Facile in situ generation of bismuth tungstate nanosheet-multiwalled carbon nanotube composite as unconventional affinity material for quartz crystal microbalance detection of antibiotics

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ABSTRACT

Overuse and thus a constant presence of antibiotics leads to various environmental hazards and health risks. Thus, accurate sensors are required to determine their presence. In this work, we present a mass-sensitive sensor for the detection of rifampicin. We chose this molecule as it is an important antibiotic for tuberculosis, one of the leading causes of deaths worldwide. Herein, we have prepared a carbon nanotube reinforced with bismuth tungstate nanocomposite material in a well-defined nanosheet morphology using a facile in situ synthesis mechanism. Morphological characterization revealed the presence of bismuth tungstate in the form of square nanosheets embedded in the intricate network of carbon nanotubes, resulting in higher surface roughness of the nanocomposite. The synergy of the composite, so formed, manifested a high affinity for rifampicin as compared to the individual components of the composite. The developed sensor possessed a high sensitivity toward rifampicin with a detection limit of 0.16 μM and excellent specificity, as compared to rifabutin and rifapentine. Furthermore, the sensor yielded statistically good recoveries for the monitoring of rifampicin in human urine samples. This work opens up a new horizon for the exploration of unconventional nanomaterials bearing different morphologies for the detection of pharmaceuticals.

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1. Introduction

The increasing drug market binds pharmaceutical companies to have strict regulations to ensure quality and stability for appropriate formulations of designed drug [1]. In this context, the selective and sensitive detection of drugs is of high demand based on their clinical importance. Particularly important is the detection of antibiotics as their overuse and constant presence (especially at lower concentrations) leads to the evolution of resistant bacterial strains which cannot be killed any more by antibiotics. Being one of the top 10 causes of deaths worldwide, tuberculosis is arguably the most important disease to target. This situation gets worse as about 480,000 people were reported to develop multidrug-resistant tuberculosis merely in 2015 [2,3]. In this connection, rifampicin (RIF) is an important drug as it is widely used in the treatment of bacterial infections to treat or prevent tuberculosis [4]. Although, the rifamycins include rifampicin, rifapentine, and rifabutin. Of these, rifampicin is most commonly used, either as first-line therapy (in combination with other agents) for treatment of mycobacterial disease (including tuberculosis). In contrast, rifabutin is used in those patients who cannot tolerate rifampicin. While rifapentine is less preferred due to hepatotoxicity [5]. Therefore, rifampicin is arguably the most important drug in the treatment of tuberculosis (TB). Stability studies showed that rifampicin is stable in the blood and urine for only a few hours. Blood plasma or urine can be used as a matrix to check for the presence of rifampicin [6].

Rifampicin basically inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase [7]. More common side effects include fever, gastrointestinal disturbances, rashes, and immunological reactions [8]. Although the drug has been administered for many decades, but there are still questions about the appropriateness of dosage regimes, especially in specific patient groups such as those co-infected with HIV and young children [9]. In order to address these health concerns, there is a stringent need to develop suitable selective and sensitive analytical method, contrary to equipment-based expensive techniques like high performance liquid chromatography (HPLC), enzyme linked immune sorbent assay (ELISA), interferometric reflectance imaging sensor (IRIS) [10–12].

We decided to use nanostructured tungstate for sensor development due to its remarkable electrical conductivity (10−7 to 10−8 S cm−1) [13,14]. Among the metal tungstate, bismuth tungstate (Bi2WO6) takes precedence due to its high chemical stability, higher catalytic activity, as well as molecular and electronic versatility. This material has been explored extensively and has excellent performance for nonlinear dielectric susceptibility [15], acoustic insulators [16], ferroelectric [17], efficient catalysts [18], pyro electricity [19], photonic building blocks [20], oxide anion conducting [21], piezoelectricity [22], photocatalytic activity [23,24], decontamination [25], sustaining drug releasing agents [26], etc.

Carbon nanotubes (CNTs) offer remarkable physical and chemical properties owing to their small dimensions, strength, and other special structural features. Generally, CNTs have been applied in sensing devices and in particular electrochemical biosensors [27,28]. CNT-based transducers offer substantial improvements in the performance of electrochemical sensors, immunosensors, and nucleic-acid sensing devices [29]. Apart from sensing applications [30] they are also valued in nano-electronics or as tips for scanning probe microscopes, photo catalysts [31], gas storage materials [32], strength enhancing materials [33], composite materials [34], in self-healing technologies [35], and drug delivery [36]. The properties of CNTs can be ameliorated by their association with other forms of nanomaterials, where, the resultant synergy can produce very exciting nanomaterials with interesting properties [37].

A quartz crystal microbalance (QCM) as a transducer element constitutes a robust and sensitive detection platform [38]. Furthermore, the technology can be adapted to various applications by altering the electrode geometry or the electrode material and hence a large variety of coating materials allow for sensitive monitoring of various analytes in real time [39,40]. Conventionally, either bare QCM electrodes are employed or the electrodes are coated with metal nanoparticles of varying morphologies or polymer films. However, the use of nanocomposite as a transducer layer over QCM is a less explored area [41].

In the present study, we introduce an entirely new and an innovative way to prepare unconventional nanomaterials with unique morphology for mass sensitive detection of antibiotics. To the best of our knowledge, this is the first report about the synthesis and investigation of MWCNTs and bismuth tungstate nanosheets, for mass-sensitive measurements. We designed this study focusing many aspects (i) in situ development of 2D Bi2WO6 nanosheets onto 1D CNTs, (ii) to study whether such composite can be used as a QCM transducer, in contrast to traditional smooth layers, and (iii) to evaluate 1D CNTs and 2D Bi2WO6 composite for the real-time monitoring of RIF, where electrostatic interactions have played the main directive force to generate selectivity. On one hand, the development of a material is studied, whereas the synergy effects of MWCNTs and bismuth tungstate nanosheets as a transducer material of QCM are explored. This allowed for the label-free, real-time detection of the RIF drug with high sensitivity and selectivity in comparison to other drugs of similar chemical nature and function. This work opens up new avenues to prepare exceptional nanomaterials of different morphologies than conventional materials.

2. Materials and methods

2.1. Chemicals and apparatus

All chemicals were purchased either from Merck or Sigma–Aldrich. These were of analytical reagent grade or of higher available synthetic grade and were utilized without further purification. MWCNTs with an average outer diameter of 25–50 nm and 10–15 μm of average length were provided by Shenzhen Nanotech Port. Co. Ltd. All solutions were prepared using ultrapure water (ρ = 18 MΩ cm) from a Millipore-Milli Q system. Rifampicin (RIF) capsules were purchased from Merck & Co. This drug was used without further purification. All the solutions and modified QCM devices were stored at room temperature when not in use.

2.2. Preparation of MWCNTs-Bi2WO6 nanocomposite (MWCNTs/Bi2WO6)

First CNTs were functionalized to possess amine groups. In a typical preparation, 0.03 g of MWCNTs were dispersed in polyethyleneimine (1% v/v) followed by sonication for about 2 h. After keeping them at room temperature for 12 h and removing excess PEI, MWCNTs were dispersed in 40 mL of methanol to produce a stable colloid. Afterwards, a freshly prepared (20 mL of 2.5 M in acetic acid) solution of Bi(NO3)3·5H2O was added, followed by the drop wise addition of the aqueous solution of Na2WO4·2H2O (5 mL of 0.05 M). Afterwards, it was centrifuged, rinsed with 5 mL each of methanol and H2O, and dried under vacuum at 50 °C. Finally, the obtained product was stored in desiccators.

The stock solution of RIF was prepared in 1 M dimethyl sulfoxide (DMSO) and further dilutions of this solution were prepared in phosphate buffer saline (pH 7.4), when required. To investigate selectivity, the stock solutions of rifabutine (RIB) and rifapentine (RIP) were prepared and diluted in the similar way.

2.3. Characterization of nanomaterials

The morphology of MWCNTs and MWCNTs/Bi2WO6 nanocomposite was studied with a field emission scanning electron microscope (FESEM) (JSM-7500F-JEOL, Japan). For this purpose, each of these materials were dispersed in deionized water (1 mg mL−1) and 10 μL out of this dispersion were dropped onto carbon coated copper grids (200 mesh).
Atomic force microscope (AFM) (SHIMADZU WET-SPM 9600, Japan) was used to study surface and related roughness parameters (RMS). To prepare samples, 10 μL out of the above prepared suspensions were coated onto a 0.5 cm² glass support for AFM analysis. Three samples of each material were imaged for at least three different areas using the same tip. To get scans, silicon nitride atomic force microscopy (AFM) probes (model OMCL-TR800PSA-1) having micro cantilevers, 100 μm thickness and force constants of 0.57 N m⁻¹ were used. All the scans were carried at ambient temperature in contact mode. For the data analysis, software (SPM Manager) provided by the AFM system supplier was used. The same software was applied to calculate surface roughness parameters for all of the prepared materials.

Powder X-ray diffraction (PXRD) spectra of the MWCNTs/Bi₂WO₆ nanocomposite were recorded using a X’pert Pro PAN analytical (Germany) diffractometer using monochromatic CuKα radiation (λ = 1.5406 Å) operating at 40 kV and a current of 30 mA. The data for the nanocomposite were collected over a range of 10–80° 2θ.

Dynamic light scattering (DLS) experiments were carried out to determine the surface charge of MWCNTs/Bi₂WO₆ nanocomposite. For that purpose, a Zeta Sizer (Nano-ZS; Malvern Instruments) with its software, Dispersion Technology Software (DTS) was used.

For FTIR qualitative analysis, materials were studied using the horizontal attenuated total reflectance (HAT) mode of a FTIR spectrometer (PerkinElmer Frontier™ with Touch™ software). To acquire spectra, a premium HATR plate with flat top and fitted with a ZnSe 45° crystal was used as it allows for simple sampling of solids, polymer films, and powders. This equipment provides one method of measuring the IR spectra in transmission with relatively long pathlength and, together with C-H bands, thus gives the greatest sensitivity. To obtain profiles, 0.5 mg of the MWCNTs and MWCNTs/Bi₂WO₆ nanocomposite were dissolved in 1 mL of methanol. To study the functional groups of RIF, and interaction of nanocomposite with RIF (MWCNTs/Bi₂WO₆-RIF), RIB (MWCNTs/Bi₂WO₆-RIB), and RIP (MWCNTs/Bi₂WO₆-RIP), 500 μL of each drug (0.5 mM in DMSO) was mixed with 10 μL of the composite suspension (1 mg mL⁻¹ in methanol).

2.4. Quartz crystal microbalance measurements

The QCM200 System consists of a thin AT-cut, α-quartz disk of 5 MHz, 1 inch in diameter, with circular gold electrodes (100–1000 mm thick coating). Circular electrodes were patterned on both sides, i.e. front side (12 mm diameter for the liquid surface) and on the rear side (5 mm diameter for the electrical contact purpose). The large diameter of the crystal, and the relatively much smaller oscillation area, assures good separation between the active electrode pads and the mounting structure of the holder. This minimizes the coupling of other resonant modes to the thickness shear oscillation as well.

The exposed area of the front electrode in contact with the liquid is ∼1.37 cm², however, the active electrode oscillation (i.e. displacement area) is mostly restricted to ∼0.40 cm². This extremely sensitive sensor is capable of measuring mass changes in the ng/cm² range with a wide dynamic range extending into the 100 μg/cm² range [42].

2.5. Preparation of QCM devices

Before modification, all QCM devices used in this study were cleaned by piranha solutions (a mixture of 3:1 sulfuric acid and 30% hydrogen peroxide) to remove residues from the substrate. After washing, 10 μL of MWCNTs/Bi₂WO₆ nanocomposite dispersion (1 mg mL⁻¹) in methanol was drop-coated. By spinning the substrate (speed 1000 RPM; 15 s) the droplet was forced to spread out while the solvent evaporated. The resultant device was dried at 60 °C for 3 h and then at room temperature for 24 h. Following this procedure, a very thin layer of MWCNTs/Bi₂WO₆ of about 18–20 nm on the quartz crystal was achieved (calculated by AFM).

2.6. Mass-sensitive measurements

The modified QCM device was mounted in a crystal holder’s head on a flat surface, with its crystal cavity pointing up. The crystal cavity contains two spring-loaded contacts (POGO® pins), which connect the two contact surface circuits of the crystal to the BNC connector on the opposite end of the probe. The device was exposed to the desired concentration of analyte in batches through a series of load/rinse exchanges. This was further connected to frequency, output port (BNC) on the front panel for connection to an external frequency counter. For data acquisition and storage, SRS QCM200 software was used. All mass changes were observed in terms of frequency shifts as a function of time, at 25 ± 2 °C with three separately MWCNTs/Bi₂WO₆ modified QCM devices. All solutions for sensor measurements were prepared in phosphate buffer saline and the required concentrations were obtained by the appropriate dilutions of the respective stock solutions.

3. Results and discussion

3.1. Characterization of MWCNTs/Bi₂WO₆ nanocomposite

The overall process for the fabrication of MWCNTs and the Bi₂WO₆ nanocomposite is schematically depicted in Fig. 1. In order to generate in situ, a facile method was carried out that is in itself improved than many reported procedures [43–47] for the synthesis of different nanostructures of bismuth tungstate. The facile and cost-efficient growth

![Fig. 1. Schematic illustration for designing MWCNTs/Bi₂WO₆ nanocomposite for the detection of rifampicin.](image-url)
of nanosheets bears much significance when very expensive methods like chemical vapor deposition techniques are in vogue \[12,48\]. Moreover, the process of migration of growing seeds on another support is very important to develop composite nanosheets \[49\]. We have carried out their successful in situ self-seeding growth in presence other structures like CNTs a very facile, solution based method. The applications of CNTs in general, and specifically as sensor transducer material, are considerably limited by their negligible suspension into aqueous and milder organic solution owing to the powerful intermolecular van der Waals bonds between tubes resulting in agglomerations \[50\]. To produce stable dispersion and to provide active centers for the growth of sheets over tubes, in the first, step carbon nanotubes were functionalized to furnish amine group. Amine modified CNTs have been studied since the amine group has great reactivity and can interact and allow for the adsorption of bismuth tungstate sheet on its modified surface. In the second step, bismuth salt was introduced, presumably making a complex with amine moieties of CNT/PEI dispersion. When the tungstate salt was added, it resulted in the formation and deposition of Bi\(_2\)WO\(_6\) nanosheets. In fact, bismuth tungsten oxide grows in supersaturated Bi(NO\(_3\))\(_3\) and Na\(_2\)WO\(_4\) solutions, and fine seed particles can act as the precursor to deposit nanosheets \[51\]. Such direct deposition of the nanomaterial over the CNTs is more suitable for sensor applications because of the better distribution of material characteristics over the network of nanotubes.

The size, shape, and morphology of the MWCNTs/Bi\(_2\)WO\(_6\) nanocomposite were studied by recording FESEM images (Fig. 2A and B) which actually provides a comparison of CNTs alone and the CNTs with deposited Bi\(_2\)WO\(_6\). Morphologies are significantly different in the two images. Bi\(_2\)WO\(_6\) sheets can be seen embedded in the network of MWCNTs strands in Fig. 2B. The diameter of MWCNTs was about 20–25 nm whereas, nanosheets were \(~100–200\) nm in length and of similar dimension in width. Based on these observations, it was concluded that the prepared composite sheets are square shaped. After the formation of the composite, the network became more crowded and entangled due to the presence of nanosheets. FESEM in TEM mode images (Fig. 2C and D) further confirmed the presence of square shaped nanosheets anchored on the tubular structures of CNTs.

MWCNTs and the MWCNTs/Bi\(_2\)WO\(_6\) nanocomposite were characterized by AFM and the resulting representative topographies are displayed in Fig. 3. Here, the strands of MWCNTs (about 25 nm) can be seen (Fig. 3A). However, after the formation of the composite, the height and width of the nanotubes were increased to about 50–100 nm (Fig. 3B). This can be credited to the agglomeration of Bi\(_2\)WO\(_6\) nanosheets over their surface which is shown as an increase in AFM height. The dimensions of sheets were estimated to be 100–200 nm in length and width. The height of the sheets was found to be 1–2 nm which validates the sheet structures of Bi\(_2\)WO\(_6\) after the formation of the composite (Fig. 3C and D). However, at various places, the sheets were stacked in bundles of \(~100–500\) nm, embedded in the intricate network of MWCNTs. The surface roughness parameter (RMS) for MWCNTs was calculated to be 15 nm, while its value for the MWCNTs/Bi\(_2\)WO\(_6\) nanocomposite was 35 nm. This high increase in roughness clearly indicated the surface modification of MWCNTs. Comparing Figs. 2 and 3, we found that AFM images are in good agreement with FESEM and TEM results, in turn corroborating the surface modification of MWCNTs by the formation of the MWCNTs/Bi\(_2\)WO\(_6\) nanocomposite.

The phase structure, crystallinity, and purity of the as-obtained Bi\(_2\)WO\(_6\) nanosheets over MWCNTs were examined by XRD measurements. Fig. 4A is depicting the XRD pattern of the MWCNTs/Bi\(_2\)WO\(_6\) nanocomposite, different characteristic peaks at 25°, 28.3°, 32°, 46° and 55° correspond to the (002), (113), (200), (220) and (313) inter-planar spacing of MWCNTs and Bi\(_2\)WO\(_6\) nanosheets, respectively. No peaks of
Fig. 3. Surface characterization (A) AFM images showing morphology of MWCNTs, (B) MWCNTs/Bi$_2$WO$_6$ nanocomposite, (C) Bi$_2$WO$_6$ nanosheets, and (D) profile showing the height and width of nanosheets. For these experiments, the parameters are scan size = 10μm and 5μm, scan rate = 1 Hz, image data = height and sample lines = 512.

Fig. 4. Structural characterization (A) XRD patterns for Bi$_2$WO$_6$ and MWCNT/Bi$_2$WO$_6$ nanocomposite and (B) HATR-IR profiles of the MWCNTs, Bi$_2$WO$_6$ nanosheets and MWCNTs/Bi$_2$WO$_6$ nanocomposite.
other impurities are detected, indicating the purity of the products. The wide diffraction peaks show that the size of the crystalline grain is small. The average crystalline size estimated from the (131) peak is about 80 and 120 nm, according to the Scherrer equation for samples and MWCNTs/Bi2WO6 nanocomposites. In the XRD pattern of MWCNTs/Bi2WO6 nanocomposite, all characteristic peaks of Bi nanosheets are present, which strongly validates the loading of the Bi2WO6 nanosheets on MWCNTs surface.

Fig. 4B shows the HATR-IR spectra of the MWCNTs, Bi2WO6 and MWCNTs/Bi2WO6 from 1000 to 4000 cm−1. In the case of MWCNTs, the spectrum showed a broad peak at 3349 cm−1 which represents the presence of CH–H. The presence of a noticeable band at 1414 cm−1 corresponds to the C–H stretching. While in case of pure Bi2WO6 nanosheets, no bands were observed. If we compare the IR profile of the nanocomposite with that of MWCNTs, we can see that main backbone of carbon is consumed during the formation of the composite. In fact, MWCNTs can adsorb PEI (cationic polyelectrolyte) due to electrostatic interactions. A large number of imine groups present on the PEI polymer chain can coordinate with the Bi(NO3)3·5H2O salt. With the addition of (Na2WO4·2H2O), it leads to the formation Bi2WO6 nanosheets [52]. The change of this carbon, carbon network of CNTs occurred as the result of the modification of the CNT backbone (−C=−C=−) with PEI and further by the formation of nanosheets.

The mechanism of the reaction was further supported by investigating zeta-potentials. The respective zeta potential graphs of Bi2WO6 nanosheets and nanotubes wrapped with Bi2WO6 are displayed in Fig. S1 (supplementary information). Bi2WO6 plain nanostructures possessed a positive potential (1.8 mV) in contrast to MWCNTs wrapped with Bi2WO6 which showed a negative potential (−18.5 mV). This change of surface charge can be attributed to the presence of a MWCNTs network that holds an inherent negative charge.

3.2. Basic sensor signals

Mass-sensitive measurements were carried out by recording the frequency responses of bare and different modified QCM devices, Bi2WO6 nanosheets, MWCNTs, and MWCNTs/Bi2WO6 nanocomposite, toward 0.5 mM of RIF. The resultant sensor signals are displayed in Fig. 5A. MWCNTs/Bi2WO6 showed a response of 150 ± 0.02 Hz as compared to the bare gold electrode exhibiting a frequency shift of 25 ± 0.02 Hz. The significantly higher net sensor response (125 Hz) shown toward RIF template as compared to the bare gold signifies the capability of the prepared nanocomposite toward the detection of the RIF. The increase of sensor responses due to the use of MWCNTs and Bi2WO6 alone has also been calculated. The coatings prepared by individual MWCNTs displayed a response of 50 ± 0.02 and if we compare that with the frequency shift of the nanocomposite, the resultant 100 Hz advocates the strength of using MWCNTs for the composite synthesis. This increase in sensor response is due to the addition of MWCNTs which can be expressed as the composite effect. The enhancement of sensor response due to a combination of CNTs and Bi2WO6 nanosheets, can also be calculated as the sensor layer of plain Bi2WO6 nanosheets generating a 46 ± 0.02 Hz frequency shift, which means a net sensor response of 104 Hz. Undoubtedly, this improvement in sensor response is the result of collaborative effects produced by MWCNTs and Bi2WO6. This can be assigned as a synergistic factor. This not only improves the surface properties as accessibility and diffusion, which in turn affects the affinity interactions of RIF with the sensor layer.

The highest increase of sensor response by the use of the composite was supported by the HATR-IR studies (Fig. S2) where the peaks within the 2000–1000 cm−1 wavelength can be assigned due to the interaction of RIF with the composite. Fig. S2 of the HATR-IR of rifampicin showed an absorption band within the region of 2600 to 3699 cm−1 corresponding to CH–H, =C–H and OH bonds [53]. The broadened shape of the band indicates the greater number of these chemical moieties. The characteristic sharp peaks at about 1527 cm−1 for acetyl C=O, at 1649 and 1562 cm−1 representing the C=O of furanone and C=O of amide group, respectively [54]. These bands were found in areas of major changes as the result of interaction of nanocomposite with RIF. Here, the HATR-IR spectra of the suspension showed a strong transmission at 2942 for aromatic C–H and at 2829 cm−1 for aldehyde C–H stretch, and a distinct sharp band was observed at 1416 cm−1 for alkane C–H [55]. It can be assumed that the carbonyl moieties of the assorted chemical nature present in RIF might have interacted with the composite, which in turn resulted in higher frequency shifts, when used as a QCM transducer layer.

![Fig. 5. Sensor characteristics (A) QCM sensor signals of bare gold, Bi2WO6 nanosheets, MWCNTs and MWCNTs/Bi2WO6 modified electrodes toward 0.5 mM rifampicin, (B) Frequency shift profile of MWCNTs/Bi2WO6 modified QCM device toward rifampicin (0.001–0.7 mM), in PBS (pH = 7.4); inset (C) is showing the calibration curve with regression value.](image-url)
3.3. Analytical performance

Since the designed composite exhibited a greater response toward RIF, further analytical parameters were studied by exposing the modified QCM devices to varying concentrations of RIF (from 0.001 mM to 0.7 mM). Fig. 5B reveals the steady increase of frequency shift with increasing concentrations of rifampicin. The corresponding decrease in frequency was found to be statistically significant. The correlation between the frequency shifts due to mass accumulation on the surface is attributed to the interaction of RIF molecules with the composite. It strongly indicates affinity interactions based detection of RIF when the MWCNTs/Bi₂WO₆ nanocomposite is used as a receptor. The linear sensor characteristics were found to have a correlation coefficient of $R^2 = 0.9912$. Two important analytical parameters are LoD (lower limit of detection) and LoQ (lower limit of quantification) are studied. To calculate the LoD and LoQ, the following formulas were used:

\[
\text{LoD} = 3.3\sigma / \text{Slope}
\]
\[
\text{LoQ} = 10\sigma / \text{Slope}
\]

where: $\sigma$ = the standard deviation of the response at low concentrations, Slope = the slope of the calibration curve.

The slope may be estimated from the calibration curve of the analyte. The estimate of $\sigma$ is typically the root mean squared error (RMSE) or standard deviation of the residuals taken from the regression line. The limit of detection was estimated to be 0.16 μM (S/N = 3) whereas, the limit of quantification was 0.50 μM. This demonstrates exceedingly high sensitivity of the designed sensor as compared to other reported sensors for detection of RIF as shown in Table 1 [56–60].

3.4. Repeatability and stability

Two further key parameters for the practical usefulness of sensors are stability and ruggedness: to assess these, we exposed sensors by recording three experiments of equilibration and regeneration cycles to 0.5 mM of RIF. The resulting frequency signals are displayed in Fig. S3. First of all, signals are stable. Moreover, each cycle is achieved with stability and ruggedness: to assess these, we exposed sensors by recording three experiments of equilibration and regeneration cycles to 0.5 mM of RIF. The resulting frequency signals are displayed in Fig. S3. First of all, signals are stable. Moreover, each cycle is achieved with statistically significant regeneration. It showed that the prepared composite yielded sensor coatings that can be used for the reliable detection of RIF.

To investigate long term stability, we exposed the prepared devices toward 0.5 mM RIF at different days, i.e. sensor measurements were recorded at the 1st day, and then after two weeks. During time intervals, the devices were stored at room temperature. The resultant sensor responses are shown in Fig. S4. The values of frequency were calculated to be 152 ± 0.03 and 150 ± 0.02, which means only a very small loss of mass from the surface coating. It indicates that the MWCNTs/Bi₂WO₆ nanocomposite QCM sensors are highly stable, when used for the detection of the drug.

Table 1

<table>
<thead>
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<th>Serial no.</th>
<th>Method</th>
<th>Device</th>
<th>LoD (μM)</th>
<th>Reference</th>
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<td>β-Cyclodextrin/polyaniline platinum electrode</td>
<td>1.69</td>
<td>[56]</td>
</tr>
<tr>
<td>2</td>
<td>SWAdSV</td>
<td>DyNWP/CPE</td>
<td>0.5</td>
<td>[57]</td>
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<tr>
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<td>LSV</td>
<td>Ni(OH)₂–RGO</td>
<td>4.16</td>
<td>[58]</td>
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<tr>
<td>4</td>
<td>DPP</td>
<td>HMDE</td>
<td>10</td>
<td>[59]</td>
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<tr>
<td>5</td>
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<td>[60]</td>
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<td>6</td>
<td>QCM</td>
<td>MWCNTs/Bi₂WO₆</td>
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<td>This work</td>
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3.5. Selectivity

The cross-sensitivity of the prepared QCM devices was also investigated. Binding features or inherent material interactions can be implemented to design artificial receptor matrices [29,61]. In this respect, a range of different non-covalent interactions can be employed depending on the chemical properties of the respective analyte, such as affinity-based ones, hydrophobic interactions, hydrogen bonds, and polar interactions [30]. Obviously, the interactions between the analyte and matrix play a key role in controlling the recognition ability of the material in any case [32]. In our study, we have used electrostatic interactions between the MWCNTs/Bi₂WO₆ composite and RIF as the directing or driving force. We have emphasized that the resulting selectivity should be influenced by the nature and extent of mutual interactions. For this purpose, we selected a couple of drugs which are very similar in their actions to RIF and are alternatively used to treat various infections. We took a systematic approach in the way that one of the drugs chosen for this study was very similar in structure to RIF, i.e. rifapentine (RIP) with the notable substitution of a methyl group for a cyclopentane (C₅H₈) group.

Rifabutin is an antibiotic which is used to treat tuberculosis and to prevent the tuberculosis complex. It is typically only administered to those who cannot tolerate rifampicin such as people with HIV/AIDS on anti-retrovirals [11]. Therefore, its use is less common as compared to rifapicin. Rifapentine is an antibiotic which is used in the treatment of tuberculosis in combination with other antibiotics [5]. Like in active tuberculosis it is given together with other anti-tuberculosis medications. Rifapentine possess a higher rate of hepatotoxicity. Its use is also not as common as rifapicin. However, the diagnosis of RIF resistant has been traditionally difficult, because it requires sophisticated bio-safety and laboratory infrastructure, which is hardly available [4,58].

The crystal structures of both these antibiotics indicate a heterocyclic structure containing a napthoquinone core which is spanned by an aliphatic ansa chain. The ansa bridge and the naphtol ring form a cavity like structure with four critical hydroxyl groups lying alongside the ring which form hydrogen bonds with amino acid residues on the protein during the inhibitory binding of bacterial RNA polymerase. We presumed that a similar binding mechanism should exist with the composite material prepared in this work with a larger steric hindrance coming from the additional cyclopentane group in RIP blocking the cavity and the access to the hydroxyl groups. In order to confirm this presumption, we selected another drug having similar size and molar mass, but differ significantly in the structure from RIF, i.e., rifabutin (RIB) which is also used to treat tuberculosis, but only in those patients who cannot tolerate RIF due to a compromised immune system due to HIV infections. Again, the crystal structure indicates that there are no accessible hydroxyl groups forming hydrogen bonds, so the mechanism of action is somewhat different.

The sensor responses were obtained for 0.5 mM solutions of both of the selected drugs, in addition to RIF, and the resultant sensor profiles are shown in Fig. 6A. Evidently, the MWCNTs/Bi₂WO₆ nanocomposite showed almost no response to RIB as compared to RIF, which produced a normalized frequency shift of 100 Hz. Contrarily, RIP showed some binding effect but still much smaller than RIF due to presumed steric hindrance in binding. This indicates that the MWCNTs/Bi₂WO₆ nanocomposite can provide higher selectivity for RIF even in the presence of compounds that possess similar characteristics in functional groups, shape, and size while only differing in some specific binding capabilities. Undoubtedly, this extraordinary selectivity is generated by the MWCNTs/Bi₂WO₆ synergistic effects in the first place. However, to bring our understanding of the mechanism further, we investigated the recognition process using HATR-IR by preparing three different suspensions of RIB, RIP, and RIF MWCNTs/Bi₂WO₆ nanocomposite

Square-wave adsorptive stripping voltammetry (SWAdSV); dysprosium nanowires modified carbon paste electrode (DyNWP/CPE); nickel hydroxide nanoparticle-reduced graphene oxide nanosheets (Ni(OH)₂–RGO); linear sweep voltammetry (LSV); differential pulse polarographic (DPP); hanging mercury drop electrode (HMDE); electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV); poly-melamine/gold nanoparticles nanocomposite (PMel-Au).
Recovery results of RIF determination at MWCNTs-Bi$_2$WO$_6$ nanocomposite modified QCM devices in human urine sample (n = 3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked (μM)</th>
<th>Found$^a$ (μM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1.01 ± 4.1</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.7 ± 3.3</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>101.3 ± 2.1</td>
<td>101.3</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.84 ± 2.9</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.7 ± 3.2</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>90.9 ± 1.7</td>
<td>90.9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.83 ± 2.2</td>
<td>88.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.48 ± 2.2</td>
<td>84.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>87 ± 1.8</td>
<td>87</td>
</tr>
</tbody>
</table>

$^a$ Average of three determinations.
4. Conclusion

In summary, we developed MWCNTs/Bi2WO6 nanocomposite as a sensor coating for the mass-sensitive detection of rifampicin. Morphological and structural characterization showed that the prepared composite consisted of an intricate network of carbon tubes embracing nanosheets of Bi2WO6. This nanocomposite was employed as a transducer layer for RIF onto the QCM sensor and multiple studies were directed to bring out the sensing characteristics of the material as well as to have a preliminary understanding of the selectivity. The developed sensor showed high sensitivity toward rifampicin with a detection limit of 0.16 μM while having excellent reproducibility and stability. These responses were significantly higher than the individual components of the composite demonstrating that a synergy effect was generated by the developed nanosheets. Additionally, MWCNTs/Bi2WO6 nanocomposite coated QCM sensors bear high specificity toward rifampicin and RNA polymerase genetic engineering, J. Biotechnol. 202 (2015) 640–646.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhazmat.2019.03.054.

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