

Full Paper

Development of Trypan Blue Polymer Film Based Electrochemical Sensor for the Determination of Dopamine and its Simultaneous Detection in Presence of Ascorbic Acid and Uric acid: A Voltammetric Method

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Received: 10 May 2014 / Accepted: 5 August 2014 / Published online: 31 August 2014

Abstract- This paper deals with the development of an electrochemical sensor base on Trypan Blue (TB) towards the determination of Dopamine (DA). An electrochemical sensor was fabricated by the electropolymerization of TB onto the Carbon paste electrode (CPE) using cyclic voltammetry (CV) technique. The polymerized TB modified CPE (Poly(TB)MCPE) was characterized using Electrochemical impedance spectroscopy (EIS) and CV techniques. The sensor Poly(TB)MCPE showed good sensitivity and catalytic activity in comparison with bare CPE. Electrochemical behaviour of Dopamine (DA) was investigated in 0.1 M PBS in alkaline medium (pH 8.0) using Poly(TB)MCPE. Moreover, simultaneous determination of DA in presence of Ascorbic acid (AA) and Uric acid (UA) was examined using Differential pulse voltammetry (DPV) technique. Several kinetic parameters such as diffusion coefficient (D_0), area of the electrode (A), surface concentration of modifier on Poly(TB)MCPE (Γ), limit of detection (LOD) and limit of quantification (LOQ) were calculated.

Keywords- Dopamine, Trypan blue, Cyclic voltammetry, Differential pulse voltammetry Electrochemical impedance spectroscopy, Electrochemical sensor

1. INTRODUCTION

Dopamine is an important widely investigated neurotransmitter and plays a potential role in the functions of mammalian central nervous, hormonal, cardiovascular and renal systems. High concentration of DA is found in a particular region of brain so called caudate nucleus (50 nmol g^{-1}) and very little concentration of DA is found in extra cellular fluids ($0.01\text{--}1 \text{ }\mu\text{M}$). High level concentration of DA causes euphoria whereas low level causes many neurological disorders, such as schizophrenia (Mental disorder), Parkinson's disease, Huntington's disease and Senile dementia [1-3].

Ascorbic acid (AA) (Vitamin C) is naturally occurring organic compound in both plant and animal kingdoms and is important vitamin in the diet of humans. It was originally called L-hexuronic acid. The present name, Ascorbic acid is derived from A- (means 'no') and scorbutus (means 'scurvy'), the disease caused by a deficiency of vitamin C. It functions as a redox co-factor catalyst in a broad array of biochemical reactions and in its processes. It is present in mammalian brain along with DA and other neurotransmitters. It has been used in the prevention and treatment of common cold, mental illness, cancer, infertility [4,5].

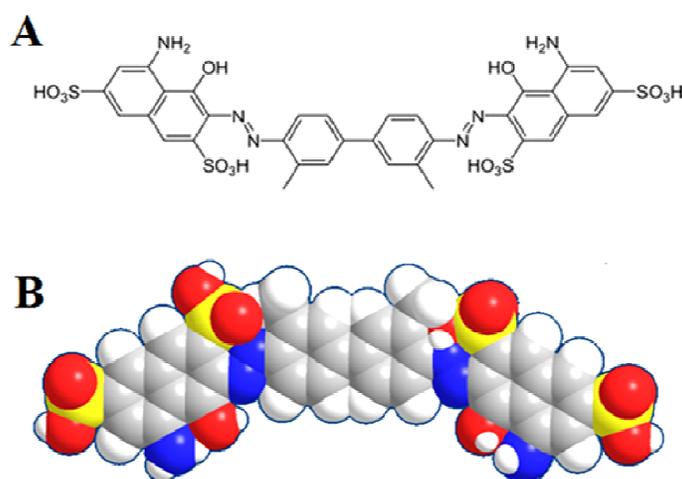
Uric acid (2,6,8-trihydroxypurine) (UA) is one of the important final products of purine metabolism in the human body. It is prepared by the xanthine oxidase catalyzed conversion from xanthine. Disorders of purine biosynthesis and/or purine catabolism, such as hyperuricemia, gout and Lesch-Nyhan syndrome are generally considered to be the indication of the irregular concentrations of UA dissolved in human urine and blood [6].

The monitoring of DA has a great significance in the early diagnosis of several neurological disorders. Therefore, it is necessary to develop reliable methods towards the determination of DA for diagnostic, neurological and industrial applications quantitatively. Over the past years, various analytical methods have been used to determine DA such as liquid capillary electrophoresis [7], chromatography [8,9] and fluorescence [10]. However, these techniques are often required high expensive equipments, over analysis time. Instead of these techniques, electrochemical methods have attained a great significance due to their high sensitivity, often associated with high selectivity, environmentally friendly detection and low cost, rapidity, precision for quantification of essential compounds in biological point of view. DA is electrochemically active and it is usually coexists with AA and UA and considered as important molecules for physiological processes in human metabolism. However, the major problem associated towards the simultaneous determination of DA, AA and UA is typically challenging because of their very similar oxidation potentials. Thus, simultaneous determination of these compounds is a special interest at electrochemical sensors [11].

To overcome this problem, various modified electrodes have been fabricated such as ruthenium oxide modified electrode [12], zinc oxide composite film [13], carbon ionic liquid electrode [14], silver doped poly(L-valine) modified GCE [15], carbon ceramic electrode prepared by sol-gel technique [16], palladium nanoparticle-loaded carbon nanofibers

modified electrode [17], Pt-Au hybrid film modified electrode [18]. Now-a-days, electropolymerized modified electrodes have also been used to determine DA and/or DA, AA and UA simultaneously, such as poly(vinyl alcohol) [19], poly(solochrome dark blue) [20], poly(chromotrope 2B) [21], poly(4-(2-pyridylazo) resorcinol) [22], poly(Rhodamine B) [23], poly(evans blue) [24]. The successful route to overcome the problems of selectivity is to modify the electrode surface, because the modified electrode could decrease the over voltage, improve the velocity of mass transfer and effectively enhance the selectivity of the analyte [25,26].

Trypan blue is an azo, ionic compound and is blue-grey coloured powder. The structure of this compound was shown in Scheme.1. However, it is used as a dye for dyeing of textiles, leather, paper, a discharge-print for cotton, a vital stain for the majority living cells. Formerly, it was used as a diagnostic agent for ocular diseases and in the treatment of sleeping sickness.



Scheme 1. (A) The molecular structure of Trypan Blue and (B) 3D molecular structure of Trypan Blue

Herein, we study the determination of DA at newly developed electrochemical sensor Poly(TB)MCPE. The Poly(TB)MCPE was fabricated by electropolymerisation of Trypan blue using CV technique. This modified electrode shows better sensitivity and selectivity for the determination of DA at low concentrations and also in presence of AA and UA. To the best of our knowledge and literature survey regarded that no research work has been carried out towards the determination of DA in optimum pH 8.0 at Poly(TB)MCPE in PBS supporting electrolyte.

2. EXPERIMENTAL

2.1. Chemicals and materials

Dopamine was purchased from Merck Specialities Pvt. Limited, Mumbai, $K_3[Fe(CN)_6]$ from MerckSpecialities Pvt. Limited, Mumbai. $K_4[Fe(CN)_6]$ from Fisher Scientific, Mumbai. KCl from Qualigens fine chemicals, Mumbai. Trypan blue from Himedia Laboratories Pvt. Limited, Mumbai. Perchloric acid from SDFCL, Mumbai, Ascorbic acid and Uric acid were purchased from Merck Specialities Pvt. Limited, Mumbai and used without any further purification. The stock solution of 10 mM Dopamine was prepared and stored at cooled place, working solution was prepared by diluting the stock solution with buffer solution. Graphite powder of 60 mm particle size was purchased from Loba chemie, Mumbai and silicon oil from S.D. Fine Chemicals Limited, Mumbai. 0.1 M phosphate buffer solution (PBS) was prepared from $NaH_2PO_4 \cdot 2H_2O$ and Na_2HPO_4 . All reagents are analytical grade.

2.2. Instrumentation

CHI 1200A electrochemical analyzer was used for the measurement of CV, CHI 660D electrochemical work station was used for the measurement of DPV and EIS. A conventional three electrode system was employed, which consists of a modified carbon paste electrode as working electrode, Ag/AgCl as a reference electrode to measure the cell potentials and platinum wire as an auxiliary electrode to measure current. The pH values were measured using Elico U 120 pH meter and a combined pH CL 51 B electrode.

2.3. Preparation of carbon paste electrode and Poly(Trypan blue) modified carbon paste electrode

The carbon paste electrode was prepared by hand grinding of 85% graphite power and 15% of silicon oil using agate mortar for 30–40 minutes to get homogeneous paste. This carbon paste was incorporated into of Teflon tube (diameter: 2 mm) and electrical contact was made through a copper wire from the tube. The modified electrode was smoothed on the piece of soft filter paper before measurement. The 0.1 M aqueous TB was placed in the electrochemical cell along with 0.1 M PBS solution of pH 8.0. The CPE was scanned for 10 multiple cycles, between the potential ranges from -1200 to +2000 mV. After the completion of the process the electrode was washed with double distilled water for further use.

3. RESULTS AND DISCUSSION

3.1. Electropolymerization of Trypan blue on the carbon paste electrode

Fig. 1 illustrates the continuous cyclic voltammograms (CVs) of electrochemical polymerization of TB on the surface of CPE. Poly(TB)MCPE was fabricated by electrochemical polymerization of 1.0 mM TB on CPE in 0.1 M PBS (at pH 8.0) using CV technique. Electropolymerization was achieved between the potential window -1.2 to 2.0 V for 10 cycles at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$. The continuous overlapping of CVs to each other indicates that conductive polymer film was achieved on the surface of the electrode.

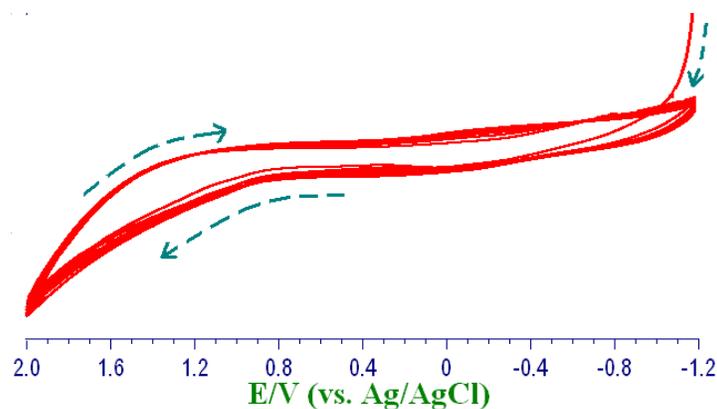


Fig. 1. Continues cyclic voltammograms for the electrochemical polymerization of Trypan Blue onto the CPE at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$

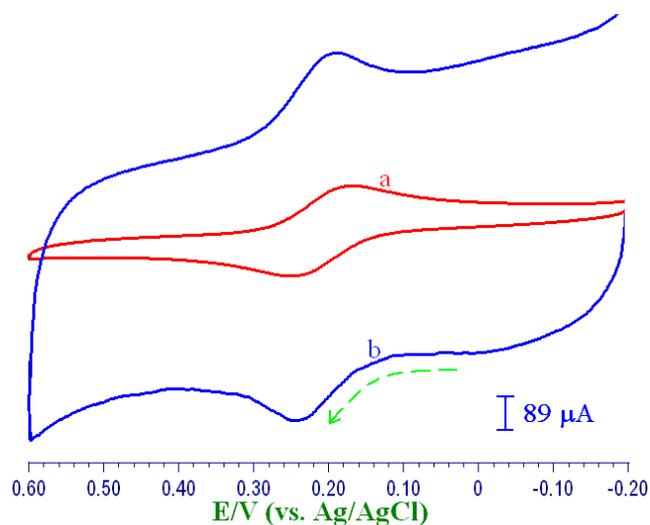


Fig. 2. Cyclic voltammograms for the electrochemical response of $1 \text{ mM K}_4[\text{Fe}(\text{CN})_6]$ in 0.1 M KCl at bare CPE (a), Poly(TB)MCPE (b) at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$

3.2. Electrochemical Characterization of Poly(TB)MCPE

Fig. 2 shows the CVs of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl supporting electrolyte at bare CPE (peak a) and Poly(TB)MCPE (peak b). The potential difference between anodic and cathodic peak (ΔE_p) was 120 mV for bare CPE meanwhile ΔE_p was greatly reduced to 56 mV for Poly(TB)MCPE. Moreover, the modified electrode exhibited enhanced redox peak currents. These results indicate that the modified electrode has good electrochemical reaction ability and faster electron transfer kinetics.

By using the Randles-sevcik equation for reversible process (1) [27-29], we have calculated the surface area of the electrode (A)

$$I_p = 0.4463 \left(\frac{F^3}{RT} \right)^{\frac{1}{2}} n^{\frac{3}{2}} \nu^{\frac{1}{2}} D_0^{\frac{1}{2}} A C_0 \quad (1)$$

At $T=300$ K (at this temperature the experiments were conducted), the above equation becomes

$$I_p = (2.69 \times 10^5) n^{\frac{3}{2}} \nu^{\frac{1}{2}} D_0^{\frac{1}{2}} A C_0 \quad (2)$$

The surface area of the electrode (A) was found to be $7.6311 \times 10^{-4} \text{ cm}^2$. In the above equations, I_p is the peak currents (A), n is the number of electrons involved ($n=1$ for $K_3[Fe(CN)_6]$) [20], ν is the rate (V.s^{-1}), F is Faraday's constant ($96,485 \text{ C mol}^{-1}$), R is the universal gas constant ($8.314 \text{ J.mol}^{-1} \text{ K}^{-1}$), C_0 is the concentration of $K_3[Fe(CN)_6]$, T is the absolute temperature (K), and D_0 is the diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$).

The surface concentration of TB on the developed Poly(TB)MCPE can be calculated using the equation (3) [31]

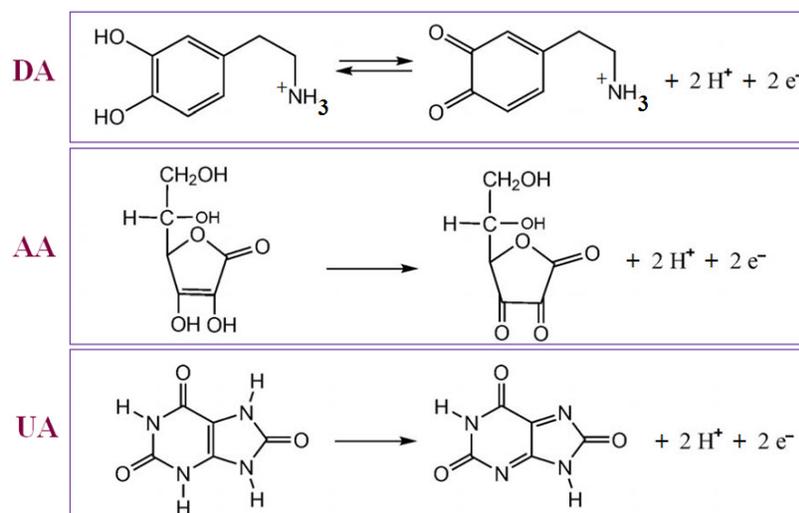
$$I_p = \frac{n^2 F^2 \Gamma \nu}{4RT} \quad (3)$$

Where n is the number of electrons transferred [20], F is the Faraday constant ($96,485 \text{ C mol}^{-1}$), Γ is the surface concentration (mol.cm^{-2}). A is the surface area of the electrode ($7.6311 \times 10^{-4} \text{ cm}^2$), ν is the scan rate (100 mV.s^{-1}), R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T is the absolute temperature (300 K). The value of the surface concentration of TB (Γ) on the electrode was found to be $1.25 \times 10^{-6} \text{ mol.cm}^{-2}$.

3.3. Electrochemistry of dopamine at Poly(TB)MCPE

The electrochemical behaviour of 1 mM DA in 0.1 M PBS at pH 8.0 was examined at bare CPE and newly fabricated Poly(TB)MCPE and it can be seen in the Fig. 3, at bare CPE (peak a) DA exhibited poor current response, whereas a well defined redox system of DA was observed at Poly(TB)MCPE (peak b). Moreover, when compared to redox peak currents at

bare CPE, 15 folds enhancement of the redox peak currents at Poly(TB)MCPE was observed. The prominent catalytic activity of Poly(TB)MCPE accompanied by the negative shift in oxidation potential and positive shift in reduction potential, great improvement in the peak currents and good reversibility, may be due to electrostatic interaction between anionic TB electrode surface and cationic DA, which leads to the increase in the concentration of DA around the surface of Poly(TB)MCPE. The redox mechanism of DA was shown in Scheme 2.



Scheme 2. Proposed mechanism of the electrochemical redox system of DA and oxidation of AA,UA at the Poly(TB)MCPE

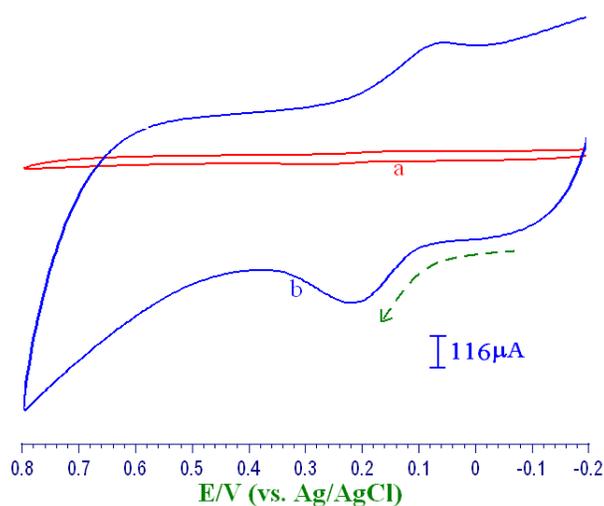
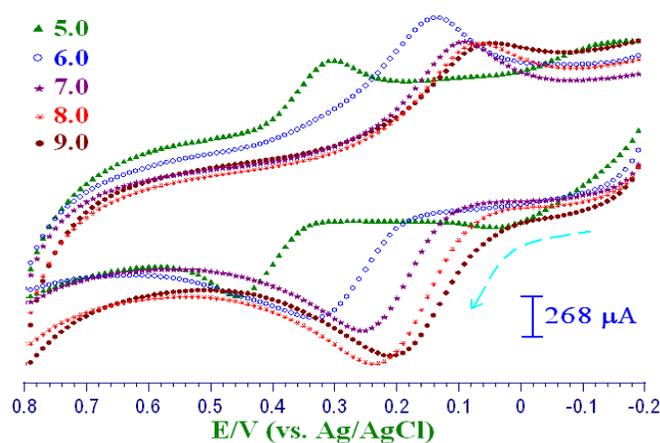
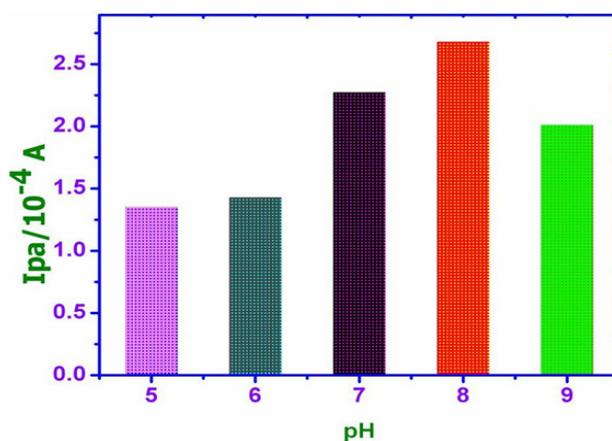


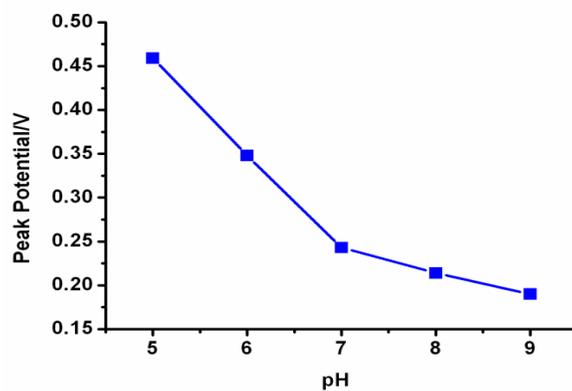
Fig. 3. Cyclic voltammogram for the electrochemical response of 1 mM DA at bare CPE (a) and Poly(TB)MCPE (b) in 0.1 M PBS pH (8.0) at a scan rate of 100 mV s^{-1}



A



B



C

Fig. 4. A) Cyclic voltammograms recorded at Poly(TB)MCPE in 0.1 M PBS solution in pH values of (a) 5.0 (b) 6.0 (c) 7.0 (d) 8.0 (e) 9.0; B) Bar diagram for the oxidation peak current (I_{pa}) of Dopamine in different pH solution ranging from 5.0 to 9.0; C) Dependence of anodic peak potential (E_{pa}) of Dopamine on pH solution in the range 5.0 to 9.0

3.4. Effect of solution pH

The pH of supporting electrolyte plays an important influence in the determination of DA at Poly(TB)MCPE. The electrochemical behaviour of 1 mM DA in PBS at a potential scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$ was examined at different pH solutions (pH: 5.0 to 9.0). As can be seen in the Fig.4A and B the maximum peak current was observed at pH 8.0. Thus, buffer solution of pH 8.0 was chosen to carry out this work. Moreover, from Fig. 4C, one can observe that, all the anodic peak potentials for the oxidation of DA shift negatively with increase in pH, this indicates the involvement of protons in the electrode reaction.

3.5. Effect of scan rate

In order to investigate the kinetics of the electrode reaction and to verify the diffusion controlling factor for mass transfer at the electrode surface, CVs of 1 mM DA in PBS pH 8.0 at Poly(TB)MCPE were performed at different scan rates (Fig. 5A). With the increase of the scan rate, the redox peak currents also increased gradually, signifying a direct electron transfer between DA and the Poly(TB)MCPE surface. Moreover, the plot of square root of scan rate and the anodic peak current (Fig.5B) was found to be linear with a correlative coefficient (R) of 0.9943 with a linear equation of

$$I_{pa} (\text{A}) = -0.27887 + 0.19713 v^{1/2} (\text{mV/s})^{1/2}$$

This suggests the electron transfer reaction was controlled by diffusion with a diffusion coefficient (D_0) of $1.8845 \times 10^{-6} \text{ cm}\cdot\text{s}^{-1}$.

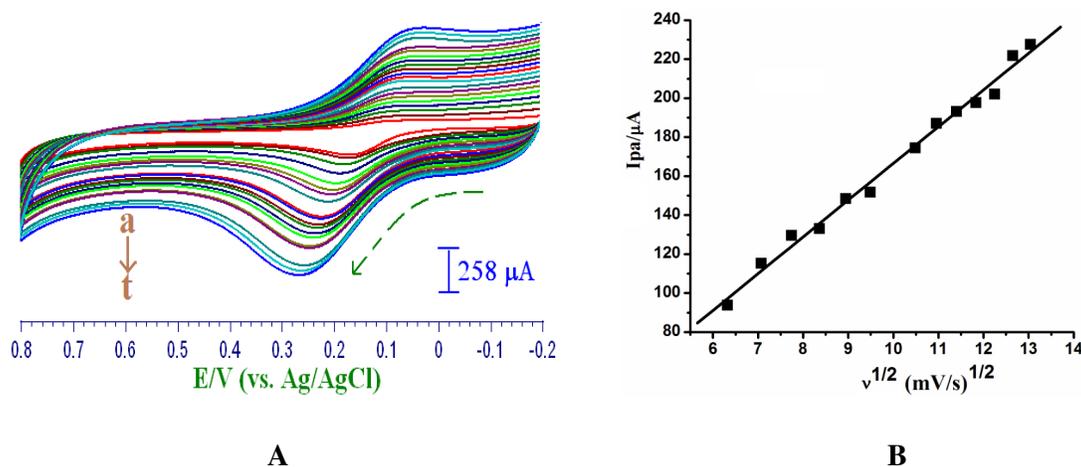


Fig. 5. A) Cyclic voltammograms of variation of scan rate for Dopamine (a to t, $10\text{--}200 \text{ mV}\cdot\text{s}^{-1}$) in PBS solution of pH 8.0; B) Calibration plots for the square root of scan rate vs. the oxidation peak current

3.6. Effect of DA concentration

To study the effect of DA concentration CV technique was used. The concentration of DA in 0.1 M PBS (pH 8.0) was increased from 4.0×10^{-5} M to 3.2×10^{-3} M and the voltammograms were recorded and was shown in Fig. 6A. The peak current increased with increase in the concentration of DA. By plotting a graph between concentration of DA and anodic peak current, two linear equations were resulted with correlation coefficients of $R=0.99609$ and 0.99464 respectively (Fig. 6B). The linear equations were found to $I_{pa}(\mu A)=1.3526+0.04363[DA](\mu M)$, and $I_{pa}(\mu A)=17.90029+0.01191[DA](\mu M)$.

The Limit of Detection (LOD) and the Limit of Quantification (LOQ) were calculated using the following equations [32-34]:

$$LOD = \frac{3S}{M} \quad (4)$$

$$LOQ = \frac{10S}{M} \quad (5)$$

In the above equations (4) and (5), 'S' is the standard deviation of anodic peak currents and 'M' is slope of calibration curve. From the above equations, LOD and LOQ values were found to be 2.3048×10^{-5} M and 7.6827×10^{-5} M respectively.

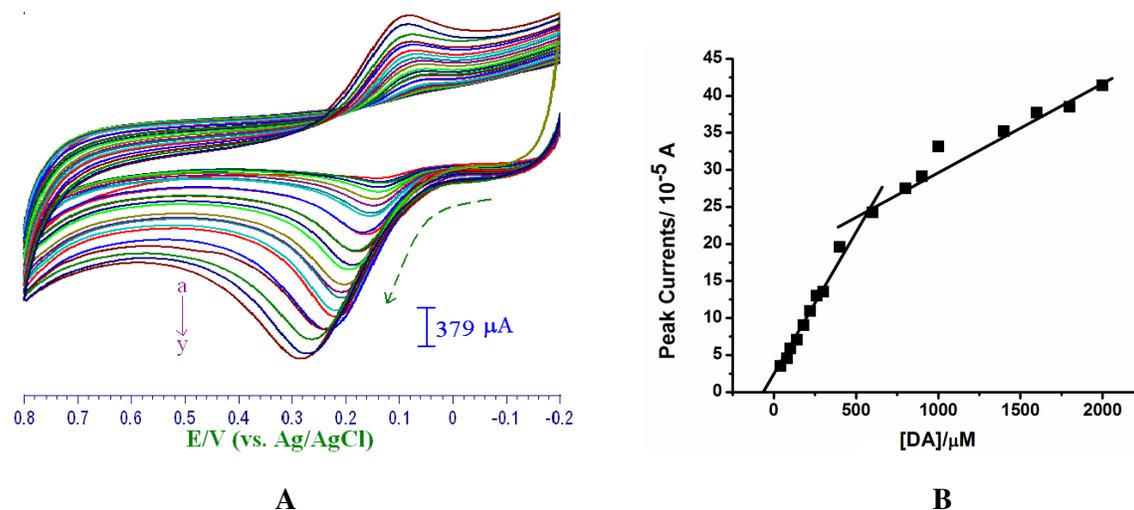


Fig. 6. A) Cyclic voltammograms of DA for the different concentrations in PBS at pH 8.0 (a) 4.0×10^{-6} M (b) 6.0×10^{-6} M (c) 8.0×10^{-6} M (d) 1.0×10^{-5} M (e) 1.4×10^{-5} M (f) 1.8×10^{-5} M (g) 2.2×10^{-5} M (h) 2.6×10^{-5} M (i) 3.0×10^{-5} M (j) 4.0×10^{-5} M (k) 5.0×10^{-4} M (l) 6.0×10^{-4} M (m) 7.0×10^{-4} M (n) 8.0×10^{-4} M (o) 9.0×10^{-4} M (p) 1.0×10^{-4} M (q) 1.2×10^{-4} M (r) 1.4×10^{-4} M (s) 1.6×10^{-4} M (t) 1.8×10^{-4} M (u) 2.0×10^{-4} M (v) 2.4×10^{-4} M (w) 2.8×10^{-4} M (x) 3.2×10^{-4} M (y) 3.6×10^{-4} M; B) Calibration plot of DA concentration

3.7. Electrochemistry of AA and UA

The electrochemical oxidation of AA, UA was investigated using CV technique at bare CPE and Poly(TB)MCPE. Electro-oxidation of 1 mM AA in 0.1 M PBS (pH 8.0) at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$ was recorded shown in the Fig. 7A. As can be seen in the Fig.7A, AA has a broad peak with poor current response at about 0.294 V at bare CPE. However, at the Poly(TB)MCPE, AA has a good oxidation peak with enhanced peak current at about -0.230 V. The oxidation peak of AA shifted towards more negative side at Poly(TB)MCPE in comparison with bare CPE. The negative shift at Poly(TB)MCPE suggests fast electron transfer kinetics towards the oxidation of AA.

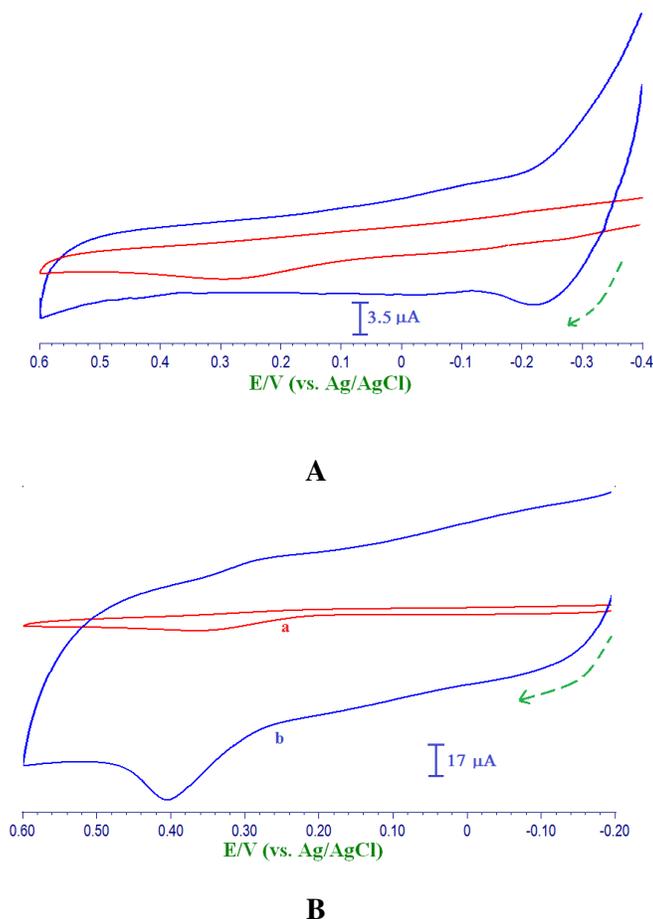


Fig. 7. A) Cyclic voltammogram for the electrochemical response of 1 mM AA at bare CPE (a), Poly(TB)MCPE (b) in 0.1 M PBS pH (8.0) at a scan rate of 100 mV s^{-1} ; B) Cyclic voltammogram for the electrochemical response of 1 mM UA at bare CPE (a), Poly(TB)MCPE (b) in 0.1 M PBS pH (8.0) at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$

Fig.7B shows CVs of oxidation peaks of 1 mM UA in 0.1 M PBS (pH 8.0). Peak 'a' and peak 'b' correspond to the responses at bare CPE with poor current signal at 0.365 V and at Poly(TB)MCPE with an enhanced peak current response at 0.405 V respectively. About 4

times growth in peak currents was observed at Poly(TB)MCPE, when compared with bare CPE. Hence, improved current response at Poly(TB)MCPE suggests that the modified electrode has good electro catalytic activity towards the oxidation of UA. Electro-oxidation mechanisms of AA and UA were shown in Scheme 2.

3.8. Simultaneous determination of AA, DA and UA

To verify the feasibility of the simultaneous determination of AA, DA and UA at Poly(TB)MCPE, the voltammograms were recorded in their mixture with DPV technique due to its high sensitivity and better resolution than the CV technique. As illustrated in the Fig.8 broad overlapped peaks corresponding to oxidation of AA, DA and AA with poor current response was observed at CPE (line a). However, a clearly resolved oxidation peaks were resulted at Poly(TB)MCPE with highly enhanced current response at the potentials of -0.072, 0.112 and 0.232 V respectively (line b) [35,36].

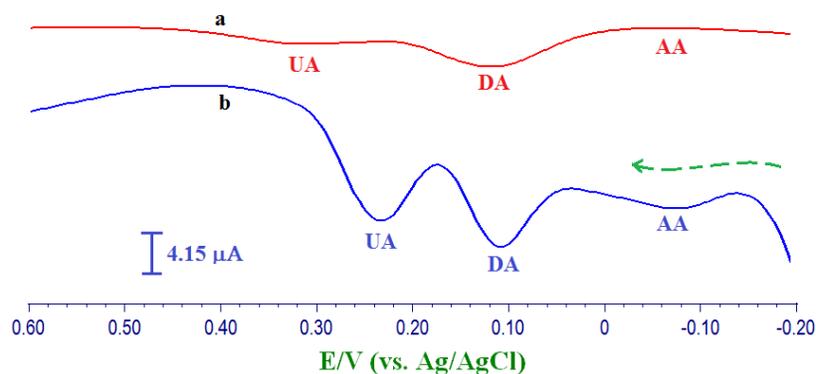
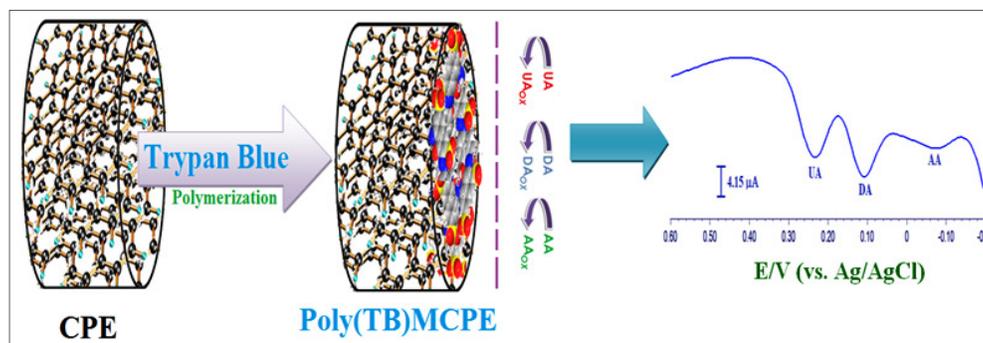


Fig. 8. Differential Pulse Voltammograms for the simultaneous separation of 1 mM AA, 0.1 mM DA, 1mM UA. (a) run with CPE (b) run with Poly(TB)MCPE in 0.1 M PBS solution of pH 8.0



Scheme 3. Newly developed electrode (Poly(TB)MCPE) mechanism towards the simultaneous determination of AA, DA and UA

Hence, Poly(TB)MCPE can be used for the simultaneous determination of DA, AA and UA. Mechanism of simultaneous separation of AA, DA and UA at Poly(TB) was shown in Scheme 3.

3.9. Repeatability and Stability of the electrode

To examine the repeatability of the Poly(TB)MCPE, the sensor was examined in 1mM DA solution in 0.1 M PBS at pH 8.0 at a scan rate of 100 mV.s⁻¹ using CV technique. For a 25 successive measurements, there was no apparent decrease in the current response for DA at Poly(TB)MCPE. From this observation, we can confirm that the developed electrochemical sensor possesses remarkable repeatability. Furthermore, the Poly(TB)MCPE sensor showed a comparable stable activity when stored at 4 °C in 0.1 M PBS (pH 8.0) for about 15 days.

3.10. Electrochemical impedance spectroscopy (EIS) for characterization of Poly(TB)MCPE

Electrochemical impedance spectroscopy (EIS) has proven as a powerful technique for the experimental interfacial characterization of surface of the modified electrode. The electrochemical impedance measurements were carried out with 1mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 0.1 M KCl solution for bare CPE and Poly(TB)MCPE. Fig. 9 illustrates complex planes (Nyquist plots) of bare CPE and Poly(TB)MCPE and include a semicircle portion at higher frequencies correspond to the restricted electron transfer process and a linear part at lower frequency range represents the ascribe diffusion process [36-40]. Electron transfer rate at the surface of the electrode was influenced by the surface charge transfer resistance (R_{ct}) of the electrode. The charge transfer resistance (R_{ct}) is equal to the diameter of semicircle. The increase in R_{ct} could be related to the electrode coverage according to the following equation.

$$(1-\theta) = R_{ct}^0/R_{ct}^1 \quad (6)$$

Where 'θ' is the apparent electrode coverage, R_{ct}^0 and R_{ct}^1 are charge transfer resistance at CPE and Poly(TB)MCPE respectively. Fig. 9 reveals that R_{ct} (equal to diameter of the semicircle) was higher at Poly(TB)MCPE (peak b) when compared to CPE (peak a). The R_{ct} of Poly(TB)MCPE was higher than that of CPE suggesting high electrode coverage. Furthermore, when the electrode coverage was high, then the decrease in ΔE_p value was observed for the redox probe of [Fe(CN)₆]^{3-/4-}. However, the EIS of Poly(TB)MCPE showed a much higher interfacial electron transfer resistance in comparison with bare CPE, indicating

obstruction of the electron transfer of the electrochemical redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ by the TB layer [41].

Randles equivalent circuit was fitted and shown in Fig. 9 as inset. In this circuit, R_s , R_{ct} and R_p are the solution resistance, charge transfer resistance and polarization resistance respectively, C_{dl} is the double layer capacitance and Q is the constant phase element.

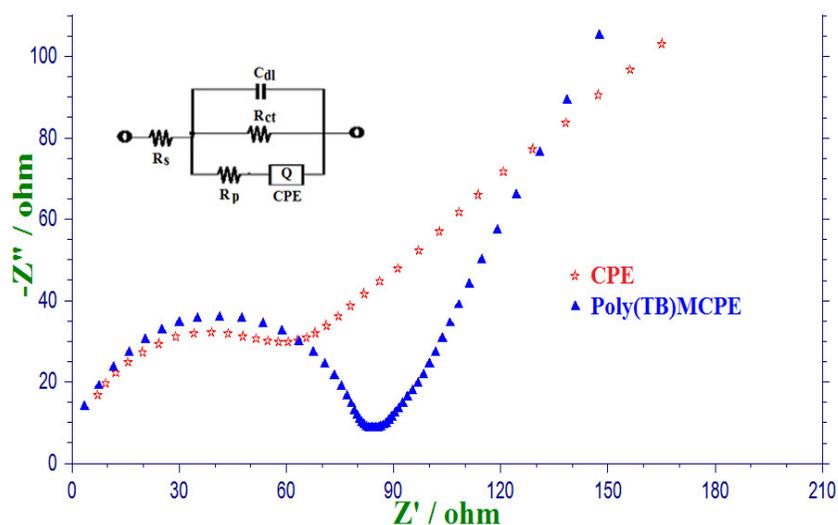


Fig. 9. EIS spectrum for 1 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ in 0.1 M KCl, (a)CPE and (b)Poly(TB)MCPE, inset is Randles equivalent circuit at Poly(TB)MCPE

4. CONCLUSION

In summary, This work describes the electrochemical determination of DA at newly fabricated Poly(TB)MCPE in alkaline medium supporting electrolyte (PBS) and results show that the system was reversible and controlled by diffusion process. Satisfyingly, Poly(TB)MCPE could effectively avoid the interferences among the DA, AA and UA. Due to good stability and repeatability of Poly(TB)MCPE, it has the potential for the future development of TB sensors for clinical point of view.

Acknowledgements

The authors gratefully acknowledge to Sri Venkateswara University, Tirupati, India for providing the necessary facilities.

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