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Electrochemical assay of rifampicin in pharmaceutical preparations via carbon nanotubes anchored meso-tetrakis(4-hydroxyphenyl) porphyrinatocobalt(II) composite

5.1 Introduction

Rifampicin is a semi-synthetic, essential antibiotic which is extensively used for chemotherapy due to its significant properties such as low toxicity, possibility for oral administration, well-tolerated and efficient activity.\textsuperscript{1-3} It is one of the most effective anti-tuberculosis drugs used in many countries for common, serious and lethal infections.\textsuperscript{2-4} However, rifampicin can be obtained from certain strains of \textit{Streptomyces mediterranei} and \textit{Streptomyces mediterranei} mutants.\textsuperscript{4} Due to its significant applications in various pathological processes, several analytical techniques have been reported in previous studies for rifampicin determination.\textsuperscript{3-7} Among these, electrochemical techniques have attracted great interest because of their fast, simple and high sensitivity detection without any pretreatments.\textsuperscript{3}
On the other hand, porphyrins are a group of naturally occurring compounds, which exhibit significant role in living systems. Porphyrin has four pyrrole rings linked via methine bridge (tetra dentate ligand) and holds a maximum diameter of approximately 3.7 Å for coordination to a metal centre.\textsuperscript{8-10} It forms neutral metal complex, when divalent metal ions such as Co(II), Ni(II) and Cu(II) are coordinated. Among these, the preparation and characterization of cobalt complexes of tetraphenyl porphyrins and their applications in modified electrodes have been studied for more than three decades\textsuperscript{8-13} Chemically modified electrodes based on cobalt porphyrins have demonstrated excellent electrocatalytic behavior for many analytes\textsuperscript{8-10, 14} and oxygen reductions reactions.\textsuperscript{15} Further, functionalization of cobalt porphyrins enhances the electron transfer reaction and improves the catalytic performance as compared to non-functionalized cobalt porphyrins.\textsuperscript{14-16}

Recently, multiwalled carbon nanotubes (MWCNTs) are emerging as one of the significant materials in several fields of research including electroanalytical chemistry.\textsuperscript{17-19} It has profound impact on the electroanalysis of important drugs, environmental pollutants and biologically important molecules.\textsuperscript{19-21} MWCNTs are extensively used in the electroanalysis due to low cost, high potential window, low chemical reactivity and suitability for various analytes.\textsuperscript{16, 22} Accordingly in the present chapter, we report a simple and versatile approach for the fabrication of meso-tetrakis(4-hydroxyphenyl)porphyrinato cobalt(II) on the surface of MWCNTs for rifampicin determination.
5.2 Experimental

5.2.1 Chemicals and Apparatus

Standard solution of rifampicin was prepared by weighing rifampicin (Sd fine, India) and dissolving in methanol. The meso-tetrakis(4-hydroxyphenyl)porphyrin(H$_2$THPP) was prepared according to the reported procedure.$^{23}$ Cobalt(II) acetate tetrahydrate was procured from Himedia and used as received. Multiwalled carbon nanotubes (MWCNTs, >90%) was procured from Sigma Aldrich, India. MWCNTs were pretreated by refluxing it in concentrated HNO$_3$ to remove the metal impurities. N,N-dimethylformaamide (DMF) was procured from Sd fine, India. Rest of the chemicals were of analytical grade and used as received. Triple distilled water was used for all experimental needs unless otherwise mentioned.

The electrochemical measurements were recorded with CHI-660C (CH Instruments, USA) electrochemical workstation. Nitrogen gas was purged each time to eliminate the oxygen from electrochemical cell solution. FT-IR spectra were collected from PerkinElmer spectrophotometer with KBr pellets. UV-Vis absorption studies were performed in UV 1700 PharmaSpec, (Shimadzu) spectrophotometer. Transmission electron microscopy (TEM) images and selected area electron diffraction (SAED) were collected using TECNAI G$^2$ 20 S-TWIN (FEI Netherlands) Atomic force microscopy (AFM) experiments were carried out at NOVA-MDT (Russia) microscope. Scanning electron microscopy (SEM) images and energy dispersive X-ray analysis (EDAX) mapping were recorded from SEM-VEGA 3 TESCAN with EDAX (Brucker) instrument.
5.2.2 Preparation of modified electrode

Synthesis of *meso*-tetrakis(4-hydroxyphenyl)porphyrinato cobalt(II) (CoTHPP) was accomplished by a reported procedure.\textsuperscript{16, 24} The CoTHPP immobilized MWCNTs was prepared by the following procedure.\textsuperscript{16} Briefly, a solution of CoTHPP (20 mL, 1.0 mM) in DMF was prepared. To it 20 mg of MWCNTs was dispersed and ultra-sonicated for 30 min. Further this mixture was stirred for 24 h, filtered, washed, and dried in vacuum. The prepared material is abbreviated as MWCNTs-CoTHPP. Glassy carbon (GC) electrodes were cleaned with alumina powder on Buhler-felt pad, washed with water and sonicated in water for 5 min. A 5.0 µL of MWCNTs-CoTHPP (1.0% colloid in DMF) was drop casted on the vertically mounted clean GC electrode (surface area = 0.07 cm\(^2\)), dried for 2 h at room temperature condition and abbreviated as GC/MWCNTs-CoTHPP. Similar procedure was followed for the preparation of GC/MWCNTs using MWCNTs colloid. GC/CoTHPP electrode was prepared by drop casting of 10 µL of CoTHPP solution (1.0 mM in DMF) on GC electrode and dried for 2 h at room temperature condition.

5.3 Result and discussion

5.3.1 Spectroscopic and morphological characterization of material

The immobilization of CoTHPP on MWCNTs is investigated by Raman spectroscopy (Fig. 5.1A). The D band at around 1340 cm\(^{-1}\) corresponds to disordered amorphous carbon structures, while G band at about 1576 cm\(^{-1}\) is attributed to tangential vibrations of the graphite carbons of MWCNTs.\textsuperscript{25-27} The degree of graphitization can be evaluated from the ratio of intensities of the D and G bands (I\(_D\)/I\(_G\)). The I\(_D\)/I\(_G\) ratio of the MWCNTs is 1.06, which is lower than that of MWCNTs-CoTHPP (1.16), indicating the increased disorder of carbon skeleton due to the presence of CoTHPP.\textsuperscript{26-28} UV-vis spectrum of
CoTHPP (Fig. 5.1B) exhibits one B band (430 nm) and two Q bands (544 and 586 nm) in methanolic solution representing the characteristic features of CoTHPP. The MWCNTs-CoTHPP also displays the absorption band of CoTHPP, indicating the existence of CoTHPP in the composite material. Further, FT-IR spectrum (Fig. 5.2) of MWCNTs-CoTHPP (using KBr pellet) displays the presence of bands at 1660, 858 and 1701 cm\(^{-1}\) which are attributed to C=N, C-H and C=O groups respectively, indicating that CoTHPP is well immobilized on MWCNTs. These small changes in the UV-vis absorption and FT-IR spectral studies between the neat components and the composite indicate the interaction between MWCNTs and CoTHPP by conjugation or \(\pi-\pi\) stacking.

![Raman and Absorbance Spectra](image)

**Fig. 5.1** (A) FT-Raman spectra of MWCNTs (a) and MWCNTs-CoTHPP (b). (B) UV-vis spectra of MWCNTs (a), CoTHPP (b) and MWCNTs-CoTHPP (c) in methanolic solution/suspension. (B) UV-vis spectra of MWCNTs (a), CoTHPP (b) and MWCNTs-CoTHPP (c) in methanolic solution/suspension.
Fig. 5.2 FT-IR spectra of MWCNTs (a), CoTHPP (b) and MWCNTs-CoTHPP (c) using KBr pellets.

The morphology of MWCNTs and MWCNTs-CoTHPP was investigated by SEM and TEM (Fig. 5.3 and 5.4, respectively). SEM images display clear view of nanotubes, which consists ordered arrays of MWCNTs with less or no amorphous carbon (Fig. 5.3a). As shown in SEM and TEM images, MWCNTs possess long nanotubes of several micrometers while the tube diameter is around 8-20 nm. Demonstrated features obtained from SEM and TEM for MWCNTs are greatly supported by literature works. For example, these results are very similar to the work of Silva et al.,29 Lee et al.30 and others.20, 26, 31 SEM images of the MWCNTs-CoTHPP show some granular appearance, which are probably due to the presence of CoTHPP. In order to further assess the elemental composition and distribution of CoTHPP in the material, energy dispersive X-
ray (EDAX) measurements were carried out. EDAX mapping of MWCNTs represents the homogeneous distribution of carbon and oxygen only (Fig. 5.3c, d and e), while MWCNTs-CoTHPP displays the presence of cobalt, carbon and oxygen, indicating the uniform distribution of CoTHPP (Fig. 5.3f, g, h and i).

**Fig. 5.3** SEM images of MWCNTs (a) and MWCNTs-CoTHPP (b) and respective EDAX mapping (c-i).
In addition, the granular appearance in the SEM images is more clearly observed in the TEM images of MWCNTs-CoTHPP, indicating the successful immobilization of CoTHPP (Fig. 5.4). Inset of Fig. 5.4 shows the respective SAED pattern of MWCNTs and MWCNTs-CoTHPP. These studies provide the evidence for the presence of CoTHPP in the MWCNTs-CoTHPP material.

**Fig. 5.4** TEM images of MWCNTs (a) and MWCNTs-CoTHPP (b). Inset represents the respective SAED pattern.

### 5.3.2 Electrochemical characterization

Electrochemical characterization of GC/MWCNTs-CoTHPP and GC/MWCNTs are performed in 0.1 M acetate buffer (pH 4.5) at room temperature condition. Fig. 5.5 represents the cyclic voltammetry (CV) response of GC/MWCNTs-CoTHPP at different scan rates from 10-500 mVs\(^{-1}\). CV response of GC/MWCNTs-CoTHPP displays two redox peaks where the anodic and cathodic peaks are observed at 0.40 and 0.34 V with their respective peak currents of 2.75 and -2.64 µA (scan rate: 20 mVs\(^{-1}\)). The oxidative and reductive peaks correspond to the conversion of Co(II) to Co(III) and Co(III) to
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Co(II), respectively. The ratio of anodic and cathodic peak currents (I\textsubscript{pa}/I\textsubscript{pc}) was found to be 1.04, which is close to 1, indicating that the observed process is a reversible redox process.\textsuperscript{32} The peak potential separation (ΔE\textsubscript{p}) and E\textsubscript{1/2} values for the redox process is calculated to be 60 mV and 0.37 V, respectively (at 20 mVs\textsuperscript{-1} scan rate). The ΔE\textsubscript{p} value in an electrochemically reversible reaction is a measure of number of electrons, n involved in that electrode reaction. Further, I\textsubscript{pa} and I\textsubscript{pc} increase with increase in scan rates and exhibit linear relationship between square root of scan rates and peak currents (inset of Fig. 5.5). These results indicate the existence of diffusion controlled one electron reversible process at the GC/MWCNTs-CoTHPP electrode.\textsuperscript{32}

![Graph showing CV response of GC/MWCNTs-CoTHPP at different scan rates from 10-500 mVs\textsuperscript{-1} in 0.1 M (pH 4.5) acetate buffer. Inset represents the plot between peak currents and square root of scan rates.]

Fig. 5.5  CV response of GC/MWCNTs-CoTHPP at different scan rates from 10-500 mVs\textsuperscript{-1} in 0.1 M (pH 4.5) acetate buffer. Inset represents the plot between peak currents and square root of scan rates.
5.3.3 Rifampicin oxidation at GC/MWCNT and GC/MWCNTs-CoTHPP electrodes

Electrochemical oxidation of rifampicin is carried out by CV technique in 0.1 M (pH 4.5) acetate buffer at the scan rate of 20 mVs⁻¹. Rifampicin generally exhibits two oxidation signals with peak potentials around 0.2 V (E_{pa1}) and 0.8 V (E_{pa2}) in aqueous medium. E_{pa1} represents for two electron and two proton involved process which is due to the oxidation of 6,9-dihydroxynaphthalene moiety to naphthoquinone, similar to the hydroquinone-quinone redox system. E_{pa2} belongs to the oxidation of piperazinyl-imino moiety of the molecule. GC/CoTHPP electrode exhibits two peaks for rifampicin oxidation (inset Fig. 5.6) at E_{pa1} (0.19 V) and E_{pa2} (0.75 V) with low currents (I_{pa1} = 0.8 µA and I_{pa2} = 1.9 µA). The observed very less current may be due to the sluggish electron transfer. The rifampicin oxidation responses are significantly improved at GC/MWCNTs and highly improved at GC/MWNTs-CoTHPP (Fig. 5.6). GC/MWCNTs electrode displays high currents (2.5 and 4.5 µA) over the GC/CoTHPP at the same potential. However, GC/MWCNTs-CoTHPP electrode exhibits rifampicin oxidation peaks at 0.18 and 0.74 V with peak currents of 9.4 and 15.9 µA, respectively, which are much higher than that observed at GC/CoTHPP (inset of Fig. 5.6) and GC/MWCNTs (Fig. 5.6). This increase in rifampicin oxidation current at GC/MWCNTs-CoTHPP will increase the sensitivity of rifampicin determination as compared to other two electrodes studied in this work. The decreased E_{pa1} and E_{pa2} values with high I_{pa1} and I_{pa2} values at GC/MWCNTs-CoTHPP are attributed to the efficient electrocatalytic oxidation of rifampicin by CoTHPP. Immobilization of CoTHPP on MWCNTs increases the absorptive sites and improves the electrical communication between the active sites resulting in considerable increase in oxidation peak current. The increased
performance of MWCNTs-CoTHPP could be due to the facile electron transfer process between electrode and rifampicin made possible by the MWCNTs. It may also due to the more number of active sites created by oxygen containing functional groups present on MWCNTs.\(^6\)

**Fig. 5.6** CV responses at GC/MWCNTs (a, a’) and GC/MWCNTs-CoTHPP (b, b’) in absence (a, b) and presence (a’, b’) of 1.0 mM rifampicin (scan rate 20 mVs\(^{-1}\), in 0.1 M acetate buffer pH=4.5). Inset of Fig. 5.6 shows the CV response of GC/CoTHPP in 0.1 M (pH 4.5) acetate buffer in absence (a) and presence (b) of 1.0 mM of rifampicin at the scan rate of 20 mVs\(^{-1}\).
5.3.4 Electrochemical sensing of rifampicin at GC/MWCNTs-CoTHPP

Linear sweep voltammetry (LSV) investigations are performed in 0.1 M (pH 4.5) acetate buffer with a scan rate of 20 mVs\(^{-1}\) (Fig. 5.7A). Similar to CV response, LSV response also signifies the two oxidation peaks for rifampicin. The oxidation peak currents increase with increasing solution concentration of rifampicin (Fig. 5.7A). Using the anodic peak currents (\(I_{pa1}\) and \(I_{pa2}\), Fig. 5.7A) as the analytical response, calibration curves are constructed for rifampicin determination (Fig. 5.7B).

![Graph](image_url)

**Fig. 5.7** (A) LSV recorded at GC/MWCNTs-CoTHPP with different additions of rifampicin (1.0 µM to 5.0 mM) in 0.1 M (pH 4.5) acetate buffer. (B) Calibration plot for rifampicin determination from 1.0 µM to 5.0 mM based on the two peaks observed for rifampicin oxidation.

In the concentration range of rifampicin from 1.0 µM to 5.0 mM the increase in current is linear with two different slopes. Although two different slopes are observed in the calibration curve, the second segment linear portion becomes less sensitive as the
concentration of rifampicin increases which may be due to the saturation of active sites at high concentrations of rifampicin.\textsuperscript{2-4} First segment of the calibration curve is from 1.0 to 500 µM with high sensitivity and the second segment is from 500 µM to 5.0 mM with low sensitivity. The sensitivity for calibration plot based on \( I_{pa1} \) and \( I_{pa2} \) are 245 and 334 µA mM\(^{-1}\) cm\(^{-2}\), respectively. The calculated limit of detection is 0.62 and 0.54 µM, respectively. Therefore, this sensor can be utilized potentially for electrochemical sensing of rifampicin in aqueous samples with high sensitivity and low limit of detection.

5.3.5 \textit{Real sample analysis in pharmaceutical formulation}

This analytical method is applied to detect rifampicin present in a pharmaceutical capsule (R-cinex, Lupin Ltd., Aurangabad, India). The capsule is dissolved in water and diluted appropriately. The amount of rifampicin in the capsule sample is verified by recovery analysis. The recovery and relative standard deviation (RSD) by this method for the rifampicin determination in the capsule is listed in Table 5.1. The recovery of rifampicin at GC/MWCNT-CoTHPP is found around 89-91% and the RSD is less than 7.0% (\( n = 3 \)). The results of the recovery and RSD indicate that, GC/MWCNTs-CoTHPP can be used proficiently for the rifampicin determination in pharmaceutical formulations.
Table 5.1  Real sample analysis for rifampicin determination at GC/MWCNTs-CoTHPP using LSV technique.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Claimed value (µg)</th>
<th>Standard added (µg)</th>
<th>Total (µg)</th>
<th>Found (µg)</th>
<th>Recovery %</th>
<th>RSD % (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-cinex, Lupin Ltd.</td>
<td>593.3</td>
<td>-</td>
<td>593.3</td>
<td>526.4</td>
<td>89.2</td>
<td>6.63</td>
</tr>
<tr>
<td>Aurangabad, India</td>
<td>82.0</td>
<td>675.3</td>
<td>607.6</td>
<td>90.3</td>
<td>3.38</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>164.0</td>
<td>755.2</td>
<td>640.4</td>
<td>84.8</td>
<td>2.66</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>246.0</td>
<td>837.2</td>
<td>763.8</td>
<td>91.2</td>
<td>2.03</td>
<td></td>
</tr>
</tbody>
</table>

% recovery = ([rifampicin found] / [total rifampicin present]) × 100

5.3.6 Reproducibility studies

The reproducibility of the analysis is tested at GC/MWCNTs-CoTHPP using LSV in presence of rifampicin (0.1 mM) in 0.1 M (pH 4.5) acetate buffer at 20 mVs⁻¹ (Fig. 5.8).

Three different electrodes are analyzed for 100 µM rifampicin determination with four individual measurements. For four successive determinations at three different electrodes, an RSD (n = 12) of 0.2% is obtained indicating an excellent reproducibility of this method. Thus, this sensor can be utilized for electrochemical sensing of low concentration levels of rifampicin with high sensitivity and excellent reproducibility.
Fig. 5.8 Reproducibility test were performed at GC/MWCNTs-CoTHPP using LSV (at three different electrodes, E₁, E₂ and E₃) for the determination of rifampicin (0.1 mM) in 0.1 M (pH 4.5) acetate buffer at 20 mVs⁻¹.

5.3.7 Interference and stability

Interference studies are generally performed to assess the knowledge regarding the selectivity of the sensor. They also provide information regarding the alteration in the original signal in presence of various electroactive interferents such as isoniazid, pyrazinamide and glucose which may present in biological samples. The electrocatalytic oxidation current of 0.1 mM rifampicin was measured in the presence of compounds like L-alanine (0.1 mM), L-arginine (0.1 mM), glucose (0.1 mM), dopamine (0.1 mM), pyrazinamide (0.1 mM) and isoniazid (0.1 mM) with no or negligible variation.
in the response current (Fig. 5.9). These results indicate the high selectivity for rifampicin determination.

**Fig. 5.9** Amperometric I-t response curves indicating the influence of various possible interferents (0.1 mM) during the determination of 0.1 mM rifampicin at GC/MWCNTs-CoTHPP. Addition of rifampicin is indicated as a, while b-g indicate the additions of isoniazid (0.1 mM), pyrazinamide (0.1 mM), alanine (0.1 mM), glucose (0.1 mM), arginine (0.1 mM) and dopamine (0.1 mM), respectively.

Stability of GC/MWCNTs-CoTHPP electrode is tested using LSV technique by keeping it for 15 days under normal air and dry conditions (Fig. 5.10), representing the small decrease in the oxidation current compared to the first day response. The response on the 1st day is compared with the response measured under exactly similar conditions on the
10\textsuperscript{th} and 15\textsuperscript{th} days. On comparison of the 1\textsuperscript{st} day response (100\%) with 10\textsuperscript{th} and 15\textsuperscript{th} days response, GC/MWCNTs-CoTHPP retains oxidation response up to 84.1 and 84.2\%, respectively.

Fig. 5.10 LSV oxidation peak currents for rifampicin in 0.1 M (pH 4.5) acetate buffer at GC/MWCNTs-CoTHPP electrode against the number of storage days to understand the stability of the electrode.

5.4 Conclusion

In summary, MWCNTs-CoTHPP material is prepared and characterized with spectroscopic, microscopic and electrochemical techniques. The electrochemical behavior of rifampicin at GC/MWCNTs-CoTHPP electrode is systematically investigated. Combination of the unique properties of MWCNTs (like fast electron transfer) with the catalytic properties of CoTHPP, offered the GC/MWCNTs-CoTHPP electrode a noticeable decrease in the overvoltage and enhanced electrochemical response
for rifampicin oxidation in 0.1 M (pH 4.5) acetate buffer. The high surface area, enhanced conductivity and oxygen containing functional groups present on the MWCNTs make it as a convenient support to hold the catalyst, CoTHPP. The GC/MWCNTs-CoTHPP electrode demonstrates a high sensitivity and a remarkable low detection limit in a wide determination range by LSV technique. Furthermore, the sensor is utilized for the determination of rifampicin in a real sample demonstrating that the MWCNTs-CoTHPP can be a promising material for the determination of rifampicin in pharmaceutical, chemical and biological samples.


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**References**


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