Chapter 2

Experimental Techniques

This chapter describes the theoretical background of the instrumentation and different experimental techniques used for the characterization of synthesized materials. The nanomaterials and their nanocomposites are extensively characterized by several instrumental techniques like UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffractometer (XRD), Scanning Electron Microscope (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive Spectroscopy (EDX), and X-ray Photoelectron Spectroscopy (XPS). UV-visible spectrophotometer has been used for optical sensing of different analytes and cyclic voltammetry (CV) setup has been used for electrochemical characterization. Differential Pulse Voltammetry (DPV) and Electrochemical Impedimetric Spectroscopy (EIS) have been used for the electrochemical sensing of different analytes. A brief background of every technique is discussed in this proceeding chapter.

2.1 Characterization Techniques:

2.1.1 Optical absorption spectroscopy (UV-vis absorption spectroscopy):

A UV-vis spectroscopy is a very usual and primary instrument that is used for the identification of absorbance or reflectance band of an analyte such as biological macromolecules, extremely conjugated organic compounds, and transition metal ions, present in a liquid medium by passing monochromatic radiation of UV or visible light through the sample. This technique finds is very helpful in studying the functionalization of the surface of the nanomaterials synthesized.

Lambert-Beer's law states that the absorbance of a solution is directly proportional to the concentration of the absorbing analytes in the solution and the path length. Thus, for a secure path length, UV-Vis spectroscopy can be used to govern the concentration of

the absorber in a solution. According to this law, the fraction of incident monochromatic beam absorbed by a homogenous medium is proportional to the number of absorbing molecules confined in a given solution. Lambert-Beer's law can be expressed as:

$$\mathrm{Log}_{10}\, I_0 / I = A = \varepsilon c l \qquad \text{(Eq. 2.1)}$$

Where I_0 is the incident intensity, I is the transmitted beam intensity through a given sample solution, A is measured absorbance, ε is a constant known as the absorptivity or extinction coefficient, c is the concentration of the absorbing species, and l is the path length through the sample. The wavelengths of absorption spectra can be related to the categories of bonds in a given conjugated compound and are resources in determining the functional groups within a molecule.

UV-Vis spectrum is essentially a graph of light absorbed versus wavelength in a range of UV or visible regions. Such a spectrum can often be produced directly by using a UV-Vis spectrophotometer. 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 (I_0). The ratio I/Io is called the transmittance and is usually expressed as a percentage (%T). The relation between the absorbance, A, and % transmittance is given by Eq. 2.2.

$$A = \quad 2 - \log \%T \qquad \text{(Eq. 2.2)}$$

A spectrophotometer (shown in Figure 2.1) comprises a light source, a sample holder, a diffraction grating or monochromator to separate distinctive wavelengths of light, and a detector. The radiation source is often a tungsten filament, a deuterium arc lamp, and more recently light-emitting diodes and xenon arc lamps. The detector is typically a photodiode or a charged coupled device (CCD). A spectrophotometer can either be based on a single beam or a double beam. In a single beam instrument, a fixed wavelength light source or a continuous source is utilized. In either type, the instrument

is calibrated with a reference cell containing only solvent to determine the I_0 value necessary for an absorbance instrument. The dual-beam design greatly simplifies this process by simultaneously measuring the I and I_0 of the sample and reference cells, respectively as shown in Figure 2.2.

Figure 2.1 UV-Visible spectrophotometer

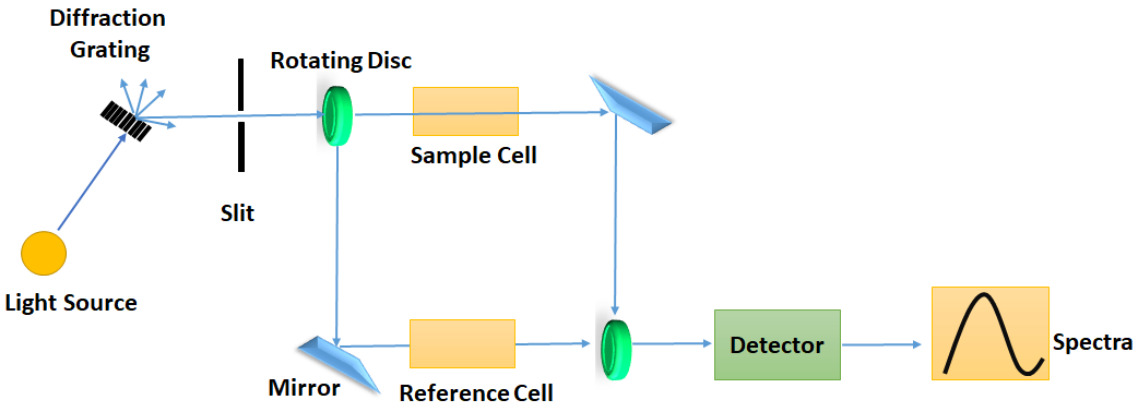

Figure 2.2 Schematic representation of dual-beam UV-Vis spectrophotometer.

2.1.2 Fourier transform infrared spectroscopy (FTIR):

An infrared spectrophotometer focuses a compound on infrared radiation in the range of 5000-450 cm^{-1}. While this radiation is weak, it does supply enough energy for bonds in the molecules to vibrate by stretching or bending. The atoms of a molecule can be measured as linked by springs that are set in motion by the application of energy. When molecules are exposed to the individual wavelength in the 5000-450 cm^{-1} range, it is absorbing only those having accurately the energy essential to cause a definite vibration and transmit some other frequencies. The functional group presents a permanent dipole moment then that molecule is infrared active. Therefore, this instrument is very useful for identifying structure and bonding in molecules due to different functional groups having different vibrations and which is characterized by only infrared spectroscopy. Functional groups attached to the surface of the nanomaterial show different FTIR patterns than those of free groups, hence FTIR provides information about the surface chemistry of nanomaterials. Identification of specific types of chemical bonds or functional groups based on their unique absorption signatures is possible by infrared spectroscopy.

Figure 2.3 FTIR spectrometer

Infrared spectrometers employing an interferometer and having no monochromator are used now a day. These non-dispersive instruments, known as Fourier transform (FT) spectrometers (Figure 2.3), have increased sensitivity and can document spectra much more rapidly than the dispersive type [Skoog et al., 2010; Harvey et al., 1956]. A particular frequency that matches the characteristic frequency of a specific bond will be absorbed, get reflected in a spectrum. The FTIR spectrometer is typically made of an IR light source, sample compartment, interferometer, detector, signal amplifier, and display unit. The IR radiation generated from the light source strikes the sample traveling through the interferometer and reaches the detector. Finally, the signal is amplified and converted to readable form (interferogram) by the involvement of the amplifier and display unit. Eventually, the spectrum is generated from the interferogram through the fast Fourier transform algorithm. [Khan et al., 2018] Typical block diagram for FTIR instrumentation as shown in Figure 2.4.

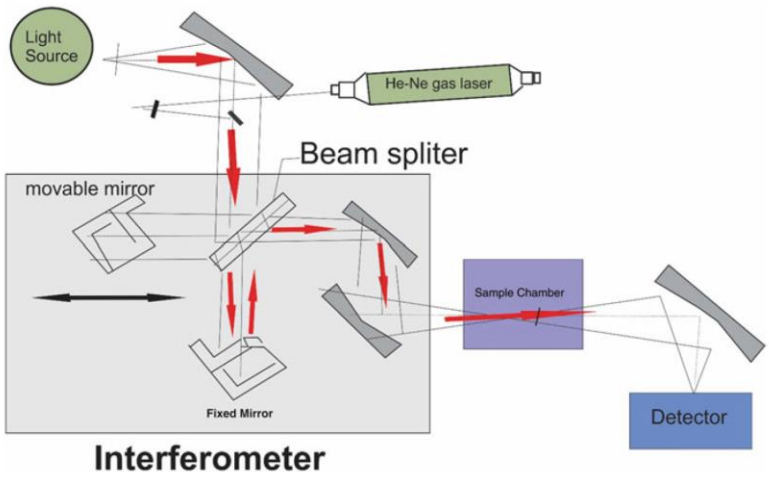

Figure 2.4 Block diagram for FT–IR. [Khan et al., 2018].

FT–IR plot is (%) transmittance vs vibrational frequency in wavenumbers (cm^{-1}). The FT–IR measurements of film and powder samples were conducted in transmission mode.

2.1.3.X-ray Diffractometer (XRD)

X-ray Diffractometer (XRD) is a versatile technique that provides detailed information about the chemical composition as well as crystallographic structure of natural and manufactured materials. The technique's major use is the identification and characterization of substances based on their diffraction pattern. Due to their uniform spacing, the atoms of a crystal produce an interference pattern of the waves present in an incoming beam of X rays in X-ray diffraction as shown in Figure 2.5.

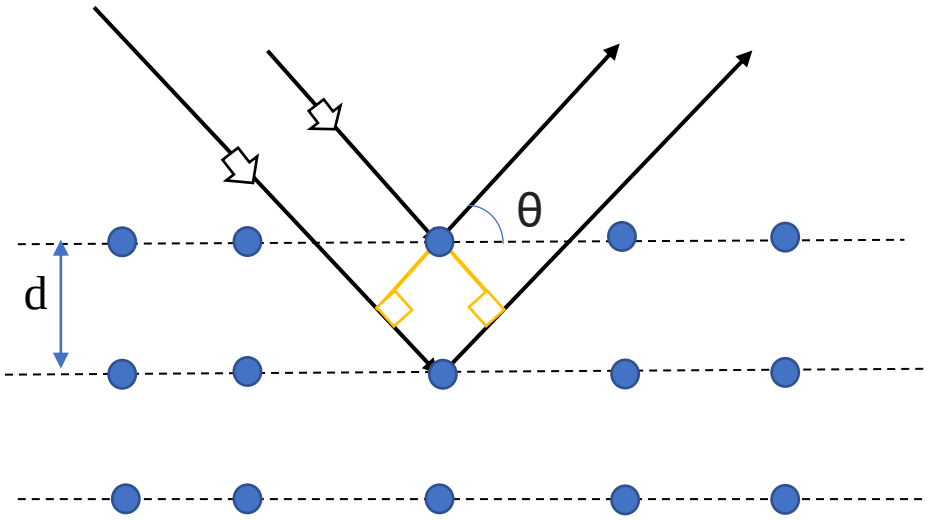

Figure 2.5 Schematic representation of X-ray beam incident on a crystallographic material.

The atomic planes of the crystal act on the X rays in the same manner as an evenly controlled grating operates on a light beam. When an incoming beam of monochromatic X-rays interacts with a target material, the major effect is the scattering of those X-rays from atoms within the target material. The dispersed X-rays undergo constructive and destructive interference in materials with regular structure (i.e. crystalline). This is the diffraction process. Bragg's Law, $n\lambda=2d\sin\theta$, describes the diffraction of X-rays by

(theta). Where λ is the wavelength of the X-rays, d is the spacing of the layers and θ is the incident angle of the photons. By varying the angle θ, Bragg's Law conditions are satisfied by different d-spacing in polycrystalline materials. Plotting the angular positions and intensities of the resultant diffracted peaks of radiation produces a pattern, which is the characteristic of the sample. Where a mixture of different phases is present, the resultant diffractogram is formed by the addition of individual patterns. Most materials, however, are not single crystals, but rather aggregates or powders comprising numerous small crystallites in all conceivable orientations. When an X-ray beam passes through a powder with randomly oriented crystallites, it sees all potential interatomic planes. All conceivable diffraction peaks from the powder will be recognized if the experimental angle is varied systematically. Powder X-ray diffraction (XRD) uses X-rays to investigate and quantify the crystalline nature of materials by measuring the diffraction of X-rays from the planes of atoms within the material. It is sensitive to both the type and relative position of atoms in the material as well as the length scale over which the crystalline order persists. Therefore, it can be used to measure the crystalline content of materials, identify the crystalline phases present (including the quantification of mixtures in favourable cases), and determine the spacing between lattice planes and the length scales over which they persist, and study preferential ordering and epitaxial growth of crystallites. In essence, it probes length scales from approximately sub angstroms (Å) to a few nanometers (nm) and is sensitive to ordering over tens of nanometers.

The prepared mechanically milled and rapidly solidified samples were characterized by using Rigaku Smart Lab X-ray Diffractometer (Figure 2.6). The latter is attached to an X-ray generator operated at 45 keV and 40 mA in most cases. In the present investigation, Cu-Kα (λ=1.5405Å) has been used. The diffractometer was calibrated

using silicon powder. Single crystal silicon was used as a standard for obtaining the accurate value of 2θ for the calculation of the quasi lattice parameter. The XRD patterns were collected from a cross-section of as-cast, powder, and ribbon samples typically over 10-80° in 2θ, using a step size of 3 °/min (these parameters were also varied as per need). The XRD characterization was used routinely to identify the structural phases present in the samples.

Figure 2.6 Miniflex 600 X-ray Diffractometer

1.1.4 X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy is an analytical technique that is surface sensitive. In XPS, the common way of spectroscopic surface measurement comprises the irradiation of a sample with a primary beam made up of photons, electrons, and the impact of this on the surface results in the formation of the secondary beam from the substrate which is measured/detected by the spectrometer. In this technique, X-rays are bombarded at the material surface and the kinetic energy of the ejected electrons is measured. The two major features of this technique are its surface sensitivity and its ability to investigate the chemical state and oxidation state of the elements in the sample. All elements except

helium and hydrogen can be identified. XPS spectrum is the plot of intensity (count/sec) vs. Binding energy (eV). Wide scan XPS spectrum called survey spectrum which generally scans from 0 to 1200 eV binding energy. In the present thesis, we have utilized the XPS spectrum for elemental analysis and identification of the oxidation state of the synthesized nanomaterials and nanocomposites [Skoog et al., 2010; Harvey et al., 1956].

2.1.5 Scanning Electron Microscopy (SEM)

Scanning electron microscopy, (SEM) is one of the most versatile instruments available, which produces magnified images of an object by scanning its surface using a focused stream of electrons to create a high resolution of the image. It gives information about the topography, chemical compositions, and microstructure morphology of both man-made and naturally occurring materials. The adaptability of SEM becomes ideal for a broad range of scientific, research, industrial and commercial applications including biological science, forensics, medical science, electronics and materials science, etc.

A column structure of a conventional SEM includes major components:

1. Electron Guns
2. Electron lenses (Condenser and objective)
3. Apertures
4. Scanning coils
5. Detectors (different for SEs and BSEs)

Electrons are produced from a heated tungsten filament or Lanthanum hexa Boride (LaB_6) single crystals are accelerated by a voltage commonly in the range of 20 V to 30 kV and directed down the centre of an electron optical column usually consisting of different magnetic lenses to generates a focused stream of electrons to strikes the

surface of the specimen. The position of the electron beam on the sample is controlled by scanning coils, which allow the beam to be scanned over the sample surface, situated above the objective lens. Image formation in the SEM is dependent on the acquisition of signals produced from the electron beam and sample interaction. These are inelastic interactions (secondary electrons, SEs) and elastic interactions (backscattered electrons, BSEs) as shown in Figure 2.7 and basic component is shown in Figure 2.8.

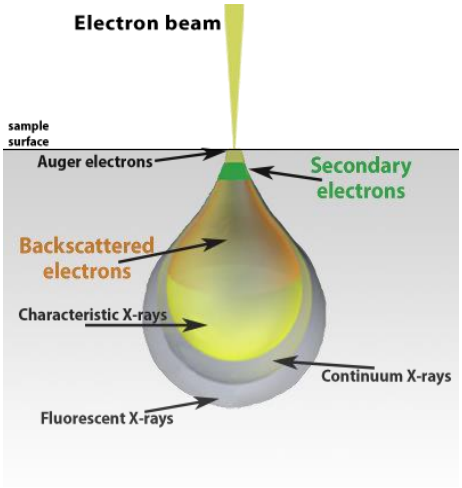

Figure 2.7. Illustration of several signals generated by the electron beam–sample surface interactions

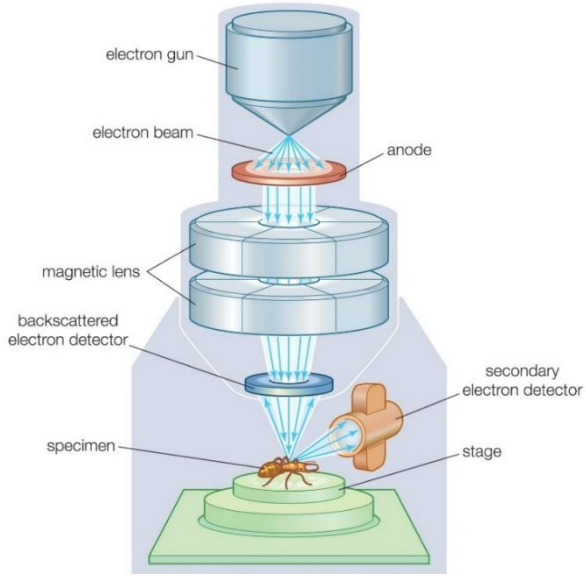

Figure 2.8. Basic components of SEM [Encyclopedia Britannica].

BSEs produced from deeper areas of the sample which provides both topographic and compositional information. SEs originate from surface regions. Hence it gives information about topographic contrast with good resolution for the visualization of surface texture and the roughness. Most nanomaterials (conductive nature) can be observed by SEM directly by loading them on carbon tape. Non-conductive samples (Bioorganic nanomaterials) need metal coating (Gold, Siver, Platinum…etc). In SEM instruments backscattered and secondary electrons are used to construct an image [Skoog et al., 2010; Harvey et al., 1956]. SEM instrument used for characterization is shown in Figure 2.9.

Figure 2.9 Scanning electron microscope.

2.1.6 Transmission Electron Microscopy (TEM)

Transmission Electron microscopy (TEM), is one of the most powerful characterization methods, described as the analysis of the interaction between high-energy beam of electrons (60-300 keV) and a very thin electron transparent sample (<100nm). this interaction generates a number of different signals. In TEM, the transmitted signals, generated during the electron beam and sample interaction, produced a highly resolved and magnified image of the sample. TEM techniques can provide information on the sample's microstructure, chemical composition, and electronic properties at the nanoscale.

A column structure of a conventional TEM includes major components:

1. Electron Gun
2. Condenser and scanning lenses
3. Sample holder
4. Objective lenses
5. Signals detections

The heated tungsten filament or electron gun will start to release electron beams. A condenser lens with a high aperture eliminates all the high angle electrons and focused all the electron beams into a thin small beam. The high-speed electron beams are now transmitted through the specimen and focused into an image with the help of objective lenses. The electron beams are projected onto a phosphorescent screen which creates an image of the specimen. All the images are captured by a CCD camera which is located underneath the screen.

Transmission Electron Microscopy (TEM) is an electron microscopy technique in which high-resolution images of the thin samples. It provides an approach to

characterizing morphology, dimensions, crystalline phases, and distribution of the nanomaterials in the sample. In a typical TEM, a static beam of electrons at 100 – 400 KeV accelerating voltage passes through a region of an electron transparent specimen which is immersed in the objective lens of the microscope [Williams et al., 2010; Skoog et al., 2010] One of the important advantages of the transmission electron microscopy is the capability of TEM for exploring the materials in reciprocal space as well as in real space, i. e. in diffraction and imaging modes. We have utilized a transmission electron microscope (TEM)-FEI-Tecnai 20 U Twin with EDX and Tecnai 20 G2 as shown in Figure 2.10 operated at 200 KeV, for the structural characterization of different nanomaterials.

Figure 2.10 Transmission electron microscope with EDX

2.1.8 Electrochemical Characterizations

In the present work, the electrochemical characterizations of the fabricated electrodes and the electrochemical response have been done by using cyclic voltammetry (CV), Differential Pulse Voltammetry (DPV), Electrochemical Impedance Spectroscopy (EIS), on Autolab (PGSTAT101, Metrohm, Netherlands) (shown in Figure 2.11) using three-electrode assembly and measuring the current potential.

The potential of one of the electrodes is sensitive to the analyte's concentration and upon which electrochemical redox reaction takes place so-called working or indicator electrode. The second electrode, which is called the counter electrode, serves to complete the electric circuit. The third electrode, which is called the reference electrode whose potential is known as w.r.t. the standard hydrogen electrode (0.00 V) and measures the potential of the working electrode w.r.t. reference electrode and which is irrespective of analyte concentration [Skoog et al., 2010].

Cyclic voltammetry (CV) is a multipurpose potentiodynamic electroanalytical technique that has been used to investigate the electrochemical properties of electroactive species. In a cyclic voltammogram, the corresponding spectrum of a CV is a measure of the change in current measured corresponding to changing voltage in the solution. The microelectrodes and an unstirred solution are used in this electrolytic method, thereby the measured current is limited by analyte diffusion at the electrode surface.

The current response over a range of potentials is measured in a CV experiment, starting at an initial value and varying the potential in a linear manner to a pre-defined limiting value. As in CV measurements, until the voltage reaches the oxidation potential of the analyte, the current increases, and after that, it falls off as the concentration of the

analyte depletes close to the electrode surface. At this switching potential, the direction of the potential scan is reversed in which the same potential window is scanned in the opposite direction (thereby the term cyclic). When reversing the applied potential, a potential will be reached where the reduction of the product resulting during the forward scan starts producing a current which is of reversed polarity. The reduction peak also usually has a similar shape as that of an oxidation peak in the other direction. Further, in this case, some non-symmetric peaks are generated, which may attribute to the quasi-reversible reaction. Along with if the process is completely irreversible, then the anodic peak does not appear in the potential region. These all result the information regarding the redox potential and the nature of the electrochemical reactions.

In the differential pulse voltammetry (DPV) technique, small amplitude, and short pulses are superimposed on a linear ramp. Current is obtained prior to and at the end of the application of each pulse. The difference between the currents is plotted against potential. This procedure minimizes the background current and results in a Faradaic current free of most capacitive current. The major advantage is low capacitive current, which leads to higher sensitivity. The smaller step sizes in the DPV technique, give rise to narrow peaks and therefore DPV can be used to distinguish analytes that have closer oxidation potential.

EIS is an electrochemical technique that uses a small amplitude, and alternating current (AC) signal to study the impedance characteristics of an electrochemical cell. The AC signal is scanned over a wide range of frequencies to produce an impedance spectrum of the electrochemical cell under study. EIS differs from direct current (DC) techniques in that it allows the study of inductive, capacitive, and diffusion processes occurring in the electrochemical cell. It is used for the study of interfacial properties related to bio-

recognition events occurring at the electrode surface, such as substrate–enzyme interaction, antibody-antigen recognition, or whole-cell capturing. Thus, EIS could be used in different important biomedical diagnosis and sensing applications.

Figure 2.11 Autolab (PGSTAT101, Metrohm, Netherlands)