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Electrochemical Determination of Isoniazid at Electrochemically reduced graphene oxide modified Electrode

*Srikanth Cheemalapati, Selvakumar Palanisamy, Shen-Ming Chen**

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology,
No. 1, Section 3, Chung-Hsiao East Road, Taipei 106, Taiwan, ROC
*E-mail: smchen78@ms15.hinet.net

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The electrochemical determination of Isoniazid (INZ) was reported based on the electrochemically reduced graphene oxide (ERGO) modified glassy carbon electrode (GCE). ERGO was prepared by electrochemical reduction of graphene oxide (GO) in the pH 5.0 solution. As prepared ERGO was characterized by Scanning electron microscopy and electrochemical impedance spectroscopy. Linear sweep voltammetry was employed for the electrochemical determination of INZ. ERGO modified GCE has superior electrocatalytic activity towards INZ than GO and Bare GCE electrodes. Moreover, the linear range for INZ has been found from 2 µM to 70 µM with a LOD of 0.17 µM. Furthermore, the sensitivity of the proposed electrode towards INZ was found as 3.987 µA $\mu M^{-1} cm^{-2}$. Additionally, ERGO/GCE modified electrode has exhibits good reproducibility, repeatability with storage stability.

Keywords: Electrochemically Reduced Graphene Oxide, Glassy carbon electrode, Isoniazid, electrocatalytic activity, Modified electrodes, pharmaceutical applications.

1. INTRODUCTION

Isoniazid (INZ) is one of the widely used effective tuberculosis drug, which has acted against mycobacterium strains (1). INZ is generally has giving to the patient's single or fixed dose combinations with Pyrazinamide, Rifampicin and Ethambutol Hcl (2, 3). The regular intake of higher dosage Isoniazid tablets may leads to severe liver problems. It is necessary to monitor the INZ dosage levels at regular intervals during the treatment (4). The reason for that, during the INZ metabolism hydrazine will form, which induces hepatotoxicity and it could causes death to the patients (5). Moreover, the INZ treating patients were very critical to monitor in all the time, since the importance of this drug in clinical chemistry makes necessity step to develop the good analytical methods to determine the INZ. In order to develop a sensitive method to determine the INZ in the pharmaceutical as well as in research and development centers (6), researchers are focusing on the simple and accurate methods. For the last few decades, many methods have been developed for the accurate determination of INZ such as including titrimetry, spectrophotometry, chemiluminescence, High performance liquid chromatography, fluorimetry, capillary electrophoresis and electrochemical methods [7-14]. On the other hand, electrochemical methods are simple to determination of INZ with accuracy; moreover other traditional methods are generally complicated to operate rather time consuming one. However, many reports have been found for the accurate determination of INZ by electrochemical determination. Especially, nanomaterial modified electrode has been used as a good sensing platform for the INZ determination with better sensitivity [15-21].

Since, there were only few reports are available for the electrochemical determination of INZ. Among various nanomaterials, reduced graphene oxide (RGO) is one of the good conductive materials which have more surface area than carbon nanotubes. Many methods have been used to prepare the RGO from the graphene oxide, such as electrochemical methods, chemical reduction and thermal reduction and so on. Among all these methods electrochemical methods is very simple one for the preparation of electrochemically reduced graphene oxide (ERGO), while other methods have used toxic reducing agents or high temperature operating one [22-23]. However, RGO has not been used for the electrochemical determination of INZ, and also it not yet been studied detailed.

Here, we have reported electrochemical determination of Isoniazid at ERGO modified glassy carbon electrode in pH 7 solution. While, ERGO was prepared by simple electrochemical reduction of GO modified electrode. The high conductivity of ERGO which is more helpful for the accurate determination of INZ with good sensitivity. Moreover, the practicality of the proposed sensor also been studied in detail. The chemical structure of the INZ has been shown in Fig.1.

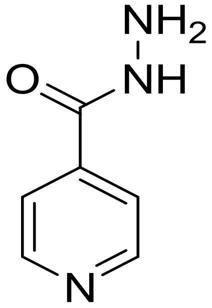

Figure 1. Chemical structure of Isoniazid

2. EXPERIMENTAL

2.1 Chemicals

Isoniazid (4-Pyridinecarboxylic acid, hydrazide) was purchased from Sigma-Aldrich. The supporting electrolyte used for all experiments was pH 7 phosphate buffer solutions (PBS), which was prepared by using 0.05 M Na_2HPO_4 and NaH_2PO_4 solutions. All other chemicals were of analytical grade and used without any further purifications.

2.2 Apparatus

CHI 1205 and 750A electrochemical workstations (CH Instruments) were employed for the cyclic voltammetry and linear sweep voltammetry studies. Hitachi S-3000 H, scanning electron microscope (SEM) was used to investigate the surface morphology of the composite. A conventional three-electrode system consisting of a ERGO modified glassy carbon electrode (GCE) was used as a working electrode (active surface area = 0.079 cm^{-2}), an Ag/AgCl electrode (Sat. KCl) as reference electrode and a platinum wire with 0.5 mm diameter as the counter electrode was employed for electrochemical experiments. All measurements were carried out at ambient conditions.

3. RESULTS AND DISCUSSION

3.1. Electrochemical fabrication of ERGO modified GCE

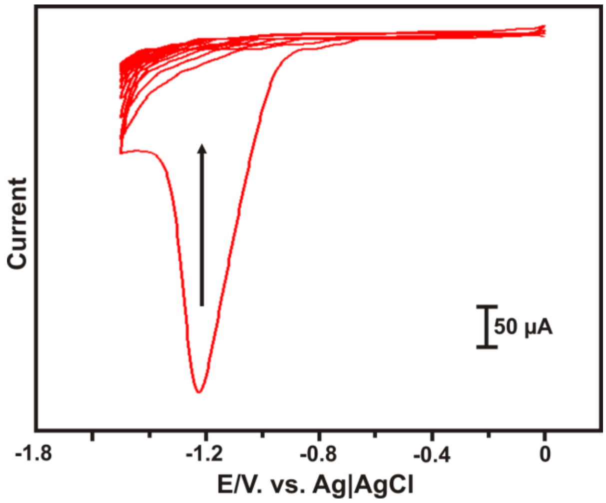

Figure 2. 30 successive cyclic voltammograms were performed in GO modified GCE at 0 to -1.5 V in pH 5 solution at the scan rate 50 mVs^{-1}.

Graphite oxide was prepared by Hummers method [24]. The prepared graphite oxide (0.5 mg ml^{-1}) was dispersed in the water, and sonicated further for 2 hr, finally exfoliated to graphene oxide (GO). Prior to the electrode modification, the GCE was polished by alumina slurry and continue polish with Buehler polishing cloth. The polished GCE was washed with deionized water and sonicated for 3 minutes in ethanol followed by gently rinsed with deionized water to remove adsorbed alumina powder at the electrode surface. About 6 µl of GO was drop casted on the pre-cleaned GCE and dried at room temperature. Then the GO modified GCE was transferred to an electrochemical cell which is containing 0.05 M PBS (pH 5) solution. 30 successive cyclic voltammograms were performed in the potential range of 0 to -1.5 V at the scan rate of 50 mVs^{-1} (Fig.2). During the first cathodic potential scan, a large cathodic peak appears at -1.0 V with an onset potential of -0.71 V, which was attributed for oxygen functional groups at the GO surface [25-28]. Further cycles, the cathodic peak was disappeared completely, attributed to the reduction of oxygen moieties at the GO basal plane [29]. Already few reports were available that GO sheets are mostly decorated with epoxy and hydroxyl groups on the basal plane, while carbonyl and carboxyl groups are located at the edges [30]. After performing the electrochemical reduction of GO modified GCE, it was named as ERGO modified GCE. Finally, the fabricated ERGO containing GCE was kept at the room temperature to dry.

3.2. Surface morphology of the proposed ERGO modified electrode

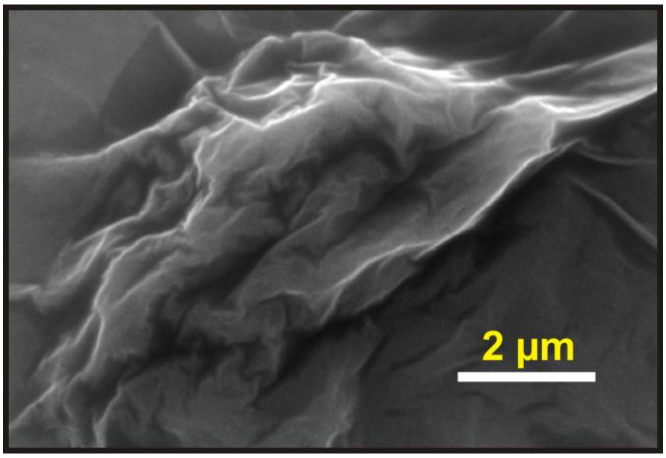

Figure 3. SEM image of ERGO

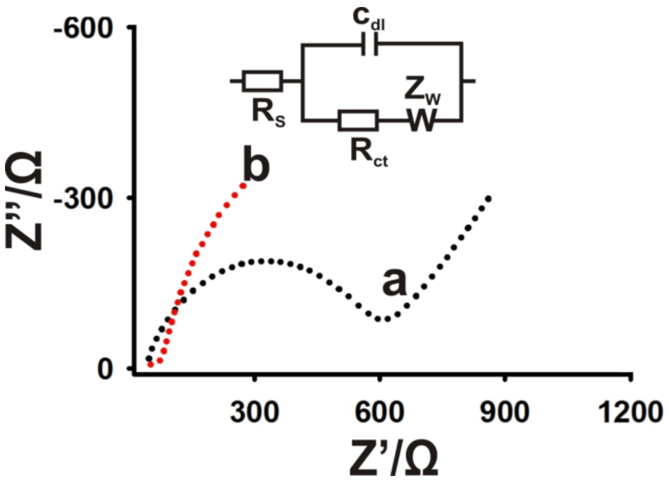

Figure 4. EIS of bare GCE (a) and ERGO/GCE (b) modified electrodes in 5 mM Fe(CN)$_6^{3-/4-}$ containing 0.1 M KCl. Inset is the Randles equivalent circuit. R$_s$, C$_{dl}$, R$_{ct}$ and Z$_w$ represent the resistance of the electrolyte solution, double layer capacitance, charge–transfer resistance and the Warburg impedance, respectively. The frequency range is from 0.1 Hz to 100 kHz.

The surface morphology of the ERGO modified GCE was investigated by scanning electron microscopy (SEM). As shown in Fig.3, ERGO are formed like closely arranged thin crumbled sheets, and the average diameter of each sheets were observed as 100 nm. Moreover, each nano sheets well connected with each other formed like wrinkled morphology. The SEM result confirms that, the electrochemical method is an efficient method for the preparation of ERGO and it's close agreement with previously published same studies. Further, the electron transfer ability of the different modified electrodes was investigated by electrochemical impedance spectroscopy (EIS). The EIS of different modified GCEs as shown in Fig.4. In Fig 4a, for bare GCE shows more R_{ct} value than ERGO modified GCE (b). Whereas ERGO modified GCE has less R_{ct} when compared with other modified electrodes mentioned in Fig.4. While, only GO (figure not shown) containing GCE has higher R_{ct} value than bare and ERGO modified GCEs. Since, the oxygen moieties are located at the GO surface which it makes repulsion with probe of $Fe^{2+/}Fe^{3+.}$ From the results demonstrate that ERGO has high electron transfer ability than other modified electrodes, the due to the reason of high surface area of the ERGO electrode.

3.3. Electrochemistry of Isoniazid at ERGO/GCE modified electrode

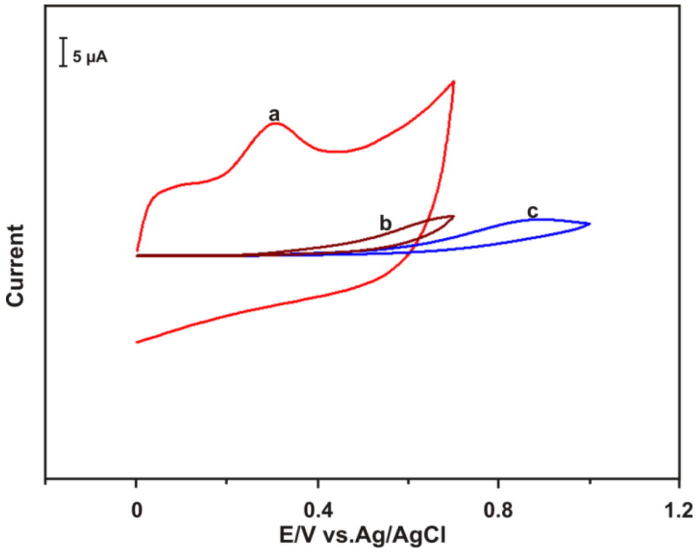

Figure 5. Cyclic voltammograms of ERGO/GCE (a),GO/GCE (b), and bare GCE (c) modified electrodes in the presence of 60 µM INZ in deoxygenated PBS at 100 mV s^{-1} scan rate.

Cyclic voltammogram was employed to evaluate the electrochemical activity of INZ at different modified GC electrodes. The electrochemical oxidations of INZ at different electrodes were examined in PBS (pH 7) which contains the 60 µM of INZ. Fig.5. shows the cyclic voltammograms (CV) obtained at bare GCE (c), GO/GCE electrode (b) and ERGO/GCE modified electrode (a) at the scan rate of 100 mV s^{-1}. Cyclic voltammograms were recorded in the potential range of 0 to 0.7 V for GO/GCE, ERGO/GCE modified electrode and for bare electrode the potential range was fixed from 0 to 1 V. In the voltammograms good oxidation peak with maximum current was appeared at 0.307 V for INZ at ERGO/GCE modified electrode. Whereas, for INZ at GO and bare GCEs were appeared at 0.345 and 0.835 V respectively. However, the oxidation peak current of INZ at ERGO/GCE modified electrode has five times higher than that of bare and GO/GCE modified electrode and the peak potential also observed in the lower potentials. The high conductivity of the ERGO electrode which leads to makes better electron transfer at the electrode surfaces. From the results confirmed that, ERGO/GCE can be effectively used for the INZ detection at lower potential with high sensitivity.

3.4. Effect of scan rate and pH

The effect of scan rate on the voltammetric response of 60 μM INZ at ERGO/GCE modified electrode in deoxygenated PBS also has been studied as shown in Fig.6

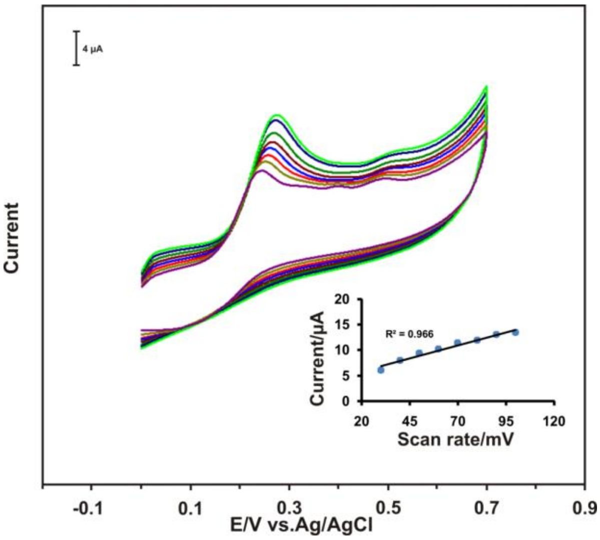

Figure 6. Cyclic voltammograms recorded at ERGO/GCE in 60 μM INZ in deoxygenated PBS at different scan rates. The scan rates from inner to outer are: 30 to 100 mV s^{-1}. Inset shows the linear dependence of I_{pa} vs scan rate (30 to 100 mV s^{-1}).

Upon increasing the scan rates, the oxidation peak current increased linearly. Meanwhile oxidation peak also shifted towards the positive side. The oxidation peak current linearly increased with the increasing the scan rate between 0.03 and 0.1 Vs^{-1} as shown in Fig.6. These results confirmed that, the scan rate played major role and also the oxidation of INZ at ERGO/GCE modified electrode was a typical adsorption-controlled process [31].

The effects of pH also played an important role and need to evaluate the electrocatalytic activity of INZ in different pH solutions. The Fig.7 shows the effect of pH on ERGO/GCE modified electrode in 70 μM INZ contain various buffer solutions (pH 4 to 9). We observed the oxidation peak current of INZ in each buffer solutions, moreover the utmost current response was observed at pH 7 when compare with other pH solutions, the INZ peak shows very good enhancement in the pH 7. Hence pH 7 solution is the optimum pH for INZ determination and we used it for the further analysis.

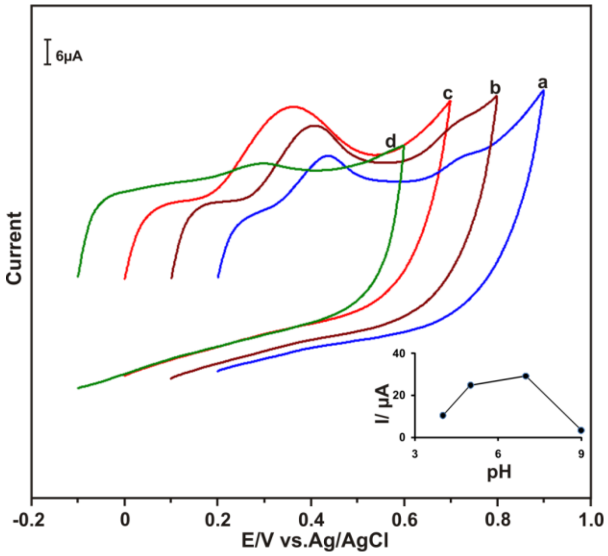

Figure 7. Cyclic voltammograms obtained at ERGO/GCE in 60 μM INZ in deoxygenated various pH solutions (4–11) at the scan rate of 100 mV s^{-1}.

3.5. Determination of INZ by Linear Sweep Voltammetry at ERGO modified electrode

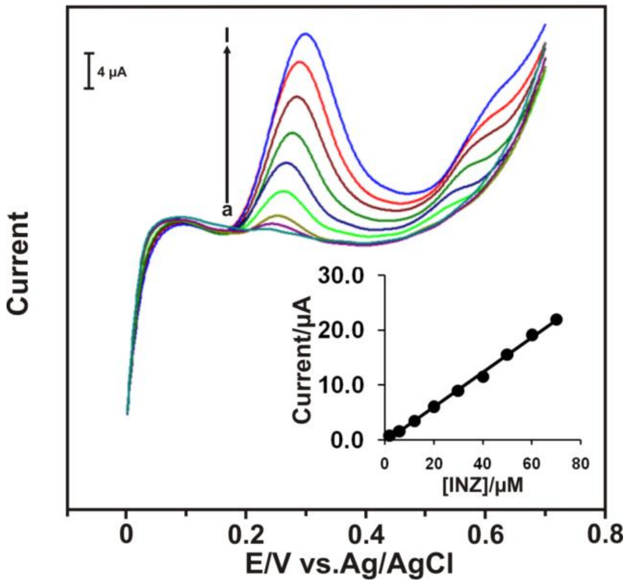

Figure 8. LSV of ERGO modified GCE in different concentration of INZ (a-i) in PBS at the scan rate of 100 mV s^{-1}. Inset plot shows the linear dependence of I_{pa} vs. [INZ]

Fig.8 shows the LSV of ERGO/GCE modified electrode in the presence of different concentrations of INZ in PBS (pH 7). In the presence of INZ, a good oxidation peak was appeared at 0.307 V at the electrode surface. Upon increasing the concentration, the oxidation current also increased gradually up to 70 µM. Moreover, the oxidation peak current has increased linearly with successive addition of different concentrations of INZ (a-i). Furthermore, the calibrations curve from LSV, the linear ranging was found for INZ from 2 to 70 µM with a correlation coefficient of 0.997 (inset). The LOD was found to be 0.17 µM. The sensitivity was found to be 3.987$\mu A \mu M^{-1} cm^{-2}$. These results validates that the ERGO/GCE modified electrode has possess good electrocatalytic activity towards INZ with good sensitivity and wider linear range.

3.6. Real sample analysis

The real sample analysis is very important to evaluate the practicability of the proposed electrode with acceptable recovery of the analytes. For real sample analysis, commercially available INZ tablets (100 mg LC) were used for real sample analysis. Prior to the analysis, the tablets were crushed to fine powder with mortar and pestle. For the preparation of 0.001M concentration of INZ real sample, the crushed tablet powder was accurately weighed and transferred into 10 ml volumetric flask, 5 ml of pH 7 (0.05 M PBS) solution was added followed by ultra sonication in cold water bath about 20 minutes, finally the solution was made up to 10 ml mark with the buffer solution. The real sample analysis was carried out by LSV technique similar like Sec.3.5.

Table 1. Determination of INZ in commercially available INZ tablets by linear sweep voltammetry at ERGO modified GCE.

Sample labeled	Added (µM)	Found (µM)	Recovery (%)
1	20	19.82	99.1
2	25	24.90	99.6
3	30	30.1	100.3

The analytical performances of INZ at ERGO/GCE modified electrode were summarized in table 1. From Table 1, it was evident that the modified electrode has possessed good recovery results towards INZ tablets, and it could be used at pharmaceutical industries in near future.

3.7. Stability, repeatability and reproducibility

In order to examine the storage stability of the modified electrode towards INZ oxidation, it was stored in N_2 saturated PBS (pH 7) containing 30 µM of INZ at 4°C, and the oxidation current response was monitored every 4 hours up to 20 hours using CV study. The modified electrode retained about 94 % of INZ initial sensitivity at 4°C, representing the modified electrode has good storage stability towards the INZ detection. The repeatability and reproducibility of the proposed sensor were evaluated by CV studies. The three electrodes fabricated independently showed an acceptable reproducibility of 3.22 % and also it has acceptable repeatability about 3.6 % for 10 successive measurements for 30 µM INZ. These results validating that, the modified electrode has the good repeatability and reproducibility for INZ detection.

4. CONCLUSIONS

In summary, a simple electrochemical technique was performed for the determination of the Isoniazid at the ERGO/GCE modified electrode. While, ERGO was fabricated by simple electrochemical reduction of GO. Moreover the proposed sensor towards the INZ has the good linear range of detection from 2 µM to 70 µM with a LOD of 0.17 µM. Besides that, INZ at ERGO modified electrode has possessed an acceptable reproducibility, repeatability with good sensitivity and storage stability. The real sample analysis also validates that, the modified electrode has good recovery results towards pharmaceutical tablets. Further, this method could be used for accurate detection of INZ in pharmaceutical industries in near future.

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