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Niacin Film Coated Carbon Paste Electrode Sensor for the Determination of Epinephrine in Presence of Uric Acid: A Cyclic Voltammetric Study

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Abstract: Voltammetric resolution of neurotransmitter epinephrine (EP) in presence if uric acid (UA) was achieved at niacin film coated carbon paste electrode (niacin/CPE) in 0.2 M PBS of pH 7.4 by cyclic voltammetry (CV) technique. The fabricated electrode remarkably enhanced an electrocatalytic activity towards the oxidation of epinephrine, resulting in the irreversible general 2-electrontransfer adsorption controlled phenomenon. The effect of several experimental parameters were discussed. Such as, scan rate, pH and concentration. Under optimal conditions, the anodic peak current (Ipa) was proportional to epinephrine concentration over the range 20.66-174.4 μM, (r^2=0.9976) with a detection limit of 11.3 nM by CV technique. The sensitive, selective and reproducible result obtained at the niacin/CPE. Finally, the developed method was successfully applied for determination of epinephrine in pharmaceutical samples.

Key words: Epinephrine, uric acid, cyclic voltammetry, niacin film coated carbon paste electrode.

Introduction

Now a days, the detection of biomolecules and analysis of pharmaceutical products by electrochemical methods using polymeric conducting films modified electrodes is an important area in the electroanalytical technique. To step up high performance of the adopted method, in order to control the concentration of organism toxics. Therefore it requires simple, sensitive, rapid and accurate methods for the determination organic molecules including drugs [1-13].

Epinephrine (EP) ([(R)-4-(1-Hydroxy-2-(methylamino)ethyl)benzene-1,2-diol] is also known as adrenaline (Table 1), a benzene derivative with two hydroxyl groups and an alkylamine chain, is a type of catecholamine neurotransmitter. EP plays an important role in the mammalian central nervous system. It was used as a medicament in common emergency health-care medicine [14]. EP is an important hormone synthesized

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from L-tyrosine and exuded by the medulla of the adrenal gland in human body along with norepinephrine [15].

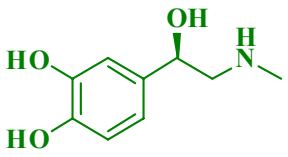
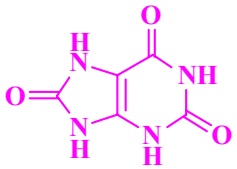
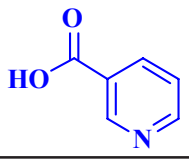
R.N. Goyal *et. al.,* [16] studied EP is used to stimulate heartbeat and to treat emphysema, vasoconstrictor, cardiac stimulator and bronchodilator and other allergic conditions. EP is also plays an important role in health and disease: as well as in the treatment of the eye disease, glaucoma and Parkinson's disease as a result of low level of EP. Therefore, investigation on EP was an important significance to medicine and life science. The normal level of epinephrine in humans is 0.037 ± 0.006 ng/ml and various physiologic conditions can alter the concentration of epinephrine. Hence the resolve of EP strength in human fluids such as urine and plasma plays an important key role in the laboratory diagnosis of some diseases [17]. Therefore, several methods were reported for the individual determination of EP in both pharmaceutical preparation and in biological fluids including various techniques such as liquid chromatography [18], fluorescence [19], flow injection electrogen-erated chemiluminescence [20], capillary electrophoresis [21], fluorimetry [22, 23], various sensors analysis [24-26] and chromatography coupled with several types of detection methods [27, 28].

Uric acid (UA) (2,6,8-trihydroxypurine) is a heterocyclic compound of carbon, nitrogen, oxygen and hydrogen (Table 1), is the primary product of purine nucleosides in the human body and is formed in liver and tissues during the oxidation of aminopurines. However, the antioxidant uric acid molecule acts as both beneficial and injurious effects on human body [29]. P. Kalimuthu *et. al.,* [30] reported normally physiological UA present in a blood is about 240-520 µmol/Land urinary excretion is in general 250-750 mg per 24 hour. Higher levels UA in the serum leads to many clinical disorders on human body like gout, kidney and cardiac problems endothelium, bronchial wall and heart [31].

Niacin (nicotinic acid, vitamin B_3, pyridine 3-carboxylic acid) is a biological organic compound (Table 1). Wu *et. al.,*[32] studied niacin is a water-soluble vitamin that is easily lost when boiled in water and it can't be stored in the human body. Niacin is primarily used to treat hypercholesterolemia and pellagra. It is a precursor to the two forms of nicotinamide adenine dinucleotide (NAD+ and NADH) and nicotinamide adenine dinucleotide phosphate (NADP+ and NADPH), which play essential metabolic roles in living cells. It is important for converting food to energy [33]. It is an essential component of mammalian diet and deficiency leads to anaemia, headaches and tiredness [34].

In recent years the development of biosensor or electrochemical sensor technology is a powerful and conventional electroanalytical technique

Table 1. The structural and molecular formula of epinephrine, uric acid and niacin

Biomolecules	Molecular formula	Structure
Epinephrine (EP)	$C_9H_{13}NO_3$	
Uric acid (UA)	$C_5H_4N_4O_3$	
Niacin (Vitamin B_3)	$C_6NH_5O_2$	

due to its time saving operation and accuracy in the results [35-46]. Graphite powder is a novel conducting material and has attracted unique advantages in the fabrication of electrode material for biosensor application. Due to their unique mechanical and electrochemical properties carbon paste electrode (CPE) were widely used in the fabrication of biosensors. This present work describes the modification of CPE by using niacin as a modifier. The niacin film coated carbon paste electrode (niacin/CPE) was used for the electrochemical determination of epinephrine (EP) in presence of uric acid by cyclic voltammetric technique. The results indicated that electrochemical responses in the determination of EP and uric acid (UA) at niacin/CPE shows good enhancement, high sensitivity, selectivity, low cast when compared to bare carbon paste electrode.

Materials and methods

Instrumentation

The electrochemical experiments were carried out using a model CHI-660c (CH Instrument-660 electrochemical workstation, USA) coupled with a conventional three-electrode cell with saturated calomel electrode (SCE) as a reference, platinum wire as a counter electrode and a self-made bare carbon paste electrode (BCPE)or niacin film coated carbon paste electrode (niacin/CPE) as working electrode. All the oxidation potentials were recorded with respect to SCE and were performed at an ambient temperature of 25±0.1ºC.

Reagents and chemicals

Epinephrine (EP) was purchased from Sigma Aldrich Ltd., India, (M_{wt}=183.20 g/mol, purity >97 %), niacin was obtained from Sigma Aldrich Ltd., India, (M_{wt}=123.10 g/mol, purity >99.5 %) and uric acid (UA) was purchased from Himedia, (M_{wt}= 168.11 g/mol, purity ≥99 %). All the experiments were carried out at room temperature. The EP and UA stock solutions (C_{EP}=25×10^{-4} M and C_{UA} = 25 × 10^{-4} M) were prepared by dissolving in 0.1 M perchloric acid and 0.1 M NaOH respectively. Phosphate buffer solution (PBS) of same ionic strength was prepared (0.2 M) and desired pH was obtained by mixing appropriate ratio of $NaH_2PO_4 \cdot H_2O$andNa_2HPO_4. Graphite powder of average particle size 50 µM purchased from Merck and silicon oil from Himedia was used to prepare carbon paste electrode (CPE). All chemicals were of analytical grade and used as received without any further purification. All the aqueous solutions were prepared with double distilled water.

Preparation of niacin/CPE

The bare carbon paste electrode (BCPE) was prepared as reported in the literature [47]. Electrochemical polymerisation of niacin on the surface of BCPE was carried out by using cyclic voltammetric technique. The BCPE was scanned for ten multiple cycles in an electrochemical cell containing the solution of 1.0 mM niacin monomer in 0.2 M PBS of pH 7.4 between the potential window of -1.0 V to +1.8 V with the scan rate of 0.1 Vs^{-1}. After that the niacin/CPE was rinsed thoroughly with double distilled water and used for the electroanalysis of EP.

Result and discussions

Electrochemical polymerisation of niacin on the surface of CPE

Electrochemical polymerisation of electroactive molecules by voltammetric technique is a more promising method in the fabrication of a stable biosensor [48]. The electrically conducting niacin/CPE was fabricated by cyclic voltammetric technique by multiple sweeping the electrode system between the potential windows of -1.0 V to +1.8 V in a positive direction. The rate of sweeping is fixed to 0.1 Vs^{-1} for ten consecutive cycles in 0.2 M PBS of pH 7.4 until a stable cyclic voltammogram was observed. The Fig. 1 showed after the 10 multiple cycles the increase in the voltammograms tends to be almost constant, reflecting saturation level of the chain growth [49]. The probable electropolymerisation mechanism of niacin and its electrocatalysis interaction with epinephrine is described in Scheme 1. Such types of mechanisms have been proposed in earlier reported literatures [10, 48].

Electrochemical characterization of niacin/CPE

The electroactive surface area of niacin/CPE

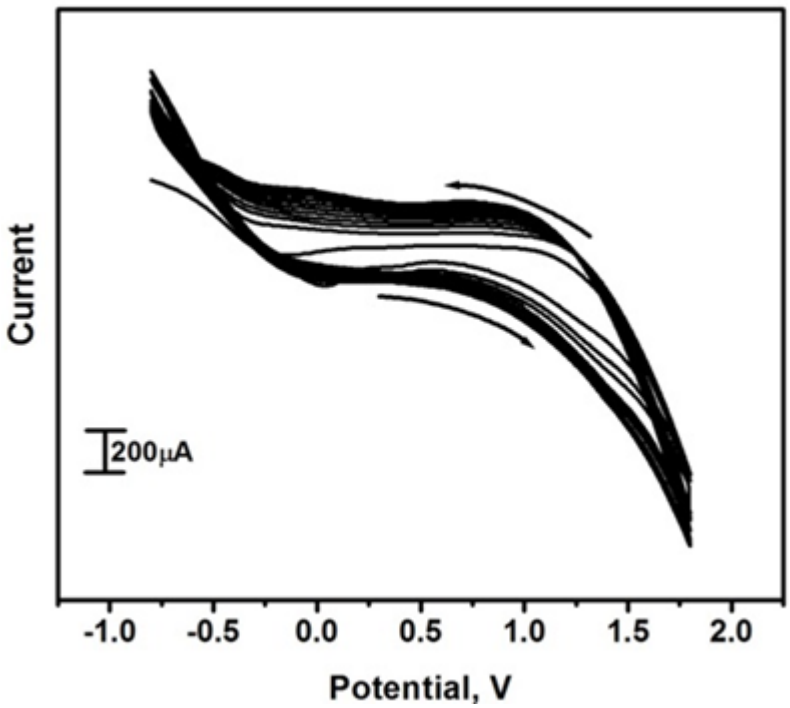
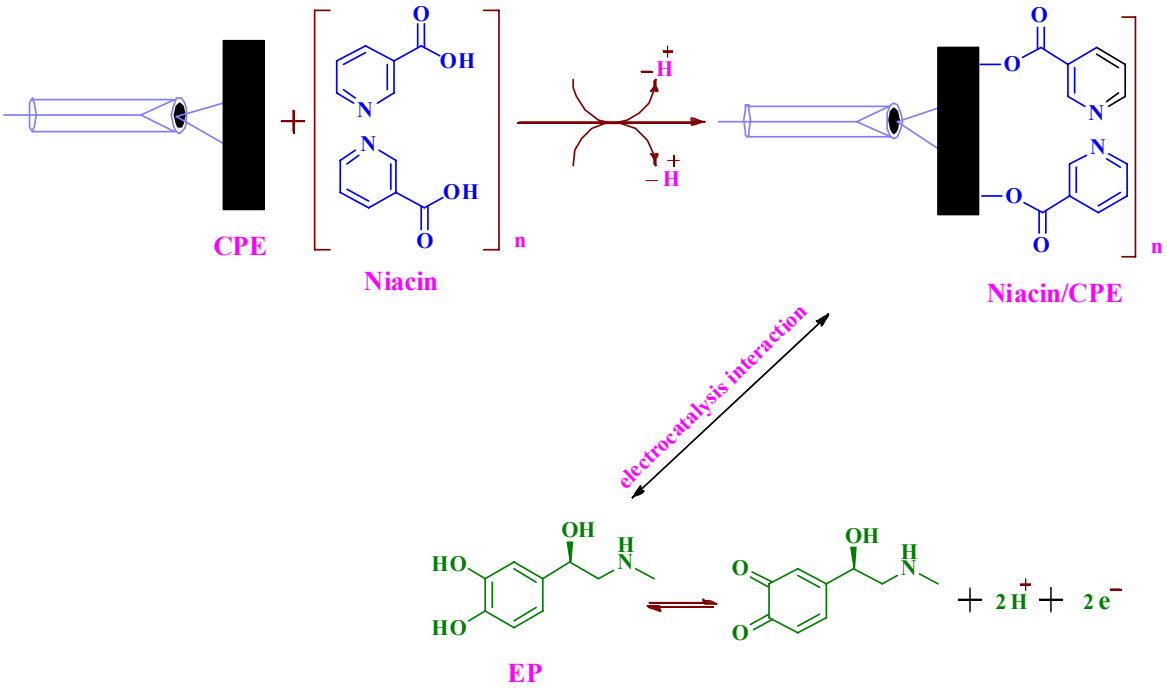


Fig. 1. Cyclic voltammograms of fabrication of niacin/CPE. 1.0 mM niacin solution in 0.2 M PBS of pH 7.4 at 10 cycles with scan rate of 0.1 Vs^{-1}



Scheme 1. Mechanism of electropolymerisation of niacin on the surface of BCPE and electrocatalytic interaction of epinephrine with niacin/CPE

was determined by cyclic voltammetric method. The cyclic voltammograms were recorded for the oxidation of 1.0 mM potassium ferrocyanide in 1 M KCl as a supporting electrolyte with the scan rate 0.05 Vs^{-1}as shown in the Fig. 2. The voltammogram obtained at BCPE (dashed line)

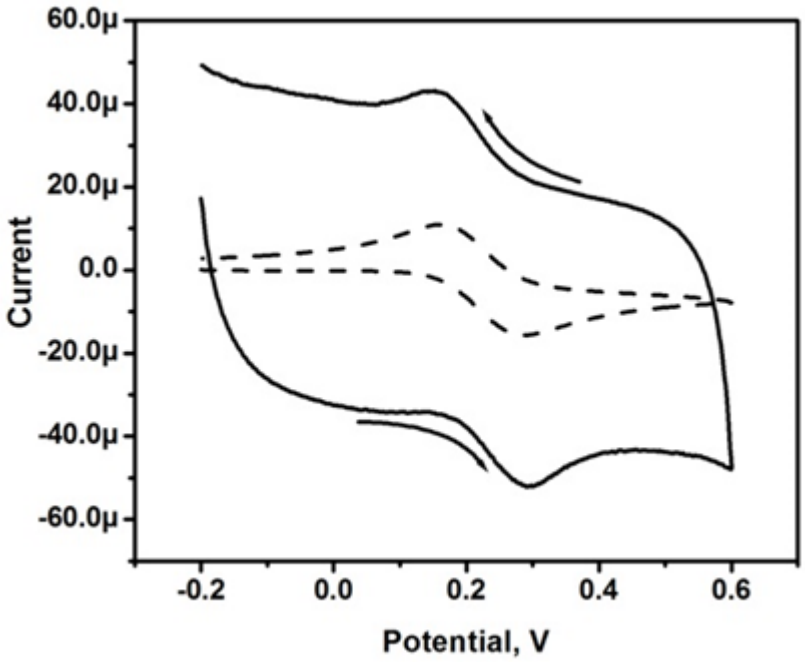


Fig. 2. Cyclic voltammograms of 1.0 mM potassium ferrocyanide at BCPE (dashed line) and niacin/CPE(solid line) at scan rate of 0.05 Vs^{-1}

was with less sensitivity. On the other hand, in the same identical condition niacin/CPE (solid line) shows remarkable refinement in the electron transfer process. The improved result obtained at niacin/CPE confirms there is a change in the surface property of the fabricated electrode. The total active surface area available for reaction of species in solution can be estimated by the Randles-Sevcik equation (1) [46, 10].

$$Ip = 2.69\times10^{5}\ n^{3/2} A\ D^{1/2}\ C_0 \upsilon^{1/2} \qquad (1)$$

where, Ip refers to 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^2s^{-1}), and υ is the scan rate (Vs^{-1}), A is the electroactive surface area (cm^2). For niacin/CPE electroactive surface area is maximum (0.0457 cm^2) as compared with BCPE (0.0287 cm^2).

Electrochemical response of EP at niacin/CPE

Fig. 3 shows the cyclic voltammetric response for the anodic oxidation of 0.1 mM EP in 0.2 M PBS of pH 7.4 at BCPE and niacin/CPE with the scan rate 0.05 Vs^{-1}. The electrochemical behaviour of EP at BCPE (dashed line) was poor in sensitivity; a broad voltammogram was obtained due to the slow electron transport phenomenon. The oxidation peak potential occurred at 0.243 V (versus SCE). However, in the same experimental condition niacin/CPE (solid line) showed significant increment in current signals and the oxidation peak potential was observed at 0.209 V. This confirms the electro capability of niacin/CPE toward the oxidation of EP.

Effect of scan rate on the peak current of EP

The effect of scan rate was studied on niacin/CPE for the oxidation of 0.1 mM EP in 0.2 M PBS of pH 7.4 by CV technique. According to Randles-Sevcik equation the peak current is directly proportional to scan rate. The Fig. 4A shows, the peak current increased with a slight positive shift in the peak potential with increase in the scan rate from 0.02 to 0.120 Vs^{-1} [50]. The plot of logarithm anodic peak current (log Ipa) versus logarithm scan rate (log υ) was plotted and the obtained graph was a straight line with good linearity as shown in Fig. 4B. The linear regression equation can be expressed as log Ipa (A) = 0.706 log υ (Vs^{-1}) - 3.196; (r^2= 0.9967). The slope of 0.706 suggests the electrode process is adsorption controlled. The graph of Ipa versus square

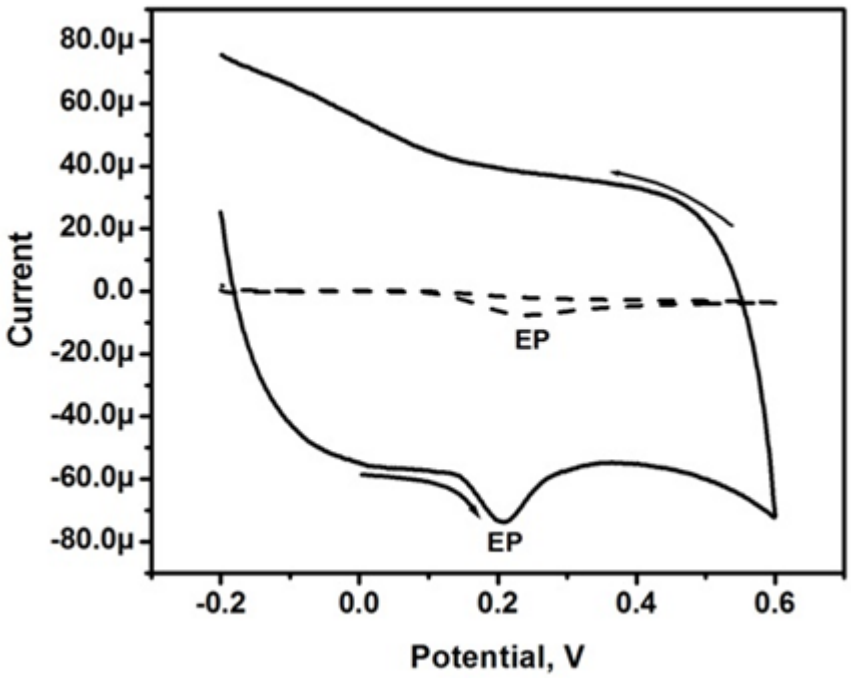


Fig. 3. Cyclic voltammograms for 0.1 mM EP at BCPE (dashed line) and niacin/CPE(solid line) in 0.2 M PBS of pH 7.4 at scan rate 0.05 Vs^{-1}

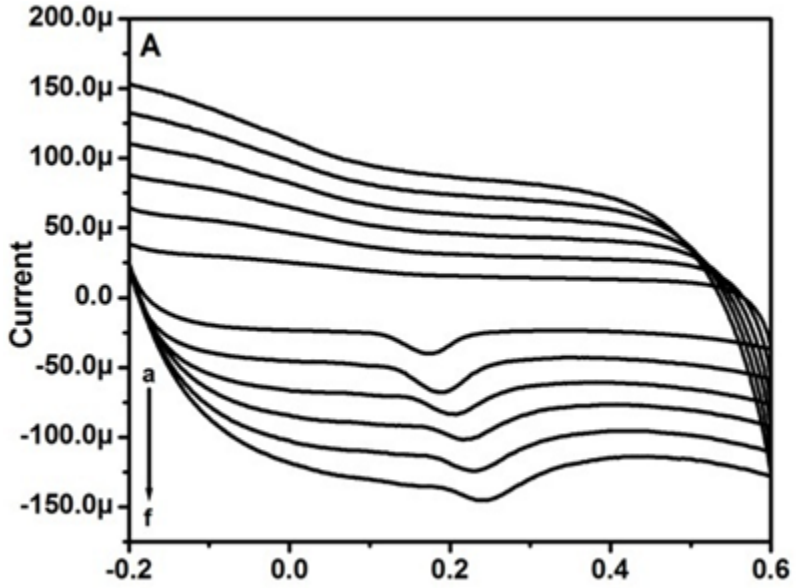


Fig. 4A. Cyclic voltammograms for 0.1 mM EP at niacin/CPE in 0.2 M PBS of pH 7.4 at different scan rate (a-f; 0.020to 0.120 Vs^{-1})

root scan rate ($\upsilon^{1/2}$) was plotted as shown in Fig. 4C, the linear regression equation can be expressed as follows, Ipa(A) = $5.082 \times 10^{-4}\ \upsilon^{1/2}$ (Vs^{-1}) - 3.517×10^{-5}; ($r^2 = 0.9914$). Which confirms the adsorption controlled electrode process at niacin/CPE[16]. For an adsorption-controlled and irreversible electrode process, according to Laviron [51], E_p is defined by the following equation,

$$E_p = E^{\circ} + \frac{2.303RT}{anF}\log\frac{RTk^0}{anF} + \frac{2.303RT}{anF}\log\upsilon \quad (2)$$

Where α is the charge transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred;

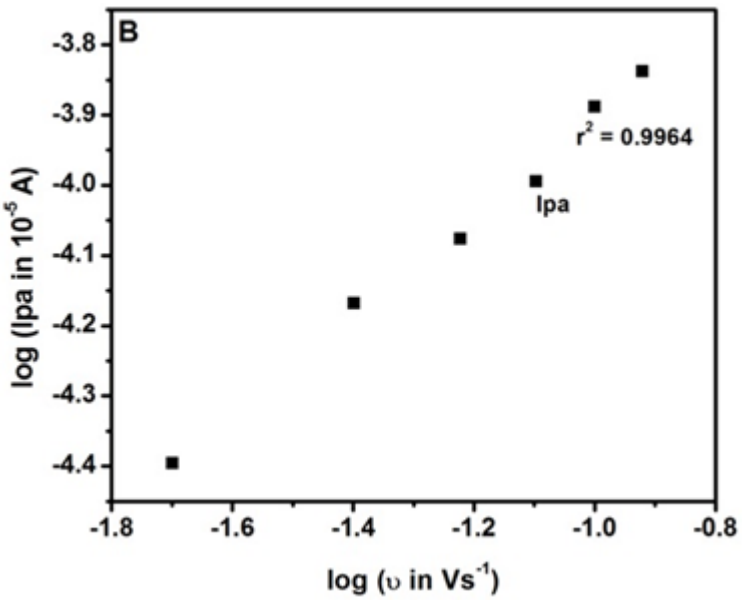


Fig. 4B. Graph of logarithm anodic peak current (log Ipa) versus logarithm scan rate (log u)

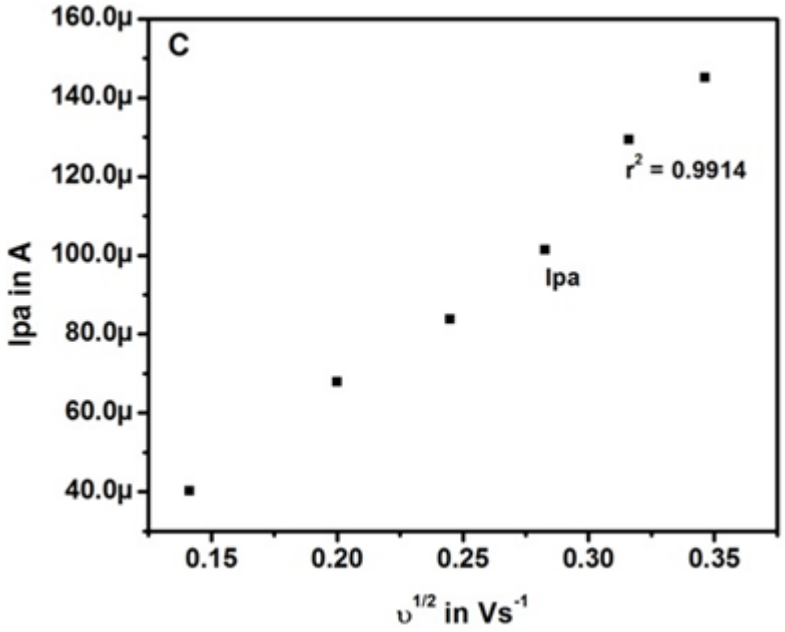


Fig. 4C. Graph of anodic peak current (Ipa) versus square root of scan rate ($\upsilon^{1/2}$)

υ is the scan rate and E° the formal redox potential. Other symbols have their usual meanings. Thus, the value of αn can be easily calculated from the slope of E_p vs. log υ (data not shown). In this system, the slope was 0.1004, taking T = 298 K, and substituting the values of R and F, αn was calculated to be 0.83. Generally α is assumed to be 0.5 in total irreversible electrode process [52]. Further, the number of electron (*n*) transferred in the electro oxidation of EP was calculated to be 1.66 ≃ 2. The value of k^0 can be determined from the intercept of the above plot if the value of E° is known. The value of E° in Eq. (2) can be obtained from the intercept of E_p vs. υ curve by extrapolating to the vertical axis at υ = 0 [53]. In our system the intercept for E_p vs. log υ plot was 0.337 and E° was found to be 0.16; k^0 was calculated to be $1.304\times 10^3\ s^{-1}$.

Effect of pH value on the determination of EP at niacin/CPE

The pH of the supporting electrolyte has significant effect on the oxidation of the analytes. Cyclic voltammetry was used to investigate the influence of PBS pH in the determination of EP at niacin/CPE. The Fig. 5 illustrates the oxidation of 0.2 mM EP at niacin/CPE in the pH range of 5.5 to 8.0 at a scan rate of 0.05 Vs^{-1}. The result shows, by increasing the pH of 0.2M PBS the oxidation peak potential was shifted to more negative side. This clearly indicates the dependence of EP oxidation on the solution pH [54].

Effect of EP concentration

The cyclic voltammograms were recorded for the electrocatalytic oxidation of EP with varying concentration in the linear range of 20.66 to 174.4 µM in 0.2 M PBS of pH 7.4 with the scan rate 0.05 Vs^{-1} at niacin/CPE as show in Fig. 6A. By increasing the concentration of epinephrine Ipa goes on increasing with shifting Epa towards less positive side. The graph of Ipa versus concentration of EP justifies the reason of increase in anodic peak current, which is due to increase in the concentration of EP as shown in Fig. 6B. The graph showed almost straight line with good linearity, with the linear regression equation of Ipa (10^{-5}A)=0.0196 (C_oµM/L) + 3.8489, (r^2=0.9976). The limit of detection (LOD) was calculated using the following equation (3),

$$LOD = 3S/M \quad (3)$$

Where, S is the standard deviation of the six blank current measurements and M is the slope of the calibration curve. The calculated detection limit of epinephrine at niacin/CPE was found to be 11.3 nM. The detection limits reported for different classical methods and electrodes are tabulated in Table 2. This method was better compared to other reported methods [15, 17, 50, 55-69].

Electrocatalytic oxidation of UA at niacin/CPE

The cyclic voltammograms were recorded for the oxidation of 0.1 mM UA at BCPE (dashed line) and niacin/CPE (solid line) in 0.2 M PBS of pH 7.4 with the scan rate 0.05 Vs^{-1} as showed in the Fig. 7. The voltammogram obtained for the irreversible oxidation of UA at BCPE was broad and less sensitive. The anodic peak potential was located at 0.286 V. On the other hand; the niacin/CPE exhibited an enhanced and sharp oxidation potential at 0.293 V. The enhancement in the current response suggests the fabricated niacin/CPE can be used for the determination of UA at physiological pH. The effect of variation in the concentration of UA was studied at niacin/CPE. The

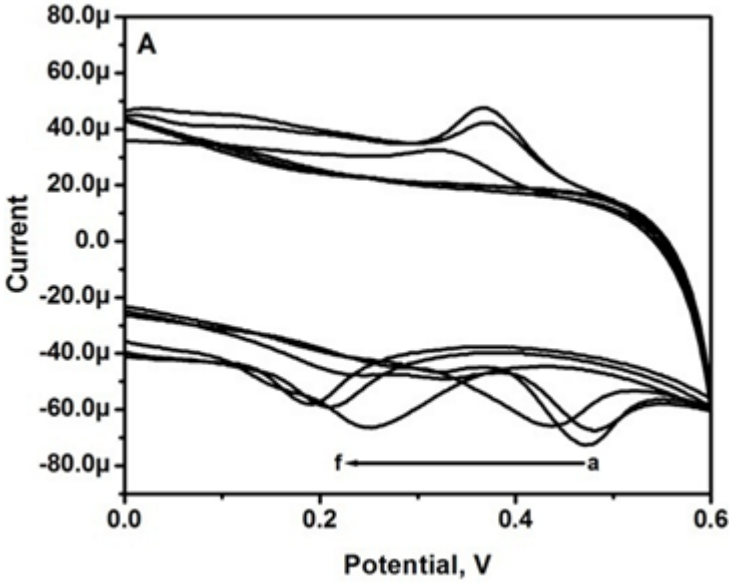


Fig. 5. Cyclic voltammograms obtained for the oxidation of EP at niacin/CPE in 0.2 M PBS solution of different pH values (a-f: 5.5 to 8.0) at scan rate of 0.05 Vs^{-1}

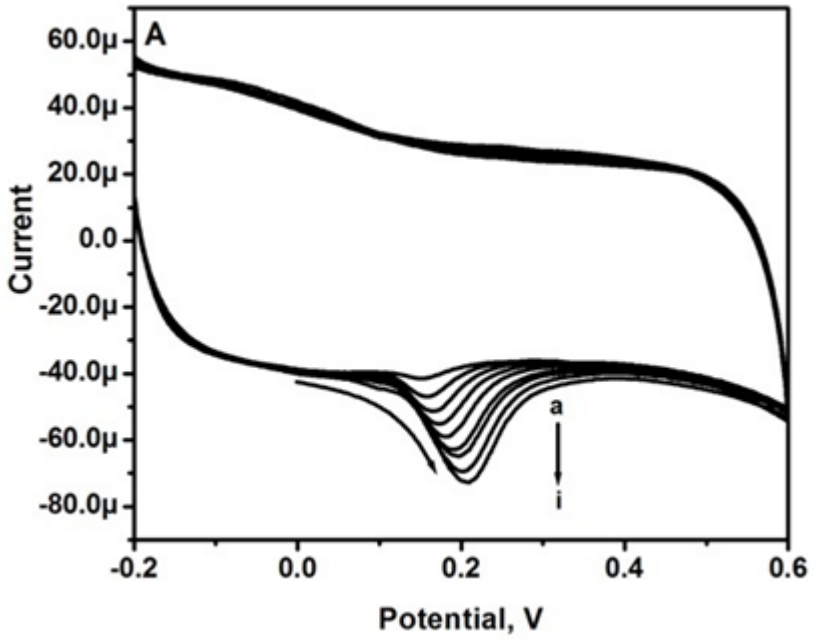


Fig. 6A. Cyclic voltammograms of EP in 0.2 M PBS solution of pH 7.4 at niacin/CPE with different concentrations (a-i: 20.66 to174.4 µM) at scan rate of 0.05 Vs^{-1}

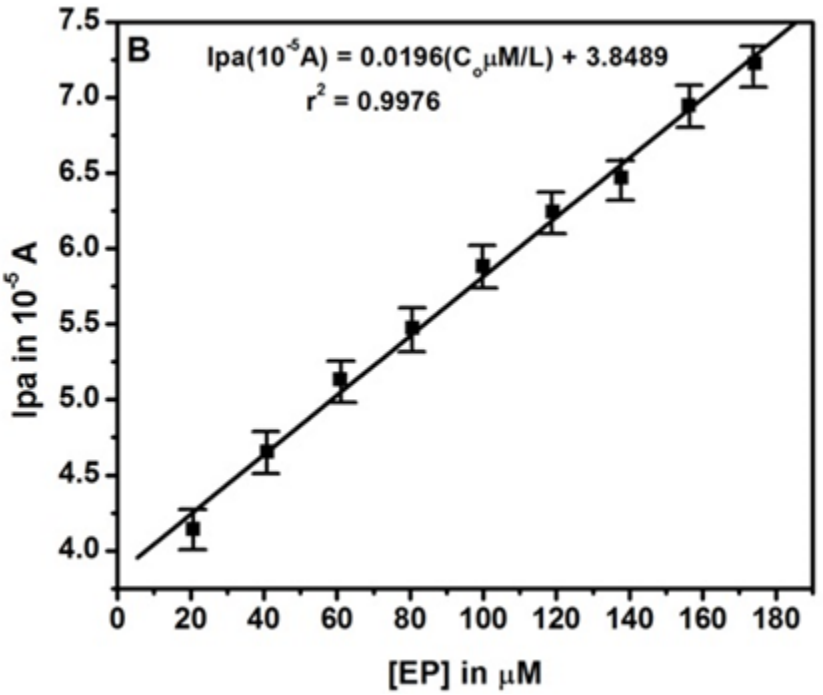


Fig. 6B. Graph of anodic peak current versus concentration of EP

cyclic voltammograms were recorded in the linear concentration range of 40.98 to 137.79 µM as showed in the Fig. 8A. The calibration graph of Ipa versus concentration of UA shows a good linearity (Fig. 8B). The linear regression equation can be expressed as Ipa(10^{-5}A)=0.0110 (C_o µM/L) + 3.4577, (r^2=0.9969).

Simultaneous determination of EP and UA

The cyclic voltammograms were recorded for the simultaneous determination of 0.1 mM EP and 0.1 mM UA in 0.2 M PBS of pH 7.4 at scan rate

Table 2. Comparison of linear range and detection limits for EP with different classical methods and working electrodes

Working Electrode	Classical methods	Linear working range (µM)	Detection limits(M)	References
Pen SAM-MAuE	CV	100-0.1	0.1×10^{-6}	15
poly(caffeic acid)MGCE	CV	2-300	0.6×10^{-6}	17
MWCNT/CFE	DPV	up to 100	0.900×10^{-6}	50
P(1-methylpyrrole)GCE	SWV	0.75-200	0.168×10^{-6}	55
p (taurine)ME	DPV	2-600	0.3×10^{-6}	56
FePc-ME	CV	1-300	0.5×10^{-6}	57
TTABMCPE	DPV	0.15-30	0.12×10^{-6}	58
DH-CN/CPE	DPV	5.0-20	1.0×10^{-6}	59
		20-600		
GCE-MWCNT-CoTSPc	Amp	3.0-15	0.45×10^{-6}	60
MnO_2/Nafion/GCE	CV	0.5-100	0.100×10^{-6}	61
		100-700		
	DPV	0.03-10	0.005×10^{-6}	
		10-100		
Paraffin/MWCNT/CoPc	DPV	1.3-5.5	0.016×10^{-6}	62
CNT/GCE	CV	1.0-50	0.100×10^{-6}	63
CNT/SSE	DPV	2.0-100	2.000×10^{-6}	64
GME/GCE	CV	0.4-13	0.089×10^{-6}	65
		13-109		
GR/Au/GCE	CV	0.05-8.0	0.007×10^{-6}	66
PolyCafA/GCE	CV	2.0-80	0.200×10^{-6}	67
RuOHCF/MWCNT/GCE	DPV	0.1-10	0.052×10^{-6}	68
2PHCMCNPE	SWV	0.05-550	9.4×10^{-9}	69
Niacin/CPE	CV	20.66-192.30	11.3×10^{-9}	Present work

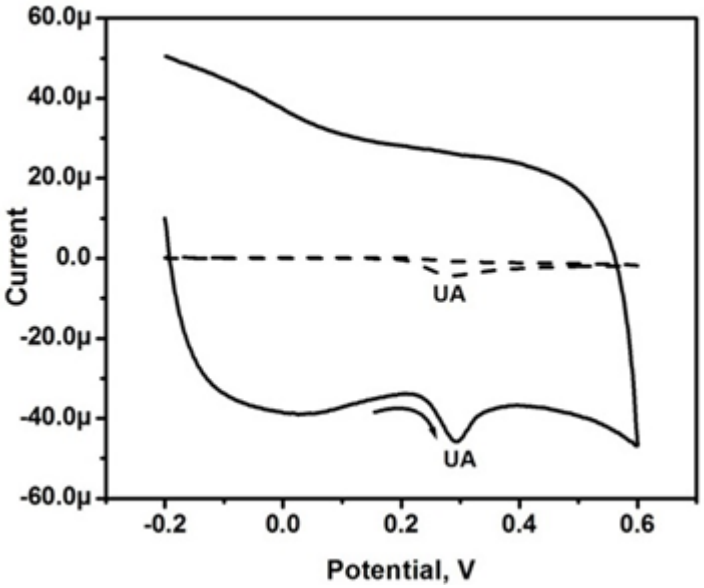


Fig. 7. Cyclic voltammograms of 0.1 mM UA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and niacin/CPE(solid line) at scan rate of 0.05 Vs^{-1}

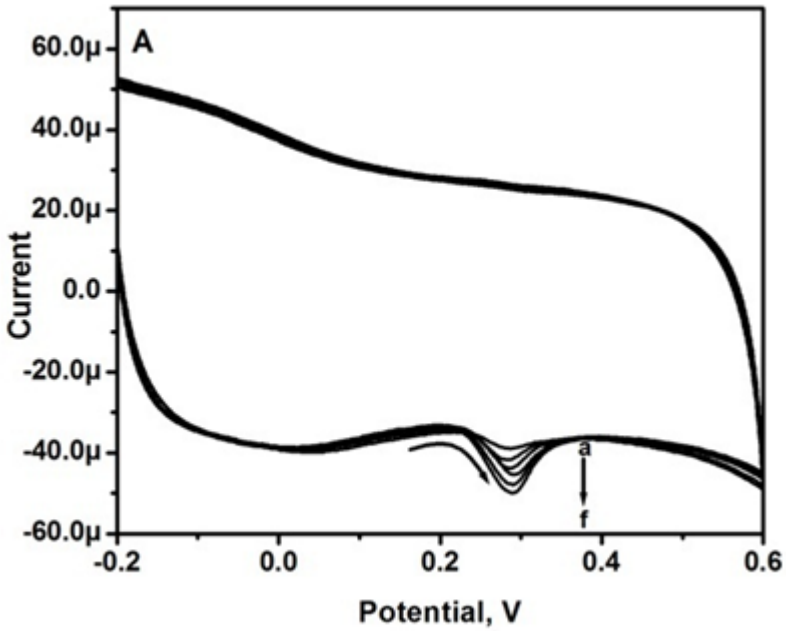


Fig. 8A. Cyclic voltammograms of UA in 0.2 M PBS solution of pH 7.4 at niacin/CPE with different concentrations (a-f: 40.98 to 137.79 µM) at scan rate of 0.05 Vs^{-1}

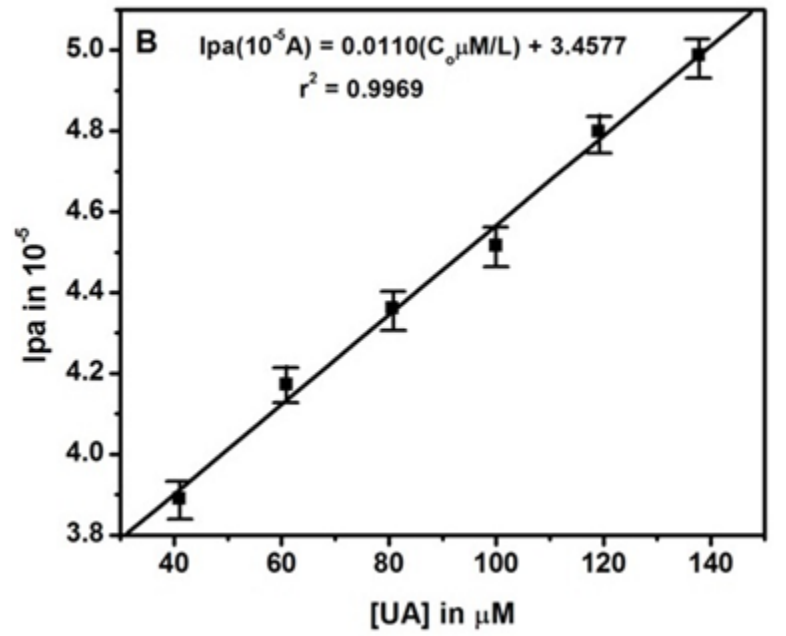


Fig. 8B. Graph of anodic peak current versus concentration of UA

of 0.05 Vs^{-1}. The Fig. 9 reveals the voltammetric response of the equimolar mixture of analytes at BCPE (dashed line) and niacin/CPE. The simultaneous oxidation of these two species at BCPE was poor in sensitivity and partially overlapped signal was observed, which makes its individual identification difficult. However, on the other hand voltammetric signal observed at niacin/CPE was sharp in sensitivity, and the oxidation of EP and UA was appeared at 0.171 V and 0.290 V respectively. This is as same as in the individual determination. The peak to peak separation between the two analytes was 0.119 V by cyclic voltammetry technique. This result was good enough to make out and resolve oxidation peak of EP in the presence of UA at niacin/CPE

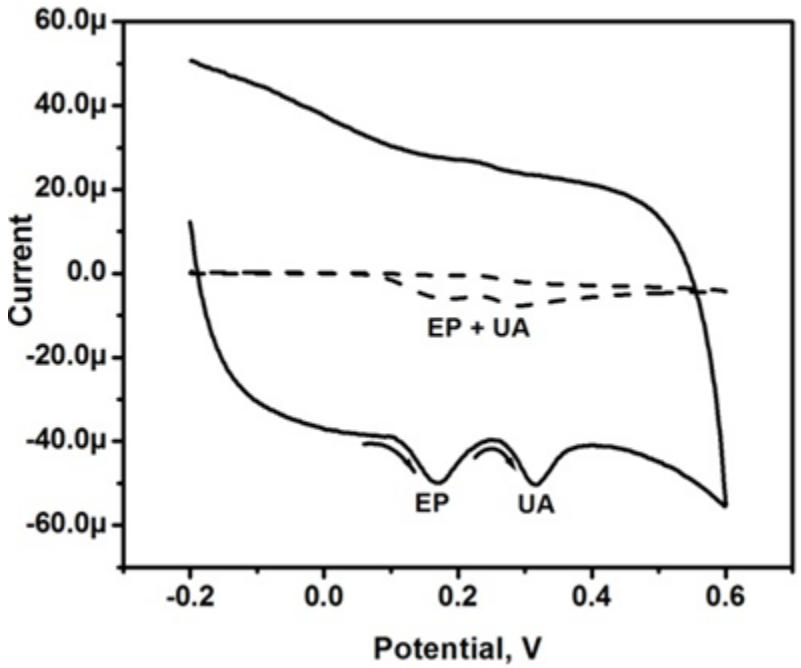


Fig. 9. Cyclic voltammograms for simultaneous determination of 0.1 mM EP and 0.1 mM UA at BCPE (dashed line) and niacin/CPE (solid line) at scan rate of 0.05 Vs^{-1}

Detection of EP in Injections

The niacin/CPE was applied for the determination of EP in injection sample (1 mg/ml, Hindustan India Pharmaceutical Limited). The appropriate amount of the sample was transferred into the electrochemical cell for the determination. The obtained analytical results are summarized in Table 3 the recovery ranged from 97.20 % to 102.80 %. The relative standard deviation (n=5) was less than 1.6 %. The results are generally acceptable and attributed to the good reproducibility of niacin/CPE, showing that the proposed method could be effectively used for the determination of EP in the pharmaceutical and clinical laboratory as a biosensor for diagnosing the disease caused by the deficiency of EP.

Conclusion

In this work, the niacin modified carbon paste electrode was successfully used for electrochemical oxidation of epinephrine in presence of uric acid at physiological pH by cyclic voltammetry techniques. The physic-chemical parameters like scan rate, pH and concentration of analytes were studied. The modified electrode also showed a lower limit of detection of 11.3 nM respectively. This method has been successfully used to determine epinephrine in the pharmaceutical injection sample. Overall, this method showed sensitive, selective, reproducible and practically reliable sen-

Table 3. Determination of epinephrine in injection sample by niacin/CPE

Sample	Added (µM)	Found (µM)	RSD (%)	Recovery (%)
1	5	4.86	1.41	97.20
2	10	10.28	1.48	102.80
3	15	14.78	1.58	98.54
4	20	19.98	1.38	99.90
5	25	24.68	1.32	98.72
6	30	29.80	1.38	99.34

sor for the determination epinephrine.

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Conflict of interest

The authors declare there is no conflict of interest with this research work.

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