The characterization techniques we have used to look into the structural, morphological, optical, and electrochemical properties of synthesized materials are briefly summarized in this chapter, along with the experimental setup. Numerous instrumental techniques are used to thoroughly characterize nanomaterials and their nanocomposites, including Ultraviolet-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). Different analytes have been optically recognized using a UV-Vis spectrophotometer, and electrochemical characterization has been done using a cyclic voltammetry (CV) system.

2.1 Characterization techniques

2.1.1 Ultraviolet-Visible Spectroscopy

Ultraviolet-visible (UV-Vis) spectrophotometry is a characterization method for finding out the amount of light absorbed through the UV-Vis region. In this method, Incident light can be transmitted, absorbed, or reflected depending on the substance. The absorption of radiation in the UV-Vis range leads to an atomic excitation, that is, the change of a molecule from a low-energy ground state to an excited state. This approach has been proven to be quite helpful in researching how synthesized nanomaterials' surfaces have been functionalized.

According to Lambert-Beer's law, the path length and concentration of the absorbing analytes in the solution have a direct relationship with the absorbance of the solution. This law states that the proportion of an incident monochromatic beam that is absorbed by a

homogeneous medium is proportional to the number of absorbing molecules confined in a particular solution.

The mathematical expression for Lambert-Beer's law is

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

I_0 = incident intensity,

I = the transmitted beam intensity through a specific sample solution,

A = measured absorbance (unitless),

c = concentration of the absorbing sample (mol L^{-1}),

ε = absorptivity or extinction coefficient (L mol^{-1} cm^{-1}),

l = path length through the sample (cm).

The following figure 2.1 shows the parts of a conventional spectrometer. Wherein a prism or grating is used to separate UV-Vis rays into their individual wavelengths. Further, a device with a half-mirrored surface divides each monochromatic (single wavelength) beam into two beams of equal intensities. One beam, known as the sample beam (I = intensity of the sample rays), travels across a tiny, transparent cuvette containing a solution of the substance being examined in a clear solvent. The other beam, known as the reference beam (I_0 = intensity of the reference beam), travels through a similar cuvette that only contains the solvent. Then, electronic detectors measure and compare the intensity of these rays. The amount of light that passes across a sample is measured by a spectrophotometer. Hence, by varying the analytical wavelengths and path length, UV-Vis spectrophotometers can consequently be used to measure the concentration of certain analytes in a micro-volume.

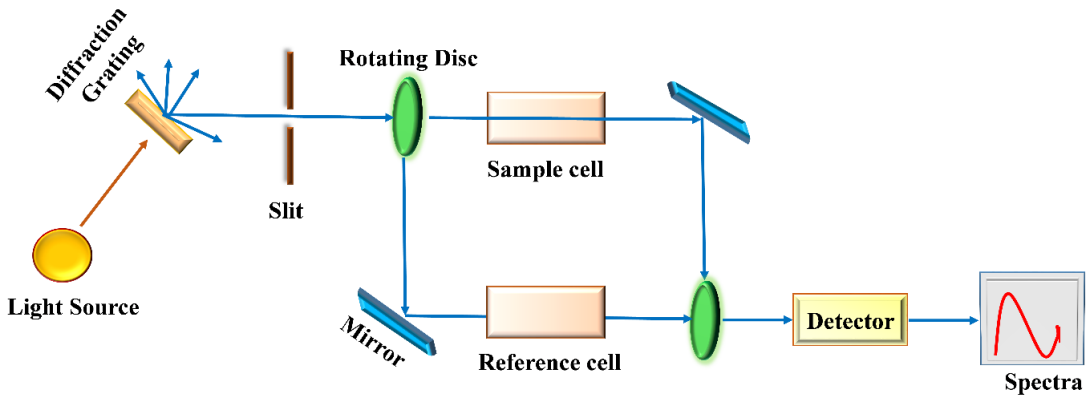

Figure 2.1 Schematic diagram of UV-Vis spectrophotometer.

Figure 2.2 UV-Vis spectrometer (EPOCH microplate reader (Biotek, Courtesy IIT BHU))

2.1.2 FT-IR (Fourier Transform Infrared)

IR (Infrared) spectroscopy is a technique that is mostly used by the researcher to know the composition of solids, liquids, and gases. IR spectra are recorded by an instrument known as an IR spectrometer. It is used to study comprehensive information about the structure of a compound. The IR region is categorized into three parts: the near-IR, mid-IR, and far-IR. The range for mid-IR wavenumber is 4000-400 cm^{-1}. Organic compounds absorb IR radiation, which causes them to change into molecular vibrational energy. Infrared

radiation is exposed to a molecule in IR spectroscopy. The absorption will occur when the energy of radiation equals the specific molecular vibrational energy.

Fourier Transform Infrared (FT-IR) spectroscopy has been developed to solve the drawbacks of dispersive devices. It required a lot of time and was tedious to scan. It was necessary to find a way to measure each infrared frequency simultaneously rather than separately. An interferometer is a fairly upfront optical instrument through which the signal can be measured very quickly, typically within one second. The main components of the FT-IR source are the interferometer and the detector. The main component of FT-IR is the interferometer (which consists of a beam splitter), a stationary mirror, a moving mirror, and a laser. With the help of a beam splitter, light from a source is divided into two paths, one path going to an immovable mirror and the other to a moving mirror (figure 2.3). As a result, when the interferogram is measured, all frequencies are measured concurrently.

The interferogram is converted into an IR absorption spectrum that is commonly identifiable with absorbance (A) or % transmittance (T) plotted against by wavenumber. The following equation represents the relation between absorbance and transmittance

$$A = -\log_{10} T \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(Eq. 2.2)}$$

The ability of the sample to absorb energy from infrared light at various wavelengths is used to determine the molecular structure and composition of the substance. A database of reference spectra is used to compare the spectrum to find unknown compounds.

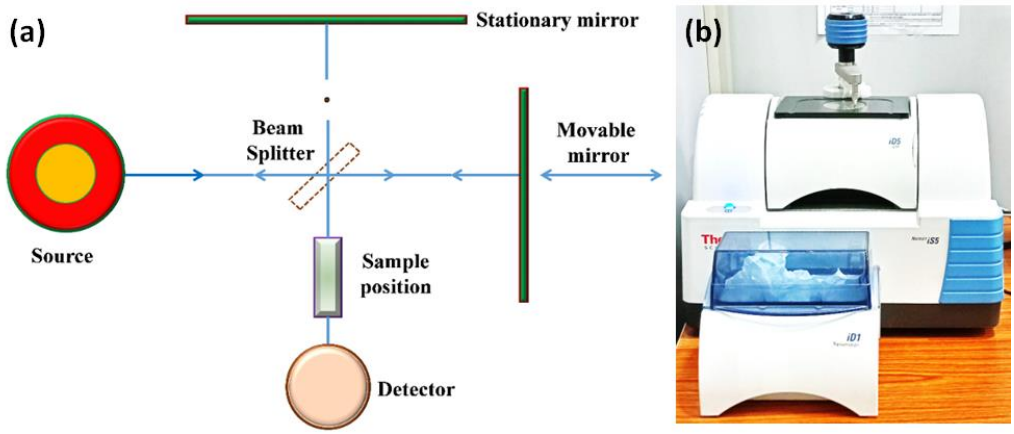

Figure 2.3 (a) Michelson Interferometer **(b)** FTIR Spectrometer (NICOLET iS5 Thermo Scientific, Courtesy CIFC IIT BHU)

2.1.3 X-ray diffractometer (XRD)

In materials science, to identify the crystallographic structure of a substance, X-ray diffraction analysis (XRD) is used, and it measures the intensities and scattering angles of X-rays when a material is exposed to incident X-rays (figure 2.4). The findings allow one to determine the distance between atoms inside the specimen's crystal lattice. These X-rays contribute constructively in a small number of precise directions, as indicated by Bragg's law, but in the majority of directions, they cancel each other out through destructive interference.

$$n\lambda = 2dsin\theta \qquad \text{.....(Eq. 2.3)}$$

Wherein, n = integer (n represents the order of reflection), d = distance between diffracting planes, λ = wavelength, θ = angle between an incident beam X-ray and crystallographic reflecting plane

By measuring the X-ray diffraction from atomic planes within the supplied material, powder XRD analyses and measures the nature of materials using X-rays. It relies on the type and relative positions of the atoms as well as how long the crystalline arrangement lasts. Thus, it determines the distance between lattice planes, the crystalline character of

materials, crystalline phases, the length scales, preferential ordering, and the epitaxial growth of crystallites.

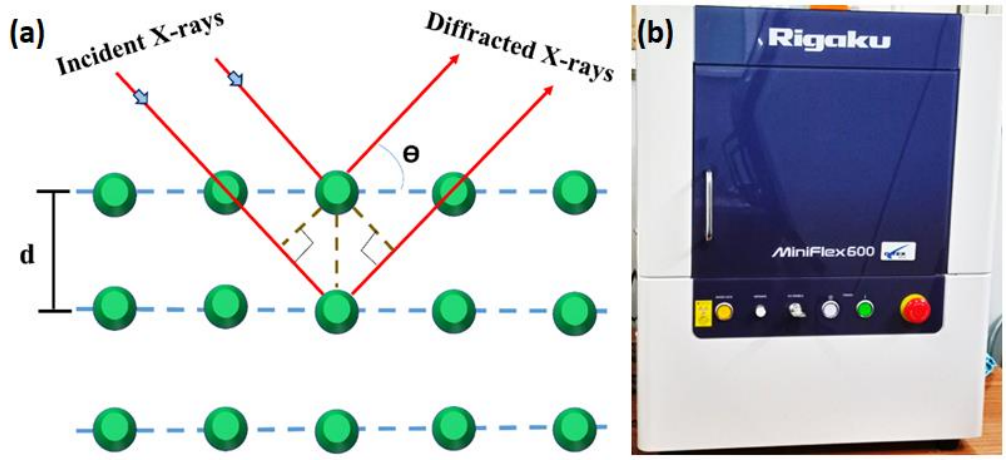

Figure 2.4 (a) Schematic diagram for X-ray diffraction (b) Miniflex 600 X-ray Diffractometer (Rigaku, Courtesy CIFC IIT BHU)

2.1.4 X-ray Photoelectron Spectroscopy

The surface analysis method, "X-ray photoelectron spectroscopy," abbreviated as XPS, can analyze about every type of material and reveal information on its elemental and chemical properties.

A material's atomic state, chemical composition, and electronic state can all be determined using XPS. Due to the relationship between the kinetic energy of an emitted electron and its binding energy, a variety of emitted (ejected) electrons with different binding energies (and kinetic energies) will be produced in this scenario and provide an XPS spectrum (figure 2.5). This is due to the fact that atoms have numerous orbitals with different energies. The equation below shows how these relationships are related.

$$E_{kinetic} = E_{photon}(hv) - E_{binding} - \varphi \quad \ldots\ldots\ldots\ldots\ldots\ldots(Eq.\ 2.4)$$

Where $E_{kinetic}$ = Photoelectron's measured kinetic energy, E_{photon} = Energy of the incident X-ray, $E_{binding}$ = Electron's binding energy, and φ = work function.

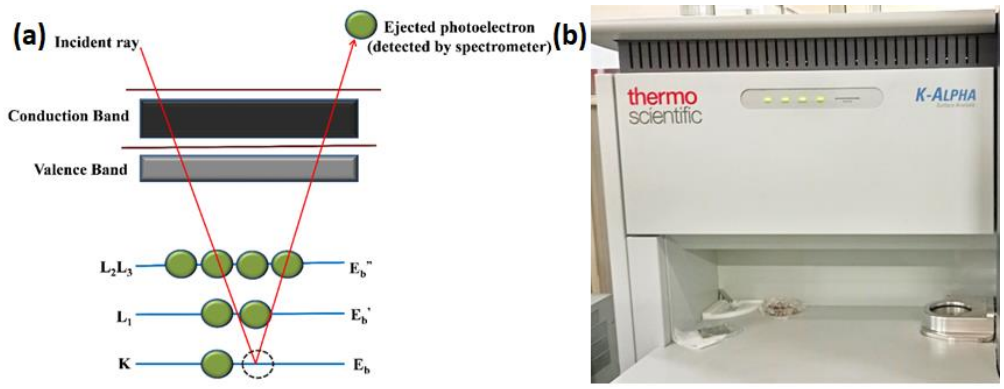

Figure 2.5 (a) X-ray photoelectron spectrometer (b) Schematic representation of X-ray Photon spectroscopy (K-alpha model of Thermo Fischer Scientific, Courtesy CIFC IIT BHU)

2.1.5 Scanning Electron Microscopy (SEM)

Scanning electron microscopy, abbreviated as "SEM," is one of the most useful characterization tools available, which provides a magnified image of an object by focusing a stream of electrons on scanning its surface for a high-resolution image. It provides details on both man-made and naturally existing materials' topography, chemical composition, and microstructure morphology. The adaptability of SEM is advantageous for a plethora of scientific, research, industrial, and commercial applications, including biology, forensics, medicine, electronics, and materials science.

SEM uses a comparatively low-intensity, focused electron beam as an electron probe that periodically scans across the sample. SEM is a form of electron microscope used for the in-depth study of solid object surfaces. Major elements of a conventional SEM's column structure include:

1. Electric source
2. Electron lenses (Condenser and objective)
3. Apertures
4. Scan coils

5. Detectors (different for SEs and BSEs)

In scanning electron microscopy, the sample is scanned by the electron beams that is produced at the tip of the column, driven downward, and then focused into a beam that reaches the sample's surface after passing through a number of lenses and apertures. As a sample is positioned on a stage in the chamber region, the chamber and column are both evacuated using a variety of pumps.

Figure 2.6 shows that scanning coils located above the objective lens govern where the electron beam will impact the material. 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 [Zhou et al., 2007]. Data can be collected on a specific region of the sample using this beam scanning. Several signals are produced as a result of the electron's interaction with the material is necessary for image creation in the SEM. The proper detectors are used to find these signals.

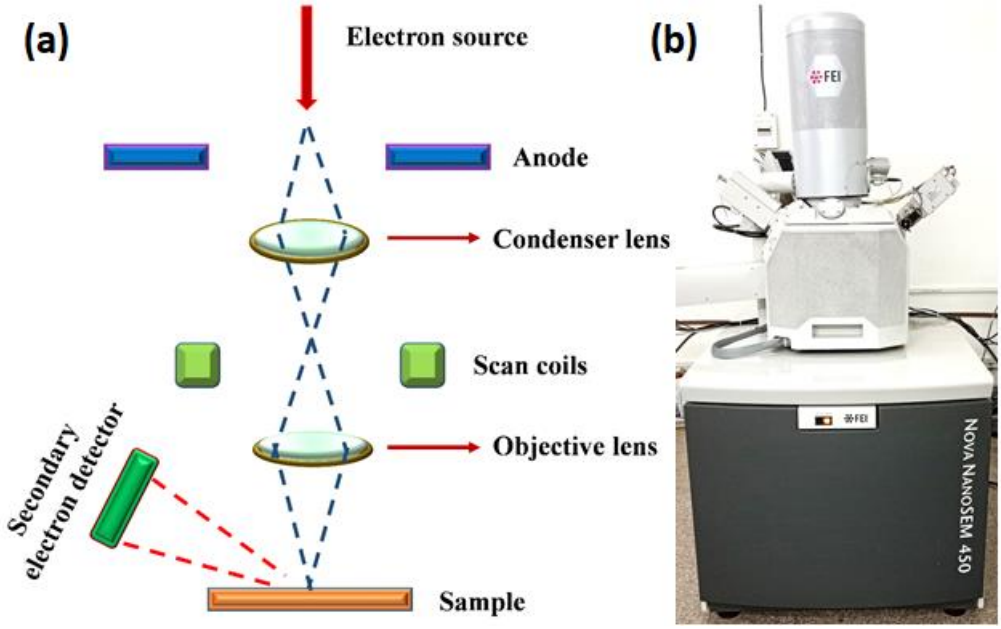

Figure 2.6 (a) Scanning electron microscope' Schematic presentation (b) Scanning electron microscope (FEI NOVA NANO SEM 450, Courtesy CIFC IIT BHU)

Secondary electrons (SEs) are inelastic interactions, and Backscattered electrons (BSEs) are elastic interactions between the primary electron beam and the sample. BSEs were created in the sample's deeper regions, which revealed information on the sample's topography and composition. SEs come from areas near the surface. As a result, it provides topographic contrast information with high resolution for the observation of surface texture and roughness. By putting them on carbon tape, the majority of nanomaterials (conductive in nature) can be directly viewed by SEM. Metal coatings (gold, silver, platinum, etc.) are required for non-conductive samples (bioorganic nanomaterials).

2.1.6 Transmission Electron Microscopy (TEM)

An extremely effective instrument for characterizing materials is the transmission electron microscope which is used for examining the characteristics of incredibly small materials. A scientist can see features like structure and morphology using the technology, which involves an accelerated electron beam that passes through a very thin object. The microstructure, chemical composition, and nanoscale electrical characteristics of the material can all be revealed via TEM techniques. Figure 2.7 reveals that a TEM consists of three core parts:

1. An electron gun for producing the beam of electrons and a system for condenser lenses that focuses the electron beam onto the object

2. A system for producing images, which includes an objective lens, a moving sample stage, intermediate lenses, and projector lenses

3. A system for recording images

From the electron gun, the electrons strike at the sample and get scattered, and focus on the magnetic lenses to create a resolved image. The user can see the image because the light is produced when the image hits the fluorescent screen. The image's darker portions

correspond to the sample's regions where electron transmission was lower, while its lighter areas correspond to the regions where electron transmission was higher. A CCD camera that is positioned underneath the screen is used to record each image. Any field of research and technology that wants to examine the atomic-level interior structure of specimens can use a TEM microscope.

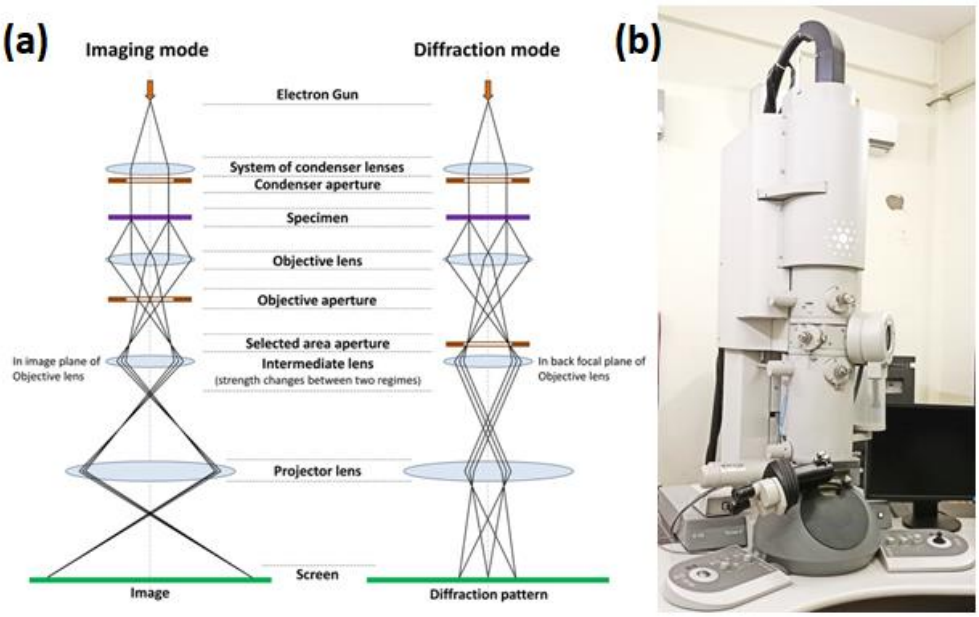

Figure 2.7 (a) Schematic representation of different modes in TEM (Image credit: Black Tubus) (b) Transmission electron microscope with EDX (FEI, TECHNAI G^2 20 TWIN)

2.1.7 Cyclic Voltammetry (CV)

Among various types of voltammetry (a group of electroanalytical methods in which current (I) is function of applied potential (V)), cyclic voltammetry is a widely used potentiodynamic electrochemical technique to investigate not only the redox behaviour of electroactive species but also inestimable for the understanding of chemical reactions that are the result of electron transfer (includes catalysis).

CV is performed by ramping the potential back and forth (negative to positive and vice versa) between the chosen range and measuring the resulting current using three electrodes

(counter electrode, reference electrode, and working electrode) combination cell containing electrolyte solution.

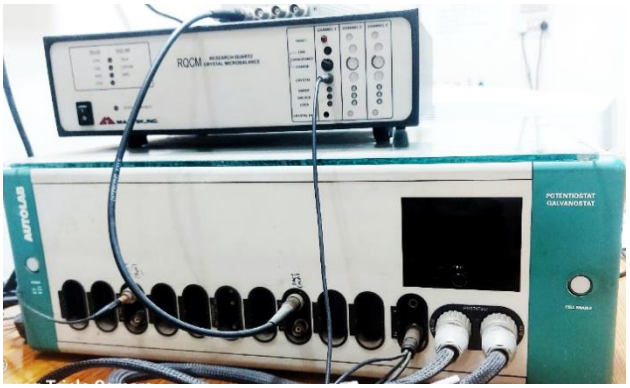

Figure 2.8 Autolab (PGSTAT 101, Metrohm, Netherlands)

The working electrode is the electrode whose potential is sensitive to the concentration of analyte and on which the redox process takes place. A counter electrode is used to complete the electric circuit, and the third electrode, whose standard potential is known and measures the potential of the working electrode with respect to this. This is independent of the analyte's concentration. The spectrum of CV is called cyclic voltammogram (response of the change in current corresponding to changing potential) in the solution. The cyclic voltammogram of a reversible redox process is analyzed using the following variables:

1. The peak potential difference [$E_p=E_{pc} – E_{pa}$] =59.2/n mV @ 25°C

2. The ratio of peak current $i=i_{pa}/i_{pc}$ =1 (for all scan rates)

3. The peak current function $i/v^{1/2}$

4. The peak current $i_p =2.69*10^5 n^{3/2} ACD^{1/2} v^{1/2}$

Wherein n is the number of the transferred electrons, A is the surface area of the electrode in cm^2, C is the analyte concentration (mol cm^{-3}), and D is the diffusion coefficient ($cm^2 s^{-1}$).