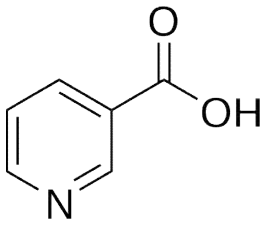
CHAPTER III

EXPERIMENTAL SECTION

III.1. NAME, STRUCTURE, PHYSICAL PROPERTIES AND APPLICATIONS OF THE BIOLOGICALLY ACTIVE MOLECULES, CYCLODEXTRINS, IONIC LIQUIDS, CROWN ETHERS AND SOLVENTS USED IN THE RESEARCH WORK

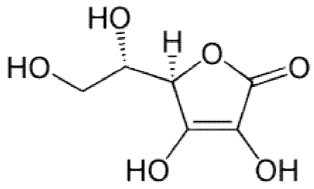
III.1.1. Biologically active molecules:

Nicotinic acid: Nicotinic acid is an organic compound and one of the essential human nutrients. Together with nicotinamide it makes up the group known as vitamin B3 complex. It belongs to the group of the pyridinecarboxylic acids.



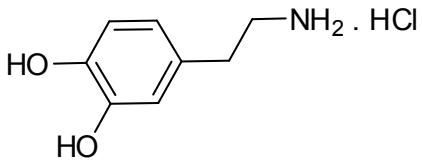
CAS Number	59-67-6
Chemical formula	$C_6NH_5O_2$
Molar mass	123.1094 g mol^{-1}
Appearance	White, translucent crystals
Melting point	510 K
Solubility in water	18 g L^{-1}
pKa	2.0, 4.85
Std enthalpy of formation	−344.9 kJ mol^{-1}
Routes of administration	Intramuscular, Oral
Biological half-life	20–45 min

Ascorbic acid: Ascorbic acid, known as vitamin C and L-ascorbic acid, is a vitamin found in food and used as a dietary supplement. As a supplement it is used to treat and prevent scurvy. It may be taken by mouth or by injection.



CAS Number	50-81-7
Chemical formula	$C_6H_8O_6$
Molar mass	176.12 g mol^{-1}
Appearance	White or light yellow solid
Melting point	463 K
Solubility in water	330 g L^{-1}
pKa	4.10, 11.6

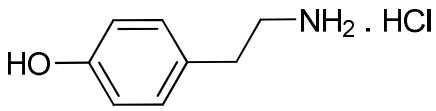
Dopamine hydrochloride: Dopamine is an organic chemical of the catecholamine and phenethylamine families that plays several important roles in the brain and body. It is an amine synthesized by removing a carboxyl group from a molecule of its precursor chemical L-DOPA, which is synthesized in the brain and kidneys. Dopamine is also synthesized in plants and most animals. In the brain, dopamine functions as a neurotransmitter - a chemical released by neurons to send signals to other nerve cells.[1]



CAS Number	62-31-7
Chemical formula	$C_8H_{11}NO_2 \cdot HCl$
Molar mass	189.639 g mol^{-1}
Appearance	Light tan powder

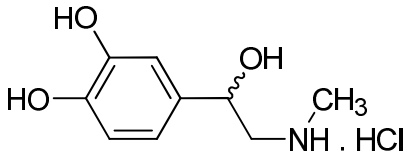
Melting point	521 K
Solubility in water	600 g L^{-1}

Tyramine hydrochloride: Tyramine is a naturally occurring trace amine derived from the amino acid tyrosine. Tyramine acts as a catecholamine releasing agent. Notably, it is unable to cross the blood-brain barrier, resulting in only non-psychoactive peripheral sympathomimetic effects following ingestion. A hypertensive crisis can result from ingestion of tyramine-rich foods in conjunction with monoamine oxidase inhibitors.[2]



CAS Number	60-19-5
Chemical formula	$C_8H_{11}NO \cdot HCl$
Molar mass	173.64 g mol^{-1}
Appearance	White crystalline powder
Melting point	526 K
Solubility in water	50 g L^{-1}

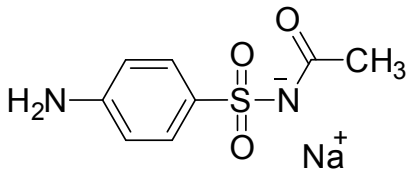
(±)-Epinephrine hydrochloride: (±)-Epinephrine is a hormone and neurotransmitter. It is normally produced by both the adrenal glands and certain neurons. It plays an important role in the fight-or-flight response by increasing blood flow to muscles, output of the heart, pupil dilation, and blood sugar.[3]



CAS Number	329-63-5
Chemical formula	$C_9H_{13}NO_3 \cdot HCl$
Molar mass	219.665 g mol^{-1}

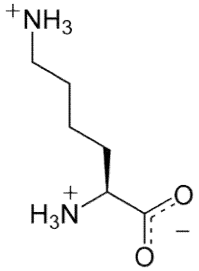
Appearance	White crystalline powder
Melting point	453 K
Solubility in water	50 g L^{-1}

Sulfacetamide sodium salt monohydrate: Sulfacetamide, also known as acetosufamine belongs to the class of organic compounds known as aminobenzenesulfonamides. These are organic compounds containing a benzenesulfonamide moiety with an amine group attached to the benzene ring.



CAS Number	6209-17-2
Chemical formula	$C_8H_9N_2NaO_3S \cdot H_2O$
Molar mass	254.24 g mol^{-1}
Appearance	White powder
Solubility in water	50 g L^{-1}

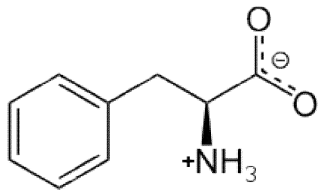
L-Lysine: L-Lysine is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group, an α-carboxylic acid group, and a side chain lysyl ($(CH_2)_4NH_2$), classifying it as a charged, aliphatic amino acid. It is essential in humans, meaning the body cannot synthesize it and thus it must be obtained from the diet.



CAS Number	56-87-1
Chemical formula	$C_6H_{14}N_2O_2$
Molar mass	146.19 g mol^{-1}

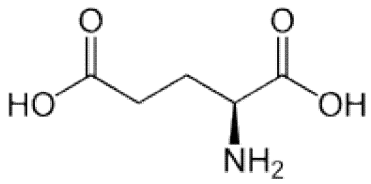
Appearance	White crystalline powder
Melting point	488 K
Solubility in water	1.5 kg L^{-1}
pKa	2.18, 8.95

L-Phenylalanine: L-Phenylalanine is an α-amino acid. It can be viewed as a benzyl group substituted for the methyl group of alanine, or a phenyl group in place of terminal hydrogen of alanine. This essential amino acid is classified as neutral and nonpolar because of the inert and hydrophobic nature of the benzyl side chain. Phenylalanine is a precursor for tyrosine; the monoamine neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline); and the skin pigment melanin.



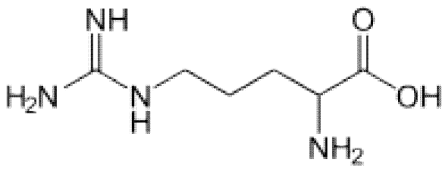
CAS Number	63-91-2
Chemical formula	$C_9H_{11}NO_2$
Molar mass	165.19 g mol^{-1}
Appearance	White crystalline powder
Melting point	548 K
Solubility in water	29.6 g L^{-1}
pKa	1.83, 9.13

L-Glutamic acid: L-Glutamic acid is an α-amino acid. It is usually abbreviated as Glu or E in biochemistry. Glutamic acid is used by almost all living beings in the biosynthesis of proteins.



CAS Number	56-86-0
Chemical formula	$C_5H_9NO_4$
Molar mass	147.13 g mol^{-1}
Appearance	White crystalline powder
Melting point	472 K
Solubility in water	7.5 g L^{-1}
pKa	2.10, 4.07, 9.47

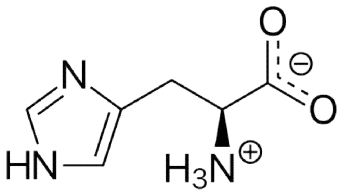
L-Arginine: L-Arginine is classified as a semi-essential or conditionally essential amino acid, depending on the developmental stage and health status of the individual. Preterm infants are unable to synthesize or create arginine internally, making the amino acid nutritionally essential for them. Most healthy people do not need to supplement with arginine because it is a component of all protein-containing foods and their body produces sufficient amounts.



CAS Number	74-79-3
Chemical formula	$C_6H_{14}N_4O_2$
Molar mass	174.20 g mol^{-1}
Appearance	White crystals
Melting point	533 K
Solubility in water	148.7g L^{-1}
pKa	12.488

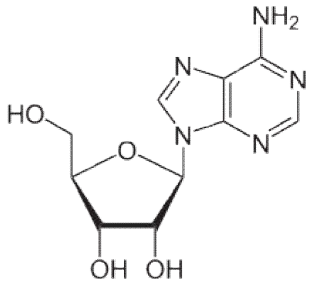
L-Histidine: L-Histidine is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group, a carboxylic acid group and an imidazole side chain, classifying it as a positively charged amino acid at physiological pH. Initially thought essential only for

infants, longer-term studies have shown it is essential for adults also. It is also a precursor to histamine, a vital inflammatory agent in immune responses.



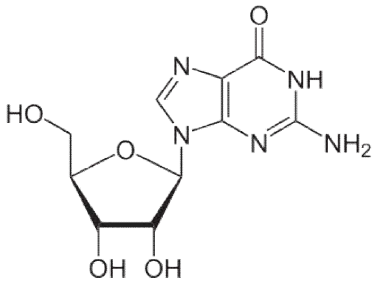
CAS Number	71-00-1
Chemical formula	$C_6H_9N_3O_2$
Molar mass	155.16 g mol^{-1}
Appearance	White crystals
Melting point	555 K
Solubility in water	41.9 g L^{-1}
pKa	6.0

Adenosine: Adenosine is a purine nucleoside composed of a molecule of adenine attached to a ribose sugar molecule moiety via a β-N_9-glycosidic bond. Adenosine is widely found in nature and plays an important role in biochemical processes, such as energy transfer—as adenosine triphosphate (ATP) and adenosine diphosphate (ADP)—as well as in signal transduction as cyclic adenosine monophosphate (cAMP). It is also a neuromodulator, believed to play a role in promoting sleep and suppressing arousal. Adenosine also plays a role in regulation of blood flow to various organs through vasodilation.[4]



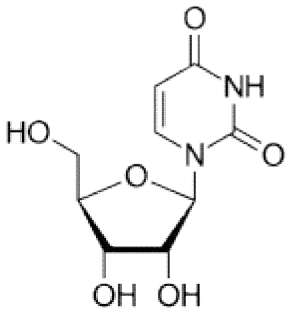
CAS Number	58-61-7
Chemical formula	$C_{10}H_{13}N_5O_4$
Molar mass	267.24 g mol^{-1}
Appearance	White powder
Solubility in water	7 g L^{-1}
Melting point	508 K
Routes of administration	Intravenous
pK_a	3.5, 12.5
pK_b	10.74

Guanosine: Guanosine is a nucleoside, which is of purine type. It is linked to a ribose residue by β-N_9-glycosidic linkage. Guanosine monophosphate, guanosine diphosphate and guanosine triphosphate can be found by phosphorylation of guanosine. These have very important biological functions.



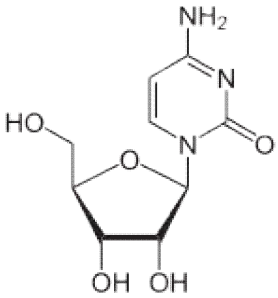
CAS Number	118-00-3
Chemical formula	$C_{10}H_{13}N_5O_5$
Molar mass	283.24 g mol^{-1}
Appearance	White powder
Solubility in water	0.7 g L^{-1}

Uridine: Uridine is a glycosylated pyrimidine-analog containing uracil linked to ribose residue by β-N_1-glycosidic linkage. It is one of the five standard nucleosides which make up nucleic acids. Uridine is found in RNA and not DNA.



CAS Number	58-96-8
Chemical formula	$C_9H_{12}N_2O_6$
Molar mass	244.20 g mol^{-1}
Appearance	White powder
Solubility in water	50 g L^{-1}
Melting point	440.3 K

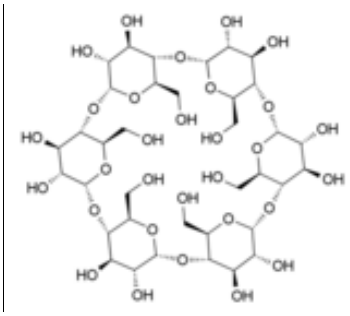
Cytidine: Cytidine is a nucleoside molecule that is formed when cytosine is linked to ribose residue by β-N_1-glycosidic linkage. Cytidine is a component of RNA. If cytosine is attached to a deoxyribose ring, it is known as a deoxycytidine.



CAS Number	65-46-3
Chemical formula	$C_9H_{13}N_3O_5$
Molar mass	243.22 g mol^{-1}
Appearance	White powder
Solubility in water	50 g L^{-1}
Melting point	489 K

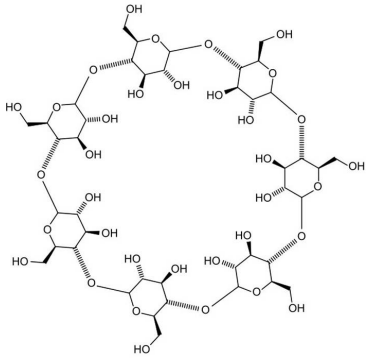
III.1.2. Cyclodextrins:

***α*-Cyclodextrin:** alpha-cyclodextrin is a polysaccharide of six glucose units that are covalently attached end to end via α-1,4 linkages.[5]



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}

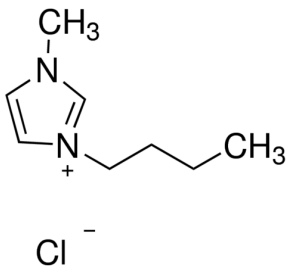
***β*-cyclodextrin:** beta-cyclodextrin is a polysaccharide of seven glucose units that are covalently attached end to end via α-1,4 linkages.[5]



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}

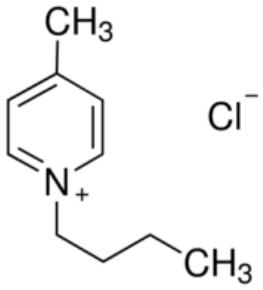
III.1.3. Ionic liquids:

1-butyl-3-methylimidazolium chloride [BMIm]Cl:



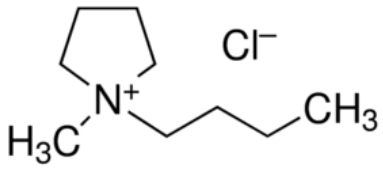
CAS Number	79917-90-1
Chemical formula	$C_8H_{15}ClN_2$
Molar mass	174.67 g mol^{-1}
Appearance	Colorless crystal
Melting point	343 K

1-butyl-4-methylpyridinium chloride [BMPy]Cl:



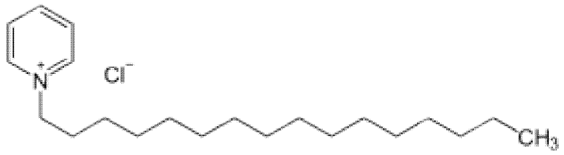
CAS Number	112400-86-9
Chemical formula	$C_{10}H_{16}ClN$
Molar mass	185.69 g mol^{-1}
Appearance	Colorless crystal
Melting point	431 K

1-butyl-1-methylpyrrolidinium chloride [BMP]Cl:



CAS Number	479500-35-1
Chemical formula	$C_9H_{20}ClN$
Molar mass	177.72 g mol^{-1}
Appearance	Colorless crystal

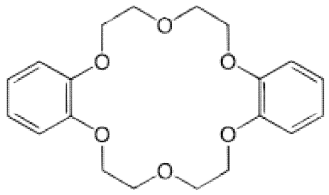
Cetylpyridinium chloride: Cetylpyridinium chloride is a cationic quaternary ammonium compound used in some types of mouthwashes, toothpastes, lozenges, throat sprays, breath sprays and nasal sprays. It is an antiseptic that kills bacteria and other microorganisms. It has been shown to be effective in preventing dental plaque and reducing gingivitis. It has also been used as an ingredient in certain pesticides.[6]



CAS Number	123-03-5
Chemical formula	$C_{21}H_{38}ClN$
Molar mass	339.99 g mol^{-1}
Appearance	White solid
Melting point	350 K

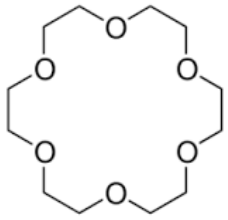
III.1.4. Crown ethers:

Dibenzo-18-crown-6: Dibenzo-18-crown-6 is a benzannulated crown ether. This compound may be synthesized from catechol and bis(chloroethyl) ether. This crown ether, like other crown ethers, has strong complexing abilities and has high affinity for alkali metal cations.



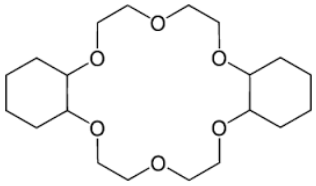
CAS Number	14187-32-7
Chemical formula	$C_{20}H_{24}O_6$
Molar mass	360.40 g mol^{-1}
Appearance	White
Melting point	435 K

18-crown-6: 18-Crown-6 is an organic compound. Like other crown ethers, 18-crown-6 functions as a ligand for some metal cations. The point group of 18-crown-6 is S_6. The dipole moment of 18-crown-6 varies in different solvent and under different temperature. The synthesis of the crown ethers led to the awarding of the Nobel Prize in Chemistry to Charles J. Pedersen.



CAS Number	17455-13-9
Chemical formula	$C_{12}H_{24}O_6$
Molar mass	264.315 g mol^{-1}
Appearance	white, hygroscopic crystalline solid
Melting point	310 K

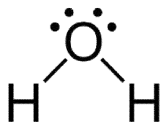
Dicyclohexano-18-crown-6:



CAS Number	16069-36-6
Chemical formula	$C_{20}H_{36}O_6$
Molar mass	372.50 g mol^{-1}
Appearance	white, hygroscopic crystalline solid
Melting point	320 K

III.1.5. Solvents:

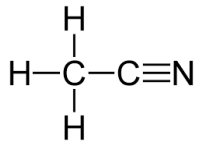
Water: Water is a polar inorganic compound that is at room temperature a tasteless and odorless liquid, nearly colorless with a hint of blue. This simplest hydrogen chalcogenide is by far the most studied chemical compound and is described as the "universal solvent" for its ability to dissolve many substances. This allows it to be the "solvent of life".



CAS Number	7732-18-5
Chemical formula	H_2O
Molar mass	18.015 g mol^{-1}
Appearance	Almost colorless, transparent, with a slight hint of blue liquid
Melting point	273.15 K
Boiling point	373.13 K
pKa	13.995

Density	0.9998396 g/mL at 0 °C 0.9970474 g/mL at 25 °C
Refractive index	1.3330 (20°C)
Viscosity	0.890 cP
Dipole moment	1.8546 D
Specific heat capacity	75.375 ± 0.05 J/mol K

Acetonitrile: Acetonitrile is a chemical compound. This colourless liquid is the simplest organic nitrile. It is produced mainly as a byproduct of acrylonitrile manufacture. It is used as a polar aprotic solvent in organic synthesis and in the purification of butadiene. In the laboratory, it is used as a medium-polarity solvent that is miscible with water and a range of organic solvents, but not saturated hydrocarbons. It has a convenient liquid range and a high dielectric constant of 38.8. It dissolves a wide range of ionic and nonpolar compounds.



CAS Number	75-05-8
Chemical formula	C_2H_3N
Molar mass	41.05 g mol^{-1}
Appearance	Colorless liquid
Melting point	227 to 229 K
Boiling point	354.4 to 355.2 K
pKa	25
Density	786 kg m^{-3}
Refractive index	1.344
Viscosity	0.3470 (298 K)
Dipole moment	3.92 D
Specific heat capacity	91.69 J/K mol
UV-vis (λ_{max})	195 nm

III.2. EXPERIMENTAL METHODS

III.2.1. Preparation of Solutions

A sock solution for each salt was prepared by mass, and the working solutions were obtained by mass dilution. The uncertainty of molarity of different salt solutions was evaluated to be ±0.0003 mol dm^{-3}.

The research works have been carried out with binary or ternary solvent systems with water or acrylonitrile as primary solvents and cyclodextrins or crown ethers as co-solvents. The biologically active solutes or ionic liquids have been dissolved in the above solvent systems.

For the preparation of solvent mixtures, pure components were taken separately in glass stoppered bottles and thermostated at the desired temperature for sufficient time. When the thermal equilibrium was ensured, the required volumes of each component were transferred in a different bottle 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. It was then stoppered and the mixed contents were shaken well before use. While preparing different solvent mixtures care was taken to ensure that the same procedure was adopted throughout the entire work. The physical properties of different pure and mixed solvents have been presented in the respective chapters.

III.2.2. Preparation of Solid Inclusion Complexes

The two solid inclusion complexes (SS + α-CD and SS + β-CD) have been prepared in 1:1 molar ratio of SS and CD. For each complex, 1.0 millimole SS and 1.0 millimole CD were dissolved in 20 mL water separately and stirred for 4 hours. Then the aqueous solution of SS was added drop wise to the aqueous solution of CD. The mixture was then stirred 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 12 hrs. The resulting suspension was filtered to get white polycrystalline powder, which was washed with ethanol and dried in air.

III.3. DESCRIPTION AND USE OF THE INSTRUMENTS INVOLVED IN THE RESEARCH WORK

III.3.1. Mass Measurement

Mass measurements were made on digital electronic analytical balance by Mettler Toledo, AG 285, Switzerland.

It can measure mass to a very high precision and accuracy. The weighing pan of a high precision (0.0001g) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation.

III.3.2. Water Distiller:

Water was distilled by an auto-connected distiller unit by Borosil Glass Works Limited, India.

A heating element in the boiling chamber heats the water until it boils. The steam rises from the boiling chamber. Volatile contaminants (gases) are discharged through a built-in vent. Minerals and salts are retained in the boiling chamber as hard deposits or scale. The steam enters a coiled tube (condenser), which is cooled by cool water. Water droplets form as condensation occurs. The distilled water is collected in a storage tank.

III.3.3. Magnetic Stirrer for Preparation of Solution and Solid Inclusion Complexes

The solutions of various biologically-active 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.3.4. Temperature Controller

All the measurements were carried out in digital thermostatic water bath maintained with an accuracy of ± 0.01 K of the desired temperature.

Digital thermostatic water bath is a system in which a glass vessel containing the material to be heated is placed into it containing water and quickly heat or cools it. This laboratory equipment controls temperature uniformity with high accuracy.

III.3.5. Density Measurement

The density was measured with the help of Anton Paar density-meter (DMA 4500M) with an accuracy of 0.0005 g cm^{-3}.

In the digital density meter, the mechanic oscillation of the U-tube is electromagnetically transformed into an alternating voltage of the same frequency.

Modern instruments employ suitable measures to compensate various influences on the measuring result, e.g., the influence of the sample's viscosity and the non-linearity. The instrument was calibrated by double-distilled water and dry air.

III.3.6. Viscosity Measurement

The solution viscosities (η) were measured using a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42. The viscosities were obtained using the following equation

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

where, *RPM*, *TK* (0.09373) and *SMC* (0.327) are the speed, viscometer torque constant and spindle multiplier constant respectively. The instrument was calibrated

against the standard viscosity samples supplied with the instrument, water and aqueous $CaCl_2$ solutions.

The temperature was maintained to within ±0.01K using Brookfield Digital TC-500 thermostat bath. The viscosities were measured with an accuracy of ±1%. Each measurement reported here is an average of triplicate reading with a precision of 0.3%.

III.3.7. Solution pH Measurement

pH values of the experimental solutions were measured by Mettler Toledo Seven Multi pH meter with uncertainty 0.009. The measurements were made in a thermostated water bath maintaining the required temperature.

Seven Multi dual pH meter has automatic, manual or timed endpoint formats with 3 selectable stability criteria which allows rapid and accurate measurement value

determinations with reproducible results. It is calibrated up to 5 calibration points by using supplied buffers. This instrument has automatic buffer recognition ability within the 8 predefined pH buffer groups. It has an integrated pH electrode that checks the slope, offset, drift and response time of electrodes without changing current calibration.

III.3.8. Ultrasonic Speed Measurement

The ultrasonic speed was measured with an accuracy of 0.2% using single-crystal variable-path ultrasonic interferometer (Model M-81 Mittal Enterprises, New Delhi) operating at 4 MHz which was calibrated with water, methanol and benzene at required temperature.

The principle used in the measurement of the ultrasonic speed (u) is based on the accurate determination of the wavelength (λ) in the medium. Ultrasonic waves of known frequency (f) are produced by a quartz crystal fixed at the bottom of the cell. These waves are reflected by a movable metallic plate kept parallel to the quartz crystal. If the separation between these two plates is exactly a whole multiple of the sound wavelength, standing waves are formed in the medium. This acoustic resonance gives rise to an electrical reaction on the generator driving the quartz crystal and the anode current of the generator becomes a maximum.

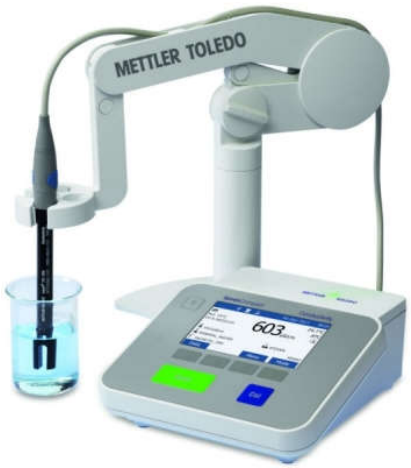
If the distance is now increased or decreased and the variation is exactly one half of wave length ($\lambda/2$) or integral multiples of it, anode current becomes maximum. From the knowledge of the wave length (λ), the speed (u) can be obtained by the relation.

$$\text{Ultrasonic speed } (u) = \text{Wave Length } (\lambda) \times \text{Frequency } (f)$$

The ultrasonic interferometer consists of the following two parts, (i) the high frequency generator, and (ii) the measuring cell. The measuring cell is connected to the output terminal of the high frequency generator through a shielded cable. The cell is filled with the experimental liquid before switching on the generator. The ultrasonic waves move normal from the quartz crystal till they are reflected back from the movable plate and the standing waves are formed in the liquid in between the reflector plate and the quartz crystal. The micrometer is slowly moved till the anode current on the meter on the high frequency generator shows a maximum. A number of maxima readings of anode current are passed and their number (n) is counted.

III.3.9. Conductivity Measurement

Conductivity of the solution was measured by Mettler Toledo Seven Multi dual conductivity meter with uncertainty ±0.001 $mS \cdot m^{-1}$.



It has automatic, manual or timed endpoint formats with 3 selectable stability criteria which allow rapid and accurate measurement value determinations with reproducible results. The instrument was standardized using 0.1 M KCl solution. This instrument has special features like linear & non-linear temperature correction, multipoint conductivity calibration, automatic standard recognition of the 5 predefined conductivity standards, entry and display of cell constant. Seven Multi provides a special mode for measuring conductivity according to USP and EP (United States / European Pharmacopeia) methods.

III.3.10. Refractive Index Measurement

Refractive index was be measure with the help of Digital Refractometer (Mettler Toledo 30GS).

Calibration was performed by measuring the refractive indices of double-distilled water, toluene, cyclohexane, and carbon tetrachloride at defined temperature. The accuracy of the instrument is +/- 0.0005. 2-3 drops of the sample was put onto the measurement cell and the reading was taken. The refractive index of a sample depends on temperature. During measurement, refractometer determines the temperature and then corrects the refractive index to a temperature as desired by the user.

III.3.11. Surface Tension Measurement

The surface tension experiments were completed by platinum ring detachment method using a Tensiometer (K9, KRŰSS; Germany) at the experimental temperature.

The precision of the measurement was within ±0.1 $mN \cdot m^{-1}$. Temperature of the system has been preserved by circulating auto-thermostated water (within ± 0.01 K) through a double-wall glass vessel holding the solution.

III.3.12. UV-Visible Spectra Measurement

Compounds that absorb Ultraviolet and/or visible light have characteristic absorbance curves as a function of wavelength. Absorbance of different wavelengths of light occurs as the molecules move to higher energy states. UV-visible spectra were recorded by JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. The measuring temperature was held constant by an automated digital thermostat.

The UV-VIS spectrophotometer uses two light sources, a deuterium lamp for ultraviolet light and a tungsten lamp for visible light. After bouncing of a mirror, the light beam passes through a slit and hits a diffraction grating. The light beam hits a second mirror before it gets split by a half mirror. One of the beams is allowed to pass through a reference cuvette (which contains the solvent only), the other passes through the sample cuvette. The intensities of the light beams are then measured at the end.

III. 3.13. FT-IR Spectra Measurement

FTIR spectra were recorded in a Perkin Elmer FT-IR spectrometer with a resolution of ± 0.25 cm^{-1} in the region of 400-4000 cm^{-1} at room temperature (25 ^{0}C). This KBr optics based instrument records data in different modes (KBr pellets, non-aqueous solutions).

III.3.14. ^{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 aspects of biological, organic and inorganic substances in liquid phases. Configured for high resolution NMR experiments, it is invaluable to chemists, molecular biologists and material scientists.

The 500 MHz NMR Spectrometer's specifications include an 11.746 Tesla Ascend superconducting 54 mm bore magnet system; 3-Channel Magic Angle Spinning (MAS) probe for bio-molecular 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 ^{1}H observation or decoupling. It includes a 3 channel MAS system with triple resonance probe system for X-Y correlation spectroscopy, 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.3.15. High Resolution Mass Spectrometry Measurement

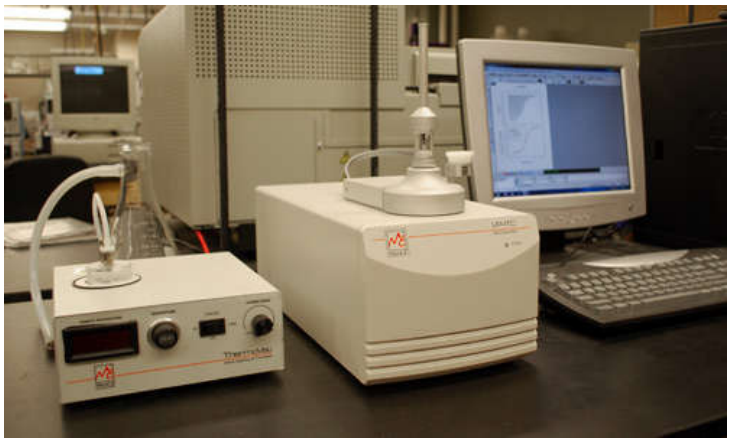
HRMS analyses were performed by Q-TOF high resolution instrument with positive mode electro-spray ionization. This high performance high resolution MS system is designed for routine use. It is sensitive to easily detect maximum residue levels, which linearity quantify up to 4 orders of magnitude.

III.3.16. Isothermal Titration Calorimetry

Isothermal titration calorimetry was employed to find out the association constants at 298 K using a MicroCal VP-ITC (MicroCal, Inc., Northampton, MA, USA). The saturation

curve for kcal/mol of the injectant against molar ratio was calculated by integration, using Origin 7.0 software to provide the heat associated with the injection. The association-affinity and thermodynamic properties of the binding phenomenon were found out by fitting the integrated heats of binding to the one site binding model to give the association constant (K_a^C), stoichiometry (N^C), binding enthalpy (ΔH^{oC}) and the entropy (ΔS^{oC}).

Isothermal titration calorimetry (ITC) is a powerful analytical techniques for in-depth characterization of molecular binding events and structural stability. Thermodynamic binding signatures not only reveal the strength of a binding event, but the specific or nonspecific driving forces involved. This instrument provides the performance, reliability and ease-of-use required for the most demanding applications in drug discovery, protein-protein interactions, structure-function characterization and more.



MicroCal VP-ITC is designed for ease-of-use, delivering fast, accurate analysis and outstanding data sensitivity for academic and research environments.

It is widely used in the life sciences and drug discovery with key applications in Characterizing biomolecular interactions, to Confrm binding and activity, Determine stoichiometry and thermodynamic parameters, Study structure activity relationships and Studying the interaction of any two biomolecules including Proteins, nucleic acids, lipids, drugs and inhibitors.