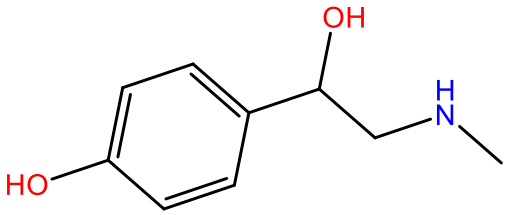
CHAPTER III

EXPERIMENTAL SECTION

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

III.1.1. Biologically active molecules:

Synephrine: Alkaloid synephrine, occurring naturally in some animals, plants and in approved drugs products. Its m-substituted derivative is known as neo-synephrine. m-synephrine and p-Synephrine are known for their longer acting adrenergic effects compared to norepinephrine. This substance is found to various foodstuff at low concentration.[1,2]



Synephrine

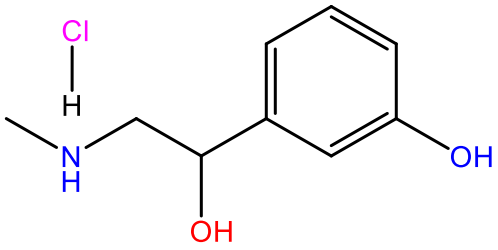
CAS Number	94-07-5
Chemical Formula	$C_9H_{13}NO_2$
Molar mass	167.21 g/mol
Appearance	Colourless solid
Melting Point	187 °C
Solubility in water	soluble

Source: Sigma Aldrich, Germany

Purification: Used as purchased. The purity of the chemical is ≥ 98%

Application: A number of studies of the effects of synephrine in humans, most of them focusing on its cardiovascular properties, have been performed since its introduction as a synthetic drug around 1930. Stockton and co-workers describes the effects of racemic synephrine in humans. Thus, it was shown by these investigators that intramuscular injections (average effective dose = 200 mg) of the drug produced an increase in systolic blood pressure and pulse rate, without affecting the diastolic pressure. However, the topical application of 1–3% solutions of the drug to the nasal mucosa of patients with sinusitis did produce a beneficial constriction without local irritation.

Phenylephrine hydrochloride: Phenylephrine is a medication primarily used as a decongestant, to dilate the pupil, to increase blood pressure, and to relieve haemorrhoids. However, it is used as a decongestant also recommended for hay fever. It can be taken by orally, by injection into muscle or applied to the skin.[3]



Phenylephrine hydrochloride

CAS Number	61-76-7
Chemical Formula	$C_9H_{13}NO_2.HCl$
Molar mass	203.67 g·mol^{-1}
Appearance	Solid powder
Melting Point	144 to 147°C
Solubility in water	soluble

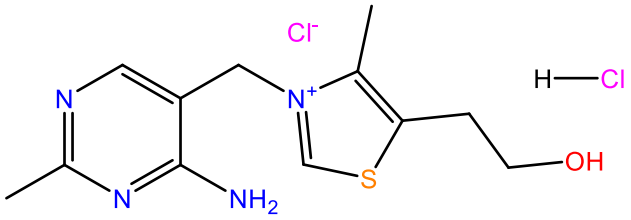
Source: Sigma Aldrich, Germany

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

Application: Phenylephrine is used as a nasal spray. In the United States, it is recommended as ingredient over-the-counter decongestants. Other decongestants

include oxymetazoline and pseudoephedrine. Phenylephrine is an alternate option for pseudoephedrine in decongestant medicines. But, it is not as much efficient as placebo for relieving sinus congestion.

Thiamine hydrochloride (Vitamin B_1): Thiamine Hydrochloride is a vitamin essential for cell growth, aerobic metabolism and acetylcholine synthesis. Upon hydrolysis, it gives active thiamine pyrophosphate (TPP). TPP is a coenzyme that controls enzymatic activities involving amino acid, fatty acid and carbohydrate metabolism.[4]



Thiamine hydrochloride (Vitamin B_1)

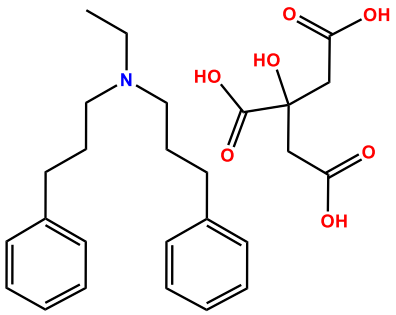
CAS Number	67-03-8
Chemical Formula	$C_{12}H_{17}ClN_4OS{\cdot}HCl$
Molar mass	$337.27 g{\cdot}mol^{-1}$
Appearance	Solid powder
Melting Point	164°C
Solubility in water	soluble

Source: Sigma Aldrich, Germany

Purification: Used as purchased without further purification. The purity of the chemical is > 99.0%

Application: Thiamine (vitamin B_1) is used to prevent or treat low levels of vitamin B_1 in people who do not get enough of the vitamin from their diets. Most people taking normal diet do not need to have extra vitamin B_1. However, alcoholism causes low levels of vitamin B_1. It maintains the health of the nerves and the heart. Deficiency of vitamin B_1 may cause nerve problems.

Alverine citrate: Alverine citrate is the citrate salt of alverine, resulting from the reaction of equimolar amounts of alvarine and citric acid. It acts directly on intestinal and uterine smooth muscle, treating irritable bowel syndrome. It also plays a role as a cholinergic antagonist drug. It is a citrate salt and an organo-ammonium salt.[5]



Alverine citrate

CAS Number	5560-59-8
Chemical Formula	$C_{20}H_{27}N{\cdot}C_6H_8O_7$
Molar mass	473.56 $g{\cdot}mol^{-1}$
Appearance	Solid powder
Melting Point	100 to 102°C
Solubility in water	soluble

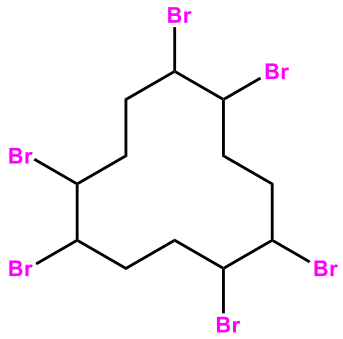
Source: Sigma Aldrich, Germany

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

Application: Alverine acts directly on the muscle in the gut, causing it to relax. This helps us to get rid of the muscle spasms, which occur in the gut in conditions such as diverticular disease and irritable bowel syndrome. Diverticular disease cause the formation of small pouches in the gut lining. Foods undergo trapping into these pouches and become inflamed and painful. The muscle spasms result in symptoms such as abdominal pain and bloating, constipation or diarrhoea. Alverine citrate relaxes the gut muscles, smooth muscle in the womb and relieves the pain. Therefore, it also treats painful menstruation, causing by muscle spasms in the uterus.

III.1.2. Water pollutant molecule:

1,2,5,6,9,10-Hexabromocyclododecane (HBCDD): HBCDD, the water pollutant, being present in biological samples undergoes long-range environmental transportation. So, it is classified as Persistent, Bio accumulative and Toxic (PBT). All the 16 possible stereoisomers of HBCDD has different biological activities. HBCDD generally, found to contain three main diastereomers namely alpha (α-HBCDD), beta (β- HBCDD) and gamma (γ- HBCDD) with traces of others.[6,7]



1,2,5,6,9,10-Hexabromocyclododecane

CAS Number	3194-55-6
Chemical Formula	$C_{12}H_{18}Br_6$
Molar mass	641.70 g·mol^{-1}
Appearance	Solid powder
Melting Point	186°C
Solubility in water	3.4 µg/L

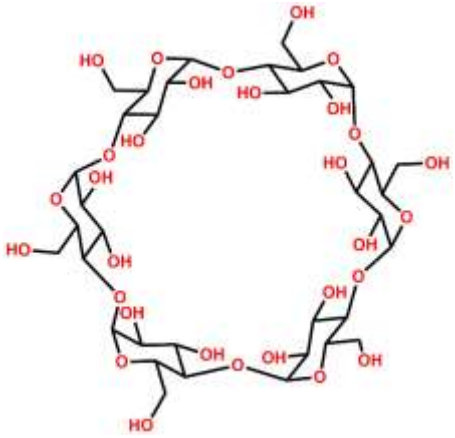
Source: Sigma Aldrich, Germany

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

Application: Hexabromocyclododecane (HBCD or HBCDD) is a brominated flame retardant. Its major application is in expanded (EPS) and extruded (XPS) polystyrene foam. Other uses are, automobile interior textiles, upholstered furniture and insulation blocks in packaging material, trucks, videocassette recorder, housing and electronic equipment.

III.1.3. Cyclodextrins:

α-Cyclodextrin (α-CD): α-cyclodextrin is a cyclic oligosaccharide composed of 6 glucose groups. This is white amorphous solid with a cylinder like molecular structure. Its versatility due to its structural arrangement. The properties are widely used in industry for various purposes.



α-Cyclodextrin

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
Number of glucose unit	6
Internal diameter (Å)	4.7-5.2
Depth	6.7

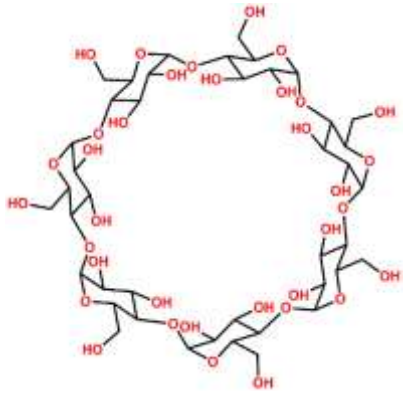
Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is 99.98%.

Application: α-Cyclodextrin is a new substance with high solubility in water and which has wide application in medicinal chemistry, food-processing industry. Moreover, it is extensively used in modification of cosmetics, foodstuffs etc.; whose function is to improve stability, solubility and good smell. In the production of medicine, it can strengthen the stability of medicine without being oxidized and resolving. On the other hand, it can improve the solubility and the effect on living of

medicine, lower the toxic and side-effect of medicine and cover the strange and bad smell. In the food industry, it is used to cover strange and bad smell of food, improve the stability of perfume and the condiment and keep food dry or wet at will. α-CD with a cavity diameter of 4.7-5.3Å, is of the good interest because it is easily available in market and its cavity size allows to encapsulate many common guest moieties like hormones, vitamins, and many compounds. This capability has also been of assistance for different applications in medicines, cosmetics, food technology, pharmaceutical, and chemical industries as well as in agriculture and environmental engineering.

β-Cyclodextrin (β-CD): β-Cyclodextrinis white amorphous solid compound composed of 7 glucose groups having a cylinder like molecular structure. The function of β-Cyclodextrin depends on its molecular structure which can be easy to integrate other materials. That feature is applied widely in industry.



β-Cyclodextrin

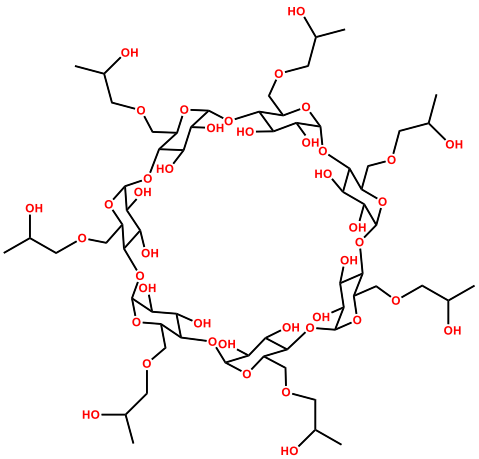
CAS Number	7585-39-9
Chemical Formula	$C_{42}H_{70}O_{35}$
Molar mass	1134.98 g·mol^{-1}
Appearance	white powder
Solubility in water	18.5 g/L
Number of glucose unit	7
Internal diameter (Å)	6.4 – 7.5
Depth	6.7

Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is 99.98%.

Application: β-Cyclodextrin is a new stuff which can be widely applied in production of medicine and food. And the effect on living of medicine, lower the toxic and side-effect of medicine and cover the strange and bad smell. In the production of food, it can mainly cover strange and bad smell of food, improve the stability of perfume and condiment and keep food dry or wet at will. β-CD with a cavity diameter of 6.4-7.5 Å, is the most interest because its cavity size allows for the best special fit for many common guest moieties. For this reason, β-cyclodextrin is widely used as host in the host-guest chemistry with various biologically active molecules such as hormones, vitamins, drug molecules and various compounds commonly used in tissue and cell-culture applications. This capability has also been of assistance for different applications in medicines, cosmetics, food technology, pharmaceutical, and chemical industries as well as in agriculture and environmental engineering as an encapsulating agent to protect sensitive molecules in hostile environment.

(2-Hydroxypropyl)-β-Cyclodextrin (HP-β-CD): HP-β-CD is a 2-hydroxypropyl beta-cyclodextrin which is highly soluble derivative of beta-cyclodextrin. (HP-β-CD) exists as a mixture of isomers having various degrees and pattern of hydroxyl propylation. (2-hydroxypropyl) beta-cyclodextrin (HP-β-CD) is a highly soluble derivative of beta-cyclodextrin. That's why, it is used as stabilizer and solubilizer for oral and parenteral formulations.



(2-Hydroxypropyl)-β-Cyclodextrin

CAS Number	128446-35-5
Chemical Formula	$C_{63}H_{112}O_{42}$

Molar mass	1541.5 g·mol^{-1}
Appearance	white powder
Solubility in water	soluble

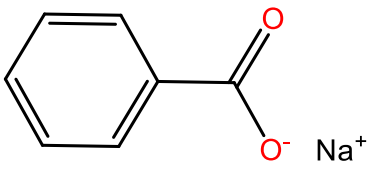
Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is 99.98%.

Application: It has enhanced solubility and less toxicity. Recently, its pharmacological activity has been documented in several diseases. The increasing applications need a closer gaze to the structure-activity relationship. Recently, the anticancer effect of HP-β-CD has been discovered and proved in vivo in mouse model of leukemia.

III.1.4. Food preservative molecules:

Sodium benzoate: Sodium benzoate is an extensively used food preservative. Reaction of benzoic acid with sodium hydroxide produces sodium benzoate, i.e. sodium salt of benzoic acid.[8,9]



Sodium benzoate

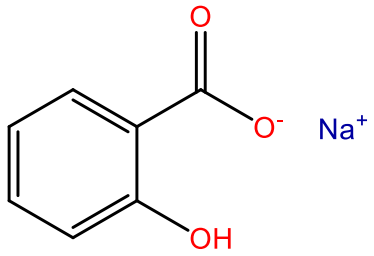
CAS Number	532-32-1
Chemical Formula	$C_7H_5NaO_2$
Molar mass	144.10 g·mol^{-1}
Appearance	white crystalline powder
Melting Point	410 °C
Solubility in water	62.87 g/100mL at 30 °C

Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is 99.98%.

Application: Sodium benzoate is a preservative and widely used in acidic foods such as carbonated drinks, salad dressings, jams, pickles and fruit juices, condiments and frogurt toppings. It is also preserves cosmetics and medicines. due to its poor water solubility is not used directly. Potassium sorbate is used in the majority of soft drinks in place of sodium benzoate. Sodium benzoate able to bind with amino acid and it is used to treat urea cycle disorders.

Sodium salicylate: Sodium salicylate is the sodium salt of salicylic acid. It can be prepared from carbon dioxide and sodium phenolate under higher temperature and pressure. It has been synthesized in presence of excess of sodium hydroxide by refluxing methyl salicylate.[10,11]



Sodium salicylate

CAS Number	54-21-7
Chemical Formula	$C_7H_5NaO_3$
Molar mass	160.10 g·mol^{-1}
Appearance	white crystalline powder
Melting Point	200°C
Solubility in water	124.6 g/100g at (25 °C)

Source: Sigma Aldrich, Germany.

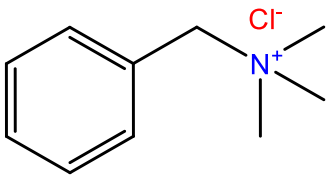
Purification: Used as parched. The purity is 99.98%.

Application: Sodium salicylate is of the salicylate group and this compound is known to cause Reye's Syndrome in adults and children, generally following a viral infection such as chicken pox or influenza. It is used in medicine as an antipyretic and analgesic. Sodium salicylate also acts as NSAID and brings necrosis and apoptosis in

cancer cells.[12] It is also a potential replacement for aspirin for people sensitive to it. It may also be used as a phosphor for the detection of vacuum ultraviolet radiation and electrons.

III.1.5. Ionic liquids:

Benzyltrimethylammoniun chloride (BTMACl): Benzyltrimethylammoniun chloride is a quaternary ammonium based ionic liquid. It can execute some sort of anti-microbial effects for instance positive charge on the Nitrogen atom of these ionic liquids attracts naturally the negatively charged species, such as bacterial proteins and consequently disorganization in the protein chain makes it denature.[13]



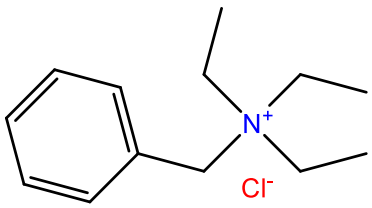
Benzyltrimethylammoniun chloride

CAS Number	56-93-9
Chemical Formula	$C_{10}H_{16}ClN$
Molar mass	185.69 g/mol
Appearance	Crystalline solid
Melting Point	239^0C
Solubility in water	soluble

Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity is 97.0%.

Benzyltriethylammoniun chloride (BTEACl): Ionic liquids, benztltriethylammonim chloride is also a quaternary ammonium based ionic liquid and shows anti-microbial activity when used along with the food preservatives.[13]



Benzyltriethylammoniun chloride

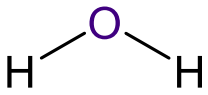
CAS Number	56-37-1
Chemical Formula	$C_{13}H_{22}ClN$
Molar mass	227.77 g/mol
Appearance	Crystalline solid
Melting Point	190 to 192°C
Solubility in water	soluble

Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity is 99.0%.

III.1.6. Solvents:

Water: Water is the basis of life as life arose from water. Water is a universal chemical substance is made up of hydrogen and oxygen and is crucial for all well-known forms of life. In distinctive treatment, water refers only to its liquid form or state, but the matter also exists as solid state or gaseous state e.g. ice and steam respectively. Water is a high quality solvent and is frequently referred to as the Universal Solvent.[14]



Water

CAS Number	7732-18-5
Chemical Formula	H_2O
Molar mass	18.015 g/mol
Appearance	Almost colourless, 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 at 20°C
Viscosity	0.891 cP
Dipole moment	1.8546 D
Specific heat capacity	75.375 ± 0.05 J/mol K

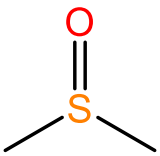
Source: Distilled water, distilled from fractional distillation method in Laboratory.

Purification: Water was first deionised 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 extensively used in chemical reactions as a solvent or reactant and less usually as a solute or catalyst. In inorganic reactions, water is a ordinary solvent, dissolving many ionic compounds. Supercritical water has newly been an important topic of research work. Oxygen saturated supercritical water combusts organic pollutants powerfully. It with no trouble forms hydrogen bond with other molecules and has appropriate polarity to freeze a numerous number of molecules and hence, it is said the universal solvent. Water is the most important constituent of life in the earth. Not only an elevated percentage of living substances, both plants and animals are set up in water, all forms of life on earth is consideration to have arisen from water and the biochemic bodies of all living organisms are composed mainly of water. About 70 to 92 percent of all organic matter is water. The biochemical reactions in all plants and animals that sustain life take place occur in water medium. Water not only gives the medium to create these life-satisfying reactions plausible, but water itself is often an essential reactant or product of all these reactions. In short, Biochemistry i.e. the 'Chemistry of life' is nothing but the "chemistry of water" in living bodies.[15,16]

Dimethyl solfoxide: Dimethyl sulfoxide (DMSO) is an organosulfur compound with the formula $(CH_3)_2SO$. This colorless liquid is an significant polar aprotic solvent and

able to dissolves both nonpolar and polar compounds and it has a great miscibility in a wide range of organic solvents as well as water. It has a comparatively high boiling point. DMSO has the unfamiliar property that many entities observe a garlic-like taste in the mouth.[17]



Dimethyl sulfoxide

CAS Number	67-68-5
Chemical Formula	C_2H_6OS
Molar mass	78.13 g/mol
Appearance	Colourless liquid
Melting Point	19°C
Boiling point	189°C
pKa	35
Density	1.1004 g/cm^3
Refractive Index	1.479
Viscosity	1.996 cP at 20°C
Solubility in water	Miscible

Source: Sigma Aldrich, Germany.

Purification: Used as purchased. The purity is 99.0%.

Application: DMSO is an aprotic polar solvent and is less toxic than other members of this class, such as dimethylacetamide, dimethylformamide, HMPA and N-methyl-2-pyrrolidone. DMSO is regularly used as a solvent for chemical reactions concerning salts, most remarkably nucleophilic substitutions and Finkelstein reactions. It is also comprehensively used as an extractant in cell biology and biochemistry. At normal atmospheric DMSO evaporates slowly due to its high boiling point, 189 °C. After dissolution in DMSO the samples cannot be easily recovered compared to other

solvents since, it is very hard to remove all traces of DMSO by orthodox rotary evaporation.[18-20]

III.2. EXPERIMENTAL METHODS

III.2.1. Preparation of solutions:

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

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

III.2.2. Preparation of multicomponent liquid mixtures:

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

(i) Mole fraction

(ii) Mass fraction

(iii) Volume fraction

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

III.2.3. Preparation of Inclusion Complexes:

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

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

III.3.1. Measurement of mass:

Digital electronic analytical balance Mettler Toledo, AG 285, Switzerland, was employed to measure mass.

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

III.3.2. Water distiller:

Distillation of water was made by using glass distillation unit, Bionics Scientific Technologies (P). Ltd.

A water distillation system is designed to purify water quickly, cheaply and effectively. To distil water, we actually need a condenser and a heat source. Since water has a lower boiling point than contaminants and minerals like salt, bacteria, heavy metals, calcium and phosphorus, when untreated water is boiled, the water turns into vapour and leaves everything else behind. We heated water to the required minimum temperature to boil the water such that the undesirable elements cannot undergo vaporization along with water. The water vapour is routed through the condensing coil where reverts back to liquid form while the undesirable elements stay in the boiling tank

III.3.3. Thermostat:

Temperature of experimental solutions were controlled using Brookfield TC-550 thermostatic water bath with an accuracy of ± 0.01 K of the desired temperature.

Laboratory water bath has a vessel containing the material to be heated is placed into the one containing water that heats it. It has digital control with greater temperature uniformity, durability, heat retention and recovery.

III.3.4. Magnetic stirrer:

Magnetic stirrer cum hot plate made by IKA was used for the preparation of solutions and the solid inclusion complexes.

III.3.5. Density Measurement:

The solvent as well as the solution densities were measured with vibrating-tube density meter (Anton Paar, DMA 4500M), maintained at 298.15 to 318.15 K. Calibration of the instrument was done with doubly distilled water and dry air. The uncertainty in density was estimated to be ±0.00001 g cm^{-3}.

The sample is introduced into a U-shaped tube made from borosilicate glass that is excited to oscillate at its characteristic frequency which is directly related to the density of the sample. Getting a stable oscillation, then switched off the excitation and the oscillation gets fade out freely. This fade-out sequence and excitation is recurring continuously. After evaluation this pattern, we obtain a highly precise density.

III.3.6. Viscosity Measurement:

Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42 was employed to measure viscosities of the solutions. The viscosities were obtained using following programmed equation

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

Where SMC (0.327), RPM, TK (0.09373) stands for spindle multiplier constant, speed, viscometer torque constant respectively. Calibration of the instrument before starting experiment was done with provided standard viscosity samples, water and aqueous $CaCl_2$ solutions.

III.3.7. Refractive Index Measurement:

Refractive index can be measured with the help of Digital Refractometer (Mettler Toledo 30GS). The refractometer was calibrated by measuring the refractive indices of double-distilled water, cyclohexane, toluene 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. During measurement,

refractometer determines the temperature and then corrects the refractive index to a temperature as desired by the user.

III.3.8. Conductivity Measurement:

METTLER-TOLEDO Seven Multi conductivity meter was employed to have specific conductivity values with an uncertainty of ±1.0 μS m^{-1}.

Temperature during the experiment was kept constant at the specific value with an auto-thermostatic water bath. HPLC-grade water having a specific conductance of 6.0 μS m^{-1} was used for conductivity measurement. Freshly prepared aqueous 0.01 M KCl solution was utilized for the calibration of the Systronics Type CD – 30 conductivity cell.

III.3.9. Surface Tension Measurement:

Surface tension of experimental solutions with the accuracy of ±0.1 mN m^{-1} were obtained by employing K9 digital TENSIOMETER (Krüss GmbH, Hamburg, Germany) which uses the platinum ring detachment technique.

The tensiometer is a precision instrument which will only perform reliably on a solid and vibration-free base. It places the same demands on its surroundings as a laboratory balance with a resolution of 0.1 mg. In addition surface tension measurements require a clean and dust-free atmosphere as atmospheric pollutants could directly falsify the results.

III.3.10. FT-IR Spectra Measurement:

With the help of Perkin-Elmer FTIR spectrometer FTIR data were collected in the scanning range of 4000–400 cm^{-1}. KBr disks were made in 1:100 ratios of sample and KBr according to the KBr disk method.

III.3.11. UV-Visible Spectra Measurement:

Compounds that absorb Ultraviolet and/or visible light have characteristic absorbance curves as a function of wavelength. Absorbance of altered wavelengths of light arises as the molecules moves to higher energy state. Utilizing JASCO V-530 and Agilent 8453 UV-Visible Spectrophotometer, UV−visible spectra were recorded with a wavelength accuracy of ±0.5 nm. Cell temperature during the experiment was controlled from 298.15K to 308.15K with a digital thermostat.

Agilent 8453 UV-Visible Spectrophotometer

Both the spectrophotometers uses two light sources, a deuterium lamp for ultraviolet light and a tungsten lamp for visible light. JASCO V-530 is a double beam spectrophotometer, one the beams passes through the reference cuvette containing solvent and the another beam goes through the cuvette containing experimental solution. On the other hand Agilent 8453 is a single beam spectrophotometer.

III.3.12. ^{1}H NMR and 2D ROESY Spectroscopic Measurement:

2D ROESY as well as ^{1}H NMR spectra were recorded in D_2O solvent at 400 MHz in Bruker Avance instrument at 298.15 K. The chemical shifts data, δ values are presented in parts per million (ppm) where, the residual protonated signal (HDO, δ 4.79 ppm) was used as internal standard.

Nuclear magnetic resonance spectroscopy, generally known as NMR spectroscopy, is a spectroscopic technique to understand local magnetic fields around atomic nuclei. After placing the sample in a magnetic field, the NMR signals were recorded by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is spotted with sensitive radio receivers. Intramolecular magnetic field around an atom in a molecule has an influence on the resonance frequency that gives details of the electronic structure of a molecule and the different functional groups. As the

fields are exclusive or extremely characteristic to different compounds, in modern organic chemistry practice, NMR spectroscopy is the decisive method to detect monomolecular organic compounds. Similarly, biochemists use NMR to identify proteins and other complex molecules. Besides identification, NMR spectroscopy provides detailed information about the dynamics, structure, reaction state, and chemical environment of molecules.

III.3.13. Fluorescence Spectra Measurement:

The Bench top spectrofluorimeter from photon technologies International (Quantamaster-40, USA) was used to record fluorescence spectra at room temperature. Hellma quartz cuvette having optical path length 1.0 cm was also used. Fluorescence is the momentary absorption of electromagnetic wavelengths from the visible light spectrum by fluorescent molecules, and the subsequent emission of light from the higher to the lower energy level. Fluorescence of a molecule takes place, when an orbital electron, nanostructure, relaxes to its ground state by emitting a photon from an excited singlet state. Different competing pathways relax a molecule in S_1. It can get relaxed through non-radiative relaxation releasing heat (vibrations) to the solvent. It can also undergo relaxation via conversion to a triplet state, i.e. via phosphorescence.

A second molecule can relax an excited molecule through fluorescence quenching. The triplet state of the molecular oxygen is a tremendously efficient quencher of fluorescence.

III.3.14. Differential Scanning Calorimetry (DSC):

The DSC thermograms of the samples were recorded with the help of Perkin-Elmer DSC-6 differential scanning calorimeter at the heating rates of 10°C min^{-1}. The thermograms were taken by heating near about 1 mg of samples in aluminium-crimped pans under nitrogen gas flow.

To undergo a physical transformation like, phase transitions, the more or less heat will necessary to flow to it than the reference to keep both at the same temperature. Whether more or less heat flows to the sample depends on whether the process is endothermic or exothermic. For example, when a solid sample melts to a liquid, it will need more heat flowing to the sample to increase its temperature at the same rate as that of the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid.

III.3.15. Powder X-Ray Diffraction (PXRD):

Powdered X-Ray Diffraction (PXRD) patterns of the pure compound and ProC were recorded by using Cu-Kα radiation (D8 Advance Bruker).

The waves produced by a diffractometer at a known frequency that is governed by their source. The source is frequently x-rays, as they are the only kind of energy with the correct frequency for inter-atomic-scale diffraction. Nevertheless, the common sources are neutrons and electrons, where, the frequency can be determined by the de Broglie wavelength. After reached to the sample, the incoming beam is either reflected or can enter the lattice and get diffracted by the atoms present in the sample. To get constructive interference the atoms must be arranged symmetrically such that the path-length difference $2d\sin\theta$ is equal to an integral multiple of the wavelength, producing a diffraction maximum in accordance with Bragg's Law.

III.3.16. Scanning Electron Microscopy (SEM):

The Scanning Electron Microscope (SEM), JEOL JSM IT 100 was used to determine the surface topography of the samples at various resolutions. Samples were prepared on a small piece of double adhesive carbon-coated tape attached to brass stubs and then a coating of ultra-thin layer of gold ions was put in a gold-ionization chamber.

SEM samples should be small enough to adequate in the specimen stage, and special preparation is required to increase their electrical conductivity and to stabilize them, so that they can resist the high-energy beam of electrons and high vacuum conditions. Samples are commonly attached strictly on a specimen holder or stubbed by conductive adhesive. SEM is used widely for defect analysis of semiconductor wafers, and the instruments made by the manufacturer can be examined any part of a 300 mm semiconductor wafer. There are instruments that have chambers can tilt an object of that size to 45° and provide continuous 360° rotation.

III.3.17. High Resolution Mass Spectrometric Measurement:

Quadrupole time-of-flight (Q-TOF) high-resolution instrument with positive-mode electrospray ionization was employed to have HRMS spectra of the solid ICs, taking the methanol solution of the solid ICs.

The ion source ionizes the material under analysis (the analyte). The high voltage maintained by magnetic or electric fields makes the ions to undergo transportation to the mass analyzer. Depending on the types of samples under analysis, different mass spectrometric techniques should be used. Chemical ionization and electron ionization are used for vapours and gases.