CHAPTER III

EXPERIMENTAL SECTION

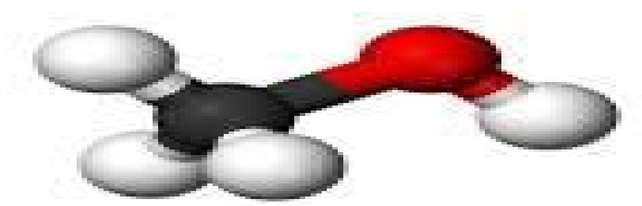
3.1. NAME, STRUCTURE, PHYSICAL PROPERTIES, PURIFICATION AND APPLICATIONS OF THE SOLVENTS AND SOLUTES USED IN THE RESEARCH WORK

3.1.1. SOLVENTS

The liquid solvents used in my research work are depicted below

i. Methanol

Methanol or Carbinol is a simplest primary alcohol with the formula CH_3OH. It is produced naturally in small amounts during many fermentation processes as well as catalytic process. This is colorless, flammable and highly toxic liquids with distinct odor.



***Source*:** Sigma Aldrich, Germany

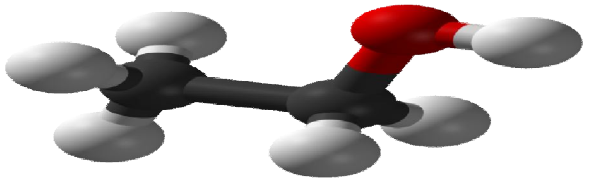
***Purification*:** It was dried by passing molecular sieve and then distilled by appropriate method [1].

Appearance:	**Colourless liquid**
Molecular Formula:	**CH_4O**
Molecular Weight:	**32.04 g/mol**
Boiling Point:	**337.7 K**
Melting Point:	**175.40 K**
Dielectric Constant:	**32.70 at 293.15 K**

Application: It is used for solvent, fuel and producing biodiesel. About 40% methanol converted to formaldehyde and from which various products are obtained like, plastics, plywood, textile and paint industries. Methanol is also used as a energy carrier.

ii. Ethanol

Ethanol is an organic compound with formula C_2H_6O and mainly used as a solvent. It is a colorless clear liquid with a characteristics odor. It is a psychoactive substance and is the principal type of alcohol found in alcoholic drinks.



***Source*:** Sigma Aldrich, Germany

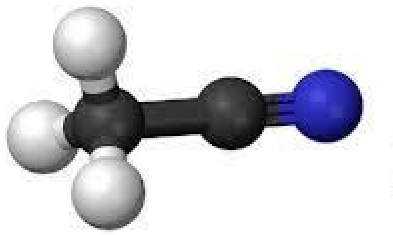
***Purification*:** It was dried by adding drying agent (e.g. $CaSO_4$, molecular sieves etc.) followed by filtration and then distilled [1].

Appearance:	**Colourless liquid**
Molecular Formula:	**C_2H_6O**
Molecular Weight:	**46.07 g mol^{-1}**
Boiling Point:	**351.37 K**
Melting Point:	**158.9 K**
Dielectric Constant:	**25.08 at 298.15K**

Application: Ethanol is a grain alcohol that can be blended with gasoline and used in motor vehicles. Many gasoline stations provide a blended fuel, which typically is 10% ethanol and 90% gasoline. Vehicles do not need any modifications to use this blend of fuel.

iii. Acetonitrile

Acetonitrile is a simplest organic nitrile and colorless liquid. It is a polar protic solvent. It is produced mainly as a byproduct of acrylonitrile manufacture.



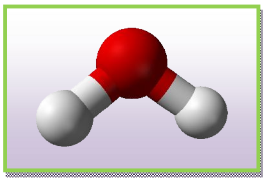
Appearance:	Liquid
Molecular Formula:	C_2H_3N
Molecular Weight:	41.05 g mol^{-1}
Boiling Point:	355 K
Melting Point:	228 K
Dielectric Constant:	37.50 at 293.15 K

Source: Sigma Aldrich, Germany

Purification: It was dried from P_2O_5 and then from CaH_2 distillation procedure [2].

Application: Its low viscosity and low chemical reactivity make it suitable for chromatographic purpose. It is widely used in battery industries because of the relatively high dielectric constants. Also it used as a common solvent for organic synthesis.

iv. Water



Water is a ubiquitous chemical substance that is composed of hydrogen and oxygen and is essential for all known forms of life. Commonest form of water is liquid built water has also other two for solid and gas. In solid state it forms ice and in gaseous state it form vapor of water. Water is a good solvent and is often known as the universal solvent.

Source: Doubly distilled water.

Purification: Water was firstly deionised and then distilled by 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 doubly distilled water had specific conductance less than 1×10^{-6} $S.cm^{-1}$ [1].

Appearance:	**Liquid, colorless**
Molecular Formula:	**H_2O**
Molecular Weight:	**18.02 g mol^{-1}**
Boiling Point:	**100 ^0C**
Melting Point:	**0^0C**
Dielectric Constant:	**78.35 at 298.15 K**
pKa	**13.995**
Density	**0.9998396 g/mL(0 °C) 0.9970474 g/mL (5 °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**

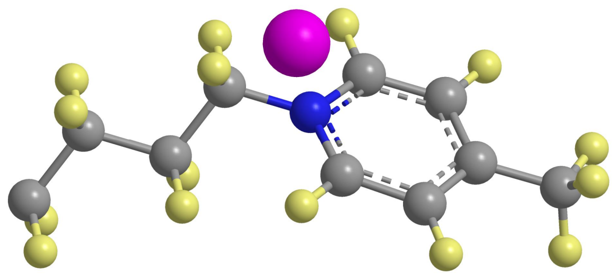
Application: Water is also a good solvent due to its polarity. The solvent properties of water are vital in biology, because many biochemical reactions take place only within aqueous solutions due to the universal solvent property. In addition, water is used to transport biological molecules. The most important use of water in agriculture is for irrigation. Water fit for human consumption is called drinking water. Water is widely used in chemical reactions as a green solvent and minutely as catalyst. In inorganic reactions, water is a common major solvent, solubilizes many ionic compounds. In organic reactions, it is not usually used as a reaction solvent, because it does not dissolve the organic substances. Nevertheless, these properties are sometimes desirable. Also, water accelerates the Diels-Alder reactions. Supercritical water has recently been a topic of research interests.

3.1.2. SOLUTES (ELECTROLYTES AND NON-ELECTROLYTES)

The electrolytes like ionic and salts of biologically active molecules liquids and the non-electrolytes, surface active molecules, cyclodextrins and crown ethers have been described below.

i. 1-Butyl-4-methylpyridinium chloride

1-butyl-4-methylpyridinium chloride is the pyridine based ionic liquid, of molecular formula $C_{10}H_{16}ClN$, containing butyl group with one active nitrogen atom in the six member ring, exist as a molten liquid phase with the melting point ≥ 70^{o} C. .



Source: Sigma Aldrich, Germany

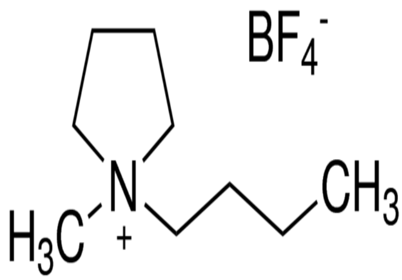
Purification: Used as purchased. The purity of the chemical is >99.0%

Appearance:	**Colorless Crystalline**
Molecular Formula:	**$C_{10}H_{16}ClN$**
Molecular Weight:	**185.69 g mol^{-1}**
Melting point	**431 K**
CAS Number	**112400-86-9**

Application: The ionic liquid are good examples of neoteric solvents, or older materials that are finding new applications as solvents, which is environmentally friendly because they are less hazardous for human body as well as less toxic for living organisms, used as recyclable solvents for organic reactions and separation processes, lubricating fluids, heat transfer fluids for processing biomass and electrically conductive liquids as electrochemical device in the field of electrochemistry.

ii. 1-butyl-1-methylpyrrolidinium tetrafluoroboarte

1-butyl-1-methylpyrrolidinium tetrafluoroboarte is the pyrrolidinium based ionic liquid, of molecular formula $C_9H_{20}BF_4N$ containing methyl and butyl group with one positive charge nitrogen atom in the ring, exist as solid.



Source: Sigma Aldrich, Germany

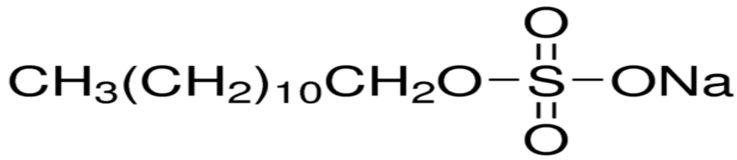
Purification: Used as purchased. The purity of the chemical is >99.0%

Appearance:	**Solid**
Molecular Formula:	**$C_9H_{20}BF_4N$**
Molecular Weight:	**229.07 g mol^{-1}**

Application: The ionic liquid are good examples of neoteric solvents, new types of solvents, or older materials that are finding new applications as solvents, which is environmentally friendly (or eco-friendly) because they are less hazardous for human body as well as less toxic for living organisms, used as recyclable solvents for organic reactions and separation processes, lubricating fluids, heat transfer fluids for processing biomass and electrically conductive liquids as electrochemical device in the field of electrochemistry (batteries and solar cells)

iii. Sodium dodecyl sulfate

Sodium dodecyl sulfate is a popular anionic surfactant of molecular formula $NaC_{12}H_{25}SO_4$, containing sodium counterion, dodecyl sulfate part, exist as a solid phase with the melting point 206^{O}C (403^{O}F; 479K).



Source: Sigma Aldrich, Germany

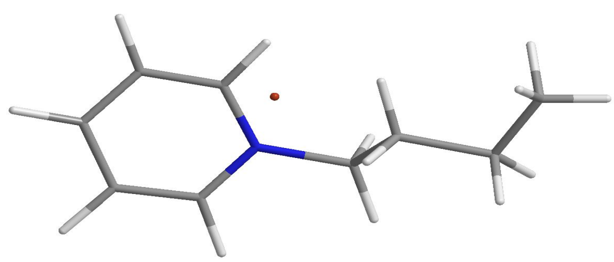
Purification: Used as purchased. The purity of the chemical is >98.0%

Appearance:	**Solidified mass (off white)**
Molecular Formula:	**$NaC_{12}H_{25}SO_4$**
Molecular Weight:	**288.38 g mol^{-1}**

Application: Sodium dodecyl sulfate (SDS) detergent is used in Biotechnology for frequent applications, most commonly, the strongly anionic detergent SDS is used in blend with a reducing agent and heat to dissociate the proteins before they are loaded on the gel. Sodium dodecyl sulfate is a widely used surfactant in cleaning products, cosmetics, and personal care products. SLS is a highly effective anionic surfactant used to remove oily stains and residues.

iv. 4-Methylpyridine

4-Methylpyridine is the organic compound with the formula $CH_3C_5H_4N$. It is one of the three isomers of methylpyridine, containing methyl group at 4 position of pyridine. Nitrogen atom is present in the six member ring, exist as liquid.



Source: Sigma Aldrich, Germany

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

Appearance:	**Liquid**
Molecular Formula:	**C_6H_7N**
Molecular Weight:	**93.13 g mol^{-1}**
Melting Point:	**275.5K**

Application: It is used as a solvent in synthesis of pharmaceuticals, resins, dyestuffs, rubber accelerators and pesticides. It is also used in the production of antituberculosis substance, isoniazid; waterproofing agents fo fabrics; as a laboratory reagent, catalyst and curing agent.

v. Trigonelline hydrochloride

This is a pyridine alkaloid has been claimed to have hypocholesterolemic activity along with potential hypoglycemic effect. This is also a natural glucosidase inhibitor, which may help to reduce synthetic drug dependence for diabetic treatment and also help reduce the resulting side effects

Appearance:	**Crystalline white**
Molecular Formula:	$C_7H_8ClNO_2$
Molecular Weight:	**173.60 g/mol**
Melting Point:	**260 ^{o}C**

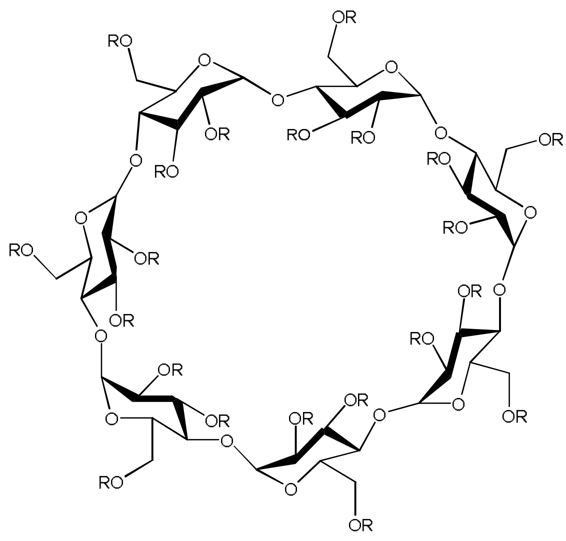
Source: Sigma Aldrich, Germany

Purification: Used as purchased.

Application: Trigonelline (*N*-methylpyridinium-3-carboxylate) is a pyridine alkaloid known to contribute diverse regulatory functions such as plant cell cycle regulation, nodulation, oxidative stress, growth of the plant. The importance of trigonelline has also been well documented as a precursor of flavor and aroma compounds.

vi. (2-Hydroxylpropyl)-β-cyclodextrin

(2-Hydroxylpropyl)-β-cyclodextrin is a hydroxypropyl derivative of β-cyclodextrin. The advantage of this chemical is advance water solubility compare to the precursor β-cyclodextrin. It is non toxic to human body and its inclusion complex is much more soluble compare to the inclusion complex of pure β-cyclodextrin.



Source: TCI, Japan

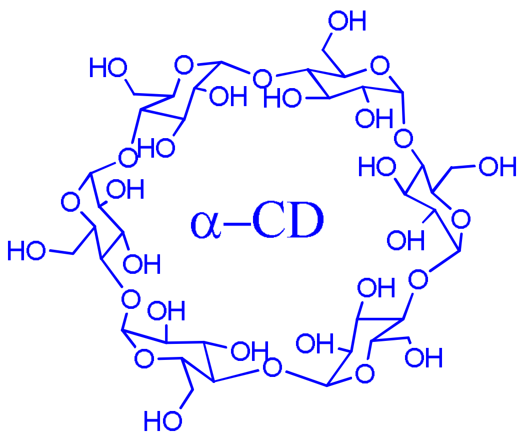
Purification: Used as purchased

Appearance:	**Amorphous white**
Molecular Formula:	$C_{63}H_{112}O_{42}$
Molecular Weight:	**1541.54 g/mol**
Melting Point:	**278 °C**

Application: HP (2-Hydroxylpropyl)-β-CD has been successfully used to improve the pharmaceutical properties of active pharmaceutical Ingredients (APIs) and exhibits a better potential for application than β-CD. HP-β-CD has a significant potential to enhance the solubility, stability, and bioavailability of active components separated from herbs. HP-β-CD is safe excipients without toxicity and well tolerated by oral, intravenous, dermal, ocular and parenteral. It is included in the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) pharmacopoeias and it is cited in the EMA's list of inactive pharmaceutical ingredients.

vii. Alpha Cyclodextrin (α-CyD)

α- CyD is naturally occurring polysachharides of six glucose units and they are covalently attached via end to end α-1,4 linkage. It has hydrophobic inner cavity and hydrophilic outer surface. In aqueous medium hydrophobic inner core allow it to form host-guest inclusion complex with suitable hydrophobic molecules. Most of the cases it forms 1:1 inclusion complex due to its small cavity volume.



Source: Sigma Aldrich, Germany

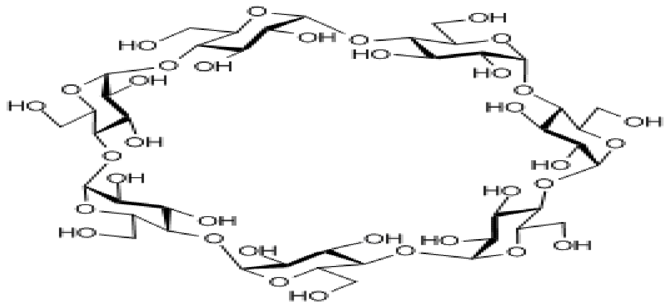
Purification: Used as parched. The purity is 99%.

Appearance:	**Crystalline Powder**
Molecular Formula:	$C_{36}H_{60}O_{30}$
Molecular Weight:	**972.84 g/mol**
Melting Point:	**>551 K**
Solubility in water	**145 g/L**
CAS Number	**10016-20-3**

Application: α-Cyclodextrin is widely applied in manufacture of medicine and food. It is used in cosmetics, paint, and textile industries as well. 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 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. α-cyclodextrin is commonly used as a complexing agent in hormones, vitamins, and many bioactive compounds frequently used in tissue and cell culture applications.

viii. Beta Cyclodextrin (β-CD)

β- Cyclodextrin is finely made from pure provision material-starch and translate enzyme, which is white powder and the molecular structure is like a cylinder compounded from seven glucose unit attached with the glycosidic linkage. The function of β-Cyclodextrin depends on its cylinder molecule structure which can be easy to integrate other materials. That feature is applied extensively in industry



***Source**:* Sigma Aldrich, Germany

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

Appearance:	**Crystalline Powder**
Molecular Formula:	**$C_{42}H_{70}O_{35}$**
Molecular Weight:	**1134.98 $gmol^{-1}$**
Melting Point:	**563.15-573.15 K**
Boiling Point	**1814.33 K**
Relative Density	**1.44 $g.cm^3$ at 200^0C**
CAS Number	**7585-39-9**
Solubility in water	**18.5 gL^{-1}**

Application: β-Cyclodextrin is a new stuff which can be widely applied in production of medicine and food. It can be applied widely in production of medicine, food and cosmetics, whose functions are improved stability, solubility and good smelled. 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 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. 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 most commonly used as a complexing agent in hormones, vitamins, and many compounds. This potential has also been supports for different applications in medicines, cosmetics, food technology, pharmaceutical, and chemical industries as well as in agriculture and environmental industries [8].

3.2. EXPERIMENTAL METHODS

3.2.1. PREPARATION OF SOLUTIONS

A stock 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 $mol \cdot dm^{-3}$.

3.2.2. PREPARATION OF SOLVENT MIXTURES

The research work has been carried out with binary or ternary solvent systems with, methanol, acetonitrile and cellosolves etc. as primary solvents with some polar, weakly polar and non-polar solvents as well as with some electrolytes (ionic liquids & other electrolytes) and non-electrolytes (cyclodextrins and their derivative, crown ethers etc).

For the preparation of solvent mixture, pure components were taken separately in glass stopper 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.

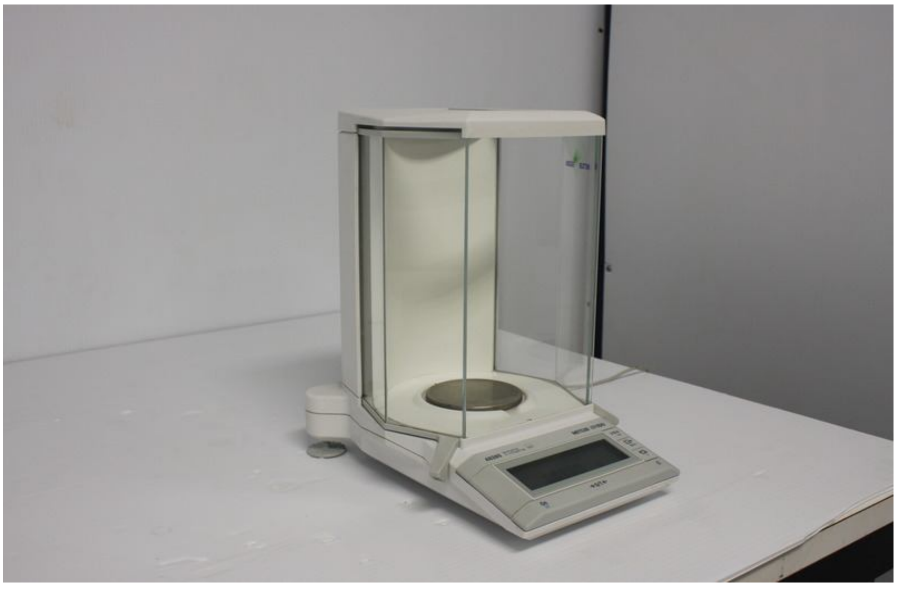
The following different binary and ternary solutions have been prepared and used for my research studies.

Binary Solutions:
Aqueous mixture of α-Cyclodextrins
Aqueous mixture of β-Cyclodextrins
Aqueous mixture of HP-β-Cyclodextrins

Ternary Solutions:
Trigonelline hydrochloride + Aqueous mixture of α-Cyclodextrins
Trigonelline hydrochloride + Aqueous mixture of β-Cyclodextrins
1-butyl-4-methylpyridinium lauryl sulfate + β-Cyclodextrins
1-dodecyl-4-methylpyridiniumiodide + β-Cyclodextrins
1-dodecyl-4-methylpyridiniumiodide + HP-β-Cyclodextrins

3.2.3. MASS MEASUREMENT

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



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.

3.2.4. DENSITY MEASUREMENT

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

In the digital density meter, the mechanic oscillation of the U-tube is e.g. electromagnetically transformed into an alternating voltage of the same frequency. The period τ can be obtained by the following relation with ρ of the sample in the oscillator [10]:

$$\rho = A \cdot \tau^2 - B \quad (1)$$

A and B are the respective instrument constants of each oscillator. The values are calculated by calibrating with two substances of the precisely known densities with ρ_1 and ρ_2. Modern instruments calculate and store the constants A and B after the two calibration measurements, which are checked with air and water condition. The instrument was calibrated by double-distilled water and dry air.

3.2.5. VISCOSITY MEASUREMENT

The 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

$$\eta = (100 / RPM) \times TK \times \text{torque} \times SMC \quad (2)$$

Here, *RPM*, *TK* (0.09373) and *SMC* (0.327) are speed in rpm, viscometer torque constant and spindle multiplier constant respectively. The instrument was calibrated by the standard samples supplied with the instrument and compared with the standard solutions of aqueous $CaCl_2$ solutions. The temperature was maintained to within ± 0.01°C using Brookfield Digital TC-500 thermostat bath. The viscosities were measured with an accuracy of ± 1 %. Each measurement reported herein is an average of triplicate reading with a precision of 0.3 %.

3.2.6. TEMPERATURE CONTROLLER

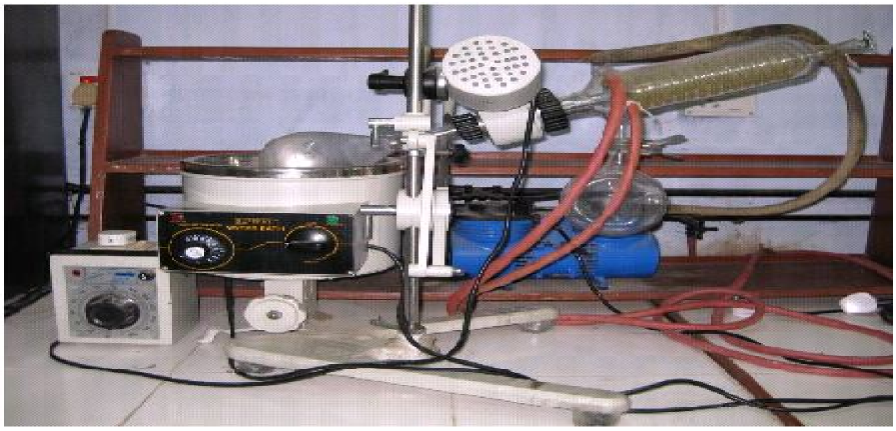
Experimental measurements were carried out in thermostatic water bath (Science India, Kolkata) maintained with an accuracy of ± 0.01 K of the desired temperature.

Laboratory water bath is a system in which a container containing water and the investigated solution is placed over this container to quickly heat. These laboratory equipments are available in different volumes and construction with both digital and analogue controls and greater temperature uniformity, durability, heat retention and recovery. The chambers of water bath lab products are manufactured using highly resistant stainless steel and other metals.

Water Distiller (Borosil Glass Works Limited, India):

Water Distiller is usually made by the Borosil Glass material. A distilining solvent in the boiling chamber heats the water until it boils off. The vapour rises from the boiling chamber. Volatile contaminants are discharged through a built-in vent tube. Minerals and salts are retained in the boiling chamber as hard deposits. The steam passed into the condenser, which is condensed by cool water. Droplets of water poured into the collecting container.

Rotary Vacuum Flash Evaporator (Superfit, An ISO 9001:2000 Certified Company)



Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents such as n-hexane or ethyl acetate from solid or mix compounds at atmospheric conditions. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there and a sufficient diversity in boiling points at the selected temperature and reduced pressure.

3.2.7. CONDUCTIVITY MEASUREMENT

Systronics Conductivity TDS meter-308 is used for measuring specific Conductivity of electrolytic solutions. It can provide both automatic and manual temperature compensation.

The conductance measurements were carried out on this conductivity bridge using a dip-type immersion conductivity cell of cell constant 1.11cm^{-1}. The entire conductance data were reported at 1 KHz and was found to be ±0.3 % precise. The instrument was standardized using 0.1(M) KCl solution. The cell was calibrated by the method of Lind and co-workers. The conductivity cell was sealed to the side of a 500 cm^3 conical flask closed by a ground glass fitted with a side arm through which dry and pure nitrogen gas was passed to prevent admission of air into the cell when solvent or solution was added. The measurements were made in a thermostatic water bath maintained at the required temperature with an accuracy of ± 0.01 K by means of mercury in glass thermo regulator [11].

Several solutions were prepared by weight precise to ± 0.02 %. The weights were taken on a Mettler electronic analytical balance (AG 285, Switzerland). The molality being converted to molality as required. Due correction was made for the specific conductance of the solvents at desired temperatures. The following figure shows the Block diagram of the Systronics Conductivity-TDS meter 308.

3.2.8. REFRACTIVE INDEX MEASUREMENT

Refractive index was being measure with the help of Digital Mettler Toledo 30GS Refractometer.

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.

3.2.9. FT-IR MEASUREMENT

Fourier transform Infrared spectra (FTIR) were recorded in KBr pellets & ethanol with a PerkinElmer FT-IR spectrometer (RX-1)

with a resolution of ± 0.25 cm^{-1} in the region of 400-4000 cm^{-1} at room temperature (25 ^{0}C) with 49-54 % humidity. This KBr optics based instrument records data in different modes (KBr pellets, Nujol mull, and non-aqueous solutions).

The intensity of light (I_0) entering through a blank is measured. The intensity of light (I) entering through the sample solution is measured. The investigated data is used to estimate two quantities: the transmittance (T) and the absorbance (A).

$$T = \frac{I}{I_0} , \quad A = -\log_{10} T \qquad (3)$$

The transmittance is simply the fraction of light in the original beam that passes through the sample and reaches the detector section of the instrument.

3.2.10. SURFACE TENSION

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·m^{-1}.

Temperature of the system has been preserved by circulating auto-thermostated water (within ± 0.01K) through a double-wall glass vessel holding the solution.

3.2.11. UV-VIS 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.

The UV-VIS spectrophotometer uses two light sources, a deuterium (D2) lamp for ultraviolet light and a tungsten (W) lamp for visible light. After bouncing of a mirror, the light beam passes through a slit and hits a diffraction grating. The grating can be rotated allowing for a specific wavelength to be selected. At any exact orientation of the grating, only monochromatic or single wavelength successfully entrances through a slit. A filter is used to eliminate unnecessary higher orders of diffraction. The light beam strikes a second mirror before it gets split by a half mirror (half of the light is reflected and the other half passes through). One of the beams is allowed to pass through a reference cuvette, the other passes through the sample cuvette. The intensities of the light beams are then considered at the end. Regarding this the Beer-Lambert law has been stated below.

Beer-Lambert Law

The change in intensity of light (dI) after passing through a sample should be proportional to the following:

(i) Path length (b), the longer the path, more photons should be absorbed

(ii) Concentration (c) of sample, more molecules absorbing means more photons Absorbed (iii) Intensity of the incident light (I), more photons means more opportunity for a molecule to see a photon. Thus, dI is proportional to bcI or $dI/I = -kbc$ (where k is a proportionality constant, the negative sign implies decrease in intensity of the light, this makes b, c and I the entire time positive. Integration of the above equation leads to Beer-Lambert's law:

$$- \ln I/I0 = kbc \qquad (4)$$

$$- \log I/I0 = 2.303kbc \qquad (5)$$

$$\varepsilon = 2.303k \qquad (6)$$

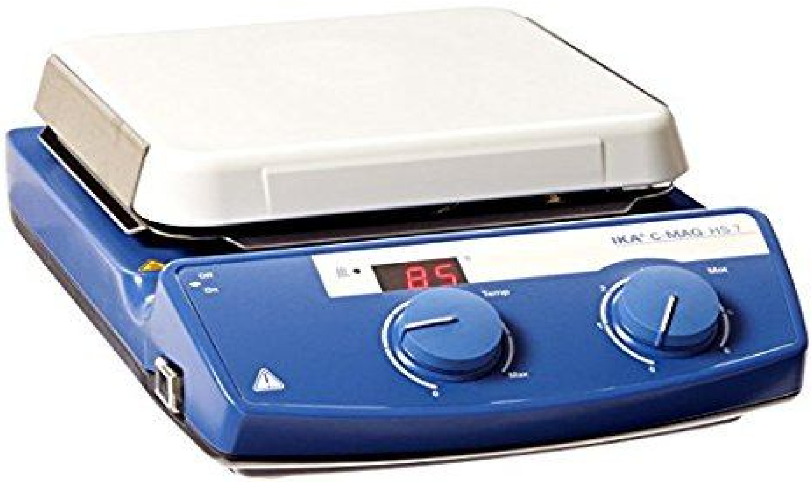
$$A = - \log I/I0 \qquad (7)$$

$$A = \varepsilon bc \qquad (8)$$

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

3.2.12. MAGNETIC STIRRER FOR PREPARATION OF SOLUTION AND SOLID INCLUSION COMPLEXES

The solutions of various bio-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.



3.2.13. NUCLEAR MAGNETIC SPECTRA MEASUREMENT

Nuclear Magnetic Spectra (NMR) spectroscopy is used to study the structure of molecules, the kinetics or dynamics of molecules and the composition of mixtures of biological or synthetic solutions or composites ^{1}H NMR spectra were recorded at 400MHz and 500 MHz using Bruker Avance instrument. Signals are quoted as δ values in ppm using residual protonated solvent signals as internal standard (D_2O: δ 4.79 ppm). Data are reported as chemical shifts.

3.2.14: POWDER X-RAY DIFFRACTION SPECTROSCOPY

When an atom is bombarded with sufficiently high energy electrons, electrons of atom are knocked out from their shell (excited state, unstable), leads to the transition of electrons to fill up the vacancy (ground state, stable). Each electron transition generates X-rays of a specific energy (with wave length in range from 0.1Å to 100Å) equivalent to that shell. Rigaku, Model: Micromax-007HF was aided to perform the analysis.

3.2.15. 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™ S47 - dual meter pH / conductivity meter

Reproducible results

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

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

Professional calibration

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

Electrode test

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

3.2.16: SCANNING ELECTRON MICROSCOPY

Structure determination along with tiny electron beam scanned across surface of specimen, backscattered or secondary electrons detected, signal output to synchronized display. Scanning electron microscope (SEM) instrument used was of Jeol JSM-IT 100, connected with EDS compartment with detector input area of 20 mm^2 (Oxford).

3.2.17: TRANSMISSION ELECTRON MICROSCOPY

Transmission Electron Microscope (JEM2100) was aided to collect the data's in the investigations. Real (Image) and reciprocal space (diffraction pattern) information can be obtained from the same region of sample. Lower resolution/large area techniques was initially performed to get a 'broad picture' about sample. This includes XRD, SEM. We can start with optical microscopy. On 'usual' samples conventional TEM was performed before trying out HRTEM. Thus we can get spectroscopic information and use it for forming images or diffraction patterns.