CHAPTER -III

Experimental section

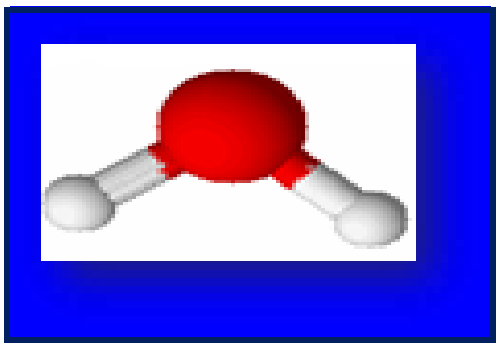
III. 1 NAME AND THEIR STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES, PURIFICATION AND APPLICATIONS OF THE CHEMICALS USED IN THE RESEARCH WORK

III.1.1 SOLVENTS

The details of the aqueous solvent used in the research work is described below.

Water (H_2O):

Water is a pervasive chemical substance is composed of two element, hydrogen and oxygen and it is very essential for all kinds of known forms of life. In typical convention, water states only to its liquid form or state, but also the substance exists as solid state, ice, and a gaseous state, water vapour or steam. Water is used as a good solvent and is often referred to as the collective or universal solvent.



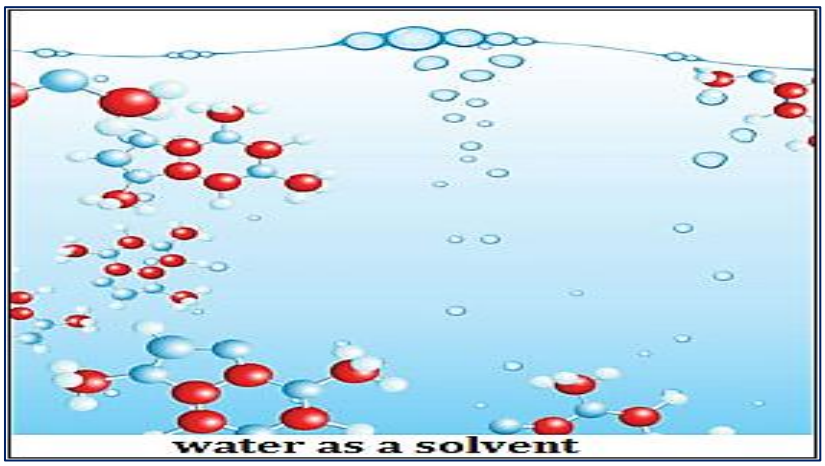
Structure of Water (H_2O)

Physical appearance	Liquid
Molecular formula	H2O
Molecular weight	18.01528 $g.mol^{-1}$
Density value	0.999700 $g.cm^3$(298.15K)
Refractive index	1.3333
Viscosity	0.890mP.s
Ultra sonic speeds	1500ms^{-1}
Dielectrical constant	78.35(298.15K)

Source: Distilled water used for experimental purpose, distilled from fractional distillation method in the Lab.

Purification*:* At first, water was deionized and then distilled in an all glass distilling set along with alkaline $KMnO_4$ solution was used to remove any organic matter in that. Finally, the doubly distilled water was distilled using an all glass distilling set for making perspective solution. Accurate Precautions were taken to prevent contamination from CO_2 and other contaminations. Specific conductance of the triply distilled water was less than one $\times 10^{-6}$ $S \cdot cm^{-1}$.

Application: Water is broadly used as a solvent or reactant in many chemical reactions and less commonly used as a solute or catalyst. Water is a conjoint solvent, dissolving many ionic compounds for solvation. Recently, Supercritical water has been a focus of research field of science. Oxygen saturated supercritical water combusts organic pollutants competently. In various industries also uses water for their many purposive work. It is a fantastic solvent, generally taken as the common solvent, due to tendency to form hydrogen bonds with other molecules and as well as the marked polarity of the water molecule. Water is so precise that our life on earth totally depends on it. Not for only a high percentage of living thing remain water, but also both plants and animals are found in water, all life on earth is believed to have stand up from water and the bodies of all living organisms are tranquil largely of water. Ln organic matter About 70 to 90 percent water is present. The chemical reactions in all types of plants and animals that provision or supportive of our life take place in a water medium. Water not only brings the medium to make these life-sustaining reactions possible, but water itself is often vital reactant or product of these reactions. In short, the chemistry of life is named as water chemistry.



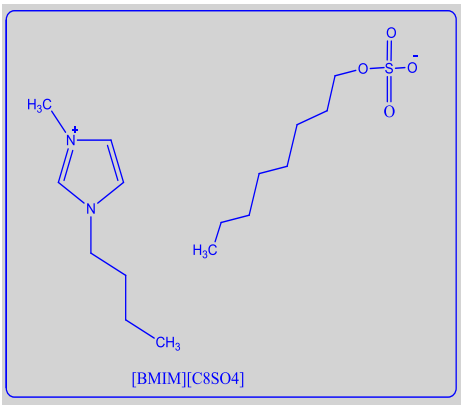
III.1.2 ELECTROLYTES AND NON-ELECTROLYTES

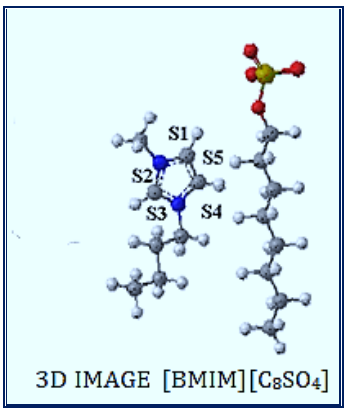
The ionic liquids,(electrolytes) and amino acids, (non-electrolytes) an two categories that are used in the research work which have been describing in the follow:

III.1.2.1 Ionic Liquids

1-butyl-3-methylimidazoliumoctylsulphate [BMIM][C_8SO_4](CASNO-445473-58-5)

1-Butyl-3-methylimidazolium octyl sulfate is a ionic liquid which is free to halogen element. This ionic liquid is a hydrolysis –stable compound. The critical micelle concentration (CMC) is above 0.031M ,it behaves as surfactant.





The Physical Property of [BMIM][C_8SO_4] is given as follow

Molecular formula	**$C_{16}H_{32}N_2O_4S$**
Density	**1.07 g/cm3**
Melting point	**37^0 C**
Boiling point	**N/A**
Molecular weight	**348.50**
Colour	**Beige**
PH-Value	**2-3 (H2O, 20℃)**
Solubility	**water**

Source*:* Sigma Aldrich(Germany)

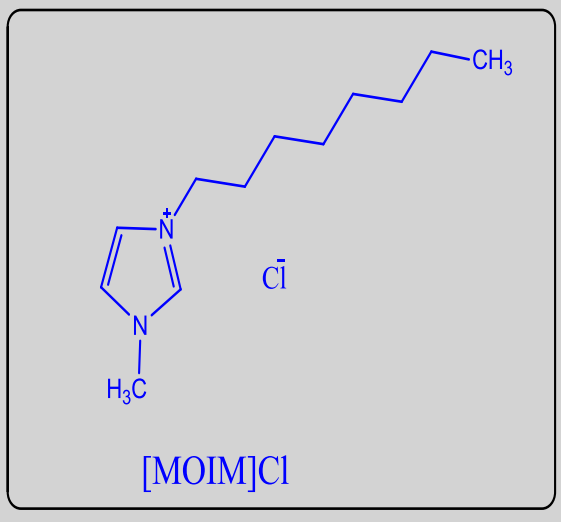
Purification*:* It is used as purchased. The purity of the chemical that used for experiment was about ≥95.0%

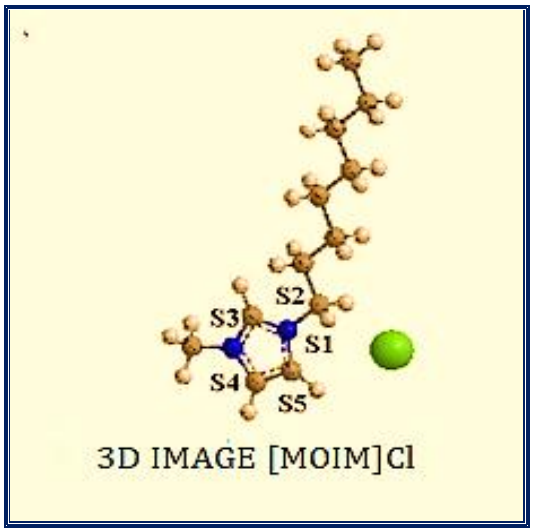
Application: In the presence of a rhodium catalyst,1-Butyl-3-methylimidazolium octyl sulfate[BMIM][C_8SO_4] can be used as a solvent during the hydro formylation reaction to convert 1-octene to form nonana . The technical accessibility and the well-documented

toxicology of the octylsulfate anion behaves this ionic liquid as a highly interesting candidate for industrial purpose.

1-methyl-3-octylimidazolium chloride [MOIM]Cl (CASNO-64697-40-1)

1-Methyl-3-octylimidazolium chloride is a ionic liquid containing imidazolium ring in this structure. The molecular formula of this ionic liquid is $C_{12}H_{23}ClN_2$.





The Physical property of [MOIM]Cl is given as follow

	$C_{12}H_{23}ClN_2$
Density 20^{0}C	**1.071g/Ml at**
Melting point	**< RT**
Conductivity	**0.09 mS/cm (30 °C)**
Molecular weight	**230.78**
Refractivity	**1.505-1.515**
Viscosity value	**3690cP(35° C)**
Solubility	**water**

Source*:* Sigma Aldrich purchased from Germany.

Purification: It is used as purchased. The purity of the using chemical was ≥ 97.0%

Application:1-Methyl-3-octylimidazolium chloride ionic liquid ([MOIM]Cl) can be used as: In the presence of a metal chloride as a catalyst and HCl in the reaction medium for the hydrolysis of sucrose to form hydroxymethylfurfural. It is used as a structure-directing agent during the preparation of organized mesoporous alumina.

1-ButylpyridiniumBromide(BTPB)(CAS NO-874-80-6)

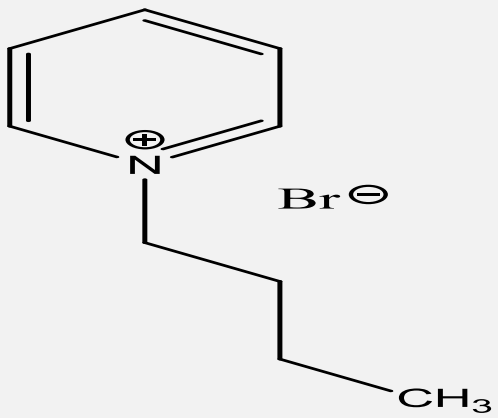
1-Butylpridinium bromide is the ammonium based ionic liquid, containing bulky alkyl (n-butyl) group of molecular formula($C_9H_{14}BrN$) exists as a molten solid phase (white crystalline) with the melting point 96-99ºC.

Source*:* Germany (Sigma Aldrich)

Purification: It is used as purchased. The purity of the using chemical was ±99.0%

Application*:*1-Butylpryidinium Bromide is widely used as electrolytes in electrochemical frames when control of electrode potentials is required source. The ionic liquid may be used in dye sensitized-cells, organic synthesis and also used as bio-catalysis batteries, electrochemical application and phase transfer catalyst, etc.

(BTBA)

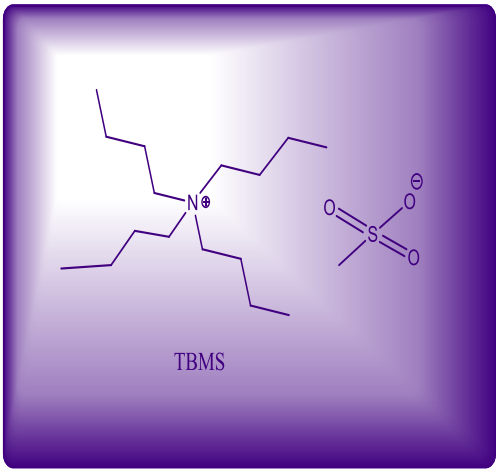


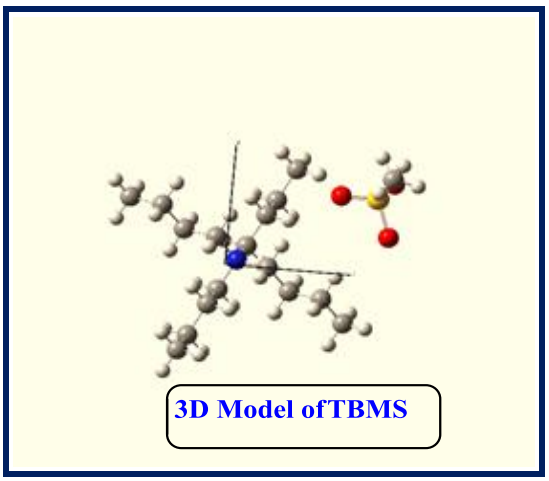
The physical property of PBr is given as follow

Appearance	:White Crystalline
Molecular Formula	:$C_9H_{14}BrN$
Molecular Weight	:216.12 g·mol^{-1}
Melting Point	:369-372 K
Relative Density	:No data available

TETRA BUTYL AMMONIUM METHANE SULFONATE(TBMS):(CASNO-65411-49-6)

Tetra butyl ammonium methane sulfonate is the also ammonium based ionic liquid, containing bulky alkyl (n-butyl) group of molecular formula exists as a molten solid phase (white crystalline) with the melting point 96-99ºC.





The Physical property of TBMS is given as follow

Appearance	:White Crystalline
Molecular Formula	:$C_{17}H_{39}NSO_3$
Molecular Weight	:337.56 g·mol^{-1}
Melting Point	:78-80 C
Relative Density	:NotData Available
Ionic radii	:4.42 (Å) of Bu_4N^+ 2.83(Å) of $CH_3SO_3^-$

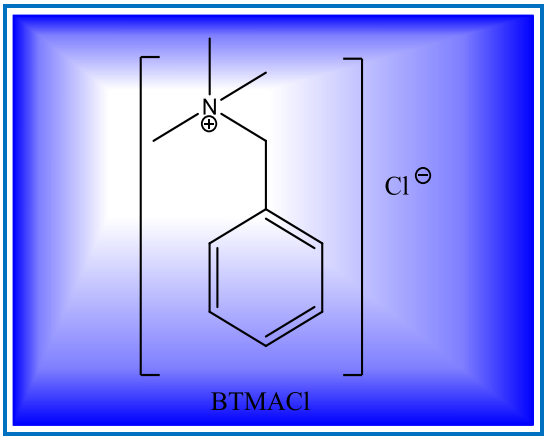
Source*:* Sigma Aldrich, Germany

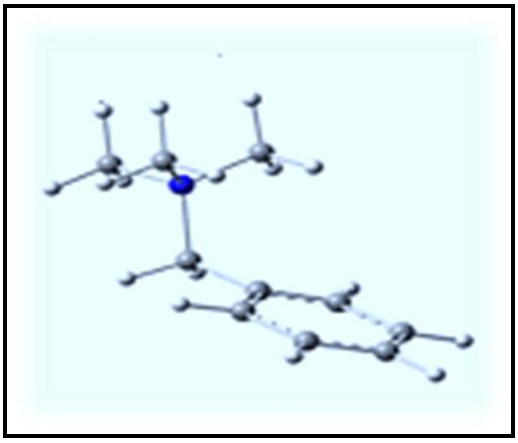
Purification*:* It is used as purchased. The purity of the chemical was about >99.0%

Application:-Tetrabutylammonium methane sulfonate (TBAMS) is perhaps used as same as 1-butyl bromide as electrolytes in electrochemical windows when switch of electrode potentials is required source. The ionic liquid may be used in bio-catalysis organic creation or synthesis an, dye sensitized-cells, batteries, electrochemical application and phase transfer catalyst, etc.

BENZYL TRI METHYL AMMONIUM CHLORIDE (BTMAC) (CAS NO-56-37-1)

It is a ammonium based ionic liquid containing three alkyl chain in its structure. It is a white crystalline solid and Faint Almond like odor. The molecular formula of the ionic liquid isC10H16ClN.





Stick and ball model of BTMAC (3D Image)

The physical property of BTMAC is given in the follow:

Physical state –solid	**density-1.07(gm/cm3)**
Molecular weight-185.70	**PH value-6-8**
Boiling point-105C	**Evaporation rate-<1**
Melting point-239c	**solubility- water**
Vapour pressure-<0.001hPa at 20c	

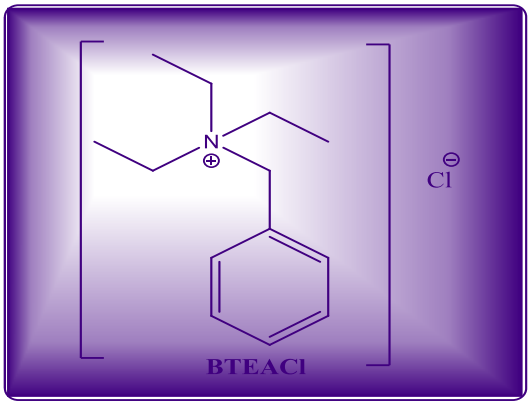
SOURCE: It was purchase From Japan, sigma Aldrich

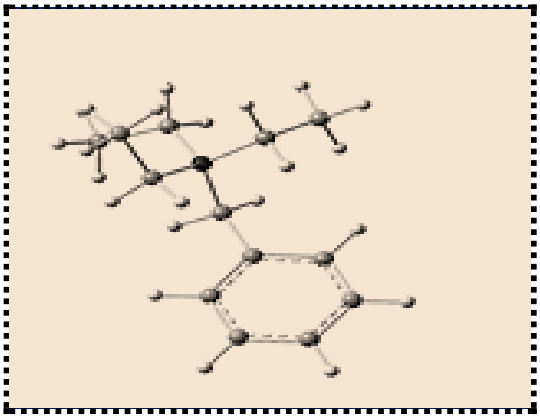
PURIFICATION: The purity of salt was taken 0.99. further purification was not taken**.**

APPLICATION: BATC is used for many reactions as a versatile phase transfer catalyst. It is also used as the field of industries purpose such as polymer, agrochemical, pharmaceutical etc.In oilfield it is used as a corrosion inhibitor.

BENZYL TRIETHYL AMMONIUM CHLORIDE: (BTEAC)(CAS NO-56-93-9)

It is also an ammonium based ionic liquid. It has three-alkyl chain (ethyl). The molecular formula is C13H22ClN. It is a white crystalline solid.





Stick and ball model of BTEAC (3D)

The physical properties of this ionic liquid is describes as the follows**.**

Physical state- solid	**density- 700kg/m3**
Flash point ->275C	**melting point-185^{0}c**
Ignition Temperature-300°c	**solubility- 630g/L**

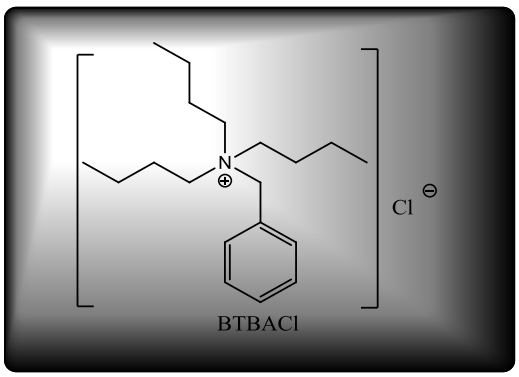
SOURCE*:* Sigma Aldrich, purchased from japan**.**

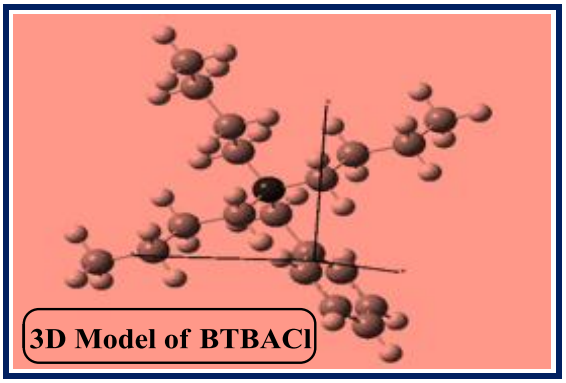
PURIFICATION: The purity of mass of the taken salt was 0.98. Further purification was not needed.

APPLICATION: Benzyl tri ethyl ammonium chloride is applied for the making of consumer products. It is used for plastic products, industry for plastic, plastic additives, manufacturing of plastics. It is also can be used as an Accelerators, activators, oxidation agents, reducing age.

BENZYL TETRA BUTYL AMMONIUM CHLORIDE (BTBAC)(CASNO-23616-79-7)

Tri butyl ammonium chloride is an ammonium based long alkyl chains ionic liquid organic chloride salt containing of a tri butyl ammonium cation and chloride anion. It is a tri butyl ammonium salt and also name as a an organic chloride salt. It is used as a purifying or cleansing agents, normally the salts of long-chain aliphatic bases or acids, that exert cleansing (oil-dissolving) and antimicrobial effects through a surface action that depends on holding both hydrophilic and hydrophobic properties of the ionic liquid.





Physical state	**Solid**
Molecular formula	**$C_{19}H_{34}ClN$**
Melting point	**(155-165)°C**
Solubility	**water(easily)**
Molecular weight	**311.94 gm/mole**

SOURCE: It was Purchased from Sigma ALDRICH (CHINA)

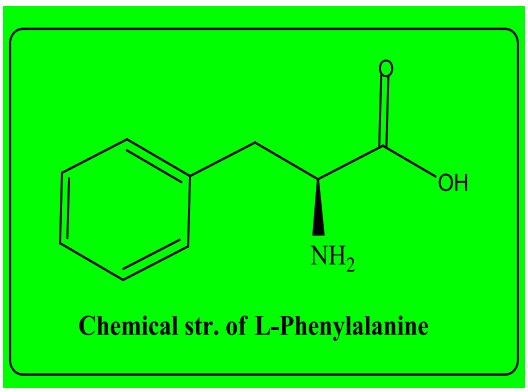
PURIFICATION: The purity of taken salt was 0.98.

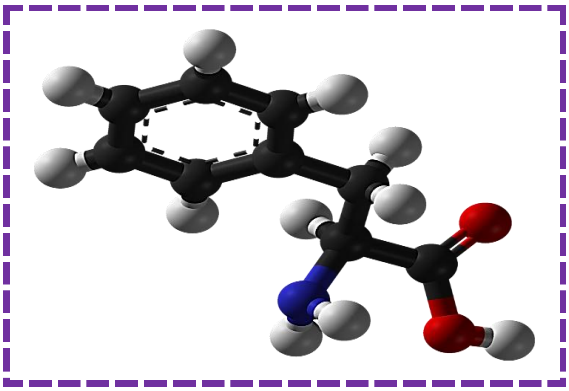
APPLICATION: Benzyl tri butyl ammonium chloride is used for the manufacturing of many industrial goods. It is used in laboratory for chemical purpose. It is successfully applicable of the above ionic liquid for the purpose of Accelerators, activators, oxidation agents, reducing agents, etc

AMINO ACIDS:

L-PHENYLALANINE: (CAS NO-63-91-2)

Phenylalanine is an essential amino acid (containing aromatic ring) in humans (provided by food). It is an Amino acid of erythrose4-phosphate/phosphoenolpyruvate family. It is also designated as a protein genic amino acid, a phenylalanine and a L-alpha-amino acid, Phenylalanine take plays a very vital role in the biosynthesis of other amino acids .It is very important in the structure and function of many proteins and enzymes. L-phenylalaninium and L-phenylalaninate are the conjugate base and conjugate acid respectively of L-phenyl alanine . L-Phenyl alanine is the enantiomer of a D-phenylalanine. It is also exist as a tautomer of a L-phenylalanine zwitterion.





3D IMAGE OF L-PHYNYL ALANINE

The Physical property of L-phenyl alanine is listed in the table form

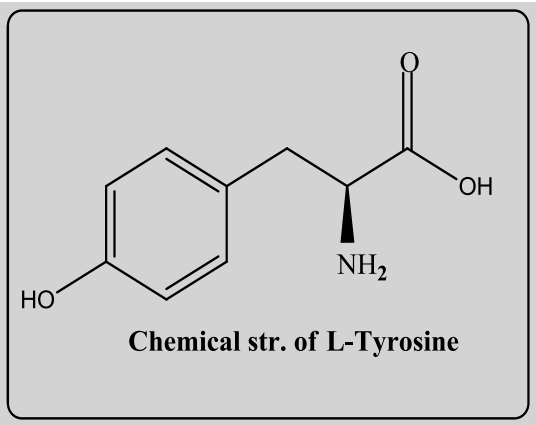
Molecular formula	**$C_9H_{11}O_2$**
Molecular weight	**165.19 gm/mole**
Physical state	**Solid(White) better in taste**
PH Value	**5.4-6(1% aqueous solution)**
Solubility	**26.9 gm/L**
Melting point	**541ºF(760mm Hg)**
Boiling point	**563º F(760mm Hg)**

SOURCE*:* Sigma Aldrich, from German.(purchased)

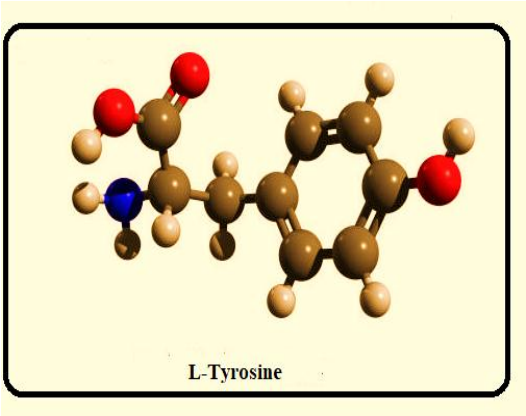
PURIFICATION: The mass purification of this salt was≥ 0.97%

APPLICATION: The phenylalanine is used to make food and also for drinking products.It is a source of nutritional supplement. It is used as antidepressant and analgesic agent. It is also a source of dietary supplement. Phenyl alanine also used as a blood- brain barrier.

L-TYROSINE: (CAS NO-60-18-4)



Chemical str. of L-Tyrosine



L-Tyrosine

Physical properties of L-Tyrosine is listed as bellow

Chemical formula	$C_9H_{11}NO_3$
Molar mass	181.191 gram/mole
Solubility	0.453 gm/100Ml(water)

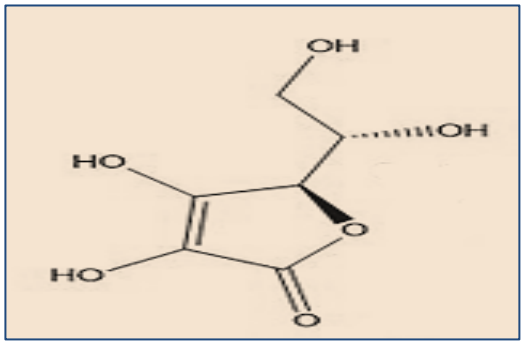
SOURCE: This salt was purchased from Sigma Aldrich (Germany)

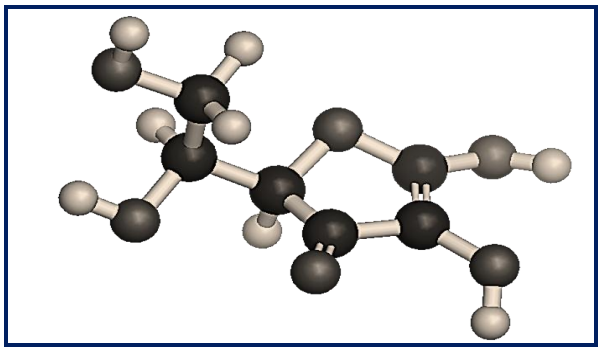
PURIFICATION: The purification of mass of this salt was≥ 0.98%

APPLICATION: Since tyrosine has contain -0H functional group at the virtue of phenol and therefore it has very especially role. It is a receiver of phosphate group and it has great role in the signal transduction process. The activity of a particular protein changes when phosphorylation of hydroxyl group take place. In case of photosynthesis the tyrosine residue has took place very important role and in the process of reduction Of oxidized chlorophyll, tyrosine residue act as a electron donor. It is also used as a ancestor to hormones and neurotransmitters.

L-ASCORBIC ACID :(CAS NO-50-81-7)

Vitamin C (Ascorbic acid) is water soluble molecules continuing many –OH functional group in their structure.





Chemical str. of L-Ascorbic acid **3D IMAGE OF L-ASCORIC ACID**

The physical property of L-Ascorbic acid is listed as below

Physical state- White or light yellow solid	**density- 1.65gm/cm3**
Chemical formula->C6H8O6	**melting point-190^{0}C-192°C**
Molecular weight-176.16 g.mole $^{-1}$	**solubility- 330g/L(In water)**

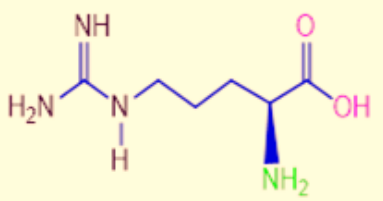
SOURCE: This salt was taken from sigma Aldrich.

PURIFICATION: ≥ 99.7%was the purity of mass. The solute molecule was placed in the dessicators over phosphorous pentaoxide for keeping the molecule in dry atmosphere. No further purification was carried out for experiments.

APPLICATION: L-Ascorbic acid helps to save and treat from the very dangerous disease scurvy and common cold. This is very need for the making of collagen, neutron transmitters and creatinine(5) due to very essential of vitamin C for mankind ,this can be used in several field of analytical chemistry such as food and also pharmaceutical applications. It is also used for the transport properties since the body fluid is always circulating. Since this is very simple molecule and it is very essay to mix with other solute molecules so it has dissolution effect.

L-ARGININE (CASNO-74-79-3)

Arginine or L-Arginine is a alpha- amino acid. In this structure there contain alpha-amino group , alpha-COOH group, three carbon aliphatic chain and also contain at the ending of straight chain a guanidine group.



Chemical str. of L-Arginine

Stick and ball model of L-Arginine (3D)

The physical property of L-Arginine is listed as bellow

Appearance	**Crystalline(white)**
Odour	**odourless**
Melting point	**high(260^{0}c)**
Boiling point	**high(368^{0}c)**

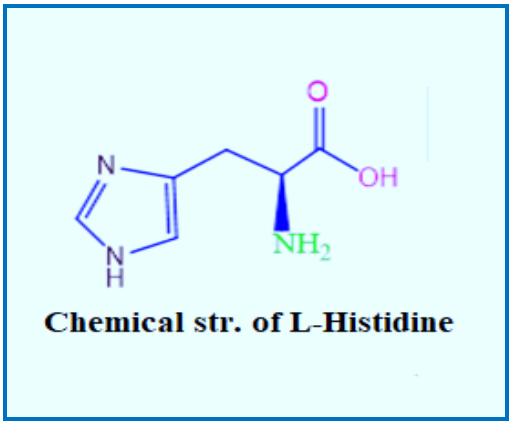
SOURCE*:*The salt was obtained from SRL, India**.**

PURIFICATION: The mass purification of salt was ≥ 0.99%. The amino acid was dried in vacuum over blue silica gel for72hr at room temperature.

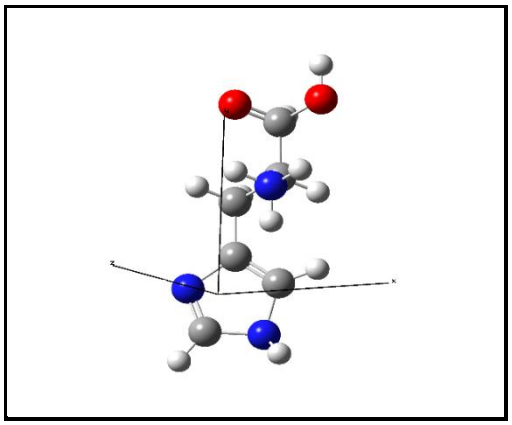
APPLICATION : L-Arginine is used as biosynthesis of proteins. It is used as a precursor for the biosynthesis of nitric oxide and making it very important in the directive of blood pressure. It takes very important role for cell division and wound healing. It is function as removing ammonia from the body and it help to remove the hormone. Arginine also used as a precursor for ornithine, agmatine and urea. It is also used for making polyamines. It is also used as a dietary sources. Since arginine remove the blood vessels, so it has cardiovascular benefits. It is also used to improve the symptoms of kidney inflammation and assist the function of kidney after a replacement.

L-HISTIDINE: (CAS NO-71-00-1)

In 1984 the German physician Albrecht Kossel and Sven Gustaf Hedin was first isolatated this amino acid (L-Histidine). L-His is a alpha amino acid. In this molecule, contain an alpha amino group and carboxylic group. Under biological condition the –NH2 group protonated to form $NH3^+$ and same condition –COOH group deprotonated to from $–COO^-$. In this molecule their contain a imidazole ring residue.



Chemical str. of L-Histidine



Stick and ball model of L-Histidine (3D)

The physical property of L-Histidine is listed below

Chemical formula	**$C_6H_9N_3O_2$**
Molar mass	**155.16 gm/mole**
Solubility	**Water(easily)**

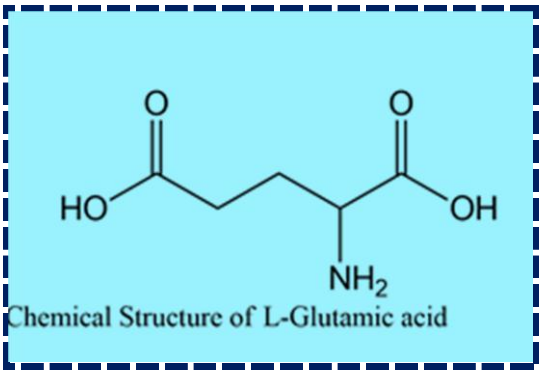
SOURCE*:* This Salt was purchased from TLC, Japan.

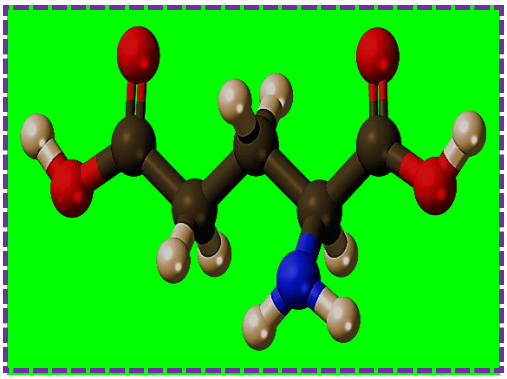
PURIFICATION*:* Purification of mass of this salt was ≥ 0.99%. The amino acid was dried in vacuum over blue silica gel for72hr at room temperature

APPLICATION: L-Histidine undergoes to complex formation with some metal ions. The side chain ring residue (imidazole) of l-Histidine behaves as a ligand in metalloproteins. The microorganisms, plants, animals take l-Histidine for their proper growth. It act as a precusor for histaminie that isused in the body for inflammation. It protect the muscale from damage. The l-hisidine take important role to regulation in the biosynthesis process. We use Histidine as a medicine. It is used for some very series diseases in our body such as allergic ulcers, rheumatoid arthritis and anemia that caused by failure of kidney i.e. dialysis of kidney.

L-GLUTAMICACID (CAS NO-56-86-0)

L-Glutamic acid are both water soluble polar aliphatic amino acids having very weak dipole–ion interaction. In this molecule, contain an alpha amino group and carboxylic group. Under biological condition the –NH2 group protonated to form $NH3^{+}$ and same condition –COOH group deprotonated to from $–COO^{-}$





3D MODEL OF L-GLUTAMIC ACID

The physical property of L-Glutamic acid listed in the table form

Appearance	**White crystalline**
Density	**1.4601 gm/cm3**
Melting point	**199°C**
Solubility	**7.5gm/L(20°C)**

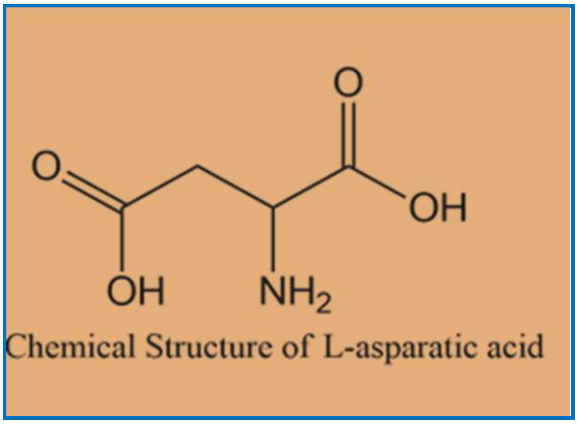
SOURCE*:* It was purchased from TLC, Japan

PURIFICATION*:* No required for further purification of this salt. Purity of mass was 0.99

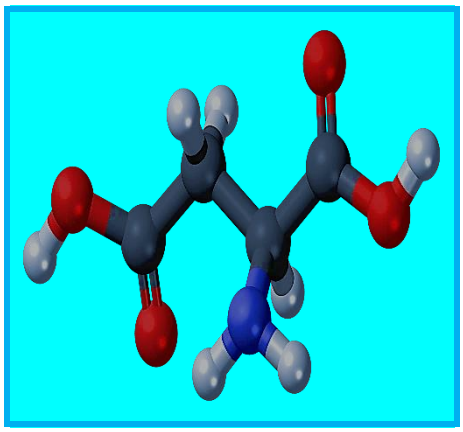
APPLICATION: L-Glutamic acid is an α-amino acid. It is used for all living beings in the biosynthesis of proteins. It is also used an excitatory neurotransmitter in the vertebrate nervous system.

L-ASPARATIC ACID(CAS NO-56-84-8)

L-Asparatic acid are both water soluble polar aliphatic amino acids having very weak dipole–ion interaction. In this molecule, contain an alpha amino group and carboxylic group. Under biological condition the –NH2 group protonated to form $NH3^+$ and same condition –COOH group deprotonated to from $–COO^-$



Chemical Structure of L-asparatic acid



3D MODEL OF L-ASPARATIC ACID

The physical property of L-aspartic acid is listed bellow

Appearance	Colourless
Density	1.79gm/cm^3
Melting point	270°C
Boiling point	324° C

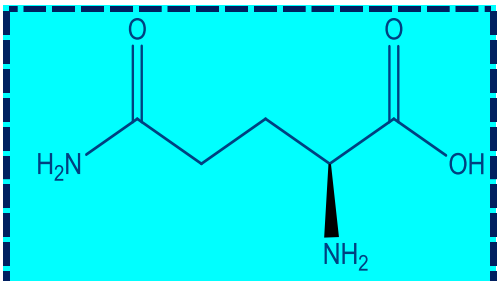
SOURCE: purchased from SRL, India

PURIFICATION: Purity of mass was 0.99. this salt was used for experiment.

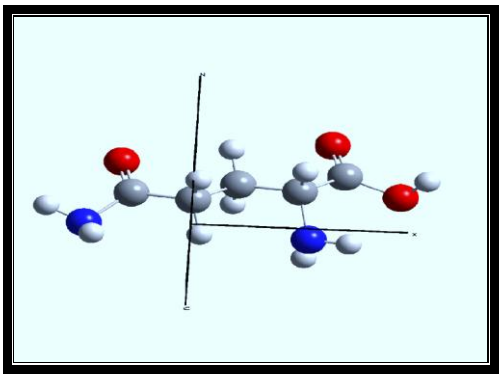
APPLICATION: L-Aspartic acid is an α-amino acid that is used in the biosynthesis of proteins. Aspartic acid is commonly used to reduce feelings of tiredness in our body, improve athletic performance, and increase the size and strength of muscles.

L-GLUTAMINE: (CASNO-56-85-9)

L-Glutamine is an alpha amino acid. The chemical formula of this amino acid is $C_5H_{10}N_2O_3$. This amino acid is a nutrient that help to synthesize the protein moeti in the human body for nutrition. hey can be found in protein-rich foods, including those from both plants and animals.



Chemical structure of L-Glutamine



Stick and ball model of L-glutamine

The physical properties of L-Glutamine is listed below in the table form

Chemical formula	**$C_5H_{10}N_2O_3$**
Molar mass	**146.146g.mol^{-1}**
Solubility	**soluble in water**
Melting point	**approximate 185^{0}C**
Appearances	**colourless solid**
Acidity(pk$_a$)	**2.2(carboxyl), 9.1(amino)**
Density	**0.99700 g/cm3**
Dielectric constant	**38.35 at 298.15K**
Refractive index	**1.3333**

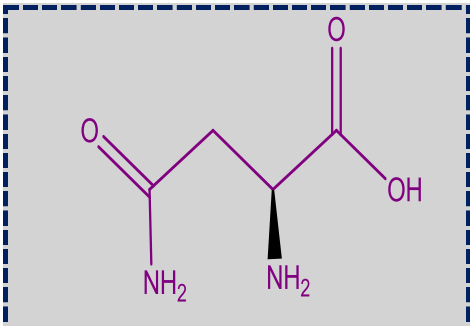
SOURCE*:* Sigma Aldrich, TLC (Product from Brazil)

PURIFICATION : The mass purity of this compound was 0.99 . Further purification of this compound is not required for experiment.

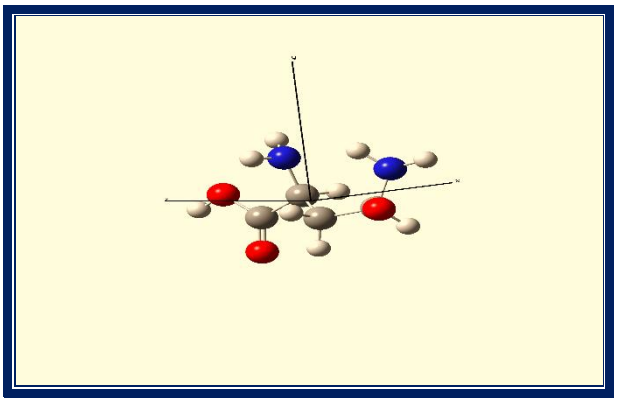
APPLICATION : Glutamine provides the most suitable example of the versatility of amino acid metabolism and immune function among the 20 amino acids detailed in the genetic code. Glutamine is such a amino acid in our body that it is most abundant and versatile amino acid and is of fundamental significance to intermediary metabolism, change the nitrogen among the organ via ammonia (NH_3) transport between tissues, and pH homeostasis.(Nutrients. 2018 Nov; 10(11): 1564).

L-ASPARAGINE (CASNO-70-47-3)

L-Asparagine is an alpha amino acid. L-Asn is written as symbolic form of L-Asparagine. In the structure of L-Asn contain alpha amino group. Under biological condition, amino group is protonated and carboxylic acid is deprotonated. It has carboxyamide group as a side chain. It is a non –essentials amino acid so it is synthesis in the human body.



Chemical structure of L-Asparagine



3DStructure of l-Asparagine (stick and ball model)

The physical properties of L-Asparagine is listed below in the Table

Chemical formula	**$C_4H_8N_2O_3$**
Appearance	**White crystals**
Molar mass	**132.119 gm.mol^{-1}**
Density	**1.543 gm/cm^3**
Solubility	**soluble in water(2.94 g/100mL)**
Log P	**-3.82**
Magnetic susceptibility	**-69.5·10^{-6} cm^3/mol**
Melting point	**234ºC (453 °F; 507 K)**
Boiling point	**438 °C (820 °F; 711 K)**

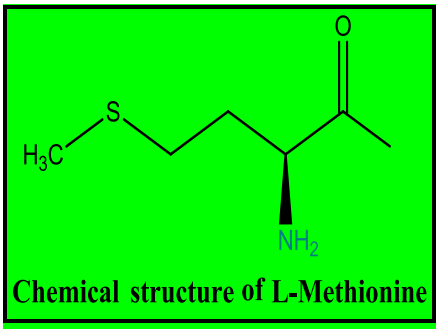
SOURCE : Sigma Aldrich TC L (USA)

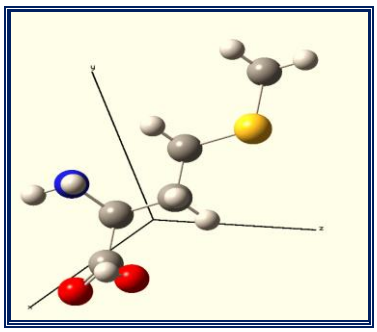
PURIFICATION*:* 98 was the purity of mass, without further purification was not required.

APPLICATION*:* L- Asparagine is used for biosynthesis of protiens. The oxaloacetate is the precursor of l-asparagine in the biosynthesis process of protein. It takes very key role to development of brain.

L-METHIONINE: (CASNO-63-68-3)

Methionine (L-Methionine) is an essential amino acid which is found in our body. It is the supplement of nutrition. It is very need for normal growth and make or repair the tissue of our body. This amino acid can not made by the body , but it must get from the diet; i.e. why it is consider as a essential amino acid in human body. Two types of methionine are found one is naturally occurring(L) another is D-methionine. The chemical make up is same for both types of amino acid. But only one is mirror image to another.(NCBI. PUB CHEM DATABASE)





3D Structure of l-methionine (stick and ball model)

The physical property of L-Methionine is listed bellow

Appearence	**White crystalline**
Density	**1.340gm/cm^3**
Melting point	**281°C**
Solubility	**water(easily)**

SOURCE*:* It was purchased from TLC, Japan.

PURIFICATION*:* The mass purity of the salt was 98. Further purification was nor required for experiment.

APPLICATION: The Sulphur containing amino acid is methionine; it has very importance application in different field of science. It is used as improve the tone and elasticity of skin, it strong the strength of healthy hair, nail. Although it is very effective in the treatment of Tylenol (acetaminophen) poising. It is also used for protecting the cell from pollutants, Facilitating the detoxify process, aging proceed, helping and adding

the adsorption of nutrients , excoriation of heavy metal(lead ,mercury) respectively. (author ,Park Bk, Dear JW, Antoine).

BY acting it as a lipotropic agent that prevent to increase the excess fat in the liver, lowering the cholesterol level by increasing production of lecithin production in the liver. It protect liver from damage, it also prevent the colon cancer(a 2013 meta analyst report) according to Study report 2016 among ten essential amino acid it is best for Brest cancer cell treatment.(CavuotoP,Fench MF Areview).

Although the amino acid is, help to improvement the memory function of brain. According to study (Molecular Neuro degeration) essesive use of this, which causes some types of diseases as heart disease, type 2 diabetes, certain type of cancer.

111. 2. EXPERIMENTAL METHODS

III.2.1 PREPARATION OF SOLUTIONS

Mass measurements for stock solutions were done on a Mettler Toledo AG-285electronic balance with a precision of±0.0003gm. The experimental density values was used to conversion of molarity into molality. The uncertainty in molality of solution is estimated to be ±0.0001mole/kg.

III.2.2. PREPARATION OF MULTICOMPONENT LIQUID MIXTURES

The preparation of binary or multicomponent liquid mixture can be done by the any one methods which is discussed below::

(a)Mole fraction (b) Weight fraction(c)volume fraction

(a) **Mole fraction: for the preparation of multicomponent liquid mixture the relation of mole fraction is used**: the relation is given in the following

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

where w_i, M_i are signify the weight and molecular weight of ith component, respectively. The number of component is apply for the formation of the solution mixture is depends on the value of i .

(a) **Weight fraction: The following given relation(mole fraction** w_i**) is utilize for the formation of** multicomponent liquid mixtures:

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

Volume fraction: The different three methods are used for evaluation or determination of the volume fraction (ϕ_i) of the multicomponent liquid mixtures.

i. Using volume: The volume fraction (ϕ_i) of the multicomponent liquid mixtures using the volume of pure liquid can be prepared by following relation

$$\phi_i = \frac{V_i}{\sum_{i=1}^{n} V_i}$$, Vi is volume of pure liquid

ii. Using molar volume: For the preparation of multicomponent liquid mixtures the volume fraction (ϕ_i^l) of the multicomponent liquid mixtures can be expressed by the following relation

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

where V_{mi} denotes the molar volume of pure liquid i.

iii. Using excess volume: The volume fraction (ϕ_i^{ex}) of the multicomponent liquid mixtures can also be obtained by following relation

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

where V^E is symbolizes the excess volume of the liquid mixture.

III.2.4 MEASUREMENTS OF EXPERIMENTAL PROPERTIES:

III.2.4.1 MASS MEASUREMENT:

A digital electronic analytical balance (Mettler Toledo, AG 285, from Switzerland) were made for the mass measurement of the sample.

This instrument can measure of mass with very high precision and accuracy. The considering cooking pail of a high precision (0.0001g) is inside a translucent enclosure with doors so that dust does not accumulate and so any air currents in the room do not affect the balance's operation.

Mettler Toledo, AG 285

Specification of Instruments:

Extreme capacity	: 210 g/81g/41g
Readability	: 0.1 mg/ 0.01mg
Taring range	: 0. . . . 210 g
Repeatability	: 0.1 mg/ 0.05 mg
Linearity	: ±0.2 mg/±0.1 mg
Stabilization time	: 3 s to15 s
Adjustment with external weights	:200 g
Sensitivity	: ±0.003%
Display	: LCD
Interface	: Local CAN universal interface
Weighing	: Φ 85 mm, stainless steel
Effective height above pan	: 240 mm
Dimensions(w/d/h)	: 205×330×310 mm
Net wt/with packaging	: 4.9 kg/7.25 kg

III.2.4.2 CONDUCTIVITY MEASUREMENT:

Systronics Conductivity TDS meter-308 was used to measurements of Conductivity of taken samples. It can provide both automatic and manual temperature compensation. Also another instrument was used to conductivity measurements(METTLER TOLEDO).

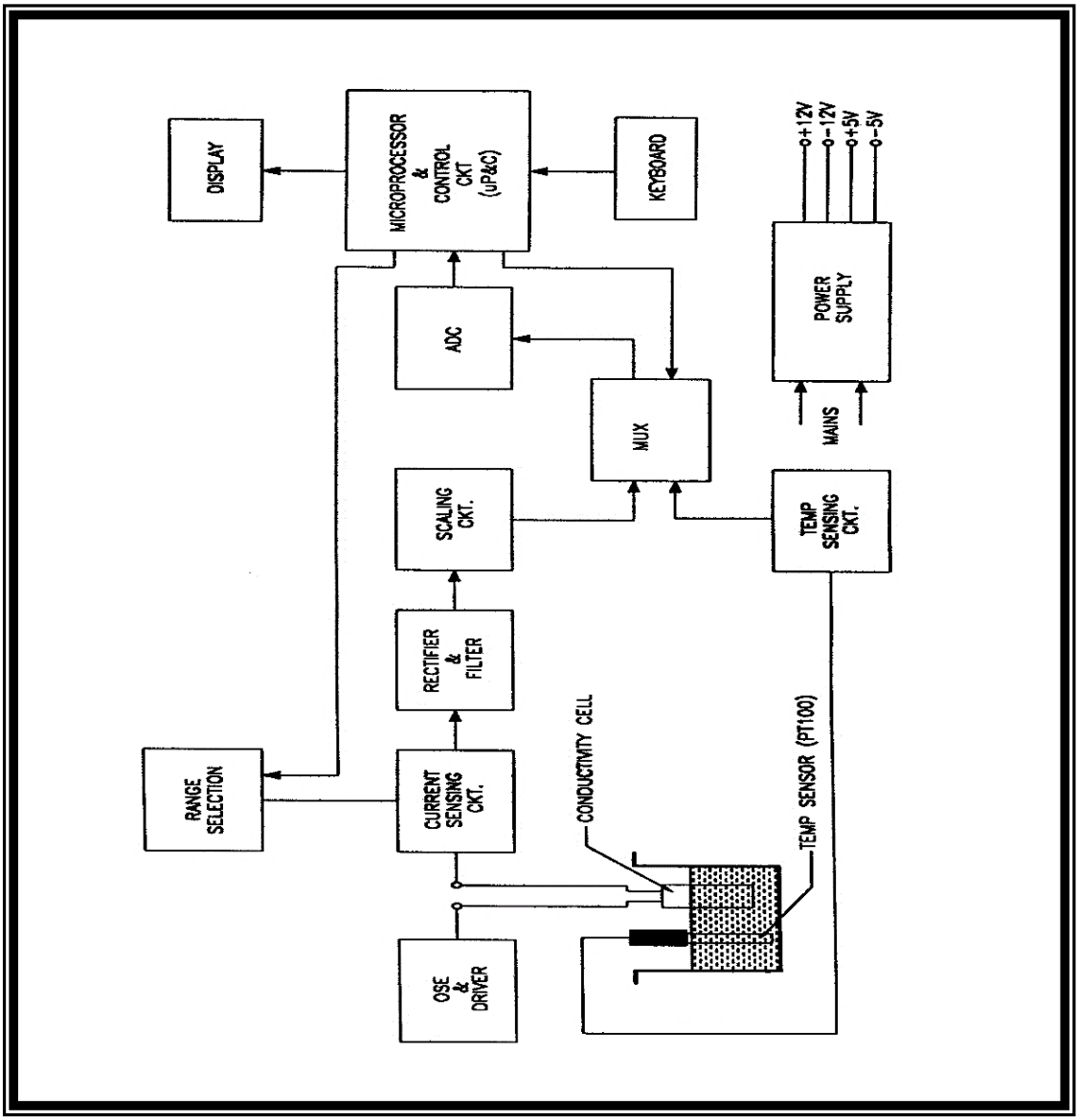
Systronics Conductivity-TDS meter 308 is a microprocessor based instrument used for measuring specific conductivity of solutions. It can provide both automatic and manual temperature compensation. The instrument shows the conductivity of the solution under test at the existing temperature or with temperature compensation. Provision for storing the cell constant and the calibrating solution type, is provided with the help of battery back-up. This data can be further used for measuring the conductivity of an unknown solution, without recalibrating the instrument even after switching it off.

Systronic-308 Conductivity Bridge:

Measurements of conductivity of experimental solutions were determined on this conductivity bridge of accuracy ±0.01%, using a dip-type immersion conductivity cell, CD-10 having a cell constant of nearly (0.1±0.001) cm^{-1}. Measurements were made in a thermostat water bath kept at T = (298.15 ± 0.01) K. The cell was calibrated by the technique proposed by Lind et al.[III.1] 0.01 M aqueous KCl solution was used to measure the cell constant.[III..2] Throughout the conductance measurements, cell constant was kept within the range 1.10–1.12 cm^{-1}. The conductance data of the experimental solutions were measured at a frequency of 1 kHz and the accuracy was ±0.3%. The conductivity cell was sealed to the side of a 500 ml 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 to the cell. The measurements of the

solutions were made in a thermostatic water bath continued at the required temperature with an accuracy of ± 0.01 K by means of mercury in glass thermoregulator.[III.3]

Experimental Solutions were prepared by weight precise to ± 0.02 %. All the weights were taken on a Mettler electronic analytical balance (AG 285, Switzerland). The molarity being transformed to molality as required. Several independent solutions were prepared and runs were made to ensure the reproducibility of the results. Due correction was completed for the specific conductance of the solvents at desired temperatures. The figure in the following displays the Block diagram of the Systronics Conductivity-TDS meter 308.



Block Diagram of the Instrument

III.2.4.3 DENSITY MEASUREMENT

The density measurement was accomplished with the help of Anton Paar DMA 4500M digital density-meter. The precision of the instrument was ±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 measured with high resolution and stands in simple relation to the density ρ of the sample in the oscillator

Anton Paar DMA 4500M digital density-meter

In the digital (Anton Paar DMA4500M) density meter, the mechanic oscillation of the U-tube is e.g. electromagnetically oscillation is converted into an alternating voltage of the same frequency. To calculate the period τ with high resolution and standpoints in simple relation to the density ρ of the sample in the oscillator is represented in the following:

$$\boxed{\rho = A \cdot \tau^2 - B} \qquad \text{(III.1)}$$

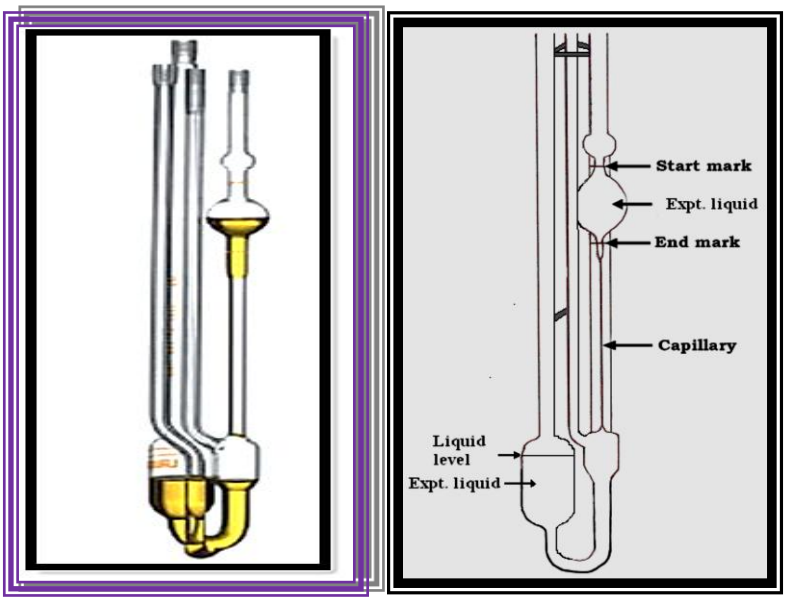
where A and B are denoted the individual instrument constants of each oscillator. With the help of two known density values ρ_1 and ρ_2 of two substances calibrating was done precisely. This Modern instruments was carried out to calculate and store the constants A and B after the two calibration measurements, which are mostly performed with air and water. They associated appropriate measures to compensate the various effects on the measuring result, e.g. the influences of the sample's viscosity and the non-linearity created by the measuring instrument's finite mass. Triply distilled water and dry air were used to calibration of the instrument.

Instrument Specification:

Density	: 0 to 1.5 g.cm^{-3}
Pressure	: 0 to 6 bar
Temperature	: 15ºC to 25ºC
Repeatability Standard Deviation	
Density	: 0.00001 g.cm^{-3}
Temperature	: 0.01 ºC
Additional information	
Minimum sample volume	: approx. 2 ml
Dimensions (L×W×H)	: 400×225×231 mm
Weight	: approx. 15 kg
Automatic bubble detection	: yes
Interfaces	: 2×CAN
Power	: Supplied by the master instrument

III.2.4.4 VISCOSITY MEASUREMENT

A Suspended level Ubbelohde Viscometer was used to measure the kinematic Viscosity. A stop watch was used to measure the flow of time. In their water bath, the viscometer was always kept in vertical position. The correction of kinetic energy was not needed for the viscometer.



Suspended-level Ubbelohde Viscometer

By Ubbelohde-type viscometer: Firstly Solution viscosity (η) was measured by means of suspended Ubbelohde type viscometer, calibrated with the help of triply distilled water, purified it with methanol and dry air with dryer. A cleaned and perfectly dried viscometer (Bose Panda Instruments Pvt. Ltd.) which was filled with experimental solution was placed vertically in a glass-walled thermostat kept to ±0.01K of the desired temperature. After achieving thermal equilibrium, efflux times of flow were recorded with a stop watch. The flow of times were exact to ±0.1s. At minimum three repetitions of each data reproducible to ± 0.1s were taken to average the flow of times. Suitable precautions were taken to minimize the evaporation loses during the actual measurements.

Instrument specification:	
Universal size number	**: 1**
Product Type	**:Glass Capillary**
Accuracy	**: ±0.2%**
Approximate constant	**: 0.01 cSt/sec**
Sample volume needed	**: 11 mL**
Range	**: 2 to 10 cSt**
Model	**: 9721-R59**
Brand	**: Cannon**

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

$$\gamma = \left(K \cdot t - \frac{L}{t} \right) \qquad \text{(III.2)}$$

$$\eta = \gamma . \rho \qquad \text{(III.3)}$$

Where, t denotes the time of flow, ρ is the density of experimental solution K and L are the characteristic constants of the particular viscometer. The accuracy of the viscosity measurement was ± 0.003 %. In all cases, the experiments were carried out in at least three replicates and the results were taken as the average of the triplicates.

The relative viscosities (η_r) were calculated by using the following equation:

$$\eta_r = \frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0} \qquad \text{(III.4)}$$

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

By Brookfield DV-III Ultra Programmable Rheometer A Brookfield DV-III Ultra Programmable Rheometer was used to measure the viscosity(η) with fitted spindle size-42. The viscosities were obtained by using the following equation

$$\eta = [100 / RPM \times TK \times torque \times SMC] \qquad \text{(III.5)}$$

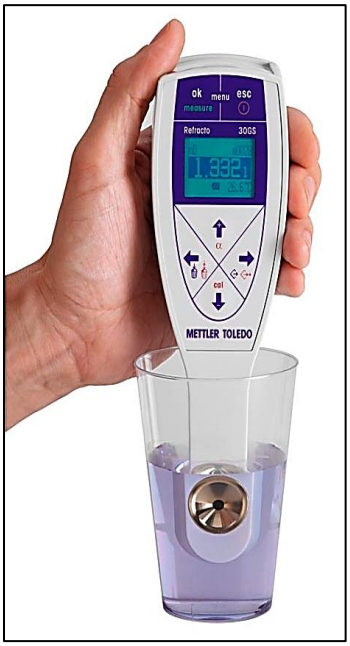
In the above mentions equation(III.5) where RPM,TK (0.09373) and SMC (0.327) are the speed, viscometer torque constant and spindle multiplier constant of the instrument respectively. The calibrated of instrument was against the standard viscosity samples provided with the instrument, water and aqueous $CaCl_2$ solutions.[I11.4] The temperature was kept constant throughout the experiments within ± 0.01°C using Brookfield Digital TC-500 thermostat bath. The viscosities of the solutions were done with an accuracy of ± 1 %. Each measurement of data of solutions reported herein is an average of triplicate reading with a precision of 0.3 %.

Speed Range of the instrument	**: 0-250 RPM, 0.1 RPM increments**
Viscosity Accuracy of the instrument	**: Full scale range (±1.0%) for a specific spindle running at a specific speed.**
Temperature sensing range	**: -100°C to 300°C (-148°F to 572°F)**
Temperature accuracy	**: ±1.0°C from -100°C to 150°C** **±2.0°C from +150°C to 300°C**
Analog Torque Output	**: 0 - 1 Volt DC (0 - 100% torque)**

III.2.4.6 REFRACTIVE INDEX MEASUREMENT

Measurements of refractive index data was carried out with the help of Digital Refractometer (Mettler Toledo 30GS).

Calibration of refractometer instrument was done by measuring the refractive indices of double-distilled water, cyclohexane, toluene, and carbon tetrachloride at defined temperature. The accuracy of the instrument is ±0.0005. 3-4 drops of the sample was place onto the measurement cell and the reading was taken. The refractive index of a sample are temperature depended. During measurement of the experimental solutions, refractometer determines the temperature and then corrects the refractive index to a temperature as wanted by the user.



REFRACTOMETER 30GS

Specifications-Refracto 30GS- extended RI measuring range

Model	: Refracto 30GS
Measurement range	: 1.32 -1.65
Accuracy	: +/- 0.0005
Resolution	: 0.0001
Measurement range BRIX of the refractometer	: 0 - 85 Brix%
Resolution	: 0.1 Brix%
Accuracy	: +/- 0.2 Brix%
Temperature range	: 10 - 40°
Resolution of temperature	: 0.1°
display	: °C or °F
Trade Name	: 51324660

The index of refraction is defined as the ratio of the speed of light in a vacuum to the speed of light in another substance is given as

$$Refractive\,index\,of\,the\,substance\,(n_D) = \frac{Speed\,of\,light\,in\,a\,vacuum}{Speed\,of\,light\,in\,substance} \qquad \text{(III.6)}$$

When light passes from one medium to another medium not only change it speed but also change the direction of the path i.e., it is refracted that shown in the(Figure 1). (In the special case when the light traveling perpendicular to the boundary there is no change in direction upon entering the new medium.) The relationship between the speed of light in the two mediums (V_A and v_{ibe}), the angles of incidence ($^{\theta}A$) and refraction ($^{\theta}B$) and the refractive indexes of the two mediums (n_{an} and n_{ab}) is shown below:

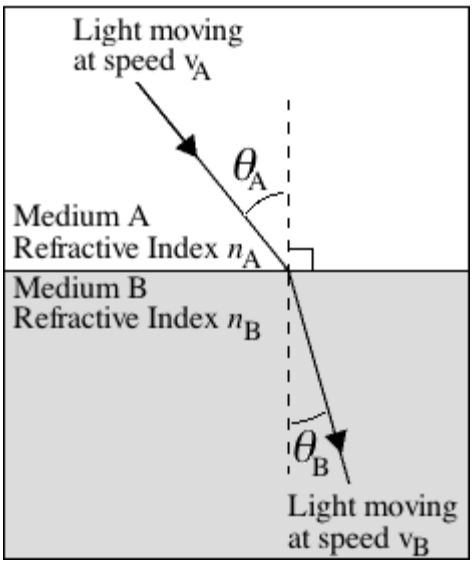


Figure 1. Light passage from any transparent medium into another in which it has a different speed, is refracted, i.e., bent from its original path (except when the direction of travel is perpendicular to the boundary). In the above case shown, the speed of light in medium A is greater than the speed of light in medium B.

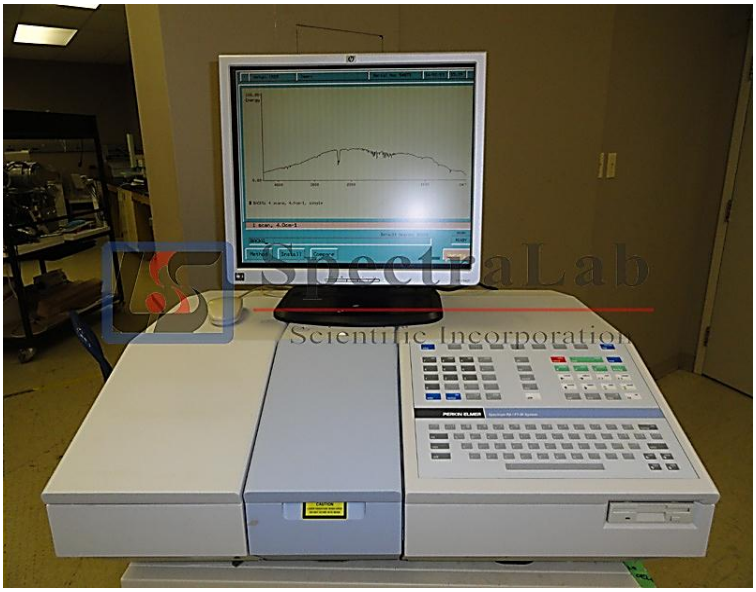
$$\frac{V_A}{V_B} = \frac{\sin\theta_A}{\sin\theta_B} = \frac{n_B}{n_A} \qquad \text{(III.7)}$$

Indeed, it is not very necessary to measure the speed of light in a sample in order to determine its index of refraction. Instead, measurement of angle of refraction and knowing the index of refraction of the sample that is contact the layer of the sample and determination of refractive index of the sample very precisely. The principle of all the refractometer is same but only difference is their optical defines. (III.5)

Source of a light is projected through the illuminating prism, the bottom surface of the prism which is designated as ground (i.e., roughened like a ground-glass joint), it is thought that each point on this surface can generating the light rays which traveling in all directions. Examination of Figure 2 displays that light when traveling from point A to point B will have the largest angle of incidence (q_i) and hence angle of refraction of the sample (QR) would be largest possiblily. All other rays of light that entering the refracting prism will have smaller $q_{tr.}$ value and hence this is lie to the left of point C. Thus, a detector sited on the backside of the refracting prism would display a light region to the left and a dark region to the right.

III.2.4.7 FTIR MEASUREMENT

FTIR (PerkinElmer, spectrumRXI) was used to determination or recorded the Infrared spectra.



Specification of FTIR spectrometer

21 CFR Part 11 Compatible	**Yes**
Wave Length	8
Wave Length Range	8,300 – 350 cm-1
Weight	13.0 kg
Width	45.0 cm

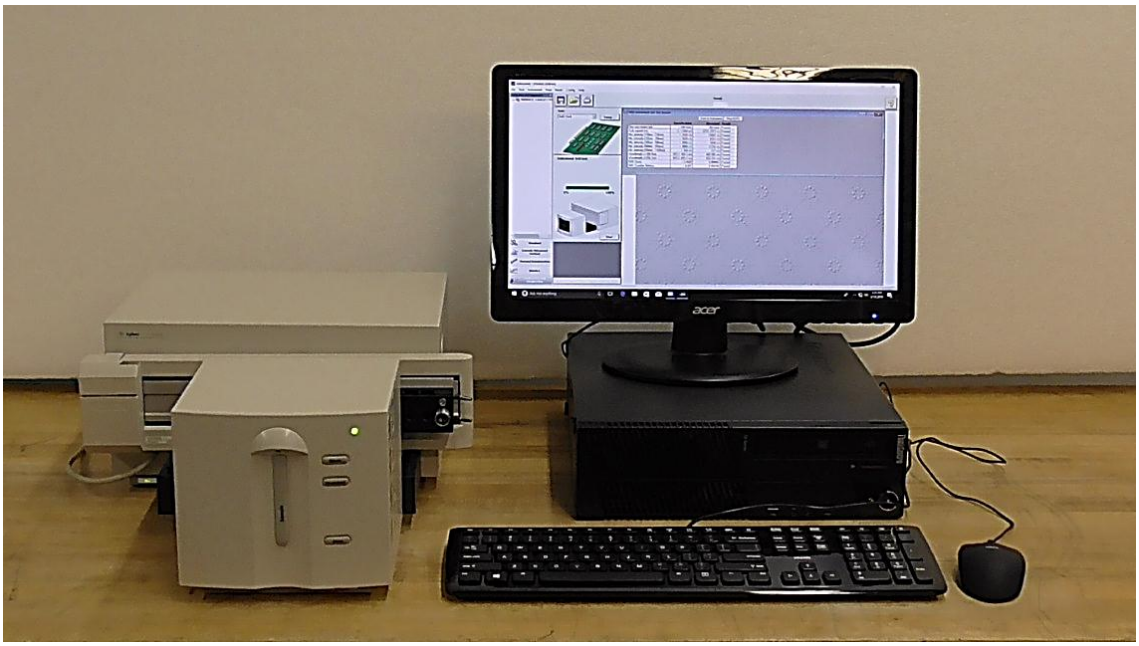
The intensity of incident light (I_0) passing through a blank is measured. The intensity is defined as the number of photons per second. The blank means is a solution that is identical to the sample solution except that the blank does not contain the solution that absorbs light. The measurement of the intensity of light (I) were done of the sample solution very carefully. (Indeed, instrument not only measures the power but also the intensity of the light. The power is defined as the the energy per second, which is the product of the intensity (photons per second) and the energy per photon.The transmittance (T) and the absorbance (A) can be calculated from the experimental data.

$$T = \frac{I}{I_0}; \qquad A = -log_{10} T \qquad \text{(III.8)}$$

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

III.2.4.8 UV Measurement:

When compounds absorb the Ultra violet and or visible light that gives the characteristic absorbance curves as a function of wavelength. Variation of absorbance occurred due to the molecules get excited to higher energy state. UV visible spectra were recorded with a wavelength accuracy of $\pm$0.5nm by Utilizing JASCO V-530 and Agilent 8453UV- visible spectrometer. With the help of a digital thermostat, the temperature of cell during the experiment was maintained from 298.15K to308.15K.



Agilent 8453 UV-Visible Spectrophotometer

A deuterium lamp for ultra violet light and a tungsten lamp for visible light uses two light source for both the using spectrophotometer. Agilent 8453 spectro photometer is a single beam spectrophotometer whereas JASCO V-530 is a double beam spectro photometric instrument containing a reference cuvete for solvent and another cuvete for measurement of experimental solution.

JASCO V-530 Spectrophotometer

III.2.4.9 Surface Tension Measurement:-

K9 digital TENSIOMETER(Kruss GmbH,Hamburg,Germany) instrument was used to determination the Siurface Tension of given experimental solutions.The accuracy of the instrument was $\pm$0.1. For determination of surface Tension the platinium ring detachment technique was used.

This tensiometer is very good precision instrument in which contain a solid and vibration-free base. This instrument put on such a place those same demands on its neighbor as a laboratory balance with a resolution of 0.1mg. In addition a clean and dust –free atmosphere is needed for measurements of surface Tension.

III.2.4.10. ^{1}H NMR Spectroscopic Measurement

^{1}H NMR spectra were recorded at 400MHz in Bruker Advance instrument in D_2O solvent at 298.15K. Values of chemical shift (δ)are presented in ppm (parts per million), (HDO, δ 4.79ppm) the residual protonated signal was used as an internal standard.

NMR spectroscopic technique gives the idea about the local magnetic field around the atomic nuclei. When the sample is placed in magnetic field, nuclei of the sample get excited with radio waves into nuclear magnetic resonance that can be recorded by NMR signal. The nature of electronic structure and as well as the functional groups in a molecule are known from the resonance frequency that obtained by intramolecular magnetic field. This field are exclusive for characterize the different compounds. In modern organic chemistry, NMR is very good technique to determine the monomolecular organic compounds. Also to identify the different complex molecules and proteins by the NMR technique according to biochemist. Over all NMR spectroscopy gives total information about the nature of dynamic, structure, state of reaction, and chemical environment around of molecules.

III.2.4.11. Fluorescence Spectra Measurement

Fluorescence Spectra were recorded by the bench top spectro fluorimeter (Quanta master -40, USA) at room temperature. In this spectrofluorimeter, a Hellma quartz cuvette was used whose optical path length was 1.0cm. Fluorescent molecules absorb the electromagnetic wave length from visible light spectrum and subsequent it can emit of light from the higher energy to lower energy state. Fluorescence of a molecule occurs when an orbital electron undergoes to its ground state by proton emitting from an excited singlet state. Different path take place for the relaxation of molecules. It can also relaxed by release heat through non-radiative process. This also undergo relaxation by the conversion to triplet to singlet state.

By the fluorescence quenching effect an excited molecule can get relax by another molecule. Avery good example of fluorescence quenching is the triplet state of molecular oxygen.

Other Instruments Used:

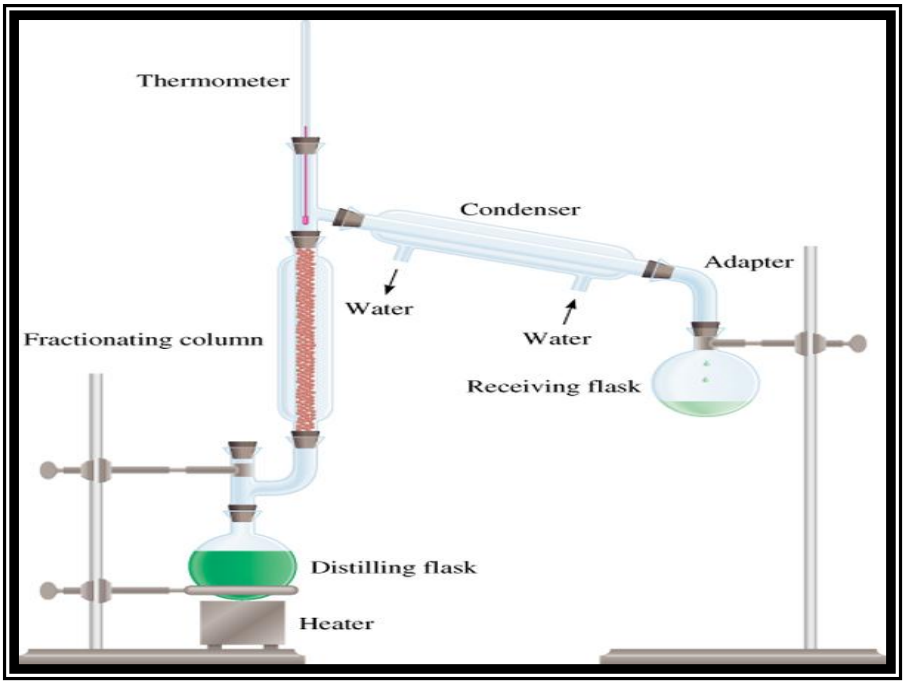
III.2.4.12. WATER DISTILLER

Water was distilled by using glass distillation unit, bionics Scientific technology private limited.

In the process of water distillation the water purify very quickly, cheaply as well as effectively. For the purpose of water distillation, basically we need a condenser and source of heat. Since boiling point of water is low therefore minerals like salt, contaminants, heavy metal, bacteria, calcium and phosphorous containing in untreated water is carried out to boiled , water easily turns to vaporize and everything leaves behind it. The undesirable elements which can not undergo by the vaporization process along

with water. Through the condensing coil vapor or water is routed such a process that the vapor reverts to the liquid form and the elements that undesirable stay in the boiling tank.

Fractional Distillation Apparatus



III.2.4.13. THERMOSTAT WATER BATH (Science India, Kolkata):

The measurements of experimental solution were carried out in thermostatic water bath and controlled with an accuracy of ± 0.01 K of the desired temperature. Brook field TC-550 also another thermostatic water bath ,this also used for the same purpose.

Laboratory working water bath is a system in which the material is placed into the vessel containing water to be heated quickly. Both digital as well as analogue control s are available to this laboratory equipment's for the purpose of the heating of the experimental solutions . it has greater temperature uniformity, heat retention, durability and recovery. Manufacturation of chambers of water bath lab products was leak proof and highly resistant stainless steel and other lab supplies.

TC-550