

Electrochemical Studies of Paracetamol and Folic Acid in the Presence of Epinephrine at Poly (Eosin) Modified Electrode: A Voltammetric Study

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Abstract

In this work, Poly eosin modified carbon paste electrode was prepared by electropolymerisation method by using cyclic voltammetry technique. This poly (Eosin) modified CPE exhibited an excellent electrocatalytic activity towards the oxidation of PA and FA in presence of EP in 0.2M PBS at pH 7.0. The effect of pH and sweep rate was studied. The limit of detection of PA and FA were found to be 0.06 and 0.04 μM , respectively. An electrochemical simultaneous separation was observed in a binary mixture containing PA, FA and EP and interference study was done by differential pulse voltammetry technique. Finally, the prepared fabricated sensor exhibits good sensitivity, and selectivity.

Keywords: Paracetamol, epinephrine and folic acid; Eosin; Cyclic electrocatalytic activity; Simultaneous determination

Introduction

Paracetamol or acetaminophen is an analgesic medicament similar to acetylsalicylic acid lacking anticoagulatory properties and gastric irritation. It is widely used as an active ingredient in pharmaceutical preparations as it is not considered to be carcinogenic at therapeutic doses. Paracetamol products give relief for all kinds of mild to moderate pain, including a headache, rheumatic pains, pains from minor injuries and all the everyday aches of normal life [1-3]. Overdose ingestions of Paracetamol lead to accumulation of toxic metabolites, which may cause severe and sometimes fatal hepatotoxicity and nephrotoxicity [4-5], which in some cases associate with renal failure. Thus, the development of a simple, precise and accurate procedure for the determination of this drug in pharmaceutical products is very useful. Many methods have been so far reported for its determination of paracetamol such as spectrophotometry [6], high performance liquid chromatography [7], and voltammetry [8]. These techniques are generally expensive and time-consuming. There are several number of modified electrodes like titanium oxide nanoparticle MPE, nafion/ TiO_2 - graphene modified GPE, ZrO_2 nano particle modified CPE, single walled carbon nanotube.

MCPE have been reported for the determination of acetaminophen by using voltammetric techniques [9-13]. On the other hand, Epinephrine (EP), also called adrenaline, is an important catecholamine neurotransmitter in the mammalian central nervous system [14] which exists as an organic cation in the nervous tissue and biological body fluid. Epinephrine is synthesized naturally in the body from L-tyrosine by the action of different enzymes. Many life phenomena are related to the concentration of EP in blood. It is also served as a chemical mediator for conveying the nerve pulse to different organs. Medically, EP has been used as a common emergency healthcare medicine [15,16]. The presence of EP in the body affects the regulation of blood pressure and the heart rate, lipolysis, immune system, and glycogen metabolism. It elevates the blood sugar level by increasing catalysis of glycogen to glucose in the liver, and at the same time begins the breakdown of lipids in fat cells [17]. These important actions of EP also make it a potent doping agent and hence, it is also banned in competitive games by the World Anti-Doping Agency [18,19]. Low levels of EP have been found in patients with Parkinson's disease [20-22]. From the point of view of medicine, it is a drug for emergency treatment in severe allergic reaction, cardiac arrest and sepsis [23]. Therefore, the quantitative

determination of EP concentration in different human fluids, such as plasma and urine, is important for developing nerve physiology, pharmacological research and life science [24].

FA, N-[p-[(2-amino-4-hydroxy-6-pteridinyl) methyl] amino] benzoyl]-L-glutamic acid, often regarded as a part of vitamin B complex, possesses the considerable biological importance for general human health, especially during periods of rapid cell division and growth [25,26]. Deficiency of folic acid is a common cause of anaemia and it is thought to increase the likelihood of heart attack and stroke. Many studies suggest that diminished folate status is associated with enhanced carcinogenesis as folic acid with vitamin B_{12} participates in the nucleotide synthesis, cell division and gene expression [27]. Periconceptual supplementation of folic acid has been demonstrated to reduce significantly the incidence and reoccurrence of neural tube defects, such as spina bifida of women [28]. In January 1998, the US Food and Drug Administration introduced mandatory fortification of cereal grain products with folic acid at a concentration of 140 mg/100 g [29]. In the UK, the Department of Health proposed fortification of flour with folic acid at 240 mg/100 g [30-31].

In the present day's carbon paste electrode was very much attracted towards the determination of biologically active molecules because of the easy preparation of modified electrode, renewability, low background current and fast response. However, Polymer modified electrodes (PMEs) have received great attention in recent years. As the polymer film which is deposited onto the surface of the electrode by electropolymerisation have good stability, reproducibility, more active sites, homogeneity and strong adherence to the electrode surface. Electropolymerisation is a good approach to immobilize polymers to prepare PMEs [13,32-33].

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Materials and Methods

Cyclic voltammetric experiments were performed on a model CH660c (CH instrument). All the electrochemical experiments were carried out in a three-electrode cell system, which contained a bare carbon paste electrode (BCPE)/Poly (Eosin) film coated MCPE as the working electrode, a platinum wire and saturated calomel electrode as the counter and the reference electrode.

Reagents and chemicals

Epinephrine (EP), Paracetamol (PA), Folic acid (FA), and Eosin was obtained from Himedia. 25×10^{-4} M PA was prepared in double distilled water, 25×10^{-4} M FA was prepared in 0.1 NaOH solution, 25×10^{-4} M EP was prepared in 0.1 M perchloric acid (HClO_4), 25×10^{-4} M Eosin was prepared in double distilled water and Phosphate buffer solution (PBS) of same ionic strength was prepared (0.2 M) by mixing appropriate ratio of sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) disodium hydrogen phosphate (Na_2HPO_4). Graphite powder of 50 mm size was purchased from Loba and silicon oil was purchased from Himedia. All the chemicals are of analytical grade quality and were used as supplied without further purification.

Preparation of bare carbon paste electrode

Bare carbon paste electrodes (BCPE) were made with silicon oil (30%), and graphite powder (70%). The two components were thoroughly mixed in an agate mortar for about 30 min. The BCPE was packed into a homemade Teflon cavity having a current collector and was polished on a weighing paper.

Result and Discussion

Electropolymerisation of eosin at carbon paste electrode

Polymer film modified carbon paste electrode was fabricated by electropolymerization of eosin at the surface of the CPE by CV method containing in 0.5 mM Eosin in 0.1 M NaOH solution as supporting electrolyte. Electropolymerization was conducted between the potential windows of -0.2 V to +1.4 V at the sweep rate of 100 mV/s for ten cycles. In the process of recording the multiple cycles, the voltammograms were slowly decreased with increasing in cyclic time as shown in Figure 1. It indicates that, a thin layer of poly (eosin) was deposited on the surface of CPE.

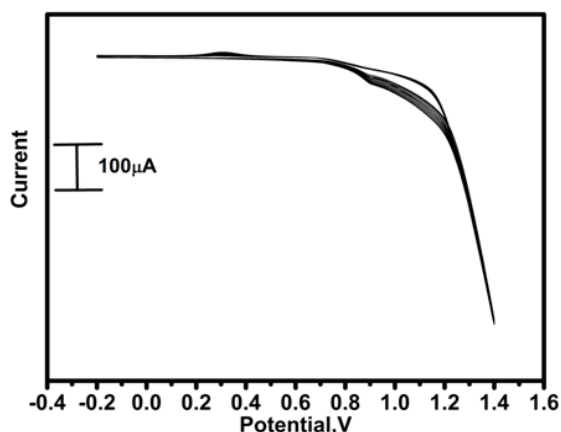


Figure 1: Electropolymerization of 0.5 mM of Eosin at 0.1 M NaOH as supporting electrolyte for 10 cycles with the sweep rate of 100 mV/s.

Effect of multiple cycles in the preparation of poly (Eosin) MCPE

From the above experimental results, the thickness of the film has a major contribution on the electrocatalytic property of the poly (Eosin) MCPE. The formation of the layer was controlled by varying the number of cycles on the BCPE (from 5 to 20) and corresponding electrocatalytic activity towards the oxidation of 0.1 mM PA in 0.2 M PBS of pH 7.0. The graph of anodic peak current (I_{pa}) versus the number of cycles was constructed and the graph shows linearly increasing the number of cycles from 5 to 20 as shown in Figure 2. However, a better catalytic performance was observed for the electrode which was modified by 10 consecutive cycle scans [13]. Therefore, ten cycles were selected for the electropolymerization of Eosin as a favourable outcome from all the parameters (Figures 1 and 2).

Electrocatalytic reaction of PA at poly (Eosin) modified CPE

Figure 3 depicts the cyclic voltammetric response for electrochemical oxidation of 0.1 mM PA at bare CPE (dashed line) and poly (Eosin) modified CPE (Solid line) in 0.2 M PBS of pH 7 recorded at a sweep rate of 100 mV/s. At bare CPE, PA shows only one oxidation peak was observed and it is an irreversible behaviour. The oxidation peak potential was observed at 408 mV. The excellent response is observed at poly (Eosin) modified CPE which shows a well-defined oxidation peak potential at

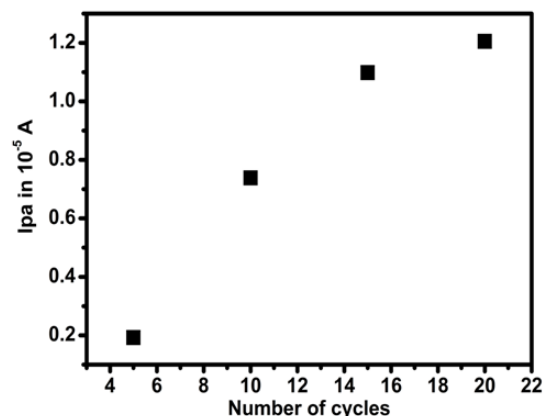


Figure 2: Graph of anodic peak current versus the number of cycles.

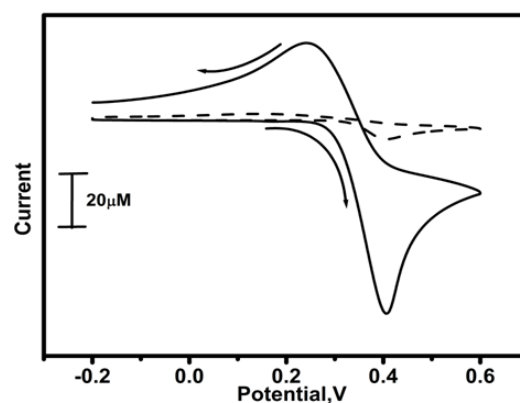


Figure 3: Cyclic voltammograms of 0.1 mM PA in 0.2 M PBS solution of pH 7.0 at BCPE (dashed line) and poly (Eosin) modified CPE (Solid line) at the sweep rate of 100 mV/s.

406 mV and corresponding reduction peak potential at 242 mV with a significant enhanced in the redox peak current. These results clearly reveal that, poly (Eosin) modified CPE acts as a very efficient promoter to enhance the kinetics of the electrochemical oxidation of paracetamol [34] (Figures 3 and 4).

Effect of PA and FA sweep rate at Poly (Eosin) modified CPE

The effect of varying sweep rates on the cyclic voltammograms of 0.1 mM paracetamol at poly (Eosin) modified CPE as shown in Figure 5a. Based on a plot the observation shows that increased in the sweep rates the redox peak current also increased within the range from 50 to 400 mV/s. To calculate the electrode process, the graph of I_p versus sweep rates was constructed. The obtained graph was the good linear relationship between the peak currents as shown in Figure 5b. The correlation coefficient value was found to be 0.9997 and 0.9967, respectively. While at the same time, the graph of I_p versus square root sweep rates were plotted (Figure 5c). Having a correlation coefficient of 0.9935 and 0.9982, respectively. It indicates that the nature of electrode reaction is adsorption controlled [34-40] (Table 1).

The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, eqn. (1) was

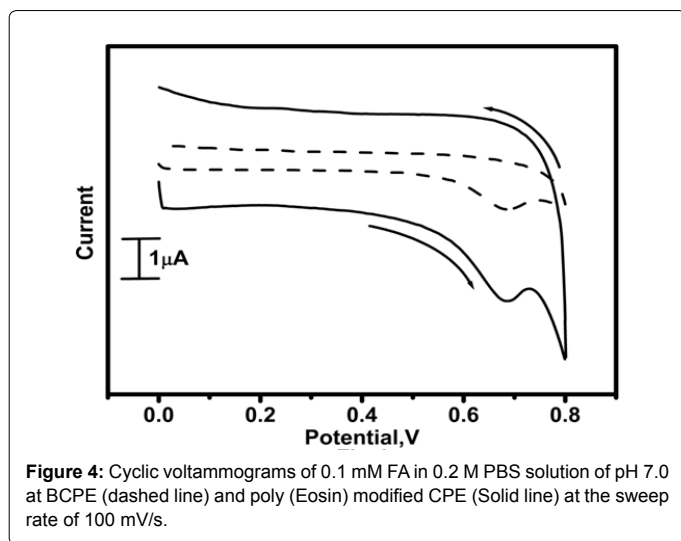


Figure 4: Cyclic voltammograms of 0.1 mM FA in 0.2 M PBS solution of pH 7.0 at BCPE (dashed line) and poly (Eosin) modified CPE (Solid line) at the sweep rate of 100 mV/s.

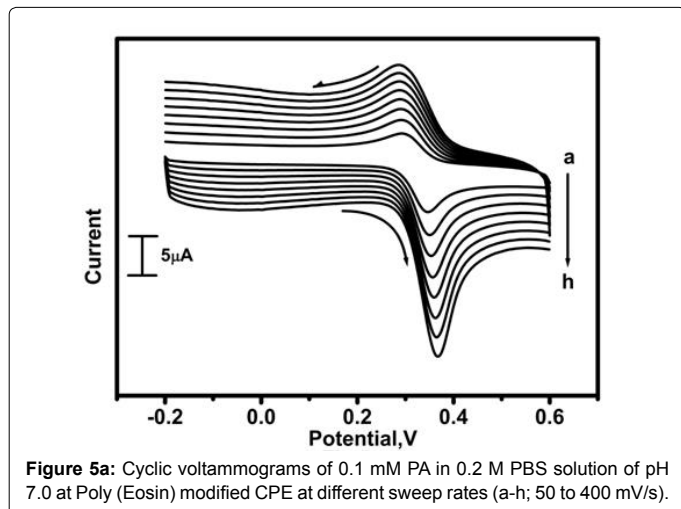


Figure 5a: Cyclic voltammograms of 0.1 mM PA in 0.2 M PBS solution of pH 7.0 at Poly (Eosin) modified CPE at different sweep rates (a-h; 50 to 400 mV/s).

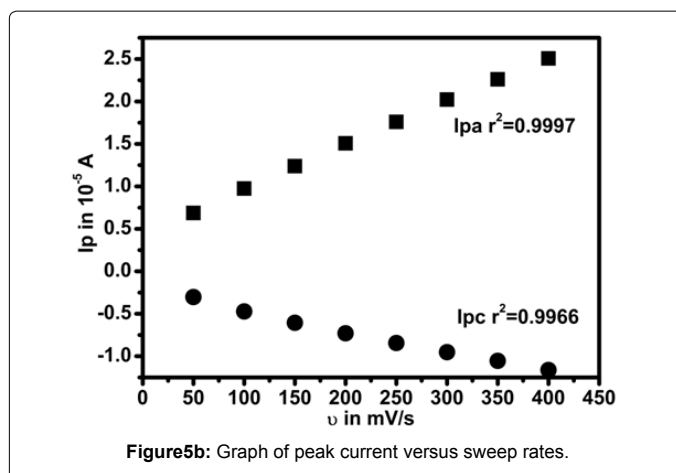


Figure 5b: Graph of peak current versus sweep rates.

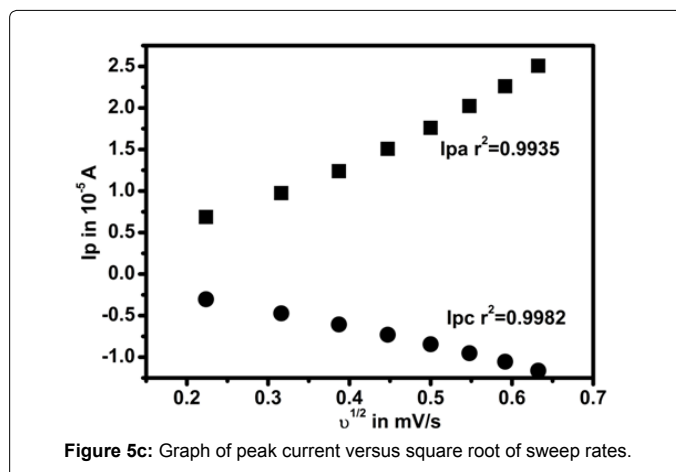


Figure 5c: Graph of peak current versus square root of sweep rates.

v mV/s	ΔE_p (V)	k^0 (s ⁻¹)
50	55	0.8452
100	59	1.1618
150	63	2.2290
200	67	2.9512
250	71	3.4673
300	73	4.0738
350	80	4.3651
400	81	5.0350

Table 1: Electrochemical parameters of PA at different sweep rates.

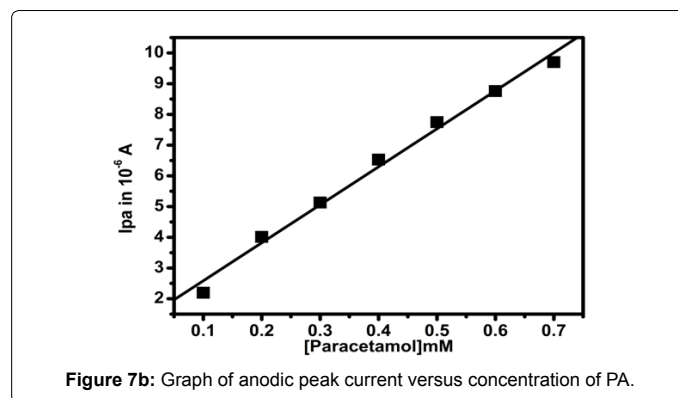
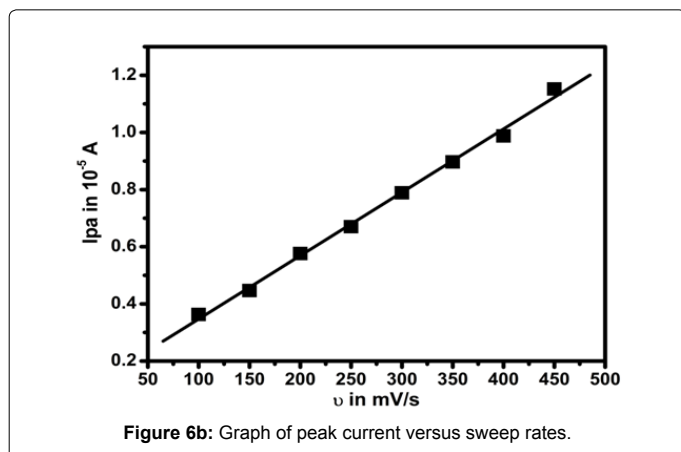
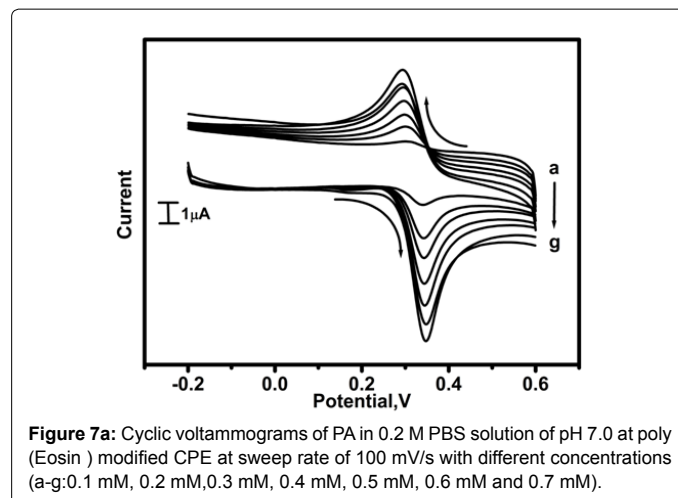
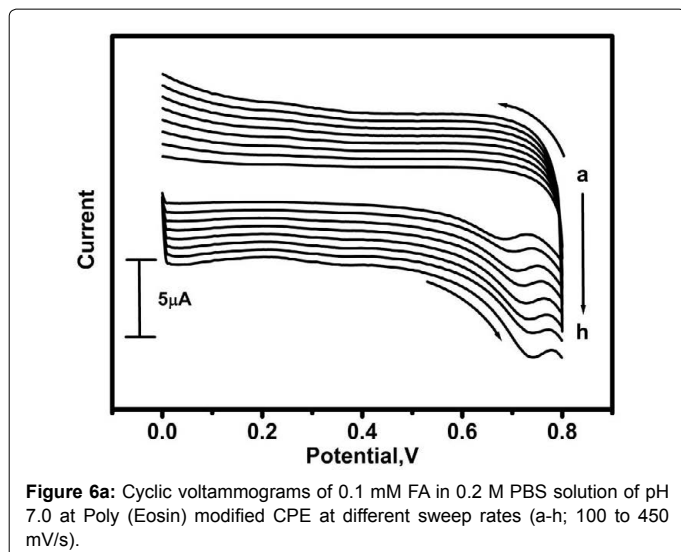
used for such voltammograms whose ΔE_p values are greater than 10 mV [42-49]

$$\Delta E_p = 201.39 \log (v/k^0) - 301.78 \quad (1)$$

From the experimental ΔE_p values as shown in Table 1 and eqn. (1); the values of the k^0 for the PA oxidation was determined. All the parameters are tabulated in Table 1.

Effect of PA and FA sweep rate at Poly (Eosin) modified CPE

The effect of sweep rate on the anodic peak current of 0.1M FA at poly (Eosin) modified CPE was studied by cyclic voltammetry. Figure 6a shows an increase in the anodic peak current by increasing the sweep rate from 100 to 450 mV/s. Figure 6b shows the plot of the I_{pa} versus the sweep rates shows almost straight line with a good linearity as shown having correlation co-efficient 0.9980. It indicates that

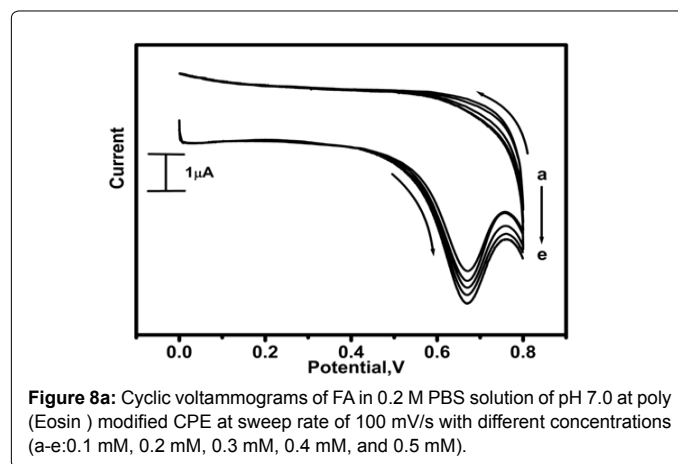


irreversible oxidation process of FA at the poly (Eosin) modified CPE was adsorption controlled electrode reactions [37].

Effect of PA and FA concentration at Poly (Eosin) modified CPE

The electrocatalytic oxidation of PA was carried out by varying its concentration at poly (Eosin) modified CPE. Figure 7a depicts the cyclic voltammograms of various concentrations of paracetamol (0.1 to 0.7 mM). By increasing the concentration of PA, the electrochemical redox peak current goes on increasing. The graph of I_{pa} versus concentration of PA was constructed as shown in Figure 7b. The correlation coefficient value was found to be 0.9954. The limit of detection of PA is 0.06 μM and in the same time Figure 8a indicates that the cyclic voltammograms of successive improvement of peak current on increasing concentration of PA. The graph of anodic peak current versus the concentration of PA from the range of 0.1 to 0.5 mM. The obtained graph has good linearity and almost straight line as shown in Figure 8b having a correlation coefficient of 0.9964. The limit of detection was calculated [38] and it was found to be 0.06 μM [PA] and 0.04 μM [FA], respectively. The performance of modified electrode exhibited lower detection limit than other reported and given it in the Tables 2 and 3.

$$\text{LOD} = 3S/M \quad (2)$$



Where S is standard deviation and M is the slope.

PA, FA and EP electrochemical determination by Simultaneous method

The main aim is the simultaneous determination of PA, FA and EP in a 0.2 M phosphate buffer solution of pH 7 at poly (Eosin) modified CPE. Figure 9 shows the simultaneous determination of PA, EP and FA by poly (Eosin) MCPE and BCPE in a mixture of solution. As can be seen the BCPE, there is failed to separate the voltammogram in the backward scan is indistinguishable but oxidation takes place only (solid line), however, in the same condition poly (Eosin) MCPE

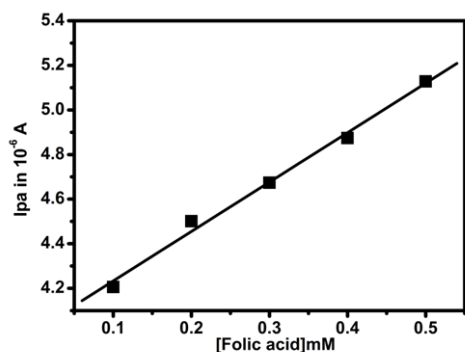


Figure 8b: Graph of anodic peak current versus concentration of FA.

S. No	Electrode	Detection limit (μM)	Techniques	Reference
1	SPE/PEDOT	1.39	DPV	43
2	Nafion/ROPCME/GCE	1.2	SWV	44
3	BDDE	0.81	DPV	45
4	GCE/f-MWCNT	0.6	DPV	46
5	Poly (Eosin) modified CPE	0.06	CV	Present work

Table 2: Comparison of some modified electrodes for determination of PA.

S. No	Modified Electrode	Detection limit (μM)	Techniques	Reference
1	ZrO ₂ nanoparticles	9.86×10^{-6}	DPV	47
2	BH ⁺ & TiO ₂ nanoparticles	2.5×10^{-5}	DPV	48
3	SAOSMCPE	2.88×10^{-5}	CV	49
4	PANI/TPA	3.0×10^{-7}	DPV	50
5	Poly (Eosin) modified CPE	0.04	CV	Present work

Table 3: Comparison of some modified electrodes for determination of FA.

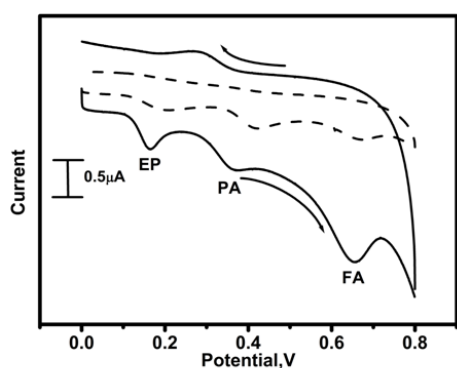


Figure 9: Cyclic voltammograms for simultaneous determination of 0.1 mM PA, 0.1 mM FA at BCPE (dashed line) and poly (Eosin) modified CPE (solid line) at sweep rate of 100 mV/s.

can separate the overlapped cyclic voltammogram, among three electroactive species PA shows reduction takes place in the reverse scan (dashed line). Also the peak current of oxidation of these three substances significantly increases at the poly (Eosin) MCPE [40].

Interference

The differential pulse voltammetric technique was used for simultaneous determination because of its more sensitivity and selectivity with negligible background current [40]. In this study, by altering the concentration of one analyte while the other two analytes were kept constant and vice versa. The concentration PA was varied

from 0.1 to 0.6 mM while other two species concentration of EP was 0.1 mM and FA was 0.1 mM was kept constant (Figure 10).

Similarly in Figures 11 and 12 explain the concentration effect of EP from 0.1 to 0.4 mM and FA from 0.1 to 0.5 mM respectively. This result shows that the oxidation of PA, FA and EP is autonomous to each other. By this method three voltammetric peaks can be separated.

Conclusion

In conclusion, the poly (Eosin) modified CPE was fabricated by

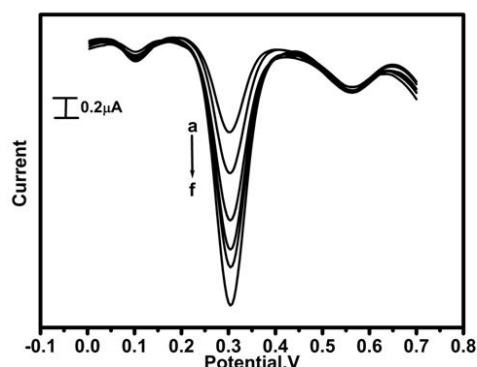


Figure 10: Differential pulse voltammograms of (a) 0.1 mM (b) 0.2 mM, (c) 0.3 mM, (d) 0.4 mM, (e) 0.5 mM and (f) 0.6 mM PA in 0.2 M PBS of pH 7.0 in the presence of 0.1 mM FA and 0.1 mM EP at poly (Eosin) modified CPE .

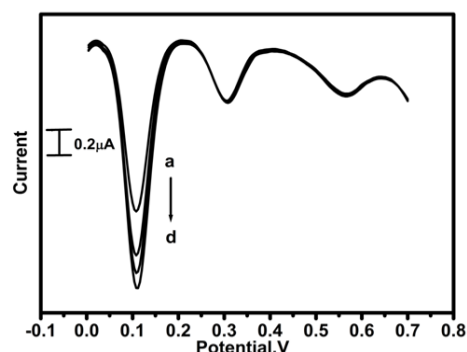


Figure 11: Differential pulse voltammograms of (a) 0.1 mM (b) 0.2 mM, (c) 0.3 mM and (d) 0.4 mM EP in 0.2 M PBS of pH 7.0 in the presence of 0.1 mM PA and 0.1 mM FA at poly (Eosin) modified CPE .

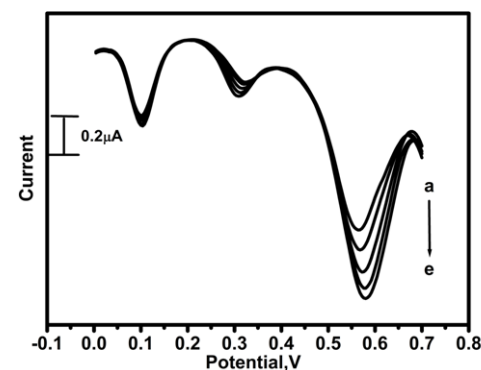
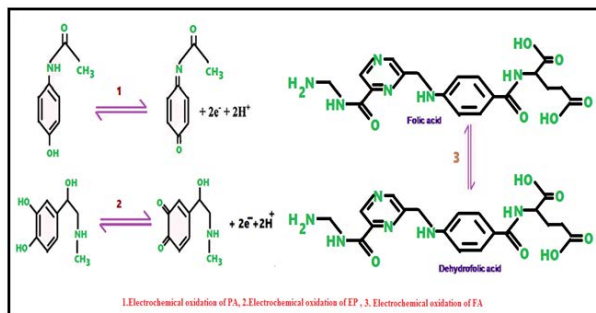


Figure 12: Differential pulse voltammograms of (a) 0.1 mM (b) 0.2 mM, (c) 0.3 mM (d) 0.4 mM and (e) 0.5 mM FA in 0.2 M PBS of pH 7.0 in the presence of 0.1 mM PA and 0.1 mM EP at poly (Eosin) modified CPE .

using CV technique. The poly (Eosin) modified CPE exhibits highly selectivity and electrocatalytic activity to PA, FA in the presence of EP. This method clearly separated the overlapped peaks into well separated individual peaks. Hence, it is good selectivity and sensitivity, it is expected that poly (Eosin) MCPE could hold great application in the fields of electro analytical chemistry and biosensors.



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