

Determination of Dopamine in Presence of Uric Acid at Poly (Eriochrome Black T) Film Modified Graphite Pencil Electrode

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Abstract

A simple commercial graphite pencil electrode was used to investigate the electrochemical oxidation of dopamine. The electropolymerised film of eriochrome black T was prepared on the surface of graphite pencil electrode by using cyclic voltammetry technique. The prepared electrode exhibits an excellent electrocatalytic activity towards the determination of dopamine. The effects of concentration, pH and scan rate were investigated. Simultaneous detection of dopamine and uric acid was investigated by using both cyclic voltammetric and differential pulse voltammetry technique. The modified electrode was also used for the detection of dopamine in injection.

Keywords: Poly (Eriochrome Black T), Graphite Pencil Electrode, Dopamine, Uric Acid, Cyclic Voltammetry, Differential Pulse Voltammetry

1. Introduction

Dopamine is an important neurotransmitter in the amygdala, a phylogenetically older structure of the brain, which is thought to play a critical role in limbic, cognitive and neuroendocrine functions [1,2]. Serious diseases such as Schizophrenia and Parkinsonism may result by loss of DA-containing neurons [3-8]. Patient with this disease shows a low level of DA. Therefore, determination of DA concentration has become important. Many methods were introduced to determine DA, such as spectroscopy, chromatography and electrochemistry [9-13]. Because of its electrochemical activity, DA can also be determined with electrochemical method [7,14]. Uric acid (UA) is the primary end product of purine metabolism in the human body [15]. In a healthy human being, the typical concentration of UA in urine is around 2 mM and in the blood is in between 120 μ M to 450 μ M ranges [16,17]. Extreme abnormalities of UA levels are symptomatic of several diseases, such as, cardiovascular disease [18], hyperuricaemia, uric acid stones [19], gout and Lesch-Nyhan syndrome [20]. Thus accurate determination of UA concentration is of great importance. Recently, electrochemical sensors have attracted much attention

due to their advantages of simplicity, cheapness, fast analysis along with high sensitivity and selectivity [21]. The oxidation potential of DA and UA are very close such that, the bare electrode often suffers from fouling effects. The chemically modified electrodes have the ability to detect both DA and UA selectively [22,23].

The electropolymer film coated electrodes are playing an important role in sensor field. Ongera *et al.* studied the simultaneous determination of dopamine in presence of ascorbic acid at electropolymer modified carbon paste electrode [3,4,21]. Gabriela Broncova *et al.* used poly (neutral red) modified electrode for determination of citrate in soft drinks [24]. M. Pandurangachar *et al.* prepared poly (patton's and reeder's) film coated carbon paste electrode for simultaneous detection of dopamine [7]. Sarah M. Kirwan studied the electrochemical properties of AA and H₂O₂ at Poly (*o*-phenylenediamine) film modified Platinum-iridium electrodes alloy wires [25].

In the present work, poly (EBT) film coated graphite pencil electrode is used for the selective detection of DA in the presence of UA. The electrochemical study of EBT was done by our research group [26]. Many electrochemical experiments have been done by electropolymerizing the EBT indicator and discussing their voltam-

metric behavior by modifying at glassy carbon electrode [27-29]. The structure of EBT was shown in **Scheme 1**. The graphite pencil electrode (GPE) has been successfully applied to analyses of certain compounds in recent years [30-34]. The GPE is relatively new type of carbon electrode, it is less expensive, more convenient, and renewable compare to the commonly used CPE or GCE [35]. The electropolymerization film of eriochrome black T (EBT) was coated on the surface of GPE by cyclic voltammetry (CV). The electrode had high concentration of negative-charged function group $-\text{SO}_3^-$ and electron rich oxygen atom on its surface. The poly (EBT) film coated GPE showed excellent electrocatalytic activity towards the selectivity and sensitivity of DA in the presence of UA in the range of pH 5.0 in acetate buffer solution. The peak to peak separation between DA and UA was 158 mV. This was large enough to identify the DA and UA individually.

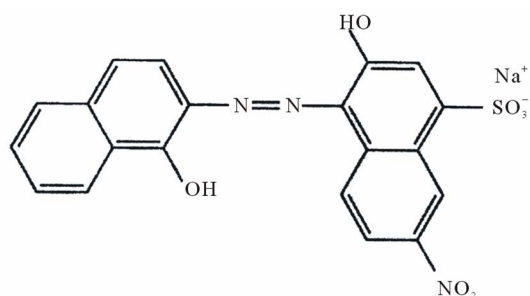
2. Experimental Section

2.1. Reagents

The pencil-lead rods were HB 0.5 mm in diameter and 6 cm length purchased from local bookstore. 25 mM eriochrome black T stock solution was prepared in double distilled water. 10×10^{-4} M DA stock solution was prepared by dissolving in 0.1 M perchloric acid solution. 10×10^{-3} M stock solution of UA was prepared by dissolving in 0.1 M sodium hydroxide solution. Sodium acetate and acetic acid were used to prepare the 0.2 M acetate buffer solution. Chemicals mentioned above were all of analytical grade. The water used in the preparation of solutions was double distilled water.

2.2. Apparatus

The electrochemical experiments were carried out using a model-201 electroanalyser (EA-201 chemilink system). All the experiments were carried out in a conventional three electrochemical cell. The electrode system contained a working electrode was bare GPE and poly (EBT)



Scheme 1. Structure of eriochrome black T.

filmmodified GPE (0.5 mm in diameter), a platinum wire as counter electrode and saturated calomel electrode as reference electrode.

2.3. Preparation of Poly (EBT) Modified GPE

The 1 mM EBT was placed in the electrochemical cell with 0.05 M H_2SO_4 . The GPE was scanned by immersing 3 mm length in that solution (from -400 to 1400 mV) at 100 mVs^{-1} for 20 times. After this, the same GPE was enforced under sweeping from -400 to 1400 mV at 100 mVs^{-1} for multiple cycles (20 cycles) in the solution containing 1 mM EBT with 0.01 M NaOH.

3. Results and Discussion

3.1. Electrochemical Polymerization of EBT on GPE

Figure 1 showed the cyclic voltammogram for electropolymerisation of EBT on the surface of GPE in the range from -400 to 1400 mV at the sweep rate of 100 mVs^{-1} at 20 multiple cycles. Before the electropolymerisation process the GPE was pretreated by scanning in the solution containing 1×10^{-3} M EBT in 0.05 M H_2SO_4 for 20 times in the same potential range. After this the electrode was made to undergo multiple cycles in 0.01 M NaOH containing 1×10^{-3} M EBT. During the process of multiple cycles the voltammogram has gradually descended with increase of cyclic time. This indicates that the poly (EBT) film was formed and deposited on the surface of GPE [27-29].

3.2. Electrocatalytic Response of DA at Poly (EBT) Modified GPE

DA being an easily oxidizable catecholamine, showed

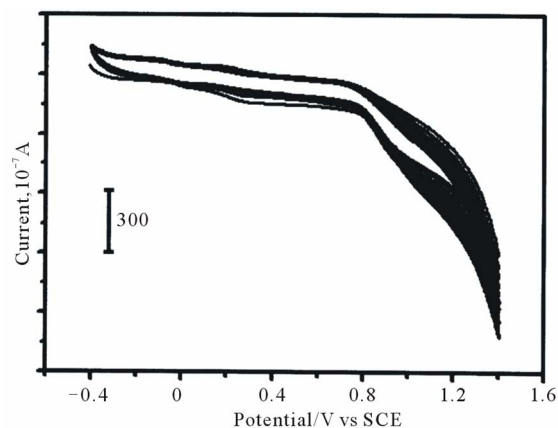


Figure 1. Cyclic voltammogram of preparation of poly (EBT) film coated GPE containing 1 mM EBT in 0.01 M NaOH at 20 cycles with sweep rate of 100 mVs^{-1} .

quasi-reversible voltammogram with supporting electrolyte 0.2 M acetate buffer of pH 5.0 at $50 \text{ mV}\cdot\text{s}^{-1}$ scan rate for bare GPE in the potential range of -200 to 600 mV. **Figure 2** showed a pair of redox peak for bare GPE (dashed line), with E_{pa} 346 mV and E_{pc} 198 mV (vs SCE). The separation of redox peaks (ΔE_p) was found to be 148 mV and the ratio of redox peak current (I_{pa}/I_{pc}) was 1.625, which were the characteristics of a quasireversible electrode process. The formal peak potential (E^0), which is the midpoint of E_{pa} and E_{pc} was obtained as 272 mV. However, at the poly (EBT) modified GPE a pair of redox peaks are obtained with strong increased in both I_{pa} and I_{pc} (solid line). The poly (EBT) modified GPE reduced the over potential which occurred for bare GPE. The E_{pa} and E_{pc} were located at 275 mV and 229 mV respectively. At poly (EBT) modified GPE the E_{pa} was shifted negatively upto 71 mV and E_{pc} was positively upto 31 mV. The ΔE_p was found to be 40 mV, which was on accordance with a Nernst reversible behaviour and identified that number of electrons involved in the reaction was about equal to two. The ratio (I_{pa}/I_{pc}) was 1.1 and the E^0 was 252 mV. The shifting of redox peak potentials and approaching of I_{pa}/I_{pc} towards 1, were shows the excellent electrocatalytic activity of poly (EBT) film coated GPE for detection of DA. Thus, the voltammogram obtained for DA at poly (EBT) modified GPE was reversible with excellent enhancement of oxidation and reduction peak currents.

3.3. The Effect of Scan Rate

The effect of scan rate on the anodic peak current of DA was studied at poly (EBT) modified GPE by using CV technique (**Figure 3(a)**). The scan rate was increased from 50 to 400 mV/s. the anodic peak current was increased with increase in scan rate. The graph of I_{pa} vs scan rate was plotted (**Figure 3(b)**). The resulted graph

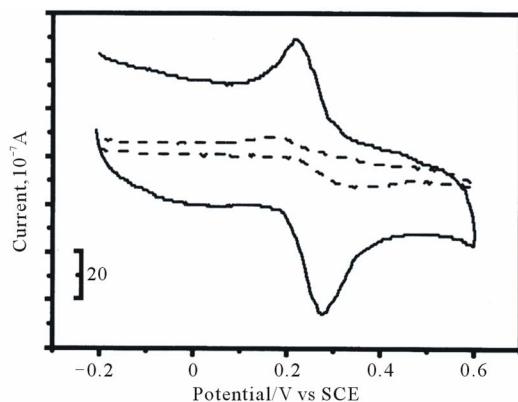
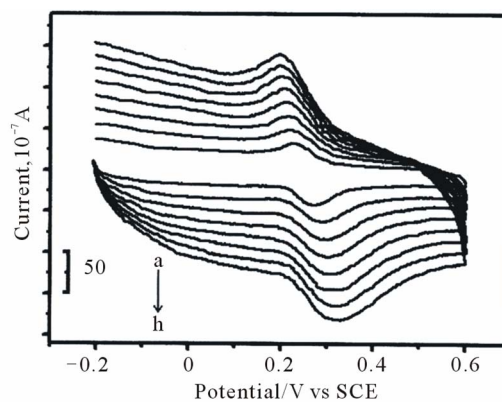
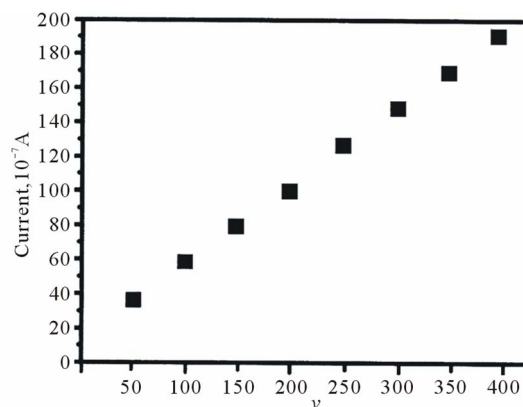


Figure 2. Cyclic voltammogram of 1×10^{-4} M DA in 0.2 M acetate buffer solution of pH 5.0 at bare GPE (dashed line) and poly(EBT) film coated GPE (solid line).



(a)



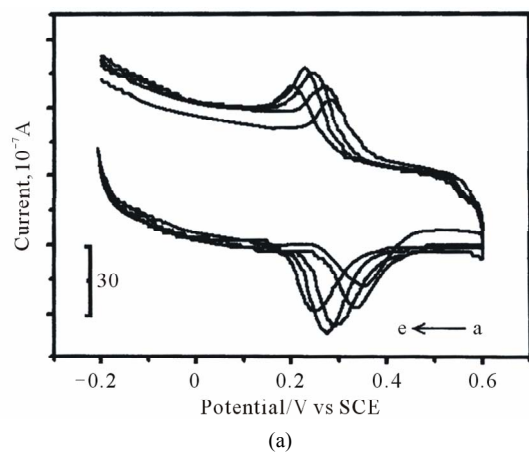
(b)

Figure 3. (a) Variation of scan rate for DA at poly (EBT) film coated GPE (a-h; $50 \text{ mV}\cdot\text{s}^{-1}$ to $400 \text{ mV}\cdot\text{s}^{-1}$). (b) Graph of current vs square root of scan rate.

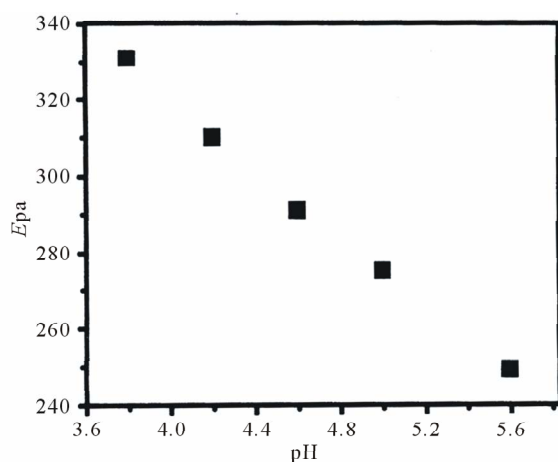
showed excellent linearity with correlation co-efficient of 0.9997. This result showed that the electrode process was adsorption controlled.

3.4. Effect of pH

The electrochemical response of DA at poly (EBT) film coated GPE is generally pH dependent. The voltammograms of DA were recorded at 0.2 M acetate buffer solutions of different pH by cyclic voltammetric method. **Figure 4(a)** demonstrates the pH dependence of DA at poly (EBT) film coated GPE at sweep rate of $50 \text{ mV}\cdot\text{s}^{-1}$. The both anodic and cathodic peak potentials were shifted to less positive side with increasing in the pH values. The anodic peak potential of DA shifted from 331 mV to 249 mV with respect the pH from 3.8 to 5.6. The potential diagram was constructed by plotting the graph of calculated E^0 vs pH of the solution (**Figure 4(b)**). The graph has good linearity with a slope of 45 mV/pH this behaviour is nearly obeyed the Nernst Equation for two electron and two proton transfer reaction.



(a)



(b)

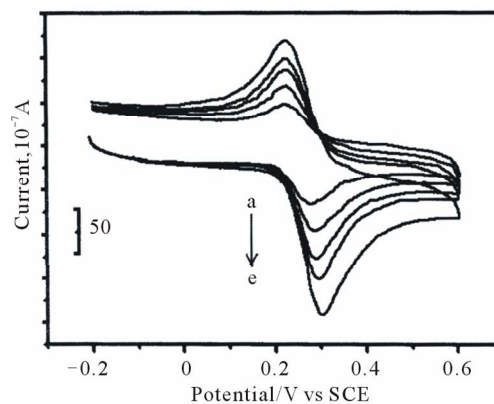
Figure 4. (a) Cyclic voltammogram of DA at different pH (a - e; 3.8, 4.2, 4.6, 5.0, 5.6 pH). (b) Graph of E^0 vs pH.

3.5. Effect of Concentration of DA

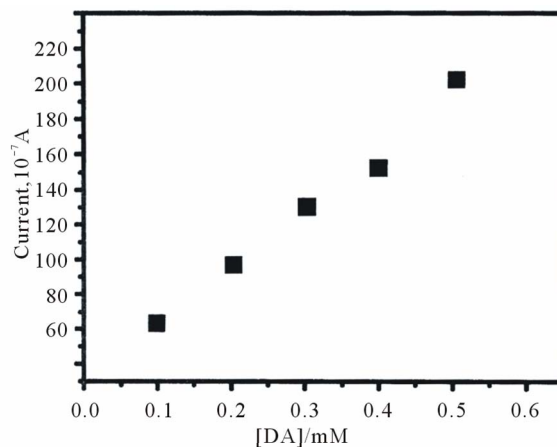
The electrocatalytic oxidation of DA was carried out by varying its concentration at poly (EBT) modified GPE. **Figure 5(a)** showed that, by increasing the concentration of DA, the electrochemical anodic and cathodic peak current goes on increasing with shifting E_{pa} towards positive and E_{pc} towards negative direction. DA from 0.1×10^{-3} to 0.5×10^{-3} concentrations showed the E_{pa} was increased from 275 mV to 305 mV. The graph of anodic peak current vs concentration of DA was plotted (**Figure 5(b)**). The anodic peak current was proportional to concentration of DA in the above range.

3.6. Simultaneous Determination of DA and UA by Cyclic Voltammetry

UA is present along with DA in mammalian brain. The concentration of UA is much higher than that of DA. Since, the oxidation potential of UA is nearly same as



(a)



(b)

Figure 5. (a) Cyclic voltammogram of DA at different concentration (a-e; $0.1, 0.2, 0.3, 0.4, 0.5 \times 10^{-3}$ M). (b) Graph of current vs concentration of DA.

that of DA result in an overlapped voltammetric response at bare CPE. However, the poly (EBT) modified GPE has ability to separate the oxidation peak potentials of DA and UA. **Figure 6(a)** showed the voltammograms were recorded for individual UA at both bare poly (EBT) film modified GPE at sweep rate of $50 \text{ mV}\cdot\text{s}^{-1}$ in the potential range from -200 mV to 700 mV . The voltammogram of UA has E_{pa} , which was found to be 452 mV in a pH 5.0 acetate buffer solution at bare GPE (dashed line). However the E_{pa} was shifted at 424 mV at poly (EBT) modified GPE (solid line) with strong enhancement of I_{pa} . The plot of E_{pa} vs pH was linear and slope was found to be -0.054 mV . The I_{pa} of uric acid was proportional to the ν with correlation coefficient (r) 0.9995 (data was not shown).

The **Figure 6(b)** showed the voltammogram for solution containing mixture of both 0.1×10^{-3} DA and 0.5×10^{-3} M UA in pH 5.0 acetate buffer solution at sweep rate of $50 \text{ mV}\cdot\text{s}^{-1}$ from potential range 0 to 700 mV . The bare GPE (dashed line) showed only one broad anodic

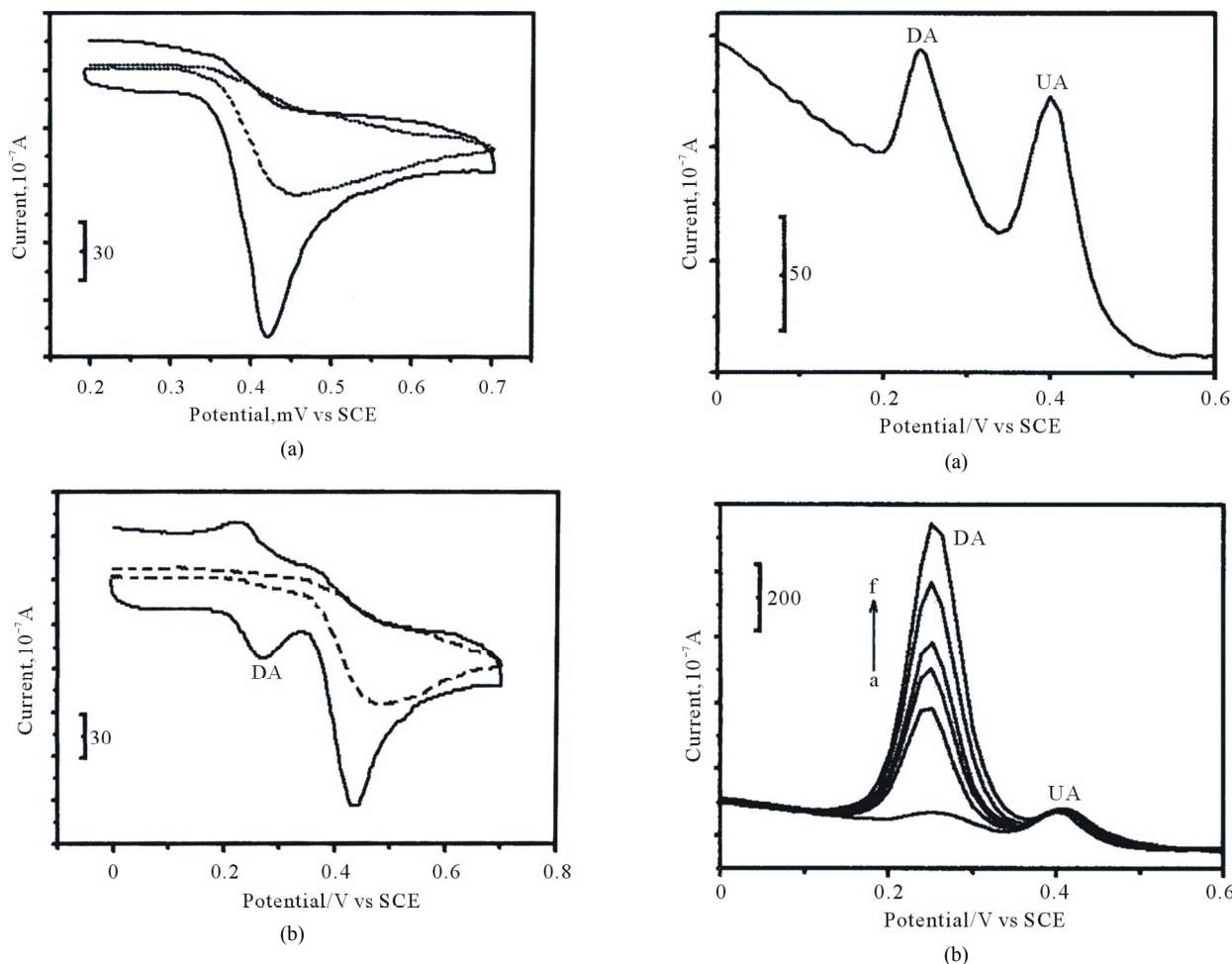


Figure 6. (a) Cyclic voltammogram of 0.5×10^{-3} M UA at bare GPE (dashed line) and at poly (EBT) film coated GPE (solid line) in 0.2 M acetate buffer solution of pH 5.0. (b) Simultaneous determination of 1×10^{-4} M DA and 0.5×10^{-3} M UA at bare GPE (dashed line) and at poly (EBT) film coated GPE (solid line).

peak but not cathodic peak. The poly (EBT) modified GPE has able to separate the oxidation peaks of DA and UA by showing two well separated anodic peaks and one cathodic peak (solid line). The electrocatalytic anodic peak of DA was obtained at 271 mV and UA was found to be at 429 mV. The cathodic peak for dopamine was found to be 235 mV. The separation between DA-UA was found to be 158 mV.

3.7. Simultaneous Detection of DA and UA by Differential Pulse Voltammetry

DPV was used for the determination of DA and UA at poly (EBT) modified GPE because of its higher current sensitivity and better resolution than CV. The simultaneous study was carried out in the potential range from 0 to 600 mV (**Figure 7(a)**). The DPV showed the simultaneous

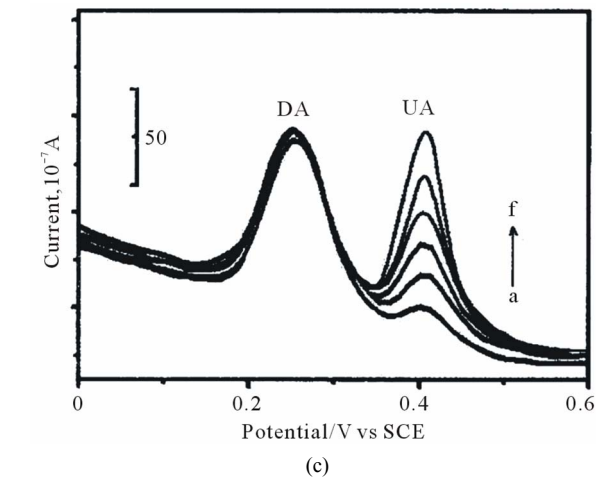


Figure 7. (a) Differential pulse voltammogram for simultaneous detection of 0.1 mM DA and 0.5 mM UA at poly (EBT) film coated GPE. (b) DPVs of (a-f; $0.1, 0.15, 0.17, 0.2, 0.25$ and 0.3×10^{-3} M) DA in 0.2 M ABS of pH 5.0 in the presence of 1×10^{-3} M UA at poly (EBT) film modified GPE. (c) DPVs of (a-f; 0.2, 0.4, 0.6, 1.1, 1.6 and 2.0×10^{-3} M) UA in 0.2 M ABS of pH 5.0 in the presence of 0.1×10^{-3} M DA at poly (EBT) film modified GPE.

determination of DA and UA with well separated two anodic peaks corresponding to their oxidation could be possible at poly (EBT) modified GPE. The 0.1×10^{-3} M DA showed its E_{pa} at 252 and 0.5×10^{-3} M UA was at 414 mV. The peak separation between DA and UA was 162 mV which was greater when comparing to peak separation occurred by CV.

The simultaneous determination of DA and UA in the mixture was carried out at poly (EBT) film coated GPE when concentration of one species changed, whereas another one remained kept constant. From the **Figure 7(b)**, it can be seen that the peak current of DA was proportional to its concentration, which was increased from 0.1×10^{-3} M to 0.3×10^{-3} M when keeping the concentration of UA 1×10^{-3} M. There was no change in the peak current and peak potential occurred for UA. Similarly in the **Figure 7(c)** keeping the concentration of DA constant, the UA concentration was varied from 0.2×10^{-3} M to 2.0×10^{-3} M. The oxidation peak current of UA increases with increase in its concentrations. The detection limit was calculated and found to be $0.08 \mu\text{M}$ for DA concentration [36].

3.8. Analytical Application

The modified electrode was applied to the determination of dopamine hydrochloride injection. The DA injection sample purchased from sterile specialities India Private Ltd with a specified content of DA of 40.0 mg/mL. The sample was used after suitable dilution. The results were shown in **Table 1**. The recovery and R.S.D. were acceptable, showing that the proposed methods could be efficiently used for the determination of DA in injections with recovery in the range 99.25% - 101.25%.

4. Conclusions

In this work, poly (EBT) film coated GPE was used to investigate the electrochemical response of an interesting neurotransmitter DA. The poly (EBT) film coated GPE enhanced both anodic and cathodic peak current strongly. The poly (EBT) film coated GPE showed excellent selective and electrocatalytic activity towards the oxidation of DA in the presence of and UA. The poly (EBT) film coated GPE has very low detection limit $0.08 \mu\text{M}$. Hence,

poly (EBT) modified GPE is acting as a good sensor for the detection of DA. We hope our modified electrode can be used for investigation of other neurotransmitters. The proposed methods can be applied to the detection of DA in injection.

5. References

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Table 1. Detection of DA in injection samples (n = 5).

Sample	Content (mg/mL)	Found (mg/mL)	RSD (%)	Recovery (%)
1	4.0	3.97	2.4	99.25
2	4.0	3.99	2.0	99.75
3	4.0	4.05	2.2	101.25

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