Chapter 2

Methodology, Characterization Techniques and Properties Measurements of Polymers and their Composites

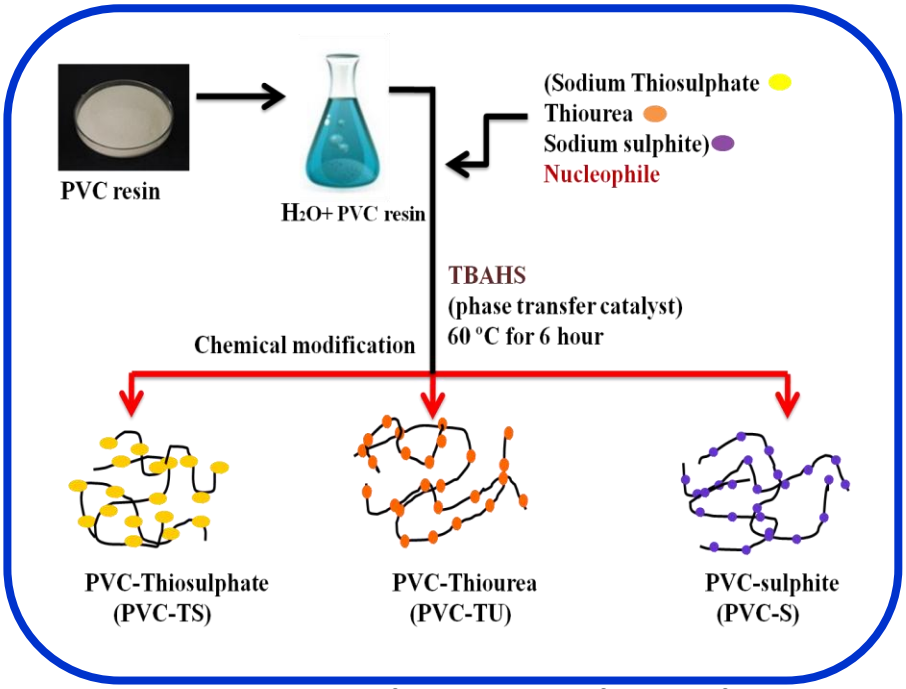
2.1 Synthesis of functionalized PVC nanocomposites

Materials: Commercially available Poly(vinyl chloride) resin was obtained from Ottokemi Mumbai, India. Sodium thiosulphate, thiourea and sodium sulphate were obtained from Merck Ltd., Mumbai, India. Tetrahydrofuron (THF) from GlaxoLtd. Mumbai, India was used as solvent for chemical modification of PVC. Magnesium nitrate hexahydrate of (99% purity) (Himedia) Aluminum nitrate nonahydrate (98% purity) (Himedia), Sodium carbonate (99%) purity (Himedia), and Sodium hydroxide (Merck India Ltd.) were used for synthesis of LDHs and double distilled water was prepared in the laboratory.

Sodium thiosulphate ($Na_2S_2O_3.5H_2O$), thiourea (H_2NCSNH_2) and sodium sulphate are soluble in cold water and their molecular weights are 248.17, 76.12 and 126.04 respectively. Tetrabutyl ammonium hydrogen sulphate (TBAHS) ($C_{16}H_{37}NO_4S$) is light sensitive, soluble in cold water and it acts as a phase transfer catalyst. Its melting point is 173 ºC and its molecular weight is 339.54. Magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$), Aluminum nitrate nonahydrate ($Al(NO_3)_2.9H_2O$), Sodium hydroxide (NaOH) are also soluble in cold water and their molecular weights are 256.41, 375.13and 40 respectively.

2.1.1 Chemical modification of Poly (vinyl chloride) (PVC)

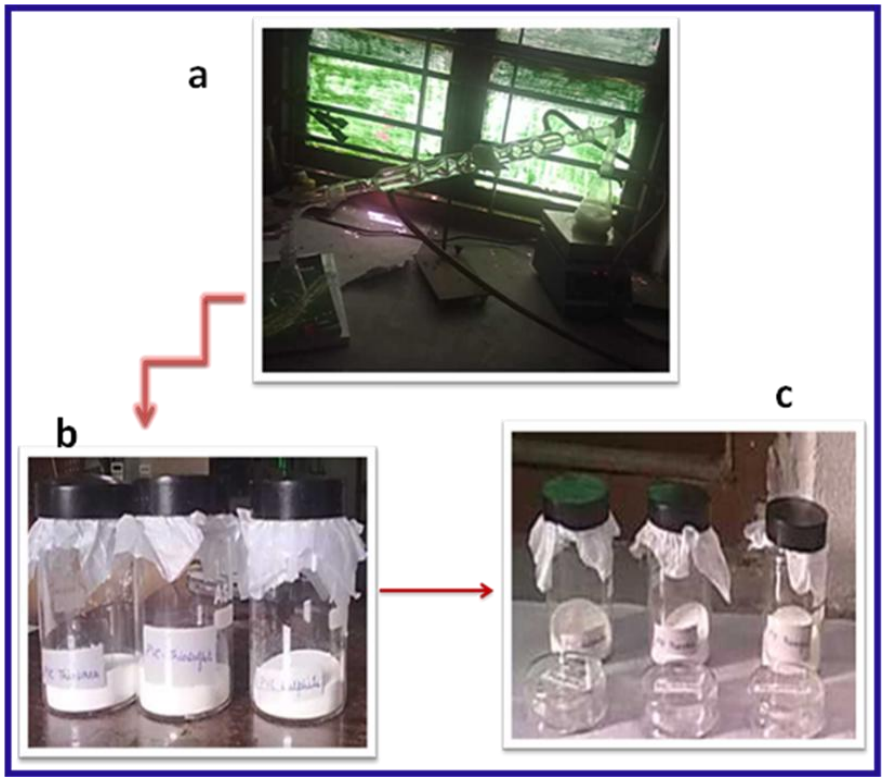
Chemical modification of polymers is one of the methods used to synthesize new polymeric compounds. It is the most active fields of research in polymer science and enables us to introduce functional or reactive groups into polymers, to alter polymer surfaces. Several different chemical modification methods have been reported by many workers to alter the physicochemical properties of polymers and make them antibacterial [Kameda et al. (2011); Herreo et al. (2006); Balazs et al. (2003); James et al. (2003)] to plasticize resistant [Reddy et al. (2010)] etc. Methods like, surface modification by nucleophilic substitution [Kameda et al. (2010)], solvent and non-solvent mixtures [SacristÃ¡n et al. (2000); Braun (2004)] have been already reported. The present modification focuses on the improvement of an approach to reduce bacterial adhesion based on the surface and chemical modification of the polymer and their difference. Functionalization of PVC can be synthesized by wide variety of techniques ranging from surface to chemical processes. Chemical modification of PVC provides stable structure after modification. Scheme 2.1 describes chemical modification of PVC with TBAHS at 60 ºC.



Scheme 2.1: Schematic representation of chemical modification of PVC.

2.1.1.1 Synthesis of thiosulphate-PVC

3M sodium thiosulphate was dissolved in 100 ml double distilled water and 10gm PVC was added in it. Heated the reaction mixture on stirrer at 60-65 ^{o}C. When the temperature stabilizes, 0.15M TBAHS was added. Connected the condenser after maintaining temperature at 60-65 ^{o}C. Next day, the reaction mixture was heated for five-six hours. The reaction mixtures was filtered and washed with double distilled water followed by methanol. Dried the residue in vacuum for 24hours.



Scheme 2.2: A photograph show reaction setup of chemical modification of PVC with different nucleophiles and their film preparation technique. (a) Reaction setup; (b) Functionalized PVC; (c) Film preparation of functionalized PVC.

2.1.1.2 Synthesis of Thiourea – PVC

7M thiourea was dissolved in 100ml distilled water and 10gm PVC was added in it. Heated the reaction mixture on stirrer at 60-65 ^{o}C. When temperature stabilizes, 0.15M

TBAHS was added. Connected the condenser after maintaining temperature at 60-65 ^{o}C. Heated the reaction mixture for 5-6 hours continuously. After 12 hours or next day the reaction mixture was filtered it and washed with double distilled water and then methanol. Dried the residue in vacuum for 24hours.

2.1.1.3 Synthesis of sulphate- PVC

7M sodium was dissolved in 100ml distilled water and 10gm PVC was added in it. Heated the reaction mixture on stirrer at 60-65 ^{o}C. When temperature stabilizs, 0.15M TBAHS was added. Connected the condenser after maintaining temperature at 60-65 ^{o}C. Heated the reaction mixture for 5-6 hours continuously. After 12 hours or next day the reaction mixture was filtered it and washed with double distilled water and then methanol. Dried the residue in vacuum for 24hours.

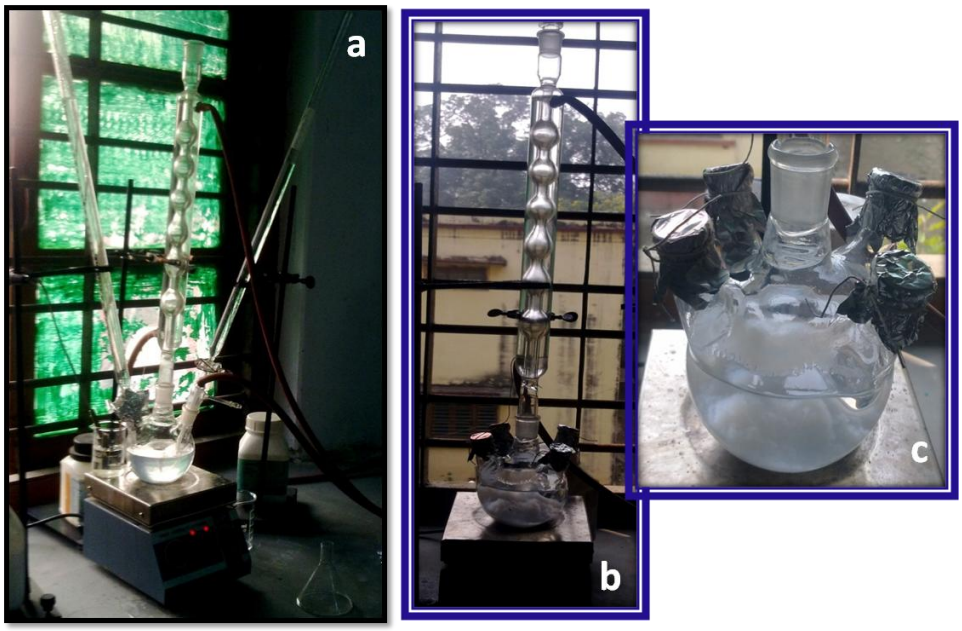
Henceforth, notations of PVC, PVC-TS, PVC-TU, and PVC-S will be used for pure polymer and the modified polymers, respectively.

2.1.2 Synthesis of Layered double Hydroxide

The preparation of LDH was performed by co precipitation method [Govinda et al. (2012)] (Scheme 2.3 shows the reaction setup). A mixed metal solution was prepared by adding Mg $(NO_3)_2.6H_2O$ (1.567 mg) and Al $(NO_3)_2.9H_2O$ (1.147 mg) in 75 mL of double distilled water with molar ratio of 2:1. Sodium carbonate solution was prepared by adding 0.24 gm Na_2CO_3 in 250 ml double distilled water. 100 ml 1M NaOH solution was also prepared.

Firstly Na_2CO_3 solution placed in five neck flask and purged with N_2 gas for 5 minutes. Simultaneously metal and NaOH solution was added drop wise under nitrogen atmosphere and stable pH 10-11 along with condenser and continuous stirring. White precipitate starts appearing as the metal and NaOH solution react with sodium carbonate. The reaction solution was covered and kept at this position with continuous stirring at 60-65 ^{o}C for overnight.

Next day, the white precipitate was seen settled down at the bottom of the flask. The precipitate was separated from solution and washed with double distilled water thrice and then centrifuged. The obtained white paste was dried under vaccum at 45 ºC for 2 days. Obtained white flakes were crushed in mortar with pestle and further used for characterization and polymer composite preparation.



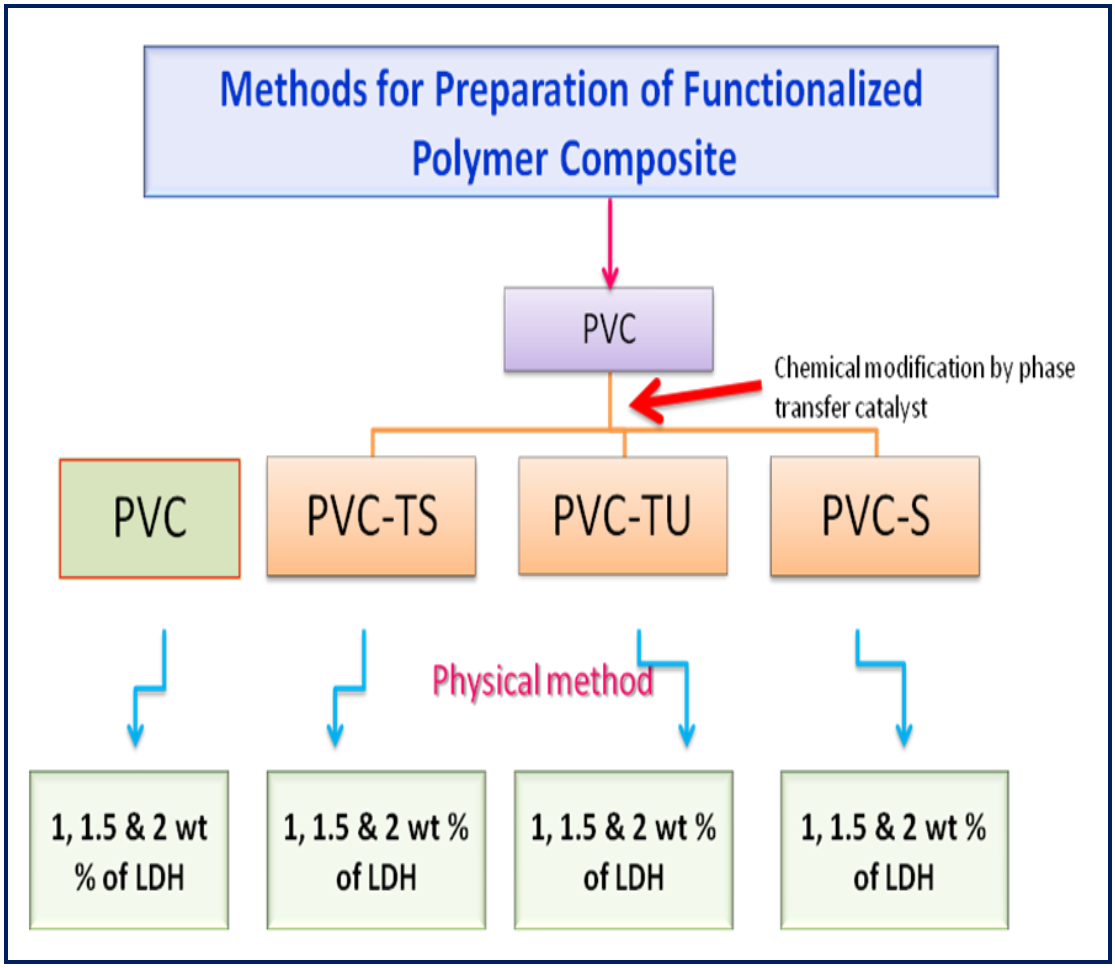
Scheme 2.3: A photograph of LDH reaction setup in Laboratory; (a) Starting phase of experimental setup of LDH**;** (b) after continuous stirring for overnight; (c) before the centrifugation of LDH.

2.1.3 Preparation of functionalized PVC/LDH nanocomposite

The PVC/LDH, PVC-TS/LDH, PVC-TU/LDH and PVC-S/LDH nanocomposites have been prepared by solution intercalation method [Costa et al. (2008)]. The desired amount of LDH was firstly sonicated in THF. One gm each of PVC, PVC-TS, PVC-TU and PVC-S were dissolved separately in THF; and then PVC/THF, PVC-TS/LDH, PVC-TU/LDH and PVC-S/LDH solution has been mixed in this LDH/THF solution for 6 h at

room temperature with continuous stirring. THF was allowed to evaporate and transparent films of unfunctionalized and functionalized polymer composites were obtained.

Hereafter, notations of PVC-1%, 1.5% & 2% for PVC/LDH composites, PVC-TS-1%, 1.5% & 2% for PVC-TS/LDH composites, PVC-TU-1%, 1.5% & 2% for PVC-TU/LDH composites and PVC-S-1%, 1.5% & 2% for PVC-S/LDH will be used in further text.



Scheme 2.4: Schematic flow chart representation of functionalization of PVC and their different weight percentage polymer composites with LDH.

2.2 Characterizations techniques for prepared materials

In order to validate the structure, size and morphology of the synthesized materials and to confirm whether it can be helpful for a particular application, it is important to recognize the different fundamental and extrinsic properties of the materials methodically. Although synthesized functionalized polymer and their composites may demonstrate miscellaneous well-known and unknown properties, some of the basic features necessary to understand include structural/microstructural features, optical and vibrational spectroscopic properties. For this purpose, various characterization tools have been employed such as X-ray diffraction for structural characterization, Scanning Electron Microscopy/Transmission Electron Microscopy for nanostructural characterization, UV Vis spectroscopy for the study optical properties and Fourier Transform Infrared spectroscopy for the confirmation and orientation study of different functional groups. All these techniques are contemporary and very good for material characterizations. A brief description of these techniques, their instrumentation and principles are explained in different sub-sections further.

2.2.1 Proton Nuclear Magnetic Rasonance (^{1}H NMR) of the functionalized PVC resins

NMR spectroscopy is widely used in chemical studies. This technique is very selective and can distinguish among a large number of atoms within a molecule or collection of molecules of the same type which, differ only in their chemical environment. Different signals in ^{1}H NMR spectrum of an organic molecule indicate the different type of protons present in the molecule and the area of peaks determine the number of protons of each type. The position of the signals i.e. their chemical shift values, tells about the electronic environment of a particular proton.

Principle-

The atomic nucleus is a spinning charged particle, and it generates a magnetic field. Without an external applied magnetic field, the nuclear spins are random and spin in random directions. But, when an external magnetic field is present, the nuclei align themselves either with or against the field of the external magnet.

In the present study, ^{1}H NMR was used to quantitatively determine the functional group present in PVC chain. The spectra of PVC, PVC-TS, PVC-TU and PVC-S were recorded on a *JEOL AL 300* spectrometer using d_6-DMSO and $CDCl_3$ as the solvents and references respectively. Each spectrum was recorded after the sample was equilibrated in the magnetic field for at least 10 min. The chemical shifts are reported in parts per million relative to trimethylsilane (TMS).

2.2.2 Fourier Transform of Infrared Spectroscopy (FTIR)

Infra Red (IR) spectroscopy is one of the most regular spectroscopic techniques, accustomed to find out the structure of organic compounds. The main purpose of this investigation is to find out the different functional groups present in an organic compound or polymer chain. Each functional group absorbs different but characteristic frequency of IR radiation. IR spectrum represents a fingerprint of a material with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the compound. Since each different compound is an exclusive arrangement of atoms and no two compounds formulate exact same IR spectrum, IR spectroscopy can be effectively used in qualitative analysis of every different class of synthesized material. Additionally, the size of the peaks present in the spectrum is directly proportional to quantity of desired functional moiety in the material. The advantage of an FTIR spectrometer over a conventional dispersive IR spectrometer is that this offers faster and more sensitive analysis and reproducibility.

Principle-

In FTIR, a single optical device called interferometer is used and employs a beam splitter which 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 which 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 [Kumar (2013)]**.**

In the present study, FTIR has been done to determine the structures of the synthesized polymers. Infrared spectra were recorded using a *Thermo Nicolet 5700* and shown in figure 2.1. FTIR spectrometer was with a resolution of 4 cm^{-1}. Thin film of PVC, PVC-TS, PVC-TU and PVC-S and their corresponding composites have been used for this FTIR study.

Figure 2.1: Photograph of FTIR used in material analysis (Thermo Nicolet 5700).

2.2.3 Ultraviolet-visible Spectroscopy (UV-Vis)

UV-Vis spectroscopy is quantitative determination and needs to be carried out much more precisely and produce reproducibly. This involves measurement of the consequences of interaction of electromagnetic radiations in visible region with the absorbing species like, atoms, molecules or ions. In such determinations the extent to which radiation energy is absorbed by a chemical system as a function of wavelength, as well as, the absorption at a fixed predetermined wavelength of the radiation is measured. Since such measurements need an instrument called spectrometer, the technique is known as UV-Vis spectrometry. The UV-Vis spectrometry is one of the oldest instrumental techniques of analysis and is the basis for a number of ideal methods for the determination of micro and semi-micro quantities of analysis in a sample.

Principle-

The greater the number of molecules that absorb light of a given wavelength, the greater the extent of light absorption and higher the peak intensity in absorption spectrum. If there are only a few molecules that absorb radiation, the total absorption of energy is less and consequently lower intensity peak is observed. This makes the basis of Beer-Lambert's Law, which states that 'the fraction of incident radiation absorbed is proportional to the number of absorbing molecules in its path'. When the radiation passes through a solution, the amount of light absorbed or transmitted is an exponential function of the molecular concentration of the solute and also a function of length of the path of radiation through the sample. Therefore,

$$\text{Log } I_o / I = \varepsilon c\, l$$

Where Io= Intensity of the incident light (or the light intensity passing through a reference cell)

I = Intensity of light transmitted through the sample solution

c = concentration of the solute in mol l^{-1}

l = path length of the sample in cm

ε = molar absorptivity or the molar extinction coefficient of the substance whose light absorption is under investigation. It is a constant and characteristic of a given

absorbing species (molecule or ion) in a particular solvent at a particular wavelength. ε is numerically equal to the absorbance of a solution of unit molar concentration (c = 1) in a cell of unit length (l = 1) and its units are liters.moles^{-1}.cm^{-1}. However, it is customary practice among organic chemists to omit the units.

The ratio I / Io is known as transmittance T and the logarithm of the inverse ratio Io/ I is known as absorbance A.

Therefore

$$- \text{Log } I / Io = - \log T = \varepsilon c\, l$$
$$\text{and Log } Io / I = A = \varepsilon c\, l$$
$$\text{or } A = \varepsilon c\, l$$

For presenting the absorption characteristics of a spectrum, the positions of peaks are reported as λ max (in nm) values and the absorptivity is expressed in parenthesis [Kumar (2006)].

In the present study, the UV-Visible measurements were carried out using Shimadzu (UV-1700), Pharma Speck, UV-Vis spectrophotometer as shown in Figure 2.2 operating in the in the spectral range of 200–1100 nm. Transparent thin films of functionalized and unfunctionalized PVC as well their corresponding composites were prepared by dissolving in THF solvent in a petri dish.

Figure 2.2: Photograph of UV-Visble used in material analysis (Shimadzu (UV-1700), Pharma Speck).

2.2.4 X-Ray diffraction (XRD)

X-ray diffraction technique (XRD) is a significant instrument to scrutinize, quantifying and characterize the phase arrangement, preferred crystal orientation and other structural parameters such as lattice parameters, crystallite size. The diffractometer consists of a goniometry for measuring diffraction angles and a number of electronic circuits for determining the intensity of diffracted beam at any angle.

Principle-

X-rays are electromagnetic radiation of exactly the same nature as light but of very much shorter wavelength lying approximately in the range 0.5 – 2.5 Å. X-rays interact with electrons in matter. Matter absorbs X-rays in two distinct ways, by scattering and by true absorption. When a beam of ray impinges on the material it is scattered in various directions by the electron cloud of the atoms. If the wave length of X-rays is comparable to the separation between the atoms then interference can occur. X-ray diffraction peaks are produced by constructive interference of monochromatic light scattered by each set of lattice plans at specific angles. The peak intensities are determined by the atomic decoration in the lattice plans. For an ordered arrays of scattering centres (such as atoms or ions in a crystalline solid), this can give rise to interference maxima and minima.

Generally, interaction of waves with crystalline structures produces diffraction effect, if the wavelength and the periodicity of the crystals have similar magnitude. Diffraction redistributes intensity from the whole scattering sphere into distinct direction and hence intensity of peaks arises in certain direction. The directions of observation of such peaks are named as reflections (Bragg reflection). As shown in Fig.2.3 constructive and hence so called Bragg reflection is obtained when the path of the wavelet scattered in the lower of the two planes is longer by an integer number of wavelengths λ than that of the wavelength scattered off the upper plane. It is mathematically represented as-

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

where λ is the wavelength of the radiation, n is an integer number, θ is the angle between the lattice planes and the incident beam and d is the distance of the lattice planes for which the peak occurs. The crystallite size is determined from the X-ray line broadening using Scherrer formula-

$$L = 0.9\lambda/\beta Cos\theta$$

where L is the average crystallite size, λ is the X-ray wavelength, β is the angular line width of half maximum intensity and θ is the Bragg's angle. The instrumental line width correction has been taken into consideration while determining the crystallite size [Kumar (2013)].

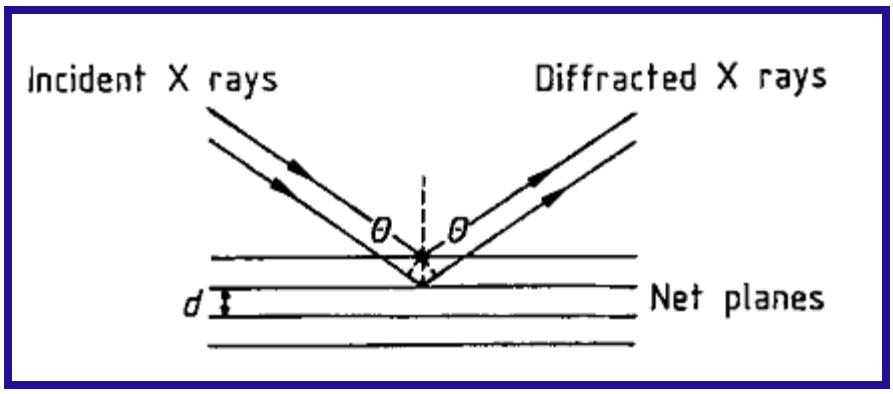


Figure 2.3: Geometrical derivation of Bragg's law [Günzler (2006)].

In the present study, the crystal structure of the synthesized nanoclay and the degree of intercalation and/or exfoliation of nanoparticles in presence of polymer matrix was examined. This technique has been used in two stages. In the first phase, nano clay has been examined in powder form while in second phase, thin sheets of the samples were placed on a quartz sample holder at room temperature and were scanned at diffraction angle 2θ from 1° to 50° at scan rate of 1°/ min. The confirmation of intercalation and/or exfoliation of LDH in the presence of polymer matrix were examined by using Brucker D8 Advance X-ray diffractometer (Figure 2.4) with Cu Ka radiation

and a graphite monochromator having wavelength, 0.154. The patterns were compared with JCPDS reference data for phase identification.

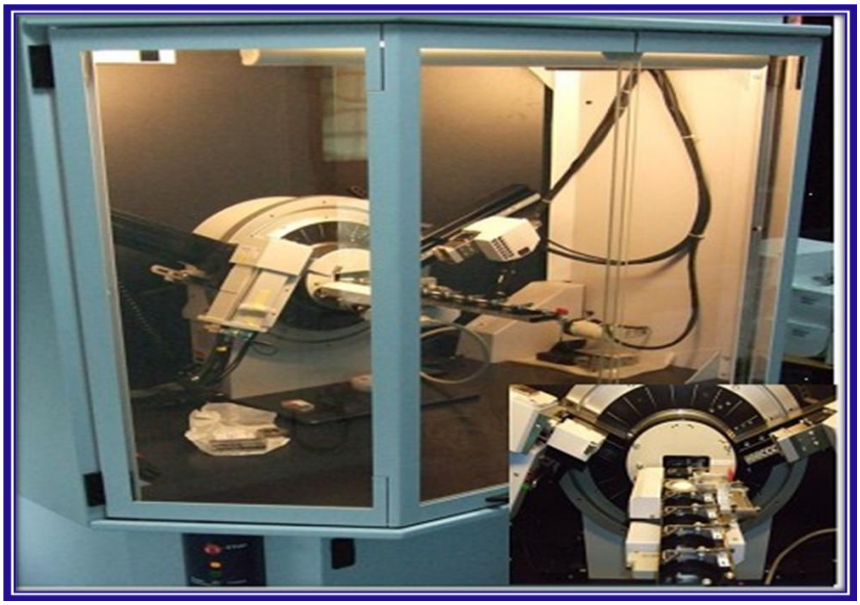


Figure 2.4: Photograph of XRD used in material analysis (Brucker D8 Advance).

2.2.5 Thermo Gravimetric Analysis

Thermo gravimetric analysis (TGA) is an analytical technique which is used to determine thermal stability of a material and its fraction of volatile components by monitoring the weight change that occurs with respect to temperature. The measurement is normally carried out in air or in an inert atmosphere such as nitrogen or argon and the weight loss % is recorded as a function of increasing temperature.

Principle-

"A group of techniques in which a physical property of a substance and/or its reaction products is measured as a function of temperature while the substance is subjected to a controlled temperature programme".

Thermogravimetry analysis (TGA) is used to measure variations in mass as a function of temperature (or time) of material.TGA can also be used as a means of probing a system to obtain other types of information, such as composition. Processes amenable to study in this way are listed in Table 2.1. TG is one of the most powerful TA techniques from the standpoint of quantitative data, and for this reason it is often employed in combination with other measurements [Gunzler and Williams (2002)].

Table 2.1 Different types of processes can be studied by TGA

Process	**Weight gain**	**Weight loss**
Ad- or absorption	*	
Desorption		*
Dehydration/desolvation		*
Sublimation		*
Vaporization		*
Decomposition		*
Solid-solid reactions		*
Solid-gas reactions	*	

Figure 2.5: Photograph of TGA used in material analysis (Mettler-Toledo).

2.2.6 Contact angle

There are three phase contact line observed when a drop of water interacts with a surface. A small contact angle is observed when the liquid spreads on the surface, a large contact angle is observed when the liquid beads on the surface. More specifically, a contact angle less than 90° indicates that wetting of the surface is favorable, and the fluid will spread over a large area on the surface; while contact angles greater than 90° generally means that wetting of the surface is unfavorable so the fluid will minimize its contact with the surface and form a compact liquid droplet. For example, complete wetting occurs when the contact angle is 0°, as the droplet turns into a flat puddle. For super hydrophobic surfaces, water contact angles are usually greater than 150°, showing almost no contact between the liquid drop and the surface (Figure 2.6) [Yuan and Lee (2013)].

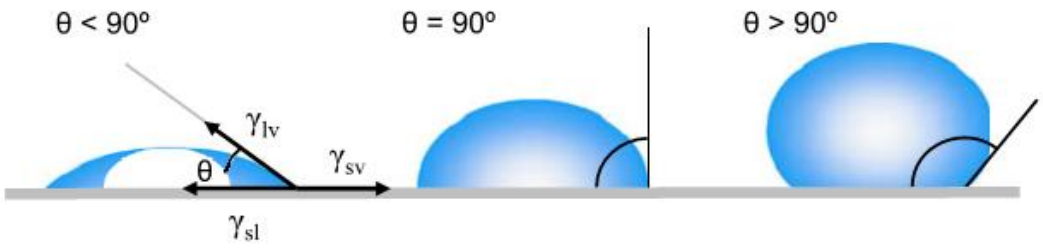


Figure 2.6: Contact angles formed by sessile liquid drops on a smooth homogeneoussolid surface.

Principle-

The contact angle is defined as the angle formed by the intersection of the liquid-solid interface and the liquid-vapor interface (geometrically acquired by applying a tangent line from the contact point along the liquid-vapor interface in the droplet profile).The interface where solid, liquid, and vapor co-exist is referred to as the "threephase contact line".

Figure 2.7: Photograph of Tensiometer used in identified wettability of different material analysis (Kruss F-100 tensiometer).

In the present study, contact angle measurements of polymer and polymer composite surface were used to determine the change of wettability with respect to de-ionized water. For this purpose the advancing contact angle was determined using a Kruss F-100 tensiometer shown in figure 2.7 system with plate method. To intend for contact angle, modified and pure PVC samples were dissolved in THF for preparation of thick polymer films ($1 \times 10 \times 20$ mm^3). Estimation of free energy was performed using double distilled water. The contact angles were obtained by taking mean of three average values measurement. This property is very important for biomaterial as it gives details about either hydrophobicity or hydrophillicity of material.

2.2.7 Scanning Electron Microscopy

Scanning electron microscopy (SEM) uses a focused electron probe to extract structural and chemical information point-by-point from a region of interest in the

sample. The high spatial resolution of an SEM makes it a powerful tool to characterize a wide range of specimens at the nanometre to micrometer length scales.

Principle-

Accelerated electrons in SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (cathodoluminescence–CL), and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination). X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete orbitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element). Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same material repeatedly.

Here, the surface morphology of the polymer resin of PVC, PVC-TS, PVC-TU and PVC-S polymers were investigated by Quanta 200 F SEM as shown in figure 2.8.

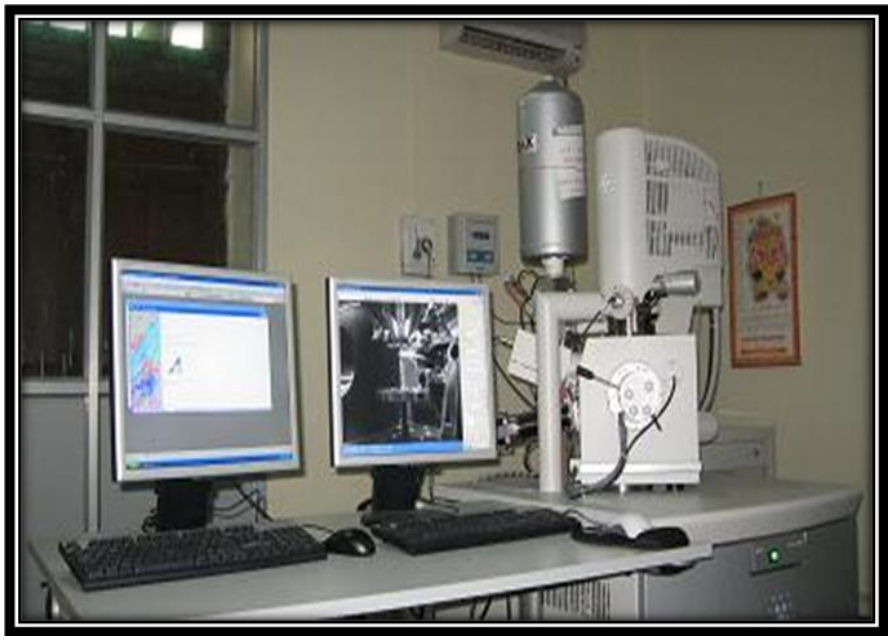


Figure 2.8: Photograph of SEM used in identified morphology of different polymer analysis (Quanta 200 F).

2.2.8 Transmission Electron Microscopy (TEM)

In transmission electron microscopy, a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as they pass through. An image is formed from the interaction of the electrons transmitted through the specimen, which is magnified and focused onto an imaging device, such as a fluorescent screen or to be detected by a sensor [Ashwani (2013)].

Principle-

The scattering processes experienced by electrons during their passage through the specimen determine the kind of information obtained. Elastic scattering involves no energy loss and gives rise to diffraction patterns. Inelastic interactions between primary electrons and sample electrons at heterogeneities such as grain boundaries, dislocations, second phase particles, defects, density variations, etc., cause complex absorption and scattering effects, leading to a spatial variation in the intensity of the transmitted

electrons. In TEM one can switch between imaging of the sample and viewing its diffraction pattern by changing the strength of the intermediate lens.

One short coming of TEM is its limited depth resolution. Electron scattering information in a TEM image originates from a three-dimensional sample, but is projected onto a two dimensional detector. Therefore, structure information along the electron beam direction is superimposed at the image plane. Although the most difficult aspect of the TEM technique is the preparation of samples, it is less so for nanomaterials.

Figure 2.9: Photograph of TEM used in identification of morphology of different polymer composites.

In the present study, TEM micrographs were observed by JEOL JEM 200 CX electron microscope operated at 200 kV as shown in figure 2.9. Samples were prepared by making clear dispersion of the nano particles in isopropyl alcohol using ultra sonication bath and placing a drop of solution through capillary on a carbon coated copper grid. The solvent was allowed to evaporate under an IR lamp.

2.2.9 Mechanical properties

Young's modulus and toughness were determined using dog bone shaped samples, prepared by compression molding technique, using *Instron 3369* tensile testing machine. Samples were stretched uniaxially at a rate of 5 mm/min at room temperature. Several samples were tested for good error estimation.

2.3 Bacterial viability assay

For the bacterial culture, *E. coli* (ATCC 25922) was obtained from the American Type Culture Collection (ATCC), and their clinical strains were preserved at the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. Fresh bacterial broth cultures were prepared before the screening procedure. Strain was hydrated and streaked for isolation on a LB agar. Following growth, a single isolated colony was selected and used to inoculate 3 mL of LB broth media [Amit et al. (2012)]. The bacteria culture was grown on a shaking incubator set at 150 rpm for 18 hours at 37°C. The resulting suspension was then adjusted to have an optical density at 480 nm (OD^{480}) of 0.42, corresponding to a bacterial density of 10^9 colony forming units (CFU) per mL. Thereafter, the solution was serially diluted over a 3-log range to a bacterial density of 10^6 CFU/mL.

Modified and unmodified polymer films were cut into small segments (1.0 x1.0 cm pieces) with a sterile pinch cutter. All samples were initially surface treated to eliminate epiphytic microorganisms. The samples were immersed in 70% ethanol for 1-3 min and then sterilized with an aqueous sodium hypochlorite (4% available chlorine) for 3-5 min and then final rinsing was done with sterilized double distilled water. Each sample was then dried under aseptic conditions.

1mL of the 10^6 CFU/mL solution of *E. coli* was pipetted into each well tube, while ensuring complete submersion of the sample. The well tube was then placed in a stationary incubator at 37°C. After 24 h, samples were taken out from the well tube, washed with deionized water and then immersed in 1 ml of saline water. Samples were further vortex mixed for a few seconds to remove all the bacteria attached on the surface.

Finally, 0.02 µl of the resulting bacterial suspension was used for streaking the culture plate.

2.4 Blood Biocompatibility

2.4.1 Hemolysis assay

The hemolytic activity of various polymers was investigated according to the standard procedure described by Kapusetti et al.(2012) using acid citrate dextrose (ACD) human blood. ACD blood (5 ml) was prepared by adding 4.5 ml of a fresh human blood to 0.5 ml ACD. ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of trisodium citrate dehydrated and 1.84 g of dextrose monohydrate to 75 ml of distilled water. Polymer films were cut into 0.5×0.5 cm pieces, equilibrated in a phosphate buffered solution for 30 min at 37^{o}C in desiccators. In positive and negative controls, distilled water and a buffer solution were used, respectively. Thereafter, 0.2 mL ACD blood was added to each test tubes that were finally kept for 1 h in an incubator at 37^{o}C. The test tubes were centrifuged for 8 min at 800 rpm. Optical density of the supernatant was measured at 545 nm. The percentage of haemolysis was calculated as follows:

$$\%\ of\ hemolysis = \frac{OD\ of\ the\ test\ sample - OD\ of\ the\ negative\ control}{OD\ of\ the\ positive\ sample - OD\ of\ the\ negative\ control} \times 100$$

2.4.2 Thrombogenicity assay

Polymer films were hydrated by equilibrating them with saline water and kept at 37ºC in petri dishes. ACD human blood (0.2 ml) was placed onto each film. Blood clotting was initiated by adding 0.02 ml of 0.1 M KCl solution followed by proper mixing with a Teflon stick. Clotting process was stopped by adding a 5 ml of distilled water after 30 min. Clot formed was fixed in a 5 ml of 3.6% formaldehyde solution for 5 min. Fixed clot was washed with distilled water, blotted between tissue papers and weighed.

2.5 Cellular Biocompatibility

2.5.1 Cell culture studies

The mouse mesenchymal stem cell line, C3H10t1/2, was used for all the experiments. The cells were cultured in 25 cm^2 flasks at 37 °C in a humidified atmosphere with 5% CO_2. Dulbecco's modified Eagle's medium (DMEM)-high glucose medium in combination with 10% foetal bovine serum (FBS), and 1% antibiotic/antimycotic solution was used for culturing cells. The cells were seeded onto samples at an equal density of 2×10^3 cells per surface (10×10 mm^2) for all cell-based assays.

2.5.2 Specimen for cell culture studies

Films of PVC and its various derivatives were prepared by a solution casting method in Petri dishes. Prepared films were placed between two Teflon sheets clamped for 10 min for obtaining the plane surface of materials. Cured specimens were removed from the molds and their edges were smoothened with an emery paper. Specimens were stored at a room temperature. A specimen size of 10×10 mm^2 was selected for in vitro cell culture studies. Before performing the cell-based studies, the specimens were washed with isopropanol for removing the attached debris. For surface sterilization, each specimen was washed thrice with phosphate buffered saline (pH~7.2), and exposed under UV light for 8 h.

2.5.3 Cell adhesion

The ability of the samples to support cell adhesion was determined by staining the cells adhered to their surfaces with crystal violet. The cells were seeded on to the surface of the samples at an equal density and incubated at 37 ºC in a humidified atmosphere with 5% CO_2 for 4 h. Prior to the addition of a dye, the culture medium was aspirated,

cells were washed twice with cold phosphate buffered saline (PBS) pH 7.2, and fixed using a 4% formaldehyde solution. After the addition of the dye, the cells were incubated at a room temperature for 30 min and then washed three times with a cold PBS. Endogenous crystal violet was then extracted using absolute methanol and the absorbance of the solution was measured at 544nm using a Fluostaroptima (BMG Labtech, Germany) microplate reader. Cells adhered to the surface of the samples were quantified using the formula,

$$Percentage\ of\ Adhesion = 100\ x\ \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}$$

2.5.4 Cell viability

The MTT assay is a colorimetric test for measuring the activity of enzymes that reduce 3-[4,5-dimethylthiozol- 2-yl]-2,5 diphenyltetrazolium bromide, (MTT) to formazon, giving a purple color appearance. Cytotoxicity of the samples was assessed by the MTT assay as described previously [Tim et al. (1983)]. The samples were cut into small pieces (10×10 mm^2) and placed into 12 well tissue culture plate (Corning, Germany) followed by their sterilization. $2x10^3$ cells in 20 µl of medium were seeded onto the samples and cultured for three different time intervals. On the 1^{st}, 3^{rd} and 5^{th} days following culture, the cells grown on each sample were assayed by the addition of 5 mg/ml MTT and incubating them for 4 h at 37°C. Only viable cells have the ability to reduce the yellow water-soluble MTT tetrazole complex into dark blue crystals of formazan, insoluble in water. After 4 h, the MTT-containing medium was then aspirated and 1 ml of ethanol-DMSO (Himedia, India) (1:1) was added to lyse the cells and solubilise the water insoluble formazan. Viable cells on the surface of the samples were quantified spectrophotometrically by measuring the absorbance of the lysates at 570 nm, using a Fluostaroptima (BMG Labtech, Germany) microplate reader. The percentage of live cells on each sample was evaluated by comparing the absorbance of the samples to

that of a control well, where cells were seeded onto the surface of a polystyrene tissue culture plate.

$$Percentage\ of\ Cell\ viability = 100\ x\ \frac{Absorbance\ of\ sample}{Absorbance\ of control}$$

2.5.5 Nuclear Staining

The ability of the samples to support the proliferation of cells was assessed by staining of cells with 4',6-diamidino-2-phenyindole (DAPI, Sigma) after incubation period of 24 h. The cells were seeded on to the surface of samples at an equal density and incubated at 37°C in a humidified atmosphere with 5% CO_2. Prior to the addition of a dye, the culture medium was aspirated; the cells were washed twice with cold phosphate buffered saline (PBS) pH 7.2, and fixed using a 4% formaldehyde solution. The cells were then permeabilized using a 0.1% solution of Triton X 100 (Himedia, India) for 45 seconds and incubated with the dye at 37°C for 5 min. Images of intact cellular nuclei stained with the dye were captured with a fluorescence microscope.

2.5.6 Statistical analysis: Statistical analyses were performed on the means of the data obtained from three independent experiments by using GRAPH PAD PRISM for Windows software. The results are expressed as mean values (±SE). The analysis of variance followed by a post hoc Dennett's testing was performed for contact angle, hemolysis assay and cell adhesion assay for one-way analysis of variance (ANOVA). In addition, Bonferroni's method was used in cell viability for multiple comparison tests in ANOVA. In all cases, p value was obtained from the ANOVA table; the conventional 0.01 level was considered to express the statistical significance.

2.5.7 Microscopic Fluorescence image system

Cells were cultured on the polymeric material surface with standard conditions. Cells were stained with a DAPI dye for nuclei and observed using a Zeiss, Axiovert 25 inverted fluorescence microscope equipped with an objective of 100x magnification.