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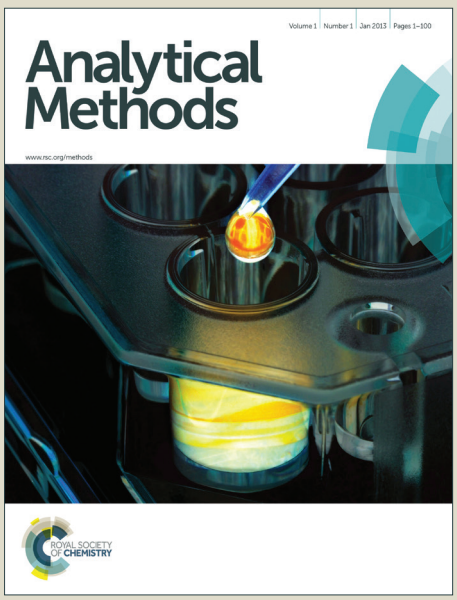
Accepted Manuscript



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Multiwall Carbon Nanotube Ensembled Biopolymer Electrode for Selective Determination of Isoniazid *in-vitro*

M. Satyanarayana, K. Koteshwara Reddy, K. Vengatajalabathy Gobi*

Department of Chemistry, National Institute of Technology, Warangal, Andhra Pradesh 506004, India.

* *Corresponding Author:*

Dr. K. V. GOBI

Associate Professor,

Department of Chemistry,

National Institute of Technology,

Warangal, Andhra Pradesh 506 004,

INDIA.

E-mail: drkvgobi@gmail.com (K.V. Gobi)

Tel.: +91-870-2462674.

Abstract

A reagent-free electrochemical biosensor is fabricated for sensitive determination of the important anti-tubercular drug isoniazid (INH). The electrochemical response of the fabricated multiwall carbon nanotube (MWCNT) – chitosan (chit) nanocomposite modified glassy carbon electrode (MWCNT-chit/GCE) towards the detection of INH is investigated by cyclic voltammetry, electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). The carbon nanotube–chitosan nanocomposite electrode exhibits an excellent electrocatalytic effect towards the oxidation of INH. The overpotential for the electrochemical oxidation is reduced largely by ~ 800 mV to +0.17 V vs. Ag|AgCl at MWCNT-chit/GCE compared to +0.97 V vs. Ag|AgCl at bare GCE, and the electrocatalytic current is enhanced nearly four orders of magnitude. Applying DPV method at optimized conditions, linear calibration plot is achieved over the concentration range of 1.0×10^{-7} M – 1.0×10^{-5} M INH and the biosensor could detect as low as 5.5×10^{-8} M INH in ~ 12 s. The modified electrode shows very good selectivity towards the specific recognition of INH in the presence of important biological interferents. The electrochemical biosensor detects INH *in-vitro* directly from spiked drug formulations and undiluted urine sample as low as 5×10^{-7} M with recovery limits of 102 % and 101.4 %, respectively.

Keywords: Isoniazid, Carbon nanotubes, Chitosan, Electrocatalysis, Voltammetric determination, Electrochemical impedance spectroscopy

1. Introduction

Carbon nanotubes (CNTs) have been recognized as an important material in recent years in various fields due to their unique electrical, mechanical and structural properties. CNTs can easily promote electron transfer between the electroactive species and electrode surface due to its unique long and tubular geometry [1,2]. The remarkable property of CNTs conductivity to the surface adsorbates permits the use of CNTs in the fabrication of highly sensitive nanoscale sensors. Their use as electrode modifiers can lead to a decrease of the overpotential, a decrease in the response time, enhanced electrocatalytic activity and an increase in available active surface area in comparison with conventional carbon electrodes. The electrocatalytic effect of CNTs has been attributed to the activity of edge-plane-like graphite sites at the CNT ends and it would be further increased by functionalization of CNTs. CNTs also reduce the electrode fouling which can greatly improve the reuse of such sensors [3,4]. The low solubility of CNTs in most solvents is the major problem to control for their use as modifiers in the fabrication of chemical sensors and/or biosensors. In order to overcome it, several strategies have been proposed for effective immobilization of CNTs on electrochemical transducers, like dispersion in different solvents or polyelectrolytes or incorporation in composite matrices using distinct binders [5–8].

Chitosan (chit) is a linear β-1,4-linked polysaccharide (similar to cellulose) that is obtained by the partial deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi [9]. It possesses many advantages such as excellent strong film forming ability but with high permeability towards water, biocompatible, good adhesion and high mechanical strength. In this investigation, chitosan has been used as a dispersant to bind CNTs with the electrode surface and to form a stable CNT–chitosan composite film on the GCE surface for sensing isoniazid *in-vitro*.

Isoniazid (Isonicotinylhydrazine, INH) is one of the most widely used first-line clinical drug for the treatment of all kinds of tuberculosis. Overdoses of the drug during the chemotherapy can cause hepatotoxicity, and there has been a global increase in the prevalence of drug–resistant tuberculosis. The large scale therapeutic use of this drug necessitated the need for the development of rapid, simple and on-site analytical method for determining isoniazid in drug formulation for quality control and in biological fluids for medical diagnosis. Various analytical methods based on titrimetric [10], spectroscopic [11–13], chromatograghic [14,15], chemiluminescence [16,17], and electrochemical techniques[18–20] have been aimed for quantitative determination of isoniazid. Electrochemical methods are interesting as they require in expensive, miniaturized portable equipment, and the analytes could be detected in trace levels without any preconcentration steps and without adding any special reagents. The major problem towards the detection of isoniazid using an electrochemical method is the large overpotential required for oxidation/reduction of isoniazid at bare electrodes. Various mediator and polymer based electrodes have been used to decrease the overpotential [21]. Gao et al. have investigated electrocatalytic oxidation of isoniazid using a ferrocenyl derivative as electrocatalyst at Pt electrode [22]. Recently, Jena and Raj have reported nanoAu decorated sol-gel based Au electrode for amperometric detection of isoniazid and the overpotential is reduced here by ~ 450 mV [23]. Carbon nanomaterials are highly interested for tailoring of electrode surface due to the fact that they provide low background currents, low-cost and wide-potential windows. Shahrokhian et al. [24] have investigated CNT based electrode for the determination of isoniazid and have obtained good sensitivity; but the overpotential was not much reduced. Very recently, Yan et al. [25] have investigated hexagonally ordered mesoporous carbon incorporated Nafion polymer electrode for amperometric detection of isoniazid. In this present work, multiwall carbon nanotubes have been ensembled into chitosan biopolymer matrix for the fabrication of a simple and cost

effective electrochemical sensor of isoniazid. MWCNT is functionalized and dispersed in chitosan solution to form a stable thin film on GCE. Optimum conditions for highly sensitive and selective determination of isoniazid are established, and this method has been effectively applied to determine isoniazid in drug formulations and physiological liquids.

2. Experimental:

2.1. Chemicals and materials

Isoniazid and ascorbic acid were purchased from Tokyo chemical industry, Japan. Chitosan of low molecular weight range (from crab shells, 60 – 120 kDa, minimum 85 % deacetylation) was obtained from Sigma Aldrich, USA. MWCNTs (95%, 20–50 nm OD and 2–5 μm length) were purchased from Sisco research laboratories, India. All other chemicals were of analytical grade (>99.5 % purity) and were used without further purification. Britton-Robinson buffer (B-R buffer) was prepared using a mixture of 0.04 M CH_3COOH, 0.04 M H_3BO_3 and 0.04 M H_3PO_4. The desired solution pH was obtained by adding 0.1 M NaOH. Artificial urine solution was prepared according to the procedure provided by Brooks and Keevil [26]. The artificial urine solution was prepared using a mixture of 1.1 mM lactic acid, 2.0 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2.0 mM magnesium sulfate, 10 mM sodium sulfate, 7.0 mM potassium dihydrogen phosphate, 7.0 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride in distilled water. The pH of the solution was adjusted to 6.0 through the addition of 1.0 M hydrochloric acid. All aqueous solutions were prepared using double distilled water.

2.2. Functionalization of MWCNTs

MWCNTs were functionalized with –COOH group by using a method similar to that described by Gouveia-Caridade et al. [27]. MWCNTs (120 mg) were added to 10 mL of 3 M nitric acid solution and stirred for 24 h at 60 ^oC. The black solid product was filtered and then washed several times with double distilled water until the filtrate solution became neutral

(pH = 7). The obtained solid product was collected in a petri dish and dried in an oven at 80 ^{o}C for 24 h. Nitric acid oxidizes CNTs and introduces -COOH groups at the ends and at the sidewall defects of the nanotube structure, which increases the electrocalytic activity of CNTs. In order to characterize the functionalized MWCNT, the number of –COOH groups per gram of functionalized MWCNT has been determined by acid-base back titration method and it is 2.14 ± 0.08 mmol/g (n = 4).

2.3. *Preparation of MWCNT-chtiosan modified electrodes*

At first, GCE (3 mm diameter) was polished with alumina slurry (down to 0.04 μm), and then washed thoroughly with double distilled water, then sonicated in 1:1 aq. HNO_3, ethanol and double distilled water consecutively and finally dried at room temperature. A solution of chitosan (1 % w/v) was prepared by dissolving 1 g of chitosan powder in 100 mL aq. acetic acid (1 % v/v) solution and sonicated for 30 min. Different amounts (1, 2, 3 and 4 mg) of oxidized MWCNTs were added to 1 mL chitosan solution and sonicated for 1 h. Then, 10 μL of the resulting homogeneous suspension was cast on the surface of cleaned GCE and dried for 24 – 30 h at room temperature and the resulting electrodes were denoted as MWCNT-chit/GCE.

2.4. Electrochemical experiments and Scanning electron microscopy(SEM)

Cyclic and differential pulse voltammetry measurements were carried out using CHI 619d electrochemical analyzer, and electrochemical impedance measurements were carried out using Zahner-elektrik workstation (Model IM6e, GmbH, Germany) equipped with Thales 3.08 USB software. All the electrochemical measurements were performed in a conventional electrochemical cell of 20 mL with bare or modified GCE as working electrode, Pt spiral wire as auxiliary electrode and Ag|AgCl (3 M KCl) electrode as reference. All the potentials were referred against Ag|AgCl (3 M KCl) electrode throughout the manuscript. Electrochemical experiments were carried out in B-R buffer at room temperature, and the

experimental solution was purged with nitrogen gas for 10 min prior to the start of the experiment to de-aerate the solution.

Scanning electron microscope (SEM) images of MWCNT-chitosan nanocomposite film were recorded using TESCAN VEGA 3 scanning electron microscope. MWCNT-chitosan nanocomposite film was prepared by casting 1 % w/v chitosan solution dispersed with MWCNT at 4 mg/mL level. A thin layer of gold was sputtered on the nanocomposite film to avoid the charging during SEM analysis.

3. Results and Discussion

3.1. Morphology of MWCNT-chitosan nanocomposite electrode

Figure 1 shows the scanning electron microscope (SEM) images of the MWCNT-chitosan nanocomposite electrode at two different magnifications. As shown in Fig. 1(a), the MWCNT-chitosan film is of porous nature with large surface area, and thus it could enhance the electrodic current for an analyte and thus the sensitivity. The nanocomposite has been distributed uniformly and homogenously all over the electrode. SEM image obtained at the higher magnification (Fig. 1b) clearly reveals that the MWCNTs are well dispersed on the electrode surface and have formed a good network on the electrode, which could promote a facile electron transfer. Fine individual strips of MWCNTs with a range of 20 to 50 nm diameter are seen. The porous nature with well dispersed homogenous structure all over the surface is expected to favour the fabrication of reliable and reproducible nanocomposite film on the electrode.

Position for Figure 1

3.2. Electrocatalytic oxidation of isoniazid

Cyclic voltammograms (CVs) obtained for the oxidation of isoniazid at bare GCE and MWCNT-chit/GCE in B-R buffer of pH 6.0 are shown in Fig. 1. Isoniazid exhibits irreversible CV with an anodic peak at both GCE and MWCNT-chit/GCE and no peaks are observed in the reverse scan. While the anodic peak is obtained at ~ + 0.97 V at bare GCE, it is obtained at a very less positive potential of + 0.17 V at MWCNT-chit/GCE. A large decrease in overpotential for the oxidation of isoniazid is obtained at MWCNT-chit/GCE, and the decrease in overpotential is as much as 800 mV compared to bare GCE. Moreover, the anodic peak current of isoniazid at MWCNT-chit/GCE is about 3 times larger than that of bare GCE. These phenomena are clear evidences for electrocatalytic effect of the MWCNT-chitosan modified electrode towards isoniazid oxidation. These results suggest that the MWCNT-chitosan film might be forming a better electron conducting pathway on the electrode surface and the formed film is able to accelerate the rate of isoniazid electron transfer.

Position for Figure 2

3.3. Optimization of pH and the amount of MWCNT

The effect of pH on the electrochemical response of 0.1 mM isoniazid at MWCNT-chit/GCE was investigated in B-R buffer solution of different pH (pH 4.0 to 10.0) by cyclic voltammetry as shown in Fig. S1 (supporting information). The solution pH obviously influenced the oxidation peak of isoniazid. The peak current is maximum in the pH range of 5.0 – 6.0, and it decreased to minimum at pH 9.0 and 10.0. Moreover, in the pH range of 8.0 to 10.0, isoniazid shows an additional irreversible anodic peak with low intensity and the peak currents are relatively low. In the overall pH range of 4.0 to 10.0, the peak currents are very high at pH 6.0. Considering these observations, B-R buffer of pH 6.0 has been chosen for all further experiments.

Different amounts of MWCNTs were dispersed in chitosan solution to prepare the modified electrodes, and electrochemical studies have been carried out to optimize the amount of MWCNTs for modification of GCE. Figure S2 (supporting information) shows the CVs observed for the oxidation of isoniazid on these modified GCEs. The oxidation peak current is increased with increasing amount of MWCNTs (1 - 3 mg/mL). At further higher amounts (above 3 mg) of MWCNT, solidification of MWCNT suspension takes place which inturn results in less stable MWCNT-chitosan film on the electrode surface. From these results, 3 mg/ml of MWCNTs is found to be optimum for the efficient oxidation of isoniazid at MWCNT-chit/GCE.

The influence of scan rate on the electrochemical response of 0.1 mM isoniazid at MWCNT-chit/GCE was investigated by cyclic voltammetry, and the respective results are shown in Fig. S3 (supporting information). The oxidation peak currents are gradually increased with increasing scan rate, and the peak current is linearly proportional to the square root of scan rate in the range of 20 to 500 mV s^{-1} (Fig. S3(A)). When peak current values were plotted against the square root of scan rate ($\upsilon^{1/2}$), a linear relationship with a regression coefficient of 0.995 was obtained (Fig. S3(B)). This behavior suggests that the oxidation of isoniazid at nano-biocomposite MWCNT-chitosan modified electrode is diffusion controlled and that the permeation of isoniazid and electrolyte across the nanocomposite film is very facile (*vide infra*).

3.4. Electrochemical Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) is a powerful tool for studying the interfacial properties of surface-modified electrodes [28,29]. The charge transfer resistance (R_{ct}) of the electrode provides vital insight into the nature of interface. EIS analysis of bare GCE, chit/GCE and MWCNT-chit/GCE is carried out in B-R buffer (pH 6.0) in the presence of 0.1 mM isoniazid in the frequency range of 60 kHz to 10 mHz. The results were found to best fit

with a simple Randles equivalence circuit. The Randles circuit consists of the ohmic resistance (R_s) of the electrolyte solution, the double layer capacitance (C_{dl}), electron transfer resistance (R_{ct}) and the Warburg impedance (Z_w) resulting from the diffusion of analyte molecules from the bulk of the electrolyte to the interface. The resultant Nyquist plots are shown in Fig. 3. The Nyquist plots of GCE, Chit/GCE and MWCNT-chit/GCE comprise a semicircular pattern followed by a linear portion. The diameter of the semicircle represents the magnitude of R_{ct} at the electrode surface. Interestingly, the R_{ct} value of the chit/GCE is nearly equal to that of bare GCE indicating that the chitosan biopolymer film allows the permeation of isoniazid and electrolyte across the biopolymer interface. The R_{ct} values of MWCNT-chit/GCE is 173 Ω, which is quite smaller than that of chit/GCE and of bare GCE, indicating that the CNTs–chitosan composite film allows facile electron transfer and greater permeation for oxidation of isoniazid.

Position for Figure 3

The cyclic voltammetric and EIS experimental results show that the CNTs–chitosan composite film formed a better electron conduction pathway on the electrode surface. That is to say, the CNTs played an important role as electron-transfer mediator thus made the electron-transfer easier. This is due to the nano level surface structural and morphological features of the modified CNTs, the large surface area and excellent electrical conductivity. The fabricated MWCNT-chit/GCE is investigated further for quantitative analysis of isoniazid.

3.5. Differential pulse voltammetry (DPV)

The voltammetric response of the MWCNT–chit/GCE electrode to the presence of INH was investigated by DPV. At the MWCNT-chitosan modified electrode, the anodic peak at +0.15 V was monitored and a better voltammetric profile was obtained with scan rate of 50 mV s^{-1}, pulse amplitude of 50 mV and pulse period of 5 ms. After optimizing the operating conditions, differential pulse voltammetric measurements were carried out at the MWCNT–chit/GCE in B-R buffer containing different INH concentrations and the results are shown in Fig. 4(A). The dependence of the oxidation peak currents on the concentration of isoniazid is shown in Fig. 4(B). The results showed that the oxidation peak current (i_p) linearly increased with the concentration of isoniazid. The linear regression equation is expressed as: i (μA) = 1.1104 + 1.673 $C_{isoniazid}$ (μM) with the regression coefficient R^2= 0.9876; the detection limit is found to be 5.5×10^{-8} M (S/N = 3) which is equivalent to ~ 7.5 ng mL^{-1} with a linear range of 1.0×10^{-7} M to 1.0×10^{-5} M. The performance of the present electrochemical sensor is compared with other electrochemical sensors and also with other analytical methods reported in the literature (Table 1). The low-detection-limit of INH obtained by the present electrochemical sensor using MWCNT-chit/GCE is highly significant and superior compared to the detection-limits reported previously by using various electrochemical[20,30,31], chromatographic[32,33] and spectrophotometric[12,34] methods.

Position for Figure 4 and Table 1

3.6. Interferences

Electrochemical response of INH in the presence of the possible electroactive physiological components such as ascorbic acid (AA), uric acid (UA), and dopamine (DA), has been carried out at MWCNT-chitosan modified electrode using DPV method. Figure 5 shows the DPVs at MWCNT-chitosan modified electrode at different concentrations of INH

in the presence of ~ 4 times higher concentration of 20 μM of AA, and the results show that the concentration of INH could be determined accurately even in the presence of higher concentrations of AA. Higher concentrations of other interferents UA and DA also did not influence the current response of INH significantly (signal change < 5%). From the experimental observations, it is clearly evident that the proposed electrochemical method using MWCNT-chit/GCE can be used effectively for the detection of INH even in the presence of higher concentrations of possible interferents.

Position for Figure 5

3.7. Repeatability and Reproducibility

Reusability of the MWCNT-chitosan modified electrode towards the electrochemical oxidation of INH was investigated by repetitively recording DPV at a fixed INH concentration of 5 μM. The relative standard deviation (RSD) for the anodic peak currents in six determinations is only 2.5 %, indicating excellent reusability of the nanocomposite modified electrode. Furthermore, the anodic peak currents for the determination of INH in multiple experiments over a time period of 10 days decreased only by 3.0 %. Also, no much change is observed in the oxidation peak potential of INH at modified electrode even after one month of its fabrication though the modified electrode kept at ambient conditions. Moreover, the reproducibility of the MWCNT-chitosan nanocomposite electrode was investigated by analyzing the DPV response of five different electrodes prepared independently. The peak potential for the oxidation of isoniazid is identical with all the electrodes, and the peak currents of the DPVs recorded by using the five independent electrodes at the isoniazid concentration of 1 μM vary only a little with a standard deviation of 2.2 %, indicating that the MWCNT-chitosan nanocomposite electrode highly reproducible.

From the results, it is clear that the MWCNT-chitosan composite formed a stable and reproducible nanobiocomposite film on GCE for the determination of isoniazid.

3.8. Determination of INH in pharmaceutical and artificial urine samples:

Pharmaceutical samples of isoniazid (300 mg/tablet), which were diluted appropriately, were analyzed with the proposed electrochemical method by the standard addition method. In Fig. 6, the curve "e" shows the DPV of analytical sample containing 0.5 μM of pure INH and 0.5 μM of INH from pharmaceutical tablet at MWCNT-chit/GCE, which gives a good recovery of 102 % with low RSD (1.6 %). The recovery of isoniazid from tablet samples at different concentrations was listed in Table 2 and it varies from 96.7 % to 102.0 %. Recovery of INH in artificial urine was also examined by direct addition of INH in undiluted artificial urine without any buffer. Isoniazid metabolizes rapidly with a half-life of 1 – 3 h. Isoniazid and its derivatives are excreted in urine with 75 – 95% of the drug excreted in 24 h. The concentration of isoniazid in urine 10 h after administration of the drug decreases to as low as 0.26 μg mL^{-1}, i.e., 1.9 μM[32,35]. Lactic acid and uric acid present in urine would interfere effectively in the electrochemical analysis. Artificial urine sample was prepared with the presence of Lactic acid and uric acid, etc. at normal urine concentrations levels (see Experimental section) and was examined by DPV analysis. The MWCNT-chit/GCE did not show any peak to the artificial urine sample in the absence of isoniazid (fig. 5c). It clearly shows that the fabricated nano-biocomposite electrode did not respond to any of the electroactive interferents present in the urine sample. The nanocomposite matrix comprising –COOH groups in the functionalized MWCNTs might have strongly retarded the electroactive interferences in urine such as lactic acid and uric acid. The electrode was then investigated for detection of isoniazid directly in artificial urine sample. DPV of MWCNT-chit/GCE electrode in artificial urine sample with the presence of 0.5 μM INH was recorded

(Fig. 6d), and it shows one peak corresponding to the oxidation of isoniazid. These results clearly indicate that the developed sensor chip could detect INH selectively from urine sample as low as 5 x 10^{-7} M. This detection limit is nearly four times superior compared to the concentration of isoniazid in urine 10 h after administration, and thus diluted urine samples could also be investigated for the determination of isoniazid by the present method. The recovery of isoniazid from undiluted artificial urine samples at different concentrations (5.0 – 15.0 x 10^{-7} M) was listed in Table 2, and it varies from 97.0 % to 101.4 %. From the results of the recovery analysis, we conclude that the fabricated sensor can be used efficiently for selective determination of isoniazid from pharmaceutical formulations and from urine samples *in-vitro*.

Position for Figure 6 & Table 2

4. Conclusions

In this work, we fabricated a stable and effective electrochemical sensor for sensitive determination of INH with MWCNT-chitosan nanocomposite modified electrode using simple drop and cast method. MWCNT-chitosan nanocomposite film remarkably enhances the voltammetric signal response and lowers the oxidation overpotential of INH. In this nanocomposite modified electrode, the MWCNTs act as good electrocatalytic mediator and MWCNT–chitosan composite film generates a better electron conduction pathway on the GCE surface. The nanocomposite film was highly stable for multiple analysis over a long period due to the unique binding character of chitosan biopolymer. The fabricated MWCNT-chitosan modified electrode can be used for the detection of ppb levels (ng/mL) of INH in the presence of biological interferents. The proposed sensor has good stability, high sensitivity and simple fabrication procedure. From all these advantages, we conclude that this

nanocomposite electrode could be extended to the determination of pharma drugs in biological fluids and pharmaceutical formulations.

Acknowledgements

The authors acknowledge gratefully the National Institute of Technology, Warangal and the Ministry of Human Resource Development, India for Junior Research Fellowship to MS and financial support.

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Captions:

Fig. 1. SEM images of MWCNT-chitosan nanocomposite at two different magnifications.

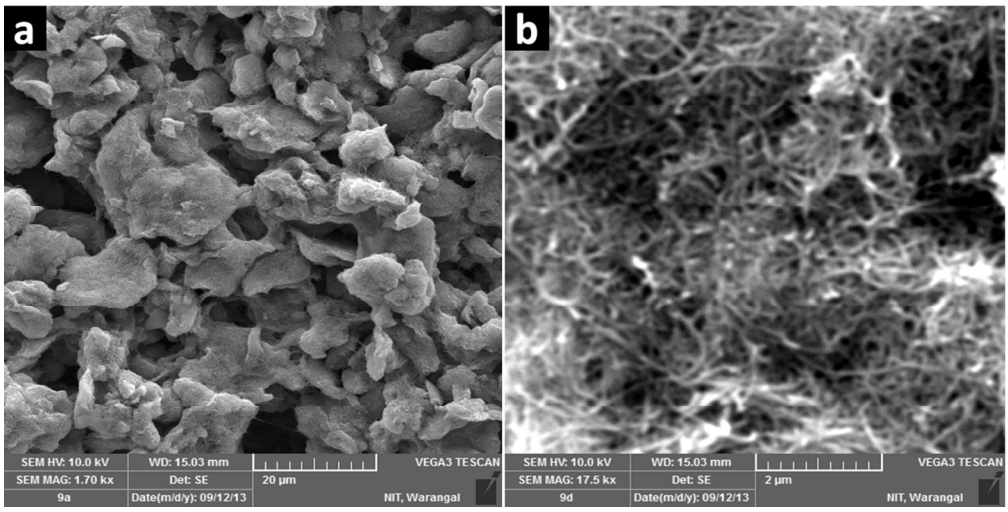
Fig. 2. CVs recorded at (a, b) bare glassy carbon electrode and at (c) MWCNT-chit/GCE in the (a) absence and (b, c) presence of 0.1 mM isoniazid in B-R buffer (pH 6.0). Scan rate = 100 mV s^{-1}.

Fig. 3. The Nyquist plots for EIS measurements of bare GCE, chit/GCE, and MWCNT-chit/GCE in B-R buffer (pH 6.0) in the presence of 0.1 mM INH. Inset: Randles equivalent circuit.

Fig. 4. (A) DPVs of different concentrations of isoniazid in B-R buffer (pH 6.0) using MWCNT-chit/GCE. (B) Plot of the peak current against the concentration of isoniazid. Inset shows the plot of peak current at higher concentrations of isoniazid.

Fig. 5. DPVs of different concentrations of isoniazid (5 μM, 10 μM, 15 μM, 20 μM) in B-R buffer (pH 6.0) using MWCNT-chit/GCE in the presence of 20 μM ascorbic acid.

Fig. 6. DPVs in the (a, c) absence and (b, d) presence of 0.5 μM INH in (a, b) B-R buffer of (pH 6.0) and in (c, d) artificial urine. e) Mixture of 0.5 μM pure INH and 0.5 μM INH from tablet in B-R buffer (pH 6.0) at MWCNT-chit/GCE.



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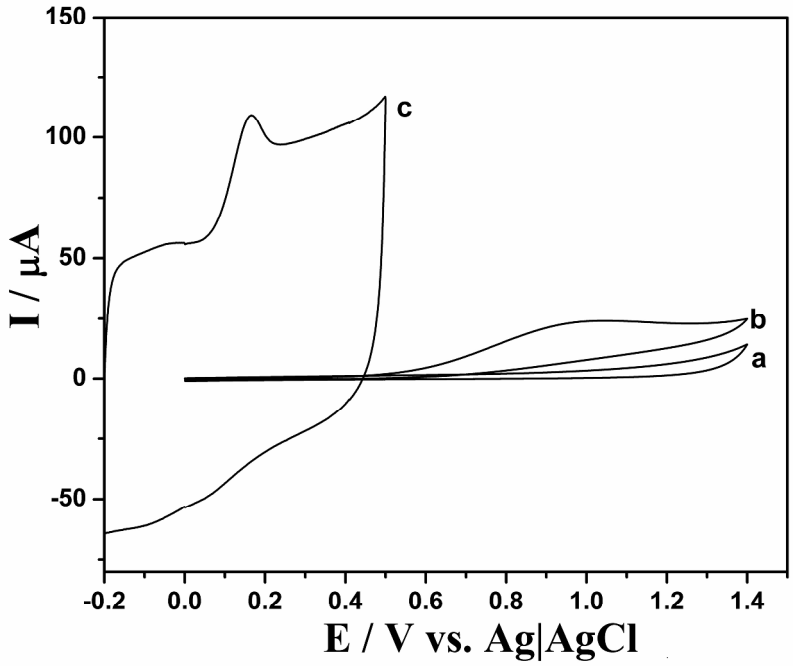

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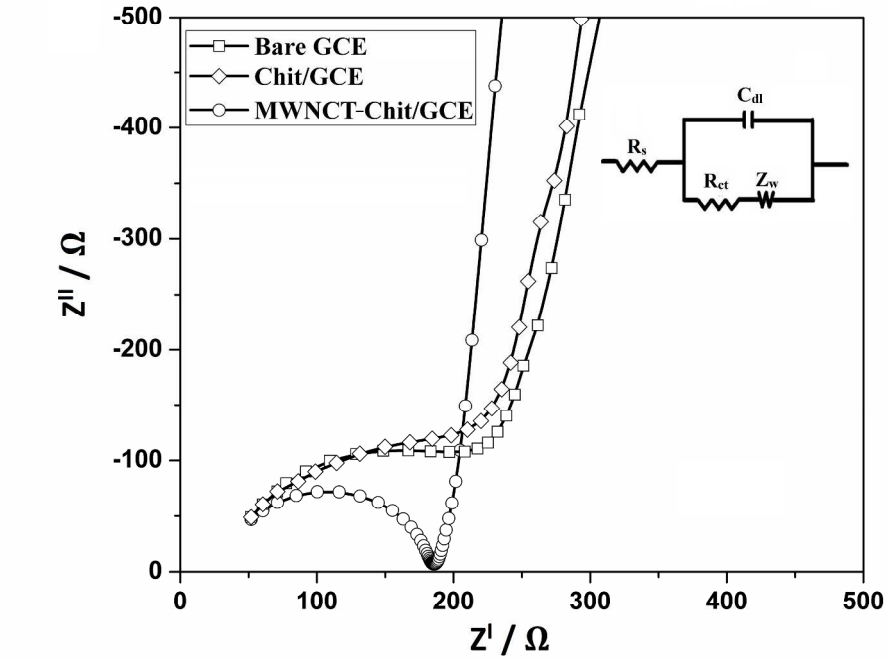

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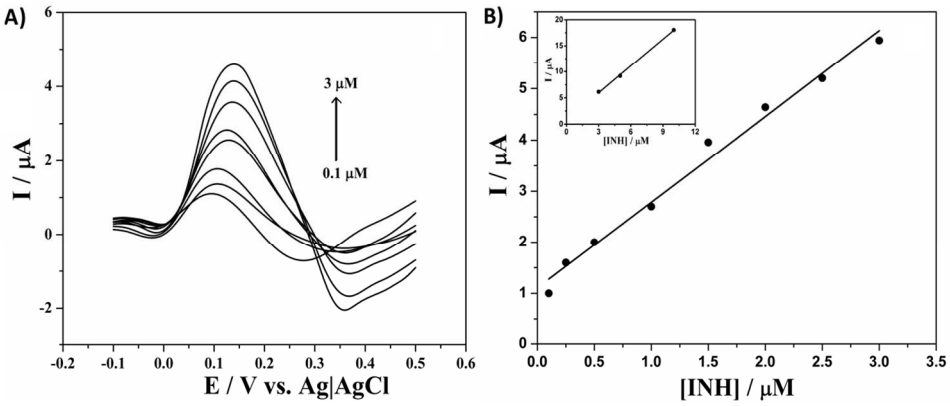

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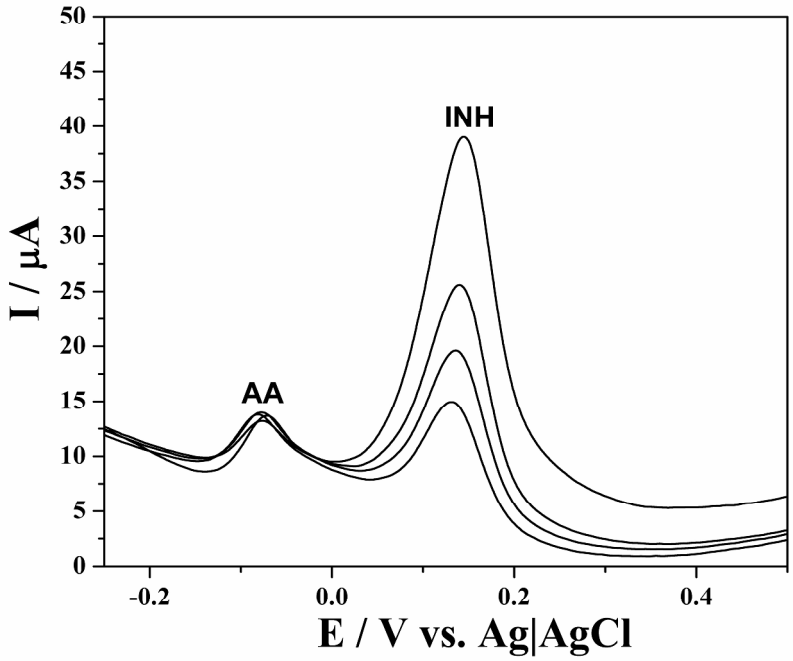

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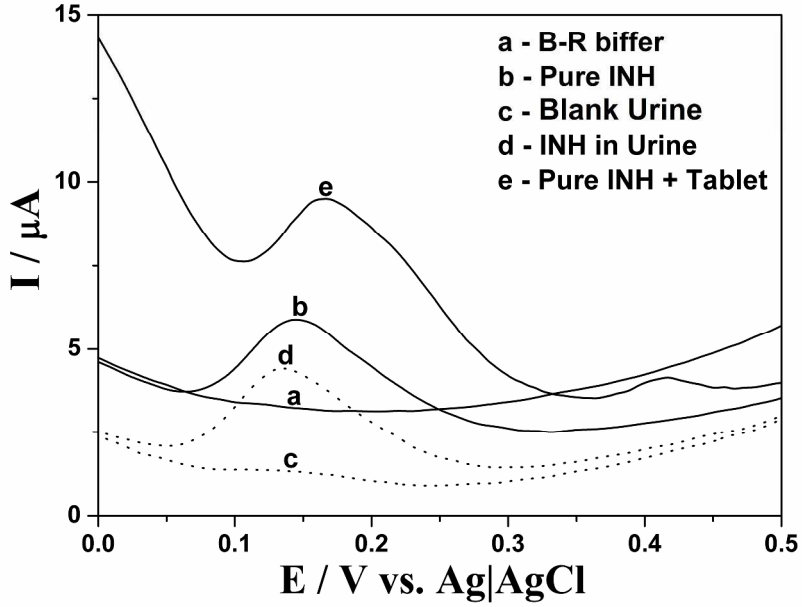

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Table 1: Detection of INH using electrochemical and various analytical methods

Method	Electrode	Decrease in overpotential (mV)	Linear range	Limit of detection	Reference
DPV	Carbon paste / Poly-L-histidine		$5.0 \times 10^{-7} – 1.1 \times 10^{-4}$ M	1.7×10^{-7} M	Bergamini et al., 2010 [30]
SWV[a]	Pt / Ferrocene mediator		$5.0 \times 10^{-5} – 6.0 \times 10^{-4}$ M	--	Gao et al., 2006 [22]
DPV	MWCNT / Thionine		$1.0 \times 10^{-6} – 1.0 \times 10^{-4}$ M	5.0×10^{-7} M	Shahrokhian et al., 2010 [31]
Amperometry	GC / Polypyrrole	345	$4.0 \times 10^{-6} – 2.0 \times 10^{-3}$ M	3.2×10^{-6} M	Majidi et al., 2006 [20]
Amperometry	GC / Nafion-Ordered Mesoporous carbon	480	$1.0 \times 10^{-7} – 3.7 \times 10^{-4}$ M	8.4×10^{-8} M	Yan et al., 2011 [25]
DPV	GC / MWCNT-carbon paste	150	$1.0 \times 10^{-6} – 1.0 \times 10^{-3}$ M	5.0×10^{-7} M	Shahrokhian et al., 2007 [24]
HPLC	n.a.	n.a.	0.002 – 20 μg mL^{-1}	0.5 ng mL^{-1}	Zhou et al., 2009 [32]
MEKC	n.a.	n.a.	3.0 – 100.0 mg mL^{-1}	1.0 mg mL^{-1}	Nemutlu et al., 2007 [33]
UV-VIS Spectrophotometry	n.a.	n.a.	0.3 – 3.5 μg mL^{-1}	0.26 μg mL^{-1}	Safavi et al., 2004 [12]
UV-VIS Spectrophotometry	n.a.	n.a.	0.6 – 6.2 μg mL^{-1}	0.15 μg mL^{-1}	Safavi et al., 2008 [34]
DPV	GC / MWCNT-chitosan	800	$1.0 \times 10^{-7} – 3.0 \times 10^{-6}$ M (0.014 – 0.411 μg mL^{-1})	5.5×10^{-8} M (7.5 ng mL^{-1})	Present Work

[a] SWV – Square-wave voltammetry, MEKC – micellar electrokinetic capillary chromatography, n.a. – Not applicable

Table 2: Determination of INH in pharmaceutical tablet and in artificial urine samples using MWCNT-chit/GCE.

Sample	INH ($\times 10^{-7}$ M)	Tablet Added ($\times 10^{-7}$M)	[a]Found ($\times 10^{-7}$M)	Average Recovery (%)	[a]RSD of recovery (%)
Tablet (Solonex, 300 mg)	5.0	5.0	10.20	102.0	1.6
	5.0	10.0	14.50	96.7	2.4
	5.0	15.0	20.10	100.5	1.8
Urine Sample	5.0	-	5.07	101.4	0.8
	10.0	-	9.80	98.0	1.2
	15.0	-	14.55	97.0	3.1

[a] Mean value of six measurements.