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Fabrication of PEDOT-PSS modified glassy carbon electrode for Biosensor and its performance in determining L-dopa in the presence of Ascorbic acid

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ABSTRACT

Poly (3,4-ethylenedioxythiophene)-(Poly(4-styrenesulfonate)) was synthesized on the surface of glassy carbon electrode by electrochemical method and used to determine biologically important L-dopa in the presence of a large excess of ascorbic acid by differential pulse voltammetry. The PEDOT-PSS surface was characterized by Scanning electron microscopy and ATR-IR. The oxidation peaks of L-dopa and ascorbic acid (AA) were well separated at PEDOT-PSS modified electrode in phosphate buffer solution (PBS, pH 7.0). A linear relationship between the peak current and concentration of L-dopa was obtained with the correlation co-efficient of 0.999 with the detection limit of 1.6 µM (S/N=3) in the presence of 5mM AA. The proposed method was successfully applied for the determination of L-dopa in pharmaceutical samples.

Key words: PEDOT, PSS, modified electrodes, L-dopa, Ascorbic acid

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease caused by low concentration of dopamine in substantia nigra of the mid-brain. This will also cause dyskinesia, tremors, rigidity and poor balance. This can be overcome by taking L-dopa (levodopa, 3, 4-dihydroxy-L-phenylalanine) orally. This drug can be metabolized to dopamine by dopa-decarboxilase enzymatic reaction. The increase in concentration of plasma L-dopa level, leads to side effect such as vomiting, nausea, gastritis, paranoia and cardiac arrhythmias [1–4]. Thus L-dopa has to be given accurate in both pharmaceutical formulations and biological fluids. Several methods have been proposed for the determination of L-dopa such as high performance liquid chromatography [5, 6], spectrophotometry [7, 8] and titration [9]. Now a day's electrochemical method is found to be of great interest in current research because of its selective determination and high sensitivity. Unfortunately, most unmodified conventional solid electrodes show a slow electron transfer for the electrochemical oxidation of some small biomolecules like L-dopa and ascorbic acid. The oxidized product of these biomolecules easily adsorbs at bare electrode which leads to poor reproducibility and repeatability. The concept of surface modification of bare electrode is one of the exciting developments in the field of electroanalytical chemistry. The modification may lead to increase in the electron transfer rate of biomolecules oxidation, reduction of overpotential and also increase in the selectivity and sensitivity for the determination of biomolecules [10–13]. The conducting polymers have attracted immense attention because of their electrochromic nature, low cost, ease of processing, high electronic conductivity, and robust switching capability [14–17]. Among the conducting polymers, PEDOT-PSS has attracted significant interest due to its high conductivity, electroactivity, permselectivity, electrochromism and a wide application in electrocatalysis [18–23] Thus; it is significant to develop a PEDOT-PSS modified GCE for L-dopa determination in the presence of ascorbic acid. In this work

electrocatalytic behaviour of PEDOT-PSS film towards the oxidation of L-dopa in the presence of ascorbic acid is described, in addition, the kinetics of mediated electro oxidation of L-dopa at the PEDOT-PSS modified glassy carbon electrode is investigated using cyclic voltammetry. Pulse technique is used to find detection limit of L-dopa. The PEDOT-PSS modified GCE not only exhibits strong catalytic activity towards L-dopa and ascorbic acid but also provides a stable and quantitative analytical reproducible performance.

EXPERIMENTAL SECTION

Reagents and solutions

3,4-ethylenedioxythiophene (EDOT) and Poly(4-styrenesulfonic acid) were purchased from Aldrich Chemicals Germany. $LiClO_4$ and L-dopa were obtained from High media Laboratories and Loba chemie, India. All other reagents were used of analytical grade and used as received. Aqueous solutions were prepared using double distilled water.

Instrumentations

The electrochemical experiments were carried out using CHI6041C (CH Inc., USA) coupled with a conventional three-electrode cell. The three electrodes namely the working electrode, the auxiliary electrode, and the reference electrode were GCE, Pt wire and Ag/AgCl electrode, respectively. All the potentials in this communication were given against Ag/AgCl. The surface morphology and PSS over PEDOT was characterized by SEM (FEI Quanta FEG 200-High resolution scanning electron microscope) and Perkin Elmer ATR-IR. An (EI-1L model 34 kHz) ultrasonic bath was used for cleaning the electrodes and to prepare a homogeneous mixture

Preparation of PEDOT-PSS modified electrode

Before modifying, the GC electrode was polished with 0.3 and 0.05 µm of alumina slurries for 2 min each, followed by thorough rinsing with double distilled water; the electrode was then sonicated with ethanol and distilled water for 2 min each. After sonicating electrode, it was rinsed with double distilled water and was examined by cyclic voltammetry using standard 1 mM potassium ferric cyanide solution (by evaluating the oxidation and reduction peak potential). The PEDOT-PSS polymer film was electrochemically prepared by electrodeposition from an aqueous solution of 10^{-3}M EDOT and 10^{-4} M PSS mixture in 0.1M of $LiClO_4$ as supporting electrolyte. Then solution mixture was sonicated for few minutes and left without any disturbance for few hours. The electrochemical synthesis was carried out by using cyclic voltammetry technique by scanning towards positive potentials from -0.1 to 1.6 V versus Ag/AgCl at a scan rate of $100mVs^{-1}$ a typical deep blue colour was observed after 8 cycles. Hereafter, this electrode was named as PEDOT-PSS modified electrode and kept in 0.1M phosphate buffer of pH 7.0 until further use.

RESULTS AND DISCUSSION

Electropolymerization of PEDOT-PSS

The electropolymerization of PEDOT-PSS has been carried out between –1.0 and +1.6 V on the surface of the GCE at a sweep rate of 100 mV s^{-1} for 8 cycles. (Figure. 1) shows the growth of film during this process of multiple cycles, and current gradually increases with an increase in cyclic time. This indicates PEDOT-PSS polymerization over the surface of GCE. It is clear from the cyclic voltammogram, two oxidation peaks were absorbed namely P1 and P2 at 1.38 V vs Ag/AgCl and 1.25 V vs Ag/AgCl. In conclusion, polymerization of PEDOT-PSS follows two steps. Adsorption of oxidized species over the electrode surface occurs in the first step. The dimer or oligomers which are formed in the first step get further oxidized in the second step [24, 25].

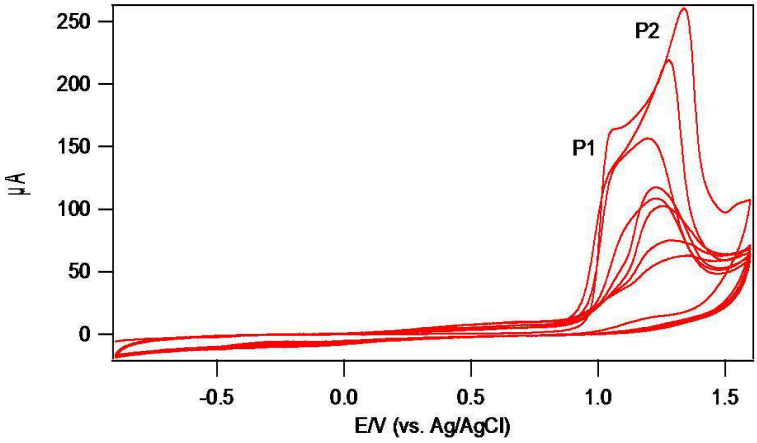


Figure 1 Cyclic voltammogram of PEDOT-PSS

Characterization of PEDOT-PSS film by ATR-IR and Scanning Electron Microscopy (SEM)

To address the surface distribution of the system, we have examined and compared morphologies of PEDOT (Figure. 2a) and PEDOT-PSS films (Figure. 2b) using scanning electron microscopy (SEM). PEDOT surface looks like irregular granular covered with micro fibers, whereas PEDOT- PSS shows a mixture of micro rings and wrinkles over the surface. This confirms the PEDOT-PSS films on the surface of the GCE [26].

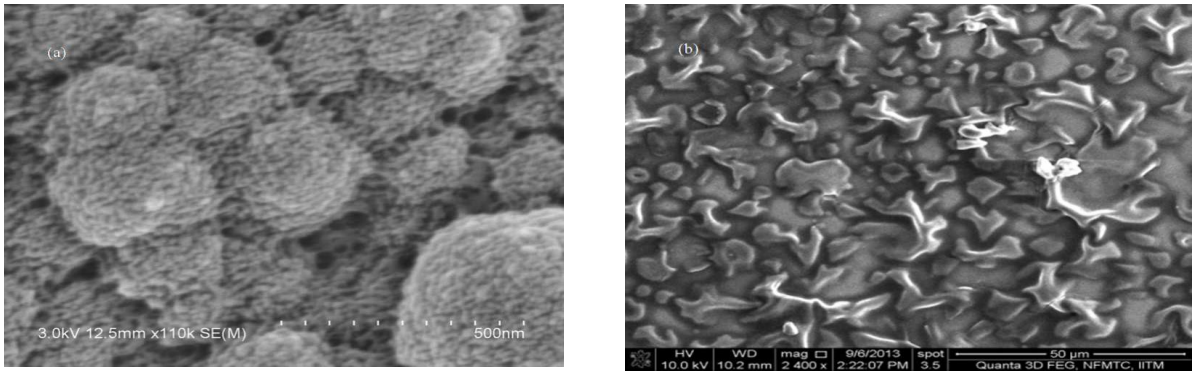


Figure 2 SEM image of (a) PEDOT, (b) PEDOT-PSS

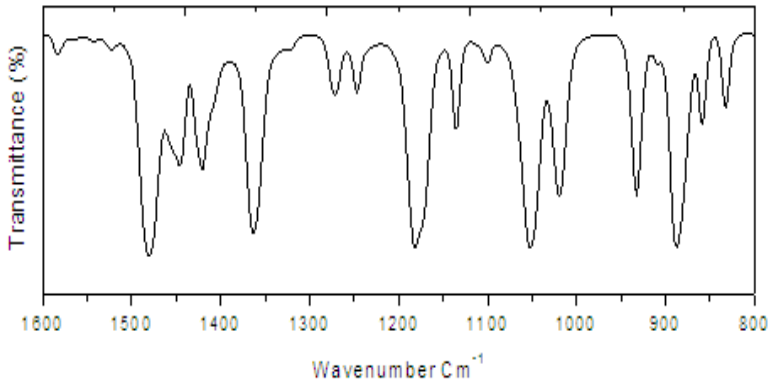


Figure 3 ATR-IR spectra of EDOT monomer

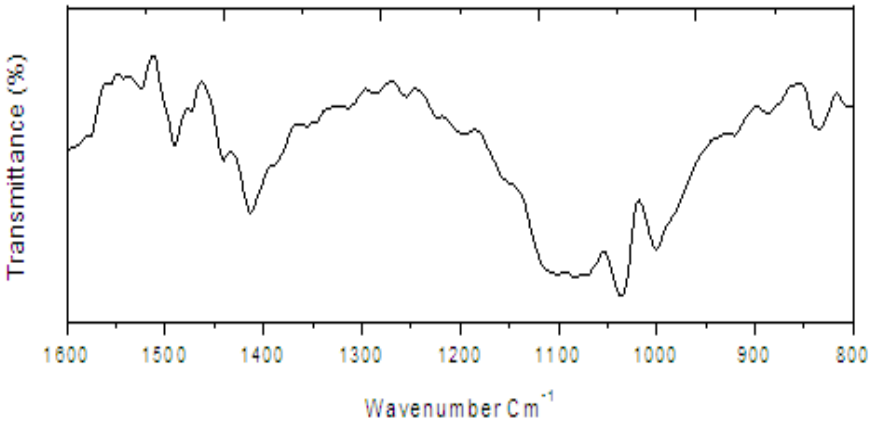


Figure 4 ATR-IR spectra of PEDOT-PSS

Further, Figures 3 and 4 show the FTIR reflectance spectra of EDOT, PEDOT-PSS. The disappearance of bending mode of C-H bond at 885 cm^{-1}, (Figure 4) confirms formation of α-α'-coupled PEDOT molecular chain. Moreover, the peaks at 1201cm^{-1} and 934 cm^{-1} in (Figure 4) are due to PSS stretching frequencies. This result coincides with the earlier reports [24, 26–28]. This clearly confirms the successful formation PEDOT-PSS polymer over GCE.

Electrochemical oxidation of L-dopa

The electrochemical behavior of L-dopa was investigated with CV in 0.1 M phosphate buffer solution at pH 7.0. As shown in (Figure. 5) a weak response and slow electron transaction for L-dopa were observed on a bare electrode. In the +0.40 to 0.5 V region, an oxidation peak of L-dopa at +0.56 vs Ag/AgCl was noted. The PEDOT-PSS modified electrode shows a good electrocatalytic oxidation toward L-dopa indicating that the PEDOT-PSS modified electrode can effectively decrease the oxidation potential of L-dopa to 0.35 V vs Ag/AgCl from 0.46 V vs Ag/AgCl.

Furthermore, the oxidation peak current (Ipa) of L-dopa at PEDOT-PSS modified electrode is three times higher than that of bare electrode with a better reversibility. These results confirm that the PEDOT-PSS on the surface of bare electrode can effectively accelerate the electrochemical redox behavior of L-dopa and significantly increase oxidation current at modified electrode. Thus, the obtained higher oxidation current may be due to π bond attraction in PEDOT-PSS

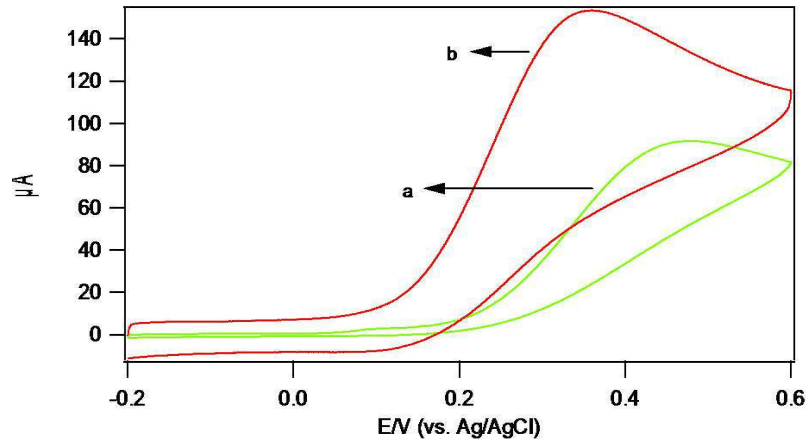


Figure 5 Cyclic voltammogram of 5mM L-dopa, (a) bare GCE (b) PEDOT-PSS GCE at pH 7.0

Effect of scan rate

The effect of scan rate on the oxidation of 5mM L-dopa in PEDOT-PSS modified electrode in phosphate buffer solution of pH 7.0 (Figure. 6). As observed in Figure. 7 the oxidation current increased linearly while increasing the

scan rate from 50 mVs^{-1} to 500 mVs^{-1} with a correlation coefficient of 0.990 suggesting that the oxidation of L-dopa at PEDOT-PSS modified GCE's was diffusion controlled process [29].

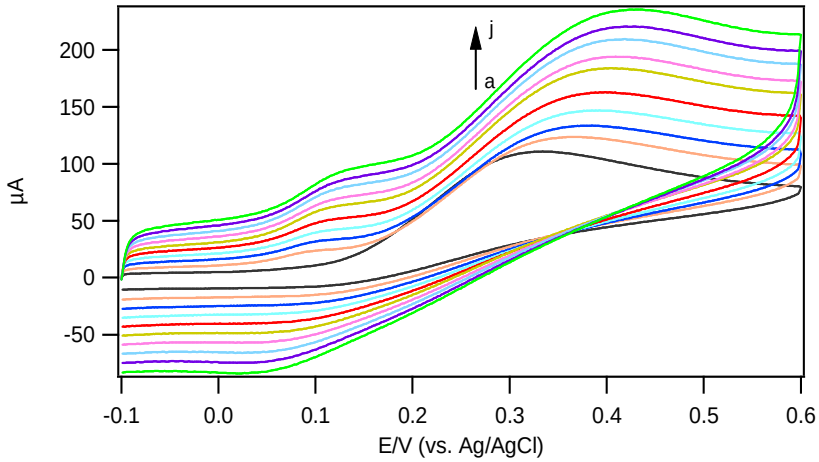


Figure 6 CV of the 5mM L-dopa in PBS (pH 7.0) at PEDOT-PSS modified GCE with different scan rates (a–j: 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mVs^{-1})

Effect of pH

The CV method was used to study electrochemical response of L-dopa in various pH at PEDOT-PSS modified GCE. Figure. 8a shows anodic peak current of L-dopa in the pH range of 3.0- 8.0. The maximum peak current was observed at 7.0pH. Further increasing of pH shows a nearly saturated current. Thus by considering the biological pH and increasing current, the pH 7.0 was fixed for further study. Moreover (Figure. 8b) demonstrates the Epa of L-dopa at a scan rate of 100mVs^{-1} in PEDOT-PSS GCE. The Epa of L-dopa shifts towards less oxidation potential. With the increasing of pH, the graph has good linearity with a slope of 53mV/pH. This behaviour nearly obeyed Nernst Equation for equal number of proton and electron transfer reaction [30, 31]. The electrochemical redox process of L-dopa to give dopaquinone is described in Scheme 3.1.

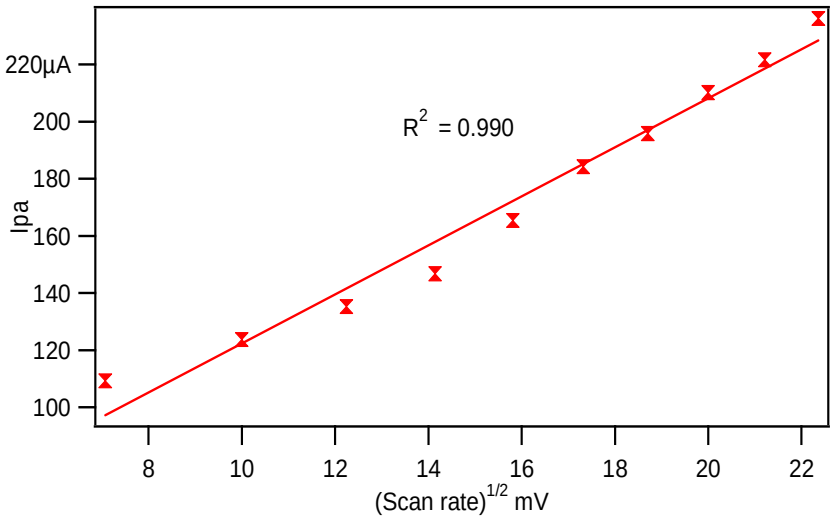


Figure 7 Square root of scan rate vs. anodic peak current of L-dopa in PEDOT-PSS modified GCE

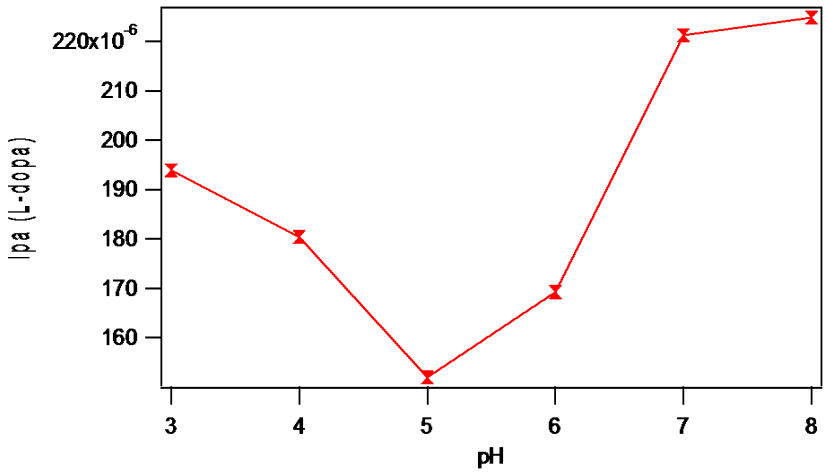


Figure 8 Ipa of L-dopa in different pH (from 3.0 to 8.0) at PEDOT –PSS GCE

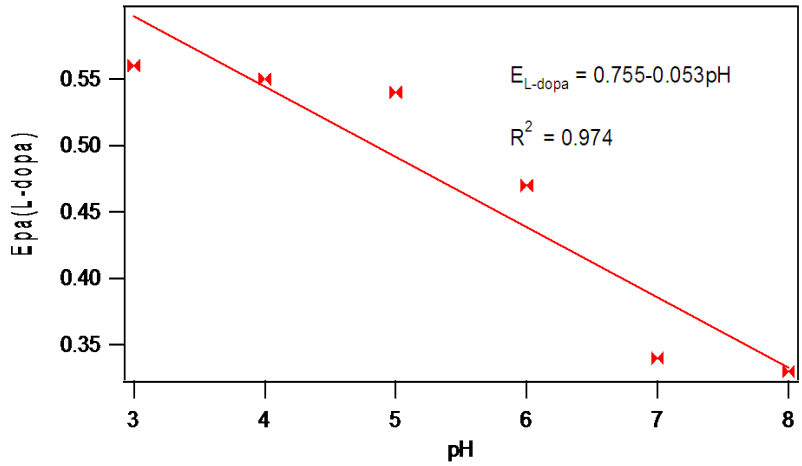
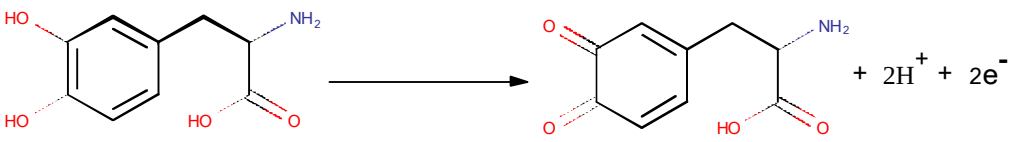


Figure 9 Plot of Epa vs different pH for 5mM L-dopa in PEDOT-PSS modified GCE



Scheme 1 Electro oxidation mechanism L-dopa

Determination of L-dopa by DPV

The determination of biomolecules needs high sensitivity. This can be achieved by DPV method normally [32]. Figure 9 shows DPVs obtained from 50µM to 247 µM L-dopa in the presence of 5mM AA at pH 7.0. A clear voltammetric signal was observed for 50µM L-dopa even in the presence of 100 fold higher concentration of AA respectively, which revealed that detection of low concentration of L-dopa is possible even at high concentration of AA.

While varying the concentration of L-dopa in the presence of 5mM AA by standard addition method the oxidation current of L-dopa increased linearly with correlation of co-efficient 0.999 (Figure. 10). Thus, the present modified electrode can be used to determine L-dopa even in the presence of high concentration of AA.

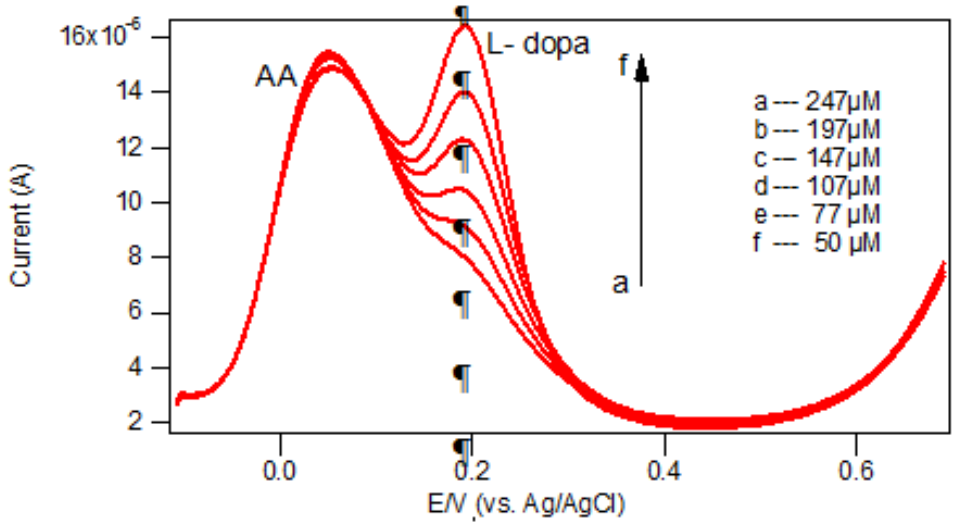
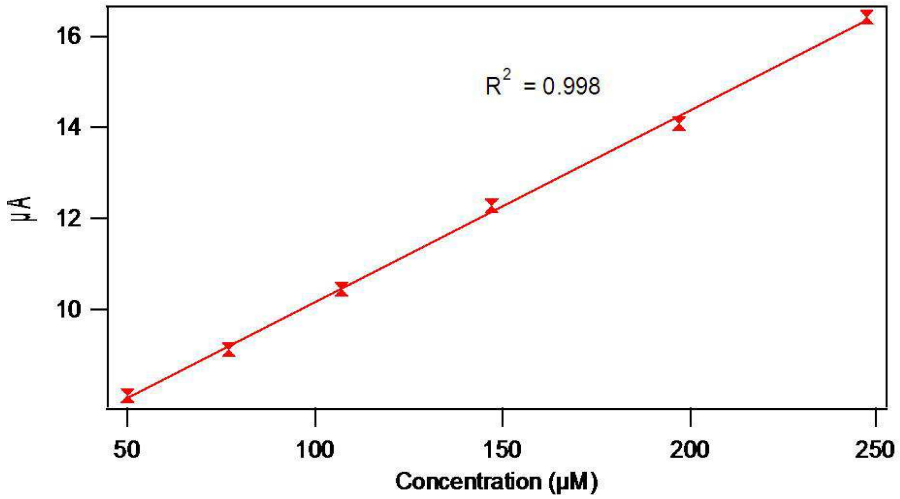


Figure 10 DPV of 5mM AA at PEDOT-PSS GCE in the presence of different concentration of L- dopa: (a) 50.0, (b) 77.0, (c) 107.0 (d)



147.0 (e) 197.0, (f) 247.0 μM.

Figure 11 Plot of i_{pa} vs Concentration of L-dopa.

Interference study

The possible influence of foreign species which coexist with L-dopa in the determination of body fluids have been investigated, in comparison with other foreign species considering the electroactive molecules like UA, AA, dopamine, epinephrine, tryptophan and tyrosine are more important. Among all AA and UA plays a serious interferes with the determination of L-dopa because of its electrode fouling nature and nearly same oxidation potential of L-dopa.

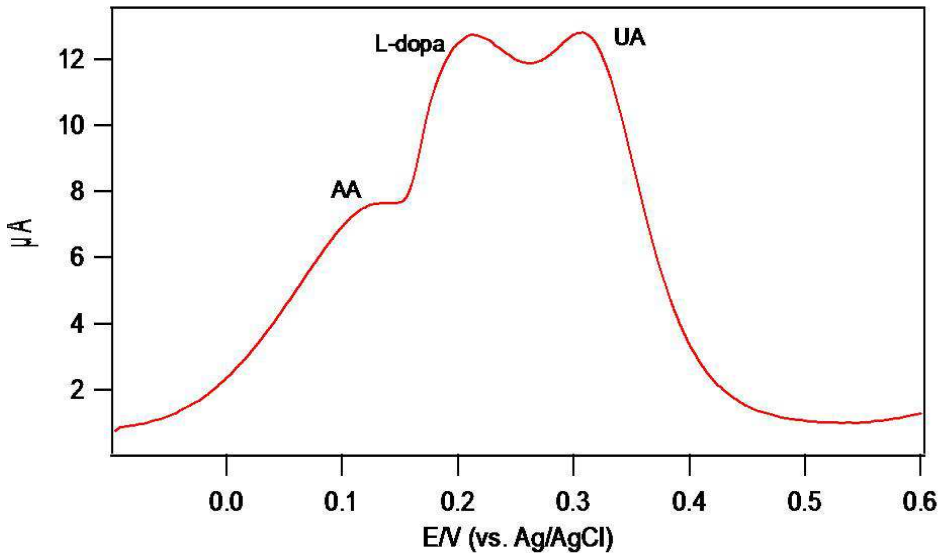


Figure 12 DPV of 1mM L-dopa, AA and UA in PEDOT-PSS GCE

Figure.12 shows the PEDOT-PSS modified GCE capability to separate the solution mixture of UA, AA and L-dopa. Were as for tryptophan and tyrosine it has highest oxidation potential when comparing with L-dopa, so this eliminates tryptophan and tyrosine interfere in the determination of L-dopa [3338]. According to G. Hu et al though amines like dopamine and epinephrine are electroactive molecule, because of their low concentration in body fluids they do not affect the accurate L-dopa determination [13]. Finally the other foreign species like (100) glucose, (100) urea and (500) NH^{4+}, (500) K^{+}, (500) Na^{+}, (500) Mg^{2+}, (500) Ca^{2+} were added to (50) L-dopa. The change in peak current was observed with tolerance limit of 5%. The data in the bracket are concentrations of the interfering species in µM

Stability and reproducibility

The PEDOT-PSS was stable and reproducible. However the electrode had to be well treated to remove adsorbed contaminant to maintain the reproducibility. It was found that electrode can be renewed by washing with de-ionized water and continuous scanning in the buffer 0.1M PBS of pH 7.0 after each experiment. Generally, 12 cycles of scanning could be recycled. The PEDOT-PSS had good storage stability in PBS for two days.

Analytical application

The modified electrode was applied to determination of L-dopa in commercial tablets (Syndopa 110); the tablets were purchased from Indian pharmaceuticals. A portion of 0.2 g was weighed accurately and dissolved in 1 mL of 5% acetic acid and made up to 100 mL using 0.1 M PBS at pH 7.0. The standard addition method was used for the determination of L-dopa; the results are shown in Table 1. The average recovery is 98%, indicating applicability and reliability of the proposed method.

Table1 Detection of L-dopa in commercial tablets

Powder tablets Samples	Concentration of L-dopa (µM)		Recovery (%)
	Added	Found	
Sample I	90µM	88µM	97.7%
Sample II	90µM	87µM	96.6%

CONCLUSION

We have demonstrated that PEDOT-PSS modified electrode can be used to determine concentration of L-dopa in the presence of AA with excellent sensitivity and selectivity. This modified electrode was confirmed by ATR-IR and SEM analysis. The characteristic behaviour was tested by cyclic voltammetry and the differential pulse voltammetry

technique, was used to find the detection limit. In comparing with bare electrode, the PEDOT-PSS modified electrode shows higher oxidation current for both L-dopa and AA. This was attributed to greater surface area of PEDOT-PSS. Moreover, the PEDOT-PSS modified electrode having an ability to detect 1.6µM L-dopa in the presence of 5mM AA. Further, it was found that this modified electrode is able to detect L-dopa in tablets with satisfactory recovery percentage.

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