INTERACTION OF NANOMATERIALS WITH MICROBES AND THEIR CHARACTERIZATION

A DISSERTATION

Submitted in partial fulfillment of the requirements for the award of the degree of

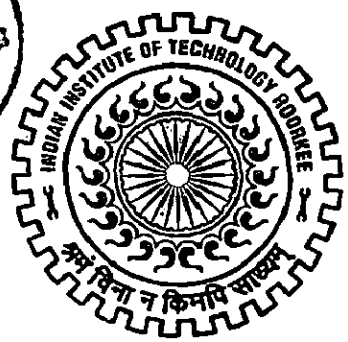
MASTER OF TECHNOLOGY

in

ADVANCED CHEMICAL ANALYSIS

By

KHUSHWANT SINGH



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Candidates Declaration

I hereby declare that the work which is being presented in this dissertation report, entitled **"Interaction of Nanomaterials with Microbes and Their Characterization"** is submitted in partial fulfillment of the requirements for the award of the degree of **Master of Technology** with specialization in **"Advanced Chemical Analysis"** to the Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee. It is an authentic record of my own work from July 2007 to June 2008 under the esteemed guidance of Dr. R.K. Dutta, Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University/Institute.

Dated: 30 JUNE 2008

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Certificate

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

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Contents

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ABSTRACT

Two different types of zinc oxide nanomaterials were synthesized, one capped with poly-ethylene glycol (PEG) and other was bare which was synthesized by solution free method. These nanoparticles were characterized by XRD, UV-Visible, SEM-EDAX, TEM and AFM. A commercially procured - Aldrich standard reference material of ZnO nanopowder of size less than 100 nm was used. The toxicity of the two types of synthesized ZnO nanomaterial and standard nanopowder, towards *E.coli* was studied by following the growth curve of *E.coli* with increasing concentrations (5 - 15 mg of nanomaterial per 100 ml of growth media) of nanomaterials where inhibition of growth was observed for higher concentrations. Inhibition in growth of *E.coli* was also complemented by Transmission Electron Microscopy and Atomic Force Microscopy, where the physical interaction of nanomaterial and *E.coli* was observed. The cell deformation due to ZnO nanomaterial treatment could be observed from TEM and AFM images. In the presence of higher concentration of these nanomaterials cell rupture was observed under TEM. The PEG coated nanomaterial showed lesser anti-microbiological activity as compared to uncoated ZnO nanomaterial.

Chapter 1

Introduction and Literature Review

1.1 Nanotechnology and Nanomaterials

The term nanotechnology describes the creation and exploitation of structures, devices and systems by controlling shape and size at nanometre scale. Nanotechnologies have the potential to transform many aspects of our life though at present theoretical modelling and research & development are dominating much of the work in nanotechnologies [1]. It promises to be the next industrial revolution but the major point of concern is the rapidly growing commercial exploitation of these innovations, without having enough data of their effect on human health. This concern has risen a new field of study called *Nanotoxicology* [2].

1.1.1 Classification of nanomaterials

A nanometer (nm) is one billionth of a meter, or 10^{-9} m. Materials having at least one dimension in this range are classified under nanomaterials. Materials in this size range exhibit different physical, chemical properties as compared to bulk materials. For example , crystals in nanometer scale have a low boiling point, the surface energy plays a significant role in the thermal stability. Bulk semiconductors become insulators when the characteristic dimension is in the order of nanometers [3]. There are four main types of nanomaterials:

1. **Carbon based nanomaterials** are composed mostly of carbon, mostly taking the form of hollow spheres, ellipsoids or tubes. Spherical and ellipsoidal materials are referred to as fullerenes, and cylindrical ones are called nanotubes.

2. **Metal based nanomaterials** include quantum dots, nanogold, nanosilver and metal oxides. A quantum dot is a closely packed semiconductor crystal comprised of hundreds or thousands of atoms. Optical properties of optically active quantum dots can be tailored by changing their sizes and shapes.

3. **Dendrimers** are nanosized polymers built from branched units, their surface has numerous chain ends which can be tailored to perform specific chemical functions. Three dimensional dendrimers contains cavity which could be used for drug loading.

4. **Composites** combine nanoparticles with other nanoparticles or with bulk materials. Nanosized clays are composite nanomaterials with properties of increasing mechanical strength and thermal, barrier, flame- retardant properties.

1.2 Applications of Nanomaterials

Nanaotechnology has broad range of potential applications in areas like electronics, optical communications, biological systems and many more. It has already delivered products like self cleaning glass, protection creams, medical dressings, antimicrobial technologies in electrical appliances and others such as higher capacity hard disks, super strong light materials are further to come. Different properties on which the application of nanomaterials is based are:

1. The interesting physical properties of nanomaterials, e.g. gold nanoparticles have a red color and are used as inorganic dye to introduce color into glass.
2. The large surface area.
3. The small size that accommodates multiple functions.

Due to these properties the nanomaterials behave different from the bulk materials and have many applications in the field of medicine, chemistry, energy, information and communication and consumer goods. A few types of applications is discussed below:

(a) Nanomedicine

One of the most significant applications using nanoparticles or nanomaterials has been nanomedicine. Nanomedicine can be broadly classified as repair, monitoring, construction and control of human biological systems at the molecular level with help of nanodevices and nanostructures. Nanomedicine has further functional areas as drug delivery system, gene delivery system, imaging, molecular diagnostics, cardiac therapy, dental care, orthopedics [4]. In drug delivery the drug is targeted to the cell/tissue of choice, with the help of nanotechnology the drug can be delivered to the right place and at right time [5]. The recent work regarding enhanced permeability and retention effect and tumor-specific targeting is discussed by Peppas and Blanchet [6]. Biodegradable nanoparticles formulated from poly (D,L-lactide-co-glycoside) (PLGA) are used for targeted/localized delivery of different agents including plasmid DNA, protiens and peptides, these nanoparticles rapidly escape into cytosol and hence help in enhanced uptake [7]. In gene delivery systems the the viral vectors will be replaced by potentially less toxic nanomaterials; (a) liposomes are potential candidates due to their nontoxic properties [8], (b) colloidal particles of the range 10 to 1000 nm formulated using PLGA, (c) dendrimers, macromolecular compounds made up of series of branches around an inner core have been shown to be

effective as DNA conjugates [9]. Nanotechnology has provided techniques for sensing chemically relevant markers, molecular disease imaging and therapeutic agents [10]. In the field of molecular diagnostics, microfluidics or lab on a chip technology which is one of the branch of nanotechnology is being applied to over come the limitations of biochip technology [11].

(b) Nanochemistry

In the field of chemistry the nanotechnology has introduced nanocatalysts and nanofiltration membranes. Nanocatalysts like gold nanoparticles deposited on transition metal oxides such as Fe_2O_3, TiO_2 and Co_3O_4 are very active at room temperature oxidation of carbon monoxide as compared to Au/Fe_2O_3 catalysts were active only after calcination at $\approx$ 400 °C as demonstrated by Khoudiakov [12]. Nanocatalysts have overcome the many complications observed in simple catalysts such as need for pre treatment, large induction time periods before reaching maximum catalytic activity and low efficiency. Nanofiltration membranes offers the physical separation techniques which is an environmental friendly technique. Nanofiltration helps in removal of metal ions from water, where the metal ion rejection is effected by the pH, temperature and are used for cleaning-up of contaminated water [13].

(c) Nanotechnology in Energy

Nanotechnology has large potential to contribute to energy systems, through their more efficient use and by providing new and novel sources and systems. In light harvesting, solar photovoltaics employ the use of nanotechnology where a dye sensitized solar cell is made with TiO_2 nanomaterial coated with a dye and entraps solar energy. Electrochemical devices also employ the nanotechnology, fuel cells the electrochemical devices convert fuel such as hydrogen to electricity through an electro-catalytic process. The electro-catalytic process takes place at the electrodes in fuel cell, which should have a high surface/volume ratio, a durable material and low resistance transport from catalyst to external circuit which are fabricated and characterized with help of nanotechnology. In photocatalysis photons are absorbed on the semiconductor surface leading to electronic excitations, which then induce desirable chemical reaction on the surface of semiconductor, nanostructures have minimized the distance over which charge has to survive to reach to surface and has increased the efficiency of the process [14].

(d) Nanotechnology in Communication

In the recent advances in the field of communication, nanomachines and nanonetworks have shown the potential to replace traditional technologies which are large in size and have higher power consumption. Nanomachines [15], cannot execute complex task by themselves, the flow of information between them enables them to perform complex tasks which is done by using nanonetworks. In nanonetworks the message is encoded using molecules, where as in traditional communication networks the information is encoded in electromagnetic, acoustic or optical signals [16]. Magnetic rings are promising material for data storage, logic and sensing devices. These rings have two different magnetic states which are reproducible and fast, these properties make them suitable candidate for magnetic random access memory (MARM). Fabrication and their use for MARM of cobalt nano-rings is demonstrated by Luo [17]. The displays with low energy consumptions could be made by use of carbon nanotubes with high efficiency for field emission displays. Molecular magnets are used as the building blocks for quantum computers which are more powerful than the classical computers [18].

(e) Nanotechnology in Food Industry

There is a great impact of nanotechnology on the protective coatings and suitable packaging in food industry as it helps in increasing the shelf lie of many food products. To reduce the global environmental problem due to packaging polymers the bio degradable polymers are used but due to their low mechanical strength the hybrid of organic-inorganic structures at nano scale could be used [19]. Products like self cleaning glass are available in the market, it uses the solar energy to break down organic dirt on it and then could be easily washed away by water. In textile industry TiO_2 nanoparticles are used to make wool and silk resistant to stains, where TiO_2 nanoparticles has a photocatalytic activity and helps in degradation of organic molecules. TiO_2 nanoparticles are also used in sunscreen lotions to protect skin from UV radiations in the sun light. The ZnO nanoparticles are embedded in medical dressings to slow down the growth of unwanted bacteria and to reduce the chances of contamination.

1.3 Toxicity of Nanomaterials

The nanoparticles or nanomaterials are finding more and more applications in various spheres of our lifestyle. The demand of such materials obviously has led to the

anti microbial agents [25].

A study on the antimicrobial effects of surgical mask coated with a mixture of silver nitrate and titanium dioxide nanoparticles is carried out by Li et al. [26]. The tests were performed on *E. coli* and *S. aureus* and a complete inhibition was observed when the coated mask material treated with microbes was incubated for 48 hrs in favorable growth conditions for bacteria in both cases. The inhibitory effect is proposed due to damage of bacterial enzymes or plasma membrane and the death of bacterial cell takes place due to impaired metabolic pathways and leakage of cytoplasmic contents. The change in morphology of bacterial cells was observed with help of electron microscopy when treated with nanoparticles (Fig. 1.1).

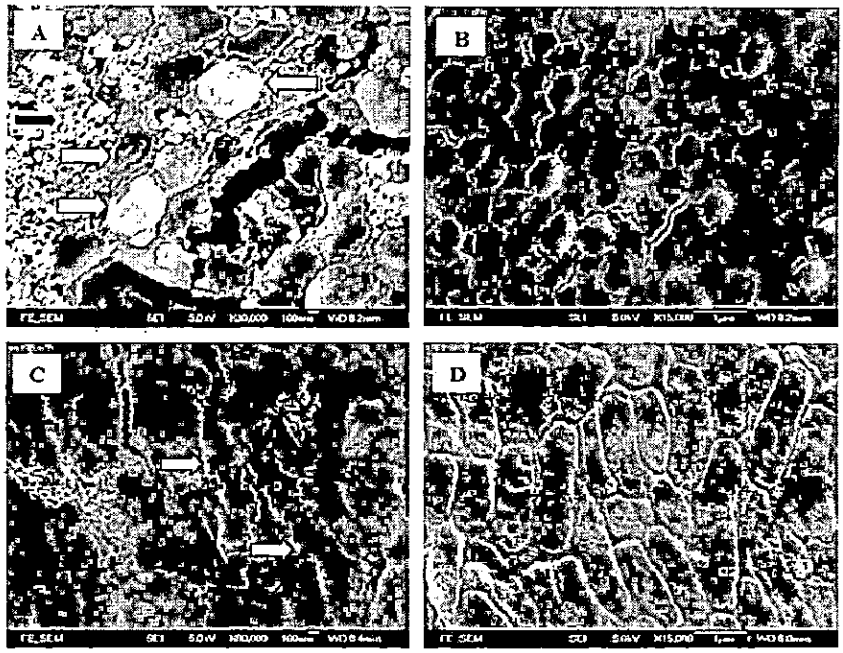
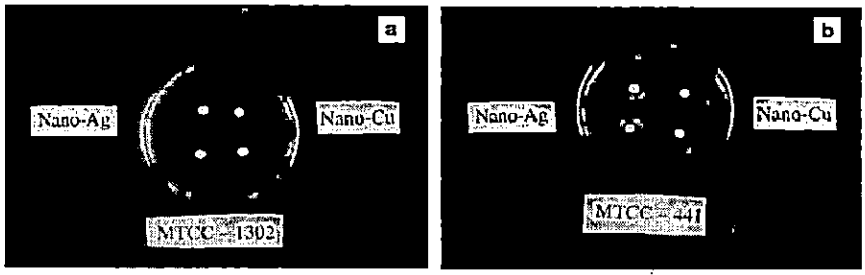


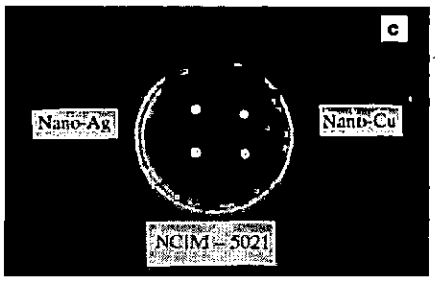
Figure 1.1: Effect of nanoparticles on *S. aureus* and *E. coli*. After treatment with nanoparticles (black arrow), cell surface depressions (white arrows) are seen in *S. aureus* (A). Untreated normal staphylococcal cells are spherical (B). Surface irregularities (white arrows) are seen in *E. coli* treated with nanoparticles (C). Untreated *E. coli* cells have a smooth cell surface (D). Magnification of (A) and (C) is 30 000 X, and that of (B) and (D) is 15 000 [26].

Ruparelia et al. [27] have investigated the antimicrobial activity of nanoparticles of silver and copper against three bacterial strains; *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* . The nanoparticles used in the study were of 3 nm for silver and 9 nm for copper. The bactericidal effect were compared using inhibition zone disk diffusion test and the minimum bactericidal concentration was calculated in nutrient broth cultures. In disk diffusion test the nanopaticles were immobilized on the filter paper disks and then placed in nutrient agar plates along with bacterial cells, the

zone of inhibition around the disk was used for comparison of inhibition effect of nanomaterials.



(a) *E. coli* (MTCC 1302) (b) *B. subtilis* (MTCC 441)



(c) *S. aureus* (NCIM 5021).

Figure 1.2: Representative images of agar plates containing silver and copper nanoparticle impregnated disks [27].

The strain susceptible to nanoparticles exhibit larger diameter of inhibition (Fig. 1.2). The inhibition was also demonstrated by growth curve.

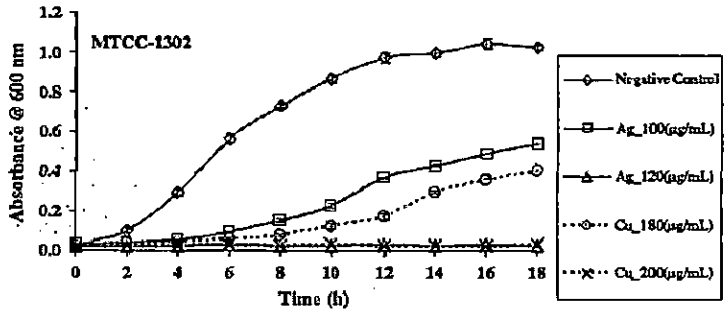


Figure 1.3: Representative batch growth profile of *E. coli* in presence of varying concentration of silver/copper nanoparticles [27].

The decrease in growth is reported with increasing concentration of nanoparticles, and *E. coli* cells are more susceptible to silver nanoparticles. Negative control in the experiment was batch culture without nanomaterials (Fig 1.3).

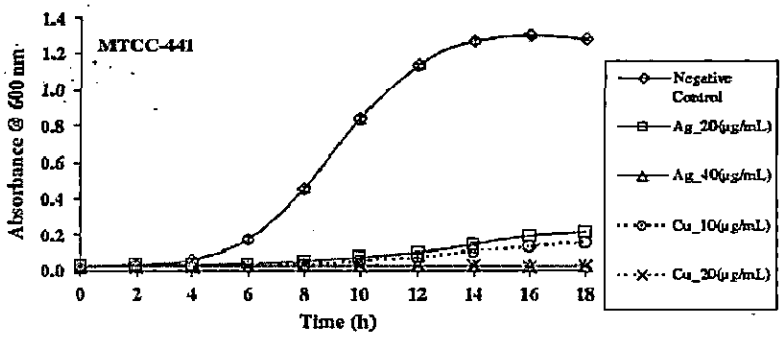


Figure 1.4: Representative batch growth profile of *B. subtilis* in presence of varying concentration of silver/copper nanoparticles [27].

B. subtilis was more susceptible to copper nanoparticles (Fig. 1.4). *S. aureus* is reported to be most resistant to nanoparticles of the three bacteria considered and was more susceptible to silver nanoparticles (Fig 1.5).

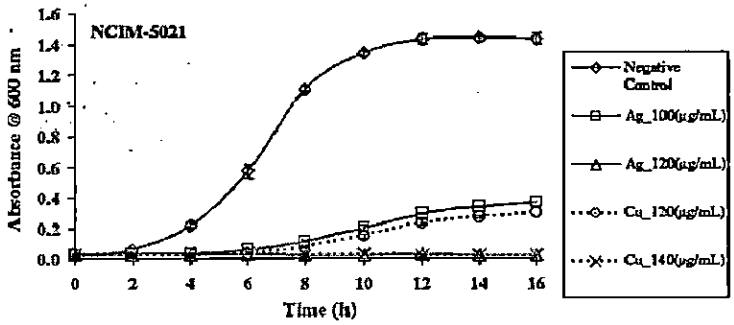


Figure 1.5: Representative batch growth profile of *S. aureus* in presence of varying concentration of silver/copper nanoparticles [27].

Sondhi et al. [28] have shown the antimicrobial effect of silver nanoparticles taking *E. coli* as a model for gram negative bacteria. The scanning electron microscopy was used to study the morphological changes in bacteria on treatment with nanoparticles, the major change in the cell wall was observed as pits in the cell wall of microbes (Fig. 1.6). It is proposed that silver nanoparticles somehow interact with building elements of the outer bacterial membrane, causing structural changes and degradation and a membrane with such a morphology exhibits a significant increase in permeability, resulting in cell death.

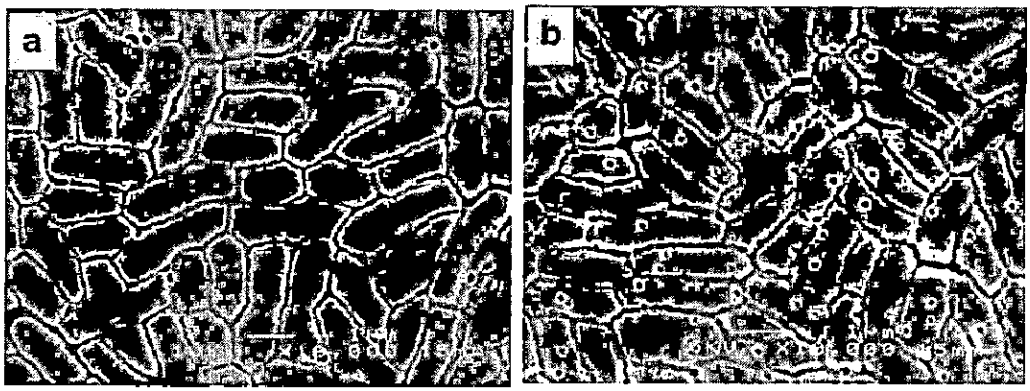


Figure 1.6: Scanning electron micrographs of (a) native *E. coli* cells and (b) cells treated with 50 μg cm^{-3} of silver nanoparticles in liquid LB medium for 4 h [28].

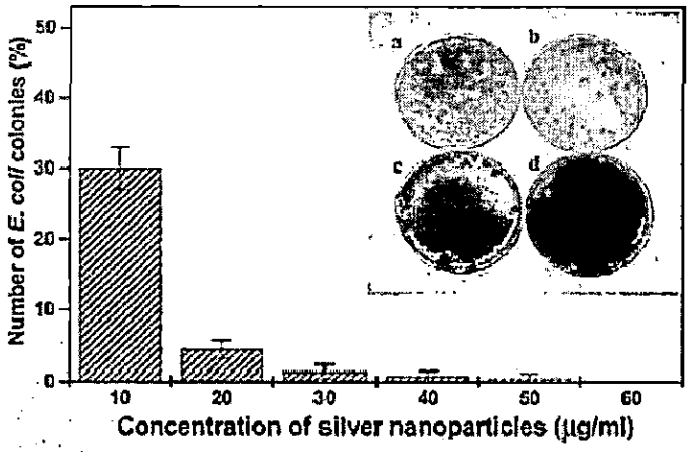


Figure 1.7: Number of *E. coli* colonies as a function of the concentration of silver nanoparticles in LB agar plates expressed as a percentage of the number of colonies grown on silver-free control plates. The photograph inserted in the upper right corner shows LB plates containing different concentrations of silver nanoparticles: (a) 0, (b) 10, (c) 20, and (d) 50 μg cm^{-3} [28].

They studied the colony forming units on Luria-Bertani (LB) agar plates with increasing concentration of silver nanoparticles and found that concentrations above 50 μg cm^{-3} were completely inhibiting the growth of cells in the LB agar medium (Fig. 1.7). However the complete inhibition of bacteria also depends on the initial cell number, with large number of initial cells a greater quantity of nanomaterial would be required. But very high number of bacterial cells is rarely found in real life systems so these nanoparticles have good biocidal effect and effectiveness in reducing cell growth for practical applications.

From the transmission electron microscopy it is showed that the nanoparticles were accumulated in the membrane and some were able to penetrate it (Fig 1.8).

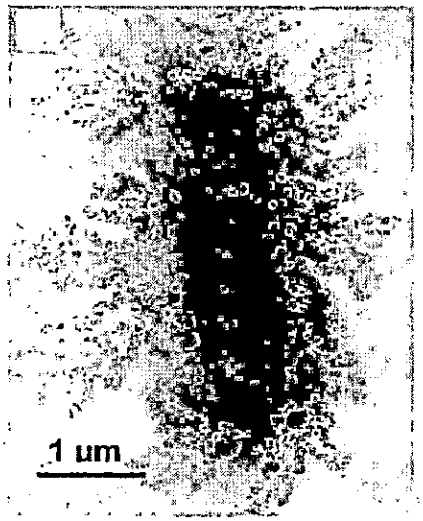


Figure 1.8: Transmission electron micrograph of *E. coli* cell treated with silver nanoparticles [28]

Huang et al. [29] investigated the bactericidal activity of nano-MgO and compared it with TiO_2, a common photoactive bactericidal material. Nano-MgO exhibits high activity against bacteria, spores due to formation of superoxide anion on its surface. Bactericidal efficacy of MgO of about 8 nm was found to be 99.9% against *S. aureus* a gram positive spore forming bacteria which can cause diseases like pneumonia, meningitis, minor skin infections and 91.6% for *B. subtilis* which is a gram positive microbe and is known to cause food poisoning in some cases. Comparison of activity of MgO and TiO_2 revealed that TiO_2 nanoparticles require light radiation for activation whereas MgO nanoparticles do not require any light activation, also the activity of MgO nanoparticles was more (94.5%) in case of *B. subtilis* as compared to TiO_2 nanoparticles (68.0%).

Kim et al. [30] have shown the toxicity of silver nanoparticles towards yeast, *E. coli* and *S. aureus* and they have proposed the possible mode of bacterial toxicity of nanoparticles as the free radical generation at the nanoparticle surface and then the disruption of biological membrane due to uncontrolled free radical generation.

Deposition of antimicrobial silver nanoparticles on nylon and silk fibres is demonstrated by Dubas et al. [31], where the deposition is carried out with a layer by layer method in which the nylon and silk fibers are dipped in dilute solutions of poly(diallydimethylammonium chloride) (PDAMAC) and silver nanoparticles coated with poly(methacrylic acid). The coated fibers were tested for their antimicrobial activity by interacting them with (S. aureus), upto 80% inhibition was observed in case

of silk fibers as silk is a natural protein based fiber, which enhanced the adhesion of PDADMAC on its surface where as in case of nylon fibers the antimicrobial activity was found to be upto 50%.

The toxicity of ZnO nanoparticles is reported by Reddy et al. [32], where they have showed that the ZnO particles of size $\approx$ 13 nm completely inhibit the growth of *E. coli* and *S. aureus* at concentrations above 3.4 mM and 1 mM respectively. It is demonstrated that the colony forming units were reduced to zero after 12 hrs incubation of *E. coli* with ZnO nanoparticles and same for *S. aureus* (Fig. 1.9).

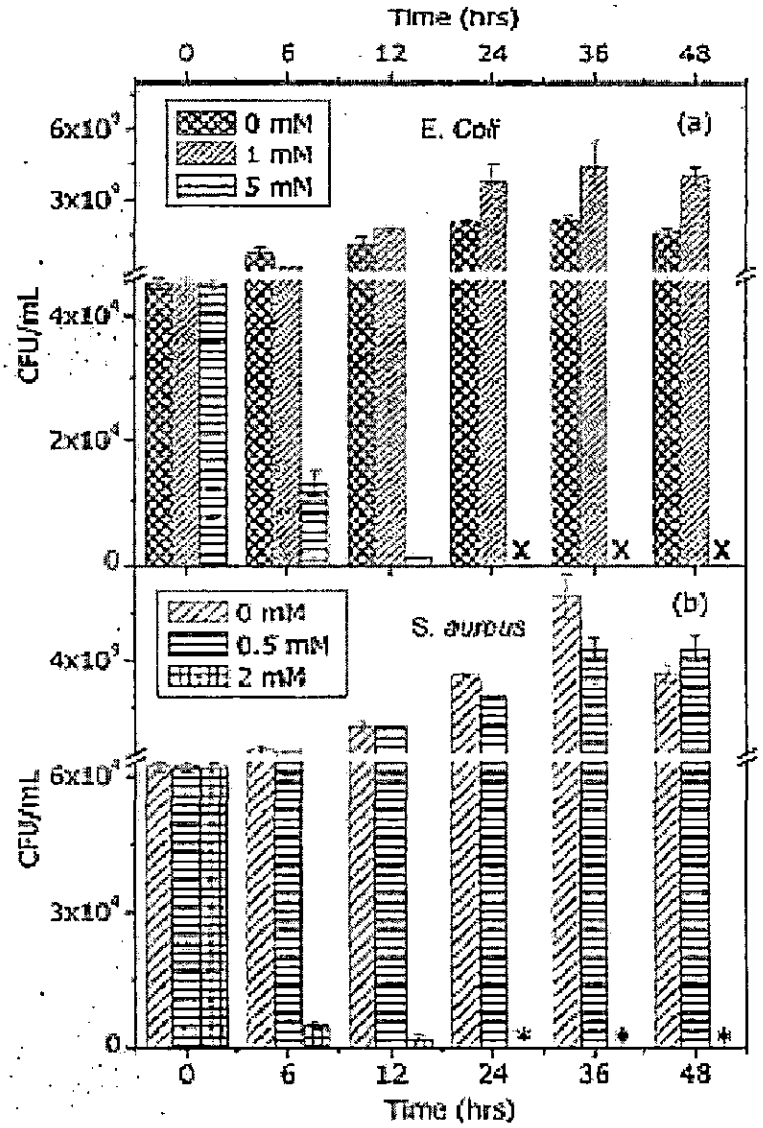


Figure 1.9: Effect of ZnO NP exposure time on the viability and growth of *E. coli* and *S. aureus* [32].

Brayner et al. [33] have reported on the toxicological impact on *E. coli* in ultra fine ZnO nanoparticles colloidal medium where they have shown the complete inhibition

of growth at concentration greater than 2.6 mM ZnO. It is also reported that at concentrations of 1.5 mM and 1 mM the growth was not inhibited and that may be because bacteria can metabolize Zn^{+2} as oligoelement. Damage of the cell wall and internalization of ZnO nanoparticles is showed with help of TEM.

The water suspensions of TiO_2, SiO_2 and ZnO nanoparticles are tested for their eco-toxicity against *E. coli* and *B. subtilis* by Adams et al. [34] and the trend of increasing toxicity is reported as SiO_2 to TiO_2 to ZnO, and *B. subtlis* being the most susceptible to nanoparticles. Effect of light on the antimicrobial activity is studied and is observed that there is increase in the activity for TiO_2, but not for SiO_2 and ZnO. It is also proposed that by photocatalytic activity the reactive oxygen species are formed leading to toxicity.

Ghule et al. [35] have reported the toxicity of ZnO nanoparticles coated on paper on illumination with 534 nm, 1000 lux light i.e. household fluorescent tube. The mode of action of ZnO nanoparticles is reported to be due to formation of H_2O_2, and it being a powerful oxidizing agent acts as an antibacterial agent.

It has been seen that the nanomaterials of different chemical forms do not exhibit the same toxicity effect. Looking at the diverse applications of ZnO nanoparticles, it is necessary to study the effect of these nanoparticles on cellular system. In this regard, *E.coli* has been chosen as the cellular system in the present study, as it is a well characterized model system.

Chapter 2

Materials and Methods

2.1 Synthesis of Zinc Oxide (ZnO) Nanoparticles

Zinc oxide (ZnO) nanoparticles were formed following tow different methods, one was solution free method method and other was based on dispersion method.

2.1.1 Chemical Synthesis of Zinc Oxide (ZnO) Nanoparticles

The ZnO nanoparticles were synthesized by the procedure given by Shen et al. [36], which is a solution free mechanochemical method. It is a two step synthesis procedure, where in the first step zinc oxalate dihydrate ($ZnC_2O_4.2H_2O$) is formed by grinding a powder mixture of 0.1 mol zinc acetate ($Zn(CH_3COO)_2$) (Sisco Research Lab. Ltd.)and 0.12 mol oxalic acid dihydrate ($H_2C_2O_4.2H_2O$) (Merk) in mortar-pestle. Zinc oxalate was thermally decomposed to zinc oxide at 450°C for 30 min.

2.1.2 Chemical Synthesis of Capped Zinc Oxide (ZnO) Nanoparticles

Capped ZnO nanoparticles were prepared by procedure given by Ashtaputre et al. [37]. Poly ethylene glycol (PEG) was used as a coating agent instead of thioglycerol. The chemical synthesis of ZnO nanoparticles was carried out in alcoholic media using methanol. The alcoholic media was used because the growth of oxide particles in this media is known to be slow and controllable [38].

A 20 ml of 0.1 M zinc chloride ($ZnCl_2$) (Rankem), 100 ml of 0.1 M sodium hydroxide (NaOH) (Merk) and 0.01 M PEG solutions were prepared in methanol. A 0.5 ml of PEG solution was added to NaOH solution on continuous stirring, the resulting solution was stirred for one hour at room temperature. To the above solution $ZnCl_2$

solution was added after mixing of NaOH and PEG. The solution was stirred for few a hours until the solution turned milky white. Precipitation of particles formed was done by adding 10 ml of acetone to the solution and then allowed to settle down. The precipitate was washed with methanol and methanol was allowed to evaporate at room temperature to obtain powder of ZnO nanoparticles.

The nanoparticles thus formed were characterized by X-ray diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Energy Dispersive Analysis of X-Rays (EDAX) was employed to check the presence of impurities.

2.2 Characterization

The various characterization methods were used for characterizing nanomaterials, UV-Visible Spectroscopy was used to determine the blue shift in the spectrum of nanomaterial compared to bulk material and it was also used to make the growth curve of *E. coli.* X-ray Diffraction provided the data for the reflecting planes in the nanoparticles formed and was used to identify the product on comparison with standard. Scanning Electron Microscopy (SEM) was employed to study the surface morphology of nanoparticles formed. Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) were used to study the cell morphology and the nanoparticles.

2.2.1 UV-Visible Spectroscopy

SHIMADZU 1601 PC, a UV-Visible Spectrophotometer was used to record the optical density of the cells at 600 nm.

UV-Visible Spectroscopy deals with the study of electronic transitions between orbitals or bands of atoms, ions or molecules in gaseous, liquid or solid state. Band gap of all semiconductor materials increases with decrease in size. This phenomena takes place in the domain of extremely small sizes and hence forms the basis for characterization of nanomaterials using this technique. The band gap increase is observed in UV- Visible spectra as the absorption edge shifts systematically to lower wavelengths [39].

For optical density of bacterial cells, a monochromatic light is used and absorption takes place according to Beer's law:

$$Absorbance = log(I_o/I_t) = \epsilon cl$$

where, I_o is the intensity of the incident light, I_t is the intensity of the transmitted light, ϵ is the molar absorption coefficient, c concentration of the absorbing species, l is the thickness or length of the light pass through the absorbing medium.

Sample Preparation and Experimental Conditions

For nanomaterial, sample is suspended in alcoholic media used for their washing and sonicated for 20 min. And the spectrum is obtained against the blank containing media for suspension and the coating agent (in case of coated nanomaterials) at room temperature.

Optical density of bacterial cells is observed using the fresh brothmedia + bacterial cells at fixed time intervals against the blank containing fresh autoclaved media only at room temperature.

2.2.2 X-ray diffraction (XRD)

X-ray diffraction powder method was used for the determination of crystallinity, crystal structures of nanoparticles on *AXS D8 Advance Bruker Instrument.*

A monochromatic X-rays of fluctuating electric field displaces the electrons of an atom upon striking and results into vibrations of the electrons of the same frequency as that of the X-rays. The electrons of an atom, therefore, absorb and re-emit X-rays, and in accordance the atoms are said to scatter X-rays.

A lattice array of atoms can be regarded as an infinite stack of parallel, equally spaced planes. In crystallography, a lattice plane of a given Bravis lattice is a plane whose interactions with the lattice points is periodic. All lattice planes can be described by a set of integer Miller indices {h k l}.

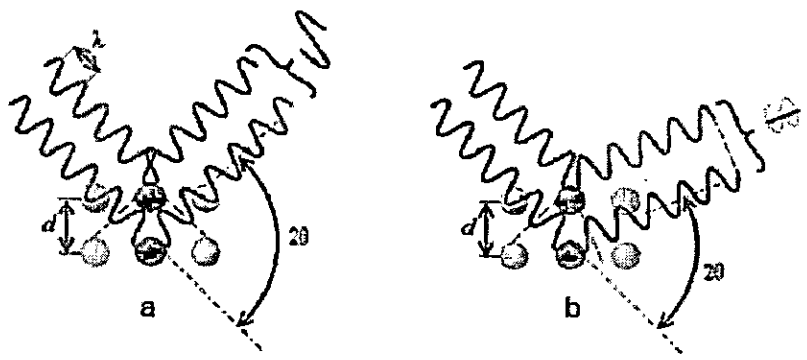


Figure 2.1: According to the 2θ deviation, the phase shift causes constructive (a) or destructive (b) interferences.

The condition for scattering in phase (Fig. 2.1) by stack of planes is known as

Bragg's law, which is represented as:

$$\lambda = 2d_{hkl}sin\theta_{hkl}$$

where d is the spacing between atomic planes in the crystalline phase and λ is the X-ray wavelength.

The locations of the reflection of a crystal depends on the shape and type of its unit cell; the relative intensities of these reflections depend on the arrangement of atoms with in the cell.

The intensity of the diffracted X-rays is measured as a function of the diffraction angle 2θ and the specimens orientation. The diffraction pattern obtained is used to identify the material, its crystallinity and to measure its structural properties. X-ray diffraction technique is nondestructive and does not require elaborate sample preparation but its sensitivity is directly proportional to the molecular weight of the sample as the intensity of the diffracted X-rays decrease with decrease in molecular weight [3].

X-ray diffraction broadening analysis has been widely used to determine the crystal size of nanomaterials. The average size D can be estimated from the peak width using Debey-Scherrer's formula:

$$D = 0.89\lambda/(\beta cos\theta_\beta)$$

where λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of a diffraction peak, θ_β is the diffraction angle.

Experimental Conditions

The experimental conditions employed to obtain the diffraction pattern of nanomaterials were:

- Copper Target, Wavelength $\lambda = 1.54$ Å
- Goniometer Speed =1°/min
- Range = 5° to 70°
- Temperature = 25°C

2.2.3 Scanning Electron Microscopy (SEM) and Energy Dispersive Analysis of X-Rays (EDAX)

Quanta 200 FEG (IIC, IITR Roorkee), a 20 KeV Scanning Electron Microscope with EDAX was used to study the surface characteristics and to confirm the material constituents from X-ray spectrum and also to check the presence of impurities in the nanomaterials synthesized and commercially procured.

Scanning electron microscopy is one of the most widely used techniques used in characterization of nanomaterials. SEM approaches the resolution of few nanometers with magnification variable over a range of 10 to 300,000X. In SEM the source of electrons is focused into a sharp beam of spot size ~5 nm and having energy ranging from few hundred eV to 50KeV, and in this case it was 20KeV. As the electron beam strikes the sample surface a number of physical processes, out of which one process leads to emission of electrons from the sample. However there are two types of electron emission, namely secondary electrons and backscatter electrons. The images are produced by collecting the emitted electrons on a cathode ray tube (CRT). Another outcome of electron beam - sample interaction is the emission of X-rays of characteristic energies.

A field emission gun is used as electron source in FESEM, an important feature of it is that the emitted electrons have very well defined energies. Where as the electrons from a thermionic source inevitably have a energy spread of 1-2 eV, the electrons from a cold field emission gun have a much smaller energy spread, usually less than 0.5 eV. Thus for both analytical and high resolution electron microscopy, field emission sources are important because they provide a high brightness beam and a clean monochromatic supply of electrons.

When a high energy primary electron interacts with an atom it undergoes either inelastic scattering with atomic electrons or elastic scattering with atomic nucleus. In an inelastic collision, the primary electron transfers part of its energy to the other electron, if the transfer is large enough the other electron will emit from the sample. If the emitted electron has energy less than 50 eV it is referred to as secondary electron. Electrons emitted due to elastic scattering are backscattered electrons, and essentially possess the same energy as primary electrons. The third possibility is that the primary electron ejects a core electron as a result the excited atom will decay to its ground state by emitting either a characteristic X-ray photon or Auger electron (Fig. 2.2), both of which have been use for chemical characterization [3].

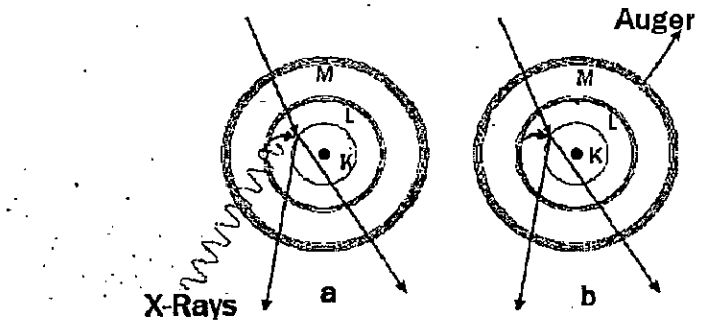


Figure 2.2: Two ways in which inner shell excited atom can relax. In both cases a K-shell electron has been knocked out - in (a) a characteristic (K_α) X-ray is emitted while in (b) an Auger electron (KLM) is ejected.

Energy dispersive Analysis of X-Rays is an analytical technique which uses the above mentioned X-ray photons emitted by the element. The X-ray energy emitted by an element is characteristic to the element which is due to the fundamental principle that an element has a unique atomic structure and energy levels. This helps in the elemental analysis of sample by drawing both qualitative information (X-ray energy) as well as quantitative information (intensity of X-ray peak).

Sample Preparation

Powdered samples were mounted on a mounter using a carbon tape, and the surface of sample was coated with gold using *BALTEC SCD 500, Sputter Coater* for making the sample conducting. Samples were then analyzed using *Quanta 200 FEG.*

2.2.4 Transmission Electron Microscopy (TEM)

TECNAI 2G S TWIN (IIC, IITR Roorkee), a 200 KeV Transmission Electron Microscope was used to study the morphology of nanomaterials and to study the interaction of nanomaterials with bacterial cells. In TEM, electrons are accelerated to 200KeV and projected on the sample by means of a condenser lens system. It has a magnification range of 50 to 10^6, and has the ability to provide both image and diffraction information from a single sample.

In Transmission Electron Microscope the source of the electrons is a field emission gun which can generate very fine electron beams, of the order of few nanometers at the specimen. Below the electron gun is condenser system which demagnifies the beam emitted by the gun and controls its diameter as it hits the specimen and hence the area of specimen exposure and intensity of illumination can be controlled. After condenser system is the specimen chamber, it is one of the crucial parts of the

microscope as the specimen must be held precisely in the correct position inside the objective lens. The placing of the specimen in correct position is achieved by using a side entry specimen rod, which holds a 3 mm diameter specimen between the pole pieces of the objective lens. The objective lens produces first intermediate image and diffraction pattern, one of which is enlarged by the subsequent projector lenses and displayed on the viewing fluorescent screen. Selected area diffraction is achieved by inserting an aperture in the first image produced by the objective lens [40].

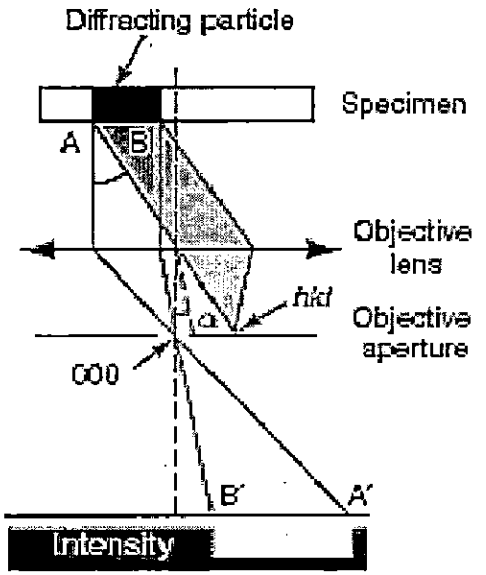


Figure 2.3: The image formation in bright field TEM.

The images were taken with *bright field* imaging technique (Fig. 2.4). The contrast in bright field image is obtained because in the regions of the specimen which are thicker, or of high density will scatter more strongly and will appear dark in the image.

The strongly scattered electrons are unable to cross the objective aperture and hence lesser number of electrons reach to detector leading to formation of an image.

As the electrons penetrate the sample they undergo either elastic scattering involving no energy loss, giving rise to diffraction patterns or inelastic interactions between primary electrons and sample electrons at heterogeneities such as dislocations, grain boundaries, defects intensity variations, etc., cause spatial variation in the intensity of the transmitted electrons. Images are formed because different atoms absorb and interact with electrons to different extent [3].

Sample Preparation

A few drops of liquid sample were put a carbon coated copper grid and was allowed to dry at room temperature. The samples were then analyzed by TEM.

2.2.5 Atomic Force Microscopy (AFM)

NTEGRA TS-150 (IIC, IITR Roorkee), was used to study the morphology of the bacterial cells and nanomaterials.

AFM is a highly sensitive surface imaging technique that provides real-space images and allows spatially localized measurement of structural properties. AFM is capable of imaging the surface of all kinds of solids and with various modifications of tips and operating conditions, AFM can be used to measure local chemical and physical properties of the sample.

In the present, study the AFM measurements were carried out by non contact mode. Here the AFM tip does not touch the sample surface, instead the tip is influenced by the long range van der Waals forces and the movement of tip is optically sensed by the laser. The advantage of this mode is that even the softest sample is not destroyed during measurement and is ideal for measuring biological specimens.

Sample Preparation

For the study the bacterial cell were adhered to the glass slide using two different agents; Chitosan and Polyethylene glycol. Bacterial cells were adhered on coated glass slide and allowed to dry at room temperature. Chitosan was found to be a better adhering agent, whereas in polyethylene glycol the cells were found standing in the 3-D images.

2.3 Cell Culture

Bacterial cells were cultured and maintained in nutrient broth. Culturing was done in nutrient broth with and without nanomaterials and log phase of growth curve was observed to check the toxicity of nanomaterials. The bacteria employed to do this study was *Escherichia coli (E.coli)* and nanomaterial was ZnO with and without capping agent.

Growth of a bacterial population increase exponentially by geometric progression : 2^o, 2^1, 2^2, 2^3. When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time in a batch culture,

plotting the yield a typical growth curve. Four characteristic phases of growth cycle are recognized:

1. **Lag Phase** - Immediately after inoculation of cells into the fresh medium, the population remains temporarily unchanged. In this phase the grow in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in cell metabolic activity.

2. **Exponential (log) Phase** - In this phase the cells divide regularly by binary fission and are growing by geometric progression. The rate of exponential growth of a bacterial culture is expressed as *generation time*, also the doubling time of the bacterial population. Generation time is defined as time per generation.

3. **Stationary Phase** - In this phase the growth rate becomes constant (in a batch culture) as the population growth is limited many factors as exhaustion of available nutrients, accumulation of inhibitory metabolites or end products, etc.

4. **Death Phase** - If incubation continues after the population reaches stationary phase. a death phase follows, in which the viable cell population declines.

2.3.1 Preparation of Media

Liquid media (nutrient broth) was prepared by dissolving 1.3 gm of nutrient broth (Hi-Media) in 100 ml of distilled water in a 500 ml of conical flask. A cellulose plug was used as a stopper to flask, and mouth was covered with aluminium foil to avoid any entrance of condensed water during autoclaving. Flasks were autoclaved at a pressure of 15 lb/in^2, 121 °C for 15 min.

Solid Media was prepared by dissolving 2.8 gm of nutrient agar (Hi-Media) in 100 ml of distilled water in 500 ml of flask, plugged, covered its mouth with aluminium foil and autoclaved at 15 lb/in^2, 121 °C for 15 min. It was allowed to cool to approximately 45 °C and then poured to petri plates in laminar air flow hood, maintaining the sterile conditions [41].

2.3.2 Maintenance of a Pure Culture

The bacterial cells can be preserved on nutrient agar plates at 4 °C for a few weeks. They are revived from plates using liquid media (broth) like nutrient broth, a non-chemically defined media containing hydrolyzed proteins and vitamin extracts.

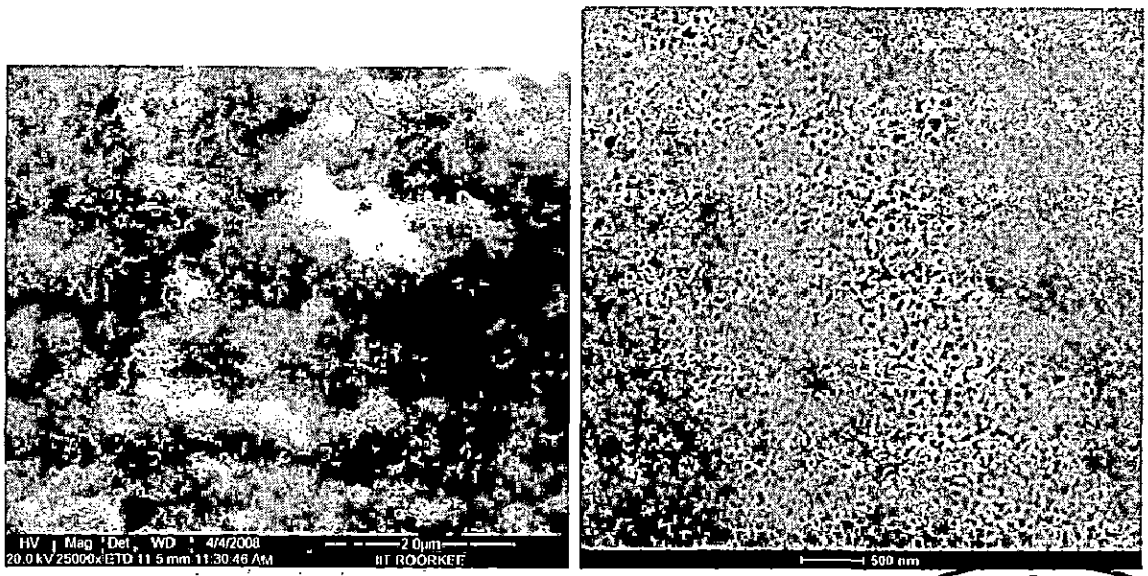
Chapter 3

Results and Discussion

3.1 Characterization of ZnO Nanomaterials

3.1.1 Commercially procured ZnO nanopowder

The SEM and TEM images of standard ZnO nanopowder suggested that the particle size is less than 100 nm, (Fig. 3.1(a), Fig. 3.1(b)). The particles are found to be agglomerated in SEM image. A much improved image of particle sizes could be seen in the TEM image. It was also noted that the particle have more or less similar sizes.



(a) SEM image (b) TEM image

Figure 3.1: Electron Microscopic images of standard ZnO nanopowder.

The purity of the material was examined using EDAX attachment with SEM. The X-ray spectrum obtained from the standard ZnO nanopowder did not show any characteristic X-ray peak corresponding to any other material except zinc, (Fig. 3.2). However it should be borne in mind that the detection limit of EDAX using electron as a probe is poor and it may be considered that if any impurity is present in the standard ZnO, it is below 500 ppm (where 500 ppm is considered as a detection limit).

The powder X-ray diffractogram revealed the planes of ZnO (100, 002, 101, 102, 110, 103, 200, 112, 210) (Fig. 3.3), suggesting the presence of hexagonal wurtzite structure of ZnO [36]. Using Debye Scherrer formula the particle size was found to be about 375 nm.

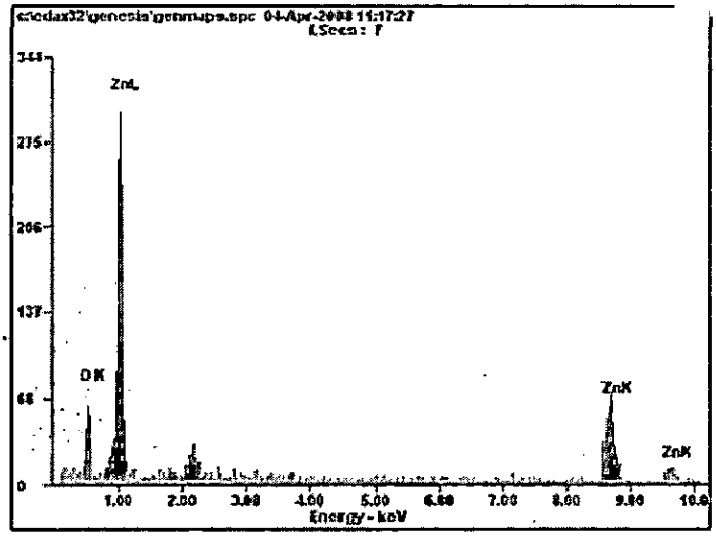


Figure 3.2: X-ray spectrum of Standard ZnO nanopowder taken from FESEM-EDAX.

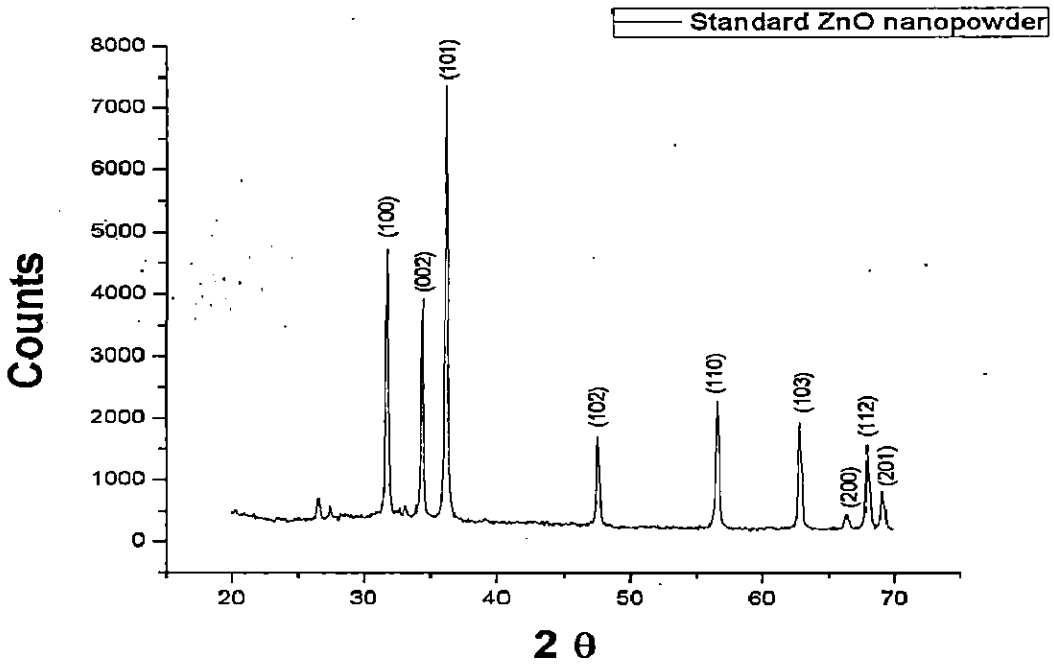


Figure 3.3: X-Ray diffractogram of Standard ZnO nanopowder.

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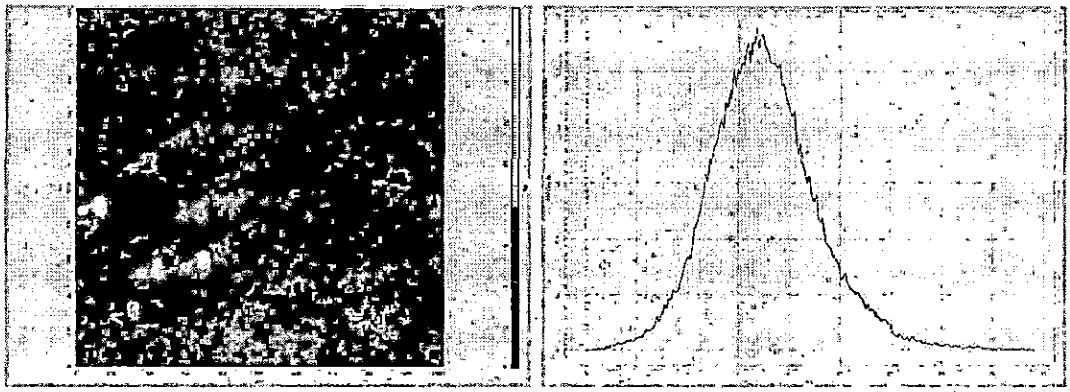
The nanopowder was dispersed in methanol and a drop of it was taken on a clean cover slip for AFM measurement. A particle size distribution was obtained and the average particle size was found to be about 40 nm spatially (Fig. 3.4(a)). However size distribution histogram (Fig. 3.4(b))suggests an average particle size to be less than 10 nm. This data possibly corresponds to the height of nanoparticles (due to vertical shift in the AFM tip during scan) Such observation could not be revealed from TEM measurement, probably particles agglomerated during sample preparation for SEM, EM. It is likely that the AFM results are more appropriate as far as particle size concerned because the probe size is much smaller and therefore sensitive. Though ıe particles tend to agglomerate but AFM probe can distinguish from one another.



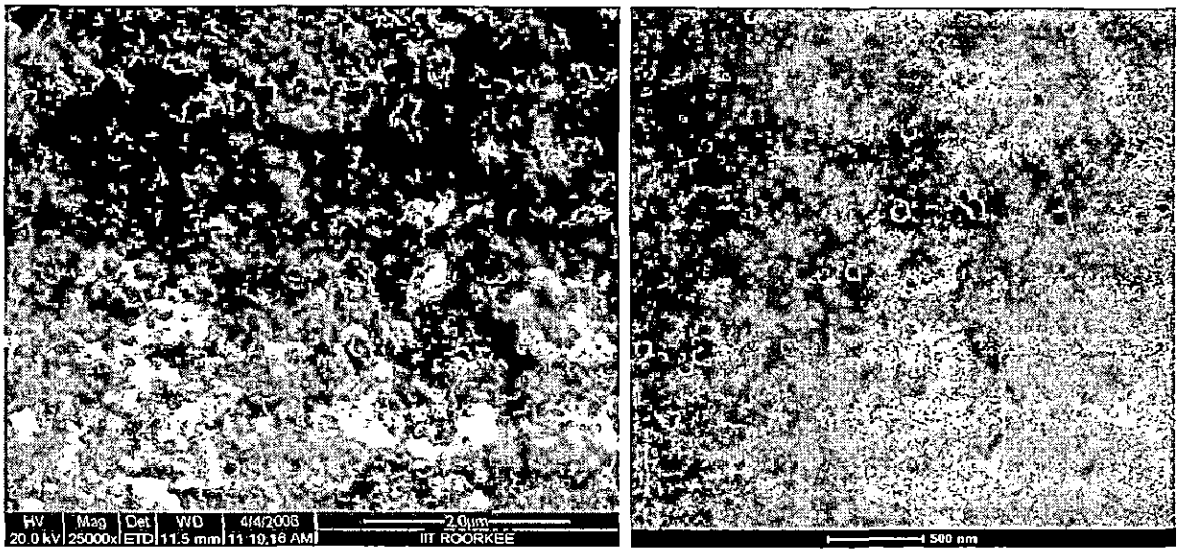
(a) Characteristic 2-D spatial AFM image of ZnO nanopowder

(b) Size distribution histogram

Figure 3.4: AFM (non contact mode) of standard ZnO nanopowder.

3.1.2 ZnO nanomaterial synthesized by solution free method

The SEM and TEM images of ZnO nanomaterial synthesized by solution free method revealed that the particle sizes are less than 100 nm (Fig. 3.5(a), Fig. 3.5(b)), which are of similar dimensions observed for standard ZnO nanopowder. Since the method involved calcination at 450 °C for 30 min for converting zinc oxalate into zinc oxide, the nanomaterial of zinc oxide has attained crystallinity as observed in the XRD spectrum. The temperature and time of calcination is a control for growth of this nanomaterial.



(a) SEM image (b) TEM image

Figure 3.5: Electron Microscopic images of ZnO nanomaterial synthesized by solution free method.

The purity of the material was examined from X-ray spectrum obtained from EDAX attachment with the SEM. The X-ray spectrum displayed only the characteristic X-ray peak corresponding to zinc and oxygen. (Fig. 3.6). A small peak at 2.126 keV that appears in the spectrum corresponds to Au M X-ray line which is due to an ultrathin gold layer deposited on the sample for making the sample conducting.

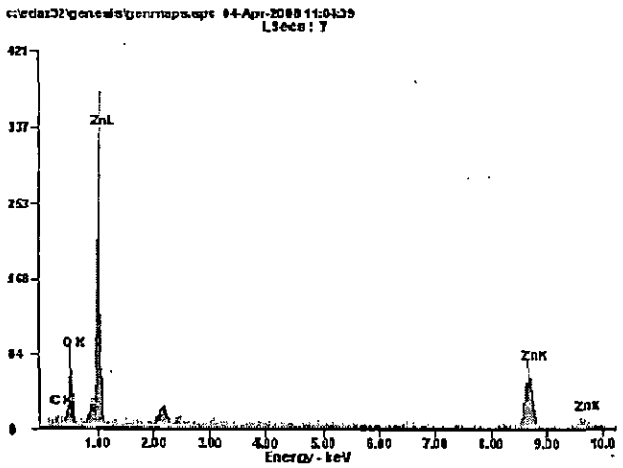


Figure 3.6: X-ray spectrum of ZnO nanomaterial (solution free mechanochemical method) taken from FESEM-EDAX.

The powder X-ray diffractogram revealed al the planes for hexagonal wurtzite structure as seen for the standard. Using Debye Scherrer formula the particle size was found to be about 206 nm.

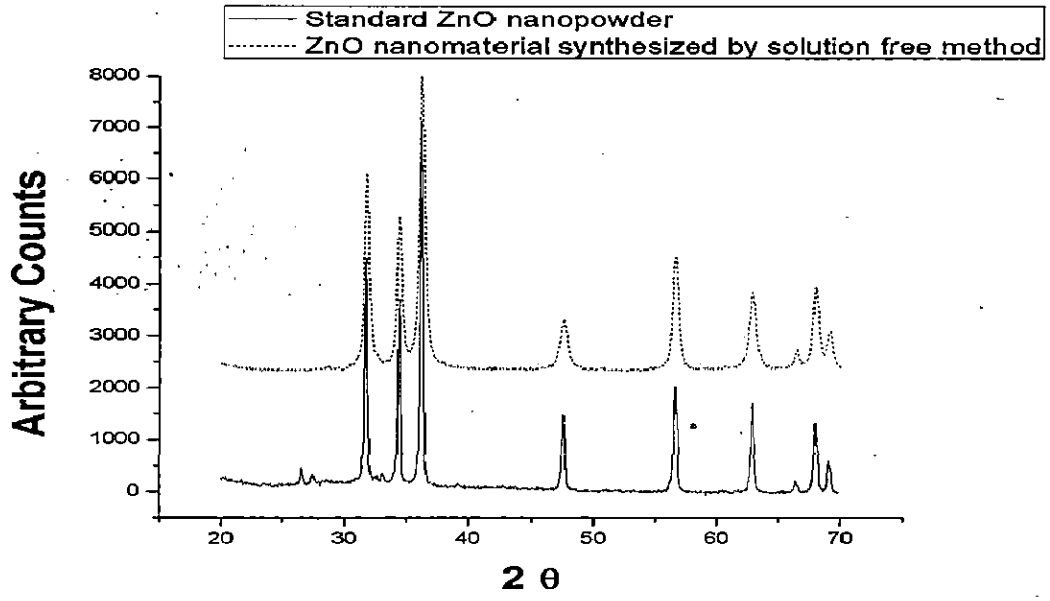
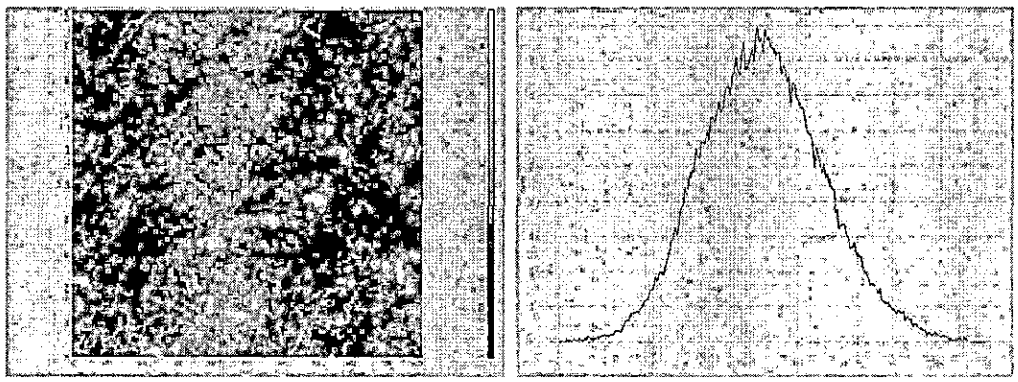


Figure 3.7: X-Ray diffractogram of ZnO nanomaterial synthesized by solution free method compared with ZnO nanopowder.



(a) Characteristic AFM image (b) Size distribution histogram

Figure 3.8: AFM of ZnO nanomaterial synthesized by solution free method.

However the AFM measurements of this nanomaterial were found to be about 30 nm spatially (Fig. 3.8). This observation is similar to the AFM measurement taken for standard ZnO nanopowder.

3.1.3 Synthesized ZnO nanomaterial coated with PEG

The SEM images of coated ZnO nanomaterial suggested that the particle size is less than 100 nm (Fig. 3.9), which is same as observed for the standard ZnO nanopowder. The purpose of coating is to avoid agglomeration and to reduce the toxic effect of ZnO nanomaterial, the toxicity is most likely to be contributed from the surface activity of the nanomaterial. Furthermore, PEG is known to be non-toxic to the cellular system. It is discussed and illustrated in section 3.2.3.

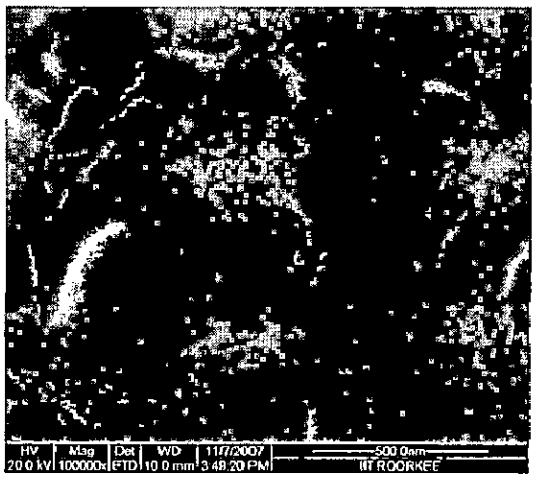


Figure 3.9: Electron Microscopic images of PEG coated ZnO nanomaterial.

The X-ray spectrum obtained in EDAX from the capped ZnO nanomaterial showed characteristic X-ray peak corresponding to zinc, oxygen, carbon (of coating agent, poly ethylene glycol) and chlorine (of starting material zinc chloride) (Fig. 3.10).

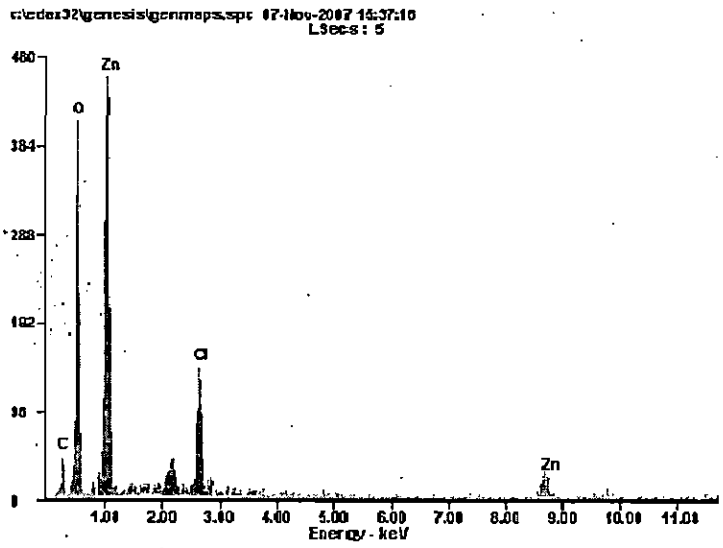


Figure 3.10: X-ray spectrum of PEG coated ZnO nanomaterial taken from FESEM-EDAX.

The X-ray and XRD spectrum suggested that the pure ZnO might not have crystallized in the PEG medium, the presence of chlorine and no trace of sodium in X-ray spectrum indicates there could be some untreated zinc chloride in the system along with ZnO nanomaterial.

Ashtaputre et. al. [37] used the above discussed method for ZnO synthesis in thioglycerol medium and they have reported the presence of the reflection planes associated ZnO material. However in this study, the thioglycerol was substituted with PEG as latter is also a good choice for biological system and it is a better biodegradable material. The X-Ray diffraction pattern of PEG coated ZnO nanomaterial revealed a number of different planes which do not correspond to standard ZnO nanopowder, is probably due to the fact that there could be presence of other phases or substoichoimetric phases of ZnO. Moreover the average particle size of this PEG coated ZnO nanomaterial was calculated to be about 271 nm, using Debye Scherrer formula.

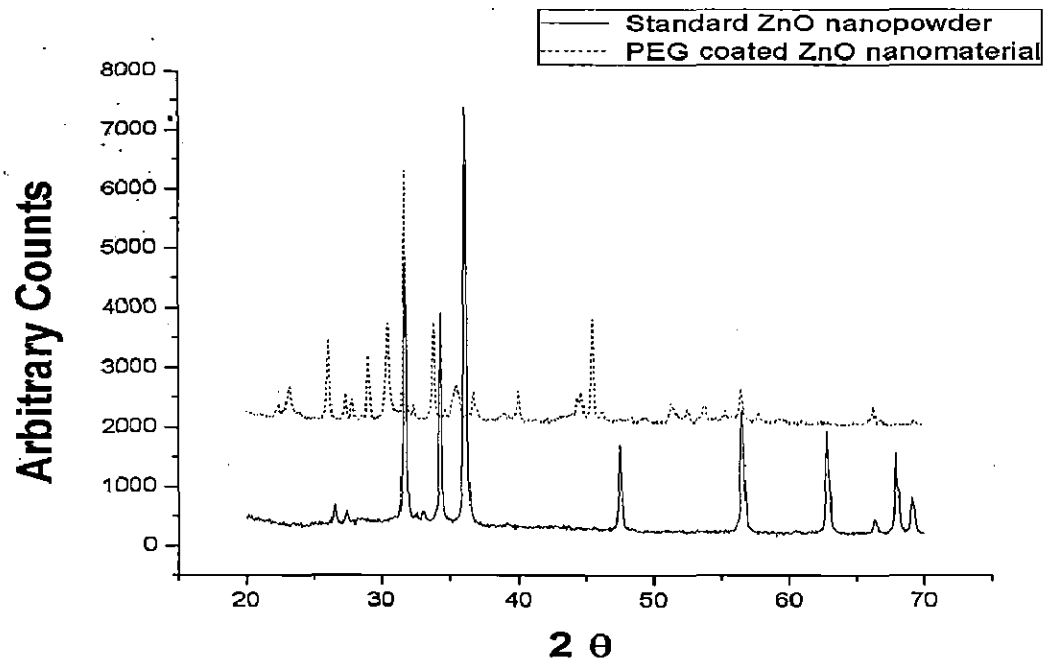
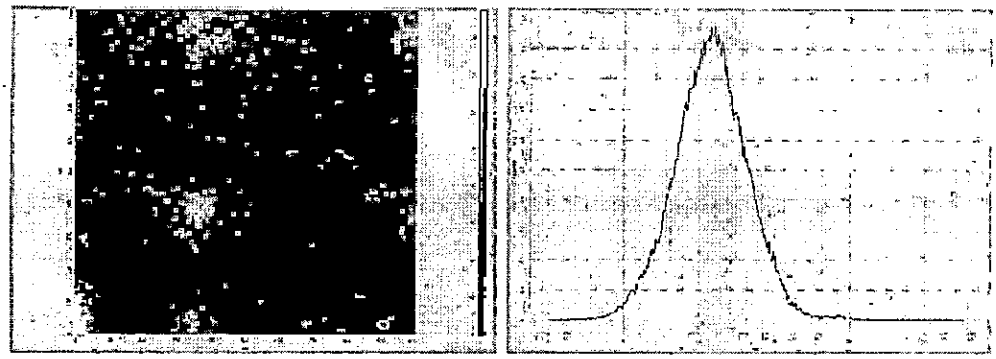


Figure 3.11: X-Ray diffractogram of PEG coated ZnO nanomaterial compared with ZnO nanopowder.

The AFM measurements of PEG coated ZnO nanomaterial reveal two types of morphology. The one corresponding to brighter area are most likely to be the inorganic material, i.e. ZnO nanomaterial. These are found to be surrounded by less brighter region which indicates softer material, and in this situation it is due to PEG



(a) AFM image (b) Size distribution histogram

Figure 3.12: AFM of PEG coated ZnO nanomaterial.

coating. So it may be concluded that the synthesized ZnO nanomaterial is coated with PEG, which is referred to as a composite nanomaterial of ZnO. A careful investigation of the AFM image of this system reveals that the size of composite system is about 100 nm spatially (Fig 3.12).

The formation of ZnO nanomaterial was evident from the UV-Visible spectrum which showed the blue shift of the excitonic peak with reference to the peak of the bulk particles at 375-400 nm (Fig. 3.13). The observed peak was at around 330 nm, confirming the formation of nanomaterial [38].

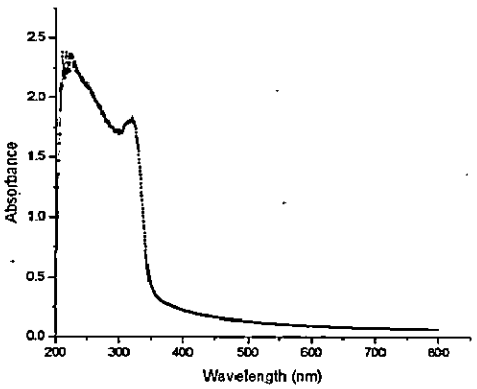


Figure 3.13: UV-Visible spectrum of PEG coated ZnO nanopmaterial.

3.2 Nanomaterial Interaction with *E.coli*

3.2.1 Interaction of standard ZnO nanopowder with *E.coli*

Untreated growth curve (shown in figure 3.14) is considered as a standard growth of *E.coli* in the chosen media. The growth rate of the bacteria treated with ZnO nanopowder was found to decrease with increasing concentration of nanopowder (Fig. 3.14).

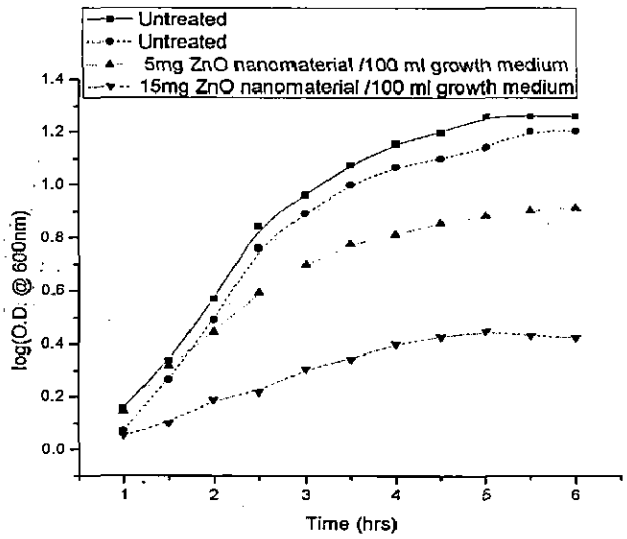


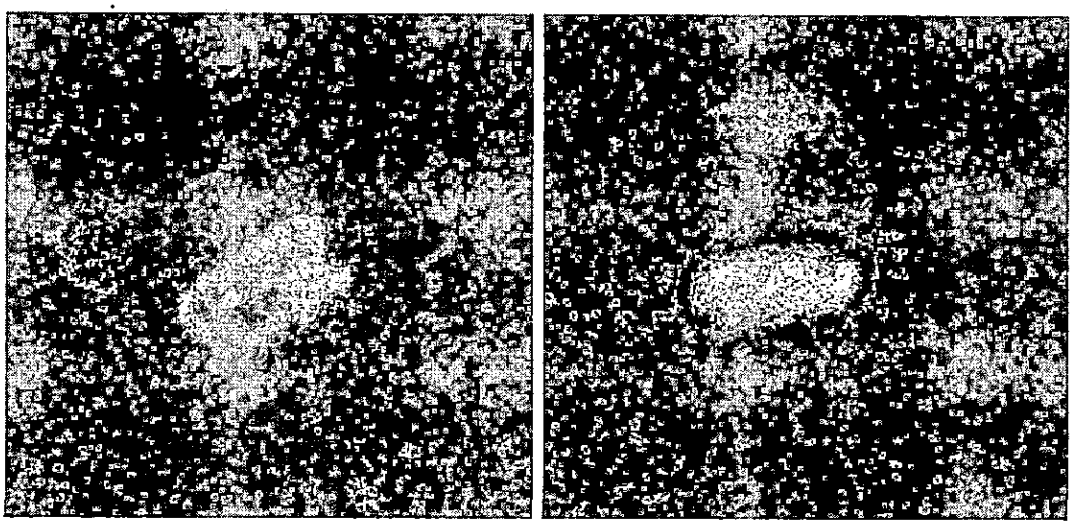
Figure 3.14: Growth curve of *E.coli*.

When *E.coli* was treated with 15 mg of nanopowder per 100 ml of nutrient media, the growth of the bacteria was remarkably low as compared to the untreated one [27]. While the one treated with 5 mg of nanopowder per 100 ml of nutrient broth though showed certain order of growth inhibition (35-40% reduced growth taking stationary phase into consideration), but the growth of bacteria was four fold higher than one treated with 15 mg of nanopowder per 100 ml of nutrient broth.

The exponential growth of bacteria with 5 mg of nanopowder per 100 ml of nutrient broth was found to be simillar to that of untreated in the initial part of the growth curve. As the concentration of nanopowder was increased, a lesser number of bacteria survived and therefore the inhibition of growth was profound.

It is a point of interest to understand how *E.coli* interacts in presence of nanopowder of ZnO, to cause inhibition in their growth. Transmission Electron Microscopy was used to study the morphology of these cells and it was found that the cells were engulfed with nanopowder of ZnO (Fig. 3.15(a)) and thereby cuts off the cells from

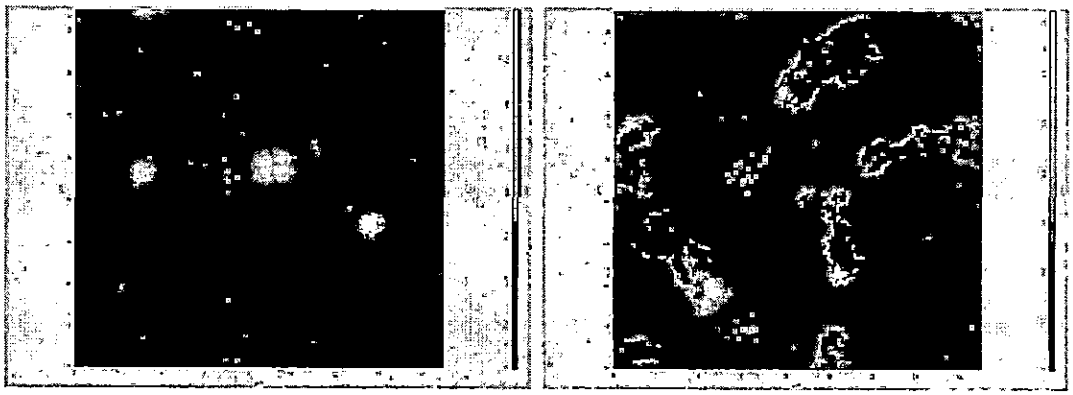
the external media from which it derives its nutrients for its growth [28]. Furthermore the *E.coli* cells tend to deform when more amount of nanopowder get attached to the cell wall (Fig.. 3.15(b)).



(a) *E.coli* surrounded by nanomaterial (b) Image with membrane deformation of *E.coli*

Figure 3.15: TEM images of *E. coli* with nanopowder.

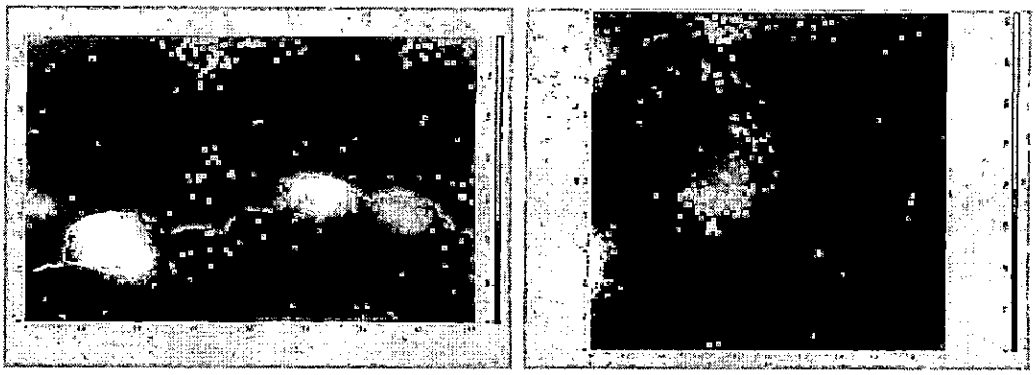
In the presence of nanopowder a fewer number *E.coli* cells and also single cell were observed (Fig. 3.16a) against higher number of *E.coli* cells for untreated ones (Fig. 3.16b).



(a) (b)

Figure 3.16: AFM images of *E.coli* with (a) and without (b) ZnO nanopowder.

Furthermore the AFM measurement of a single cell which was cultured in presence of nanopowder showed change in surface morphology as against the untreated ones (Fig. 3.17). The change in the morphology is due to the adherence of the nanomaterials on the cell which complements the TEM measurement (Fig 3.15)



(a) Image of *E.coli* without nanomaterial

(b) Image of a single *E. coli* cell treated with ZnO nanopowder showing deposition of these nanomaterials on cell membrane

Figure 3.17: Higher resolution AFM images of *E.coli* treated without (a)and with (b) nanopowder.

3.2.2 Interaction of ZnO nanomaterial (synthesized by solution free method) with *E.coli*

A similar trend of decrease in growth rate and finally lesser number of cells as in previous section was observed with synthesized nanomaterials (Fig. 3.18).

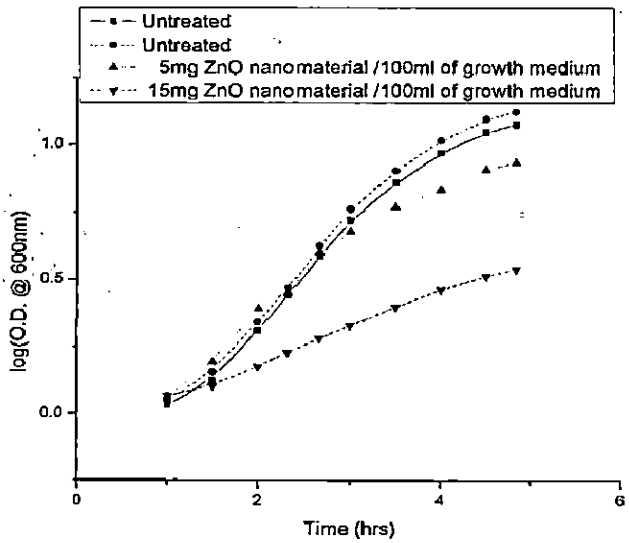
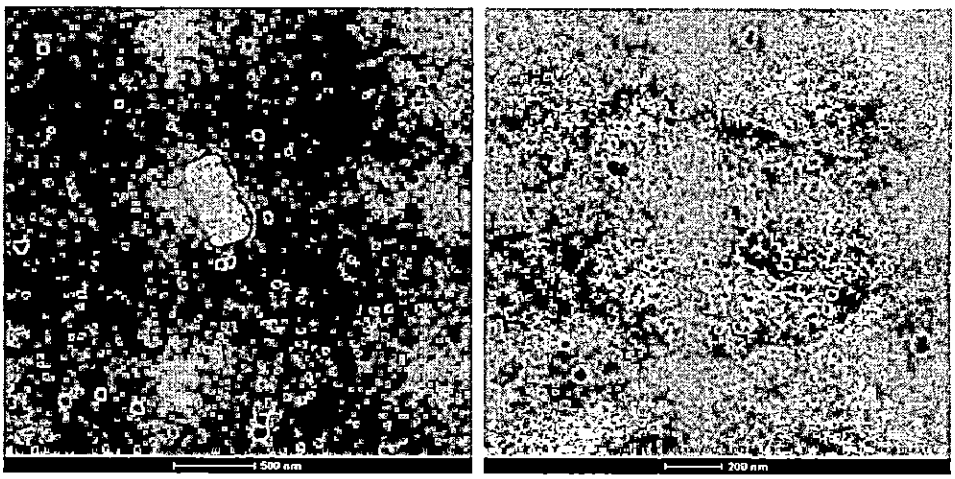


Figure 3.18: Growth curve of *E.coli*.

When *E.coli* was treated with 15 mg of nanomaterial per 100 ml of nutrient media, the growth of the bacteria was 50-60% low as compared to the untreated one. While the one treated with 5 mg of nanomaterial per 100 ml of nutrient broth showed certain order of growth inhibition, 15-20% reduced growth taking stationary phase

into consideration. The exponential growth of bacteria with 5 mg of nanomaterial per 100 ml of nutrient broth was found to be similar to that of untreated in most part of the growth curve. As the concentration of nanomaterial increased, lesser number of bacteria survived and therefore the growth phase was smaller.

TEM image of the treated *E.coli* clearly shows that the nanomaterial engulf these bacteria (Fig. 3.19(a)) and thereby cuts off the cells from the external media from which it derives its nutrients. Furthermore the TEM measurement revealed that the cell was completely damaged and ruptured in certain parts in presence of ZnO nanomaterials. It was interesting to observe here that the nanomaterials were located mostly on the inner cell wall region (Fig. 3.19b).



(a) *E.coli* surrounded by nanomaterial (b) Image of ruptured *E.coli* due to ZnO nanomaterial loading

Figure 3.19: TEM images of *E. coli* treated with synthesized ZnO nanomaterial.

It may be argued here that, due to excessive nanomaterial with high surface activity around a cell, it first distort the cell followed by eventual cell wall rupture and cell death.

3.2.3 Interaction of ZnO nanomaterial (PEG coated) with *E.coli*

Similar to the observation made in section 3.2.1, PEG coated ZnO nanomaterial was also found to show inhibition of growth when added to the culture medium. But it could be noted that the O.D. values for this condition of growth are higher than the O.D. values in the previous two sections. This can be explained from the fact that the effective weight of ZnO nanomaterial in these coated nanomaterials is lesser than the un-coated ones and moreover PEG do not show toxicity effect. So the combined

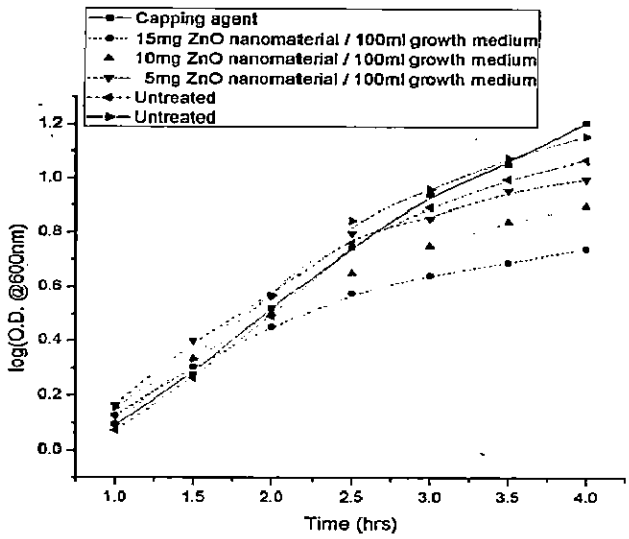


Figure 3.20: Growth curve of *E.coli* treated with PEG coated ZnO nanomaterial, where PEG is the capping agent.

effect of coating agent and ZnO leads to lesser toxicity.

When *E.coli* was treated with 15 mg of nanomaterial per 100 ml of nutrient media, the growth of the bacteria was nearly 30% low and the one with 10 mg of nanomaterial per 100 ml of nutrient media showed nearly 20% low growth compared to the untreated one. While the one treated with 5 mg of nanomaterial per 100 ml of nutrient broth showed growth inhibition nearly 10% reduced growth taking stationary phase as reference (Fig. 3.20).

The exponential growth of bacteria with 5 mg of nanomaterial per 100 ml of nutrient broth was found to be similar to that of untreated in the most part of the growth curve. As the concentration of nanomaterial was increased, inhibition of growth was appreciable, but to a lesser extent as compared to the un-coated ZnO. The capping agent (PEG) was found to show no toxic effect on growth of bacteria.

Chapter 4

Conclusion

Use of nanomaterial is increasing day by day which has raised an important question whether these materials pose any threat to living systems. Very few data is available in this context. The present study was undertaken to understand any toxic effect of ZnO nanomaterial to living system. ZnO nanomaterial was chosen as it is one of the most widely used in R&D and industry. And *E. coli* was chosen to model the living system. The present study showed that ZnO nanomaterials are toxic to living system as it caused inhibition in the growth of *E. coli*. Following conclusions were drawn:

1. The nanoparticles of ZnO were toxic to the selected cellular model of *E. coli* in the conditions favorable for the growth of *E. coli*.

2. The toxicity of ZnO nanparticles synthesized was similar to the toxicity of commercially obtained ZnO nanoparticles.

3. PEG coated nanoparticles showed reduced toxicity as compared to bare nanoparticles.

4. TEM and AFM study could show the interaction of nanoparticles with cell.

5. The mode of action of nanoparticles leading to cell death was by their interaction with the cell wall and finally disintegrating the cell wall structure.

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beginning of large scale production of these materials. The negative side of this is the controversy of potential toxicity due to its exposure to human body via our cellular system. Broadly, two classes of population are exposed to nanoparticles: (a) the end user of the product (b) one involved in manufacturing these materials in industries or in research laboratories.

Though the boom of nanoparticle based products has been quite a recent phenomenon, but there has been yet another class of materials named nanosized particles (NSP) which has been under study in connection to toxicity based on dose-response effects. These particles are particularly important with respect to environmental issues. These are usually referred to as ultra-fine particles which are released to the atmosphere from various sources and are susceptible to be inhaled by human beings and therefore may find access to tissues and cellular levels, which may pose threat due to toxicity. Particles of nanosized dimension are known to interact with cell membranes and sub-cellular structures resulting in biological/toxicological responses. The effect is likely to change if these particles are surface modified, inducing change in solubility or by surface coating. It was found that 20 nm of TiO_2 and Al_2O_3 particles induce significantly greater inflammatory responses than same mass dose of larger sized TiO_2 and Al_2O_3 particles are treated intratracheally to the lungs of rats. It should be noted that all nanomaterials are not bactericidal. For example un-coated and peptide wrapped [20] and ssDNA-wrapped Single walled carbon nanotubes [21] are not toxic to *E. coli* up to several ppm levels, which are the limits of their water solubility. The toxicity of nanosized particles have been experimented on animal model to understand the possible pathway of these particles into blood circulation [22] and to the brain [23]. The probable mechanism of toxicity effect of nanosized particles on human beings have been shown indirectly by Nemmar et al. [24]. They have discussed that the nanosized particles translocates from lungs into blood circulation and affect blood coagulation. It is a matter of academic interest to study the effect of synthetic nanoparticles especially size dependent ones on the cellular system in order to understand if these particles pose threats to human health. So that further measures can be taken up to minimize, if not eliminate the toxicological threat.

1.4 Interaction of Nanonaterials with Microbes

Microbial contamination is a major area of concern in health and food industry, many developments in the field of antimicrobial agents are observed from past many years. But due to mutations in the microbial DNA, there are now antibiotic resistant infections which have risen the concern for use of inorganic materials as alternative