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Simultaneous Electroanalysis of Norepinephrine, Ascorbic Acid and Uric Acid at Poly(niacinamide) Modified Carbon Paste Electrode

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Abstract- Niacinamide was electropolymerised on the surface of carbon paste electrode by cyclic voltammetric technique. The modified electrode showed an excellent electrocatalytic activity towards the oxidation of norepinephrine (NE) and ascorbic acid (AA). The response of the sensor was evaluated towards the different NE concentration in the range 40.98 to 244.3 μM, the oxidation peak current was linearly increased with varying NE concentration and the detection limit was calculated to be 0.21 μM. The modified electrode showed excellent selectivity in the determination of NE in presence of large excess of AA and UA. The voltammetric responses were stable and resistant to fouling. The proposed electrode can be used for the real sample analysis in medical, pharmaceutical and biotechnological sectors.

Keywords- Norepinephrine, Ascorbic acid, Uric acid, Electroanalysis, Voltammetry

1. INTRODUCTION

In recent years, so many efforts have been exerted in the development of voltammetric sensors for the electroanalysis of norepinephrine (NE) in the presence of common interferences like ascorbic acid (AA) and uric acid (UA) [1-3]. NE is one of the derivative of catecholamine plays a multiple significant role including as a hormone and neurotransmitter [4]. NE is commonly used as a drug of choice as a vasoconstrictor, cardiac stimulator and bronchodilator. It exists in protonated form at physiological pH. It is synthesized in the human body from L-tyrosine and secreted by the medulla of the adrenal gland along with epinephrine. It affects muscle and tissue control, stimulates arteriole contraction, decreases peripheral circulation and activates lipolysis in adipose tissue [5-6]. It is also critical in the neurological disease, heart failure; DNA breaks in cardiac myoblast cells and diabetes. Recent reports have indicated that NE enhances adhesion of human immune deficiency virus-1 (HIV-1)-infected leukocytes to cardiac micro vascular endothelial cells and also accelerates HIV replication via protein kinase [7-8]. Hence, it is very necessary to develop a sensitive, selective and practically reliable method for the determination of NE concentration to monitor the physiological activities and also diagnosing diseases [9-10].

Uric acid (UA) is the primary product of purine metabolism in the human body and a major nitrogenous compound in the urine [11]. Its abnormality in human body leads to many severe diseases, such as gout, hyperuricaemia and Lesch–Nyan disease [12-13]. Increased urate level also leads to the phenumonia and leukaemia [14]. Ascorbic acid (AA) is commonly known as vitamin-C which was readily soluble in water that take part in maintaining many important life processes. It has been used as a medicine for the treatment of common cold, mental illness and cancer [15]. AA can be chemically or electrochemically oxidized to dehydroascorbic acid [16]. Hence, monitoring the concentration of these biological compounds is very important in clinical diagnosis. There are so many endless reports for the voltammetric determination of these molecules [17-22].

NE, AA and UA usually coexist together in the biological fluids [23]. The concentration of NE is hundred times lower the concentration of AA and UA. The direct electrochemical oxidation of these biomolecules was difficult due to the same oxidation potential [24]. The voltammetric response of these biomolecules at bare working electrodes is indistinguishable and gives overlapped voltammetric response. Moreover, an oxidation requires high over potential due to fouling of the electrode surface by the adsorption of oxidized products [25]. Therefore, it is necessary to develop the modification methodologies to fabricate a stable working electrode.

Niacinamide is a derivative of pyridine and a main constituent of water soluble vitamin B complex. It can be converted into the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) [26]. In the present work, the *in-situ* electropolymerisation of niacinamide on the surface of carbon paste electrode (CPE) was

achieved by cyclic voltammetric (CV) technique followed by its characterisation. The fabricated electrode not only showed an electrocatalytic activity towards the oxidation of NE, AA and UA, it also resolved an overlapped response into three well distinguishable peaks by CV and differential pulse voltammetric (DPV) techniques.

2. EXPERIMENTAL SECTIONS

2.1. Reagents and instrumentation

Norepinephrine (L-Noradrenaline hydrochloride) (NE), UA and AA were purchased from Himedia. Niacinamide was obtained from sigma Ltd., India. The stock solution 25×10^{-4} M NE, 25×10^{-4} M UA, 25×10^{-3} M AA was prepared in 0.1 M perchloric acid, 0.1 M NaOH, and double distilled water respectively. Graphite powder of 50 µm particle size was purchased from Merck and silicone oil from Himedia was used to prepare the carbon paste electrode (CPE). All other reagents and solvents used were of analytical grade. All electrochemical experiments were performed using a model CHI-660c (CH Instrument-660 electrochemical workstation). A conventional three electrode system was used in a single compartment electrochemical cell with a saturated calomel electrode (SCE) as a reference, a platinum counter electrode, and bare carbon paste electrode (BCPE) or poly (niacinamide) modified carbon paste electrode (MCPE) as a working electrode. All potentials are reported with respect to SCE at an ambient temperature of 25±0.5 °C.

2.2. Fabrication of the working electrode

The bare carbon paste electrode (BCPE) was prepared according to the reported literature [21]. Electrochemical polymerization of niacinamide on the surface of carbon paste electrode was carried out by cyclic voltammetric technique in an aqueous solution containing 1 mM of niacinamide monomer in 0.2 M PBS of pH 9.0. The *in-situ* electropolymerisation was achieved by the formation of a thin film that grew between -0.8 V to +1.6 V at the scan rate of 0.1 Vs^{-1} for 15 cycles. After that the electrode was rinsed thoroughly with double distilled water.

3. RESULT AND DISCUSSION

3.1. The *In-situ* electropolymerisation of niacinamide on BCPE

The *in-situ* electropolymerisation of niacinamide on BCPE was achieved by placing 1.0 mM of niacinamide monomer in 0.2 M PBS of pH 9.0 in an electrochemical cell over the potential range of -0.8 V to +1.6 V with scan rate 0.1 Vs^{-1}. It can be observed from the Fig. 1A the anodic peak current enhanced gradually in the cyclic voltammogram, which indicates the formation and growth of an electroactive layer on the surface of BCPE. After some

successive sweeps the increase of this peak current tended to be almost virtually constant and becomes steadier; suggesting that the growth of polymerization was reached the level of saturation [27].

The thickness of the electroactive layer has a significant contribution on the electrocatalytic property of the modified electrode, and it can be controlled by the electrochemical input parameters. The extended level of thickness was also calibrated. The coating was controlled by varying the number of multiple cycles on the CPE (from 5 to 25 multiple cycles) and corresponding electrocatalytic activity towards oxidation of 0.2 mM of NE in 0.2 M PBS of pH 7.4 was investigated. The Fig. 1B showed that at 15 multiple cycles the anodic peak current (Ipa) was maximum. Therefore fifteen cycles was chosen for the *in-situ* electropolymerisation of niacinamide. With the increase in the polymerisation cycles, the electrocatalytic response of NE increased at first. But when the polymerizing cycles is more than fifteen, the peak currents begin to decrease. This was due to an increase in thickness of the film would prevent the electron transfer process [21,27]. Therefore, 15 cycles was used for all the electrochemical analysis. The probable electropolymerisation mechanism of niacinamide on the surface of CPE was depicted in scheme 1.

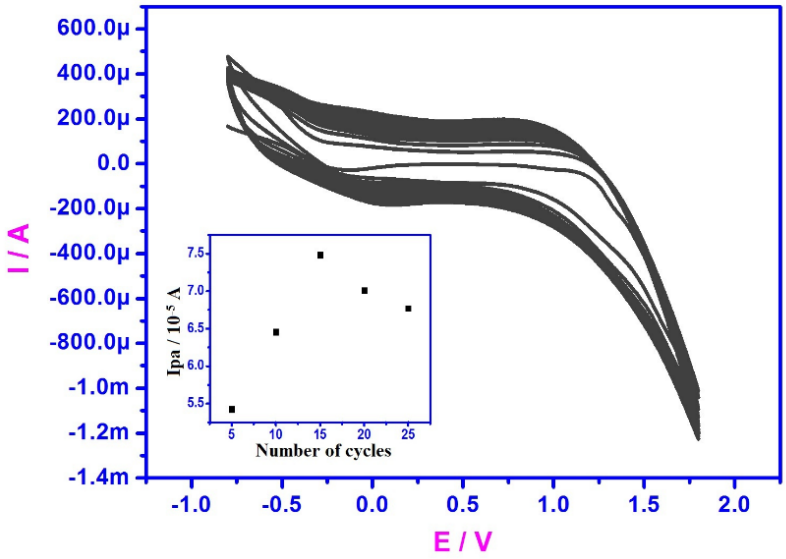
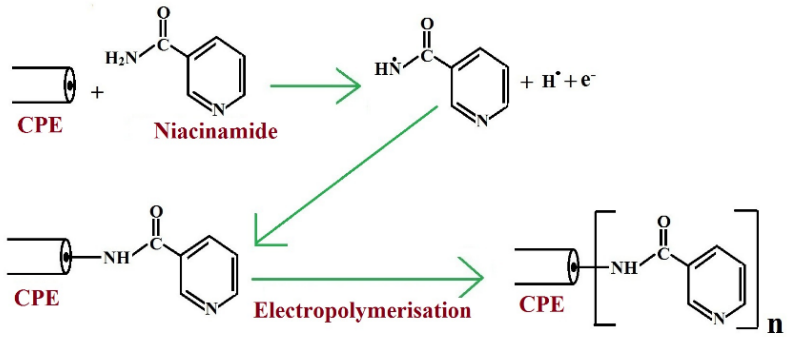


Fig. 1. (A) Cyclic voltammograms of preparation of poly (niacinamide) MCPE. 1.0 mM aqueous solution in 0.2 M PBS of pH 9.0 at 15 cycles with scan rate 0.1Vs^{-1}; **(B)** Graph of anodic peak current versus number of polymerisation cycles



Scheme 1. Electropolymerisation of niacinamide on the surface of carbon paste electrode

3.2. Characterization of poly (niacinamide) MCPE

The freshly prepared stock solutions of 1 mM potassium ferrocyanide with 1 M KCl were placed in an electrochemical cell. The Fig. 2a showed the cyclic voltammograms recorded for the oxidation of 1 mM potassium ferrocyanide at both BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at the scan rate 0.025 Vs^{-1}. The low redox peak currents response was obtained at BCPE but in the same identical condition poly (niacinamide) MCPE exhibited stable enhancement of redox peak currents and also it showed the fast rate of electron transfer kinetics. This refinement in the voltammetric response of potassium ferrocyanide at poly (niacinamide) MCPE suggest that, the surface property of the modified electrode has been significantly changed and also the results demonstrates that the electrocatalytic activity of the poly (niacinamide) MCPE. The total active surface area available for reaction of species in solution can be estimated by the Randles-Sevick's equation (1) [28].

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

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 in cm^2s^{-1}, and υ is the scan rate (Vs^{-1}), A is the electroactive surface area (cm^2). For poly (niacinamide) MCPE the electroactive surface area is maximum (0.0435 cm^2) as compared with BCPE (0.0285 cm^2).

The scanning electron microscopy (SEM) was used to study the surface morphology of BCPE and poly (niacinamide) MCPE. The Fig. 2b shows surface of BCPE is of irregular shape (A). After the electropolymerisation of niacinamide (B), the surface of CPE was covered with the thin film of niacinamide, which contains numerous uniformly aligned ridges and valleys on the surface, which can increase the accessible surface area of poly (niacinamide) MCPE. This morphological feature is having more advantage because of the

availability of large surface area, which will permit an active platform for the electroanalysis of the targeted neurotransmitter molecule [21,22].

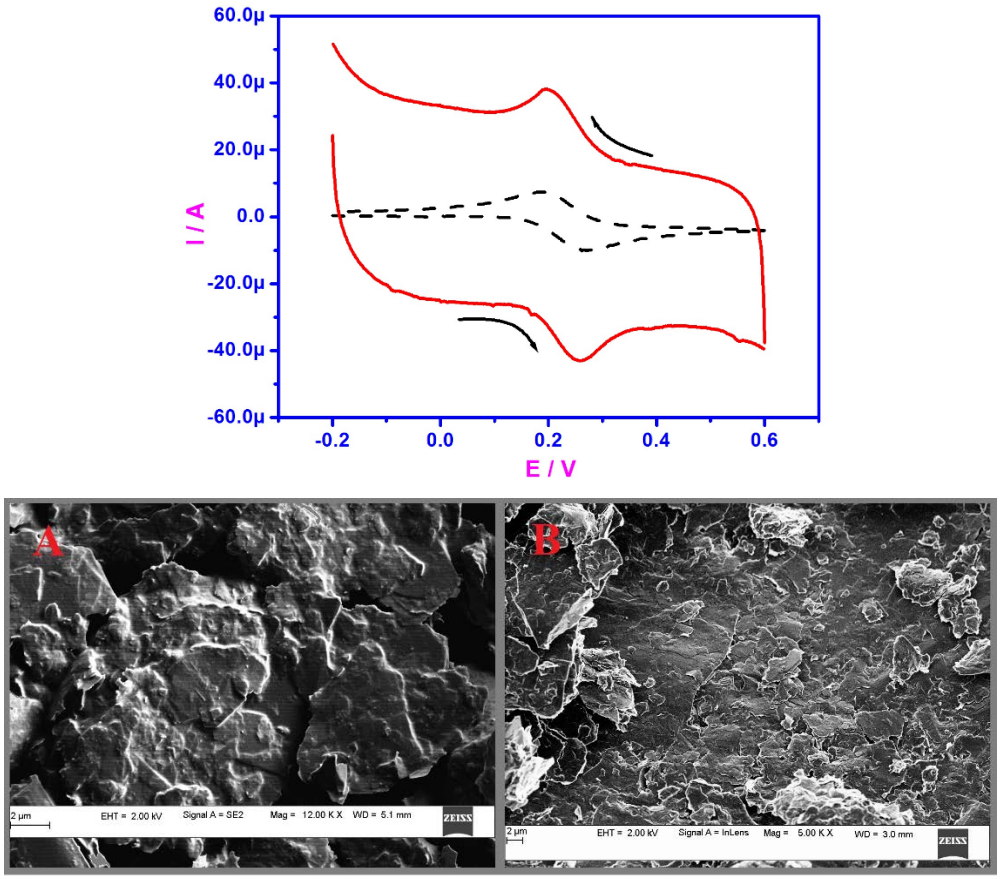


Fig. 2. (a) Cyclic voltammograms of 1.0 mM potassium ferrocyanide at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at scan rate of 0.025 Vs^{-1}; **(b)** SEM images of BCPE **(A)** and poly (niacinamide) MCPE **(B)**

3.3. Electrocatalytic oxidation of NE at poly (niacinamide) MCPE

The cyclic voltammograms were recorded for the oxidation of 0.1 mM of NE at BCPE and poly (niacinamide) MCPE in 0.2 M PBS of pH 7.4 with the scan rate of 0.05 Vs^{-1} as showed in the Fig. 3. The NE shows oxidation and reduction current with poor response. Oxidation potential was observed at 0.232 V (*versus* SCE) in a low current signal. However, in the same identical condition poly (niacinamide) MCPE (solid line) showed significant empowerment in the current signals and oxidation potential was located at 0.197 V. The minimization of over potential in the oxidation process and enhancement of redox peak current indicates the electrocatalytic activity of poly (niacinamide) MCPE towards the detection of NE.

The Fig. 4A showed by increasing the concentration of NE from 40.98 to 244.3 μM, the I_{pa} and I_{pc} goes on increasing with shifting E_{pa} towards less positive and E_{pc} towards least negative side. A linear relationship was observed between the I_{pa} and different concentration of NE as showed in Fig. 4B with the linear regression equation of $I_{pa}(10^{-5}A)=0.03039(C_0$ μM/L)+6.4401, (r^2=0.9994).

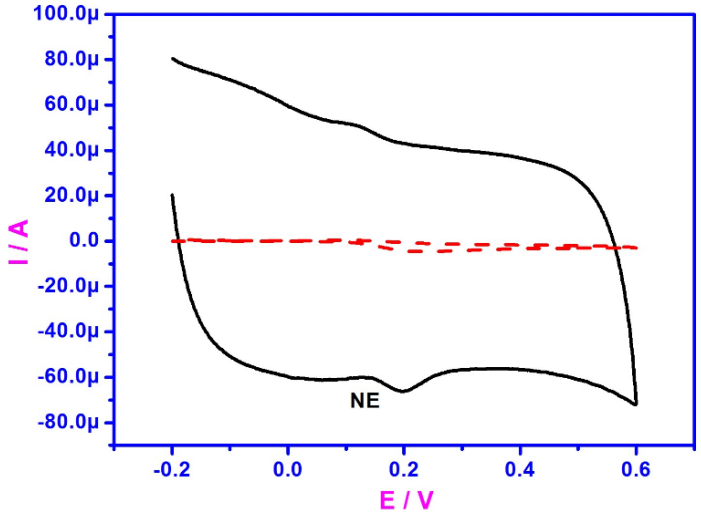


Fig. 3. Cyclic voltammograms of 0.1 mM NE in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

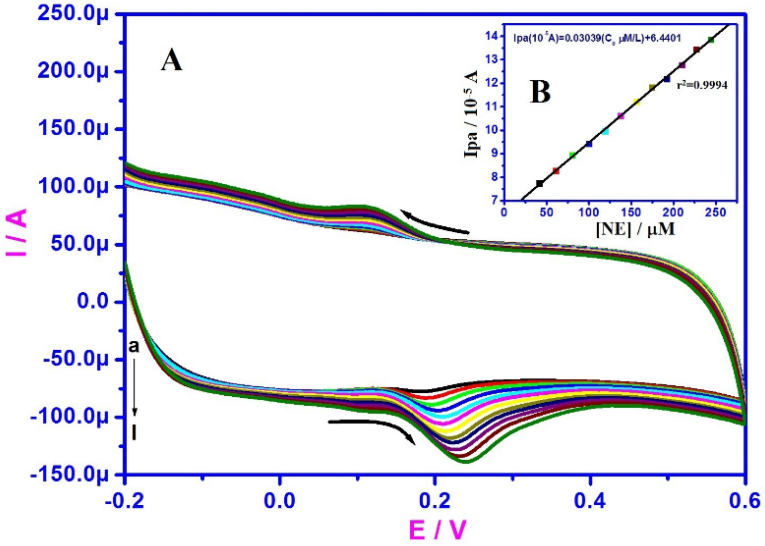


Fig. 4. (A) Cyclic voltammograms of NE in 0.2 M PBS solution of pH 7.4 at poly (niacinamide) MCPE at scan rate of 0.05Vs^{-1} with different concentration (a–l; 40.98 μM, 60.97 μM, 80.64 μM, 100.00 μM, 119.04 μM, 137.79 μM, 156.25 μM, 174.41 μM, 192.30 μM, 209.92 μM 227.2 μM and 244.3 μM); **(B)** Graph of anodic peak current versus concentration of NE

The limit of detection was calculated according to the reported method [29] and the detection limit in the lower concentration range for NE was calculated to be 0.21 μM for the poly (niacinamide) MCPE and limit of quantification was 0.70 μM. The proposed electrode exhibited a relatively lower detection limit than those reported previously as shown in the Table 1 [22,30-33].

Table 1. Comparision of detection limits of different modified electrodes

Working Electrode	**Detection limit (μM)**	**pH of supporting electrolyte used**	**Technique**	**Ref.**
(TLA/Au)	**2.0**	5.91	CV	[30]
TX-100/CPE	**5.0**	7.0	CV	[22]
ME/Au SAMs	**0.7**	5.5	SWV	[31]
Ppy/b-CD-ME	**0.8**	7.4	CV	[32]
BHa and TiO2 nanoparticles	**0.5**	8.0	DPV	[33]
poly (glutamic acid) MCPE	**0.43**	7.4	CV	[21]
poly(niacinamide)MCPE	**0.21**	7.4	CV	This work

3.4. The effect of scan rate on peak current of NE

The effect scan rate on the peak current response of 0.1 mM of NE in 0.2 M PBS of pH 7.4 was evaluated by CV technique at poly (niacinamide) MCPE as showed in Fig. 5A. The experimental results obtained at poly (niacinamide) MCPE showed increase in the redox peak currents with increase in the applied scan rate and they are directly proportional to each other according to Randles-Sevick's equation. The observation while recording the voltammogram showed that there is a shifting of anodic peak potential (E_{pa}) to positive side, and cathodic peak potential (E_{pc}) to the least negative side. In order to get the information on the electrode process, the graph of I_{pa} versus scan rate (υ) was plotted and the obtained graph was a straight line with good linearity in the range from 0.01-0.12 Vs^{-1} as showed in Fig. 5B the correlation coefficient (r^2) was 0.9994. The I_{pa} versus square root scan rate ($\upsilon^{1/2}$) was plotted as showed in Fig. 5C with the correlation coefficient of (r^2) 0.9874, this suggests that the electrode process was adsorption controlled. In support to this logarithm of anodic peak current (log I_{pa}) *vs*. logarithm of scan rate (log υ) (Fig. 6) was plotted and the determined slope was 0.8565 which confirms the electrode process was adsorption controlled process [34]. This was again supported by previously reported literatures [35].

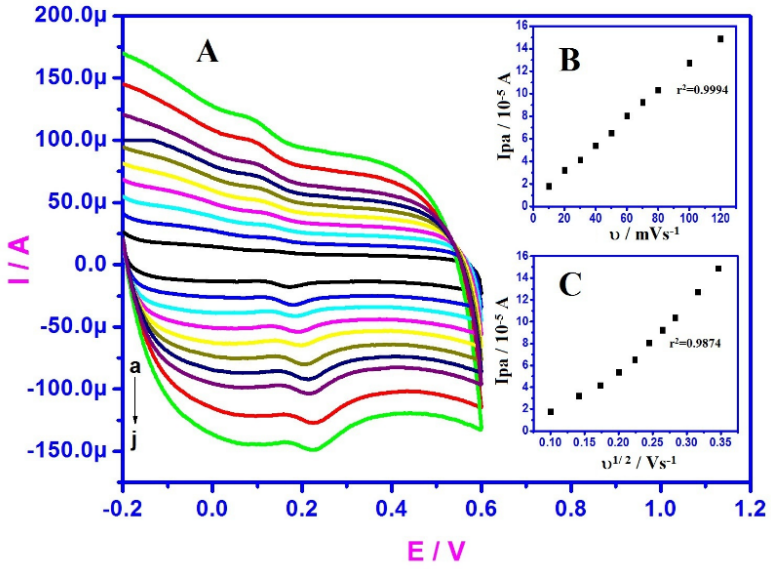


Fig. 5. (A) Cyclic voltammograms of 0.1 mM NE in 0.2 M PBS solution of pH 7.4 at poly (niacinamide) MCPE at different scan rate (a–j; 0.01 Vs^{-1} to 0.12 Vs^{-1}); **(B)** Graph of anodic peak current versus scan rate; **(C)** Graph of anodic peak current versus square root of scan rate

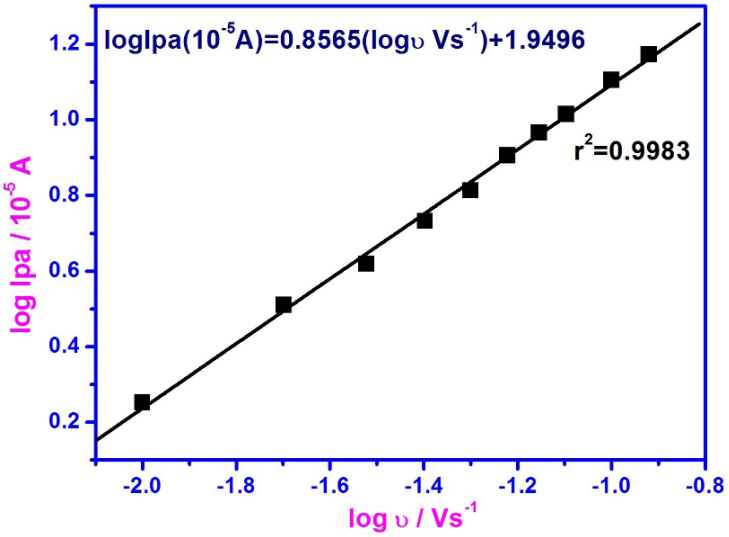


Fig. 6. Graph of logarithm of anodic peak current versus logarithm scan rate

According to an equation previously reported [21,29]. The heterogeneous rate constant (k^0) values was determined from the experimental peak potential difference (ΔE_p) data's, equation (2) was used for such voltammograms whose ΔE_p values are greater than 10 mV.

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

From the experimental ΔEp values as shown in Table 2 and equation (2) the values of the k^0 for the NE oxidation was determined. The value of k^0 obtained at a scan rate of 0.12 Vs^{-1} for the poly (niacinamide) MCPE exhibits larger heterogeneous rate constant compared with those determined in other scan rate variation studies. All the parameters are tabulated in Table 2.

Table 2. Variation of the voltammetric parameters gathered from the plots shown in Fig. 5 as a function of the potential scan rate

υ/ Vs^{-1}	**ΔE_p / mV**	**k°/ s^{-1}**
0.01	0.040	0.2002
0.02	0.057	0.3304
0.03	0.064	0.4534
0.04	0.079	0.5121
0.05	0.092	0.5497
0.06	0.105	0.5536
0.07	0.109	0.6694
0.08	0.120	0.6466
0.10	0.136	0.6629
0.12	0.146	0.7148

3.5. Effect of pH value on the determination of NE

The pH of the PBS has a significant contribution on the electrocatalytic oxidation of NE at poly (niacinamide) MCPE by affecting both peak current and peak potential. The effect of the PBS pH value on the oxidation of NE at poly (niacinamide) MCPE was carefully evaluated in the pH range of 5.5-8.0. The Fig. 7A showed cyclic voltammograms recorded for 2.27×10^{-4} M of NE at poly (niacinamide) MCPE. The oxidation peak potential shifts to a more negative potential with increasing pH. The E_{pa} versus pH graph clearly indicated that the E_{pa} depends linearly on the pH value in the range of 5.5-8.0 with a slope of 0.0663 V/pH (r^2=0.9952) as showed in an inset Fig. 7B and suggesting there is an equal number of protons and electrons are involved in the redox mechanism and this was consistent with the reported literature [21].

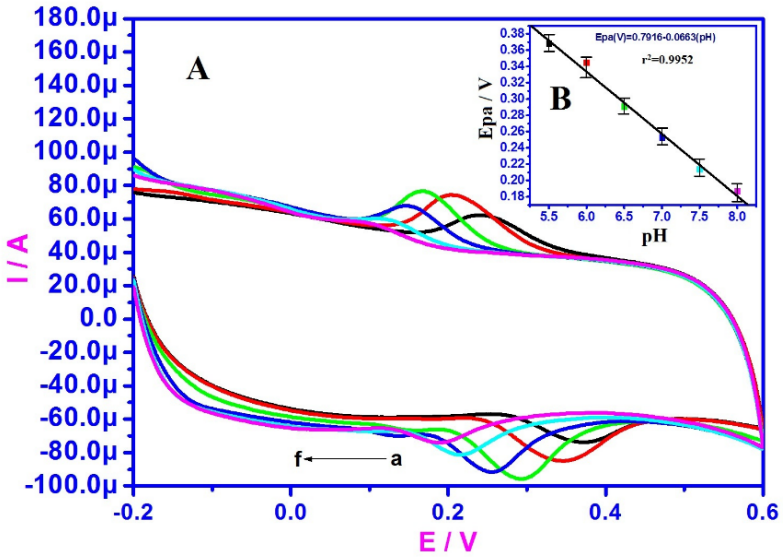


Fig. 7. (A) Cyclic voltammograms of the poly (niacinamide) MCPE in 0.2 M PBS solution at different pH (a-f: 5.5 to 8.0) at scan rate of 0.05 Vs^{-1}; **(B)** The effect of pH on the peak current response of 2.27×10^{-4} M NE in 0.2 M PBS solution

3.6. Electrocatalytic oxidation of AA at poly (niacinamide) MCPE

The cyclic voltammograms were recorded for the oxidation of 2 mM of AA at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) in 0.2 M PBS of pH 7.4 with the scan rate 0.05 Vs^{-1}as showed in the Fig. 8. At the BCPE the oxidation peak occurred at 0.258 V and it was generally irreversible, critically broad and required high over potential for oxidation due to fouling of the electrode surface by the adsorption of oxidized product of AA. However, at the poly (niacinamide) MCPE the oxidation peak potential of AA was obtained at -0.045 V, which is shifted to more negative potential and showed faster electron transfer kinetics of AA when compared to that of BCPE. This result indicated that the poly (niacinamide) MCPE lowers the over potential in oxidation and facilitates the oxidation process of AA.

The electrocatalytic oxidation of AA was carried out by varying its concentration (1.00 to 2.57 mM) in 0.2 M PBS of pH 7.4 at poly (niacinamide) MCPE by CV technique with the scan rate of 0.05 Vs^{-1} as showed in the Fig. 9A. This result showed, there is an increase in the anodic peak current due to the increase AA concentration. The plot shown in the inset of Fig. 9B showed the linear relationship between I_{pa} and the concentration of AA with the linear regression equation of I_{pa} (10^{-5}A)=3.0153(C_0 mM/L)+5.634, r^2=0.9972. The detection limit on the lower concentration range for AA was calculated to be 0.30 μM at poly (niacinamide) MCPE and the limit of quantification was 1.01 μM.

The effect of scan rate was studied for 1.0 mM of AA in 0.2 M PBS of pH 7.4 in the scan range from 0.04 to 0.12 Vs^{-1} at poly (niacinamide) MCPE as showed in Fig. 10A. The graph

of log I_{pa} versus log υ was plotted in the range from 0.04 to 0.12 Vs^{-1}. The graph obtained was linearly straight line with the slope of 0.7467 (inset Fig. 10B). This suggests there was an adsorption of analytes on the surface of the poly (niacinamide) MCPE [34-35].

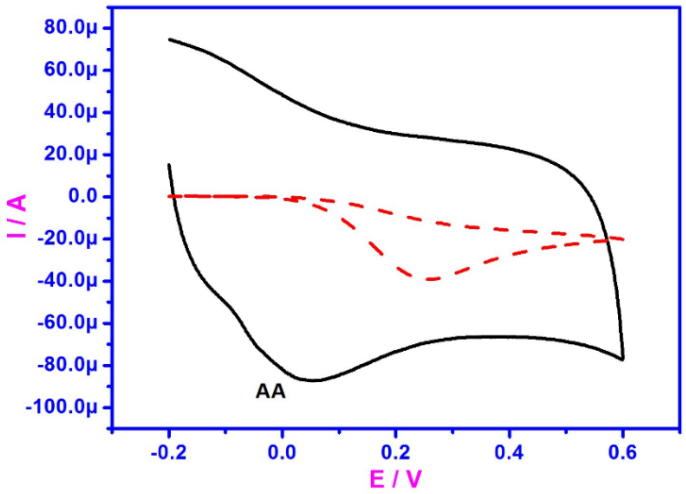


Fig. 8. Cyclic voltammograms of 2 mM AA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

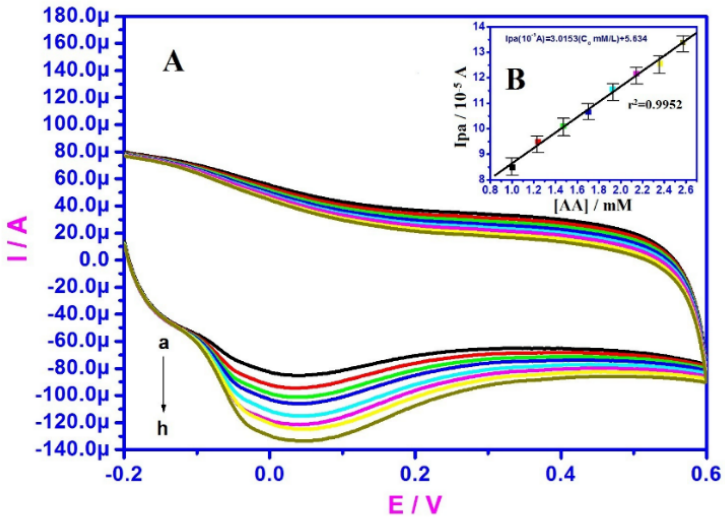


Fig. 9. (A) Cyclic voltammograms of AA in 0.2 M PBS solution of pH 7.4 at poly (niacinamide) MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a-h:1.00 mM, 1.24 mM, 1.47 mM, 1.69 mM, 1.92 mM, 2.14 mM, 2.35 mM and 2.57 mM); **(B)** Graph of anodic peak current versus concentration of AA

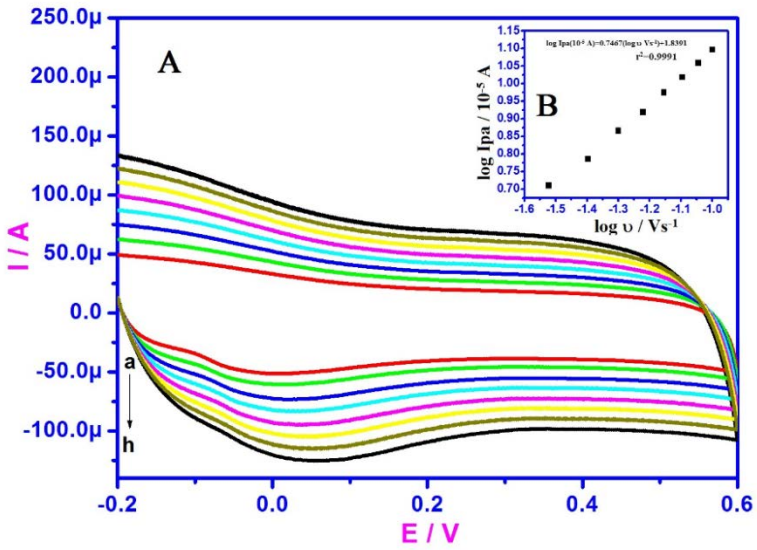


Fig. 10. (A) Cyclic voltammograms of 1.0 mM AA in 0.2 M PBS solution of pH 7.4 at poly (niacinamide) MCPE at different scan rate (a–i; 0.04 Vs^{-1} to 0.12 Vs^{-1}); **(B)** Graph of logarithm of anodic peak current versus logarithm scan rate

3.7. Electrochemical response of UA at poly (niacinamide) MCPE

Fig. 11 showed the cyclic voltammograms recorded for 0.1 mM of UA at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) in 0.2 M PBS solution of pH 7.4 with the scan rate 0.05 Vs^{-1}. The voltammogram obtained at BCPE was less sensitive and showed poor electrochemical response. However, the poly (niacinamide) MCPE evinces the oxidation peak current response with an improved electron transfer rate and E_{pa} was located at 0.302 V. By this result, it can be suggested that the poly (niacinamide) MCPE acts as a good electrochemical sensor for UA.

The Fig. 12A showed by increasing the concentration of UA from 20.66 to 70.85 μM, the I_{pa} and I_{pc} goes on increasing with shifting E_{pa} towards less positive and E_{pc} towards least negative side. The inset graph of I_{pa} *vs.* concentration of UA shows almost straight line with good linearity as showed in the Fig. 12B. The linear regression equation can be expressed as $I_{pa}(10^{-5}A)=0.049(C_0$ μM/L)+4.074, (r^2=0.9979). The detection limit in the lower concentration range for UA was calculated to be 0.18 μM for the poly (niacinamide) MCPE and limit of quantification was 0.60 μM. The variation of scan rate was studied for 0.1 mM of UA in 0.2 M PBS of pH 7.4 in the scan rate ranges between 0.04 to 0.12 Vs^{-1} at poly (niacinamide) MCPE as showed in Fig. 13A. A linear relationship was observed between log I_{pa} versus log υ with the slope of 0.8245 as showed in Fig. 13B. It suggests the electrode process involves the adsorption phenomenon [34-36].

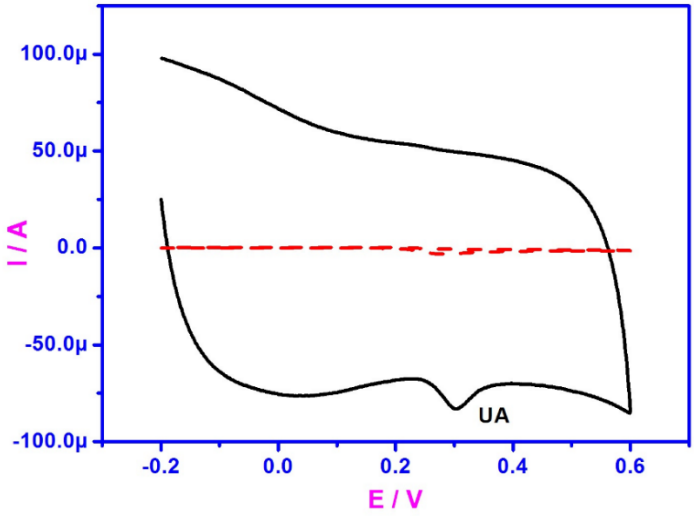


Fig. 11. Cyclic voltammograms of 0.1 mM UA in 0.2 M PBS solution of pH 7.4 at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

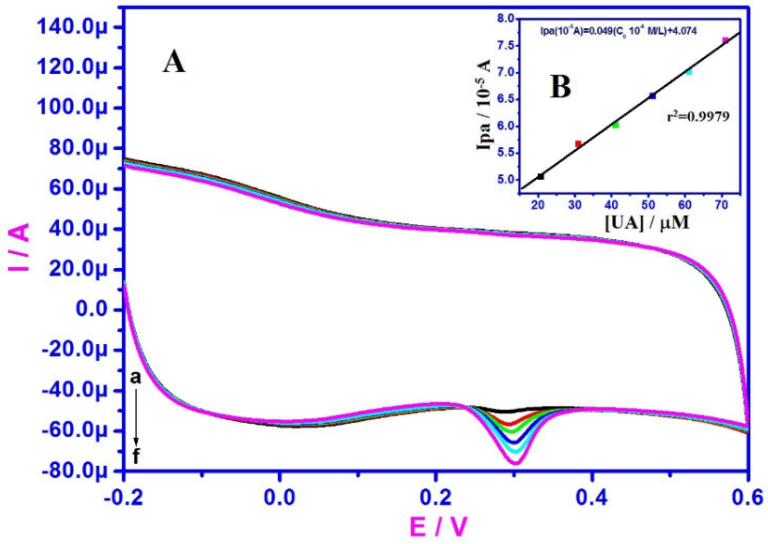


Fig. 12. (A) Cyclic voltammograms of UA in 0.2 M PBS solution of pH 7.4 at poly (niacinamide) MCPE at scan rate of 0.05 Vs^{-1} with different concentration (a–l; 20.66 μM, 30.86 μM, 40.98 μM, 51.02 μM, 60.97 μM and 70.85 μM); **(B)** Graph of anodic peak current versus concentration of UA

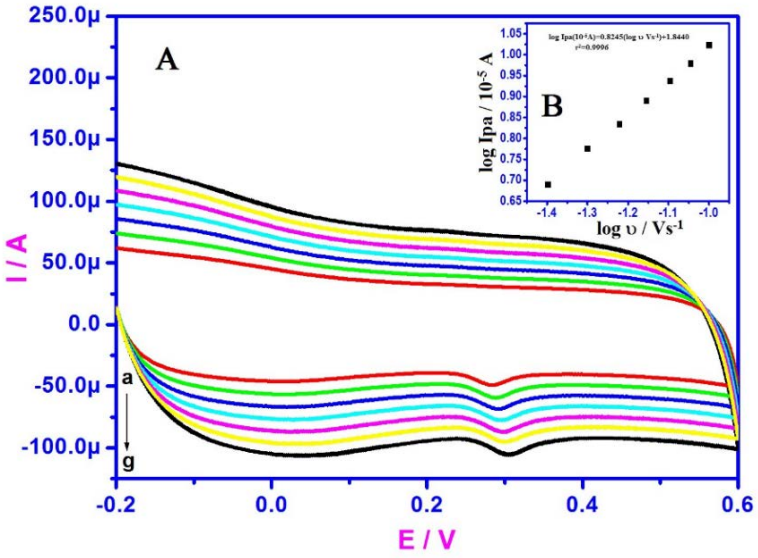


Fig. 13. (A) Cyclic voltammograms of 0.1 mM UA in 0.2 M PBS solution of pH 7.4 at poly (niacinamide) MCPE at different scan rate (a-g; 0.04 Vs^{-1} to 0.1 Vs^{-1}); **(B)** Graph of logarithm of anodic peak current versus logarithm scan rate

3.8. Simultaneous electroanalysis of NE in presence of interferences

In order to establish a selective and sensitive method for the electroanalysis of NE in presence of probable interferences like AA and UA the poly (niacinamide) MCPE was used.

The Fig. 14 showed the cyclic voltammograms recorded for the ternary mixture of 0.13 mM of NE in presence of high concentration of 0.91 mM AA and 0.45×10^{-4} M UA in 0.2 M PBS of pH 7.4 at the scan rate of 0.05 Vs^{-1} at both BCPE and poly (niacinamide) MCPE. The voltammetric response obtained for the oxidation of all the three analytes was broad, less sensible and given overlapped potential at 0.268 V. This leads to their individual identification impossible (dashed line). However, in the same identical conditions the poly (niacinamide) MCPE has an ability to overcome this difficulty and resolved the oxidation potential of all three analytes in the mixture. Three well defined oxidation peak potential of NE, AA and UA were observed and are located at 0.218 V, 0.0375 V and 0.334 V respectively (solid line). The oxidation peak to peak separation of NE-AA was 0.180 V and that of NE-UA was 0.115 V. This result was more than enough to identify and determine NE in the presence of high concentration of UA and AA at poly (niacinamide) MCPE.

Differential pulse voltammetry (DPV) was used for its higher current sensitivity and absence of background current. The Fig. 15 showed the selective determination of 0.13 mM of NE, 0.91 mM of AA and 0.45×10^{-4} M of UA in 0.2 M PBS of pH 7.4 at poly (niacinamide) MCPE. The oxidation peak potential of NE, AA and UA were situated at 0.141

V, -0.087 V and 0.252 V respectively. The peak to peak separation between NE-AA was 0.228 V and that of NE-UA was 0.111V respectively.

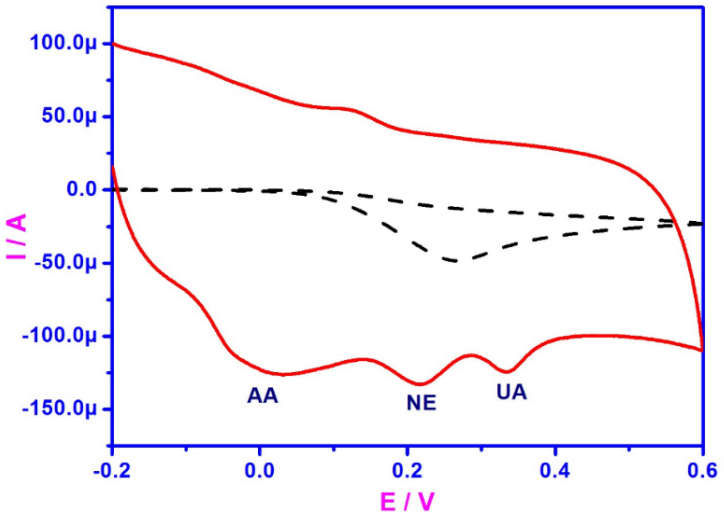


Fig. 14. Cyclic voltammograms for simultaneous determination of 0.13 mM NE, 0.91 mM AA and 0.45×10^{-4} M UA at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

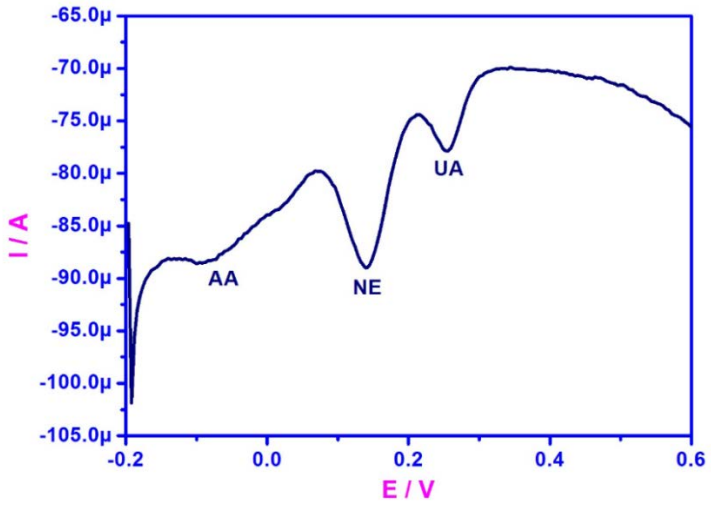


Fig. 15. Differential pulse voltammogram for simultaneous determination of 0.13 mM NE, 0.91mM AA and 0.45×10^{-4} M UA at BCPE (dashed line) and poly (niacinamide) MCPE (solid line) at scan rate of 0.05 Vs^{-1}

3.9. Interference study

The interference study was conducted in a ternary mixture containing NE, AA and UA at poly (niacinamide) MCPE. The concentration of one species is changed by keeping the concentration of the other two species constant. From the Fig. 16 it can be seen that the peak current of NE was increased because of increased concentration from 9.76 to 57.91 μM by keeping the concentration of 0.97 mM AA and 49.01 μM UA constant.

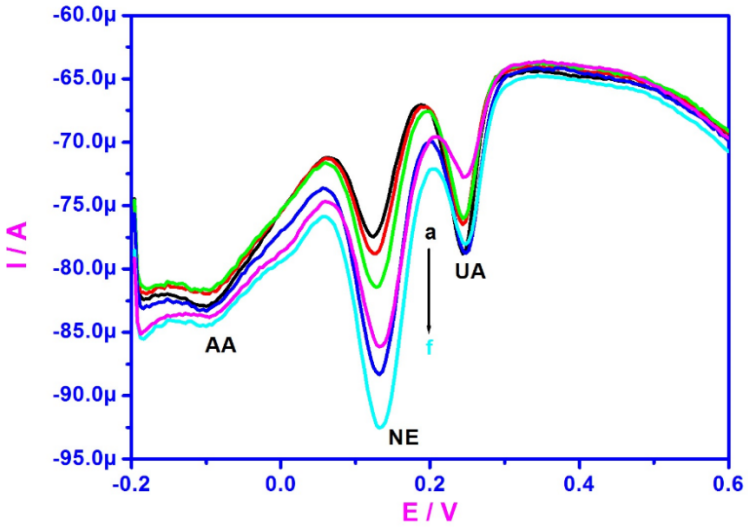


Fig. 16. Differential pulse voltammograms of (a) 9.76 μM (b) 19.45 μM (c) 29.06 μM (d) 38.75 μM (e) 48.44 μM (f) 57.91 μM in 0.2 M PBS of pH 7.4 in presence of 0.97 mM AA and 49.01 μM UA

3.10. Analytical applications

The poly (niacinamide) MCPE was applied to the determination of NE in norepinephrine bitartarate injection sample (1 mg/ml, Norad, Neon laboratories 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 96.62% to 100.62%. The relative standard deviation (n=5) was less than 2.0%. The results are generally acceptable and attributed to the good reproducibility of poly (niacinamide) MCPE, showing that the proposed method could be effectively used for the determination of NE in the pharmaceutical and clinical laboratory as a biosensor for diagnosing the disease caused by the deficiency of NE.

Table 3. Determination of NE in noradrenaline bitartarate injection sample by poly (niacinamide) MCPE

Sample	Added (μM)	Found (μM)	RSD (%)	Recovery (%)
1	10	10.06	1.21	100.62
2	20	19.32	1.18	96.62
3	30	29.42	1.24	98.07
4	40	38.69	1.28	96.73
5	50	48.58	1.30	97.16

4. CONCLUSION

In the present work, the electropolymerisation of niacinamide on the surface of carbon paste electrode produced a stable conducting polymeric film. This study showed that the poly(niacinamide) MCPE not only exhibited strong electrocatalytic activity towards the oxidation of NE, AA and UA, it also resolved the overlapping oxidation peaks of NE, AA and UA into three-well resolved peaks. The high sensitivity and selectivity together with the very easy method of preparation and reproducibility in the results made this modified electrode a very useful in the construction of sample devices for the simultaneous determination of NE, AA and UA in the ternary mixture. The interference and analytical application of the modified electrode made this electrode to be used as biosensors in the medicine field for the diagnosis of norepinephrine deficiency.

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