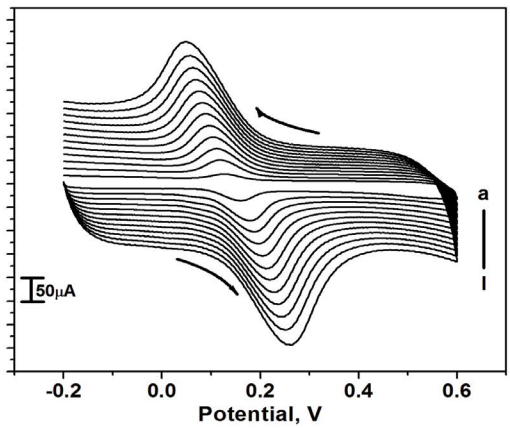

Figure 5: Cyclic voltammograms of 0.2 mM DA in 0.2 M PBS solution of pH 7.4 at poly (brilliant blue) MCPE at different Scan rate (a –l; 0.05 Vs-1 to 0.6 Vs^{-1}).

υ/ Vs^{-1}	ΔEp / V	k°/ s^{-1}
0.05	0.0328	1.090
0.10	0.0667	1.480
0.15	0.0770	1.977
0.20	0.0913	2.234
0.25	0.1100	2.254
0.30	0.1265	2.244
0.35	0.1381	2.290
0.40	0.1544	2.172
0.45	0.1687	2.074
0.50	0.1830	1.954
0.55	0.1982	1.811
0.60	0.2137	1.655

Table 1: Variation of the voltammetric parameters gathered from the plots shown in Fig 5 as a function of the potential scan rate.

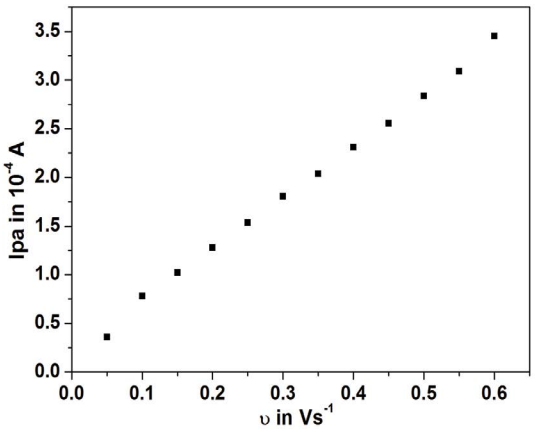

Figure 5a: Graph of anodic peak current Vs Scan rate of DA.

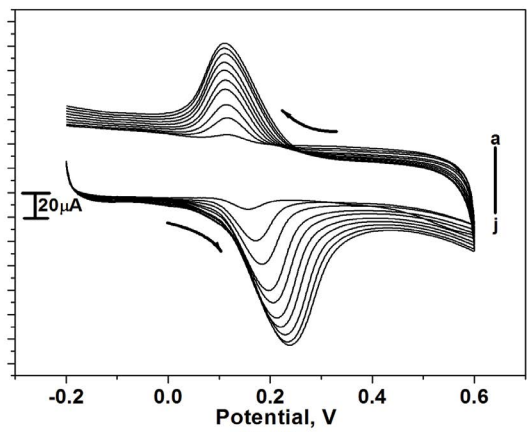

Figure 6: Cyclic voltammograms of DA in 0.2 M PBS solution of pH 7.4 at poly(brilliant blue) MCPE at Scan rate of 0.1 Vs-*1* with different concentration (a –j ; 0.510 × 10^{-4}, 1.000 × 10^{-4}, 1.470 × 10^{-4}, 1.923 × 10^{-4}, 2.358 × 10^{-4}, 2.777 × 10^{-4}, 3.1818 × 10^{-4}, 3.5714 × 10^{-4}, 3.947 × 10^{-4}, 4.310 × 10^{-4} M).

BCPE was broad and poor in sensitivity (dashed line) the anodic peak potential was located at around 0.216 V. however, at poly (brilliant blue) MCPE the oxidation peak potential was shifted towards negative side by minimizing the over potential with enhancement in peak current, the anodic peak potential was located at -0.065 V. This shifting of oxidation peak potential and enhancement of peak current signal confirms the electrocatalytic activity of poly (brilliant blue) MCPE towards AA.

The oxidation of 1 × 10^{-3} M UA at BCPE and poly (brilliant blue) MCPE in 0.2 M PBS of 7.4 at the scan rate 0.1 Vs^{-1} is shown in Figure 8. At BCPE the oxidation peak potential was located at 0.293 V with less sensitivity. But in the same condition at poly (brilliant blue) MCPE the cyclic voltammogram obtained was with increase in current signal and oxidation peak potential was located at 0.350 V. Therefore the enhancement of current signal showed good sensor activity towards the detection of UA.

Simultaneous detection of DA, AA and UA

AA and UA were present along with DA in mammalian brain and the concentrations of these were much higher than that of DA. Since the oxidation potential of both AA and UA was nearly same as that of DA results in an overlapped voltammetric response at BCPE. The Figure 9 shows the cyclic voltammetric response of 0.5 × 10^{-4} M DA in presence of high concentration of 1.0 × 10^{-3} M AA and 0.5 × 10^{-3} M UA in PBS of pH 7.4 at the scan rate of 0.1 Vs^{-1} at both BCPE and poly (brilliant blue) MCPE. The cyclic voltammogram obtained for the mixture of DA and AA at BCPE was broad, less sensible and overlapped potential at 0.201 V (dashed line). However the poly (brilliant blue) MCPE has an ability to overcome this difficulty and achieved the separation of all three analytes in the mixture. The resulted cyclic voltammogram at poly (brilliant blue) MCPE has three well defined peak potential of DA, AA and UA at different potentials. The oxidation peak potential of DA, AA and UA were at 0.155 V, -0.060 V and 0.357 V respectively (solid line). The peak to peak separation of DA-AA was 0.251 V and that of DA-UA was 0.202 V. This result was more enough to identify DA in presence of High concentration of UA and AA at poly (brilliant blue) MCPE.

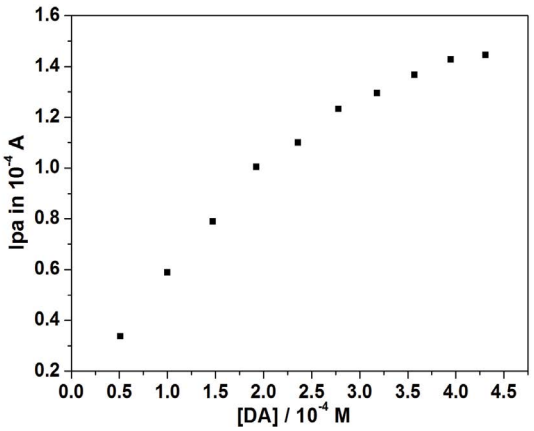

Figure 6a: Graph of anodic peak current Vs concentration of DA

Electrode	Detection limit (mol/L)	Method	Reference
Bicopper complex modified GCE	1.4 × 10^{-6}	DPV	[42]
Self-assembled gold nanoparticle modified Gold electrode	9.0 × 10^{-5}	DPV	[43]
Ionic liquid modified Carbon paste electrode	7.0 × 10^{-7}	CV	[44]
αCD/CNT/CE	5.0 × 10^{-7}	DPV	[45]
Poly(brilliant blue) modified CPE	6.7 × 10^{-7}	CV	This work

Table 2: Comparision of detection limits of different modified electrodes

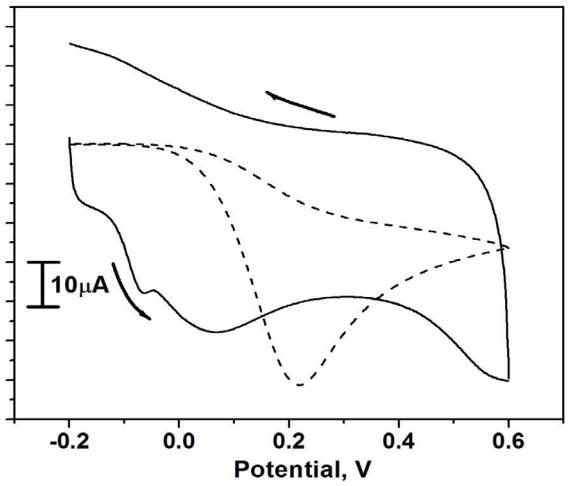

Figure 7: Cyclic voltammograms of 2 mM AA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (brilliant blue) MCPE (solid line) at scan rate of 0.1 Vs^{-1}

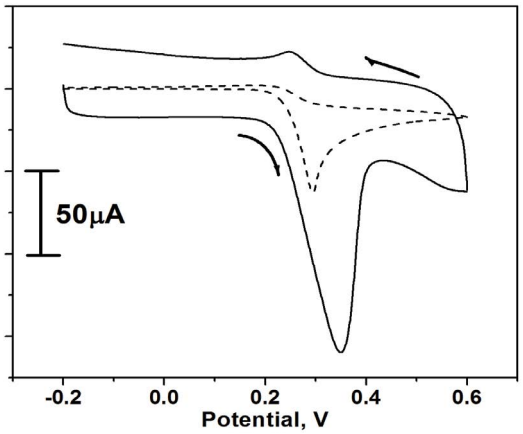

Figure 8: Cyclic voltammograms of 1 mM UA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (brilliant blue) MCPE (solid line) at scan rate of 0.1 Vs^{-1}.

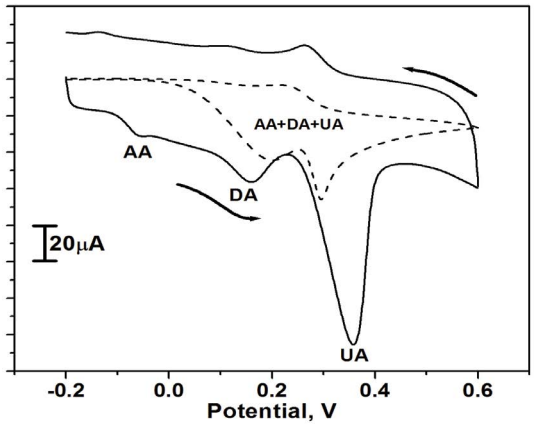

Figure 9: Cyclic voltammograms for simultaneous determination of 0.5 × 10-4 M DA, 1.0 × 10-3 M AA, 0.5 × 10-3 M UA at BCPE (dashed line) and poly (brilliant blue) MCPE (solid line) at scan rate of 0.1 Vs^{-1}.

Interference study

The interference study was carried out in the mixture of samples containing DA, AA and UA. In their mixtures was performed at the poly (brilliant blue) MCPE when the concentration of one species is changed, whereas the concentration of the other two species remained constant. From the Figure 10 it can be seen that the peak current of DA was increased from 0 to 3.57×10^{-4} M when keeping the concentration of AA and UA at 1.0×10^{-3} M and 0.5×10^{-3} M respectively. From the Figures 11 and 12 it is seen that by keeping the concentration of other two analytes constant the anodic peak current of AA or UA increased upto certain range.

Stability of the poly (brilliant blue) MCPE

The stability of poly (brilliant blue) MCPE was studied for the reversible redox couple of Fe^{2+}/Fe^{3+} in 1 M KCl at the scan rate of 0.1 Vs^{-1} by cyclic voltammetric technique. The result in Figure 13 shows that the redox peak current of potassium ferrocyanide remained virtually constant throught the 30 potential cycles and reflecting the stability of the poly (brilliant blue) MCPE.

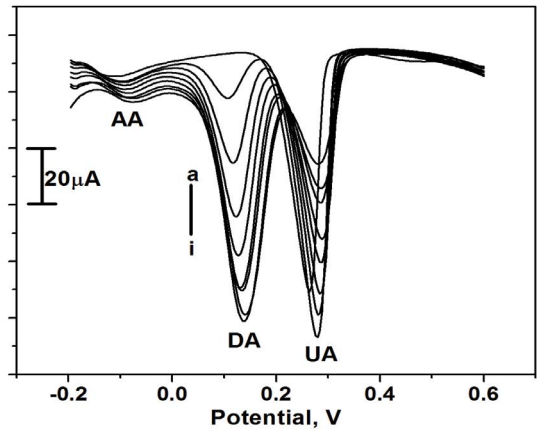

Figure 10: Differential pulse voltammograms of (a) 0.0 M (b) 0.5×10^{-4} (c) 1.0×10^{-4} (d) 1.47×10^{-4} (e) 1.92×10^{-4} (f) 2.35×10^{-4} (g) 2.77×10^{-4} (h) 3.18×10^{-4} (i) 3.57×10^{-4} M DA in 0.2 M PBS of pH 7.4 in presence of 1.0×10^{-3} M AA, 0.5×10^{-3} M UA at poly (brilliant blue) MCPE.

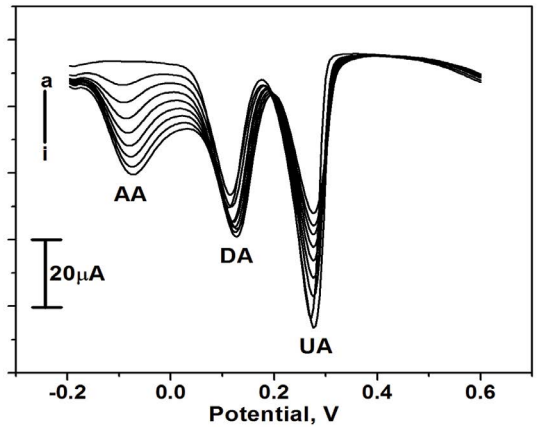

Figure 11: Differential pulse voltammograms of (a) 0.0 M (b) 0.5×10^{-3} (c) 1.0×10^{-3} (d) 1.47×10^{-3} (e) 1.92×10^{-3} (f) 2.35×10^{-3} (g) 2.77×10^{-3} (h) 3.18×10^{-3} (i) 3.57×10^{-3} M AA in 0.2 M PBS of pH 7.4 in presence of 1.0×10^{-4} M DA, 0.5×10^{-3} M UA at poly (brilliant blue) MCPE.

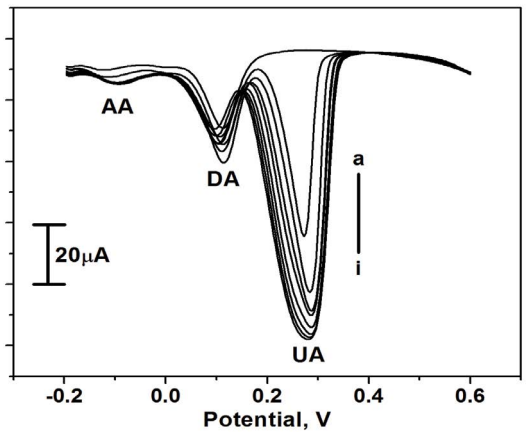

Figure 12: Differential pulse voltammograms of (a) 0.0 M (b) 0.49 × 10^{-3} (c) 0.96 × 10^{-3} (d) 1.38 × 10^{-3} (e) 1.81 × 10^{-3} (f) 2.23 × 10^{-3} (g) 2.63 × 10^{-3} (h) 3.07 × 10^{-3} (i) 3.44 × 10^{-3} M UA in 0.2 M PBS of pH 7.4 in presence of 1.0 × 10^{-4} M DA, 1.0 × 10^{-3} M AA at poly (brilliant blue)MCPE.

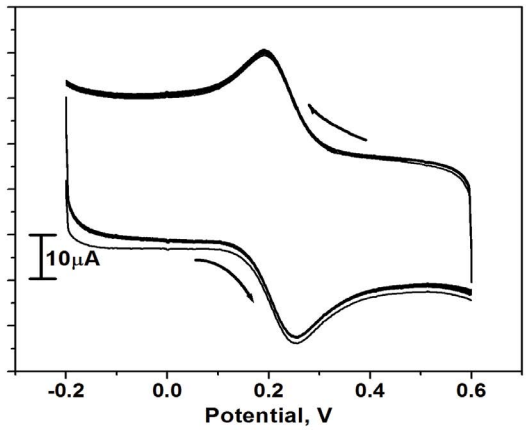

Figure 13: Cyclic voltammograms of 30 multiple cycles for 1mM potassium ferrocyanide in 1M KCl at poly (brilliant blue) MCPE at the scan rate of 0.1 Vs^{-1}.

Conclusion

The prepared poly (brilliant blue) MCPE shows excellent electrocatalytic activity towards the detection of DA electrochemically in the mixture of solutions contains large excess of AA and UA at physiological pH of 7.4 by using both CV and DPV techniques. The oxidation peak to peak separation of DA-AA was 0.251 V and that of DA-UA was 0.202 V. This result was more enough to identify DA in presence of large excess of AA and UA. The prepared modified electrode has detection limit of 6.7 × 10^{-7} M for DA by CV technique. It has excellent sensitivity, selectivity, stability, reproducibility and antifouling properties. This modified electrode can be applied for other neurotransmitters and also for some electroactive pharmaceutical compounds.

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