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Development of Electrochemical sensor based on Poly (xylenol orange) film towards the determination of L-Dopa and its simultaneous resolution in the presence of Uric acid: A cyclic Voltammetric study

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Abstract

The surface of carbon paste electrode (CPE) was modified based on electropolymerization of xylenol orange (XO) using cyclic voltammetry. This poly (XO) modified carbon paste electrode (poly(XO)MCPE) exhibited good electrocatalytic activity towards the quantification of L-dopa in 1mM phosphate buffer solution (PBS) at pH 5.0. The method was used to study the factors such as effects of pH, scan rate and concentration. Kinetic parameters such as diffusion coefficient (D), area of the modified electrode (A), limit of detection and limit of quantification of L-dopa were calculated. Simultaneous determination of L-dopa in the presence of uric acid (UA) was studied by differential pulse voltammetry (DPV). The interfacial electron transfer behaviour of L-dopa was studied by means of electrochemcal impedance spectroscopy (EIS). The practical analytical application of poly(XO)MCPE towards the detection of L-dopa in a commonly available commercial tablet sample has also been evaluated.

Keywords: L-dopa, Xylenol orange, cyclic voltammetry, differential pulse voltammetry, electrochemcal impedance spectroscopy, electrochemical kinetic parameter.

Introduction

According to James Parkinson, Parkinson disease (Paralysis agitans) is an age related neurological non-genetic disorder associated with progressive degeneration of dopamine (DA) resulted in hyperuricemia and occasionally gout[1,2]. L-dopa (levodopa, 3,4-dihydroxy-1-phenylalanine) is a chemical messenger, well–known biogenetic amine, one of the catecholamine and direct precursor of DA. In 1960s, L-dopa has been considered as an effective agent to increase the DA levels in patients suffering from Parkinson's disease, since it can cross the blood brain barrier whereas DA itself cannot[3]. Once L-dopa has entered into the central nervous system then it is converted into DA by enzymatic decarboxylation process[4]. It has been found that the L-dopa shows very strong electrochemical activity towards oxidation process as levodopa quinine and then cyclizes to cyclodopa[5]. From the literature, L-dopa is a powerful toxin and is lethal to the culture of neurons. Many animal research studies had resulted that chronic L-dopa may be toxic in vivo also[6]. Therefore, determination of L-dopa concentration level is of much interest in analytical chemistry.

Uric acid (2,6,8-trihydroxypurine, UA) is the end product of protien and purine metabolism and is present in blood and urine[7]. If there is an increase in the production of UA or if the kidneys do not remove enough of UA from the body, then the levels of UA will build up in the blood (the condition called hyperuricemia)[2]. As discussed above, both L-dopa and UA interrelated to hyperuricaemia and gout. Therefore, their simultaneous determination is reasonably necessary in the diagnosis and treatment of diseases.

Over the past decade, several analytical methods have been employed for the determination of L-dopa such as gas chromatography (GC)[8], high performance liquid chromatography (HPLC)[9], spectrophotometry[10], flowing injection analysis[11], capillary zone electrophoresis[12] and chemiluminesence[13]. The above said methods often require expensive equipments and long time analysis; in this point of view alternative technique is needed. Electrochemical methods have received great importance due to their good sensitivity, often associated with high selectivity, low cost, rapidity and precision for quantification of important compounds in biological and clinical point of view[14]. Chemically modified electrodes (CMEs) play an essential role in reducing the high over voltage for the determination of analyte. There are several research articles describing the electrochemical determination of L-dopa at CMEs[15-17].

Now-a-days, Polymer modified electrodes have attained a great importance. In addition to, the electro polymer film coated electrodes with organic dyes exhibits high stability, more active sites, reproducibility and homogeneity in electrochemical deposition[18]. Numerous redox dyes are available as artificial electron donors[19]. Xylenol orange (XO) [*N*,*N*^{1}-[3H-2,1-Benzoxathiol - 3- ylidenebis- [(6-hydroxy-5-methy l-3,1-phenylene)methylene]] bis[*N*-(carboxymethyl)glycine] *S*,*S*-dioxide] is an important organic dye, is capable to undergo

electropolymerization in aqueous solutions and produces redox stable active layers[20]. Hence it can be used as modifier for the electrocatalytic activity towards different biologically important compounds[21].

The aim of the present study describes the quantification of L-Dopa at poly(XO)MCPE by cyclic voltammetry technique. The modified electrode showed better sensitivity and selectivity towards the determination of L-dopa at low level concentration and in presence of UA. Literature survey resulted that no examination has been done towards the determination of L-dopa at poly(XO)MCPE. The practical application of poly(XO)MCPE towards the determination of L-dopa was successfully demonstrated.

Material and Methods

Instrumentation: A CHI 1200A electrochemical analyzer was used for the cyclic voltammetry measurements. CHI 660D electrochemical work station was used for the measurements of Electrochemical impedance spectroscopy (EIS). A conventional three electrode system was employed, which consists of a modified CPE as working electrode; Ag/AgCl as reference electrode to measure cell potentials and platinum wire as an auxiliary electrode to measure current. The pH values were measured with the help of Elico U 120 pH meter combined with pH CL 51 B electrode.

Reagents: L-dopa was purchased from Merck Specialities Pvt. Limited, Mumbai. $K_3[Fe(CN)_6]$ from Merck Specialities Pvt. Limited, Mumbai. KCl from Qualigens fine chemicals, Mumbai. Xylenol orange from Himedia Laboratories Pvt. Limited, Mumbai. Perchloric acid from SDFCL, Mumbai, Uric acid was purchased from Merck Specialities Pvt. Limited, Mumbai. All of them were used without any further purification. The stock solution of 10 mM L-dopa was prepared and stored in cool 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 and silicon oil from S.D. Fine Chemicals Limited, Mumbai. 0.1 M PBS was prepared from $NaH_2PO_4.2H_2O$ and Na_2HPO_4. All reagents are of analytical grade.

Preparation of CPE/ poly(XO)MCPE: The CPE was prepared by hand grinding of 85% graphite power and 15% of silicon oil using agate mortar for 30–35 min to get homogeneous paste. This carbon paste was incorporated into 2 mm diameter of Teflon tube and electrical contact was made by means of a copper wire. The CPE was smoothed on a piece of soft filter paper before the measurement. The 1 mM aqueous XO was placed in the electrochemical cell along with 0.1 M PBS solution of pH 5.0. The CPE was scanned for 10 multiple cycles, between the potential windows from -600 to +1400 $mV.s^{-1}$. After the completion of the process the electrode was washed with double distilled water for further use.

Preparation of real sample: 250 mg of L-dopa of a tablet was taken and grinded well by using agate mortar to prepare finely powder. The white powder was dissolved in 100 mL of 0.1 M perchloric acid. The mixture was shaken well and filtered into volumetric flask and then used for the formulation studies.

Results and Discussion

Characterization of poly(XO)MCPE: The $[Fe(CN)_6]^{3-}$ probe was selected as a marker to examine the changes of the electrode behaviour before and after the modification. Figure-1(a) illustrates the cyclic voltammograms (CVs) of 1mM $K_3[Fe(CN)_6]$ in 1 M KCl at bare CPE (a) and poly(XO)MCPE (b) at a scan rate of 100 $mV.s^{-1}$. The peak to peak separation (ΔEp) was 120 mV at bare CPE whereas it was greatly reduced to 70 mV at poly(XO)MCPE. Moreover, enhanced peak currents was observed at poly(XO)MCPE. This result suggests that the modified electrode has good electrochemical reaction ability and faster electron transfer kinetics.

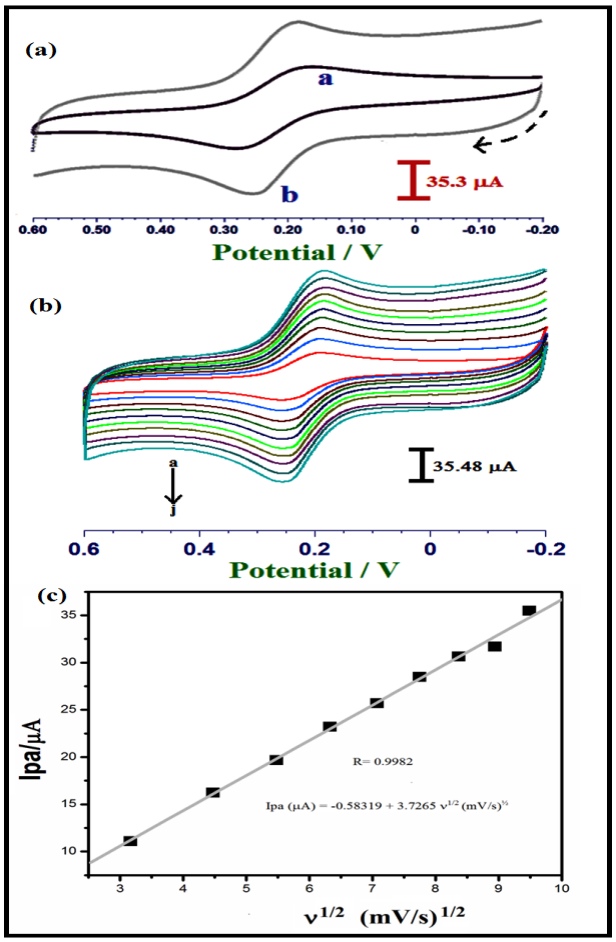


Figure-1
(a) CVs of 1mM $K_3[Fe(CN)_6]$ in 1M KCl at bare electrode (a) and poly(XO)MCPE (b) at a scan rate of 100 $mV.s^{-1}$.(b). CVs of variation of scan rate (a to j, 10–100$mV.s^{-1}$) in 1M KCl. (c). Calibration plots for the anodic peak current vs the square root of scan rate

Effect of scan rate on the peak currents at $K_3[Fe(CN)_6]$: To study the effect of scan rate at poly(XO)MCPE, 1 mM $K_3[Fe(CN)_6]$ was dissolved in 1M KCl electrolyte and recorded CVs with scan rates between 10-100 mV.s^{-1}. Figure-2(b) shows that the anodic peak current as well as cathodic peak current increased linearly with increase in the scan rate. From Figure-2(c), the relationship between square root of the scan rate and anodic peak current reveals (correlation coefficient, R = 0.99824) that the electrode reaction was controlled by diffusion (D =3.726×10^{-6} cm^{2}s^{-1}) and linearly dependent on square root of scan rate with a linear equation of Ipa (µA) = -0.58319 + 3.7265 $\nu^{1/2}$ (mV/s)^{½} [22]. The scan rate of 100 mV.s^{-1} was used for all subsequent experiments in this work because this scan rate made the faradic current maximum and non-faradic charging current minimum.

By using Randles-Sevcik eq. (i), the effective surface area of the modified electrode was calculated[23,24].

$$Ip = (2.69 \times 10^5)\, A\, D^{1/2}\, n^{3/2} \nu^{1/2}\, C_0 \qquad (i)$$

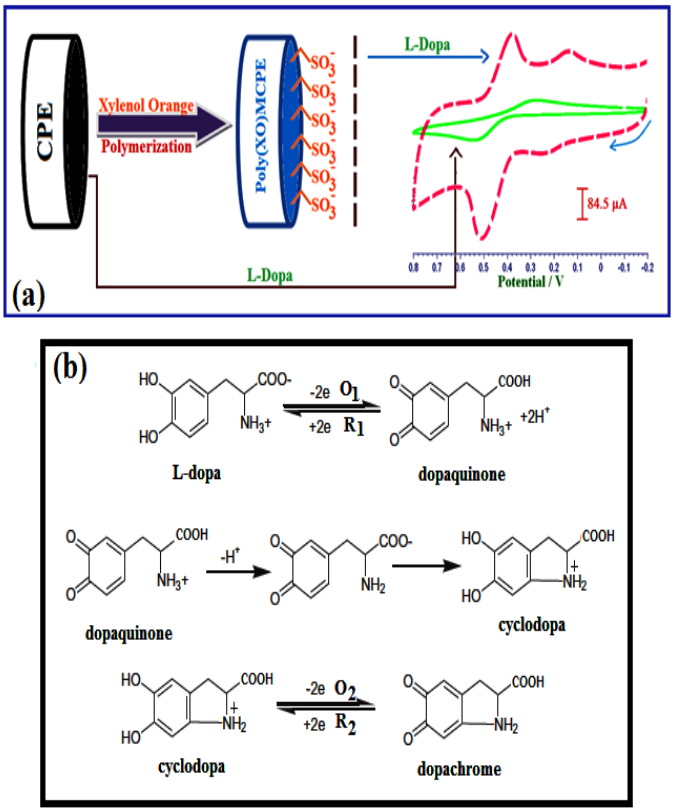
From the above eq. 'Ip' is the peak current (A), 'n' is the number of electrons involved (n =1 for $K_3[Fe(CN)_6]$), 'ν' is the scan rate (V.s^{-1}), 'C_0' is the concentration of $K_3[Fe(CN)_6]$ (mole.L^{-1}) and 'D' is the diffusion coefficient (cm^{2}s^{-1}). The area of poly(XO)MCPE was calculated as 2.1497×10^{-4} cm^2.

The concentration of the XO layer on the electrode surface was estimated using Brown–Anson model[42] based on the following eq. (ii):

$$Ip = n^2 F^2 A\, \Gamma \nu / 4RT \qquad (ii)$$

Where 'n' is the number of electrons transferred (n =1), 'F' is the Faraday constant (96,500 C mol^{-1}), 'Γ' is the surface concentration (mol.cm^{-2}). 'A' is the surface area of the electrode (2.1497×10^{-4} cm^2), 'ν' is the scan rate (V.s^{-1}), 'R' is the gas constant (8.314 J mol^{-1} K^{-1}) and 'T' is the absolute temperature (300 K). The value of the surface concentration of XO on the electrode surface was found to be 1.759×10^{-6} mol.cm^{-2}.

Voltammetric studies of L-dopa at poly(XO)MCPE: The cyclic voltammogram (CV) of 1 mM L-dopa in 0.1M PBS of pH 5.0 at a scan rate of 100 mV.s^{-1} at bare and poly(XO)MCPE was recorded and shown in Figure-2(a). The electrochemical response of L-dopa at bare CPE (b) was less intense, were as an enhanced current response was observed at poly(XO)MCPE (c). However, for the oxidation of L-dopa at the bare CPE requires a relatively large overvoltage. The electrocatalytic activity of poly(XO)MCPE at L-dopa was represented in Scheme-1(a). The proposed mechanism for the catalytic activity of poly(XO)MCPE was as follows: XO coating was uniformly spread over the electrode surface and it has anionic SO_3^- groups, these anionic sites have more affinity towards the cationic moiety of L-dopa and can effectively facilitate the electron transfer kinetics towards the electro-oxidation of L-dopa. The CV of L-dopa shows a quasi redox reversible system. The mechanism of L-dopa at poly(XO)MCPE proceeds as follows: in the first cycle, L-dopa was oxidized to dopaquinone (O_1) and then reduces to dopaquinone (R_1) in the reverse scan. In the subsequent cycles an additional redox system was observed and indicated as R_2 and O_2. The O_2 corresponds to the oxidation of cyclodopa to dopachrome and R_2 peak corresponds to the reduction of dopachrome to cyclodopa, Figure-2(b)[25]. The schematic representation of mechanism of L-dopa was shown in scheme-1(b). The electrochemical results showed that the poly(XO)MCPE was a good electrode for the electrocatalytic activities.



Scheme-1
(a) The electrode mechanism at bare CPE and poly(XO)MCPE towards L-dopa. (b)The electrochemical redox mechanism of L-dopa in acidic media at poly(XO)MCPE

Effect of pH of supporting electrolyte: In electrochemical reactions, pH of supporting electrolyte plays an important role. Cyclic voltammetric behavior of 1 mM L-dopa at poly (XO) MCPE in 0.1M PBS ranging 5.0 to 9.0 pH was investigated. From figure-2(c) the maximum peak current was attained at pH 5.0 and as the pH of the electrolyte increases further, the currents of redox system decreases drastically and also the system shifted towards less positive values. Thus, PBS of pH 5.0 was chosen and carried out for all subsequent electrochemical studies in this work.

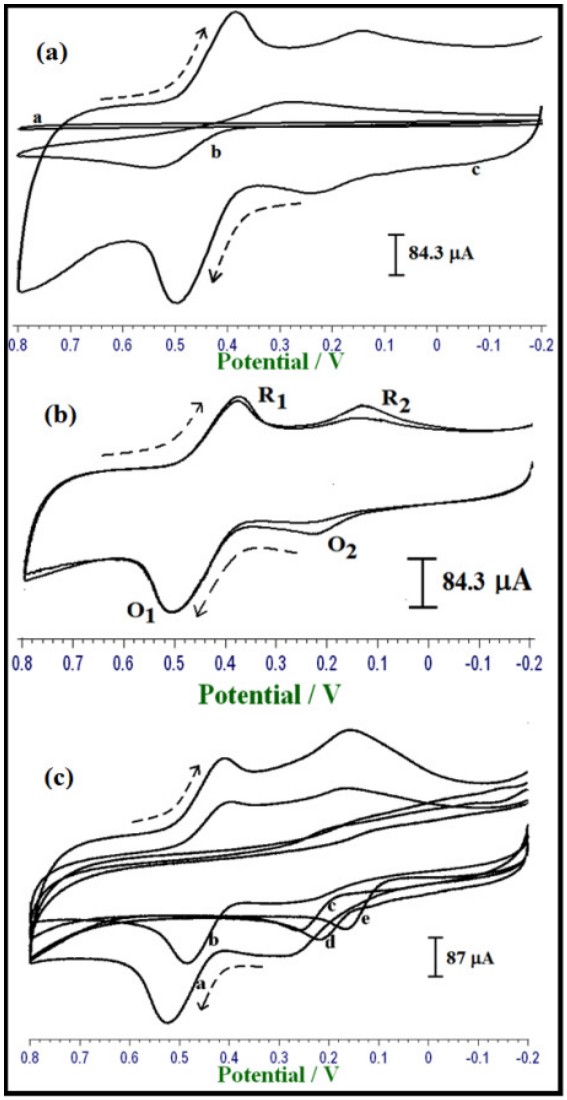


Figure-2

(a) CVs for the electrochemical response of 1mM L-dopa at (a) bare CPE (solid line) and (b) poly(XO)MCPE (dashed line) in 0.1M PBS of pH 5.0 at a scan rate of 100 mV.s^{-1} (b). CVs of 1mM L-dopa at poly(XO)MCPE with a scan rate of 100 mV S^{-1} in M PBS solution of pH 5.0. First scan cycle (a), 5th scan cycle (b). (c). CVs obtained at poly(XO)MCPE in 0.1 M PBS solution of pH values (a) 5.0 (b) 6.0 (c) 7.0 (d) 8.0 (e) 9.0

Effect of scan rate at L-dopa: Figure-3(a) shows the CVs of 1mM of L-dopa in 0.1 M PBS at pH 5.0 at various potential scan rates. With the increase of scan rates, the redox peak currents also increased gradually, signifying a direct electron transfer between DA and the poly(XO)MCPE surface. Moreover, the plot of square root of scan rate vs the anodic peak currents (figure-3(b)) reveals a linear equation of Ipa(10^{-5}A) = $0.55559+0.68339\nu^{1/2}(mV)^{1/2}$ with a correlative coefficient (R) of 0.99836. From these results a conclusion was made that the electrode reaction was controlled by the diffusion process.

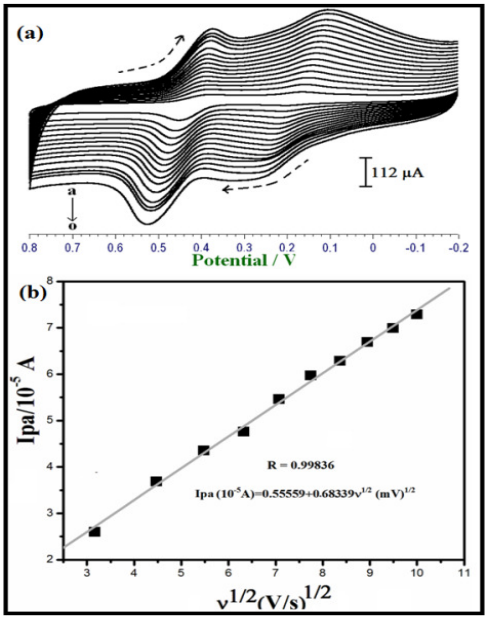


Figure- 3

(a) CVs of L-dopa at poly(XO)MCPE in 0.1 M PBS solution of pH 5.0 at different scan rates (a to o, 10–150 mV.s^{-1}). (b). Calibration plots for the anodic peak current vs the square root of scan rate

Effect of concentration: The concentration of L-dopa between 2×10^{-6} and 6×10^{-4} M was investigated by employing cyclic voltammetry technique (figure-4(a)). From the figure-4(a), the peak current increased linearly with increase in the concentration level of L-dopa. A graph was drawn between peak current (µA) vs concentration of L-dopa (µM) (Figure-4(b)) it was resulted with three linear equations with correlation coefficients of R=0.99838, 0.99349, 0.9900 respectively. The linear equations were found to to be as I_{pa} (µA) = 0.09535 + 0.02357[L-dopa](µM), I_{pa} (µA) = 1.64602+0.01089[L-dopa] (µM), I_{pa} (µA) = 4.0 +0.0034[L-dopa](µM) respectively[26]. The decrease in the sensitivity of 2nd and 3rd linearities is may be due to the kinetic limitations[27]. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) was calculated using the following equations.

$$LOD = \frac{3S}{M} \qquad \text{(iii)}$$

$$LOQ = \frac{10S}{M} \qquad \text{(iv)}$$

Where 'S' is the standard deviation of anodic peak current and 'M' is the slope of calibration curve. From the above equations, LOD and LOQ values were found to be as 5.24×10^{-5} M and 17.467×10^{-5} M respectively[28].

Determination of L-dopa in the presence of UA: In order to understand the electrocatalytic activity of poly(XO)MCPE, simultaneous determination of L-dopa and UA was carried out

using CV technique. The electroactive biomolecule UA has similar oxidation potential as that of L-dopa. Thus, there is a possibility of serious interference of UA with L-dopa. Hence, it is important to investigate this interference towards the determination of L-dopa. Figure-5(a) shows CVs of two systems, corresponding to L-dopa and UA and their individual anodic peak potentials were 419, 570 mV respectively. This corresponds to two distinct oxidation peaks due to the oxidation of L-dopa and oxidation of UA. These results indicate that it is possible to detect L-dopa in the presence of excess of UA[16].

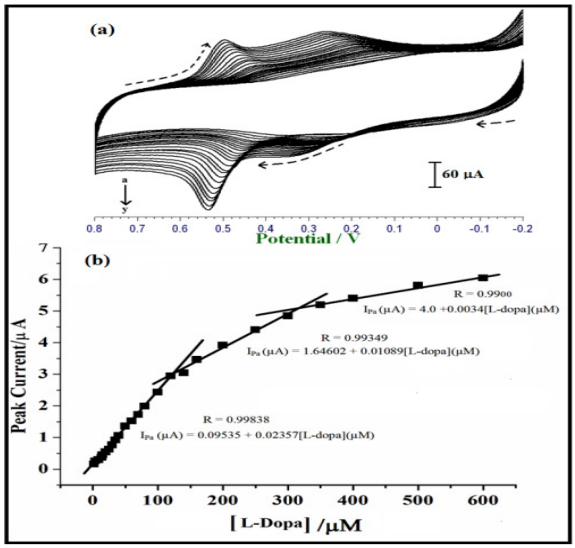


Figure-4

(a) CVs of L-dopa for the different concentrations in PBS at pH 5.0 (a) 2 × 10^{-6} M (b) 4 × 10^{-6} M (c) 9 × 10^{-6} M (d) 14 × 10^{-6} M (e) 15 × 10^{-6} M (f) 2 × 10^{-5} M (g) 2.5 × 10^{-5} M (h) 3 × 10^{-5} M (i) 3.5 × 10^{-5} M (j) 4 × 10^{-5} M (k) 5 × 10^{-5} M (l) 6 × 10^{-5} M (m) 7 × 10^{-5} M (n) 8 × 10^{-5} M (o) 1×10^{-4} M (p) 1.2 × 10^{-4} M (q) 1.4 × 10^{-4} M (r) 1.6 × 10^{-4} M (s) 2 × 10^{-4} M (t) 2.5 × 10^{-4} M (u)3 × 10^{-4} M (v) 3.5 × 10^{-4} M (w) 4 × 10^{-4} M (x) 5 × 10^{-4} M (y) 6 × 10^{-4} M (b).Calibration plots of L-dopa concentration

Stability and repeatability of modified electrode: The long-term stability of poly(XO)MCPE was investigated by examining its current response when stored in a refrigerator at 4°C for 30 days. The poly(XO)MCPE exhibited no obvious decrease in peak current response and maintained 95% of its initial value during 30 days of time. The relative standard deviation (%RSD) of the poly(XO)MCPE in response to 1mM L-dopa for successive measurements was found to be 4.5%. This indicates good stability and free from surface fouling. The poly(XO)MCPE was used for twenty parallel measurements of 1mM L-dopa in PBS. The measurements at poly(XO)MCPE exhibited constant readings suggesting good repeatability of the fabricated electrode.

Electrochemical impedance spectroscopy (EIS) studies of L-dopa: EIS is a useful and important technique, to study the electron transfer properties on the surface of chemically modified electrodes. This technique was used to investigate the efficient electron transfer rate of L-dopa at bare and modified electrodes[29]. EIS examination at the CPE and poly(XO)MCPE was studied in the range of 1 and 10^5 Hz with 0.38 V equilibrium potential, for the electrochemical reaction of L-dopa in PBS at pH 5.0 (figure-5(b)). It was observed that at CPE (a) showed a slant line which indicates high resistance whereas poly(XO)MCPE (b) appeared as nearly vertical line due to low resistance and was shown in figure-5(b). The modified electrode exhibited high electron transfer rate between the electrode and L-dopa because the polymer film acted as a good mediator. Randles equivalent circuit was shown in figure-5(b) as inset and this was selected to fit the EIS experimental results. In this circuit, R_s,,R_{ct} and R_p are the solution resistance, charge transfer resistance and polarization resistance respectively, C_{ld} is the double layer capacitance and Q is the constant phase element.

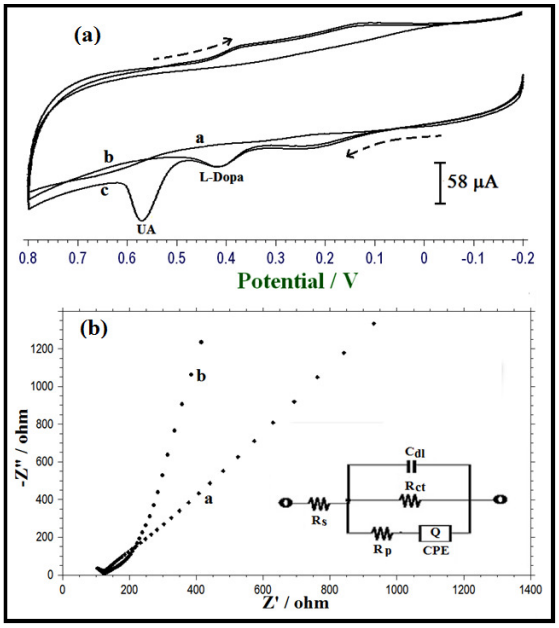


Figure- 5

(a) CVs for the determination of 0.1mM L-dopa in 0.1 M PBS solution of pH 5.0, (a) Blank run with poly(XO)MCPE, (b) at poly(XO)MCPE without UA, (c) at poly(XO)MCPE with UA. (b).EIS spectrum of L-dopa (a) CPE (b) poly(XO)MCPE, inset equivalent circuit at poly(XO)MCPE

Pharmaceutical analytical applications: The practical application of poly(XO)MCPE was used to determine L-dopa in tablet sample. L-dopa tablet sample equivalent to 250 mg was weighed accurately and dissolved in 100 ml of 0.1 M perchloric acid. A standard addition method was employed to evaluate the L-dopa in 0.1 M PBS solution. Recoveries were found to be between 98.3 to 101.25% with RSD lower than 1.5%. The obtained results are shown in table 1 and these results confirmed that the poly(XO)MCPE has good precision and reliability for the quantification of L-dopa in the pharmaceutical formulations.

Table-1
Determination of L-dopa in commercially available tablet samples

Sample	Add (mg)	Expected (mg)	Detected (mg)	Recovery (%)	SD (%)	RSD	Bias
Tablet	0.0	-	5.5	-	-	-	-
	2.5	8.0	8.1	101.25%	0.112	1.187	+1.25
	5.0	13.0	12.98	99.8%	0.208	1.457	-0.15
	7.5	15.5	15.6	100.6%	0.04	0.26	+0.64
	10.0	18.0	17.7	98.3 %	0.004	0.27	-1.66

Conclusion

The fabricated modified electrode poly(XO)MCPE, showed good electrocatalytic activity, stability and sensitivity towards the determination of L-dopa. The electrochemical kinetic parameters such as diffusion coefficient (D), area of the working electrode (A), amount of the modifier deposited on the electrode (*Γ*□□ LOD, LOQ were calculated. EIS technique confirmed the good electron transfer rate of L-dopa at the fabricated electrode. Simultaneous resolution of L-dopa in presence of UA at modified electrode was also determined. The fabricated poly(XO)MCPE was found to be efficient towards the electrochemical determination of L-dopa in pharmaceutical formulations with good satisfactory results.

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