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Anti-tuberculosis Drug Pyrazinamide Determination at Multiwalled Carbon Nanotubes/Graphene Oxide Hybrid Composite Fabricated Electrode

Sivakumar Mani, Srikanth Cheemalapati, Shen-Ming Chen[*] *and Balamurugan Devadas*

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 (R.O.C).
[*]E-mail: smchen78@ms15.hinet.net

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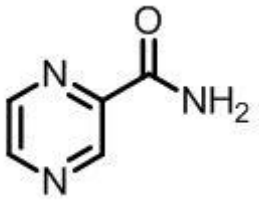
Herein we report a simple electrochemical approach for preparing multiwalled carbon nanotubes (MWCNTs)/graphene oxide (GO) composite obtained by dispersion of MWCNTs and GO. The obtained composite modified glassy carbon electrode (GCE) has to sensitive perform for the electrochemical reduction of pyrazinamide (PZM). The surface morphological results by transmission electron microscope (TEM) confirmed that MWCNTs were enfolded with GO sheets. The chief reason for the detection of PZM at nanocomposite was the highly interaction process effect between MWCNTs and GO. The electrochemical reduction of PZM was employed using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The response of PZM is linear over the concentration range from 37.5 – 1800 µM, with the detection limit (S/N = 3) of 5.54 µM and the sensitivity was found to be 0.038 µA µM -1 cm -2. The proposed sensor exhibits good sensitivity, selectivity and has shown potential for the detection of PZM in real samples with appreciable consistency and precision. In addition, the proposed electrochemical sensor showed good results towards the commercial pharmaceutical formulated PZM samples.

Keywords: Graphene oxide, multiwalled carbon nanotubes, pyrazinamide, reduction, serum.

1. INTRODUCTION

Since, the year1985 pyrazinamide (PZM) attention was increased due to its vital usage for the tuberculosis (TB) therapy [1]. The TB is generally caused by the Mycobacterium tuberculosis, a leading causing adult death rate across the globe, since 2006 the death rate was decreased [2,3]. In order to prevent TB, PZM has been prescribed in single or combination with drugs such as isoniazid,

rifampicin and ethambutol Hcl at fixed dosages [4,5]. The chemical structure of PZM was shown in Scheme.1 and the regular intake of PZM drug causes cutaneous adverse drug reactions and severe hepatic damages [6,7].



Scheme 1. Chemical structure of PZM

Therefore, it is highly essential to monitor the changes in the PZM level in human body fluids for providing effective therapeutic treatments [8]. Owing to the increased use of this drug in clinical analysis, it is important to develop a sensitive method for the determination of PZM in pharmaceutical and biological samples. Untill now, there are several techniques for the detection of PZM, such as UV–visible method [9], capillary-electrophoresis [10], chromatographic methods [11,12], square-wave polarography method[13] and electrochemical methods[14-16]. Nonetheless, the reporting electrochemical method has the advantages of very simple, highly sensitive and less expensive than other time consuming traditional methods [17,18]. Besides, the bare GCE has poor sensitivity and selectivity towards the pharmaceutical drug determinations. Hence to avoide these difficulties, modified electrodes were employed with various materials, such as nanoparticles [19,20] and integrated nanomaterials[21] respectively.

In recent years Graphene oxide (GO) dispersed Multiwalled carbon nanotube (MWCNTs) have been widely studied for its exceptional properties in various fields such as dye electrochemical sensors[22,23], biosensors[24], proton exchange membrane fuel cell[25], dye sensitized solar cells[26], super-capacitors[27] and biofuel cells[28]. Graphene is a monolayer of sp2 bonded carbon atoms and packed closely which looks like a honeycomb lattice structure and it has a large surface area with good mechanical strength GO is an oxygenated derivative of graphene and it is an amphiphilic molecule containing epoxy, hydroxyl and carboxyl groups on its surface[29]. Multiwalled carbon nanotubes (MWCNT) have exclusively used in the electroanalytical field due to its good electrical conductivity, mechanical strength, high surface area, and chemical stability [30]. The special properties of MWCNT have been used for developing of various electrochemical sensors including the biological and drug molecules [31-33]. As a result, the superior performance of the MWCNT/GO nanocomposite is due to the synergy and high edge density [23] between GO and MWCNT. Being a versatile dispersant, GO has an exceptional ability to form stable aqueous dispersions with MWCNTs. The exceptional properties of the prepared composite could enhance the electron transfer ability to electrode as well as reduce the fouling effect of MWCNT. The unique properties of these two different nanomaterials could enhance the PZM reduction than only pristine GO or MWCNT.

Here in, we developed an electrochemical sensor to determine PZM in human blood serum samples by DPV method. MWCNT/GO nano- composite modified GCE exhibited good electrocatalytic reduction towards PZM; this could be ascribed to the synergistic effect of both MWCNT and GO. Further the morphological and electrochemical studies were carried out by Transmission Electron Microscope (TEM) and Electrochemical Impedance spectroscopy (EIS). To the best of our knowledge, no reports were available for MWCNCT/GO/GCE to determine the PZM in human blood serum samples. In addition, the practicality of the proposed sensor was evaluated in commercially available BPH tablets

2. EXPERIMENTAL

2.1. Reagents and Apparatus

MWCNTs with the lengths of 0.1-10 μm (Purity 90%) was purchased from Sigma Aldrich. Graphite powder was obtained from Alfa Aesar, USA and Pyrazinamide procured from Sigma Aldrich. The supporting electrolyte used for all the experiments was pH 7 (0.05 M PBS), prepared by using 0.05 M Na_2HPO_4 and NaH_2PO_4. All the other pH solutions were adjusted with 0.5 M H_2SO_4 and 0.5 M NaOH. Sulphuric acid obtained from J.T.Baker and all other chemicals used in this study were of analytical grade and used without any further purification.

The electrochemical studies were employed with CHI worl stations 1205A and CHI 900 respectively. Transmission electron microscopy (TEM) JEM 2007 model was used for morphology studies. Electrochemical impedance spectroscopy (EIS) studies were performed using IM6ex ZAHNER (Kronach, Germany). Three electrode electrochemical cell system having GCE as a working electrode, Ag/AgCl electrode (Sat. KCl) as a reference electrode and a platinum wire with 0.5 mm diameter as a counter electrode was employed for electrochemical experiments. All the electrochemical measurements were carried out at room temperature and electrolyte cell solutions were kept under a nitrogen (N_2) atmosphere.

2.2. Synthesis of graphene oxide and Preparation of MWCNT/GO hybrid nanocomposite

GO was prepared by the Hummer's modified method[34]. Briefly, the starting material graphite powder was oxidized into graphite oxide by treating with a mixture of concentrated sulfuric acid, sodium nitrate and potassium permanganate. The synthesized brown color graphite oxide was exfoliated through continuous ultra-sonication in water (1 mg/ml) for 2h to get GO. Finally the GO was centrifuged for 15 min to remove the un-exfoliated graphite oxide.

The prepared graphite oxide was exfoliated via ultrasonication for 2 h to get GO. For the preparation of MWCNT/GO nanocomposite, MWCNTs were added into 0.5 mg/ml of GO aqueous solution (1:2, V:V %) and sonicated for 1 h to obtain a homogeneous dispersion[35,36]. Finally, the MWCNT/GO nanocomposite was centrifuged to remove the un bounded MWCNTs and washed several times with deionized water. The as-purified MWCNT/GO composite was dried overnight in the hot oven and redispersed in water, further used for the experiments.

2.3 Fabrication of MWCNT/GO nanocomposite modified GCE

Prior to fabrication process, the bare GCE was well polished with 0.05µm alumina slurry on BAS pad and sonicated with ethanol to remove the alumina particles, further the GCE was washed with deionized water and dried in oven. Then, pre-cleaned GCE kept at room temperature. About 6 µl (optimized concentration) of the MWCNT/GO dispersion was pipetted out on GCE surface and dried at room temperature. As fabricated, MWCNT/GO nanocomposite modified GCE surface was rinsed with water to remove the loosely bounded MWCNT/GO composite and used further.

2.4 Procedure of sample preparation

2.4.1 Lab sample

To sample preparation, 0.01 M of PZM was taken in a 25 ml volumetric flasks containing pH 7.0 PBS and this solution was sonicated for 10 minutes in cold water bath. Then as-prepared sample solution was stored at 4°C when not in use. The sample was prepared 30 minutes preior to the analysis.

2.4.2 Real sample (Human blood serum)

To prepare real blood serum sample, 6 ml of human blood was collected from a healthy male person aged around 25 years and the sample was collected in a test tube. The collected blood sample was kept at room temperature for 30 minutes and then centrifuged for 15 minutes at 1300 rpm. Finally, the supernatant was collected in a new test tube and stored at 4°C in refrigerator when not in use. 1 ml of the supernatant was further diluted 10 times with pH 7.0 PBS to reduce the complex interferences. Aliquot of sample was prepared before 45 minutes prior to analysis. The blood serum was attentively transferred into the 25 ml electrochemical cell and analyzed without any further pretreatment.

2.4.3 Pharmaceutical sample (Tablets)

PZM (500 mg), tablets were collected from the near pharmacy, followed by crushed with mortar and pestle. The powdered samples were exactly weighed and make it for equalize the 0.01M concentration of PZM and carefully transferred to 25 ml volumetric flask and further dissolved with 0.05 M PBS. All the samples were prepared (crushed) before 30 minutes prior to analysis and were stored in refrigerator at 4°C in a closed container when not in use.

3. RESULTS AND DISCUSSION

3.1. Characterization of MWCNT/GO Morphological studies

The as synthesized MWCNT/GO hybrid nanocomposite morphology was studied by using TEM. The TEM morphology of MWCNTs clearly showed in the Fig.1, the MWCNTs are appeared as

a tubular like morphology and GO was observed like an thin flat sheets. The TEM image of MWCNT/GO hybrid nanocomposite showed that the MWCNTs walls were wrapped well with thin sheets of GO. This could be due to the π- π stacking interaction between the sidewalls of MWCNT and hydrophobic regions of GO. Hence, which is clear that the prepared composite was nanocomposite, and the MWCNTs formed a hybrid composite with GO.

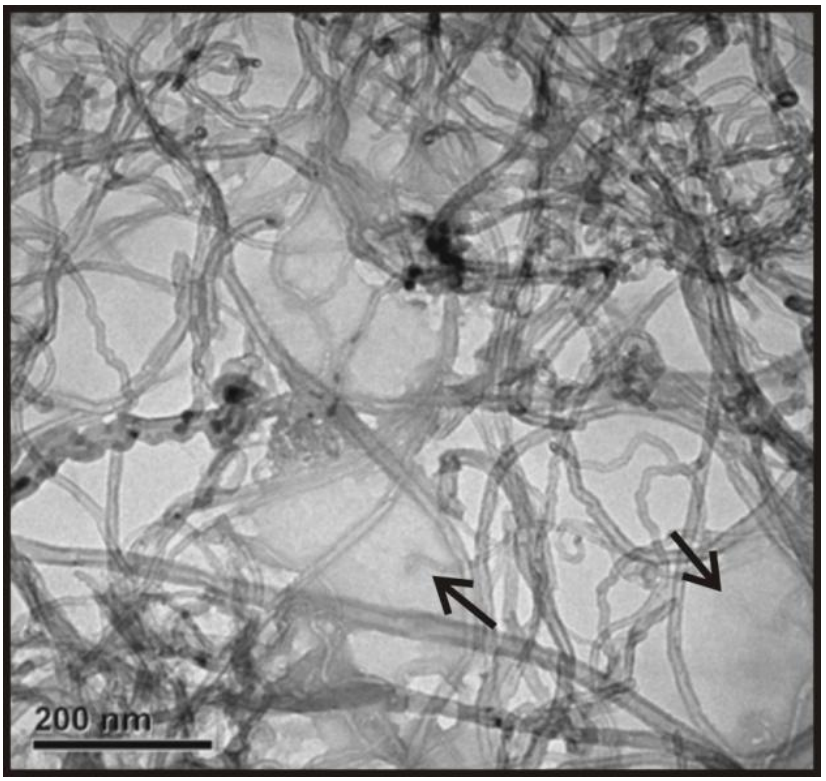


Figure 1. TEM image of MWCNT/GO composite, the marked arrows shows GO sheets wrapped on MWCNTs.

The GO nanosheets fold together with MWCNTs and make more accessible surface area, this high surface area could serve as a good path way for electron conductivity for PZM electrocatalysis.

3.2. Electrochemical impedance spectroscopy (EIS) studies

The electrochemical impedance behavior at MWCNT/GO/GCE, bare GCE, GO/GCE and MWCNT/GCE surfaces have been studied by using EIS technique. EIS is used to study specifically the interfacial properties of surface-modified electrodes in electrochemical fields; the obtained EIS data gives us useful information of the electrochemical impedance changes on the modified electrode surface between each step.

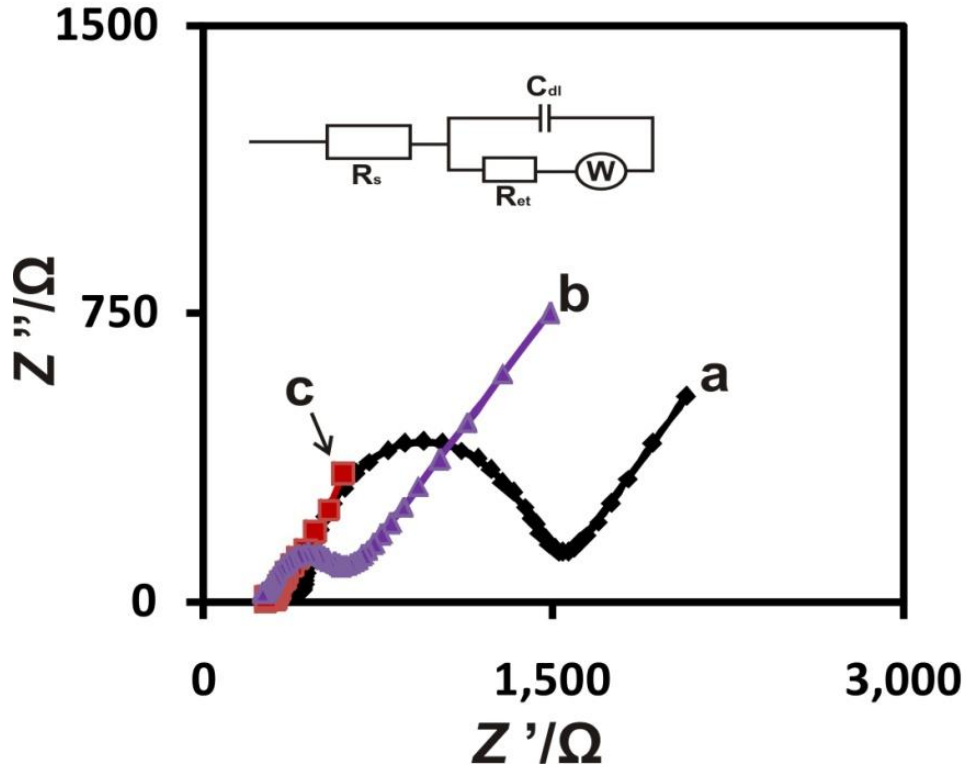


Figure 2. EIS of bare GCE (a), MWCNT/GCE (b) and modified MWCNT/GO/GCE (c) in 5 mM Fe $(CN)_6^{3-/4-}$ solution. Inset is the Randles equivalent circuit model. R_s, C_{dl}, R_{ct} and Z_w represent the resistance of the electrolyte solution, double layer capacitance, charge–transfer resistance and Warburg impedance respectively. The frequency range is from 0.1 Hz to 100 kHz.

And the Fig.2 shows the results of bare GCE (a), MWCNT/GCE (b), MWCNT/GO/GCE (c) and GO/GCE (Fig.S1) in PBS with 5 mmol L^{-1} $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$.

In the current study, EIS of bare GCE exhibits a larger semicircle part with R_{ct} value of 979.1 Ω as shown in Fig.2, is equivalent to the electron transfer resistance and the linear part corresponds to the diffusion process, which exhibits hindered electron transfer at the surface of the GCE. The EIS spectra include a semicircle and linear portions, indicating that the semicircle part corresponds to the electron transfer resistance (Rct) at higher frequencies and the linear part at the lower frequencies corresponding to the diffusion electrochemical process [37, 38] modified GC electrodes. Inset (Fig.2) is the Randles equivalence circuit model used for fitting the EIS data of the above mentioned electrodes. From the Randles equivalence circuit model, we have calculated the electron transfer resistance (R_{ct}), double layer capacitance (C_{dl}), Warburg impedance (W) and electrolyte resistance (R_s) values for the bare GCE, GO/GCE, MWCNT/GCE and MWCNT/GO/GCE. From the Fig.2, a smaller semicircle observed for MWCNT/GO/GCE with R_{ct} value of 10.22 Ω. This indicates that higher electron transfer occurs at MWCNT/GO/GCE due to the synergic effect and excellent conductivity of MWCNT, which increase the conductivity between GO and the electrode surface, when compared with GO/GCE R_{ct} value of 218.6 Ω and MWCNT/GCE has R_{ct} value of 300.3 Ω.

3.3 Effect of scan rate and pH studies at the fabricated electrode

The effect of scan rate was investigated in order to study the nature of the modified electrode process occurring at the electrode surface. Cyclic voltammograms were performed and recorded in the presence of 750 µM PZM prepared in the deoxygenated PBS. The different scan rates were performed from 10 to 200 mVs^{-1} (Figure not shown here).

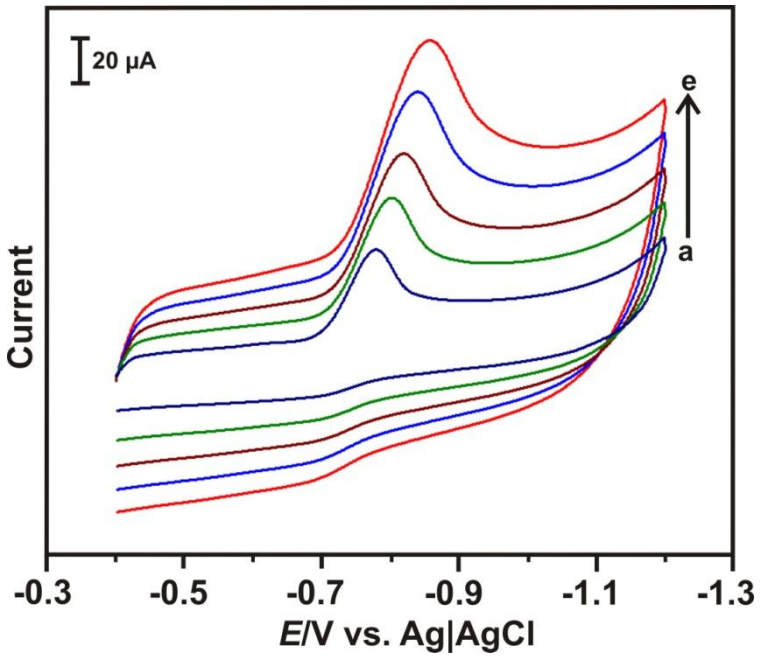


Figure 3. CVs recorded at MWCNT/GO/GCE in 750 µM of PZM in deoxygenated PBS at different scan rates (10 to 50 mV s^{-1}).

Upon increasing the scan rates, the I_{pc} current was increases and also the I_{pa} current also slightly increases shown in the Fig.3 (10 to 50 mVs^{-1}). Hence, the reaction was reversible process and meanwhile the cathodic peak of PZM slightly shifted towards the more negative direction. Furthermore these results confirmed that, the reduction of PZM at MWCNT/GO/GCE was a diffusion controlled electrochemical process [39, 40].

The effect of pH on modified electrode was investigated, Fig.4 depicts the effect of pH for MWCNT/GO/GCE in 750 µM PZM for various buffer solutions (pH 4 to 11). The influence of pH on the reduction of PZM was studied clearly. The peak current shows clear enhancement at pH 5.5 when compared to pH 4 and pH 7, further the cathodic peak current was increasing linearly from pH 7 to pH 11. Moreover, the maximum cathodic current response was observed at pH 11. Based on the physiological and analytical perspective we preferred pH 7 to carry out our remining analytical experiments. The peak potential E_{pc} shifts more negative potential side when increseing the pH and the obtained correlation coefficient is 0.996 (Inset Fig.4).

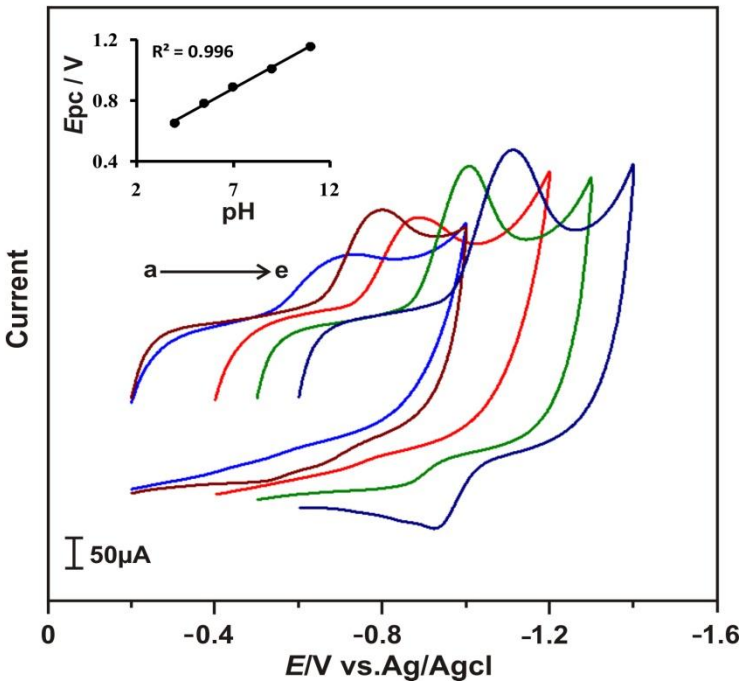
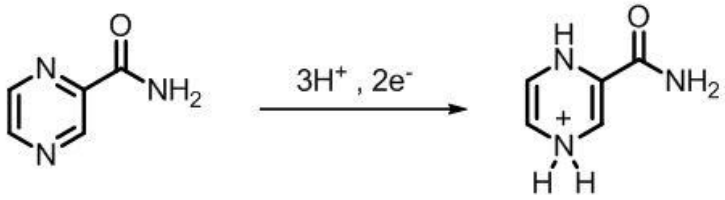


Figure 4. CVs obtained at MWCNT/GO/GCE in 750 µM concentration of PZM in deoxygenated buffer solutions pH 4 (a), pH 5.5 (b), pH 7 (c),pH 9 (d) and pH 11 (e) at the scan rate of 100 mV $s^{-1.}$



Scheme 2. The schematic representation of the redox reaction of PZM leads to 1, 4-hidro-pirazinium ion formation.

According to the Nernstian equation for equal number of proton and electron transfer process is $\Delta E/\Delta$ pH = (59.1 mV/n) * N^{+}_{H} (n=2) and the obtained slope value for the curve in our study is -70.0 mV pH^{-1}, hence we suggest that the number of protons (N^{+}_{H}) transfer could be three, the above results were coincide with the electrochemical behavior of pyrazine in the mercury electrode[41, 42], the redox reaction of the PZM leads to formation of 1, 4-hidropiraziniumion (Shwon in Scheme-2) the mechanism was clearly explained by the Bergamini et al[15].

3.4. Cyclic voltammetry of PZM at MWCNT/GO nanocomposite modified electrode

Cyclic voltammetry was employed to evaluate the electrochemical activity of PZM at different modified GC electrodes. The electrochemical reduction of PZM at different electrodes were examined

in PBS (pH 7) which consists of 1500 μM PZM. Fig.5 shows the cyclic voltammograms (CV) obtained at MWCNT/GO/GCE (a) and bare GCE (b), inset shows the GO/GCE (c), MWCNT/GCE (d) and MWCNT/GO/GCE (a') at the scan rate of 100 mV s^{-1}. In the voltammograms good reduction peak with maximum current was appeared at -0.978 V for PZM at MWCNT/GO/GCE. Whereas, for PZM at GO/GCE and bare GCEs were appeared at -0.842 V and -1.128 V respectively, similarly for MWCNT/GCE PZM cathodic peak appeared at -1.01V.

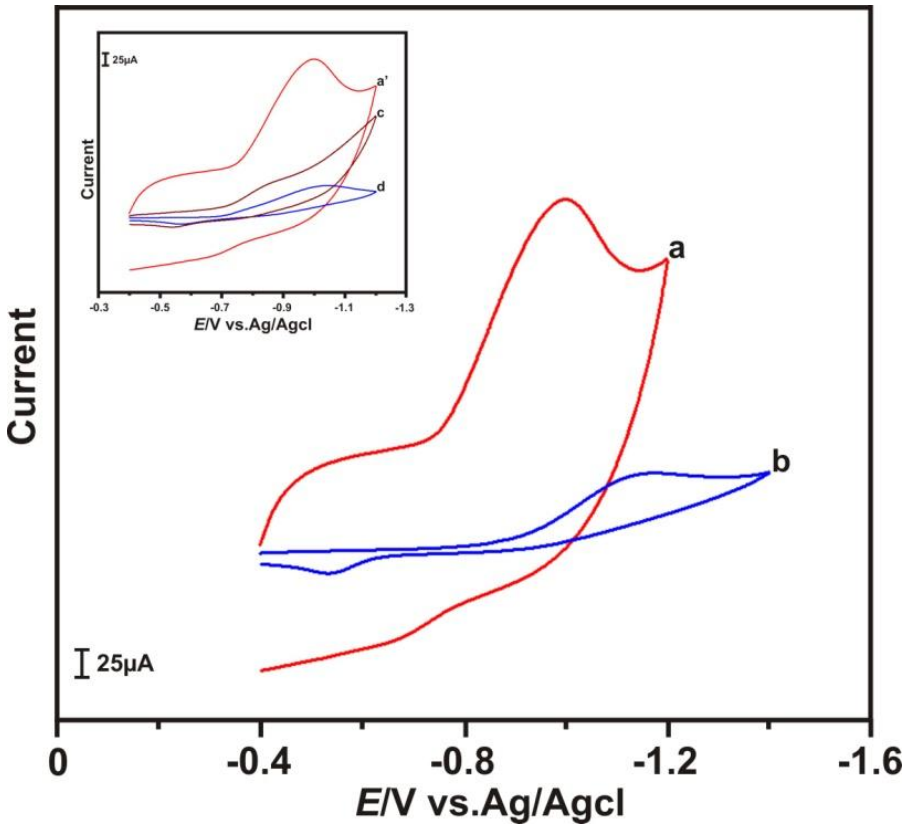


Figure 5. CVs obtained in 1500 μM PZM at bare GCE (a), MWCNT/GO/GCE (b), inset figure showed that MWCNT/GO/GCE (a'), GO/GCE (c) and MWCNT/GCE (d) recorded in the N_2 purged PBS pH 7.0, Conditions: scan rate 100 mVs^{-1}.

When compare to bare and MWCNT/GCE, the MWCNT/GO/GCE and GO/GCE showed a slightly less positive potential. All the modified electrode showed cathodic peaks strongly and responded less peak for oxidation process, hence it exhibits a redox reaction property as reported earlier in the literatures due to the formation of 1, 4-hidropiraziniumion [15]. When compared to other modified electrode our proposed electrode MWCNT/GO/GCE shwoed well cathodic peak with great enhancement which is due to the the excellent catalytic activity of the nanocomposite and surface negative charges due to the presence of COOH groups at basal plane of the GO ,which easily attracts the positively charged PZM and the high conduit is due to the synergic effect of MWCNT/GO. The superior behavior of the GO/MWCNT nanocomposite exhibits a promising platform for the electrochemical determination of PZM.

3.5. Electrochemical determination of PZM by DPV

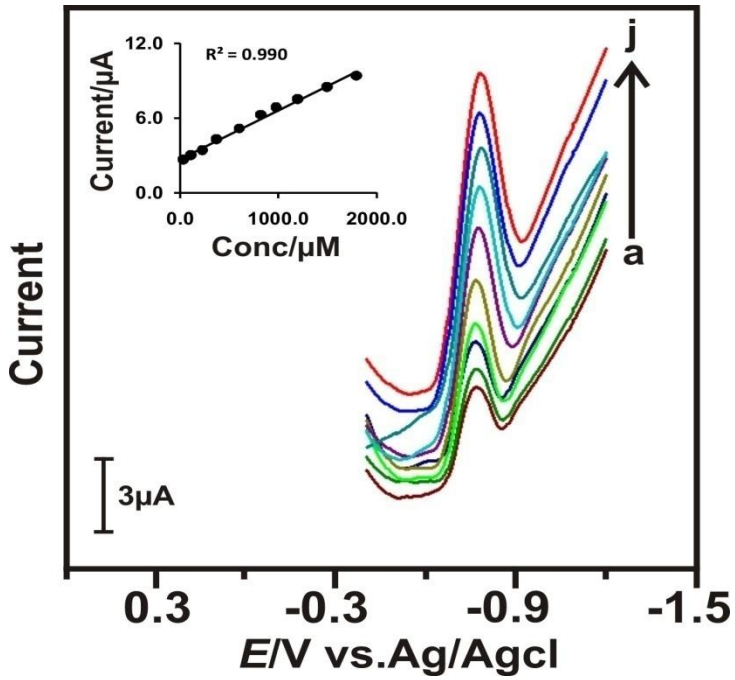


Figure 6. DPVs of MWCNT/GO/GCE obtained in a linear range of 37.5 to1800 µM (a-j) of PZM in PBS (pH 7) at 100 mV s^{-1} scan rate. Inset plot shows the linear dependence of I_{pc} vs. [PZM].

The DPV technique has been followed for the detection of PZM in the potential range of -0.4 to -1.2 V and the following parameters of 50 mV for pulse amplitude, 0.05 ms for pulse width and 200 ms for pulse period was used for this study. In the Fig.6 we have shown the DPV catalysis voltammograms and the resultant calibration. The well defined cathodic peak (Ipc) was appered at -0.772 V, and the obtained results clearly indicating that, our proposed modified GCE shown a good linearity range for PZM as 37.5 to 1800µM range with a linear regression equation R2 value was found as 0.990. The low detection limit (S/N = 3) for PZM was found to be 5.54 µM with a ssensitivity of 0.038 µA µM-1 cm^{-2}.

3.6. Anti-interference studies

In order to investigate the possible interfering electro active species on the fabricated MWCNT/GO/GCE, we monitored the response of proposed electrode by DPV technique with an addition of each 50 µM of Isoniazid, Rifampicin, Oseltamivir phosphate, Ascorbic acid, Uric acid and Ethambutol in the electrochemical cell contains 200 µM of PZM. We investigated the possible interference of these pharmaceutical drugs with PZM peak, it has been noted that no other peak response was observed for each addition of the interfering species, because they are repelled well by the negatively charged MWCNT/GO nanocomposite surface. Additionally few metal ions such as Na^{+}, Ca^{2+}, K^{+}, Mg^{2+} was evaluated by each 50 µM addition. The addition of each electro active interfering species brought out hardly discernible current response. It was concluded that, interfering effect caused

by these electroactive species is quite negligible and that the presence of these substances do not affect the peak potential and current of PZM reduction at the modified electrode. Further, it validating that PZM determination at the MWCNT/GO nanocomposite film was good selective.

3.7. Stability, repeatability and reproducibility

In order to investigate the film stability of proposed sensor, MWCNT/GO nanocomposite modified electrode after an each experiment the modified electrode was washed gently with pH 7 buffer and stored at 4°C, and its background current was tested for almost 30 days. The sensitivity retained about 93.6% and the background current was also 94.2% stable even after 200 consecutive cycles performed by CV technique, further these obtained results clearly indicating, that the excellent film stability of the MWCNT/GO/GCE. The repeatability and reproducibility of the proposed sensor were evaluated by DPV studies and the experimental conditions were similar to the section 3.5. The five electrodes fabricated independently showed an acceptable with a relative standard deviation (RSD) of 3.1 for 200 µM of PZM concentration measurements. The RSD for 10 successive measurements of each 200 µM of PZM is 2.9 revealing the good consistency of the proposed sensor.

3.8. Analytical applications

3.8.1 PZM determination in human blood serum samples

The DPV technique has been used for PZM detection in human blood serum samples to demonstrate the selectivity of biological interference and applicability of the proposed method. The analytical conditions were similar to section 3.5 and the sample preparation stated in the section 2.4.2. As we know that blood serum contains several biological components and complex minerals, but our proposed method shown good agreement of recovery results for PZM detection, further the recovery shows 97.6 and 98.5% respectively for both the samples. It is clear evident that the proposed method could be used for the PZM detection in blood serum in clinical and in pharmaceutical fields and the results were summarized in Table-1.

Table 1. Analytical recovery of PZM for blood serum samples.

Sample labeled	Added (µM)	Detected (µM)	Recovery (%)
1	250	244	97.6
2	400	394	98.5

3.8.2 Pharmaceutical analysis of PZM

Table 2. Recovery of PZM for pharmaceutical formulated tablets

Sample labeled	Added (μM)	Found (μM)	Recovery (%)
1	250	245	98.0
2	400	393	98.2

To confirm the applicability of the proposed method for the analysis of pharmaceutical samples were discussed here. The DPV technique has been employed for PZM determination with the commercially pharmaceutical tablets. The preparation of the samples was clearly mentioned in the section 2.4.3. The concentration of PZM in the pharmaceutical formulations was determined by standard additions method and the obtained results were satisfactory, the results were summarized in Table-2. It is noting that the obtained results obviously shows that the existing drug excipients in the commercially available tablets do not notably interfere with the proposed method. The good agreement with the method is a promising feature for the applicability of the modified electrode for direct determination of PZM in pharmaceutical samples.

4. CONCLUSIONS

In this paper, we have successfully fabricated a MWCNTs/GO composite modified glassy carbon electrode for PZM determination, which exhibits a remarkable electrocatalytic activity for the reduction of PZM. MWCNT/GO nanocomposite was prepared simply by simple sonication of MWCNT with GO aqueous solution. The strong π - π stacking interactions between MWCNT and GO provides good stability to the dispersion.The proposed sensor possesses good sensitivity, selectivity, a low detection limit and good stability due to the synergistic effect of GO and MWCNT, which makes it very promising for the determination of PZM in real samples. The practicality of the proposed modified electrode was evaluated by sensing of PZM in human blood serum and pharmaceutical formulated tablets.

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References

1. Y. Zhang, M. M. Wade, A. Scorpio, H. Zhang, Z. Sun, *J. Antimicrob. Chemother.*, 52 (2003) 790.
2. S. N. M. Hanif, A. J. Hickey, L. G. Contreras, *J. Pharm. Biomed. Anal.*, 88 (2014) 370.

3. M. D. J. A. Khan, Z. Ahmed, *International Journal of General Medicine and Pharmacy.*, 2 (2013) 2319.
4. P. Nagaraja, K. C. S. Murthy, H. S. Yathirajan, *Talanta.,* 43 (1996) 1075.
5. M. Y. Khuhawar, F. M. A. Rind, *J. Chromatogr. B.,* 766 (2002) 357.
6. R. V. Hest, H. Baars, S. Kik, P. V. Gerven, M. C. Trompenaars, N. Kalisvaart, S. Keizer, M. Borgdorff, M. Mensen, F. Cobelens, *Clinical Infectious Diseases.*, 39 (2004) 488.
7. S. Tafazoli, M. Mashregi, P. J. O'Brien, Toxicol. *Appl. Pharmacol.*, 229 (2008) 94.
8. *Tuberculosis.,* 88 (2008) 141.
9. J. Madan, A. K. Dwivedi, S. Singh, *Anal. Chim. Acta.,* 538 (2005) 345.
10. A. F. Faria, M. V. N. Souza, R. E. Bruns, M. A. L. Oliveira, *Talanta.,* 333 (2010) 82.
11. Z. Gong, Y. Basir, D. Chu, M. M. Tipton, *J. Chromatogr. B.,* 877 (2009) 1698.
12. J. W. Wu, H. H. Shih, S. C. Wang, T. H. Tsai, *Anal. Chim. Acta.,* 522 (2004) 231.
13. H. M. Maher, R. M. Youssef, *Chemom. Intell. Lab. Syst.,* 94 (2008) 95.
14. M. A. Alonso Lomillo, O. Domınguez Renedo, M.J. Arcos Martınez, *Anal. Chim. Acta.,* 449 (2001) 167.
15. M. F. Bergaminia, D. P. Santos, M. V. B. Zanoni, *J. Electroanal. Chem.,* 690 (2013) 47.
16. S. Cheemalapati, B. Devadas, S. M. Chen, *Journal of Colloid and Interface Science.,* 418 (2014) 132.
17. Y. Umasankar, B.Unnikrishnan, S. M. Chen, T. W. Ting, *Int.J.Electrochem.Sci.,* 7 (2012) 484.
18. 18 R. N. Goyal,V. K.Gupta, M. Oyama, N. Bachheti, *Electrochem.Commun.,* 7 (2005) 803.
19. C. R. Raj, T. Okajima, T. Ohsaka, *J.Electroanal.Chem.,* 543 (2003) 127.
20. S. Reddy, B. E. K. Swamy, H. Jayadevappa, *Electrochim.Acta.,* 61, (2012) 78.
21. Q. Zhang, S. Yang, J. Zhang, L. Zhang, P. Kang, J. Li, J. Xu, H. Zhou, X. M. Song, *Nanotechnology.,* 22 (2011) 494010.
22. S. Woo, Y. R. Kim, T. D. Chung, Y. Piao, H. Kim, *Electrochim. Acta.,* 59 (2012), 509.
23. S. Cheemalapati, S. Palanisamy, V. Mani, S. M. Chen, *Talanta.,* 117, (2013) 297.
24. V. Mani, B. Devadas, S. M. Chen, *Biosensors and Bioelectronics.,* 41 (2013), 309.
25. R. I. Jafri, T. Arockiados, N. Rajalakshmi, S. Ramaprabhua, *J. Electrochem. Soc.,* 157, (2010) 874.
26. M. Y. Yen, M. C. Hsiao, S. H. Liao, P. I Liu, H. M. Tsai, C. C. M. Ma, N. W. Pu, M. D. Ger, *Carbon.,* 49 (2011) 3597.
27. X. Dong, G. Xing, M. B. C. Park, W. Shi, N. Xiao, J. Wang, Q. Yan, T. C. Sum, W. Huang, P. Chen, *Carbon.,* 49 (2011) 5071.
28. B. Devadas, V. Mani, S. M. Chen, *Int. J. Electrochem. Sci.,* 7 (2012) 8064.
29. D. R. Dreyer, S. Park, C. W. Bielawski, R. S. Ruoff, *Chem. Soc. Rev.,* 39 (2010) 228.
30. S. Cheemalapati, S. Palanisamy, S. M. Chen, *J Appl Electrochem.,* 44 (2014) 317.
31. J. Wang, *Electroanalysis.,* 17 (2005) 7.
32. C. B. Jacobs, M. J Peairs, B. J Venton, *Anal Chim Acta.,* 662 (2010) 105.
33. H. Zarei, H. Ghourchian, K. Eskandari, M. Zeinali, *Anal Biochem.,* 421 (2012) 446.
34. W. S. Hummers, R. E. Offeman, *J.Am.Chem.Soc.,* 80 (1958) 1339.
35. S. H. Aboutalebi, A. T. Chidembo, M. Salari, K. Konstantinov, D. Wexler, H. K. Liu, S. X. Dou, *Energy Environ.Sci.,* 4 (2011) 1855.
36. Y. Li, T. Yang, T. Yu, L. Zheng, K. Liao, *J. Mater.Chem.*, 21 (2011) 10844.
37. S. Palanisamy, S. Cheemalapati, S. M. Chen, *Mater. Sci. Eng.,* C., 34 (2014) 207.
38. N. F. Atta, A. Galal, E. H. El-Ads, *Analyst.,* 137 (2012) 2658.
39. D. K. Gosser, Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms; *Wiley−VCH Publishers, New York.,* (1993) 27–45.
40. M. Arvand, T. M. Gholizadeh, M. A. Zanjanchi, *Mater. Sci. Eng. C.,* 32 (2012) 1682.
41. Y. Ni, S. Kokot, M. Selby, M. Hodgkinson, *Electroanalytical.,* 4 (1992) 713.
42. F. C. Anson, J. M. Saveant, K. Shigeharat, *J. Am. Chem. Soc.,* 105 (1983) 1096.

SUPPLEMENTARY INFORMATION

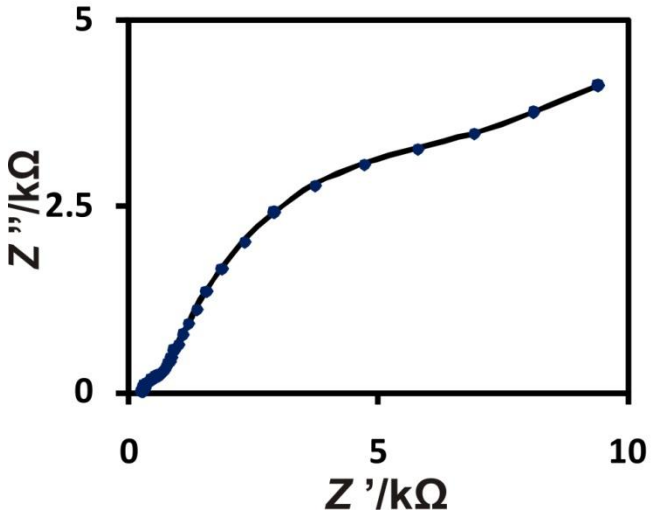

Figure S1. EIS of GO/GCE in 5 mM Fe $(CN)_6^{3-/4-}$ solution and the frequency range is from 0.1 Hz to 100 kHz.