Chapter 2

Synthesis and Characterization
Chapter 2

2.1 Synthesis

2.1.1 Materials

Metal salts used for the metallic and bimetallic nanoparticles are listed below:

**Ag:** Silver Nitrate (AgNO₃), from Merck Mumbai, India.

**Au:** Tetrachloroauric (III) acid (HAuCl₄), from Sigma-Aldrich, Steinheim, Germany.

**Pd:** Palladium Chloride (PdCl₂), from Merck Germany.

**Pt:** Platinum Chloride (PtCl₄), from Merck Germany and Chloroplatinic acid (H₂PtCl₆), from Sigma-Aldrich, Steinheim, Germany.

Stabilizing agents used for the preparation of metallic and bimetallic nanoparticles

**Surfactants:** TritonX-100, from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India and Sodium dodecyl sulphate (SDS), from Qualigen, Mumbai, India.

**Polymers:** Polyacrylamide (Mw = 10,000) and Polyvinylpyrrolidone (PVP) (Mw = 10,000), from Sigma-Aldrich, Steinheim, Germany.

Reducing agents used for the preparation of metallic and bimetallic nanoparticles

Sodium borohydride (NaBH₄), from S.D. Fine-Chemicals, Mumbai, India.

Sodium citrate (Na₃C₆H₅O₇·2H₂O; M. wt = 294.10), from Qualigen, Mumbai, India.

Hydrazine hydrate (N₂H₄·H₂O), from S.D. Fine-Chemicals, Mumbai, India.

Ethanol (C₂H₅OH)

Other chemicals used

Cyclohexane, 1-hexanol, and sodium chloride, from S.D. Fine-Chemicals, Mumbai, India.

Throughout the work double-distilled deionised water was used. All the chemicals were used as received.
2.1.2 Methods of preparation of nanoparticles

Through out the work chemical reduction method in "bottom up" approach was opted. For the synthesis of metallic and bimetallic nanoparticles, the corresponding metal ions were reduced with suitable reducing agent and using suitable stabilizing agents. In simple chemical reduction method the metal ions are reduced to form zero valent metal particles. Moreover, for stability and controlled size and shape metal colloidal particles need suitable stabilizing agent. The stabilizing agent can be surfactant, polymer, long chain thiol, amine, citrate etc. The general equation for the formation of metal nanoparticles is given below.

$$M^{n+} + ne^{-}\text{(reductant)} \rightarrow M^0\text{(nanoparticles)}$$

In the present work, $M^{n+} = Ag^+, Au^{3+}, Pt^{4+},$ and $Pd^{2+};$ and reducing agents are borohydride, citrate, hydrazine and alcohol. Based on the stabilization of the particles, three different systems are selected for the preparation of metallic or bimetallic nanoparticles. They are

(a) Synthesis in water-in-oil microemulsion and micellar solution using surfactant as a stabilizing agent.
(b) Synthesis in aqueous polyacrylamide and ethanolic polyvinylpyrrolidone solution as a stabilizing agent.
(c) Template free synthesis without polymer or surfactant stabilization.

2.1.2 (a) Stabilization by surfactant

Water-in-oil microemulsion

One of the best definitions of microemulsions by Danielsson and Lindman\textsuperscript{1} is "A microemulsion is a system of water, oil and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution". Microemulsions are colloidal ‘\textit{nano-dispersions}’ of water in oil (or oil in water) stabilized by a surfactant film. These thermodynamically stable dispersions can be considered as truly nanoreactors, which can be used to carry out chemical reactions in particular, to synthesize nanomaterials. Under appropriate conditions in an oil-water-
surfactant system, the surfactant molecules form spherical aggregates containing water. The microstructure of these aggregates exhibits surfactant head groups enclosing an aqueous core of water, thereby separating water from the non-polar solvent which is continuous phase. These structures are known as reverse micelles. The diameter of the reverse micelle, typically ranges between 20 and 100 Å, is characterized by the molar ratio of water to surfactant \((w_0)\) which ranges from 0 to 45\(^2\). The schematic representation for the formation of metal nanoparticles in water-in-oil (w/o) is given in Fig 2.1.

![Fig 2.1 Diagram of formation of metal nanoparticles in w/o microemulsion system](image)

**In the present work**, metallic and bimetallic Ag, Au, Pd, Pt and Au-Ag, Au-Pd, Au-Pt and Ag-Pt nanoparticles are synthesized in nonionic water-in-oil (w/o) microemulsion systems. The components of the water-in-oil (w/o) microemulsion are TritonX-100 (surfactant), water and cyclohexane (oil) and 1-hexanol (co-surfactant). When two w/o microemulsion systems (Fig 2.1), one containing reducing agent and other containing metal ion solution, were mixed under stirring, a newly formed w/o microemulsion contains the product in the water core. A change in colour due to the formation of metallic/bimetallic nanoparticles was almost instant after mixing of two microemulsions. The size of the water core of the microemulsion monitors the size of the particles. The size of
the water core was tuned by changing water-to-surfactant molar ratio \((W_o)\). The reactions were carried out at water-to-surfactant ratio \((W_o) = 1, 3, 5, \text{ and } 7\). Metallic particles were prepared by using different metal ion concentrations and bimetallic particles were prepared using different ratios of metal ion concentrations. The separation of metal nanoparticles from the microemulsion droplets is a crucial step. The w/o microemulsion systems are highly stable and particles were also stable without sign of agglomeration at room temperature \((25\pm2^\circ C)\) for more than six months. Surfactant free metal nanoparticles from these microemulsion systems were obtained by adding ethanol and then centrifugation. The concentrations of the metal ions, reducing agents and co- surfactant used in the present study are given in Table 2.1
Table 2.1 Concentrations of metal ions, reducing agents and co-surfactant used in the synthesis of metallic/bimetallic nanoparticles in w/o microemulsion.

<table>
<thead>
<tr>
<th>Metal/Bimetal</th>
<th>( W_0 )</th>
<th>Metal ion concentration in ( 10^{-4}\text{M} )</th>
<th>( \text{NaBH}_4 (10^{-3}\text{M}) )</th>
<th>Volume of 1-hexanol (( \mu\text{L}/5\text{ ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>3</td>
<td>2.70 and 1.35</td>
<td>1.43</td>
<td>27</td>
</tr>
<tr>
<td>Au</td>
<td>3</td>
<td>2.70 and 1.35</td>
<td>1.43</td>
<td>27</td>
</tr>
<tr>
<td>Pd and Pt</td>
<td>3</td>
<td>2.70</td>
<td>1.43</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.50</td>
<td>2.37</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.30</td>
<td>3.23</td>
<td>63</td>
</tr>
<tr>
<td>Au-Ag*</td>
<td>3</td>
<td>2.70 and 1.35</td>
<td>1.43</td>
<td>27</td>
</tr>
</tbody>
</table>

\( \text{Au}^{3+} \) | \( \text{Pt}^{4+} \)

| Au-Pt        | 3         | 1.30                                  | 1.40                   | 1.43 | 27 |
|--------------|-----------|----------------------------------------|------------------------|------------------------|

| Au-Pd        | 3         | 1.30                                  | 1.40                   | 1.43 | 27 |

| Ag-Pt        | 3         | 1.30                                  | 1.40                   | 1.43 | 27 |

\*Au-Ag alloy nanoparticles were synthesized by using the above two concentrations with varying Au mole ratio (%) 38, 50 and 62.

Micellar solution

Micellar solution can also provide a good environment for the stabilization of monodispersed metallic and bimetallic nanoparticles depending on the concentration and orientation of surfactant molecules in the medium. In the present work water soluble surfactant sodium dodecyle sulphate (SDS) is used. With SDS we could get highly stable metallic and bimetallic nanoparticles at high concentration in aqueous system. Use of aqueous method is the main advantage over the above w/o microemulsion method, where non-aqueous medium is required. Microwave and thermal heating both were used as the sources of heating for particular reactions. One \( \text{(w/v)} \) aqueous solution of SDS was used for all the reactions carried out in micellar solution. For thermal heating the reactions were
carried out at 90°C for 1h stirring. For microwave heating the reactions were completed in 30 s. Silver, gold and gold-silver bimetallic nanoparticles were prepared using $5 \times 10^{-4}$ M metal ion concentration and aqueous sodium citrate (2% w/v) solution as a reducing agent. The reactions were carried out at 90°C for 1h. Gold nanoparticles were also prepared using 0.1 to 1mM aqueous HAuCl₄ solution and sodium citrate solution as a reducing agent and heating the reaction mixture in microwave for 30 s.

2.1.2 (b) Stabilization by polymer

Like surfactants polymer also plays an important role in the preventing agglomeration of nanoparticles. A primary purpose of introduction of polymer is to protect the nanoparticles from growing and agglomeration. The adsorption of large molecules like polymer on the surface of the colloidal particles provides steric stabilization. The formation of stable metallic nanoparticles in polymer matrix is achieved by a combination of a low concentration of solute and adhereness of polymeric monolayer to the growing surface. Both low concentration and polymeric monolayer would hinder the diffusion of growing species from the surrounding solution to the growing surface. As a result, the diffusion process becomes the rate-limiting step of subsequent growth of the initiating nuclei, resulting in the formation of uniformly sized nanoparticles.

Polyacrylamide (PAM)

Aqueous polyacrylamide solution is used for the stabilization of metallic silver, gold, palladium, platinum and bimetallic silver-gold, silver-palladium, silver-platinum, gold-palladium and gold-platinum nanoparticles. Microwave heating mode was used for all the reactions in polyacrylamide solution. Aqueous solution of polyacrylamide was used for the preparation of nanoparticles. In a typical reaction procedure 10 ml reaction volume was taken in 25 ml closed conical flask and put it under the microwave. Various concentrations of reactants used in systems are given in Table 2.2.
**Table 2.2 Various concentrations of reactants used in the synthesis of metallic and bimetallic nanoparticles in polyacrylamide solution**

<table>
<thead>
<tr>
<th>Metal/Bimetal</th>
<th>Reducing agent</th>
<th>Stabilizer (PAM)</th>
<th>Metal ions con. in 10^{-4}M</th>
<th>MW heating time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>Hydrazine</td>
<td>1% w/v,</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Au</td>
<td>Sodium citrate</td>
<td>2% w/v,</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Hydrazine</td>
<td>1% w/v,</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Pd</td>
<td>Hydrazine</td>
<td>0.25 M</td>
<td>0.1</td>
<td>3</td>
</tr>
<tr>
<td>Au-Ag</td>
<td>Hydrazine</td>
<td>1% w/v,</td>
<td>10</td>
<td>3*</td>
</tr>
<tr>
<td>Pt</td>
<td>Hydrazine</td>
<td>0.25 M</td>
<td>0.1</td>
<td>3</td>
</tr>
<tr>
<td>Au-Pd</td>
<td>Hydrazine</td>
<td>0.25 M</td>
<td>0.1</td>
<td>(1.5+1.5) = 3*</td>
</tr>
<tr>
<td>Au-Pt</td>
<td>Hydrazine</td>
<td>0.25 M</td>
<td>0.1</td>
<td>(1.5+1.5) = 3*</td>
</tr>
<tr>
<td>Ag-Pt</td>
<td>Hydrazine</td>
<td>0.25 M</td>
<td>0.1</td>
<td>(1.5+1.5) = 3*</td>
</tr>
</tbody>
</table>

* Three different Au : Ag mole ratios [0.16:0.84 ; 0.5:0.5 ; 0.33:0.64 ] were taken keeping metal ion concentration at 3×10^{-4}M; † Concentration; * bimetallic composition 50:50.

**Polyvinylpyrrolidone**

Well-dispersed metallic and bimetallic nanoparticles were prepared by chemical reduction method, using Polyvinylpyrrolidone (PVP) as dispersant. In a typical procedure 1 % (w/v) PVP in ethanol was used for the stabilization of metallic (Ag) and bimetallic (Ag-Au) nanoparticles. For the preparation of silver nanoparticles, a 25 ml conical flask containing 10 ml PVP-ethanol solution with 0.2 ml 0.1 M Ag^{+} ions was kept for 5 s under microwave. The colourless solution was observed to turn yellow instantaneously confirming the formation of silver nanoparticles. Au-Ag bimetallic particles were obtained by adding aqueous HAuCl_{4} solution to the above colloidal silver solution through the galvanic replacement method. The reaction was carried out adding 20, 40, 60, 80 and 200μL HAuCl_{4} solution of 0.01M concentration to the 1 ml above mentioned silver nanoparticles solution.
2.1.2 (c) Template free synthesis

The template free synthesis of nanoparticles is based on electrostatic stabilization. The electrostatic stabilization involves use of charged capping agents. Sodium citrate is a commonly used capping agent\(^3\). Citrate- capped nanoparticles are negatively charged and they attract positively charged counter-ions from the solution. This arrangement results in the formation of a diffuse electrical double layer and consequently a Coulombic repulsion between the particles. As long as the electric potential associated with the double layer is high, electrostatic repulsion between the particles will prevent agglomeration. However, the double layer is very sensitive to changes in temperature, and in particular the ionic strength of the solution. An increase in the ionic strength by the addition of a salt causes a compression of the double layer and shortens the range of repulsion. Reduction of the charge on the colloid by the addition of a neutral, strongly binding adsorbate, which displaces the adsorbed citrate anions, also results in agglomeration\(^4\).

Through citrate stabilization methods Au-Ag alloy nanoparticles were prepared by using 2 % (w/v) aqueous solution of trisodium citrate. In 100 ml round bottom flask 49 ml water was taken. To it 0.5 ml of 2% w/v aqueous solution of trisodium citrate was added. The reaction mixture was heated to 90±2°C. To it 0.5 ml of gold (10\(^{-2}\)M) or gold (10\(^{-2}\)M) + silver (10\(^{-2}\)M) with 0.1, 0.2, 0.4, 0.5, 0.6, 0.8 and 0.9 Au mole fraction was added. Total metal ion concentration was maintained at 10\(^{-4}\) M. The refluxion was carried out at 90±2°C for 1 h. The formation of nanoparticles was instantaneous and was noticed through the change in colour. In this method citrate acts as a reducing as well as stabilizing agent.

2.1.3 Microwave as a heating device

For the preparation of metallic and bimetallic nanoparticles, instead of thermal heating, microwave heating (MW) shows a better promise due to its rapid dielectric heating. MWs are electromagnetic radiation with frequency range 300 MHz to 300GHz. The commonly used frequency for heating is 2.45 GHz. The
principle of MW heating of polar molecules is shown in Fig 2.2 for H₂O molecules. In the microwave frequency range, polar molecule such as H₂O tries to orient along with the electric field. When the dipolar molecules try to re-orient with respect to an alternating electric field, they lose energy in the form of heat by molecular friction. The MW power dissipation per unit volume in a material (P) is given by equation;

\[ P = cE^2 f \varepsilon'' = cE^2 f \varepsilon' \tan \delta \]

where, \( c \) is a velocity of radiation, \( E \) is an electric field in the material, \( f \) is frequency of radiation and \( \varepsilon' \) and \( \varepsilon'' \) are the dielectric and dielectric loss constants, respectively. \( \varepsilon' \) represents the relative permittivity, which is a measure of the ability of a molecule to be polarized by an electric field and \( \tan \delta = \varepsilon'' / \varepsilon' \) is the energy dissipation factor or loss tangent. The above equation indicates that \( \varepsilon'' \) is most important physical parameter that describes the ability of a material to heat in the MW field. Water and alcohols have high dielectric losses and a high reducing ability. Therefore, they are the ideal solvents for microwave heating. The boiling point of ethanol is 78°C and \( \varepsilon'' \) and \( \varepsilon' \) values are 6.08 and 24.3 respectively.

![Fig 2.2 Heating mechanism of H₂O by microwave irradiation.](image)

The physical parameters of typical solvents used in MW heating for synthesis of metallic nanostructures are listed in Table 2.3.
Table 2.3 Physical parameters of typical solvents used for microwave heating.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>B.p. [°C]</th>
<th>$\varepsilon'$</th>
<th>$\varepsilon''$</th>
<th>tan$\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100</td>
<td>78.3</td>
<td>12.3</td>
<td>0.175</td>
</tr>
<tr>
<td>Methanol</td>
<td>65</td>
<td>32.7</td>
<td>20.9</td>
<td>0.639</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78</td>
<td>24.3</td>
<td>6.08</td>
<td>0.200</td>
</tr>
<tr>
<td>N,N-dimethyl formamide (DMF)</td>
<td>153</td>
<td>36.71</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethylene glycol (EG)</td>
<td>198</td>
<td>41.0</td>
<td>41.0</td>
<td>1.000</td>
</tr>
<tr>
<td>N-methyl pyrrolidone</td>
<td>202</td>
<td>32.0</td>
<td>8.85</td>
<td>0.277</td>
</tr>
</tbody>
</table>

The desired solution mixture in a closed conical flask was reacted in a LG make microwave oven model MG 605 AP operated at the 100% power of 1350W and frequency 2450 MHz.
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2.2 Characterization Techniques

Synthesized nanoparticles were characterized using techniques based on optical spectroscopy (UV-Visible and infrared) electron microscopy (transmission and scanning), atomic force microscopy, dynamic light scattering (DLS), X-ray diffraction method (XRD), and energy dispersive X-ray (EDX).

2.2.1 UV-Visible spectroscopy

The total energy of a molecule is the sum of its electronic, vibrational and rotational energy. The magnitude of these energies decreases in the following order: \( E_{\text{elec}} > E_{\text{vib}} > E_{\text{rot}} \) Energy absorbed in the UV-visible region produces changes in the electronic energy of the molecule resulting from transitions of valence electrons in the molecule. The relationship between the energy absorbed in an electronic transition and the frequency (\( v \)), wavelength (\( \lambda \)) and wavenumber (\( \tilde{\nu} \)) of radiation producing the transition is

\[
\Delta E = h\nu = \frac{hc}{\lambda} = h\tilde{\nu}c
\]

where, \( h \) is Planck’s constant, \( c \) is the velocity of light and \( \Delta E \) is the energy absorbed in an electronic transition in a molecule from a low-energy state (ground state) to a high energy state (excited state). The energy absorbed is dependent on the energy difference between the ground state and the excited state. Smaller the difference in energy, longer the wavelength of absorption. Since UV-visible energy is quantized, the absorption spectrum arising from a single electronic transition should consist of a single, discrete line. A discrete line is not obtained since electronic absorption is superimposed by rotational and vibrational sublevels as shown in Fig 2.3. The principal characteristics of an absorption band are its position and intensity. The position of an absorption band corresponds to the wavelength of radiation whose energy is equal to that required for an electronic transition. The intensity of absorption is largely dependent on two factors: the probability of interaction between the energy of radiation and the electronic mater and its concentration. The intensity of absorption may also be expressed as transmittance (\( T \)), defined as

\[
T = \frac{I}{I_0}
\]
where $I_0$ is the intensity of the radiant energy striking the sample, and $I$ is the intensity of the radiation emerging from the sample. A more convenient expression of absorption intensity is that derived from the Lambert-Beer law, which establishes a relationship between the absorbance, the sample thickness and the concentration of the absorbing species. The relationship is expressed as $A = abc$; where, $A$ is the measured absorbance, $a$ is absorptivity, $b$ is the cell-path length, and $c$ is the analyte concentration.

\[ \alpha = \frac{18\pi \times 10^5 \ M \ h \ \lambda}{\ln 10 \ \rho \ \left( \varepsilon_1 + 2n_0^2 \right) + \varepsilon_2^2} \]

where, $\lambda$ is the wavelength of light in nanometers, $M$ and $\rho$ are the molecular weight and density of the metal, $n_0$ is the refractive index of the solvent and $\varepsilon_1$ and $\varepsilon_2$ are the dielectric constants of the metal and the solvent, respectively.

**Fig 2.3** Energy level diagram of a diatomic molecule.
\( \varepsilon_2 \) are the real and imaginary parts of the dielectric constant of the metal. When the particles size becomes smaller than the mean free path of the electrons, the absorption bands are broadened; this is accounted by using size-corrected values of \( \varepsilon_2 \).

\[
\varepsilon_2 = \varepsilon_2^{(\text{bulk})} + \left( \frac{\omega_p^2}{\omega_0^3} \right) \left( \frac{V_F}{R} \right)
\]

where \( \omega \) is the light frequency, \( \omega_p \), the plasmon frequency, \( V_F \), the electron velocity at the Fermi level, \( R \) the particle radius and \( R/V_F \) mean time of the free movement of the electrons. 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. For example, gold particles possess plasmon resonances in the visible range at \( \sim 524 \) nm. When a small spherical metallic nanoparticle is irradiated by light, the oscillating electric field causes the conduction electrons to oscillate coherently. This is schematically pictured in Fig 2.4.

![Figure 2.4](image_url)

**Figure 2.4** Polarization of a spherical metal particle by the electrical field vector of the incoming light

When the electron cloud is displaced relative to the nuclei, a restoring force arises from Coulombic attraction between electrons and nuclei that results in oscillation of the electron cloud relative to the nuclear framework. The oscillation frequency is determined by four factors: the density of electrons, the effective electron mass, and the shape and size of the charge distribution. The collective oscillation of the electrons is called the dipole plasmon resonance of the particle (sometimes denoted as “dipole particle plasmon resonance” to distinguish from plasmon excitation that can occur in bulk metal or metal surfaces). Thus, the electron density within a surface layer, the thickness of which is about equal to the screening length of a few angstroms, oscillates, whereas the density in the interior of the particle remains constant ("surface
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plasmon"). Therefore, any changes in the electron density of this surface layer will lead to changes in the plasmon absorption.

UV–visible spectra of all the samples in the present study were recorded on a Perkin-Elmer Lambda 35 UV–vis spectrophotometer operated at resolution of 1 nm.

2.2.2 Spectrofluorometry

At room temperature most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to excited states. Excitation can result in the molecule reaching any of the vibrational sublevels associated with each electronic state. Having reached one of the higher vibrational levels of an excited state, the molecule rapidly loses its excess of vibrational energy by collision and falls to the lowest vibrational level of the excited state. In addition, almost all molecules occupying an electronic state higher than the second undergo internal conversion and pass from the lowest vibrational level of the upper state to a higher vibrational level of a lower excited state which has the same energy. From there the molecules again lose energy until the lowest vibrational level of the first excited state is reached. From this level, the molecule can return to any of the vibrational levels of the ground state, emitting its energy in the form of fluorescence. Transition, that from the lowest vibrational level in the ground electronic state to the lowest vibrational level in the first excited state, i.e 0 - 0 transition, is common to both the absorption and emission phenomena, whereas all other absorption transitions require more energy than any transition in the fluorescence emission. We can therefore expect the emission spectrum to overlap the absorption spectrum at the wavelength corresponding to the 0 – 0 transition and the rest of the emission spectrum to be of lower energy, or longer wavelength. A diagram for fluorescence emission is given in Fig 2.5.
**Fig 2.5 Transitions giving rise to absorption and fluorescence emission spectra**

Emission spectra of the solutions were recorded by using spectrofluorometer from JASCO, Japan.

### 2.2.3 Fourier Transform- Infrared Spectroscopy (FTIR)

FTIR analysis was carried out using Perkin-Elmer RX1 IR spectrophotometer (Massachusetts, USA). The spectra were collected in the range 4000 to 400 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ in the transmittance mode. Solid samples were finely ground, dispersed in spectroscopic grade KBr and made into pellets.

### 2.2.4 Transmission Electron Microscopy and Scanning Electron Microscopy

*What are Electron Microscopes?*

Electron microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects at a very atomic scale, to get topographic, morphological composition, and crystallographic information.
Why Electron Microscopy?

Electron microscopes were developed due to the limitations of optical microscopes, which are limited, by the physics of light to 500X or 1000X magnification and a resolution of 0.2 micrometers. In the early 1930's this theoretical limitation was noticed, when there was a scientific desire to see the finer details of the interior structures of organic cells (nucleus, mitochondria...etc.). This required 10,000X plus magnification, which was just not possible using optical microscopes. The Transmission Electron Microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the optical microscope. It was developed by Max Knoll and Ernst Ruska in Germany in 1931. The first Scanning Electron Microscope (SEM) was debuted in 1942 and the first commercial instruments around 1965. The late development was due to the electronics involved in "scanning" the beam of electrons across the sample.

Transmission electron microscopy (TEM) is a powerful tool for examining the internal structure of materials at high resolution. In the microscope, a high-energy electron beam is focused onto a specially prepared site-specific thin specimen. The transmitted electrons carrying structural information of the specimen travel through a series of electrostatic lenses, finally forming an enlarged image on a phosphorus-covered screen. The TEM has been used as a research tool for many years as it is the only analytical instrument that provides microstructural details down to an atomic level. Much of the TEM work performed to date has centred on determining structure-property relationships for a wide range of materials, including metals/alloys, semiconductors, ceramics, and polymers. TEM has been extensively used in the fields of material science, semiconductor/ICs, medicine, and biology.

Scanning electron microscopy (SEM) is one of the most widely used techniques used in characterization of nanomaterials and nanostructures. In a typical SEM, a source of electrons is focused into a beam, with a very fine spot size of ~5 nm and having energy ranging from a few hundred eV to 50 KeV. As the electrons strike and penetrate the surface, a number of interactions occur that result in the emission of electrons and photons from the samples, and SEM images
are produced by collecting the emitted electrons on a cathode ray tube (CRT). Various SEM techniques are differentiated based on what is subsequently detected and imaged. The principle images produced in the SEM are of three types:

- secondary electron images,
- backscattered electron images
- elemental X-ray maps.

When a high-energy primary electron interacts with an atom, it undergoes either inelastic scattering with atomic electron or elastic scattering with atomic nucleus. In an elastic collision with an electron, the primary electron transfers part of its energy to the other electron. When the energy 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 a secondary electron. Backscattered electrons are the high-energy electrons that are elastically scattered and essentially possess the same energy as the incident primary electrons.

Size, shape and particle size distributions were determined using a JEOL JEM-2011 transmission electron microscope operated at an accelerating voltage of 200 KeV. Images were recorded using a Gatan DualVision 600t CCD camera attached to the microscope and were analyzed using Gatan Digital Micrograph Version 3.11.1. The TEM was calibrated for diffraction and imaging mode using standard samples. The resolution of the system was calibrated with manganese. Samples were prepared for TEM analysis by placing a drop of the solution on a carbon coated copper grid and drying in air.

2.2.5 Atomic force Microscopy (AFM)

Since its birth in 1986, Atomic force microscopy (AFM) has become a very important characterization technique\(^1\). In, AFM a tip connected to a cantilever interacts with a surface. The interaction could be by Van der Waals, electrical, magnetic, short range, capillarity, or any other electromagnetic force. It is composed of several parts to be able to extract topography from surfaces. There are components that are not missed in any system like a very small radius tip on a
cantilever with a low spring constant, a technique to measure cantilever deflections produced by forces between the tip and the surface, a scanning capability in three dimensions that makes the tip move relatively on the surface, a feedback system to obtain topographic or other type of information, and a software that can interpret data as image. Now a days great number of cantilevers made of silicon and silicon nitride are used. There are silicon cantilevers that have rectangular shape with spring constants from 0.1N/m to 40 N/m. The triangular silicon nitride cantilevers more suitable for contact mode have spring constants from 0.01 to 0.58 N/m\textsuperscript{12}. The three most used techniques are the laser reflection method, interferometric method, and the piezoresistive method. The three basic modes of AFM in which topography can be obtained are: contact mode, non-contact mode, and tapping mode. Topography can be recorded in air or liquid by contact and tapping mode, whereas in vacuum by contact and non-contact mode. In any mode to obtain topography, the tip is moved along with the surface. This motion or scanning is done by piezoscanners. Scanner also moves the cantilever up or down to obtain topography of the samples. The most AFM imaging modes involve a feedback loop which regulates the tip-sample distance, as shown in the Fig 2.6.

\textbf{Fig 2.6} Laser reflection method and schematics of a feedback loop. Any vertical deflection of the cantilever is measured by the signal composed by subtracting the photocurrent on the bottom of the detector (3+4) from the photocurrent on the top of the detector (1+2)\textsuperscript{12}.
In the contact mode, there are two basic ways to obtain topography: **constant force mode**, which is a constant deflection mode, and **variable force mode**, which is a free deflection mode. The schematics for the contact force mode is shown in Fig.2.7.

![Fig 2.7 Contact modes.](image)

(a) Constant force mode. The deflection signal \((1+2)-(3+4)\) is taken for the error signal. The circuit maintains that signal equal to the setpoint. To do that the feedback loop circuit changes the piezovoltage to maintain a constant deflection. The piezovoltage is taken as topography.

(b) Variable force mode. In this mode, the feedback loop is disabled. When the tip scans the surface, the cantilever is free to deflect following the surface. The deflection of the cantilever is taken as topography.

In the non-contact mode region, Van der Waals forces are in effect. Some other forces such as electrical and magnetic are also in action. However, if the surface is not charged and if the surface and tip are not ferromagnetic, the predominant forces are Van der Waals. The schematic of non-contact mode is given in Fig. 2.8.
AFM is not only used for topographic measurement but also used for non-topographic measurements. The physical properties that have been measured using AFM include mechanical, electrical, and magnetic. Force-distance plots of the interaction of the tip and surface can be presented in three different modes. Among the mechanical properties that have been obtained by AFM, friction is one of the most studied properties. Friction can be obtained by three techniques: lateral force mode, lateral force modulation, and torsional resonance mode. Lateral force mode is a widely used technique. Some electrical properties have also been obtained by AFM. In contact mode, conductivity measurements have been realized by applying a potential between the tip and the bottom electrode of the sample and measuring the current through the tip by a low-noise amplifier. In tapping mode, also conductivity measurements have been done based on the same principle that the tip is in contact for some time.

Ultrasonically cleaned 20×20 mm², 0.2-mm-thick glass slides were covered with 0.1 mL of precursor solution, and spin-coated at 2000 rpm for 30 s to form the polymer/metal nanoparticle composite layer. The texture and morphology of the film materials were investigated by atomic force microscopy (Digital Instruments AFM Nanoscope III) in tapping mode.
2.2.6 Dynamic Light Scattering and Zeta-potential

What causes scattering of light?

Light is an electro-magnetic wave. The oscillating electromagnetic field induces oscillations of the electrons in a particle.

Over the years many features of the scattered light have been used to determine particle size. One of these features is fluctuations about the average intensity. This phenomenon is the basis for quasi elastic light scattering (QELS). The random motion of small particles in a liquid gives rise to fluctuations in the time intensity of the scattered light. The fluctuation is processed by forming the autocorrelation function, $C(t)$, $t$ being the time delay. As $t$ increases correlation is lost, and the function approaches the constant background term $B$. For short times the correlation is high. In between these two limits the function decays exponentially for a monodisperse suspension of rigid, globular particles and is given by

$$C(t) = Ae^{-2\pi} + Bt$$

where, $A$ is an optical constant determined by the instrument design and $\tau$ is related to the relaxation of the fluctuations by $\tau = Dq^2$

The value of $q$ is calculated from the scattering angle $\theta$, the wavelength of the laser light $\lambda_o$, and the refractive index of the suspending liquid, using following equation

$$q = \frac{2\pi n}{\lambda_o}2\sin\left(\frac{\theta}{2}\right)$$

The translational diffusion coefficient, $D$, is the principle quantity measured by QELS. It is an inherently interesting property of particles. Particle size is related to $D$ for simple common shapes like a sphere, ellipsoid, cylinder and random coil. Of these, the assumption for spherical particles is most useful in the greatest number of cases. For a sphere, $D = \frac{K_BT}{3\pi\eta(t)d}$; where “$d$” is the diameter of the particle, $K_B$ is Boltzmann’s constant ($1.38054\times10^{-16}$ ergs/deg), $T$ is the temperature in °K, $\eta(t)$ (in centi poise) is the viscosity of the liquid in which the particle is moving.
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The theory behind the zeta-potential is when particles dispersed in a liquid have a charge on the surface, on application of an electric field in the liquid these charged particles will move towards either the positive or negative pole of the applied field. The direction they select is a clear indication of the sign of the charge they carry. The velocity with which they translate is proportional to the magnitude of the charge and size. Thus from the direction and the velocity of the particles under the influence of a known electric field mobility and zeta-potential is calculated.

The particle size distributions and zeta-potential of the synthesized particles were measured using a 90 Plus DLS unit from Brookhaven (Holtsville, USA) with 15mW solid laser (660 nm) and scattering angle 90°. Appropriate dilutions were made as and when required.

2.2.7 X-Ray Diffraction (XRD)

XRD is a very important technique that has long been used to address issues related to the crystal structure of solids, including lattice constants and geometry, identification of unknown materials, orientation of single crystals etc. In XRD a collimated beam of X-rays, with a wavelength typically ranging from 0.7 to 2 Å, is incident on a specimen and is diffracted by the crystalline phases in the specimen according to Bragg's law: \( \lambda = 2d \sin \theta \); where \( d \) is the spacing between atomic planes in the crystallite and \( \lambda \) is the wavelength of the X-ray used. The intensity of the diffracted X-rays is measured as a function of the diffraction angle \( 2\theta \) and the specimen's orientation. XRD is a nondestructive method and does not require elaborate sample preparation, which partly explains the wide use of XRD in material characterization.

Diffraction peak positions are accurately measured with XRD, which makes it the best method for characterizing homogeneous and heterogeneous strains\textsuperscript{13}. Homogeneous or uniform elastic strain shifts the diffraction peak positions. From the shift in peak positions, one can calculate the change in d-spacing, which is the result of the change of lattice constants under a strain.
Heterogeneous strains vary from crystallite to crystallite or within a single crystallite and this cause a broadening of the diffraction peak that increases with \( \sin \theta \). Peak broadening is also caused by the finite size of crystallites, but here the broadening is independent of \( \sin \theta \). When both crystallite size and heterogeneous strain contribute to the peak width, these can be separately determined by careful analysis of peak shapes. If there is no inhomogeneous strain, the crystallite size, \( D \), can be estimated from the peak width using Scherrer's formula:

\[
D = \frac{K\lambda}{B \cos \theta_B}
\]

where \( \lambda \) is wavelength of X-ray, \( B \) is the full width of height maximum (FWHM) of a diffraction peak, \( \theta_B \) is the diffraction angle, and \( K \) is the Scherrer's constant of the order of unity for usual crystal.

The X-ray powder diffraction patterns of materials were recorded with a computer-controlled RIGAKU-DMAX-2200 apparatus over the range 15- 70° 2\( \theta \) range at a scan rate of 1° min\(^{-1} \) using CuK\( \alpha \) radiation monochromatize with a graphite crystal.

### 2.2.8 Energy Dispersive X-ray (EDX) Analysis

EDX is sometimes referred also as energy dispersive x-ray spectroscopy (EDS) analysis. It is a technique used in identifying the elemental composition of the specimen, or an area of interest thereof. The EDX analysis system works as an integrated feature of an electron microscope (EM), and can not operate on its own without the latter.

During EDX Analysis, the specimen is bombarded with an electron beam inside the electron microscope. The bombarding electrons collide with the electrons of the specimen’s atom, knocking some of them off in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. To be able to do so, however, the transferring outer electron must give up some of its energy by emitting an X-ray. The amount of energy released by the transferring electron depends on which shell it is transferring from, as well as which shell it is transferring to. Furthermore, the atom of every element releases X-rays with unique amounts of energy during the
transferring process. Thus, by measuring the amounts of energy present in the X-rays being released by a specimen during electron beam bombardment, the identity of the atom from which the X-ray was emitted can be established. The principle of EDX is given in Fig 2.9.

Fig 2.9 Principle of EDX

The output of an EDX analysis is an EDX spectrum (Fig 2.10). The EDX spectrum is just a plot of how frequently an X-ray is received for each energy level. An EDX spectrum normally displays peaks corresponding to the energy levels for which the most X-rays had been received. Each of these peaks is unique to an atom, and therefore corresponds to a single element. The higher a peak in a spectrum, the more concentrated the element is in the specimen.
Figure 2.10 Example of an EDX Spectrum

The energy dispersive X-ray analysis was undertaken with a Princeton Gamma Tech Prism 1G system with a 10 mm² silicon detector attached to the TEM and the peaks were analysed with Imix 10.594 software.
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2.3 Reference: