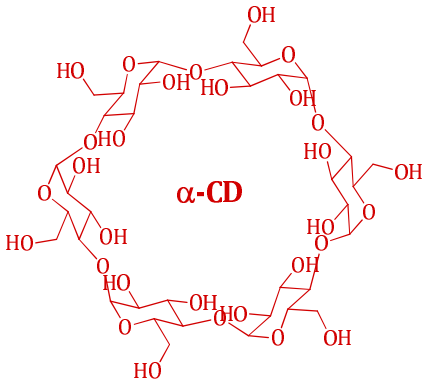
CHAPTER III

EXPERIMENTAL SECTION

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

III.1.1: α-Cyclodextrin:

α-Cyclodextrin, a cyclic oligosachharide, is well known in supramolecular chemistry as molecular host.[1] 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.



Source: Sigma Aldrich, Germany.

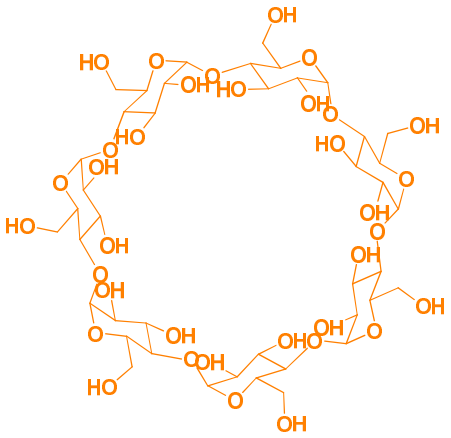
Purification: Used as purchased. The purity of the chemical is 98.0%.

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., 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: 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.



Source: Sigma Aldrich, Germany.

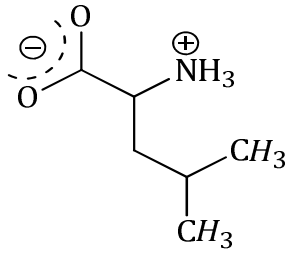
Purification: Used as purchased. The purity of the chemical is 98.0%.

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., arginine, histidine, lysine, phenyl alanine, glutamic acid ionic liquids e.g., 1-butyl-4-methylpyridinium iodide, RNA nucleosides etc. as guest molecules.

III.1.3: L- Leucine:

L-Leucine is an essential amino acid. Its appearance is white powder. It is soluble in water. It consists of one α-amino group (which is in the protonated –NH3+ form under biological conditions), an α-carboxylic acid group(which is in the deprotonated –COO- form under biological conditions) and an isobutyl side chain, classifying it as a non polar amino acid. an. Molecular formula of this acid is $C_6H_{13}NO_2$. It is essential in humans-meaning the body cannot synthesize it. It must be obtained from the diet.



L-Leucine

Source: Sigma Aldrich, Germany.

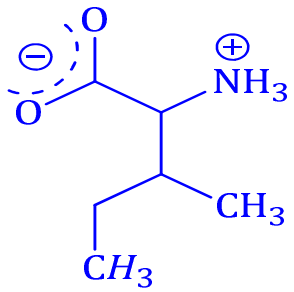
Purification: Used as purchased. The purity of the chemical is 98.0%.

CAS Number	61-90-5
Chemical formula	$C_6H_{13}NO_2$
Molar mass	131.17
Appearance	White Powder
Melting point	293°C
Solubility in water	21.5g/L
pKa	2.35

Application: L-Leucine is helps in the biosynthesis of various important proteins and therefore considered as essential in humans, *which means* our body is not able to produce it and thus it have to be integrated from some other source, this may be possible using α and β-Cyclodextrins as carriers. It helps to form a part of the subunits in ferritin, astacin etc. proteins. L-Leucine is also activates mTOR; the later can be explained as the only nutritional amino acid that has the capability to immediately kindle muscle protein synthesis. It is also present in the liver, adipose tissue and mussle tissue.[4] Adipose and mussle tissue uses L-Leucine in the creation of sterols. This amino acid is quickly reached in the brain and the astrocytes present there translate it to alpha-ketoisocaproate through the path of transmission of alpha-ketoglutarate to glutamate.

III.1.4: L-Isoleucine:

L-isoleucine is an essential amino acid. Its appearance is white powder. It is soluble in water. It contains an α-amino group (which is in the protonated $-NH_3+$ form under biological conditions), an α-carboxylic acid group (which is in the deprotonated COO form under biological conditions), and a hydrocarbon side chain, classifying it as a non-polar, uncharged (at physiological pH), aliphatic amino acid. It is essential in humans, meaning the body cannot synthesize it, and must be ingested in our diet. Isoleucine is synthesized from pyruvate employing leucine biosynthesis enzymes in other organisms such as bacteria.Molecular formula of this acid is $C_6H_{13}NO_2$.



Source: Sigma Aldrich, Germany.

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

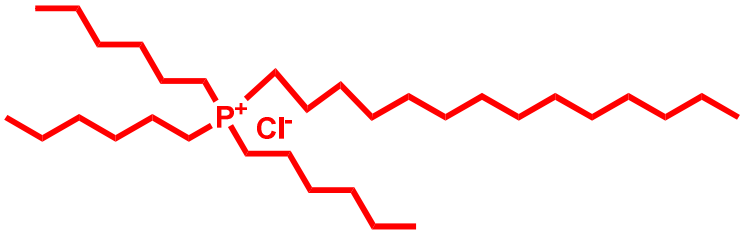
CAS Number	73-32-5
Chemical formula	$C_6H_{13}NO_2$
Molar mass	131.17
Appearance	White Powder
Melting point	285.5°C
Solubility in water	34.4g/L
pKa	2.37

Application: L-Isoleucine helps in biosynthesis of proteins in human and considered as essential in humans. L-Isoleucine is prepared using pyruvate utilizing leucine biosynthesis enzymes in various microorganisms such as bacteria.[5] Isoleucine is known to be a glucogenic and a ketogenic amino acid. The process of trans amination with alpha-ketoglutarate, the carbon skeleton has a tendency to convert into either succinyl CoA, and become a part of the TCA cycle for oxidation or can be transformed to oxaloacetate and used in gluconeogenesis. The same process can be done by transforming into acetyl CoA and enter into the TCA cycle by condensation with oxaloacetate forming citrate. For mammals Acetyl CoA is unable to transform again to carbohydrate but helps in the synthesis of ketone bodies or fatty acids, and that's why considered as ketogenic. Biotin, commonly known as vitamin B7 or vitamin H, is a necessary obligation for the complete catabolism of isoleucine (also for leucine). Without sufficient biotin, the humans are not able to fully break down isoleucine molecules.

III.1.5. Trihexyltetradecylphosphonium chloride:

It is a phosphonium based ionic liquid. Trihexyltetradecylphosphonium chloride is liquid at room temperature and appears as colourless. It is dense and freely soluble in water. It contains four long hydrocarbon chains among them three are hexyl chain and other is tetradecyl chain which helps it to incorporate in the

hydrophobic cavity of cyclodextrin.[6] Molecular formula of this ionic liquid is $C_{32}H_{68}ClP$.



Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

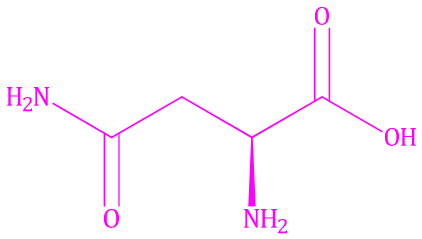
CAS Number	258864-54-9
Chemical formula	$C_{32}H_{68}ClP$
Molar mass	519.32
Appearance	Colourless Liquid
Melting point	-50°C
Solubility in water	Soluble

Application: Ionic liquids are generally constituted with a large organic cataion and a small anion. They have vast applications in various chemical industries because of their green nature. They produce less hazardous compounds during their use.Phosphonium based ionic liquids are less toxic and more thermally stable than nitrogen based ionic liquids. This ionic liquid is highly used in separation of different dyes including methylene blue from aqueous media. This has also application as additives to improve the yield of essential oils in the hydrodistillation process.[7]

III.1.6: Asparagine:

Asparagine or 2-amino-3-carbamoylpropanoic acid is a natural amino acid. Its appearance is white powder. It is soluble in water. It contains an α-amino group (which is in the protonated NH_3+ form under biological conditions), an α-

carboxylic acid group (which is in the deprotonated COO^- form under biological conditions), and a side chain carboxamide, classifying it as a polar (at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it. Molecular formula of this amino acid is $C_4H_7N_2O_3$.



Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

CAS Number	70-47-3
Chemical formula	$C_4H_8N_2O_3$
Molar mass	132.11
Appearance	White Powder
Melting point	234-235°C
Solubility in water	29.4 g/L
pKa	2.02

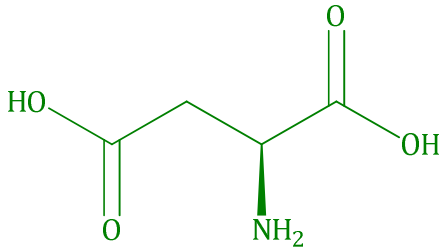
Application: It helps in biosynthesis of proteins in humans. L-Asparagine is also necessary for the improvement of brain and has a vital role in the preparation of ammonia. Usually the reaction between asparagine and some reducing carbohydrates or other compounds using carbonyls fabricates acrylamide in food after heating to optimum temperature. The products thus formed are present in baked goods such as French fries, potato chips, and toasted bread.

The asparagine amino acids form long chains by the hydrogen bond interactions with the peptide backbone, this amino acid residues are commonly found at the starting of alpha-helices as asx turns and asx motifs, and in similar turn motifs, or as amide rings, in beta sheets. Its main function is to cap the hydrogen bond

communications that can be also fulfilled by the polypeptide backbone. The Glutamines having an extra methylene group have higher entropy due to conformation and hence they are less capable for capping. Asparagine also helpful providing free sites for N-linked glycosylation, amendment of the protein chain with the accumulation of carbohydrate chains. Naturally, a carbohydrate side chain can exclusively be summed up to an asparagine residue if it is edged on the C side by X-serine or X-threonine, here X is any amino acid with the exception of proline.[8]

III.1.7: Aspartic Acid:

L-Aspartic acid or 2-aminobutanedioic acid is a natural amino acid. Its appearance is white powder. It is soluble in water. It is a α-amino acid that is used in the biosynthesis of proteins. Similar to all other amino acids it contains an amino group and a carboxylic acid. Its α-amino group is in the protonated $-NH_3^+$ form under physiological conditions, while its α-carboxylic acid group is deprotonated COO under physiological conditions. Aspartic acid has an acidic side chain (CH_2COOH) which reacts with other amino acids, enzymes and proteins in the body. Under physiological conditions (pH 7.4) in proteins the side chain usually occurs as the negatively charged aspartate form, COO .It is a non-essential amino acid in humans, meaning the body can synthesize it as needed. Molecular formula of this amino acid is $C_4H_6NO_4$.



Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

CAS Number	56-84-8
Chemical formula	$C_4H_7NO_4$
Molar mass	133.10

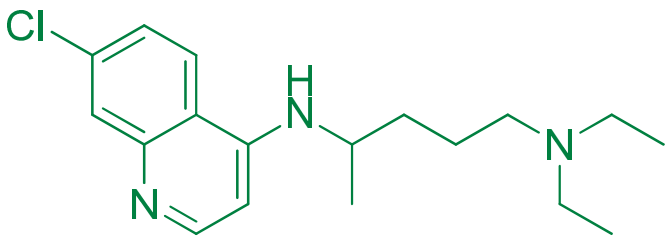
Appearance	White Powder
Melting point	270°C
Solubility in water	5.3g/L
pKa	2.01

Application: It helps in biosynthesis of proteins. L-Aspartic acid is the precursor of many essential amino acids and plays a vital role in gluconeogenesis process in animals.[9] aspartate is usually prepared in humans all the way through the transamination process of oxaloacetate. The biological synthesis of aspartate is favoured by an aminotransferase enzyme which can be explained as the transport of an amine group from another molecule namely alanine or glutamine forming aspartate and an alpha-keto acid. Aspartate is again produced in the urea cycle.

Aspartate is the precursor of many amino acids, for plants and bacterias. It also synthesis four amino acids that are essential for humans: methionine, threonine, isoleucine, and lysine. Before conversion of aspartate to other amino acids there is reduction of it to "semialdehyde," having formula $O_2CCH(NH_2)CH_2CHO$. Besides those this amino acid has many other biochemical roles. It acts as a metabolite in the urea cycle and plays important role in gluconeogenesis. It carries reducing equivalents in the malate-aspartate shuttle, which utilizes the ready interconversion of aspartate and oxaloacetate, which is the oxidized (dehydrogenated) derivative of malic acid. Aspartate donates one nitrogen atom in the biosynthesis of inosine, the precursor to the purine bases. In addition, aspartic acid acts as hydrogen acceptor in a chain of ATP synthase.

III.1.8. chloroquine diphosphate:

Chloroquine diphosphate is a medicine that is primarily used to prevent and treat malaria. It is freely soluble in water. It appears as white powder. It is a member of drug class 4-aminoquinoline. It comprises of quinoline moiety. The molecular formula of this drug is $C_{18}H_{32}ClN_3O_8P_2$.



Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

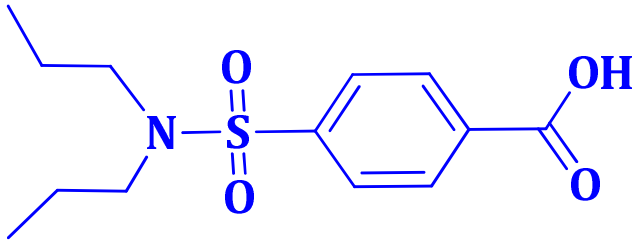
CAS Number	50-63-5
Chemical formula	$C_{18}H_{32}ClN_3O_8P_2$
Molar mass	515.86
Appearance	White Powder
Melting point	87°C
Solubility in water	0.14mg/L
pKa	10.1

Application: Chloroquine diphosphate is mostly used to treat malaria. Many categories of complex cases of malaria characteristically need some special medication.[10] This drug is frequently used for the treatment of amebiasis, rehumatoid artharitis and lupus erythematosus. Women can safely take this during pregnancy time. It is useful to prevent the asexual type of malaria within the red blood cell. Chloroquine is widely used in along the field of medicines, this can be supplied to the materialization and extend of resistance. There is still recommendation to verify whether chloroquine is helpful in the province prior to using it. However in areas where conflict is present, additional antimalarials, as for example mefloquine or atovaquone, can be used . The Centers for Disease Control and Prevention suggest in the healing of malaria with chloroquine alone owing to higher efficient combinations. In order to cure amoebic liver abscess, this drug may be used with other efficient medications in case of failure of development with metronidazole or another nitroimidazole with a time limit of 5

days or bigotry to metronidazole or a nitroimidazole. This drug have the capability to mildly restrain the immune system, it has use in some autoimmune disorders, namely rheumatoid arthritis and lupus erythematosus.[11]

III.1.9: Probenecid:

The chemical name of probenecid is 4-(dipropylsulfamoyl) benzoic acid. It is a drug which helps to lower uric acid level in human. It is insoluble in water but freely soluble in basic solution. It appears as white powder. Probenecid comprises of an aromatic moiety, carboxyl acid group, sulpher and nitrogen atoms. The molecular formula of this drug is $C_{13}H_{19}O_4SN$.



Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

CAS Number	57-66-9
Chemical formula	$C_{13}H_{19}NO_4S$
Molar mass	285.36
Appearance	White Powder
Melting point	381-385°C
Solubility in water	27.1mg/L
pKa	3.4

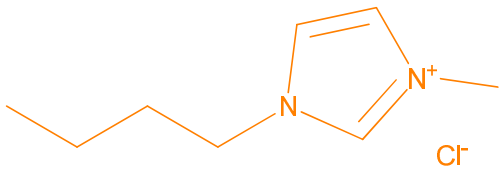
Application: Probenecid is a vital drug which is particularly used to prevent gout and hyperurecemia. Besides this the drug is also helpful to restrain renal secretion of some other drugs, resulting an increase in the concentration of plasma and extending their functions. Probenecid is often employd to enhance

the attentiveness of some antibiotics and also defend the kidneys by giving with cidofovir. Particularly, a few evidences support the employ of intravenous cefazolin only one time inspite of using it three times a day in combined with probenecid. The other function of probenecid is masking agent, particularly assisting athletes by performing as enhancing substances to shun recognition by drug tests.

Probenecid perhaps has various pharmacological functions, such as blocking pannexins. Probenecid is very efficient in the treatment of gout and the method of action is considerd to be centered on the kidney. Probenecid has the main action on kidneys' organic anion transporter (OAT),that regains uric acid from the urine and precedes it to the plasma. If this drug is present, the OAT is attached particularly to it (without binding to uric acid), it prevent the reaccumulation of the uric acid. Consequently, the urine contains more uric acid, decreasing uric acid concentration in the cell fluid.

III.1.10: 1-butyl-3-methylimidazolium chloride:

It is an ionic liquid. 1-butyl-3-methylimidazolium chloride is liquid at room temperature and appears as colourless. It contains an imidazolium ring and butyl group as side chain and a methyl group at 1and 3 positions respectively of the imidazolium ring. Molecular formula of this ionic liquid is $C_7H_{15}N_2Cl$.



Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

CAS Number	79917-90-1
Chemical formula	$C_8H_{15}ClN_2$

Molar mass	174.67
Appearance	Colourless Liquid
Melting point	70°C
Solubility in water	Soluble

Application: This ionic liquid has vast applications in chemical reactions, synthesis, cellulose processing, nuclear fuel reprocessing, waste recycling, metal air batteries etc. They are considered as green solvents as they do not produce any environmental hazards.[13] Because of its distinctive properties they are attracting increasing attention in many fields such as organic chemistry, electrochemistry, catalysis, physical chemistry and applied supramolecular chemistry.

III.1.11: 1-butyl-1-methylpyrolidinium chloride:

It is an ionic liquid. 1-butyl-1-methylpyrolidinium chloride is liquid at room temperature and appears as colourless. It contains a pyrolidinium group and butyl group as side chain and a methyl group at 1 position of the pyrolidinium ring. Molecular formula of this ionic liquid is $C_9H_{20}ClN$.

Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity of the chemical is 99.0%.

CAS Number	479500-35-1
Chemical formula	$C_9H_{20}ClN$
Molar mass	177.71
Appearance	Molten powder

Solubility in water	Soluble
pH	8

Application: The ionic liquid are good examples of neoteric solvents, new types of solvents, or older materials that are finding new applications as solvents, which is environmentally friendly (or eco-friendly) because they are less hazardous for human body as well as less toxic for living organisms, 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 (batteries and solar cells)[14].

III.2. EXPERIMENTAL METHODS

III. 2. 1. Preparation of Solutions

The stock solutions for different solid compounds were prepared by mass. The uncertainty of molarity of different salt solutions was approximately ± 0.0003 mol·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. The guest ionic liquid molecules, amino acid molecules and drug molecules were dissolved in the above mentioned solvent systems.

Pure solid compounds were taken in glass stoppered bottles and thermostated at the experimental temperature to prepare solution mixtures. 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. Conversion of required mass of the respective solvents to volume was accomplished by using experimental densities of the solvents at experimental temperature. 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. For each solid inclusion complex 1.0 millimole guest molecules and 1.0 millimole CD were dissolved in 20 mL water separately and stirred for 4 hours. 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).

Measurement using this device has high accuracy and precision. 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 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 both automatic 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.[15] 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.[16]

Several solutions were prepared by weight with precision of ± 0.02 %. The weights were taken on a Mettler electronic analytical balance (AG 285, Switzerland). The molarity is converted to molality with requirements. Due correction was made for the specific conductance of the solvents at desired temperatures.

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. The solid inclusion complexes have also been prepared on the magnetic stirrer cum hot plate made by IKA.

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

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

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. the influence of the sample's viscosity and the non-linearity caused by the measuring instrument's finite mass. The instrument was calibrated by double-distilled water and dry air.

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

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

Here *RPM*, *TK* (0.09373) and *SMC* (0.327) represent the speed, viscometer torque constant and spindle multiplier constant, respectively. 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 %. Each measurement reported herein is an average of triplicate reading with a precision of 0.3 %.

III.2.9. TEMPERATURE CONTROLLER

For this research work all the experiments were done with in thermostatic water bath (Science India, Kolkata) at the required temperature(accuracy ± 0.01 K).


The material used in the experiment is placed in a vessel and it is placed in the water bath. These instruments are available in different shapes and volumes. Both digital and analogues controls are used. The chambers of water bath lab products are manufactured using rugged, leak proof and highly resistant stainless steel and other lab supplies.

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 (*dl*) 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. Thus, *dI* is proportional to *bcI* or $dI/I = - kbc$ (where *k* is a proportionality constant, the negative sign indicates that there is a decrease in intensity of the light, this makes *b*, *c* and *I* always positive. Integration of the above equation leads to Beer-Lambert's law:

$- \ln I/I0 = kbc$

$- \log I/I0 = 2.303kbc$

$\varepsilon = 2.303k$

$A = - \log I/I0$

$A = \varepsilon bc$

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. The blank sample contains the solution that does not absorb light. The intensity of light (I) passing through the sample solution is measured. (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.

III. 2. 14. Solution pH Measurement

The pH values of the amino acid solutions are measured with Mettler Toledo Seven Multi pH meter having uncertainty 0.009. All the experiments were done in a thermostated water bath maintaining the required temperature.

SevenMulti™ S47 - dual meter pH / conductivity

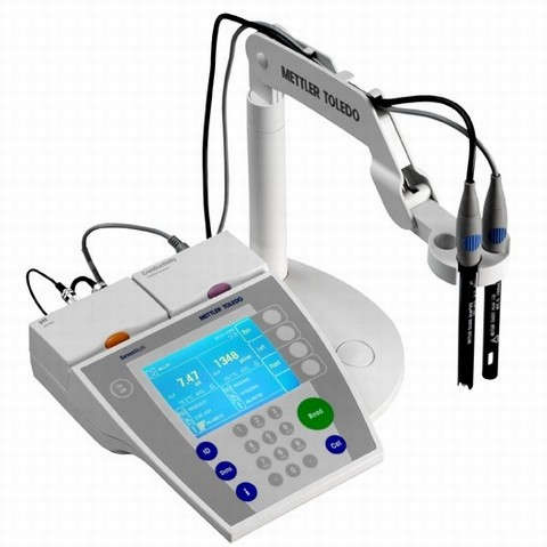
Reproducible results

Automatic, manual or timed endpoint formats with 3 selectable stability criteria allow rapid and accurate measurement value determinations with reproducible results.

- Linear & non-linear temperature correction
- Selectable reference temperature (20°C or 25°C)
- Procedure for automatic α -coefficient determination

Professional calibration

- User-definable buffers and standards including their temperature dependence
- Up to 5 calibration points with linear or segmented algorithms
- Multipoint conductivity calibration
- Automatic buffer recognition within the 8 predefined pH buffer groups
- Automatic standard recognition of the 5 predefined conductivity standards
- Entry and display of cell constant



Electrode test

- An integrated pH electrode test checks the slope, offset, drift and response time of your electrodes without changing your current calibration.
- Complying with USP/EP standards
- SevenMulti™ provides a special mode for measuring conductivity according to USP and EP (United States / European Pharmacopeia) methods.

III. 2. 15. ^{1}H NMR and 2D ROESY Spectroscopic Measurement

2D ROESY and ^{1}H NMR spectra were recorded in D_2O at 500 MHz, 400 MHz and 300 MHz in Bruker Avance 500 MHz, Bruker Avance 400 MHz and Bruker Avance 300 MHz instrument respectively at 298 K. Signals are cited as δ

values in ppm using residual protonated solvent signal as internal standard (HDO: δ 4.79 ppm). Data are presented as chemical shift.

The spectrometers use nuclear magnetic resonance to provide information on the molecular and structural dynamics of biological, organic and inorganic substances in solid and liquid phases. Configured for high resolution NMR experiments, it will be invaluable to chemists (for structural elucidation and kinetics), molecular biologists (protein structure) and material scientists (solid state NMR).

500 MHz Avance III HD NMR Spectrometer The 500 MHz NMR Spectrometer's specifications include an 11.746 Telsa Ascend superconducting 54 mm bore magnet system; 3-Channel Magic Angle Spinning(MAS) probe for biomolecular studies; Software licensed for data acquisition, processing, management and mailing; and software for DOSY experiments.

The spectrometer is equipped with a BBFO and a BBI probes optimized for broadband and flourine observation and 1H observation or decoupling. It includes a 3 channel MAS system with triple resonance probe system for X-Y correlation spectroscopy (useful for protein characterization), cryogenic probe and a sample automation system for up to 24 samples. The NanoBay incorporates the highly sophisticated AVANCE III HD electronics, which delivers

unprecedented RF switching speed and flexibility, making it ideal for both simple and highly advanced NMR experiments.

III. 2. 16. High Resolution Mass Spectrometry Measurement

The high performance high resolution MS/MS system designed for routine use.

Sensitivity to easily detect maximum residue levels.

Resolving power to remove interference from complex matrices.

Linearity to quantify over up to 4 orders of magnitude.

Mass accuracy to identify compounds following regulatory guidelines.