Synthesis Characterization of TiO$_2$ doped Ferric Oxide Nanoparticles and their Antibacterial Activity

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Abstract:
The present work outlines the antibacterial activity of TiO$_2$ doped Ferric oxide nanoparticles synthesized through chemical combustion method where ferric nitrate is used as precursor material and urea as fuel with the assistance of Tween 80, a non-ionic surfactant. The obtained Fe$_3$O$_4$ nanoparticles were characterized by X-ray diffraction, SEM with EDAX. From XRD and it was found to be 43–50 nm. The study aims to determine the antibacterial activity of TiO$_2$ doped Ferric oxide nanoparticles. The synthesized metal oxide nanoparticles were analyzed for antibacterial activity by disc diffusion method for zone of inhibition and by micro dilution plate method for MIC and showed strong antibacterial activity against bacterial species.

Keywords: TiO$_2$ doped Ferric oxide, antibacterial activity, Escherichia coli, Staphylococcus aureus.

I. INTRODUCTION

‘Nanotechnology- there is plenty of space at the bottom’, this sentence was never thought to be as powerful as much it could revolutionize the worldwide industries. The world is now turning towards enormous applications of nanotechnology especially with the inorganic materials [1]. Due to its high aspect ratio, nanoparticles are used not just for electrical or optical applications but also for biological utilities [2]. Free radicals are generated in human body due to pollutants, radiation and pesticides. These radicals will attack biomolecules such as proteins and lipid and hence these radicals need to be scavenged in order to prevent the deleterious effect in body [3].

1. Materials and methods

1.1 Synthesis of TiO$_2$ doped Ferric oxidennanoparticles

The synthesis of magnetite (Fe$_3$O$_4$) Nanoparticles was done by chemical combustion. The required amount of ferric nitrate (0.1 M) was dissolved in 20 ml of deionized water under the magnetic stirrer for 10 min. The fuel urea and ammonia (0.1 M) were dissolved separately in 30 ml of distilled water respectively. The surfactant TWEN80 (0.07 M) was dissolved in 20 ml of distilled water and was kept under stirring for 10 min separately. Fuel solution was mixed with oxidizer solution which was under stirring followed by mixing of surfactant solution. The whole solution was kept under stirring for 15 min for stirring. The solution was placed on a hot plate to initiate the reaction. When the temperature had started to increase, the solution boiled and fumes gushed forth from the solution; as the temperature increased above 100 °C, the solution started to evaporated leading to an increase in the viscosity of the liquid and smouldering started eventually self-ignition took place forming the final product (Fe$_3$O$_4$). The powder was collected from the beaker and calcinated for 1 h at 400 °C [4].

1.2 Synthesis of TiO$_2$ doped Ferric oxidennanoparticles

Stoichiometric ratio of synthesized magnetite (Fe$_3$O$_4$) Nanoparticles was dissolved in 20 ml of ethanol sonicated for 20 minutes to form solution A. Then Solution B was formed by dissolving pre calculated amount of Titanium iso Propoxide in 10 ml of deionized water, 10 ml of ethanol and 1 ml of acetone. The Solution B is transferred to Solution A under vigorous stirring, sonicated for 10 minutes then stirred at 70 °C for 4 hours. Precipitate was separated by Magnet, washed with deionized water and ethanol. Dried at 80 °C for 6 hours and calcined at 400 °C for 2 hours. The nanoparticles were collected for characterization and antibacterial application.

1.3 Characterization of prepared samples

TiO$_2$ doped Fe$_3$O$_4$ nanoparticles were characterized by XRD to find out the phase formation. X-ray diffraction patterns were obtained from Rigaku Ultima IV X-ray Diffract meter with CuKα radiation and diffraction angle range 2θ = 10° to 70° operating at 45 kV and 30 mA. To study the size and morphology of the product, SEM was performed on a Vega 3 Tescan scanning electron microscope.

1.4 Antibacterial studies

Antibacterial activity assessment of TiO$_2$ doped Fe$_3$O$_4$ nanoparticles were done using agar well diffusion method as per CLSI guidelines [5] for Zone of Inhibition (ZOI) and MIC. The lawn of bacterial inoculum was spread uniformly on a sterile Muller Hinton Agar plates and wells were made using sterile borer. In each well, 100 µl of TiO$_2$ doped Fe$_3$O$_4$ nanoparticles with 1000, 500 and 250 µg/ml was added separately and the plates were incubated at 37 °C for 24 h, after which ZOI was measured and readings were taken in triplicate against the standard antibiotic i.e., ciprofloxacin. For determination of antibacterial activity by Minimum Inhibitory Concentration, a100 µl of sterile CAMHB broth was added to all 96 wells except first column three wells of the microtitre plate A$_1$B$_1$C$_1$ to which only 200 µl TiO$_2$ doped Fe$_3$O$_4$ nanoparticles were added. From first three wells (A$_1$B$_1$C$_1$) of plate, 100 µl of the TiO$_2$ doped
Fe₃O₄ nanoparticles were double diluted till A₁₂B₁₂C₁₂. Then 10μl bacterial suspension of approximately 10⁶CFU/ml was added to each dilutions. A growth control (bacterial cell suspension + 100μl broth medium) from G₁ to G₁₂ and broth control (only broth medium 100μl) from H₁ to H₁₂ were also done on plate. The positive control consists of the 0.1%ciprofloxacin was also placed in the plate D₁E₁F₁ to D₁₂E₁₂F₁₂. The plates of gram –ve Escherichia coli and gram +ve Staphylococcus aureus were then incubated at 37° C for 24hours. After incubation, 10μl of working solution of resazurin was added to all wells. The plates were wrapped with aluminum film and incubated at 37°C for 1hour. The change in color was then assessed visually i.e., from purple to pink or colorless was recorded as positive (growth). The lowest concentration at which there is no colour change occurred was taken as the MIC value.

2. Result and discussions

2.1 Characterization

TiO₂ doped Fe₃O₄ nanoparticles from x-ray diffraction (XRD) confirms crystalline structure with particle size 43-50nm. The XRD pattern of TiO₂ doped Fe₃O₄ nanoparticles is shown in Figure 1. The SEM image of TiO₂ doped Fe₃O₄ nanoparticles is shown in Figure 2. The EDAX image of the presence of Iron and Titanium in the synthesized TiO₂ doped Fe₃O₄ nanoparticles shown in Figure 3. 

Figure 1. The X-ray Diffraction pattern of TiO₂ doped Fe₃O₄ nanoparticles

The structural characterization was performed using SEM. The nanoparticles were readily identifiable, the particle dimension increased, size distribution was wide and nanoparticles were homogenously distributed. The SEM pattern of TiO₂ doped Fe₃O₄ nanoparticles are shown in Figure 2. The EDAX confirms the presence of Iron and Titanium in the synthesized TiO₂ doped Fe₃O₄ nanoparticles shown in Figure 3.
2.2 Antibacterial studies

The E. coli and S. aureus cells were exposed to TiO2 doped Fe3O4 nanoparticles for antibacterial activity by ZOI method [6-9] and the results are as shown in Table 1 with inhibition from TiO2 doped Fe3O4 nanoparticles against S. aureus as shown in Figure 4.

Table 1. ZOI antibacterial activity of TiO2 doped Fe3O4 nanoparticles

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Conc. (µg)</th>
<th>ZOI# (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO2 doped</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>Fe3O4</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>nanoparticles</td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1000</td>
<td>36</td>
</tr>
</tbody>
</table>

# The zone of inhibition is exclusive of zone of well diameter.

Figure 4. ZOI for standard and TiO2 doped Fe3O4 nanoparticles against S. aureus bacterium.

The Minimum Inhibitory Concentration [10-12] readings were taken from concentration ranging from 1000 µg to 0.488 µg for TiO2 doped Fe3O4 nanoparticles. MIC of TiO2 doped Fe3O4 nanoparticles were found to be 250 µg against E.coli whereas 125 µg for S. aureus.

Table 2. MIC antibacterial activity of TiO2 doped Fe3O4 nanoparticles

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Conc. (µg/ml)</th>
<th>MIC (µg or µl/0.1ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO2 doped</td>
<td>1000-0.488</td>
<td>250</td>
</tr>
<tr>
<td>Fe3O4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nanoparticles</td>
<td></td>
<td>125</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&lt;0.488</td>
<td>&lt;0.488</td>
</tr>
</tbody>
</table>

* MIC value is expressed as mean of triplicate, n = 3

3. CONCLUSIONS

We have demonstrated that TiO2 doped Fe3O4 nano particles showed a significant antibacterial activity against gram negative E. coli and gram positive S. aureus organisms as the concentrations increased (i.e., 250, 500 and 1000 µg) and its prevalent that TiO2 doped Fe3O4 nanoparticles had better antibacterial activity from both ZOI and MIC test methods.

4. ACKNOWLEDGEMENT:

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5. REFERENCES