

Research Article

Voltammetric Detection of Dopamine in Presence of Ascorbic Acid and Uric Acid at Poly (Xylenol Orange) Film-Coated Graphite Pencil Electrode

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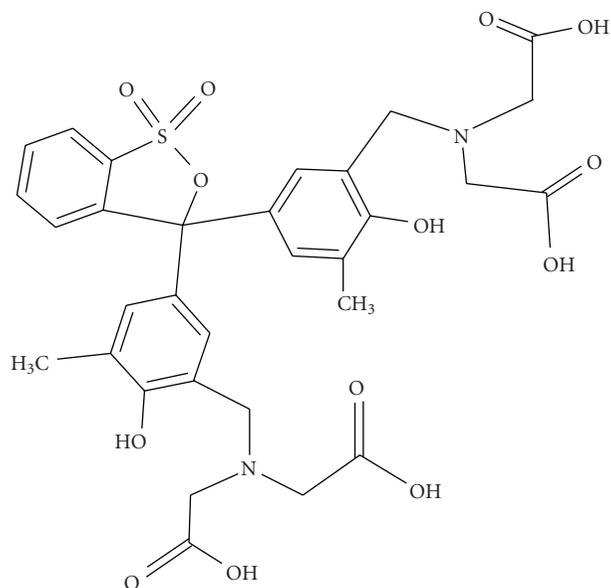
Poly (xylenol orange) film-coated graphite pencil electrode was fabricated for the detection of dopamine in the presence of ascorbic acid and uric acid in phosphate buffer solution of pH 7. The redox peaks obtained at modified electrode shows a good enhancement. The scan rate effect was found to be a diffusion-controlled electrode process. The electrochemical oxidation of dopamine was depended on pH, and the limit of detection was found to be 9.1×10^{-8} M. The simultaneous study gave an excellent result with great potential difference between dopamine and other bioactive organic molecules by using both cyclic voltammetric and differential pulse voltammetric techniques. The present modified graphite electrode was applied to the detection of dopamine in the injection samples, and the recovery obtained was satisfactory.

1. Introduction

The graphite pencil electrode (GPE) has been successfully acting as a biosensor in modern electroanalytical field. A porous composite is consisting of graphite particles, polymeric binder and other additives such as clay. Due to high electrochemical reactivity, high electrical conductivity, good mechanical rigidity, low cost, low technology, high electrochemical reactivity, ease of modification, renewal, low background current, and miniaturization, the GPE has good application in analysis of neurotransmitter and in the detection of traces of metal ions [1–4]. GPE has a larger active electrode surface area and is therefore able to detect low concentrations and/or volume of the analyt [5]. This type of electrode has been successfully applied to design various biosensors [6–9].

In recent days the electropolymer modified carbon paste electrode works with very much excellent effort in acting as a sensor for dopamine [10–12]. Especially, the electropolymer film-coated electrodes with dyes have good stability, reproducibility, more active sites, homogeneity in

electrochemical deposition [13, 14]. The electropolymerisation generally results in polymer film which is uniform and strongly adherent to the electrode surface. In addition the polymer film can be deposited onto the small area with high degree of geometrical conformity and controllable thickness. Several redox dyes are known to artificial electron donors [15]. Such dyes are able to undergo electropolymerisation from aqueous solution producing stable redox active layer [16, 17]. Dopamine (DA) is a well-known biogenic amine acting as a neurotransmitter in the brain. It has received considerable attention because of its suspected role in a variety of neuropsychiatric disorders such as Parkinson's disease and Schizophrenia [18–21]. It has been found that the dopamine possesses very strong electrochemical activity by giving dopamine-*o*-quinone as oxidation product. However the determination remains a challenge because of the presence of large excess of ascorbic acid (AA) and uric acid (UA). It is generally believed that direct redox reactions of these species at bare electrode are irreversible and therefore requires high over potential [22]. Moreover the direct redox reactions of these species at bare electrodes take place at



SCHEME 1: Structure of xylénol orange.

very similar potential and often suffer from a fouling effect, which results in rather poor selectivity and reproducibility. The ability to determine DA, UA, and AA selectively has been a major goal of electroanalytical research [23]. Development of both sensitivity and selectivity are of equal importance in voltammetric procedure and also this would help in the prevention and treatment of several neurodegenerative diseases [24].

The aim of our work was to fabricate stable electrode by electropolymerising xylénol orange (The structure of xylénol orange was shown in Scheme 1) on the surface of graphite pencil electrode to achieve the challenge of simultaneous determination of DA in the presence of AA, and UA in physiological pH. In this investigation electrochemical method was chosen for the detection of DA because, the electrochemical methods have proved to be selective, sensitive, reliable, and cost effective [25]. Although no examination of the detection of dopamine in the presence of both ascorbic acid and uric acid in physiological pH at poly(xylénol orange) film-coated graphite pencil (poly(XO)/GPE) electrode has been reported. This work discussed about sensitivity, selectivity and reproducibility of neurotransmitter at poly(XO)/GPE at physiological pH.

2. Experimental

2.1. Materials. The pencil-lead rods were (HB 0.5 mm in diameter and 6 cm length) purchased from local bookstore. 25×10^{-3} M xylénol orange stock solution was prepared in double-distilled water. 10×10^{-5} M DA stock solution was prepared in 0.1 M perchloric acid solution. 10×10^{-3} M UA stock solution was prepared in 0.1 M NaOH and 10×10^{-3} M AA was prepared in double-distilled water. Buffer used was 0.2 M phosphate buffer solution (PBS) and chemicals

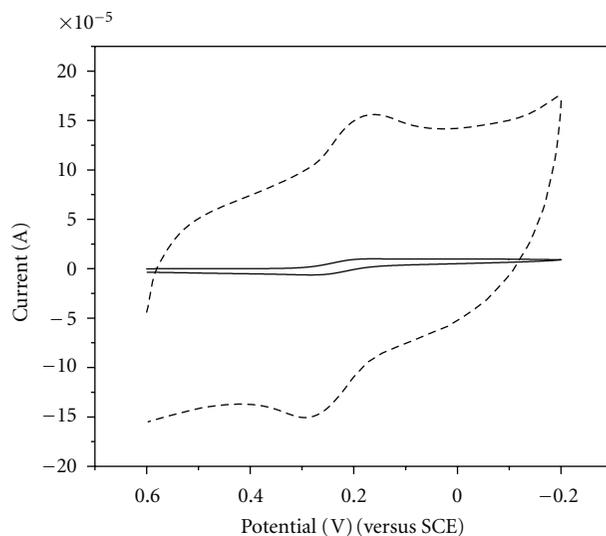


FIGURE 1: Cyclic voltammogram of 1 mM potassium ferricyanide at BGPE (solid line) and at poly(XO)/GPE (dashed line) in 1 M potassium chloride at scan rate of 50 mV s^{-1} .

mentioned above were all analytical grade used without purification.

2.2. Apparatus. The electrochemical experiments were carried out using a model-201 electroanalyser (EA-201 chemilink system). The electrode system contained the working electrodes were bare graphite pencil electrode (0.5 mm in diameter), platinum counter electrode and saturated calomel reference electrode.

2.3. Preparation of Poly(XO)/GPE. The poly(XO)/GPE was prepared by placing 5×10^{-4} M XO with 0.2 M PBS at pH = 10 as supporting electrolyte in an electrochemical cell. The potential was maintained between -400 mV to 2000 mV with 10 multiple cycles at scan rate of 100 mV s^{-1} . The poly(XO) film could be formed uniformly on the surface of GPE. After that, the electrode was rinsed with double-distilled water and kept in the 0.2 M PBS at pH 7.

3. Results and Discussion

3.1. Electrochemical Characterization of Poly(XO)/GPE Using Standard Potassium Ferricyanide System. The freshly prepared 1×10^{-3} M potassium ferricyanide and 1 M potassium chloride solutions were placed in the electrochemical cell. Figure 1 shows the cyclic voltammograms recorded for the 1×10^{-3} M potassium ferricyanide at both BGPE (solid line) and at poly(XO)/GPE (dashed line) at scan rate of 50 mV s^{-1} . The low redox current signals were observed at BGPE. The anodic and cathodic peak potentials were located at 171 mV and 283 mV. The redox peak potentials difference (ΔE_p) was 112 mV. But the poly(XO)/GPE showed significant improvement in the redox peak current signals. The anodic and cathodic peak potentials were found at 169 mV and

289 mV. The ΔE_p was 120 mV. This shows the electrocatalytic property of poly(XO)/GPE.

3.2. Electrocatalytic Response of DA at Poly(XO)/GPE. Figure 2(a) explains the cyclic voltammogram of 0.5×10^{-5} M DA in 0.2 M PBS at pH 7 at BGPE and poly(XO)/GPE at 50 mV s^{-1} sweep rate. The poor electrochemical response was observed at BGPE (solid line). The oxidation peak potential (E_{pa}) was located at 240 mV and reduction peak potential (E_{pc}) response at 110 mV were observed at BGPE. The potential difference (ΔE_p) was found to be 130 mV. However the strong improvement and reversible redox peaks current were observed at poly(XO)/GPE. During the first cycle (dashed line) the E_{pa1} and E_{pc1} were located at 200 mV and 160 mV, respectively, and another reduction peak E_{pc2} was found at -250 mV. The ΔE_p (E_{pa1} and E_{pc1}) was 40 mV. This is the clear evidence that our electrode has better electrocatalytic activity by exposing large surface area for electrochemical oxidation of DA. In the same condition, the poly(XO)/GPE was scanned again. The resultant voltammogram (dotted line) shows another anodic peak E_{pa2} at -200 mV. The oxidation mechanism of DA at poly(XO)/GPE could be explained as shown in Scheme 2. During the first scan the DA was oxidized and converted to dopa-*o*-quinone (E_{pa1}) in forward scan. While in reverse scan this was reduced to DA (E_{pc1}). The E_{pc2} was appeared by next to the E_{pc1} . This is because, the electron-rich nitrogen atom becomes deprotonated leads to formation of cyclized product known as lucodopachrome. By applying the second scan E_{pa2} appeared because of the lucodopachrome oxidized to dopachrome [9, 26–30].

To understand whether the E_{pc2} and E_{pa2} were exist or not, the experiment was carried out at poly(XO)/GPE by applying successive multiple cycle in the same above mentioned solution and condition. The voltammogram was recorded for 20 multiple cycles (Figure 2(b)). The voltammogram shows negligible decrease for second and third cycles and almost constant for further cycles and the results shows E_{pc2} and E_{pa2} were existing strongly. This confirms that DA was undergoing ring cyclization, that is, lucodopachrome and that was oxidizing to dopachrome.

3.3. Effect of Scan Rate towards the Electro-Oxidation of DA at Poly(XO)/GPE. To study the scan rate effect at poly(XO)/GPE the voltammograms were recorded for different scan rate. The cyclic voltammogram recorded for 0.5×10^{-5} M DA in 0.2 M PBS at pH 7.4 at different scan rates at poly(XO)/GPE, respectively. The redox peak currents were increased with increase in the scan rate from 50 to 400 mV s^{-1} . The graph of redox peak current versus square root of scan rate was plotted (Figure 3). The graphs shows linear relations ships between redox peak currents and scan rates. The correlation coefficient occurred was $r^2 = 0.999$ suggested diffusion-controlled electrode process. Further the graph of E_p versus $\ln \nu$ was plotted to calculate the heterogeneous rate constant and number of electron transferred in the electrochemical oxidation of DA. These

parameters were calculated by using the equation given below [31, 32]

$$E_{pa} = E^0 + m \left[0.78 + \ln \left(\frac{D^{1/2}}{k^0} \right) - 0.5 \ln m \right] + 0.5m \ln \nu, \quad (1)$$

$$m = \frac{RT}{[(1 - \alpha)nF]}, \quad (2)$$

$$E_{pc} = E^0 - m' \left[0.78 + \ln \left(\frac{D^{1/2}}{k^0} \right) - 0.5 \ln m' \right] - 0.5m' \ln \nu, \quad (3)$$

$$m' = \frac{RT}{[\alpha nF]}, \quad (4)$$

where E^0 is the formal potential, D is the diffusion coefficient, k^0 is the heterogeneous rate constant, α is the energy transferred coefficient and n is the number of electrons transferred. R , T , and F are the universal gas constant, absolute temperature and Faraday constant, respectively.

The k^0 was calculated from the above equation and it was found to be 0.0055 cm s^{-1} for forward electrochemical reaction and 0.0048 cm s^{-1} for backward electrochemical reaction at poly(XO)/GPE. The average k^0 was $0.00515 \text{ cm s}^{-1}$. This calculated rate constant was higher when compared to the rate constant for BGPE 0.0005 cm s^{-1} . The graph of $\ln I_p$ versus $(E_p - E^0)$ were plotted for the poly(XO)/GPE. The slope values from the above graph were used to calculate the energy transfer coefficient (α) using the equation (5) [31, 32]

$$I_p = 0.227FAC_0k^0 \exp[-\alpha f (E_p - E^0)], \quad (5)$$

where A is the surface area of the electrode, C_0 is the concentration of DA and $f = F/RT$.

The calculated average energy transfer coefficients were 0.662 for BGPE and 0.635 for poly(XO)/GPE. From this energy transfer coefficients the number of electrons transferred in the electrochemical redox process for DA were calculated using (2) and (4).

In our case the number of electrons transferred were $n_a = 1.93$ for anodic peak and $n_c = 1.75$ for cathodic peak when working electrode used was poly(XO)/GPE. Hence from this evidence this was confirmed that the electrochemical redox process of DA at poly(XO)/GPE was carried by transferring the two electrons.

3.4. Effect of Solution pH. The electrochemical response of DA at poly(XO)/GPE was generally pH dependent. The both redox peak potentials of DA were shifted to less positive side with increasing in the pH values. The anodic peak potential of DA shifted from 300 mV to 90 mV with respect the pH from 4 to 10. The potential diagram was constructed by plotting the graph of E_{pa1} versus pH of the solution (Figure 4). The graph has good linearity with a slope of 54 mV/pH suggested, equal number of electron and proton transfer reactions [33–35].

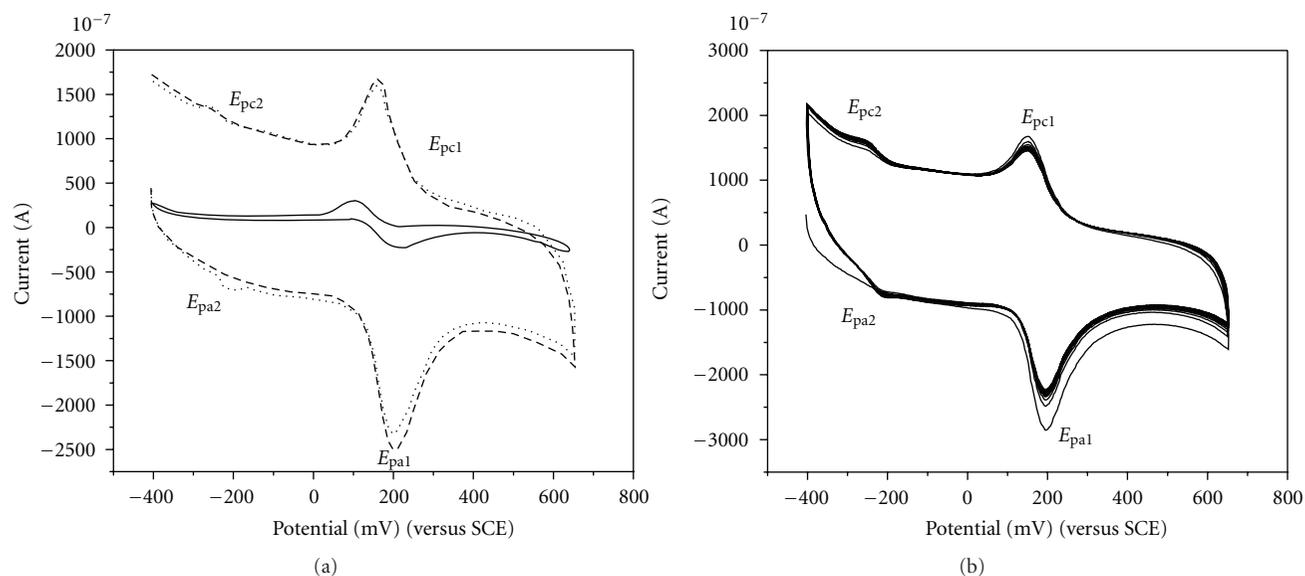
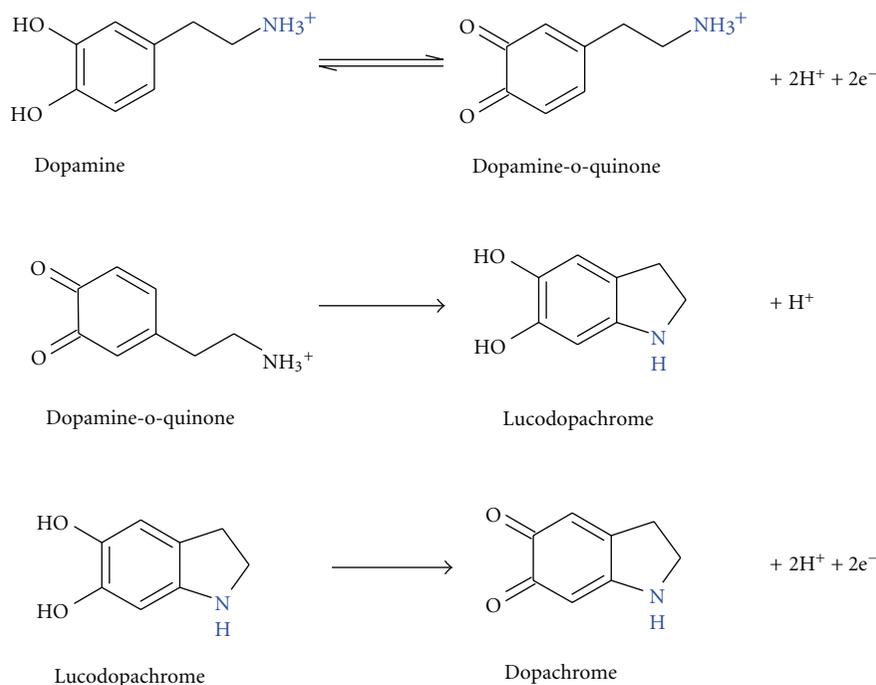


FIGURE 2: (a) Cyclic voltammograms of 0.5×10^{-5} M dopamine at BGPE (solid line) and at poly(XO)/GPE; First cycle (dashed line) and Second Cycle (dotted line) in 0.2 M PBS of pH 7 at sweep rate 50 mV s^{-1} . (b) Cyclic voltammograms of 0.5×10^{-5} M dopamine for 20 multiple cycles in 0.2 M PBS at poly(XO)/GPE at sweep rate 50 mV s^{-1} .



SCHEME 2: Electrocatalytic oxidation mechanism of DA at the poly(XO) GPE surface.

3.5. Simultaneous Determination of DA, AA, and UA. Further our poly(XO)/GPE was introduced for analysis of DA in the mixture containing large excess of AA and UA. As shown in Figure 5(a), the cyclic voltammogram at BGPE (solid line) appear the seriously overlapped peak at around 240 mV was observed for the mixture containing 0.5×10^{-5} M DA, 1×10^{-4} M AA, and 5×10^{-5} M UA in 0.2 M PBS at pH

7. However, resolved voltammetric peaks were obtained at poly(XO)/GPE (dashed line) with great enhancement in current signals. The anodic peak potentials (E_{pa1} and E_{pa2}) of DA were found at 205 and -205 mV. The corresponding E_{pc1} and E_{pc2} were located at 160 and -250 , respectively. The anodic peak potentials of AA and UA were found at 10 mV and 350 mV, respectively. The difference of anodic

TABLE 1: Comparison of different modified electrodes for DA determination.

Electrode	Detection limit ($\mu\text{mol/L}$)	Method	Reference
Triazole Self-Assembled Monolayer-Modified Gold Electrode	0.5	DPV	[36]
Metallothioneins self-assembled gold electrode	6	CV	[37]
IL-CPE	0.7	CV	[41]
Poly(L-methionine) Modified Electrode	0.42	CV	[38]
P-pTSA modified electrode	0.6	DPV	[39]
poly(XO)/GPE	0.091	DPV	This work

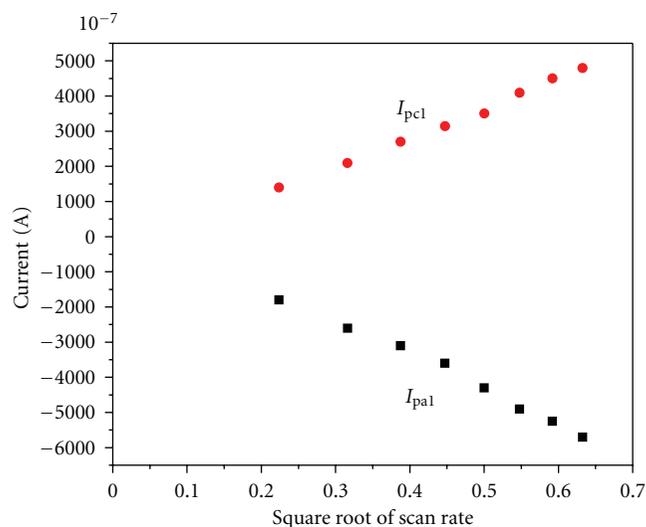
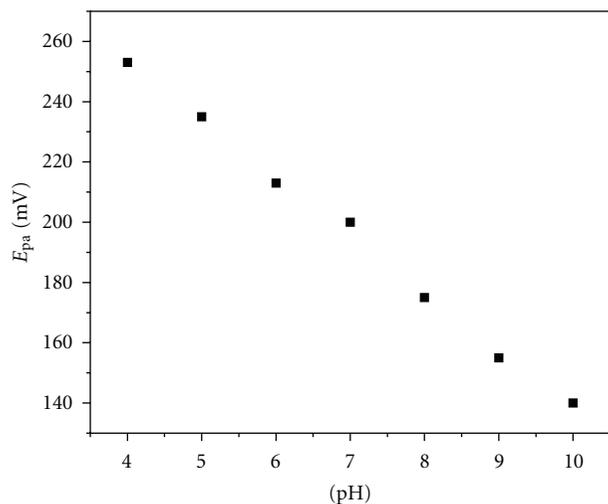


FIGURE 3: Graph of redox peak current of DA versus different scan rate.

FIGURE 4: Graph of anodic peak potential (E_{pa1}) of DA versus different pH.

peak potential between DA (E_{pa1})-AA was 195 mV and to that of UA-DA (E_{pa1}) was 145 mV by CV technique.

DPV technique was used for the determination of DA, AA, and UA at both BGPE and poly(XO)/GPE because to get higher current sensitivity and better resolution. Figure 5(b)

shows the DPVs recorded for the mixture of the same samples and condition mentioned above. The DPV recorded at BGPE shows a single and broad peak (solid line) and well-separated three anodic peaks of DA, AA, and UA were occurred at poly(XO)/GPE. The oxidation peaks of DA, AA, and UA were located at 190 mV, -20 mV and 360 mV, respectively. The anodic peak potential differences between DA-AA and UA-DA were 210 mV and 170 mV, respectively. The peak separation were greater when comparing to peak separation occurred by CV.

3.6. Calibration of DA Concentration. The incorporation study of DA was carried out in its mixture at poly(XO)/GPE when the concentration of DA was increased whereas the concentration of AA and UA were keep constant. From Figure 6(a), it can be seen that the concentration of DA was increased from 0.2×10^{-5} M to 0.9×10^{-5} M when keeping the concentration of UA 5×10^{-5} M and AA 1×10^{-4} M. The anodic peak current was proportional to concentration of DA and there were no change in the peak current and peak potential occurred for AA and UA. The graph of anodic peak current of DA was plotted against its concentration (Figure 6(b)). The correlation coefficient was found to be 0.9989. The linear regression equation was $I_{pa} (\mu A) = 47.416(\mu M/L) + 23.833$. The limit of detection and limit of quantification were calculated by using the equations given below (6) and (7), respectively, [18, 40]. The limit of detection and quantification were 9.1×10^{-8} M and 3.03×10^{-7} M, respectively, and this was compared with the other literatures [36–39, 41] (Table 1)

$$\text{LOD} = 3 \frac{S}{M}, \quad (6)$$

$$\text{LOQ} = 10 \frac{S}{M}, \quad (7)$$

where S is standard deviation and M is the slope of calibration plot.

3.7. 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 using 0.2 M PBS. The recovery and R.S.D. were acceptable ($n = 5$), showing that the proposed methods could be efficiently used for the determination of DA in injections with recovery in the range 98.5–100.05% (Table 2).

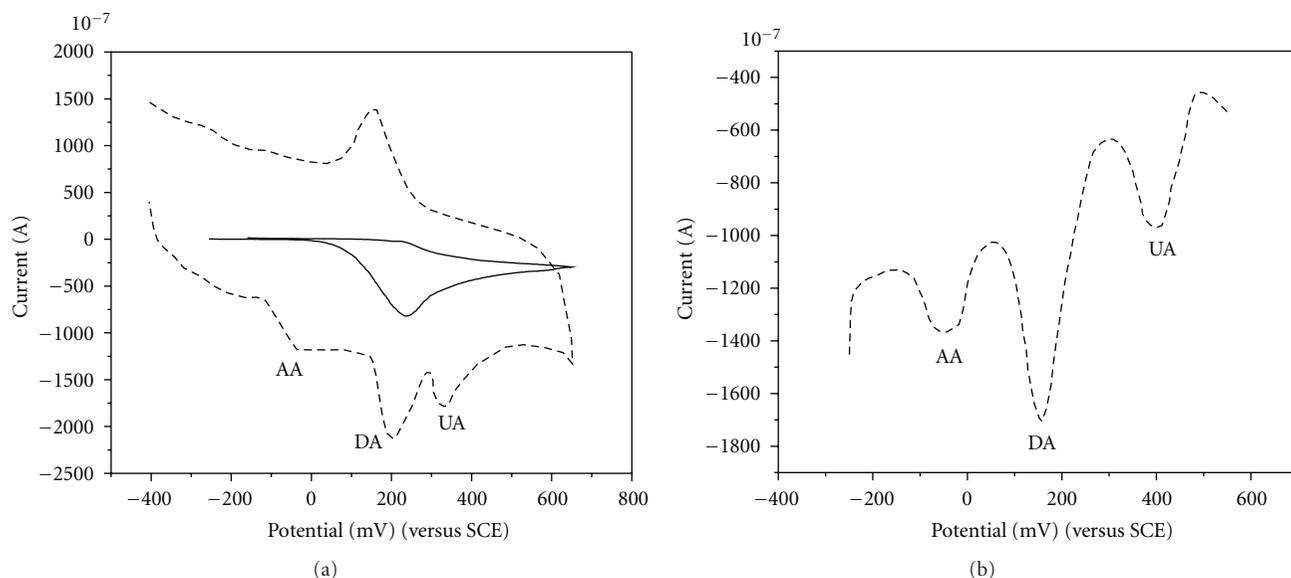


FIGURE 5: (a) Simultaneous detection of 0.5×10^{-5} M DA, 1×10^{-4} M AA, and 5×10^{-5} M UA at BGPE (solid line) and at poly(XO)/GPE (dashed line) by CV at scan rate of 50 mV s^{-1} . (b) Simultaneous detection of 0.5×10^{-5} M DA, 1×10^{-4} M AA, and 5×10^{-5} M UA at BGPE (solid line) and at poly(XO)/GPE (dashed line) by DPV at scan rate of 20 mV s^{-1} .

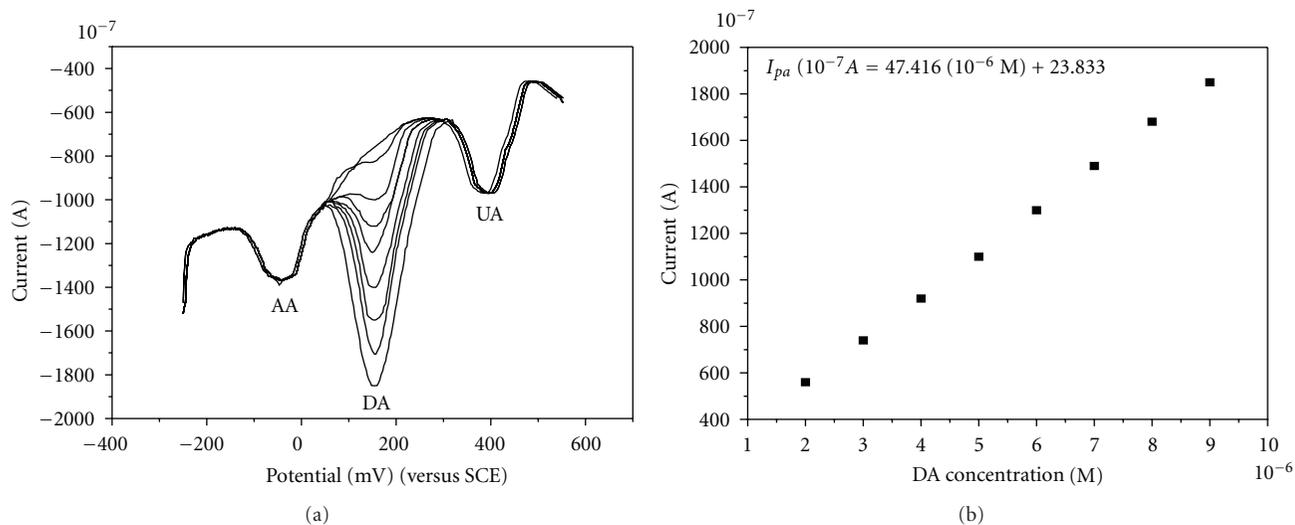


FIGURE 6: (a) Differential pulse voltammograms of different concentration of DA (a-g; $0.2, 0.3, 0.4, 0.5, 0.5, 0.6, 0.7, 0.8$ and 0.9×10^{-5} M in 0.2 M phosphate buffer solution of pH 7.0 in the presence of 1×10^{-3} M AA, and 5×10^{-4} M UA at poly(XO)/GPE. (b) Graph of anodic peak current versus different concentration of DA.

TABLE 2: Detection of DA in injection samples ($n = 5$).

Sample	Content (mg/mL)	Found (mg/mL)	RSD (%)	Recovery (%)
1	4.0	3.94	2.4	98.5
2	4.0	3.968	2.0	99.2
3	4.0	4.002	2.2	100.05

4. Conclusion

DA can be detected electrochemically in mixture of solution, at physiological pH, containing large excess of AA and UA at

poly(XO)/GPE. The prepared modified electrode detection limit was 9.1×10^{-8} M and it has excellent sensitivity, selectivity and antifouling properties. The proposed methods can be applied to the detection of DA in injection. This approach can readily be applied to the development of electrochemical sensors for dopamine and related neurotransmitters.

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