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Electrocatalytic Determination of Isoniazid by a Glassy Carbon Electrode Modified with Poly (Eriochrome Black T)

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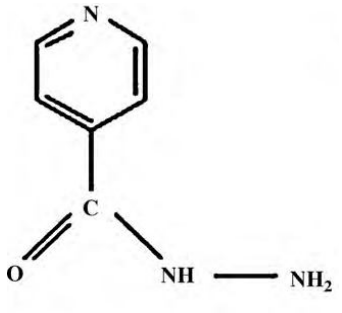
In this work, poly eriochrome black T (EBT) was electrochemically synthesized on the glassy carbon electrode as electrode modifier. On the modified electrode, voltammetric behavior of isoniazid (INH) was investigated. The poly (EBT)-modified glassy carbon electrode has excellent electrocatalytic ability for the electrooxidation of isoniazid. This fact was appeared as a reduced overpotential of INH oxidation in a wide operational pH range from 2-13. It has been found that the catalytic peak current depends on the concentration of INH and solution pH. The number of electrons involved in the rate determining step was found to be one. The diffusion coefficient of isoniazid was also estimated using chronoamperometry technique. The experimental results showed that the mediated oxidation peak current of isoniazid is linearly dependent on the concentration of isoniazid in the ranges of 8.0×10^{-6}-1.18×10^{-3} M and 2.90×10^{-5} M-1.67×10^{-3} M with differential pulse voltammetry (DPV) and amperometry methods, respectively. The detection limits (S/N = 3) were found to be 6.0 µM and 16.4 µM by DPV and amperometry methods, respectively. This developed method was applied to the determination of isoniazid in tablet samples with satisfactory results.

Keywords: Isoniazid, Poly (eriochrome black T) modified electrode, Voltammetric behavior, Determination

INTRODUCTION

According to the World Health Organization (WHO), Tuberculosis (TB) is a public health disease that produces several million deaths annually worldwide [1]. Isoniazid (pyridine-4-carboxilic acid hydrazide or isonicotinic acid hydrazide) is an effective drug in the treatment of tuberculosis, which is used alone or in combination with some other drugs, such as rifampicin and pyrazinamide [2]. Isoniazid (INH) is used as a first line treatment of tuberculosis and is synthesized from the reaction of ethyl isonicotinate with hydrazine (HZ) (Scheme 1) [3].

IHN metabolism is associated with the hydrazine production and subsequently hepatotoxicity which is known as a major cause for death in patients. Therefore, it is necessary to control the level of INH at regular intervals. Thus, from the clinical point of view, it is necessary to explore a stable and reliable analytical method for the quantification of INH. Several analytical methods have been developed for the determination of INH, such as titrimetry [4,5], spectrophotometry [6-13], chemiluminescence [14-16], fluorimetry [17,18], chromatography (HPLC, GC and HPTLC) [19-26], capillary electrophoresis [27,28] and electroanalytical [29-37]. Among the various techniques



Scheme 1. The structure of isoniazid

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mentioned, electroanalytical techniques were advantageous due to fast response, practicality, simplicity, low-cost, good sensitivity, precision, free from organic solvents and no separation techniques involved. However, at the bare unmodified electrodes large overpotential is required for oxidation of isoniazid. In general, to overcome this problem, various types of the modified electrodes are used to detect isoniazid [33-35]. Carbon electrodes, especially glassy electrodes, are widely used in electrochemical investigations because of their low background current, wide potential windows (anodic and cathodic), chemical inertness, low cost and suitability for detection of various organic and biological compounds [38]. Among these, glassy carbon electrodes due to unique characteristics, *e.g*., versatility of chemical modification, renewability of the electrode surface and compatibility with various electron mediators has been extensively studied.

In this work, an electropolymerized film of eriochrome black T (EBT) was prepared on the surface of a glassy carbon electrode by cyclic voltammetry (CV). The experimental results showed that poly (eriochrome black T) film performed a good electrocatalytic activity toward the oxidation of isoniazid. This method was successfully applied to the determination of isoniazid in pharmaceutical formulations with satisfactory results.

EXPERIMENTAL

Reagents and Instrumentation

All reagents were of analytical grade and used without any further purification. EBT coming from Merck is stored in a refrigerator and prevented from light. INH tablets were taken from an Iranian pharmaceutical company with a claimed value of 300 mg INH. Deionized water was used for the preparation of all solutions.

The 0.1 M ammonia buffer solution made up of NH_3 and NH_4Cl was employed as a supporting electrolyte. All electrochemical experiments were performed using an Electro Analyzer system (Sama 500, Iran) running on a PC. Measurements of pH were made with a Metrohm 827 pH-meter using a combined glass electrode. A glassy carbon disk electrode with 2 mm diameter was used as a working electrode. A platinum wire was employed as counter electrode and a saturated calomel electrode (SCE) served as the reference electrode and all potentials in the text refer to it (all electrodes obtained from Azar Electrode Co., Urmia, Iran). All experiments were performed at room temperature without removing the dissolved oxygen.

Preparation of the Modified Electrode

The glassy carbon electrode was polished with 0.05 μm alumina in water slurry using a polishing cloth, and then rinsed with deionized water and sonicated in ethanol and deionized water (1:1) for 5 min each time to remove alumina particles and other possible contaminants. The polished electrode was electrochemically pretreated by cycling the potential scan between -0.4 V and 1.5 V in 0.1 M H_2SO_4 at the scan rate of 100 mV s^{-1} for 25 times and then was scanned in 0.1 M NaOH under the same conditions for 25 times to obtain the pretreated electrode. The poly (EBT)-coated electrode was fabricated on the same conditions with the pretreated electrode but in the presence of 0.1 M NaOH containing 0.4 mM EBT.

RESULTS AND DISCUSSION

Electrochemical Polymerization of EBT on GCE

Poly (EBT) film was prepared by cyclic voltammetry method. Before the electro-polymerization process, the GCE was pretreated by scanning in the 0.1 M H_2SO_4 solution and then scanned in 0.1 M NaOH in the same potential range for 25 times. Then, the electrode was made to undergo multiple cycles in 0.1 M NaOH containing 0.4 mM EBT. During the process of multiple cycles the voltammogram has gradually descended with increase of cyclic time. This shows that the poly (EBT) film has been formed and deposited on the surface of GCE [39-41]. The oxidation mechanism of EBT was described with Yao *et al.* [40]

Electrocatalytic Response of INH at Poly (EBT) Modified GCE

Differential pulse voltammetry was employed to evaluate the electrochemical activity of INH at poly (EBT) modified GC electrodes. Figure 1 shows the cyclic voltammograms of 1 mM INH at a bare (a) and modified (b) glassy carbon electrode at pH 9 NH_3/NH_4Cl buffer solution. The voltammogram of c is the background. At the bare and

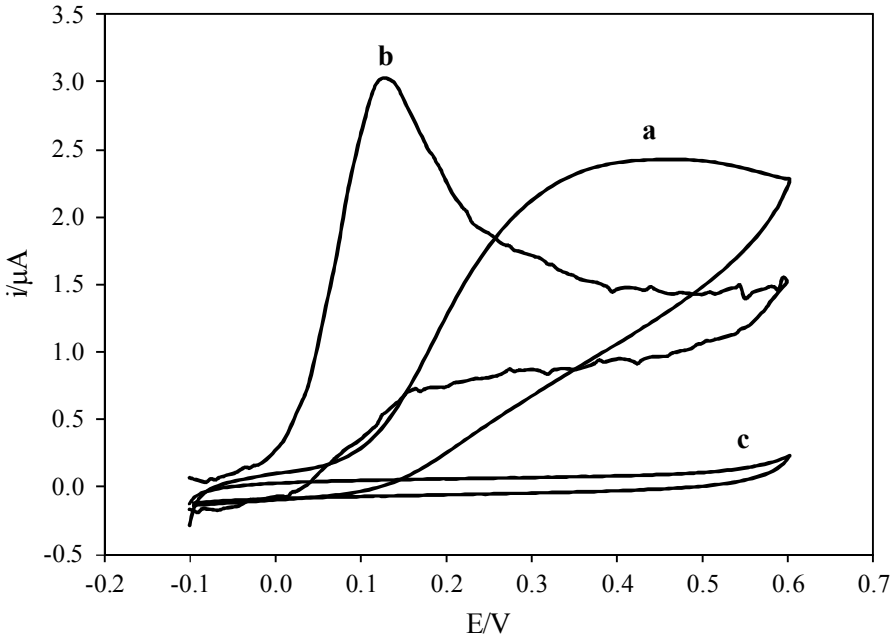


Fig. 1. Cyclic voltammograms of 0.25 mM INH at a bare (a) and modified (b) glassy carbon electrode in pH 9 NH_3/NH_4Cl buffer solution. The voltammogram of (c) is the background.

without pretreatment GC electrode, INH shows a featureless voltammogram (Fig. 1a). However, at the poly (EBT) modified electrode, it appears at 105 mV (Fig. 1b). In comparison with the data at the bare GC electrode, an increase in peak current and a decrease in the overpotential of INH are obtained at the poly (EBT) modified glassy carbon electrode. These results show that the kinetic of the charge transfer rate of modified electrode is higher than that of the bare electrode. This fact shows the effect and role of poly (EBT) as a suitable modifier and excellent intermediate charge transfer.

Effect of pH and Scan Rate

The effects of pH played an important role, so the electrochemical behavior of INH with Poly (EBT) modified glassy carbon electrode was studied with Britton–Robinson buffer over the pH range of 2.0-13.0. Figure 2 represents the differential pulse voltammograms of 1 mM INH at poly (EBT)/GCE in the pH range from 2-13 at a scan rate of 50 mV s^{-1}. The peak potential shifts towards negative potential with the increase in pH. The plot of peak potential *vs*. pH shows two linear ranges. It depends on the pK_a of isoniazid and were confirmed by Majidi *et al.* [33]. The following reactions could be considered for electrooxidation of isoniazid at different pHs [33]:

At the pH < pKa the electrooxidation of isoniazid is:

$$PyCoNHNH_3^+ + H_2O \rightarrow PyCoOH + N_2 + 5H^+ + 4e \quad (1)$$

And at the pH > pKa

$$PyCoNHNH_2 + H_2O \rightarrow PyCoOH + N_2 + 4H^+ + 4e \quad (2)$$

At pH 9 the peak current is high and peak potential is low. Therefore, all subsequent experiments were carried out at pH 9. The mechanism of oxidation INH on the modified electrode was shown as Scheme 2 (at pH > pKa).

The effect of the scan rate varying from 5 to 500 mV s^{-1} on the voltammetric response of poly (EBT)/GC electrode in a solution containing 1 mM INH was studied (Fig. 3). The anodic currents increase and the peak potential shifts as the scan rate increases. When peak current values were plotted against $v^{1/2}$, a linear relationship with $R^2 = 0.998$ was obtained. This behavior suggests that the oxidation process

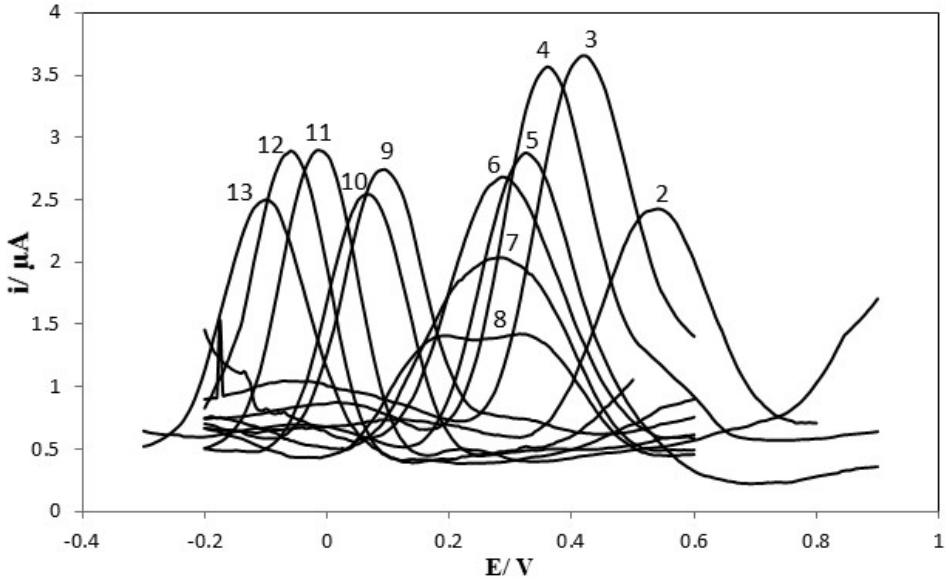
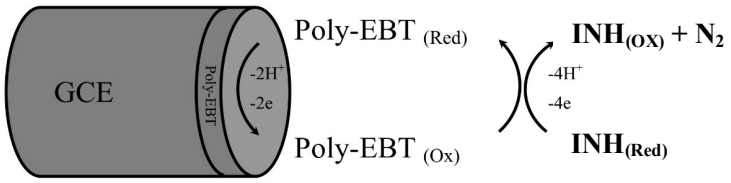


Fig. 2. Differential pulse voltammograms recorded for a 1 mM concentration solution of INH at scan rate of 50 mV s^{-1} in 0.04 M Britton-Robinson buffer at various pH values from 2 to 13.



Scheme 2. Mechanism of INH oxidation on the poly-EBT GC modified electrode

is controlled by diffusion. For determination of the transfer coefficient (α) and the number of electrons involved in the rate-determining (n_α), Tafel plot was drawn using the data from the raising part of the current-voltage curve at a scan rate of 10 mV s^{-1}. The Tafel slope was 10.167 V $decade^{-1}$ and by considering that α *is* equal to 0.4, the results indeed suggest one-electron ($n_\alpha = 0.999 \approx 1$) transfer process at the rate-determining step for the electrocatalytic oxidation of INH. The Tafel slope could be calculated by another method, *i.e.* it can be determined from the following equation [42]:

$$Ep = b/2 \log(v) + k \quad (3)$$

where b is the Tafel slope and k is a constant value. Figure 3 (inset C) shows the plot of Ep *vs*. $\log(v)$. The slope of this plot is 0.0524 V, therefore, the slope becomes 0.1048 V $decade^{-1}$. This result is close to that obtained from the rising part of voltammogram recorded at the 10 mV s^{-1}. If α was assumed to be 0.4, n_α would be 0.94, close to 1.0 [43].

Determination of Isoniazid

The electrocatalytic oxidation of INH at the poly (EBT)/GC electrode was studied by chronoamperometry. Chronoamperometry is used for the estimation of the diffusion coefficient of INH. For an electroactive material with diffusion coefficient D, the current corresponding to

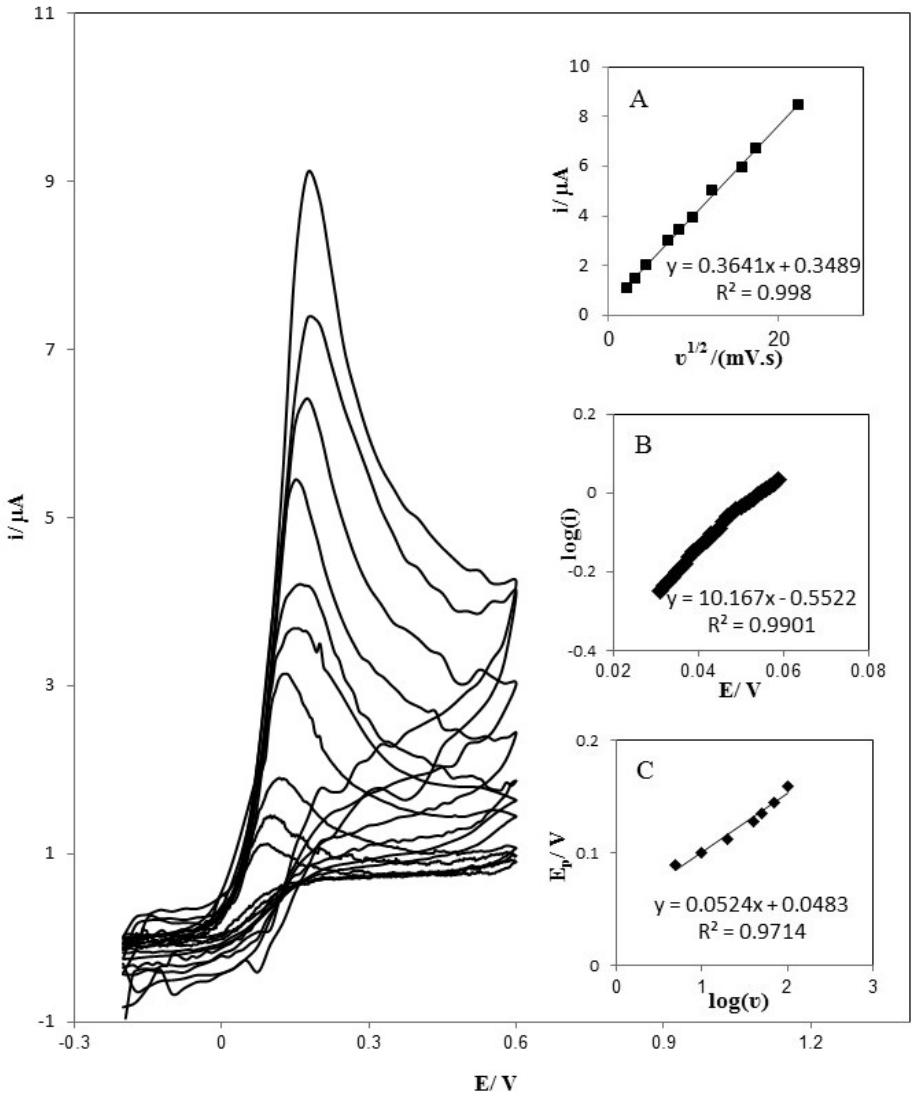


Fig. 3. Dependence of the cyclic voltammetric response at a poly (EBT) modified glassy carbon electrode in 0.1 M ammonia buffer (pH 9) containing 1 mM INH. Scan rate: 5, 10, 20, 50, 70, 100, 150, 250, 300 and 500 mV s^{-1}, respectively. Inset A: Dependence of the peak current with square root. Inset B: Tafel plot derived from the rising part of voltammogram recorded at a scan rate 10 mV s^{-1}. Inset C: plot of Ep *vs*. log*v*.

the electrochemical reaction (under diffusion control) is described by Cottrell's equation [43].

$$I = nFAD^{1/2}C/\pi^{1/2}t^{1/2} \quad (4)$$

where D is the diffusion coefficient ($cm^2\ s^{-1}$) and C is the bulk concentration (mol cm^{-3}). The experimental plots of I *vs*. $t^{-1/2}$, which are linear, are illustrated in Fig. 4A. The plot of the slopes of these lines in Fig. 4A, *vs*. the concentration of isoniazid can be used to obtain the value of D, (Fig. 4B). The diffusion coefficient was calculated as 1.706×10^{-6} cm^2 s^{-1} for isoniazid which is comparable to the reported value

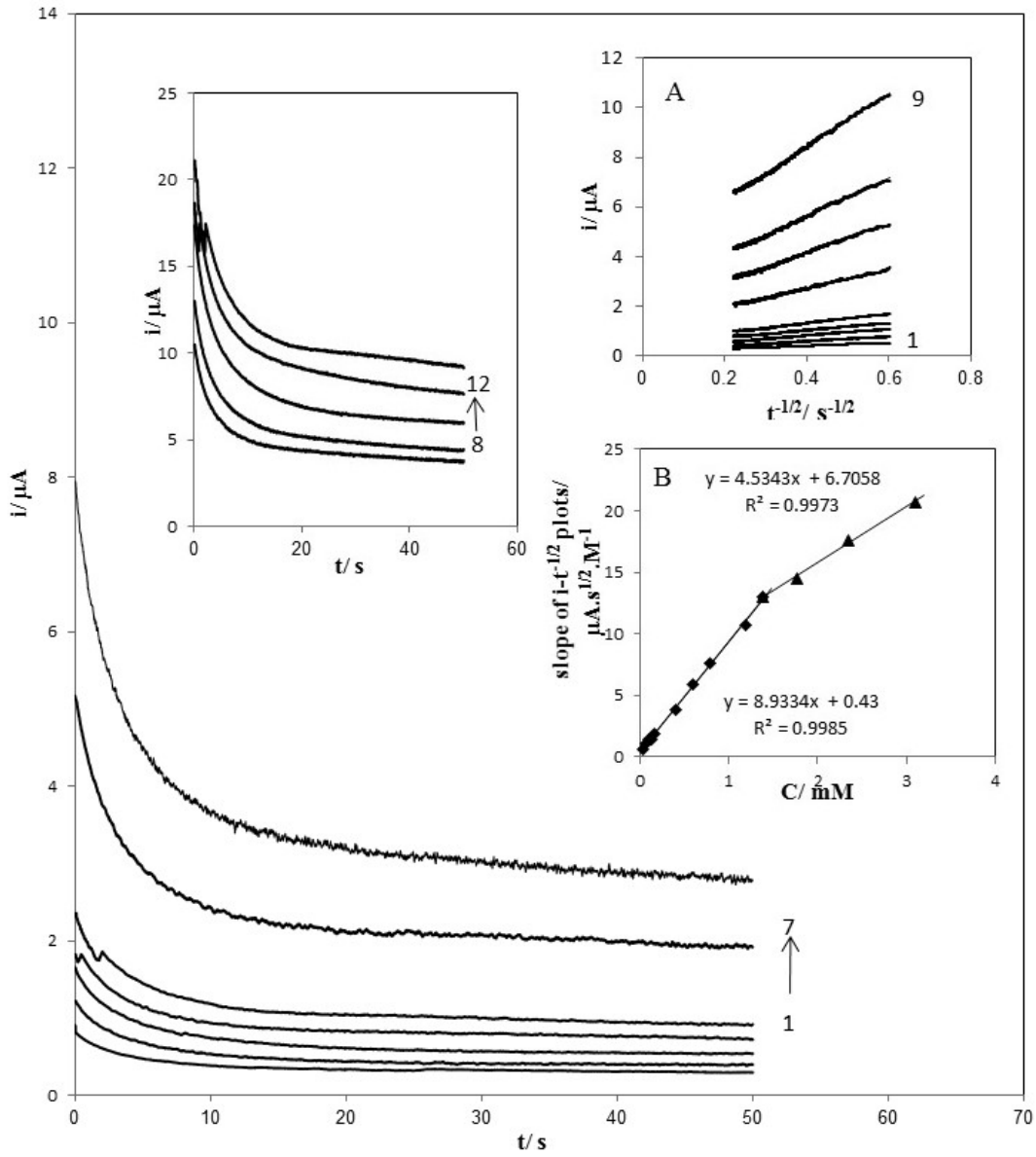


Fig. 4. Chronoamprometric response of a poly (EBT)/GCE in 0.1 M ammonium buffer solution (pH 9) containing different concentrations of INH for a potential step of 400 mV *vs*. SCE. The numbers 1-12 correspond to 2.90×10^{-5}-3.10×10^{-3} M of INH. Insets: Plots of *Ip vs*. $t^{-1/2}$ (A) and the relationship between the slope of the linear segments *vs*. the INH concentration (B).

[44].

DPV technique was used for determination of isoniazid at poly (EBT) modified glassy carbon electrode in static solutions. Figure 5 shows the differential pulse voltammograms obtained for various concentrations of INH at poly (EBT)/GCE in pH 9. The INH concentration in the solution was increased by adding several volumes of INH solution. The linear dependence of *I*p with the concentration of INH is given in Fig. 6, which was linear in the concentration range of 8.0×10^{-6}-1.18×10^{-3} M INH with

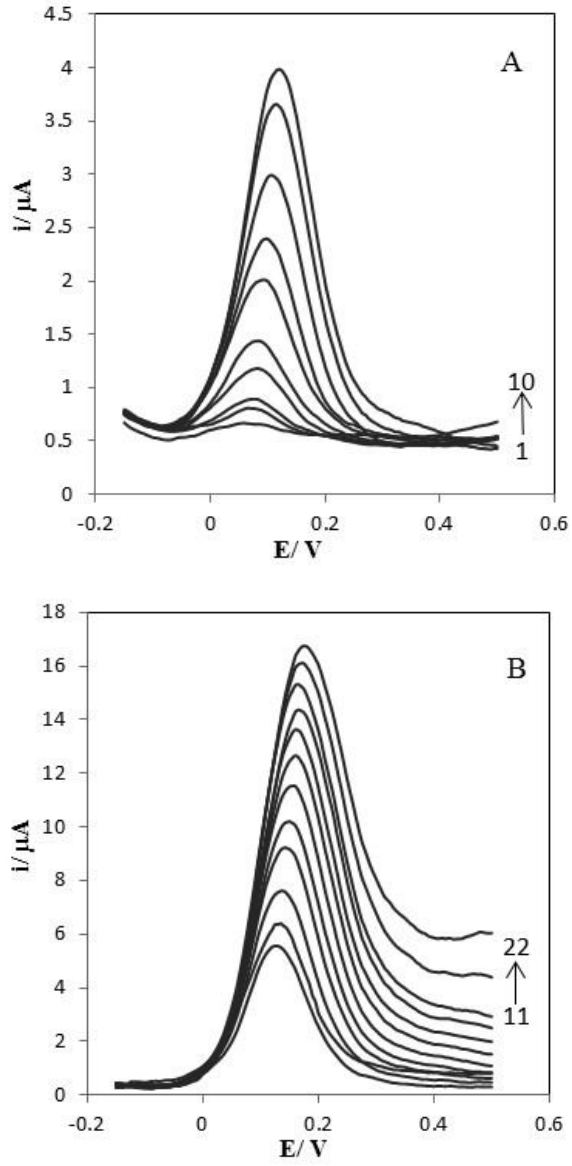


Fig. 5. Differential pulse voltammograms for increasing concentrations of INH from (1) 8.0×10^{-6} to (21) 1.18×10^{-3} M in buffer solution (pH 9.0) on poly (EBT)/GC electrode. Scan rate was 50 mV s^{-1}.

the following regression *I*p (μA) = 10.667 C (mM) + 0.2367, R^2 = 0.997. The detection limit calculated from the calibration graph was 6.0 μM INH. Table 1 indicates the linear calibration range reported for several INH sensors [33-36,44] together with other properties. This clearly explains that the present work reports the reasonable detection limit for INH determination.

The isoniazid analysis was quantified by using amperometric (i-t) measurement. Figure 7A represents the amperometric response of INH recorded from stirred solutions under conditions where the poly (EBT) modified electrode potential was kept at 400 mV during the successive addition of INH. The current dependence to INH concentration in the range of 2.9×10^{-5} M-1.67×10^{-3} M is

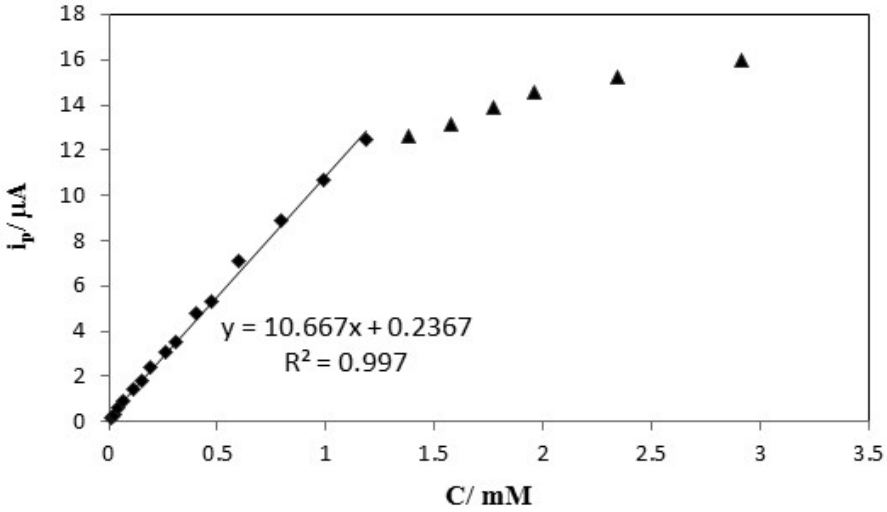


Fig. 6. Calibration plot for concentrations of INH from differential pulse voltammograms.

shown in Fig. 7B and there are two linear segments with different slopes, corresponding to two different ranges of substrate concentration linking this to a change in catalytic reaction conditions arising from the formation of nitrogen gas bubbles at the surface of the electrode. Indeed, at low substrate concentrations, gas formation is negligible, and has no effect on the diffusion of INH towards the electrode surface. While, at high concentrations of INH, gas evolution at the electrode surface slackened to some extent the normal diffusion of substrate [33]. The limit of detection (LOD) is 16.4 µM and has been calculated based on the signal to noise ratio (S/N) of 3. This result shows a good stability of the proposed electrode and the efficient amperometric detection of INH.

Real Sample Analysis

The real sample analysis is very important to evaluate the practicability of the proposed electrode. The proposed method was applied to the determination of INH in tablets by using differential pulse voltammetry with the standard addition method and gave mean values of 309.051 ± 0.3 mg of INH per tablet for three replicates. The same samples were analyzed using the United States Pharmacopoeia (USP) method [2], recommending a direct spectrophotometric measurement at 267 nm for the assay of INH and 303.421 mg was found for each tablet, which is in a good agreement with the declared value of 300 mg, and indicating that present electrode can be used for the determination of INH present in the pharmaceutical formulations.

CONCLUSIONS

This study showed that INH can be determined using the differential pulse voltammetry and amperometry techniques on the basis of its oxidation process at a polymerized film of eriochrome black T (EBT) modified glassy carbon electrode. The overall number of electrons involved in the oxidation of INH, the number of electrons involved in the rate-determining step and the diffusion coefficient of INH were calculated. The proposed modified electrode was successfully applied to the determination of INH in pharmaceutical formulations with adequate reproducibility and sensitivity. A faster analysis for INH can be performed by direct measurement from the standard addition method.

ACKNOWLEDGEMENTS

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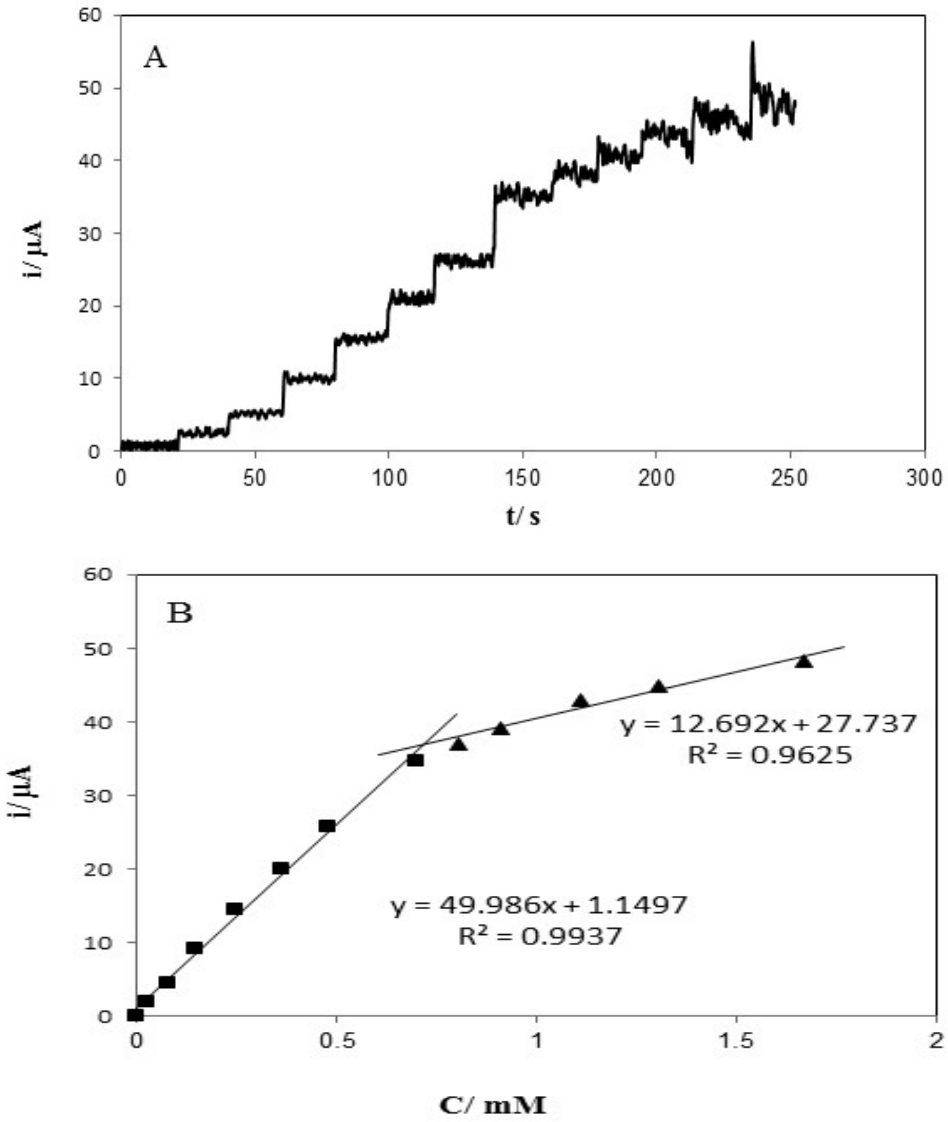

Fig. 7. A) Amperometric response of a poly (EBT) modified glassy carbon electrode kept in 400 mV in 0.1 M ammonium buffer solution pH 9 containing different concentrations of INH from 2.90×10^{-5} to 1.67×10^{-3} M. B) Calibration plot of INH.

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