

CHAPTER III

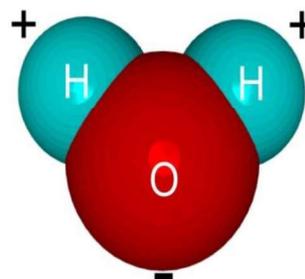
EXPERIMENTAL SECTION

3.1. Detailsdescription of Different Solvents and Solutes used in the Research Work

i. Water



Appearance:	Liquid
Molecular Formula:	H ₂ O
Molecular Weight:	18.02 g/mol
Boiling Point:	100 °C
Melting Point:	0 °C
Dielectric Constant:	78.35 at 298.15 K



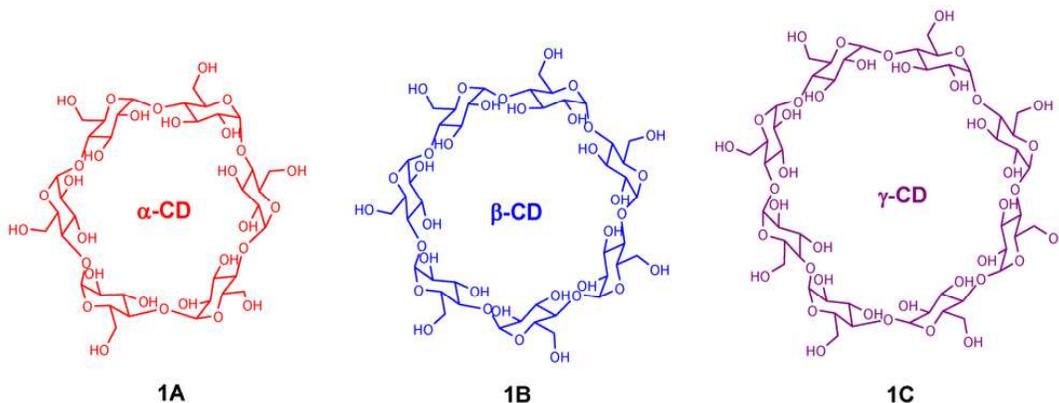
Water is a omnipresent substance that is composed of hydrogen and oxygen and is essential for living organism. Water is known as universal solvent as it can dissolve most organic and inorganic compounds.

Source: distillation of water.

Purification: Water was deionised using suitable process and then distilled doubly using distilling set along with alkaline KMnO_4 . Contamination from CO_2 and other impurities are avoided by adopting sufficient precautions.

Application: Water is polar and amphoteric in nature. In addition to that, presence of H-bond made water as universal solvent. Water is essential in living organism because most of the biochemical process take place in aqueous medium. Water in our biological systems acts as biomolecule carrier especially in blood, cellular membrane and body fluid etc. Water is used in organic and inorganic reactions as a green solvent.

ii. Alpha Cyclodextrin (α -CD)



Appearance:	Crystalline Powder
Molecular Formula:	$\text{C}_{36}\text{H}_{60}\text{O}_{30}$
Molecular Weight:	972.84 g/mol
Melting Point:	>551 K

Alpha Cyclodextrin (α -CD) is a polysaccharides of six glucose units joined with α -1,4 linkage. Its inner cavity is of hydrophobic character and outer surface is of hydrophilic character. It forms host-guest inclusion complex with suitable molecule which has a hydrophobic part. It may form inclusion complex of 1:1, 1:2, 2:1 stoichiometry depending upon nature and size of the guest molecule.

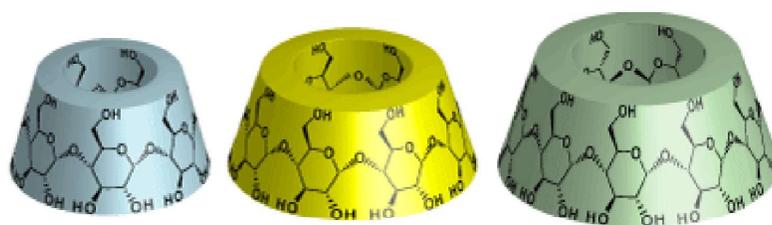
Source: Sigma Aldrich, Germany

Purification: The mass purity is 99.98% and used as purchased.

Application: α -Cyclodextrin has wide applicability in formulation of drugs and food in pharmaceutical industries. It also used in others industries such as cosmetics, textile, and paint industries. It improves the solubility of medicine, increases the stability of medicine. In food industries it is used to remove bad smell of food and to improve the stability of food. It is also used as a complexing agent for bioactive compounds such as hormones, vitamins etc. used for tissue and cell culture purposes.

iii. Beta Cyclodextrin (β -CD)

Beta Cyclodextrin (β -CD) is a polysaccharides of seven glucose units joined with α -1,4 linkage. Its inner cavity is of hydrophobic character and outer surface is of hydrophilic character. It forms host-guest inclusion complex with suitable molecule which has a hydrophobic part. It may form inclusion complex of 1:1, 1:2, 2:1 stoichiometry depending upon nature and size of the guest molecule.

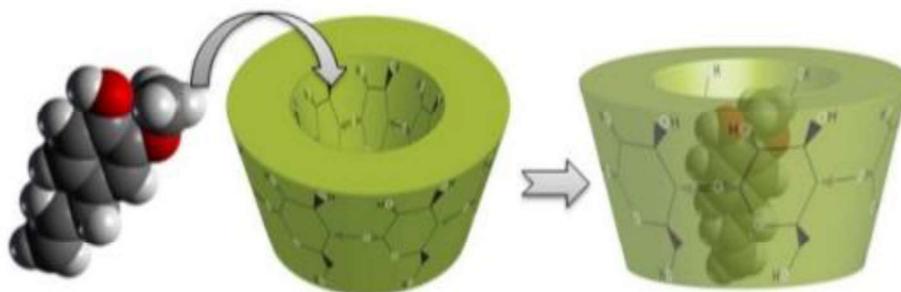


	α -CD	β -CD	γ -CD
No. of Glucose Units	6	7	8
Cavity Diameter (nm)	0.47	0.60	0.75
Height of Torus (nm)	0.79	0.79	0.79

Source: Sigma Aldrich, Germany

Purification: The mass purity is 99.98% and used as purchased.

Appearance:	Crystalline Powder
Molecular Formula:	C₄₂H₇₀O₃₅
Molecular Weight:	1134.98 g/mol
Melting Point:	563.15-573.15 K
Boiling Point	1814.33 K
Relative Density	1.44 g.cm³ at 200 C



Application: β -Cyclodextrin has wide applicability in formulation of drugs and food in pharmaceutical industries. It also used in others industries such as cosmetics, textile, and paint industries. It improves the solubility of medicine, increases the stability of medicine. In food industries it is used to remove bad smell of food and to improve the stability of food. It is also used as a complexing agent for bioactive compounds such as hormones, vitamins etc. used for tissue and cell culture purposes [3-6].

iv. Paracetamol

Properties	
Chemical formula	C ₈ H ₉ N ₀₂
Molar mass	151.163 g mol ⁻¹
Appearance	White
Density	1.263 g cm ⁻³
Melting point	169°C
Solubility in water	18 g L ⁻¹
Refractive index (n_D)	1.4936
Dipole moment	0.1271305813 D



Source: Sigma Aldrich, Germany

Purification: The mass purity is 99.98% and used as purchased.

Application: It is a drug used to take care of pain and fever. It is naturally used for gentle to modest pain relief. Evidence for its use to get alleviate fever in children is mixed. It is often sold in recipe with other medication such as in many cold medications. In blend with opioid pain medication, paracetamol is also used for ruthless pain such as cancer pain and pain after surgery. It is classically used either by mouth or rectally, but is also obtainable intravenously. Effects last between 2 and 4 hours.

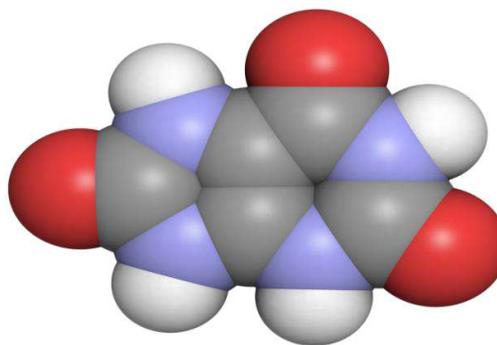
Paracetamol is normally safe at suggested doses. The suggested maximum daily dose for an mature is 3 or 4 grams. Higher doses may cause toxicity, including liver failure. Solemn skin rashes rarely may occur. It appears to be safe during pregnancy and when breast feeding. In those with liver sickness.. It is confidential as a mild analgesic. It does not have major anti-inflammatory activity and how it works is not completely clear.

v. Uric Acid

Properties	
Chemical formula	$C_5H_4N_4O_3$.
Molar mass	$168.1103 \text{ g mol}^{-1}$
Appearance	White
Density	1.87 g cm^{-3}
Melting point	300°C
Solubility in water	60 mg L^{-1}

Source: Sigma Aldrich, Germany

Purification: The mass purity is 99.98% and used as purchased.



About Uric Acid:Uric acid is a heterocyclic compound having molecular formula $C_5H_4N_4O_3$. It forms ions and salts known as urates such as ammonium acid urate. Uric acid is a product of the metabolic collapse of purine nucleotides, and it is a normal constituent of urine.

Causes of high uric acid:

- Diet may be an important factor.High consumption of dietary purine,and table sugar can increase amount of uric acid.
Uric acid from serum can be elevated by reduced excretion via the kidneys.
- Fasting or rapid weight loss may can temporarily elevate uric acid levels.

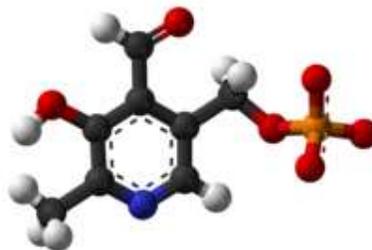
Gout

Excess blood uric acid can cause gout, a painful condition resulting from needle-like crystals of uric acid depositing in joints, capillary, skin, and other tissues.In humans, purines, are metabolized into uric acid which is then released in the urine. Use of some types of purine-rich foods, particularly meat and sea-food, increases gout risk. Gout may arise from regular

use of meats, such as liver, kidney, and sweet-breads, and certain types of sea-food including anchovies, sardines, mussels, haddock, mackerel and tuna.

vi. Pyridoxal Phosphate

Properties	
Chemical formula	C ₈ H ₁₀ NO ₆ P
Molar mass	247.142 g mol ⁻¹
Appearance	Yellowish
Density	1.638 g cm ⁻³
Melting point	142°C
Solubility in water	5 g L ⁻¹



Source: Sigma Aldrich, Germany

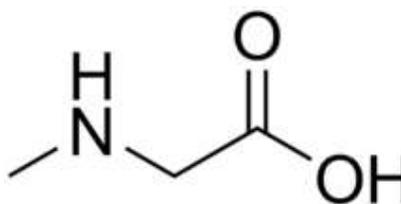
Purification: The mass purity is 99.98% and

used as purchased.

About Pyridoxal 5'-phosphate (PLP): It is one of the most important biologically active form of vitamin B6. Vitamin B6 is an necessary nutrient in humans with many precise biochemical function that generally involve PLP's use as a cofactor in the metabolism of amino acids, glucose and lipids.

vii.N-Methyl Glycine

Properties	
Chemical formula	C ₃ H ₇ NO ₂
Molar mass	89.093 g mol ⁻¹
Appearance	White
Density	1.093 g cm ⁻³
Melting point	212°C
Solubility in water	89.09 g L ⁻¹



Source: Sigma Aldrich, Germany

Purification: The mass purity is 99.98% and used as purchased.

About N-methyl glycine:Sarcosine, also known as *N-methylglycine*, is an intermediate and consequence in glycine synthesis and humiliation Sarcosine is metabolized to glycine by the enzyme sarcosine dehydrogenase, while glycine-*N*-methyl transferase generates sarcosine

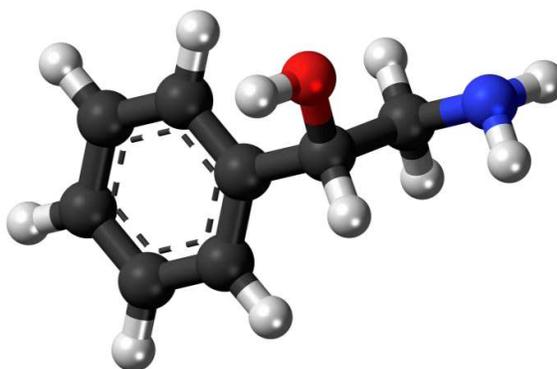
from glycine. Arccosine is an amino acid derivative that is naturally found in muscles and other body tissues.

Preparation: In the laboratory, it may be prepared from chloro-acetic acid and methyl amine through substitution reaction. Sarcosine is originate naturally as an intermediary in the metabolism of choline to glycine. Sarcosine is sugary to the taste and dissolve in water.

Uses: It is used in built-up biodegradable surfactants and toothpastes as well as in other applications.

viii. Phenyl Ethanol Amine (PEA)

Properties	
Chemical formula	C ₈ H ₁₁ N ₀
Molar mass	137.18 g mol ⁻¹
Appearance	Pale yellow
Melting point	57°C
Solubility in water	Soluble



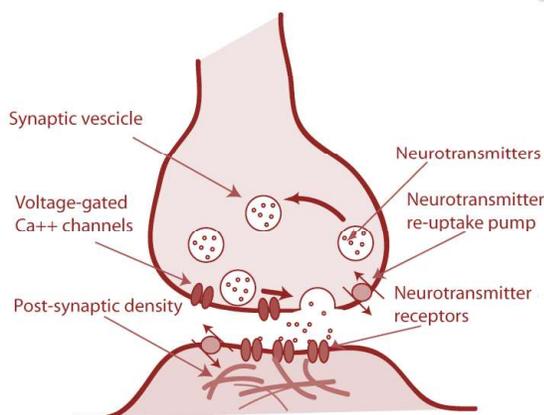
Source: Sigma Aldrich, Germany

Purification: The mass purity is 99.98% and used as purchased.

About PEA: Phenylethanolamine is known as β -hydroxyphenethylamine (PEOH) is a neurotransmitter which is structurally similar to others trace amine like norepinephrine, dopamine and epinephrine etc. It is endogenous chemical which transmit signals transversely a chemical synapse from one neuron to another neuron. The neurotransmitters produced from synaptic vesicles are received by specific neurotransmitter receptors on the cell membrane of the postsynaptic neuron as shown in **Scheme-1**. This neuron may be connected to many more neurons and pass the information to adjacent neuron.

Application of PEA: The neurotransmitters are clinically used for the treatment of several neurological and psychiatric disorders such as schizophrenia, Parkinson's disease, bipolar disorder, attention deficit, Huntington's disease, hyperactivity disorder. It regulates the blood pressure, respiration, and body temperature, the secretion of hormones from the pituitary gland, the regulation of α_2 -adrenoceptors in the hypothalamus etc. Phenylethanolamine is

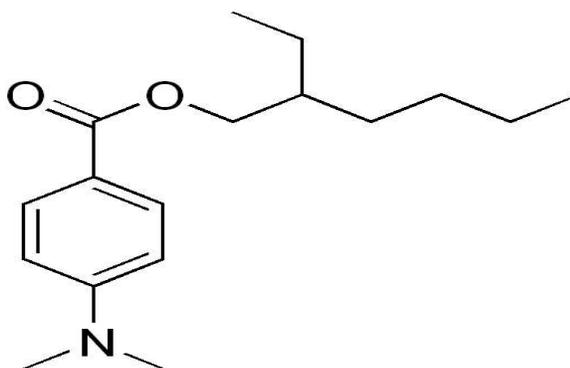
responsible for the production of enzyme phenylethanolamine-N-methyl transferase for the conversion of norepinephrine into epinephrine. Phenylethanolamine-N-methyl transferase catalyses the biosynthesis of epinephrine from norepinephrine by transferring methyl group from S-adenosyl-L-methionine.



Scheme-1

x. Pademate-O(PMO)

Properties	
Chemical formula	C ₁₇ H ₂₇ N ₀₂
Molar mass	277.408 g mol ⁻¹
Appearance	Yellowish
Density	0.99 g cm ⁻³
Melting point	362°C
Solubility in water	0.0355 g L ⁻¹



Source: Sigma Aldrich, Germany

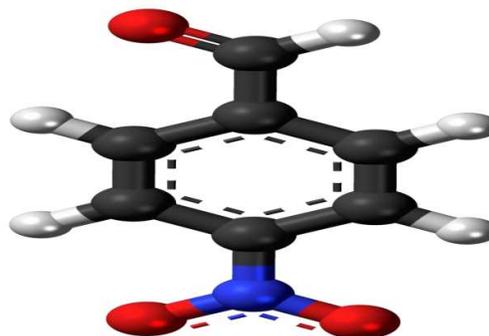
Purification: The mass purity is 99.98% and used as purchased.

Application: Padimate-O (PMO) is a sunscreen agent.

It can protect us from many problems mainly skin cancer, sunburn and photo-aging.

ix. Para Nitro Benzaldehyde (PNB)

Properties	
Chemical formula	C ₇ H ₅ NO ₃
Molar mass	151.12 g mol ⁻¹
Appearance	Yellowish crystalline solid
Density	1.546 g cm ⁻³
Melting point	106°C
Solubility in water	Soluble



Source: Sigma Aldrich, Germany

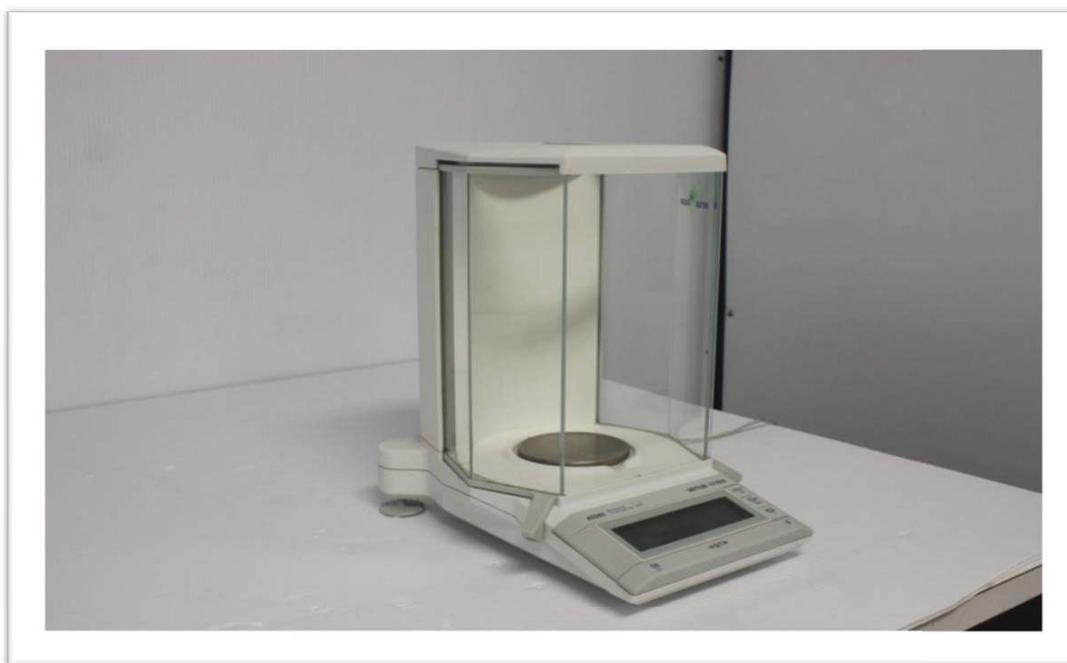
Purification: The mass purity is 99.98% and used as purchased.

Application; It is used as an important drug in medicinal chemistry for the treatment of Hepatitis.

3.2. Experimental Methods

3.2.1. Mass Measurement

Weights of different compounds were taken by digital Mettler Toledo, AG 285, Switzerland analytical balance.



It can measure weight to a high degree precision. The weighing pan is surrounded by transparent enclosure with doors so that any air currents in the room do not disturb the operation of balance.

3.2.2. Density Measurement

The density of different solutions were taken from Anton Paar density-meter (DMA 4500M) which has accuracy of 0.0005 g.cm^{-3} .



The mechanic oscillation of the U-tube filled solution is transformed into an alternating voltage of the some frequency. The can be measured from the period of oscillation τ as follows [11]:

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

A and B are the instrumental constants for each oscillator. The A and B values will be obtained by calibrating with two substances of the precisely known densities. Each measurement is carried after proper calibration of the instrument with double-distilled water and dry air.

3.2.3. Viscosity Measurement

The viscosities (η) were measured using a Brookfield DV-III Ultra Programmable Rheometer having spindle size-42 (precision of 0.3 %) which follows equation;

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

Here, RPM, TK and SMC are speed in rpm of spindle, viscometer torque constant and spindle multiplier constant respectively. The instrument was calibrated using the standard samples of precisely known viscosity. Brookfield Digital TC-500 thermostat with precision $\pm 0.01^\circ\text{C}$ was used to maintain the temperature of solution.

The temperature was maintained to within $\pm 0.01^\circ\text{C}$ using Brookfield Digital TC-500 thermostat bath. The viscosities were measured with an accuracy of $\pm 1\%$. Three successive measurements were taken for each sample to get the average viscosity.



3.2.4. Temperature Controller

All the experimental measurements were performed in a thermostatic water bath (Science India, Kolkata) having heater and cooler system with an accuracy of $\pm 0.01\text{ K}$.



In laboratory water bath the investigated solution is placed over the water container for quickly heating or cooling. These laboratory water baths should have greater temperature uniformity, heat retention, durability and recovery system

3.2.5. Water Distiller (Borosil Glass Works Limited, India):

Water Distiller is a set of a heater, round bottom flask, condenser and collecting container. Volatile contaminants are removed through a built-in vent tube. Minerals and salts remains in the boiling chamber as hard deposits. The circulation of cool water condenses the steam which passes through the condenser. Water droplets are collected in the collecting container.

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



Rotary evaporation is used to separate "low boiling" solvents such as n-hexane or alcohol or ethyl acetate from their mixtures. However, careful applications at reduced pressure and higher temperature also allow removal of a solvent from a sample containing a liquid compound if they have considerable difference in boiling points.

3.2.6. Conductivity Measurement

Systronics Conductivity TDS meter-308 was used to measure conductivity of electrolytic solutions at certain temperature.



The conductance was measured using a dip-type immersion conductivity cell of cell constant 1.11cm^{-1} . The instrument was calibrated using 0.1M KCl standard solution before the conductance measurements of any sample. The measurements were made in a thermostatic water bath maintained at certain temperature with an accuracy of $\pm 0.01\text{ K}$. by means of mercury in glass thermo regulator [12].

3.2.7. Refractive Index Measurement

Refractive indices were measured with accuracy ± 0.0005 by Digital Mettler Toledo 30GS Refractometer.



The instrument was calibrated with double-distilled water at certain temperature. Just 2-3 drops solution is sufficient for getting the refractive index of the sample. The refractive index values obtained at certain temperature may be converted to the refractive index any temperature.

3.2.8. FT-IR Measurement

Infrared spectra of liquid or solid compounds were recorded by Perkin Elmer 8300 FT-IR spectrometer (Shimadzu, Japan) having resolution of $\pm 0.25 \text{ cm}^{-1}$ at room temperature (25°C) with 49-54 % humidity.



The KBr disk of the solid compounds was prepared by mixing 100 mg of the KBr and 1 mg of the compound under study thoroughly. For liquid compounds Nujol mull was used for the preparation of the film.

The transmittance (T) or the absorbance (A) of a sample is estimated from the intensity of light entering (I_0) and the intensity of transmitted light (I) entering from the following equation.

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

3.2.10. Surface Tension

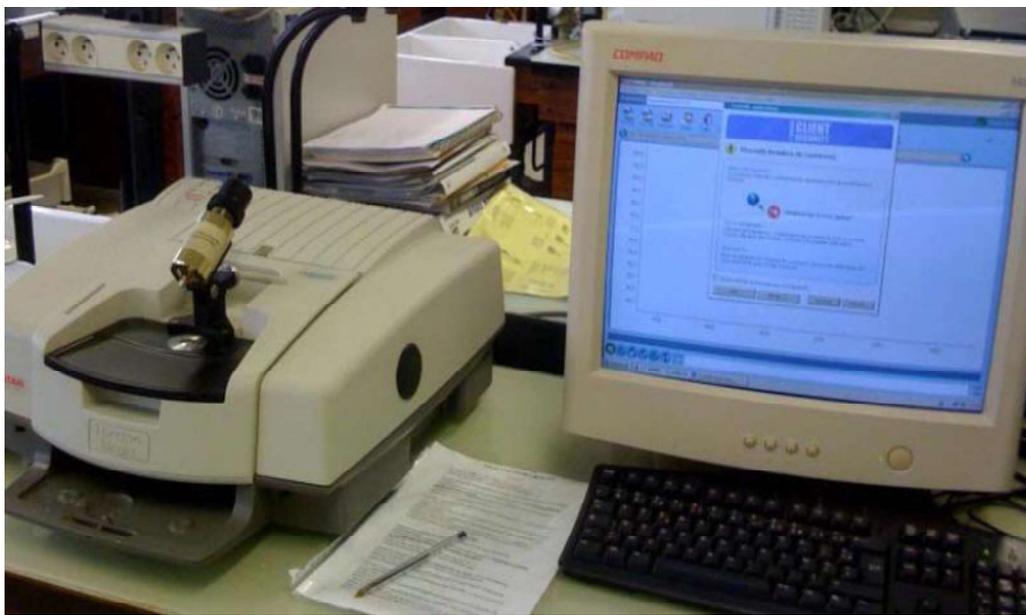
The surface tension measurements with precision within $\pm 0.1 \text{ mN}\cdot\text{m}^{-1}$ of surface active compounds in their aqueous solution were done by platinum ring method using a Tensiometer (K9, KRÜSS; Germany).



Temperature of the system was kept constant by circulating auto-thermostated water bath (precision within $\pm 0.01\text{K}$) through a double-wall glass vessel container holding the solution. The calibration of the tensiometer was done with Millipore water. The platinum plate used for measurements was thoroughly cleaned with double-distilled water.

3.2.9. UV-VIS Measurement

UV-Vis absorption spectra were recorded at 298.15 K by JASCO V-530 UV-VIS Spectrophotometer for the compounds which absorb Ultraviolet and/or visible light. Suitable probe may be used for the compounds that do not absorb at in the UV and VIS range. The characteristic absorbance graph was recorded as a function of wavelength.



In UV-VIS spectrophotometer two light sources, a deuterium (D2) lamp for ultraviolet light and a tungsten (W) lamp for visible light were used.

3.2.10. Nuclear Magnetic Spectra Measurement

Nuclear Magnetic Spectra (NMR) spectroscopy is used to study the structure of molecules, the kinetics of various reaction, intermolecular interactions prevailing in solution etc. ^1H NMR spectra were recorded at 400MHz using Bruker Advance spectrometer in suitable solvent.



3.2.11. Sem (Scanning Electron Microscope)

Images of samples are taken by the following machine of JEOL to find out the morphological changes during the formation of inclusion complexes.

