Chapter 5

Synthesis of 3-APTES (3-aminopropyltriethoxysilane) embedded ZnO nanostructures and its characterization along with the antibacterial activity study

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5.1 INTRODUCTION

Bacteria are present in a wide range of geologic and aquatic environments. The cell wall tends to be the first cellular structure to come into contact with the antibacterial agents. A variety of surface functional groups on bacterial cell walls such as carboxyl’s, phosphonomoesters, phosphodiesters, amines and hydroxyls have been suggested to be directly responsible for the reactivity of bacterial cells [1-3]. Among them carboxyl and phosphoryl groups are considered to be particularly important in metal complexation based chemistry and modeling studies [4-7]. The 3-aminopropyltriethoxysilane binds with ZnO surface through the hydroxyl group of ZnO and finally forms Zn-O-Si- bond. This coupling agent has amine group on it’s other end that helps for attachment to the bacterial cell surface.

Here, it had been initiated to synthesized ZnO nanoparticles by a co-precipitation method with in situ capping by 3-APTES under room temperature. Co-precipitation method has an advantage of room temperature growth and the fabrication process is inexpensive and environmentally friendly. The interaction type between ZnO and 3-APTES is that the hydroxyl group may interact with the silanol group of 3-APTES and finally gives the surface functionalized ZnO nanoparticles [8]. The probable schematic diagram of the interaction type between ZnO & 3-APTES had been drawn in Fig1. In the present study functionalized ZnO nanomaterials were obtained by chemical reaction between water solution of Zinc acetate precursor and 3-APTES in the presence of NaOH. Initially Zinc hydroxide had been formed & finally the hydroxyl group may interact with the silanol group of 3-APTES to synthesize the capped nanomaterial.
Reaction between surface OH groups of ZnO and Hydrolyzed 3-APTES

Removal of H₂O molecule

3-APTES capped ZnO spherical nanoparticles

Fig 1: Schematic representation of functionalized ZnO nanoparticles by 3-APTES
The objective of this study was to thoroughly examine the significance of 3-APTES embedded ZnO nanoparticles on bacterial cell wall destruction and the subsequent status of these nanomaterials after getting entered into the bacterial cell. Actually in this study, an attempt had been done to investigate the exact antibacterial activity mechanism behind the already reported antibacterial property of ZnO nanomaterials. Generally, the antimicrobial mechanism of chemical agents like 3-APTES embedded ZnO nanoparticles can be understood by studying the specific binding on the surface of the agent with the microorganism and the consequent mode of action against those bacteria. It was believed that the amine group present in the 3-APTES network leads to bacterial cell surface attachment.

### 5.2 Experimental Procedure

The 3-APTES embedded ZnO spherical nanoparticles were synthesized by co-precipitation technique. The general procedure was involved by addition of Zinc acetate dehydrate (99% purity from Sigma Aldrich) to a given volume of double distilled water. The pH of the solution was maintained at 10 by drop wise addition of 1M NaOH (GR grade from MERCK). After the formation of white colloidal solution, the 3-APTES (From Sigma Aldrich) capping agent was added and stirred vigorously. The white precipitation was collected after one day by centrifuging the samples at 3000 rpm and washed several times by water and ethanol and finally dried in hot air oven at slightly warm condition. By changing the molar concentration of this capping agent i.e. 3-APTES, 10 different samples were prepared (from 0.1M-1.0M). The uncapped nano ZnO was synthesized at the same time based on the above mentioned technique. The schematic diagram of synthesis process is given in Fig2 as a flow chart.
5 gm Zinc Acetate dissolved in 100 cc double distilled water

Magnetic stirer

Room temperature

1M NaOH add

pH = 10

Transparent solution

White colloidal solution

Centrifuge at 3000 rpm

Dried in hot air oven at little warm condition

APTES capped nano ZnO

Fig2: Schematic diagram of synthesis process for preparing 3-APTES embedded ZnO spherical nanoparticles
5.3 RESULTS AND DISCUSSION

5.3.1 XRD analysis of uncapped & 3-APTES capped ZnO nanoparticles

The synthesized uncapped & 3-APTES capped ZnO nanoparticles were characterized by X-ray powder diffraction (XRD, Rigaku Ultima III, Cu Kα radiation) method in the range of 2θ values from 10°-80° at a scan rate of 5°/min. Fig3 had shown the XRD plots of 3-APTES embedded ZnO spherical nanoparticles with the variation of capping agent concentration[9]. The sharp intense peaks of ZnO had confirmed the good crystalline nature of ZnO and the peaks originated from (100), (002), (101), (102), (110), (103), (200), (112) and (201) plane had reflected the presence of hexagonal ZnO. No peaks for Zn or other impurities were detected in the spectrum, revealing the phase purity of the products. The d values were computed and compared with standard d values of ZnO (JCPDS No. (036-1451)) resulting in excellent matching for all embedded samples. The average crystal size was calculate separately by the Debye Scherrer’s formula \( D = \frac{k\lambda}{\beta \cos \theta} \), where K is a constant equal to 0.89, \( \lambda \) the X-ray wavelength (0.1540595 nm), \( \beta \), the full wavelength at half maximum (FWHM) and \( \theta \), the half diffraction angle. The broadening of the FWHM value had confirmed the formation of nanomaterial. The average crystallite size of the monophase samples were calculated from x-ray line broadening (\( d_{101} \)) and it was summarized in Table 1. The computed d values of all samples and standard d values of ZnO had been summarized in Table 2.

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Table1: Crystallite size of 3-APTES embedded ZnO spherical nanoparticles
**Figure 3:** XRD analysis of 3-APTES capped ZnO spherical nanoparticles

XRD analysis: standard ZnO with 3-APTES embedded ZnO spherical nanoparticles (different molar ratio of 3-APTES)

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XRD analysis: standard ZnO with 3-APTES embedded ZnO spherical nanoparticles (different molar ratio of 3-APTES)

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Table2: The computed d values of APTES capped ZnO spherical nanoparticles comparison with standard d values of ZnO
5.3.2 UV-VIS Spectroscopy analysis of uncapped and 3-APTES capped ZnO nanoparticles

The optical transmission spectra were taken in the UV-VIS range by using UV-VIS spectrophotometer (Perkin Elmer, lambda 35 UV-VIS spectroscope). The transmission of control sample i.e. bare ZnO nanomaterial was about 367 nm wavelength, but it was changed its transmission into 353 nm after getting attach with 0.1M 3-APTES. The transmission was further shifted towards blue region after capping with 0.2M and 0.3M 3-APTES and the transmission fall was 348nm and 339nm wavelength but the transmission fall was increased into 360 nm wavelength after getting attached with 0.4M capping agent i.e. 3-APTES. Finally it was fixed the transmission at about 360 nm wavelength and it did not show any change with increasing the molar concentration of the capping content up to 1.0M (Fig4).

![Graph showing transmission spectra](image)

**Fig4:** UV-VIS spectroscopy analysis of 3-APTES embedded ZnO spherical nanoparticles
The band gap was calculated from the transmittance for all samples along with the control i.e. bare ZnO nanomaterial. Calculated value of bare ZnO was about ~3.93eV which was significantly higher than the band gap of bulk ZnO (~3.37eV). Calculated band gap value of all 3-APTES capped ZnO spherical nanoparticles were 4.47eV (0.1M APTES), 4.61eV (0.2M APTES), 4.66eV (0.3M APTES), 4.24eV (0.4M APTES) [10]. Further increase of capping content, molar concentration did not affect any significant change for band gap (Fig5).

**Fig5:** Band gap calculation of 3-APTES embedded ZnO spherical
Fig5 continued: Band gap calculation of 3-APTES embedded ZnO spherical nanoparticles
The experimental results had concluded the fact about the wider band gap compare to that of narrower. This explanation had signified the characteristics of materials at nano dimension. An internal force had created inside the nanomaterial and due to this force it was difficult for an electron to jump from valence band to the conduction band.

5.3.3 FTIR Spectroscopy analysis of uncapped and 3-APTES capped ZnO nanoparticles

The interaction between ZnO with APTES was confirmed by the FTIR measurement. The experimental results had shown the evidences of the modification of amino groups to the surface of ZnO. In Fig6, the FTIR absorption characteristics associated with APTES had been distinguished easily. The band at 2920 cm\(^{-1}\) was due to the stretching vibration of C-H bonds; the appearance of these bands had indicated that functional groups in modifier (propyl or aminopropyl group) existed on modified surface [8]. The band at 1520 cm\(^{-1}\) was due to the contribution of Zinc-carboxylate group and such a band was familiar in the IR spectrum of ZnO synthesized from zinc acetate precursor [11,12]. Furthermore, the peak at 893 cm\(^{-1}\), which had indicated that the condensation reaction between silanol groups of APTES and surface hydroxyl group of ZnO (Zn-O-Si) had occurred [13]. Both NH\(_2\) and OH stretching bond had appeared at around 3423 cm\(^{-1}\). The band corresponding to Zn–O–Zn bond generally appeared in the region between 400 cm\(^{-1}\) to 700 cm\(^{-1}\). Band at 538 cm\(^{-1}\),558 cm\(^{-1}\),569 cm\(^{-1}\),485 cm\(^{-1}\) had corroborated the IR absorption of Zn-O stretching vibration. Decreasing size of the capped nanoparticles had caused the shifting of Zn-O stretching band [14].
5.3.4 Morphological analysis by SEM study

The nanoparticles morphology was obtained by SEM analysis. Fig7 showed SEM images of ZnO nanomaterials obtained with capping molecule, 3-APTES. Three different molar concentration of capping content i.e. 0.1M, 0.4M and 1.0M was used for SEM analysis. Lowest capping content (0.1M), middle capping content (0.4M) and highest capping content (1.0M) ZnO nanoparticles had showed same spherical like morphology but in case of 0.1M sample, spherical nanoparticles were found without formation of any networking whereas, this regular spherical like structures had assembled themselves in a bridge like morphology were observed by addition of 0.4M and
1.0M capping agent APTES. SEM images had confirmed the morphology of these capped samples.

![SEM images](image)

**Fig7: SEM analysis:**

- **A)** 0.1M APTES capped ZnO spherical nanoparticles
- **B)** 0.4M APTES capped ZnO spherical nanoparticles start attaching to each other in a bridge-like manner
- **C)** 1.0M APTES capped ZnO spherical nanoparticles attach to each other and finally obtained bridge-like structure

### 5.3.5 Morphological analysis by HRTEM study

APTES capped ZnO nanoparticles (0.1M) detected by high-resolution transmission electron microscope (HRTEM) analysis was polycrystalline spherical nanoparticles, whereas 0.4M and 1.0M capping content APTES had shown polycrystalline spherical nanoparticles attached to each other leading to bridge-like morphology. The silanol group of APTES and the hydroxyl group had been attached with each other which were already discussed in FTIR analysis. Fig8 had given the morphology of capped ZnO nanomaterials in detail. It had been seen from the selected-area electron diffraction (SAED) patterns of both APTES capped ZnO nanocrystals that the
diffraction spots corresponded to wurtzite ZnO. In the insets of Fig8A and 8B, the spotted
diffraction rings had shown the crystalline capped ZnO. 0.1M APTES embedded ZnO
nanostructures had formed spherical morphology and this was retained up to 0.3M APTES,
whereas this structure was started attaching with each other at the molar concentration of 0.4M.
Finally it had been seen from the TEM image Fig8C that 1.0M capping content APTES gave
bridge like morphology which was made of several ZnO spherical nanoparticles assembled to
each other. The particle size was approximately 20-30 nm in diameter.

![TEM images](image)

**Fig8: TEM analysis:** A) 0.1M APTES capped ZnO spherical nanoparticles, B) 0.4M APTES
capped ZnO spherical nanoparticles start attaching to each other in a bridge like manner, C) 1.0M APTES capped ZnO spherical nanoparticles attach to each other and finally obtained
bridge like morphology.
5.3.6 Optical property study by Photoluminescence spectroscopy analysis

The optical property of the ZnO nanostructures was characterized by the PL spectra measurement at room temperature. Fig9 had showed the PL analysis of 3-APTES embedded ZnO nanomaterial. It had indicated that the PL spectrum of ZnO nano crystal was mainly composed of two emission bands. One was ultraviolet emission (UVE) band and the other was deep-level emission (DLE) band. The DLE in ZnO was considered to be related to intrinsic defects such as oxygen vacancy eVOT or Zn interstitial eZnIT [15-17].

To understand the influence of the molar concentration of the capping agent i.e. APTES on the optical property of the ZnO nanoparticles, photoluminescence of the ten samples were conducted, along with the bare ZnO nanomaterial, as shown in Fig9. Two characteristic photoluminescence peaks were found in all the samples. In bare ZnO, a relative lower ultraviolet emission peak was observed around 398 nm, corresponding to the near-band-edge emission of ZnO. Two intensive broad peaks at 544 and 573 nm were detected from the ZnO nanoparticles, ascribed to the single ionized oxygen vacancy or zinc interstitial in ZnO [18], which is under debate.

There was the occurrence of changing photoluminescence when the ZnO had prepared in presence of capping agent 3-APTES. The ratio between the UVE and the DLE was changed with changing of molar concentration of the capping agent. In case of 0.1M 3-APTES capped ZnO nanoparticles, the UVE was blue shifted to 388 nm wavelength. The resulting blue shifts were important indicators that attested to an increasing electronic interaction between the ZnO and 3-APTES, which had signified that the ZnO nanoparticles were, decreased its size after getting attached with 0.1M 3-APTES. Here two intensive broad DLE peaks positioned at 544 and 573 nm (bare ZnO) were degenerated and emitted at 534 nm wavelength. Such visible
emission in 0.1M APTES capped ZnO nanoparticles might be related to the surface oxygen vacancies. Recently, Dijken et al. had figured that the particle surface played an important role in the visible emission [19]. The UVE was further blue shifted to 386 nm, when the ZnO was synthesized in presence of 0.2M 3-APTES. It was confirmed the further size decrease of the capped ZnO nanoparticle [20]. The visible emission was unchanged for the 0.2M and 0.3M capping content sample. From the SEM and TEM analysis it had been found that the ZnO nanoparticles had started initializing bridging morphology (0.4M 3-APTES) and these different structures were responsible for changing the ratio between the UVE and the DLE. The strong peak at 386 nm had corresponded to near band edge emission. Meanwhile the band at 533 nm was related to single ionized oxygen vacancy. It was found that the band edge emission was much higher in intensity than the deep level emission in case of 0.4M and 0.5M capped sample, suggested the positive role of 3-APTES capping. For the visible emission, the intensity of the broad photoluminescence band decreased drastically when the ZnO nanoparticles were capped with 0.4M and 0.5M 3-APTES. This result had suggested that this visible emission originated mainly from the deep surface traps, which can be removed through surface passivation by higher amount of 3-APTES [21]. The visible emission may be due to the amount of O$_2$ on the ZnO surface which might be in the form of OH$^-$ ions. The lower intensity of DLE had suggested that the surface OH ions of ZnO were blocked due to the interaction with 3-APTES. The molar ratio 0.6M and 0.7M had showed the higher intensity in DLE peaks, suggested that higher amount of capping agent had remarkably enhanced the deep level emission & finally caused the structural defect. From 0.1M to 0.7M capping content concentration, the UVE was unchanged in its position but there was an occurrence of its intensity change. From 0.8M to 0.9M APTES it was gradually increased the DLE with subsequently decreasing UVE. It was further blue shifted its
UVE from 386nm (0.7M APTES) to 378nm (0.8M APTES) and finally reached at 370 nm (1.0M APTES). This blue shifting was due to smaller size crystalline formation. From the above study it had been concluded that there was no further capping had occurred from 0.6M capped sample but the changing morphology from particles to bridge like structure had been continued. It had been already revealed that the surface modifier agent had restricted the agglomeration but in this research work it was also responsible for changing the morphology from particles to bridge like structure. This special chain like manner was due to the higher molar concentration of capping agent and this higher capping agent induced more compact structure to the ZnO. Due to the strain created by higher molar concentration of APTES the inner core ZnO nanoparticles became smaller in size.
Fig9: PL analysis of 3-APTES capped ZnO spherical nanoparticles
**Fig9 continued:** PL analysis of 3-APTES capped ZnO spherical nanoparticles
5.3.7 Interaction of 3-APTES capped ZnO nanoparticles with E.coli (Gram-Ve) and S.aureus (Gram+Ve) and it’s antibacterial activity study

In previous chapter it had been already discussed about the proposed antibacterial mechanism of chitosan capped ZnO nanorod. Here, an attempt had been drawn to determine the mechanism of damaging the bacterial cell with the help of 3-APTES capped sample. The main objective of this research work was to thoroughly examine the significance of capped ZnO nanomaterial on bacterial cell wall destruction and the subsequent status of this nanomaterial after getting entered into the bacterial cell. Bacterial cell surface was the main contact point where these nanomaterials got attached. After capping with 3-APTES, the ZnO had obtained the nanospherical particle shape and this morphology was changed to the new structure after increasing the molar concentration of capping content. FTIR analysis revealed the information about the binding of these nanoparticles with the cell surface. Transmission electron microscopy (TEM) analysis will help to study the antibacterial mechanism and finally zone of inhibition study will help to confirm the antibacterial activity quantitatively.

Tests for assessment of antibacterial activity;

5.3.7.1 Kirby-Bauer test (Cup plate method)

Gram-negative bacteria E. coli and Gram-positive bacteria S.aureus were used for the evaluation of antibacterial activity testing. Culture broth (Nutrient broth from HIMEDIA) and culture agar (Nutrient agar from HIMEDIA) were used as culturing nutrient sources. Both E. coli and S.aureus were grown at 37 °C in an incubator [22]. The antibacterial activity study of uncapped ZnO, 3-APTES capped ZnO nanoparticles with amoxicillin was carried out.
5.3.7.2 Sample preparation for antibacterial activity study

Both *E.coli* and *S.aureus* bacterial cell were incubated at 37°C, under continuous shaking at 120 rpm. At the exponential phase bacteria were harvested by centrifugation at 4000g for 10 min at 4°C followed by washing twice with 10mM phosphate buffer saline (PBS, pH 7.2). The bacteria were suspended in PBS. Separately, 0.1M, 0.4M and 1.0M 3-APTES capped ZnO nanostructures (0.02 gm) were mixed in the bacterial medium & were incubated under same condition. At the exponential phase, the bacteria with nanomaterial were harvested separately. Finally the bacteria attached to those nanoparticles were dried in vacuum drier.

5.3.7.3 Study of attachment of 3-APTES capped ZnO nanoparticles with the *E.coli* and *S.aureus* bacterial cell analyzed by FTIR

Fig10 had revealed the comparative FTIR spectral analysis of untreated *E.coli*, *S.aureus* and both the bacteria treated with the sample (1.0M APTES capped ZnO nanostructures). The absorption peak at 1084 cm⁻¹ is typical for P=O stretching in peptidoglycan layer of *E.coli* and *S.aureus* whereas the bands at 3316 cm⁻¹, 3327 cm⁻¹ denote the reflected stretching vibrations of O-H and NH (the functional group in polysaccharides and proteins). Meanwhile, the bands at 1678 cm⁻¹ & 1651 cm⁻¹ are the result of C=O and C-N (Amide I) stretching. The bands at 1583 cm⁻¹ & 1557 cm⁻¹ reflect a combination of N-H bending and C-N (Amide II) stretching (Amide I and Amide II corresponded to the functional groups in proteins) of *E.coli* and *S.aureus* [23, 24, 25] (Fig10A and 10B).

After treatment with 3-APTES embedded ZnO nanoparticles the bands, which were observed at 1084 cm⁻¹, 1678 cm⁻¹ and 1583 cm⁻¹ in untreated *E.coli*, shifted to lower values 1048 cm⁻¹, 1665 cm⁻¹ and 1547 cm⁻¹. Whereas the bands at 1084 cm⁻¹, observed in
untreated \textit{S.aureus}, were shifted to lower values $1135 \text{ cm}^{-1}$. The bands at $1651 \text{ cm}^{-1}$ and $1557 \text{ cm}^{-1}$ were remaining unchanged. The relative shift of these bands had suggested that polysaccharides and proteins of the bacterial cells were involved in the attachment which may cause the ultimate destruction of bacterial cell (Fig10C and 10D). The FTIR analysis had confirmed that the interaction of these nanoparticles with \textit{E.coli} bacteria was stronger than interaction with \textit{S.aureus}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{images/ftir_analysis.png}
\caption{A, B) FTIR analysis of untreated \textit{E.coli} and \textit{S.aureus}, C, D) FTIR analysis of sample treated \textit{E.coli} and \textit{S.aureus}}
\end{figure}
5.3.7.4 Study of attachment of 3-APTES capped ZnO nanoparticles with the E.coli and S.aureus bacterial cell analyzed by HRTEM

The attachment of APTES embedded ZnO nanomaterials on both S.aureus as well as E.coli bacterial cell was further presented in TEM analysis. Fig11 had demonstrated the intact E.coli structure. The SAED pattern had been confirmed the amorphous nature of bacterial cell. TEM analysis had also allowed direct visualization of morphological changes resulting in the microorganisms upon contact with APTES embedded ZnO nanomaterials. E. coli cell had been grown in the presence of functionalized ZnO samples & these had showed preliminary results of no cellular internalization in case of 0.4M and 1.0M APTES sample. But cellular internalization had occurred in lowest capping content i.e. 0.1M APTES sample (nanoparticles). The dotted ring, shown from the SAED pattern, had confirmed the nanoparticles were present inside the bacterial cell. From this study it had been observed that lowest capping content sample, which was in nanoparticles range, were got entered inside the cell [26]. But for the other two samples, where the morphology had changed to bridge like pattern, the particles could not enter inside it (in case of E.coli). APTES embedded ZnO nanoparticles (0.4M and 1.0M) had distorted the E.coli cell wall and ultimately damaged the whole cell but the mechanism was different from the lowest capping content sample which was spherical in nature. SAED pattern of this sample did not prove any cell internalization. No any dotted ring had been found for both untreated as well as treated E.coli but the cell wall disorganization as well as the whole cell destruction had been found. From the TEM analysis it had been confirmed that the lowest capping content nanospherical sample had easily got entered through cell internalization into the E.coli cell whereas the middle and highest capping content sample did not get scope for cellular
internalization. One more interesting thing was observed in case of 3-APTES capped ZnO nanoparticles, it had assembled the all *E.coli* cells through their amine linker.

![Intact E.coli](image)

**Fig11:** **A)** 0.1M APTES capped ZnO nanoparticles internalized into the *E.coli* cells, inset SAED shows presence of crystalline ZnO, **B)**, **C)** 0.4M and 1.0M APTES capped ZnO nanoparticles (initialized branched pattern) assembled *E.coli* cells, **D)** Higher magnification TEM analysis of Fig C)

Peptidoglycan layer is much thicker in case of *S.aureus*. Fig12 had showed the exact spherical structure of this bacterial cell which had been destructed after getting attaches with 0.1M, 0.4M and 1.0M APTES capped ZnO nanoparticles. The entire bacterial cell had been connected
through the APTES linker which had covered the ZnO nanoparticle externally. Through this linking, the bacterial cell had suffered a partial pressure which gave the shrinking surface texture to the *S. aureus* cell and ultimately caused cell damage. All of the SAED pattern of these untreated and sample treated *S. aureus* cell were almost same. It did not show any crystallinity inside the cell after getting attached with all samples & it had corroborated that there was no cellular internalization occurred for all samples.

**Fig 12:** **A)** Intact *S. aureus*, **B)** 0.1M APTES capped ZnO nanoparticles does not internalized into the *S. aureus* cells, inset SAED shows absence of crystalline ZnO, **C), D)** 0.4M and 1.0M APTES capped ZnO nanoparticles (initialized branched pattern) assembled *S. aureus* cells.
The probable antibacterial mechanism of APTES embedded ZnO nanoparticles against both *E. coli* and *S. aureus* is as follows: The ZnO had been present inside the APTES linker very firmly. This strong bond between the ZnO and APTES linker did not give the opportunity to ZnO to come out from the embedded condition and internalized into the bacterial cell. Actually higher molar concentration of 3-APTES had been created the main barrier between ZnO and the bacterial cell surface and due to this reason cell internalization had not occurred. SAED pattern of TEM analysis had been revealed the above fact.

5.3.7.5 *Antibacterial activity test through zone of inhibition study*

Antibacterial effect of 3-APTES capped ZnO nanoparticles had been analyzed by the study of inhibition zone in agar plate with respect to known antibiotic Amoxicillin. All tests had been repeated thrice after culture incubation at 37°C overnight and the average zone diameter were given in Table 3, which had revealed the antibacterial zone due to the presence of known antibiotic (amoxicillin) and different structured ZnO nanomaterial respectively. From the table it was confirmed that larger inhibition zone created by 0.1M APTES capped ZnO nanoparticles. As the molar concentration of capping content increased, the morphology of the nanomaterials had changed from spherical particles to assembled spherical particles and as a result the inhibition zone was gradually decreased.
Here, the amine functional groups were successfully attached to ZnO nanomaterials through 3-Aminopropyltriethoxysilane encapsulation. This 3-APTES containing amine groups helped the attachment. The X-ray diffraction (XRD) patterns had confirmed the presence of pure single crystalline ZnO nanomaterials. No other impurities had occurred due to the 3-APTES and the FTIR study was used to understand the formation of ZnO nanomaterials with the presence of surface amine functional groups. Here in this study, the process had been developed and this

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>( E. coli ) (mm)</th>
<th>( S. aureus ) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>~21.83</td>
<td>~20.54</td>
</tr>
<tr>
<td>Uncapped ZnO</td>
<td>~18.88</td>
<td>~17.50</td>
</tr>
<tr>
<td>0.1M APTES capped ZnO (spherical nanoparticle)</td>
<td>~20.56</td>
<td>~20.50</td>
</tr>
<tr>
<td>0.4M APTES capped ZnO (spherical nanoparticles initialize branch pattern)</td>
<td>~19.00</td>
<td>~18.00</td>
</tr>
<tr>
<td>1.0M APTES capped ZnO (spherical nanoparticles bridge like morphology)</td>
<td>~18.85</td>
<td>~17.85</td>
</tr>
</tbody>
</table>

**Table 3:** Analysis of zone of inhibition study

5.4 Conclusion

Here, the amine functional groups were successfully attached to ZnO nanomaterials through 3-Aminopropyltriethoxysilane encapsulation. This 3-APTES containing amine groups helped the attachment. The X-ray diffraction (XRD) patterns had confirmed the presence of pure single crystalline ZnO nanomaterials. No other impurities had occurred due to the 3-APTES and the FTIR study was used to understand the formation of ZnO nanomaterials with the presence of surface amine functional groups. Here in this study, the process had been developed and this
innovative method was responsible for the structural modification of ZnO nanomaterials which were identified by SEM and HRTEM images.

Finally, the study had been undertaken on the antibacterial efficacy for the E.coli, S.aureus bacteria and at the same time it had been trying to find out the probable mechanism behind the antibacterial effect. All nanomaterials had showed good antibacterial effect for the both bacteria and the spherical nanomaterials had shown better result among the all. The TEM result had visualized directly the morphological changes resulting in the microorganisms upon contact with APTES embedded ZnO nanomaterials. Cellular internalization had occurred in lowest capping content i.e. 0.1M APTES sample (spherical nanoparticles) only for E.coli bacteria. Whereas the bridge like morphology did not enter inside the bacterial cell. APTES embedded ZnO nanoparticles in bridge like morphology (0.4M and 1.0M) had distorted the E.coli cell wall and ultimately damaged the whole cell but the mechanism was different. In case of S.aureus all the bacterial cells had been suffered a partial pressure which gave the shrinking surface texture to the entire cell and ultimately the cell was destroyed.
5.5 REFERENCES


