

Preparation and Application of Keggin Silicomolybdic acid Modified Electrode

Miao Liu

Jinzhou Medical University

Mingxuan Jia

Jinzhou Medical University

Dong Hui Li (✉ lidonghuilx@sina.com)

Jinzhou Medical University

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Abstract

An innovative method for the determination of isoniazid tablets is studied through electrochemical method for the modification of glassy carbon electrode (GCE). Polyoxomolybdate, with stable structures, has not been widely used for the determination of substance. In this study, the mentioned polyoxomolybdate was characterized by Fourier transform infrared spectroscopy (FT-IR), UV-vis, X-ray diffraction (XRD), Atomic force microscope (AFM) and X-ray photoelectron spectroscopy (XPS), and used to modify the glassy carbon electrode. The electrochemical performance of the polyoxomolybdate@GCE was investigated with cyclic voltammetry (CV) and differential pulse voltammetry (DPV), compared with the unmodified electrode, the proposed polyoxomolybdate modified electrode exhibited strong electro-catalytic activities towards isoniazid (INH). Under the optimized conditions, there was linear relationships between the DPV peak currents and the concentrations in the range of 1×10^{-7} g/L to 3×10^{-7} g/L for INH ($R^2 = 0.9979$), with the limit of detection (LOD) of 0.024 μ g/L (based on $S/N = 3$). The modified electrode has proper reproducibility ($RSD < 5\%$), stability, response time (< 3 min) and lifetime (up to 6 days).

Introduction

It is well known that tuberculosis (TB) is a chronic disease caused by mycobacterium tuberculosis (MTB). According to the report of the World Health Organization for 2017, it is the ninth leading cause of death in the world and the leading cause from a single infectious factor. In 2016, 6.3 million new medical cases of illness of TB were reported (increased from 6.1 million in 2015), account for 61% of the estimated frequency of 10.4 million [1]. Isoniazid (INH) has a powerful bactericidal effect on the active growth of intracellular and extracellular MTB, so it is one of the most effective tuberculosis inhibitor drug that has been generally used for the treatment of clinical tuberculosis [2,3]. Of course, it is often combined with other anti-tuberculosis agents to resist the mycobacterium strains. It is undeniable that isoniazid is an indispensable essential medicine for treating tuberculosis in medicine [4-7]. Unfortunately, even in excellent effects on treatment of tuberculosis, there has not avoid to cause pains from a number of side effects, toxicity such as hepatotoxicity and multiple drug resistances, sometimes it can even cause death [8,9]. Therefore, it is of great significant to establish a sensitive, rapid and reliable method for the determination of isoniazid in clinical.

Until now, many methods for measuring isoniazid have been reported, such as electrochemiluminescence [10,11], electrochemical technology [12,13], performance liquid chromatography [14,15], capillary electrophoresis [16-18], spectrophotometry [19-21] and liquid chromatography-mass spectrometry [22,23]. Among these methods mentioned, although their doubtless accuracy and effectiveness, the boundedness of these technologies are worth considered, such as difficulty in operation, poor stability, high experimental cost and so on. Therefore, a simple and environment-friendly alternative is beneficial that enable isoniazid to be detected in a simple and cost-effective manner. In recent years, the electrochemical technique has generated abundant interests because of its fast response, easy handling, excellent conductivity and low costs. However, the

electrochemical detection of isoniazid on bare electrode was not best choice since the bare surface of electrode is difficult to transfer electrons and sensitive to influence. To work out these problems, various modified electrodes have been designed, such as the graphene oxide–Au nanomaterials modified glassy carbon electrode [24], cadmium sulfide nanoparticles modified silver electrode [25], the immobilization of phosphovanadomolybdate onto N-doped carbon nanotubes modified glassy carbon electrode [26], and using carbon paste electrode [27].

Polyoxomolybdates (POMs), a type of cluster compounds, are formed by condensation polymerization of high oxidation state of oxygen atom and pre-transition metal (Mo, W, V, Nb, Ta) with MO_x as unit through common angle, common edge connection. There is a well-known classification in this category as the Keggin type, which is a tetrahedral structure with a heteroatom surrounded twelve molybdenum octahedrons in the center. Keggin-type hetero-poly acid anions not only can be used as electron acceptors, but also as electron donors, which depend on its multi-faceted applications, such as catalytic chemistry, medicinal chemistry, electrochemistry [28-30]. Nevertheless, the difference between in multi-domain applications depends on the type of heteroatoms in the center. The common Keggin-type hetero-poly acids are $\{PMo_{12}\}$, $\{GeMo_{12}\}$, $\{SiMo_{12}\}$ and so on, the rare Keggin-type hetero-poly acids are $\{MoMo_{12}\}$, $\{CoMo_{12}\}$, $\{NiMo_{12}\}$ [31,32]. It also includes the differences in the application fields caused by the differences of Keggin-type hetero-poly acid anions. We found that the specificity of $\{SiMo_{12}\}$ in the field of electrochemistry has aroused our research interest. Additionally, polyoxomolybdate compounds can also be used as solid electrolytes due to their proton conductivity. They also play an important role in catalyzing many organic reactions, such as homogeneous or heterogeneous acid catalysis, redox catalysis, photocatalytic oxidation [33-37].

In this experiment, the polyoxomolybdate also considered to be a simple substitution for the nanomaterials. we combined with innovative polyoxomolybdate and Nafion PFSA Polymer solution to develop a modified glassy carbon electrode. It was found that the oxidation peak current of isoniazid on modified electrode was much higher over that on bare GCE. The proposed electrode was successfully employed to detect the concentration of isoniazid in the detection of pharmaceutical preparations. Compared to traditional methods, the sensor shows high stability and sensitivity. It was expected that the sensor can also be used for facile analysis of other drugs with high sensitivity.

Methods

Experimental

Reagents

We obtained the nanomaterials by rapid evaporation method according to the method of reference (chemical formula was $[Himi]_y[H_{30}]_x[SiMo_{12}O_{40}] \cdot nH_2O$), which was named Compound 1 [38]. Isoniazid was obtained from Meilun Biological Technology Co., Ltd. (Dalian, China, purity $\geq 99\%$), and the isoniazid tablet was supported by Yunpeng Pharmaceutical Co., Ltd. (Shanxi, China). All experimental reagents

were analytical reagents, and all solutions were prepared with distilled water. Diluted hydrochloric acid solution was bought from City Chemical reagent Co., Ltd. (Jinzhou, China). N,N-dimethylformamide was bought from Tianjin Guangfu Science and Technology Development Co., Ltd. (purity $\geq 99.5\%$). Nafion PFSA Polymer (5 wt% solution) from Dupont Co. Ltd. (USA). All reagents were stored at room temperature.

Apparatus

The infrared spectroscopy (IR) was recorded by a Fourier transform infrared spectroscopy (FT-IR, Shantou Keyi Instrument Equipment Co., Ltd.) at the range from 4000 to 500 cm^{-1} . All the electrochemical measurements including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out on a CHI 650D electrochemical workstation (Chenhua Corp Shanghai, China), using a three-electrode system consisting of a working electrode, a reference electrode (a saturated calomel electrode), and a counter electrode (a platinum wire). In addition, XRD patterns were all collected by using an X-ray diffractometer (XRD, Dantong Tongda Science and Technology Co., Ltd.). X-ray photoelectron spectroscopy (XPS) measurements were carried out with a Thermo Fisher K-Alpha (USA). UV-vis absorption spectra were recorded on a Mipuda UV-1600 spectrophotometer (Shanghai, China). Atomic force microscopy images (AFM-6800, Suzhou Fei Shiman Precision Instrument Co., Ltd.) were taken on silicon slides using a Nanoscope instrument (Digital Instruments).

Fabrication of modified electrodes

A number of methods have been reported for preparing chemically modified electrodes [39]. In this experiment, dipping method was adopted. Before preparing the modified electrodes, glassy carbon electrode was polished sequentially with 0.3 and 0.05 μm $\alpha\text{-Al}_2\text{O}_3$ powder, followed by ultrasonic cleaning with ethanol and rinsing thoroughly with ultrapure water. 0.0200 g of Compound 1 and 40 μL N,N-dimethylformamide solution were added into a mortar and ground completely, which was named mixture 1. 0.1% Nafion aqueous solution and mixture 1 were captured on the glassy carbon electrode respectively. Afterward the electrode was dried at a cool temperature under dark condition for about 12 hours. Finally, 0.1% Nafion aqueous solution was taken on the modified electrode and allowed to dry at room temperature.

Sample pretreatment

Before preparing samples, we needed to make electrolyte, prepare different acid solutions respectively and a 0.1% Nafion aqueous solution. As a prescription drug, isoniazid tablets and one isoniazid active pharmaceutical ingredient were purchased from domestic pharmacies. CV scanning from a potential range of 0.1–0.4 V was recorded to stabilize the positive volt-ampere curve characteristics with a 100 $\text{mV}\cdot\text{s}^{-1}$ scan rate in 0.1 M HCl. The electrochemical analysis was carried out by running differential pulse voltammetry (DPV) from 0.1 to 0.4 V with scan rate of 100 $\text{mV}\cdot\text{s}^{-1}$ in 0.1 M HCl. All electroanalytical measurements were taken at room temperature.

Results And Discussion

IR spectroscopy of Compound 1

Compound 1 was examined by IR spectroscopy, and the infrared spectra was analyzed and compared with the positions of characteristic absorption peaks of the spectral map in the published paper (as shown in Table 1) [38,40]. The absorption band of Si-O was 1092 cm^{-1} , and the absorption peak measured by the experiment was 1092 cm^{-1} . Symmetric vibration and asymmetric vibration of $\text{Mo}=\text{O}_t$ were 904 cm^{-1} and 953 cm^{-1} respectively, while the peak values measured by the experiment were 904 cm^{-1} and 955 cm^{-1} respectively. $\text{Mo}=\text{O}_{\mu/\nu}$, symmetry and asymmetry vibration, respectively, 791 cm^{-1} , 862 cm^{-1} , experimental measured absorption peak at 793 cm^{-1} , 863 cm^{-1} . These results indicated that there was basic consistent with the literature, it can be determined in the laboratory of synthesis of material for Compound 1.

Table 1. Compound 1 was compared with the position of characteristic absorption peak in the spectrogram of literature

Characteristic group	Vibration frequency in the literature/ (cm^{-1})	Vibration frequency in experiment/ (cm^{-1})
$\delta(\text{Si-O})$	1092	1092
$\nu_{\text{as}}(\text{Mo}=\text{O}_t)$	953	955
$\nu_{\text{s}}(\text{Mo}=\text{O}_t)$	904	904
$\nu_{\text{as}}(\text{Mo}=\text{O}_{\mu/\nu})$	791	793
$\nu_{\text{s}}(\text{Mo}=\text{O}_{\mu/\nu})$	862	863

UV-vis spectra of Compound 1

UV-vis spectroscopy was used to monitor the Compound 1. We studied that the UV-vis spectra of aqueous solutions of Compound 1 at various concentrations and the UV-vis spectra of hydrochloric acid solution of Compound 1 at varieties of pH. It can be seen from the figure that the concentration increases, the absorbance also increases and is linear. The two figures demonstrated that the concentration and acidity have little effect on Compound 1, which means that the nanomaterial was stable.

XRD spectroscopy and X-ray photoelectron spectroscopy of Compound 1

Compound 1 was characterized by XRD, and the intensities data were collected over a 2θ range of 5° to 50° . In order to identify the elemental composition of the Compound 1, we measured the XPS of the Compound 1. Although the XPS measurement gives only semi-quantitative elemental composition, the existence of C, O, N, Si and Mo in the film was confirmed. We regarded that the peak intensities of the $\text{Mo}_3\text{d}_{5/2}$ (at 232.8 eV) and $\text{Mo}_3\text{d}_{3/2}$ (at 235.9 eV) [41]. In addition, the peak intensity of the Si_{2p} (at 101.8 eV), consistent with published paper [42], in agreement with the XRD pattern.

AFM micrographs and size distribution of Compound 1

For further study, Compound 1 was characterized by AFM and its particle size distribution was plotted as shown in Figures 1 and 2. As is described, the size of compound 1 was small, and according to statistical results, the average particle size of the compound 1 was less than 20 nm, which was basically the consistent with the characteristics of AFM.

[Figure 1]

[Figure 2]

Preparation of modified electrode and electrochemical oxidation of isoniazid in modified GCE

The detailed reaction mechanism and the fabrication of the modified electrode were shown in Schematic 1. As a comparison with the study of Subramanian Nellaiappan and

Annamalai Senthil Kumar, it was predicted that isoniazid reacts on the electrode [43].

Schematic1. Schematic illustration of electrochemical of fabricating modified electrodes and oxidation of isoniazid at the modified GCE

Electrode properties

Electrochemical behaviors of electrodes

Electrochemical characterization of Compound 1 was done by CV and DPV. CV behaviors of the electrodes were investigated in 0.1 M HCl, in the potential range between 0.1 and 0.4 V with scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$. In figure 3, all of these three types of electrodes have oxidation and reduction peak currents, the bare one (a) presents no obvious peak current, while the modified one (c) presents a stronger peak current than the modified one (b). It suggests that the response of the Compound 1 modified electrode in 0.1 M HCl solution has been significantly enhanced, and the modified electrode was more stable due to the last layer of Nafion aqueous solution, the Compound 1 was also difficult to fall, which was beneficial to the experimental operation. In other words, the electronic transfer rate of modified electrode has been accelerated in 0.1 M HCl solution after modifying. The result shown in Figure 3 might consider that the Compound 1 improves the electron transfer rate on the surface of the modified electrode.

[Figure 3]

Type of electrolyte

In order to obtain an appropriate electrolyte, the modified electrode was placed in HCl, H_2SO_4 , H_3PO_4 , HNO_3 and HClO_4 at $\text{pH}=1.0$ for CV measurement (scan rate as $100 \text{ mV}\cdot\text{s}^{-1}$). As evidenced in Figure 4, it was obvious that the sensitivity and stability of the modified electrode in HCl solution was obtained by scanning, and the response of the current was also relatively high. So the determination of modified electrode was selected the most suitable electrolyte was HCl, and carried out the following experiment.

[Figure 4]

Effects of pH value

Figure 5 was a cyclic voltammetry plot of modified electrode in HCl solution at pH 1.0 ~ 7.0 (scan rate as $100 \text{ mV}\cdot\text{s}^{-1}$). As shown in Figure 5, it can be seen that along with the pH value increases, the current response of the modified electrode was instead reduced. And can be clearly seen when pH = 1.0, the peak current was relatively largest. Therefore, we determined the optimum pH of the modified electrode was selected pH = 1.0.

[Figure 5]

Effects of scan rate

For the sake of obtain detailed information of the reaction dynamics on the modified electrode surface, as evidenced in Figure 6 A, the influence of scan rate was explored at the range from 50 to 300 $\text{mV}\cdot\text{s}^{-1}$ by CV on the modified electrode in 0.1 M HCl solution. As evidenced in Figure 6 B, the peak currents (I_{pc}) were increased linearly with the scan rate (ν); the equations can be expressed as $I_{pc} = -0.3328 \nu - 80.762$ ($R^2 = 0.998$). Another way to understand that the peak currents (I_{pc}) were increased linearly with the square root of scan rate ($\nu^{1/2}$), as shown in Figure 6 C; the equation was expressed as $I_{pc} = -8.2040 \nu^{1/2} - 33.763$ ($R^2 = 0.994$). This result showed that the electrochemical reaction of Compound 1 was a diffusion-controlled process at the modified electrode surface, the slope values were obtained as 8.20. We can also be expressed like this: the electrode reaction was to draw the figure of $\log I_{pc}$ on $\log \nu$, as shown in Figure 6 D; the equation was expressed as $\log I_{pc} = -0.35 \log \nu - 1.36$ ($R^2 = 0.984$). The result demonstrated the diffusion controlled process, which had been certified in Figure 6 D.

[Figure 6]

Standard curve and detection line

By optimizing the optimal conditions for the modified electrode above, we have collected the optimum conditions for this modified electrode. Under this condition, we drew a standard curve for the isoniazid bulk drug, and the relationship between current response value (I_{pc}) and isoniazid concentration were investigated by DPV method. The results shown that in the range of 1.0×10^{-7} g/L to 3×10^{-7} g/L, the isoniazid concentration has a good linear relationship with the peak current (as show in figure 7 and figure 8). The linear equation was $I_{pc} = 39.22 c + 39.12 \times 10^{-6}$ ($R^2=0.9979$), the detection limit was 0.24×10^{-7} g/L (based on $S/N = 3$), compared with the methods reported in the literature (Table 2), the method has a lower detection limit and excellent sensitivity.

[Figure 7]

[Figure 8]

Table 2. Comparison of different methods for determination of isoniazid

Method	Liner range	Limit of detection	Reference
High performance liquid chromatography	0.1-20 $\mu\text{g/mL}$	0.023 $\mu\text{g/mL}$	[44]
UV spectrophotometry	5-25 $\mu\text{g/mL}$	5 $\mu\text{g/mL}$	[45]
capillary electrophoresis	0.7-30 $\mu\text{g/mL}$	0.3 $\mu\text{g/mL}$	[46]
RP-LC	18.75-150 $\mu\text{g/mL}$	3.5 $\mu\text{g/mL}$	[47]
Polyoxomolybdate modified electrode	0.1-0.3 $\mu\text{g/L}$	0.024 $\mu\text{g/L}$	This work

Reproducibility and stability

The repeatability was one of the important factor to assess the electrode performance. Under the optimal conditions, isoniazid was repeatedly measured by DPV for five times, the relative standard deviation (RSD) of repeatability measurements by one modified electrode

with the isoniazid solution of concentration from 1.0×10^{-7} g/L to 3.0×10^{-7} g/L shown in Table 3 was totally below 5%, indicated the modified electrodes have proper reproducibility.

Table 3. Reproducibility of polyoxomolybdate modified electrode (n=5)

C/(g/L)	Average/ μ A	SD/ μ A	RSD/%
1.0×10^{-7}	39.63	0.61	1.54
1.5×10^{-7}	40.60	1.03	2.5
2.0×10^{-7}	43.75	0.83	1.9
2.5×10^{-7}	46.31	0.53	1.15
3.0×10^{-7}	50.95	0.14	0.28

Actual sample analysis

Further investigate the utility of the modified electrode, the method was applied to the determination of isoniazid tablets. Detected by DPV method, and the standard recovery test was carried out. As shown in Table 4, the recovery rate was between 97.8% and 103.3%, indicating that this method can be used for the detection for isoniazid tablets.

Table 4. Determination of the recovery of isoniazid tablets

Sample	Original(μ g/L)	Added(μ g/L)	Total found(μ g/L)	Recovery(%)	RSD(% n=5)
1	0.10	0.10	0.1978	97.8	1.62
2	0.15	0.10	0.2550	103.3	1.02
3	0.20	0.10	0.2980	99.0	0.17

Conclusions

So far, electrochemical sensors have been widely putted into biotechnology and environmental monitoring, the electrode in electrochemical sensors methods are often modified with a variety of materials to improve the performance of potential sensors. Polyoxomolybdates have been widely used in electrochemistry as a class of polyoxometalate compounds formed by oxygen linkage of transition metal ions. Using the characteristics of electrochemical detection, we have established a method for rapid determination of isoniazid. In the meantime, all the factors that need to be considered were further exploration of the factors affecting the performance of the modified electrode, such as lifetime, stability, proper electrolyte, proper pH value and repeatability, at last the best experimental conditions response time were obtained. The results of this paper shown that the detection range of Keggin-type silicomolybdic acid modified electrode was 1.0×10^{-7} g/L to 3.0×10^{-7} g/L, LOD of 0.024 μ g/L. In addition, the analysis of the actual samples also has demonstrated that the recovery rate was 97.8% ~ 103.3%. Therefore, the Keggin-type silicomolybdic acid modified electrode can be used as an effective electrochemical method. This method can also be used as a reference for rapid determination of isoniazid. However, the modified electrodes prepared still have some limitations compared to other experimental methods. As a supplement, a further research on the scope of the electrode performance and the detection stability should be discussed in the following work, and the results will be appeared in our future papers.

Abbreviations

GCE: glassy carbon electrode; FT-IR: Fourier transform infrared spectroscopy; XRD: X-ray diffraction; AFM: Atomic force microscope; XPS: X-ray photoelectron spectroscopy; CV: cyclic voltammetry; DPV: differential pulse voltammetry; INH: isoniazid; LOD: limit of detection; RSD: relative standard deviation; SD: standard deviation.

Declarations

Availability of data and materials

All data and materials could be available upon the request.

Authors' contributions

HDL participated in the design of this study. XMJ collected important background information and performed the statistical analysis. ML tested the performance of different modified electrodes and drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests

All authors declare that they have no competing or conflict of interests.

Author details

*Correspondence: lidonghuilx@sina.com

¹ The Center Laboratory of Pharmaceutical analysis, Pharmaceutical College, Jinzhou Medical University, Jinzhou, People's Republic of China

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Figures

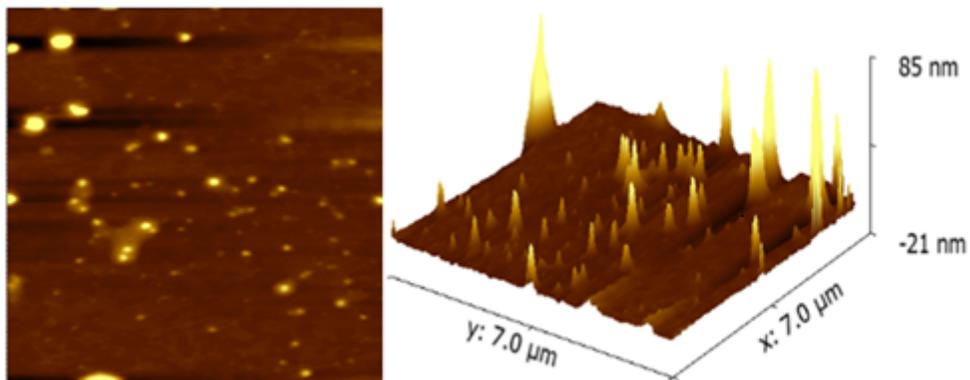


Figure 1

Tapping mode AFM images and three-dimensional view of Compound 1

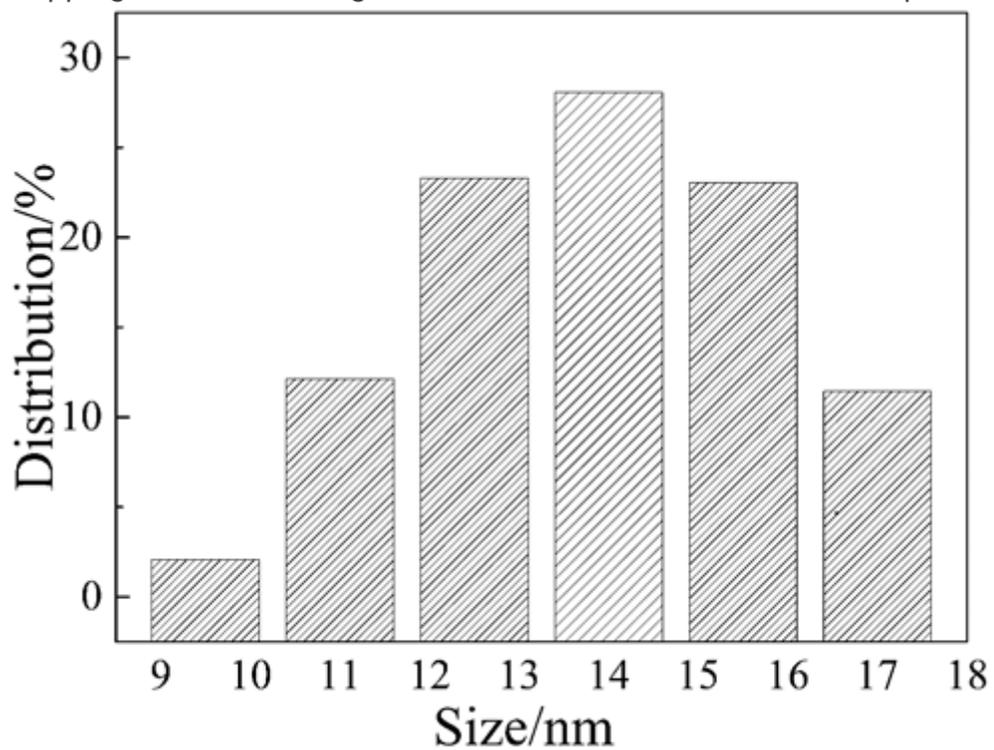


Figure 2

The size distribution of Compound 1

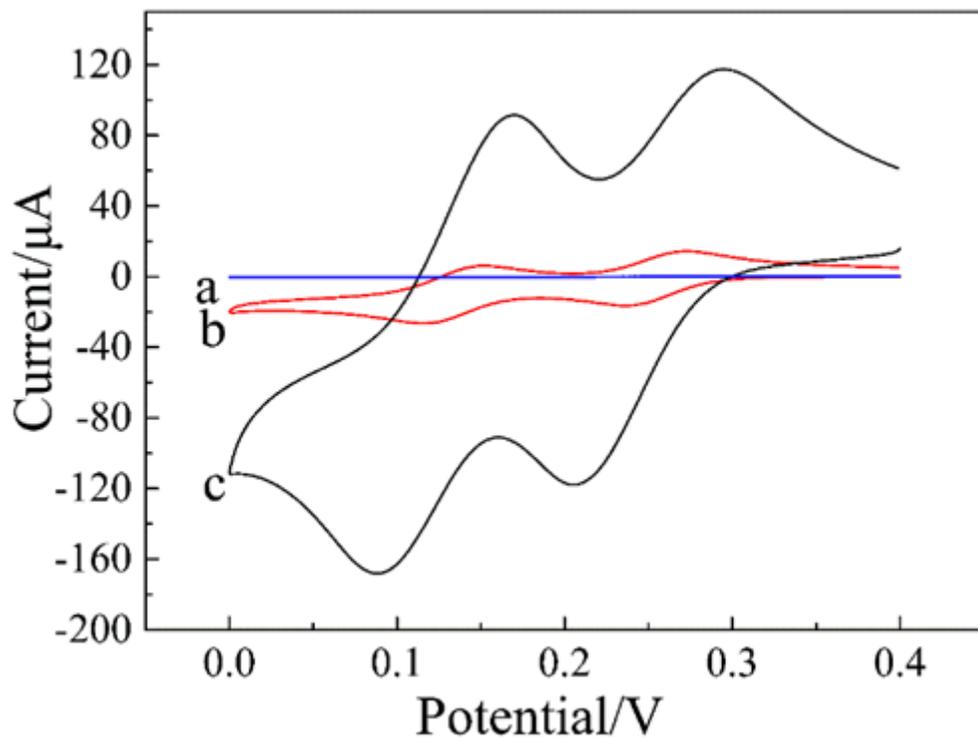


Figure 3

CV graph of the bare electrode (a), the GCE@Nafion@Compound 1 electrode (b), and the GCE@Nafion@Compound 1@Nafion modified electrode (c) in 0.1 M HCl solution at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$.

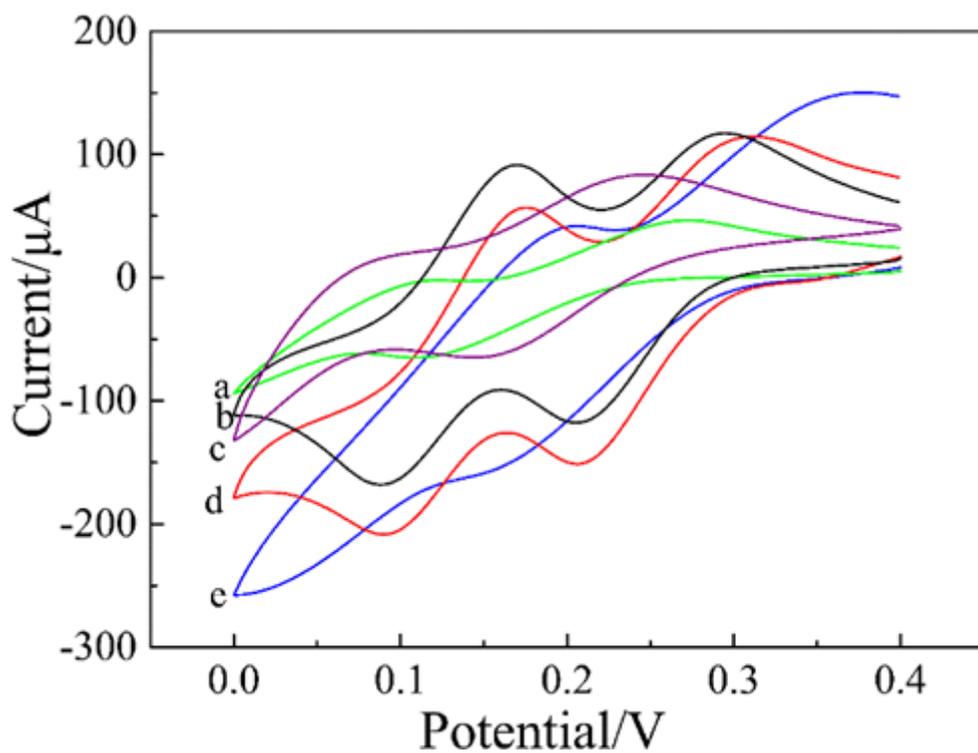


Figure 4

CV of modified electrode scanned in diverse acids at pH=1.0 (From a to e: HNO₃, HCl, H₃PO₄, H₂SO₄ and HClO₄).

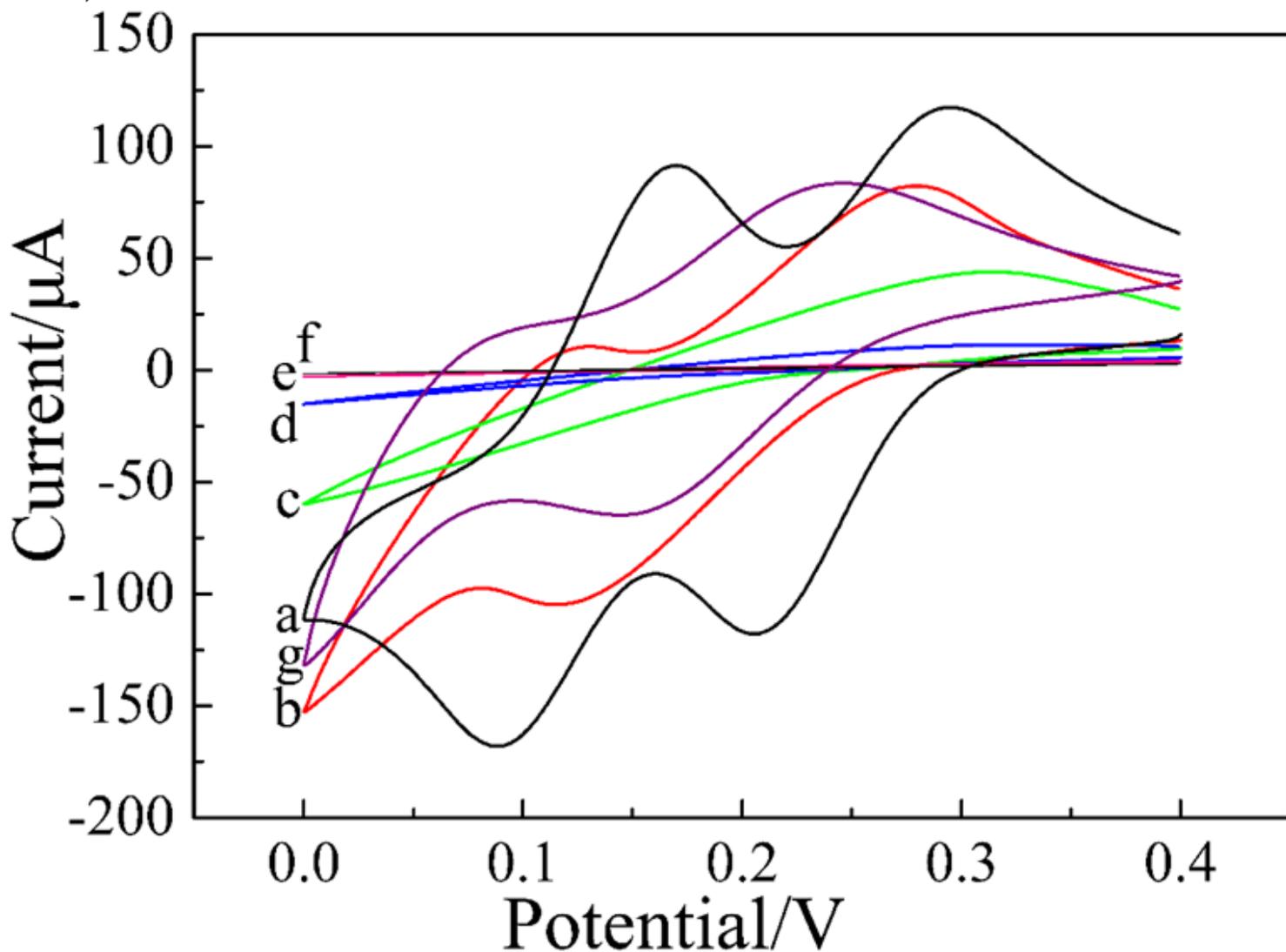


Figure 5

CV curves of modified electrode in different pH values of HCl solution (From a to g: 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0).

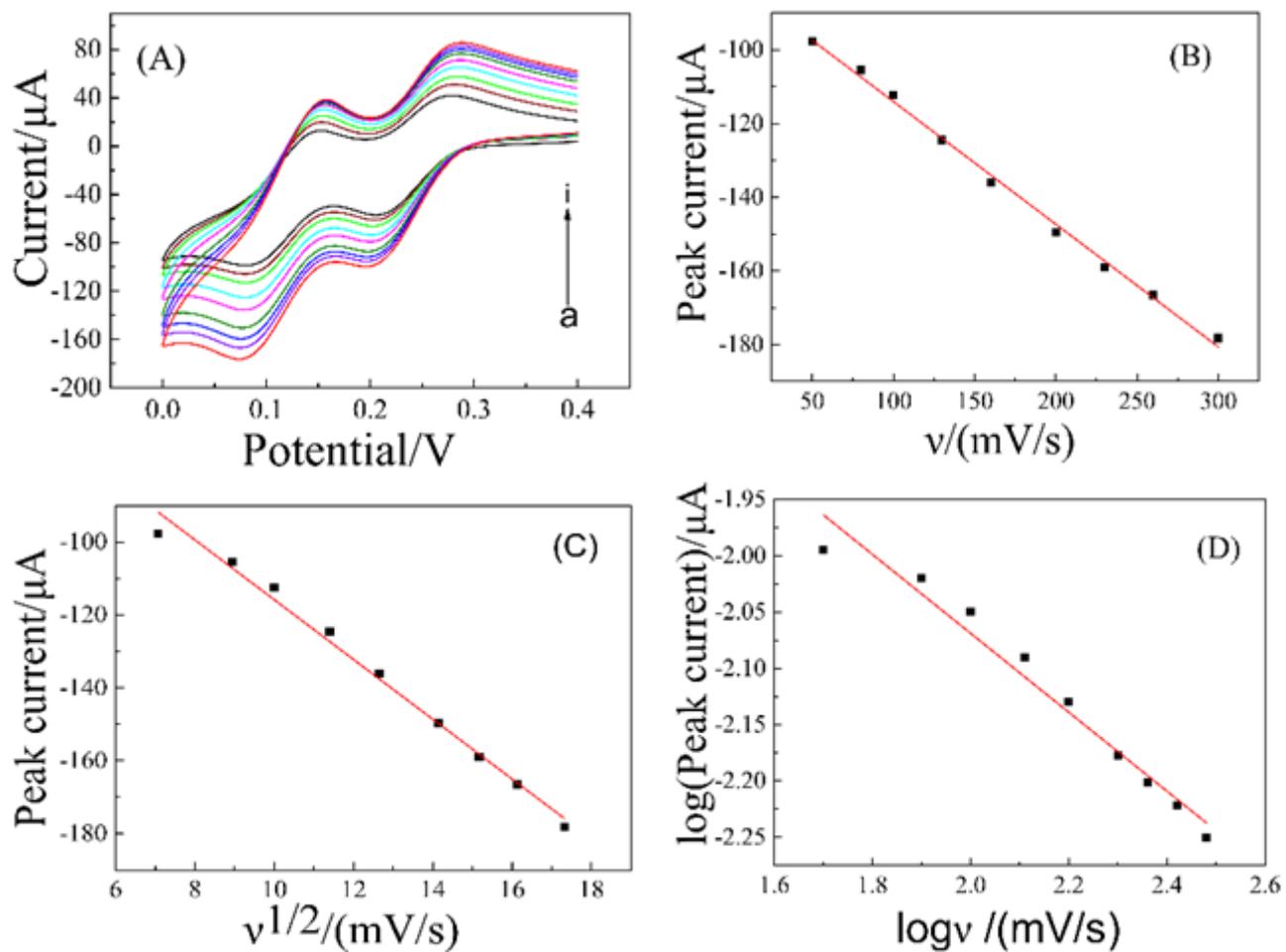


Figure 6

A, CV records of 0.1 M HCl solution for various scan rates (a-i): 50, 80, 100, 130, 160, 200, 230, 260 and 300 $\text{mV}\cdot\text{s}^{-1}$ on the modified electrode. B, Peak currents versus the scan rate. C, Peak currents versus the square root of scan rate. D, Log peak current versus log scan rate.

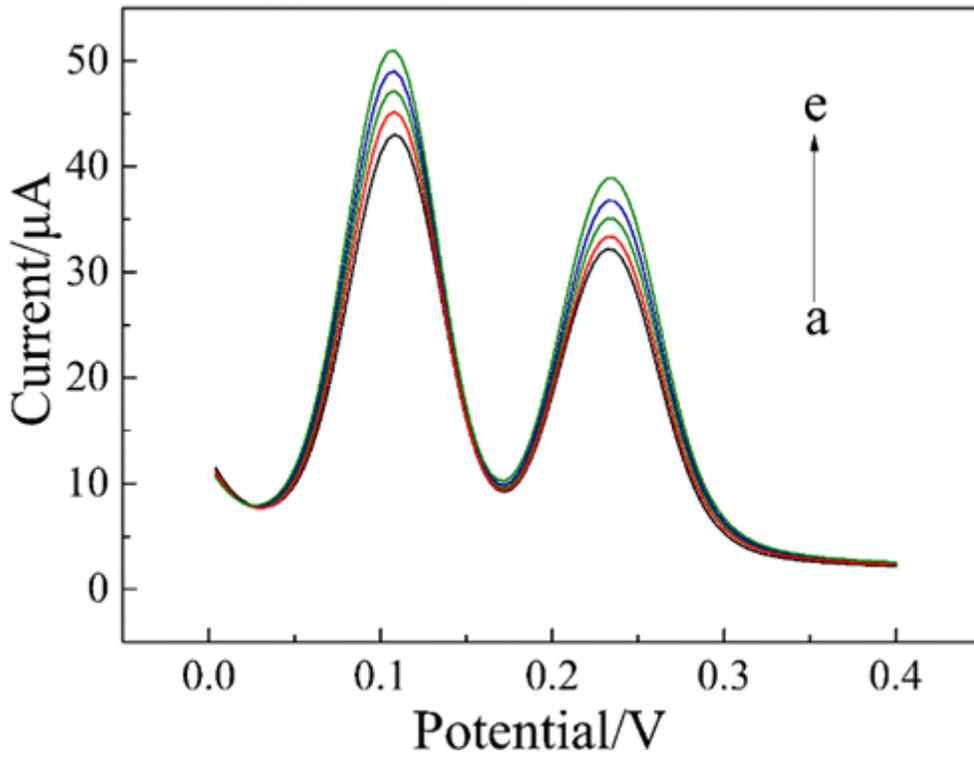


Figure 7

isoniazid reduction peak current in the range

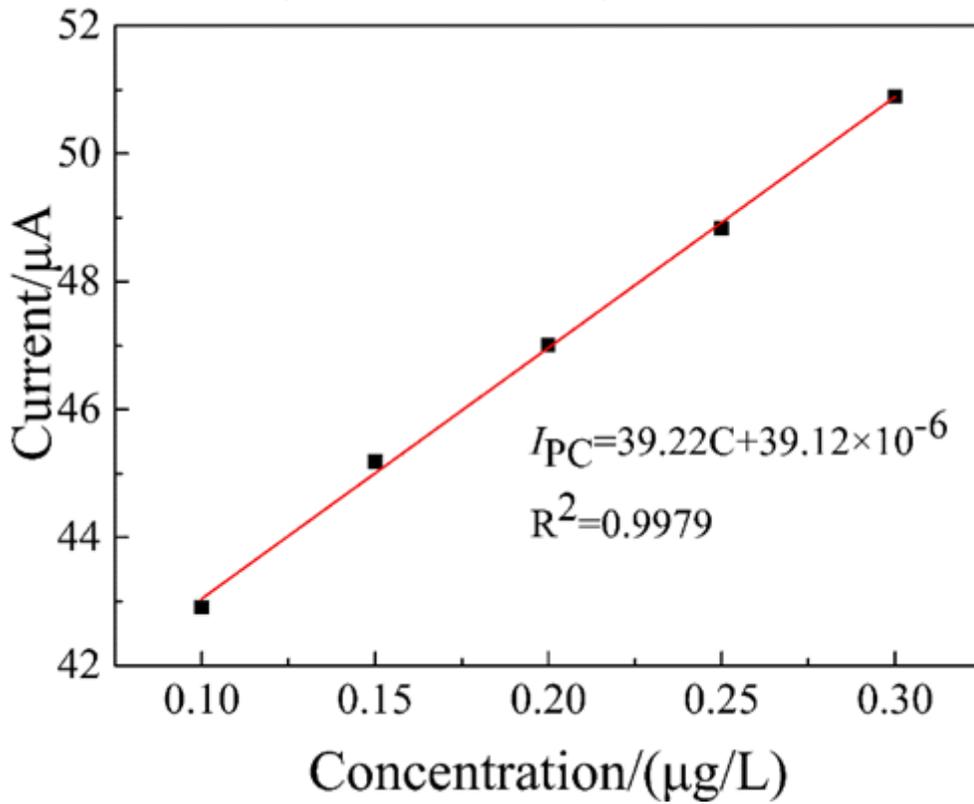


Figure 8

Plot of I_{pc} versus concentrations of isoniazid

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