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Poly (4-Amino-3-Hydroxynaphthalene Sulfonic Acid)-Modified Glassy Carbon Electrode for Electrochemical Detection of Ephedrine in Human Urine

Meareg Amare, Worku Lakew and Shimelis Admassie*

Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

*Corresponding Author, Tel:+251-111-23 94 66 Fax: +251-111-23 42 96

E-mail: shimadm@chem.aau.edu.et , shimadm09@gmail.com

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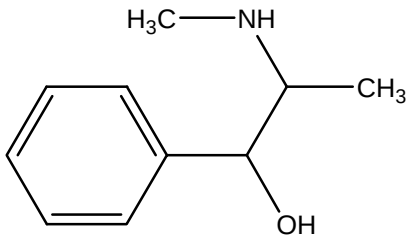
Abstract- A polymer modified glassy carbon electrode was fabricated by electropolymerizing 4-amino-3-hydroxynaphthalene sulfonic acid (AHNSA) in a 0.1 M HNO_3 and was characterized by cyclic voltammetry. The polymer modified electrode showed an excellent electrocatalytic activity towards the electrochemical oxidation of ephedrine. The oxidative peak current response of ephedrine at the polymer modified-glassy carbon electrode was found to show linear dependence on the concentration of ephedrine in the range of 8.0×10^{-6} to 1.0×10^{-3} mol L^{-1} with a linear regression equation, correlation coefficient and limit of detection (according to S/N=3; for n=4) of $Ip_a(\mu A)=0.971+0.042$ C (μM), 0.99913 and 7.9×10^{-7} M, respectively. The proposed method was tested for the determination of ephedrine in human urine. Recoveries ranging between 92.06–93.15% for filtered and 81.15–86.30% for unfiltered human urine were achieved validating the developed method.

Keywords- Square Wave Voltammetry, Ephedrine, Britton-Robinson Buffer Solutions, 4-Amino-3-Hydroxynaphthalene Sulfonic Acid

1. INTRODUCTION

Ephedrine, (1R, 2S)-(+)-α-(1-methylaminoethyl) benzyl alcohol (scheme 1) is a natural alkaloid obtained by extraction from several plants of the genus *Ephedra* or by synthesis [1,

2]. It has been used at therapeutic doses of 15–60 mg in the treatment of bronchial asthma, allergic states, catalepsy and myasthenia gravis, as a nasal decongestive, as an antidote for poisoning by central nervous system (CNS) depressants and in spinal anaesthesia [1,3]. About 60% of the ephedrine taken is excreted free in urine, ca. 10% being metabolized [4]. It is a sympathomimetic drug that stimulates both α- and β-adrenergic receptors in the central nervous system [1, 2]. It causes a rise of systolic and diastolic pressure, bronchodilation and mild stimulation of the CNS [3,4].



Scheme. 1. The chemical structure of ephedrine

The stimulant properties of ephedrine are well established and the alkaloid has been exploited in numerous over the counter (OTC) medicines [5]. Another problem that has arisen in recent years has been its potential of drug to impair driving ability [5-8]. This concern is compounded by the exploitation of ephedrine's stimulant properties by transport workers, especially those involved in long distance routes, where they can be used to alleviate the effects of fatigue [7]. Ephedrine has been characterized as a prohibited compound by the International Olympic Committee [2,9]. As ephedrine is an ingredient of common anti-cold preparations and various nutritional supplements, athletes tested positive for ephedrine often claim to have received it while using products without indication [3]. The extensive use of this chemical is of great concern for the international athlete associations and has generated significant interest for development of methods for its rapid detection in health foods, pharmaceutical products and human fluids of athletes. The international Olympic committee and most international sports federations have put ephedrine on the list of prohibited substances and have adopted urinary threshold concentrations above which an athlete is regarded as positive. The threshold amount is 10 μg/mL of ephedrine in urine [10]. Therefore, fast, simple and reliable method for the quantitative determination of ephedrine in human urine is needed.

High performance liquid chromatography [11-16], gas chromatography [17] and gas chromatography-mass spectrometry [18] techniques have been used to determine ephedrine samples. These techniques, however, are very expensive, highly sophisticated and tedious which also need organic solvents for separation aggravating environmental pollution. Therefore, the determination of ephedrine in biological fluids and pharmaceutical

preparations *via* a simple and reliable method is of great interest. Electrochemical methods have been of great interest due to several advantages, including high sensitivity, simplicity, rapid response and low cost [19]. However, there are few reports on the application of electrochemical methods for the determination of ephedrine using modified electrodes [1,3-5,9].

In recent years, polymer- modified electrodes (PMEs) have received attention [20-22] due to their good stability, reproducibility, increase in active sites, homogeneity in electrochemical deposition and strong adherence to electrode surface [23,24]. Compared to metal electrodes, glassy carbon electrode (GCE) has been widely used due to its biocompatibility with tissue, low residual current over a wide potential range and minimal propensity to show deteriorated response as a result of electrode fouling [23,25]. Hence, in this work we report the use of poly-(4-amino-3-hydroxynaphthalene sulfonic acid) modified glassy carbon electrode for quantitative determination of ephedrine in human urine samples, which to our knowledge have not been communicated previously.

2. EXPERIMENTAL

2.1. Apparatus and reagents

4-amino-3-hydroxynaphthalene sulfonic acid (AHNSA) was purchased from Sigma-Aldrich and ephedrine hydrochloride (EPH) with purity of 100.85% from Emmellen Biotech Pharmaceuticals Limited. Britton-Robinson buffer solutions (RBS) were prepared using equi-volume mixtures of 0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M CH_3COOH and adjusted to the desired pH with 2.0 M NaOH using Jenway 3345 ion meter. 50.0 mM EPH stock solution was prepared using pH 11.0 RBS and stored under refrigeration. All experiments were carried out at room temperature and all solutions were prepared from distilled water except the sample used for recovery which was prepared with urine. The chemicals used were all of analytical grade and were used without further purification.

All electrochemical measurements were performed using CHI760D Electrochemical Workstation, CHI Instruments (Austin, Texas, USA). A conventional three-electrode system consisting of bare GCE (3 mm in diameter) or poly (AHNSA)-modified GCE as a working electrode, saturated silver-silver chloride (Ag/AgCl, KCl saturated) as a reference electrode and platinum wire as a counter electrode was employed.

2.2. Preparation of urine samples

Human urine collected from a volunteer was divided in to two portions. The one portion was suction filtrated using a 0.45 μm pore size filter paper. The filtrate was then diluted with pH 10.5 RBS in a 1:5 volume ratio. Then, 80, 100, and 200 μM EPH solutions were prepared from the stock solution using the diluted filtrated urine. The same procedure has been followed to prepare solutions for the unfiltrated urine portion.

2.3. Electrode preparation

The GCE was polished with 1.0, 0.3 and 0.05 micron size powder of alumina on a polishing cloth before modification. After it was rinsed with distilled water, poly (AHNSA) was grown at the GCE potentiodynamically by scanning the potential between -0.8 and +2.0 V at a scan rate of 0.1 Vs^{-1} for fifteen cycles in a 2.0×10^{-3} mol L^{-1} AHNSA/0.1M HNO_3. After modification, the modified electrode was rinsed with distilled water and the cyclic voltammogram of the modified electrode was run between -0.8 to +0.8 V at 100 mVs^{-1} in monomer free 0.5 M H_2SO_4 until a stable voltammogram was obtained.

3. RESULTS AND DISCUSSION

3.1. Electropolymerization of AHNSA at the GCE surface

A polymer film on the electrode surface was obtained from a solution of 2.0×10^{-3} M of AHNSA in 0.1 M HNO_3 potentiodynamically by cycling the potential between -0.8 to +2.0 V (Fig 1). During the first cycle, two oxidative (*b'* and *a*) and one reductive peaks (*b*) appeared at about +0.23, +0.65 and -0.30 V respectively. In the consecutive scans, two new additional peaks, (*c'* and *c*) begun to appear at about +0.03 and -0.14 V, respectively. With increasing number of cycles, the peak currents for all the peaks increased except for the anodic peak (*a*). This increment of peak currents with increasing number of scanning cycles indicates the formation of a polymer film on the surface of the GCE. The cyclic voltammogram of a stabilized polymer modified GCE (Fig. 1. (Inset-B)) in a monomer free 0.5 M H_2SO_4 shows three redox couples designated by a-a', b-b' and c-c' which confirms the deposition of the polymer at the GCE.

3.2. Electrochemical characterization of poly (AHNSA)-modified GCE

Fig. 1 (insets A and B) depict the CVs of bare GCE (*A*) and the poly (AHNSA)-modified GCE (*B*) in a monomer free 0.5 mol L^{-1} of H_2SO_4 solution. The poly (AHNSA)-modified GCE (inset B), shows three redox couples designated by *a, a'*; *b, b'* and *c, c'* at potentials (+0.055, +0.074), (+0.190,+0.215) and (+0.302,+0.357) V, respectively. This electrochemical behavior of the modified electrode in a monomer free supporting electrolyte clearly indicates the formation of a polymer film at the electrode surface. The effect of scan rate on the peak currents has also been investigated and is shown in Fig. 2. The cathodic (Ip_c) and anodic (Ip_a) peak currents of *a* and *a'* were linearly dependent on the scan rate (ν) in the range 10–300 mVs^{-1} with correlation coefficients of 0.99854 and 0.99789, respectively (Fig. 2. inset), indicating that the process of the electrode reaction is a surface-confined process.

In the case of surface-confined redox processes, the peak current and charge passed during the electrolysis are given by equations 1 and 2, respectively [26] which could be rearranged to give equation 3:

$$i_p = \frac{n^2F^2}{4RT}\upsilon A\Gamma \quad (1)$$

$$Q = nFA\Gamma \quad (2)$$

$$n = \frac{4IRT}{FQv} \quad (3)$$

Where n is the number of electrons transferred, F is the Faraday's constant, R is the gas constant, T is the temperature (K), A is the electrode area (cm^2) and Γ is the surface coverage of the species (mol cm^{-2}). From these equations, n and Γ, were found to be 1.933 and 1.135×10^{-10} mol cm^{-2}, respectively. From these observations, the electropolymerization reaction mechanism of AHNSA on GCE seems to follow the reaction mechanism already proposed for the electropolymerization of *para*- aminobenzene sulfonic acid on glassy carbon electrode [27].

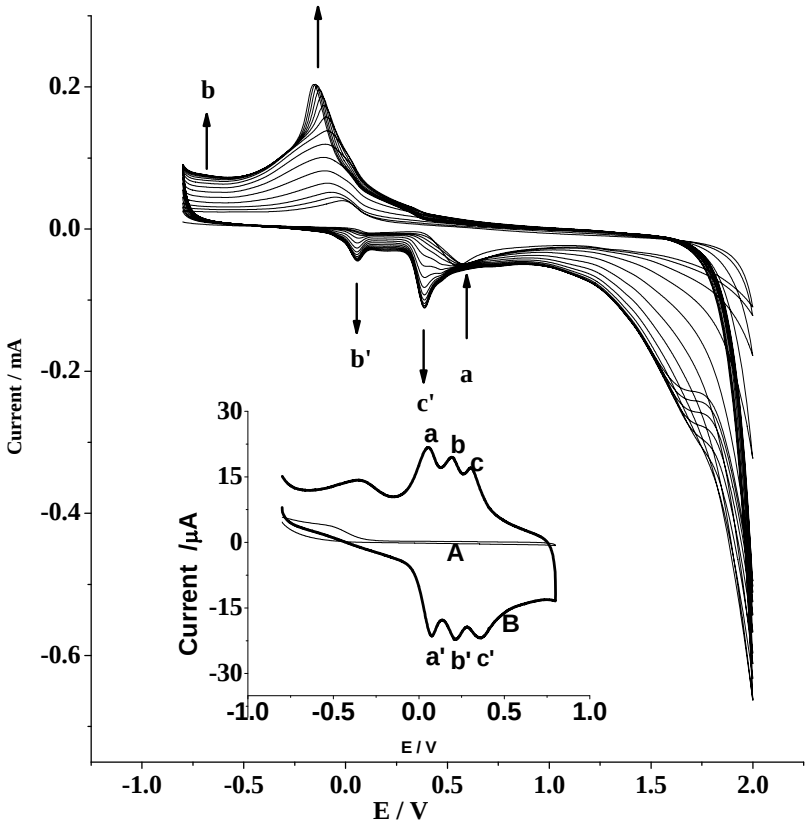


Fig. 1. Cyclic voltammograms of 2.0×10^{-3} $molL^{-1}$ of 4-amino-3-hydroxynaphthalene sulfonic acid (AHNSA) in 0.1M HNO_3 at GCE when the potential is scanned between -0.8 V to 2.0 V at 100 mV/s for 15 cycles. Inset: cyclic voltammograms of (A) bare GCE and (B) poly (AHNSA)-modified GCE in a monomer free 0.5 M H_2SO_4 scanned between -0.8 and +0.8 V at 100 mVs^{-1}

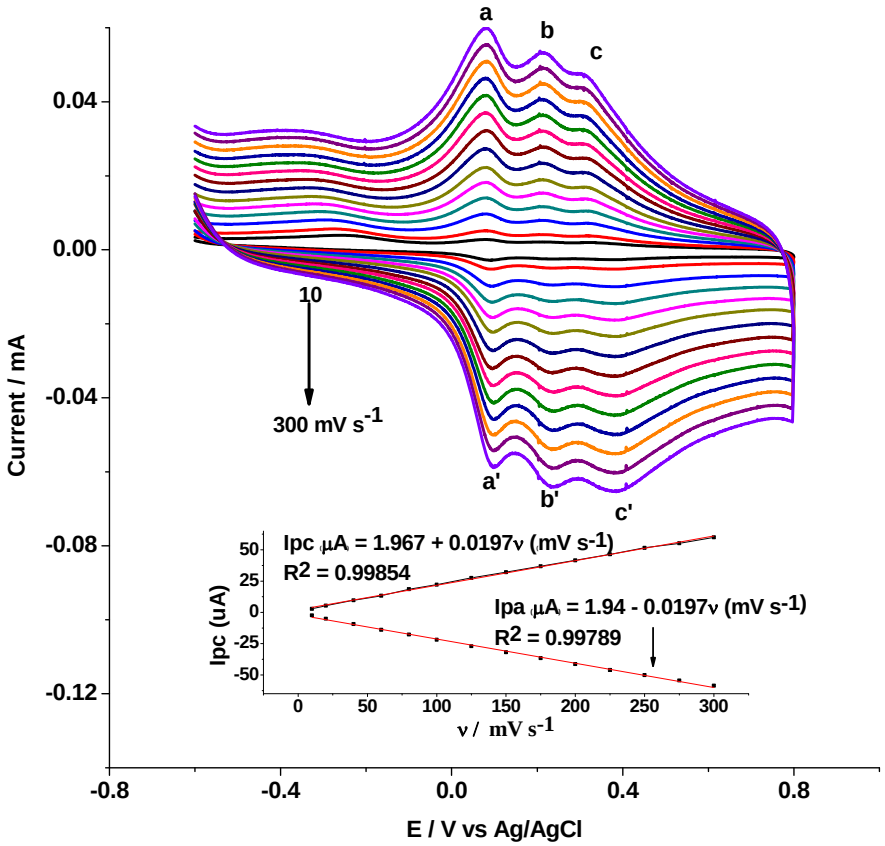


Fig. 2. Cyclic voltammograms of poly (AHNSA)-modified GCE in 0.5 M H_2SO_4 at different scan rates. Inset: Plot of peak current versus scan rate

3.3. Electrocatalytic oxidation of EPH at the poly (AHNSA)-modified GC electrode

Fig. 3 shows the cyclic voltammograms obtained for 1.0 mM EPH in pH 11.0 RBS at a bare GCE (Fig. 3a) and poly (AHNSA) modified GCE (Fig. 3b). At the bare GCE, EPH is observed to undergo an irreversible oxidation reaction at a large positive potential as reported elsewhere [1,4-5]. Similar irreversible oxidation behavior was observed at the poly (AHNSA)/GCE (Fig. 3b) except that the oxidative peak current of EPH is enhanced and peak potential is shifted to a less positive potential indicating the electrocatalytic activity of the poly (AHNSA)-modified GCE.

The effect of scan rate on the anodic peak current of EPH at poly (AHNSA)-modified GCE was studied (Fig. 4). The anodic peak current (Ip_a) was found to increase with increasing scan rate. The linear relationship observed between Ip_a and $\nu^{1/2}$ with a correlation coefficient (r^2) of 0.99832 suggests that the reaction of EPH at the poly (AHNSA)-modified GCE is a diffusion-controlled process (Fig. 4. Inset).

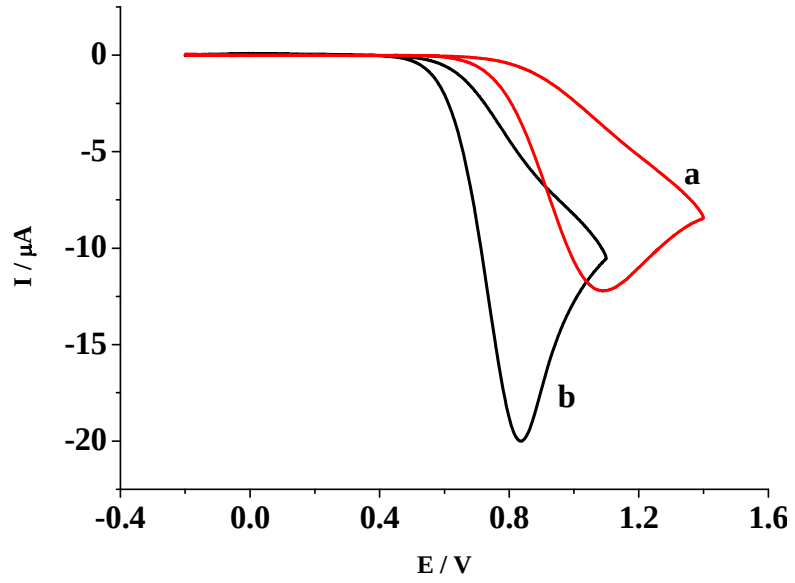


Fig. 3. Cyclic voltammograms of 1.0×10^{-3} mol L^{-1} ephedrine in pH 11.0 RBS (a) at bare GCE and (b) at poly (AHNSA)-modified GCE at a scan rate of 100 mVs^{-1}

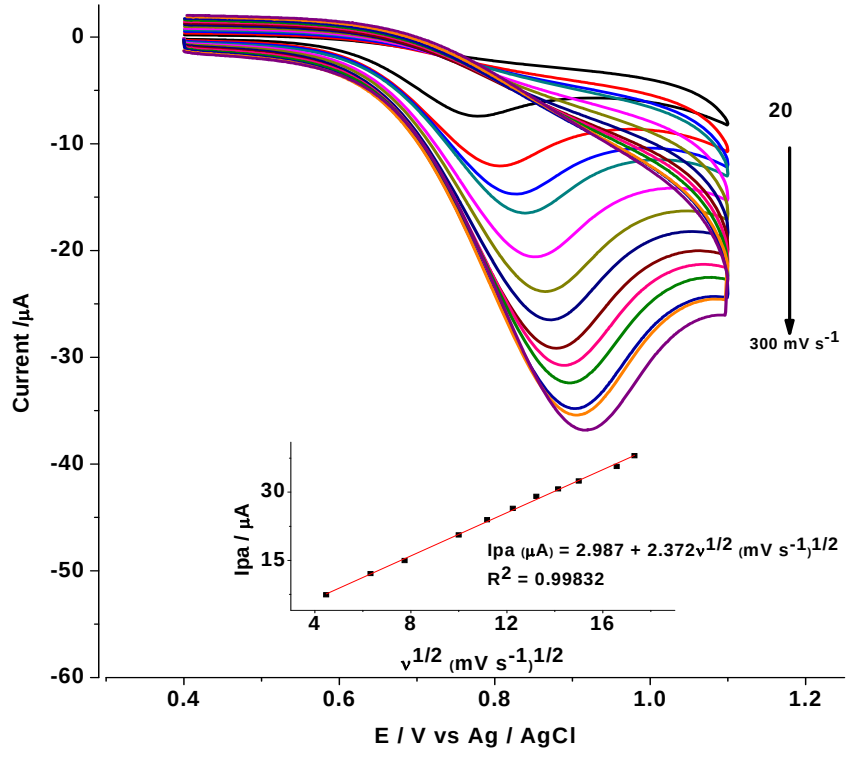


Fig. 4. Cyclic voltammograms of 1.0×10^{-3} mol L^{-1} ephedrine in pH 11.0 RBS at poly (AHNSA)-modified GCE at different scan rates. Inset: Plot of anodic peak current *versus* square root of scan rate

3.4. Effect of solution pH on the peak potentials and peak currents

The cyclic voltammograms of 1.0×10^{-3} M EPH at poly (AHNSA)-modified GCE in RBS at different pHs are shown in Fig. 5. With increasing the pH of the solution (in the range of 7.5–11.5) the peak potential shifted in the negative direction indicating that protons are involved in the electrode reaction [28]. The anodic peak current gradually increased from pH 7.5 to 10.0 and levels off between 10.0 and 10.5. At pH higher than 10.5, the peak current sharply decreased. Hence, pH 10.5 is used as the optimum pH of the buffer solution in the subsequent experiments. A linear relationship between the peak potential and solution pH was obtained (Fig. 5. inset) with a linear equation and correlation coefficient of $Ep_a(V)=1.526–0.0684pH$ and $R^2=0.99419$, respectively. According to the Nernst equation (T=293 K), the slope of 0.0684 V/pH suggests that the number of protons taking part in the electrode reaction are similar to the number of electrons [29]. Hence, a reaction mechanism (scheme 2) was proposed for the oxidation of ephedrine at the poly (AHNSA)-modified GCE.

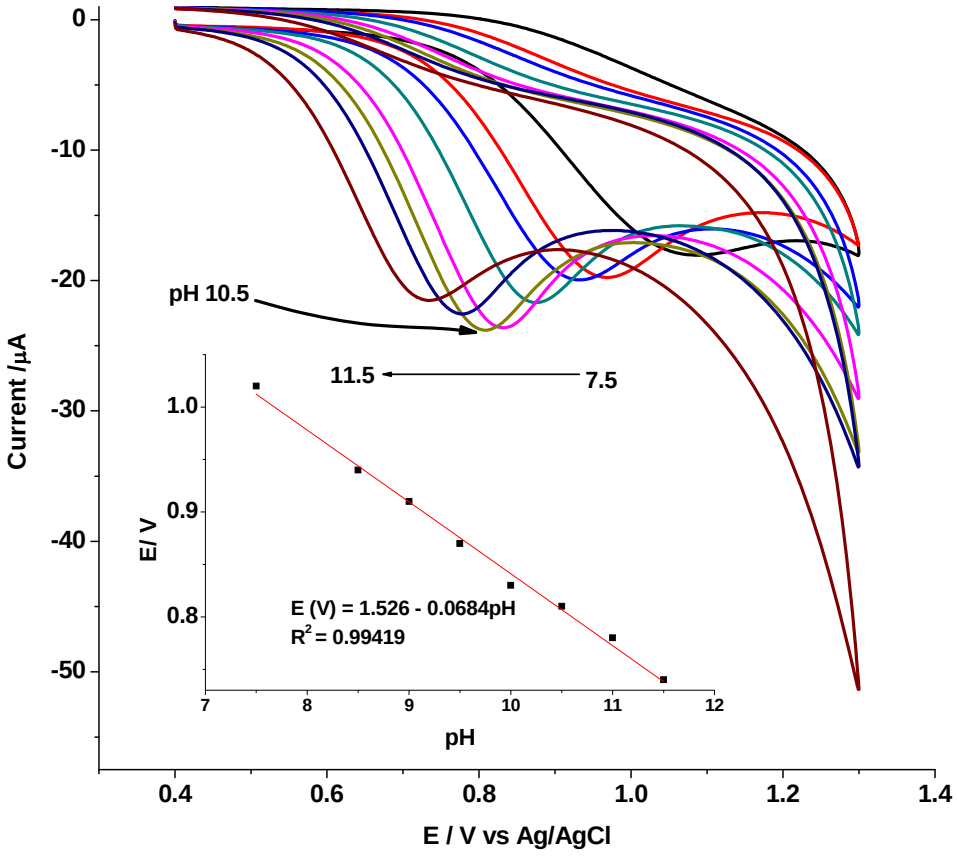
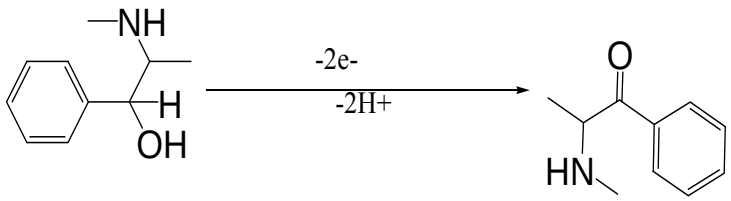


Fig. 5. Cyclic voltammograms of 1.0×10^{-3} M ephedrine at poly (AHNSA)-modified GCE at different pHs (7.5–11.5) of RBS at a scan rate of 100 mV s^{-1}. Inset: Variation of Ep_a *vs.* the pH. (C) Plot of anodic peak current *vs*. Ph



Scheme. 2. Proposed reaction mechanism for the oxidation of ephedrine at the polymer modified GCE

3.5. Amperometric studies of EPH at poly (AHNSA)-modified GCE

Amperometric experiments were carried out for 20 s at a potential of +0.90 V for different concentrations of EPH (100.0, 200.0 and 400.0 μM) in pH 10.5 RBS. Fig. 6 shows the plots of I *vs.* time for different concentrations of EPH. The slopes of the plots of I *vs.* $(t)^{-1/2}$ (inset of Fig. 6.) for each concentration were used to estimate the diffusion coefficient of EPH in the polymer film using the Cottrell equation: $i_{pa} = nFAD^{1/2}C^*/(\pi t)^{1/2}$ [30], where ip_a, n, F, A, D, C* and t represent the anodic peak current (A), number of electrons transfer, Faraday's constant (C mol^{-1}), area of the electrode (cm^2), diffusion coefficient (cm^2 s^{-1}), initial concentration of EPH (mol cm^{-3}) and time (s) respectively. By taking the value of n as 2 and A as 0.0707 cm^2, the diffusion coefficient of EPH was found to be 7.011×10^{-11} $\pm 3.15\times10^{-11}$ cm^2 s^{-1}. As can be seen from the curves, the time needed to reach the minimum concentration is too short which indicates that the response of the polymer to EPH is rapid and hence the polymer can be used as an amperometric sensor of EPH.

3.6. Performance of the poly (AHNSA)-modified GCE

Square wave voltammetry (SWV) has been used to study the application of the poly (AHNSA) -modified GCE for the quantitative analysis of EPH. Fig. 7 shows the DPVs of EPH at the bare glassy carbon electrode and the poly (AHNSA)–modified GCE. The curves (a) and (b) correspond to the oxidation of 1.0×10^{-3} mol L^{-1} EPH in pH 10.5 RBS at the bare GCE and the poly (AHNSA)–modified GCE, respectively.

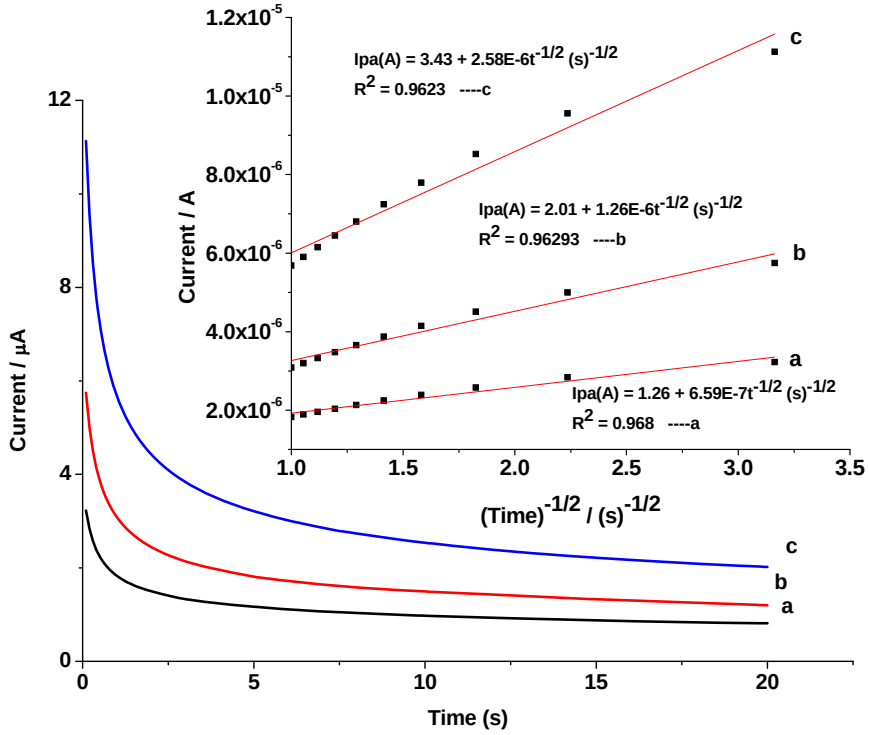


Fig. 6. i–*t* curves of (a) 100, (b) 200 and (c) 400×10^{-6} mol L^{-1} of ephedrine at poly (AHNSA)-modified GCE in pH 10.5 RBS. Insets: Plot of current *vs*. $(time)^{-1/2}$

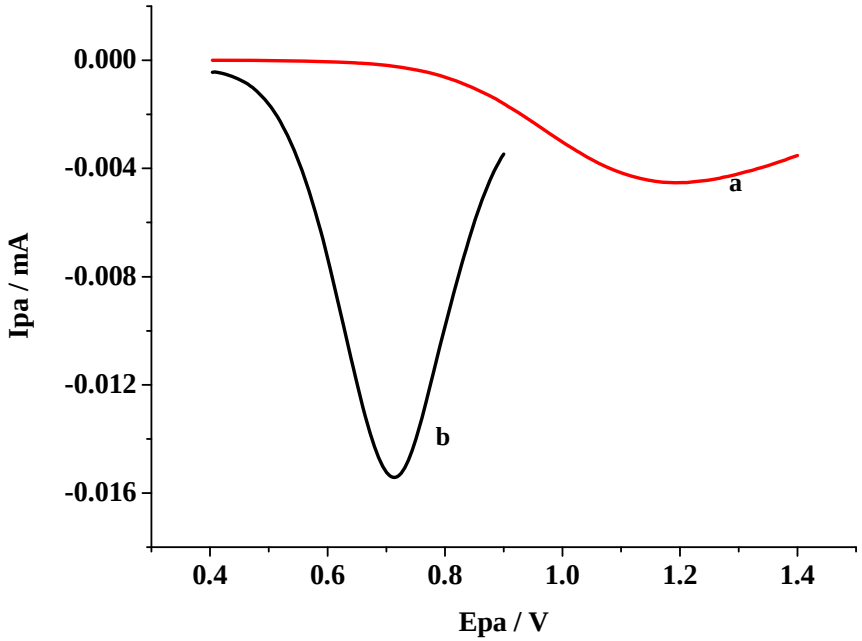


Fig. 7. Square wave voltammograms of 1.0×10^{-3} $molL^{-1}$ ephedrine (pH 10.5 RBS) at bare GCE (a) and at poly (AHNSA)-modified GCE (b). Pulse amplitude: 50 mV; Step potential: 4 mV

It can be observed from the figure that, EPH exhibits better electrochemical response at the at the poly (AHNSA)-modified GCE where the peak potential of EPH has shifted towards less positive potential with enhancement of the peak current indicating the catalytic effect of the polymer.

Using the optimized pH (pH 10.5 RBS) and selected SWV parameters (potential step, pulse amplitude and frequency of 12 mV, 75 mV and 15 Hz respectively), a calibration curve was constructed for quantitative determination. Fig. 8 depicts the variation of the square wave peak current with concentration and the linear dependence of the peak current on concentration in the range 8.0×10^{-6}–1.0×10^{-3} mol L^{-1}. The linear regression equation, linear regression coefficient and limit of detection (according to S/N=3; for n=4) were found to be Ia(μA)=0.971+0.042 C(μM), 0.99913 and 7.9×10^{-7} M respectively.

The stability of poly(AHNSA)/GCE was also examined. After keeping it in refrigerator for one week, the current response of the electrode for 1.0 mM EPH in pH 10.5 RBS did not remarkably change, showing that poly (AHNSA)/ GCE has good stability. The reproducibility of the current response of the polymer-modified electrode for ephedrine sample was also studied. As can be seen from Table 1 below, the maximum R.S.D (%) recorded for triplicate measurements was 2.11% showing the reproducibility of the response of the developed electrode for ephedrine in human urine.

Table 1. Percentage recovery of ephedrine from human urine samples

[EPH] (μM)	Filtered urine sample				Unfiltered urine sample			
	Added (mg)	Detected (mg)	Recovery (%)	R.S.D (%)	Amount expected (mg)	Amount detected (mg)	Recovery (%)	R.S.D (%)
80	16	14.73	92.06	1.49	16	13.20	82.50	2.11
100	20	18.63	93.15	1.07	20	16.23	81.15	1.87
200	40	37.12	92.8	1.9	40	34.52	86.3	1.98

* Average of triplicate measurements using one modified electrode

The applicability of the poly (AHNSA)-modified GCE for the determination of ephedrine was investigated by the determination of ephedrine in human urine. Known concentrations (80.0, 100 and 200.0 μM) of ephedrine in urine samples were prepared using the filtered and unfiltered human urine (1:5 diluted with pH 10.5 RBS). Square wave voltammetry under the optimized parameters was employed to determine the anodic current response for the urine samples at poly(AHNSA)/GCE and the results are summarized in Table 1. Excellent recoveries have been achieved both for the unfiltered and filtered urine samples. The significantly low R.S.D (%) values indicate the reproducibility of the developed method.

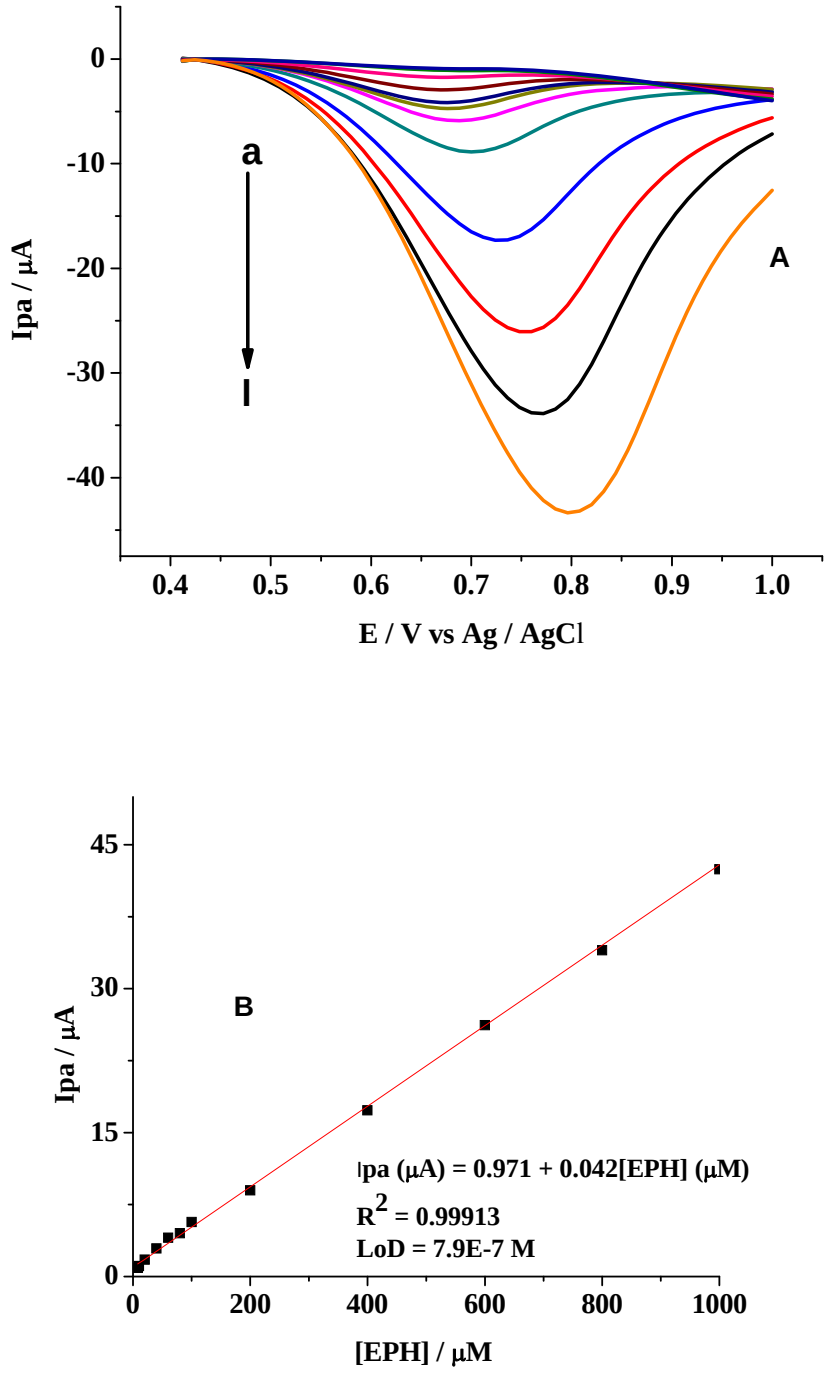


Fig. 8. (a) Square wave voltammograms of different concentrations of ephedrine at poly (AHNSA)-modified GCE in pH 10.5 RBS. (b) Calibration curve for the determination of ephedrine at poly (AHNSA)-modified GCE. Step potential: 12 mV; pulse amplitude: 75 mV; Ephedrine concentrations: (a) 8.0, (b) 10.0, (c) 20.0, (d) 40.0, (e) 60.0, (f) 80.0, (g) 100.0, (h) 200.0, (i) 400.0, (j) 600.0, (k) 800.0 and (l) 1000.0 µM

Table. 2. Comparison between the newly developed method and other reported methods

Electrode	Linear range	Detection limit	Method	Ref.
GCE	3. 47-9.916×10^{-5} mol L^{-1}	4.96×10^{-6} mol L^{-1}	DPV	1
CoPC/CPEs	1.0-100×10^{-6} mol L^{-1}	8 ×10^{-7} mol L^{-1}	HDV	3
Dropping Hg	4.0×10^{-8}-4×10^{-6} mol L^{-1}	8×10^{-8} mol L^{-1}	DP	4
Poly (AHNSA)/GCE	8.0×10^{-6}-1.0×10^{-3} mol L^{-1}	7.9×10^{-7} mol L^{-1}	SWV	This work

4. CONCLUSION

The approach taken in this work provides a simple method to detect ephedrine using an environmentally friendly electrode-modifier unlike the other electroanalytical methods developed using mercury electrode. A wide linear response up to 8.0 μM and a low limit of detection of 7.9×10^{-7} M was observed (Table 2), which is well below the current acceptable threshold limit by the International Olympic Committee for this alkaloid in athlete urine. It was also employed for the direct detection of EPH in human urine prior to filtration with good recoveries. Therefore, the developed method will be very useful for the direct analysis of the ephedrine content in human urine.

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