



Poly (Nigrosine) Modified Electrochemical Sensor for the Determination of Dopamine and Uric acid: A Cyclic Voltammetric Study

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Abstract: A new modified electrode based on poly (Nigrosine) has been prepared and applied on the electrochemical determination of biomolecules such as dopamine [DA] and uric acid [UA] using voltammetric methods. In this study, the poly (Nigrosine) modified carbon paste electrode [PNMCPE] gives greater current responses for DA compared with bare carbon paste electrode [BCPE]. The effect of pH, thickness, scan rate, detection limit and concentration of dopamine on the peak current was determined. The oxidation peak current of DA showed linear dynamic range 1×10^{-6} to 3.2×10^{-5} and 3.2×10^{-5} to 1×10^{-4} M with a detection limit of 8.9×10^{-7} , by cyclic voltammetry [CV] method. It has been successfully applied to the determination of dopamine in dopamine hydrochloride injection with recoveries ranging from 99.5% to 102.25%. The proposed method possesses the distinct advantages of simple, appropriate for operation, good reproducibility, and cheap instrument.

Keywords: poly (Nigrosine), Carbon paste electrode, Dopamine, Uric acid.

Introduction

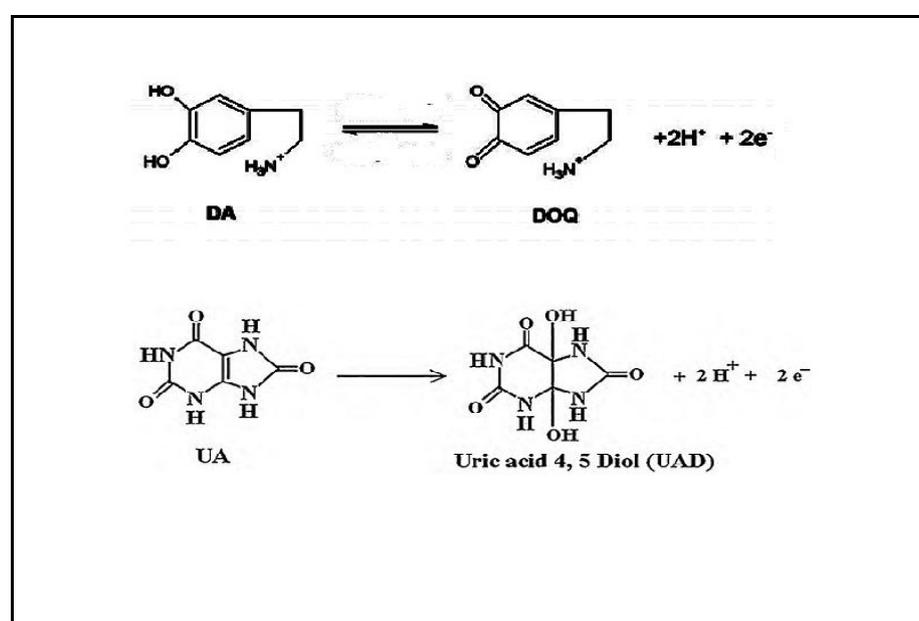
Many analytical methods have been proposed to estimate DA and UA such as chromatography, spectroscopy and electrochemistry [1-6]. Since the two molecules are electroactive compounds they can be detected by electrochemical methods based on oxidation processes, however, in general at bare electrodes their reactions are irreversible and therefore require high over-potentials. Additionally, their direct redox reactions take place at very similar potentials at bare electrodes, which results in rather poor selectivity. Consequently, modification of electrode surface with suitable electrocatalysts should improve their sensitivity and the selectivity. Therefore, the construction of new electrode provides increased sensitivity and selectivity is extremely important.

Dopamine is a neurotransmitter that plays a big role in the central nervous system [7]. Very low levels of DA result in brain disorders such as schizophrenia and Parkinson's disease [8-9]. Uric acid (UA) is the final oxidation product of urine metabolism and is excreted in urine [10]. Abnormal concentrations of UA lead to development of symptoms of several diseases such as Lesch-Nyhan syndrome and hyperuricemia [11, 12] DA, and UA usually coexist in the extracellular fluids of the central nervous system and serum in mammals that play a potential role in the metabolic system. So, simultaneous determination of these two species is of great importance not only in biomedical chemistry and neurochemistry but also for diagnostic and pathological purposes. At bare carbon paste electrodes, the selective determination of DA and UA is impossible because their oxidation potentials are very close. Besides, stability and reproducibility cannot be achieved at bare carbon

paste electrodes due to the surface fouling caused by the adsorption of oxidized products on electrode surface [13-16]. To come out these problems, many modified electrodes have been fabricated such as self-assembled iodine-layer modified palladium electrode [17], poly(3,4-ethylene dioxythiophene) modified Pt electrode in the presence of sodium dodecyl sulphate [18], Pd nanoparticles modified indium tin oxide electrode [13], Pd and Pt nanoclusters modified poly(3-methylthiophene), poly(N-methylpyrrole), and polyfuran film-coated platinum electrode [19–22], and Pd nanoparticles modified poly(3,4-ethylene dioxythiophene) electrode [23]. The redox mechanism equations of DA were shown in Scheme 1.

In this paper, we report a electrochemical method to fabricate the poly (Nigrosine) on carbon paste electrode. The obtained results with large surface area and high electron transfer ability was used to detect DA and UA. For comparison, the electrocatalytic performance of the bare CPE and PNMCPPE toward DA and UA detection were also studied.

In continuation of our studies concerning the preparation of modified electrodes [24-38] to the best of our knowledge, no study has reported the simultaneous electrocatalytic determination of DA and UA by using PNMCPPE film modified carbon paste electrode.



Scheme.1. The scheme of oxidation of DA and UA.

2. Experimental Details

2.1. Reagents

DA, UA, were purchased from Sigma Aldrich India, Silicone oil, Perchloric acid, Graphite powder (150 mesh) were purchased from Nice Chemicals, Nigrosine from Himedia and were of analytical grade. Phosphate buffer (PBS, 0.2 M, pH 7.0) was prepared from 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 were prepared in distilled water and mixed together for the pH 7.0 aqueous solution. All solutions were prepared with doubly distilled water.

2.2. Apparatus

Electrochemical measurements were carried out with EA-201 Chemilink system in a conventional three-electrode system. The working electrode was a PNMCPPE and bare carbon paste electrode (BCPE), having cavity of 3 mm diameter. The counter electrode was a bright platinum wire with saturated calomel electrode (SCE) as reference electrode completing the circuit.

2.3. Preparation of bare carbon paste electrode

The bare carbon paste electrode was prepared by hand mixing of graphite powder and silicon oil in the ratio of 70:30 (w/w) in an agate mortar until a homogenous paste was obtained. The prepared carbon paste was tightly packed into a PVC tube (3 mm internal diameter) and the electrical contact was provided by a copper wire connected to the paste at the end of the tube.

2.4. Preparation of the PNMCPPE

The polymer film-modified electrode was fabricated by electrochemical polymerization of Nigrosine by cyclic voltammetry in the potential range -400 to 1300 mV at a sweep rate of 100 mV/s in 0.05N H₂SO₄. The monomer concentration was usually 1×10^{-3} M. After 10 cycles (Fig.1), the surface of the electrode was washed with doubly distilled water to remove the physically adsorbed material and PNMCPPE film with different thickness was achieved by altering scan cycles during polymerization process (Fig.2).

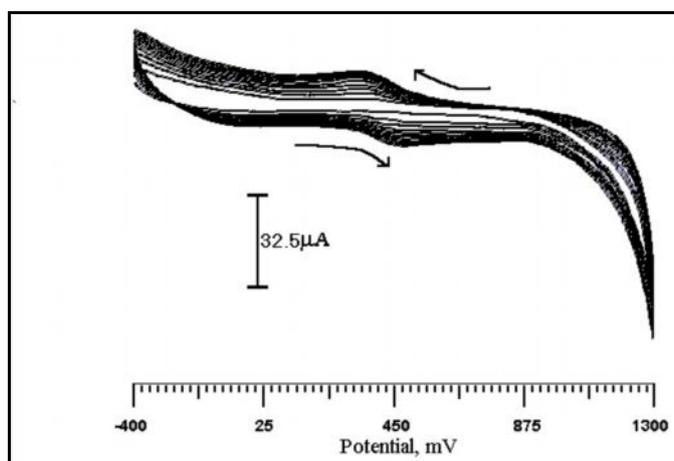


Fig.1. Continuous 10 cyclic voltammograms for the electrochemical polymerization of 1×10^{-3} M Nigrosine on a CPE in 0.05N H₂SO₄ at the scan rate 100 mV/s.

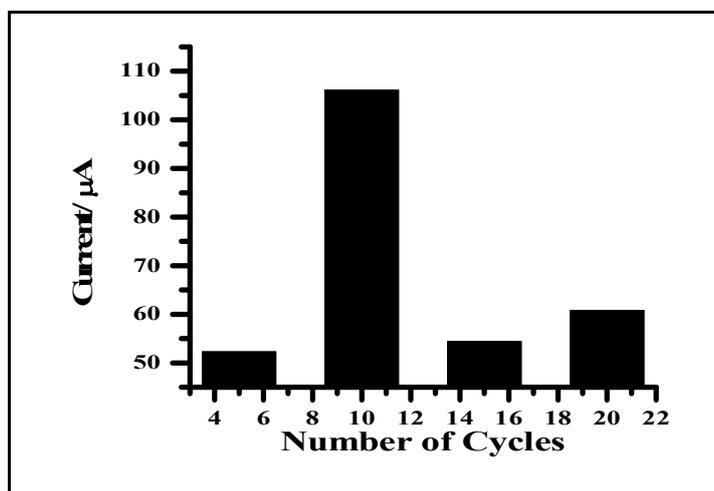


Fig.2. Dependence of the oxidation peak current of 0.2 mM DA on the number of voltammetric scans.

3. Results and Discussion

3.1. Interaction of PNMCPPE surface with potassium ferrocyanide

Cyclic voltammetry was used to estimate the electroactive surface of the modified electrode. Fig. 3 represents the CVs of BCPE (dashed line curve) and PNMCPPE, which were recorded in 1mM K₄ [Fe (CN) ₆] solution containing 1 M KCl at a scan rate of 50 mV/s. Well defined oxidation and reduction peaks due to the

$\text{Fe}^{2+}/\text{Fe}^{3+}$ redox couple were observed. It could be seen from fig. 3 that the peak-to-peak separation of BCPE (97 mV) is larger than that of the PNMCPPE (89 mV). This may be ascribed to the higher electrochemical activity of the PNMCPPE. Compared with the BCPE the peak current at PNMCPPE increased and was 1.0 times of that on BCPE. Due to increased peak current the modified electrode possesses the highest electrocatalytic activity.

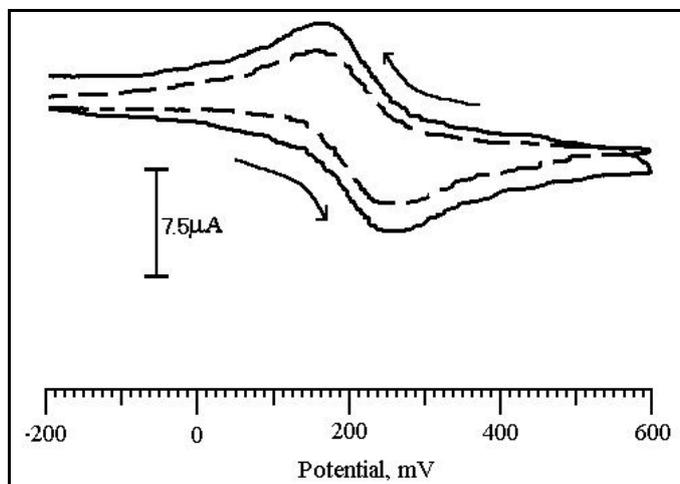


Fig.3. Cyclic voltammograms of 1 mM potassium ferrocyanide at the BCPE (solid line) and at the PNMCPPE (dashed line) in 1M KCl

3.4. Electrocatalytic Response of DA at PNMCPPE

The redox peak of 1mM DA in 0.2 M PBS (pH 7) was observed by CV in the potential range of -200 to 600 mV. As shown in fig.4, at a BCPE, the oxidation and reduction peak potential appear at 193 and 90 mV, respectively. The difference of the oxidation peak potential from the reduction peak potential was 103 mV, which suggested that the electrochemical oxidation of DA at the BCPE should be a quasireversible process. At the PNMCPPE, a well-defined redox wave of DA was obtained, with the oxidation and the reduction peak potential at 161 and 103 mV, respectively. The separation of peak potentials, $\Delta E_p (= E_{pa} - E_{pc})$, was 58 mV, which was close to $2.303RT/nF$ (or $59/n$ mV at 25°C), suggesting that the number of electrons involved in the reaction was about two. It was also observed that the anodic peak currents enhanced greatly at the PNMCPPE, which provided more evidence for asserting that the polymer on the surface of CPE possessed greatly electrocatalytic activity to the electrochemical reaction of DA.

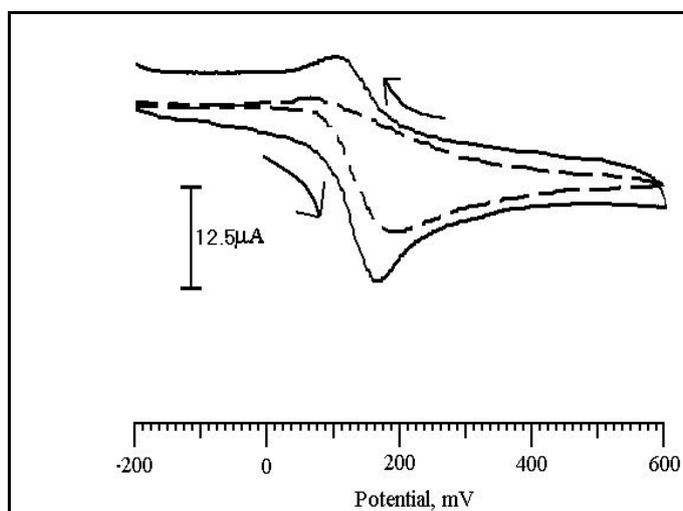


Fig.4. Cyclic voltammograms of 1mM DA in pH 7 PBS at (a) BCPE (dashed line), (b) PNMCPPE (solid line).

3.5 Effect of scan rate on the oxidation of DA

The effect of scan rate on the electrocatalytic activity of PNMCPPE toward oxidation of DA has been investigated. From fig.5, it can be seen that the peak currents and the peak potentials of DA oxidation display a close correlation with the scan rate. When the scan rate increases, peak potentials gradually shift to positive values and the oxidation peak current is proportional to the scan rates between 50 and 500 mV/s^{-1} . The linear equation is $I_{pa} (\mu\text{A}) = 22.1733 + 0.21842(\text{mV/s}^{-1})$, with linear relative coefficients 0.996 (fig.6). The above results indicate that the oxidation reaction of DA on PNMCPPE is controlled by diffusion process. In addition, the electrode reaction of DA oxidation is quasi-reversible as the redox peak potentials vary with the scan rates.

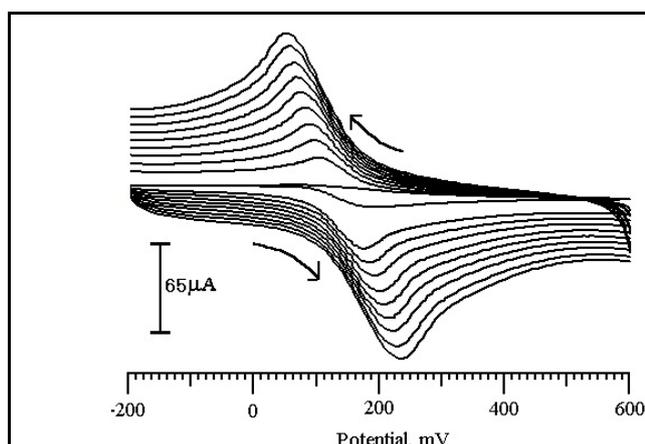


Fig.5. Cyclic voltammograms of 1 mM DA at the PNMCPPE in pH 7 PBS at various scan rates. From: 50-500 mV/s .

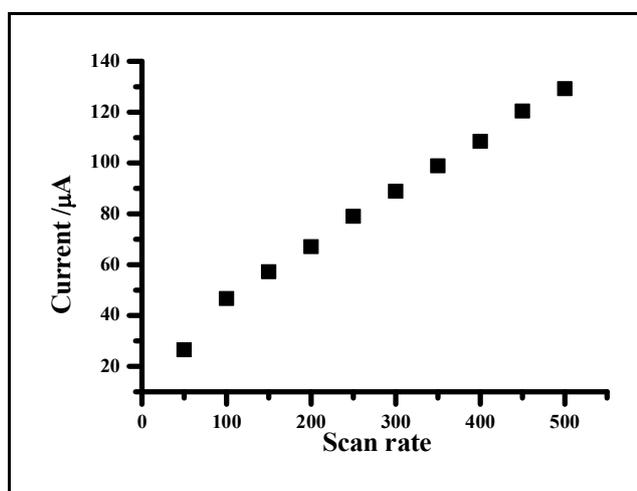


Fig.6. Plot of the anodic peak current of DA as a function of the scan rate.

3.6. Effect of pH

The effect of pH value on the CV responses was investigated in the presence of 1 mM DA, in the pH range of 5.5 to 8.0, and the results are shown in fig. 7. The peak current of DA increased with increasing pH values until the pH value reached 7.0 and then decreased when the pH increased further. The results were very close to those reported in the literature. To determine the sensitivity, pH 7.0 was chosen as the optimal experimental condition. So, all of the consequent experiments were investigated in 0.2 M PBS (pH 7.0). Fig. 9 shows that all of the peak potentials for the oxidation of DA shifted negatively with increasing pH values. The relationships between the peak potentials of DA and pH were also investigated. For DA, the linear regression equation for peak potentials and pH could be expressed as $E_{pa} = 512.86 + 56.4\text{pH}$ ($R^2 = 0.9979$). The slopes of the regression equation for DA 56.4 mV/pH , respectively, indicating that the redox reaction of DA on PNMCPPE were accompanied by proton transfer [40]. The slope of DA very close to the theoretical value of 59

mV/pH (25°), indicating that their oxidation reactions were accompanied by the transfer of an equal number of protons and electrons.

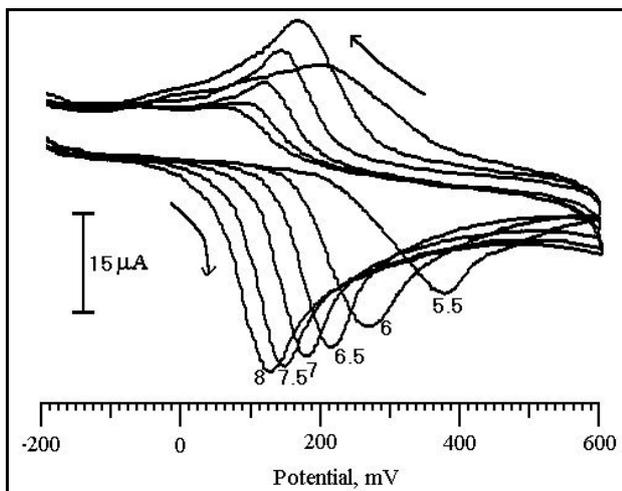


Fig.7. Cyclic voltammograms obtained at the PNMCPe in 0.2 M PBS in pH values, 5.5- 8 containing 1mM DA

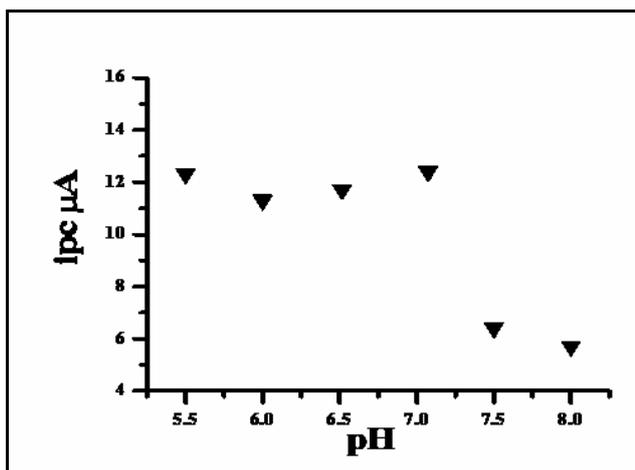


Fig.8. Plot of anodic peak current vs. pH (5.0–8.0) of 1mM DA at the PNMCPe.

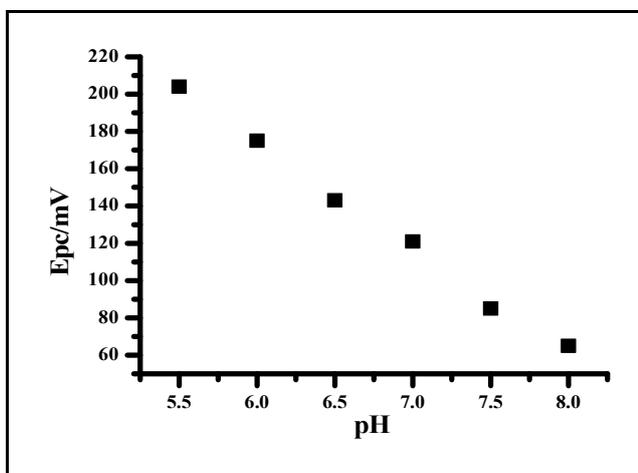


Fig.9. Plot of E_p vs. pH for DA.

3.7 Calibration curve

Fig. 10 Describes the CV recorded for various concentrations of DA at the PNMCPPE. The oxidation peak current of DA linearly increased with their concentrations. For DA, two calibration curves corresponding to two concentration ranges. The first calibration equation is $I_{pa} (A) = 0.716 \times 10^{-5} + 0.0388$ with a regression coefficient $r^2 = 0.99491$ for the concentration range from 1×10^{-6} to 3.2×10^{-5} M. The second calibration equation is $I_{pa} (A) = 1.318 \times 10^{-5} + 0.02091$ with a regression coefficient $r^2 = 0.99718$ for the concentration range from 3.2×10^{-5} to 1×10^{-4} M. The detection limit for the determination of DA were evaluated as 8.9×10^{-7} .

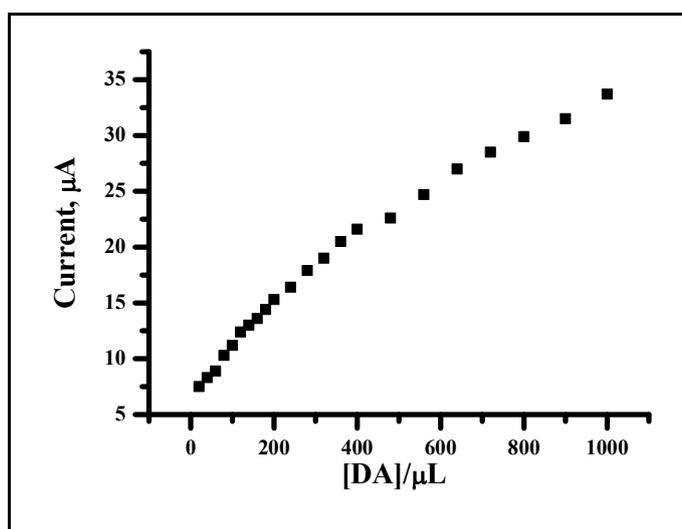


Fig.10. Calibration plot for the determination of DA at the PNMCPPE in pH 7 PBS with the scan rate 100 mV/s

3.8 Electrochemical behaviour of uric acid at PNMCPPE

Fig. 11 shows the CV responses of BCPE and PNMCPPE in 1 mM UA in 0.2 M PBS (pH 7.0). For the BCPE, oxidation peak and current is obtained at 271 mV, $i_{pa} = -2.3 \mu A$ and the current response of UA at the PNMCPPE is much better, $E_{pa} = 377$ mV, $E_{pa} = -8 \mu A$. Oxidation peak current of UA at the PNMCPPE is almost high current response, which indicates that PNMCPPE film can significantly catalyze the UA oxidation process and the electron transfer rate of UA in PNMCPPE film is much faster.

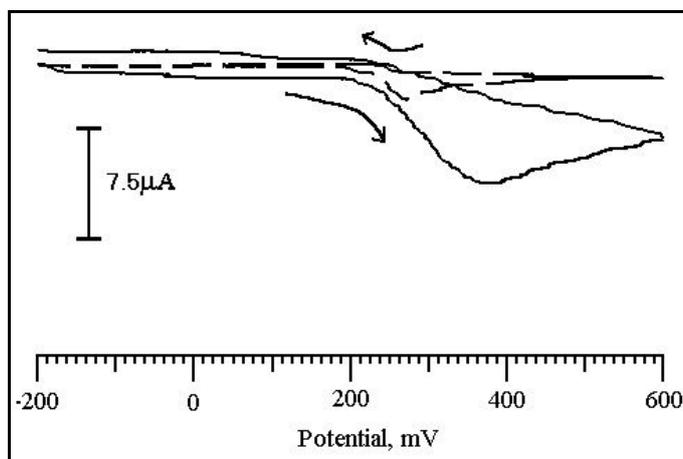


Fig.11. Cyclic voltammograms obtained for the oxidation of 1 mM UA at PNMCPPE (solid line) and BCPE (dashed line).

3.9. Simultaneous Determination of DA and UA by Differential Pulse Voltammetry [DPV]

For simultaneous determination of DA and UA at the PNMCPPE, under the optimized conditions, DPV was carried out in the potential range of -200 to 600 mV in 0.2M PBS (pH 7.0). The PNMCPPE was able to separate the defined oxidation peaks of DA and UA by showing two anodic peaks (dashed line) with significantly enhanced current responses. The electrochemical response of DA and UA showed their anodic peak potentials at 137 mV and 372 mV. The peak to peak separation of DA-UA was found to be 235 mV. This potential difference was large enough to identify the DA in presence of high concentration of UA fig.12. In these measurements, only the concentration of the target molecule was changed, while concentrations of the other one molecule remained constant. As shown in fig. 13 showed the anodic peak current of UA increase linearly with the concentration increasing in the presence of 0.2 mM DA. The linear range of UA was from 0.5 to 1.7 mM. The regression equations were $I_{pa} = 0.17 \times 10^{-5} + 2.8133$ with a regression coefficient $r^2 = 0.99797$.

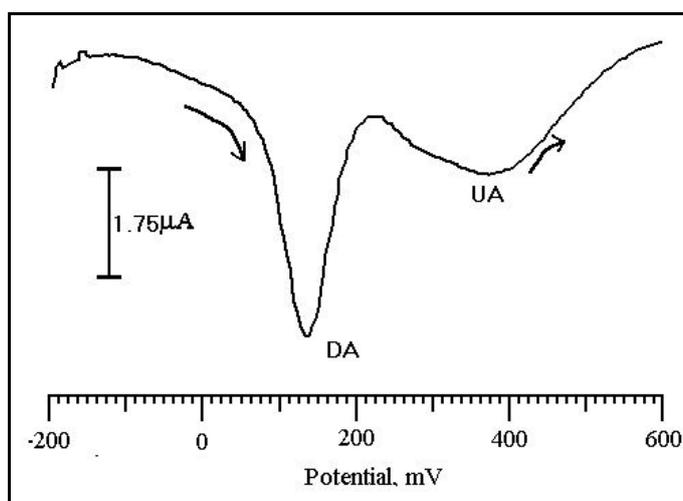


Fig.12. DPVs of mixture of 0.2 mM DA and 0.5 mM UA in 0.2 M PBS of pH 7. At PNMCPPE.

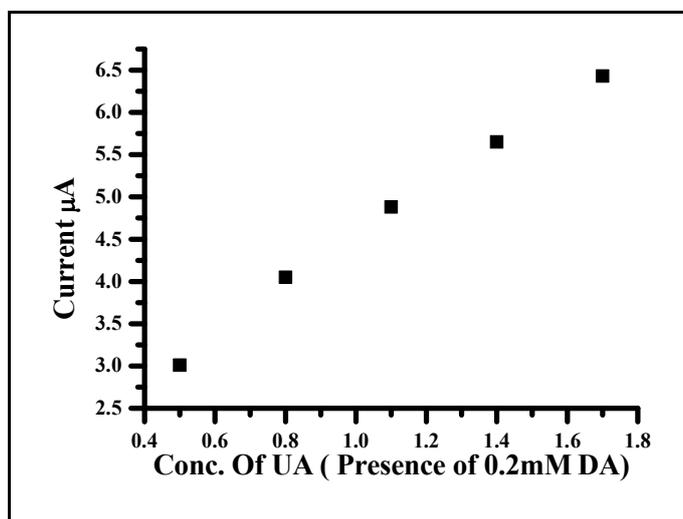


Fig.13. Plot of different concentration of UA (0.05- 0.3 mM) vs Current (DPV recordings in the presence of 0.05 mM DA)

3.11. Analytical Application

To verify our results, the modified electrode was applied to the determination of dopamine hydrochloride injection. The specified content of DA of 40.0 mg/mL. The sample was used after required dilution. Results were obtained with recovery in the range 99.5% - 102.25%, which indicates that the sensor can be applied for the analysis of these compounds and R.S.D. all so acceptable.

4. Conclusion

In this work, the PNMCPPE prepared and then was used to investigate the electrochemical behaviour of DA and UA by CV and DPV. It can be clearly concluded that the PNMCPPE had high electrocatalytic activities toward the oxidation of DA and UA by decreasing the oxidation over potentials and increasing the peak currents remarkably. When this modified electrode was used to simultaneously determine DA, and UA by DPV, the detection limit (S/N= 3) for DA were 8.9×10^{-7} . The proposed method could be applied to the determination of DA and UA in pharmaceutical formulations.

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