Chapter 2

Characterization and instrumentation techniques

2 Introduction

Characterization of the as-synthesized nanoparticles using various available instrumentation technique and careful evaluation of their various physical and chemical properties remains an integral part in the study of nanoscience. Hence, one needs to adapt and modify/develop a range of methods for the characterization of structural, electrical, magnetic and optical properties of nanostructured systems. Primarily characterization consists of two main categories: structure analysis and property measurements. Characterization techniques such as microscopy and spectroscopy etc. are used in this investigation. In this chapter, principles on the working of X-ray diffraction, electron microscopy such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) will be discussed. Also, spectroscopy techniques like UV-visible absorption, Fourier Transform Infrared Spectroscopy (FTIR), fluorescence spectroscopy, etc. And also a brief principle on thermal analysis and cyclic voltammetry is discussed.

2.1 X-ray diffraction (XRD)

X-ray diffraction is a physical phenomenon as well as an experimental method for the characterization of materials. On November 8th, 1895, Röntgen discovered by accident a new kind of radiation. While he was using a Crookes tube, he noticed a glow on a plate, covered with barium platinocyanide, and rather far away from the tube. Röntgen, who was working at the time on the cathode rays produced by Crookes tubes, immediately understood that the glow he was observing could not be caused by this radiation. Realizing the importance of his discovery, and before making it known to the scientific community, he tried for seven weeks to
determine the nature of this new kind of radiation, which he named himself X-Strahlen. On December 28th, 1895, Röntgen presented his observations before the Würzburg Royal Academy of Physics and Medicine. Immediately after this discovery, a large number of studies were launched to find out the nature of this radiation.

It was found that when matter is irradiated with a beam of X photons, it emits an X-ray beam with a wavelength equal or very close to that of the incident beam, which is referred to as scattering effect. The analysis of the diffracted intensity makes it possible to characterize the structure of the material being studied. This constitutes the core elements of X-ray diffraction.

First of all, consider an atomic plane. The paths between the two wave planes \( \pi' \) and \( \pi \) of the waves scattered by the atoms located in \( \Lambda \) and \( \Lambda_1 \) are equal if the angle between the atomic plane and the incident beam is equal to the angle between this plane and the scattered beam.

In addition to this first condition, the waves scattered by each plane of the considered family have to be in phase. As we have just seen, the waves scattered by each of the planes have the phase of the wave scattered by an arbitrary atom in these planes. The path difference between the wave scattered by the atom located in \( \Lambda' \) and the wave scattered by the atom located in \( \Lambda \) is written \( \delta = HA' + A'K \). If \( d \) is the interplanar distance that characterizes this family of planes, then \( \delta = 2d \sin \theta \), but this path difference has to be a multiple of the wavelength, hence:

\[
\hat{n} = 2d_{hkl} \sin \theta \tag{2.1}
\]

This relation is called Bragg’s law and it constitutes, along with the equation that leads to the scattered intensity, the core of radiocrystallography. It shows how
the interplanar distances in a given crystal can be calculated from the measurements of the diffraction angles. Note that if we consider a family of plane with indices \((hkl)\), for a given wavelength \(\lambda\), the diffraction angle (Bragg angle) is given and therefore we will observe a wave diffracted by this family of planes if and only if the angle between this family and the incident wave is equal to the one given by Bragg’s law.

When Bragg’s law is satisfied, the reflected beams are in phase and interfere constructively so thus diffraction peaks are formed. From the diffraction peak corresponding to \(2\theta\) values, one can find the phase of the material by comparing the literature of Powder Diffraction File (Joint Committee on Powder Diffraction Standards, Swarthmore, USA). Knowing the \(d\)-spacing and corresponding \(hkl\) planes it is possible to find the lattice parameters of the materials using the following:

(i) For cubic system,
\[
\frac{1}{d^2} = \frac{h^2+i^2+k^2}{a^2} \quad (2.2)
\]

(ii) For tetragonal system,
\[
\frac{1}{d^2} = \frac{h^2+i^2}{a^2} + \frac{l^2}{c^2} \quad (2.3)
\]

(iii) For hexagonal system,
\[
\frac{1}{d^2} = \frac{4}{3} \left( \frac{h^2+2hk+k^2}{a^2} \right) + \frac{l^2}{c^2} \quad (2.4)
\]

(iv) For monoclinic system,
\[
\frac{1}{d^2} = \frac{1}{\sin^2 \beta} \left( \frac{h^2}{a^2} + \frac{i^2 \sin^2 \beta}{b^2} + \frac{l^2}{c^2} - \frac{2bh \cos \beta}{ac} \right) \quad (2.5)
\]

Where \(a, b, c\) are lattice parameters of the crystal and \(\beta\) is angle.

The average crystal size can be calculated from the width of the diffraction curve using Scherrer equation (Eqn. 2.6).
\[
r = \frac{0.9 \lambda}{BCos \theta_b} \quad (2.6)
\]

Where,
\(d\) is the crystallite size,
\(K\) is the shape factor (usually taken as 0.9 for dimensionless shape),
\(\lambda\) is the X-ray wavelength,
\(\beta\) is the measure of broadening at full width at half maximum (FWHM) in radians, and \(\theta\) is the Bragg angle.
2.2 Scanning electron microscopy (SEM)

SEM consists of an illumination system, specimen stage, objective lens, the magnification system, the data recording system(s) and chemical analysis system. The illumination is typically made of tungsten filament, or LaB6 or Schottky emitter, or a tungsten field-emission tip. Magnification is made of magnetic lenses. There are two or three such lenses above the specimen which act somewhat like condenser lenses and final lens called objective lens which forms incident beam as small as possible (~10 nm) known as electron probe. Fig. 2.2 shows the schematic diagram of SEM. In principle, SEM is based on secondary emission of electrons working with an accelerating voltage of ~30 kV. The entire process of imaging is known as raster scanning and causes the beam to sequentially cover a rectangular area on the specimen. In this process electron probe scans horizontally across the specimen in two perpendicular (x and y) directions. The x-scan is relatively fast and is generated by a saw tooth-wave generator operating at a line frequency \( f_x \). This generator supplies scanning current to two coils, connected in series and located on either side of the optic axis, just above the objective lens. The coils generate a magnetic field in the y-direction, creating a force on an electron (traveling in the z-direction) that deflects it in the x-direction (Fig. 2.2). The y-scan is much slower and is generated by a second sawtooth-wave generator running at a
frame frequency $f_y = f_x/n$ where $n$ is an integer. In this process, the electrons lose energy by repeated random scattering and absorption within a volume of the specimen known as the interaction volume, which extends from less than 100 nm to around 5 µm into the surface. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering, emission of secondary electrons by inelastic scattering and the emission of electromagnetic radiation, each of which can be detected by specialized detectors. The electronically amplified output signal is fed to Cathode ray tubes and image is recorded using high resolution camera. In modern days, the output is digitally captured and displayed in monitor. Apart from imaging, using suitable electron voltage the emitted X-ray can be utilized to characterize the sample composition (Energy dispersive X-ray i.e. EDX). In the entire cases sample should be conductive or made it conductive.

### 2.3 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail even as small as a single column of atoms, which is tens of thousands times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEMs find application in cancer research, virology, materials science as well as pollution and semiconductor research. **Fig.2.3** shows a schematic outline of a TEM. A TEM contains four parts: electron source, electromagnetic lens system, sample holder, and imaging system. The electron source consists of a cathode and an anode (Fig. 2.3).
Characterization and Instrumentation Techniques

Fig. 2.3 Typical schematic diagram of a conventional TEM

The cathode is a tungsten filament which emits electrons when being heated. A negative cap confines the electrons into a loosely focused beam (Fig.2.3). The beam is then accelerated towards the specimen by the positive anode. Electrons at the rim of the beam will fall onto the anode while the others at the center will pass through the small hole of the anode. The electron source works like a cathode ray tube. After leaving the electron source, the electron beam is tightly focused using electromagnetic lens and metal apertures. The system only allows electrons within a small energy range to pass through, so the electrons in the electron beam will have a well-defined energy. Magnetic Lens: Circular electro-magnets capable of generating a precise circular magnetic field. The field acts like an optical lens to focus the electrons. Aperture: A thin disk with a small (2-100 micrometers) circular through-hole. It is used to restrict the electron beam and filter out unwanted electrons before hitting the specimen.

The sample holder is a platform equipped with a mechanical arm for holding the specimen and controlling its position. The imaging system consists of another electromagnetic lens system and a screen. The electromagnetic lens system contains two lens systems, one for refocusing the electrons after they pass through the specimen, and the other for enlarging the image and projecting it onto the
The screen has a phosphorescent plate which glows when being hit by electrons. Image forms in a way similar to photography.

### 2.4 Atomic force microscopy (AFM)

Atomic Force Microscopy works principally on the force of interaction between a sharp tip (probe) with the sample or specimen. Fig. 2.4 illustrates the schematic representation of AFM. AFM has the advantage over SEM that samples need not be conductive and superior topographical contrast.

![Schematic diagram of Atomic Force Microscope](image.png)

**Fig. 2.4 Schematic diagram of Atomic Force Microscope**

AFM can give the three dimensional image without expensive sample preparation. AFM consist of a cantilever with a sharp tip at its end that is used to scan the specimen surface. The cantilever is typically silicon or silicon nitride with a tip radius of curvature on the order of nanometers. It is the heart of the instrument because it is brought in closest contact with the sample surface and gives rise an image through the interaction with the surface of the sample. When the tip is brought into proximity of a sample surface, forces between the tip and the sample lead to deflection of the cantilever according to Hooke's law. Depending on the situation, forces that are measured in AFM include mechanical contact force, van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces, Casimir forces, solvation forces, etc. The interaction force between the tip and sample gives the deflection in the cantilever. The deflection in the cantilever is
monitored by reflected laser beam from the backside of the cantilever (often coated with a thin metal layer to make a mirror) onto a position sensitive photodiodes consisting of two side by side photodiodes. Images are formed by recording the effects of the interaction forces between tip and surface as the cantilever is scanned over the sample. The scanner and the electronic feedback circuit, together with sample, cantilever, and optical lever form a feedback loop set up for the purpose.

2.5 Fourier transformation infrared spectroscopy (FT-IR)

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. The original infrared instruments were of the dispersive type. These instruments separated the individual frequencies of energy emitted from the infrared source. This was accomplished by the use of a prism or grating. In order to overcome this limitation Fourier transform Infrared (FT-IR) spectrometry was developed with a device called an interferometer (Fig. 2.5).

The interferometer produces a unique type of signal which has all of the infrared frequencies “encoded” into it. The signal can be measured very quickly,
usually on the order of one second or so. Most interferometers employ a beam splitter which takes the incoming infrared beam and divides it into two optical beams. One beam reflects off of a flat mirror which is fixed in place. The other beam reflects off of a flat mirror which is on a mechanism which allows this mirror to move a very short distance (typically a few millimeters) away from the beam splitter. The two beams reflect off of their respective mirrors and are recombined when they meet back at the beam splitter. Because the path that one beam travels is a fixed length and the other is constantly changing as its mirror moves, the signal which exits the interferometer is the result of these two beams “interfering” with each other. The resulting signal is called an interferogram which has the unique property that every data point (a function of the moving mirror position) which makes up the signal has information about every infrared frequency which comes from the source. This means that as the interferogram is measured; all frequencies are being measured simultaneously. Thus, the use of the interferometer results in extremely fast measurements. Because the analyst requires a frequency spectrum (a plot of the intensity at each individual frequency) in order to make identification, the measured interferogram signal cannot be interpreted directly. A means of “decoding” the individual frequencies is required. This can be accomplished via a well-known mathematical technique called the Fourier transformation. This transformation is performed by the computer which then presents the user with the desired spectral information for analysis.

2.6 UV-visible spectroscopy

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR) ranges. The absorption/transmittance or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions from the ground state to the excited state. When an electromagnetic light beam is attenuated after passing through a material, the beam intensity attenuation $dI$ after traversing a differential thickness $dx$ can be written as
where $I$ is the light intensity at a distance $x$ into the medium and $\alpha$ accounts for the amount of reduction due to the constitution of the material. In the case of negligible scattering, $\alpha$ is called the absorption coefficient of the material. Upon integration of Eqn. 2.7, we obtain

$$I = I_0 e^{-\alpha x}$$

Fig. 2.6 represents a simple layout of UV-visible spectrophotometer which gives an exponential attenuation law relating the incoming light intensity $I_0$ (the incident intensity minus the reflection losses at the surface) to the thickness $x$. This law is known as the Lambert–Beer law. Fig.2.6 shows the representative schematic diagram of double beam UV-visible spectrophotometer. In this instrument the light coming from the source is split into two beams of equal intensity, which are directed to two different channels of, reference channel and sample channel. The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light, and a detector. The radiation source is often a Tungsten filament (300-2500 nm), a deuterium arc lamp, which is continuous over the
ultraviolet region (190-400 nm), Xenon arc lamps, which is continuous from 160-2,000 nm. The detector is typically a photomultiplier tube, a photodiode, a photodiode array or a charge-coupled device (CCD). It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (Io). The ratio I / Io is called the transmittance, and is usually expressed as a percentage (%T). The absorbance, A, is based on the transmittance:

\[ A = -\log \frac{\text{Io} \%}{100\%} \]  

The UV-visible spectrophotometer can also be configured to measure reflectance. In this case, the spectrophotometer measures the intensity of light reflected from a sample (I), and compares it to the intensity of light reflected from a reference material (Io) (such as a white tile). The ratio I/Io is called the reflectance, and is usually expressed as a percentage (%R).

### 2.7 Luminescence spectroscopy

Luminescence spectroscopy technique is in some ways inverse of the absorption which includes the luminescence processes such as photoluminescence (fluorescence, phosphorescence), chemiluminescence, electroluminescence, cathodoluminescence etc. The photoluminescence (PL) is emission of light which occurs when a light source (i.e. radiation within the optical range) excites a photoluminescent material. Fluorimeters are of two types: (i) Filter fluorometers use filters to isolate the incident light and fluorescent light and (ii) Spectrofluorometers use diffraction grating monochromators to isolate the incident light and fluorescent light. Both types use the following scheme: The light from an excitation source passes through a filter or monochromator, and strikes the sample. A proportion of the incident light is absorbed by the sample, and some of the molecules in the sample fluoresce. The fluorescent light is emitted in all directions. Some of this fluorescent light passes through a second filter or monochromator and reaches a detector, which is usually placed at 90° to the incident light beam to minimize the risk of transmitted or reflected incident light reaching the detector.
The schematic representation of a spectrofluorimeter is shown in Fig. 2.7. Various light sources may be used as excitation sources, including lasers, photodiodes, and lamps; xenon arcs and mercury vapor lamps in particular. Filters and/or monochromators may be used in fluorimeters.

A monochromator transmits light of an adjustable wavelength with an adjustable tolerance. The monochromator can then be adjusted to select which wavelengths to transmit. For allowing anisotropy measurements the addition of two polarization filters are necessary: One after the excitation monochromator or filter, and one before the emission monochromator or filter. As mentioned before, the fluorescence is most often measured at a 90° angle relative to the excitation light. This geometry is used instead of placing the sensor at the line of the excitation light at a 180° angle in order to avoid interference of the transmitted excitation light. No monochromator is perfect and it will transmit some stray light, that is, light with other wavelengths than the targeted. An ideal monochromator would only transmit light in the specified range and have a high wavelength-independent transmission. When measuring at a 90 angle, only the light scattered by the sample causes stray light. This results in a better signal-to-noise ratio, and lowers the detection limit by approximately a factor 10000, when compared to the...
180° geometry. Furthermore, the fluorescence can also be measured from the front, which is often done for turbid or opaque samples. The detector can either be single-channeled or multichanneled with a photomultiplier tube. The single channeled detector can only detect the intensity of one wavelength at a time, while the multichanneled detects the intensity at all wavelengths simultaneously, making the emission monochromator or filter unnecessary.

2.8 Thermal analysis

Thermal analysis deals with the measurement of physical and chemical properties of a material as a function of temperature. The two main analysis techniques consist of thermogravimetric analysis (TGA) and differential thermal analysis (DTA). TGA measures the change in weight of a sample as function of temperature or time and DTA is used to measure the difference in temperature between a sample and an inert reference as a function of temperature therefore detects change in heat content. In TGA, few milligram of the sample is heated with constant heating rate in the range 1 to 20 °C per min and changes in the weight of the sample is recorded over a range of temperature. In DTA the event of temperature change of the sample with reference to inert reference material is same until some thermal event, such as melting, decomposition or change in crystal structure, occurs in the sample, in which case the sample temperature lags behind (if the change is endothermic) or leads (if the change is exothermic) the reference material.

2.9 Cyclic voltammetry

Cyclic voltammetry is a method for investigating the electrochemical behaviour of a system. It was first reported in 1938 and described theoretically by Randles. In this technique current flowing between the electrode of interest (whose potential is monitored with respect to a reference electrode) and a counter electrode is measured under the control of a potentiostat. The voltammogram determines the potentials at which different electrochemical processes occur. The working electrode is subjected to a triangular potential sweep, whereby the potential rises from a start value Ei to a final value Ef then returns back to the start potential at a
constant potential sweep rate. The sweep rate applied can vary from a few millivolts per second to a hundred volts per second. The current measured during this process is often normalised to the electrode surface area and referred to as the current density. The current density is then plotted against the applied potential, and the result is referred to as a cyclic voltammogram. A peak in the measured current is seen at a potential that is characteristic of any electrode reaction taking place. The peak width and height for a particular process may depend on the sweep rate, electrolyte concentration and the electrode material. The shape of a typical cyclic voltammogram for a Nernstian electrochemical reaction is shown in Fig. 2.8. It shows the plot of current vs. potential difference over the electrode surface.

![Cyclic Voltammogram](image)

**Fig. 2.8** A typical cyclic voltammogram for a Nernstian electrochemical reaction

Cyclic voltammetry makes possible the elucidation of the kinetics of electrochemical reactions taking place at electrode surfaces. In a typical voltammogram, there can be several peaks. From the sweep-rate dependence of the peak amplitudes, widths and potentials of the peaks observed in the voltammogram, it is possible to investigate the role of adsorption, diffusion, and coupled homogeneous chemical reaction mechanisms.
References