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Poly(amidosulfonic acid) modified glassy carbon electrode for determination of isoniazid in pharmaceuticals

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ABSTRACT

Amidosulfonic acid was electropolymerized by cyclic voltammetry onto the surface of glassy carbon electrode (GCE) to fabricate the chemically modified electrode, which showed high stability, good selectivity and reproducibility for determination of isoniazid. The modified electrode showed an excellent electrocatalytical effect on the oxidation of isoniazid. Under the optimum conditions, there was a good linear relationship between anodic peak current and isoniazid concentration in the range of 5.0×10^{-8}– 1.0×10^{-5} M, and a detection limit of 1.0×10^{-8} M ($S/N = 3$) was obtained after 120 s at the accumulation potential of − 0.2 V (vs. SCE). This developed method had been applied to the direct determination of isoniazid in injection and tablet samples with satisfactory results.

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1. Introduction

Isoniazid (see Scheme 1), pyridine-4-carboxylic acid hydrazide, is an antituberculosis agent, which is now widely used together with other antituberculostatic agents for chemotherapy of tuberculosis. The importance of this drug is wide-spread use and its efficiency in the treatment of pulmonary tuberculosis. Many analytical methods have been reported for the analysis of isoniazid, such as spectrophotometry [1–3], high performance liquid chromatography [4–7], capillary electrophoresis [8], chemiluminescence [9–10] and fluorimetry [11]. Several electrochemical methods have also been studied by using different types of electrodes, such as differential pulse voltammetry at the gold electrode [12], square-wave adsorptive cathodic stripping voltammetry at the hanging mercury drop electrode [13] and cyclic voltammetry at the polypyrrole modified glassy carbon electrode [14].

A number of researchers have employed many kinds of polymeric film modified electrodes due to their features of good electrocatalysis and stability [15]. The chemically modified electrodes by polyaniline [16], poly (thiophene) [17] or polypyrrole [18] have been used to detect electroactive materials, such as dopamine, ascorbic acid, nitrate, norepinephrine and hemoglobin, and so on. To the best of our knowledge, there were very few reports about poly(amidosulfonic acid) film in the electroanalytical field [19]. In order to expend its application in electroanalytical field, the electrochemical sensor by poly(amidosulfonic acid) film modified glassy carbon electrode was fabricated. The experimental results showed that poly(amidosulfonic acid) film performed the good electrocatalytic activity toward the oxidation of isoniazid. And then an electrochemical method for the sensitive determination of isoniazid was established. This method was successfully applied to determine isoniazid in pharmaceutical formulations.

2. Experiment

2.1. Chemicals and materials

Isoniazid, which was kindly gifted by Yangzhou Institute of Drug Control, was used without further purification. A stock solution of isoniazid (1.0×10^{-2} M) was prepared with double-distilled water and kept in a refrigerator at about 4 °C. Amidosulfonic acid was obtained from Sinopharm chemical Reagent Co. (China). All of the other chemicals were of analytical-reagent grade.

2.2. Apparatus

Electrochemical measurements were performed with CHI660A Electrochemical Workstation (CHI Instruments Inc., USA). A glassy carbon electrode (GCE) modified with poly(amidosulfonic acid) was used as a working electrode, the reference electrode was a saturated calomel electrode (SCE) and the counter one was a platinum wire. All experiments were performed at 25.0 ± 0.5°C.

2.3. Fabrication of the poly(amidosulfonic acid) modified glassy carbon electrode (denoted as PASA-GCE) [19]

Prior to modification of PASA, the surface of glassy carbon electrode was polished by 0.05 μm aluminum suspension to a mirror finish. The

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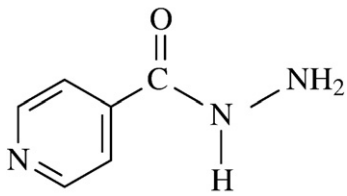

Scheme 1. Structural formula of isoniazid.

electrode was then washed with 1:1 HNO_3, acetone and doubly distilled water with ultrasonic bath, respectively. After rinsing in doubly distilled water, the electrode was activated by cyclic sweepings for 20 cycles from − 1.2 to + 1.2 V in phosphate buffer solution (pH 7.0). Finally, the film of poly (amidosulfonic acid) was deposited by cyclic sweeping from − 1.5 to + 2.5 V at 50 mV/s for 10 cycles in phosphate buffer solution (pH 7.0) containing 2.0×10^{-3} M amidosulfonic acid.

2.4. Analytical procedure

The required volume of isoniazid standard solution was added into the electrochemical cell containing 5 mL phosphate buffer solution (pH 6.5). The quantification of isoniazid was achieved by measuring their oxidation peak current by differential pulse voltammetry (DPV). The differential pulse voltammograms were recorded after accumulation of 120 s at the potential of − 0.2 V, and its parameters were selected: the increase potential of 8 mV, pulse amplitude of 50 mV, pulse width of 0.06 s, and sampling width of 0.02 s.

2.5. Preparation of tablet sample

Twenty tablets, each containing 100 mg/tablet of isoniazid, were accurately weighed and finely powdered, respectively. To accurately weighed amount of the powder equivalent to approximately 28 mg, 30 mL of water was added. The mixture was shaken for 20 min and filtered into a 100 mL volumetric flask. The residue was washed several times with water and solution was diluted to the mark.

3. Results and discussion

3.1. Electropolymerization of amidosulfonic acid at the surface of glassy carbon electrode

Fig. 1 shows the voltammograms of 2.0×10^{-3} M amidosulfonic acid in phosphate buffer solution (pH 7.0) at GCE. The multicycle curves show three major voltammetric peaks at about + 1.71 V (peak 1), − 0.57 V (peak 2) and + 0.29 V (peak 3). In the first cycle, an anodic oxidation peak 1 and cathodic peak 2 were obtained in the potential of − 1.5 to + 2.5 V. During the following cycles, a new unobvious anodic peak 3 appeared, and then larger peak was observed with the continuous scanning, indicating the continuous growth of the film. A certain blue-black substance was produced at the surface of glassy carbon electrode. It manifested that the poly (amidosulfonic acid) was electropolymerized on the surface of glassy carbon electrode.

3.2. Electrochemical behavior of isoniazid on PASA-GCE

3.2.1. Electrocatalytic effect on isoniazid

Fig. 2 shows the cyclic voltammograms of 1.0×10^{-4} M isoniazid in phosphate buffer solution (pH 6.5) at the PASA-GCE and at the bare GCE. It can be seen that the oxidation process of isoniazid is irreversible at bare GCE and PASA-GCE. However, the anodic peak current of isoniazid at the PASA-GCE was about 13 times larger than that at the bare GCE. At the same time, the more negative shift of 40 mV at PASA-GCE obtained compared to that at the bare GCE. These phenomena are clear evidences of electrocatalytic effect of the PASA modified electrode toward isoniazid oxidation. These results indicate that the PASA film is able to accelerate the rate of isoniazid electron transfer.

3.2.2. Effect of the scan rate on the peak current and peak potential

The effect of scan rate on the peak current at PASA-GCE in phosphate buffer solution (pH 6.5) was investigated by cyclic voltammetry in the presence of 1×10^{-4} M isoniazid (shown in Fig. 3a). The anodic peak current of isoniazid at PASA-GCE was proportional to square root of scan rate (see Fig. 3b), which indicates a diffusion controlled oxidation process at PASA-GCE. At the same time, the peak current increased with the increase of scan rate.

The experimental results show the good linear dependence of the oxidation peak potential upon the logarithm of the scan rate (log$v$), with a slope of 0.055 (see Fig. 3c). The peak potential also shows a linear dependence upon pH over the pH range from 5.0 to 8.0, with a slope of − 0.055 V/pH (data not shown). This behavior can be interpreted on the basis of the treatment of an irreversible electrode process by cyclic voltammetry [20], which relies on a general approach of electrode kinetics [21].

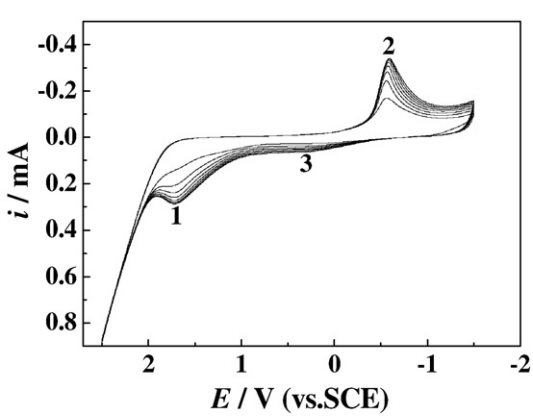

Fig. 1. Cyclic voltammograms of electropolymerization for amidosulfonic acid on the surface of GCE. Scan rate: 50 mV s^{-1}.

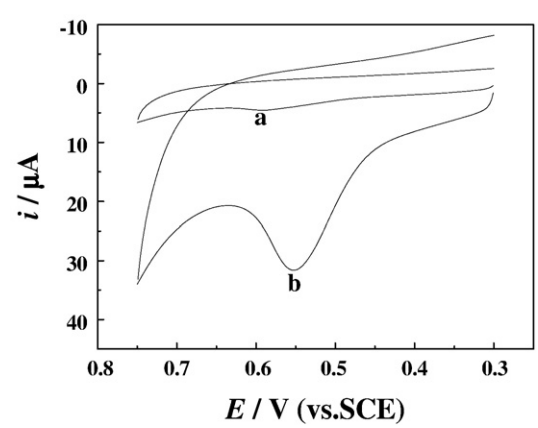

Fig. 2. Cyclic voltammograms of isoniazid at bare GCE (curve a) and PASA-GCE (curve b) in phosphate buffer solution (pH 6.5) containing 1.0×10^{-4} M isoniazid. Scan rate:100 mV s^{-1}.

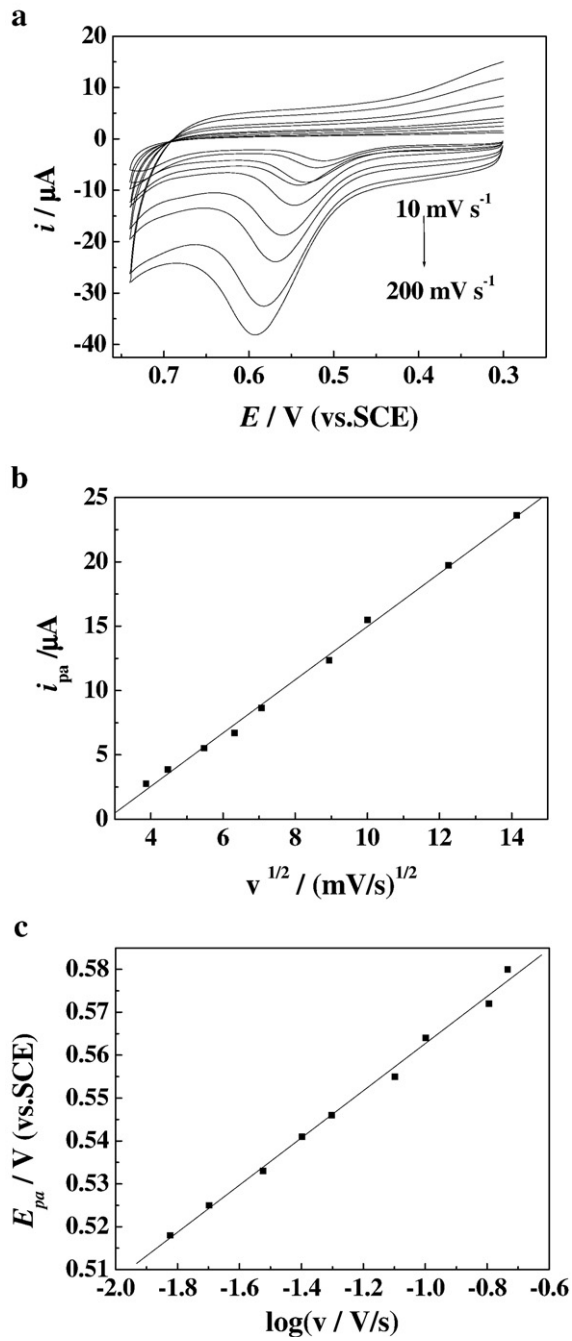

Fig. 3. (a) Cyclic voltammograms of the PASA-GCE in the presence of 1×10^{-4} M isoniazid at different scan rates from 10 to 200 mV s^{-1} in phosphate buffer solution (pH6.5). (b) The linear relationship between the peak currents and the square roots of scan rates. (c) The linear relationship between the peak potentials and the logarithm of scan rates.

The dependence of the oxidation peak potential upon the scan rate v is expressed by the equation (see Eq. (6) of Ref. [20]):

$$\left(\frac{\partial E_p}{\partial \log v}\right)_{pH} = \frac{2.303RT}{F(\vec{n}+\delta\alpha)} \quad (1)$$

and its dependence upon pH is expressed by the equation (see Eq. (7) of Ref. [20]):

$$\left(\frac{\partial E_p}{\partial pH}\right) = \frac{-2.303RTh}{F(\vec{n}+\delta\alpha)}. \quad (2)$$

Here, R, T and F have their usual significance, $\vec{n}$ transfer steps preceding the rate-determining step, δ is a parameter equal to 1 for a rate-determining electron-transfer step or to 0 for a rate-determining chemical step, $\alpha \cong 0.5$ is the symmetry factor for an electron-transfer step (if rate-determining), and h is the number of elementary deprotonation steps that precede the rate-determining step. Considering that $2.303RT / F$ equals 0.059 V at room temperature, the dependence of the oxidation peak potential upon log v and upon pH points to a unit value for $(\vec{n} + \delta\alpha)$ and to a unit value for h, in view of Eqs. (1) and (2). This implies that the rate-determining step is a chemical step ($\delta = 0$) following both the uptake of the first transferring electron ($n = 1$) and an elementary deprotonation step ($h = 1$).

3.3. Analytical performance of PASA-GCE for isoniazid

3.3.1. Choice of supporting electrolyte

The effect of the supporting electrolytes on voltammograms of isoniazid was investigated, which included 0.05 M HAc–NaAc (pH 4.8), 0.05 M NaH_2PO_4–Na_2HPO_4 (pH 6.5), 0.05 M NH_3–NH_4Cl (pH 9.8) and 0.05 M Britton-Robinson (pH 7.5) (data not shown). The experimental results showed that the oxidation peak of isoniazid could be obtained in all above electrolytes. However, in phosphate buffer solution (pH 6.5), the current of anodic peak was higher than that in other supporting electrolytes. Thus, 0.05 M phosphate buffer solution was chosen as the supporting electrolyte for further studies.

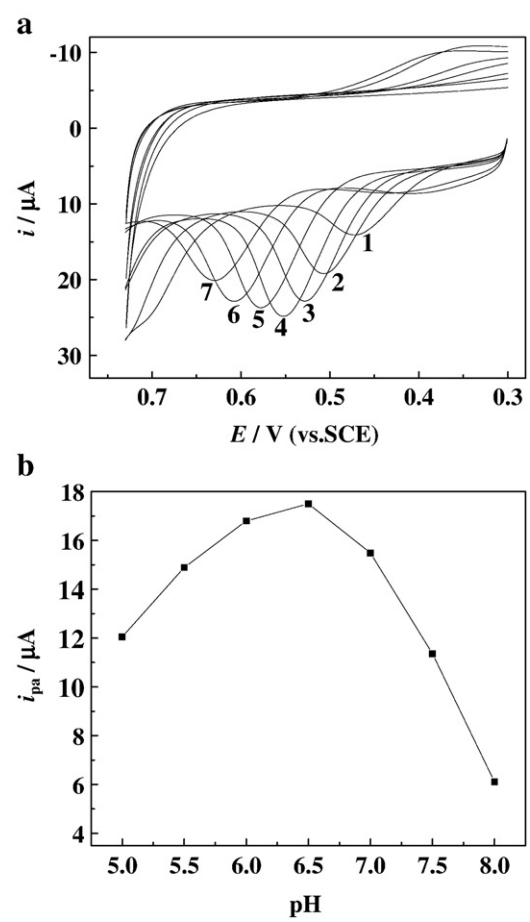

Fig. 4. (a) Cyclic voltammograms of 1×10^{-4} M isoniazid in 0.05 M phosphate buffer solution at different pH values. pH of Curve 1 to 7: 8.0, 7.5, 7.0, 6.5, 6.0, 5.5, 5.0. Scan rate 100 mV s^{-1}. (b) plot of i_{pa} vs. pH.

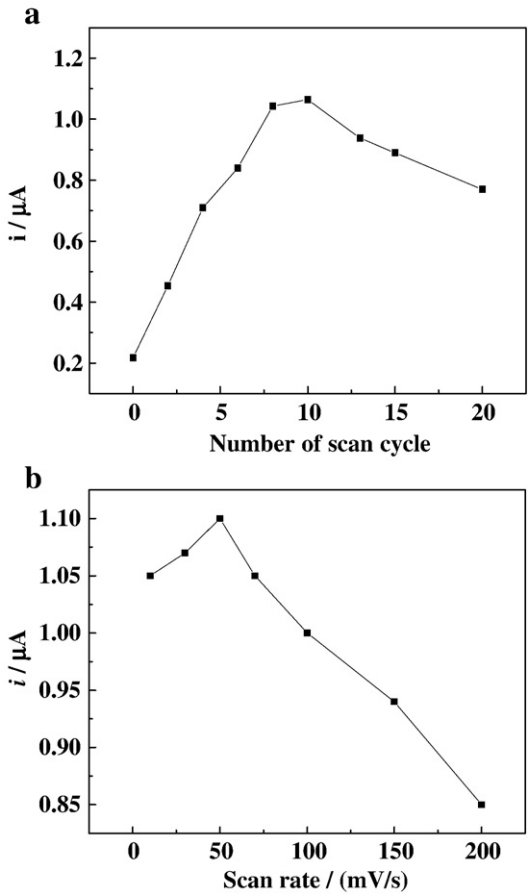

Fig. 5. Effect of scan cycle (a) and scan rate (b) during electropolymerization on the catalytic current (DPV) of 1×10^{-6} M isoniazid in phosphate buffer solution (pH 6.5). Accumulation time: 120 s; accumulation potential: −0.2 V.

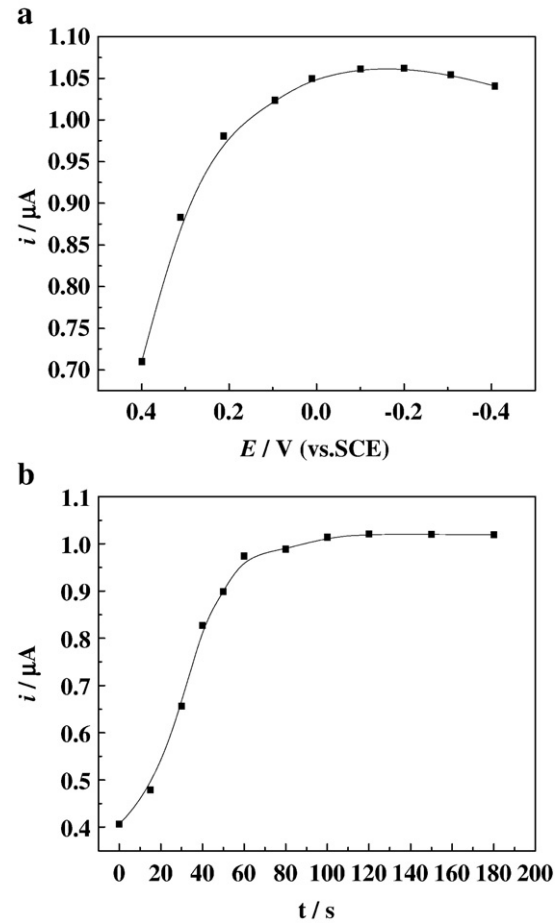

Fig. 6. Effect of accumulation potential (a) and accumulation time (b) on the peak current of 1×10^{-6} M isoniazid in phosphate buffer solution (pH 6.5).

3.3.2. Effect of solution pH on the peak currents and peak potential

The effect of solution pH on the peak potentials and the peak currents was shown in Fig. 4. With the increase of pH value, the peak potentials shifted negatively (shown in Fig. 4a). At the same time, the experimental results also showed that pH has a significant influence on the anodic peak current of isoniazid at PASA-GCE (shown in Fig. 4b). It can be seen that the values of the peak current changed with the different pH in the range of 5.0 to 8.0. Obviously, the maximum response of anodic peak current appeared at pH 6.5. In this work, the optimum pH value of 6.5 could be selected in the following experiments.

3.3.3. Effect of electropolymerization on the electrocatalytic ability of PASA-GCE

Effect of scan cycles and scan rate in electropolymerization on anodic peak current of isoniazid was investigated. Fig. 5a shows the relationship between electrocatalytic currents of 1.0×10^{-6} M isoniazid and the scan cycles of electropolymerization at the surface of GCE by differential pulse voltammetry. The oxidation peak current gradually increased with the increase of scan cycles of electropolymerization. When the cycles were beyond 10, the anodic peak current decreased. Under the optimal electropolymetric cycles, the effect of different scan rates was also studied (shown in Fig. 5b). It was found that the scan rate of 50 mV/s for electropolymerization was favorable. So scan cycles of 10 and scan rate of 50 mV/s were selected in the electropolymerization, and the maximum electrocatalytic response on isoniazid achieved.

3.3.4. Effect of accumulation potential and accumulation time

Fig. 6a shows the effect of different accumulation potentials on anodic peak current of isoniazid with accumulation time 120 s by differential pulse voltammetry. When the accumulation potential shifted from − 0.4 V to 0.0 V, the response of peak current hardly changed. So the accumulation potential of − 0.2 V was selected in this work.

The effect of the accumulation times on the peak current of isoniazid was also shown in Fig. 6b. It showed that the peak current increased when the accumulation time was less than 100 s whereas

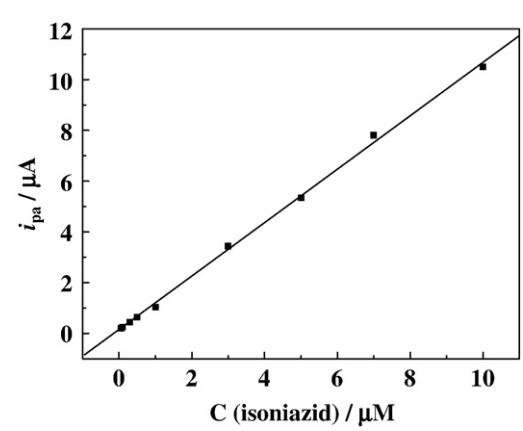

Fig. 7. The linear relationship between the peak current and concentration of isoniazid in phosphate buffer solution (pH 6.5).

Table 1
Comparison of detection limits and linear range for isoniazid at different modified electrodes

Type of electrodes	Linear range	Detection limits
Au electrode [12]	2.0×10^{-6}–2.3×10^{-4} M	9.69×10^{-8} M
Mercury electrode [13]	5.0×10^{-10}–2.0×10^{-6} M	1.18×10^{-10} M
Polypyrrole modified GCE [14]	3.99×10^{-6}–12.60×10^{-2} M	3.15×10^{-6} M
PASA-GCE [This work]	5.0×10^{-8}–1.0×10^{-5} M	1.0×10^{-8} M

the peak current hardly changed as further increasing the accumulation time. Here, the accumulation time of 120 s was selected.

3.3.5. Calibration curve for isoniazid

Under the optimum conditions, the dependence of the anodic peak currents on the concentration of isoniazid was shown in Fig. 7. The results showed that the anodic striping peak current (i_p) is proportional to the concentration of isoniazid from 5.0×10^{-8} to 1.0×10^{-5} M. The linear regression equation was expressed as: i (μA) = 0.154 + 1.052 $C_{isoniazid}$ (μM) (r = 0.999), and the detection limit was 1.0×10^{-8} M (three times of the ratio of signal to noise), which is lower than previously being reported ones except the literature [13] (see Table 1).

3.3.6. Reproducibility

1.0×10^{-6} M isoniazid was determined repeatedly at the identical surface of PASA-GCE for successive 25 times, and the average current was 1.11 μA with the RSD of 3.7%, which showed an excellent reproducibility. The precision at renewed surface of PASA-GCE was also investigated. The average peak current for 1.0×10^{-6} M isoniazid was 1.10 μA with the RSD of 4.7% (n = 10). It indicated that this modified electrode has a remarkable reproducibility.

The stability of PASA-GCE was evaluated by measuring the anodic peak current response at a fixed 1.0×10^{-6} M isoniazid over a period of 7 days. The chemically modified glassy carbon electrode by poly (amidosulfonic acid) was treated only once and stored in phosphate buffer solution (pH 6.5) after every determination. The RSD with 3.8% (n = 7) showed that the PASA-GCE has a good stability.

3.3.7. Interference

In order to evaluate the interferences of foreign species on the determination of isoniazid, some interference experiments were carried out. 1000-fold concentration of dopamine, ascorbic acid and glicose, 500-fold that of uric acid (UA) and glutamic acid almost did not influence the current response of 1×10^{-6} M isoniazid in phosphate buffer solution (pH 6.5) (signal change below 5%,). The above experimental results indicated that this proposed electrochemical method performed the ability of anti-jamming.

3.4. Determination of isoniazid in injection and tablet samples

The injection (100 mg/2 mL) and tablet (100 mg/tablet) samples of isoniazid, which were diluted appropriately, were determined with the proposed electrochemical method. The injection and tablet samples of isoniazid were analyzed by the standard addition method. The results are in agreement with that obtained by the method in China Pharmacopoeia [22] (see Table 2). The recovery of isoniazid for the injection and tablet samples was listed in Table 3 with the recovery from 98.63% to 102.00% for injection and 95.29% to 103.50% for tablet, respectively.

The results of the recovery and RSD showed that the proposed methods can be used efficiently for the determination of isoniazid in pharmaceutical formulations.

Table 2
Determination of isoniazid in samples of its injection and tablet

Samples	Pharmacopoeia method (n=5)		This method (n=6)	
	Values	RSD	Values	RSD
Injection	100.32 mg/2 mL	2.7%	99.53 mg/2 mL	3.5%
Tablet	99.34 mg/tablet	2.9%	100.98 mg/tablet	3.8%

Table 3
Recovery test of isoniazid in its injection and tablet samples

Samples	Added ($\times10^{-7}$ M)	Founded $\times10^{-7}$ M)	Recovery (%)	RSD (%)
Injection	0.00	3.06	/	2.0
	2.00	5.08	101.00	3.3
	27.00	29.69	98.63	2.9
	47.00	49.99	99.85	3.3
	67.00	71.11	102.00	2.5
Tablet	0.00	2.95	/	2.5
	2.00	5.02	103.50	3.1
	27.00	28.68	95.29	3.9
	47.00	49.07	98.13	2.6
	67.00	71.18	101.84	2.8

Number of samples assayed: 6.

4. Conclusions

This work has shown that isoniazid can be determined using electrochemical method on the basis of its oxidation process at poly (amidosulfonic acid) modified glassy carbon electrode with wider linear range and lower detection limit. The proposed methods can be applied to the detection of isoniazid in pharmaceutical formulations. The good properties of modified electrode, such as high sensitivity, easy fabrication, reproducibility, stability and anti-jamming, indicate that the proposed modified electrode will be promising for measurements of isoniazid in vivo and in vitro. At the same time, it will expand the application of this polymer film in electrochemical field.

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