CHAPTER III

This chapter provides a detailed description of all the materials and the methods as well as instruments used for the synthesis of inclusion complexes (IC) and β-cyclodextrin grafted graphene oxide (GO) based functional materials and mentions the various characterization technique and theoretical model used for studying the structure and composition of the supramolecular materials.

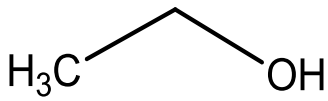
3. MATERIALS AND METHODS:

3.1 Materials:

Graphite (21 µm particle size), 98% sulfuric acid (H_2SO_4), 70% nitric acid (HNO_3), potassium permanganate (KMnO4), 30% hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), acetone, ethanol from Sigma Aldrich India Co. Ltd. India were used as received. Various drugs like Trigonelline hydrochloride, Rebamipide, Ambroxol hydrochloride, Umbelliferone are purchased from TCI INDIA PVT LTD as well as Sigma Aldrich PVT LTD. D_2O, d6-DMSO were purchased from Cambridge Isotope Laboratories, Inc. USA. All the detailed descriptions of the following chemicals are given below:

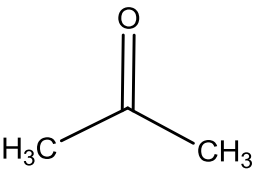
(a) Solvents:

(i) Ethanol



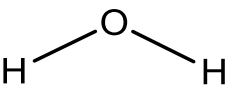
Physical Properties	Description
Appearance	Colourless liquid
Molecular Formula	C_2H_6O
Molecular Weight	44.07 g mol^{-1}
Boiling Point	351.37 K
Melting Point	158.90 K
Dielectric Constant	25.08 at 298.15 K

(ii) Acetone



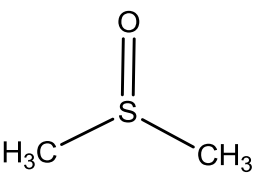
Physical Properties	Description
Appearance	Colourless liquid
Molecular Formula	C_2H_6O
Molecular Weight	58.04 g mol^{-1}
Boiling Point	329.68 K
Melting Point	178.15 K

(iii) Water



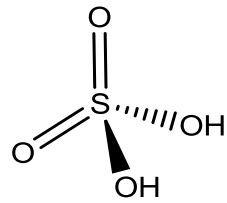
Physical Properties	Description
Appearance	Colourless liquid
Molecular Formula	H_2O
Molecular Weight	18.01 g mol^{-1}
Boiling Point	373.15 K
Melting Point	273.15 K

(iv) Dimethyl sulfoxide



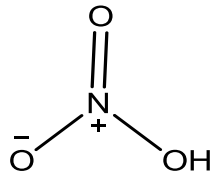
Physical Properties	Description
Appearance	Colourless liquid
Molecular Formula	C2H6OS
Molecular Weight	78.01 g mol^{-1}
Boiling Point	462.15 K
Melting Point	292.15 K

(v) Sulfuric acid



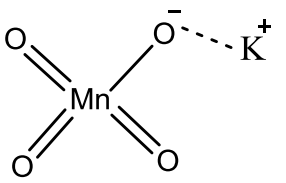
Physical Properties	Description
Appearance	Colourless liquid
Molecular Formula	H_2SO_4
Molecular Weight	98.07 g mol^{-1}
Boiling Point	660.15 K
Melting Point	283.15 K

(vi) Nitric acid



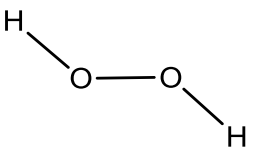
Physical Properties	Description
Appearance	Colourless liquid
Molecular Formula	HNO_3
Molecular Weight	63.01 g mol^{-1}
Boiling Point	356.15 K
Melting Point	231.15 K

(vii) Potassium permanganate



Physical Properties	Description
Appearance	purplish-black crystalline solid
Molecular Formula	$KMnO_4$
Molecular Weight	158.03 g mol^{-1}
Melting Point	513.15 K

(viii) Hydrogen peroxide:

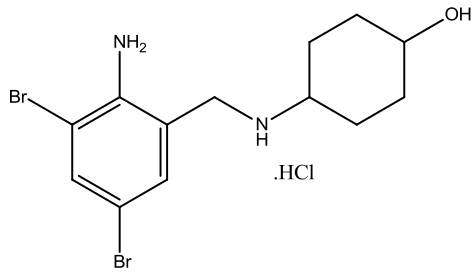


Physical Properties	Description
Appearance	very pale blue liquid
Molecular Formula	H_2O_2
Molecular Weight	34.01 g mol^{-1}
Boiling Point	423.35 K
Melting Point	272.72 K

(b) Biologically active drugs:

(i) Ambroxol hydrochloride:

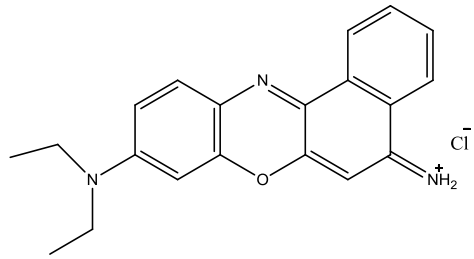
Ambroxol hydrochloride is a drug that helps to increase mucous excretion [1]. Ambroxol is a metabolite of bromhexine. It acts as a mucolytic agent during acute and chronic disorder caused by the production of excess or thick mucus and thus helps to reduce viscosity of the mucus [2,3]. It is chemically written as trans-4-{(2-amino-3,5-dibromobenzyl)amino}cyclohexanol. It is white to yellowish crystalline powder; slightly soluble in hot water, ethanol; soluble in dimethyl formamide, methanol and insoluble in benzene as well as chloroform.



Physical Properties	Description
CAS No.	23828-92-4
Appearance	Colourless crystalline powder
Molecular Formula	$C_{13}H_{18}Br_2N_2O \cdot HCl$
Molecular Weight	414.57 g mol^{-1}
Melting Point	508 K
Purity	>98%

(ii) Nile blue:

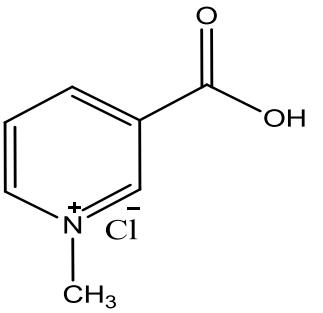
Nile blue chloride is a fluorescent dye with high quantum yield in nonpolar solvents. Derivatives of Nile blue are potential photosensitizers in photodynamic therapy of malignant tumors. Normal and premalignant tissues in animal experiments can be distinguished by fluorescence spectroscopy in fluorescence imaging [4,5].



Physical Properties	Description
CAS No.	2381-85-3
Appearance	Solid deep bluish
Molecular Formula	$C_{20}H_{20}ClN_3O$
Molecular Weight	353.85 g mol^{-1}
Melting Point	563 K
Purity	> 98%

(iii) Trigonelline hydrochloride:

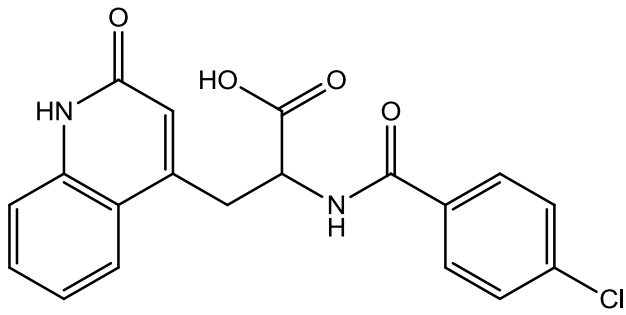
Trigonelline hydrochloride is a pyridine based alkaloid remain as a zwitterion formed by the methylation of the nitrogen atom of niacin (vitamin B_3) [6,7]. Trigonelline hydrochloride is a product of niacin metabolism that is excreted in urine of mammals.



Physical Properties	**Description**
CAS No.	6138-41-6
Appearance	Colourless solid
Molecular Formula	$C_7H_8ClNO_2$
Molecular Weight	173.60 g mol^{-1}
Melting Point	503-506 K
Purity	>98.0% (HPLC)

(iv) Rebamipide:

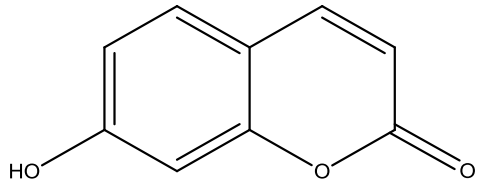
Rebamipide, an amino acid derivative of 2-(1*H*)-quinolinone, is used for mucosal protection, healing of gastroduodenal ulcers, and treatment of gastritis [8,9]. It can be used as an active agent by enhancing mucosal defense, scavenging free radicals and temporarily activating genes encoding cyclooxygenase-2.



Physical Properties	Descriptions
CAS No.	90098-04-7
Appearance	Colourless solid
Molecular Formula	$C_{19}H_{15}ClN_2O_4$
Molecular Weight	370.79 g mol^{-1}
Melting Point	563 K
Purity	>98.0% (HPLC)

(v) Umbelliferone:

The ultraviolet activity of umbelliferone led to its use as a sunscreen agent, and an optical brightener for textiles [10,11]. Umbelliferone can be used as a UV absorbing agent and selective fluorescence indicator for metal ions such as copper and calcium. It acts as a synthetic fragrance component for different food and cosmetic products.



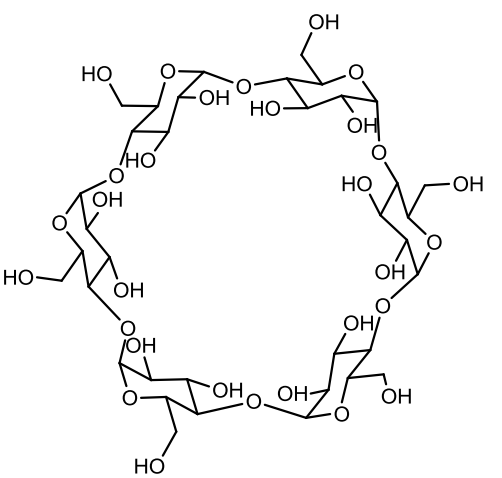
Physical Properties	Descriptions
CAS No.	93-35-6
Appearance	yellowish-white crystalline solid
Molecular Formula	$C_9H_6O_3$
Molecular Weight	162.14 g mol^{-1}
Melting Point	503 K
Purity	>98% (HPLC)

(C) Supramolecular host molecules:

Cyclodextrins (CDs) are basically supramolecular host with truncated-cone polysaccharides that are consist of six to eight D-glucose monomers linked by a-1,4-glucose bonds [12,13]. In a cyclodextrin molecule, each glucose unit form rigid chair conformation which lead to hollow truncated cone shape where the secondary hydroxyl of C-2 and C-3 are situated on the wider side and the primary hydroxyl group on C-6 are on the narrow face. They have a hydrophobic cavity and a hydrophilic outer surface and can encapsulate various organic molecules or inorganic metals to form host–guest complexes or supramolecular assembles [14,15].

(i) α-cyclodextrin:

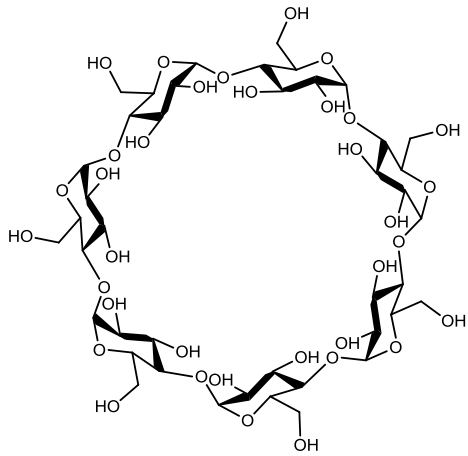
α-cyclodextrins are a class of cyclic oligosaccharides that consist of six glucose monomer units. It has the cavity diameter 4.7-5.3 Å, outer diameter 14.6±0.4 Å, height/depth 7.9±0.1 Å, approx cavity volume 174 Å [16].



Physical Properties	**Descriptions**
CAS No.	10016-20-3
Appearance	White solid
Molecular Formula	$C_{36}H_{60}O_{30}$
Molecular Weight	972.84 g mol^{-1}
Solubility in water	14.5 g/100 mL
Melting Point	>551 K

(ii) β-cyclodextrin:

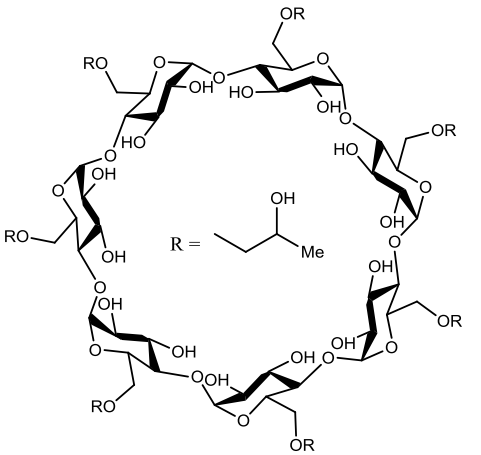
β-cyclodextrins are a class of cyclic oligosaccharides that consist of seven glucose monomer units [17]. It has the cavity diameter 6.0-6.5 Å, outer diameter 15.4±0.4 Å, height/depth 7.9±0.1 Å, approx cavity volume 262 Å [16]. βCD has been used for supramolecular catalysis in organic synthesis in ecofriendly solvent or solvent free conditions [18].



Physical Properties	**Descriptions**
CAS No.	7585-39-9
Appearance	Colourless liquid
Molecular Formula	$C_{42}H_{70}O_{35}$
Molecular Weight	1134.98 g mol^{-1}
Solubility in water	1.85 g/100 mL
Melting Point	563-573 K

(iii) Hydroxypropyl-β-cyclodextrin:

Hydroxypropyl-β-cyclodextrin (HP-β-CD), a β-CD derivative, with a higher aqueous solubility is prepared by reacting β-CD with propylene oxide in alkaline aqueous solutions [20]. HP-β-CD has better aqueous solubility compared with α-, β- and γ-CD as in HP-β-CD, all the hydrogen bonds are replaced by hydroxyl groups.



Physical Properties	Descriptions
CAS No.	128446-35-5
Appearance	Amorphous
Molecular Formula	$C_{63}H_{112}O_{42}$
Molecular Weight	1541.54 g mol^{-1}
Solubility in water	~50 g/100 mL
Melting Point	551 K

3.2 Synthesis methods:

3.2.1 Synthesis of inclusion complex:

Several methods may be applied to synthesize inclusion complex between guest and host [21]. In our cases, we have applied co-precipitation method. To prepare 1:1 molar ratio of solid inclusion complex between guest and host (αCD or βCD or other derivatives), at first, 1 molar concentration of solid guest compound should be accurately weighted and taken in a beaker and minimum volume of distilled water should be added to dissolve the solid samples and placing it in a thermostated water bath at temperature set at 323.15K with constant stirring in a magnetic stirrer. Next, accurately measured 1 molar concentration of host is then added in solid form in the same beakers slowly in presence of the constant stirring. It is kept in the thermostated water bath for 24-48 hours. Thereafter, it was collected and dried in a hot air oven & after that inclusion complexes in the solid form is obtained [22,23].

3.2.2 Synthesis of graphene oxide (GO):

GO was synthesized using modified Hummers method from purified natural graphite flakes as reported earlier [24,25]. According to the method, a mixture of graphite flakes (3.0 g, 1 wt equiv) and $KMnO_4$ (18.0 g, 6 wt equiv) were added to 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (360:40 mL), the mixture get warmed upto 40^0C showed exothermic nature. The reaction was kept at 55^0C and stirred with magnetic stirring for 12 hrs. After cooling at room temperature, the reaction mixture was poured into crust ice (~400 mL) with 30% H_2O_2 (3 mL). The solid material obtained after filtration was then wash twice successively with 200 mL of water, 200 mL of 30% HCl and 200 mL of ethanol. After that, solution was centrifuged at 4000 rpm for 10 mins and again washed with distilled water so that various chlorides, sulphate ions got free. Finally, the solution was dried by rotary evaporator under reduced pressure and obtained 1.7g of product.

3.2.3 Synthesis of GO-βCD nanocomposites:

To prepare rGO-βCD composite, a relatively greener approach was chosen rather than using highly toxic hydrazine as reducing agent [26]. Prior to the experiment, GO was ultrasonicated in distilled water for 10 mins for getting a homogenous solution. Then, 200 ml of 1 mg/mL βCD aqueous solution was mixed with 200 mL of 0.5 mg/mL GO aqueous suspension. The mixture was stirred at room temperature for 12 hrs. Then, the pH of the mixture was adjusted to 12 by adding aqueous solution of NaOH (1.0 M). Finally, the solution was heated at 75^0C and stirred at 370 rpm for 6 hrs. After the reaction, the stable black dispersion of the GO-βCD mixture was centrifuged at a relative centrifugal force 4000 rpm so that unreacted βCD got removed from the solution followed by washing with distilled water for three times. After that a solid GO-βCD nanocomposites was obtained by using rotary evaporator under reduced pressure.

3.3 Characterization techniques:

3.3.1 Spectroscopic techniques:

3.3.1.1 Fluorescence spectroscopy:

Fluorescence spectroscopy, also known as spectrofluorometry, is the observation of the emitted electromagnetic radiation; usually appear in the visible or near-infrared regions of the spectrum, when a compound is electronically excited with UV radiation. It consists of a xenon lamp as a source of excitation and a monochromator which is used to input an excitation wavelength from the light produced in the range of 200 to 800 nm. This monochromatic beam is directly strike the sample under investigation and the resulted emission spectrum is analysed, using a second monochromator, for photon energies between 200 and 900 nm.

Fluorescence spectroscopy is an important investigational tool in many areas of analytical field, such as chemical, biochemical and medical research, due to its extremely high sensitivity and selectivity. In material sciences, this is used to study structure and dynamics of surfaces. Particularly in the areas of biochemistry and molecular genetics, fluorescence spectroscopy has become a dominating technique.

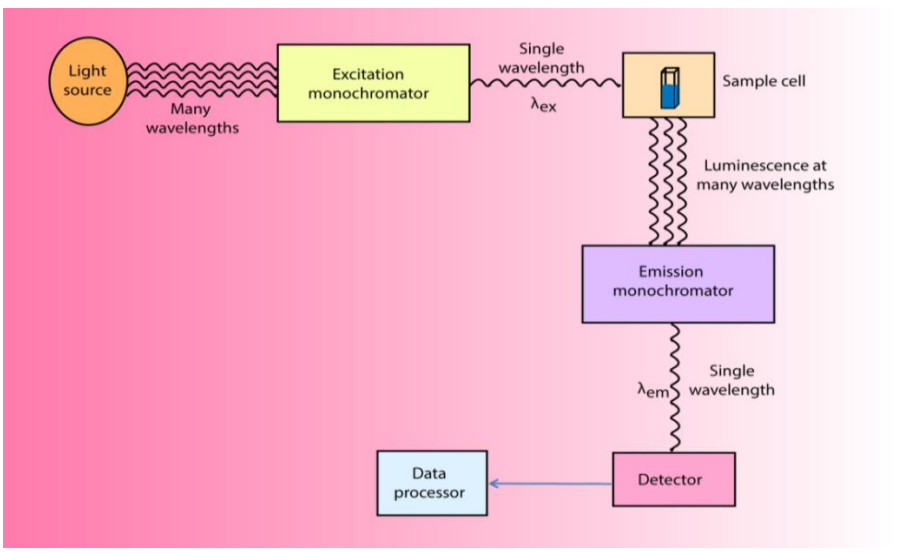


Figure 1: The basic instrumental set up of a fluorescence spectrophotometer and it's working principle

During course of this PhD thesis PTI QuantaMaster-40 fluorometer had been used. It comes with integrated FelixGX software to control both the instrument and accessories. FelixGX software is very much user friendly and easy to use includes

analytical functions for different spectral and kinetic analysis for steady-state, lifetime or anisotropy analysis. The whole system provides a full set of data acquisition protocols, and controls the hardware for all system configurations and operating modes.

3.3.1.2 Ultraviolet-visible (UV-vis) absorption spectroscopy:

UV-vis absorption spectroscopy is a preliminary but helpful spectroscopic technique for the characterization of various materials such as organic, inorganic, supramolecular or nanomaterials. According to the principle, the molecules within the sample are irradiated by electromagnetic energy, may be, UV or visible light, will undergo electronic excitation by the absorption of light from ground state to excited state. It is to be mentioned that during irradiation of sample, wavelength of UV light is continuously changed and when the wavelength matches the energy state required to excite an electron to a higher level, energy is absorbed. Then, the intensity of absorption can be obtained from Beer Lamberts law (eqn. 1):

$$A=\in cl \quad \text{..... (1)}$$

Where, 'A' denotes measured absorbance, '∈' is the molar extinction coefficient, 'l' is the path length and 'c' is the concentration of the solution.

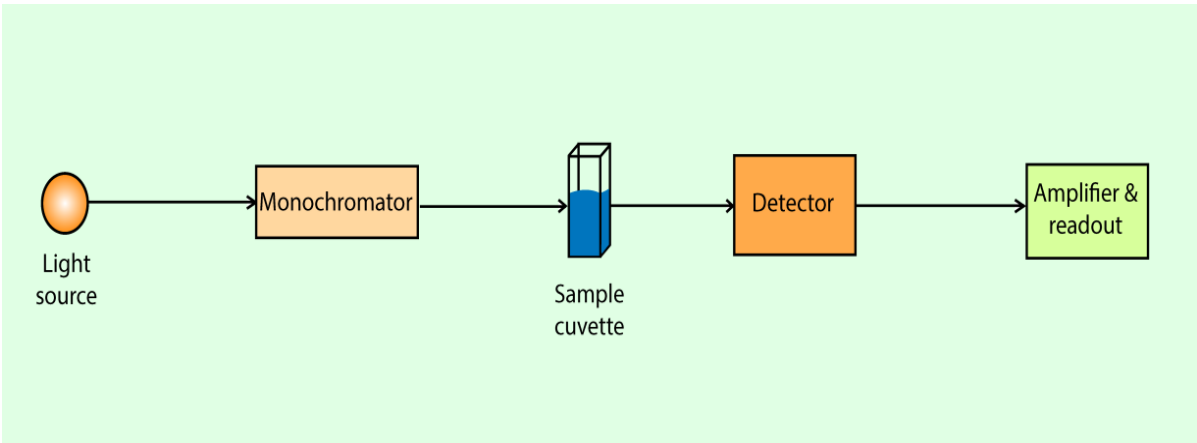


Figure 2: Basic outline of a UV-vis spectrophotometer

Deuterium discharge and tungsten-halogen lamps are commonly used light sources for UV-visible measurements and NIR measurements. In UV-visible-NIR spectrometers photomulplier tube combined with Peltier-cooled PbS IR is used as

detector. In our every study, optical absorption measurements of the samples are recorded by dispersing in respective solvent using Agilent 8453 UV-visible spectrophotometer Agilent Technologies USA.

3.3.1.3 Fourier Transform Infrared spectroscopy:

It is a spectroscopic technique based on the molecular vibration spectrum, for a compound having covalent bonds, whether organic or inorganic, absorbs different frequencies of electromagnetic radiation in the infrared region of the electromagnetic spectrum. The frequency of infrared region in the electromagnetic spectrum lies in the region from 2.5 μm to 25 μm but most of the chemists express it as wavenumber (cm^{-1}) which is reciprocal of centimetre. Although FTIR spectroscopy ranging between 12800 to 10 cm^{-1} but it can be classified in three different categories as, near infrared (NIR) (12800 to 4000 cm^{-1}), mid infrared (MIR) (4000 to 400 cm^{-1}), and far infrared (FIR) (50 to 1000 cm^{-1}) [36]. However, most of the organic chemists focus to this "MIR" in the frequency range, since, nearly all the functional groups of organic molecules falls under this fundamental vibrations region; it is the most desirable spectral range for chemical analysis of any known or unknown molecule.

The instrument that determines the absorption spectrum for a compound is called an infrared spectrometer or, more precisely, a spectrophotometer. Now-a-days, Fourier transform (FT) infrared spectrometers are in common use in the organic laboratory. Fourier transform is nothing but a mathematical operation by which time domain spectrum (intensity versus time) are easily converted into frequency domain spectrum (frequency versus time). A schematic diagram of Fourier transform infrared spectrum has been given below (fig. 3):

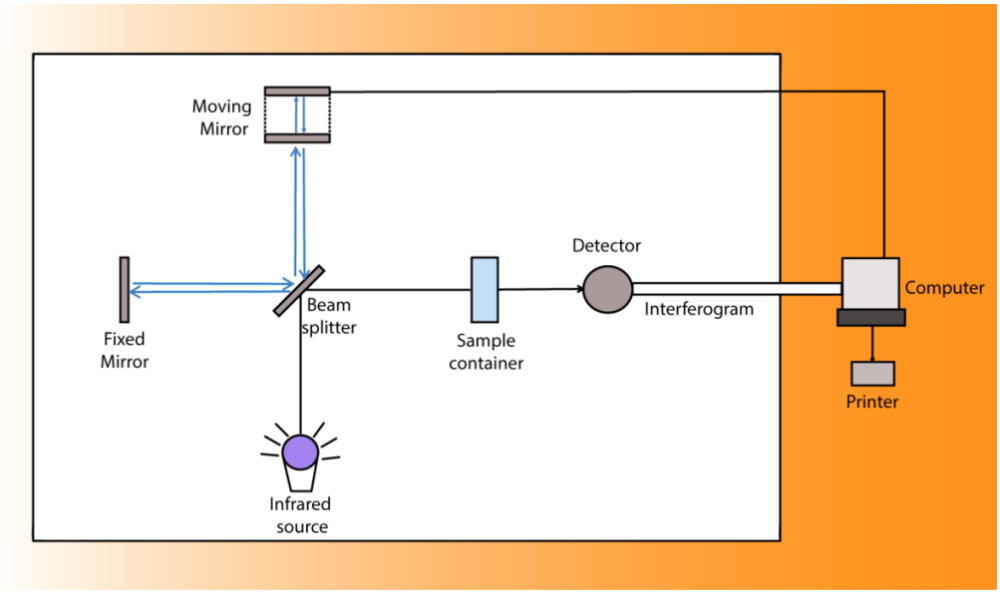


Figure 3: The layout of a typical infrared spectrophotometer

When the samples are subjected to infrared radiation, some part of the radiation is absorbed by the molecules in the sample, which vibrate at certain frequency. Rest of the radiation is transmitted according to Beer's Law and is collected by a detector. The obtained signal is processed using complex mathematical operations known as Fourier Transformations, and a unique spectrum is produced between transmittance and frequency.

The instrument used for the present study is Perkin Elmer Model spectrum 100, FTIR, USA in the wave number range of 4000-400 cm^{-1} and 1 cm^{-1} optical resolution of the instrument. The samples are diluted with KBr before the measurement and each sample is scanned 32 times.

3.3.1.4 Nuclear magnetic resonance spectroscopy (^{1}H-NMR):

Nuclear magnetic resonance (NMR) is one of the most important spectroscopic method that one synthetic chemist usually used for structural depiction. Nuclear magnetic resonance (NMR) spectroscopy works on a basic principle that when an external magnetic field is being applied to a sample which is susceptible towards magnetic field, energy is absorbed and converts the nucleus from lower-energy spin state to the higher-energy state and records the absorption spectrum. From NMR data, it is possible to assign structural details through analysis of these environments.

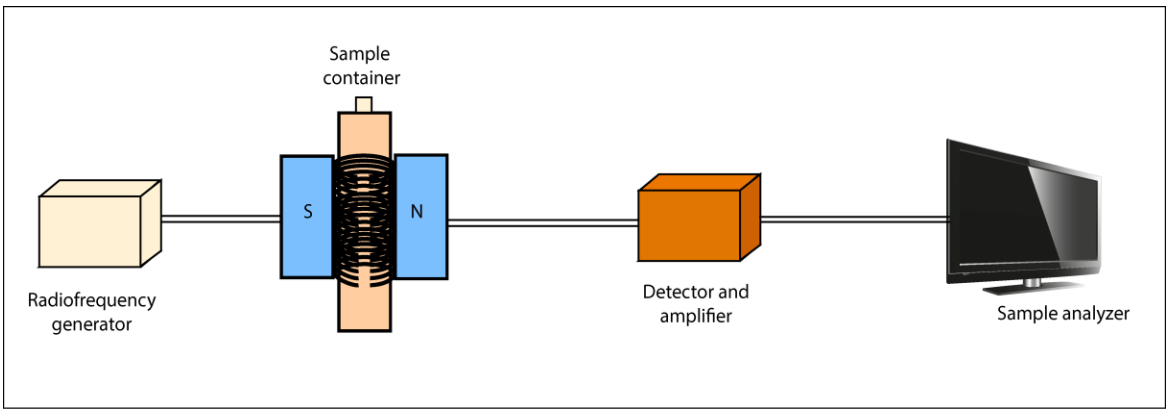


Figure 4: Schematic operation of a basic NMR spectrometer

The method is complementary to IR spectroscopy; however, timescales of the two techniques are quite different. In case of IR, the absorption of infrared energy by a molecule giving rise to a change in vibrational energy change is much faster (about 10^{-13} s), but the NMR process is much slower (about 10^{-3} s). In our study, all NMR spectra were recorded on a Bruker AVANCE spectrometer at 400 MHz and 25 ^{0}C in D_2O as well as in d6-DMSO. In host-guest chemistry, the change in position of the chemical shifts can also reveal the orientation and other useful information of the host-guest inclusion complexes.

3.3.1.5 2D-NMR spectroscopy:

2D-NMR spectroscopy allows the determination of host-guest binding by analyzing 'through space' interactions. Two-dimensional (2D) ROESY spectra were collected at 25 ^{0}C with number of scan 8, and a 2048 K time domain in F2 (FID resolution 5.87 Hz) and 460 experiments in F1. ROESY NMR is primarily works on the basis of the interaction between two protons that are closely located in space and thus produce a nuclear Overhauser effect (NOE) cross-correlation in NOE (NOESY) or ROESY spectroscopy. It is to be mentioned that two CD protons such that H3 and H5 are located at the inner cavity of the CD. In contrast, H1, H2 and H4 protons are located at the exterior of the CD. H3 is located close to the wider rim, H5 is close to the narrower rim and H6 is attached to the primary rim. When, CD protons, H3 and H5 come in close proximity with protons of the guest molecule within 0.4 nm NOE cross-peaks between protons from two species indicates spatial contacts [27]. It confirms that the guest's protons are included, or almost included, in the CD cavity.

3.3.2 Spectrometric techniques:

3.3.2.1 ESI-MS spectrometric analysis:

Mass spectrometry is a crucial analytical tool for the identification and structural characterization of small molecules to very large proteins. The information given by mass spectroscopy is sufficient for the identification of elements present and the determination of the molecular mass as well as molecular formula of the given chemical sample. The basic principle of mass spectrometry (MS) is to generate ions by the bombardment of inorganic or organic compounds by high energy electrons of around 70 electron volts (eV), or 6700 kJ/mol, with the help of different ionization method. Then, from their mass-to-charge ratio (m/z), these ions are detected qualitatively and quantitatively by their respective m/z and abundance. The ionization process may be of different types; it may be thermally, applying electric fields or by impacting high energetic electrons, ions.

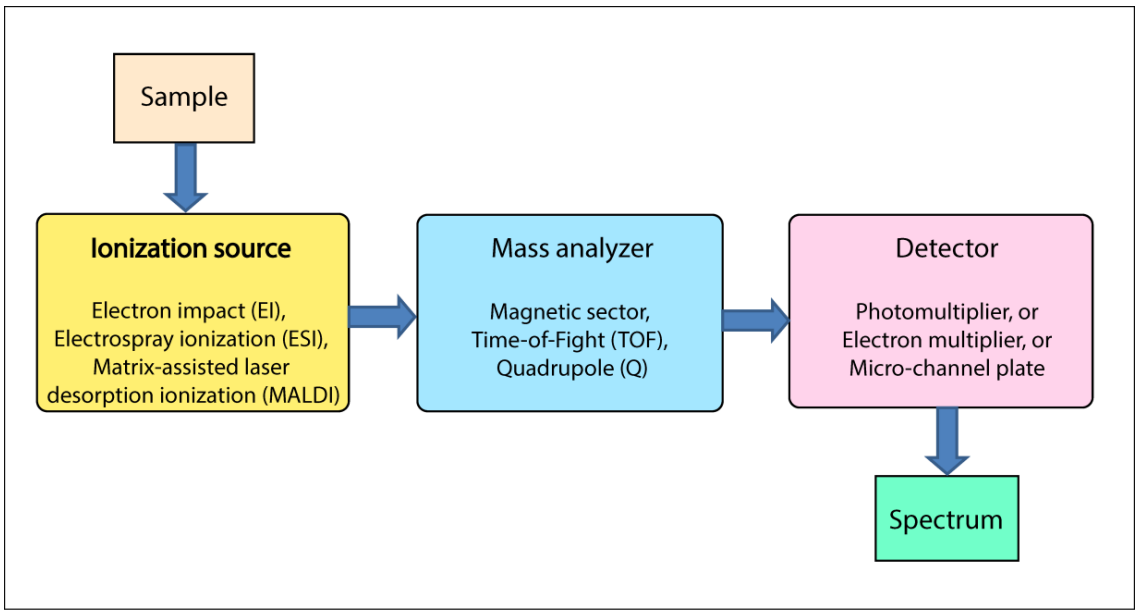


Figure 5: A schematic representation of an electron-ionization mass analyzer

A mass spectrometer consists of an ionization source, where, samples are given an electrical charge; a mass analyzer, where, ions are separated by their mass-to-charge ratio and a detector (Fig. 5), which is used for separating ions and are operated under high vacuum conditions. Mass spectrometry is destructive in nature i.e, sample cannot be recovered after successful run. A typical mass spectrum is

represented as a two-dimensional bar graph of signal intensity (ordinate) versus m/z, in which the tallest peak, with an intensity of 100%, is called the base peak and the peak with unfragment cation radical denoted as molecular ion peak (M^+). It is to be mentioned that m/z is mass to charge ratio which is dimensionless.

3.3.3 Thermogravimetric techniques:

3.3.3.1 Thermogravimetric (TGA) analysis:

Thermal analysis is the analysis of a change in a property of a sample induced by heating. The sample is usually a solid and the changes that occur include melting, phase transition, sublimation, and decomposition. Thermogravimetric analysis (TGA) measures the change in mass in terms of weight percentage (wt %) of a material as a function of time at a determined temperature (i.e., isothermal mode), or over a temperature range using a predetermined heating rate. A software with a computer records any mass gains or losses. Weight loss can be plotted against a function of time for isothermal studies and as a function of temperature for experiments at constant heating rate. Thus, this method is extremely useful in monitoring heat stability and loss of components.

3.3.3.2 Differential scanning calorimetric (DSC) techniques:

In DSC, the sample and the reference are maintained at the same temperature throughout the heating procedure by using separate power supplies to the sample and reference holders. Any difference between the power supplied to the sample and reference is recorded against the furnace temperature. Thermal events appear as deviations from the DSC baseline as either endotherms or exotherms, depending on whether more or less power has to be supplied to the sample relative to the reference. In DSC, endothermic reactions are usually represented as positive deviations from the baseline, corresponding to increased power supplied to the sample. Exothermic events are represented as negative deviations from the baseline.

3.3.4 Dynamic light scattering (DLS) measurements:

3.3.4.4 Hydrodynamic diameter and zeta potential analysis:

Dynamic light scattering (DLS) method can be used to measure the hydrodynamic diameter and zeta potential of all the prepared nanoparticles or

nanocomposites as well as host-guest inclusion complex. Particle size and size distribution of the nanomaterials are often found out extremely useful technique prior to TEM. A basic DLS instrument consists of an incident light source, typically a laser (for example gas ion, HeNe, or may be laser diodes), the light scattering cell, in most cases a cylindrical quartz glass cuvette, a detector and a signal processing computer (Fig. 6). It is based on the Brownian motion of the particles caused by random thermal density fluctuations of the solvent molecules which push the scattering particle along. It is to be pointed out that particles in suspension or dispersion undergo continuous motion and when incident light strike on these particles, it gets scattered in different directions. Scattering intensities of the particles vary with time, since the particles are in continuous motion. Laser light from the source hit the sample in the cuvette and the scattered light signal is collected at 173° (back angle) scattering angle by the detector. Refractive index and viscosity (η) of the dispersion medium is required to carry out the measurement.

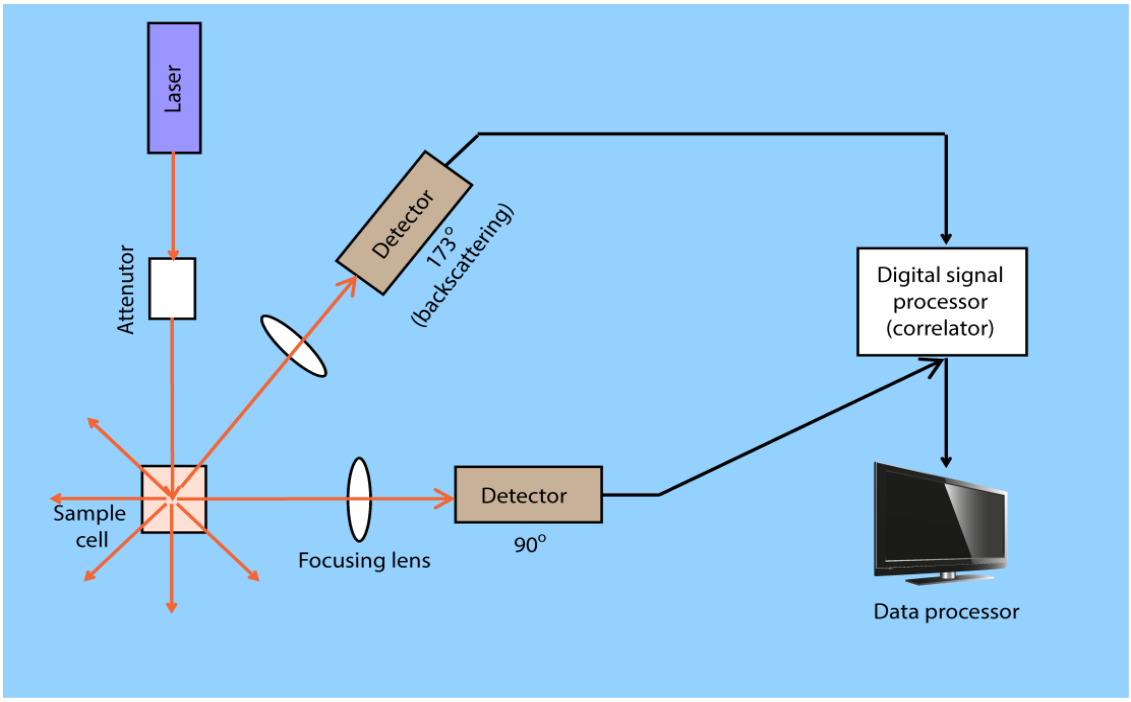


Figure 6: schematic diagram of working principle of DLS

The concentration of the samples should be as dilute as possible to minimize interparticle interactions, which may create contributions from interparticle interferences. The hydrodynamic diameter is measured from Stokes–Einstein–equation, (Eq. 2)

$$D_S = \frac{kT}{f} = \frac{kT}{6\pi\eta R_H} \quad \text{..... (2)}$$

R_H is the hydrodynamic radius of the scattering particle, D_S the selfdiffusion coefficient, T is the temperature of the sample and η is the viscosity of the solvent. f is the friction experienced during Brownian motion.

Zeta potential measurement provides an insight to the surface charge of the nanoparticles or nanocomposites. Particle surface charge plays main role in their stability in medium, agglomeration tendencies and interaction with biological systems. The surface charges control the interactions between particles and therefore determine the behavior of a sample suspension. During zeta potential measurements, an electrical field is applied across the sample and the motion or electrophoretic mobility of the particle is measured by the light scattering of the particles. Then, the zeta potential can be calculated by Henry equation as follows (**Eq. 3.2**):

$$U_E = \frac{2\varepsilon\zeta}{3\eta} f(ka) \text{ ..... (3.2)}$$

Where, U_E is the electrophoretic mobility; ε is the dielectric constant; ζ is the zeta potential; η is the viscosity; f(ka) is the Henry's function.

3.3.5 Microscopic measurement:

3.3.5.1 Scanning electron microscopy (SEM):

In scanning electron microscope, a high energy beam of electrons is used to obtain an image of the given sample in a similar fashion to optical microscopes but at much higher resolution. In an imaging electron microscope, electron beams are generally accelerated through 1–200 kV and electric as well as magnetic fields are used to focus the electrons.

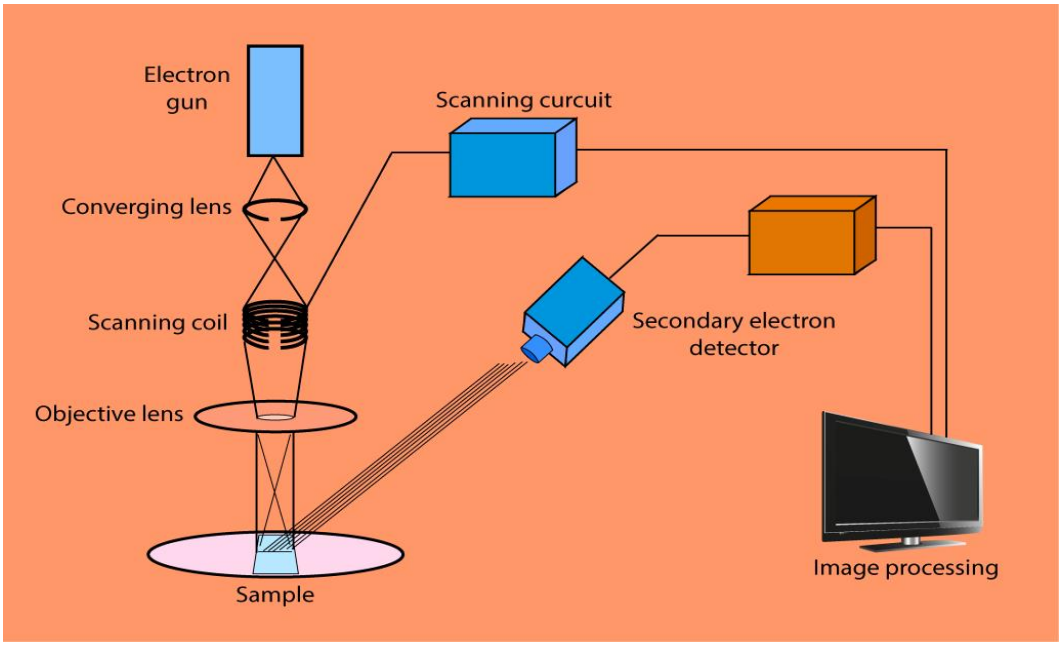


Figure 7: A systematic flow chart for scanning electron microscope

In scanning electron microscopy (SEM), the beam ejected from an electron gun is first scanned over the object and the reflected (scattered) beam is then imaged by the secondary electron detector (Fig. 7). This instrument helps to get the information concerning about the sample along with surface morphology, texture of the solid sample either may be crystalline or amorphous structure, chemical composition by EDX. Scanning of the sample can be done with area ranging from 1 cm upto 5 microns of width.

3.3.6 Conductivity measurement:

The specific conductance measurements were carried out with dip-type immersion conductivity cell of cell constant 1.11 cm^{-1} to measure the conductivity of an electrolyte solution. The entire experiments were reported at 1 KHz and were found to be ±0.3 % precise. The instrument was standardized using 0.1 (M) KCl solution. Lind and co-worker's method was used to calibrate the cell. The measurements were carried out using a thermostatic water bath maintained at the required temperature with an accuracy of ±0.01 K by means of mercury in glass thermo regulator. Conductometric study is of one the physicochemical method used

to identify the supramolecular host-guest interaction as well as the stoichiometry between host and guest in inclusion complex.

Several solutions were prepared by weight precise to ±0.02%. The weights were taken on a Mettler electronic analytical balance (AG 285, Switzerland). The molality being converted to molality as required. Due correction was made for the specific conductance of the solvents at desired temperatures. The following figure shows the Block diagram of the Systronics Conductivity-TDS meter 308.

3.4 Theoretical analysis techniques:

Computational chemistry and molecular modeling is a fast growing area which is used for the modeling and simulation of small chemical entity and biological systems in order to understand and predict their behaviour towards the system at the molecular level. It has a wide range of applications in various disciplines such as materials science, biological science, chemical engineering, biomedical engineering, etc.

3.4.1 Molecular docking:

Molecular docking studies can be carried out with our guest and supramolecular host molecules using the docking module implemented in MOE 2015 (Molecular Operating Environment) available from Chemical Computing Group Inc., http://www.chemcomp.com. Molecular docking is a computational simulation to predict the binding mode and binding affinity of an inclusion complex. Initially, all the X-ray crystallographic structures of are obtained from the Protein Data Bank. Then, all the structures are protonated with the addition of polar hydrogens, followed by energy minimization with the MMFF94x force field, with gradient: 0.05 in case of host and 0.005 in case of guest, in order to get the stable conformer. The probable binding site residues are highlighted through the “Site Finder” module implemented in the MOE software. The docking is carried out with the default parameters i.e., placement: triangle matcher, recording 1: London dG, refinement:

force field and a maximum of 5 conformations were allowed to be saved in a separate database file in a .mdb format.

After the docking process, the binding energy and binding affinity (kcal.mol^{-1}) of the optimized inclusion complexes, also termed as build-in scoring function of MOE, S-score, were calculated using a molecular mechanics GBVI/WSA dG scoring function module implemented in MOE. Inclusion complexes with lowest S score is selected for binding affinity.

3.4.2 Dynamic simulations:

Molecular dynamics and simulations studies are generally performed to obtain the information about stability of inclusion complex with respect to time, temperature, kinetic energy and potential energy. The best conformer of optimized inclusion complex obtained from molecular docking was subjected for Molecular Dynamics Simulations with the help of MOE software, Molecular Operating Environment (MOE), 2015. Usually, protein simulations are carried out for 0 femtoseconds to 1000 femtoseconds with respect to temperature, potential energy and kinetic energy. MOE dynamics simulation uses the Nosé-Poincaré-Andersen (NPA) equations of motion to identify structural and dynamic behaviour of inclusion complexes. In our inclusion complex system, default steps and protocols of the MD are selected to optimize the systems equilibrium 100 ps and production run is carried out for 500 ps. With the help of molecular dynamics calculations, several parameters can be calculated as a function of time, we can mention here: U, K, P, V.

U: The atomic potential energy function, a function of r

K: The kinetic energy of the atoms; $2K = pT M^{-1} p$

P: The real-space momenta of the atoms; heir velocities are $M^{-1} p$

V: The instantaneous volume of the system.

Although dynamic simulations are carried out in terms of RMSD or RMSF as a function of time but due to unavailability of software, molecular dynamics calculations are carried out as a change of potential energy as a function of time.

3.4.3 Potential energy calculations:

To understand the binding affinity and the energy component that is responsible for enhancing the stability of the inclusion complexes, it is important to calculate the potential energy of the ICs. Potential energy consists of several components: bond stretching energy (Str), bond angle energy (angle), stretching-bend interaction energy (Stb), out of plane bending energy (oop), dihedral torsional energy (tor), van der Waals energy (vdW), electrostatic interaction energy (ele), Solvation energy (sol). Out of those, two energy components, van der Waals energy (vdW), electrostatic interaction energy (ele) play a major role in the formation of most of the inclusion complexes.