

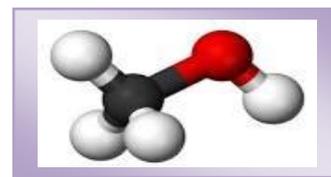
CHAPTER : III

EXPERIMENTAL SECTION

III.1. Name, Structure, Physical Properties, Purification and Applications of Diverse Solvents and Solutes Used in the Research Work

III. (1.1) Solvents particulars of the aqueous and non-aqueous solvents used in the research work are given below:

Methanol



Methanol, also known as methyl alcohol, wood alcohol, wood naphtha or wood spirits, is simplest alcohol, is a light, volatile, Colorless, flammable, liquid with a distinctive odor that is very Similar to but slightly sweeter than ethanol (drinking alcohol).^[1,2]

| | |
|------------------------------|--------------------------------|
| Structural form: Methanol | $\text{H}_3\text{C}-\text{OH}$ |
| Appearance: | Liquid |
| Molecular Formula: | CH_3OH |
| Molecular Weight: | 32.04 g/mol |
| Boiling Point: | 176 K |
| Melting Point: | 337.8 K |
| Dielectric Constant: | 32.70 at 298.15 K |

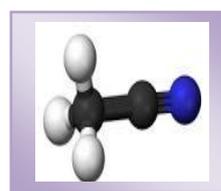
Source: - Merck, India

Purification: - It was passed through Linde 4Å molecular sieves and then distilled.

Application: - The major use of methyl alcohol by far is in making other chemicals. About 40% of methanol is converted to [formaldehyde](#), and from there into products as

diverse [plastics](#), [plywood](#), [paints](#), [explosives](#), and [permanent press textiles](#). Methanol is traditional denaturant for ethanol, thus giving the term [methylated spirit](#). Methanol is as well used as a [solvent](#), and as an [antifreeze](#) in [pipelines](#). In a few [waste water treatment plants](#), a small amount of methanol is added to [waste water](#) to provide a food source of carbon for [denitrifying bacteria](#), which converts [nitrates](#) to [nitrogen](#) to decrease the denitrification of sensitive aquifers. Methanol is used on a partial basis to fuel [internal combustion engines](#). Methanol is also functional as an [energy carrier](#). It is easier to store than hydrogen, burns cleaner than fossil fuels, and is ecological.

Acetonitrile



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

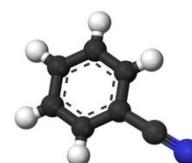
| | |
|----------------------------------|--------------------|
| Structural form: Acetonitrile | |
| Appearance: | Liquid |
| Molecular Formula: | CH ₃ CN |
| Molecular Weight: | 41.05 g/mol |
| Boiling Point: | 607 K |
| Melting Point: | 628.3 K |
| Dielectric Constant: | 35.94 at 298.15 K |

Source: - Merck, India.

Purification: - It is shaken with silica gel, refluxed with CaH₂ and distilled over P₂O₅.

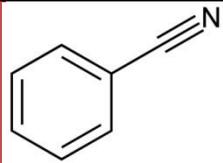
Application: - It is extensively used in battery applications for the reason that it's relatively high [dielectric constant](#) and ability to dissolve electrolytes. For similar reasons

it is a well-liked solvent in [cyclic voltammetry](#). Its low viscosity and low chemical reactivity build it a popular choice for [liquid chromatography](#). Acetonitrile plays a noteworthy role as the dominant solvent used in the manufacture of DNA oligonucleotides from monomers. Industrially, it is used as a solvent in the purification of [butadiene](#) and in manufacture of [pharmaceuticals](#) and [photographic film](#). Acetonitrile is a widespread two-carbon building block in [organic synthesis](#) as in the production of [pesticides](#) to [perfumes](#). [3,4]



Benzonitrile

[Aromatic organic compound](#) is a colorless non-aqueous liquid with a sweet almond odour. It is essentially used as a precursor to the resin benzoguanamine.

| | |
|--------------------------------------|---|
| Structural form: Benzonitrile |  |
| Molar mass: | 103.04 g/mol |
| Formula: | C ₇ H ₅ N |
| Density: | 1 g/cm ³ |
| Boiling point: | 191 °C |
| Melting point: - | 13 °C (9 °F; 260 K) |
| Solubility in water: | <0.5 g/100 ml (22 °C) |

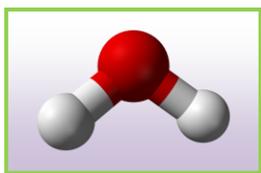
Source: - Merck, India.

Purification: - Benzonitrile was dried with CaSO₄ and distilled from P₂O₅ in an all-glass apparatus, under the reduced pressure (69 °C/10mm), collecting the middle fraction.

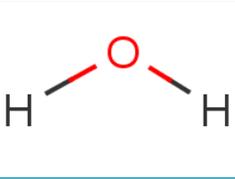
Applications Laboratory uses of Benzonitrile as a valuable solvent and a versatile precursor to many derivatives is well known. It reacts with amines to afford N-substituted benzamides subsequent to hydrolysis. It is a precursor to Ph₂C=NH (b.p. 151 °C, 8 mm Hg) by means of reaction with [phenyl magnesium bromide](#) followed

by [methanolysis](#). Benzonitrile forms [coordination complexes](#) with transition metals that are equally soluble in organic solvents and conveniently labile. For example is $\text{PdCl}_2(\text{PhCN})_2$. The benzonitrile ligands are voluntarily displaced by stronger ligands, making benzonitrile complexes useful synthetic intermediates. [5,6]

Water



Water is a ubiquitous [chemical substance](#) that is composed of [hydrogen](#) and [oxygen](#) and is essential for every known forms of [life](#). In typical usage, water refers merely to its [liquid](#) form or state, but the substance also has a [solid](#) state, [ice](#), and a gaseous state, [water vapor](#) or [steam](#). Water is a excellent [solvent](#) and is often referred to as the [universal solvent](#).

| | |
|---------------------------|---|
| Structural form: Water |  |
| Appearance: | Liquid |
| Molecular Formula: | H_2O |
| Molecular Weight: | 18.02 g/mol |
| Boiling Point: | 100 °C |
| Melting Point: | 0 °C |
| Dielectric Constant: | 78.35 at 298.15 K |

Source: - Distilled water.

Purification: - Water was first demonstrated and then distilled in an all glass distilling set along with alkaline KMnO_4 solution to remove any organic matter therein. The doubly distilled water was finally distilled by means of an all glass distilling set. Precautions were taken to avoid contamination from CO_2 and other impurities.

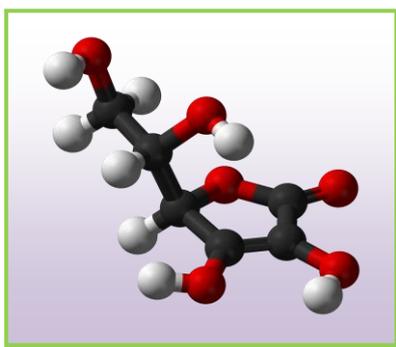
The triply distilled water had specific conductance less than $1 \times 10^{-6} \text{ S.cm}^{-1}$.

Application: - Water is a superior solvent due to its polarity. The solvent properties of water are vital in biology, because numerous biochemical reactions take place only within aqueous solutions (e.g., reactions in the cytoplasm and blood). In totaling, water is used to transport biological molecules. The most imperative use of water in agriculture is for irrigation. Water healthy for human consumption is called drinking water. Water is widely used in chemical reactions as a [solvent](#) or [reactant](#) and less commonly used as a [solute](#) or [catalyst](#). In inorganic reactions, water is a common

solvent, dissolving scores of ionic compounds. In organic reactions, it is not usually used as a reaction solvent, for the reason that it does not dissolve the reactants well and is [amphoteric](#) (acidic *and* basic) and [nucleophilic](#). Nevertheless, these properties are occasionally desirable. Also, acceleration of [Diels-Alder reactions](#) by water has been experimental [Supercritical water](#) has freshly been a topic of research. [7,8]

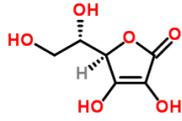
III. (1.2) Solutes which are used in the analysis are listed as follows:

Ascorbic acid



Ascorbic acid (C₆H₈O₆) is a naturally occurring organic compound amid antioxidant properties. Its appearance is white powder, soluble in water. Ascorbic acid is usually known as Vitamin-C. The name is derived from α- (meaning “no”) and scorbutus (scurvy), which is the disease caused due to deficiency of vitamin-C in the body.

[9, 10]

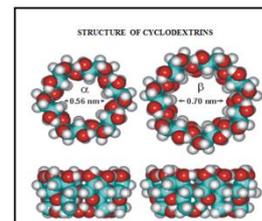
| | |
|--|--|
| Structural form: Ascorbic Acid |  |
| Appearance: | White powder |
| Molecular Formula: | C₆H₈O₆ |
| Molecular Weight: | 176.12 g/mol |
| Melting Point: | 190°C |

Source: - S.D. Fine Chemicals Ltd., Mumbai, India

Purification: - Used as purchased, no purification was required.

Application: - Ascorbic acid and its sodium, potassium, and calcium [salts](#) are generally used as [antioxidant food additives](#). Ascorbic acid is easily oxidized and so is used as a reductant in photographic developer solutions (amongst others) and as a [preservative](#). In [fluorescence microscopy](#) and related fluorescence-based techniques, ascorbic acid can be used as an [antioxidant](#) to raise fluorescent signal and chemically retard dye [photo bleaching](#).

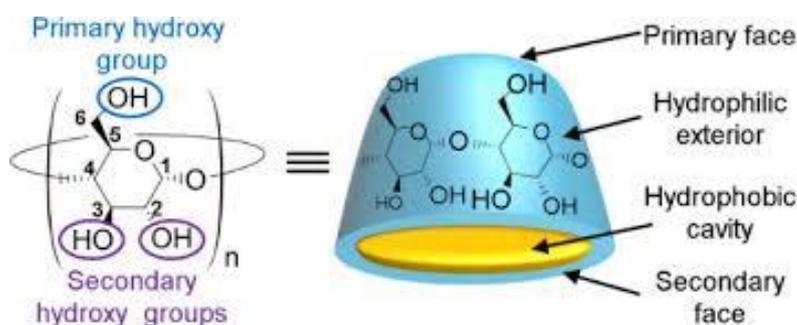
Cyclodextrins



Features → (Sometimes called [cycloamyloses](#)) are a family of compounds made up of sugar molecules bound collectively in a ring (cyclic [oligosaccharides](#)). Cyclodextrins composed of 5 or more α -D-glucopyranoside units linked 1- \rightarrow 4, as in [amylose](#) (fragment of [starch](#)). 5-membered macro cycle is not natural. Cyclodextrins are formed from [starch](#) by means of [enzymatic](#) conversion. [11-14]

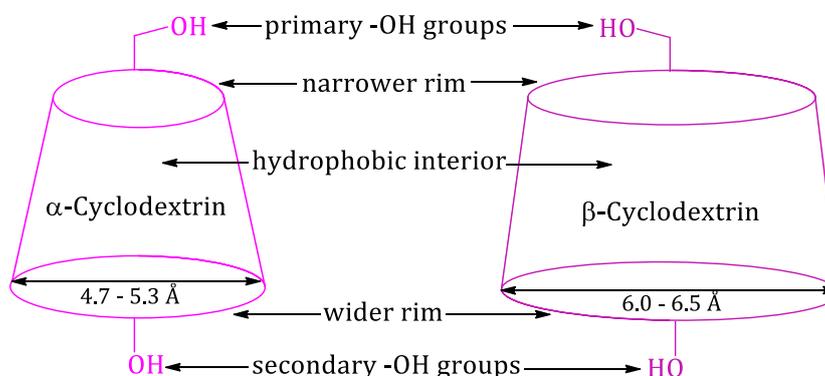
Source: - Sigma Aldrich, India.

Purification: - The purity of the sample was 0.98%, so no further purification was done.



Two types of cyclodextrins are being studied:

- **α (alpha)-cyclodextrin:** 6-membered sugar ring molecule
- **β (beta)-cyclodextrin:** 7-membered sugar ring molecule



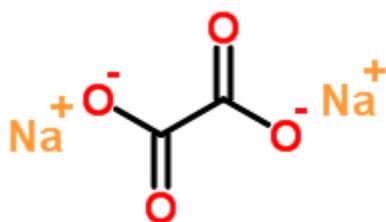
Recently, largest and finest well-characterized cyclodextrin contains 1, 4-anhydroglucopyranoside units, while as a feebly characterized mixture; at least 150-

membered cyclic oligosaccharides are well-known. Typical cyclodextrins contain a number of [glucose](#) monomers ranging from six to eight units in a ring, creating a bucket shape.

Applications: - The unique property in Cyclodextrin is having a hydrophilic outer shell and having an inner hydrophobic cavity thus it act as “Host” molecules in “Host –Guest complexation” study.

Formation of inclusion complex is established on Physicochemical and Spectroscopic Contrivances with this host molecule in our various studies. They are used in food, pharmaceutical, [drug delivery](#), bio- chemical industries, in addition to agriculture, environmental engineering, paint industry, Cosmetics and hygiene.

Sodium Oxalate



Formula: Na₂C₂O₄ (Sodium Oxalate)

Molar mass: 134 g/mol

Solubility: soluble in formic acid; insoluble in alcohol, ether

Solubility in water: 2.69 g/100 mL (0 °C); 3.7 g/100 mL (20 °C); 6.25 g/100 mL (100 °C)

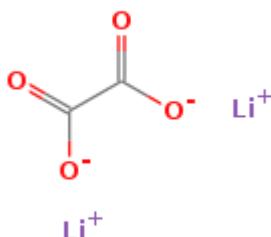
Appearance: White crystalline solid

Melting point: 260 °C (500 °F; 533 K) decomposes above 290 °C

Source & Purification: It was purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay – 400060, India) with 98% purity.

Features & Applications: - Sodium oxalate, or disodium oxalate, is the [sodium](#) salt of [oxalic acid](#) with formula Na₂C₂O₄. It is a white, crystalline, odorless solid, which decomposes over 290 °C. Disodium oxalate can act as a [reducing agent](#), and it could be used as a [primary standard](#) for standardizing [potassium permanganate](#) (KMnO₄) solutions. ^[15, 16]

Lithium Oxalate



Formula: $\text{Li}_2\text{C}_2\text{O}_4$ (Lithium Oxalate)

Molar mass: 101.898g/mol

Appearance: White crystalline solid

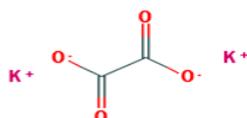
Solubility: 8gm/100g of solvent at 19.5°C. Insoluble in diethyl ether and ethanol.

Boiling point: 365.1°C at 760 mmHg.

Source & Purification: It was purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay-400060, India) with 98% purity.

Features & Applications: - It is a strong dicarboxylic acid occurring in various plants and vegetables. It is produced in the body by metabolism of glyoxylic acid or ascorbic acid. It is not metabolized but excreted. It is used as an analytical reagent as well as reducing agent. [17-20]

Potassium Oxalate



Formula: $\text{K}_2\text{C}_2\text{O}_4$ (Potassium oxalate)

Density: 2.04 g/cm³

Appearance: White crystalline solid

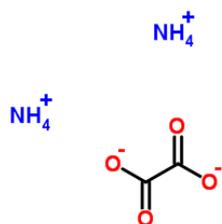
Solubility: 2.5 g/100 g, slightly soluble in alcohol

Molar mass: 128.12 g·mol

Source & Purification: It was purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay-400060, India) with 98% purity.

Features & Applications: it is the basic salt of hydrogen oxalate anion, can be obtained by reacting KOH with oxalic acid in 1:1 mole ratio. Potassium hydrogen oxalate occurs in some plants, particularly sorrel. It is commercial product used in photography, marble grinding and to remove ink stains. [21-23]

Ammonium Oxalate



Formula: C₂H₈N₂O₄ (Ammonium Oxalate)

Molar mass: 124.1 g/mol

Density: 1.48g/ cm³

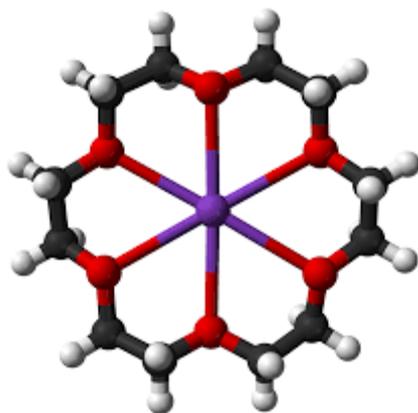
Melting point: 70 C (158 F, 343.15 K)

Appearance: White solid

Source & Purification: It was purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay-400060, India) with 98% purity.

Features & Applications: It is an oxalate salt produced in the body of vertebrates by metabolism of glyoxylic acid or ascorbic acid. It is not metabolized but excreted. It is used as an analytical reagent and general reducing agent. It is also used as anticoagulants, to preserve blood outside the body. It occurs in various plants and animals. [24]

18 crown-6



Formula: $[C_{24}H_{40}O_6]$ (18 crown-6)

Source: - Sigma Aldrich, India.

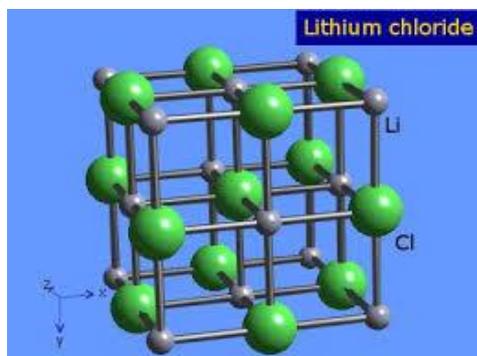
Purification: - The purity of the sample was 0.98%, so no further purification was done.

Appearance: It is a white, hygroscopic crystalline solid with a low melting point.

Features: [IUPAC](#) name of 1,4,7,10,13,16-hexaoxacyclooctadecane. Like other [crown ethers](#), 18-crown-6 functions as a ligand for some metal cations with a particular affinity for potassium cations ([binding constant](#) in [methanol](#): $10^6 M^{-1}$). The point group of 18-crown-6 is S_6 . The dipole moment of 18-crown-6 varies in different solvent and under different temperature. Less than 25 °C, the dipole moment of 18-crown-6 is $2.76 \pm 0.06 D$ in cyclohexane and 2.73 ± 0.02 in benzene. [\[25-27\]](#)

Applications: It is also used as a host molecule, i.e., acts as a carrier to bind diverse imperative compounds through assorted interactions.

Lithium Chloride



Formula: LiCl (Lithium chloride)

Molar mass: $42.39 g \cdot mol^{-1}$

Source: purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay-400060, India).

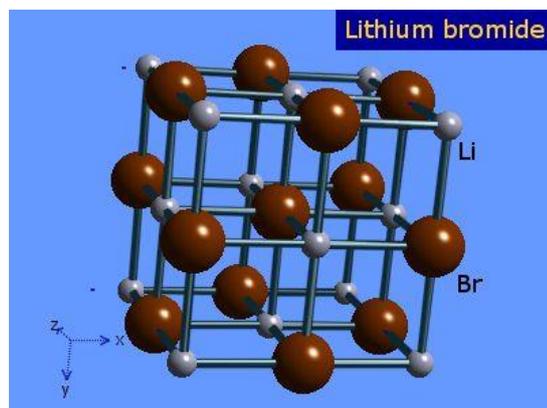
Purification: - purity of the sample was 0.98%, so no further purification was done.

Appearance: white solid, hygroscopic in nature.

Features: The [salt](#) is a typical [ionic compound](#), although small size of the Li^+ ion gives rise to properties not seen for other alkali metal chlorides, such as extraordinary solubility in polar [solvents](#) (83.05 g/100 mL of water at 20 °C) and its [hygroscopic](#) properties.

Application: Lithium chloride is mainly used for the production of [lithium](#) metal by [electrolysis](#), as a brazing [flux](#) for [aluminium](#) in [automobile](#) parts, as a [desiccant](#) for drying air streams. In more specialized applications, lithium chloride finds some use in [organic synthesis](#). Also, in biochemical applications; it can be used to precipitate [RNA](#) from cellular extracts. Molten LiCl is used for the preparation of [carbon nanotubes](#), [graphene](#) etc. [28, 29]

Lithium Bromide



Formula: Lithium bromide (LiBr)

Features and appearance: white solid, its extreme [hygroscopic](#) character makes LiBr useful as a [desiccant](#) in certain air conditioning systems.

Molar Mass: 86.845g/mol

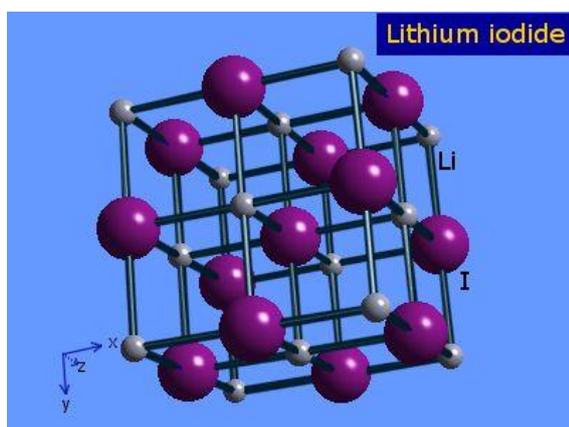
Solubility: 166.7g/ml at 20°C in water, it is also soluble in methanol, ethanol, ether, acetone, slightly in pyridine.

Source: purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay: 400060, India).

Purification: - purity of the sample was 0.98%, so no further purification was done.

Application: Lithium bromide is used in air-conditioning systems as [desiccant](#), as a salt in absorption chilling along with water, as a reagent in [organic synthesis](#). In medical applications used as a [sedative](#). [30-32]

Lithium Iodide



Formula: Lithium iodide (LiI).

Features and appearance: White crystalline solid, when exposed to [air](#), it becomes yellow in color, due to the [oxidation](#) of iodide to iodine. It can participate in various [hydrates](#).

Molar mass: 133.85g/mol

Solubility: 1670g/L (25°C), it is also soluble in ethanol, propanol, ethanediol, ammonia.

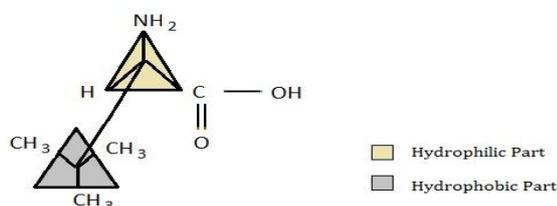
Source: purchased from SRL (Sisco Research Laboratories PVT.LTD. Bombay: 400060, India).

Purification: - purity of the sample was 0.98%, so no further purification was done.

Application: Lithium iodide is used as [electrolyte](#) for high temperature batteries. It is also used for long life batteries as required, for example, in [artificial pacemakers](#). It is also used, in complex with [Iodine](#), in [electrolyte](#) of [dye-sensitized solar cells](#). In the [organic synthesis](#), LiI is useful for cleaving C-O bonds. It was used as a radio contrast

agent for [X-ray computed tomography](#) imaging studies. Its use was discontinued due to renal toxicity, replaced by organic iodine molecules. [33, 34]

L-Tertiary-Leucine (Tert-Leucine)



Tertiary structure of Amino Acid (Leucine) representing Hydrophilic and Hydrophobic parts.

Formula: $C_6H_{13}NO_2$. (Tertiary- Leucine) also termed as 2-amino-3, 3-dimethyl butanoic acid or tert-butyl glycine.

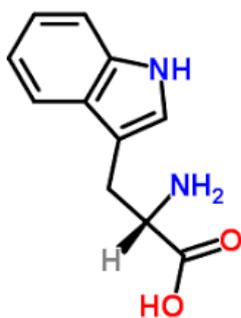
Molar Mass: 131.175g/mol

Source: Sigma-Aldrich, India.

Purification: It was 0.99% pure, so no purification was done.

Features and Applications: It is white powder, soluble in water at 125.5g/L at 20°C, 1 M HCl: 50 mg/mL. L-tert-Leucine is an essential amino acid creating up one third of our muscle protein. L-tert-leucine is imperative in developing chiral pharmaceutically active chemicals. It can be used as the food additive. It is used in formation of sterols. It can also be used as catalyst in the production of cobalt oxazoline palladacycles complex. It can also be used for production of Chiral tridentate Schiff base ligands. [35, 36]

Tryptophan



Formula: C₁₁H₁₂N₂O₂

IUPAC: (2S)-2-amino-3-(1H-indol-3-yl) propanoic acid

Solubility: 11.4g/L at 25°C, also soluble in hot alcohol, alkali hydroxides.

Molar mass: 204.23gmol⁻¹

Source: Sigma-Aldrich, India.

Purification: It was 0.99% pure, so no purification was done.

Features & Applications: It contains a α -amino group, an α -carboxylic acid group, and a side chain indole, making it a non-polar aromatic amino acid. It is essential in humans, meaning the body cannot synthesize it: it must be obtained from the diet. Tryptophan is also a precursor to the neurotransmitter serotonin and the hormone melatonin. For instance, tryptophan residues play special roles in "anchoring" [membrane proteins](#) within the [cell membrane](#). In adding up, tryptophan functions as a biochemical [precursor](#) for the following [compounds](#) :

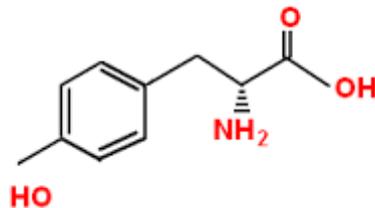
- [Serotonin](#) (which is a [neurotransmitter](#)), synthesized by [tryptophan hydroxylase](#).
- [Melatonin](#) (the [neurohormone](#)) is in turn synthesized from serotonin, via [N-acetyltransferase](#) and [5-hydroxyindole-O-methyltransferase](#) enzymes.
- [Niacin](#), also identified as vitamin B₃, is synthesized from tryptophan via [kynurenine](#) and [quinolinic acids](#).
- [Auxins](#) (a class of [phytohormones](#)) are synthesized from the tryptophan.

The disorder [fructose malabsorption](#) causes improper absorption of tryptophan in the intestine, reduced levels of tryptophan in blood, and depression.

In bacteria synthesis tryptophan, high cellular levels of this amino acid activate a [repressor](#) protein, which binds to [trp operon](#). Binding of this repressor to tryptophan operon prevents [transcription](#) of downstream DNA that codes for the enzymes involved in biosynthesis of tryptophan. So high levels of tryptophan prevent tryptophan synthesis through a [negative feedback](#) loop, when cell's tryptophan levels go down again,

transcription from trp operon resumes. This permits tightly regulated and rapid responses to changes in cells internal as well as external tryptophan levels. [38-40]

Tyrosine



Formula: C₉H₁₁NO₃

IUPAC: 2-Amino-3-(4-hydroxyphenyl)propanoic acid

Molar Mass: 181.19g.mol⁻¹

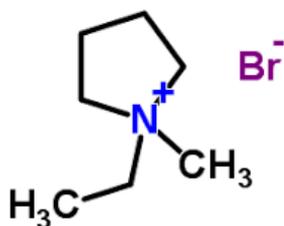
Source: Sigma-Aldrich, India.

Purification: It was 0.99% pure, so no purification was done.

Solubility: .0453g/100mL

Features & Applications: Tyrosine or 4-hydroxyphenylalanine is one of the 20 standard amino acids studied that are used by cells to synthesize proteins. It is a non-essential amino acid consisting of a polar side group. For instance, [tyrosine](#) residues play special roles in "anchoring" [membrane proteins](#) within the [cell membrane](#). A tyrosine residue also plays an imperative role in [photosynthesis](#). In [chloroplasts](#) ([photosystem II](#)), it acts as an electron donor in [reduction](#) of oxidized [chlorophyll](#). In this process, it loses hydrogen atom of its phenolic OH-group. This radical is consequently reduced in photosystem II by the four core manganese clusters. [41-44]

1-ethyl-1-methylpyrrolidinium bromide



Formula: 1-ethyl-1-methylpyrrolidinium bromide (C₇H₁₆BrN)

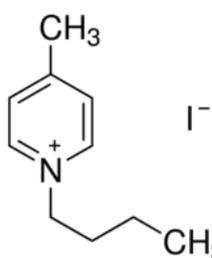
Molar mass: 194.11g

Source: Sigma Aldrich, India.

Purification: 99% pure was obtained, so no purification was done; only it was refrigerated after and before use.

Features & Applications: 1-Ethyl-1-methylpyrrolidinium bromide is an ionic liquid that can be all set by reacting *N*-methylpyrrolidine with bromoethane. It is conventionally used as a bromine-sequestering agent (BSA) in zinc or bromine redox flow batteries. 1-Ethyl-1-methylpyrrolidinium bromide may be used in the preparation of 1-ethyl-1-methylpyrrolidinium lactate, a potential solvent for separation of hexan-1-ene from hexane. It can moreover be used to prepare 1-ethyl-1-methylpyrrolidinium tribromide. It can be used as a co-catalyst in the cycloaddition of CO₂ with diverse epoxides to form cyclic carbonates in the presence of a nona-vacant Keggin-type tricarbonyl rhenium derivative catalyst. Thus it has an extensive uses and can be used as a green solvent in the supramolecular assembly formation. [45, 46]

1-Butyl-4-methylpyridinium iodide



Formula: 1-Butyl-4-methylpyridinium iodide (C₁₀H₁₆IN)

Molar mass: 277.15g

Source: Sigma Aldrich, India.

Purification: 99% pure was obtained, so no purification was done; only it was refrigerated after and before use.

Features & Applications: Ionic liquids containing pyridinium cations exhibit higher melting points, lower solubility in water, and higher polarity than those containing imidazolium cations. These compounds exhibit many outstanding physical properties such as great thermal stability and no measurable vapor pressure. So, for its outstanding benefits in physicochemical contrivances it is encapsulated in cyclodextrin in order to increase its solubility in aqueous solution and extend its applications. [46-48]

III. 2. EXPERIMENTAL METHODS

III. (2.1) PREPARATION OF SOLVENT MIXTURES

For the preparation of solvent mixtures, pure components were taken separately in glass stoppered bottles and thermo stated at desired temperature for sufficient time. When the thermal equilibrium was ensured, 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 means of experimental densities of the solvents at experimental temperature. It was then stoppered and mixed contents were shaken well prior to use.

While preparing different solvent mixtures care was taken to ensure that the same procedure was adopted throughout the complete work. The

physical properties of different pure and mixed solvents have been presented in the relevant chapters. [49-55]

III. (2.2) PREPARATION OF SOLUTIONS

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·dm⁻³.

III. (2.3) MASS MEASUREMENT

Mass measurements were prepared on digital electronic analytical balance (Mettler Toledo, AG 285, Switzerland).



It can calculate 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 don't affect the balance's operation.

III. (2.4) DENSITY MEASUREMENT

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



In digital density meter, mechanic [oscillation](#) of the U-tube is e.g. electromagnetically transformed into an alternating [voltage](#) of the same [frequency](#). The period τ can be measured with high resolution and stands in simple relation to [density](#) ρ of the sample in the oscillator [7]:

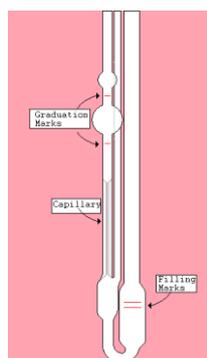
$$\rho = A \cdot \tau^2 - B \quad (1)$$

A and B is respective instrument constants of each oscillator. Their values are determined by [calibrating](#) with two substances of precisely known densities ρ_1 and ρ_2 . Modern instruments calculate and store constants A and B after the two calibration measurements, which are mostly performed with [air](#) and [water](#) respectively. They employ suitable measures to compensate various influences on the measuring result, e.g. influence of the sample's viscosity and non-linearity caused by measuring

instrument's finite mass. The instrument was calibrated by double-distilled water and dry air simultaneously. [56-58]

III. (2.5) VISCOSITY MEASUREMENT

Solvent viscosities were measured with the aid of a suspended Ubbelohde-type viscometer,



The kinematic viscosity (γ) and absolute viscosity (η) are given by the following equations.

$$\gamma = k t - l/t \quad (2)$$

$$\eta = \gamma \cdot \rho \quad (3)$$

where, t is time of flow, ρ is density and k and l are characteristic constants of the particular viscometer. The time of efflux of a constant volume of experimental liquid through the capillary was measured with aid of a Racer stop watch capable of measuring times accurate to ± 0.1 s. The viscometer was always kept in a vertical position in thermostatic bath with an accuracy of ± 0.01 K of desired temperature. The uncertainty of viscosity measurement was ± 0.003 . In every case, experiments were performed in at least three replicates and results were averaged. [59-63]

Relative viscosities (η_r) were obtained by means of the equation:

$$\eta_r = \eta / \eta_0 = \rho t / \rho_0 t_0 \quad (4)$$

where η , η_0 , ρ , ρ_0 and t , t_0 are absolute viscosities, densities and flow times for the both solution and solvent respectively.

The viscosity was also calculated with help Brookfield DV-III Ultra Programmable Rheometer fitted to a Brookfield Digital Bath TC-500.



III. (2.6) TEMPERATURE CONTROLLER

All 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 arrangement in which a vessel containing the material to be heated is placed into or over the one containing water and to quickly heat it. These laboratory equipments are available in diverse volumes and construction with both digital and analogue controls and greater temperature uniformity, durability, heat retention and recovery. The chambers of water bath laboratory products are manufactured using rugged, leak proof and highly resistant stainless steel and other lab supplies. [64]

III. (2.7) Water Distiller (Borosil Glass Works Limited, India):

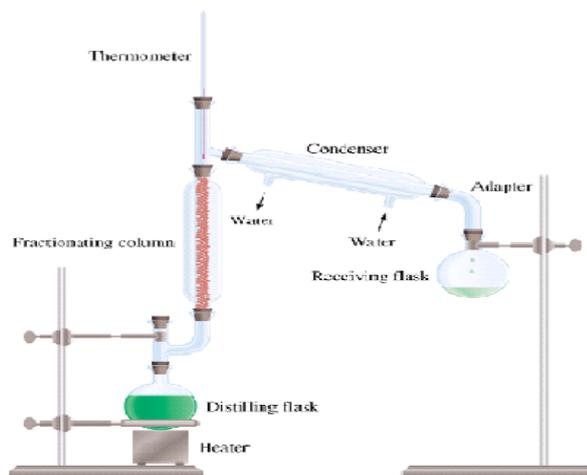
Water distillation units produce highly treated and disinfected water for laboratory usage. The distillation process removes minerals and microbiological contaminants and can reduce levels of chemical contaminants. A water distiller works by boiling water into water vapour, condensing it and then returning it to its liquid state. It is collected in a storage container.



Municipal or well water is manually or automatically fed into distiller unit's boiling chamber. A heating element in the boiling chamber heats water until it boils. The steam rises starting the boiling chamber. Volatile contaminants (gases) are discharged throughout a built-in vent. Minerals and salts are retained in boiling chamber as hard deposits or scale. Steam enters a coiled tube (condenser), which is cooled by cool water. Water droplets form as the condensation occurs. The distilled water is collected in the storage tank. [65]

III. (2.8) Fractional Distillation Apparatus:

Distillation involves 2 stages and both are physical state changes. (1) The liquid or solution mixture is **boiled to vaporise** the most volatile component in the mixture (**liquid ==> gas**). The anti-bumping granules give a smoother boiling action. (2) The vapour is cooled by cold water in the condenser to **condense** (**gas ==> liquid**) it back to a liquid (the distillate) which is collected.



In the distillation flask, vaporization of a liquid and consequent condensation of resultant gas back to liquid form. It is used to separate liquids from non-volatile solids or solutes (e.g., water from the salt and other components of sea water) or to separate two or more liquids with diverse boiling points (e.g., alcohol from fermented beers and wines). Several variations have been devised for industrial applications. An imperative one is fractional distillation, in which vapor from a heated liquid mixture is contacted by

a series of trays and condensed liquid as it rises through a vertical column. The most volatile fraction of mixture emerges from the top of the column, while less volatile fractions are withdrawn at lower points. [66]

III. (2.9) Rotary Vacuum Flash Evaporator (Super fit, an ISO 9001:2000 Certified Company):

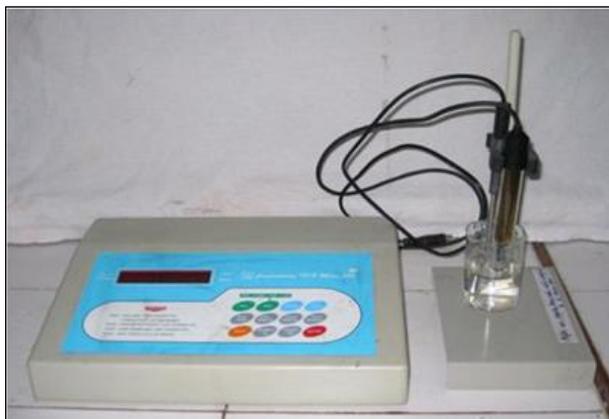
Rotary evaporation is the most often and conveniently applied to separate "low boiling" solvents such as n-hexane or ethyl acetate from compounds which are solid at room temperature and pressure. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there is minimal co-evaporation ([azeotropic](#) behavior), and the sufficient difference in boiling points at chosen temperature and reduced pressure. [67]

A rotary evaporator (or rotavap) is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (e.g., "the sample was evaporated under reduced pressure").



III. (2.10) CONDUCTIVITY MEASUREMENT

Systronics Conductivity TDS meter-308 used for measuring specific Conductivity of electrolytic solutions. It can supply 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 $\pm 0.3\%$ precise. The instrument was standardized using 0.1(M) KCl solution. The cell was calibrated by the process of *Lind and co-workers* [8]. The conductivity cell was sealed to 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 stop admission of air into the cell when solvent or solution was added. The measurements were made in a thermostatic water bath maintained at required temperature with an accuracy of ($\pm 0.01\text{ K}$) via mercury in glass thermo regulator.

Quite a lot of solutions were prepared by weight precise to $\pm 0.02\%$. The weights were taken on Mettler electronic analytical balance (AG 285, Switzerland). The molarity being transformed to molality as required. Several independent solutions were all set and runs were performed to ensure the reproducibility of the results. Due correction was made for the specific conductance of solvents at desired temperatures. The following figure shows Block diagram of Systronics Conductivity-TDS meter 308. [68, 69]

III. (2.11) REFRACTIVE INDEX MEASUREMENT

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



Calibration was performed by measuring refractive indices of double-distilled water, toluene, cyclohexane, and carbon tetrachloride at defined temperature. The accuracy of instrument is ± 0.0005 . 2-3 drops of the sample was put onto the measurement cell and reading was taken. Refractive index of a sample depends on temperature. During measurement, refractometer determines temperature and then corrects refractive index to a temperature as desired by the user. [70, 71]

III. (2.12) FT-IR MEASUREMENT

Infrared spectra were recorded via 8300 FT-IR spectrometer (Shimadzu, Japan)



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

The intensity of light (I_0) passing during a blank is measured. The intensity is number of photons per second. The blank is a solution that is identical to the sample solution except that blank does not contain the solution that absorbs light. The intensity of light (I) passing through the sample solution is calculated. (In practice, instrument measures

power rather than the intensity of the light. The power is the energy per second, which is the product of intensity (photons per second) and the energy per photon. The experimental data is used to analyze two quantities: the transmittance (T) and the absorbance (A).

$$T = \frac{I}{I_0}, \quad A = -\log_{10} T \quad (5)$$

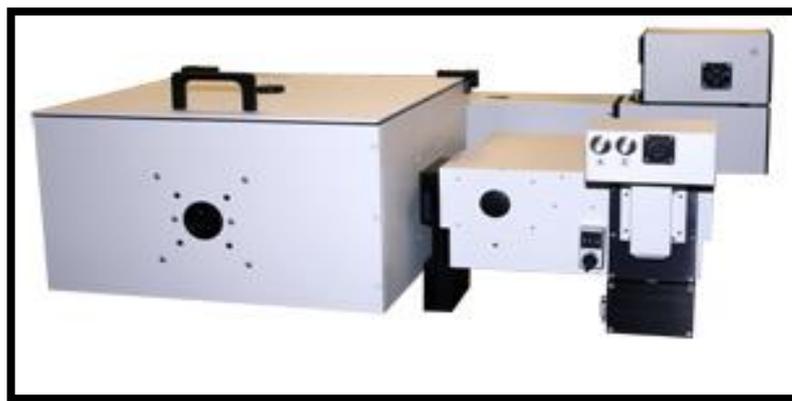
The transmittance is simply fraction of light in the original beam that passes through the sample and reaches the detector. [72, 73]

III. (2.13) Surface Tension Study

The Surface Tension experiments were done by a platinum ring detachment process via a Tensiometer (K9, KRUSS: Germany) at the experimental temperature. The temperature of system was preserved by circulating auto thermo stated water (within 0.01 K) through a double-wall glass vessel holding solutions. The accuracy of the measurement was within $\pm 0.1 \text{ m N} \cdot \text{m}^{-1}$. Surface tension (γ) is an imperative tool, providing a valuable clue about the formation of an inclusion complex for an aqueous solution. The trend of the curves for surface tensions (γ) against concentration (molality) curve clearly shows a break point in surface tension at a certain concentration, i.e., the (γ) values increase or decrease with corresponding concentration, reach a certain point (break point), and then become approximately steady, which obviously indicates formation of an inclusion complex. [74, 75]



III. (2.14) Fluorescence Measurements

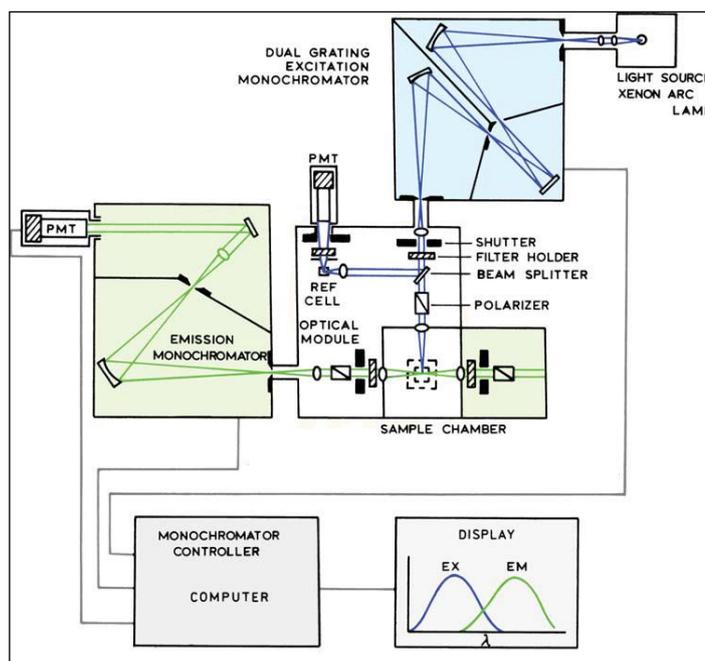


Steady state Fluorescence measurements were recorded in Quanta Master 40 Spectrofluorometer. The output range of the machine was almost 2 analog (+/- 10 volts). The phenomenal sensitivity of this technique makes routine measurements in sub-micromolar, even nanomolar (nM) range possible and hence, fluorescence spectroscopy is ideal for the determination of incredibly large association constants ($K_a > 10^6 \text{ M}^{-1}$). Fluorescence is a particularly helpful technique in the case when only one of the species in solution is fluorescently active, i.e. when either free host or guest is fluorescent “silent” or inactive and fluorescence of the remaining species is either turned “off” (quenched) or “on” upon complexation. If quenching plays a role, it is necessary to differentiate among static and dynamic (collisional) quenching, with only the former of real significance for supramolecular binding studies.

Dynamic quenching is usually measured by plotting the ratio of initial (F_0) and measured (F) fluorescence intensity ratio (F_0/F) against concentration of quencher $[Q]$ according to *Stern-Volmer relation* $F_0/F = 1 + K_{SV} [Q]$, by means of K_{SV} = Stern-Volmer constant.

Unfortunately, pure 1:1 static quenching follows a nearly identical relation: $F_0/F = 1 + K_a [Q]$, with $[Q]$ = the free concentration of quencher (guest) and K_a is association constant of interest in supramolecular binding studies.

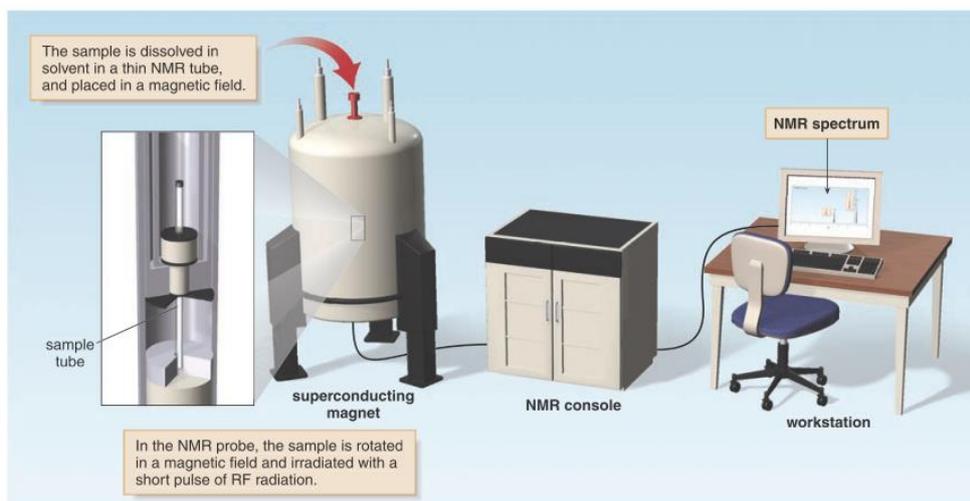
In numerous cases the observed quenching is a mixture of both static and dynamic quenching which can lead to some complication in the analysis of titration data. ^[76]



Schematic diagram of a spectrofluorometer

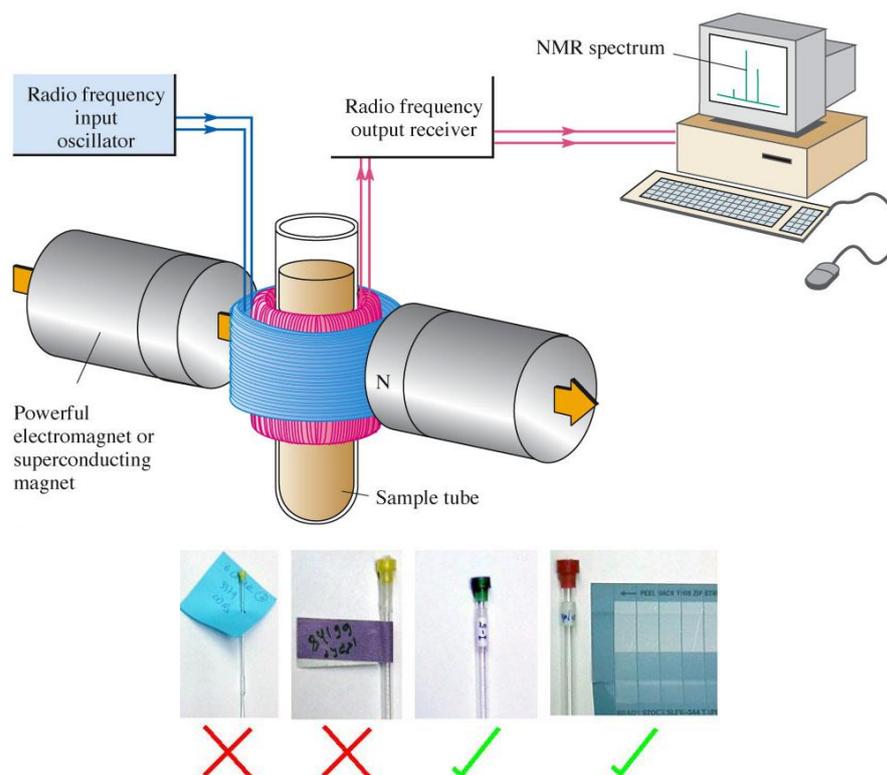
III. (2.15) NMR Spectroscopy

Nuclear magnetic resonance spectroscopy: generally referred to as NMR, is a technique which exploits the magnetic properties of certain nuclei to revise physical, chemical, and biological properties of matter. [77-79]



An NMR spectrometer. The sample is dissolved in a solvent, usually CDCl_3 (deuteriochloroform), and placed in a magnetic field. A radiofrequency generator then irradiates the sample with a short pulse of radiation, causing resonance. When the nuclei fall back to their lower energy state, the detector measures the energy released, and a spectrum is recorded. The superconducting magnets in modern NMR spectrometers have coils that are cooled in liquid helium and conduct electricity with essentially no resistance.

Components of an NMR Spectrophotometer: Powerful Magnet, Field Sweep Generator, Sample Tube, Radio-frequency Transmitter, Radio-frequency Receiver, Amplifier, Recorder, Integrator.



Instrumentation and sample handling techniques

NMR spectral data were taken in D_2O using Bruker AV-300 spectrometer operating for 1H at 300 MHz and for ^{13}C at 75 MHz. Splitting patterns of protons were described by singlet, doublet, triplet, broad and multiplet. Chemical shifts (δ) were reported in parts per million (ppm) relative to TMS (Tetra methyl silane) as internal standard (D_2O : δ 4.79 ppm).

2D-ROESY (Rotating Frame Overhauser Effect Spectroscopy) was taken in Bruker AV-300 spectrometer in D_2O . 400 MHz Nuclear Magnetic Resonance (NMR) Spectrometer was used to do the NMR titration experiments.

III. (2.16) UV-Visible spectroscopy

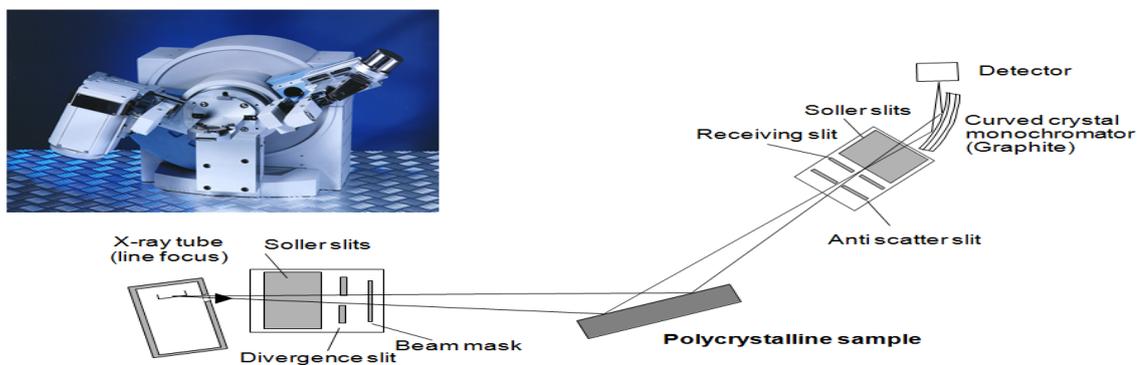
Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most imperative analytical instrument in the modern day laboratory. In several applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness. This description outlines basic principles for those new to UV-Visible spectrometry. UV-Visible absorption spectroscopy is a generally used technique for the determination of equilibrium constants, particularly in biochemical applications. For example, study relates to the binding of a cyclic-polysaccharide to a small molecular guest in lab. [80, 81]



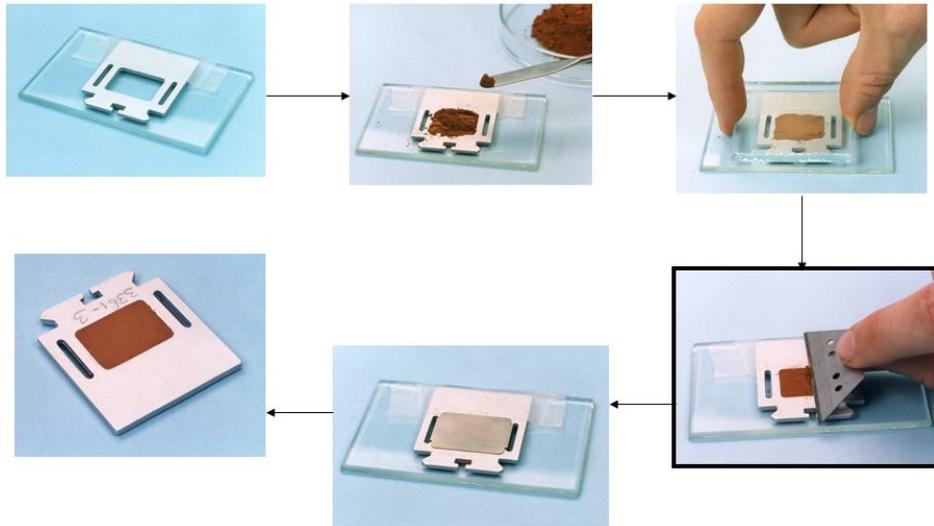
UV-Visible spectrums were noted via JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty of wavelength resolution of ± 2 nm. Measuring temperature was detained constant by a thermostat. Absorption spectra used to verify the formation of inclusion complex. In this study, absorption spectrum of studied compound was taken into considerations.

III. (2.17) Powder X-ray Diffraction spectroscopy

Geometry of a diffractometer with monochromator



Powder Sample Preparation on a Aluminium Holder



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 0.1\AA to 100\AA) equivalent to that shell. Rigaku, Model: Micromax-007HF was aided to perform the analysis. [82, 83]

III. (2.18) 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 (JSM-6360) was aided to perform the analysis and obtain the data's. [84, 85]

III. (2.19) 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 [86, 87]



Instrumentation in TEM

III. (2.20) Computational technique

Gaussian is a universal purpose [computational chemistry](#) software package initially released in 1970 by [John Pople](#), and his research group at [Carnegie Mellon University](#) as Gaussian 70. The name originates from Pople's use of [Gaussian orbitals](#) to speed up molecular electronic structure calculations as opposed to using [Slater-type orbitals](#), a choice made to advance performance on limited computing capacities of then-current computer hardware for [Hartree-Fock](#) calculations. The current version of the Quantum chemical program is Gaussian 16. Gaussian quickly became a popular and extensively used electronic structure program. The development of the package was pushed including cutting-edge research in [quantum chemistry](#) and various other fields. Ab-initio calculations are implemented through Gaussian 09W quantum chemical package in our analysis. For small molecules in the Solution phase, *ab initio* quantum chemical calculations can provide results approaching benchmark accuracy, are used routinely to complement experimental studies. A wide variety of properties, including structures,

thermo chemistry, and spectroscopic quantities of various types can be computed effectively. [88-97]

III. (2.20) Agar diffusion method to detect anti-microbial assay in Inclusion complexes

Spread plate technique was applied to inoculate organisms in Muller-Hinton agar and the compounds were applied in agar cup at 1mg/ml concentration in separate plates and incubated at 37°C for 24 hrs. Double distilled water was used as the control. Antimicrobial activity was determined by the zone of inhibition surroundings Agar cup. All those experiment was done in triplicate. [98,99]

✚ In vitro anti-inflammatory activity: TO detect the novelty of the Inclusion complexes: Experimental Animals

Wistar albino rats of both sexes, weighing 140-160 gm, were measured as experimental animals for acute and sub-chronic toxicity and pain sensation. Animals were purchased from authorized animal vendor Ghosh Enterprise, Kolkata, India and were reserved in the Departmental animal house facility, within a temperature range of $24 \pm 2^{\circ}$ C. Animals were maintained in a 12h/12h day-night cycle and were fed by means of standard pellet food obtained from Ghosh Enterprise and water *ad libitum*. All animals were acclimatized for a period of 10 days before initiation of the experiments and 4 or fewer rats were kept per cage during the total period. The experiments were approved for period 2013-16 by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) with registration number 840/ac/04/CPCSEA, dated 01/01/2004 of University of North Bengal, West Bengal, India prior to the commencement of experiments. [100-105]

III. (2.21) Acute Toxicity Test

In acute toxicity test, three rat groups consisting of mature rats were chosen, each containing 6 rats (3 males and 3 females). The first group was considered as normal (N) which wasn't fed with *Aloe vera* gel. The extra two were experimental groups, designated as TA1 and TA2. TA1 animals were fed with 2 g *Aloe* crude gel/kg b. w. and TA2 were fed with *Aloe* crude gel at the rate of 5g/kg b. w. The animals were fasted overnight prior to feeding with a single dose of gel homogenate. The mortality rate, salivation, fur irritation, sleep/dizziness, lethargy, and diarrhea were observed for next 24 hours. This one-day long observation was further followed by 7-day long screening

for any long-term toxicological effects. All the experiments of the investigations were done according to OECD guideline 423 (Adopted 17th December 2001) for the acute oral toxicity study in rodents.

III. (2.22) Sub-chronic Toxicity

For the purpose of sub-chronic toxicity, animals were divided into four groups. All the groups enclosed 6 rats each (3 male and 3 females). The first group was considered as Normal (N), the second, third and fourth groups were treatment groups with a 28 day long daily feeding schedule of desired inclusion complex (designated as T1, T2, and T3 respectively). After completion of 28 day-long schedule, animals were sacrificed through anesthesia; blood from each animal was collected separately by puncturing their hearts with the aid of sterile needles. Kidney and livers tissues were collected by separating out the organ from body and were fixed in 4% formalin for histological processing. Blood was collected in EDTA vials to assess the entire blood profile. The clear serum was collected by allowing blood samples to clot. Hemoglobin was calculated immediately after the blood collection. All the experiments were prepared according to the standard guidelines with slight modifications.

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