Int. J. Electrochem. Sci., 7 **(2012)** 9138 - 9149

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Oxidation and Amperometric Determination of Isoniazid at Functionalized Multiwalled Carbon Nanotube Modified Electrode

Wan-Chun Chen, Binesh Unnikrishnan, Shen-Ming Chen[*]

Electroanalysis and Bioelectrochemistry Lab, 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

Received: 2 August 2012 / *Accepted:* 7 September 2012 / *Published:* 1 October 2012

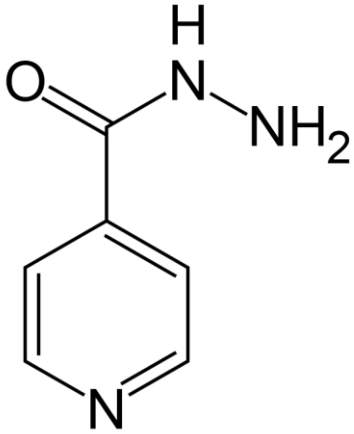
In this work we report the fabrication of an electrochemical sensor for the amperometric determination of isoniazid (ISN) based on functionalized multiwalled carbon nanotube (f-MWCNT) modified glassy carbon electrode (GCE). f-MWCNT has been synthesized by acid treatment of MWCNT by ultrasonication. The voltammetric behavior of ISN at various carbon nanomaterials such as MWCNT, f-MWCNT, graphene oxide, reduced-graphene oxide, bare GCE, etc have been studied by cyclic voltammetry (CV). ISN undergoes electrochemical oxidation at all the aforementioned nanomaterials modified electrodes. However, f-MWCNT film modified GCE shows the maximum peak current for ISN among the various carbon nanomaterials, which is 7.11 times higher than that obtained at MWCNT film. ISN undergoes an irreversible oxidation at f-MWCNT film in pH 4 at 0.4V which is well defined and it can be utilized for electroanalytical purposes. ISN showed fast amperometric response at f-MWCNT modified GCE with a good linear range of detection 1 to 70 μM ISN. The film showed good background current stability and excellent performance in pharmaceutical sample analysis with good reproducibility.

Keywords: Isoniazid, multiwalled carbon nanotube, electrochemical oxidation, cyclic voltammetry, amperometry, electrochemistry.

1. INTRODUCTION

Isoniazid (ISN) is used worldwide as an effective drug in the treatment of tuberculosis. Isoniazid (isonicotinic acid hydrazide) is administered in combination with rifampicin and pyrazinamde to fight the drug-sensitive strains of Mycobacterium *tuberculosis* [1]. Toxicity such as hepatotoxicity of ISN [2], therapeutic failure, relapse and multiple drug resistance [3] associated with

ISN intake have been reported [4]. Hydrazine is produced during the isoniazid metabolism which induces hepatotoxicity with inflammation and it can lead to death [5]. Therefore, the determination of ISN in clinical and pharmaceutical samples is very important. Several methods have been employed for the determination of ISN. Segovia et al. reported the simultaneous determination of ISN and acetylisoniazid in plasma by high performance liquid chromotography [6,7,8]. Techniques such as electrochemiluminescence [9, 10, 11], titrimetry [12], chemometry [13], spectroflurimetry [14], colorimetry [15] and electrochemical methods such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) have also been used successfully for the determination of ISN [16]. Oliveira et al. demonstrated the flow injection amperometric determination of ISN using screen-printed carbon electrode modified with silver hexacyanoferrates nanoparticles [17]. Amperometry [18, 19], chronoamperometry [20], differential pulse voltammetry [20, 21] and linear sweep voltammetry [22] also have been successfully demonstrated. Among the various techniques mentioned, electrochemical techniques are advantageous due to fast response, easy handling, do not use organic solvents and no separation techniques involved. Several carbon based materials modified electrodes have been used for the electrochemical determination of ISN. Poly-L-histidine modified screen-printed carbon electrode shows good performance in the determination of ISN in human urine samples [23]. ISN shows good response at multiwalled carbon nanotube (MWCNT) paste electrode [24]. Thionine immobilized multi-walled carbon nanotube modified carbon paste electrode [25], Iron-tetrasulfophthalocyanine functionalized graphene nanosheets [26], ordered mesoporous carbon modified electrode [19], etc. Silicate network decorated with Au nanoparticles are efficient in ISN oxidation and its amperometric sensing in PBS (pH 9.2) with high sensitivity at low overpotential [18]. The reports [18] and [23] shows oxidation at low overpotential and other reports are at higher overpotential in higher pH. Chemically ISN is pyridine-4-carboxylic acid hydrazide. The chemical structure of ISN is given in scheme 1.



Scheme 1. Chemical structure of ISN

Carbon nanotubes (CNT) have been extensively used in electrode modification for electrochemical studies [27-37] due to their high specific surface area, excellent structural, electronic and mechanical properties. Previous reports show that electrodes modified with pretreated CNT exhibit very good catalytic activity towards the electrochemical oxidation of substances like phenolic compounds [38-40], alkaloids [41] etc. CNT modified electrodes also provide a faster electron transfer rate and catalytic activity towards many important biomolecules [42-44]. In this work we report the electrochemical oxidation and amperometric determination of ISN at f-MWCNT modified electrode in pH 4. A comparison of the electrochemical oxidation of ISN at various carbon nanomaterials has been also made.

2. EXPERIMENTAL

2.1 Apparatus

The cyclic voltammetric experiments were carried out using a CHI 1205 B model electrochemical workstation. A conventional three electrode system with f-MWCNT or MWCNT, graphene oxide and reduced-graphene oxide modified GCE as working electrodes, a thin Pt wire as counter electrode and Ag/AgCl (sat. KCl) as reference electrode was used for electrochemical studies. The electrode surface area of GCE was 0.0706 cm^2. Electrochemical impedance spectroscopy (EIS) measurements were done using IM6ex ZAHNER (Kroanch, Germany). Scanning electron microscopy (SEM) was performed using a Hitachi S-3000 H Scanning Electron Microscope. The hydrodynamic experiments were carried out using a 1205 B potentiostat with AFMSRX analytical rotator (PINE Instruments, USA). All the experiments were carried out at room temperature (25 ±2 °C). Purified nitrogen gas was used for deoxygenation of solutions.

2.2 Materials and reagents

MWCNT with O.D. 10 – 15 nm, I.D. 2–6 nm and length 0.1–10 μm and Isoniazid (Pyridine-4-carboxylic acid hydrazide) was purchased from Sigma–Aldrich. 0.1 M acetate buffer solution (pH 4) was prepared from 0.1 M acetic acid and 0.1 M sodium acetate in doubly distilled deionized water. Real sample, ISN tablets (50 mg/tablet) was purchased from local pharmaceutical company in Taiwan. Inert atmosphere was set by passing N_2 over the solution during experiments.

2.3. Preparation of f-MWCN modified electrode

The functionalization of MWCNT was done following the procedure reported earlier [45, 46]. 150 mg of MWCNT was heated at 350 ºC for 2 h to remove amorphous carbon and volatile impurities and allowed to cool down to room temperature. The purified MWCNT was treated by ultrasonication in a mixture of sulfuric acid and nitric acid in 3:1 ratio for 6 h. The treatment with the acid mixture removes the catalyst impurities present in the MWCNT. The carbon atoms at the edge plane as well as at the defect sites on the basal plane undergo oxidation during acid treatment producing –COOH groups. It was then washed several times with deionized water until the filtrate was neutral and then dried. The functionalized MWCNT is denoted as f-MWCNT. A uniform dispersion of f-MWCNT in deionized water was prepared by dispersing 1 mg of f-MWCNT in 1mL of water by ultrasonication for 30 min. Prior to the electrode modification the GCE was polished using 0.05 μm alumina slurry and Buehler polishing cloth. The GCE was washed and then ultrasonicated in deionized water and ethanol for 5 min each to remove any adsorbed alumina particles on the electrode surface. To fabricate f-MWCNT modified GCE 6 μL (84.6 μg/cm^2) of f-MWCNT dispersion was drop casted onto the well polished GCE surface and dried at 50 ºC. Electrochemical properties of ISN at unmodified MWCNT, graphene oxide and reduced graphene oxide (RGO) modified GCEs were also prepared. Graphene oxide (GO) was prepared modified Hummer's method [47, 48]. The reduced graphene oxide modified electrode was prepared by electrochemical reduction of GO in pH 5 by CV [49] for 30 cycles.

3. RESULTS AND DISCUSSION

3.1 Characterization of f-MWCNT modified electrode by SEM and EIS

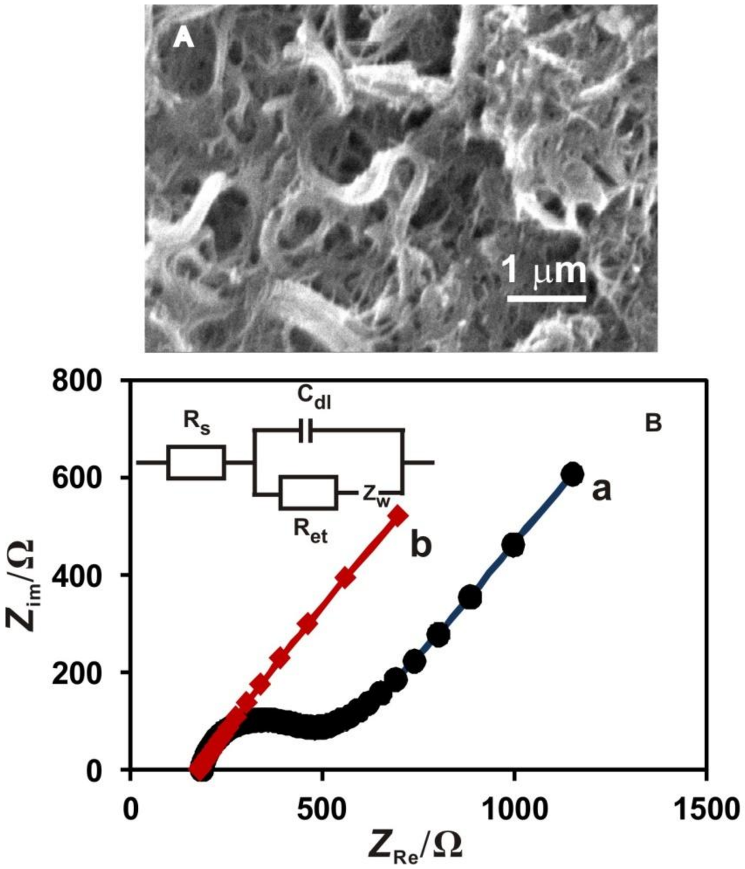


Figure 1. A) SEM image of f-MWCNT modified electrode and B) EIS of a) bare GCE and b) f-MWCNT modified GCE in 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in 0.1 M KCl. Applied AC voltage: 5 mV, frequency: 0.1 Hz to 100 kHz.

Fig. 1 (A) shows the SEM image of f-MWCNT film coated on indium tin oxide (ITO) electrode. f-MWCNT film was prepared by drop casting from a 1 mg/mL f-MWCNT dispersion in water. The surface morphological characterization of the modified electrode by SEM shows that f-MWCNT appears as thin nanofibers with random distribution on the ITO. The sonication process helps to unlock the MWCNT bundles into more simple strands of MWCNTs. f-MWCNT is formed as a porous matrix which helps to increase the diffusion of the analyte and thereby increasing the electrocatalytic properties of the electrode. The electrochemical impedance properties of the bare GCE and f-MWCNT modified GCE were recorded in 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in 0.1 M KCl and are represented as Nyquist plot (Z_{im} vs. Z_{re}) in Fig. 1(B). The inset of Fig. 1 (B) shows the Randles equivalence circuit model used to fit the experimental data. Where R_s is the electrolyte resistance, R_{et} is charge transfer resistance, C_{dl} double layer capacitance and Z_w is Warburg impedance. The semicircle appeared in the Nyquist plot indicates the parallel combination of R_{et} and C_{dl} resulting from electrode impedance. The semicircles obtained at lower frequency represent a diffusion limited electron transfer process and those at higher frequency represent a charge transfer limited process. Bare GCE shows a larger semicircle of than that of f-MWCNT modified GCE. GCE shows a charge transfer resistance of around 300 Ω. However, f-MWCNT shows very small semicircle in the Nyquist plot indicating very low charge transfer resistance. The low resistance exhibited by f-MWNT is due to the high conducting nature of MWCNT.

3.2 Electrochemical behaviour of ISN at different electrodes

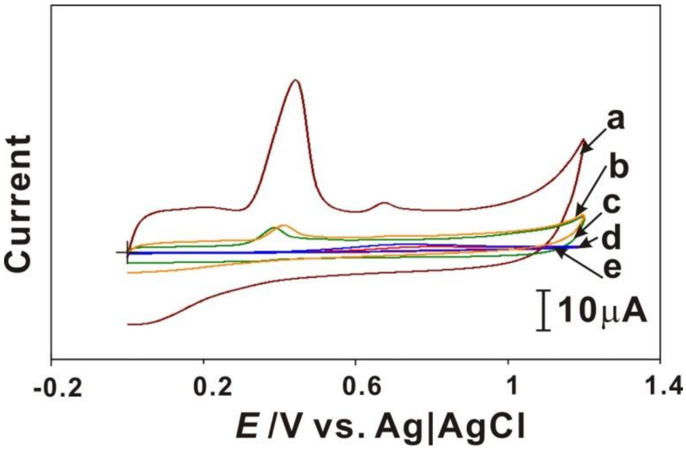


Figure 2. Cyclic voltammograms of 1 × 10^{-4} M ISN in N_2 saturated pH 4 acetate buffer solution a) f-MWCNT/GCE, b) MWCNT/GCE, c) graphene oxide/GCE, d) reduced-graphene oxide/GCE and e) bare GCE. Scan rate: 0.05 Vs^{-1}.

Fig. 2 shows the cyclic voltammetric behavior of ISN at various electrodes in acetate buffer (pH 4). Cyclic voltammograms were recorded in the potential range of 0 to 1.2 V vs. Ag/AgCl reference electrode at a scan rate of 0.05 Vs^{-1}. In prior to each experiment, purified N_2 was purged into acetate buffer containing 1 × 10^{-4} M ISN for 10 min. ISN shows an irreversible oxidation peak at bare GCE at 0.8 V with an anodic peak current (I_{pa}) of 2.3 µA at 0.8 V. At graphene oxide modified GCE the peak appears at 0.75 V with I_{pa} of 9.25 µA. At reduced graphene oxide modified GCE the oxidation peak appears at 0.41 V with a peak current of 0.7 µA. MWCNT/GCE also shows the oxidation peak at 0.4 V with a higher peak current 9.25 µA. f-MWCNT/GCE shows the oxidation peak at same potential, however, with a very high peak current of 65.85 µA which is 7.11 times higher than that of MWCNT/GCE. As evident from the EIS results in section 3.2, f-MWCNT exhibits very low electron transfer resistance. This provides a fast electron transfer thereby increasing the efficiency of the electrode for the electrocatalysis of ISN. Shahrokhian et *al*. reported the oxidation peak of ISN at 0.849 V in pH 4 [24]. In our work 400 mV shift in the peak potential at f-MWCNT/GCE and the high increase in peak current reveal the excellent electrocatalytic and conducting properties of f-MWCNT. Therefore, we observed that f-MWCNT is the best among the above mentioned carbon materials for ISN electrocatalysis. At low pH the positively charged cationic ISN [22] can make contact with the negatively charged functionalized MWCNT. This helps to increase the electrocatalytic activity.

3.3 Effect of pH on the electrochemical behavior of ISN at f-MWCNT/GCE

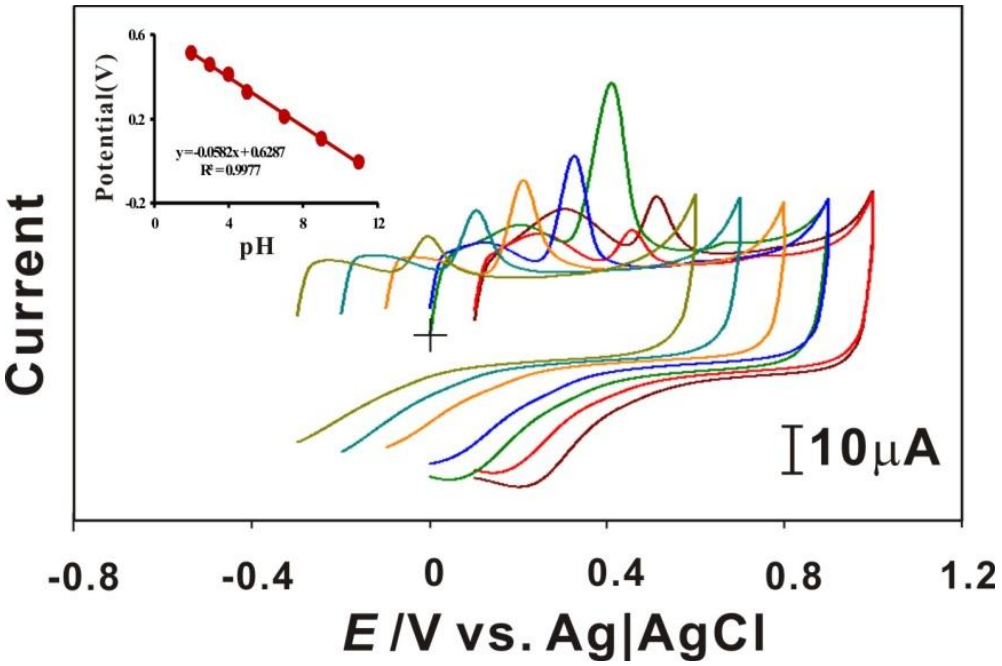


Figure 3. Cyclic voltammograms of 1 × 10^{-4} M ISN at f-MWCNT/GCE in different pH solutions a) 2, b) 3, c) 4, d) 5, e) 7, f) 9 and g) 11 at a scan rate of 0.05 Vs^{-1}. Inset the pH vs. peak current.

Fig. 3 represents the cyclic voltammograms of 1 × 10^{-4} M ISN at f-MWCNT/GCE in the pH range from 2 - 11 at a scan rate of 0.05 Vs^{-1}. ISN exhibits well defined anodic peak in all pH. The peak potential shifts towards negative potential with the increase in pH. The inset of Fig. 4 shows the plot of peak potential vs. pH. The change in peak potential with pH is 58 mV/pH. This result indicates that the electrooxidation of ISN taking place at the f-MWCNT/GCE involves equal number of electron and proton transfer process. As can be seen from Fig. 4, the peak has the highest current in pH 4. Therefore, pH 4 has been chosen for all the electrochemical experiments in this work.

3.4 Different scan rate studies for f-MWCNT/GCE

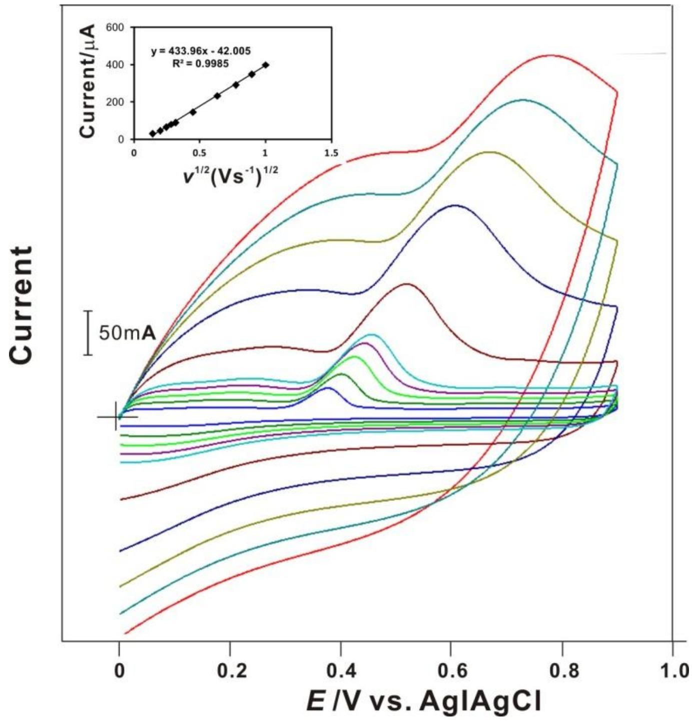


Figure 4. Cyclic voltammograms recorded at f-MWCNT/GCE in N_2 saturated acetate buffer (pH 4) in presence of 5 × 10^{-5} M ISN at different scan rates. From inner to outer: 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 Vs^{-1}. The inset shows the plot of I_{pa} vs. $\nu^{1/2}$.

Different scan rate (ν) experiment has been conducted to understand the nature of electrochemical process taking place at the electrode surface. The different scan rate studies were conducted for f-MWCNT/GCE using CV in acetate buffer (pH 4) containing 5 × 10^{-5} M ISN in the potential range of 0.0 – 0.9 V. Fig. 4 shows the cyclic voltammograms obtained for ISN at different scan rates from 0.01 to 1.0 Vs^{-1}. The anodic peak current increases with the increase in scan rate. The linear dependence of the peak current with ν is given in the inset of Fig. 4. I_{pa} increases linearly with $\nu^{1/2}$. The linear regression equation can be written as I_{pa} (μA) = 433.96 ($\nu^{1/2}$) – 42.005, R^2 = 0.9985. The effect of scan rate on the peak current of ISN oxidation can be described by Randles–Sevcik equation as given in Eq. (1) [50]

$$I_p = 2.99 \times 10^5\, n\, [(1-\alpha)n_\alpha]^{1/2}\, A C_b D^{1/2} \nu^{1/2} \qquad (1)$$

Where, I_p is the forward peak current, n is the number of electrons exchanged per molecule, α is the electron transfer coefficient, n_α is the number of electrons involved in the rate-determining step, A is the area of the electrode, C_b is the bulk concentration of ISN, D is the diffusion coefficient of ISN and ν is the scan rate in Vs^{-1}. The peak potential also shifts towards positive potential with the increase in ν. These results indicate that the electrochemical oxidation of ISN at the f-MWCNT/GCE is irreversible and diffusion controlled process [24].

3.4 Electrocatalytic behavior of ISN at f-MWCNT/GCE

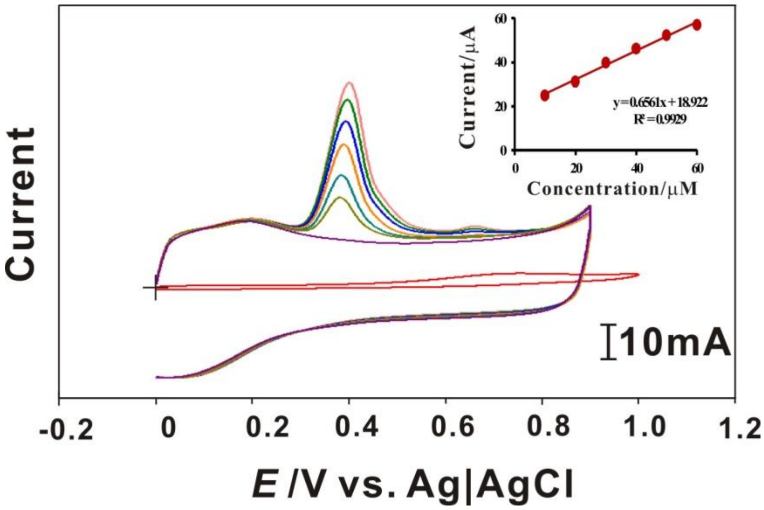


Figure 5. Cyclic voltammograms obtained for ISN in N_2 saturated acetate buffer (pH 4) at f-MWCNT/GCE for various concentrations inner to outer: 0.0, 10, 20, 30, 40, 50 and 60 µM. Scan rate: 0.05 Vs^{-1}. Inset shows the plot of I_{pa} vs. ISN concentration.

Fig. 5 shows the cyclic voltammograms obtained for various concentrations of ISN at f-MWCNT/GCE in pH 4. The ISN concentration in the solution was increased by adding several volumes of 1 × 10^{-2} M ISN solution. The anodic peak current increases with the increase in concentration of ISN from 10 – 60 µM. The linear dependence of I_{pa} with the concentration of ISN is given in the inset of Fig. 5. I_{pa} increases linearly with ISN concentration as per the following linear regression equation I_{pa} (µA) = 0.6561 C (µM) + 18.922, and the linear regression coefficient, R^2 = 0.996. The above result shows the good stability of the film for electrocatalytic applications in which the analyte is added continuously for many additions. Thus the film shows promising properties for its application for electroanalytical purposes. Amperometric technique could improve the sensitivity and linear range of the sensor.

3.5 Amperometric detection of ISN at f-MWCNT/GCE

Rotating disc electrode is a hydrodynamic electrochemical technique which involves the convective mass transport of reactants and products at the electrode surface, when the electrode is rotated at a specific speed [50]. Fig. 6 represents the amperometric response of ISN at f-MWCNT modified RDE with surface area of 0.19625 cm^2. The experiment was conducted in pH 4 (acetate buffer) at an applied potential of 0.4 V with a rotation rate of 1500 RPM. 10 µL of 1 × 10^{-2} M ISN solution was added at regular intervals of time (50 s). Electrocatalytic oxidation of ISN occurs at RDE and a quick response was observed for every addition. The oxidation current increases linearly up to 3650 s which is equal to 70 µ M ISN. The inset of Fig. 6 shows the plot of dependence of current response with the concentration of ISN. The f-MWCNT/GCE shows a good linear range of detection from 1 to 70 µM ISN. The linear regression equation for the current – concentration relationship can be written as I_{pa} (µA) = 0.9463 C (µM). The correlation coefficient (R^2) is 0.9767. The sensitivity of the sensor is 4.82 µA μM^{-1} cm^{-2}.

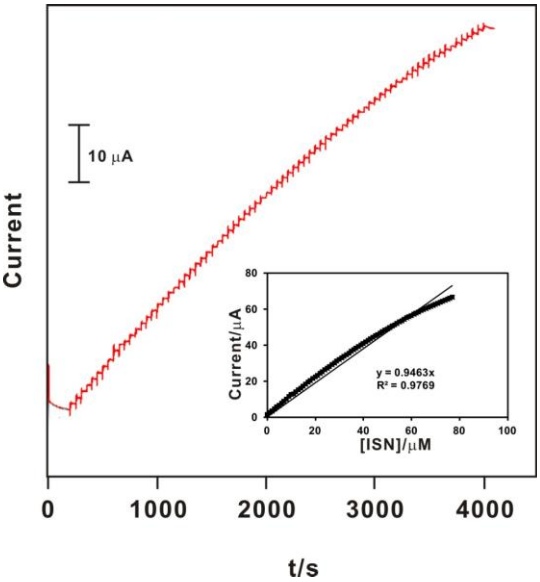


Figure 6. Amperometric response at f-MWCNT modified rotating disc GCE (RDE) for the addition of 1.0 to 79 µM ISN in N_2 saturated acetate buffer (pH 4). Applied potential: 0.4 V; Rotation rate: 1500 RPM. Inset shows the plot of dependence of current response with the concentration of ISN.

The limit of detection (LOD) is 0.27 µM and has been calculated from the equation LOD = $3S_b/S$ and the limit of quantification (LOQ) is 0.91 µM. The LOQ is calculated as LOQ = $10S_b/S$. This result shows the good stability of the proposed electrode and the efficient amperometric detection of ISN.

3.6 Selectivity studies of the modified electrode by amperometry

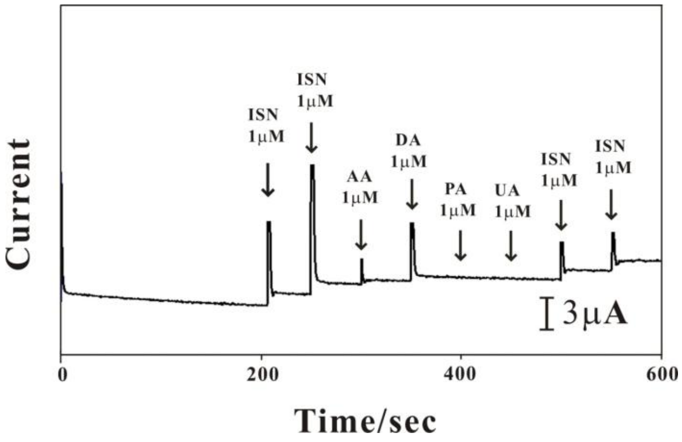


Figure 7. Amperometry response of some common interfering species in N_2 saturated pH 4. Applied potential: 0.4 V. rotation speed: 1500 rpm.

In order to determine ISN in real samples, selectivity study is mandatory for the developed electrochemical sensor. Biological fluids contain biomolecules like ascorbic acid (AA), uric acid (UA), dopamine (DA), paracetaminophen (PA) etc. [51,52] which may interfere the performance of the electrode. Therefore, selectivity of the developed GCE/f-MWCNT electrode was evaluated in the presence of above said interfering species. The technique utilized for the selectivity study was amperometry. Fig. 7 shows the rapid, well defined amperometric responses obtained at GCE/f-MWCNT for 1.0 µM ISN additions. Very little response was obtained for 1.0 µM addition of AA. DA shows a small response for 1.0 µM addition. However, PA and UA did not show any response. Again well defined response was obtained for 1.0 µM ISN additions.

The practical feasibility of the proposed method has been evaluated by the determination of ISN from commercially available tablets. 10 tablets (100 mg ISN/tablet) were powdered well in a mortar using pestle. Then, aliquots of the sample solutions were prepared in pH 4 using calculated amounts of the powdered sample. Amperometry experiments were conducted under the same experimental conditions as mentioned in section 3.3. The proposed film shows good amperometric response for ISN in real sample. The results are shown in table 1. ISN has a recovery of 97.43 to 98.0%.

Table 1. Determination of ISN in pharmaceutical sample

No.	Added (µM)	Found (µM)	Recovery (%)
1	1.0	0.98	98.0
2	3.0	2.933	97.76
3	5.0	4.87	97.43
4	7.0	6.846	97.8

4. CONCLUSION

We demonstrated the electrochemical oxidation of isoniazid at different carbon nanomaterials modified glassy carbon electrode. The EIS studies reveal that f-MWCNT has very less electron transfer resistance which facilitates the fast electron transfer. The negatively charged functional groups in the f-MWCNT help the easy diffusion of ISN to the electrode surface and make the reaction faster and thus increasing the current enormously higher than that of other carbon nanomaterials. pH 4 is very suitable for ISN determination considering its stability and higher peak current. ISN shows good amperometric response in pH 4 with a sensitivity of 4.82 µA μM^{-1} cm^{-2}. The good film stability and stable background current during amperometric experiments at a rotation speed of 1500 rpm shows f-MWCNT is a promising material for other sensor applications.

ACKNOWLEDGEMENT
This project was supported by the National Science Council and the Ministry of Education of Taiwan (Republic of China).

References

1. D. L. Combs, R. J. O'Brien and L. J. Geiter, *Ann., Intern. Med.*, 112 (1990) 397.
2. C. M. Nolan, S. V. Goldberg and S. E. Buskin, *J. Am. Med. Assoc.* 281 (1999) 1014-1018.
3. D. E. Snider and W. L. Roper, *N. Engl. J. Med*., 326 (1992) 703.
4. J. Ray, I. Gardiner, and D. Marriott, *Intern. Med. J.*, 33 (2003) 229-234.
5. S. Tafazoli, M. Mashregi and P. J. O'Brien, *Toxicol. Appl. Pharmcol*., 229 (2008) 94-101.
6. R. M. Swgovia, G. P. Flores, J. D. T. Tirado, X. H. Ramírez, M. V. Pérez and S. R. Moreno, *Acta Chromatogr.*, 19 (2007) 110-118.
7. L. Huang, F. Marzan, A. L. Jayewardene, P. S. Lizak, X. Li and F. T. Aweeka, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 877 (2009) 285.
8. Z. Zhou, L. Chen, P. Liu, M. Shen and F. Zou, *Anal. Sci*., 26 (2010) 1133-1138.
9. B. Wu, Z. Wang, Z. Xue, X. Zhou, J. Du, X. Liu and X. Lu, *Analyst*, 137 (2012) 3644- 3652.
10. L. Hu, J. Gao, Y. Wang and G. Xu, *Anal. Methods*, 3 (2011) 1786-1789.
11. Z. Guo, Y. Xue and X. Zheng, *J. Electroanal. Chem.*, 625 (2009) 47–52.
12. B. S. A. Andrews and A. V. D. N. Kumar, *Res. J. Pharm. Biolo. Chem. Sci.*, 2 (2011) 937-946.
13. E. Dinc, O. Ustundag and D Baleanu, *Drug Test. Anal.*, 2 (2010) 383–387.
14. J. A. G. Bautista, J. V. G. Mateo and J. M. Calatayud, *Anal. Lett*. 31 (1998) 1209 1218.
15. R. Kashyap, E. V. S. Subrahmanyam, A. R. Sharbaraya, *Int. J. Pharm. Pharm. Sci.* 4 (2012) 688-695.
16. U. P. Azad and V. Ganesan (2012) DOI: 10.1007/s10008-012-1724-5.
17. P. R. de Oliveira, M. M. Oliveira, A. J. G. Zarbin, L. H. M. Junior and M. F. Bergamini, *Sens. Actuators, B*, 171-172 (2012) 795-802.
18. B. K. Jena and C. R. Raj, *Talanta*, 80 (2010) 1653-1656.
19. X. Yan, X. Bo and L. Guo, *Sens. Actuators, B*, 155 (2011) 837–842.
20. M. A. Karimi, A. H. Mehrjardi, M. M. Ardakani, R. B. Ardakani, M. H. Mashhadizadeh, S. Sargazi, *Int. J. Electrochem. Sci.*, 5 (2010) 1634 - 1648.
21. H. Y. Xia and X. Y. Hu, *Anal. Lett*. 38 (2005) 1405–1414.
22. N. F. Atta, A. Galal and R. A. Ahmed, *Int. J. Electrochem. Sci.*, 6 (2011) 5097 - 5113.
23. M. F. Bergamini, D. P. Santos and M. V. B. Zanoni, *Bioelectrochemistry*, 77 (2010) 133–138.
24. S. Shahrokhian and M. Amiri, *Michrochim. Acta* ,157 (2007) 149-158.
25. S. Shahrokhian and E. Asadian, *Electrochim. Acta*, 55 (2010) 666–672.
26. N. Li, M. Zhu, M. Qu, X. Gao, X. Li, W. Zhang, J. Zhang and J. Ye, *J. Electroanal. Chem.*, 651 (2011) 12–18.
27. K. C. Lin, Y. C. Lin, S. M. Chen, *Analyst*, 137 (2012), 1378 – 1383.
28. B. Unnikrishnan, Y. Umasankar, S. M. Chen, C. C. Ti , *Int. J. Electrochem. Sci.* 7(2012) 3047-3058.
29. Y. Umasankar, B. Unnikrishnan, S. M. Chen, T. W. Ting, *Int. J. Electrochem. Sci.* 7(2012) 484-498.
30. A. P. Periasamy, Y. H. Ho, S. M. Chen, *Biosensors and Bioelectronics*, 29(2011) 151-158.
31. Y. Li, J. Y. Yang, S. M. Chen', *Int. J. Electrochem. Sci.* 6(2011) 4829-4842
32. Y. Umasankar, S. H. Wang, S. M. Chen, *Analytical Methods*, 3 (11)(2011), 2604 – 2610
33. Y. Umasankar, T. Y. Huang, S. M. Chen, *Analytical Biochemistry*, 408 (2011) 297-303
34. Y. Li, S. Y. Yang , S. M. Chen, *Int. J. Electrochem. Sci.* 6 (2011) 3982-3996
35. J. Y. Yang, Y. Li, S. M. Chen, K. C. Lin, *Int. J. Electrochem. Sci.* 6(2011)2235-2245
36. K. C. Lin, T. H. Tsai, S. M. Chen, *Biosensors and Bioelectronics*, 26(2010) 608-614
37. S. Thiagarajan, T. H. Tsai, S. M. Chen, *Biosensors and Bioelectronics*, 24(2009) 2712-2715
38. H. Qi and C. Zhang, *Electroanalysis.*, 17 (2005) 832.
39. Z. Wang, S. Li and Q. Lv, *Sens. Actuators B*, 127 (2007) 420.
40. Y. Umasankar, A. P. Periasamy and S. M. Chen, *Anal. Biochem.*, 411 (2011) 71.

41. S. F. Wang and Q. Xu, *Anal. Lett.*, 38 (2005) 657.
42. L. Agui, P.Y. Sedeno and J. M. Pingarron, *Anal. Chim. Acta*, 622 (2008) 11.
43. U. Yogeswaran and S. M. Chen, *Electrochim. Acta*, 52 (2007) 5985.
44. U. Yogeswaran and S. M. Chen, *Sens. Actuators, B*, 130 (2008) 739.
45. H. Su, R. Yuan, Y. Chai, Y. Zhuo, C. Hong, Z. Liu and X. Yang, *Electrochim. Acta*, 54 (2009) 4149.
46. C. G. Caridade, R. Pauliukaite and C. M. A. Brett, *Electrochim. Acta* 53(2008) 6732–6739.
47. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc*. 80 (1958) 1339.
48. G. Wang, X. Shen, J, Yao and J, Park, *Carbon*, 47 (2009) 2049-2053.
49. H. L. Guo, X. F. Wang, Q. Y. Qian, F. B. Wang, X and H. Xia, *ACS nano* 3 (2009) 2653-2659.
50. A. J. Bard, L. R. Faulkner, Electrochemical Methods, Fundamentals and Applications, Wiley, New York, 1980.
51. S. M. Chen, K. T. Peng, *J. Electroanal. Chem.* 547(2003), 179-189.
52. P. Shakkthivel, S. M. Chen, *Biosensors and Bioelectronics*, 22 (2007) 1680-1687