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Electrochemical Behaviour of Norepinephrine in the Presence of Paracetamol and Folic Acid at Poly (Congo red) Modified Carbon Paste Electrode

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Abstract- Herein, Poly (Congo red) modified carbon paste electrode was prepared by electropolymerization method. The poly (Congo red) modified carbon paste electrode showed an excellent electrocatalytic effect on the oxidation of Norepinephrine (NE) in phosphate buffer solution (pH 7.4) at the sweep rate 100 mV/s. Some electrochemical parameters like sweep rate, different concentrations and effect of pH were studied. The limit of detection of NE was found to be 0.29 µM in the linear range of 30 to 90 µM by CV method. The simulations determination of NE, PA and FA in their sample mixture was analyzed by using cyclic voltammetric technique. The differences of the oxidation peak potentials for NE–PA and PA–FA were about 0.212 V and 0.323 V in cyclic voltammetric at 100 mV/s respectively. The interference studies showed that the modified electrode exhibits excellent selectivity in the presence of Paracetamol (PA) and Folic acid (FA). This work provides a simple and easy approach to selectively determine NE in the presence of PA and FA.

1. INTRODUCTION

Norepinephrine an endogenous adrenoceptor agonist is present mainly in mammalian central nervous systems. It is released supra-spinally, spinally and peripherally at sites expressing adrenoceptor such as neurons in the spinal cord [1]. It is used for treating myocardial infarction hypertension, bronchial asthma and organic heart disease. Extreme abnormalities of NA concentration levels may lead to the occurrence of many diseases such as ganglia neuroblastoma, ganglion neuronal, paraganglioma and Parkinson' disease [2-3]. NE is also a significant transmitter in many parts of the central nervous system, where it is engrossed in emotional arousal, blood pressure regulation, and mood disorders. Recent reports have indicated that NE enhances adhesion of human immunodeficiency virus-1-infected leukocytes to cardiac microvascular endothelial cells and also accelerates HIV replication via protein kinase [4-5]. Various methods including spectrophotometry, capillary electrophoresis and high-performance liquid chromatography, (HPLC) have been employed for the determination of NE. Because NE is an electroactive compound, its electrochemical detection has been the focus of research for electroanalytical researchers and neurochemists and some modified electrodes have been used to determine NE [6-8].

Paracetamol is widely used as an antipyretic and analgesic drug. It is an effective and safe analgesic agent used for the relief of mild to moderate pain associated with headache, backache, arthritis and postoperative pain [9]. It is also used for reduction of fevers of viral and bacterial origin. Paracetamol relieves pain and fever by inhibiting prostaglandin's synthesis in the central nervous system and sedating hypothalamic heat-regulating center [10-11] Overdose of paracetamol leads to hepatic toxicity and in some cases associated with liver and kidney damage and even death [12,13]. Thus several methods have been used for the determination of PA in pharmaceutical formulations and biological fluids including spectrophotometry, flow-injection and chromatographic methods. Also, PA is an electroactive molecule (PA contains hydroxyl and NH groups on its aromatic rings), and its electrochemical behaviour has been studied extensively. The ease of PA oxidation led to development of electrochemical procedures for measuring PA levels at various electrodes and using various electrochemical methods [14-18].

Folic acid is a water-soluble vitamin B_9 and can act as coenzyme in the transfer and utilization of one-carbon groups and in the regeneration of methionine from homocysteine [19-20]. Folate is also found in vegemite or Marmite. Folate is also synthesized in bacteria. Several important nutrients serve as building blocks of a healthy pregnancy. FA is one such nutrient of great importance, especially for women planning for pregnancy. Adequate folate intake during the preconception period helps protect against a number of congenital malformations [21]. The most notable birth defects that occur from folate deficiency are neural tube defects, which result in malformations of the spine (spina bifida), skull, and brain anencephaly [22,23]. As FA is an electroactive component, some electrochemical methods

have been reported for its determination [24-26]. Comparing with other technologies, electrochemical method is more desirable because of its convenience and low [27].

PA administration is known to increase brain serotonin (5-HT) levels as a result of liver tryptophan-2,3-dioxygenase (TDO) inhibition [28] and 5-HT is known to play a role in NE release in the brain [29-30]. Also, FA, the synthetic form of the B vitamin folate, works primarily in the brain and nervous system and is necessary for the production of NE and serotonin in the nervous system. Also, some substances like nonsteriodal anti-inflammatory drugs such as aspirin, ibuprofen and AC can inhibit FA from being absorbed or used by the body. Likewise, when taken for long periods of time, AC and other anti-inflammatory medications can also increase the need for FA [31]. Therefore simultaneous determination of NE, PA and FA is important Comparing with other technologies, electrochemical method is more desirable because of its convenience and low cost [32].

Congo red (CR) is a benzidine-based anionic diazo dye prepared by coupling tetrazotised benzidine with two molecules of napthionic acid [33]. It is a complex chemical structure, high solubility in aqueous solution. Congo red is the first synthetic azo dye produced that is capable of dying cotton directly. Congo red containing effluents are generated from a number of industrial activities: textiles, printing and dyeing, paper, rubber, plastics industries [34,35] the presence of amino biphenyl group and azo bonds, two features generally considered as xenobiotic [36-38].
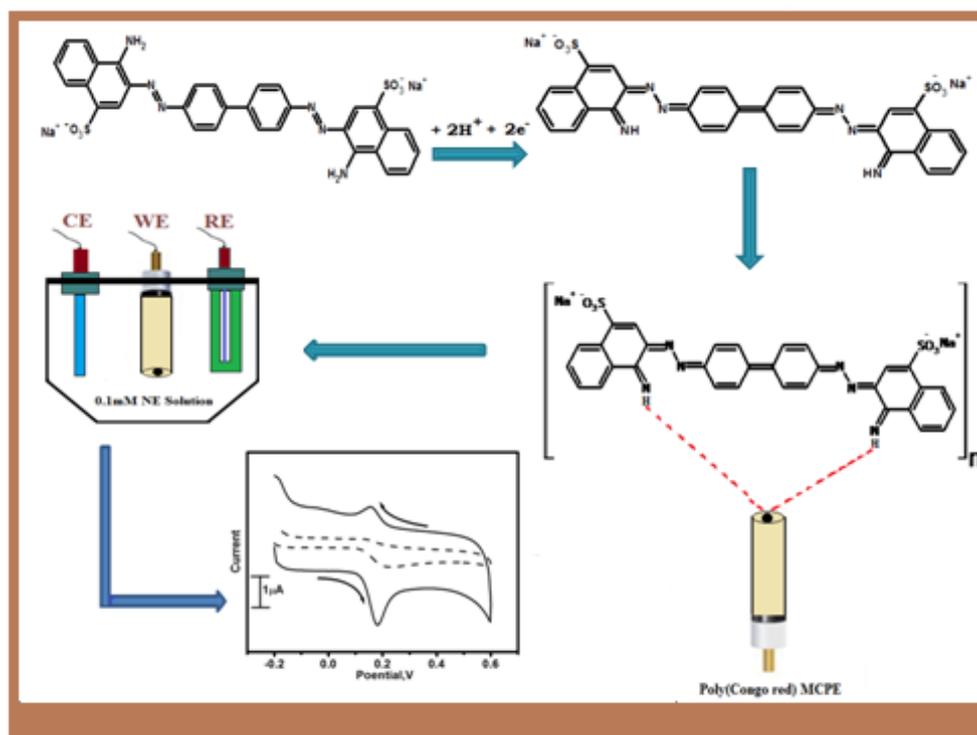
Scheme. 1. The detailed oxidation mechanism of Norepinephrine

During the last few decades the modification of electrode by the application of electropolymerisation technique is of great importance in constructing electrochemical sensors and biosensors [39-41] because of their characteristics like film thickness, permeation and charge transport can be controlled by adjusting the electrochemical parameters. Therefore PME's have many advantageous such as improved electrocatalysis, absence of surface fouling and prevention of undesirable reactions competing kinetically with the desired electrode process [42-43]. In future these modified electrocatalytic electrodes which acts as sensors can be used in the medicine and biotechnology field.

In the present work, fabricated stable electrode by electropolymerising Congo red on the surface of carbon paste electrode to determine the NE in the presence of PA and FA at physiological pH. This modified electrode shows better electrochemical properties compared to BCPE (scheme 1).

2. EXPERIMENTAL PART

2.1. 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 (CR) film coated MCPE as the working electrode, a platinum wire and saturated calomel electrode as counter and reference electrode.

2.2. Reagents and Chemicals

Norepinephrine (NE) was purchased from Himedia (Molecular weight=169.2, purity 99%), Paracetamol (PA) was purchased from Himedia (Molecular weight=151.64, purity 99%), Folic acid (FA) was purchased from Himedia (Molecular weight = 441.39, purity 99%) and Congo red (CR) was Obtained from Sigma Ltd., India (molecular weight=696.66, purity 99%). Graphite powder of average particle size 50 μM was purchased from Merck and silicon oil from Himedia was used to prepare carbon paste electrode (CPE). All chemicals are of analytical grade quality and were used as supplied without further purification. 25×10^{-4} M NE was prepared in 0.1 M Perchloric acid (HClO$_4$), 25×10^{-4} M PA was prepared in double distilled water, 25×10^{-4} M FA was prepared in 0.1 M Sodium hydroxide (NaOH), 25×10^{-4} M Congo red (CR) was prepared in double distilled water. Phosphate buffer (0.2 M) was prepared by mixing appropriate quantity of aqueous sodium dihydrogen phosphate monohydrate and aqueous disodium hydrogen phosphate. All solutions were prepared with double distilled water.

2.3. 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 minutes. The BCPE was packed into a homemade Teflon cavity having a current collector and was polished on a weighing paper.

2.4. Preparation of poly (CR) modified carbon paste electrode

Electropolymerisation of Congo red at CPE was optimized and carried out in 0.1 M NaOH solution containing 1 mM Congo red solution. The polymer growth was achieved by the formation of film that grew between -0.2 to 1.5 V at the sweep rate of 100 mV/s for 10 cycles by using cyclic voltammetric technique. After every measurement, the modified electrode was rinsed with doubly distilled water and used for further electrochemical analysis. The resulted electrode was denoted by Poly (CR) MCPE.

2.5. Electropolymerisation of poly (CR) MCPE at CPE surface

1 mM Congo red was taken in the electrochemical cell which contains 0.1 M NaOH as a supporting electrolyte undergoing electropolymerization by the formation of film that grew between -0.2 to 1.5 V at the sweep rate of 100 mV/s for 10 cycles by using cyclic voltammetry. During the process of multiple cycles the voltammogram has gradually descended with increase of cyclic time increasing as shown in Figure 1. This indicates that the poly (CR) film was formed and deposited on the surface of carbon paste electrode [44]. The thickness of the layer also affects the electrocatalytic property of the electrode and it can be maintained by changing the sweep segments. The electrode was then stored at room temperature.

2.6. Effect of multiple cycles in the preparation of poly (CR) MCPE

Figure 2A shows the cyclic voltammograms for the different cycles of electropolymerized Congo red modified carbon paste electrode at the sweep rate of 100 mV/s, with a Nor epinephrine concentration of 0.3 mM in 0.2 M phosphate buffer solution (pH 7.4) with increment in the peak current. The plot anodic peak current (Ipa) verses number of polymerisation cycles were plotted. By increasing the number of polymerisation cycles from 5-20 as shown in Figure 2B. From the graph it was found that current will shows linearly increase. The thickness of the electroactive layer directly affects the electrocatalytic activity of the fabricated electrode. As shown in the obtained results, maximum current response is obtained for 25 multiple cycles. However, a better catalytic performance was observed for the electrode which was modified by 10 consecutive cycle scans [45]. Therefore 10 cycles was

chosen for the electropolymerization of Congo red as optimum for the study of all other parameters.
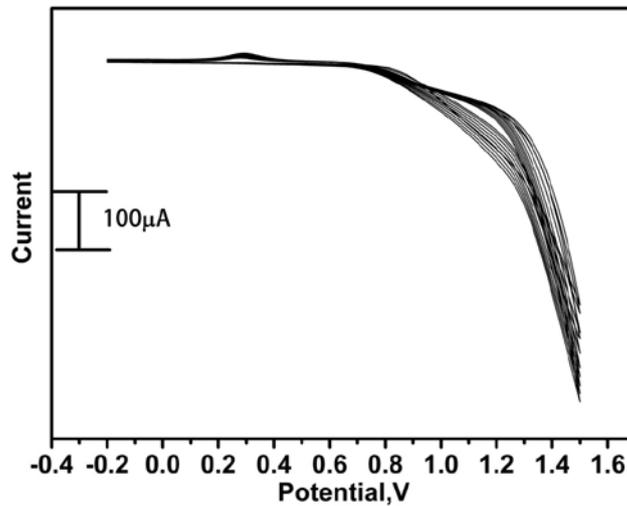
Fig. 1. Electropolymerization of 0.5 mM of Congo red at 0.1 M NaOH as supporting electrolyte for 10 cycles with Sweep rate of 100 mV/s
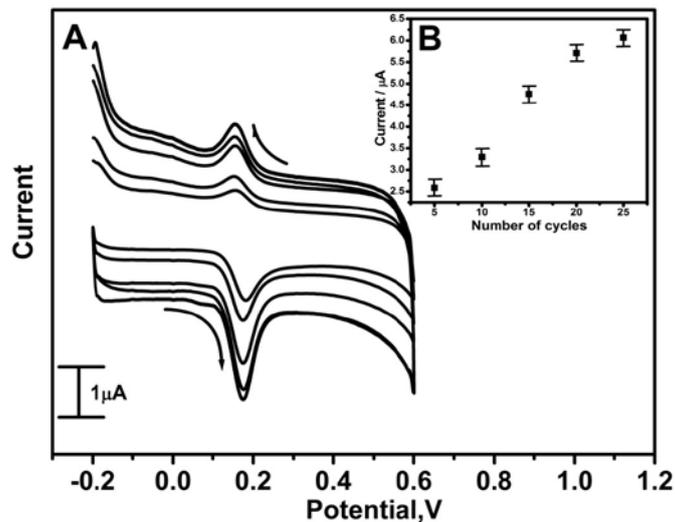
Fig. 2. (A) Cyclic voltammograms recorded for 0.3 mM NE at different cyclised poly (CR) MCPE (5, 10, 15, and 20) in PBS at pH 7.4; **(B)** Graph of anodic peak current versus number of polymerisation cycles

2.7. Electrochemical characterization of poly (CR) MCPE using standard potassium ferrocyanide system

Potassium ferrocyanide was selected to evaluate the performance of the proposed electrode. Figure 3 shows the electrochemical response of potassium ferrocyanide at the

BCPE (dashed line) and Poly (CR) MCPE (solid line) in 1 M KCl as supporting electrolyte containing 1 mM $K_4[Fe(CN)_6]$ at 50 mV/s. The small redox peak currents response was obtained at BCPE. The potential difference (ΔE_p) was found to be 0.089 V. On the other hand, in the same identical condition poly (CR) MCPE shows a stable improvement of redox peak currents and also showed faster electron transfer kinetics with the ΔE_p of 0.060V. As ΔE_p is a function of the rate of electron transfer, the lesser ΔE_p, the higher electron transfer rate. Based on the above observations the surface property of the modified electrode has been dramatic changed and also result proves electrocatalytic activity of the Poly (CR) MCPE. The total active surface area available for reaction of species in solution can be estimated by the Randles-Sevcik equation (1) [46].

$$I_p = 2.69 \times 10^5 \, n^{3/2} \, A \, D^{1/2} \, C_0 \, v^{1/2} \tag{1}$$

Where, I_p is the peak current in A. C_0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electrons exchanged, D is the diffusion-coefficient (cm^{2}s^{-1}), and v is the sweep rate (V/s), A is the electroactive surface area (cm^2). The maximum surface area was obtained for Poly (CR) MCPE. (0.0431 cm^2) as compared with BCPE (0.0324 cm^2)
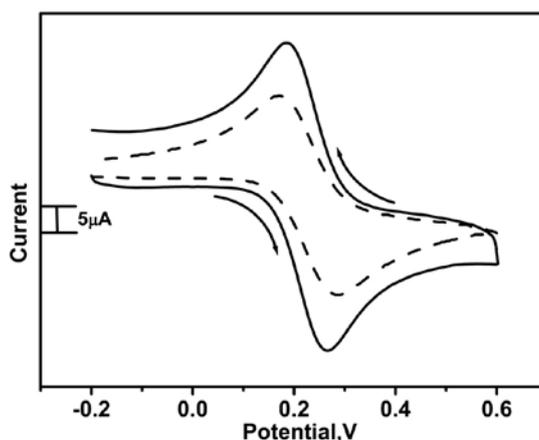
Fig. 3. Cyclic voltammograms of 1 mM potassium ferrocyanide at BCPE (dashed line) and poly (CR) MCPE (solid line) at scan rate of 50 mV/s

2.8. Electrochemical response of NE at poly (CR) MCPE

Figure 4 depicts the cyclic voltammograms responses for the electrochemical oxidation of 0.3 mM NE at BCPE (dashed line) and a poly (CR) MCPE (solid line) in 0.2 MPBS solution of pH 7.4 with sweep rate 100 mV/s. In BCPE a pair of redox peak showed deprived electrocatalytic activity due to slow electron transport phenomenon, and the oxidation potential was observed at 0.226 V. Conversely, the voltammetric response of NE at poly (CR) MCPE showed a good increment in redox peak current as compared to the BCPE and

oxidation potential was sited at 0.186 V. Due to the improvements in the reversibility of the electron transfer processes. This suggests that our poly (CR) MCPE has better electrocatalytic activity by exposing huge surface area for electrochemical oxidation of NE.
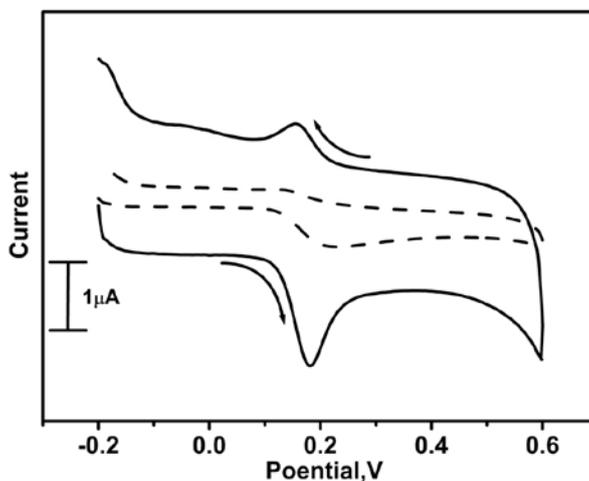
Fig. 4. Cyclic voltammograms of 0.3 mM NE in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (CR) MCPE (Solid line) at scan rate of 100 mV/s

2.9. Effect of sweep rate on the peak current of NE

In order to study, the nature of the electrochemical reaction of 0.3 mM NE in 0.2 M PBS of pH 7.4 was examined at poly (CR) MCPE by cyclic voltammetric technique as shown in Figure 5A. The obtained results showed that the anodic peak current increased linearly with the increase of sweep rate in the range of 50–300 mV/s. The graph of current (I_{pa}) verses sweep rate (υ) was plotted (inset Figure 5B) and it was good linearity. The correlation coefficient was originated to be 0.9998 which indicate the electrode transfer reaction was adsorption controlled. Additional the graph of current (I_{pa}) versus square root of sweep rate ($\upsilon^{1/2}$) was plotted (inset Figure 5C). The obtained graph was good linearity in the assortment of 50–300 mV/s with correlation coefficient 0.9922; it reveals that electrode process is diffusion controlled taking place at the poly (CR) MCPE. From the above observation it is clear that the electrode process was controlled by both adsorption and diffusion process consecutively.

The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, Eq. (2) was used for such voltammograms whose ΔE_p values are greater than 10 mV [47]

$$\Delta E_p = 201.39 \log (\upsilon/ k^0) - 301.78 \tag{2}$$

From the experimental ΔE_p values as shown in Table 1 and Eq. (2); the values of the k^0 for the NE oxidation was determined. The values of k^0 obtained at the sweep rate of 300 mV/s for the poly (CR) MCPE exhibits larger heterogeneous rate constant compared with those determined in other sweep rate variation studies. All the parameters are tabulated in Table 1.
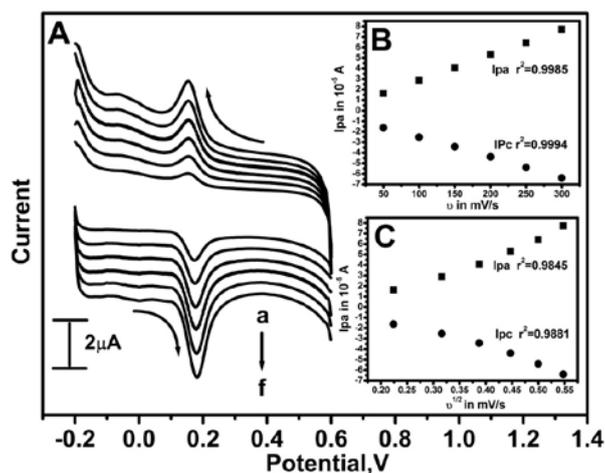
Fig. 5. (A) Cyclic voltammograms of 3 mM NE in 0.2 M PBS solution of pH 7.4 at Poly (CR) MCPE at different sweep rates (a–h; 50 to 300 mV/s); **(B)** Graph of peak current versus sweep rate; **(C)** Graph of peak current versus square root of sweep rate

Table 1. Variation of the voltammetric parameters gathered from the plots shown in Figure 5 as a function of the potential sweep rate

mVs^{-1}	ΔE_p(V)	k^0(s^{-1})
50	25	1.188
100	29	2.254
150	30	2.870
200	32	4.255
250	33	5.423
300	34	6.456

3. RESULTS AND DISCUSSION

3.1. Effect of NE concentration

The concentration effect of NE was carried out by altering its concentration at poly (CR) MCPE. Figure 6A showed that, by escalating the concentration of NE from 30 to 90 µM the anodic peak current and cathodic peak current goes on increasing.
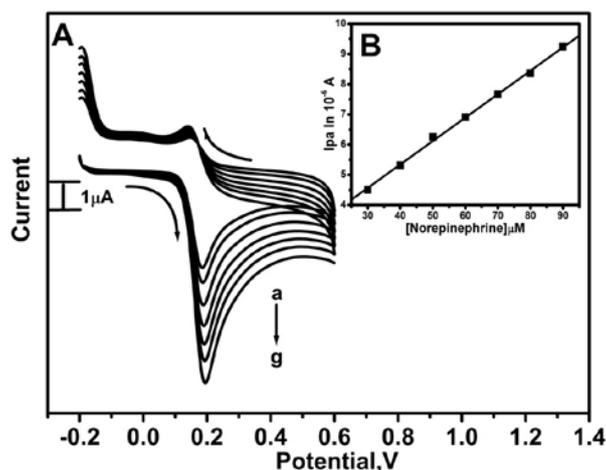
Fig. 6. (A) Cyclic voltammograms of NE in 0.2 M PBS solution of pH 7.4 at poly(CR) MCPE at scan rate of 100 mVs^{-1}with different concentrations (a–g: 30 µM, 40 µM, 50 µM, 60 µM, 70µM, 80µM, 90µM); **(B)** Graph of anodic peak current versus concentration of NE

Table 2. The performance of modified electrode was compared with other reported modified electrodes

Sl.No	Electrode	Modifier	pH	Detection limit (µM)	Techniques	Ref.
1	Carbon paste	5ADMBCNPE	7.0	8.0	SWV	[49]
2	Gold	ME/Au SAMs	5.5	0.7	SWV	[50]
3	Carbon paste	TiO$_2$ nanoparticles carbon paste	8.0	0.5	DPV	[51]
4	Gold	Thiolactic	5.9	2.0	CV	[52]
5	Carbon paste	TX-100/CPE	7.0	5.0	CV	[53]
6	Glassy carbon	SWNT-modified electrode	5.72	6.0	CV	[54]
7	Gold	C$_{60}$-[dimethyl-(β cyclodextrin)]$_2$/Nafion	6.0	8.0	CV	[55]
8	Glassy carbon	Poly(2,4,6-trimethylpyridine)	7.4	8.0	CV	[56]
9	Glassy carbon	Ppy/b-CD-ME	7.4	0.8	CV	[57]
10	Carbon paste	poly(glutamic acid) MCPE	7.4	0.43	CV	[59]
11	Carbon paste	Poly (CR) MCPE	7.4	0.29	CV	This work

The graph of anodic peak current verses dissimilar concentration of NE was plotted (inset Figure 6B) and results showed that the concentration was proportional to I$_{pa}$ with good linearity and correlation coefficient 0.9995. The linear regression equation was found to be I$_{pa}$ (10^{-6}A)=0.77561(C$_0$ µM/L) +3.7908.The detection limit for DA was found to be 0.29 µM and quantification limit was 0.99 µM. The limit of detection (LOD) and limit of quantification

(LOQ) were calculated by using the formula (1) and (2) respectively [48]. Where S is the standard deviation and M is the slope obtained from the three calibration plots. The performance of modified electrode was compared with other reported modified electrodes as shown in Table 2 [49-57,59].

$$LOD = 3S/M \tag{1}$$
$$LOQ = 10S/M \tag{2}$$

3.2. Effect of pH value on the determination of NE at poly (CR) MCPE

The pH of the phosphate buffer solution has a significant contribution on the electrocatalytic oxidation of NE at the poly (CR) MCPE by affecting peak potentials.
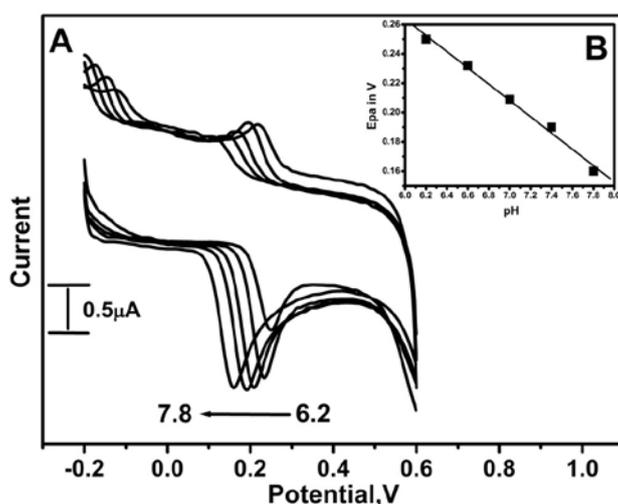
Fig. 7. (A) Cyclic voltammograms of the poly (CR) MCPE in 0.2 M PBS solution at different pH values (a–e: 6.2 to 7.8) at scan rate of 100 mV/s; **(B)** Graph of the anodic peak potential versus pH

The voltammograms of NE were recorded at 0.2 M PBS of different pH by cyclic voltammetric technique as shown in Figure 7A The graph of anodic peak potential (E_{pa}) verses pH was clearly indicated that the E_{pa} depends linearly on the pH value in the range of 6.2–7.8 with a slope of 55.5 mV/pH (r^2=0.9985) (inset Figure 7B) and its value nearly obeys the Nernst Equation for equal number of electrons and protons transfer reaction. This was consistent with that reported in literature [58]. As shown in Figure 7, the proportion of the electron and proton involved in the reaction was 1:1 [59]. As NE oxidation was a two electron process, the number of protons involved in the electrochemical oxidation was predicted to be two.

3.3. Simultaneous determination of NE, PA and FA

The consecutive determination of NE, PA and FA has been recorded at poly (CR) MCPE by cyclic voltammetric technique. Figure 8 shows the simultaneous determination of NE, PA and FA by BCPE (dashed line) and poly (CR) MCPE (solid line) in a mixture of solution. In BCPE, there is failed to separate the voltammogram in the reverse scan is indistinguishable but oxidation take place only (dashed line), However in the same circumstance at poly (CR) MCPE can separate the overlapped cyclic voltammogram, leads to their NE and PA reduction takes place in the reverse scans (solid line). The anodic peak potentials were located at 0.178 V, 0.390 V and 0.713 V corresponding to the oxidation of NE, PA and FA respectively, with a potential difference NE-PA was 0.212 V and PA-FA was 0.323 V. In addition the peak current of oxidation of these three substances appreciably increases at the poly (CR) MCPE.
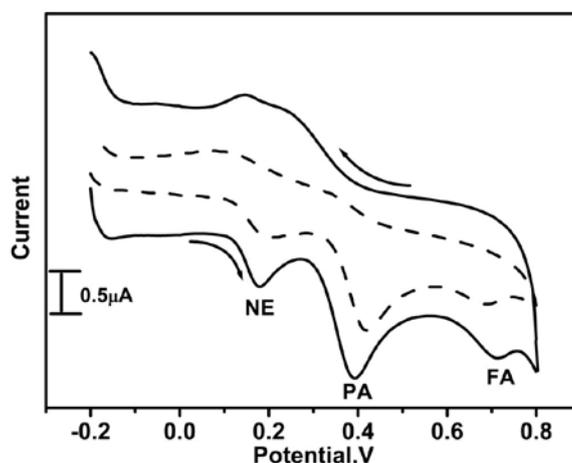
Fig. 8. Cyclic voltammograms for simultaneous determination of 0.3 mM NE, 0.1 mM PA and 0.1 mM FA in 0.2 M PBS (pH 7.0) at BCPE (dashed line) and poly (CR) MCPE (solid line) at scan rate of 100 mV/s

3.3. Interference investigation

The interfering analysis was performed in the mixture of samples containing NE, PA and FA. When the concentration of one analyte changed, whereas other two analyte remained same at poly (CR) MCPE. Figure 9 shows the differential pulse voltammograms obtained by mounting the concentrations of NE from 0.1 mM to 0.6 mM the other two analyte 0.2 mM PA and 0.2 mM FA unvarying. Similarly on mounting the concentrations of PA (0.1 to 0.6 mM) in the presence of 0.2 mM NE and 0.2 mM FA exhibited DPV responses as shown in Figure 10 and Figure 11 shows the differential pulse voltammograms of different concentration of FA (0.4 to 0.8 mM) in the presence of 0.2 mM PA and 0.2 mM NE and there is no shifting and enhance in the oxidation peak of PA and NE. These experimentally obtained results have shown that NE, PA and FA exist autonomously in their mixture samples at Poly (CR) MCPE.
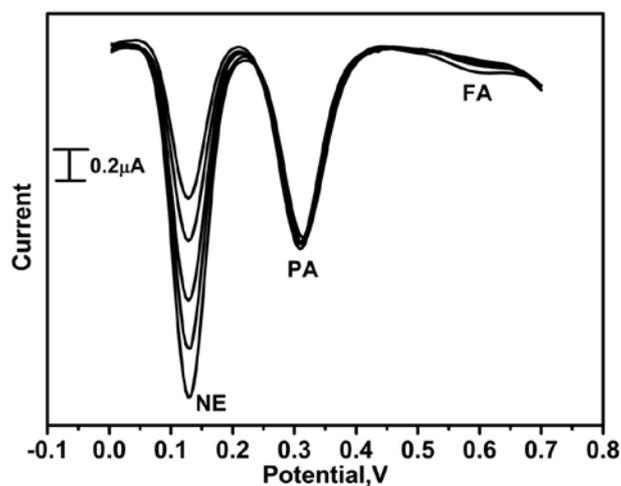
Fig. 9. Differential pulse voltammogram recordings for varying concentration of NE (a-e: 0.1-0.5 mM) in presence of constant 0.2 mM PA and 0.2mMFA at poly (CR) MCPE
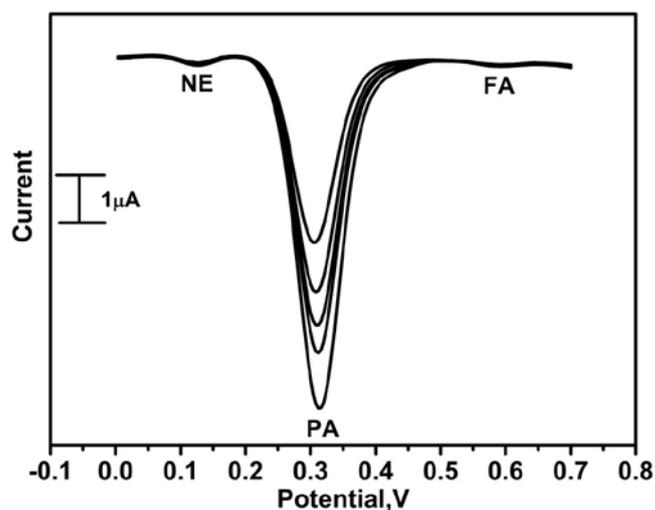
Fig. 10. Differential pulse voltammogram recordings for varying concentration of PA (a-e: 0.1-0.5 mM) in presence of constant 0.2 mM NE and 0.2 Mm FA at poly (CR) MCPE
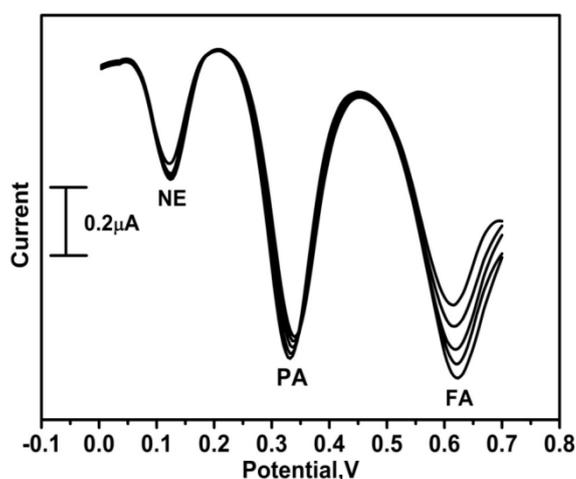
Fig. 11. Differential pulse voltammogram recordings for varying concentration of PA (a-e: 0.4-0.8 mM) in presence of constant 0.2 mM NE and 0.2 Mm PA at poly (CR) MCPE

3.4. Stability of the modified electrode

The consistency of poly (CR) MCPE was studied for the 0.3 mM NE at the sweep rate of 100 mV/s by cyclic voltammetric technique. The reproducibility of the MCPE was shown in the Figure 12 with the ten successive voltammograms were taken to check the consistency at poly (CR) MCPE. Even after ten cycles there is slight decrease in the peak current and no change in peak potential. The overall study reveals that excellent catalytic activity and reproducibility of poly (CR) MCPE towards the electroactive species.
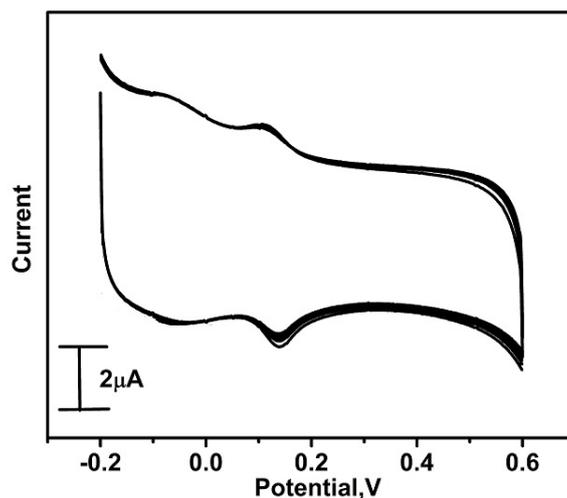
Fig. 12. Cyclic voltammograms for 10 multiple cycles of 0.3 mM NE in 0.2 M phosphate buffer solution pH 7.4 at Poly (CR) MCPE at the sweep rate of 100 mV/s

3.5. Analytical applications

The poly (CR) film was applied in the analysis of NE containing injection samples. The NE injection sample was purchased from Thrikaa Pharmaceuticals Ltd. with a specified content of NE 2.0 mg/mL and the sample was used after suitable dilution. 0.2 M phosphate buffer was used for diluting the injection samples [60]. The results have been shown in Table 3. The recovery was acceptable, showing that the proposed methods could be efficiently used for the detection of NE in injections with recovery in the range 94–103.7%

Table 3. Determination of Norepinephrine using injection sample (n=3)

NE added(µM)	Found(µM)	Recovery%
10	10.37 ±0.30	103.7
20	20.40 ±0.4	102.1
30	28.20 ±0.8	94

4. CONCLUSION

Carbon-paste electrode was modified by electropolymerising the Congo red monomer by cyclic voltammetric technique and used for the determination of NE. The poly (CR) MCPE strongly enhanced both anodic and cathodic peak currents of NE. The electrochemical process was found to be adsorption and followed by diffusion controlled process and detection limit is 0.29 μM for NE. The pH study reveals that same number of protons and electrons are involved in the catalytic oxidation of NE. The modified electrode showed highly selective and electrocatalytic activity towards the oxidation of NE in the mixture of solutions containing higher concentrations of PA and FA at physiological pH of 7.4 by using both CV and DPV techniques. This method can also be used for some other bio molecules.

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