

Electrochemical Determination Of Dopamine In Presence Of Uric Acid At Eucalyptus Oil (Eu-O) Modified Carbon Paste Electrode: A Cyclic Voltammetric Study

Harshavardhan A^a, Prakash K K^b, S Raghavendra^c, Manjappa Sidlinganahalli^d, Santhosh Bullapura Matt^{e*}

^aDepartment of chemistry, sharanabasveshwara College of Science, kaluburgi, India

^bDepartment of Biotechnology, G.M. Institute of Technology, Davangere, India

^cDepartment of physics, Adichunchanagiri Institute of Technology, Chikkamagalur India

^dDepartment of chemistry, University BDT College of Engineering, Davangere, India

^eDepartment of chemistry, G.M. Institute of Technology, Davangere, India

*Corresponding Authors

Santhosh Bullapura Matt, ^aResearch Centre, Department of chemistry, G.M. Institute of Technology, Davangere, India

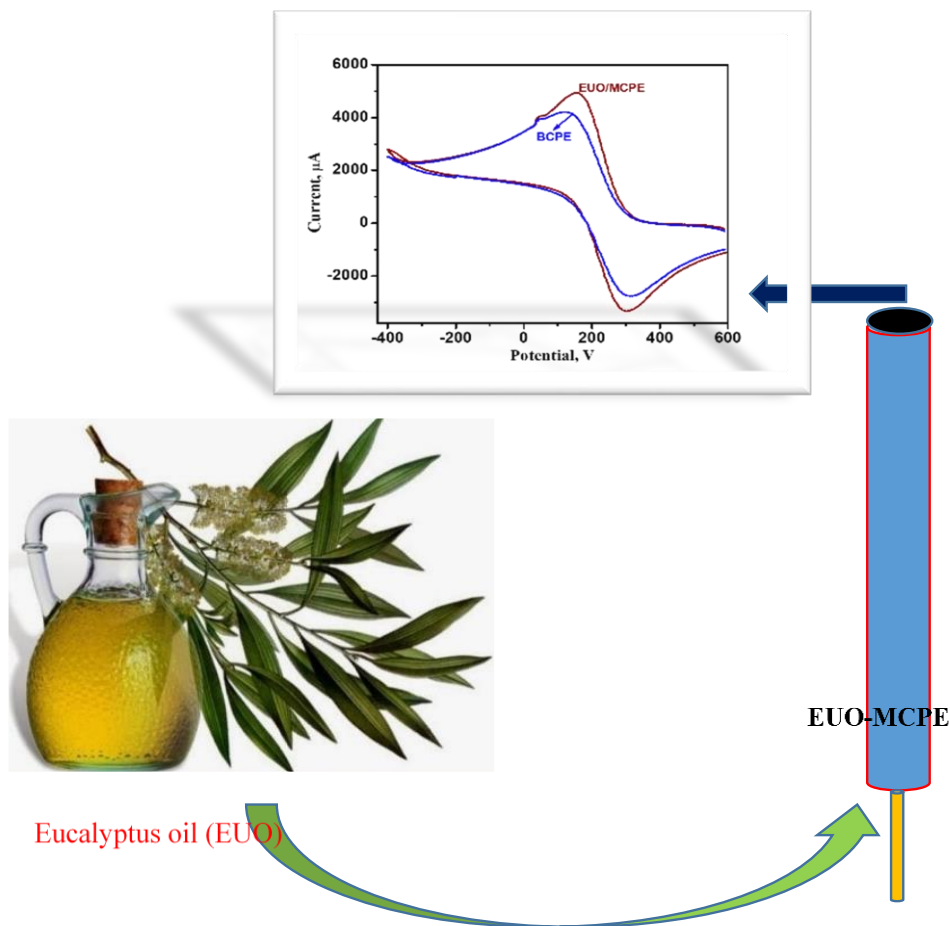
E-mail: santhoshamma041987@gmail.com

Abstract

Eucalyptus oil (Eu-O) is explored as a modifier for the fabrication of Carbon paste electrode (CPE) for the quantification of dopamine (DP), a nuero-transmitters in the presence of uric acid (UA) in 0.2 M phosphate buffer solution (PBS) of pH 7.2. The Eu-O based carbon paste electrode enhances the peak current when compared to bare carbon paste electrode (BCPE). Eu-O modified CPE exhibit excellent electro-catalytic activity for selective detection of DP in presence of UA. The surface morphology of the electrode material was studied by SEM and shows that the material is agglomerated with distinct morphology. Some electrochemical parameters such as, Scan rate, effect of pH and different concentrations of DP and UA were considered at modified CPE and the results are presented. Eu-O modified carbon paste electrode (Eu-O/MCPE) works like selective electrochemical sensor for Quantification of DP and UA.

Keyword: Dopamine; Uric acid; Carbon paste electrode; Eucalyptus oil;

Graphical abstract:



1. Introduction

DA is also known as 3, 4-Dihydroxy phenyl ethylamine, is one of the most significant neuro-transmitters in the mammalian central nervous system. Deficiency of dopamine in the human body leads to malfunctioning of the brain cells. Deterioration of the function of the brain and motor symptoms failure, leads to decrease the effective functioning of the body and brain [1,5]. Additionally, it also causes aging, relapse, death and failure of the body organs. DP plays vital role as a medicine during the treatment of schizophrenia Parkinson's disease, Huntington's disease, HIV infection and Senile dementia [6].

UA is a compound of boundless biomedical benefits and has vital roles in human metabolism. The end metabolic product of purine *via* the liver is UA and present in urine and blood. Regular checking of UA in urine or blood can be used as an identifier for an initial cautioning sign of kidney disorders. Abnormal UA level in a human body can produced numerous illnesses, such as gout, hyperuricemia, chronic renal disease and cardiovascular disorder [7,12]. UA and DA are together present in biological fluids, such as blood and urine. Consequently, it is significant to grow new methods for the selective quantification of Dopamine [13,14] along with the presence of UA in a repetitive analyze.

Eu-O is a leaf infusion of plant *eucalyptus globulus* it is a medicinal plant, it comes under *myrtaceae* family, the major constituents present in eucalyptus oil are Monoterpene 1,8- cineole /eucalyptol (60-85%) and also small amount of β -Pinene, α -Pinene, Limonene and terpinen-4-ol [15,18]. Which gives oil soothe and cooling vapor and hence it is used to treat symptoms of antibacterial properties of pathogenic bacteria in respiratory path, influenza and cold, also it is used for treatment of asthma and bronchitis. Eu-O has widespread range of applications such as antiseptic, pharmaceutical, cosmetics flavoring, industrial uses, fragrance and repellent [19,24]. Eu-O will be given in a very low and minimum dosage in therapeutic substances, for an adult the dosage varies from 0.05 to 0.5 mL/kg of weight of a body.

Present work describes that, EU-O/MCPE is responsible for the quantification of DP and UA by using electrochemical technique such as cyclic voltammetric and differential pulse voltammetry (DPV). Most of the works do not reported the quantification of UA and DA using Eu-O as modifier. Eu-O modified electrode depicts low detection limit (LOD), good sensitivity and selectivity for the quantification of DP along with the presence of UA.

2. Material and methodology

2.1 Reagents and chemicals

All chemical and reagents procured was analytical grade to avoid further purification in this study. Eu-O was supplied from Sigma Aldrich, Bangalore, India. Dopamine, uric acid, Sodium dihydrogen phosphate, Potassium ferrocyanide, potassium chloride and disodium hydrogen phosphate were obtained from Avara synthesis Pvt. Ltd., Hyderabad, India. DP stock solution was prepared in 0.1 M per-chloric acid. Solutions of potassium chloride and Potassium ferrocyanide were prepared by Milli-Q water. silicon oil was purchased from Spectrochem, Bangalore, India, and Graphite powder of 50 mm size was bought from Loba chemical, Bangalore, India. The PBS was made by mixing of disodium hydrogen phosphate and Sodium dihydrogen phosphate of 0.2 M concentration as a supporting electrolyte.

2.2 Preparation of Standard solutions:

EU-O sample is dissolved in hexane in a ratio of 6:200 (V/V), for the standard solutions, 0.5 mg of camphor, 0.5 μL of β -pinene, 0.5 μL of α - pinene, 0.5 μL of α -phellandrene, 5 μL of cineole 1 μL of limonene were liquefied in 1 mL of hexane.

2.3 Instrumentation

Analysis of Eucalyptus oil was done by using Gas Chromatograph (GC) (Agilent 7890A) with a Flame Ionization Detector (GC-FID) method. The oven temperature was set 60°C - 200 °C at a heating rate of 8 °C/min. The carrier gas used is Helium (He) with a flow rate of He is adjusted to 1 mL/min. The temperature of the detector and injector respectively were set at 260 °C and 210 °C. The analyte samples were injected in a volume of 1 μL , by split sampling method, in the ratio of 1:50. The percentage compositions were obtained by means of the standardization method.

EA-201 (Chemilink system) model was used to record the cyclic voltammetry (CV) experimentations. 3-electrode system is used to conduct all the experiments. One of the electrodes

in the three-electrode system is Eu-O/MCPE and BCPE as working electrode, saturated calomel as a reference electrode and platinum wire as a counter electrode.

2.4 Preparation BCPE and EU-O /MCPE

The BCPE was prepared by uniform mixing of silicon oil and graphite powder in 30:70 (w/w) ratio in an agate mortar to obtain a uniform and homogenous paste. The obtained carbon paste was strongly packed into the cavity then the carbon paste electrode was refined the surface by brass rubbing on a weighing paper.

The EU-O/MCPE was prepared by using 20 μ L eucalyptus oil by (10 wt %), silicon oil and graphite powder at a ratio of 10:25:65 (w/w) an agate grout by hand mixing for about 20 min to get homogenous paste. The gained EU-O/MCPE paste was strongly crowded into the cavity of a CPE then refined the outward surface by weighing paper. The fabricated electrodes were dried out in hot air oven at a temperature at 60°C for 12 hours.

3. Result and discussion

3.1 Characterization of Eucalyptus oil (EU-O)

GC-FID chromatogram of EU-O is as shown in Figure-1 which shows the all-possible peaks with the standards as shown in the Table -1. 1,8-cineole is the main compound noted as the major peak at the retention time (RT) at 9.10 minutes and constitutes 72.80% and limonene constitutes 11.06 % with RT 8.902 minutes. Other compounds are also present in small quantity and its RT is given in the Table-1[19-24] .EU-O GC-FID analysis indicates the 1,8-Cineole and limonene is major compounds are present in the EU-O.1,8-Cineole is a terpene and also it is a strong antioxidant, hence EU-O is responsible for the detection neurotransmitter.

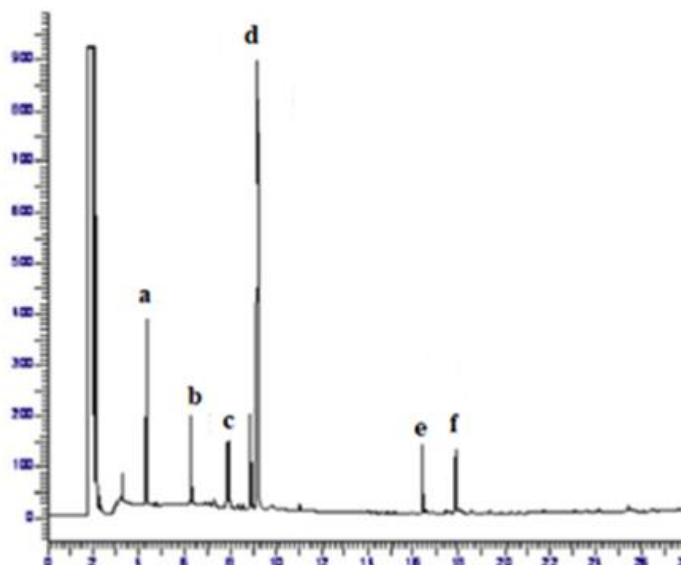


Figure 1: GC-FID chromatogram of oil a) α -Pinene b) β -Pinene c) Limonene d) 1,8-Cineole e) Sabinene and f) Camphor

Table 1: Retention time (RT) of the standard solution		
Peak position	Substances	RT
A	α -Pinene	4.286
B	β -Pinene	6.308
C	Limonene	8.902
D	1,8-Cineole	9.10
e	Sabinene	16.409
f	Camphor	17.849

3.2 Consequence of modifier concentration on the surface of CPE

The modifier used in the present study is Eucalyptus oil, it acts as a good modifier and it was used in different ratios for the fabrication of Eu-O/MCPE. During the fabrication of MCPE the concentration of the Eu-O was varied from 5 μ L to 40 μ L and the anodic peak current was

recorded. The varied in the redox peak potentials was recorded and the same is predicted in the Figure 2. It is also observed that, peak currents at anode and cathode were decreased as the amount of Eu-O was increased in the Eu-O/MCPE. Among the different ratios of Eu-O concentration, highest sensitivity was found with 20 μL of EU-O/MCPE, henceforth 20 μL (10% wt) EU-O was optimised to fabricate the electrode for further electrochemical studies [25,28].

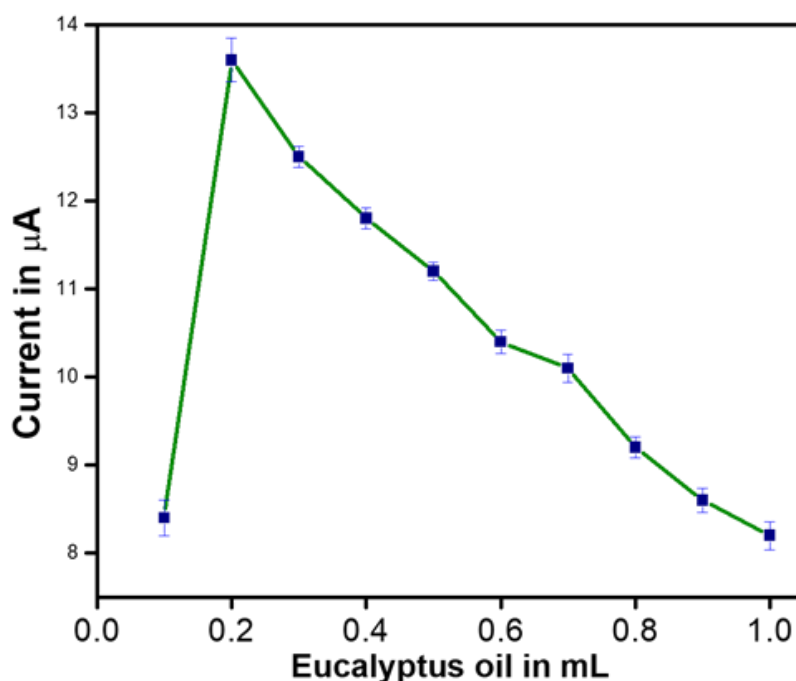


Figure-2: Graphical representation of current v/s quantity of EU-O/MCPE

3.3 Characterization of $[\text{K}_4\text{Fe}(\text{CN})_6]$ at EU-O/MCPE

The electrochemical behavior of the EU-O/MCPE as well as BCPE was recorded in the freshly prepared 1mM $[\text{K}_4\text{Fe}(\text{CN})_6]$ solution and 1M KCl was used as supporting electrolyte in the medium. The CV response of the EU-O/MCPE was recorded at a scan rate of 100mVs^{-1} and the same is presented in Figure 3. Blue coloured line represents the CV is for BCPE and the red coloured line for the EU-O/MCPE. In Figure 3, for BCPE decrease in the redox peak current was observed whereas, for EU-O/MCPE shows increase in the peak current response [29,30]

The surface morphology of BCPE and Eu-O/MCPE can be found in figure 4 and 5 using scanning electron microscopy (SEM). The surface of BCPE was haphazardly molded with the flakes of graphite. Though, the Eu-O/MCPE has characteristic uniform arrangement of Eu-O molecules on the surface of CPE. This confirms that the CPE modified by Eu-O leads to the modification in the surface action of the EU-O/MCPE [31].

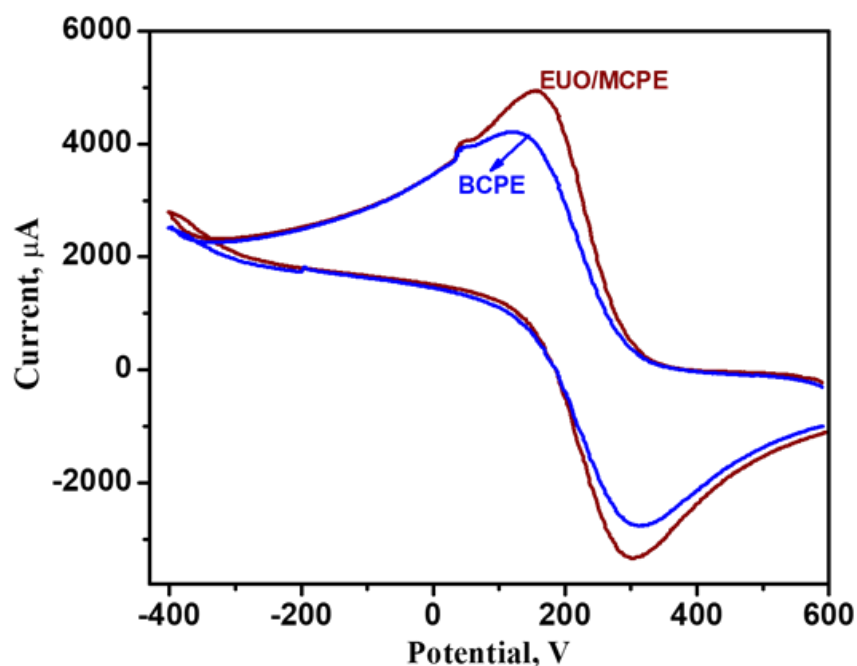


Figure-3: Cyclic voltammograms of 1 mM $K_4Fe(CN)_6$ at BCPE (solid line) and at EU-O / MCPE (dashed line), supporting electrolyte 1 M KCl at scan rate 100 mVs^{-1}

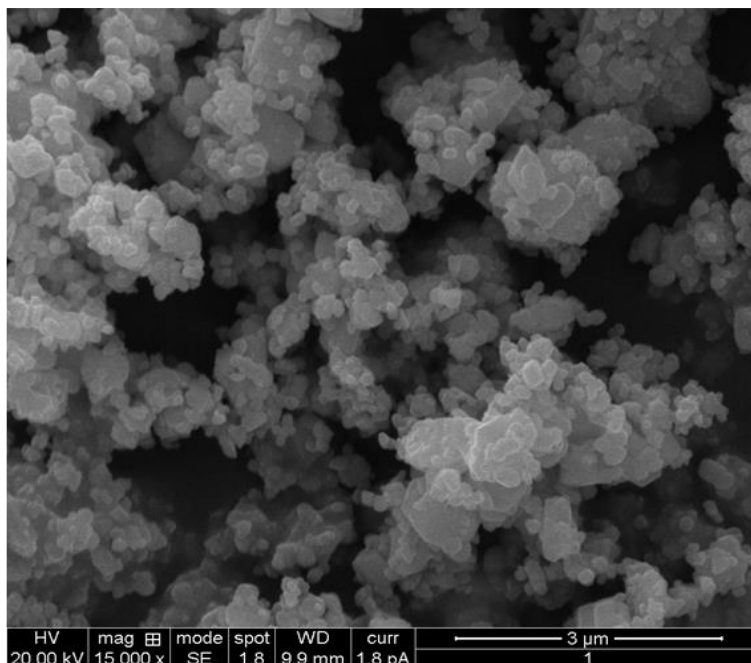


Figure-4: SEM image of the BCPE

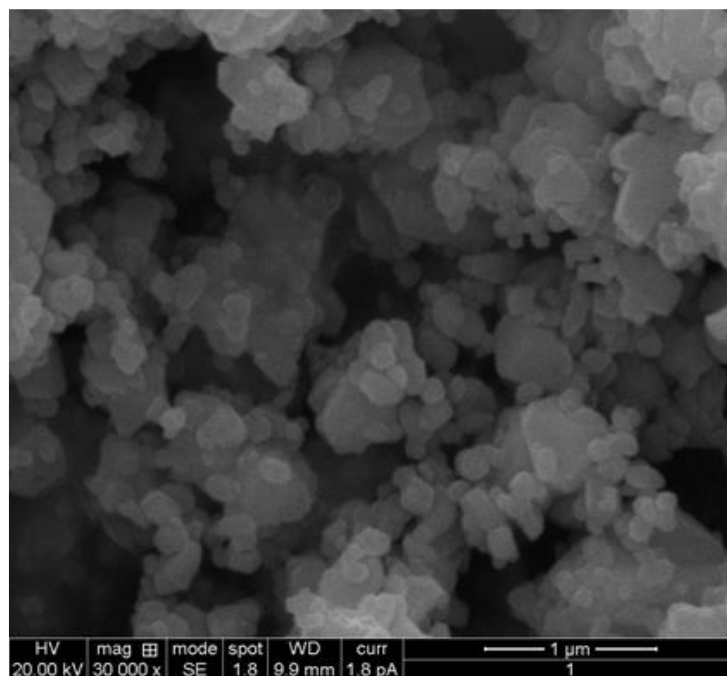


Figure-5: SEM image of the Eu-O/MCPE

3.4 Characterization of Dopamine at EU/MCPE

Cyclic voltammograms (CV) found for electrochemical reaction of DP at BCPE and Eu-O/MCPE in 0.2 M PBS at pH 7.2 holding 0.05 mM DA at 100 mVs⁻¹ scan rates revealed in figure 6. The CV found at the BCPE showed very less current compare to EU-O/MCPE. The CV of DP at Eu-O/MCPE specifies larger enhancement in redox current when equated with the BCPE. Basically, improved redox current proves the better electrochemical property of EU-O/MCPE to the DP Quantification.

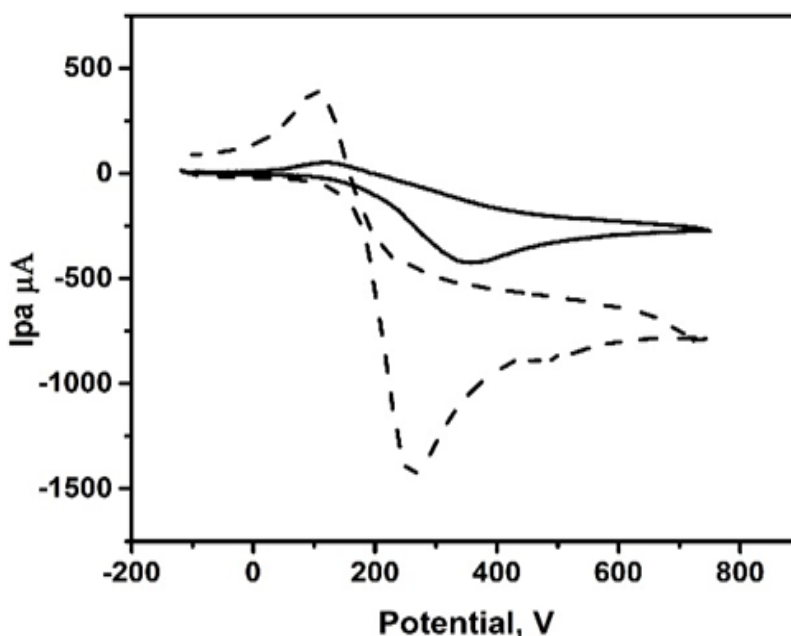


Figure 6: CV of 0.05 mM DA in 0.2M PBS pH 7.2 at BCPE (solid line) and EU/MCPE (dashed line) with scan rate of 100 mV/s.

3.4 Effect of pH

Under different pH with scan rate 50 mVs⁻¹ the electrochemical response of EU-O/MCPE towards DP was studied. when pH increases from 4.5 to 9.0 the increase with increasing pH and it shows maximum anodic peak current at pH 7.2, later it decreases gradually as revealed in Figure 7. Hence, pH of 7.2 was carefully chosen for all the electrochemical parameters analysis

of DP and UA. The relativity of anodic peak vs. pH is displayed in Figure 10. It was noted at around pH range of 4.5-9.0 a direct relationship between them was found. [32]

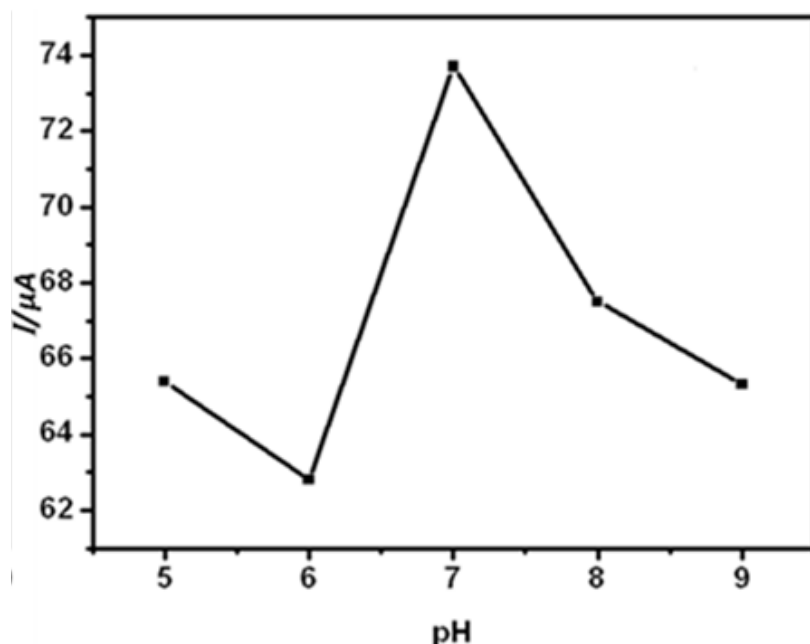


Figure 7: Graph of anodic peak current v/s pH (4.5 – 9.0) of at the 1×10^{-5} M DA at EU/MCPE.

3.5 Effect of Scan rate

For EU-O/MCPE scan rate was recorded at different potentials at room temperature, which shows the effect of applied potential in the redox peak current in DP. According to Randles-Siviks equation the peak current is proportional and directly depends on scan rate. The scan rate was increased from 50-250mVs⁻¹, the peak current was increased continuously during the electrochemical reaction. This increase in the peak current with increase in the scan rate is because of the electron transfer between the surface of the EU-O/MCPE and DP molecule. The CV for EU-O/MCPE at a pH of 7.2 for DP at different scan rates is shown in the Figure 8(a). A correlation graph of scan rate with anodic peak current shows the linearity with correlation

coefficient and is found to be 0.9936 is as shown in Figure 8(b). From all the above results, it is confirmed that the electrode process is a diffusion controlled.

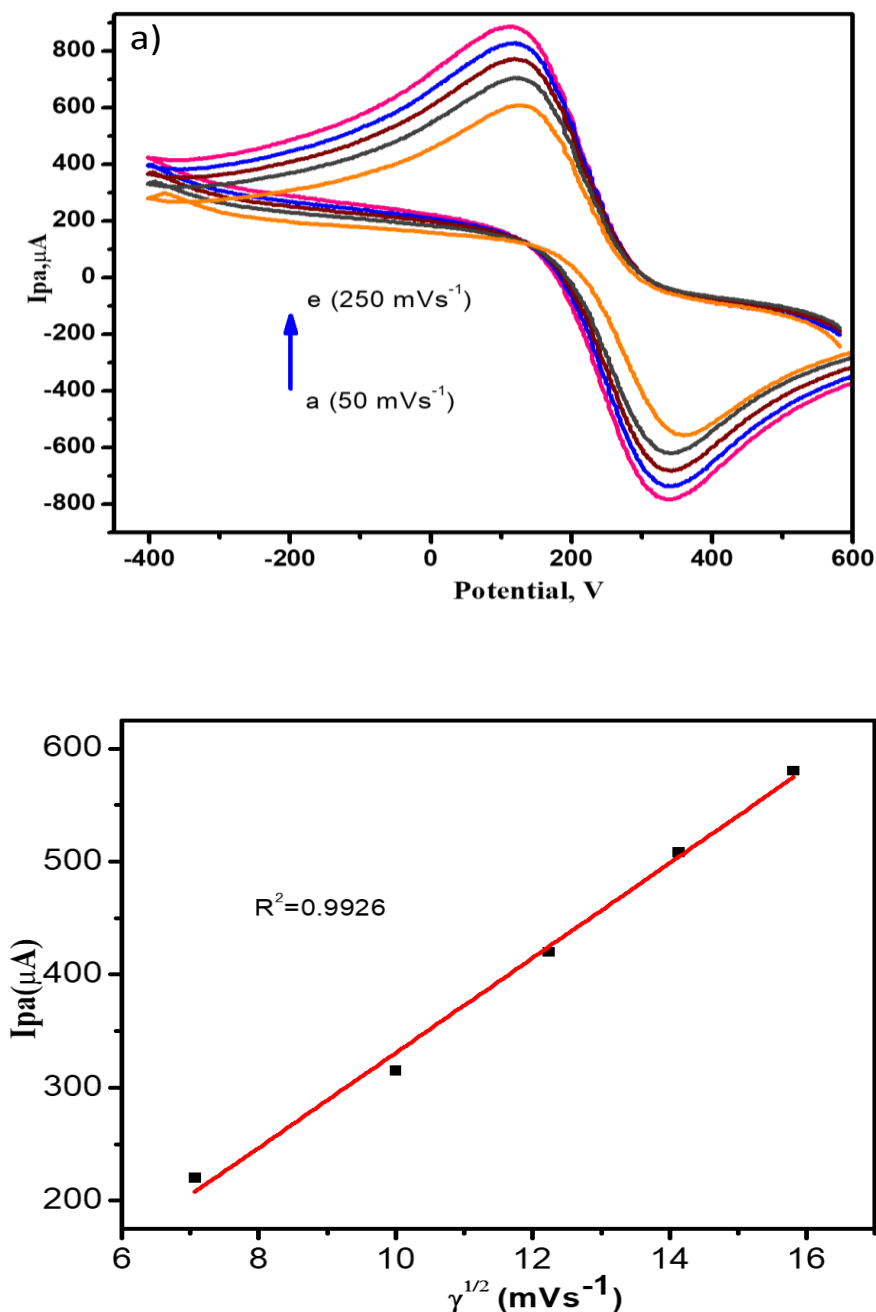


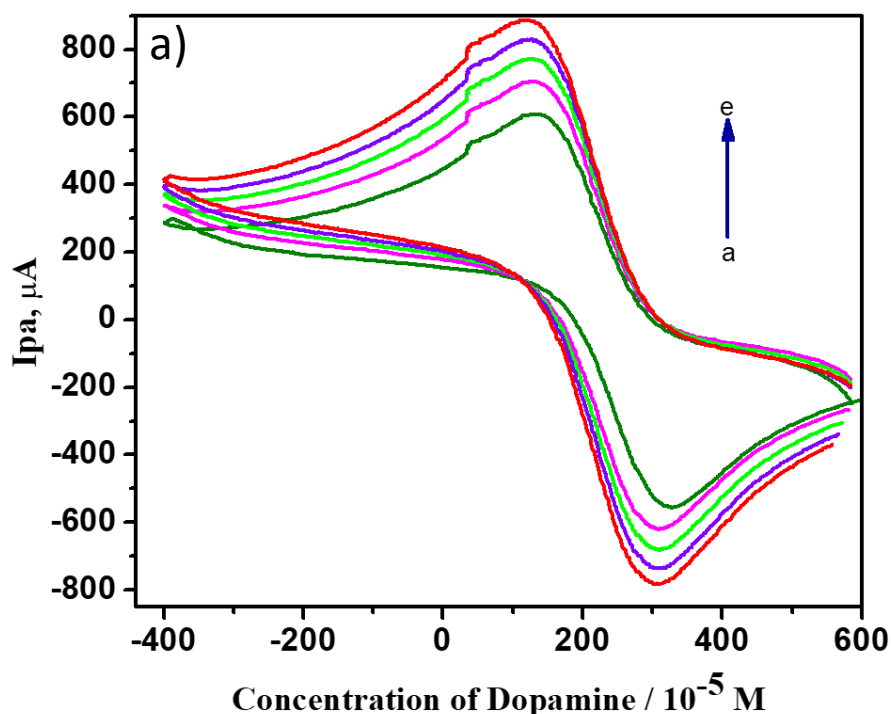
Figure 8 (a): CV of DA at EU/MCPE with dissimilar scan rate were (a) 50 to (h)250 mVs^{-1}
b) Plot of anodic peak current (I_{pa}) v/s square root of scan rates of DA at EU/MCPE

3.6 Effect of Concentration

The electrochemical investigation of various concentrated DP at Eu-O/MCPE was shown in figure 9. By increasing the concentration of DP to 5×10^{-5} M from 1×10^{-5} , then the redox peak currents of DP was improved. A step-by-step increase in the result of the anodic and cathodic peak current is achieved by carefully moving the Epa (anodic peak potential) towards the positive, the vice versa was done for the anodic peak current.

3.7 Simultaneous detection of dopamine and uric acid

The CV of UA and DA mixture at BCPE and MCPE/EU-O in 0.2M of PBS was shown in figure 10. The CV found for BCPE (dashed line) and (solid line) resemble to the oxidation of 1×10^{-5} M DA and 4×10^{-5} M UA at EU-O/MCPE mixture correspondingly. As projected, a deprived current retort was detected at BCPE. Exactly two anodic peaks at the potential of 398 mV and 287 mV were detected for UA and DA oxidation corresponding to EU-O/MCPE at a pH of 7.2 [33,37].



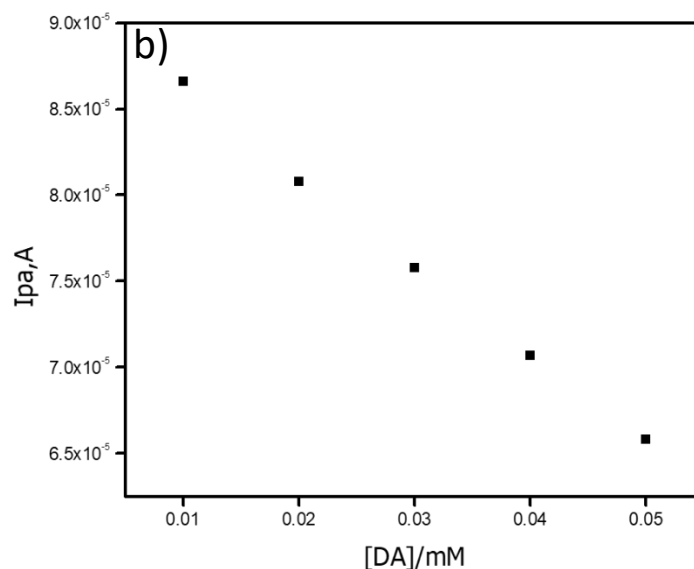


Figure 9: Effect of difference concentration of DA (a) 1×10^{-5} M, (b) 2×10^{-5} M, (c) 3×10^{-5} M, (d) 4×10^{-5} M, (e) 5×10^{-5} M on anodic peak current at EU/MCPE; $v=50 \text{ mVs}^{-1}$.

3.8 Real sample analysis:

Drugs of DA which are pharmaceutically framed and available commercially, was accepted in our medical vicinity. On the basis of the weight percentage given on the label the analyte concentration of the commercial sample was determined.

In order to validate the performance of the sensor, the respective analyte solution of 50mM concentration was mixed up with PBS and the sensor signal was measured and the values are tabulated in the Table 2. The relative standard deviation (RSD) and recovery was acceptable, which showed that the proposed methods could be efficiently used for the determination of DA in real sample with recovery in the range ~96.26 to 99.18% for DA analysis [34].

Table-2 : Real sample analysis of DA				
Sample	DA added (μM)	Found (μM)	Recovery (%)	RSD (%)
1	10	9.926	99.26	1.42
2	20	19.814	99.07	1.40
3	30	29.59	98.6.	1.82

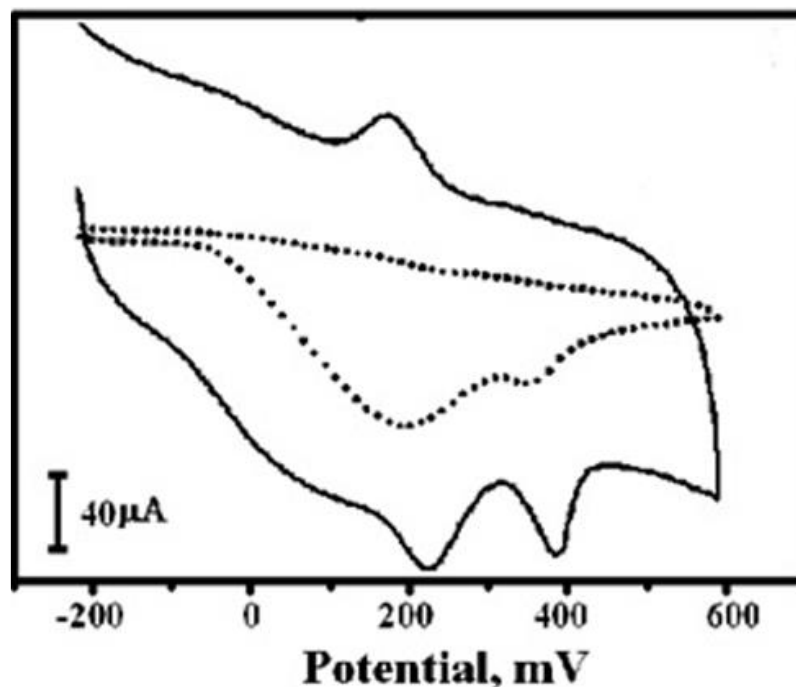


Figure 10: CV for simultaneous determination of 1×10^{-5} M DA and 4×10^{-5} M UA at BCPE (Dashed line) and EU/MCPE (solid line) in 0.2 M PBS at a scan rate 50 mVs^{-1}

4. CONCLUSION:

Eucalyptus oil modified carbon paste electrode electrochemical sensor is developed for simultaneous detection for the DA in presence of UA. Eu-O/MCPE Fabricated electrode exhibited significant electrocatalytic activity for the oxidation of DA and UA and gives a distinct separation of peaks for DA and UA. Eu-O/MCPE shows good stability and reproducibility along with the good selectivity and sensitivity for the simultaneously or individual detection of some of the pharmaceutically important compounds such as DA and UA. Also, these electrodes can be used satisfactorily towards real sample analysis. The LOD is significantly good and it was found to be $0.048 \mu\text{M}$. Based on these facts and excellent performance of this electrochemical method using these modified electrodes routine analysis of DA and UA can be performed in clinical tests.

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