Research Article

Cyclic Voltametric Determination of Dopamine, Uric acid and Ascorbic acid by using Emodin Modified Carbon Paste Electrode

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Abstract

In the present work, Emodin modified carbon paste electrode (EMCPE) was fabricated for the simultaneous determination of Dopamine (DA), Uric acid (UA) and Ascorbic acid (AA). EMCPE has exhibited high selectivity, sensitivity electrochemical catalytic activity towards the detection limit of DA in 0.1 M Phosphate buffer solution at pH 7.0.The effect of pH, scan rate, concentration of Dopamine were studied by using Emodin modified C.P.E. The EMCPE showed a good detection limit of 1.7484 X 10^{-6} over the linear dynamic range of 2.3 µm - 5.3µm which is very lower than the reported methods. This EMCPE shows good stability, high sensitivity and low detection limit towards the determination of DA.

Keywords: Emodin, Dopamine, Ascorbic acid, cyclic voltammetry, differential pulse voltammetry

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Introduction

In recent years, efforts have been put forwarded in the development of voltammetric [1] methods for the determination of DA, UA and AA. Ascorbic acid (AA) and Dopamine (DA) are electro active compounds which play an important role in the maintenance of human health. Dopamine is one of the physiological neurotransmitters [2, 3] that is involved in the function of Cardiovascular, renal, hormonal and nervous systems. Abnormal dopaminergic transmission can cause some neurological diseases such as Alzheimer's disease [4], Parkinson's disease [5], Schizophrenia [6, 7], Huntington's disease [8], tardive dyskinesia disorders. Uric acid is a heterocyclic compound which is a primary end product of the urine metabolism and formed by the breakdown of the purine nucleotides.

Extreme abnormalities i.e high levels of UA [9] in the blood can lead to Gout which is an inflammatory disease [10, 11], Hyperuricemia and are associated with other medical conditions like kidney stones. Ascorbic acid is very popular by its anti oxidant properties. Moreover a number of studies have been investigated to know the function of AA and suggested that it can be helpful in gene expression and as a co-substrate of many important deoxygenizes. Therefore determination of DA, AA and UA in the biological samples and pharmaceutical preparation is of great interest. But, they have similar oxidation potential at most solid electrodes. So, simultaneous detection is very difficult with unmodified electrodes. Thus, there is a need for the development of simple, reliable and efficient electrodes with enhanced characteristics to distinguish DA, AA and UA in mixtures. The determination of DA in presence of UA and AA is always challenging because DA and AA always occur together in the biological environment. To overcome these problems, various modified electrodes have been fabricated. Among these modified electrodes, EMCPE are highly advantageous in the detection of bio molecules and also act as a good sensor in the determination of DA, AA and UA due to their extra sensitivity, excellent selectivity and homogeneity in the electro chemical deposition. Several analytical techniques which can be used in the determination of DA, AA and UA are capillary electrophoresis [12], gas chromatography [13, 14], florescence [15] etc. But they are very expensive and can take long time for analysis. So, the usage of electrochemical methods has attained a great significance due to their selectivity, sensitivity [16], low cost and environmentally friendly detection. The main aim of this study is the preparation of a stable modified CPE by polymersation to perform simultaneous determination of DA, AA and UA in 0.1 M phosphate buffer at pH 7.0 with good accuracy, low detection limits, excellent sensitivity and selectivity with great reproducibility [17, 18].

Figure 1 shows Emodin(1,3,8 – trihydroxy -6-methyl anthracene-9,10-dione) is a orange solid, also purgative resin extracted from the herbs Rhubarb(Himalayan), Buckthorn and also found in Aloe. Barbadensis Mill, Aloe.barbadensis var. Emodin is one of the medicine which reduces the impact of type –II diabetes, used as enzyme Inhibitor, treated as a Anticancer agent such as pancreatic cancer [19], Adrenal cortex cancer pancreatic neuroendocrine tumours, Emodin also fight against toxicity [20]. Emodin has a strong stimulant-laxative action. Emodin is non carcinogenic, hence modified electrode is a environment eco friendly electrode.

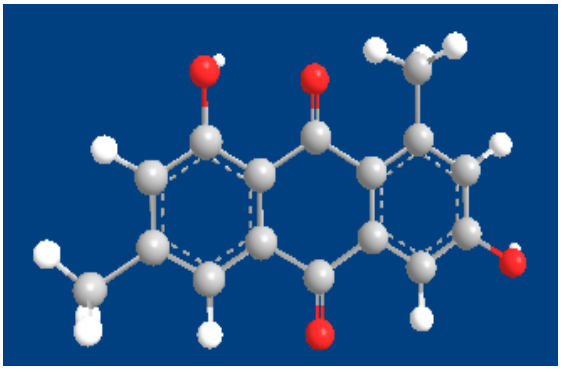

Figure 1 Emodin chemical structure

Apparatus

Cyclic voltammetric experiments were performed with a CH-instrument model No: CH 1610D. Electro chemical work station with a connection to a personal computer with a conventional three electrode cell (a platinum wire as a counter electrode, Ag/Agcl (salt) as reference electrode, a bare C.P.E/ Emodin MCPE (3.0 mm diameter) as working electrode). All measurements were taken at room temperature with Elico Li 120 pH meter.

Chemicals

Analytical grade Dopamine HCl (DA), Ascorbic acid (AA), Uric acid (UA), Sodium dihydrogen phosphate (NaH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4), Emodin Graphite powder (20 µmg), and Silicon oil was supplied by Sigma Aldrich chemicals. All chemicals supplied, were used without further purification. The ascorbic acid and uric acid stock solution was prepared by dissolving in 0.1 M NaOH and in distilled water obtained from the Millipore water purification system. Phosphate buffer (pH=7) was prepared as per literature with 0.1 M NaH_2PO_4 solution with Millipore water.

Preparation of bare CPE and Emodin polymer modified Emodin (CPE)

The bare carbon paste electrode was prepared by adding the graphite powder and silicon oil in the ratio of (70%:30%) in an agate mortar to obtain a homogenous carbon paste. The polymer film modified CPE was prepared by the electrochemical polymerisation of Emodin on a carbon paste electrode in 0.1 M phosphate buffer solution of pH 7.0 containing 1.0 mm of emodin with cyclic voltammetric sweeps in the potential range of -0.8V to 1.2 V at the scan rate of 50 mV/s. The surface of the electrode was washed with distilled water.

Results and Discussion

Electrochemical polymerisation of poly Emodin carbon paste electrode

During polymerisation process an oxidation peak at 443 mV was obtained. The oxidation peak current is directly proportional to the number of cyclic voltammetric scans, indicating that the electro conductive polymer film was formed on the electrode surface. **Figure 2** demonstrates the effect of cycles of polymerisation on the oxidation peak current (IPa) of 25 mM DA in phosphate buffer solution (pH 7.0) in which the current response was enhanced up to 30 cycles and decreased thereafter. Hence in this study, we have chosen 30 cycles as the optimum number for the formation of a polymer film on the electrode surface area.

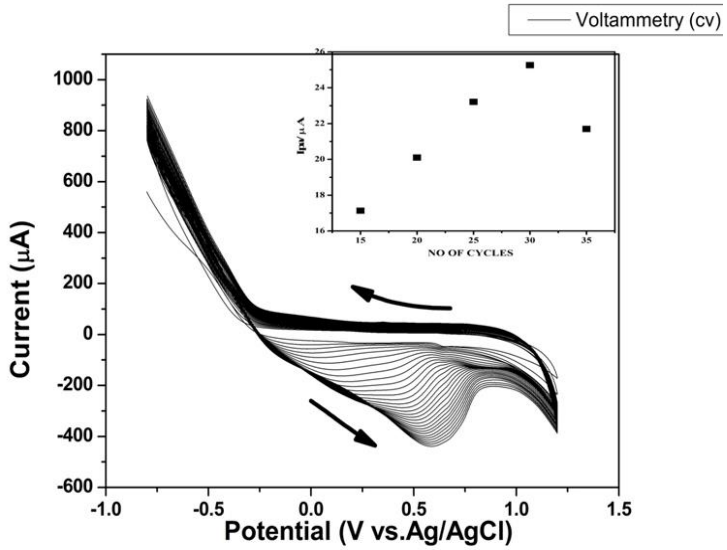

Figure 2 Cyclic voltamogramme for the electrochemical polymerization of 1.0 mm Emodin on a CPE at the scan rate 50 mV s^{-1}. Inner picture represents the dependence of the oxidation peak current of 0.025 mm DA on the number of voltammetric scans

The response of DA at the bare CPE & EMCPE

Figure 3 shows the cyclic voltammograms of 0.1mm DA at the bare carbon paste electrode (dotted line) and the EMCPE with the scan rate of 50 mV/s. When compared with bare CPE, modified CPE showed 3 folds increase in anodic & cathodic peak currents for DA.The peak potential difference (EP) of 69.9 mV was obtained which indicates that the same number of electrons and protons [21] are involved in the reaction mechanism. This concludes that the Emodin modified carbon paste electrode (Solid line) exhibited an excellent sensitivity for the determination of DA.

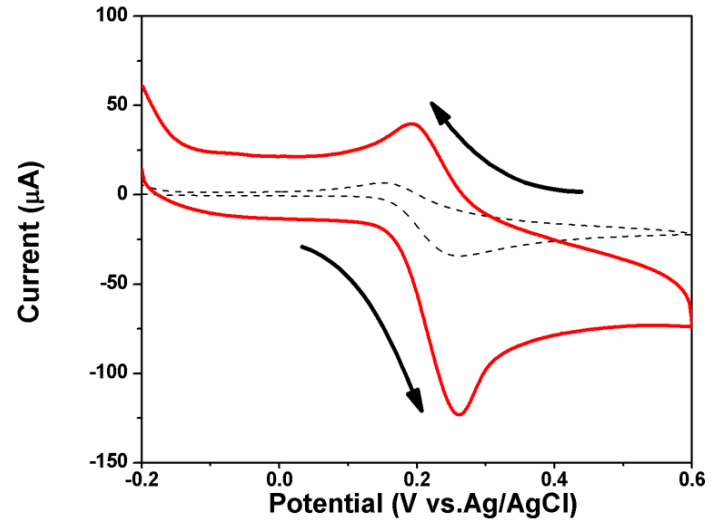

Figure 3 Cyclic voltamogramme of bare (dotted line), poly emodin MCPE using 0.1 mm DA in 0.1 M PBS (pH=7.0) at the scan rate 50 mV s^{-1}

The electro catalytic activity of is due to the presence of electron releasing groups such as -CH_3 and -OH on the surface of CPE. Due to the existence of electron rich groups, EMCPE shows a good affinity towards the DA and it can easily undergo oxidation by exchanging positive ions with the electron rich groups in EMCPE.

The effect of scan rate

The effect of scan rate for 0.1 mM DA in 0.1M PBS at pH 7.0 was studied by CV by using Emodin modified CPE. The linear relationship between the redox peak current and the scan rates (**Figure 4**) clearly indicate that the electrochemical oxidation of DA at the poly emodin CPE is an adsorbtion controlled process [22].

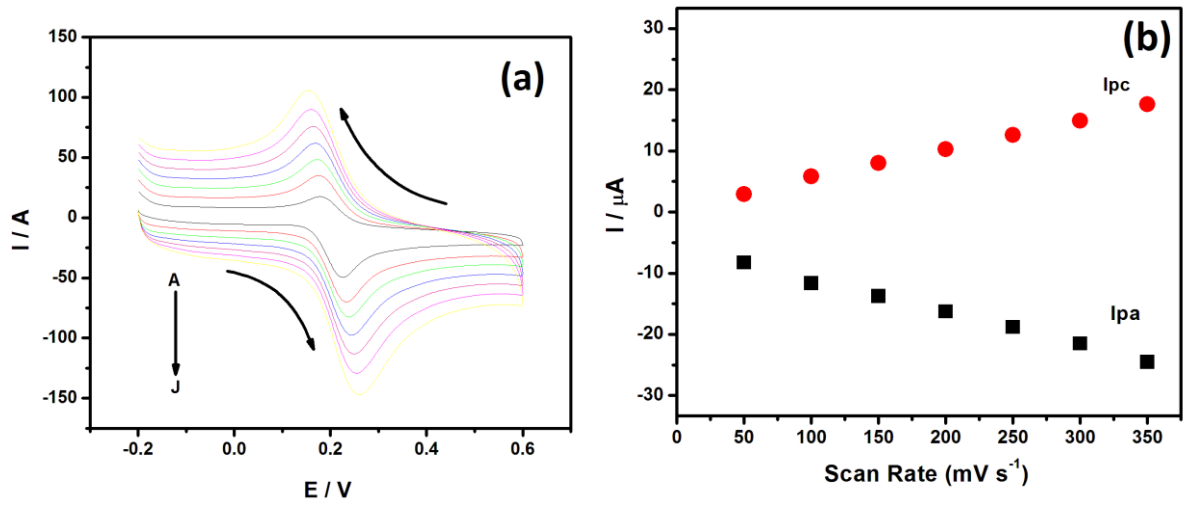

Figure 4 (a) Cyclic voltammogramme obtained for poly emodin CPE in the presence of 0.1 mm DA in 0.1 M PBS (pH=7.0) with various scan rates (50, 100, 150, 200, 250, 300, 350 mV s^{-1}), 3(b) Calibration plot of cathodic and anodic peak current Vs various scan rates from 50-350 mV s^{-1}

Effect of pH

The electrochemical response of DA at poly Emodin modified CPE was tested for its pH dependency. The voltammogrames of DA were recorded at 0.1 M phosphate buffer solution of different pH values from 5.5 - 8.0 by cyclic voltammetric method. **Figure 5** clearly demonstrates the dependency of the DA anodic peak current and formal potential on the pH of buffer solution. It could be seen that the anodic peak current of DA increases with increasing pH values until it reaches 8.0 (shown with symbol) and then there is a decrease in the peak current of DA until it reaches 8.0. The formal potential of DA shifts towards lower potential with the increase in pH value of the solution and depends linearly on the pH values in the range of 5.5 - 8.0 with R=0.9941 (shown with closed circles).

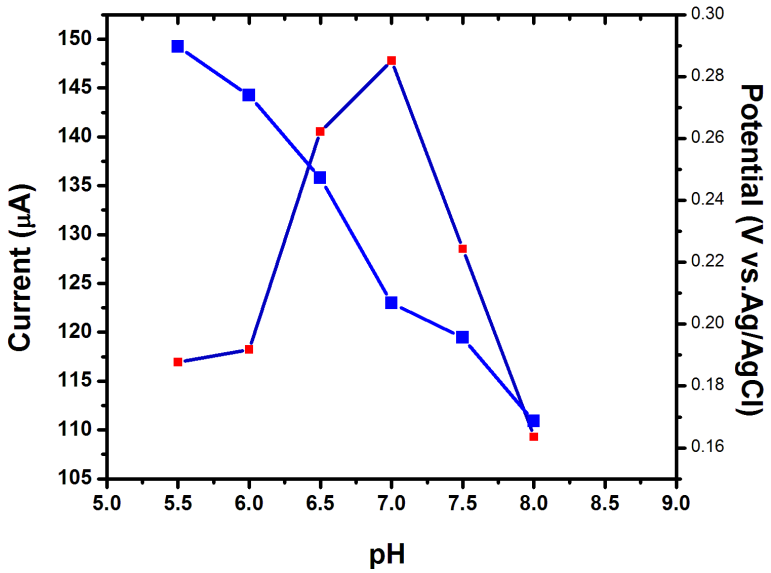

Figure 5 Effect of pH on anodic peak current (IPa), anodic peak potential (EPa) of 0.1 mm DA in 0.1 M PBS

Effect of DA concentration:

It involves the determination of the detection limit of DA in the presence of poly Emodin MCPE. The differential pulse voltammetric (DPV) technique was applied due to its sensitivity. **Figure 6** shows the anodic peak current observed at the modified CPE at various concentrations of DA. Inner diagram is the graph in between IPa/A Vs concentration of DA showing the linear dynamic range (LDR) from 2.3 µm – 5.3 µm. The detection limit 1.7484 X 10^{-6} calculated as and by using the following formula

$$DL = 3 \times SD/B \quad (1)$$

Where SD= Standard deviation, B is the slope obtained from the calibration plots. This detection limit is superior than the detection limits reported in the literature.

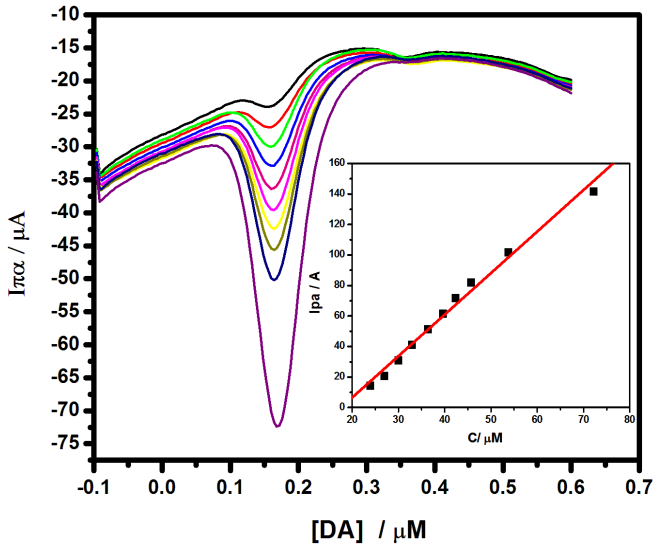

Figure 6 The effect of DA concentration

Simultaneous determination of DA, AA &UA

Figure 7 represents the DPV of the solution mixture containing 0.25 mM of DA, 0.25 mM of UA and 0.05 mM of AA. From Figure 5 it was well understood that, by increase in the concentration 3 prominent oxidation peaks were observed. When the concentration of DA was increased in the presence of 5 mM of AA, 0.28 mM of UA, an excellent selectivity was shown by the Emodin modified carbon paste electrode.

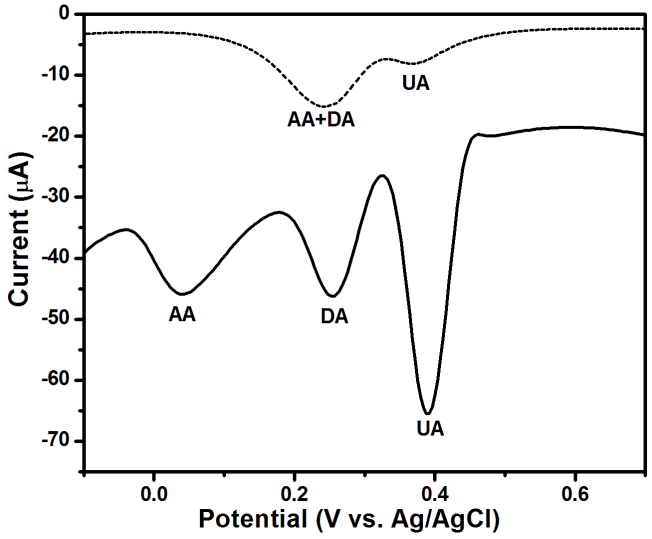

Figure 7 Simultaneous determination of DA, AA, and UA

Analytical applications to real systems

Practical application of modified electrode was validated by the quantitative determination of DA in human blood serum samples (Obtained from the health centre, S.V University, Tirupati, A.P). The procedure followed the human serum, 2 mL of the sample without any pre-treatment was diluted to 100 mL with pH 7.0 PBS. Different volumes of this solution were mixed with a known volume concentration of DA solution and also of known concentration to obtain different concentrations of spiked DA. Similarly, a drug injection capsule containing 200 mg of Dopamine HCl suitably diluted in 5 ml sterilized water was used to provide standard concentrations of DA which were analysed by DPV using the EMCPE. Each experiment was carried at least three times and the results were presented in the **Table** 1. The obtained recovery & relative standard deviation (RSD) were in good, agreement indicating the efficiency of EMCPE.

Table 1 Determination of DA in injection samples and human serum (n= 3)

Sample	Spiked DA sample (mm)	Found (mm)	Recovery	RSD (%)
Drug Injection	0.1	0.092	92	3.5
	0.2	0.165	82.5	3.2
	0.3	0.3	100	1.4
Blood Serum	0.1	0.092	92	3.0
	0.2	0.179	89.5	2.5
	0.3	0.265	88.3	1.1

Conclusion.

In this we have developed Poly Emodin CPE for sensing DA. The polyemodin shows both anodic and cathodic peak current remarkably. The polyemodin CPE showed an electro catalytic activity, selectivity and sensitivity towards the oxidation of DA, UA and AA. The poly emodin CPE has given some satisfactory results with detection limit of 1.748 X 10^{-6} µm for DA. Hence poly Emodin CPE can acts as an excellent sensor for the simultaneous determination of AA, DA, UA and so can be applied for the detection of neurotransmitters in the real sample analysis.

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