Chapter 2
Experimental Techniques

This chapter illustrates the theory behind the different experimental techniques used and their instrumentation for the characterization of the developed materials. The synthesized nanomaterials and their composites are characterized by various tools such as UV-visible spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and X-ray Photoelectron Spectroscopy (XPS). CV and Electrochemical Impedance Spectroscopy (EIS) were utilized for the electrochemical characterization. CV as well as DPV were employed for the electrochemical determination of different analytes. The theoretical background of each of these techniques is covered in this proceeding chapter.

2.1 Characterization Techniques:

2.1.1 UV-visible spectroscopy or Electronic Spectroscopy

Spectroscopy is the branch of science that deals with the interaction of an electromagnetic wave with matter. When an electromagnetic wave interacts with the atoms/molecules, it results in a change in the atoms/molecules which can be rationalized as:

- X-rays: excite the core electrons
- UV-visible rays: excite the valence electrons
- IR rays: induce molecular vibrations
- Radio waves: induce the change in nuclear spin states under the influence of a magnetic field.

UV-visible spectroscopy is a significant tool that helps to identify the absorption or reflectance band of species such as biological molecules and macromolecules, metal ions, and organic compounds present in a solution by illuminating the sample with

monochromatic UV or visible radiation. This technique also finds application in materials chemistry. It can help to investigate the surface functionalization of the developed materials.

This spectroscopic technique obeys Lambert-Beer's law which states that when a monochromatic radiation passes through a solution of light-absorbing species, the rate of depletion in the intensity of the incident radiation with the width of the test solution is directly proportional to the intensity of the incident radiation and concentration of the solution of the absorbing species. Hence, for a fixed path length of the absorbing medium, UV-visible spectroscopy can also be used to estimate the concentration of the absorbing species present in the test solution. The formula for Lambert-Beer's law is as follows:

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

where A = absorbance,

I_0 = intensity of light incident over the sample,

I = intensity of the transmitted light leaving the sample,

ε = molar extinction coefficient or molar absorptivity,

c = concentration of the light-absorbing species,

l = path length of the light through the sample,

It is observed that the greater the number of light-absorbing species, the greater the extent of absorption of light. This is the basic principle behind the UV absorption technique. Further, the specific wavelengths of absorption spectra can give information about the type of bonds in a particular conjugated organic compound and they can also help to determine the functional groups present in a molecule. Moreover, this technique is widely used for the characterization of noble metal nanoparticles since the optical properties of the nanoparticles are directly linked to the shape, size, and concentration

of the nanoparticles and their dielectric environment (Skoog et al., 2010; Harvey et al., 1956).

A UV- visible spectrum is a graph between the intensity of light absorbed versus the wavelength of light in the UV-visible region. This spectrum is recorded by a UV-visible spectrophotometer. It records the light intensity before passing through the sample (I_0) and after passing through the sample (I) and calculates the ratio I/I_0 which is termed transmittance (T). The transmittance is usually conveyed in terms of percentage transmittance (%T). The absorbance A and %T can be related as:

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

The UV-visible spectrophotometer is composed of a source of light, a monochromator or a diffraction grating for separating a distinct wavelength, a sample holder, and a detector (Figure 2.2). The instrument may be of two types- single beam and double beam spectrophotometer.

Figure 2.1 A UV-visible spectrophotometer

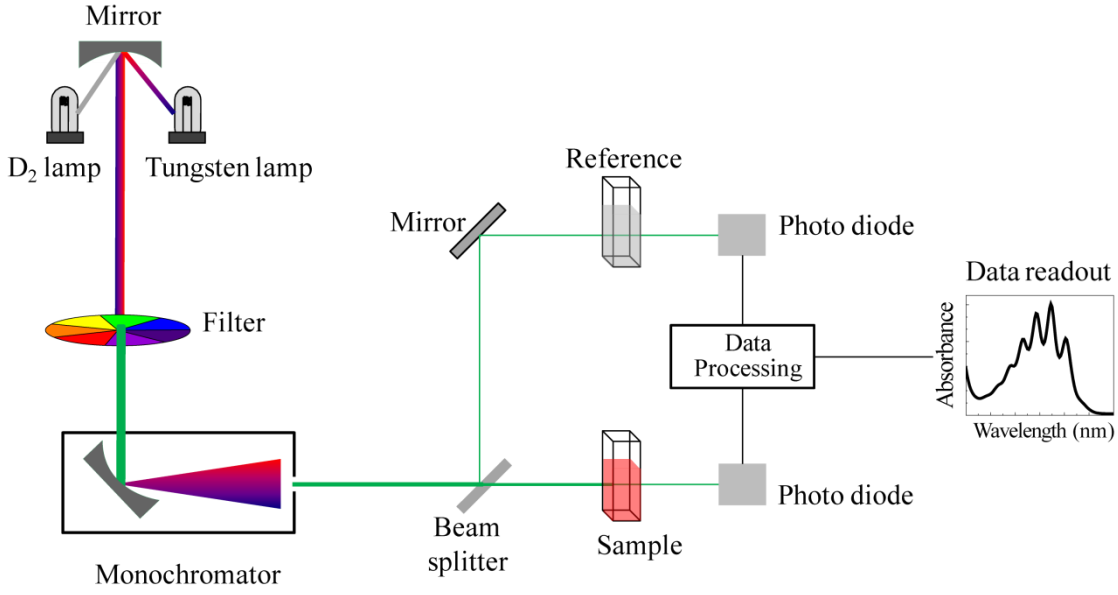

Figure 2.2 Block diagram of a double beam UV-visible spectrophotometer [agora.cs.wcu.edu]

2.1.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy deals with infrared (IR) radiation having wavenumber in the range 450-5000 cm^{-1}. IR radiation induces sufficient energy for chemical bonds in a molecule to vibrate (stretching or bending). When IR radiation falls on a molecule, the molecule absorbs only those wavelengths having energy equal to the energy required to cause a definite vibration in a bond, transmitting the other wavelengths. In other words, the IR radiation of a particular frequency, which matches the frequency of a bond associated with a molecule, gets absorbed and displayed in the resulting spectrum. Further, the molecules with the permanent dipole moment are considered to be IR active.

FTIR spectroscopy is an important tool to identify the bonding and structure of molecules with various functional groups having a certain vibrational pattern. With respect to the nanomaterials, the existing functional groups on their surface exhibit different FTIR spectra in comparison to the corresponding free groups. Therefore, the

surface chemistry of these materials can be investigated via FTIR spectroscopy, and the bonding pattern and the functional groups can be characterized and identified [Skoog et al., 2010; Harvey et al., 1956].

Figure 2.3 An FTIR spectrophotometer

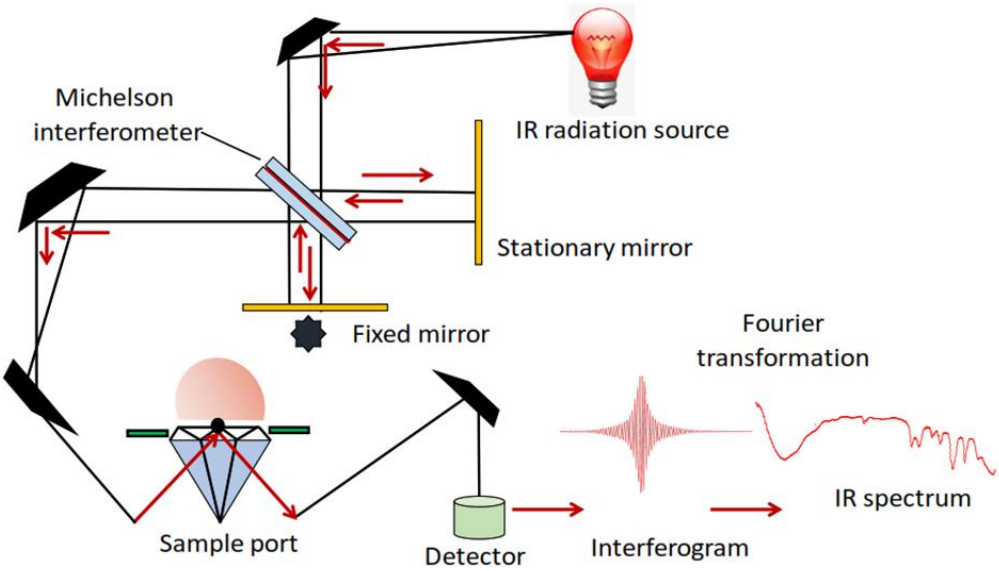

Figure 2.4 Schematic representation of FTIR spectrophotometer [easytocalculate.com]

A typical FTIR spectrophotometer is composed of a light source, sample cell, interferometer, detector, signal amplifier, and display unit. The IR radiation from the light source is incident on the sample, passes through the interferometer, and reaches the detector. Then, the obtained signal gets amplified and converted into a readable form which is presented in the display unit and referred to as an interferogram. A typical block diagram of the FTIR spectrophotometer is shown in Figure 2.4.

2.1.3 X-ray Diffraction (XRD)

It is a significant analytical tool that is used to examine the crystallographic structure as well as the chemical composition of the materials. Here, the characterization of the materials is mainly based on their diffraction pattern. As a result of a uniform arrangement in a crystal, the constituent atoms generate an interference pattern of the waves when an X-ray beam is incident on the sample. The X-ray diffraction from the atoms is illustrated in Figure 2.5.

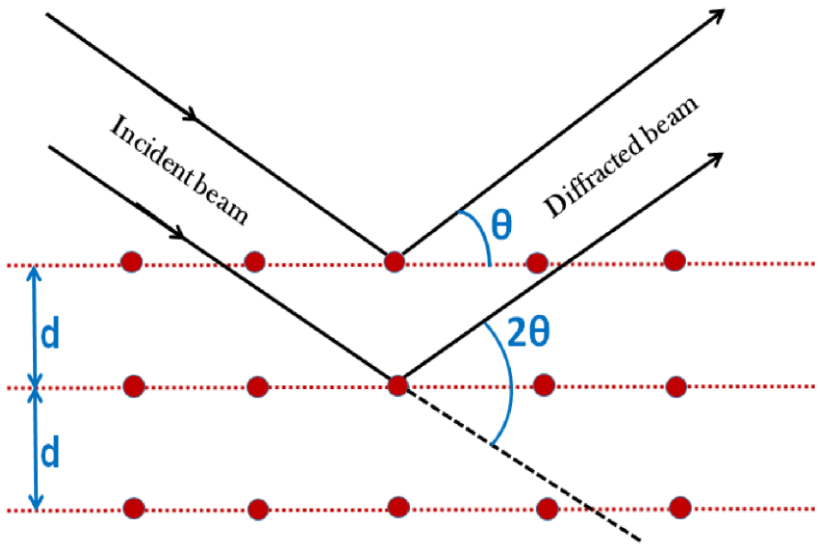

Figure 2.5 Schematic illustration of an X-ray beam getting diffracted by a crystallographic material

When a monochromatic beam of X-rays is incident on the sample, most of the X-rays get scattered by the atoms present in the material. These scattered rays experience constructive and destructive interference in the materials with a regular arrangement of atoms (i.e. crystalline). This is known as diffraction. This diffraction of X-rays is governed by Bragg's Law which is as follows:

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

where λ is the wavelength of incident X-rays, d is the spacing of the atomic layers and θ is the incident angle of the photons.

With the variation in the angle θ, the conditions of Bragg's Law are fulfilled by different d-spacing values in polycrystalline materials. When the angular positions are plotted against the intensities of the diffraction peaks of the radiation, a pattern is developed which is the characteristic signature of the material. If the sample is composed of different phases, the emerged diffraction pattern is the amalgamation of individual patterns. However, the lion’s share of the materials is not purely crystalline, but they are powders or aggregates composed of different small crystallites with possible orientations. So, when the beam of X-rays interacts with a powder having a random orientation of crystallites, it encounters all the existing interatomic planes and if the incident angle is minutely varied systematically, all the probable diffraction peaks will be thoroughly recognized. The powder XRD helps to study and quantify the extent of crystallinity and it is also sensitive to variety, location of atoms, and length scale up to which the order of crystallinity persists. Thus, it can be employed to find the crystalline content of the sample, investigate the existing crystalline phases and deduce the ‘d’ spacing between two lattice planes and the length scales over which this order

continues. The technique covers a length scale approximately from a few angstroms to a few nanometers.

In our thesis work, the structural phases of synthesized materials were investigated by using Rigaku Smart Lab X-ray Diffractometer (Figure 2.6). The latter is attached to an X-ray generator which is mostly functioned at 45 keV and 40 mA. For the current examinations, Cu-Kα (λ=1.5405Å) has been utilized. The XRD patterns were mostly recorded over 10-80° in 2θ, at a step size of 3 °min^{-1}, but varied as per the requirements. A schematic representation of the working of an X-ray Diffractometer is presented in Figure 2.7.

Figure 2.6 Rigaku MiniFlex Benchtop 600 X-ray diffractometer

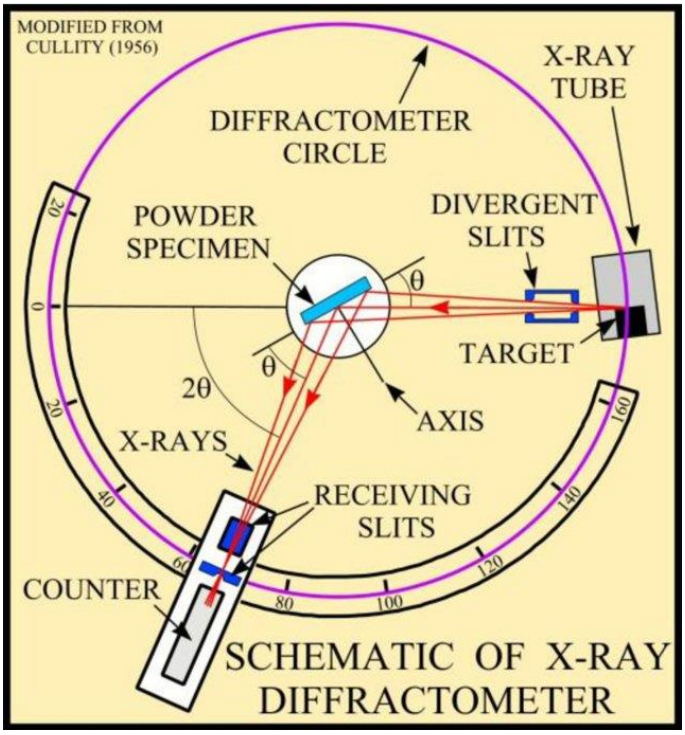

Figure 2.7 Schematic representation of the working of an X-ray Diffractometer [Mohanta, 2017]

2.1.4 X-ray Photoelectron Spectroscopy (XPS)

XPS is also termed electron spectroscopy for chemical analysis (ESCA). In this technique, the sample surface is bombarded with a beam of monochromatic X-rays of known energy, it knocks out one of the core electrons with a kinetic energy E_{kin} which is computed. Figure 2.8 depicts the physical process involved in XPS based on the concept of the photoelectric effect. The binding energy of an electron (E_b) can be determined by the following equation:

$$E_{kin} = h\nu - E_b - W_f \qquad \text{(Eq. 2.4)}$$

where E_{kin} = kinetic energy, ν = frequency of x-rays, h = Planck's constant, E_b = binding energy of electron, and W_f = work function

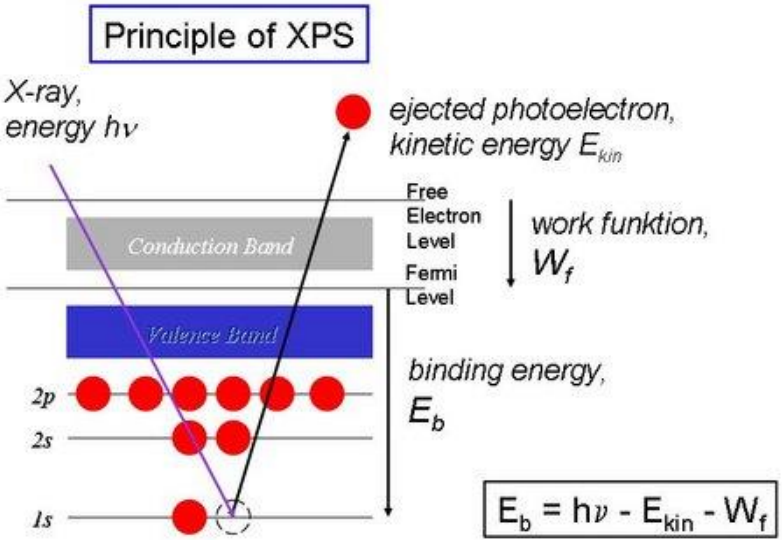

Figure 2.8 Basic principle involved in XPS [ifw-dresden.de]

A common XPS technique involves the irradiation of a sample with a primary beam made up of photons, electrons, and its impact on the sample surface leads to the generation of the secondary beam from the sample which is analyzed/detected by a spectrometer as illustrated in Figure 2.9. XPS spectrum is produced by plotting the intensity of electrons (count/sec) vs. their binding energy (eV). Wide scan XPS spectrum is termed a survey spectrum that normally scans from 0 to 1200 eV binding energy.

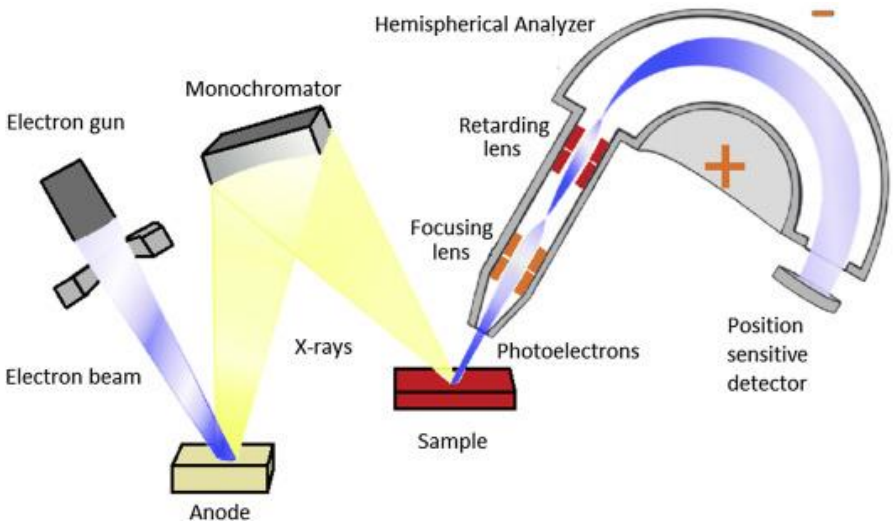

Figure 2.9 Schematic illustration of an XPS instrument [Kloprogge and Wood, 2020]

XPS is a surface-sensitive technique that can determine the constituent elements present in a material as well as its electronic state. All the elements can be recognized through this technique except hydrogen and helium.

In the present thesis, we have exploited this technique for elemental analysis and determination of the oxidation state of the elements present in the synthesized nanomaterials and nanocomposites [Skoog et al., 2010; Harvey et al., 1956].

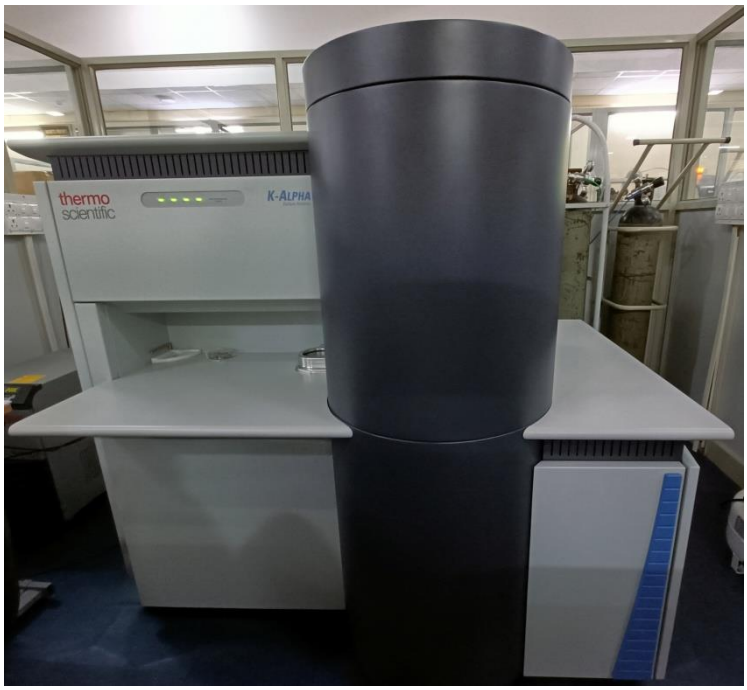

Figure 2.10 X-ray Photoelectron Spectrometer

2.1.5 Scanning Electron Microscopy (SEM)

It is a fundamental characterization tool that generates magnified high-resolution images of a specimen by scanning its exterior surface through a focused beam of electrons. It delivers details about the chemical composition, topography, and microstructure morphology of the material. A stereotyped SEM instrument comprises the following major elements:

i. Electron Gun

ii. Electron lenses (Condenser and objective)

iii. Apertures

iv. Scanning coils

v. Detector (different for SEs and BSEs)

Electrons get generated from a source i.e. lanthanum hexaboride (LaB_6) single crystals or a heated tungsten filament, and accelerated by a voltage and pointed towards the center of an electron optical column containing various magnetic lenses for producing a focused electron beam to strike the surface of the material. The focus of the electron beam is governed by scanning coils which permit the electron beam to scan over the specimen surface, placed above the objective lens. The image formation is very much dependent on the interaction between the incident electron and the sample. Such interactions are of two types- Inelastic interactions (Secondary electrons, SEs) and Elastic interactions (Backscattered electrons, BSEs) as presented in Figure 2.11.

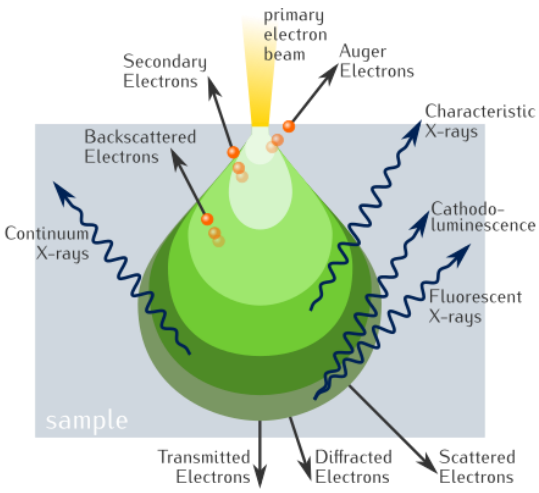

Figure 2.11 Schematic illustration of signals emitted from different regions of the sample on interaction with electron beam [https://www.wikiwand.com]

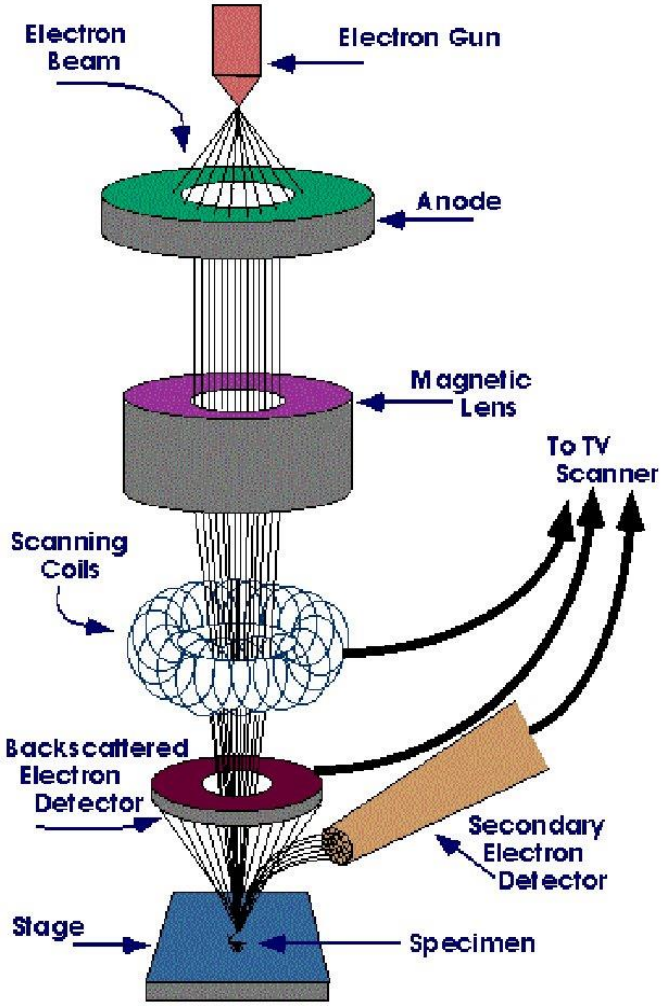

Figure 2.12 Block diagram of SEM [Mohanta, 2017]

BSEs generated from the deeper zone of the sample furnish topographical and constitutional details of the sample. SEs produced from the surface area of the sample furnish topographic contrast information and visualization of exterior surface texture. The conducting samples can be examined by loading them directly over carbon tape. For non-conducting samples, the metal coating is applied with metals such as gold, platinum, silver, etc. The SEs and BSEs ultimately lead to the development of the SEM image [Skoog et al., 2010; Harvey et al., 1956].

Figure 2.13 A Scanning Electron Microscope (SEM)

2.1.6 Transmission Electron Microscopy (TEM)

TEM is a very significant and versatile characterization technique involving the investigation of the interaction of a beam of highly energetic electrons (60-300 keV) with a thin sample (<100 nm). Such interaction leads to the development of extremely resolved and magnified images of the sample. This technique helps to explore the nanomaterials' microstructure, dimensions, chemical constitution, electronic properties, and distribution in the sample.

The major components in the column structure of a TEM instrument are:

i. Electron Gun

ii. Condenser and scanning lenses

iii. Sample holder

iv. Objective lenses

v. Signals detections

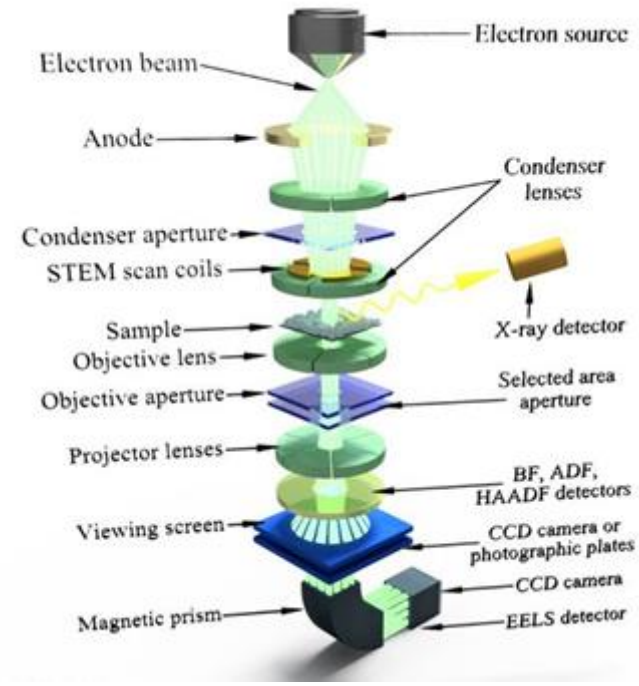

Figure 2.14 Schematic representation of a TEM instrument [Inkson, 2016]

The electron gun provides the source of electrons and a heated tungsten filament is used as an electron gun in most of the cases. It emits the electrons which travel down a column where a high aperture condenser lens gets rid of the high angle electrons and focuses the electrons in the form of a very thin beam. This beam of high-speed electrons illuminates the specimen placed on a sample holder. Depending upon the density of the sample, a few electrons get scattered and vanished from the beam. Other electrons are transmitted through the sample and focused into an image by the objective lens, followed by passing through the projective lens where the image gets magnified. Now, the electron beam is directed on a phosphorescent screen that produces an image of the

specimen. All such images of the specimen are recorded by immediate exposure over a photographic emulsion or digitally captured by a CCD camera located underneath the screen. In the case of a conventional TEM, a static beam of electrons at an accelerating voltage of around 100–400 KeV travels through a section of the specimen that is immersed in the objective lens of the microscope [Williams et al., 2010; Skoog et al., 2010].

One of the significant benefits of this technique is the capability of inspecting the materials in reciprocal space as well as in real space, as in diffraction as well as imaging modes. The imaging in conventional microscopy is conducted by selectively permitting the transmitted beam (termed Bright-field imaging) or by the diffracted beam (termed Dark-field imaging) down to the microscope column. The intensity of image contrast depends on the variation of the transmitted beam and diffracted beam which arises due to variance in diffraction conditions relying on the microstructural feature of the electron path. A schematic representation of these two kinds of imaging is shown in Figure 2.15.

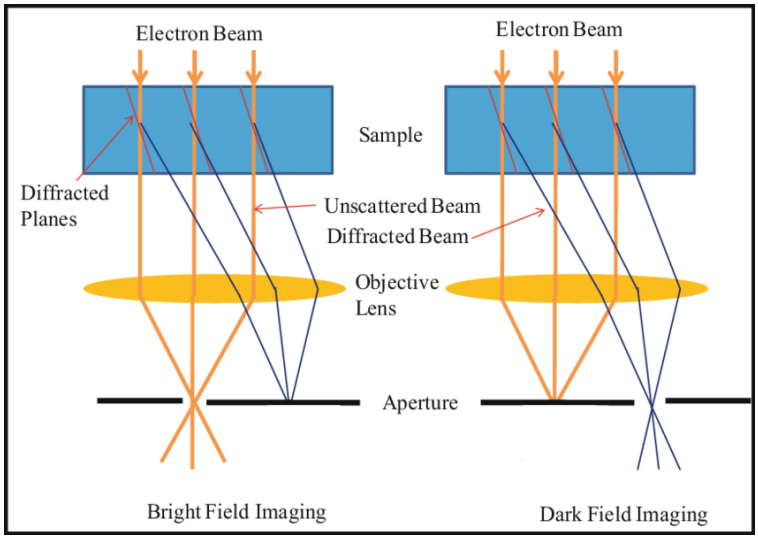

Figure 2.15 Diagrammatic illustrations of Bright-field and Dark-field imaging [Javed et al., 2018]

We have prepared our samples for TEM analysis on a Copper grid coated with Carbon and TEM micrographs were captured by a FEI-Tecnai 20 U Twin with EDX and Tecnai 20 G2 as shown in Figure 2.16 which was operational at an accelerating voltage of 200 kV, for the structural characterization of different synthesized nanomaterials.

Figure 2.16 Transmission Electron Microscope

2.1.7 Electrochemical Characterizations

For the electrochemical characterizations and investigation of the response of the prepared electrodes, CV, DPV, and EIS were employed using Metrohm Autolab [PGSTAT 302, The Netherlands], with Nova 1.11 software (shown in Figure 2.13). A

conventional three-electrode assembly was utilized where a bare/modified GCE, Pt disc electrode, and Ag/AgCl (saturated KCl) were employed as working (WE), auxiliary/counter (AE) and reference (RE) electrodes respectively.

The potential of WE depends on the concentration of the analyte or electrochemical redox reactions. The potential of RE is constant & known, and the potential of WE is measured with respect to that of RE [Skoog et al., 2010]. The AE aids to complete the electrical circuit.

Figure 2.17 Metrohm Autolab (PGSTAT 302, The Netherlands)

CV is the most extensively used electroanalytical tool for investigating the qualitative details of electrochemical reactions in a system. It gives the position of redox potentials of the electroactive species in the system. The experimental setup consists of the three-electrode assembly in a cell, connected to a power supply. Materials coated over conducting surface serve as the WE. The voltage is scanned between two sets of

potential as the lower and upper limits. After reaching a set potential during the CV scan of a system, the potential ramp of the working electrode gets inverted to give a cyclic voltammogram. The scanning with a constant scan rate between two sets of potential causes the appearance of peak(s), which gives the level of electron where the electron gets ejected or inserted (oxidation or reduction). During the forward scan, the current in the voltammogram increase as the voltage approaches the oxidation potential, thereafter it decreases since the analyte concentration gets depleted near the surface of WE. During the reverse scan, the current increases with a decrease in the applied potential, but with the reversed polarity. The reduction peak looks similar to the oxidation peak in the opposite direction. Hence, the term 'cyclic' is assigned to this technique. In some cases, non-symmetric oxidation and reduction peaks are observed which is indicative of a quasi-reversible reaction. Further, if the reaction is irreversible, it results in a single peak in the voltammogram. Thus, CV furnishes plenty of information about the nature of electrochemical species/reactions.

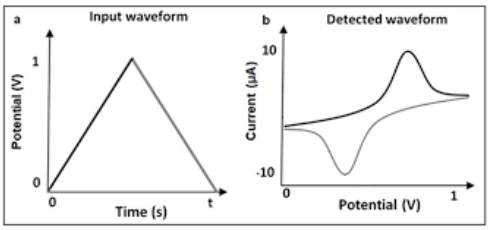

Figure 2.18 a) CV input waveform and b) a general resulting output plot [Fu et al., 2015]

EIS is a key technique to study the charge transfer kinetics at the interface of electrode and electrolyte using an alternating current (AC) signal. This AC signal is surveyed over a broad frequency range to produce an impedance spectrum of an electrochemical system. In this case, the frequencies from 10^5 Hz to 0.01 Hz were scanned at 5 mV

amplitude. Since EIS permits the examination of inductive, capacitive, and diffusion processes occurring in the electrochemical system, it is different from direct current (DC) methodologies. It is exploited for the investigation of interfacial properties concerning bio-recognition events taking place at the surface of the electrode, for instance, antibody-antigen recognition, substrate–enzyme, or whole-cell capturing. Thus, EIS serves as a significant tool in different sensing applications.

DPV offers some advantages over other techniques like CV. Here, the input waveform is a series of small amplitude short pulses that increase along a linear baseline [as shown in Figure 2.15]. The current is sampled prior to the application of the potential pulse. Then, the potential is stepped a little, and the current is sampled again at the end of the pulse. Now, the potential of WE is stepped by a lower value in comparison to the forward pulse such that the baseline potential of each pulse is stepped up throughout the sequence. This current measurement at each potential pulse minimizes the background (charging) current and leads to Faradaic current with minimum capacitive current, as a result of which, the sensitivity gets significantly enhanced. The smaller potential step size is accompanied by narrower peaks in DPV and it is a very helpful technique to distinguish the analytes exhibiting very close oxidation potential.

CV, DPV and EIS all are electrochemical techniques, but they differ in their outputs and targets. DPV is mainly an analytical technique that is conventionally employed for the quantitative analysis of some specific electroactive analyte. It is the most common pulse technique, along with SWV, and is extremely sensitive. CV and EIS, on the other hand, are generally employed for the electrochemical characterization of any electrochemical species/system, and investigation of its properties. Both are now rarely utilized for the determination of the analyte concentration. Rather, EIS gives an insight into the details

of the capacitance as well as the kinetics of manifold electrochemical processes occurring in the system.

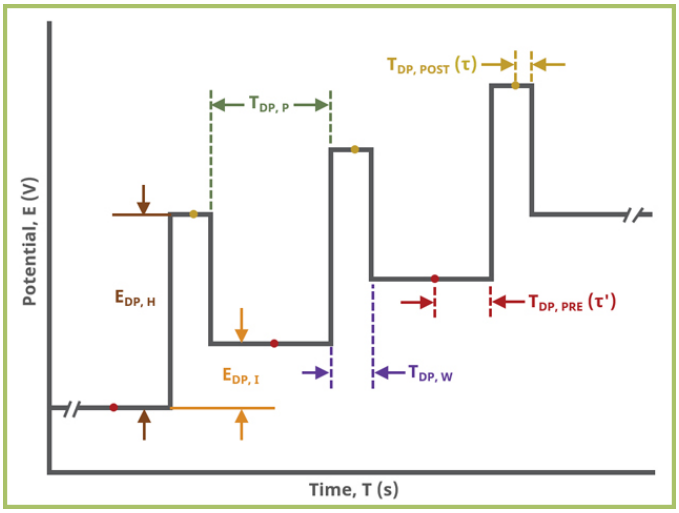

Figure 2.19 Differential Pulse Voltammetry (DPV) - Pulse Sequence Detail [pineresearch.com]