Chapter 2

A-Synthesis of nanomaterials

The various synthesis methods and experimental techniques used during the course of the present work are discussed in this chapter.
2.1 Nanomaterial synthesis

Technological progress of modern society depends on the material science and engineering, community's ability to conceive the novel materials with extraordinary combination of physical and mechanical properties [1]. The results of nanoscience are realized in nanotechnology as new material and functional facilities. At present time, nanochemistry has become one of the main growing areas of nanoscience [2]. Frequently, nanometer size metallic particles show unique and considerably different physical, chemical and biological properties compared to their bulk counterparts. This is primarily due to their high surface to volume ratio [3-6]. The various synthetic methods for nanoparticles are tabulated in Table 2.1.

**Table 2.1:** Methods for synthesis of nanomaterials

<table>
<thead>
<tr>
<th>Physical</th>
<th>Chemical</th>
<th>Biological</th>
<th>Hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Mechanical</td>
<td>2) Vapor</td>
<td>1) Colloid</td>
<td>1) CVD</td>
</tr>
<tr>
<td>I. High energy</td>
<td></td>
<td></td>
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<tr>
<td>II. Melt mixing arresting polymer</td>
<td>I. Physical</td>
<td>2) Sol-gel</td>
<td>2) Electro-chemical</td>
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<tr>
<td></td>
<td>vapor deposition</td>
<td>3) L-B Film</td>
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<tr>
<td></td>
<td>II. Laser</td>
<td>4) Inverse micelle</td>
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<tr>
<td></td>
<td>ablation</td>
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<td></td>
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<tr>
<td></td>
<td>III. Sputter</td>
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</tr>
<tr>
<td></td>
<td>deposition</td>
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</tr>
<tr>
<td></td>
<td>IV. Electric</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>arc deposition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Using biomembrane, DNA, Enzyme, Micro-organisms

1) Zeolite
Chapter 2

There are numerous techniques available for the synthesis of different types of nanomaterials in the form of colloids, clusters, powder, tubes, rods, wires, thin films etc. Some of the already existing conventional techniques to synthesize different types of nanomaterials could be optimized to get novel nanomaterials.

Nanotechnology is emerging as a cutting edge technology interdisciplinary with biology, chemistry and materials science [7]. There are, therefore, various physical, chemical, biological and hybrid techniques available to synthesize nanomaterial. This thesis describes the chemical and biological methods for the synthesis of silver nanoparticles (SNPs), zinc oxide (ZnO) and silver-zinc oxide nanocomposite.

2.2 Chemical methods

There are numerous advantages of chemical methods which are given below,

1) Chemical methods are simple, inexpensive; less instrumentation is required as compared to physical methods.
2) The synthesis requires low temperature.
3) Doping of atoms or ions is possible during synthesis.
4) Large quantity of material is obtained with variety of shapes and size.
5) Materials obtained in the form of colloids could be converted into dry powder or thin film, quite easily.
6) Self assembling or patterning is possible.

Chemical reduction is the most frequently applied method for the preparation of SNPs, as stable, colloidal dispersions in water or organic solvents. Commonly used reductants in the synthesis of SNPs are borohydride, citrate, ascorbate and elemental hydrogen. The reduction of silver ions (Ag⁺) in aqueous solution generally yields colloidal silver particles with diameters of several nanometers [8]. Colloids are known since very long time. A class of materials co-exists with at least one dimension less than a micrometer is known as colloids. Nanomaterials are a sub class of colloids, in which one of the dimensions is about 1 to 100 nm range. Colloids may be particles, plates, fibers, rods and cube etc. In this thesis, the synthesis
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of SNPs is discussed (chapter 3A and chapter 3B). The outline of the procedure is as follows,

\[
\text{Adjust pH 8-9 by ammonia} \quad 25 \text{ mL 0.01M AgCl + 20 mL 2\% PVA} \rightarrow 0.1 \text{ mL 0.1 M } \text{NH}_2-\text{NH}_2 \rightarrow \text{SNPs}
\]

where, PVA= Polyvinyl alcohol

\[
25 \text{ mL 0.001M AgNO}_3 + 0.1 \text{ g IL in 10 mL DW} \rightarrow 0.1 \text{ M, 1 mL } \text{NH}_2-\text{NH}_2 \rightarrow \text{SNPs}
\]

where, IL= Ionic liquid

2.2.1 Colloidal nanoparticles

Colloid is a two-phase heterogeneous system consisting of the dispersed phase and dispersion medium. However, colloidal particles present in small amount as the dispersed phase component behave like a solute in a solution when suspended in a solvent phase or dispersing medium, because of their small size. Since the dispersed phase in a colloidal system is uniformly distributed in the dispersion medium, the colloidal state appears homogenous to the naked eye or even by an ordinary microscope (due to particles being invisible). The heterogeneous dispersion is two immiscible phases and this is proved by viewing it under an ultra-microscope, where the light reflected by colloidal particles can be seen. Colloidal particles do not settle down under gravity: a colloidal solution of gold prepared by Faraday over 160 years ago continues to be in excellent condition even today. Colloids can pass through ordinary filter paper but do not pass through animal cell membranes [9].

2.2.2 Interaction of colloids and medium

Colloids are particles with large surface to volume ratio. Correspondingly there are large number of atoms or molecules on the surface of colloidal particle, which do not have as many neighbours as those for atoms or molecules inside the interiors. Therefore, atoms on the surface are in highly reactive state, which easily interact to form bigger particle or tend to coagulate. It is thus necessary to understand the stability of colloids i.e. how the colloids dispersed in a medium and can remain as separated particles. When fine
particles are dispersed in a medium, it is known that they undergo Brownian motion. If we are able to tag a particle in the liquid, as depicted in Fig. 2.1, it would appear as if it is making a random motion. All other particles also execute random motion, hitting each other and changing their directions of motion in the liquid.

![Brownian motion of colloidal particles](image)

**Fig. 2.1** Brownian motion of colloidal particles

The distance travelled between successive collisions is random, and average distance travelled by colloidal particle can be found as,

\[
\Delta R^2 = \left(\frac{K}{3\pi \eta}\right) \Delta t
\]

where, \(\Delta R^2\) is distance travelled by particle from its original position in time \(\Delta t\), \(K\) is Boltzmann constant, \(T\) is temperature of liquid, \(r\) is radius of particle and \(\eta\) is viscosity of liquid.

Interactions of such constantly and randomly moving particles with each other and with liquid in general would be quite complex. There are two types of interactions, attractive and repulsive. Those can be expressed as irrespective of whether there exists permanent dipole or not,

\[
dG_1 = \left(\frac{A}{R^{12}}\right) - \left(\frac{B}{R^6}\right)
\]

\(G\) is free energy, \(A\) and \(B\) are constants, \(R\) is the radius of particle.

### 2.2.3 Sol-gel method

Sol–gel processing is perhaps the most well known and extensively used method for synthesis of nanoparticles. The name itself indicates this method involves two types of components or materials, sol and gel. Sol-gel is known since the time when, M. Ebelman synthesized them in 1845. However, it is
only last one or two decades that considerable interest in it, both in scientific and industrial field, has generated due to realization of several advantages one gets as compared to some other technique. The sol-gel formation process is usually a low temperature process. This means less energy consumption and less pollution too. It is therefore, not surprising that in the nuclear fuel synthesis it is a desired process. Although sol-gel process involves highly pure, well controlled ceramics, it competes with other process like CVD or metallo-organic vapours derived ceramics. The choice, of course depends upon the product of interest, its size, instrumentation available, ease of processing etc. In some cases sol-gel can be economical route, provided precursors are not very expensive. Some of the benefits of sol-gel method are getting unique material such as aerogels, zeolites. Ordered porous solids by organic-inorganic hybridization are unique to sol-gel process. It is also possible to synthesis nanoparticles, nanorods, nanotube etc. using sol- gel technique. The starting materials used in the preparation of the “sol” are usually reactive inorganic metal salts or metal organic compounds such as metal alkoxides. In a typical sol–gel process, the precursor is subjected to a series of hydrolysis and polymerization reactions to form a colloidal suspension, or a “sol”. Sol on hydrolysis, condensation and polycondensation reactions leads to the formation of three dimensional gel (hydroxide form) which on further thermal treatment gives oxides. Thermal treatment of the sol results in the complete loss of the alkoxides as alcohol and formation of the metal oxide. By drying the liquid, it is possible to obtain powders, thin films or even monolithic solid.

Although it is not mandatary that only oxides be formed by a sol- gel process, often oxide ceramics are best synthesized by sol- gel route. For example in silica, SiO₄ group with Si at the centre and four oxygen atoms at the apexes of tetrahedron are ideal for forming sol interconnectivity through the corners of tetrahedron, creating some cavities or pores. By polycondenatation process (many hydrolysed units coming together by removal of some atoms from small molecule like, (OH) sols are nucleated and ultimately sol- gel is
formed. In this thesis sol-gel method is used for the synthesis of zinc oxide nanoparticles.

0.2 M zinc acetate in 80: 20 (ethanol: water) + 5M KOH

\[ \text{pH} \approx 10 \]

zinc oxide nanoparticles

2.2.4 Biological method

The very famous speech delivered in 1959, before the scientists of American Physical Society Nobel Laureate Richard Feynman asked the scientists to derive the inspiration from Mother Nature to make the things smaller and see the advantages of making things smaller. Indeed the biological world, animal kingdom and plants make optimum use of materials and space. Inorganic materials are produced in biological systems. A variety of mechanically strong or weak, rigid or flexible, porous or nonporous, thick or thin materials either organic or inorganic materials are abundantly produced by live cells. These materials exhibit a rich variety in their functions like providing support to body, allow body movements and in general for functioning.

Many of materials synthesized by microorganisms, animals and plants in nature can indeed be synthesized using them in laboratories even on a large scale. This is considered to be very attractive possibility so as to have eco-friendly or so called green synthesis. Green chemistry is defined as “design, manufacture, and application of chemical product and processes to reduce or to eliminate the use and generation of hazardous materials” striving for sustainable development is necessary task of mankind. Driven by the motivation of understanding biological system as well as mimicking the nano synthesis by nature’s way, scientists have been using the methods by which inorganic materials are synthesized using biomaterials like enzyme, DNA, membrane etc. A variety of metal, semiconductor and insulator nanoparticles or their assemblies have been made [62]. Synthesis of nanomaterial using biological ingredients can be roughly divided into three types.

1) Use of microorganism like fungi, yeast or bacteria and actinomycetes.
2) Use of plant extract or enzymes.
3) Use of template like DNA, membranes, virus and diatoms.

This thesis deals with the synthesis of silver nanoparticles using leaf extract of *Ocimum tenuiflorum* (Tulsi). Use of plant extracts in synthesis of nanoparticles is quite novel method of green chemistry. However, compared to use of microorganism to produce nanoparticles, use of plant extract is relatively less investigated. There are some examples where plant extracts are used for the synthesis of nanoparticles. Vaseeharan et al used tea leaf extract for synthesis of silver nanoparticles [10], Kasthuri et al used heena leaf extract (appin) for the synthesis of silver and gold nanoparticles [11], Dwivadi et al used chinopodium leaf extract for synthesis of silver and gold nanoparticles [12], Gunasaragan and Xiang et al used mangosteen leaf extract for synthesis of silver nanoparticles [13]. The nanoparticles produced by fungus and leaves have quite different size and shape.

We have explored biological method for synthesis of SNPs which is described in chapter 3C.

![20 mL 0.001M AgNO₃ + 2 mL Ocimum tenuiflorum leafs extract SNPs](image)

2.3 Antibacterial activity

Formerly classified with the fungi, bacteria were considered as primitive members of the plant kingdom, but they are now called prokaryotes, a name which means primitive nucleus. All other living organisms are called eukaryotes, a name implying a true or proper nucleus. This important division does not invalidate classification schemes within the world of bacterial, animal and plant life.

2.3.1 Methods for determination of antibacterial activity

There are two methods for testing the antibacterial activity as, well diffusion method and disk diffusion method. We have used Nathans well diffusion method [14] for testing of antibacterial activity of silver nanoparticles, zinc oxide nanoparticles and silver loaded zinc oxide nanoparticles. The experimental details are given below,
2.3.2 Test plate preparation

A Petri dish, 90 mm in diameter, was filled with 15 mL of agar containing a standard brain-heart infusion medium (BHI). Four holes, 9 mm in diameter, were formed in the agar by removing plugs cut with a cork-borer. The holes, evenly distributed on the plate, were spaced about 20 mm apart and 10 mm from the outer edge. All procedures were performed with sterile instruments to avoid contamination of the Petri dish. Each hole was filled with a definite (5, 10, 15, 20….µL) amount of a topical antimicrobial agent.

2.3.3 Plate inoculation with bacteria

Seven mL of agar of the same composition as that on the test plates was melted, and when the agar reaches approximately 45°C, organisms are added to the tubes. Test organisms can be obtained from a single colony on solid agar and transferred to the fluid agar with a wire loop. Alternatively, organisms may be tested directly from the wound by rolling a wet swab 10-20 times over the area to be sampled. The swab is extracted in 10 mL of saline. 1 mL of the saline containing the suspended organisms then added to the melted agar. The suspension of agar and organisms obtained by either method, were mixed on a mechanical agitator and poured on to the previously prepared plates containing the material to be tested. The holes were completely filled with the agar and the overlay was evenly distributed. Either of the procedures described for obtaining the organisms would usually be sufficient for the test. However, the relatively small number of organisms obtained directly from the swab may produce fewer colonies and be more difficult to evaluate. It is not necessary to determine precisely the number of organisms inoculated. If a variety of organisms are present on the wound it may be necessary to sub-culture the initial preparation so that the response of single colonies can be evaluated.

2.3.4 Incubation time

The fluid agar overlay, containing the suspension of bacteria, solidifies in about 1 hour. The test plates were inverted and incubated at 37°C for 6-24 h. With some fast-growing organisms a preliminary reading can be made at 6 h, but most organisms require 18-24 h of incubation for a secure analysis.
2.3.5 Theory of inhibition zone

The zone of inhibition is measured by scale in mm. Despite its simplicity, the well diffusion test is based on sophisticated physicochemical principles governing the dynamics of diffusion of antibacterial material simultaneous to bacterial growth in an agar system. Cooper and Woodman [15] applied the formula for diffusion of neutral particles in gases to the diffusion of antibiotics through agar gels as follows,

\[ X^2 = 4 \ D \ T \ 2.3 \ (\log \ M_0 / M) \]

where,
X= zone radius
D= diffusion coefficient
T= critical time for zone demarcation
Mo= antibiotic concentration at the reservoir
M= critical concentration of antibiotic inhibiting the organism

The critical time T can be further related to the growth characteristics of the test organism and the inoculum factor as follows:

\[ T = L + n \ \log \ N / N_0 \]

where, L= lag period, n= generation time, N= critical mass of cells formed at T, N0= inoculum density used in the test. Thus, it is clear that many drug and organism related variables directly influence the zone size. To obtain meaningful results, as many test variables as possible need to be strictly standardized such that the rate-limiting step influencing the zone size is proportional to the critical concentration of the drug (M) inhibiting the organism relative to its susceptibility or resistance.

2.3.6 The race between diffusion and growth

When an antibiotic disk comes in contact with an inoculated agar surface, the “drug–bug” race begins. Antibacterial agent diffuses out from the well into the agar, creating dynamically changing gradient of its concentrations, while the test organism starts to divide and growth progresses toward the critical mass. The zone edge is formed at the critical time where the concentration of antibacterial agent that is just able to inhibit the organism,
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reaches an overwhelming cell mass. At this point, the density of cells is sufficiently high such that the antibiotic in the immediate vicinity can be absorbed, thus maintaining concentrations to sub-inhibitory levels and enabling the test organism to grow. The critical times of most rapidly growing aerobic and facultative anaerobic bacteria vary between 3 and 6 h and should not be confused with the incubation period needed to achieve visible growth as seen by the naked eye. Besides molecular properties of the antibacterial agent such as molecular weight and size, ionic charge, and aqueous solubility, the diffusion coefficient of the antibacterial agent is also influenced by the viscosity and depth of the agar as well as the assay temperature and incubation conditions. Besides characteristics intrinsic to the organism, growth is affected by the nutritive capacity of the test medium, the density and growth phase of the inoculums, and the incubation temperature [16, 17].

2.3.7 Antimicrobial activity and its mechanism

It has been known since ancient times that, silver, zinc oxide and their compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus [18-20]. Compared with other metals, silver exhibits higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cells [21]. Nanometer-sized silver particles have been known for a long time but have been paid little attention [18]. Lately, the recent advances in research on metal nanoparticles appear to revive the use of SNPs for antimicrobial applications. Though the mode of action of SNPs on the bacteria is still unknown, it’s possible mechanism of action has been suggested according to the morphological and structural changes in the bacterial cells. The SNPs show efficient antimicrobial property compared with other salts due to their extremely large surface area, which provides better contact with microorganisms. The brief explanation of its anti-microbial mechanism can be explained as follows: Generally, metal ions destroy or pass through the cell membrane and bond to the −SH group of cellular enzymes [22]. The consequent critical decrease of enzymatic activity causes change in metabolisms of micro-organism and inhibits their growth, up
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to the cell’s death. The metal ions also catalyze the production of oxygen radicals that oxidize molecular structure of bacteria. The formation of active oxygen occurs according to chemical reaction.

\[
\text{H}_2\text{O} + \frac{1}{2} \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + (\text{O})
\]

Such a mechanism does not need any direct contact between anti-microbial agent and bacteria. The produced active oxygen diffuses from fiber to the surrounding environment, and thus the metal ions inhibit the multiplication of micro-organisms. However, bacteria are not permanently exposed to oxygen radicals and thus the ionic additive does not seem to facilitate the selection of resistant strains [23, 24]. Silver ions can lead to denaturing of protein and cell death because of their reaction with nucleophilic amino acid present in proteins, and attach to sulfhydryl, amino, imidazole, phosphate and carboxyl groups of membrane or enzyme proteins [25]. Respiration blocking and cell death also may be caused by forming R–S–S–R bonds [26, 27]. Kumar et al have proposed that bonds may be formed via reaction between silver in oxidic form and sulfhydryl (–S–H) groups [26]. Silver is also known to inhibit a number of oxidative enzymes such as yeast alcohol dehydrogenase, the uptake of succinate by membrane vesicles and the respiratory chain of \textit{E. coli}, causing metabolite efflux and interfering with DNA replication [28]. Silver can associate with the cell wall [29], cytoplasm and the cell envelope [30]. Attachment of Ag ions or nano-particles to the bacteria because of electrostatic interaction with negative charge of bacterial cell wall is known as one of the mechanisms of cell death by Ag via rupturing cell membrane [27, 31]. Generally, low concentrations of Ag\(^+\) induce a massive proton leakage through the bacterial membrane and cell death [23, 32, 33]. Moreover, nanomolar concentration of SNPs can be efficient while Ag ions are needed at the micromolecular level [31]. Recently Kim et al. suggested that the anti-microbial mechanism of SNPs is related to the formation of free radicals and subsequent free radical-induced membrane damage. They confirmed that the anti-microbial activity of SNPs and silver nitrate was influenced by N-acetylcysteine (NAC). They have also suggested that free radicals that might
have been derived from the surface of SNPs were responsible for the antimicrobial activity through electron spin resonance (ESR) [24]. Investigation of bio-innoxiousness of silver revealed that smaller-sized silver particles are less toxic to skin than larger ones at the same level of concentration. Although a small irritation has been reported by applying the colloidal silver with 30 nm particle size, the colloidal silver with 2–3 nm particle size has been known to be innoxious [53]. Pape et al developed an activated carbon fibre after-treatment with nano-silver [35]. Yeo et al applied SNPs to produce antibacterial as-spun mono-filament yarns [36].

Fig. 2.2 Mechanism of cell lysis when cell interacts with SNPs

Silva Paula et al have reported the influence of nano-silver introduction into poly (styrene-co-acrylic acid) copolymer on antibacterial activity. They believed that the carboxylic groups of acrylic acid led to increased ionic mobility in the copolymer responsible for the enhanced antibacterial surface activity of the copolymer [37]. Fernandez et al have developed the SNPs on cellulose fibers used as absorbent pad. They immersed fluff pulp and nano-structured lyocell fibers in silver nitrate and subsequent transformation to SNPs have been done by physical (thermal or UV) or chemical (sodium botohydride)
methods [38]. The effect of silver [39] and silver nano-particles on the electrical conductivity of polymeric matrices [40-44], improvement of UV protection properties and the effect of dyeing on the ultraviolet protection factor (UPF) [45] have also been investigated. Producing silver nano-wires has been expanded by Sun et al. [46]. Ilic et al. have described the anti-fungal efficiency of pretreated polyester and polyamide fabrics treated with SNPs [47]. The schematic representation of mechanism of antibacterial activity is shown in Fig.2.2
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B-Characterization techniques for nanomaterials

2.4 Introduction

In the past years the advancement in science has taken place mainly with the discovery of new novel material. Characterization is an important step in the development of materials. The complete characterization of any material consists of phase analysis, compositional characterization, structural evaluation, microstructure analysis and surface characterization, which has long bearing on the properties of materials. This has lead to the emergence of variety of advanced techniques in the field of material science.

This thesis describes the formation of silver nanoparticles, zinc oxide nanoparticle and silver loaded zinc oxide nanoparticles. The various characterization techniques such as UV-visible spectroscopy, X-ray diffraction, fourier transform spectroscopy (FT-IR), scanning electron microscopy (SEM), Energy dispersive X-ray analysis (EDAX), field emission scanning electron microscope (FE-SEM), transmission electron microscopy (TEM), photoluminescence spectroscopy (PL), dynamic light scattering (DLS) and zeta potential measurement have been used to characterize the synthesized nanomaterial. This chapter is devoted to explain different instrumentation techniques used and their basic principles, operation and working.

2.4.1 UV-visible spectroscopy

UV-visible spectroscopy is used for the confirmation of formation of silver nanoparticles, zinc oxide nanoparticles and silver loaded zinc oxide nanoparticles and also for kinetic study of formation of SNPs.

Instrument

A schematic of the components of a typical UV-visible Spectrophotometer is shown in Fig. 2.3. The beam of light from a visible or UV light source is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic beam in turn is split into two equal intensity beams by a half-mirrored device. One of the beam, the sample beam (colored magenta), passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent.
The other beam, the reference, passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as \( I_0 \). The intensity of the sample beam is defined as \( I \). Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region is normally from 200 nm to 400 nm, and the visible portion is from 400 nm to 800 nm.

If the sample compound does not absorb light of a given wavelength, \( I = I_0 \). However, if the sample compound absorbs light then \( I \) is less than \( I_0 \), and this difference may be plotted on a graph versus wavelength. Absorption may be presented as transmittance \( (T = I/I_0) \) or absorbance \( (A = \log I_0/I) \). If no absorption has occurred, \( T = 1.0 \) and \( A = 0 \).

Most of the spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance). The wavelength of maximum absorbance is a characteristic value, designated as \( \lambda_{max} \).

![Schematic of UV-visible-NIR spectrophotometer](image)

**Fig. 2.3** Schematic of UV-visible-NIR spectrophotometer
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Theory

UV-visible spectroscopy is a powerful tool for the characterization of colloidal particles. The noble metal particles are ideal candidates for study with UV-visible spectroscopy, since they exhibit strong surface plasmon resonance absorption in the visible region and are highly sensitive to the surface modification. The light absorption by small metal particles is described by Mie theory [48-50]. The peaks obtained in UV-visible spectrum are broad and their positions are size dependent. They too show blue shift with reduction in particle size. The absorption spectrum of particles in a given solvent can be calculated from optical constants of the bulk metal [51]. The absorption spectrum of spherical particles of sizes between 3 nm to 30 nm does not strongly depend on particle size. This is because the particles are below the size at which higher order term in the Mie formula for the absorption constant becomes significant. Thus, one has to regard only the dipole term, which depends only on the total metal concentration in the solution and not on particle size. The absorption coefficient $\alpha$ (mol$^{-1}$Lcm$^{-1}$) is calculated from the following relation,

$$\alpha = \frac{18 \times 10^5 \frac{Mn_0^3}{\lambda \rho} \frac{\epsilon_2}{(\epsilon_{1+2n_0^2}) + \epsilon_2^2}}{\ln 10}$$

where, $\lambda$ is the wavelength of light, $M$ and $\rho$ are the molecular weight and density of the metal, $n_0$ is the refractive index of the solvent and $\epsilon_1$ and $\epsilon_2$ are the real and imaginary parts of the dielectric constant of the metal. When the size of the particles becomes smaller than the mean free path of the electrons, the absorption bands are broadened, this is accounted by using size-corrected values of $\epsilon_2$ [52].

$$\epsilon = \epsilon_2^{(bulk)} + \left(\frac{\omega_p^2}{\omega^2}\right) \left(\frac{V_F}{R}\right)$$

where, $\omega$ is the frequency of light, $\omega_p$ the Plasmon frequency, $V_F$ the electron velocity at the Fermi level and $R$ the particle radius mean time of the free movement of the electrons).
Fig. 2.4 Schematic representation showing polarization of spherical metal nanoparticles by electric field vector of incoming light

Resonance with the incident light is reached at the wavelength where the negative value of $\varepsilon_1$ of the metal is equal to twice the dielectric constant of the medium. This is a classical effect in which electromagnetic field of light drives the collective oscillation of the free electron of nanoparticles into resonance. The observed color originates from strong absorption of light by metal nanoparticle when frequency of electromagnetic field becomes resonant with coherent electron motion [53].

Silver nanoparticles possess surface plasmon resonance at about 420 nm. Resonance is produced by the collective excitation of free electrons in the particle. Fig. 2.4 shows the movement of electrons under the influence of electric field vector of incoming light. This leads to dipole excitation across particle and the positive polarization charge acting as restoring force which makes electrons to oscillate. Thus, the electron density within surface layer, the thickness of which is equal to the screening length of few angstroms, oscillates whereas the density in the interior of the particle remains constant (“Surface Plasmon”). Therefore, any change in electron density of this surface layer will lead to change in plasmon absorption.

The UV-visible measurements were done on Shimadzu UV-visible NIR spectrophotometer (model-3600).
2.4.2 X-ray diffraction technique (XRD)

X-ray diffraction is a very powerful technique for characterizing the crystal structure of materials. It is non-destructive, non-contact technique and provides useful information, such as presence and composition of phases, crystallite size and orientation and strain state. The basic principles of X-ray diffraction are found in textbooks e.g. by Buerger [54], Klug and Alexander [55], Cullity [56], Taylor [57], Guinier [58], Barrett and Massalski [59].

Fig. 2.5 shows the schematics of X-ray diffractometer. Diffraction in general occurs only when the wavelength of the incident wave is of the same order of magnitude as the repeat distance between scattering centers. This condition of diffraction is nothing but Bragg’s law and is given as,

\[ 2d \sin \theta = n\lambda \]

where,
- \( d \) = interplaner spacing
- \( \theta \) = diffraction angle
- \( \lambda \) = wavelength of x-ray
- \( n \) = order of diffraction

**Fig. 2.5 Schematics of X-ray diffractometer**
In crystalline solids the atoms are ordered in particular repeated pattern referred as unit cell with its interatomic spacing comparable to wavelength of X-rays (0.5 to 2.5Å). Hence crystals are the best gratings for the diffraction of X-rays. The directions of diffracted X-rays give information about the atomic arrangements and hence the crystal structure and phase formation can be confirmed by X-ray diffraction studies.

The way of satisfying Bragg’s condition is devised and this can be done by continuously varying either $\lambda$ or $\theta$ during the experiment. The way in which these quantities are varied, distinguish the three main diffraction methods as tabulated in Table 2.2.

In powder method the crystal to be examined is reduced to a fine powder and placed in a beam of a monochromatic X-rays. Each particle of the powder is the tiny crystal, or assemblage of smaller crystals, oriented at random with respect to incident beam. Some of the crystals will be correctly oriented so that their (100) planes, for example, can reflect the incident beam. Other crystals will be correctly oriented for (110) reflections and so on. The result is that every set of lattice planes will be capable of reflection. This is the principle of a powder diffractometer.

### Table 2.2: X-ray diffraction methods

<table>
<thead>
<tr>
<th>Method</th>
<th>$\lambda$</th>
<th>$\theta$</th>
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<tbody>
<tr>
<td>Laue Method</td>
<td>Variable</td>
<td>Fixed</td>
</tr>
<tr>
<td>Rotating crystal Method</td>
<td>Fixed</td>
<td>Variable (in part)</td>
</tr>
<tr>
<td>Powder Method</td>
<td>Fixed</td>
<td>Variable</td>
</tr>
</tbody>
</table>

#### Theory

X-rays are electromagnetic radiations with typical photon energies in the range of 100 eV to 100 KeV. For diffraction applications only short wavelength range of few angstroms to 0.1 Å are used. Since the wavelength of X-rays is comparable to the size of atoms they are ideally suited for probing the structural arrangement of atoms and molecules in a wide range of materials. The energetic X-rays can penetrate deep into the materials and provides the
information about the bulk structure. X-rays are generated when a focused electron beam accelerated across a high voltage field and bombards a stationary or rotating solid target. As electron collides with the atoms in the target and slow down, a continuous spectrum of X-ray are emitted, which are termed Brems strahlung radiation. The high energy electron also eject inner shell electron in atoms through the ionization process. When a free electron fills a shell, X-ray photon with energy characteristic of target material is emitted. Common target used in X-ray tube are Cu and Mo, which emits 8 keV and 14 keV X-rays with corresponding wavelength of 1.54 Å and 0.8 Å, respectively.

X-rays primarily interacts with electron in atom. When X-ray photon collides with electrons, some photons from incident beam will be deflected away from the direction where they original travel. If the wavelength of these scattered X-rays did not change (X-ray photon does not lose any energy) the process is called elastic scattering where only momentum has been transferred in the scattering process. These are the X-rays we measure in diffraction experiment since the scattered X-ray carry information about the electron distribution in material. The measurements were done on on Bruker AXS D8, Cu Kα radiation.

Identification of phases

From the d-spacing, phases can be identified in a sample using the standard JCPDS powder diffraction file and the reflections can be indexed with Miller indices.

However, if the size of the diffracting tiny crystal is small, there is no more complete destructive interference at \( \theta \pm d\theta \), which broadens the peak corresponding to diffracted beam in proportion to the size of the tiny crystal. This can be used to calculate the crystallite size. The relation for the same is given by Debye Scherrer and formulated [60] as,

\[
t = \frac{0.9\lambda}{\beta \cos \theta_B}
\]

where, \( t \) = crystallite size, \( \theta_B \) = diffraction angle, \( \lambda \) =wavelength of X-rays and \( \beta \) line broadening at full width at half maxima (FWHM).
Further, the powder diffractometer can also be used for X-ray diffraction study of thin film or powder. Epitaxial or polycrystalline (may or may not be oriented) thin films can be considered as single crystal or powder (crystals or assembly of crystals spread on substrate) respectively. Hence, a typical epitaxial or oriented film may not show all corresponding reflections but only few reflections for example say, a c-axis oriented film will show only (hkl) for which h and k indices are zero and l is non zero. However, these hidden peaks can be detected by small angle X-ray diffraction technique.

2.4.3 Fourier transform infra red spectroscopy (FT-IR)

Infrared spectroscopy (IR) is a technique for qualitative and quantitative analysis of various functional groups present in the compound. Instead of recording the amount of energy absorbed when the frequency of IR light is varied (using monochromator) the IR light is guided through interferometer. Then the Fourier transform is performed on this signal from interferometer, which results in a spectrum similar to that from conventional infrared spectrometer.

**Working**

Infrared spectroscopy works on principle that the chemical bonds have characteristics frequencies at which they vibrate. The resonant frequencies are dependent on length of bond and masses of atom at either ends of it. For a diatomic molecule the natural frequency of vibration is,

\[ \omega = \left( \frac{K}{M_r} \right)^{\frac{1}{2}} \]

where, K is force constant and \( M_r \) is the reduced mass and it is given by,

\[ M_r = \frac{(m_1 m_2)}{(m_1+m_2)} \]

When the incident IR frequency matches with resonant frequency, absorption takes place, resulting in an absorption peak in the IR spectrum.

In a conventional IR spectrometer, a sample is exposed to electromagnetic radiation and the response is monitored. The energy of radiation is varied over the desired range and the response is plotted as a function of radiation energy. At a certain resonant frequencies characteristics of
specific sample, the radiation will be absorbed resulting in the series of peak in the spectrum, which can then be used to identify the sample.

Instead of varying the energy of electromagnetic radiation, Fourier transform spectroscopy exposes the sample to a single pulse of radiation and measures the response. The resulting signal, called free induction decay, contains a rapidly decaying composite of all possible frequencies. Due to resonance by the sample, resonant frequencies will be dominant in the signal and by performing a mathematical operation called Fourier transform on the signal the frequency response can be calculated. In this way the Fourier transform spectrometer can produce same kind of spectrum as conventional spectrometer, but in a much shorter time. In addition measurement of single spectra is faster for the FT-IR technique because the information of all frequencies is collected simultaneously.

For the work described in this thesis, FT-IR spectrometer, Perkin Elmer model was used. The ray diagram of the FT-IR is shown in Fig. 2.6.

Studies of the spontaneous orientation of dipole moment in semiconductors are carried out with a non destructive tool of analysis by infrared spectroscopy which can give information on atomic arrangement and inter atomic forces in the crystal lattice itself.

**Fig. 2.6** Ray diagram of FT-IR spectrometer
It is possible to investigate how the infrared vibrational frequencies and thus the inter-atomic forces are affected by the onset of the semiconductor states. If the two energy levels $E_1$ and $E_2$ are placed in an electromagnetic field and the difference in the energy between the two states is equal to a constant 'h' multiplied by the frequency of the incident radiation $\gamma$, a transfer of energy between the molecules can occur, therefore

$$\Delta E = h\gamma$$

When the $\Delta E$ is positive the molecule absorbs energy; when $\Delta E$ is negative, radiation is emitted during the energy transfer and emission spectra are obtained. When the energies are such that the above equation is satisfied, a spectrum unique to the molecule under investigation is obtained. The spectrum is usually represented as a plot of the intensity vs the frequencies and peaks occur when the above equation is satisfied. The most of spectroscopic investigation are carried out in a relatively small portion of spectrum close to visible light. This region includes UV, visible and IR region and is arbitrarily defined as being between wavelength of $10^{-6}$ cm and $10^{-3}$ cm. Both the atoms and molecules give rise to spectra but they differ from each other. The difference between the atomic and molecular spectra lies in the nature of energy levels involved in the transitions.

In the atom, the absorption represents transition between the different allowed levels for the orbital electrons. In case of molecules, however, the atoms within the molecules vibrate and the molecule as a whole rotates and the total energy contributions are represented by the following equation,

$$E_{\text{tot}} = E_{\text{elect}} + E_{\text{vib}} + E_{\text{rot}} + E_{\text{trans}}$$

where, $E_{\text{elect}}$ is the electronic energy, $E_{\text{vib}}$ is the vibrational energy, $E_{\text{rot}}$ is the rotational energy and $E_{\text{trans}}$ is the translation energy. The separate energy levels are quantized and only certain transitions of electronic, vibrational and rotational energy are possible. Translational energy is usually sufficiently small to be ignored. The vibrational spectrum of a molecule is considered to be a unique physical property and is a characteristic of the molecule. As such the
infrared spectrum can be used as a finger print for identification in support of X-ray diffraction technique for the purpose of characterization [61].

2.4.4 Photoluminescence spectroscopy (PL)

**History**

Some materials when excited with an external source of stimulus like electron, light etc. emit light in the visible, UV or IR range. This phenomenon is known as luminescence. The word luminescence was coined by E. Wiedermann in 1888 from a Latin word lumen which means light. The word luminescence includes fluorescence and phosphorescence. They differ in duration of time over which light is emitted. Broadly, the terminology fluorescence is used if emission of light takes place within $10^{-4}$ sec of stimulus. If the emission persists for a longer duration of few tens of milliseconds to 10 sec. after the stimulus is removed, it is termed as phosphorescence. It is sometimes called afterglow [62].

Photoluminescence spectroscopy is a contactless, nondestructive method of probing the electronic structure of materials. Light is directed onto a sample, where it is absorbed and imparts excess energy into the material in a process called photo-excitation. One way this excess energy can be dissipated by the sample is through the emission of light or luminescence. In the case of photo-excitation, this luminescence is called photoluminescence. The intensity and spectral content of this photoluminescence is a direct measure of various important material properties. Photo-excitation causes electrons within the material to move into permissible excited states. When these electrons return to their equilibrium states, the excess energy is released and may include the emission of light (a radiative process) or may not (a nonradiative process). The energy of the emitted light (photoluminescence) relates to the difference in energy levels between the two electron states involved in the transition between the excited state and the equilibrium state. The quantity of the emitted light is related to the relative contribution of the radiative process [63].

In order to measure photoluminescence of semiconductors, there are various requirements: (a) a stable, powerful monochromatic light source, (b)
optics to focus light on the sample, (c) sample holder, (d) collection optics, (e) monochromator and (f) detector for spectral analysis as shown in Fig.2.7

![Fig.2.7 Typical experimental set up for PL measurement](image)

**Applications**

PL can be used to identify surface, interface, and impurity levels and to gauge alloy disorder and interface roughness. The intensity of the PL signal provides information on the quality of surfaces and interfaces. Under pulsed excitation, the transient PL intensity yields the lifetime of non equilibrium interface and bulk states. Variation of the PL intensity under an applied bias can be used to map the electric field at the surface of a sample. In addition, thermally activated processes cause changes in PL intensity with temperature.

The measurements of PL were performed on spectrofluorimeter JASCO, F. P. 750, Japan.

2.4.5 **Scanning electron microscopy (SEM)**

Interaction of electrons with elements is well understood and has been extensively used for characterizing the materials. As the electrons can be focused to micron or sub-micron size, it is well suited for analyzing sub-micron sized areas or features. When an electron strikes the atom, variety of interaction products are evolved. Fig.2.8 illustrates these various products and their use to obtain the various kinds of information about the sample. Scattering of electron from the electrons of the atom results into production of back scattered
electrons and secondary electrons. Electron may get transmitted through the sample if it is thin. Primary electrons with sufficient energy may knock out the electron from the inner shells of atom and the excited atom may relax with the liberation of Auger electrons or X-ray photons. All these interactions carry information about the sample. Scanning electron microscope is an instrument that uses electron beams to observe the morphology of a sample at higher magnification; higher resolution and depth of focus of these back scattered electrons, secondary electrons and transmitted electrons give information about the microstructure of the sample. Auger electron, ejected electrons and X-rays have energies specific to the element from which they are coming. These characteristic signals give information about the chemical identification and composition of the sample.

![Diagram of interaction products](image)

**Fig. 2.8** Variety of interaction products evolved due to interaction of electron beam and sample

**Principle**

A well-focused mono-energetic (~ 25KeV) beam is incident on a solid surface giving various signals as mentioned above. Back scattered electrons and secondary electrons are particularly pertinent for SEM application, their intensity being dependent on the atomic number of the host atoms. Each may be collected, amplified and utilized to control the brightness of the spot on a
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cathode ray tube. To obtain signals from an area, the electron beam is scanned over the specimen surface by two pairs of electro-magnetic deflection coils and so is the C.C.D. beam in synchronization with this. The signals are transferred from point to point and signal map of the scanned area is displayed on a long persistent phosphor C.C.D. screen. Change in brightness represents change of a particular property within the scanned area of the specimen [64]. The ray diagram of scanning electron microscope is shown in Fig.2.9.

![Ray Diagram of Scanning Electron Microscope](image)

**Fig.2.9** The ray diagram of scanning electron microscope

Interaction of energetic electron beam with solid surface leads to several signals like elastically scattered electrons (i.e. change of direction without change of energy) from the coulomb field of the nucleus whereas some others includes inelastically scattered electrons (with change of energy) from the electrons of the host atoms giving rise to secondary electrons, auger electrons and X-rays characteristics to host lattice. The secondary electrons (signal from approximately top 100 Å) are used to get contrast from surface morphology. The scattering cross section for back-scattered electrons [60] is given as,
\[ Q = 16.2 \times 10^{-30} \left( \frac{Z^2}{E} \right) \cot \left( \frac{\phi}{2} \right) \]

where, \( Z \) is atomic number and \( E \) is electric field.

Here the cross-section is proportional to \( Z^2 \). Hence, the back-scattered electrons are used for the \( Z \) contrast or for compositional mapping.

2.4.5.1 Energy dispersive X-ray analysis (EDAX)

If the sample is made the target in an X-ray tube and bombarded with electrons of suitable energy, it emits characteristics X-rays. This is the basis of a method of chemical analysis. The emitted X-rays are analyzed in an X-ray spectrometer and the elements present in the sample are quantitatively identified by their characteristics wavelengths. Quantitative estimation is also possible by measuring relative intensities in the spectra. For compositions greater than or about 1% and elements separated by few atomic numbers, energy dispersion analysis is very useful because the intensities are increased to about 100-fold. However, the resolution of an energy dispersion instrument is as much as 50 times less than the wavelength dispersion spectrometer using a crystal; thus overlapping of lines from nearby elements may occur. The specimen must be either electrically conducting or made so by evaporating a metallic layer on the surface, otherwise surface stray electric fields may divert the incident electron beam. If a sample is irradiated with X-rays of sufficiently high energy, it will emit fluorescent radiation. The X-ray fluorescent spectra are simple and more accurate with corresponding optical spectra if the sample contains at least one percent element. In EDAX analysis, as each atom has specific energy levels so for ejecting X-ray from their shell requires particular amount of energy which is quantified and used to directly detect the present atom present.

2.4.5.2 Field emission scanning electron microscope (FE-SEM)

**History**

FE-SEM is the acronym for Field Emission Scanning Electron Microscope. It was Ernest Ruska (1906 - 1987) whom in his Ph. D. thesis mentioned the potential for electrons to be used in a microscope. In 1933, Ruska and Knoll constructed the first electron microscope and in 1935 wrote
the first work describing the concept of a SEM. In 1938 Von Ardenne built a scanning transmission electron microscope (STEM) adding coils to a transmission electron microscope. The first SEM used to study a solid surface was described by Zworykin et al (1942) working for the RCA laboratories in the United States. As a practice in the early days the gun was located in the bottom so the specimen chamber was high enough for the operator but the specimen might fall down the column. A resolution of 50 nm was achieved with this microscope. The first micrographs showing the striking three-dimensional imaging capability were obtained in Cambridge at the Engineering Department in 1952 by Dennis McMullan who was continuing the work by Ken Sander. The next important step was also in Cambridge when Oatley improved the secondary electron detector by adding a scintillator to convert electrons to photons, and electron detector, and let the way for improvement in signal to noise ratio. Now a day, three-dimensional features can be observed due to the large depth of field available in the FE-SEM. The addition of energy dispersive X-ray detector combined with digital image processing is a powerful tool in the study of materials, allowing good chemical analysis of material. The FE-SEM is a major tool in materials science research and development.

Principle

Under vacuum, electrons generated by a field emission source are accelerated in a field gradient. The beam passes through electromagnetic lenses, focusing onto the specimen. As result of this bombardment different types of electrons are emitted from the specimen. A detector catches a secondary electron and image of sample surface is constructed by comparing the intensity of these secondary electrons to the scanning primary beam. Finally the image is displayed on a monitor. The ray diagram of FE-SEM is shown in fig.2.10 and the ray diagram of emission of different type of electron during scanning is shown in Fig.2.11.
Fig. 2.10 The ray diagram of field emission scanning electron microscope

Fig. 2.11 The ray diagram of emission of different type of electron during Scanning
Basic concept

Vacuum

The FE-SEM can be classified as a high vacuum instrument. The vacuum allows electron movement along the column without scattering and helps prevent discharges inside instrument. The vacuum is designed as a function of electron source, and due to it influence on the cathode emitter lifetime.

Field emission source

The function of electron gun is to provide a large and stable current in small beam. There are two classes of emission sources: thermionic emitter and field emitter. Emitter type is the main difference in SEM and FE-SEM. Thermionic emitters use electric current to heat up a filament; the two most common material used for the filament are Tungsten (W), and Lanthanum hexaboride (LaB$_6$). When the heat is enough to overcome the work function of filament materials the electron can escape from the material. Thermionic sources have relative low brightness, evaporation of cathode material and thermal drift during operation. Field emission is a one way of generating electron that avoids these problems. A field emission source (FES) also called as cold cathode field emitter, does not heat the filament. The emission is reached by placing the filament in a huge electrical potential gradient. The FES is usually a wire of tungsten (W) fashioned into a sharp point. The significance of small tip radius is that an electric field can be concentrated to an extreme level, becoming so big that the work function of material is lowered and electron can leave the cathode. FE-SEM uses field emission source producing the cleaner image, less electrostatic distortion and spatial resolution < 2 nm. The FE-SEM S-800 has two anodes for electrostatic focusing. A voltage (0 ~ 6.3 kV) between the field emission tip and first anode, called the extraction voltage, control the current emission (1 ~ 20 mA). A voltage (1~30 KV), called the accelerating voltage, between cathode and second anode increase the beam energy and determine the velocity at electron move into column. This voltage combines with beam diameter and determines the resolution (capacity to
resolve two closely spaced point as two separate entities). As voltage increases better point to point resolution can be reached.

**Electromagnetic lenses**

To resolve a feature on a specimen surface, the beam diameter must be smaller than the feature (still containing high current density). Therefore, it is necessary to condense electron beam. To assist in the demagnification of the beam, electromagnetic lenses are employed. Since, the cross over diameter of field emission source is smaller, a lower level of beam condensation is necessary to have a probe useful for image processing. This makes FE-SEM the highest resolution instrument. Aperture variables are used to refine the beam. Increase in objective aperture causes a drop in the irradiation current. Small objective aperture size will produce better resolution, good depth of field and minimal charging. It is the responsibility of user to choose the correct aperture size. The objective lenses can focus the probe at various specimens working distance (the Z axis from the lenses to the specimen surface). Long working distance and small aperture has shown an image that appears in focus over a large change in Z. Long working distance and small aperture yields images that appear in focus over a large change in Z-axis. A common practice is to select the current in the objective lenses and move the specimen vertically until it becomes in focus. The FE-SEM capability must often used in routine microscopy.

**Electron beam and specimen interaction**

The specimen and the electron beam interact in both elastic and inelastic fashion giving different types of signals. Elastic scattering events are those that do not affect the kinetic energy of the electron even when its trajectory had been affected. Inelastic scattering events are a result of the energy transference from the electron beam to the atoms in the specimen; as a result the electrons experiences energy loss with small trajectory deviation. Some of the signals created in this way are: secondary electrons (SE), auger electrons and X-Rays. Each of these signals gets specific information about topography, crystallography, surface characteristics, specimen composition and
other properties. The FE-SEM in this thesis is used for morphological study of zinc oxide nanoparticle and silver loaded zinc oxide nanoparticles. The measurements were performed on field emission scanning electron microscope (JSM-6160) operated at room temperature.

2.4.6 Transmission electron microscope (TEM)

The conventional electron microscope is now a day’s called transmission electron microscopy (TEM). In TEM the transmitted electrons are used to create an image of sample. Scattering occurs when the electron beam interacts with matter. The ray of electrons is produced by a pin-shaped cathode heated up by current. The electrons are vacuumed up by a high voltage at the anode. The acceleration voltage is between 50 and 150 kV. The higher is acceleration voltage, the shorter are the electron waves and the higher is the power of resolution. But this factor is hardly ever limiting. The power of resolution of electron microscopy is usually restrained by the quality of the lens-systems and especially by the technique with which the preparation has been achieved. Modern gadgets have powers of resolution that range from 0.2–0.3 nm. The useful resolution is therefore around 300,000 X.

The ray diagram of TEM is shown in Fig. 2.12. The accelerated ray of electrons passes a drill-hole at the bottom of the anode. The lens-systems consist of electronic coils generating an electromagnetic field. The ray is first focused by a condenser. It then passes through the object, where it is partially deflected. The degree of deflection depends on the electron density of the object. The greater the mass of the atoms, the greater is the degree of deflection. After passing the object the scattered electrons are collected by an objective. Thereby an image is formed, that is subsequently enlarged by an additional lens-system (called projective with electron microscopes). Thus, the formed image is made visible on a fluorescent screen or it is documented on photographic material. Photos taken with electron microscopes are always black and white. TEM with resolving power in the vicinity of 1Å are now common. As a result HR-TEM is one of the most essential tools of nanoscience. For the work described in this thesis TEM is used to find out
actual particle size and morphology of silver nanoparticles, zinc oxide nanoparticles and silver zinc oxide nanocomposite. Sample for TEM analysis were prepared by placing synthesized material from liquid liquid interface onto carbon coated copper TEM grid. The measurements were performed on TEM with a JEOL TEM 2010 instrument operated at an accelerated voltage 200 kV.

![Ray diagram of transmission electron microscope](image)

**Fig.2.12** Ray diagram of transmission electron microscope

**Applications**

TEM is used heavily in both material science/metallurgy and the biological sciences. It is possible to determine the position of defects and to determine the type of defect present. The quantitative interpretation of the contrast shown in lattice images is possible. Crystal structure can also be investigated by high resolution transmission electron microscopy (HR-TEM), also known as phase contrast imaging as the images are formed due to differences in phase of electron waves scattered through a thin specimen. Typical biological applications include tomographic reconstructions of small cells or thin sections of larger cells and 3-D reconstructions of individual molecules via single particle reconstruction.
Limitations

There are a number of drawbacks to the TEM technique. Many materials require extensive sample preparation to produce a sample thin enough to be electron transparent, which makes TEM analysis a relatively time consuming process with a low throughput of samples. The structure of the sample may also be changed during the preparation process. Also the field of view is relatively small, raising the possibility that the region analyzed may not be characteristic of the whole sample. There is potential that the sample may be damaged by the electron beam, particularly in the case of biological materials.

2.4.7 Dynamic light scattering (DLS)

Dynamic light scattering is called as photon correlation spectroscopy. This is one of the foremost techniques used to measure the radius of particle or particle size distribution in medium. The motion of particle of micron or lower size is uncorrelated that is they are random. As light scatters from such particles, there will be shift in the phase of scattered light which is random and as a result, when the scattered light rays of several particles are added together, constructive or destructive interference occurs. We get the time dependant fluctuation in the intensity of scattered light. The scattering of light from particles undergoing Brownian motion also leads to a doppler shift of the radiation, modifying the wavelength of light.

The summery of theory is that when the electric field of the light interacts with the molecule in the medium, an oscillating electric field is induced. The interaction leads to a shift in frequency of light and angular distribution of scattered light, both of which are related to the size. If one can assume that the particles are in Brownian motion, one can apply Stoke-Einstein equation and get the radii of suspended particles,

$$\alpha = \frac{K_b T}{6\pi \eta D}$$

where, $K_b$ is Boltzmann constant, $D$ is diffusion coefficient, $\eta$ is viscosity and $T$ is absolute temperature.
In DLS, time dependant fluctuations in scattered light are measured. A quantitative measure of fluctuation is the correlation function. In typical experiment, only wavelength and one scattering angle are used. In principle, the technique can distinguish the nature of particle, separated or aggregated, over a range of particle size [65].

For the work described in this thesis DLS is used to find out actual particle size of silver nanoparticles, zinc oxide nanoparticles and silver loaded zinc oxide nanoparticles. Sample for DLS analysis were prepared by dispersing synthesized material in distilled water. The measurements were performed on photon correlation spectrometer (PCS) – Zetasizer 3000 HAS equipped with a digital autocorrelation from Malvern instrument UK.

2.4.8 Zeta potential

Almost all particulate or macroscopic materials in contact with a liquid acquire an electronic charge on their surfaces. Zeta potential is an important and useful indicator of this charge which can be used to predict and control the stability of colloidal suspensions or emulsions, for example. The greater the zeta potential the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. The measurement of zeta potential is often the key to understanding dispersion and aggregation processes in applications as diverse as water purification, ceramic slip casting and the formulation of paints, inks and cosmetics.

Theory

The major source of kinetic nonlability of colloids is the existence of an electric charge on surface of a particle. On account of this charge, an ion of opposite charge tends to cluster nearby, and ionic atmosphere is formed just as for ions.

We need to distinguish two regions of charge. First, there is a fairly immobile layer of ions that adhere tightly to the surface of colloidal particle and which may include water molecule. The radius of sphere that captures this rigid layer is called radius of sphere and is the major factor determining the
mobility of particle. This electric potential at radius of sphere relative to its value in the distant, bulk medium is called zeta potential. Second the charged unit attracts an oppositely charged atmosphere of mobile ions. The inner shell of charge and outer ionic atmosphere is called electric double layer [66].

Zeta potential can also be a controlling parameter in processes such as adhesion, surface coating, filtration, lubrication and corrosion. Consequently, the presence or absence of charged groups on the surface of macroscopic materials such as hair, glass fiber, paper pulp, plastic films and refractories, as revealed by their zeta potentials can directly affect their performance and processing characteristics. For the work described in this thesis zeta potential is used to find out charge stability and surface modification of silver nanoparticles, zinc oxide nanoparticles and silver loaded zinc oxide nanoparticles. The measurements were performed on photon correlation spectrometer (PCS) Zetasizer 3000 HAS equipped with a digital autocorrelation from Malvern instrument UK.

![Mechanism of measurement of zeta potential](image)

**Fig. 2.13** Mechanism of measurement of zeta potential

Malvern zetasizer uses electrophoretic light scattering and the laser doppler velocimetry (LDV) method to determine particle velocity and, from this, the zeta potential. It also offers the option of particle size analysis in the same instrument.

If your suspended samples typically have very low mobilities because they are suspended in oils or organic solvents, or because they are in a fluid
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with high salt concentration (> ~ 20 mMolar) or just because they are near the iso-electric point (IEP) then you may require the 1000x sensitivity provided by unique phase analysis light scattering. The mechanism of zeta potential measurement is as shown in Fig. 2.13.

Each particle dispersed in a solution is surrounded by oppositely charged ions called the fixed layer. Outside the fixed layer, there are varying compositions of ions of opposite polarities, forming a cloud-like area. This area is called the diffuse double layer, and the whole area is electrically neutral.

When a voltage is applied to the solution in which particles are dispersed (Fig 2.14), particles are attracted to the electrode of the opposite polarity, accompanied by the fixed layer and part of the diffuse double layer, or internal side of the "sliding surface".

Zeta potential is considered to be the electric potential of this inner area including conceptual "sliding surface". As this electric potential approaches zero, particles tend to aggregate (Fig.2.15). The zeta potential is given by equation,

$$\zeta = \frac{4\pi \eta}{\epsilon} \times U \times 300 \times 300 \times 1000$$

where, $\zeta$ = zeta potential (mV), $\eta$ = viscosity of solution $\epsilon$ = dielectric constant, $U$ = electrophoretic mobility.

**Fig.2.14** Dispersed particles in colloid
Fig.2.15 Aggregated particles in colloid


References


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