Chapter 2

Experimental methods and characterization techniques

Synthesis

Characterization

Applications
2.1 Experimental methodology

Material synthesis is the fundamental aspect of modern research and industrial development [1]. The synthesis of nanomaterials with defined dimensions, structure and composition is one of the most important pre-requisites for their applications in various fields such as bio-separation [2], drug delivery [3], catalysis [4], sensing [5] and data storage etc. [6]. Chemical synthesis represents a key approach for the large scale production of materials, which generally involves a number of steps taking place in liquid or gas phase [7].

This chapter gives the detailed procedure employed in the present work for the synthesis of the samples i.e. TiO$_2$, Nd doped TiO$_2$, Ag coated TiO$_2$, Fe$_3$O$_4$ and nanocomposites of TiO$_2$-Fe$_3$O$_4$.

Synthesis protocols used in the present work

The following methods were used in the experimental work:

- Sol-gel method
- Sol-hydrothermal method
- Sonochemical activated sol-gel method and sol-hydrothermal method
- Photochemical method of deposition
- Sonochemical method of deposition

All the chemicals used were of analytical grade. Titanium isopropoxide was supplied by Avra synthesis Pvt. Ltd. India. The salt precursor, i.e. Neodymium oxide (Nd$_2$O$_3$), was from Alfa Aesar (Johnson Matthey Chemicals) India, while Silver nitrate (AgNO$_3$) and Nitric acid (HNO$_3$) were from S. D. fine chemicals, India. The mineralizers such as Glycine, Urea, Hexamine and Sodium hydroxide were supplied by Loba Chemie Pvt. Ltd. India. High purity Ferric chloride (FeCl$_3$) and Ferrous ammonium sulphate (FAS) were from Qualigens chemicals. Double distilled water was used wherever necessary.
2.1.1 Synthesis of bare TiO$_2$

The synthesis of bare TiO$_2$ was carried out by three different procedures mentioned below

**A) Sol-gel method**

Undoped TiO$_2$ was prepared by starting with stoichiometric amount of Titanium isopropoxide dissolved in appropriate amount of ethanol under vigorous stirring. To this, 2–3 drops of nitric acid were added to obtain a clear solution; the solution was then hydrolyzed with distilled water for 45 min to obtain the sol of TiO$_2$. The sol thus formed was subjected to aging for a period of 24 h under ambient conditions. The resulting gel was then heated at 100°C by keeping it in an oven for 48 h. This dried powder was further sintered at 500 °C for 3 h to get the desired phase of TiO$_2$.

**B) Sol-hydrothermal method**

In case of sol-hydrothermal method, the same procedure was repeated up to the sol formation as in case sol-gel synthesis. The sol was then transferred to a stainless steel autoclave with a teflon lined container and subjected to hydrothermal treatment under autogeneous pressure for a period of 24 h. The temperature of the autoclave was maintained at 100 °C. The product was washed three times with double distilled water and dried in an oven at 100 °C for 1 hour.

**C) Sonochemically activated sol-gel and sol-hydrothermal method**

i) **Sonochemical activation of precursor solution**

A fixed volume of ethanol (33 ml) was taken in a beaker and kept in a bath type ultrasonicator (40 ± 3) kHz, 500 W. To this, 8 ml of Titanium isopropoxide were added dropwise with the help of a syringe. Further, 2-3 drops of nitric acid were added to obtain the clear precursor solution followed by dropwise addition of distilled water until a sol of TiO$_2$ was formed. For synthesis in presence of mineralizers (Glycine, Urea, Hexamine and Sodium hydroxide) by hydrothermal method, 20 ml of 2 M solution was added before the addition of water. The total time of sonication was around 1 h. The sol thus obtained was then divided into two parts and used for hydrothermal and sol gel synthesis.
ii) Sol-hydrothermal method
A part of the sonochemically activated sol was transferred to a stainless steel autoclave with a teflon lined container and subjected to hydrothermal treatment under autogeneous pressure for a period of 24 h. The temperature of the autoclave was maintained at 100 °C. The product was washed three times with double distilled water and dried in an oven at 100 °C for 1 hour. The sample synthesized by this method will be abbreviated as SH and in presence mineralizers e. g. Glycine, Urea, Hexamine and NaOH, samples will be abbreviated as SH(GLY), SH(URE), SH(HEX) and SH(Na) respectively.

iii) Sol - gel method
Another part of the sonochemically activated sol was subjected to aging for a period of 24 hours under ambient conditions. The resulting gel was then heated at 100° C by keeping it in an oven for 3 h. The resulting powder was further sintered at 100° C for 24 hours to obtain the desired phase of TiO₂. The sample synthesized by this method will be abbreviated as SG.

2.1.2 Synthesis of Nd doped TiO₂
Sol-gel method
Two sets of solutions were prepared initially. Solution-A consisted of stoichiometric amount of Titanium isopropoxide dissolved in appropriate amount of ethanol along with 2–3 drops of nitric acid and Solution-B consisted of stoichiometric amount of neodymium nitrate (Neodymium oxide was dissolved in nitric acid) dissolved in a mixture of ethanol and water (10:1). Solution-B was added dropwise to solution-A under vigorous stirring at room temperature. After complete addition, the mixture was stirred for additional few minutes to obtain the sol of Nd doped TiO₂. The remaining procedure was similar as mentioned above in case of sol-gel preparation of undoped TiO₂.

2.1.3 Synthesis of Fe₃O₄ nanoparticles
Sonochemically activated sol-hydrothermal method
2 mM of Ferric chloride and 1 mM of ferrous ammonium sulphate were taken in 20 ml of distilled water and placed in a bath type of ultrasonicator. Further, 2 M of
NaOH was taken in 10 ml of distilled water and kept in a ultrasonocator. After the complete dissolution of iron precursors, solution was transferred to a stainless steel autoclave with a teflon lined container. 2 M of NaOH solution was then added dropwise to the iron precursor solution. The stainless steel autoclave was subjected to hydrothermal treatment under autogeneous pressure for a period of 3 h. The temperature of the autoclave was maintained at 100 °C. The product was washed three times with double distilled water and dried in an oven at 100 °C for 1 h.

2.1.4 Synthesis of Fe$_3$O$_4$-TiO$_2$ nanocomposites

**Sonochemically activated sol-hydrothermal method**

Two sets of solutions were prepared initially. Solution-A consisted of stoichiometric amount of Fe$_3$O$_4$ in 5 ml of distilled water and Solution-B consisted of stoichiometric amount of as prepared TiO$_2$ in 20 ml of ethanol. Both the solutions were subjected to the ultrasonic treatment for about 30 min for the uniform dispersion of the solutes. Solution A was then added dropwise to solution B under ultrasonication treatment. After complete addition, the mixture was sonicated for additional few minutes and then subjected to hydrothermal treatment. The temperature of the autoclave was maintained at 100 °C. The product was washed three times with double distilled water and dried in an oven at 100 °C for 1 hour.

2.1.5 Deposition of silver on TiO$_2$ samples

The samples were coated with silver by photodeposition and sonochemical deposition method.

A) **Photodeposition method**

In this method, appropriate amount of sample was taken in a mixture of distilled water and ethanol (5:1). To this, 2 to 10 ml of 0.005 M AgNO$_3$ solution was added. This mixture was then photoirradiated under 80 Watt high pressure Hg lamp for 2 h. The reaction mixture was then filtered and the products were dried by keeping them in an oven for 3 h at 100°C.

B) **Sonochemical method**

In this method, appropriate amount of sample was taken in beaker containing a mixture of distilled water and ethanol (5:1). To this, 2 to 10 ml of 0.005 M AgNO$_3$ solution was added. The beaker was then kept in a bath type of an ultrasonicator
At a frequency of 40 ± 3 kHz and 500 power. The total time of sonication was around 2 h. The reaction mixture was then filtered, and the products were washed and dried by keeping it in an oven for 3 h at 100 °C.

2.2 Applications of synthesized nanoparticles

2.2.1 Photocatalytic activity

The photocatalytic activities of all the catalysts were tested by studying the degradation reaction of Methyl Orange (MO) dye solution. A high pressure mercury lamp (80 W) was used as a light source placed in the photoreactor surrounded with water circulated quartz jacket to avoid thermal heating. Prior to photoreaction, the suspension was magnetically stirred in dark for 30 min to establish adsorption/desorption equilibrium. For a photocatalysis experiment, 0.050 mg /200 mg of the catalyst was dispersed in 500 ml of MO (7x10⁻⁵ M) solution. The above suspension was kept under constant stirring during irradiation. About 4 ml of aliquots were sampled at regular time intervals, centrifuged to remove particles, and analyzed by recording the absorption spectra of Methyl Orange between 400-600 nm using Shimadzu 1800 spectrophotometer.

The absorption spectrum of MO dye solution (λ_max = 462 nm) after stirring the suspension in the dark for 30 mins (that is, just before the UV light exposure) was recorded (A₀) as a reference spectrum corresponding to the initial MO dye concentration (C₀). The intensity of absorbance peak (A) of MO dye solution after photocatalysis reaction, was taken as a measure of residual MO dye concentration (C). The residual MO dye concentration was calculated using the relationship as follows [8].

\[
(C/C₀)_{MO} = (A/A₀)_{462 \text{ nm}}
\]  

A schematic diagram of the indigenously fabricated photoreactor is shown in Fig. 2.1. A cylindrical reactor surrounded by a circulating water jacket maintained at a constant temperature throughout the experiment was used. High pressure mercury lamp was used as the UV light source. The solution was constantly stirred with a magnetic stirrer. The setup was kept in a closed wooden box.
2.2.2 Antibacterial activity

The antimicrobial activities of TiO$_2$, Nd doped TiO$_2$ and Ag coated Nd doped TiO$_2$ nanoparticles were tested in dark as well as in the solar light using two common bacterial strains: *E. coli* (Gram negative) and *S. aureus* (Gram positive). For this, the nanoparticles (1 mg ml$^{-1}$ for *E. coli* and 1.5 mg ml$^{-1}$ for *S. aureus*) and bacteria (10$^4$ cells ml$^{-1}$) were stirred (250 rpm) together in a nutrient broth (NB) for 24 h at 37 °C. The bacterial viable count was determined at different time intervals by plating the serial dilutions on nutrient agar plates and the number of colony forming units (CFU) were counted [9]. The survival of bacterial population was calculated by the equation:

$$\text{Survival (\%)} = \frac{(PT - PI) \times 100}{(PT - PI)} \quad (2.2)$$

Where PT - CFU after illumination time (T);
PI - CFU before illumination.

The morphological damages in the bacterial cells were investigated with the help of TEM and SEM analysis of the samples. Bacterial cells were harvested after the exposure, fixed in glutaraldehyde (2.5%) and dehydrated in a series of increasing
concentration of ethanol (50, 60, 70, 80, 90, 95, and 100%) and observed under the microscope.

The antibacterial activities for TiO$_2$, Fe$_3$O$_4$-TiO$_2$ composite and Ag coated Fe$_3$O$_4$-TiO$_2$ nanocomposites were investigated by the cell viability test using *Eschericia coli* (*E. coli*) as the model organism. For this, different concentrations of nanoparticles (viz 100, 50 and 25 µg ml$^{-1}$) were mixed with the bacteria ($10^7$-$10^8$ cells ml$^{-1}$) in saline (0.15 M NaCl) solution. The mixture was incubated at 37 °C for 2 h with intermittent shaking. After incubation, the viable bacterial count was determined by spread plate technique using nutrient agar plates. The number of colony forming units (CFU) was counted after 24 h. Corresponding controls (samples without the nanoparticles) were run simultaneously. Loss of viability was calculated by the following formula [10]:

\[
\text{Loss of viability} \% = \left[ \frac{C_c - C_s}{C_c} \right] \times 100
\]  

Where, $C_c$ is the colony count of the control and $C_s$ is the colony count of sample in presence of nanoparticles.

### 2.3 Characterization and measurement techniques

Being very small, analyzing and understanding the structural and chemical changes of these nanomaterials is extremely crucial hence; special instruments and techniques are needed for their characterization. This part of the chapter presents the basic principles of the characterization techniques that have been used in the present work. This mainly includes X-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR), Diffuse reflectance UV-Visible spectroscopy (DRUV), Photoluminescence (PL) spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and N$_2$-BET surface area analysis. Some techniques which are especially important with reference to magnetic nanoparticles such as Vibrating sample magnetometry (VSM) and Magnetic force magnetometry (MFM) are also discussed in some details. The emphasis of this chapter is laid on the
discussion of the specific strengths of different techniques in unfolding the various aspects of the physical and chemical properties of the synthesized nanoparticles.

### 2.3.1 X-ray diffraction analysis

In 1895 Röntgen published his work on the discovery of X-rays and in 1912 Laue Bragg observed diffraction of X-rays from a crystal. These two events are very important in the history of X-ray diffraction. Interaction of cones of X-rays is related to interplanar spacings in the crystalline powder according to a mathematical relation called “Bragg’s Law” [11].

\[ n\lambda = 2d \sin\theta \]  \hspace{1cm} (2.4)

Where \( n \) is an integer

\( \lambda \) = wavelength of X-rays

\( d \) = interplanar spacing generating the diffraction and

\( \theta \) = diffraction angle

\( \lambda \) and \( d \) are measured in similar units, usually angstroms. The pictorial representation of diffraction of X-rays from the crystal plane is shown in Fig. 2.2. For a powder specimen in a diffractometer having a statistically infinite amount of randomly oriented crystallites, diffraction maxima (or peaks) are measured along the 2\( \theta \) diffractometer circle.

![Figure 2.2: Representation of diffraction of X-rays by crystal planes](image)
X-ray crystallography relies on the dual wave/particle nature of x-rays to obtain information about the structure of crystalline materials. The relation between the width of X-ray diffraction line and particle size was first derived by Debye-Scherrer in 1918 [12].

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

(2.5)

According to the above equation, peak width (\(\beta\)) is inversely proportional to crystallite size (\(D\)). \(\beta\) is measured in degrees which can be converted in radians by the equation

\[
\beta = \beta' \pi/180
\]  

(2.6)

The constant of proportionality, \(K\) (the Scherrer constant) depends on how the width is determined, the shape of the crystal, and the size distribution.

**A) Instrumentation**

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons towards a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being \(K_\alpha\) and \(K_\beta\). \(K_\alpha\) consists, in part, of \(K_{\alpha1}\) and \(K_{\alpha2}\). \(K_{\alpha1}\) has a slightly shorter wavelength and twice the intensity as \(K_{\alpha2}\). The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. \(K_{\alpha1}\) and \(K_{\alpha2}\) are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction with CuK\(_\alpha\) radiation = 1.5418\(\text{Å}\). These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded.
When the geometry of the incident X-rays impinging the sample satisfies the Bragg equation, constructive interference occurs and a peak appears. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or a computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle $\theta$ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of $2\theta$. The instrument used to maintain the angle and rotate the sample is termed as a goniometer. For typical powder patterns, data is collected at $2\theta$ from $\sim5^\circ$ to $70^\circ$, angles that are preset in the X-ray scan as shown in Fig. 2.3 [13].

**B) Applications**

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical in almost all branches of science such as geology, environmental science, material science, engineering and biology. Other applications of XRD include:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions
- Measurement of sample purity, textural measurements such as the orientation of grains in a polycrystalline sample

In the present study, the XRD patterns of the powder samples were recorded using a Philips PW 1840 powder X-ray diffractometer. Silicon was used as an external standard for correction due to instrumental broadening.

2.3.2 Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a spectroscopic technique that utilizes lower energy radiations to induce vibrational and rotational excitations of atoms and groups in atoms within molecules. Molecules containing covalent bonds generally show absorption in IR to some extent. Infra-red absorption occurs when the frequency of the alternating electric field that is associated with the incident radiation matches with the change in a vibrational or rotational frequency of the absorbing molecule. When the match occurs, the electromagnetic radiations (EMR) are absorbed by the molecule causing a change in the amplitude of vibration or a change in the rate of rotation. For absorption of electromagnetic radiation by a molecule, it is necessary that the molecule undergoes a change in dipole moment during the absorption [14]. The infrared portion of the electromagnetic spectrum is divided into three regions:

- The near infra-red region - 14000 to 3600 cm\(^{-1}\)
- Mid infra red region - 3600 to 200 cm\(^{-1}\) and
- Far- infrared region - 200 to 20 cm\(^{-1}\)

A) Instrumentation

In the fourier transform spectrophotometer (Fig. 2.4), there is a source, a monochromator and a detector. The source is in the form of a filament (e.g. Nernst filament, made up of a spindle of a rare earth oxide or carborundum rod) which is heated to red or white-heat by an electric current. The monochromator filters the IR beam and focuses it on the sample.
The detectors are based on either temperature (bolometer/thermometer) or conductivity rise at a given frequency. Further, an interferometer such as Michelson interferometer is used which converts the EMR to a slower oscillating frequency; slow enough for the infra-red detector to respond to it. The data is continuously recorded for substantial time to get the desired enhancement. The amplitude of each resolved oscillation is a function of the radiation frequency. A mathematical method called a Fourier transform is used for the conversion of time domain spectrum to frequency domain spectrum [15].

B) Applications

The vibrational spectrum of a molecule is considered to be a unique physical property and is characteristic of the molecule. As such, the infrared spectrum can be used as a fingerprint of the compound. Fingerprint areas of various bonds are shown in Fig. 2.5.

In the present study, the FTIR analysis of the samples were carried out in the region ~ 4000 – 400 cm$^{-1}$ on FTIR Shimadzu 8400 instrument. KBr was used as the mulling agent for preparing the samples. Fig. 2.6 depicts the photograph of the same instrument used for the FTIR analysis in the present study.
2.3.3 UV-Visible spectroscopy – Diffuse reflectance UV-Visible spectroscopy

Ultraviolet (200-400 nm) and visible (400-800 nm) radiations are found towards the small wavelength and high frequency end of the electromagnetic spectrum. This energy range corresponds well with the energy range required to cause electronic excitations between molecular orbitals (ΔE). ΔE is defined as the energy difference between an occupied orbital (ground state) and an empty (excited state) orbital. The smaller the value of ΔE, longer is the wavelength required to excite the electron. When the energy of the incoming photon matches with ΔE, the photon is absorbed, and an electron from an occupied level "jumps" from its ground state to an empty level (also called an excited state) [16].

The electronic transitions shown in Fig. 2.7a, describe that these transitions occur from various occupied levels to empty levels, but in reality, excitation only occurs between the two lowest energy transitions, the outer electron, π > π* and the n > π* levels for the energy range of 200-800 nm associated with UV-Vis radiations. In general, these transitions occur between the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) as shown in Fig. 2.7b. When the photon is absorbed, a portion of the energy is absorbed as the electron is promoted to the excited state.
Figure 2.7: Electronic excitations of electrons between molecular orbitals

A) Instrumentation

Fig. 2.8 represents the schematic diagram of the UV-Visible spectrometer. The light source is usually a hydrogen or deuterium lamp for UV measurements and a tungsten lamp for visible measurements. The wavelengths of these continuous light sources are selected with a wavelength separator such as a prism or grating or monochromator. Spectra are obtained by scanning the wavelength separator and quantitative measurements can be made from a spectrum or at a single wavelength.

Figure 2.8: Block diagram of UV-Visible spectrophotometer

B) Diffuse reflectance spectroscopy

In general, the sample must be in liquid state for its analysis by UV-Vis spectrophotometer. However, some solid materials can be analyzed by dissolving the solid in a solvent. This process is labor intensive and can introduce many sources of error into the measurement. It is also a destructive technique. These factors make dissolution methods undesirable for most solid materials. Fortunately, there are accessories available which allow the analyst to take advantage of the reflection of the
incoming beam rather than using the standard absorption configuration like in the case of diffuse reflectance UV-Visible spectroscopy [17].

Diffuse reflectance relies upon the focused projection of the spectrophotometer beam into the sample where it is reflected, scattered and transmitted through the sample material as shown in Fig. 2.9. The back reflected, diffusely scattered light (some of which is absorbed by the sample) is then collected by the accessory and directed to the detector optics. Only the part of the beam that is scattered within a sample and returned to the surface is considered to be diffuse reflection. Usually, the sample must be ground and mixed with a non-absorbing matrix such as BaSO₄.

![Figure 2.9: Different types of reflections](image)

**C) Applications**

Diffuse UV-Vis reflectance spectroscopy is ideal for characterizing optical and electronic properties of many different materials such as films, filters, and pigments. This method can also be used for determining the band gap of a material. The determination of band gap of materials is important to obtain the basic solid state physics of the samples. Band gap indicates the difference in energy between the top of the valence band filled with electrons and the bottom of the conduction band devoid of electrons. Hence, the information related to the electric conductivity of the materials can also be obtained. In the present work, Diffuse Reflectance Spectra (DRS) of the neat samples were recorded on the Perkin Elmer LAMBDA 950 spectrophotometer. BaSO₄ was used as a standard reference.
2.3.4 Photoluminescence spectroscopy

Photoluminescence spectroscopy is a contactless, nondestructive method of probing the electronic structure of materials. Light is directed onto a sample, where it is absorbed and imparts excess energy into the material in a process called photo-excitation. One way this excess energy is dissipated by the sample is through the emission of light which is also called as luminescence. In the case of photo excitation, this luminescence is called as photoluminescence. The intensity and spectral content of this photoluminescence is a direct measure of various important material properties. Photo-excitation causes electrons within the material to move into permissible excited states. When these electrons return to their equilibrium states, the excess energy is released by the emission of light by a radiative or a non radiative process. The energy of the emitted light (photoluminescence) relates to the difference in energy levels between the two electron states involved in the transition between the excited state and the equilibrium state. The quantity of the emitted light is related to the relative contribution of the radiative process [18].

A) Instrumentation

The photoluminescence spectrophotometer contains five essential parts, a light source to provide excitation radiation; a filter or monochromator to select the wavelength of the excitation radiation; a sample cell; an emission monochromator to analyze the emitted light and finally a photo sensor to measure the intensity of the emitted light. These five units are shown in a block diagram, in Fig. 2.10.

![Schematic representation of a typical photoluminescence spectrometer](image_url)

Figure 2.10: Schematic representation of a typical photoluminescence spectrometer
B) Applications

Photoluminescence technique is very useful to determine the band gap of a semiconductor material, impurity levels, defects present in the compound and material quality. Analysis of photoluminescence also helps to understand the underlying physics of the recombination mechanism in the semiconductor material. In the present study, the room temperature - Photoluminescence Spectra (RT-PL) were recorded on Perkin Elmer-LS-55-Photoluminescence spectrophotometer. The solid samples were placed in a powder holder which has a synthetic fused silica window.

2.3.5 BET Surface area analysis

In 1938, Brunauer, Emmett, and Teller published an article about the BET theory in a journal for the first time; "BET" consists of the first initials of their family names ‘Brunauer-Emmett-Teller’ [19].

The Langmuir theory is based on the following assumptions

- All surface sites have the same adsorption energy for the adsorbate, which is usually argon, krypton or nitrogen gas. The surface site is defined as the area on the sample where one molecule can adsorb onto.
- Adsorption of the solvent at one site occurs independently of adsorption at the neighboring sites.
- Activity of adsorbate is directly proportional to its concentration.
- Adsorbates form a monolayer.
- Each active site can be occupied only by one molecule.

The Langmuir theory has a few flaws that are addressed by the BET theory. The BET theory extends the Langmuir theory to multilayer adsorption (Fig. 2.11) with three additional assumptions [20]:

- Gas molecules will physically adsorb on a solid in layers infinitely.
- The different adsorption layers do not interact.
- The theory can be applied to each layer.
Figure 2.11: Schematic diagram showing (a) the monolayer adsorption model assumed by the Langmuir theory and (b) the multilayer adsorption model assumed by the BET theory

The resulting BET equation is expressed by

$$\frac{1}{V \left[\frac{p}{p_0} - 1\right]} = \frac{1}{V_m C} + \frac{C - 1 \left(\frac{p}{p_0}\right)}{V_m C}$$  \hspace{1cm} (2.7)

$p$ and $p_0$ are the equilibrium and the saturation pressure of adsorbates respectively at the temperature of adsorption,

$V = \text{Adsorbed gas quantity (for example, in volume units)}$

$V_m = \text{Monolayer adsorbed gas quantity.}$

$C = \text{BET constant, which is expressed by}$

$$C = \exp \left(\frac{E_1 - E_L}{RT}\right)$$  \hspace{1cm} (2.8)

$E_1 = \text{Heat of adsorption for the first layer}$

$E_L = \text{Heat of adsorption for the second and higher layers and is equal to the heat of liquefaction.}$

Equation (1) is based on adsorption isotherm which can be plotted as a straight line with $V \left[\frac{p}{p_0} - 1\right]$ on the y-axis and $\phi = \frac{p}{p_0}$ on the x-axis according to experimental results. This plot is called as a ‘BET plot’. The linear relationship of this equation is maintained only in the range of $0.05 < \frac{p}{p_0} < 0.35$. The value of the slope $A$ and the y-
intercept I of the line are used to calculate the monolayer adsorbed gas quantity $V_m$ and the BET constant $C$. The following equations can be used:

$$V_m = \frac{1}{A + I} \quad (2.9)$$

$$C = 1 + \frac{A}{I} \quad (2.10)$$

The BET method is widely used in surface science for the calculation of surface areas of solids by physical adsorption of gas molecules. Total surface area $S_{\text{total}}$ and a specific surface area $S$ are evaluated by the following equations

$$S_{\text{BET,Total}} = \frac{V_m N_s}{V} \quad (2.11)$$

Where, $V_m$ is in units of volume which are also the units of the molar volume of the adsorbate gas

$$S_{\text{BET}} = \frac{S_{\text{(total)}}}{A} \quad (2.12)$$

$N = \text{Avogadro’s number}$
$s = \text{Adsorption cross section of the adsorbing species}$
$V = \text{Molar volume of adsorbate gas}$
$A = \text{Mass of adsorbent (in g)}$

Monolayer formation of gas molecules on the surface is used to determine the specific surface area, while the principle of capillary condensation can be applied to assess the presence of pores, pore volume and pore size distribution.

**A) Instrumentation**

Prior to any measurement, the sample must be degassed to remove water and other contaminants before the surface area can be accurately measured. Samples are degassed in a vacuum at high temperatures. The highest temperature possible that will not damage the sample’s structure is usually chosen in order to shorten the degassing time. IUPAC recommends that samples be degassed for at least 16 hours to ensure that unwanted vapors and gases are removed from the surface of the sample. Generally, samples that can withstand higher temperatures without structural changes
have smaller degassing times. A minimum of 0.5 g of sample is required for the BET analysis to successfully determine the surface area.

Samples are placed in glass cells to be degassed and analyzed by the BET machine. Glass rods are placed within the cell to minimize the dead space in the cell. Sample cells typically come in sizes of 6, 9 and 12 mm and in different shapes. 6 mm cells are usually used for fine powders, 9 mm cells for larger particles and small pellets of 12 mm are used for large pieces that cannot be further reduced. The cells are placed into the heating mantles and connected to the outgas port of the machine.

![Schematic representation of the BET instrument](image)

**Figure 2.12: Schematic representation of the BET instrument**

After the sample is degassed, the cell is moved to the analysis port (Fig. 2.12). Dewars of liquid nitrogen are used to cool the sample and maintain it at a constant temperature. Low temperature must be maintained so that the interaction between the gas molecules and the surface of the sample will be strong enough for measurable amounts of adsorption to occur. The adsorbate, nitrogen gas in this case, is injected into the sample cell with a calibrated piston. The dead volume in the sample cell must be calibrated before and after each measurement. To do that, helium gas is used as a blank because helium does not adsorb onto the sample [21].
B) Applications

This technique provides detailed information about the specific surface area, pore volume and pore size distributions of the sample. The sample generally includes activated carbon, zeolites, and also any type of sample like alumina, silica, metal supported catalysts, metal oxide powders, pigments, pharmaceutical powders, etc.

In the present study, the surface area of the catalyst samples were measured by using BET method on a surface area analyzer from Thermo Scientific Surfer. The same instrument can be seen along with the degassing unit in Fig. 2.13. Prior to the measurement, the samples are pre-treated at elevated temperature in vacuum or flowing gas in order to remove any contaminants.

![Figure 2.13: Photograph of BET surface area analyzer](image)

2.3.6 Scanning electron microscopy (SEM)

The scanning electron microscopy is a versatile, non-destructive technique that reveals detailed information about the morphology and the composition of natural and manufactured materials [22].

A) Instrumentation

In a typical SEM instrument (as shown in Fig. 2.14), electrons are thermionically emitted from a tungsten or LaB₆ cathode filament towards an anode. The electron beam, which typically has an energy ranging from a few keV to 50 keV, is focused by two successive condenser lenses into a beam with a very fine spot size (~ 5nm).
The beam then passes through the objective lens, where pairs of scanning coils deflect the beam either linearly or in a raster fashion over a rectangular area of the sample surface. As the primary electrons strike the surface they are inelastically scattered by the atoms in the sample. Through these scattering events, the primary beam effectively spreads and fills a teardrop-shaped volume extending about 1μm into the surface. Interactions in this region lead to the subsequent emission of electrons and x-rays, which are then detected to produce an image.

![Schematic diagram of scanning electron microscope](image)

**Figure 2.14: Schematic diagram of scanning electron microscope**

**B) Field emission scanning electron microscopy (FE-SEM)**

Type of emitter is the main difference between Scanning electron microscope (SEM) and field emission scanning electron Microscope (FESEM). In SEM, thermionic emitter is used while in case of FESEM field emitter is used [23]. Thermionic Emitters use electrical current to heat up a filament; the two most common materials used for filaments are Tungsten (W) and Lanthanum Hexaboride (LaB₆). When the heat is enough to overcome the work function of the filament material, the electrons can escape from the material itself. Thermionic sources have relatively low brightness, evaporation of cathode material and thermal drift during operation. Field emission is one way of generating electrons that avoids these problems. A field emission gun (FEG); also called a cold cathode field emitter, does not heat the filament. The emission is reached by placing the filament in a huge electrical potential
gradient. The FEG is usually a wire of Tungsten (W) fashioned into a sharp point. Some of the advantages of the FE-SEM instrument

- FESEM produces clearer, less electrostatically distorted images with spatial resolution down to 1 to 1/2 nm. That's 3 to 6 times better than conventional SEM.
- Smaller-area contamination spots can be examined at electron accelerating voltages compatible with Energy Dispersive X-ray Spectroscopy.
- Reduced penetration of low kinetic energy electrons probes closer to the immediate material surface.
- High quality, low voltage images are obtained with negligible electrical charging of samples. Accelerating voltages range from 0.5 to 30 kV.
- Need for placing conducting coatings on insulating materials is virtually eliminated.

C) Applications

The scanning electron microscope (SEM) is a type of electron microscope capable of producing high resolution images of a sample surface. It has a characteristic 3-dimensional quality and is useful for determining the surface structure, topography, morphology and composition of the sample.

![Photograph of scanning electron microscope](image)

**Figure 2.15: Photograph of scanning electron microscope**

In the present study, both SEM and FESEM have been used for the characterization of the materials. The SEM micrographs were taken using JEOL JSM 6360A (as shown in Fig. 2.15) and the Field emission micrographs were taken on TESCAN (Model-MIRA...
3 LMH) instrument. For both the analysis, the samples were sputtered with Pt and then the wafer was mounted onto a stainless steel sample holder using silver conductive paste.

### 2.3.7 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is an imaging technique whereby a beam of electrons is focused onto a specimen causing an enlarged version to appear on a fluorescent screen or a layer of photographic film, or to be detected by a CCD camera. TEM operates on the same basic principle as the light microscope but uses electrons instead of light. TEM is also useful for the determination of the lattice planes \((d\) spacing). The \(d\)- spacing between lattice planes of crystalline materials can be calculated from a SAED pattern using the relationship [24]:

\[
dr = \lambda L
\]  

\(2.13\)

where, \(L\) is the distance between the specimen and the photographic plate, \(\lambda L\) is known as the camera constant and \(r\) is the radius of diffracted rings. It is easy to measure \(r\) directly from the photographic plate, and \(\lambda L\) can be established from the instrument by calibrating it with a standard material (usually Ag), and hence one can easily get the \(d\) values. Since, each \(d\) value corresponds to a specific lattice plane for a specific crystal structure; description of the crystal structure of a crystalline specimen can be obtained from a SAED pattern. In some cases, SAED pattern is more helpful as compared to XRD, due to the limited detection limit of XRD instrument. Also, the XRD generally gives global information.

### A) Instrumentation

The line diagram of a typical TEM column is shown in Fig. 2.16. There are basically three types of electron guns used in today's transmission electron microscopes, the tungsten cathode, the lanthanum hexaboride (LaB\(_6\)) cathode, and the field emission gun. The electron beam is focused using electromagnetic lenses. The condenser lens system, which is composed of one or more lenses, determines the beam current that impinges on the sample. The probe-forming lens, often called the objective lens, determines the final spot size of the electron beam. Conventional electromagnetic
lenses are used and the electron beam is focused by the interaction of the electromagnetic field of the lens on the moving electrons. The working distance is defined as the distance between the bottom pole piece of the objective lens and the sample surface. It is typically 5 to 25 mm. As the working distance is increased, the spot size increases on the sample, given the same final aperture lens.

![Schematic diagram of a transmission electron microscope](image)

**Figure 2.16**: Schematic diagram of a transmission electron microscope

**B) Applications**

TEM is useful for determining size, shape and arrangement of the particles which make up the specimen. Direct information about the structure is obtained by high resolution transmission electron microscopy (HRTEM). The possibility for high magnifications has made the TEM a valuable tool in both medical, biological and materials research. It is highly useful for the determination of the lattice planes and the detection of atomic-scale defects localized in areas of few nanometers in diameter with the help of selected area electron diffraction (SAED) technique.

The TEM measurements in the present work were performed on a JEOL JEM-1200EX instrument operating at 120 kV, camera length of 80 cm and field limited aperture of 100 μm. Prior to TEM measurements, the samples were dispersed in a suitable organic solvent (isoamyl acetate/methanol/ acetone/ toluene, etc.) and a drop of the solution was poured on carbon-coated copper grid of 400 mesh size. The film formed on the TEM grids was allowed to dry for 2 minutes and the TEM and SAED
measurements were performed. The TEM instrument used in the present work can be seen in Fig. 2.17 [25].

![TEM Instrument](image)

**Figure 2.17:** A typical photograph of the TEM instrument along with a generalized cut away diagram of the internal structure of a transmission electron microscope

### 2.3.8 Vibrating sample magnetometry (VSM)

The magnetic characteristics of different materials as a function of the applied magnetic field at different temperatures and applied field strengths were measured using a Vibrating sample magnetometer (VSM) [26].

#### A) Instrumentation

Diagram explaining working of a vibrating sample magnetometer (VSM) is shown in Fig. 2.18. If a sample of any material is placed in a uniform magnetic field created between the poles of an electromagnet, a dipole moment will be induced. If the sample vibrates with sinusoidal motion a sinusoidal electrical signal can be induced in suitably placed pick-up coils. The signal has the same frequency of vibration and its amplitude is proportional to the magnetic moment, amplitude and relative position with respect to the pick-up coils system.
A loudspeaker transducer creates a vibrating motion of both the sample and the reference. The sample and reference pickup coils measure the flux change when the samples are vibrating. The sample signal is proportional to the rate and amplitude of the vibration, which can be obtained from the signal of the reference sample. Hence, the moment of the sample can be obtained. By sweeping the field of the magnet, hysteresis loops can be measured. Calibration of the vibrating sample magnetometer is done by measuring the signal of a pure Ni standard of known saturation magnetic moment placed in the saddle point. The VSM system used in this study has a field precision of 1 Oe and signal precision of 10^{-5} emu.

![Figure 2.18: Pictorial representation of vibrating sample magnetometer](image)

**B) Applications**

The vibrating sample magnetometry has become a widely used technique for determining the magnetic properties of a large variety of materials: diamagnetic, paramagnetic, superparamagnetic, ferrimagnetic, ferromagnetic and antiferromagnetic. In the present study, VSM analysis was done on a 7307 lakeshore model, at room temperature using a maximum field of 8000 Oe, and parameters viz. saturation magnetization (\(M_s\)), coercivity, and remanence magnetization (\(M_r\)) were evaluated for each sample.

**2.3.8 Magnetic force microscopy (MFM)**

Over the last two decades the Magnetic force microscopy (MFM) has evolved as an important technique for studying the magnetic properties of nanomaterials. The basic
principle of MFM is similar to the AFM technique. Briefly speaking, the MFM is AFM with the springy cantilever equipped with sharp magnetic probe on its end. MFM measures the magnetic forces (or the force gradient) acting between the surface of the magnetized sample and the magnetized tip. When magnetized tip is brought close to the surface of the magnetized sample (generally between units of hundred nanometers), long ranged magnetic forces (attractive and repulsive) originate. These forces deflect the cantilever stage which is detected by four section photodiode (PSPD) detector [27]. Fig. 2.19 depicts the principle of the MFM technique.

![Diagram of MFM technique](image)

**Figure 2.19: Figure depicting the working principle of magnetic force microscopic technique**

**A) Instrumentation**

A 25 μm diameter iron wire was used for cantilever as well as for the tip. The tip’s shape was created by tapering the wire to a 2 μm diameter with electrochemically etched 0.1 nm diameter tips at its end. The wire is rounded to the end of the tip in such a way that the axis of the tip is aligned perpendicular to the cantilever. Generally, two modes of operations were performed in this technique. In the first mode (dynamic regime), the magnetized tip is kept at a constant height over the specimen, whilst the magnetic field is dynamically modulated at specific frequency.
Because of the modulated magnetic field, the cantilever senses the long–ranged forces and vibrates with the same above mentioned frequency. The vibrations are detected by optical interferometer (sensitive He–Ne laser heterodyne probe). The second mode (static regime) is based on the modulation of the tip magnetization by pulsing the alternating current and detecting the deflection of the cantilever at the pulsing frequency [28].

B) Applications
MFM is an important tool for the detection of the magnetic properties of many ferromagnetic semiconductor nanomaterials. It is very useful in the field of nanobiotechnology which includes the study of the structural features of magnetotactic bacteria such as *Magnetospirillum magneticum*. The other application of MFM technique is characterization of magnetic nanoparticles which acts as an effective and sensitive detection system for many biological elements e.g. streptavidin. In the present study, the MFM micrographs were obtained from Asylum Research MFP3D instrument.
References


