CHAPTER III

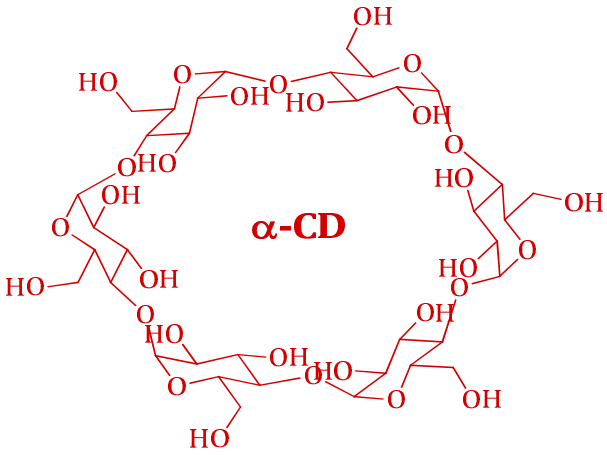
EXPERIMENTAL SECTION

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

III.1.1. HOST MOLECULES

α-Cyclodextrin:

α-Cyclodextrin, a cyclic oligosachharide, is well known in supramolecular chemistry as molecular host. They have a shape of truncated cone with a hydrophobic cavity and hydrophilic exterior rim. Primary hydroxyl groups are situated at narrow end and secondary hydroxyl groups are placed around the wider end. α-CD is composed of six glucopyranose units linked through α (1-4) bond.(1-2)



Source: Sigma Aldrich, Germany.

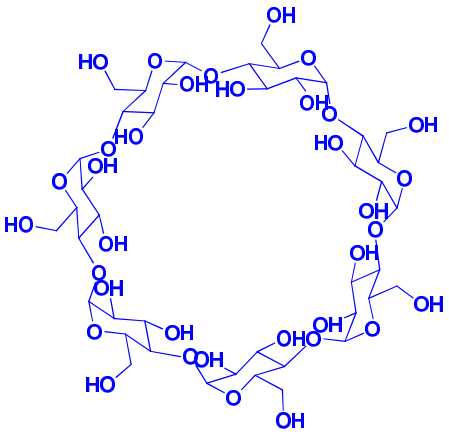
Purification: Used as purchased. The purity of the chemical is >98.0%.

CAS Number	10016-20-3
Chemical formula	$C_{36}H_{60}O_{30}$
Molar mass	972.84 g mol^{-1}
Appearance	White powder
Solubility in water	145 g L^{-1}

Application: Cyclodextrins have vast applications in the field of pharmaceuticals, pesticides, foodstuffs, toilet articles, textile processing industry, supramolecular host-guest chemistry, molecular encapsulation etc.[2] CDs form stable host-guest Inclusion Complexes with essential amino acids e.g. 3-(2-Napthyl)-D-Alanine,sodium valporate arginine, histidine, lysine, phenyl alanine, glutamic acid ionic liquids e.g., 1-butyl-4-methylpyridinium iodide, RNA nucleosides etc. as guest molecules.

β-Cyclodextrin:

β-Cyclodextrin, a cyclic oligosachharide, is well known in supramolecular chemistry as molecular host. They have a shape of truncated cone with a hydrophobic cavity and hydrophilic exterior rim. Primary hydroxyl groups are situated at narrow end and secondary hydroxyl groups are placed around the wider end. β-CD is composed of seven glucopyranose units[3] linked through α (1-4) bond.(3-4)



Source: Sigma Aldrich, Germany.

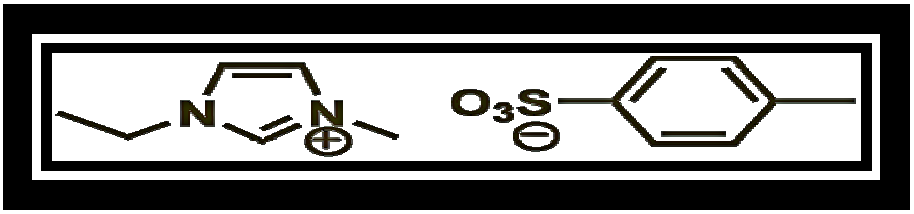
Purification: Used as purchased. The purity of the chemical is >98.0%.

CAS Number	7585-39-9
Chemical formula	$C_{42}H_{70}O_{35}$
Molar mass	1134.98 g mol^{-1}
Appearance	White powder
Solubility in water	18.5 g $.L^{-1}$

Application: Cyclodextrins have vast applications in the field of pharmaceuticals, pesticides, foodstuffs, toilet articles, textile processing industry, supramolecular host-guest chemistry, molecular encapsulation etc. CDs form stable host-guest Inclusion Complexes with essential amino acids e.g. 3-(2-Napthyl)-D-Alanine Sodium valporate, arginine, histidine, lysine, phenyl alanine, glutamic acid ionic liquids e.g., 1-butyl-4-methylpyridinium iodide, RNA nucleosides etc. as guest molecules.

III.1.2. IONIIC LIQUID

1-ethyl-3-methyl imidazolium tosylate: Ionic Liquids (ILs) having combination of organic-organic and organic-inorganic cations/anions are of great interest in the current chemical field. Their intrinsic physicochemical properties make them “designer solvents” or “green solvent”, such as the favourable solubility of organic and inorganic compounds, negligible vapour pressures, low melting points, high thermal stability, solvated many organic, inorganic and polymeric materials, adjustable polarity, selective catalytic effects, chemical stability. In addition, along with these exceptional properties. 1-ethyl-3-methylimidazolium tosylate is also imidazolium based ionic liquid, of molecular formula $C_{13}H_{18}N_2O_3S$, containing ethyl, methyl groups with two active nitrogen atoms in the imidazole or five member ring, exist as a molten liquid phase with the melting point below 313K.(5-6)



Appearance	crystalline
Molecular Formula	$C_6H_{11}N_3O_3$
Molecular Weight	173.17 g/mol
Melting Point	313.15 K
Density	1.23g/mol

Source: Sigma Aldrich, Germany

Purification: Used as purchased without further purification. The purity is >98%.

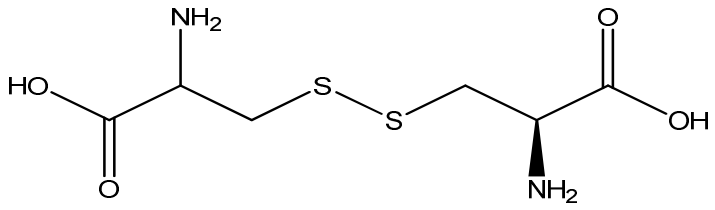
Application: It is used as recyclable solvents for organic reactions and separation processes, lubricating fluids, heat transfer fluids for processing biomass and electrically conductive liquids as electrochemical device in the field of electrochemistry. , ILs are used as heat transfer materials for processing biomass and electrically transport liquids as electrochemical tool in electrochemistry. Imidazolium cation based ILs are highly thermally stable, lager commercial available prominent bio-applications . ILs having large anions are also susceptible to additional interactions with polar solvents.

III.1.3. AMINO ACID

Amino acids are organic compounds that combine to form proteins. Amino acids and proteins are the building blocks of life. The human body uses amino acids to make proteins to help the body, e.g. Break down food, grow, repair body tissue, used as a source of energy by the body, Perform many other body functions. Amino acids are classified into three groups: Essential amino acids, Nonessential amino acids and Conditional amino acids. Out of this three categories conditional amino acids are usually essential in times of illness and stress. Few examples of conditional amino acids are arginine, cysteine, glutamine, tyrosine, glycine, ornithine etc.(7-8)

L Cystine:

L-Cystine is the oxidized dimer of the amino acid cysteine and has the formula $[SCH_2CH(NH_2)CO_2H]_2$. It is a white solid i.e. faintly soluble in water. It serves two biological functions: a location of redox reactions and a mechanical linkage that allows proteins to retain their 3-D structure.



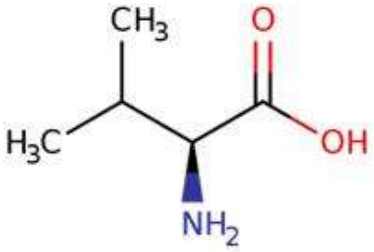
CYSTINE

Appearance	White dry powder
Molecular Formula	$C_6H_{12}N_2O_4S_2$
Molecular Weight	240.29g/mol
Solubility in water	190mg/L
Melting point	260
Boiling point	468.2^0C

Source: Sigma Aldrich, Germany

Purification: Used as purchased without further purification. The purity is >98%.

Application: L-Cysteine is an amino acid which is building block of protein.(Scheme 1) It is a powerful anti oxidant. It is also used to metabolize of lipid, boosting the immune system.L-cysteine increase malie fertility, reduce inflamination and combarts decrease osteoporosis. In the body, cysteine is also used to produce the amino acid taurine as well as coenzyme A, biotine and heparin. Cysteine is component in beta karatin and it is proved that it preserve skin elasticity. It also protects the lining of digestive system . (9)



Appearance	White crystals
Molecular formula	$C_5H_{11}NO_2$
Molecular weight	117.148 $gmol^{-1}$
Melting Point	298^0C
Solubility in water	Soluble
density	1.136g/L

L-valine:

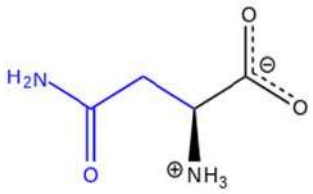
Source and purification:

L-valine was purchased from Sd. Fine Chemicals Limited, Mumbai, India. Its mass purity as supplied is 99%

Application

L-valine is a branched chain amino acid (as are L-isoleucine and L-leucine), which is important for supplying energy to muscles. The branched chain amino acids enhance energy, increase endurance and aid in muscle tissue recovery and repair. As a branched chain amino acid, L-valine is important for optimal growth in infants and children and nitrogen balance in adults. Branched chain preparations are used in sports nutrition and health foods.(10)

Asparagine:(2,4-Diamino-4-oxobutanoic acid) Asparagine is non –essential amino acid in humans,Asparagine is a beta amino derivatives of aspartic.

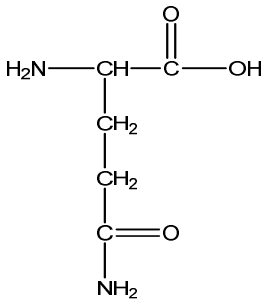


Asparagine

Appearance	White crystaline
Molecular formula	$C_4H_8N_2O_3$
Molecular weight	132.119 g/mol
Melting point	235^0C
Solubility	20g/L
density	$1.543g/cm^3$

Asparagine (Asn)is role in the biosynthesis of glycoproteins.It is essential synthesis of other protein Human nervous system also needed to this amino acid to be able to maintain an equllibrum.It required to developed and function the brain and it is alsoused to synthesis ammonia.(11)

Glutamine

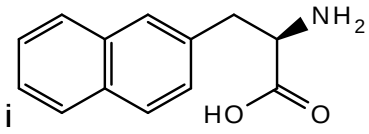


Molecular formula	$C_5H_{10}N_2O_3$
Molecular weight	146.146g/mol
Appearance	Colorless liqued
Density	$0.904g/cm^3$
Melting point	Decomposed around 185^0C

Application: Glutamine (glen) is a significant amino acid. L-Glutamine is found in animal foods, supplements and the human body. It is a part of the protein involved in the immune function of our body within the intestine [2]. Glutamine is an energy source for the gut and immune cells. It helps maintain a wall between the interior and the rest of our body. It develops properly with the intestinal cells of body.(11)

3-(2-Napthyl)-D-Alanine

The guest is amino acid derivative that has been used to synthesize cholecys to kinin analogues and as a carrier may be achieved nutritional analysis and disease diagnosis. It is used in artificial receptors for amino acid.(12)



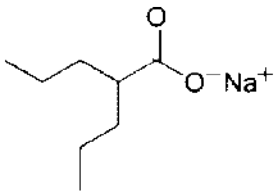
Appearance	Off White Solid
Molecular formula	$C_{13}H_{13}NO_2$
Molecular weight	215.25g/mol
Boling point	355.53^{0}C
Density	1.1242 g/mol

Source: Sigma Aldrich, Germany

Purification: Used as purchased without further purification. The purity is >98%.

III.1.4. DRUGS

Sodium Valproate



SV is an extremely hygroscopic solid and completely ionized to form highly active mode of administration[2]. Clinically high doses consideration for use that's why drug present high side effect known as black box warning for hepatotoxicity, pancreatitis and fetal abnormalities[3]. The search for lead to reduction.(13)

Molecular formula	$C_8H_{16}O_2$
Molecular weight	144.211g/mol
Appearance	Colorless liqued
Density	0.904g/cm^3
Boiling point	219.5 ^{0}C
Refractive Index	1.425

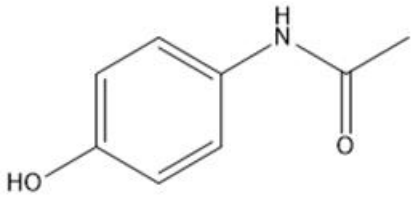
Source:Valporic acid sodium salt,of highly pure were purchased form Sigma –Aldrich .

Purification:The purities of sodium valporate, were-99.1%

Application:Sodium valproate (SV) is an anticonvulsant drug which is used in epilepsy and bipolar disorder[1]. It is also used for neuropathic pain and migraine prophylaxis.

Acetaminophen(N-acetyl paraamino phenol):

N-acetyl para aminophenol(paracetamol) is also known as acetaminophen. Paracetamol consists of a bengene ring core, one hydroxyl group and the nitrogen atom of an amide group in the *para* (1,4) position. It is an extensively conjugated system, as one lone pair on the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair, the p orbital on the carbonyl carbon, and one lone pair on the carbonyl oxygen are all conjugated. The presence of two activating groups also make the benzene ring highly reactive . (14)



Acetaminophen

Molecular formula	$C_8H_9NO_2$
Molecular weight	151.16 g.mol^{-1}
Boiling point	420^{0}C
Density	1.263g/cm^3
Melting point	169 ^{0}C
Solubility	7.21 g/kg at 0^{0}C

Source: Sigma Aldrich, Germany

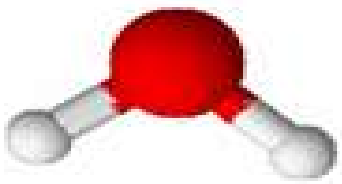
Purification: Used as purchased without further purification. The purity is >98%.

Application: Acetaminophen(APAP) is used a pain reliever and fever reducer. It is used to treatment of headache ,muscheaches,arthritis etc

III.1.5. SOLVENTS

Water (H_2O):

Water is an omnipresent chemical substance is composed of hydrogen and oxygen and is essential for all known forms of life. In typical usage, water refers only to its liquid form or state but the substance also exists as solid state, ice, and a gaseous state, water vapour or steam. Water is a good solvent and is often referred to as the universal solvent.



Appearance Appearance	**Colourless liquid**
Molecular Formula	**H_2O**
Molecular Weight	**18.02g/mol**
Dielectric Constant	**78.35**
Density	**0.99713g.cm**
Viscosity	0.891 mPs
Refractive Index	1.3333
Boiling Point	78.35 at298.15K

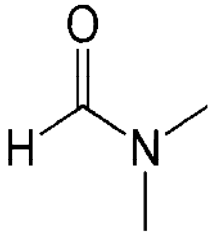
Source: Distilled water, distilled from fractional distillation method in Lab.

Purification: Water was first demonized and then distilled in an all glass distilling set along with alkaline $KMnO_4$ solution to remove any organic matter therein. The doubly distilled water was finally distilled using an all glass distilling set. Precautions were taken to prevent contamination from CO_2 and other impurities. The triply distilled water had specific conductance less than 1×10^{-6} $S \cdot cm^{-1}$.

Application: Water is commonly used in chemical reactions as a solvent or reactant, and less commonly as a solute or catalyst commonly In an inorganic reaction, water is a

common solvent, dissolving many ionic compounds. Supernatural water has recently become the subject of research. Oxygen saturated supercritical water efficiently suppresses organic pollutants. It is also used in various industries. It is a great solvent, generally accepted as a universal solvent, due to the marked polarity of water molecules and the tendency to form hydrogen bonds with other molecules. Life on earth depends entirely on water. Not only a high percentage of living things, both plants and animals are found in water, all life on earth is said to have originated from water, and the bodies of all organisms are essentially water. About 70 to 90 percent of all organic matter is water. The chemical reactions of all plants and organisms that support life occur through a water. Not only does water provide a means of producing a sustainable reaction to this life, but water itself is an important reactor or product of these reactions. In short, the chemistry of life is water chemistry.

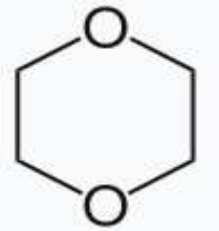
N,N-dimethylformamide (DMF)



Molecular formula	C_3H_7NO
Molecular weight	73.095 g·mol^{-1}
Appearance	Colourless liquid
Melting Point	−60.5 °C; −76.8 °F; 212.7 K
Solubility in water	Miscible
density	0.948 g mL^{-1}
Boiling point	152 to 154 °C; 305 to 309 °F; 425 to 427 K
Refractive Index	1.4305

Application : It is used in the production of acrylic fibers and plastics. It is also used as a solvent in peptide coupling for pharmaceuticals, in the development and production of pesticides, and in the manufacture of adhesives, synthetic leathers, fibers, films, and surface coatings.(15)

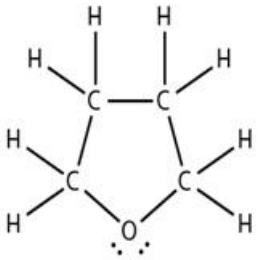
1,4-Dioxan(DO)



Appearance	White/Off White Powder
Molecular formula	$C_4H_8N_2O_2$
Molecular weight	116.12 g/mol
Melting Point	240 to 241 °C (464 to 466 °F; 513 to 514 K)
Solubility in water	Low
density	1.37 g/cm^3

Application: **is a trace contaminant of some chemicals used in cosmetics, detergents, and shampoos**.

Tetrehydrofuran (THF)

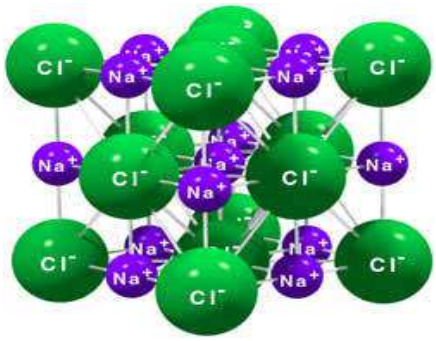


Appearance	C olorless liquid
Molecular formula	C_4H_8O
Molecular weight	72.107g/mol
Melting Point	−108.4 °C (−163.1 °F; 164.8 K)
Solubility in water	miscible
density	0.8892g/cm^3

Application: is used as a precursor to polymers. The other main application of THF is an industrial solvent for PVC and in vernishes.

III.1.6. ALKAYL HALIDE

Sodium chloride: Sodium chloride commonly known as sea salt ,chemical formula Nacl .It is ionic compound representing 1:1 Sodium and Chloride ions,it is also known as table salt.



Appearance	White crystal
Molecular formula	Nacl
Molecular weight	58.443g/mol
Melting point	800.7 ^{0}C
Boling point	1465 ^{0}C
Solubility	360g/L
Density	2.17g/cm^3

Source and purification NaCl purity as supplied is 99% of Sisco research laboratories pvt.ltd Mumbai, India. It was recrystallised twice from aqueous ethanol solution and dried under vacuum at T=348 K for 6 h. Thereafter, it was stored over P_2O_5 in a desiccator before use.

Application: It is commonly used as a Condiment and food preservatives ,large quantity use in many industrial processes. It is used major source of Sodium and chlorine compounds used feed stocks for further Chemical synthesis. Sodium chloride is de icing of road way in sub freezing weather.

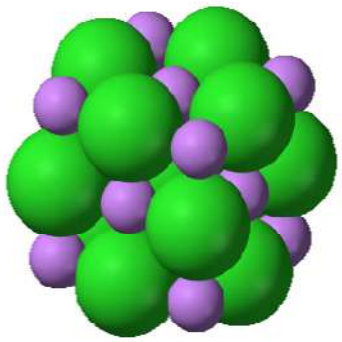
Potassium chloride: KCl is a metal halide Salt composed of Potassium and Chloride and white crystal appear. The solid readily dissolve in water.

Appearance	White crystalline solid
Molecular formula	KCl
Molecular weight	74.55g/mol
Melting point	770 ^{0}C
Boling point	1420 ^{0}C
Solubility	217.1g/L
Density	1.984g/cm^3

Source and purification KCl purity as supplied is 99% of Sisco research laboratories pvt.ltd Mumbai, India. It was recrystallised twice from aqueous ethanol solution and dried under vacuum at T=348 K for 6 h. Thereafter, it was stored over P_2O_5 in a desiccators before use.

Application: It is used as fertilizer ,medicine purpose ,in scientific application and also food processing .

Lithium chloride: LiCl is ionic compound .The small size of the Li+ ion gives rice to properties not aries the other alkali metal chloride,extra odinari soluble in polar solvent and its hygroscopic properties.



Molecular formula	LiCl
Molecular weight	42.39g/mol
Melting point	609 ^{0}C
Boling point	1382 ^{0}C
Solubility	842.5g/L
Density	2.068g/cm^3
Appearnce	White solid hygroscopic

Source and purification: LiCl, purity as supplied is 99% of Sisco research laboratories pvt.ltd Mumbai, India. It was recrystallised twice from aqueous ethanol solution and dried under vacuum at T=348 K for 6 h. Thereafter, it was stored over P_2O_5 in a desiccators before use.

Application

It is used to lithium extraction by electrolysis ,Licl is used as brazing flux for aluminum in automobile, used in organic synthesis. It is used in precipitated RNA from cellular extract, also used in hygrometer

III.2. EXPERIMENTAL METHODS

III.2. 1. PREPARATION OF SOLUTIONS

The uncertainty of molarity of different salt solutions was approximately ± 0.0003 $mol \cdot dm^{-3}$. The research works described in this thesis was generally carried out with binary or ternary solution systems taking water as primary solvent and host cyclodextrin molecules as co-solvent.

After attainment of thermal equilibrium, the required volumes of each solution were transferred in a different volumetric flask which was already cleaned and dried thoroughly. The mixed contents of the stoppered volumetric flask were shaken well before use in any experiment. Same procedure was followed in making different solvent mixtures in the entire research work. The physical properties of different pure and mixed solvents have been mentioned before in this chapter.

III.2. 2. PREPARATION OF SOLID INCLUSION COMPLEXES

Solid inclusion complexes are also prepared for some of the experimental works described in the thesis. For this purpose 1:1 molar ratio of the guest molecules (i.e., ionic liquids 1-butyl-1-methylpyrolidinium chloride, 1-butyl-3-methylimidazolium chloride and Trihexyltetradecylphosphonium chloride, drug molecules probenecid and chloroquine diphosphate) and cyclodextrin(α-CD and β-CD) were taken. After that the aqueous solution of the guest molecules were gradually added drop by drop to the aqueous solutions of the CD. The mixture was allowed to stir for 48 hrs at 50–55^{o}C and filtered at this hot condition. It was then cooled to 5^{o}C and kept for 24 hrs. The resulting suspension was filtered to get white polycrystalline powder, which was washed with ethanol and dried in air.

III.2.3. MASS MEASUREMENT

All the stock solutions are prepared by mass. The chemicals are weighed in digital electronic analytical balance (Mettler Toledo, AG 285, Switzerland).

The weighing pan with high precision of 0.0001g is placed inside a transparent enclosure. The doors do not allow any dust. So no air current in the room effect the balance's operation.

III.2.4. SURFACE TENSION

Surface tension of a series of solutions required for experiment was measured by platinum ring detachment method using a Tensiometer (K9, KRŰSS; Germany).

The precision of the measurement was within ±0.1 $mN \cdot m^{-1}$. Temperature during various mexperiments is controlled by circulating auto-thermostated water (within ± 0.01K) through a double-wall glass vessel containing the solution.

III.2.5. CONDUCTIVITY MEASUREMENT

Systronics Conductivity TDS meter-308 is used for measuring specific Conductivity of various solutions. It can provide together regular and manual temperature compensation.

The conductivity measurements were done on this conductivity bridge using a dip-type immersion conductivity cell of cell constant 1.11cm^{-1}. The conductance data are taken at 1 KHz and was found to be ±0.3 % precise. The instrument was standardized using 0.1(M) KCl solution. Calibration of the cell was done by the method of Lind and co-workers.[16] A 500 cm^3 conical flask closed by a ground glass fitted with a side arm was taken and the above mentioned conductivity cell was attached with the side of it. Dry and pure nitrogen gas was passed through it in order to prevent insertion of air into the cell when solvent or solution was added. The experiments and measurements were done in a thermostatic water bath maintained at the required temperature with an accuracy of ± 0.01 K with the help of mercury in glass thermo regulator.[17]

Several solutions were prepared by weight with precision of ± 0.02 %.

III.2.6. MAGNETIC STIRRER FOR PREPARATION OF SOLUTION AND SOLID INCLUSION COMPLEXES

The different solutions of guest molecules and cyclodextrins have been prepared on magnetic stirrer.

III.2.7. DENSITY MEASUREMENT

The density of different solutions were measured by Anton Paar density-meter (DMA 4500M) with an accuracy of 0.0005 g.cm^{-3}.

A U-shaped tube mechanically oscillates in this density meter and electromagnetically it is transformed into an alternate voltage of same frequency. The period τ can be measured with high resolution. The relationship between density ρ and period can be expressed as

$$\rho = A \cdot \tau^2 - B$$

where A and B indicate instrument constants of each oscillator respectively. The values of A and B can be calculated by calibration of two substances of the precisely known

densities ρ_1 and ρ_2. In modern instruments the calculation of this constants are usually done air and water. They employ suitable measures to compensate various influences on the measuring result, e.g.

III.2.8. VISCOSITY MEASUREMENT

The viscosities (η) of the solutions were measured with a Brookfield DV-III Ultra Programmable Rheometer having spindle size-42. Viscosity can be calculated by the following equation

Calibration of the above mentioned rheometer was done standard viscosity samples supplied with the instrument, water and aqueous $CaCl_2$ solutions. The temperature was maintained to within ± 0.01°C using Brookfield Digital TC-500 thermostat bath. The accuracy of the instrument was around ± 1 %.

III.2.9. TEMPERATURE CONTROLLER

The material used in the experiment is placed in a vessel and it is placed in the water bath.

III.2.10. WATER DISTILLER (BOROSIL GLASS WORKS LIMITED, INDIA):

Ordinary water is inserted in the distiller unit's boiling chamber. A heating system in the boiling chamber heats the water until it boils. The steam rises from the boiling chamber. The impurities that are volatile are discarded through a built-in vent. Non volatile components such as Minerals and salts are present in the boiling chamber as hard deposits or scale. The steam passes through a coiled tube (condenser), which is kept cool by cold water. Water droplets appear as condensation occurs. The distilled water is collected in a storage vessel.

III.2.11. REFRACTIVE INDEX MEASUREMENT

Refractive index for various experiments was measured with the help of Digital Refractometer (Mettler Toledo 30GS).

We calibrate the above mentioned machine by various standard solvents namely double distilled water, toluene, cyclohexane, and carbon tetrachloride at room temperature (accuracy +/- 0.0005). Few drops of the aqueous solution of various samples are added in the cell and reading was taken. Refractive index of sample is a function of temperature. System determines the temperature during experiment and accurate the refractive index to a temperature as preferred by the user.

III.2.12. UV-VIS SPECTRA MEASUREMENT

Compounds that have chromophores and auxochromes show absorbance in the ultra-violet and visible regeion. Characteristic peaks as a function of wavelength appears for this compounds.

The light source used in the UV-VIS spectrophotometer are of two types- a deuterium (D2) lamp for ultraviolet light and a tungsten (W) lamp for visible light. The light beam bounces in a mirror and then passes through a slit collides with a diffraction grating. The diffraction grating can have rotation to allow a selected specific wave length. Only monochromatic (single wavelength) are able to pass the silt for any orientation of the grating. Unwanted higher orders of diffraction are removed by filtration. Next the light beam collides a second mirror before it gets split by a half mirror (half of the light is reflected, the other half passes through). One of the beam passes through a reference cuvette containing the solvent, the other is passed through the sample cuvette. The intensities of the light beams are then measured at the end. The Beer-Lambert law has been mentioned below in this connection.

Beer-Lambert Law

The change in intensity of light (*dI*) after passing through a sample proportionates to the following:

(i) Path length (*b*), the iarger the path, more number of photons should be absorbed

(ii) Concentration (*c*) of sample, more molecules absorbing means more photons Absorbed

(iii) Intensity of the incident light.

$-\ln I/I0 = kbc$

$-\log I/I0 = 2.303kbc$

$\varepsilon = 2.303k$

$A = -\log I/I0$

$A = \varepsilon bc$

A is reffered as absorbance and it is found to be directly proportional to the path length, *b* and the concentration of the sample, *c*. The extinction coefficient is characteristic of the substance under study and of course is a function of the wavelength.

III.2.13. FT-IR MEASUREMENT

Infrared spectra were taken in 8300 FT-IR spectrometer (Shimadzu, Japan).

Resolution of the system is ± 0.25 cm^{-1}. The region of absorption is 400-4000 cm^{-1} at room temperature (25 ^{0}C) with a humidity level of 49-54 %. The instrument is able to record data in various ways such as KBr pellets, Nujol mull, and non-aqueous solutions.

At first light passes through a blank sample and the intensity (I_0) of it is measured. The intensity is proportional to the number of photons passing per second. (Actually, instrument measures the power rather than the intensity of the light. The power is defined as the energy per second, which is the product of the intensity (photons per second) and the energy per photon. The experimental data is used to evaluate two quantities: the transmittance (T) and the absorbance (A).

$$T = \frac{I}{I_0} \quad , \quad A = -\log_{10} T$$

The transmittance can be defined as the fraction of light in the original beam that passes through the sample and reaches the detector.