Synthesis, Characterization and Antibacterial Activity of Zinc Oxide Nanoparticles

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Abstract

Zinc oxide nanoparticles were synthesized by a chemical method and characterized by various analytical techniques such as UV-visible spectroscopy, SEM-EDX, XRD, and FTIR. X-ray diffraction and SEM analysis confirmed the formation of well-dispersed zinc oxide nanoparticles with average particle size 22 nm as well as revealed their spherical structure. Chemically synthesized nanoparticles were found to exhibit antibacterial activity against bacterial strains Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus using agar well diffusion method of analysis.

1. Introduction

Nanotechnology is the technology to control a matter of an atomic and molecular scale. It has extraordinary potential to change the lives by improving existing products and enabling new ones. It facilitates the development of new materials in the 1-100 nm range, comparable to the size range of biological molecules and structures [1]. Nanoparticles are used as manipulation, sensing, and detection of biological structures and systems. The principal factors which make nanomaterials different from their bulk counterparts include an increase in their relative surface area and quantum effects, which affect their physical and chemical properties [2] and due to the high surface area to volume ratio, it develops as novel antimicrobial agents and flow enthusiasm to th...
2.3.2 Agar Well Diffusion Method

Nutrient agar plates were swabbed on three axes with the sterile cotton-tipped swab, which was dipped in the freshly prepared diluted culture. Sterile cork borer was used to make the two 6 mm holes aseptically in the agar plate. ZnO NPs were prepared by diluting 10 mg of dry ZnO Nps with 100 mL sterile distilled water and dispersed in water using ultrasonicator for 20 minutes. One hole was filled with ZnO Nps, one antibiotic disc and one well filled with distilled water which serves as a control. Then plates were incubated at 30 °C for 24 h. After incubation, the plates were observed for the zone of inhibition which was measured in terms of diameter.

3. Results and Discussion

3.1 Synthesis of Zinc Oxide Nanoparticles

As sodium hydroxide (NaOH) was added to the zinc acetate(Zn(CH₃COO)₂·2H₂O), it is found to be colour changes from colorless to milky white precipitate (Fig. 1) and this precipitate confirms the formation of zinc oxide nanoparticles.

\[
\begin{align*}
&\text{Zn (CH}_3\text{COO)}_2\cdot2\text{H}_2\text{O} + 2\text{NaOH} \rightarrow \text{Zn(OH)}_2\cdot2\text{H}_2\text{O} + 2\text{CH}_3\text{COONa} \\
&\text{Zn (OH)}_2\cdot2\text{H}_2\text{O} \rightarrow \text{ZnO} \cdot \text{H}_2\text{O} + 2\text{H}_2\text{O} \\
&\text{ZnO} \cdot \text{H}_2\text{O} \rightarrow \text{ZnO} + \text{H}_2\text{O} \\
&\text{ZnO} \cdot \text{H}_2\text{O} + 2\text{H}_2\text{O} \rightarrow 2\text{ZnO} + 2\text{H}_2\text{O}
\end{align*}
\]

Fig. 1 Synthesis of Zinc Oxide Nanoparticles

3.2 Characterization of Zinc Oxide Nanoparticles

3.2.1 UV-Vis Analysis

The primary characterization of chemically synthesized nanoparticles was done with UV-Vis spectroscopy which is a very useful and reliable technique. Zinc oxide NPs have unique optical properties which make them strongly interact with specific wavelengths of light. In addition, UV-Vis spectroscopy is simple, easy, fast, required a short period of time for measurement, and to characterize the colloidal suspensions [12]. UV-Vis spectroscopy analysis of chemically synthesized ZnO NPs analyzed between the range 300 – 500 nm (Fig. 2) and showed high peak absorbance spectrum at 335 nm. Satyanarayana et al. [13] also reported a peak at 320 nm for zinc oxide nanoparticles.

Fig. 2 UV-Vis analysis of zinc oxide nanoparticles

3.2.2 SEM-EDAX Analysis

SEM analysis was conducted in order to examine the morphology and structure of the chemically synthesized ZnO nanoparticles. This reveals that the obtained nanoparticles are uniform spherical in shape and shown in Fig. 3. It can be also used for predicting the antimicrobial activity of ZnO nanoparticles. Nie et al. [14] reported that the results from scanning electron microscopy indicated the presence of nanoparticles in growing medium may cause damages to the E. coli cell membrane. Fig. 4 indicates the high purity and chemical composition of ZnO nanoparticles which are confirmed by EDX analysis.

3.2.3 X-Ray Diffraction Analysis (XRD)

The crystalline structure of chemically synthesized zinc oxide nanoparticle was obtained using XRD. Dorofeey et al. [15] reported that XRD analysis is important and act as good potential techniques for nanostructures examination because it yields information about the substructure and sizes of crystallites of the materials. Fig. 5 shows that ZnO nanoparticle was highly crystalline and all diffraction peaks are well indexed 31.687°, 34.339°, 36.172°, 47.453°, 56.507°, 62.768°, 66.281°, 67.856°, 68.981°, 76.908°, 92.670°, 95.314° and 98.472° which corresponds to 100, 002, 101, 102, 110, 103, 200, 112, 201, 202, 210, 211 and 114 crystal plane respectively (JCPDS 36-1451). Giri et al. [16] reported similar peaks for zinc oxide nanoparticle. The average crystalline size of ZnO nanoparticle is 22 nm. The crystallite domain size of XRD peaks was calculated from the width of resulted peaks, confirming that they were free from non-uniform strains by adopting Scherrer’s formula, 

\[
D = \frac{0.94\lambda}{\beta \cos \theta}
\]

where, \(D\) = average crystallite domain size, \(\lambda\) = X-ray wavelength, \(\beta\) = full width at half maximum (FWHM), and \(\theta\) = diffraction angle.

Fig. 3 SEM image of zinc oxide nanoparticles

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3.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is used to measure the vibration modes of functional groups of molecules and is sensitive to molecular structure, conformation, and environment. Therefore, in the current study, it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups. FTIR spectroscopy was analyzed in the range of 4000 - 400 cm⁻¹. The FTIR spectra of chemically synthesized zinc oxide nanoparticles were analyzed for knowing the possible functional groups (Fig. 6). This FTIR spectrum shows the purity of ZnO nanoparticles, the peak at 402.0 cm⁻¹ is the characteristic absorption of Zn-O bond and the broad absorption peak at 3459.6 cm⁻¹ can be attributed to the characteristic absorption of hydroxyl groups (Table 1). Similarly [17] reported that ZnO nanoparticles exhibit a rather broad and moderately strong band at 495 cm⁻¹, owing to Zn-O vibrational mode. The band at 3445 cm⁻¹ corresponds to O-H mode of adsorbed moisture in the annealed sample. The stretching C-O vibration is observed at 1530 cm⁻¹. The bands at 1625 and 2363 cm⁻¹ are due to CO₂ and C-O groups. Sharma et al. [18] applied the FTIR technique to characterize ZnO Nps and showed a standard peak of zinc oxide is around 464 cm⁻¹.

Fig. 4 EDAX analysis of zinc oxide nanoparticles

Fig. 5 XRD analysis of zinc oxide nanoparticle

electrostatic interactions between ZnO NPs and cell walls resulting in destroying bacterial cell integrity [20], the liberation of antimicrobial Zn²⁺ ions [21] which is related to an accumulation of ZnO NPs into the bacterial cells [22]. Meakra and Rajan [23] reported that CuO nanoparticles attach to the surface of the cell membrane disturbs its function and penetrates directly with the bacterial outer membrane and release CuO ions and smaller sized NPs can enter the mitochondria of cells through various pathways and thereby induce oxidative stress and cell damage apoptosis [24]. Thus ZnO NP may contort and harm bacterial cell layer, causing spillage of intracellular substance promoting cell death [25].

4. Conclusion

ZnO nanoparticles have been prepared using a wet chemical method. The structural characterization of synthesized nanoparticles are crystalline in structure and their diameter was around 22 nm confirmed by XRD. The synthesized ZnO nanoparticles exhibit the characteristic absorption peak at 335 nm. In FT-IR spectroscopy pure ZnO nanoparticles show stretching vibrations at 402 cm⁻¹. The chemically synthesized ZnO NPs exhibit significant antibacterial activity against all the three bacterial strains, i.e., gram -ve E.coli, Pseudomonas aeruginosa and gram +ve bacteria Staphylococcus aureus and show a significant zone of inhibition to ZnO Nps compared to the positive control penicillin. This study proves that zinc oxide nanoparticles possess good antibacterial activity.

Table 1 Functional group assignments of FT-IR characterization

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Wave number ZnO Nps (Cm⁻¹)</th>
<th>Spectral assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3459.6</td>
<td>-O-H Stretching vibration, broad</td>
</tr>
<tr>
<td>2</td>
<td>3015.1</td>
<td>=C=H Stretching vibration</td>
</tr>
<tr>
<td>3</td>
<td>2962.1</td>
<td>C-H, CH₃ and CH₂ stretching vibration</td>
</tr>
<tr>
<td>4</td>
<td>2040.3</td>
<td>CC Stretching vibration</td>
</tr>
<tr>
<td>5</td>
<td>1742.3</td>
<td>C=O Stretching vibration</td>
</tr>
<tr>
<td>6</td>
<td>1445.3</td>
<td>C=O Stretching mode, carbonate</td>
</tr>
<tr>
<td>7</td>
<td>1364.3</td>
<td>C-H bond bending</td>
</tr>
<tr>
<td>8</td>
<td>1222.6</td>
<td>C-H Deformation vibration</td>
</tr>
<tr>
<td>9</td>
<td>902.5</td>
<td>C-H Deformation vibration</td>
</tr>
<tr>
<td>10</td>
<td>402</td>
<td>Zn-O Stretching vibration, broad</td>
</tr>
</tbody>
</table>

Table 2 Antibacterial activity of ZnO Nps against pathogens

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Zone of inhibition (mm)</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10</td>
<td>18</td>
</tr>
</tbody>
</table>

Fig. 6 FT-IR analysis of zinc oxide nanoparticles

![FT-IR analysis of zinc oxide nanoparticles](image)

![Zone of Inhibition of ZnO Nps against three bacterial strains](image)

3.3 Antibacterial Activity of Zinc Oxide Nanoparticles

The antibacterial activity of chemically synthesized zinc oxide nanoparticles against three strains of pathogenic bacteria such as Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus was done by using well diffusion method. Antibacterial effect was measured by observing the zone of inhibition. The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO nanoparticles as presented in Table 2. This assay reveals that the gram-negative bacteria are sensitive to the ZnO NPs, while it shows resistance to the synthetic antibiotic (Fig. 7). Sivakumar and Senthilkumar [19] observed the similar antibacterial activity of zinc oxide nanoparticles. Literature revealed that the

References


