CHAPTER 2

Materials and Methods

2.1 List of Chemicals

All the chemicals were used in the application part of this study, which was of AR grade and used without further purification. The name of all the chemicals is listed in Table 2.1. In the current study, deionized distilled water (DW) was utilized throughout the experiments.

Table 2.1 List of chemicals

S. No.	Name	Chemical formula	Physical state	Manufacturer
1.	1-pyrenecarboxaldehyde	$C_{17}H_{10}O$	solid	Sigma Aldrich
2.	Hydrazine hydrate	$N_2H_4.H_2O$	liquid	Sigma Aldrich
3.	Thiourea	NH_2CSNH_2	solid	Sigma Aldrich
4.	Citric acid	$HOC(COOH)(CH_2COOH)$	solid	Sigma Aldrich
5.	2,6-Diaminopyridine	$C_5H_7N_3$	solid	Sigma Aldrich
6.	Methanol	CH_3OH	liquid	S D Fine Chem Ltd.
7.	Ethanol	C_2H_5OH	liquid	S D Fine Chem Ltd.
8.	Isopropyl alcohol	$(CH_3)_2CHOH$	liquid	S D Fine Chem Ltd.
9.	Acetone	$(CH_3)_2O$	liquid	S D Fine Chem Ltd.
10	Dimethyl sulphoxide	$(CH_3)_2SO$	liquid	S D Fine Chem Ltd.
11	Dimethylformamide	$HCO(CH_3)_2$	liquid	S D Fine Chem Ltd.
12	Tetrahydrofuran	C_4H_8O	liquid	S D Fine Chem Ltd.
13	Dimethyl sulphoxide-d_6	$(CD_3)_2SO$	liquid	S D Fine Chem Ltd.
14	Deuterium oxide	D_2O	liquid	S D Fine Chem Ltd.
15.	Hydrochloric acid	HCl	liquid	S D Fine Chem Ltd.
16.	Sulphuric acid	H_2SO_4	liquid	S D Fine Chem Ltd.

17.	Nitric acid	HNO_3	liquid	S D Fine Chem Ltd.
18.	Sodium acetate	CH_3COONa	solid	S D Fine Chem Ltd.
19.	Sodium hydroxide	NaOH	solid	S D Fine Chem Ltd.
20.	Sodium chloride	NaCl	solid	Alfa Aesar
21.	Sodium nitrae	$NaNO_3$	solid	S D Fine Chem Ltd.
22.	Potassium nitrate	KNO_3	solid	S D Fine Chem Ltd.
23.	Aluminium nitrate	$Al(NO_3)_3.9H_2O$	solid	S D Fine Chem Ltd.
24.	Ferric Nitrate	$Fe(NO_3)_3.9H_2O$	solid	Sigma Aldrich
25.	Ferrous chloride	$FeCl_3$	solid	Sigma Aldrich
26.	Manganese nitrate	$Mn(NO_3)_2.6H_2O$	solid	Sigma Aldrich
27.	Chromium nitrate	$Cr(NO_3)_3.9H_2O$	solid	Sigma Aldrich
28.	Potassium dichromate	$K_2Cr_2O_7$	solid	Sigma Aldrich
29.	Nickel nitrate	$Ni(NO_3)_2.6H_2O$	solid	Sigma Aldrich
30.	Cobalt nitrate	$Co(NO_3)_2.6H_2O$	solid	Sigma Aldrich
31.	Copper nitrate	$Cu(NO_3)_2.2.5H_2O$	solid	Sigma Aldrich
32.	Zinc nitrate	$Zn(NO_3)_2.6H_2O$	solid	Sigma Aldrich
33.	Cadmium nitrate	$Cd(NO_3)_2.4H_2O$	solid	Sigma Aldrich
34.	Cadmium nitrate	$Cd(NO_3)_2.4H_2O$	solid	Sigma Aldrich
35.	Mercuric nitrate	$Hg(NO_3)_2.H_2O$	solid	Sigma Aldrich
36.	Lead nitrate	$Pb(NO_3)_2$	solid	Sigma Aldrich

2.2 Experimental Methodology

This chapter highlights the methods used for synthesis and characterization of polynuclear metal clusters and cages (with the transition as well as lanthanide metals), metal-organic frameworks, and their various applications.

2.3. Synthesis of ligands and metallogels

All reagents and chemicals were purchased from commercial sources and were used without further purifications.

2.3.1 Synthesis of ligands

The procedure for the synthesis of all ligands was mentioned in the respective chapter.

2.3.2 Synthesis of metallogels

The metallogels were synthesized either by introducing them to sonication or injection addition methods, which were mentioned in respective chapters.

2.3.3 Synthesis of aggregates

Aggregates were formed upon introduction of different water fraction in methanolic solution of pyrene derived reduced Schiff base ligands

2.4 Characterization of metallogels

The ligands and complexes were characterized by the following methods.

2.4.1 IR spectroscopy

Infrared spectroscopy is an important technique in organic chemistry. It is an easy way to identify the presence of certain functional groups in a molecule. Also, one can use the unique collection of absorption bands to confirm the identity of a pure compound or to detect the presence of specific impurities. Analysis by infrared spectroscopy is based on the fact that

molecules have specific frequencies of internal vibrations. These frequencies occur in the infrared region of the electromagnetic spectrum from ~ 4000 cm^{-1} to ~ 200 cm^{-1}.

When a sample is placed in a beam of infrared radiation, the sample will absorb radiation at frequencies corresponding to molecular vibrational frequencies, but will transmit all other frequencies. An infrared spectrometer measures the frequencies of radiation absorbed, and the resulting plot of absorbed energy *vs*. frequency is called infrared spectrum of the material. Identification of a substance is possible because different materials have different vibrations and yield different infrared spectra. Furthermore, from the frequencies of the absorption, it is possible to determine whether various chemical groups are present or absent in a chemical structure. In addition to the characteristic nature of the absorption, the magnitude of the absorption due to a given species is related to the concentration of that species.

Fourier Transform Infrared (FTIR) spectrometry was developed to overcome the limitations encountered with dispersive instruments. The main difficulty was the slow scanning process — a method for measuring all of the infrared frequencies simultaneously, rather than individually, as needed. A solution was developed which employed a very simple optical device called an interferometer. The interferometer produces a unique type of signal which has all of the infrared frequencies "encoded" into it. The signal can be measured very quickly, usually on the order of one second or so. Thus, the time element per sample is reduced to a matter of a few seconds rather than several minutes. Most interferometers employ a beam splitter that takes the incoming infrared beam and divides it into two optical beams. One beam reflects off of a flat mirror, which is fixed in place. The other beam reflects off of a flat mirror, which is on a mechanism that allows this mirror to move a very short

distance (typically a few millimeters) away from the beam splitter. The two beams reflect off of their respective mirrors and are recombined when they meet back at the beam splitter. Because the path that one beam travels is a fixed length and the other is constantly changing as its mirror moves, the signal which exits the interferometer is the result of these two beams "interfering" with each other.

The resulting signal is called an interferogram, which has the unique property that every data point (a function of the moving mirror position), which makes up the signal has information about every infrared frequency which comes from the source. It means that as the interferogram is measured, all frequencies are being measured simultaneously. Thus, the use of the interferometer results in extremely fast measurements. Because the analyst requires a frequency spectrum (a plot of the intensity at each frequency) to make the identification, the measured interferogram signal cannot be interpreted directly. A means of "decoding" the individual frequencies is required. It can be accomplished via a well-known mathematical technique called the Fourier transformation. This transformation is performed by the computer, which then presents the user with the desired spectral information for analysis (Figure 2.1).

FT-IR spectra were obtained for all the complexes on a Perkin Elmer spectrum 100 spectrometers with samples prepared as KBr pellets.

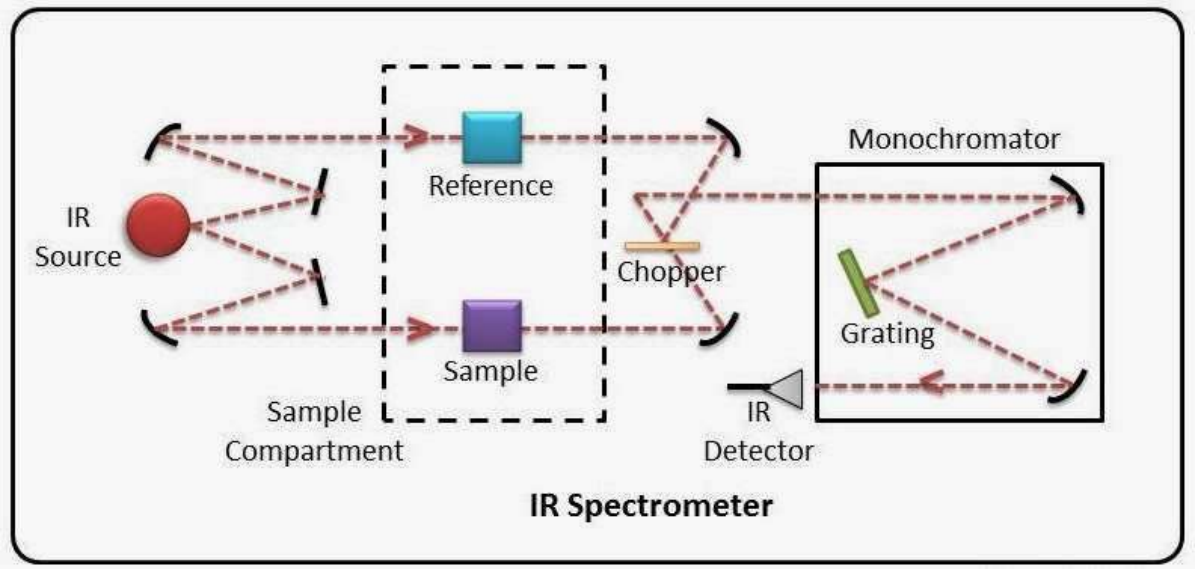

Figure 2.1 schematic representation of working of IR spectrometer

2.4.2 Elemental analysis

C, H, and N microanalyses for all the complexes were carried out with a 2400 Series-II CHN Analyzer, Perkin–Elmer, USA. Elemental analysis is an experiment that determines the amount (typically a weight percent) of an element in a compound. A CHN/O Analyzer is a scientific instrument that can determine the elemental composition of a sample. The name derives from the three primary elements measured by the device: carbon (C), hydrogen (H), and nitrogen (N) and oxygen (O). Sulfur (S) can also be measured.

2.4.2.1 Basic Principle

The capsule is injected into a high temperature (1000°C) furnace and combusted in pure oxygen under static conditions. At the end of the combustion period, a dynamic burst of oxygen is added to ensure the total combustion of all inorganic and organic substances. The resulting combustion products pass through specialized reagents to produce carbon dioxide (CO_2), water (H_2O), and Nitrogen (N_2) and oxides of nitrogen. These reagents also remove

other interferences, including halogens, sulfur, and phosphorus. The gases are then passed over copper to scrub excess oxygen and reduce oxides of nitrogen to elemental nitrogen. After scrubbing, the gases enter a mixing volume chamber to ensure a homogeneous mixture at constant temperature and pressure. The mixture then passes through a series of high-precision thermal conductivity detectors, each containing a pair of thermal conductivity cells. Between the first two cells is a water trap. The differential signal between the cells is proportional to the water concentration, which is a function of the amount of hydrogen in the original sample. Between the next two cells is a carbon dioxide trap for measuring carbon. Finally, nitrogen is measured against a helium reference.

2.4.3. NMR spectroscopy

Room temperature NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer in the DMSO solvent (Figure 2.2).

Nuclear Magnetic Resonance spectroscopy is a powerful and theoretically complex analytical tool. On this page, we will cover the basic theory behind the technique. It is important to remember that, with NMR, we are performing experiments on the nuclei of atoms, not the electrons. The chemical environment of specific nuclei is deduced from information obtained about the nuclei. Subatomic particles (electrons, protons, and neutrons) can be imagined as spinning on their axes. In many atoms (such as ^{12}C), these spins are paired against each other, such that the nucleus of the atom has no overall spin. However, in some atoms (such as ^{1}H and ^{13}C), the nucleus does possess an overall spin. The rules for determining the net spin of a nucleus are as follows if the number of neutrons and the number of protons are both even, then the nucleus has no spin. If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin (n = 1/2, 3/2, 5/2) if the

number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (n = 1, 2, 3)

The overall spin, I, is important. Quantum mechanics tells us that a nucleus of spin I will have 2I + 1 possible orientations. A nucleus with spin 1/2 will have 2 possible orientations. In the absence of an external magnetic field, these orientations are of equal energy. If a magnetic field is applied, then the energy levels split. Each level is given a magnetic quantum number, m.

Imagine a nucleus (of spin 1/2) in a magnetic field. This nucleus is in the lower energy level (i.e., its magnetic moment does not oppose the applied field). The nucleus is spinning on its axis. In the presence of a magnetic field, this axis of rotation will process around the magnetic field;

The potential energy of the processing nucleus is given by;

$$\mathbf{E = - mB\cos q}$$

Where, q is the angle between the direction of the applied field and the axis of nuclear rotation. If the nucleus absorbs energy, then the angle of precession, q, will change. For a nucleus of spin 1/2, absorption of radiation "flips" the magnetic moment so that it opposes the applied field (the higher energy state).

It is important to realize that only a small proportion of "target" nuclei are in the lower energy state (and can absorb radiation). There is the possibility that by exciting these nuclei, the populations of the higher and lower energy levels will become equal. If this occurs, then there will be no further absorption of radiation. The spin system is saturated. The possibility of saturation means that we must be aware of the relaxation processes which return nuclei to the lower energy state.

2.4.3.1 Relaxation processes

Ideally, the NMR spectroscopist would like relaxation rates to be fast - but not too fast. If the relaxation rate is fast, then saturation is reduced. If the relaxation rate is too fast, line-broadening in the resultant NMR spectrum is observed.

There are two major relaxation processes:

1. Spin - lattice (longitudinal) relaxation
2. Spin - spin (transverse) relaxation

2.4.3.2 Spin - lattice relaxation

Nuclei in an NMR experiment are in a sample. The sample in which the nuclei are held is called the lattice. Nuclei in the lattice are in vibrational and rotational motion, which creates a complex magnetic field. The magnetic field caused by the motion of nuclei within the lattice is called the lattice field. This lattice field has many components. Some of these components will be equal in frequency and phase to the Larmor frequency of the nuclei of interest. These components of the lattice field can interact with nuclei in the higher energy state, and cause them to lose energy (returning to the lower state). The energy that a nucleus loses increases the amount of vibration and rotation within the lattice (resulting in a tiny rise in the temperature of the sample).

The relaxation time, T_1 (the average lifetime of nuclei in the higher energy state) is dependent on the magneto-gyric ratio of the nucleus and the mobility of the lattice. As mobility increases, the vibrational and rotational frequencies increase, making it more likely for a component of the lattice field to be able to interact with excited nuclei. However, at extremely high mobilities, the probability of a component of the lattice field being able to interact with excited nuclei decreases.

2.4.3.3 Spin - spin relaxation

Spin - spin relaxation describes the interaction between neighboring nuclei with identical processional frequencies but differing magnetic quantum states. In this situation, the nuclei can exchange quantum states; a nucleus in the lower energy level will be excited, while the excited nucleus relaxes to the lower energy state. There is no net change in the populations of the energy states, but the average lifetime of a nucleus in the excited state will decrease, which leads to line-broadening.

2.4.3.4 Chemical shift

The magnetic field at the nucleus is not equal to the applied magnetic field; electrons around the nucleus shield it from the applied field. The difference between the applied magnetic field and the field at the nucleus is termed the nuclear shielding.

Consider the s-electrons in a molecule. They have spherical symmetry and circulate in the applied field, producing a magnetic field which opposes the applied field. It means that the applied field strength must be increased for the nucleus to absorb at its transition frequency. This upfield shift is also termed a diamagnetic shift.

Electrons in p-orbitals have no spherical symmetry. They produce comparatively large magnetic fields at the nucleus, which give a low field shift. This "deshielding" is termed as a paramagnetic shift.

In proton (1H) NMR, p-orbitals play no part (there aren't any!), which is why only a small range of chemical shifts (10 ppm) is observed. We can easily see the effect of s-electrons on the chemical shift by looking at substituted methane, CH_3X. As X becomes increasingly electronegative, so the electron density around the protons decreases, and they resonate at lower field strengths (increasing δH values).

Chemical shift is defined as a nuclear shielding/applied magnetic field. Chemical shift is a function of the nucleus and its environment. It is measured relative to a reference compound. For $_1H$ NMR, the reference is usually tetramethylsilane, $Si(CH_3)_4$.

Figure 2.2 photograph of NMR spectrometer

2.4.4 Thermogravimetric Analysis

Thermal analyses were carried out with a TA Instruments SDT Q600 under a nitrogenous atmosphere with a flow rate of 100 mL/min in a platinum crucible at a rate of 10 °C/min (Figure 2.3).

It is a simple analytical technique that measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. Measurements are used primarily to determine the composition of materials and to predict their thermal stability at temperatures up to 1000 °C. It is the most widely used thermal

method, which can characterize materials that exhibit weight loss or gain due to decomposition, oxidation, or dehydration.

2.4.4.1 Basic principle

As materials are heated, they can lose weight from a simple process such as drying, or from chemical reactions that liberate gases. Some materials can gain weight by reacting with the atmosphere in the testing environment. Since weight loss and gain are disruptive processes to the sample material, knowledge of the magnitude and temperature range of those reactions is necessary to design adequate thermal ramps and holds during those critical reaction periods. Such analysis relies on a high degree of precision in three measurements: weight, temperature, and temperature change. A plot of weight change versus temperature is referred to as the thermogravimetric curve (TGA curve), which helps in revealing the extent of purity of analytical samples and determining the mode of their transformations within the specified range of temperature. As many weight loss curves look similar, the weight loss curve may require transformation before results may be interpreted. A derivative weight loss curve can be used to tell the point at which weight loss is most apparent. Therefore, TGA curves can provide information about the composition of multi-component systems, thermal stability, and oxidative stability of materials, decomposition kinetics of materials, and the effect of reactive or corrosive atmospheres on materials and moisture content of materials.

Figure 2.3 photograph of TGA

2.4.5 Powder X-ray diffraction (PXRD)

PXRD measurements were carried out using a Bruker AXS diffractometer (D8 advance) using Cu-Kα radiation (λ= 1.5406 Å), a generator voltage of 40 kV, and current 30 mA (Figure 2.5). The sample was scanned in the range of 2θ= 0–100° with the scan rate 1s/step. X−ray Powder Diffraction (XRD) is an efficient analytical technique used determination of grain size, the composition of solid solution, lattice constants, and degree of crystallinity in a mixture of amorphous and crystalline substances. It is a common technique for the study of crystal structures, atomic spacing, crystallite sizes, stress analysis, lattice parameters, and quantitative phase analysis and can provide information on unit cell dimensions. This information is important for relating the production of a material to its structure and hence its properties.

Figure 2.4 photograph of benchtop PXRD

2.4.5.1 Basic principle

X−ray diffraction is based on the constructive interference of monochromatic X−rays and a crystalline sample. These X−rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample,. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda = 2d \sin\theta$), as shown in Figure 2.5. These diffracted X−rays are then detected, processed, and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d−spacing allows identification of the mineral because each mineral has a set of unique d−spacing. Typically, this is achieved by comparison of d−spacing with standard reference patterns, i.e., JCPDF files.

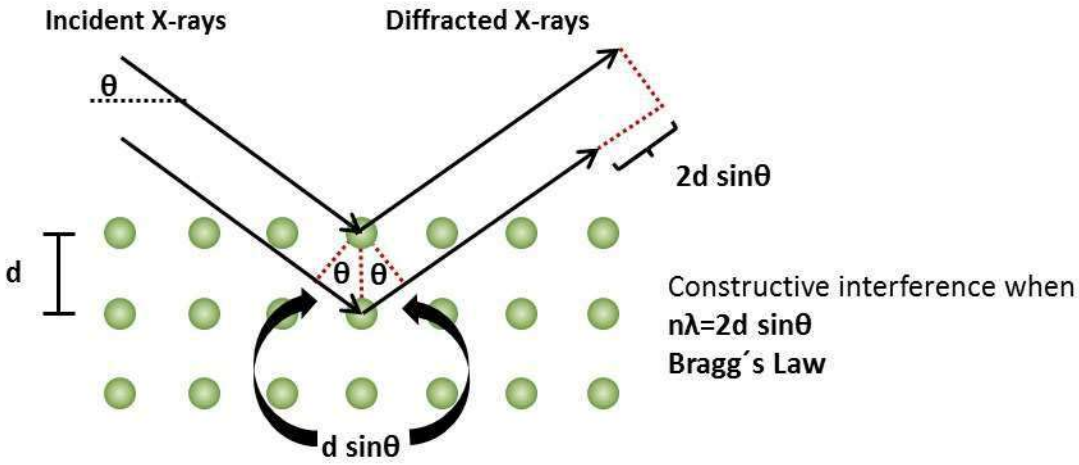

Figure 2.5 Schematic representation of Braggs Law working in PXRD

2.4.6 UV−vis Spectroscopy

UV−visible studies were performed in a PerkinElmer Lambda 950 UV−vis instrument.

Figure 2.6 photograph of UV-vis spectrometer

The instrument used in ultraviolet−visible spectroscopy is called a UV−vis−NIR Spectrophotometer. Spectrophotometer provides a means for analyzing liquids, gases, and solids through the use of radiant energy in the far and near ultraviolet, visible, and near infrared regions of the electromagnetic spectrum (Figure 2.7). Accordingly, the predetermined electromagnetic radiation wavelengths for ultra−violet (UV), visible (Vis) and near infra−red (NIR) radiation are defined as follows-

UV radiation: 300 to 400 nm

Vis radiation: 400 to 765 nm

NIR radiation: 765 to 3200 nm

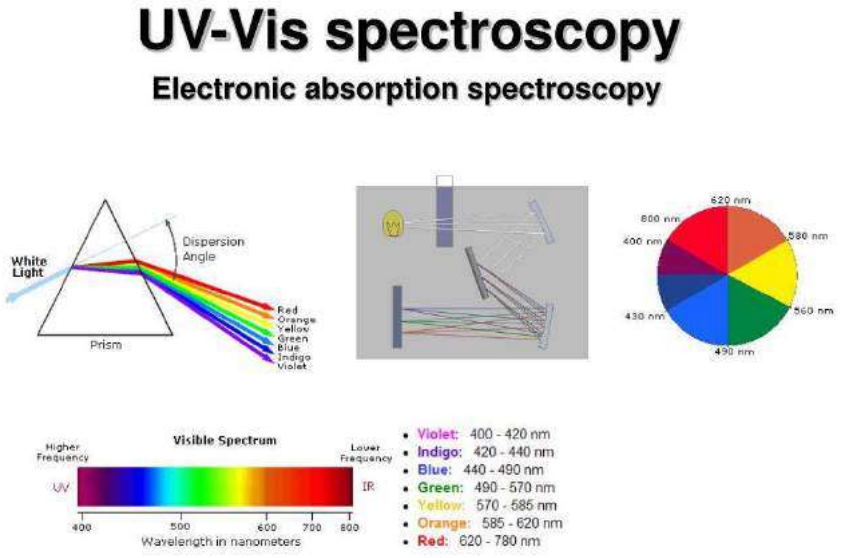

Figure 2.7 components of lights and UV-vis region

2.4.6.1 Basic principle

The instrument operates by passing a beam of light through a sample and measuring the wavelength of light reaching a detector. The wavelength gives valuable information about the chemical structure, and the intensity is related to the number of molecules, which means quantity or concentration. Analytical information can be revealed in terms of transmittance, absorbance, or reflectance of energy in the wavelength range between 160 and 3500 mili microns. Light is quantized into tiny packets called photons, the energy of which can be transferred to an electron upon collision. However, the transfer occurs only when the energy level of the photon equals the energy required for the electron to get promoted onto the next energy state, for example, from the ground state to the first excitation state. This process is the basis for absorption spectroscopy. Generally, the light of a certain wavelength and energy is illuminated on the sample, which absorbs a certain amount of energy from the incident light. The energy of the light transmitted from the sample afterward is measured using a photodetector, which registers the absorbance of the sample. A spectrum is a graphical representation of the amount of light absorbed or transmitted by matter as a function of wavelength. A UV−vis−NIR spectrophotometer measures absorbance or transmittance from the UV range to which the human eye is not sensitive to the visible wavelength range to which the human eye is sensitive. Bouguer−Beer law, as shown in Figure 2.8 is a basic principle of quantitative analysis, is also called the Lambert−Beer law The following relationship is established when light with intensity I_o is directed at material and light with the intensity I is transmitted called transmission rate (T%). The value log (1/T) = log (I_o/I) is called absorbance (Abs). $T = I/I_o = 10^{-kcl}$, Abs = log (1/T) = log(I_o/I) = −kcl. Here k is proportionality constant and & l = length of the light path through the cuvette in cm. As can

be seen from the above formulas, the transmittance is not proportional to sample concentration. However, absorbance is proportional to sample concentration (Beer's law) along with an optical path (Bouguer's law). Besides, when the optical path is 1cm, and the concentration of the target component is 1mol/l, the proportionality constant is called the molar absorption coefficient and expressed using the symbol ε. The molar absorption coefficient is a characteristic value of material under certain, specific conditions. Finally, stray light, generated light, scattered light, and reflected light must not be present for the Bouguer−Beer rule to apply.

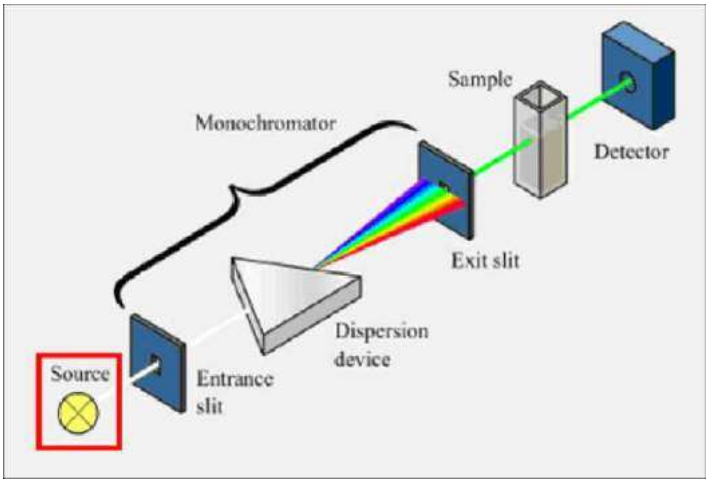

Figure 2.8 basic instrumentation Scheme of UV-vis Spectrophotometer

2.4.7 Fluorescence spectroscopy

Fluorescence spectra were recorded using a Perkin Elmer Luminescence spectrophotometer at 298 K in different solvents.

2.4.7.1 Basic principle

Photoluminescence spectroscopy is a contactless, versatile, nondestructive, powerful optical 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 the sample can dissipate this excess energy is through the emission of light, or luminescence. In the case of photo−excitation, this luminescence is called photoluminescence. Thus photoluminescence is the spontaneous emission of light from the material under optical excitation. This light can be collected and analyzed spectrally, spatially, and also temporally. The intensity and spectral content of this photoluminescence is a direct measure of various important material properties.

Photoexcitation causes electrons within the material to move into permissible excited states. When these electrons return to their equilibrium states, the excess energy is released and may include the emission of light (a radiative process) or may not (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. PL spectroscopy gives information only on the low lying energy levels of the investigated system. In semiconductor systems, the most common radiative transition is between states in the conduction and valence bands, with the energy difference being known as the bandgap. During a PL spectroscopy experiment, excitation is provided by laser light with energy much larger than the optical bandgap. The photoexcited carriers consist of electrons and holes, which relax toward their respective band edges and recombine by emitting light at the energy of the bandgap. Radiative transitions in semiconductors may also involve localized defects or impurity levels. Therefore, the analysis of the PL spectrum leads to the identification of specific defects or impurities, and the magnitude of the PL signal allows determining their concentration.

The respective rates of radiative and non-radiative recombination can be estimated from a careful analysis of the temperature variation of the PL intensity and PL decay time. At higher temperatures, non-radiative recombination channels are activated, and the PL intensity decreases exponentially. Thus photoluminescence is a process of photon excitation followed by photon emission and important for determining bandgap, purity, crystalline quality, and impurity defect levels of semiconducting material. It also helps to understand the underlying physics of the recombination mechanism. PL spectrum is quite different from the absorption spectrum in the sense that absorption spectrum measures transition from the ground state to excited state, while photoluminescence deals with transitions from the excited state to the ground state. The period between absorption and emission is typically extremely short. An excitation spectrum is a graph of emission intensity versus excitation wavelength, which looks very much like an absorption spectrum. The value of wavelength at which the molecules absorb energy can be used as the excitation wavelength, which provides a more intense emission at a red-shifted wavelength, with a value usually twice the excitation wavelength **(Figure 2.9).**

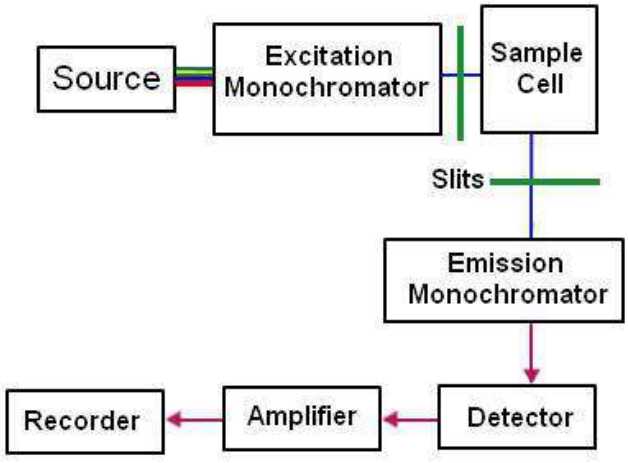

Figure 2.9 Schematic representation of working of Fluorescence spectrometer

2.4.8 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a technique used for analyzing the morphology, defects, crystallographic structure, particle size, and even composition of a specimen (Figure 2.10).

Figure 2.10 photograph of TEM

2.4.8.1 Basic principle

In this technique, a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. What you can see with a light microscope is limited by the wavelength of light. TEM use electrons as "light source," and their much lower wavelength make it possible to get a

resolution a thousand times better than with a light microscope. TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small De Broglie wavelength of electrons. It enables the instrument's user to examine fine detail even as small as a single column of atoms, which is tens of thousands of times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEMs find application in cancer research, virology, materials science as well as pollution and semiconductor research. At smaller magnifications, TEM image contrast is due to the absorption of electrons in the material due to the thickness and composition of the material. At higher magnifications, complex wave interactions modulate the intensity of the image, requiring expert analysis of observed images. Alternate modes of use allow for the TEM to observe modulations in chemical identity, crystal orientation, electronic structure, and sample induced electron phase shift as well as the regular absorption-based imaging (Figure 2.11).

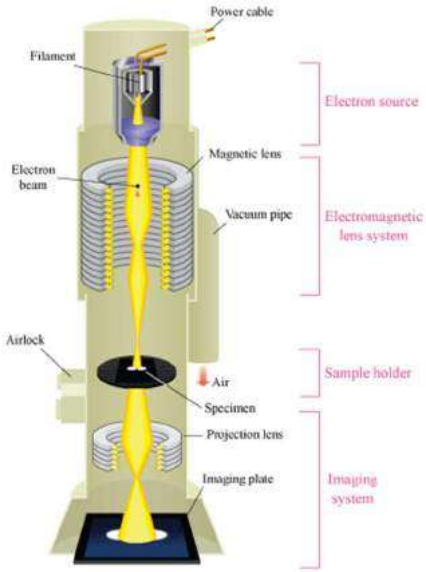

Figure 2.11 Schematic representation of working of TEM

2.4.9 Scanning electron microscope (SEM)

The SEM images were JEOL-6700F microscope instrument and the sample is prepared by dropping solution on a glass cover slip, dried under vacuum (Figure 2.12). SEM is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. In the most common SEM mode, secondary electrons emitted by atoms excited by the electron beam are detected using a secondary electron detector (Everhart-Thornley detector). The number of secondary electrons that can be detected, and thus the signal intensity, depends, among other things, on specimen topography. SEM can achieve resolution better than 1 nanometer.

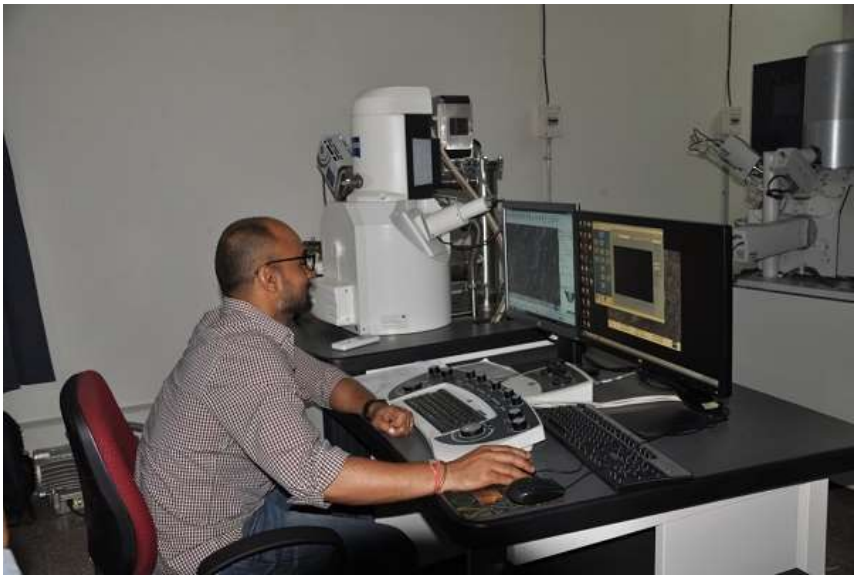

Figure 2.12 photograph of SEM

Specimens are observed in high vacuum in conventional SEM, or in low vacuum or wet conditions in variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures with specialized instruments

2.4.9.1 Principles and capacities

The signals used by a scanning electron microscope to produce an image result from interactions of the electron beam with atoms at various depths within the sample. Various types of signals are produced including secondary electrons (SE), reflected or back-scattered electrons (BSE), characteristic X-rays and light (cathode-luminescence) (CL), absorbed current (specimen current) and transmitted electrons (Figure 2.13). Secondary electron detectors are standard equipment in all SEMs, but it is rare for a single machine to have detectors for all other possible signals.

Secondary electrons have very low energies on the order of 50 eV, which limits their mean free path in solid matter. Consequently, SEs can only escape from the top few nanometers of the surface of a sample. The signal from secondary electrons tends to be highly localized at the point of impact of the primary electron beam, making it possible to collect images of the sample surface with a resolution of below 1 nm. Back-scattered electrons (BSE) are beam electrons that are reflected from the sample by elastic scattering. They emerge from deeper locations within the specimen and, consequently, the resolution of BSE images is less than SE images. However, BSE is often used in analytical SEM, along with the spectra made from the characteristic X-rays, because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen. BSE images can provide information about the distribution, but not the identity of different elements in the sample. In samples predominantly composed of light elements, such as biological specimens, BSE

imaging can image colloidal gold immuno-labels of 5 or 10 nm diameter, which would otherwise be difficult or impossible to detect in secondary electron images. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher-energy electron to fill the shell and release energy. The energy or wavelength of these characteristic X-rays can be measured by Energy-dispersive X-ray spectroscopy or Wavelength-dispersive X-ray spectroscopy and used to identify and measure the abundance of elements in the sample and map their distribution.

Due to the very narrow electron beam, SEM micrographs have a large depth of field, yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. A wide range of magnifications is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times the magnification limit of the best light microscopes.

2.4.9.2 Sample preparation

SEM samples have to be small enough to fit on the specimen stage and may need special preparation to increase their electrical conductivity and to stabilize them, so that they can withstand the high vacuum conditions and the high energy beam of electrons. Samples are generally mounted rigidly on a specimen holder or stub using a conductive adhesive. SEM is used extensively for defect analysis of semiconductor wafers, and manufacturers make instruments that can examine any part of a 300 mm semiconductor wafer. Many instruments have chambers that can tilt an object of that size to 45° and provide continuous 360° rotation.

Nonconductive specimens collect charges when scanned by the electron beam, and especially in secondary electron imaging mode, this causes scanning faults and other image

artifacts. For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge. Metal objects require little special preparation for SEM except for cleaning and conductively mounting to a specimen stub. Non-conducting materials are usually coated with an ultrathin coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation. Conductive materials in current use for specimen coating include gold, gold/palladium alloy, platinum, iridium, tungsten, chromium, osmium, and graphite. Coating with heavy metals may increase the signal/noise ratio for samples of low atomic number (Z). The improvement arises because secondary electron emission for high-Z materials is enhanced.

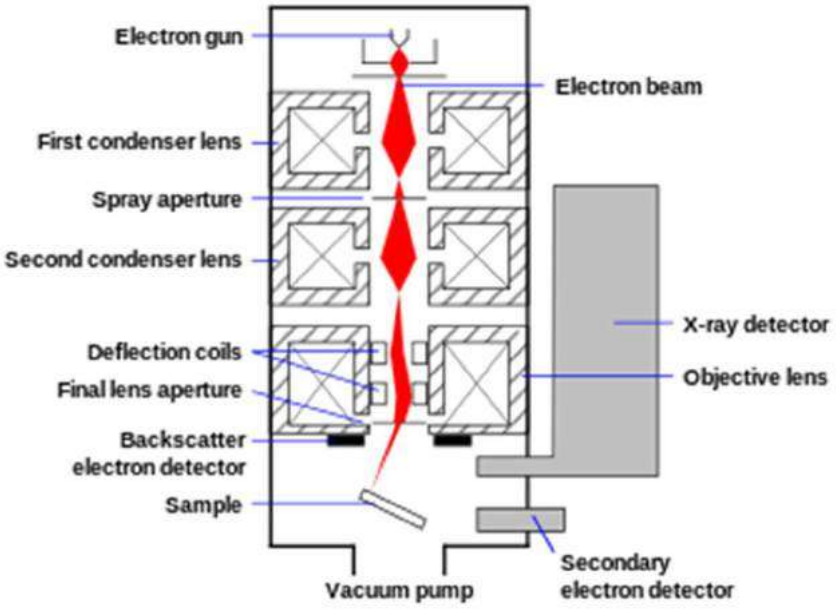

Figure 2.13 basic instrumentation scheme of scanning electron microscope

2.4.10 X-ray crystallography (XRC)

X-ray diffraction intensities for the complexes were collected either at 120K or room temperature on Bruker Smart 6K/Bruker APEX-2 CCD diffractometer using Mo-Kα

radiation and processed using SAINT, as shown in Figure 2.14. The structures were solved by direct methods in SHELXS and refined by full-matrix least-squares on F2 in SHELXL.1.

2.4.10.1 Basic principle

XRC is the experimental science determining the atomic and molecular structure of a crystal, in which the crystalline structure causes a beam of incident X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their crystallographic disorder, and various other information.

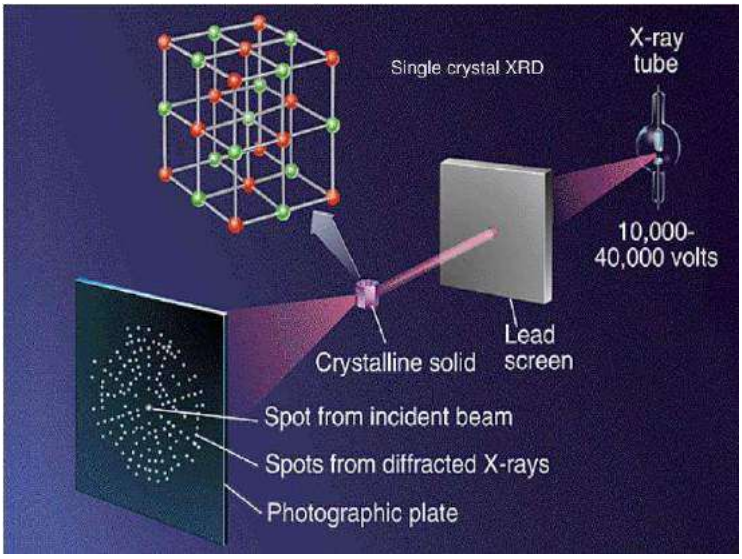

Figure 2.14 schematic representation of working of XRC

Crystals are regular arrays of atoms, and X-rays can be considered waves of electromagnetic radiation. Atoms scatter X-ray waves, primarily through the atoms' electrons. Just as an ocean wave striking a lighthouse produces secondary circular waves emanating from the lighthouse, so an X-ray striking an electron produces secondary spherical waves emanating from the electron. This phenomenon is known as elastic scattering, and the

electron (or lighthouse) is known as the scatterer. A regular array of scatterers produces a regular array of spherical waves. Although these waves cancel one another out in most directions through destructive interference, they add constructively in a few specific directions, determined by Bragg's law:

$$n\lambda = 2d \sin\theta$$

Here d is the spacing between diffracting planes, θ is the incident angle, n is any integer, and λ is the wavelength of the beam. These specific directions appear as spots on the diffraction pattern called reflections. Thus, X-ray diffraction results from an electromagnetic wave (the X-ray) impinging on a regular array of scatterers (the repeating arrangement of atoms within the crystal).

X-rays are used to produce the diffraction pattern because their wavelength λ is typically the same order of magnitude (1–100 angstroms) as the spacing d between planes in the crystal. In principle, any wave impinging on a regular array of scatterers produces diffraction

2.4.11 Atomic force microscopy (AFM)

AFM belongs to a family of instruments called scanning probe microscopes make NT-MDT NTEGRA Prima (Figure 2.15). It measures surface structure in length scale 10 nm-100 μm with high resolution and accuracy (lateral resolution~30 nm, vertical resolution~0.1 nm).

2.2.11.1 Basic principle

The principle of AFM functioning is completely different from the scanning electron microscope and provides a better analysis of nanoscale materials with minimal sample preparation (Figure 2.15). It is based on a physical scanning of samples at the sub-micron

level using a probe tip of the atomic-scale and offers an ultra-high-resolution in particle size measurement. Depending upon properties, samples are usually scanned in contact or noncontact mode.

In contact mode, the cantilever/tip is allowed to be in contact with the surface of the sample in the entire imaging process. The contact mode measures the surface topography of the sample by using a cantilever, which is made of a material having lower spring constant.

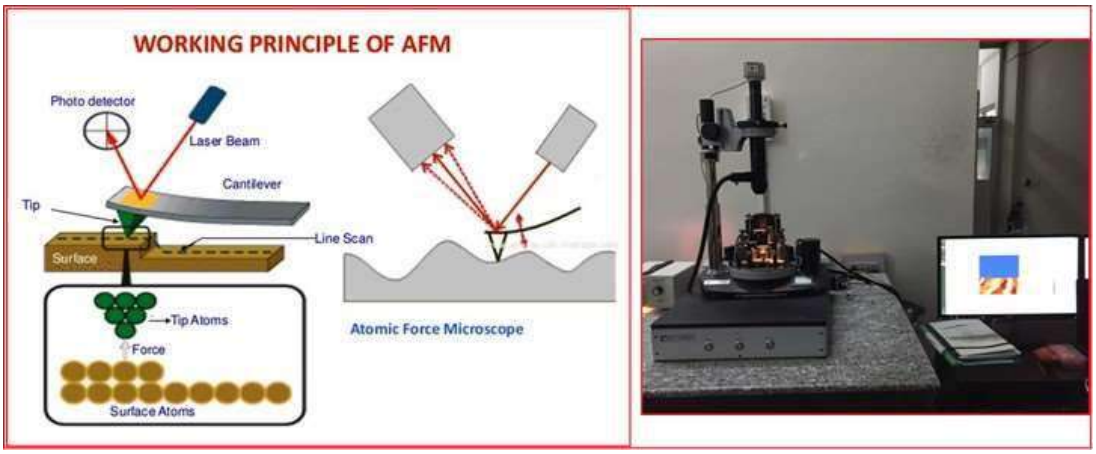

Figure 2.15 representation of principle of atomic force microscopy and the photograph of the atomic force microscope

As the cantilever remains at the surface, the tip gets deflected by changing the height of surface features. The height of the tip can be adjusted using the feedback system of AFM to follow the contour and to maintain a constant tip pressure at the surface. The use of low spring constant materials and the feedback serves to preserve the integrity of the tip while imaging the sample. During the imaging, an abrupt change in surface feature height may occur due to the incapability of the system to handle and to damage and destroy the tip. The AFM requires the samples should be mounted on a very flat surface because the height limitation of many AFMs is only in the 10-15 μm range. To avoid the damaging of the sample, tapping or intermittent contact mode imaging is preferred. In this mode, the

cantilever is driven by a piezoelectric resonator in a frequency range of 150 – 300 kHz. In this mode, cantilever used is made up of higher spring constant material, which provides higher resonant frequency to the cantilever/tip and the overall system.

One of the prime advantages of AFM is its ability to image non-conducting samples without any specific treatment. This feature allows the imaging of delicate biological and polymeric Nano and microstructures. Moreover, AFM (without any mathematical calculation) provides the most accurate description of size, size distribution, and real picture, which helps in understanding the effect of various biological conditions.

2.4.12 Characterization methods for gels

The characterization of gels is essential to obtain measurable values for comparison. The obtained values can help to conclude possible applications, too. Minimum gelation concentration, the minimum gelation concentration, MGC, defines the lowest amount of gelator, which is necessary for the gelation of a certain volume of solvent. Below MGC, a gelator is not able to build up a sufficient 3D network structure of the gelator molecules and cannot entrap the solvent molecules, which can be confirmed by the inversion of the test tube. The soft material is classified as a gel if no gravitational flow occurs. Usually, MGC is determined in $mol \cdot L^{-1}$ or wt.% (w/v).

2.4.12.1 Temporal stability

The temporal stability of a gel is defined as the period between gel formation and gel destruction. Crystallization inside the gel material or phase separation are two examples for the possible destruction of gels. The temporal stability has to be monitored under constant conditions (e. g. temperature and pressure).

2.4.12.2 Thermal stability

The thermal stability of a gel is described by the gel-to-sol phase transition temperature T_{gel}. Several different characterization methods can obtain it. The following two are the most common ones:

The "Dropping ball method" uses a small ball that is placed in the middle of the gel surface. Then the temperature is increased slowly until the gel transforms into the sol, and the ball touches the ground of the vial. It is important that the ball is inert (no reaction or destruction in contact with the gel) and not too heavy or too light to avoid dunking or swimming of the ball in the gel.

The "Inverse flow method" describes the T_{gel} measurement by a sealed vial containing the gel. The vial is immersed upside-down in a thermostated oil bath, and the temperature rises slowly until the gel breaks.

2.4.12.3 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy, FT-IR, can be a useful tool to analyze the precise roles of each different functional group of the gelator in the formation of bonds which then forms the 3D gelator network, for example, carbonyl and amine residues, amide I band and amide II band and also aromatic groups. A change in intensity, as well as a shift of the signals, can be often observed between the FT-IR spectra of a gel and a solution of gelator.

2.4.12.4 Temperature-dependent ^{1}H NMR spectroscopy

^{1}H NMR spectra can provide an insight into the stabilization of the gelator network by the involved protons. For this, gels have to be made from deuterated solvents, and ^{1}H NMR spectra must be measured at several different temperatures, including temperatures below and above $T_{gel.}$ Protons that are part of the gelator network cannot be observed by ^{1}H

NMR due to long correlation times. That means that any signal which is observed belongs to a gelator molecule that is dissolved in the immobilized solvent, either aggregated or disaggregated. With increasing temperature, the number of dissolved gelator molecules is increasing, too. That leads to a growth in signal intensity. A temperature-induced up or downfield shift of the signals can also be observed. The point of inflection of this shift corresponds to $T_{gel.}$

2.4.12.5 Morphological characterization

Scanning electron microscopy, SEM, transmission electron microscopy, TEM, and atomic force microscopy, AFM, are proven tools to gain visual insight into the microscopic morphologies of gels. The microscopy techniques make it possible to observe changes in the morphology as they are caused by the change of solvent or gelator concentration. The images also make the inclusion of particles (for example, dyes), crystals, or formation of nanoparticles visible.

For SEM samples, xerogels must be prepared first. To preserve internal structures, the gels are freeze-dried before the solvent is removed by vacuum.

The remaining xerogel has to be sputtered with metal (for example gold) before imaging. A copper grid is used to prepare the TEM samples by drop-casting a diluted sample of the gel on the grid. AFM samples are provided by the xerogels, too.

As a result, AFM provides 3D surface topographic images, height images of the gelator structure. The high resolution of TEM enables the imaging of single fibers. SEM images show the assembly of the fibers as beams, tubes, or cauliflower-like.

2.4.12.6 Rheological properties

Oscillatory rheological measurements have to be carried out to confirm the viscoelastic state of the gel material. G*, the complex modulus, is given by the ratio of the amplitudes of stress/strain in an oscillatory experiment (Figure 2.16**)**. It comprises two components-

1. The storage/elastic modulus, G′, represents the ability of a material to turn back in the Original state after deformation.

2. The loss/viscous modulus, G″, which shows the tendency of a material to flow under stress.

G′ and G″ are calculated from the measured phase angle (δ):

$$G' = G^* \cos \delta$$

$$G'' = G^* \sin \delta$$

G′ = G* (δ = 0°) for an ideal solid and G″ = G* (δ = 90°) for an ideal liquid. The rheometer directly shows the moduli in pascal. G′ > G″ is the rheological definition of a gel and shows that the elastic behavior is dominant in these materials. Three different kinds of measurements are carried out to confirm the gel nature:

1. Dynamic Strain Sweep (DSS): The capability gels to deal with mechanical stress is shown in a plot of G′ and G″ where the frequency is kept constant, and strain is increased until G′ < G″ marks the breaking of the gel.

2. Dynamic Time Sweep (DTS): DTS gives a plot of G′ and G″ with constant frequency and constant strain over time, which confirms the gel nature of a material over time.

3. Dynamic Frequency Sweep (DFS): The DFS plot shows G′ and G″ at the constant strain in dependency of increasing frequency.

Mechanical destruction of a gel into a fluid state, and it is returning to the gel state upon resting, is called thixotropy. Rheological measurements can examine this property in three steps:

1. A DTS experiment confirms the gel state, G′ > G″ at low strain.

2. The DTS is carried out again, but this time under the massive increase of the strain, which let the gel fracture into a solution, G′ < G″.

3. In the third step, the DTS experiment is using the initial strain, and for thixotropic material,

a recovering of the gel state, G′ > G″, can be observed.

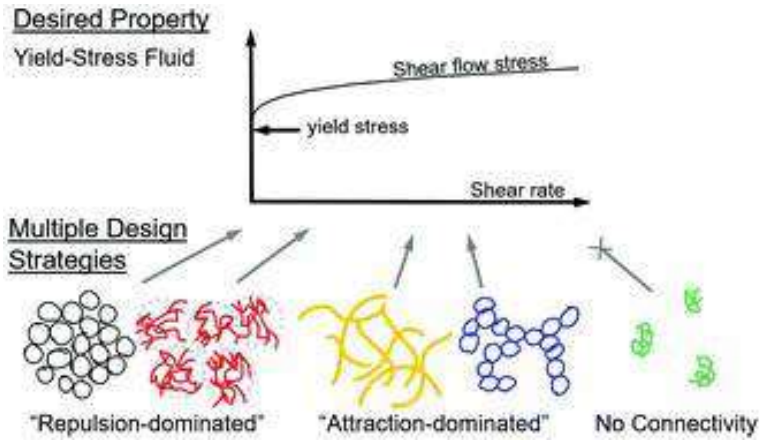

Figure 2.16 stress and shear rate dependency on physical properties

2.4.12.7 Responsiveness to stimuli

A further step in the characterization of gels is the investigation of their behavior when being exposed to external stimuli. The response of a gel to a stimulus results in a change of the microstructure, which can lead to phase separation or a change of shape or color. Further, the optical, mechanical, or electrical response may be created. Common

stimuli that can cause a response are temperature-changes, light, pH, chemical, mechanical, and electro/magnetic stimuli. It is interesting to a broad range of applications as drug delivery media or as sensors. If, for example, the gelator network collapses as a response of stimuli, incorporated pharmaceuticals can be released safely and target-oriented.