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Electrochemical Investigation of Isoniazid on Poly (*p*-Aminobenzene Sulfonic Acid) Film Modified Glassy Carbon Electrode

Esra Demirbilek, Gulsen Saglikoglu[*]*, Selehattin Yilmaz*

Canakkale Onsekiz Mart University, Faculty of Sciences and Arts, Department of Chemistry, Terzioglu Campus, 17020, Canakkale, Turkey
[*]E-mail: gulsensaglikoglu@hotmail.com

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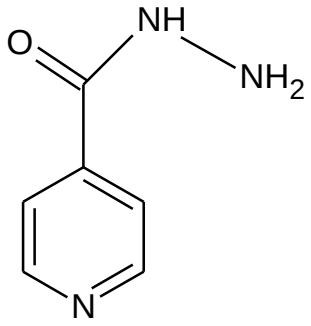
Isoniazid is a tuberculostatic agent effective active compound. A glassy carbon electrode (GCE) was modified with an electropolymerized film of *p*-aminobenzene sulfonic acid (*p*-ABSA) in phosphate buffer solution (PBS). The electrochemical properties of isoniazid on modified electrode were studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The polymer film-modified electrode was used to electrochemically detect isoniazid. The polymer film had excellent electrocatalytic activity for the oxidation of isoniazid. A linear calibration curve for DPV analysis was constructed in the isoniazid concentration range $2x10^{-5}$- $1x10^{-4}$ mol L^{-1}. Limit of detection (LOD) and limit of quantification (LOQ) were obtained as $3.05x10^{-6}$ and $1.01x10^{-5}$ mol L^{-1} respectively. Isoniazid was determined in tablet dosage form. The proposed method exhibits good recovery and reproducibility. The mechanisms of electropolymerization were also suggested**.**

Keywords: *p*-Aminobenzene sulfonic acid; Modified-glassy carbon electrode; Electropolymerization; Electrocatalytic-oxidation; Determination of isoniazid

1. INTRODUCTION

Isonicotinic acid hydrazide or isoniazid shown in Scheme 1 is a tuberculostatic agent effective against mycobacterium strains widely used for clinical purposes. Isoniazid may have bacterisotatic or bacteriocidal action, depending on the concentration the drug attains at the site of infection and the susceptibility of the infecting organism [1].



Scheme 1. Chemical structure of isoniazid.

Isoniazid has been determined in various pharmaceutical preparations, blood plasma, tissues and urine by high performance liquid chromatography–tandem mass spectrometry [2], capillary electrophoresis [3], flow injection chemiluminescence [4], and spectrophotometric methods [5]. Most of the reported methods require a long time for sample pretreatment, expensive reagents and equipment, which are not useful for routine analysis studies. Voltammetry is a practical analytical technique that offers high sensitivity, precision, and accuracy, wide linear range, low-cost instrumentation and little time.

Metabolism of isoniazid produces hydrazine which induces hyper-toxicity and sometimes may cause death too. Therefore, patients on this medicine are generally examined for their isoniazid level at regular intervals. Thus, from the clinical point of view it is necessary to have a sensitive analytical method for real-time quantification of isoniazid. It is also essential for pharmaceutical industries to determine the isoniazid level in their products for quality control purposes [6].

An inherent advantage of voltammetric measurements is that they can provide a wide range of information about the redox transformation of an analyte including thermodynamic, kinetic, and mechanical information. An additional advantage of voltammetric measurements is that many analytes are extremely sensitive to the chemical environment at the electrode surface. For example, analyte/surface interactions (e.g. adsorption and electrostatic interactions) can result in changes in the thermodynamic and kinetic behavior by stabilizing or destabilizing reactants, products, and/or intermediates in the redox reaction [7].

Chemically modified electrodes comprise a relatively modern approach to electrode systems. They find utility in a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and ionic transport phenomena in polymers. They are also used for the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic syntheses. [8]

A few electrochemical studies have been conducted on the electrochemical behavior and determination of isoniazid in pharmaceuticals and biological samples by modified electrodes. The electrochemical behavior of isoniazid was studied using different modified electrodes such as polypyrrole glassy carbon modified electrode [9], thionine immobilized multi-walled carbon nanotube

modified carbon paste electrode [10], screen-printed carbon electrode modified with poly-L-histidine [11], modified rhodium electrode [12], and ordered mesoporous carbon modified electrode [13].

One of the methods used for the modification of electrode surfaces is electropolymerization. Electropolymerization can accelerate transmission of electrons onto the surface of the electrode, it has high selectivity and sensitivity due to the film homogeneity in electrochemical deposition, and it has strong adherence to the electron surface and large surface area [14,15]. Researchers have employed polymeric film-modified electrodes to detect organic and inorganic molecules in recent years.

Isoniazid has not been studied for determination of its drug forms and electrocatalytic-oxidation behavior on poly(*p*-ABSA)-modified glassy carbon electrodes. In this study, we prepared the poly(*p*-ABSA) modified GCE [16,17] and studied the electrochemical properties of isoniazid on the modified glassy carbon electrode. Quantitative analysis was performed on the drug active substance in tablet dosage form on modified GCE electrode. It showed excellent electrocatalytic activity for oxidation of isoniazid in 0.04 mol L^{-1} Britton Robinson buffer pH 9.

2. EXPERIMENTAL

2.1. Reagents and Materials

Isoniazid and its tablet dosage forms of the brand isovit were kindly supplied by Deva (Turkey). Their stock solutions (0.01 mol L^{-1}) were prepared with methanol. *p*-aminobenzensulfonic acid (Sigma Aldrich) solution was prepared with 0.1 mol L^{-1} PBS and used without any further purification. 0.1 mol L^{-1} phosphate buffer solution pH and 0.04 mol L^{-1} Britton Robinson buffer from pH 4 to pH 10 were used as the supporting electrolytes. All aqueous solutions were prepared in twice-distilled de-ionized water and used analytical grade chemicals. To remove oxygen in experimental solutions argon gas (99% purity) was used.

2.2. Apparatus

A Model Autolab PGSTAT 101 (Netherlands) was used for the voltammetric measurements, with a three-electrode system consisting of glassy carbon working electrode (GCE) [surface area (ϕ) 7 mm; disc diameter (R) 2 mm, Metrohm], a platinum wire auxiliary electrode and Ag/AgCl (KCl 3 M, Metrohm) reference electrode. The bare GCE was polished successively with 0.3 and 0.1 diamond suspension and 0.05 μm Al_2O_3 slurry on a polishing cloth before electrochemical modification. Firstly, the deoxygenating process of the supporting electrolyte solutions was carried out with argon gas for 5 min before all experiments. Then, in the experiments the argon gas was also passed through the solutions for 60 s after the addition of each sample solution. Monomer solutions were purged with argon gas for about 30 min before polymerization and the solution was blanketed with the same gas during electropolymerization. In each new experiment, a new bare electrode surface was used. All pH measurements were made with EZDO 5011 A. All measurements were carried out at ambient

temperature of the laboratory (15-20 °C). A Wise Clean model sonicator was used to clean the surface of electrodes. The 0.055 μS/cm ultra pure water (UPW) was used throughout the experiments.

For analytical application, the following parameters were employed: pulse amplitude (pulse amplitude of the voltage pulse superimposed on the direct voltage) 50 mV; pulse time (time interval during which a voltage pulse is superimposed on the direct voltage) 0.04 s, voltage step (voltage step for direct voltage ramp) 0.009 V, voltage step time (time interval after which the voltage in the sweep is increased or decreased by the amount of the voltage step) 0.04 , potential step 10 mV (DPV); and scan rate (display of the ramp slope calculated as voltage step/voltage step time) in the range 100-300 mV s^{-1} (CV).

2.3. Preparation of poly(p-Aminobenzene Sulfonic Acid)-modified GCE

Prior to electrochemical modification, the bare GCE was polished successively with 1 μm diamond paste, 3 μm diamond paste and 0.05 μm Al_2O_3 slurry on a polishing cloth. Then it was rinsed with double-distilled water, and sonicated in 1:1 nitric acid, acetone and double-distilled water for 10 min, respectively. After being cleaned, the electrode was immersed in 2.0×10^{-3} mol L^{-1} *p*-ABSA solution and was conditioned by cyclic sweeping between −1.5 to +2.4 V at 200 mV s^{-1} for 10 scans (Fig. 1). In order to get a stable response prior to measurements, the resulting modified electrode was also continuously cycled for another few scans. Finally, the modified electrode was carefully rinsed with distilled water, and used for analysis of isoniazid and stored in air for later use [16-18].

3. RESULTS AND DISCUSSION

3.1. Electropolymerization of p-aminobenzene sulfonic acid at the GCE surface

The electropolymerization of *p*-ABSA on GC electrode surface was performed by repetitive cyclic voltammetry (Fig. 1).

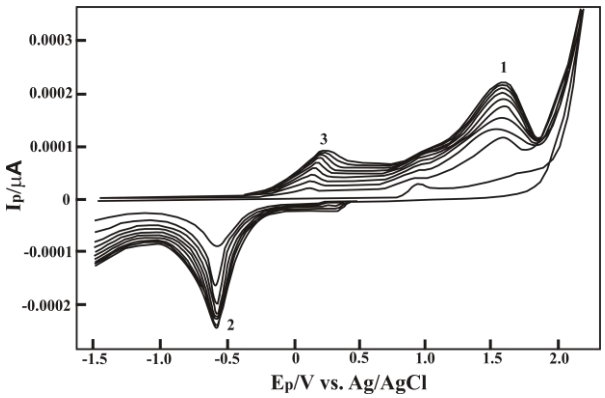
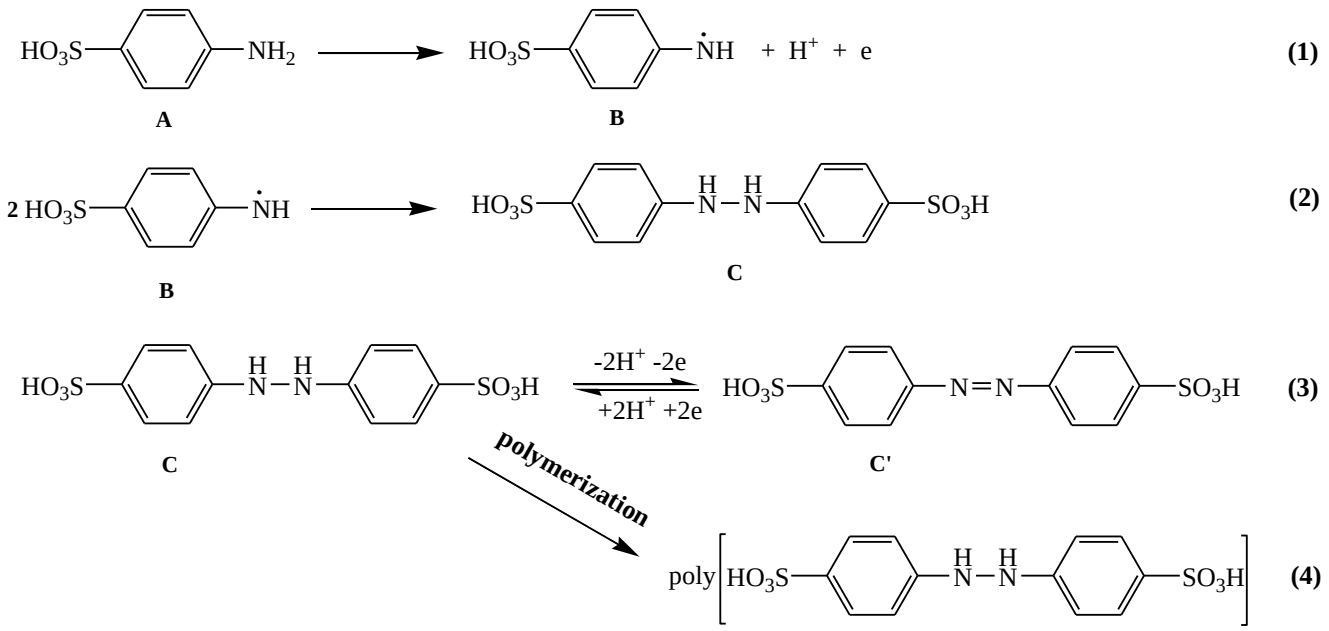


Figure 1. Repetitive cyclic voltammograms of 2.0×10^{-3} mol L^{-1} *p*-ABSA on bare GCE. Initial potential: -1.5 V; terminal potential: +2.4 V. Scan rate: 200 mVs^{-1}; supporting electrolyte: 0.1 mol L^{-1} PBS (pH 7.0).

As can be seen in Fig. 1, in the first scan, anodic peak 1 and cathodic peak 2 were observed with peak potential value at about +1.5 V and −0.65 V, respectively. From the second cycle on, anodic peak 3 appeared with potential at about +0.15 V. Then larger peaks were observed upon continuous scanning, reflecting the continuous growth of the film. These facts indicated *p*-ABSA was deposited on the surface of GCE by electropolymerization. A uniform adherent blue polymer film was formed on the GCE surface. After modification, the poly(*p*-ABSA) film electrode was carefully rinsed with doubly distilled water, then stored in pH 7.0 PBS for later use.

The electropolymerization behavior of *p*-ABSA on GCE was similar to the reported references [17-21]. We propose the reaction mechanism is similar to the literature [17-21] as in Scheme 2.



Scheme 2. Suggested electrochemical polymerization of *p*-ABSA on GCE.

As can be seen from Scheme 2, *p*-ABSA (A) was first oxidized to free radical (B) (peak 1); the free radical (B) combined together rapidly to form hydrazobenzene sulfonic acid (C); then hydrazobenzene sulfonic acid (C) was oxidized to azobenzene sulfonic acid (C′) (peak 3), and azobenzene sulfonic acid (C′) was reduced to hydrazobenzene sulfonic acid (C) (peak 2) and finally the electrode surface was covered by the formed polymer (D).

3.2. Electrochemical response of isoniazid on poly(p-ABSA)-modified GCE

Fig. 2 shows CV curves of 5.0×10^{-5} mol L^{-1} isoniazid on bare GCE (b) and poly(*p*-ABSA)-modified GCE (c) in 0.04 mol L^{-1} BR (pH 9.0), respectively.

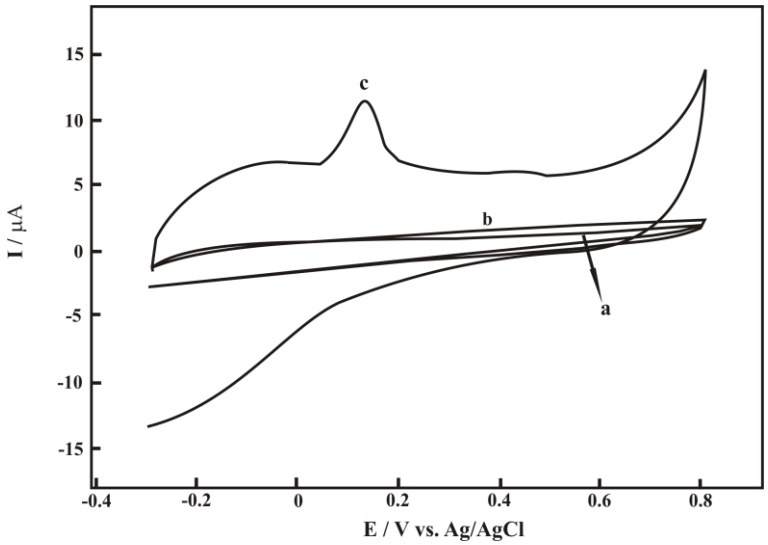
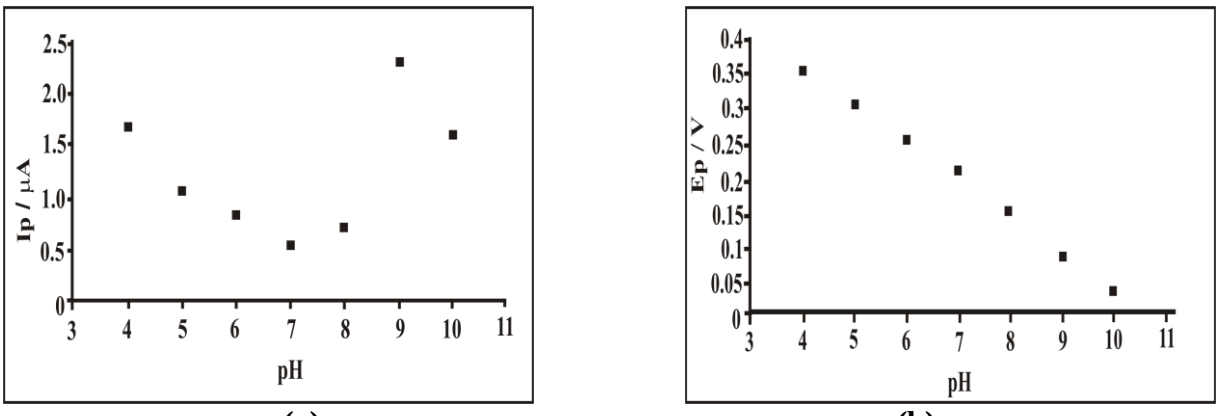


Figure 2. CV voltammograms of $5.0x10^{-5}$ mol L^{-1} isoniazid a) blank, b) at bare GCE c) at poly (*p*-ABSA)-modified GCE. Scan rate: 100 mVs^{-1}; supporting electrolyte: 0.04 mol L^{-1} BR (pH 9.0).

In blank buffer solution, no obvious peaks were observed on both bare GCE and poly(*p*-ABSA)-modified GCE. The modified electrode provides greater and more sensitive signal for the determination of isoniazid. Oxidation peak potential of isoniazid was shifted positively and peak current increased about 14 times on the modified-GCE. As result poly(*p*-ABSA)-modified GCE exhibited electro-catalytic behavior towards the electrochemical oxidation of isoniazid due to its fast electron transfer capability [19-23].

3.3. Effect of pH on oxidation of isoniazid



(a) **(b)**

Figure 3. (a) Effect of pH on DPV peak current of $5x10^{-5}$ M isoniazid. **(b)** Effect of pH on DPV peak potential of $5x10^{-5}$ M isoniazid.

In order to obtain the optimum experimental conditions, some variables affecting the peak current and peak potential, which are pH and the species of supporting electrolyte, were studied for an

isoniazid solution of $5.0x10^{-5}$ mol L^{-1} on modified-GC electrode by the proposed voltammetric techniques. The voltammograms of isoniazid on modified-GC electrode were recorded in BR buffer from pH 4 to pH 10.

The effect of pH on the electrocatalytic oxidation of isoniazid is shown in Fig. 4. Anodic peak potentials (Fig. 3b) are shifted to being less positive with increasing pH values. The anodic peak potentials of isoniazid shift from 350 mV to 33 mV with respect to pH from 4 to 10, indicating that protons take part in the electrode process. The peak current was decreased by increasing the pH from 4.0 to 7.0, and then increased from 7.0 to 9.0. After pH 9.0, the peak current was decreased. Therefore, the higher peak current and better shape of the voltammogram peak at pH 9.0 suggested this pH as the optimal pH value for our purposes [19-29].

3.4. Effect of scan rate on oxidation of isoniazid

The effect of scan rate on the oxidation peak current of $5.0x10^{-5}$ mol L^{-1} isoniazid was studied. With increasing scan rate, the anodic peak current increased. A good linearity between the square root of scan rate and peak current was obtained between the range of 100-300 mV s^{-1} (Fig. 4). The linear regression equation was $I_p(\mu A)= 0.381\upsilon^{1/2}-1.219$ with correlation coefficient (r=0.99). A linear relationship was observed between peak current and square root of scan rate with a correlation coefficient of r=0.99. The correlation coefficient is very close to 1.0 showing that the oxidation process is diffusion controlled. Also, the plot of logarithm of peak current versus logarithm of scan rate has a slope of 0.66 which is almost close to the theoretical value of 0.5. The equation was $\log I_p(\mu A)=0.66\log\upsilon-0.919$ (r=0.99) on modified electrode. The oxidation process is controlled by totally irreversible diffusion controlled processes. A plot of the sweep rate-normalized current (Ip/υ 1/2) versus sweep rate exhibits the characteristic shape typical of an EC' catalytic process [19-23].

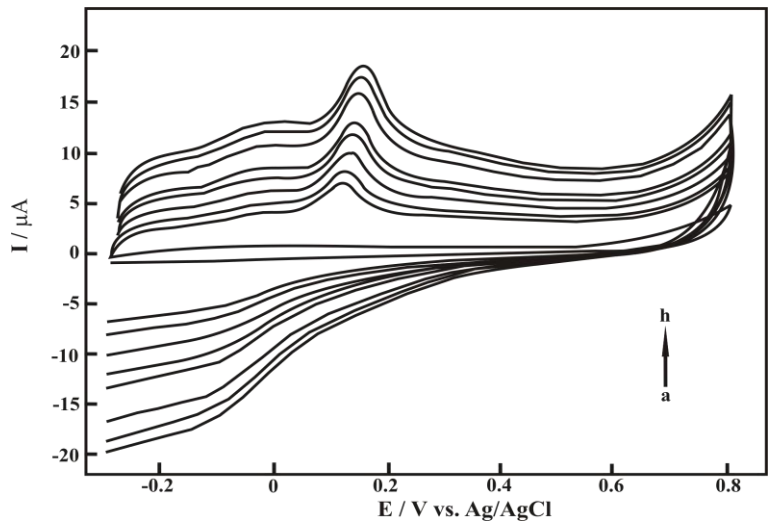


Figure 4. CV voltammograms of $5.0x10^{-5}$ mol L^{-1} isoniazid on poly(*p*-ABSA)-modified GCE; Scan rates: 100, 120, 150, 180, 200, 250, 280, and 300 mVs^{-1}; supporting electrolyte: 0.04 mol L^{-1} BR (pH 9.0).

3.5. Determination of isoniazid

The determination of isoniazid concentration on poly(*p*-ABSA)-modified *GC* electrode was performed with differential pulse voltammetry (DPV). Under the optimum analytical conditions, the determination of isoniazid was performed at different concentrations. A linear calibration curve (Fig**.** 5) was obtained for isoniazid in the range $2x10^{-5}$-$1x10^{-4}$ mol L^{-1} for 0.04 mol L^{-1} BR (pH 9.0) supporting electrolyte (Fig. 6).

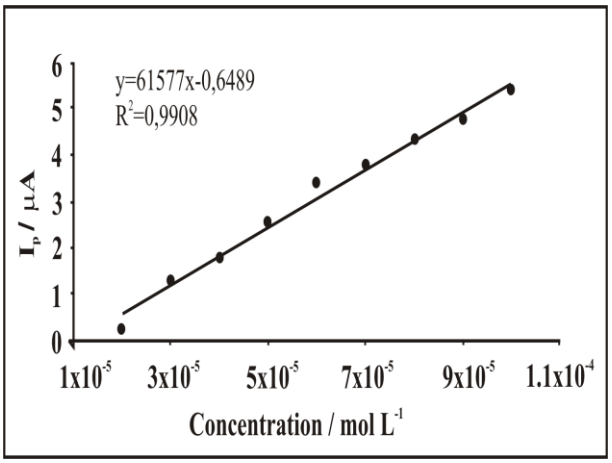


Figure 5. Plot of concentration versus current for isoniazid.

LOD and LOQ were calculated for the electro-oxidation peak current using the following equations. LOD = 3 s/m LOQ = 10 s/m; where s is the standard deviation of the peak currents (for five runs) and m is the slope of the calibration curve. The achieved LOD and LOQ were $3.05x10^{-6}$ and $1.01x10^{-5}$ mol L^{-1} for poly(p-ABSA)-modified GCE, respectively [19-21, 24-29].

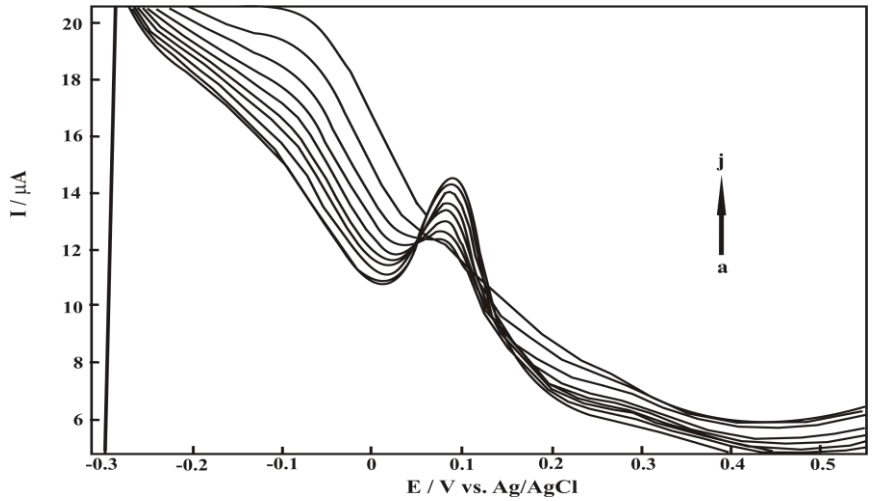


Figure 6. DPV voltammograms of isoniazid at poly(p-ABSA)-modified GC electrode; a) $2.0x10^{-5}$; b) $3.0x10^{-5}$; c) $4.0x10^{-5}$; d) $5.0x10^{-5}$; e) $6.0x10^{-5}$; f) $7.0x10^{-5}$; g) $8.0x10^{-5}$; h) $9.0x10^{-5}$ i)$1.0x10^{-4}$ mol L^{-1}. Supporting electrolyte: 0.04 mol L^{-1} BR (pH 9.0).

Validation of the procedure for the quantitative determination of isoniazid was investigated via evaluation of the limit of detection (LOD), limit of quantification (LOQ) and recovery studies by DPV technique (Table 1).

Table 1. Regression data of the calibration curve for assay of isoniazid by DPV.

Parameters	Results
Measured potential, V	0.087
Linear concentration range, mol L^{-1}	$2.0x10^{-5}$-$1x10^{-4}$ M
Slope, mA mol L^{-1}	369924
SD (Standard Deviation) of slope	11988
Intercept, nA	0.101
SD (Standard Deviation) of intercept	0.086
Correlation coefficient, r	0.994
Limit of detection (LOD), mol L^{-1}	$3.05x10^{-6}$
Limit of quantification (LOQ), mol L^{-1}	$1.01x10^{-5}$

$5.0x10^{-5}$ mol L^{-1} isoniazid was investigated repeatedly on an identical surface of poly(*p*-ABSA)-modified GCE 20 successive times. The average currents were 2.45 μA with the relative standard deviation (RSD) of 2.68%. It indicated excellent reproducibility. Also, the stability of the modified electrode was investigated. The modified electrode was stored in BS (pH 7.0) at 4 °C in a refrigerator. The peak current retained 97.4% of its initial response after storage in air for 15 days. This indicates that the modified electrode has good stability.

3.6. Analytical Applications

Table 2. Detection of isoniazid in commercial tablets and mean recoveries on poly(*p*-ABSA)-modified GCE by DPV**.**

Parameters	Results
Labeled acyclovir, mg	100
Amount of acyclovir found, mg	99.28
RSD[a], %	1.08
Bias, %	0.72
Spiked acyclovir, mg	40.00
Found, mg	39.36
Recovery, %	98.40
RSD of recovery, %	1.20
Bias, %	1.60

RSD is relative standard deviation

Five tablets of isovit®, containing 100 mg isoniazid per tablet, were accurately weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration $1x10^{-3}$ mol L^{-1}, was weighed and transferred into a 10 mL calibration flask and the volume was adjusted with methanol. The contents of the flask were centrifuged for 20 min at 4000 rpm to affect complete dissolution. The non-dissolved excipients settled on the bottom. Each solution was transferred into the voltammetric cell. The amount of isoniazid in isovit commercial tablets was calculated by reference to the appropriate calibration plots. For this reason, the proposed techniques were checked by performing recovery tests. The results obtained are given in Table 2. The proposed techniques could be successfully applied to isoniazid assay in tablets without any interference.

4. CONCLUSIONS

A poly(*p*-aminobenzene sulfonic acid)-modified GCE was fabricated by electropolymerization techniques in PBS using the cyclic voltammetry method. The modified GC electrode showed good electrocatalytic activity for the oxidation of isoniazid. The modified electrode provides greater sensitivity and selectivity in the determination of isoniazid. Moreover, the modified electrode showed easy regeneration, good reproducibility and stability. The proposed methods can be applied to the detection of isoniazid in practical drug samples.

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