CHAPTER III

EXPERIMENTAL SECTION

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

3.1.1. SOLVENTS

The information of the aqueous and non-aqueous solvents used in the research work have been specified below:

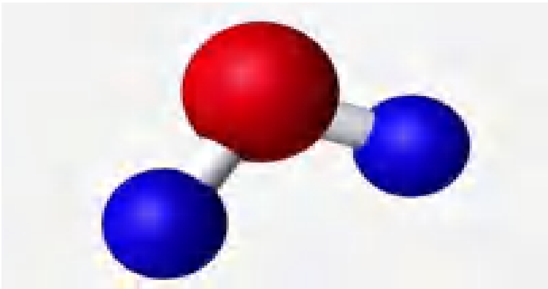
■ Water

Water, a substance existing in gaseous, liquid, and solid states, is composed of the chemical elements hydrogen and oxygen. It is one of the most plentiful but essential of compounds. It is tasteless and odorless and exists as liquid at room temperature. It has the supreme ability to dissolve many other substances. Indeed, it is the most extensively used solvent and is often referred to as the Universal Solvent. [1,2] The versatility of water as a solvent is essential to living organisms. Life is, in fact, believed to have originated in the aqueous solutions of the oceans, and, for carrying out biological processes, living organisms depend on aqueous solutions, such as blood and digestive juices.

Natural water (H_2O)

In small quantities water appears colorless, but water actually has an intrinsic blue color caused by slight adsorption of light at red wavelengths. The solid state of water is ice, while, the gaseous state is steam or water.

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



Water

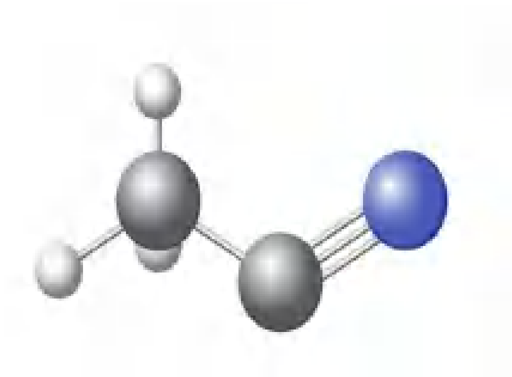
Moleculer Formula	H_2O
Molar mass	18.015 g/mol
Appearance	Colorless Liquid
Melting Point	273.15K
Boiling point	373.13K
Density	0.9970474 g/mL at 25°C
Viscosity	0.891cP
Refractive Index	1.3330 at 20°C
Dipole moment	1.8546D

Purification: At first, water was de-ionised, and then, distilled using an 'entire glass distilling set' along with some alkaline $KMnO_4$ solution so as to remove any organic constituent therein. The doubly distilled water yet again allowed for the final distillation in an all glass distilling set. The triply distilled water thus obtained had a specific conductance value less than 1.0×10^{-6} S·cm^{-1}. During the whole distillation procedure, precautions were taken to detain contamination from CO_2 and other impurities.

Application: Water is one of the most versatile of all chemicals. Owing to it's polarity as well as the ability to form hydrogen bonds, water makes itself an excellent solvent. Water can readily dissolve many different kinds of molecule. Indeed, it is capable of dissolving more substances than any other liquid. Thus, water is more commonly used as a solvent or reactant in a chemical reaction, than as a solute or catalyst. Oxygen saturated supercritical water can combust organic pollutants effectively. Water is the most important component of life, both plants and animals are set up in water. Almost all organic matter contains 70 to 90 percent of water. Most of the chemical reactions important to life take place in a watery environment inside of cells. Water not only provides the medium to carry out these life-sustaining reactions plausible, but often it is an essential reactant or product in these reactions. In short, Biochemistry i.e., the 'Chemistry of life' is nothing but the "chemistry of water" in living bodies. [3,4]

■ Acetonitrile

Acetonitrile appears as a transparent liquid with an aromatic odor. It is the simplest organic nitrile and is prepared mainly as a byproduct of acrylonitrile manufacture.



Acetonitrile

Moleculer Formula	CH_3CN
Moleculer Weight	41.05g/mol
Appearence	Liquid
Melting Point	607.5K
Boiling point	628.3K
D.C.	37.5 at 294.26K

Source: Thomas Baker, India.

Purification: It is shaken with silica gel, refluxed with CaH_2 and finally, distilled over P_2O_5. [5]

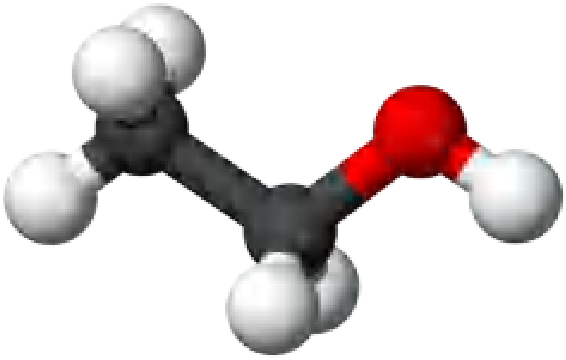
Application: Acetonitrile is a widely used, polar aprotic organic solvent. It is widely used in battery applications owing to its relatively high dielectric constant and ability to dissolve electrolytes. The low viscosity and low chemical reactivity make acetonitrile an automatic choice for liquid chromatography. In the manufacture of DNA oligonucleotides from monomers, acetonitrile plays an important role as the dominant solvent. Acetonitrile is used to make other chemicals, pharmaceuticals, photographic films, fibres, plastics, perfumes, rubber products, acrylic nail removers, and in dyeing fabrics. It is used to make synthetic medicines such as insulin, antibiotics and vitamins and also to extract fatty acids from animal and vegetable oils.

■ Ethyl Alcohol

Ethanol, also known as ethyl alcohol, grain alcohol, or sometimes as drinking alcohol, is a volatile, inflammable, colorless liquid with a slight typical odor. Ethanol produced either by fermentation of sugars by yeasts or by hydration of ethylene, is obtained as a dilute aqueous solution and must be concentrated by fractional distillation.

Source: Merck, India.

Purification: It was passed through Linde Å molecular sieves and then distilled. [5]



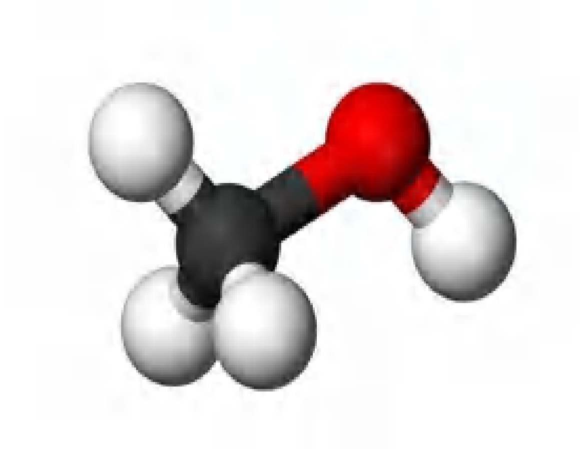
Ethyl Alcohol

Molecular Formula	**C_2H_6O**
Molecular Weight	**46.07g·mol^{-1}**
Appearance	**Colourless Liquid**
Melting point	**-114°C**
Boiling Point	**78.24°C**
Density (30^{0}C)	**0.8029g·cm^3**
Viscosity	**0.948mP·s (303.15K)**
Refractive Index	**1.361(298.15K)**
Dielectric Constant	**24.3 (298.15K)**

Application: Ethanol is an important industrial chemical. Because it can readily dissolve in water and other organic compounds, it is used as a solvent, in the synthesis of other organic chemicals. Ethanol is widely used as a solvent in the manufacturing of varnishes and perfumes. Ethanol is also the intoxicating ingredient of many alcoholic beverages such as beer, wine or brandy and distilled spirits. It has wide applicability as a preservative for biological specimens; in the formulations of essences and flavorings; in numerous medicines and drugs; as a disinfectant and in tinctures; and as a fuel and gasoline additives.

■ Methyl Alcohol

Methanol (or methyl alcohol) is the simplest primary alcohol which appears as a fairly volatile colorless liquid with a faintly sweet pungent odor similar to that of ethyl alcohol. It is a light, flammable liquid. Although, methanol is produced in small amounts throughout many fermentation processes, the modern method of preparing methanol is based on the direct combination carbon monoxide gas and hydrogen in the presence of a catalyst.



Methyl Alcohol

Molecular Formula	**CH_4O**
Molecular Weight	**32.04 g/mol**
Appearance	**Colourless Liquid**
Melting point	**175 K**
Boiling Point	**337-378K**
Density (25^{0}C)	**0.791g·cm^3**
Viscosity	**0.544 at 25^{0}C**
Refractive Index	**1.329 (20^{0}C)**
Dielectric Constant	**32.70 at 293.15K**

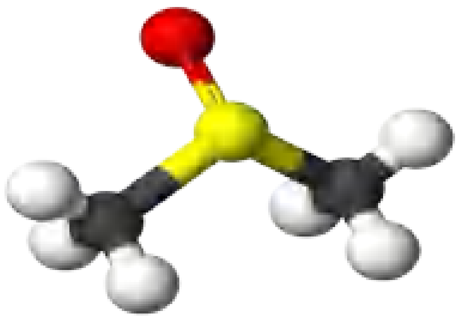
Source: Sigma Aldrich, Germany

Purification: It was dried by passing molecular sieve and then distilled by utilizing appropriate technique. [6]

Application: Methanol has versatile use for industrial purposes. It is a critical component of hundreds of chemicals like acetic acid, formaldehyde and olefins. About 40% of the worldwide production of methanol is used for the production of formaldehyde which is used for the production of plastics, plywoods, paints, explosives, permanent press textiles etc. In order to restrict the recreational consumption of ethanol, methanol is often added to it as a denaturant. Methanol has significant uses as a solvent, and as an anti-freeze in pipelines and windshield washer fluid. It is also used in sewage treatment plants since it serves as a carbon based food source for denitrifying bacteria. Methanol has accredited uses as a fuel in many internal combustion engines.

■ Dimethyl Sulphoxide

The organsulfur compound, Dimethyl Sulfoxide (DMSO) is an organosulfur compound . It is essentially odorless, and has a relatively high boiling point with a low level of toxicity. DMSO is a colorless liquid and is highly miscible in water as well as in a wide range of organic solvents. Dimethyl sulfoxide is widely used as a chemical solvent as this polar aprotic solvent has the ability to dissolve both polar and non-polar compounds.



Dimethyl Sulphoxide

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

Source: Sigma Aldrich, Germany.

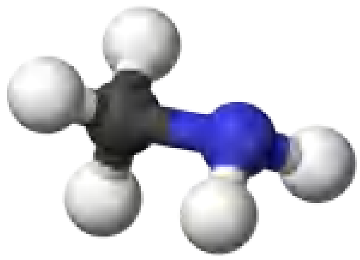
Purification: Used as purchased and the purity was 99.0%.

Application: DMSO is a potent solvent because of its highly polar nature. DMSO have been frequently used as a solvent in many chemical reactions involving salts, for example, Finkelstein reactions and various other nucleophilic substitution reactions. DMSO finds its use as an extractant in biochemistry and cell biology. The ability to penetrate biological membranes affiliate dimethyl sulphoxide to be used as a vehicle for topical application of pharmaceuticals. [7] DMSO is used as a rinsing agent in the electronic industry and, in its deuterated form (DMSO-d6), is a useful solvent in NMR due to its ability to dissolve a wide range of chemical compounds and its minimal interference with the sample signals.

■ Methyl amine

Methylamine is the simplest primary amine and appears as a colorless gas or a liquid. When liquid, it boils at a very low temperature, hence, vaporizes rapidly when unconfined. It has a pungent fishy odor resembling to the odor of ammonia.

While, the gas is extremely flammable, and may even explode if heated. Usually, methylamine is sold as anhydrous gas or as a solution in water, methanol, ethanol, or THF.



Methyl amine

Source: Sigma Aldrich, Germany.

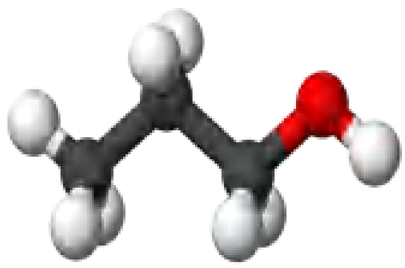
Purification: Used as purchased and the purity was 99.0%.

CAS Number	**74-89-5**
Chemical Formula	CH_5N
Molar mass	31.06g/mol
Concentrtion	40 wt. % in H2O
Appearance	Colorless to yellow liquid
Melting Point	-93.4
Boiling point	48°C
Density	0.897g/cm^3 at 20°C
Refractive Index	1.37

Application: Methylamine, aqueous solution appears as a colorless to yellow aqueous solution of a gas. The solvent properties of liquid methylamine is analogous to those of liquid ammonia. Methylamine is used as a catalyst or raw material in manufacturing many chemicals having catalytic activity, and as a building block in the production of several surfactants. It is an intermediate for production of many agricultural chemicals, such as herbicides, biocides, insecticides, etc. Methylamine also used as a component in manufacture of pharmaceuticals, such as ephedrine, theophylline etc.

■ 1-Propanol

1-Propanol also known as *n*-propanol is a primary alcohol. This colorless, transparent liquid has a typical sharp musty odor comparable to the smell of rubbing alcohol. 1-propanol is made by oxidation of aliphatic hydrocarbons. It is also formed naturally in small amounts during many fermentation processes.



1-Propanol

CAS Number	**71-23-8**
Chemical Formula	C_3H_8O
Molar mass	60.10g/mol
Appearance	Colorless liquid
Melting Point	-127°C
Boiling point	96.5-98°C
Density	0.80g/cm^3 at 20°C
Viscosity	2.256cP at 20°C
Refractive Index	1.3862 at 20°C

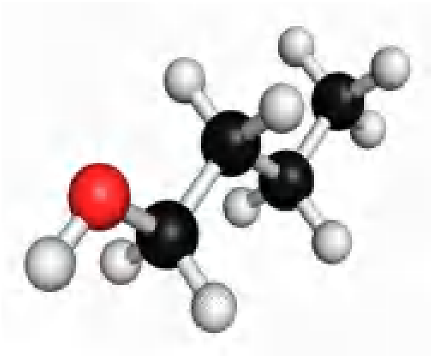
Source: Sigma Aldrich, Germany.

Purification: Used as purchased and the purity was 99.0%.

Application: 1-Propanol is used as a solvent and chemical intermediate, to prepare acetone and many other chemical products. The major use of 1-propanol is as a multipurpose solvent in industry and in the home. It is used in flexographic printing ink and textile applications, lacquer formulations, dye solutions, products for personal use, such as cosmetics and lotions, and in window cleaning, polishing and antiseptic formulations. In the pharmaceutical industry, 1-Propanol is widely used as a solvent.

■ 1-Butanol

1-butanol, also known as *n*-butanol, is a colorless liquid with a banana like mildly alcoholic odor. It is a primary alcohol. *n*-Butanol is produced naturally as a minor product during the fermentation of sugars and other carbohydrates. It is present in many foods and beverages.



1-Butanol

CAS Number	71-36-3
Chemical Formula	$C_4H_{10}O$
Molar mass	74.12g/mol
Appearance	Colorless liquid
Melting Point	-90°C
Boiling point	116-118°C
Density	0.81 g/cm^3 at 25°C
Viscosity	2.544 /2.573cP at 15°C
Refractive Index	1.399 at 20°C

Source: Sigma Aldrich, Germany.

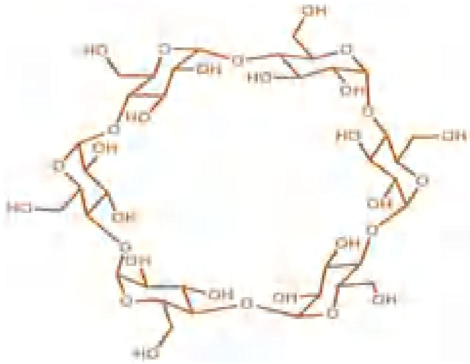
Purification: Used as purchased and the purity was 99.8%.

Application: It is primarily used as a solvent and as an intermediate in chemical synthesis. n-Butanol has been proposed to be used as a substitute for fuels like diesel and gasoline. It has a role as a human metabolite and a mouse metabolite. 1-butanol is used in the production of varnishes and a wide range of consumer products.

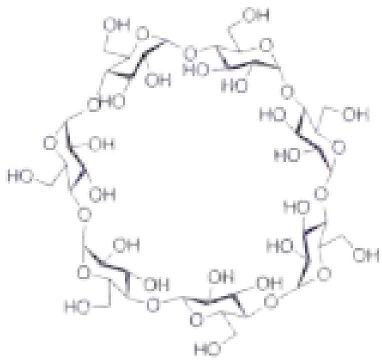
3.1.2 HOST MOLECULES

■ α- and β-Cyclodextrin

Cyclodextrins(CDs) are doughnut-shaped cyclic oligosaccharides consist of glucopyranose units connected by α-(1,4) linkages. [8] There are some types of cyclodextrins, commons amongst are having six and seven D-glucopyranose units and are known as α-, and β-CDs, respectively. The conical structure of CD consists of hydrophobic interior and hydrophilic rim having primary and secondary –OH groups. It is the structural arrangement of cyclodextrins that play the key role in making them to have wide applications in various field. Both the CDs are white, amorphous solid.



α-Cyclodextrin



β-Cyclodextrin

	α-Cyclodextrin	β-Cyclodextrin
CAS Number	10016-20-3	7585-39-9
Chemical Formula	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$
Molar mass	972.84 g·mol^{-1}	1134.98 g·mol^{-1}
Appearance	white powder	white powder
Solubility in water	145 g/L	18.5 g/L
Number of glucose unit	6	7
Internal diameter (Å)	4.7-5.2	6.4 – 7.5
Depth	6.7	6.7

Source: Sigma Aldrich, Germany.

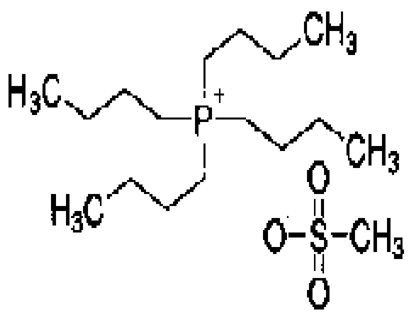
Purification: Used as parched. The purity is 99.98%.

Application: Due to the presence of cavity of hydrophobic nature, CDs show a phenomenal property of accommodating organic molecules into its cavity to form inclusion complexes. [9] While, the cavity diameter of α-CD is 4.7A˚, that for β-CD is 6.0A˚. The encapsulation occurs without any chemical modification of the guest. Sometimes it may also lead to increase the solubility of guest. The controlled release of CD has useful application in many industrial purposes e.g., in food, medicine, cosmetic, paint industry and elimination of various toxic materials, pollutants, waste products without a chemical change. [10] Cyclodextrins have been applied for delivery of a variety of drugs because they confer solubility and stability to these drugs. They can reduce toxicity and side effects of medicine and cover the strange and ugly smell. The molecular encapsulation of food ingredients with cyclodextrins improve the stability of flavors, vitamins, colorants, unsaturated fats, and other lipophilic molecules. Cyclodextrins have been extensively used in modifying cosmetics, by improving stability, solubility and good smell. Facile encapsulation with many biologically active molecules like vitamins, hormones, drug molecules and various compounds, have been used frequently in tissue and cell-culture applications. This capability has also been in favour of their diverse applications in medicine, food technology, chemical industries in addition to agricultural and environmental engineering to protect sensitive molecules in an adverse circumstance.

3.1.3 IONIC LIQUIDS AND SALTS

- **Tetrabutylphosphonium Methanesulfonate**

Tetrabutylphosphonium Methanesulfonate is an ionic liquid at ambient temperature. It is more stable than the corresponding ammonium salt.



Tetrabutylphosphonium Methanesulfonate

Source: Sigma Aldrich, Germany

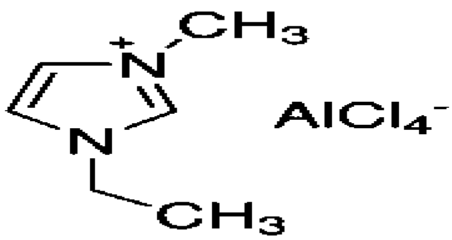
CAS Number	**98342-59-7**
Chemical Formula	$C_{17}H_{39}O_3PS$
Molar mass	354.53g/mol
Appearance	White crystalline solid
Melting Point	59-62°C
Solubility	Soluble in water

Purification: Used as purchased without further purification. The chemical had a purity level which is >98.0%

Application: Phosphonium based ionic liquids have great thermal stability. Tetrabutylphosphonium Methanesulfonate has been used as conducting material.

■ 1-Ethyl-3-methylimidazolium chloride-aluminum chloride

Ionic liquid composed of 1-Ethyl-3-methylimidazolium chloride ([EMIm]Cl) and $AlCl_3$ is a classical chloroaluminate based electrolyte having many desired properties such as non-flammability, non-volatility, low viscosity, high conductivity, and high thermal stability and chemical inertness.



1-Ethyl-3-methylimidazolium chloride -Aluminum chloride

CAS Number	5039-09-0
Chemical Formula	$C_{12}H_{22}Al_3Cl_{11}N_4$
Molar mass	693.26g/mol
Appearance	Liquid
Solubility	Soluble in water

Source: Sigma Aldrich, Germany

Purification: Used as purchased without further purification. The chemical had a purity level which is >98.0%

Application:

1-Ethyl-3-methylimidazolium chloride-Aluminum chloride can be used as a medium for the acylative cleavage of ethers, preparation of poly (p-phenylene) films via electropolymerization of benzene. It has found applicability in Al-ion batteries technology in recent times.

■ Lithium Iodide

Lithium iodide (LiI), is a compound of lithium and iodine. On exposing to air, it becomes yellow in color, owing to the oxidation of iodide to iodine. It crystallizes in the NaCl motif.

Lithium Iodide

CAS Number	10377-51-2
Chemical Formula	LiI
Molar mass	133.85g/mol
Appearance	White crystalline solid
Melting Point	446°C
Solubility	Soluble in water

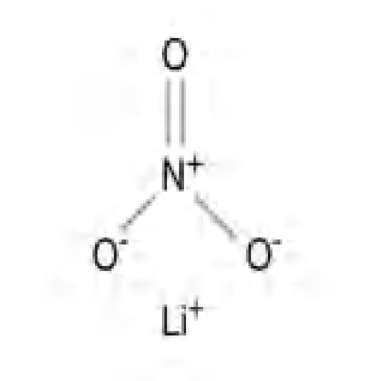
Source: Sigma Aldrich, Germany

Purification: Used as purchased without further purification. The purity of the chemical is >99.99%

Application: Lithium iodide is used as an electrolyte in high temperature batteries. It is significantly used for long-life batteries as per requirement, for example, in artificial pacemakers. The solid is very useful as a phosphor for neutron detection. It is also used, in a complex with iodine, in the electrolyte of dye-sensitized solar cells. LiI is very useful for cleaving C-O bonds in organic synthesis .

■ Lithium Nitrate

Lithium nitrate ($LiNO_3$) is the lithium salt of nitric acid. It appears as a white to light yellow colored crystalline solid. The salt absorbs water to form the hydrated form, lithium nitrate trihydrate.



Lithium Nitrate

CAS Number	7790-69-4
Chemical Formula	$LiNO_3$
Molar mass	68.95g/mol
Appearance	White crystalline solid
Melting Point	264°C
Solubility	Soluble in water

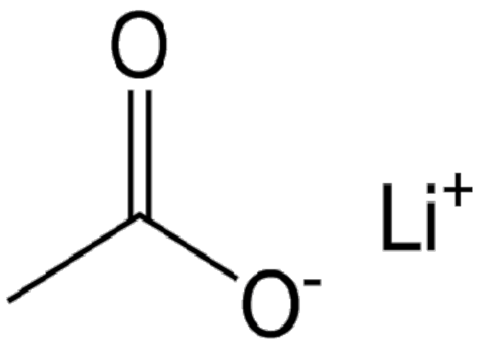
Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is 99.99%.

Application: The deliquescent, colourless salt is an oxidising agent, used for the production of red-colored fireworks and flares. The trihydrate form, can be used for thermal energy storage at its melt temperature of 303.3K. $LiNO_3$ has been used as an additive to the electrolytic solution of bis(trifluoromethane)sulfonimide in Lithium-Sulfur batteries for increasing the coulombic efficiency. It can be used in the sol-gel synthesis of spinel lthium titanate ($Li_4Ti_5O_{12}$).

■ Lithium Acetate

Lithium acetate is a white, crystalline salt of lithium and acetic acid. It is moderately soluble in water.



Lithium Acetate

CAS Number	**546-89-4**
Chemical Formula	$C_2H_3LiO_2$
Molar mass	65.99g/mol
Appearance	White crystalline solid
Melting Point	283-285°C
Solubility	Soluble in water

Source: Sigma Aldrich, Germany.

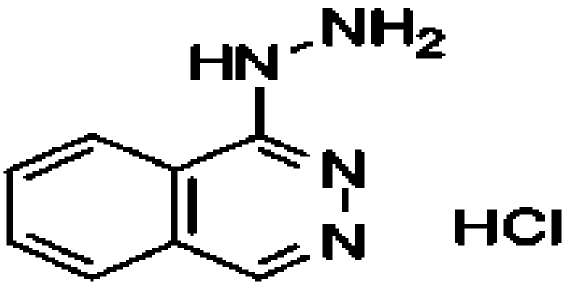
Purification: Used as parched. The purity is 99.95%.

Application: Lithium acetate is used to permeabilize the cellular wall of yeast for use in DNA transformation. In the laboratory, it is used as buffer for gel electrophoresis of DNA and RNA. It is believed that the beneficial effect of the salt is caused by its chaotropic effect, denaturing DNA, RNA and proteins.

3.1.4 BIOLOGICALLY ACTIVE MOLECULES/GUESTS:

■ 1-Hydrazinophthalazine Hydrochloride

The phthalazine derivative, Hydralazine Hydrochloride, is a hydrochloride salt of hydralazine, known to have antihypertensive and potential antineoplastic activities.



1-hydrazinophthalazine Hydrochloride

CAS Number	**304-20-1**
Chemical Formula	$C_8H_8N_4$. HCl
Molar mass	196.64g/mol
Appearance	White powder to crystal
Melting Point	273°C
Solubility	Soluble in water

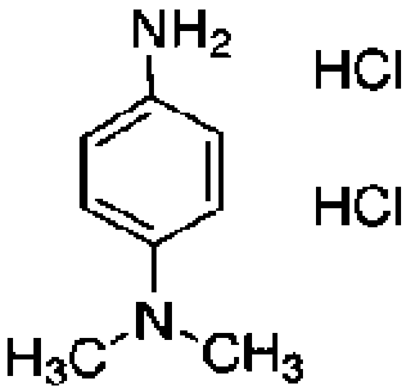
Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is >99.0%.

Application: Hydralazine is a drug of vasodilator family of medications. It is believed that the drug works by dilating the blood vessels. Hydralazine, sold under the name Apresoline among others, is a medication used to treat high blood pressure and heart failure including high blood pressure in pregnancy and a very high blood pressure causing in symptoms.

■ N, N-Dimethyl-p- phenylenediamine dihydrochloride

N,N-Dimethyl-p-phenylenediamine or more simply, Dimethyl-4-phenylenediamine is a substituted aniline and a diamine. It is a colorless to reddish-violet solid. It is made by nitrosylation of dimethylaniline followed by reduction.



N,N-dimethyl-p-phenylenediamine dihydrochloride

CAS Number	**99-98-9**
Chemical Formula	$C_8H_{12}N_2$
Molar mass	136.19g/mol
Appearance	Colorless to reddish-violet solid
Melting Point	34-36°C
Solubility	Soluble in water

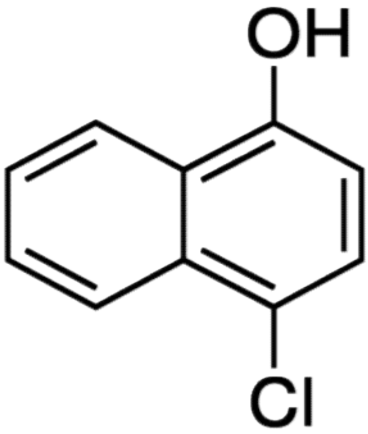
Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is >97%.

Application: N, N-Dimethyl-p-phenylenediamine is an aromatic amine mainly used as an intermediate to produce dyes and diazonium chloride salts. It is used in the production of methylene blue and photographic developer. It has been used as an accelerator for the vulcanisation of rubber and as an analytical reagent to detect chloramine in water. It can also be used for oxidase test. Its oxidation reaction with H_2O_2 in the presence of Fe(III) catalyst is used to detect trace quantities of iron(III) spectrophotometrically.

■ 4-Chloro-1-Napthol

4-Chloro-1-napthol is a needle shaped crystalline compound that may appear as off-white to gray to light brown.



4-chloro-1-napthol

CAS Number	604-44-4
Chemical Formula	$C_{10}H_7Cl$
Molar mass	178.61g/mol
Appearance	White crystalline solid
Melting Point	118-121°C
Solubility	Soluble in ethanol, acetone etc.

Source: Sigma Aldrich, Germany.

Purification: Used as parched. The purity is >98.0%

Application: 4-chloro-1-napthol is primarily used for the detection of proteins. A characteristic purple precipitate is obtained and the reactions can also be controlled easily. The chromgenic peroxidase substrate results in a precipitate that is blue to bluish purple in color and hence, can be observed visually. It can be used for western blotting and immunohistochemical staining. It was used in immunoblotting analysis during purification of native specific proteins of Taenia Grassiceps cysticerci antigens obtained by immunoaffinity chromatography. It may be used in Scanning Electrochemical Microscopy (SEM), for imaging of DNA hybridization on microscopic polypyrrole patterns. It can also be used as a substitute for benzidine compounds, which are considered to be carcinogenic.

3.2. EXPERIMENTAL METHODS

3.2.1. Preparation of solutions

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

To start with, solvent mixtures were prepared from pure components which were taken separately in glass stoppered bottles and thermostated for sufficient time at the required temperature. After the thermal equilibrium was insured, the utile volumes of each component were transferred in a different bottle which was already cleaned and dried methodically. Transformation of requisite mass of the relevant solvents to volume was skilled utilising experimental densities of the solvents at experimental temperature. Then the mixed contents were shaken well before experiments. While preparing diverse solvent mixtures care was taken to assure that similar course was adopted right through the whole work. The physical properties of varied pure and mixed solvents have been included in the relevant chapters.

3.2.2. Preparation of multi component liquid mixtures

The double and polycomponent liquid mixtures can be equipped by any one of the procedure given 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 respectively the mass and molecular mass of i^{th} component. The values of i depend on the number of components engaged 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 employing three methods as given below:

a. Using volume: By using the following relation, the volume fraction (ϕ_i) of the polycomponent liquid mixtures can be obtained

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

Where, V_i, stands for 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} signifies 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

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

Here, V^E refers to the volume of the liquid mixture.

3.2.3. Preparation of Inclusion Complexes

First of all, 20mL 1.0(mM) solutions of Hosts (α- and β-CD) were prepared separately with triply distilled, deionized and degassed water. The solutions were then allowed to stir for several hours on a magnetic stirrer. After that, 20mL 1.0(mM) solutions of guest molecules were added drop wise to the previously prepared solution of CD to make the ultimate equimolar mixture and were continued to stir for 2-3 days at 55-60°C. The suspensions obtained after cooling the mixture to 5°C, were filtered to get the white crystalline powder which were then dried in air and kept in a vacuum desiccator.

3.3. DETAILS OF THE INSTRUMENTS INVOLVED IN THE RESEARCH WORK

3.3.1. Measurement of mass

Various mass measurements were performed by using digital electronic analytical balance Mettler Toledo, AG 285, Switzerland.

Analytical balance Mettler Toledo, AG 285, Switzerland

It is efficient to determine mass with a high degree of precision and accuracy. The weighing pot is of exalted accuracy and precision (10^{-4}g) is kept inside a glass enclosed space. The sliding doors protect from dusts and air currents.

3.3.2. Water distiller

Water distiller, is a water treatment procedure used to produce contaminant free water. Water is converted into vapour before condensing it and returning it to the liquid state. Since water has a lower boiling point, during the evaporation process, impurities like bacteria, heavy metals, arsenic because are unable to turn into steam, and, hence, are eliminated. As the water transitions from a liquid to a gaseous state, these contaminants are left behind in the boiling chamber. Then the distiller cools the evaporated water, returning it to its liquid state in the form of mineral-free highly pure water. Distillation of water was made by using glass distillation unit, Bionics Scientific Technologies(P). Ltd.

Water distiller

To start the process, water is first poured into the boiling chamber. A heating element in the boiling chamber raise the temperature of water to a rolling boil. With rising the temperature of water, steam is produced. The upper part of the boiling chamber is vented, the rising steam is then allowed to travel through the vent into a condenser, leaving behind everything from fluoride to bacteria.

3.3.3. Thermostat

A Brookfield TC-550 thermostatic water bath was employed to control the temperature of experimental solutions. The temperature was maintained with an accuracy of ± 0.01 K of the desired temperature.

Brookfield TC-550 thermostatic water bath

Laboratory water baths have a digital or an analogue interface to allow users to set a desired temperature with greater uniformity, durability, heat retention and recovery. The vessel containing the material to be heated is placed into the one containing water that heats it. Their application includes reagents warming, substrates melting or cell cultures incubation, moreover, to enable certain chemical reactions to occur at high temperature. A water bath is always preferred for heating flammable chemicals instead of an open flame to avoid ignition.

3.3.4. Magnetic stirrer

A magnetic mixer or magnetic stirrer is a laboratory instrument. A rotating magnetic field is applied to cause a stir bar (or flea) dipped in a liquid to spin very rapidly, thus stirring it.

Magnetic stirrer by IKA

Magnetic stirrer cum hot plate made by IKA was employed to prepare different solutions as well as inclusion complexes.

3.3.5. Density Measurement

Densities of various solutions were measured by using an Anton Paar DMA 4500M digital density-meter. It is based on the oscillating U-tube principle and fluid densities can be measured over a wide range of temperature and pressure with a precision of $\pm 0.0005 g \cdot cm^{-3}$. The calibration of the instrument was done by using doubly distilled water and dry air.

Anton Paar DMA 4500M digital density-meter

The heart of this digital density meter is the measuring sensor (oscillator), usually a U-shaped tube made from borosilicate glass. The tube containing the sample is electronically excited to oscillate at its characteristic frequency, which depends on the density of the filled sample. Determination of true density of the sample is possible through a precise measurement of the characteristic frequency.

Either a system of magnets and coils or Piezo elements is usually used for the electronic excitation of the sensor.

3.3.6. Viscosity Measurement

Viscositiy(η) measurements were done utilising a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42. The viscosities were evaluated on the basis of the following equation,

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

Brookfield DV-III Ultra Programmable Rheometer

Where, *RPM*, *SMC* (0.327) and *TK* (0.09373) stands respectively for the speed, spindle multiplier constant and viscometer torque constant. A spindle dipped in the test fluid is allowed to drive through a calibrated spring. A rotary transducer is used to measure the spring deflection, which in turn, measures the viscous drag of the fluid against the spindle. The instrument is calibrated with standard viscosity samples, water and aqueous $CaCl_2$ solutions.

3.3.7. Refractive Index Measurement

The Mettler Toledo 30GS Digital Refractometer can meticulously determine the refractive index of liquids, providing temperature-compensated results. To get the results, either place the instrument on a flat surface and add a drop of sample onto the measurement cell or immerse the cell directly into the sample. As soon as the measurement key is pressed, the result is displayed in the desired units on the backlit LCD.

Digital Refractometer (Mettler Toledo 30GS)

The accuracy of the instrument is ± 0.0005. Calibration of the device is performed by measuring the refractive indices of double-distilled water, cyclohexane, toluene, and carbon tetrachloride at destined temperature.

3.3.8. Conductivity Measurement

The specific conductivity values were obtained by using a METTLER-TOLEDO Seven Multi conductivity meter with an uncertainty of ±1.0 μS m^{-1}.

Conductivity meter by METTLER-TOLEDO

The measurement is very simple and fast. The conductivity of a solution is highly temperature dependent, so it is important to calibrate the instrument at the same temperature as the solution being measured. HPLC-grade water with a specific conductance value of 6.0 μS m^{-1} is used for the conductivity measurement. A freshly prepared aqueous 0.01(M) KCl solution was used to calibrate the Systronics Type CD – 30 conductivity cell.

3.3.9. Surface Tension Measurement

Surface tension of various solutions were measured by using K9 digital TENSIOMETER (Krüss GmbH, Hamburg, Germany) with the accuracy of ±0.1 mN m^{-1}

Digital Tensiometer Krüss K9 (Germany)

The tensiometer uses platinum ring detachment technique (Du Noüy ring method). The process utilises slowly lifting a ring, made of platinum, from the surface of a liquid. The force required to raise the ring from the liquid's surface is measured and related to the liquid's surface tension. Measurements of surface tension necessitate a clean and dust-free atmosphere, otherwise, atmospheric pollutants could directly misstate the results.

3.3.10. FT-IR Spectra Measurement

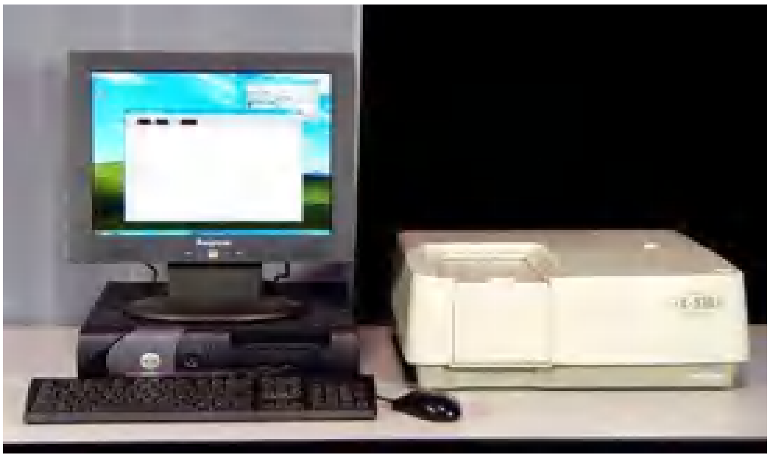
FTIR data were obtained by making use of a Perkin-Elmer FTIR spectrometer in the scanning range of 4000–400 cm^{-1}.

Perkin-Elmer FTIR spectrometer

The KBr disk method was utilized for the measurement of spectra. Disk methods utilize the disk's property of becoming clear to infrared light when pressure is applied, due to the plasticity of its alkali halides. KBr disks were prepared in 1:100 ratios of sample to KBr.

3.3.11. UV-Visible Spectra Measurement

UV-Vis spetrophotometer is used to study uitraviolet-visible spectroscopy. The intensity of light is measured after passing it through a sample, this intensity is then compared with the intensity of light before it passes through the sample. Molecules containing bonding and non-bonding electrons can absorb energy in the form of ultra-violet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The UV-visible spectra were recorded using JASCO V-530 and Agilent 8453 UV-Visible Spectrophotometer with a wavelength accuracy of ±0.5nm. Temperature was controlled using a digital thermostat during the experiment.



The UV-VIS spectrophotometer

The fundamental parts of such a spectrophotometer consists of a light source, a holder for the sample, a prism or a diffraction grating in a monochromator for separating different wave lengths of light, and a detector. A tungsten lamp is used as the radiation source for the visible light, while, it is a deuterium arc lamp for the ultraviolet region. JASCO V-530 is a double beam spectrophotometer and the light splits into two beams before it reaches the sample. One beam passes through the sample, while, the other is used as the reference. In a single-beam instrument like Agilent 8453, the cuvette containing only a solvent has to be measured first.

3.3.12. High Resolution Mass Spectrometric Measurement

Combined with fragmentation of the chemicals and accurate mass measurement of the pieces, High Resolution Mass Spectroscopy (HRMS) is invaluable to prove the identity of a chemical. HRMS spectra of the solid ICs was mesured by using Quadrupole time-of-flight (Q-TOF) high-resolution instrument with positive-mode electrospray ionization. Methanol solution of the solid ICs were employed for such spectral measurement.

High Resolution Mass Spectrometer

The ion source causes the ionisation of the material under analysis. The transportation of the ions to the mass analyzer is associated with a high voltage maintained by magnetic or electric fields. Different mass spectrometric techniques can be used depending on the type of samples under study. Chemical ionization and electron ionization are used for vapours and gases.

3.3.13. Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is used to scan a focused electron beam over a surface by creating an image. Various signals formed by the interaction of electrons in the beam with the sample can be used to get information about the surface topography and composition. JEOL JSM IT 100 was used for the determination of surface topography of the samples at different resolutions.

JEOL JSM IT 100

For standard imaging within the SEM, specimens should be electrically conductive, at least at the surface, and electrically grounded to restrict building up of electrostatic charge. If the material is non-conducting, then it is coated with an ultrathin coating of an electrically conducting material, which is finally deposited on the sample either by low-vacuum sputter coating or through the high-vacuum evaporation. Conductive materials in current used for the specimen coating includes platinum, gold, gold/palladium alloy, iridium, tungsten, chromium, osmium, and graphite. In addition to morphological, topological, and compositional data, a Scanning Electron Microscope can also spot and analyse surface fractures, examine surface contaminations, provide information in microstructures, reveal spatial variations in chemical compositions.

3.3.14. Fluorescence Spectra Measurement

Fluorescence spectra was recorded using the spectrofluorimeter Quantamaster-40 from photon technologies International, USA. A Hellma quartz cuvette with an optical path length 1.0cm was applied.

Spectrofluorimeter Quantamaster-40

Fluorescence, is a kind of luminescence due to the photons exciting a molecule, trekking it to an excited electronic state. A beam with a wavelength varying between 180 and ~800 nm passes through a solution in a cuvette. The absorption of photons promotes the singlet ground state to a singlet-excited state. On returning of the excited molecule to ground state, photon of lower energy emits. This corresponds to a longer wavelength, compare to the absorbed photon. Both an excitation spectrum and an emission spectrum can be measured by means of fluorescence spectrometry. The intensity of the emission is directly proportional to the concentration of the analyte.

3.3.15. ^{1}H NMR and 2D ROESY Spectroscopic Measurement

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

Bruker AVANCE 500

NMR Spectrometer can be used for studying the structure of molecules, kinetics or molecular dynamics, molecular interactions. Nuclei containing an odd mass number or an odd atomic number possess a net nuclear spin. Whenever a charged particle like a nucleus is in motion, it develops a magnetic field around it. When the nuclei with non-zero spins placed in a strong magnetic field with respect to the applied magnetic field with the supply of appropriate energy, these nuclei flip to a higher energy state from lower energy state. The difference of energy between the two states is governed by the applied field. The energy absorbed during such a transition is a function of the type of nucleus and the chemical environment around it within the molecule. The increasing magnetic field causes the excitation or flipping of the nuclei from an orientation to another and this can be detected as an induced voltage that results from the absorption of energy by the nucleus from the radio frequency field. The free induction decay, in time domain gives rise to an equivalent frequency domain signal on Fourier transformation. The area under the peak has been found to be proportional to the number of nuclei flipping. One can know about the structure of a molecule by observing the field strength at which absorption of energy by protons takes place.