CHAPTER II
CHARACTERISATION TECHNIQUES

2.0 Introduction

Nanoparticles are becoming increasingly important part in our everyday lives from modern electronics to medical to diagnostics to protein based therapeutics. Nanoparticles have unique physical and chemical properties. For example the electronic, optical and chemical properties of nanoparticles may be very different from their bulk. At nanoscale levels materials behave very differently as compared to larger scales. The fascinating properties of the nanoparticles have triggered a massive advance of nanomaterials, in material science and nanotechnology and its commercial applications in the last twenty years. It is thus necessary to establish an understanding and control of nanoparticle synthesis and applications. The applications of the nanoparticles are dependent upon their principal parameters like, shape, size, surface characteristics, inner structure and homogeneity. Thus nanoparticle characterization and the knowledge of the principal parameters are very essential. It is strictly due to the improvement of characterization on the nanometer scale that this area of nanotechnology has seen rapid development. Characterization is done by using a variety of different techniques, mainly drawn from materials science. The common techniques are electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy, dual polarization interferometry and nuclear magnetic resonance (NMR).

2.1 UV-visible spectroscopy

Ultraviolet-visible absorption corresponds to excitation of an electron from ground electron state to a higher electron state. Each photon of the incident radiation must possess energy that corresponds to the energetic difference between the electron levels. The energetic transition can occur from any of the rotational and vibrational
level that are associated with the lower electron state to any of the rotation and vibrational levels that are associated with higher electron state.

In order to obtain useful information from the ultraviolet visible spectrum of a compound the wavelength of maximum absorption ($\lambda_{\text{max}}$) and intensity of absorption must be measured accurately\(^1\). The compound or sample must be dissolve in a suitable solvent that does not itself absorb light in the region under investigation. The most suitable source of light in the UV region is deuterium discharge lamp. A tungsten filament lamp is generally used in the visible region.

In most cases absorption occurs when a ground state electron in a bonding or non-bonding molecular orbital is excited by incident radiation to a higher molecular orbital\(^2\).

$$\Delta E = h\nu.$$

A sigma ($\sigma$) orbital is an orbital that has cylindrically symmetrical electron density around the internuclear axis. Sigma orbitals are those that correspond to single bonds between atoms and to one of bonds in the double or triple bonds. Molecular orbitals that do not have cylindrically symmetrical electron density around the internuclear axis is a Pi ($\pi$) orbital. Pi molecular orbitals are usually formed from side by side overlap of atomic P orbitals. A cloud of electron exists above and below the internuclear axis, but not completely around the axis. These molecular orbitals are vacant in the ground or unexcited state and are commonly called as antibonding orbital. The antibonding orbitals associated with $\sigma$ and $\pi$ bonds are called $\sigma^*$ and $\pi^*$, respectively. The molecular orbital neither contributes to the stability nor lack of stability of a molecule, consisting of unshared electron pairs. The energy that is associated with the electrons in the bond is identical to the energy of the same electrons in the individual atoms that formed the molecule. An orbital of that type is a nonbonding molecular orbital and is symbolized by $n$.

The electronic transitions that are involved in the ultraviolet or visible regions are of the following type

$$\sigma \rightarrow \sigma^*, \quad \pi \rightarrow \sigma^*, \quad n \rightarrow \sigma^*, \quad \text{and} \quad \pi \rightarrow \pi^*.$$
The $\delta \rightarrow \delta^*$ transitions require the greatest energy and generally occurs in vacuum ultraviolet region and the vacuum ultraviolet region is not accessible in most of the ultraviolet visible spectrophotometers, the transitions generally are not of use for routine analysis.

Compounds that contain non bonding electrons on oxygen, nitrogen, sulphur or halogen atoms are capable of showing absorption owing to $n \rightarrow \delta$ transitions. These transitions are of lower energy than $\delta \rightarrow \delta^*$ transitions. Transitions to antibonding $\pi^*$ orbitals are associated only with unsaturated centers in the molecules, these are of still lower energy requirement and occur at longer wavelength usually well within the region of ordinary light e.g. saturated aldehydes and ketones exhibit an absorption of low intensity around 285 nm which is attributed to $n \rightarrow \pi^*$ transition and an absorption of high intensity around 180 nm which is attributed to $\pi \rightarrow \pi^*$ transitions.

2.2 Infrared spectroscopic analysis

Infrared absorption occurs when the frequency of the alternating electric field that is associated with the incident radiation matches, a possible change in a vibrational or rotational frequency of the absorbing molecule. When a match occurs EMR can be absorbed by the molecule causing a change in the amplitude of vibration or a change in the rate of rotation.

In order for electromagnetic radiation to be absorbed by a molecule it is necessary for the molecule to undergo a change of dipole moment during the absorption. The molecules that have a completely symmetrical charge distribution and in which no change in dipole moment occurs when the molecules vibrates with different amplitude or rotates at a different rate do not absorb infrared radiation. Substances that are transparent to infrared radiation are primarily monoatomic and homonuclear diatomic gases such as He, Ne, Cl$_2$, N$_2$ and O$_2$. Nearly all other substances absorb radiation in the infrared region.

The application of infrared spectroscopy to the identification of unknown organic compound is well established and widely used. Infrared spectra of organic
compounds generally exhibit sharp well defined bands, assignable to the vibrations of the individual functional groups in the molecule. However, the infrared spectra derived from inorganic compounds usually have absorption bands that are broad and overlapping, making assignments and the specific identification of a cation anion pair more difficult. FTIR spectroscopy also leads to know surface composition, as the surface properties are strongly modified according to the experimental conditions of preparation. It is a useful technique for identification of the nature and sites present. The organic compounds absorb electromagnetic energy in the infrared region of the spectrum. Infrared radiation does not have sufficient energy to cause the excitation of electrons, however, it causes atoms and groups of atoms of organic compounds to vibrate faster about the covalent bonds which connect them. The vibrations are quantized, and as they occur, the compound absorbs infrared energy in particular regions of the spectrum. The position of an infrared band is specified in frequency units by its wave numbers measured in reciprocal centimeters (cm$^{-1}$) or by its wavelength ($\lambda$) measured in micrometers.

In an infrared spectrometer infrared radiation of successively increasing wavelength is passed through the sample of the compound and the percent transmittance is measured. An infrared spectrum is the graph of percent transmittance versus either increasing wavelength or decreasing frequency.

For IR absorption to occur two major conditions must be fulfilled. First the energy of the radiation must coincide with the energy difference between the excited and ground state of the molecules. Second, the vibration must entail a change in the electrical dipole moment a restriction that distinguishes IR and Raman spectroscopy.

Many of the vibration modes in the fingerprint region depend on complex vibrations involving the entire molecule, it is impossible for any two different compounds (except enantiomers) to have precisely the same IR spectrum. In fact, this region from 600 to 1400 cm$^{-1}$ is called the fingerprint region because the pattern of absorptions in this region is unique for a particular compound. The infrared region constitutes three parts, the near infrared the middle infrared and the far infrared. The near infrared region corresponds to energies in the range 37-10 kals mole$^{-1}$. As there
are few absorptions of organic molecular in this range, it is of little use for spectroscopic purposes. Radiation in the middle infrared region $E = 10^{-1}$ kcals mole$^{-1}$ which corresponds to the differences commonly observed between vibrational states$^3$. Spectroscopy in the middle infrared region, usually 4000-650 cm$^{-1}$ is extremely useful for the study of organic compounds. The far infrared has $E = 1.0 - 0.1$ kcals mole$^{-1}$. This region is also not much use for organic spectroscopy, since only little useful absorption occurs.

<table>
<thead>
<tr>
<th>Wavenumber cm$^{-1}$</th>
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<tr>
<td>10000</td>
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<tr>
<th>Near IR</th>
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$\lambda$ (µm)

In the present work, quaternary ammonium salts are used as capping agent as well as supporting electrolyte. When electrochemical reduction reaction was carried out for preparation of nanoparticles of Au and Ag capping phenomenon was very important and necessary for obtaining the nanoparticles of different size. It was confirmed by the IR spectra of washed and dried samples of nanoparticles. Different peaks were observed corresponding to frequencies of different functional groups present in the quaternary ammonium salts. IR spectra were also obtained for the samples heated upto 800 °C. These did not show any peaks corresponding to the functional groups.

2.3 X-ray Diffraction (XRD) analysis

X-ray crystallography is used to determine the spacing between atoms in crystal. The inter-atomic spacing is used to deduce the crystalline structure. In addition to use in structural determinations, X-ray diffraction can be used in qualitative and quantitative analysis.
Interaction of X-rays with the electrons of the matter, through which they pass results in scattering. When the X-rays are scattered by the ordered environment in a crystal, interferences (both constructive and destructive) takes place among the scattered rays because the distance between the scattering centers are of the same order of magnitude as the wavelength of radiation. The phenomenon is known as diffraction. X-ray diffraction of crystals was studied by Bragg in 1912.

The Braggs equation shows that the conditions of constructive interferences of the beam at angle \( \theta \) are,

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

In other words, X-rays appear to be reflected from the crystal only if the angle of incidence satisfies the condition.

\[ \sin \theta = \frac{n\lambda}{2d} \]

where,

\( n = \) order of reflection

\( \lambda = \) wavelength of X-ray used.

\( d = \) interplanar distance

\( \theta = \) angle of diffraction

X-ray diffraction has provided a lot of important information to science and industry. X-ray diffraction pattern is unique for each crystalline substance therefore is currently of prime importance in elucidating the structures of metals, polymeric materials and complex natural products such as steroids, vitamins and antibiotics. A crystalline substance produces a good X-ray diffraction pattern if the size of the particles lies in the range \( 10^{-3} \) to \( 10^{-5} \). For larger crystals the pattern becomes spotty because the number of crystallities in the part exposed to X-ray radiation is insufficient. If the size is smaller than this then the line becomes broad.
The relation between the width of diffraction line and particle size was first derived by Scherrer in 1918. The Scherrer formula is,

\[ D = \frac{k\lambda}{\beta \cos \theta} \]

where \( k = \) shape constant (0.94), \( \lambda = 1.54 \text{ Å} \) for CuK\(_\alpha\) and \( \beta = \frac{\beta' \pi}{180} \)

where \( \beta' = \) full width at half maximum (FWHM)

The inter-planar distances of the particles were calculated by using Bragg’s equation. X-ray analysis was carried out to estimate the particle size and to determine the phase of a substance. The samples were prepared on a glass plate and scanned from 20 to 80° on an X-ray powder diffractometer PW 1840.

### 2.4 X-ray Photoelectron Spectroscopic (XPS) analysis

The X-ray photoelectron spectroscopy known as XPS has been developed in the fifties by K. Siebahn for which he was awarded the Physics Noble Prize in 1981. XPS\(^{5,6}\) is a surface science technique used to study the composition and electronic state of the surface region of a sample. Since the technique provides a quantitative analysis of the surface composition it is sometimes known by the alternative acronym ESCA (Electron Spectroscopy for Chemical Analysis).

The working principle of XPS is based on well known photoelectric effect (a single photon in / electron out process) first experienced by Einstein in 1905. Photoelectron spectroscopy uses monochromatic sources of radiation (i.e. photon of fixed energy given by relation \( E = h\nu \)). In XPS, the photon is absorbed by an atom in a molecule or solid leading to the ionization and the emission of a core (inner shell) electron. The kinetic energy distribution of the emitted photoelectrons (i.e. the number of emitted photoelectrons as a function of their kinetic energy) can be measured using any appropriate electrons energy analyzer and a photoelectron spectrum can thus be recorded. The one way to look at the overall process of photoionization is follows.
\[ A + h\nu = A^+ + e^- \]

Conservation of energy then requires that
\[ E(A) + h\nu = E(A^+) + E(e^-) \]

Since the electron energy is present solely as kinetic energy (KE) this can be rearranged to give the following expression for the KE of the photoelectron
\[ KE = h\nu - [E(A^+) - E(A)] \]

The final term in brackets, representing the difference in energy between the ionized and neutral atom is generally called the binding energy (BE) of the electron, this then lead to the following commonly quoted equation
\[ KE = h\nu - BE \]

The binding energies (BE) of energy levels in solids are conventionally measured with respect to the Fermi-level of the solid rather than the vacuum level. This involves a small correction to the equation given above, in order to account for the work function (\(\Phi\)) of the solid.
\[ KE = h\nu - BE - \Phi \]

Employing photons with fixed energy, \(h\nu\), it is obvious that if kinetic energy KE and the work function of the sample are measured it is possible to measuring binding energy of electron in solids. Binding energies being characteristic of atoms, different elements present in the sample under investigation are identified. Electrons traveling through a material have a relatively high probability of expiring inelastic collisions with locally bound electrons as a result of which they suffer energy loss and contribute to the background of the spectrum rather than a specific peak. Due to inelastic scattering process the flux of photoelectrons emerging from the sample is much attenuated.

The soft X–rays employed in XPS penetrate a substantial distance into the sample (\(\mu m\)). Thus this method of excitation imparts no surface sensitivity at the required atomic scale. However the photoelectrons can escape from only a very short distance beneath the surface (< 100 \(^0 A\)). The surface sensitivity thus arises from the emission and detection of the photo emitted electrons.
2.5 Scanning Electron Microscopy (SEM)

The scanning electron microscope is a type of electron microscope that images the sample surface by scanning it with a high energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the samples surface topography, composition and other properties such as electrical conductivity. In most applications, data are collected over a selected area of the surface of the sample and a 2-dimensional image is generated that displays special variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques. The SEM is also capable of performing analysis of selected point locations on the sample, this approach is especially useful in qualitatively or semi quantitative determining chemical composition (using EDS), crystalline structure, and crystal orientations (using EBSD).

The electron microscope consists of an electron source, an anode, magnetic lenses, apertures, specimen, stage and image recording system, all of which operate in a high vacuum. In a typical SEM, an electron beam is thermo ionically emitted from an electron gun fitted with a tungsten filament cathode. The electron beam, which typically has an energy ranging from 0.5 Kev to 40 Kev, is focused by one or two condenser lenses to a spot about 0.4 nm to 5 nm in diameter. The beam passes through pairs of scanning coils or pairs of deflectors plates in the electron column, typically in the final lens, which deflects the beam in the x and y axis so that it scans in a raster fashion over a rectangular area of the sample surface.

When the primary electron beam interacts with the samples, the electrons lose energy by repeated random scattering and absorption within a tear drop shaped volume of the specimen known as the interaction volume, which extends from less than 100 nm to around 5 μm into the surface. The size of the interaction volume depends on the electrons landing energy, the atomic number of the specimen and the specimen’s density. The exchange of energy between the electron beam and the sample result in reflection of the high energy electrons, due to the elastic scattering. The emission of the electromagnetic radiations can be detected by specialized
detectors. The beam current absorbed by the specimen can also be detected and used to create images of the distribution of specimen current. Electronic amplifiers of various types are used to amplify the signals which are displayed as variation in brightness on the cathode ray tube. The raster scanning of the CRT display is synchronized with that of the beam on the specimen in the microscope, and the resulting image is therefore a distribution map of the intensity of the signal being emitted from the scanned area of the specimen. The image may be captured by photography from a high resolution cathode ray tube, but in modern machines it is digitally captured and displayed on a computer monitor and saved to computer hard disk.

For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface and electrically grounded to prevent the accumulation of electrostatic charge of the surface. Metal objects require little special preparation for SEM except for cleaning and mounting on a specimen stub. Non-conductive specimens lend to charge when scanned by the electron beam, and especially in secondary electron imaging mode, this causes scanning faults and other image artifacts. They are therefore usually coated with an ultrathin coating of electrically conducting material, commonly gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation. Conductive materials in current use for specimen coating\textsuperscript{7,8} include gold, gold/palladium alloy, platinum, osmium, iridium, tungsten, chromium and graphite. Coating prevents the accumulation of static electric charge on the specimen during electron irradiation. The two reasons for coating, even when there is enough specimen conductivity to prevent charging, are to increase signal and surface resolution, especially with samples of low atomic number. The improvement in resolution arises because backscattering and secondary electron emission near the surface are enhanced and thus an image of the surface is formed.
Figure 2.5.1   Scanning Electron Microscope
2.6 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy\textsuperscript{9,10} is being extensively used for measuring the particle size of small metal particles. The electron microscope uses an electron beam in place of beam of visible light, by an optical microscope. The electron source can be made of various types of materials. The most common is the tungsten filament which, when heated produces electrons which are attracted by the anode and are accelerated down to the column and interacts with the specimen. The electrons are focused using magnetic lens in the column and the apertures filter out scattered electrons, so the resulting beam is monochromatic. This monochromatic beam is focused and interacts with the specimen in different ways depending upon the type of electron microscope. These interactions are detected and converted into an image by the image recording system. This system converts the radiation into permanent image either on a photographic film or into a digital image. In the TEM the focused monochromatic electron beam interacts with and is transmitted through the sample, focused into an image and projected onto a phosphor coated screen which emits visible light. The brighter areas of the image represent areas where more electrons have passed through the sample. The darker areas represent the areas where few electrons have passed through as a result of higher specimen density. The other effect produced by the striking electron emits x-ray photons, Auger electron, secondary electrons, backscattered electron, etc. Many of these effects are used for characterizing the specimen. Transmission electron microscopes record the information carried by transmitted electron, unscattered as well as elastically and inelastically scattered.

The use of high energy electrons (100 Kev or more) has the advantage that it increases the resolving power, reduces the chromatic aberration and decreases sample heating. The resolution of a commercial instrument in 10 A\textsuperscript{0} and the corresponding magnification is about 10\textsuperscript{6} X, although there exist special electron microscope with a resolution of 2A\textsuperscript{0}.

The sample is taken on the grid. The grid is a sieve woven from a thin metal wire usually nickel or copper. Initially a thin collodion film is produced on the grid, which is again coated with carbon film. Then a drop of sample is placed on the carbon film, which rests on an electron microscope grid.
Electron diffraction pattern produced in transmission in the electron microscope can be of three different types.

- Ring pattern – polycrystalline
- Spot pattern – single crystal region of the species
- Kikuchi line pairs – bands in electron diffraction

The b and c patterns often occur on the same diffraction pattern. In general, spot and Kikuchi patterns are taken from a specific area of the specimen and are known as “selected area diffraction pattern” (SADP).

de’Broglie proposed that particles could exhibit wave behavior in the fashion that was described as having both particle and wave behavior. He hypothesized that wavelength; \( \lambda \) associated with the moving particle of velocity \( v \) is given by:

\[
\lambda = \frac{h}{p} = \frac{h}{mv}
\]

where \( m = 9.11 \times 10^{-31} kg \)

if acceleration voltage is 200 kv then

\[
T = 200000 \times 1.6 \times 10^{-19} J/ev
\]

\[
T \text{ (Tesla)} = 3.2 \times 10^{-14}
\]

From de Broglie Principle

\[
T \frac{p^2}{2m} = \frac{h^2}{2m\lambda^2}
\]

\[
\lambda = \frac{6.626 \times 10^{-34} Js}{2(9.1 \times 10^{-31} kg)(3.2 \times 10^{-14})}
\]

\[
\lambda = 0.027 \, \text{Å}^0
\]

\[
\theta = \frac{D}{2L}
\]

\( D \) = diameter of diffraction ring
\( \lambda \) = wavelength of electron
\( L \) = distance of sample to the screen
\( \theta \) = diffraction angle
Figure 2.6.1  Transmission Electron Microscope
References