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Multiwall carbon nanotube ensembled biopolymer electrode for selective determination of isoniazid *in vitro*†

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A reagent-free electrochemical biosensor is fabricated for the 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 greatly reduced by ~800 mV, to + 0.17 V *vs.* Ag|AgCl at MWCNT–chit/GCE compared to + 0.97 V *vs.* Ag|AgCl at the bare GCE, and the electrocatalytic current is enhanced by nearly four orders of magnitude. Applying the DPV method under optimized conditions, a linear calibration plot is achieved over the concentration range of 1.0×10^{-7} M to 1.0×10^{-5} M INH and the biosensor could detect concentrations 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 samples at concentrations as low as 5×10^{-7} M with recovery limits of 102% and 101.4%, respectively.

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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 the electrode surface due to their unique long and tubular geometry.[1,2] The remarkable property of 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 can be further increased by functionalization of CNTs. CNTs also reduce the fouling of the electrode, which can greatly improve the reusability of such sensors.[3,4] The low solubility of CNTs in most solvents is the major problem to overcome regarding their use as modifiers in the fabrication of chemical sensors and/or biosensors. In order to overcome this problem, several strategies have been proposed for effective immobilization of CNTs on electrochemical transducers, such as 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 the cell walls of fungi.[9] It possesses many advantages, such as excellent strong film forming ability, but has high permeability towards water, is biocompatible, and shows 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 drugs for the treatment of all kinds of tuberculosis. Overdoses of the drug during 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 methods for determining isoniazid, in drug formulations for quality control and in biological fluids for medical diagnosis. Various analytical methods based on titrimetric,[10] spectroscopic,[11–13]

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chromatographic,[14,15] chemiluminescence,[16,17] and electrochemical techniques[18–20] have aimed at the quantitative determination of isoniazid. Electrochemical methods are interesting as they require inexpensive, miniaturized portable equipment, and the analytes could be detected at trace levels without any preconcentration steps and without adding any special reagents. The major problem in the detection of isoniazid using an electrochemical method is the large overpotential required for oxidation/reduction of isoniazid at bare electrodes. Various mediators and polymer based electrodes have been used to decrease the overpotential.[21] Gao *et al.* have investigated the electrocatalytic oxidation of isoniazid using a ferrocenyl derivative as an electrocatalyst at a Pt electrode.[22] Recently, Jena and Raj have reported nano-Au decorated sol–gel based Au electrodes for the amperometric detection of isoniazid, and the overpotential was reduced by ~450 mV.[23] Carbon nanomaterials are highly interesting for the tailoring of electrode surfaces due to the fact that they provide low background currents, wide-potential windows and are of low cost. Shahrokhian *et al.*[24] have investigated CNT based electrodes for the determination of isoniazid and have obtained good sensitivity; however, the overpotential was not reduced by much. Very recently, Yan *et al.*[25] have investigated hexagonally ordered mesoporous carbon incorporated Nafion polymer electrodes for the amperometric detection of isoniazid. In this present work, multiwall carbon nanotubes have been ensembled into a chitosan biopolymer matrix for the fabrication of a simple and cost effective electrochemical sensor for isoniazid. MWCNT are functionalized and dispersed in chitosan solution to form a stable thin film on the 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 simulated physiological fluids.

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 groups 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 °C. 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 °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 electrocatalytic activity of CNTs. In order to characterize the functionalized MWCNTs, the number of –COOH groups per gram of functionalized MWCNT was determined by the acid–base back titration method and found to be 2.14 ± 0.08 mmol g^{-1} ($n = 4$).

2.3. Preparation of MWCNT–chitosan modified electrodes

At first, the GCE (3 mm diameter) was polished with an 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 the 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 a CHI 619d electrochemical analyzer, and electrochemical impedance measurements were carried out using a 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 the working electrode, Pt spiral wire as the auxiliary electrode and an Ag|AgCl (3 M KCl) electrode as a reference electrode. All the potentials were referred against the 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 deaerate the solution.

Scanning electron microscopy (SEM) images of the MWCNT–chitosan nanocomposite film were recorded using a TESCAN VEGA 3 scanning electron microscope. The MWCNT–chitosan nanocomposite film was prepared by casting 1% w/v chitosan solution dispersed with MWCNT at the 4 mg mL^{-1} level. A thin

layer of gold was sputtered onto the nanocomposite film to avoid charging during the SEM analysis.

3. Results and discussion

3.1. Morphology of the MWCNT–chitosan nanocomposite electrode

Fig. 1 shows the scanning electron microscopy (SEM) images of the MWCNT–chitosan nanocomposite electrode at two different magnifications. As shown in Fig. 1a, the MWCNT–chitosan film is of a porous nature with a large surface area, and thus it could enhance the electrode current for an analyte and thus the sensitivity. The nanocomposite has been distributed uniformly and homogenously all over the electrode. The SEM image obtained at 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 ranging from 20 to 50 nm in 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 films on the electrode.

3.2. Electrocatalytic oxidation of isoniazid

Cyclic voltammograms (CVs) obtained for the oxidation of isoniazid at the bare GCE and MWCNT–chit/GCE in B–R buffer of pH 6.0 are shown in Fig. 2. Isoniazid exhibits irreversible CV with an anodic peak at both the GCE and the MWCNT–chit/GCE, and no peaks are observed in the reverse scan. While the anodic peak is obtained at ~+0.97 V at the bare GCE, it is obtained at a very much less positive potential of +0.17 V at the MWCNT–chit/GCE. A large decrease in overpotential for the oxidation of isoniazid is obtained at the MWCNT–chit/GCE, and the decrease in overpotential is as much as 800 mV compared to the bare GCE. Moreover, the anodic peak current of isoniazid at the MWCNT–chit/GCE is about 3 times larger than that of the bare GCE. These phenomena are clear evidence for an 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 electron transfer in isoniazid.

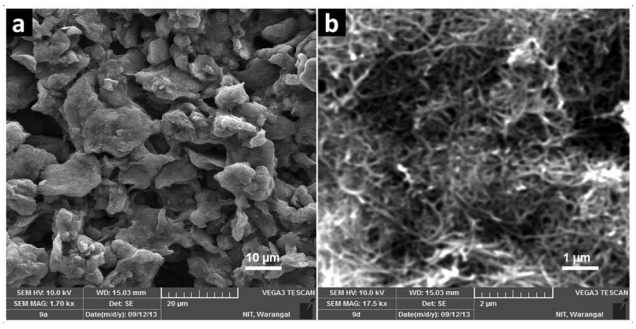

Fig. 1 SEM images of the MWCNT–chitosan nanocomposite at two different magnifications.

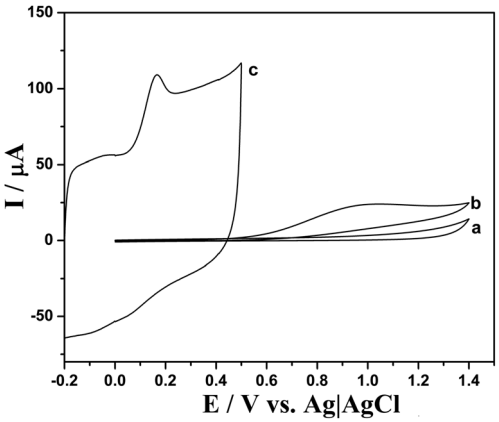

Fig. 2 CVs recorded at (a and b) bare glassy carbon electrode and at (c) MWCNT–chit/GCE in the (a) absence and (b and c) presence of 0.1 mM isoniazid in B–R buffer (pH 6.0). Scan rate = 100 mV s^{-1}.

3.3. Optimization of pH and the amount of MWCNT

The effect of pH on the electrochemical response of 0.1 mM isoniazid at the MWCNT–chit/GCE was investigated in B–R buffer solution at different pH values (pH 4.0 to 10.0) by cyclic voltammetry as shown in Fig. S1 (ESI†). The solution pH obviously influenced the oxidation peak of isoniazid. The peak current was maximum in the pH range of 5.0–6.0, and it decreased to a 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 was chosen for all further experiments.

Different amounts of MWCNTs were dispersed in chitosan solution to prepare the modified electrodes, and electrochemical studies were carried out to optimize the amount of MWCNTs for the modification of the GCE. Fig. S2 (ESI†) shows the CVs observed for the oxidation of isoniazid on these modified GCEs. The oxidation peak current increased with increasing amounts of MWCNTs (1–3 mg mL^{-1}). At greater amounts (above 3 mg) of MWCNT, solidification of the MWCNT suspension takes place which, in turn, results in less stable MWCNT–chitosan films on the electrode surface. From these results, 3 mg mL^{-1} of MWCNTs is found to be optimum for the efficient oxidation of isoniazid at the MWCNT–chit/GCE.

The influence of scan rate on the electrochemical response of 0.1 mM isoniazid at the MWCNT–chit/GCE was investigated by cyclic voltammetry, and the respective results are shown in Fig. S3 (ESI†). The oxidation peak currents gradually increase with increasing scan rate, and the peak current is linearly proportional to the square root of the 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 the 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 the 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 the interface. EIS analysis of the bare GCE, chit/GCE and MWCNT–chit/GCE was 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 fit best to 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 the 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 the bare GCE, indicating that the chitosan biopolymer film allows the permeation of isoniazid and electrolyte across the biopolymer interface. The R_{ct} value of the MWCNT–chit/GCE is 173 Ω, which is smaller than that of chit/GCE and of the bare GCE, indicating that the CNTs–chitosan composite film allows facile electron transfer and greater permeation for the oxidation of isoniazid.

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 an electron-transfer mediator, and 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 was investigated further for the quantitative analysis of isoniazid.

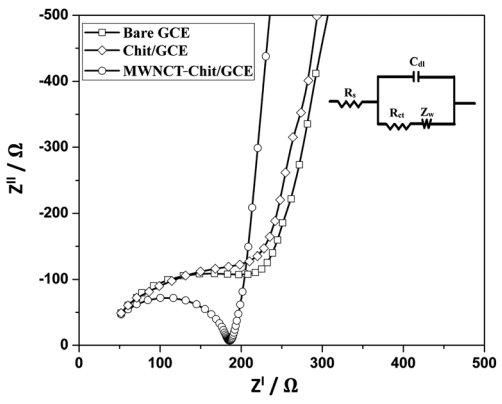

Fig. 3 The Nyquist plots for EIS measurements of the bare GCE, chit/GCE, and MWCNT–chit/GCE in B–R buffer (pH 6.0) in the presence of 0.1 mM INH. Inset: Randles equivalent circuit.

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 a 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 concentrations of INH, and the results are shown in Fig. 4A. The dependence of the oxidation peak current on the concentration of isoniazid is shown in Fig. 4B. 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 the 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.

3.6. Interferences

The electrochemical response towards INH in the presence of possible electroactive physiological components such as ascorbic acid (AA), uric acid (UA), and dopamine (DA), has been investigated at the MWCNT–chitosan modified electrode using DPV. Fig. 5 shows the DPVs at the MWCNT–chitosan modified electrode at different concentrations of INH in the presence of ~4 times higher concentrations 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 significantly influence the current response of INH (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.

3.7. Repeatability and reproducibility

Reusability of the MWCNT–chitosan modified electrode towards the electrochemical oxidation of INH was investigated by repetitively recording the 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.

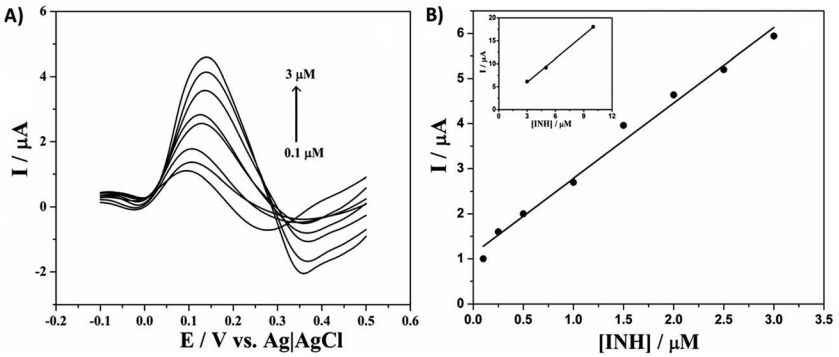

Fig. 4 (A) DPVs of different concentrations of isoniazid in B–R buffer (pH 6.0) using MWCNT–chit/GCE. (B) Plot of the peak current against the concentration of isoniazid. The inset shows the plot of the peak current at higher concentrations of isoniazid.

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, little change is observed in the oxidation peak potential of INH at the modified electrode even one month after fabrication, even though the modified electrode was kept under 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 for all the electrodes, and the peak currents of the DPVs recorded 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 is highly reproducible. From the results, it is clear that the MWCNT–chitosan composite formed a stable and reproducible nano-biocomposite film on the GCE for the determination of isoniazid.

3.8. Determination of INH in pharmaceutical and artificial urine samples

Pharmaceutical samples of isoniazid (300 mg per tablet), which were diluted appropriately, were analyzed with the proposed electrochemical method by the standard addition method. In Fig. 6, curve "e" shows the DPV of the analytical sample containing 0.5 μM of pure INH and 0.5 μM of INH from a pharmaceutical tablet at the MWCNT–chit/GCE, which gives a good recovery of 102% with a low RSD (1.6%). The recovery of isoniazid from tablet samples at different concentrations is

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	—	5.0×10^{-7} to 1.1×10^{-4} M	1.7×10^{-7} M	Bergamini *et al.*, 2010 (ref. 30)
SWV[a]	Pt/ferrocene mediator	—	5.0×10^{-5} to 6.0×10^{-4} M	—	Gao *et al.*, 2006 (ref. 22)
DPV	MWCNT/thionine	—	1.0×10^{-6} to 1.0×10^{-4} M	5.0×10^{-7} M	Shahrokhian *et al.*, 2010 (ref. 31)
Amperometry	GC/polypyrrole	345	4.0×10^{-6} to 2.0×10^{-3} M	3.2×10^{-6} M	Majidi *et al.*, 2006 (ref. 20)
Amperometry	GC/Nafion-ordered mesoporous carbon	480	1.0×10^{-7} to 3.7×10^{-4} M	8.4×10^{-8} M	Yan *et al.*, 2011 (ref. 25)
DPV	GC/MWCNT–carbon paste	150	1.0×10^{-6} to 1.0×10^{-3} M	5.0×10^{-7} M	Shahrokhian *et al.*, 2007 (ref. 24)
HPLC	n.a.	n.a.	0.002–20 μg mL^{-1}	0.5 ng mL^{-1}	Zhou *et al.*, 2009 (ref. 32)
MEKC	n.a.	n.a.	3.0–100.0 mg mL^{-1}	1.0 mg mL^{-1}	Nemutlu *et al.*, 2007 (ref. 33)
UV-vis spectrophotometry	n.a.	n.a.	0.3–3.5 μg mL^{-1}	0.26 μg mL^{-1}	Safavi *et al.*, 2004 (ref. 12)
UV-vis spectrophotometry	n.a.	n.a.	0.6–6.2 μg mL^{-1}	0.15 μg mL^{-1}	Safavi *et al.*, 2008 (ref. 34)
DPV	GC/MWCNT–chitosan	800	1.0×10^{-7} to 3.0×10^{-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.

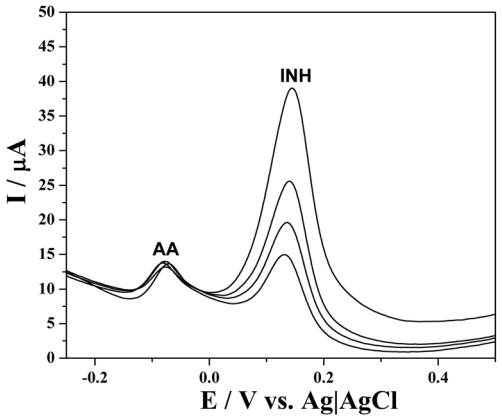

Fig. 5 DPVs of different concentrations of isoniazid (5 μM, 10 μM, 15 μM, 20 μM) in B–R buffer (pH 6.0) using MWCNT–chit/GCE in the presence of 20 μM ascorbic acid.

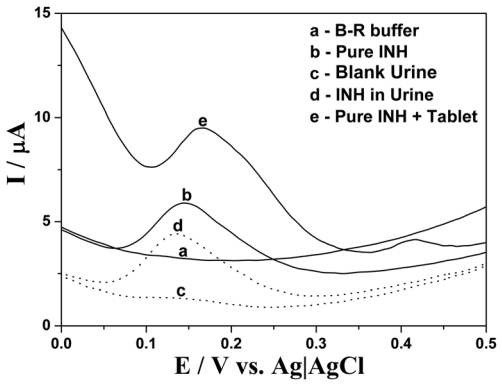

Fig. 6 DPVs in the (a and c) absence and (b and d) presence of 0.5 μM INH in (a and b) B–R buffer of pH 6.0 and in (c and d) artificial urine. (e) Mixture of 0.5 μM pure INH and 0.5 μM INH from tablets in B–R buffer (pH 6.0) at the MWCNT–chit/GCE.

listed in Table 2 and varies from 96.7% to 102.0%. Recovery of INH in artificial urine was also examined by the direct addition of INH into 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 with the electrochemical analysis. Artificial urine samples were prepared in the presence of lactic acid and uric acid, *etc.* at normal urine concentrations levels (see Experimental section) and were examined by DPV analysis. The MWCNT–chit/GCE did not show any peak for 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 the direct detection of isoniazid in the artificial urine sample. The DPV of the MWCNT–chit/GCE electrode in the artificial urine sample in 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 samples at concentrations as low as 5×10^{-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 ($5.0–15.0 \times 10^{-7}$ 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 the selective determination of isoniazid from pharmaceutical formulations and from urine samples *in vitro*.

4. Conclusions

In this work, we fabricated a stable and effective electrochemical sensor for the sensitive determination of INH with MWCNT–chitosan nanocomposite modified electrodes using a simple drop and cast method. The 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 a good electrocatalytic mediator and the 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

Table 2 Determination of INH in pharmaceutical tablets and in artificial urine samples using the MWCNT–chit/GCE

Sample	INH ($\times 10^{-7}$ M)	Tablet added ($\times 10^{-7}$ M)	Found[a] ($\times 10^{-7}$ M)	Average recovery (%)	RSD of recovery[a] (%)
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.

of the chitosan biopolymer. The fabricated MWCNT–chitosan modified electrode can be used for the detection of ppb levels (ng mL^{-1}) of INH in the presence of biological interferents. The proposed sensor has good stability, high sensitivity and a 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.

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