CHAPTER III

EXPERIMENTAL SECTION

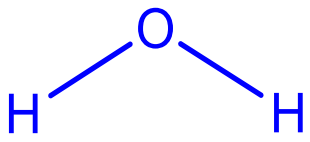
III.1. NAME, STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES, PURIFICATION AND APPLICATIONS OF THE CHEMICALS USED IN THE RESEARCH WORK

III.1.1. Solvents

Here are the detailed discussions of some aqueous and non-aqueous solvents used in our research work.

Water:

Water is the indivisible part of every living organism. The existence of life relies on it. It is basically a tasteless, colourless, odourless inorganic compound having the chemical formula H_2O. Mostly it appears as liquid but can exist in three forms depending on atmospheric pressure and temperature; solid, liquid and gas. Its use is universal as a solvent.



Source: Distilled water; distilled by fractional distillation method in laboratory.

Purification: First water was deionised and then distilled using alkaline $KMnO_4$ solution to exclude the organic materials.

Water	
Appearance	Liquid
Molar mass	18.015 g/mol
CAS number	7732-18-5
Melting point	273.15 K
Boiling point	373.15 K
Density	0.9970474 g/mL at 25^0C

Viscosity	0.890 cP
Refractive index	1.333 at 20^0C
Chemical formula	H_2O

Application: Water is very essential for every life. In industries, large quantities of water are used in the form of liquid water, ice and steam. By dissolving various substances in water , the physical and often chemical properties of those substances and their nature of interaction can be studied. Water is easily available than other solvents so it can be smartly used in plenty of projects either research based or industrial purposes. Every biochemical reaction that occurs in plants and other living bodies need water. The property of water to form emulsion is used in different processes. Its capacity of forming hydrogen bond makes other solutes dissolve in water.

Ethanol: Ethanol is an organic compound. It is volatile, flammable, colourless liquid and has a wine like pungent smell. [1, 2]

Ethanol	
Appearance	Liquid
Molar mass	46.07 g/mol
CAS number	64-17-5
Melting point	-114.1 ^{0}C
Boiling point	78.37 ^{0}C
Density	789 kg/m^3
Viscosity	1.2 mPa.s at 20^0C
Refractive index	1.3611
Chemical formula	C_2H_5OH

Source: purchased from Sigma Aldrich

Purification: Used as purchased.

Application: Ethanol is also considered as a universal solvent and it allows a plenty of polar-nonpolar, hydrophobic-hydrophilic compounds to dissolve in it due to its structural effect. Further its low boiling point helps in separating it from other media. Thus it can be used as an extracting agent. It is an essential agent for paints, vernishes

and cosmetics. It possesses antimicrobial property and used in different antimicrobial preservatives. [3]

Acetonitrile: It is an aprotic polar solvent basically a by product in the manufacture of acrylonitrile synthesis. It is the building block of many organic compounds.

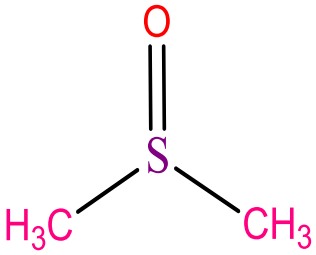
Acetonitrile	
Appearance	Liquid
Molar mass	41.053 g/mol
CAS number	75-05-8
Melting point	227K-229 K
Boiling point	354.4 K-355.2 K
Density	0.786 g/cm^3
Acidity (pk_a)	25
Refractive index	1.344
Chemical formula	CH_3CN

Source: Purchased from Sigma Aldrich

Purification: Used as purchased

Application: The use of acetonitrile is generally as solvent of modest polarity. It can dissolve many non-polar and ionic compounds and acts as the mobile phase in HPLC. High dielectric constant makes it useful in batteries and also in cyclic voltametry. For the purification of butadienes in refineries acetonitrile has outstanding industrial value.

Dimethyl sulfoxide: DMSO is a very popular aprotic polar solvent like acetonitrile. It has no colour and scores of compounds including polar , non-polar dissolve in it. DMSO is miscible in water and in other organic solvents.



DMSO	
Appearance	Colourless liquid
Molar mass	78.13 g/mol
CAS number	67-68-5
Melting point	19 ^{0}C(292 K)
Boiling point	189 ^{0}C(462 K)
Density	1.1004 g/cm^3
Viscosity	1.996 cP at 20 ^{0}C
Refractive index	1.479
Chemical formula	C_2H_6OS

Source: Sigma Aldrich, Germany

Purification: Used as purchased. (99.0% purity)

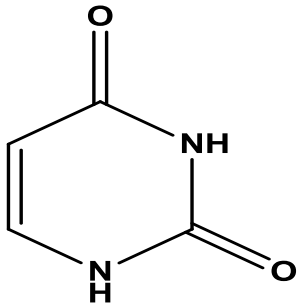
Application: It is an organosulfur compound and used in various applications. For the purpose of mild oxidation in organic syntheses, in some sulfonium based oxidation reactions, DMSO is used as an oxidant. [4]

DMSO is sometimes used as solvent in salt involving reactions and because of its less acidity it can bear with strong bases and thus use in the studies of carbanions. In biochemistry and cell biology its use is as an extracting agent. The deuterated d_6-DMSO is extensively functional as solvent in NMR spectroscopy. In the study of in vitro drug discovery or designing DMSO is broadly used to dissolve compounds under test. The in-vivo experiments are also done with DMSO as vehicle.[5] [6, 7] [8, 9]

III.1.2. The biologically significant molecules

There are a number of bioactive molecules used in the research work and a detailed information about them is as follows.

Uracil: RNA has four nucleobases, one of which is uracil. It is linked to adenine by two hydrogen bonds. Uracil is absent in DNA or it is said that it is in demethylated form which is thymine. It is a pyrimidine derivative of natural occurrence. Uracil shows tautomeric shifts, amide form to imide form. But the amide or lactum tautomer is the popular one. [10]



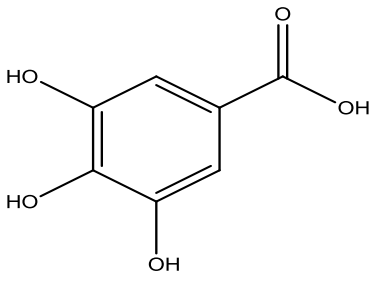
Uracil	
CAS Number	66-22-8
Molar mass	112.08676 g/mol
Appearance	solid
Melting point	335 ^{0}C (608 K)
Solubility	Soluble in water
Chemical formula	$C_4H_4N_2O_2$

Source: Sigma Aldrich, Germany

Purification: Used as purchased. Mass fraction purity is ≥ 0.99

Application: Uracil is biologically very significant molecule. In drug delivery and pharmaceuticals it is used extensively. Uracil plays vital role binding through ribose and phosphate in the synthesis of many enzymes which accelerate the function of the cell. It helps to detoxify many drugs and engages in the polysaccharide's biosynthesis. Uracil is the basic building unit of many anticancer drugs[11].

Gallic Acid: Gallic acid is a polyphenolic compound. It is trihydroxy benzoic acid. It is present in different berries, oak barks, tea leaves etc.



GALLIC ACID

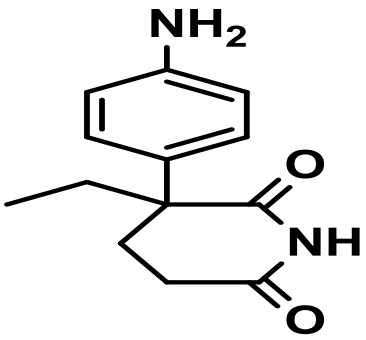
Gallic acid	
CAS Number	149-91-7
Molar mass	170.12 g/mol
Appearance	White, yellowish white
Melting point	1.694 g/cm^3
Solubility in water	1.19 g/100mL at 20 ^{0}C(anhydrous) 1.5 g/100mL at 20 ^{0}C(monohydrate)
Chemical formula	$C_7H_6O_5$

Source: Sigma Aldrich

Purification: Used as purchased. Mass fraction purity≥ 0.99

Application: Gallic acid and its derivatives are extremely valuable for their properties such as antifungal, antibacterial, anti-inflammatory, anticancer. Gallic acid esters are industrially important components for pharmaceuticals and food industries. [12]

DL-Aminogluthimide: Aminoglutethimide is a medication in cushing's syndrome, breast cancer and prostate cancer. It had been used as anticonvulsant in early 1960 's but for its proficiency of inhibiting the production of certain hormones it is used in the treatment of hormone dependent breast carcinoma generally assisted by estrogen hormone.



DL-Aminoglutethimide	
CAS Number	125-84-8
Molar mass	232.28 g/mol
Appearance	White powder

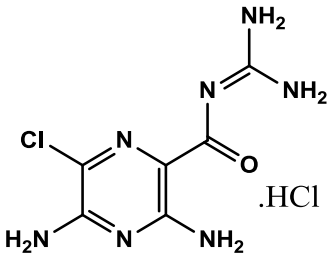
Melting point	152-154 ^{0}C
Solubility	Slightly soluble in H_2O; 0.2 mg/mL, soluble in acetonitrile
Chemical formula	$C_{13}H_{16}N_2O_2$

Source: TCI chemicals.

Purification: Used as purchased. The purity is >98%

Application: Generally **DL-AGT** is used in advanced breast and prostate cancers as a medicine. It is an anticonvulsant also. It is an aromatase inhibitory drug. For the treatment of metastatic breast cancer which are basically hormone assisted. The antitumor efficacy of the drug by suppressing the level of estrogen is due to its potency of inhibiting the conversion of cholesterols into steroid hormones.

Amiloride hydrochloride: Amiloride is taken as a medication of cardiac failure, high blood pressure and in liver cirrhosis. It works in our body by retaining potassium but excluding excess sodium with excess fluid .



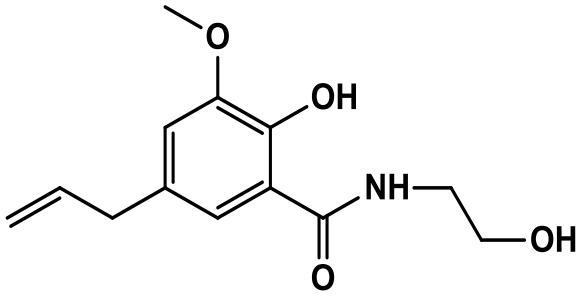
Amiloride hydrochloride	
CAS Number	2016-88-8
Molar mass	266.09 g/mol
Appearance	Yellowish white powder
Melting point	293 ^{0}C-294^{0}C
Solubility	<0.1g/100 mL in H2O at 19.5 ^{0}C
Chemical formula	$C_6H_8ClN_7O.ClH$

Source: Sigma Aldrich, Germany

Purification: Used as purchased

Application: It has natriuretic and diuretic effects though the effect is moderate i.e. by expulsion of excess sodium it can reduce the blood pressure. For the treatment of hypokalemia it has significant outcome. It can be taken along with other diuretic medicines.[13, 14]

Alibendol: Alibendol is 2-hydroxy-*N*-(2-hydroxyethyl)-3-methoxy-5-(2-propenyl)benzamide**.** Its use is as an antispasmodic medication. The use of alibendol is mostly as a constituent of some choleretic, cholekinetic drugs.



Alibendol	
CAS Number	26750-81-2
Molar mass	251.28 g/mol
Appearance	White solid
Melting point	97 ^{0}C-99 ^{0}C
Solubility	Ethanol, DMSO
Chemical formula	$C_{13}H_{17}NO_4$

Source: TCI chemicals.

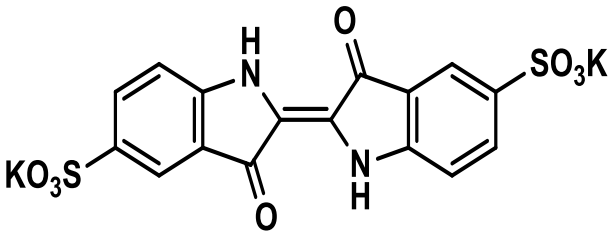
Purification: Used as purchased. Its purity is >98%

Application: It is the antispasmodic drug. It gives relief from muscle spasms in stomach, intestine and other type of muscle contractions occur due to various reasons. Sometimes it is functional in choleretic and cholekinetic diseases. Loads of choleretic drugs have this compound as the main constituent. [15, 16]

III.1.3. Dye molecule (Industrially significant)

We have chosen the dye the following dye molecule for our research work.

Indigosulfonic acid dipotassium salt: It is a derivative of indigo dye which is of natural occurrence. The intra-molecular hydrogen bond is possible in the structure of ISD and due to this, it has high melting point. It has similar structure that of indigo carmine but has potassium in place of sodium.



Indigosulfonic acid dipotassium salt	
CAS Number	13725-33-2
Molar mass	498.57 g/mol
Appearance	Blue solid
Melting point	>300 ^{0}C
Solubility	Partly in water,ethanol
Chemical formula	$C_{16}H_8K_2N_2O_8S_2$

Source: TCI chemicals.

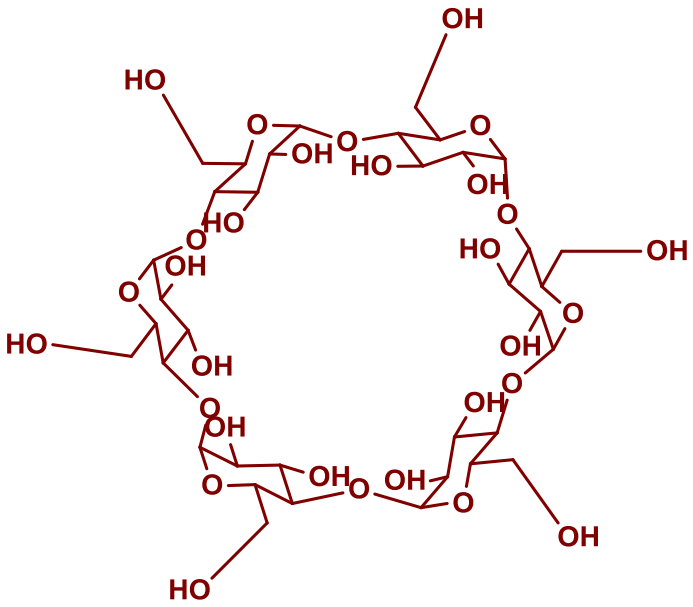
Purification: Used as purchased. Purity is >90%

Application: It has applications in colouring products, textiles and other industrial and research based uses are there. It does not have much mentioning in literature. Due to its dark blue colour it is used as a colouring agent for different purposes.

III.1.4. Cyclodextrins

The use of cyclodextrin molecules, have taken a huge part of our research work. The cyclodextrin molecules that have been taken for our convenience are α and β cyclodextrins. Their descriptions are given here as follows.

α –Cyclodextrin: It is a cyclic amylose formed by the enzymatic degradation of raw vegetable materials or starch. There are six glucose subunits are joined by glycosidic α -1,4 bonds in this molecule. It is natural in origin. The glucose units are chiral and chair conformation makes the molecule rigid and also makes it truncated cone shaped with a void inside. Here the position of the secondary hydroxyl groups on the wider rim and primary hydroxyl groups are on the narrower rim.[17]



α -Cyclodextrin	
CAS Number	10016-20-3
Molar mass	972.84 g/mol
Appearance	White solid
Melting point	>278 ^{0}C
Solubility	H_2O:50mg/mL
Chemical formula	$C_{36}H_{60}O_{30}$
Internal diameter (A^0)	4.7-5.2
Number of glucose unit	6

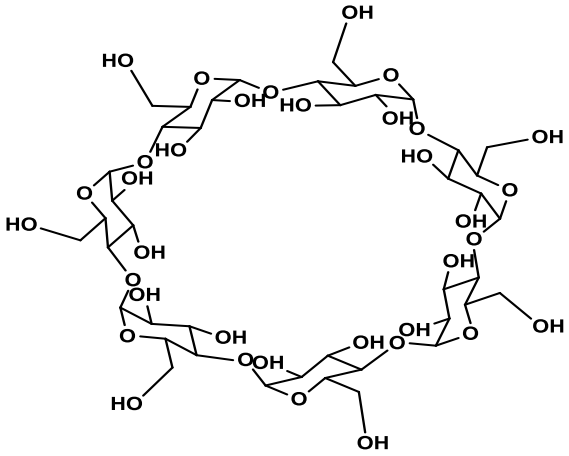
Source: Sigma Aldrich.

Purification: Used as purchased. Purity ≥98%

Application: α –CD has numerous applications in pharmaceuticals, foods, cosmetics, textiles etc. It has a specific inner hydrophobic cavity which can accommodate

small molecules and can form inclusion complexes with them by some non-bonding interactions. It has profound application in drug delivery and also in drug designs.

β-Cyclodextrin: It has the same property like α – cyclodextrin. It has seven α-(D)-glucopyranose units connected to α-1,4 bonds. It is a amorphous white solid.



β -Cyclodextrin	
CAS Number	7585-39-9
Molar mass	1134.98 g/mol
Appearance	White solid
Melting point	290-300 ^{0}C
Solubility	18.5g/L
Chemical formula	$C_{42}H_{70}O_{35}$
Internal diameter(A^0)	6.4-7.5
Number of glucose unit	7

Source: Sigma Aldrich.

Purification: Used as purchased. Purity is ≥97%

Application: Application of β-CD is marvellous. The low production cost among the cyclodextrins makes it available for various applications. β –CD has the suitable cavity size to incorporate a number of valuable molecules of interest. Different guest molecules which are of biological importance are fitted in the cavity of β –CD to change their physical and chemical properties so as to make the compound more prospective in its activity. Low toxicity makes it an ideal component for the drug delivery and design.

III.2. EXPERIMENTAL PROCEDURES

III.2.1. Preparation of Inclusion Complexes:

At first, 20 mL 1.0 (mM) solutions of α and β-CD were prepared separately with triply distilled, deionized and degassed water which, allowed to stir for several hours on a magnetic stirrer. Then,20 mL 1.0 (mM) (prepared in purely distilled water or in 20% ethanol-water or 15% acetonitrile-water mixtures) solutions of guest molecules were added drop wise to the previously prepared aqueous solution of α-CD or β-CD making the ultimate equimolar mixture and were continued to stir for 48-72 hours at 55-60°C. The suspensions obtained after cooling the mixture to 5 °C were filtered to obtain white crystalline powder, which were then dried in air and preserved in vacuum desiccators.

III.2.2. Solution preparation

A stock solution for each component was equipped (digital electronic analytical balance, Mettler Toledo, AG 285, Switzerland) by mass, and the functioning solutions were obtained by mass dilution. The doubt of molarity of dissimilar salt solutions was evaluated to be ± 0.0003 mol·dm^{-3}.

Solvent mixtures are prepared from pure components which were taken independently in glass stoppered bottles and thermostated at the required temperature for adequate time. When the thermal equilibrium was ensured, the requisite volumes of each component were transferred in a dissimilar bottle which was already cleaned and dried methodically. Translation of essential mass of the relevant solvents to volume was skilled by using experimental densities of the solvents at experimental temperature. It was then Stoppard and the mixed contents were shaken well before use. While preparing different solvent mixtures care was taken to ensure that the same process was adopted right through the whole work. The physical properties of diverse pure and mixed solvents have been offered in the relevant chapters.

III.2.3. Preparation of multicomponent liquid mixtures:

The double and polycomponent liquid mixtures can be equipped by any one of the procedures discussed below:

(i) Mole fraction

(ii) Mass fraction

(iii) Volume fraction

(i) Mole fraction: The mole fraction (x_i) of the polycomponent liquid mixtures can be equipped using the following relation:

$$x_i = \frac{(w_i / M_i)}{\sum_{i=1}^{n}(w_i / M_i)}$$

Where, w_i, and M_i are mass and molecular mass of i^{th} component, correspondingly. The values of i depends on the number of components implicated in the development of a mixture.

(ii) Mass fraction: The mole fraction (w_i) of the polycomponent liquid mixtures can be equipped using the following relation:

$$w_i = \frac{(x_i / M_i)}{\sum_{i=1}^{n}(x_i M_i)}$$

(iii) Volume fraction: The volume fraction (ϕ_i) of the poly component liquid mixtures can be equipped by following employing three methods:

(a) Using volume: The volume fraction (ϕ_i) of the polycomponent liquid mixtures can be prepared by following relation

$$\phi_i = \frac{V_i}{\sum_{i=1}^{n} V_i}$$

Where, V_i, is the volume of pure liquid i.

(b) Using molar volume: The volume fraction (ϕ_i^l) of the polycomponent liquid mixtures can be equipped by following relation

$$\phi_i^l = \frac{x_i V_{mi}}{\sum_{i=1}^{n}(x_i V_{mi})}$$

Where, V_{mi} is the molar volume of pure liquid i.

(c) Using excess volume: The volume fraction (ϕ_i^{ex}) of the polycomponent liquid mixtures can be equipped by following relation

$$\phi_i^{ex} = \frac{x_i V_i}{\sum_{i=1}^{n} (x_i V_i) + V^E}$$

Where, V^E is the excess volume of the liquid mixture.

III.3. DETAILS OF THE INSTRUMENTS INVOLVED IN THE RESEARCH WORK:

III.3.1.Measurement of mass:

To measure mass has been carried out in a digital electronic analytical balance, Mettler Toledo, AG 285, Switzerland.

It can measure mass with excessive precision and accuracy. The weighing pot is of elevated accuracy and precision (0.0001g) is kept inside a glass enclosed space with sliding doors to save from harm from dust and air currents.

III.3.2. Thermostat:

Using Brookfield TC-550 thermostatic water bath temperature of the solutions under experiment were controlled . It has an accuracy of ± 0.01 K of the desired temperature.

III.3.3. Water distiller:

The glass distillation unit of the water distiller was of Bionics Scientific Technologies (P).Ltd. It produces contaminants free water.

It converts the water into vapour by heating it. During heating the contaminants like bacteria, some heavy metals, salts cannot get into vapour phase with water so they are left as residues in the heating/boiling chamber. The evaporated water is then condensed and the liquid form of water is collected in a container. This is how a very pure, mineral free , water is produced by the water distiller in laboratory. It is the cheapest way to get distilled water rather than buying.

III.3.4. Magnetic stirrer:

In order to get homogeneous solutions and to promote heat and mass exchange in the mixture we used the Magnetic stirrer cum hot plate from IKA. Preparation of different required solutions and also to get solid inclusion complexes at a fixed temperature the magnetic stirrer was used.

III.3.5. Density Measurement:

The density measurementsof the solutions were measured by vibrating-tube density meter ,Anton Paar, DMA 4500M(temperature range 298.15 to 318.15 K). Calibration of the instrument was done with doubly distilled water and dry air. The uncertainty in measurement was ±0.00001 g cm^{-3}.

Here the mechanical oscillation of the U-tube at a frequency is converted into an AC voltage of the equivalent frequency associated to the density of the sample.

The related equation is , $\rho = A.\tau^2 - B$

Where,τ is the period; A and B are the instrument constants of each of the oscillator and ρ is the density of the sample.

III.3.6. Measurement of Viscosity

Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42 was engaged to calculate the viscosities of the solutions. With the help of the following equation the viscosities were determined.

$$\eta = (100/RPM) \times TK \times torque \times SMC$$

Where RPM is the speed, TK (0.09373) is the torque constant and SMC (0.327) is the spindle multiplier constant.

The calibration of the instrument was achieved with supplied standard viscosity samples, water and aqueous solutions.

III.3.7. Refractive Index Measurement:

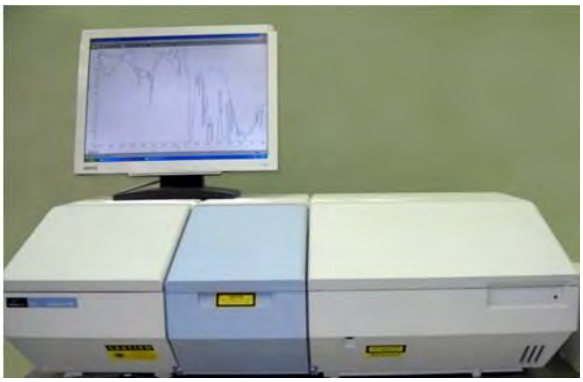
Refractive indices of the sample solutions were measured by the instrumentDigital Refractometer, Mettler Toledo 30GS of accuracy $\pm$0.0005. It can be calibrated by different solutions by measuring their refractive indices e.g. distilled water, CCl_4, toluene and cyclohexane at a specific temperature.Very small quantity of the sample was poured in sample holder pan of the machine reading was taken.

Resolution	0.0001
Accuracy	$\pm$ 0.0005
Measurement range	1.32-1.65
Temperature range	$10\text{-}40^0$

III.3.8. FT-IR Spectra Measurement:

The Perkin-Elmer FTIR spectrometer (shimadzu, Japan) has been used for all the IR data (in the scanning range of 4000−400 cm^{-1}).

In the KBr disk method the sample concentration should be very low 0.2-1 % and the disk has to be thicker than a film of liquid. Much grinding of KBr not needed because finely divided mixture of KBr absorbs more moisture.



III.3.9. Isothermal titration calorimetry:

The various thermodynamic parameters regarding the inclusion process were collected from the Microcal VP-ITC (microcal now Malvern instrument). By this instrument direct quantitative measurement of the thermodynamic parameters can be obtained.

Only liquid, non-acidic samples are allowed into this machine. For each sample there should be a minimum requirement of 2 mL of reference and sample fluid. A spinning syringe is used to mix the reactants and for injecting the ligand or the sample of interest. The operator inputs all the experimental parameters and computer runs the experiment.Then the computer do the experiment. The sensitivity is 0.1 μcal. Its normal operating range is 2 ^{0}C to 80 ^{0}C. Two coin shaped cells have been enclosed guarded by the adiabatic jacket.

III.3.10. Measurement of UV-Visible Spectra

Using JASCO V-530 and Agilent 8453 UV-Visible Spectrophotometer, UV–visible spectral data were collected. The accuracy in wavelength is ±0.5 nm. The temperature , we have used in the range of 293.15K to 313.15K controlled by a digital thermostat attached to it.

Optical Specifications	
Slit width	1nm
Typical time scan	1.5 s (full range)
Wavelength range	190-1100 nm

It is a double beam spectrophotometer. First the baseline correction is done with the solvent/reference. Then the samples are taken in a cuvette for the experiment and spectra are recorded.

III.3.11. NMR Spectroscopic Measurement:

The NMR spectra were recorded in a Bruker Avance instrument at 298.15 K. The solutions were prepared in d_6-DMSO and D_2O as per requirements of the sample. Both

the 2D ROESY and NOESY as well as ^{1}H NMR spectra were recorded at 400 MHz. The δ values (chemical shifts) are represented in ppm (parts per million, D_2O; δ 4.79ppm).

III.3.12. Fluorescence Spectra Measurement

The Quantamaster-40 spectrofluorometer was used to record fluorescence spectral data at ambient temperature. Fluorescence is the emission of radiation when a molecule returns from higher electronic to ground level.

Resolution	0.06nm
Wavelength accuracy	± 0.5nm
Light source	High power continuous xenon arc lamp
Focal length	200nm
System control	spectroscopy software (computer controlled)
Emission range	185-680 nm

III.3.13. Powder X-Ray Diffraction (PXRD)

Powdered X-Ray Diffraction (PXRD) data of the test samples were recorded by D8 Bruker Advance (Cu-Kα radiation).

It can be applied for various findings like structure determination, identifying texture, phase analysis and many more. It includes primary and diffracted beam monochromator and equipped with many other drives electronically. It is basically computer controlled.

The ray produced by the x-ray tube is diffracted by the sample; detector records it. The sample rotates at a fixed angular velocity so that the glancing angle of the primary beam changes and the detector rotates round the sample at a double angular velocity.

III.3.14. Scanning Electron Microscopy (SEM)

To get the idea about the surface morphology of the samples under test the Scanning Electron Microscope (SEM), JEOL JSM IT 100 was used. The resolutions were maintained requirement wise to acquire clear and vivid micro images.

Double adhesive carbon-coated tape (tiny piece) is used for the sample preparation. The samples are sprinkled over it and excess amount of sample is removed. Samples for this experiment should be extremely dry because of high vacuum condition inside the sample chamber.

III.3.15. High Resolution Mass Spectrometry:

The mass spectrometric measurements were done by a quadruple time-of-flight (Q-TQF) high resolution instrument (with positive mode electrospray ionization) taking the solution of the ICs in methanol. This technique is a source of information both qualitative and quantitative.

First the samples are exposed to the ionisation source to get ionised. After that the ions move to the mass analyser and get separated according to their mass to charge ratio at different parts of the detector. As soon as the ions come in get in touch with detector, signals produced. Computer records the signals.