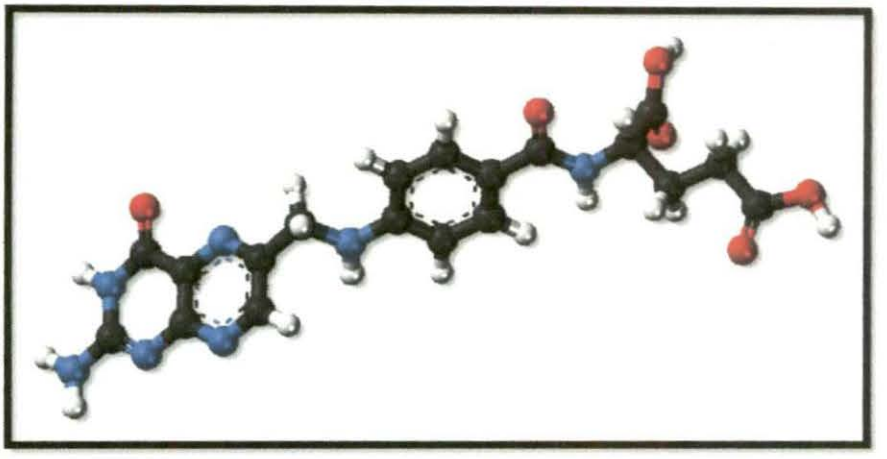
CHAPTER III

EXPERIMENTAL SECTION

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

SOLUTES

Folic Acid



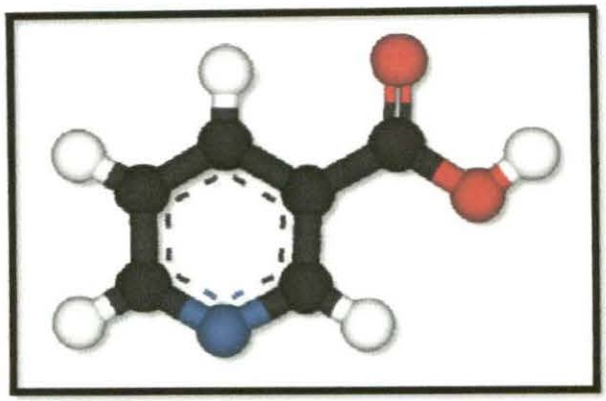
Molecular Formula : $C_{19}H_{19}N_7O_6$, **Molecular Weight**: 441.40 , **Appearance**: yellow-orange solid, **Melting Point**: 523.15K

Source: Sigma-Aldrich, Germany.

Purification: The mass purity as supplied is 0.99. It was dried from moisture at 353K for 24 h, and then cooled and stored in a desiccator prior to use.

Application: Vitamin B9 (folic acid and folate) is essential for numerous bodily functions. Humans cannot synthesize folate de novo; therefore, folate has to be supplied through the diet to meet their daily requirements. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions. It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy, and reproduction of cells, particularly red blood cells. Children and adults both require folic acid to produce healthy red blood cells and prevent anemia.[1]

Nicotinic Acid



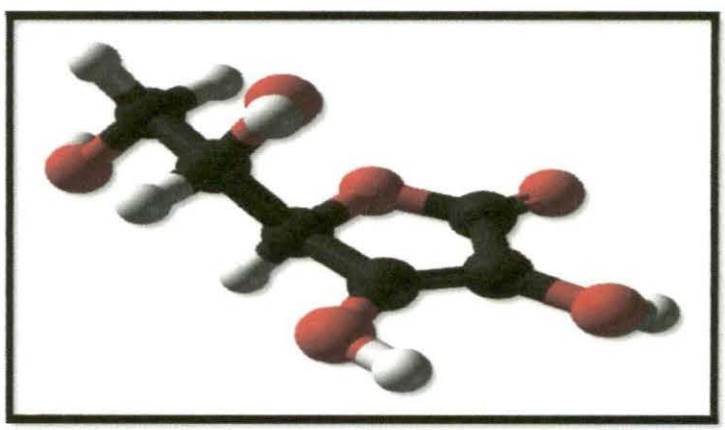
Molecular Formula: $C_6H_5O_2$, **Molecular Weight:** 123.11, **Appearance:** white solid, **Melting Point:** 510.15K

Source: Sigma-Aldrich, Germany.

Purification: It was dried from moisture at 353K for 24 h, and then cooled and stored in a desiccator prior to use.

Application: Nicotinic acid also known as Vitamin B3 is a water-soluble vitamin and an essential in micronutrient [2,3]. It is useful in pharmaceutical industries and food technology as well as in every process of the reaction occurring in protein and peptides chain.

Ascorbic Acid



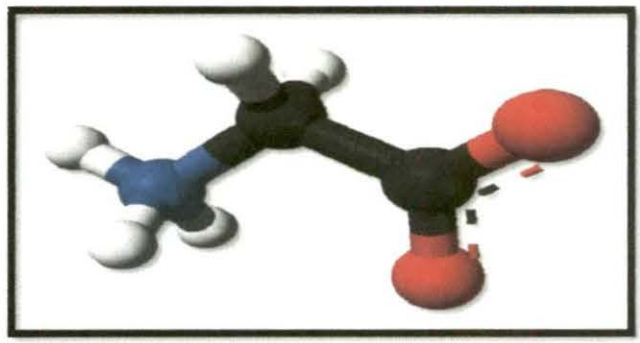
Molecular Formula: $C_6H_8O_6$, **Molecular Weight:** 176.12, **Appearance:** white solid, **Melting Point:** 463.15K

Source: Sigma-Aldrich, Germany.

Purification: Used as purchased as the purity assay of the solute was ≥98%.

Application: It is very important constituent of our physiological system. Vitamin C is required for the synthesis of collagen, the intercellular "cement" which gives the structure of muscles, vascular tissues, bones, and tendon.. It also enhances the eye's ability and delay the progression of advanced age related muscular degeneration [4]. It is helpful in formulation of many pharmaceutical products.

Glycine

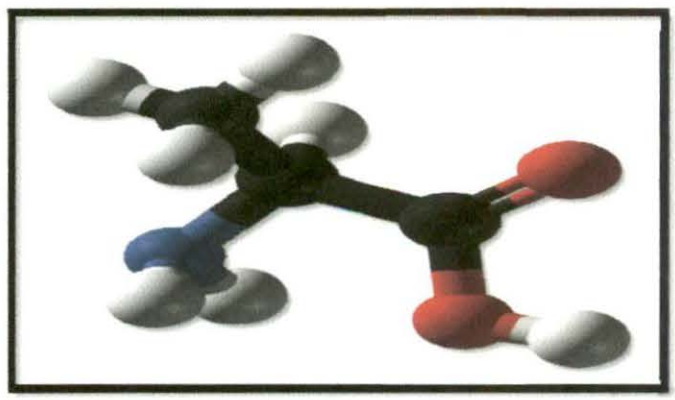


Molecular Formula: $C_2H_5NO_2$, **Molecular Weight**: 75.07, **Appearance**: white solid, **Melting Point**: 506.15K, **Source**: Sigma Aldrich, Germany

Purification: Used as parched without further purification. The purity is 99.99%.

Application: It has some pharmaceutical application. For humans, glycine is solid as a sweetener/taste enhancer. Certain food supplements and protein drinks containing glycine, for drug formulations it used to improve gastric absorption. Many miscellaneous products use glycine or its derivatives to make daily usable commodities.

L- Alanine



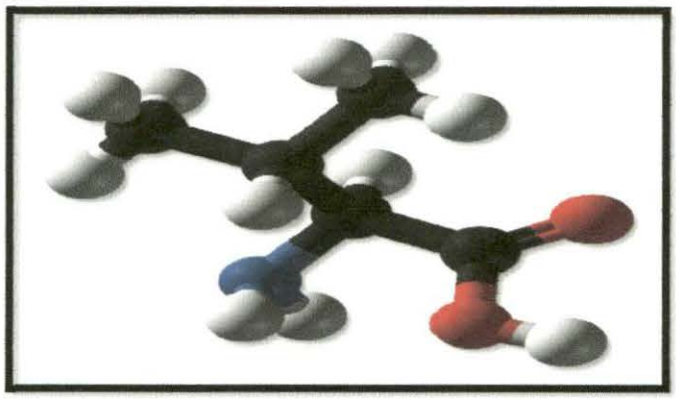
Molecular Formula: $C_3H_9NO_2$, **Molecular Weight**: 89.09, **Appearance**: white solid, **Melting Point**: 531.15K

Source: Sigma Aldrich, Germany

Purification: Used as parched without further purification. The purity is 99.99%.

Application: Alanine plays a key role in glucose–alanine cycle between tissues and liver. Sometimes it is used in case of radiotherapy. It has some medicinal use also.

L-Valine

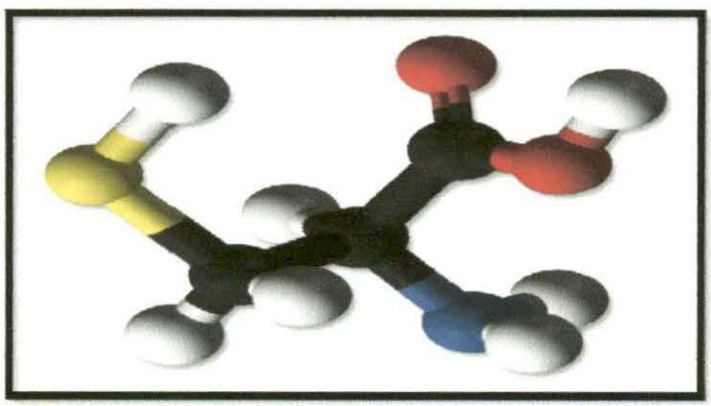


Molecular Formula: $C_5H_{11}NO_2$, **Molecular Weight**: 117.15, **Appearance**: white solid, **Melting Point**: 506.15K, **Source**: Sigma Aldrich, Germany

Purification: Used as parched without further purification. The purity is 99.99%.

Application: It is used for some pharmaceutical applications, industrial applications, food supplements and protein drinks, give out as a buffering agent in antacids, analgesics, antiperspirants, cosmetics, toiletries, production of rubber sponge products, fertilizers, metal complexants etc. Valine is an essential amino acid; hence it must be ingested, usually as a component of proteins.

Cysteine



Molecular Formula: $C_3H_7NO_2S$, **Molecular Weight**: 121.16,

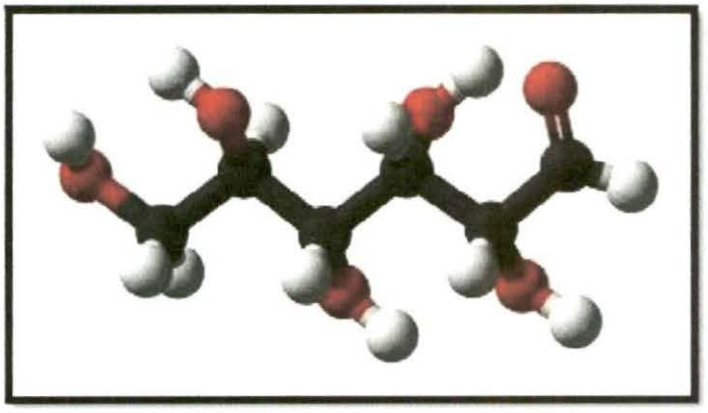
Appearance: white solid, **Melting Point**: 513.15K

Source: Sigma Aldrich, Germany

Purification: Used as parched without further purification. The purity is 99.99%

Application: Cysteine is a semi-essential amino acid, which means that it can be bio-synthesized in human body under normal physiological conditions if a sufficient quantity of methionine is available. Although classified as a non-essential amino acid, in rare cases, cysteine may be essential for infants, the elderly, and individuals with certain metabolic disease.

D-Glucose



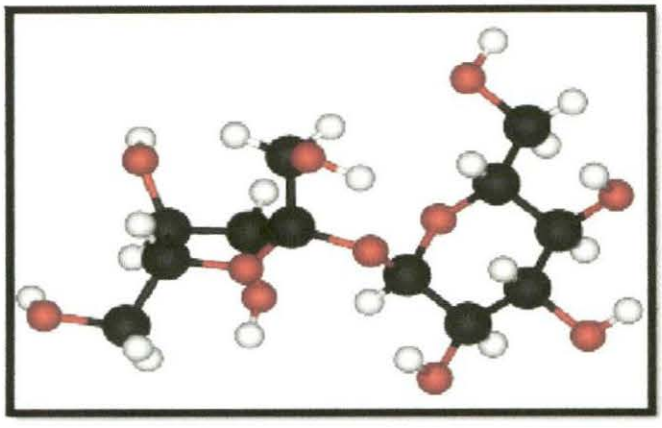
Molecular Formula: $C_6H_{12}O_6$, **Molecular Weight**: 180.16, **Appearance**: white solid, **Melting Point**: 419.15K

Source: Sigma-Aldrich, Germany.

Purification: Used as purchased as the purity assay of the solute was ≥98%.

Application: It is used as an energy source in most organisms, from bacteria to humans. Living cells use it as a secondary source of energy and a metabolic intermediate. Glucose is one of the main products of photosynthesis and fuels for cellular respiration. It is used in energy drinks and having some important pharmaceutical use.

D-Sucrose



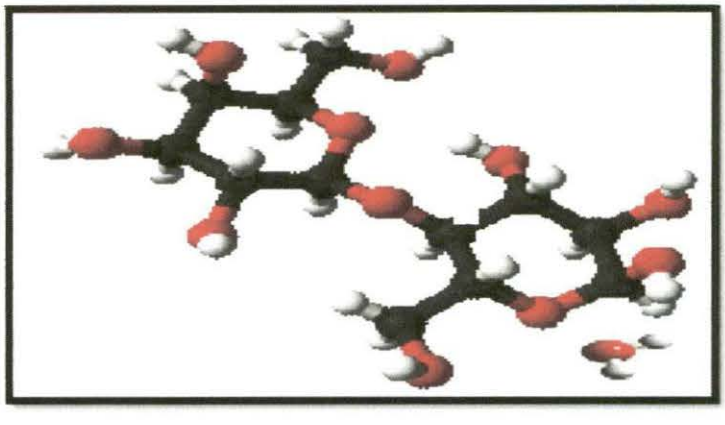
Molecular Formula: $C_{12}H_{22}O_{11}$, **Molecular Weight**: 342.30, **Appearance**: white solid, **Melting Point**: 459.15K

Source: Sigma-Aldrich, Germany.

Purification: Used as purchased as the purity assay of the solute was ≥98%.

Application: Commonly known as table sugar, cane sugar, beet sugar or, usually, just sugar. Widely used as sweetener in different food products. Acts as source of energy in human body system. Industrially used to prepare Ethyl alcohol and having some important pharmaceutical use as well.

D-Maltose monohydrate



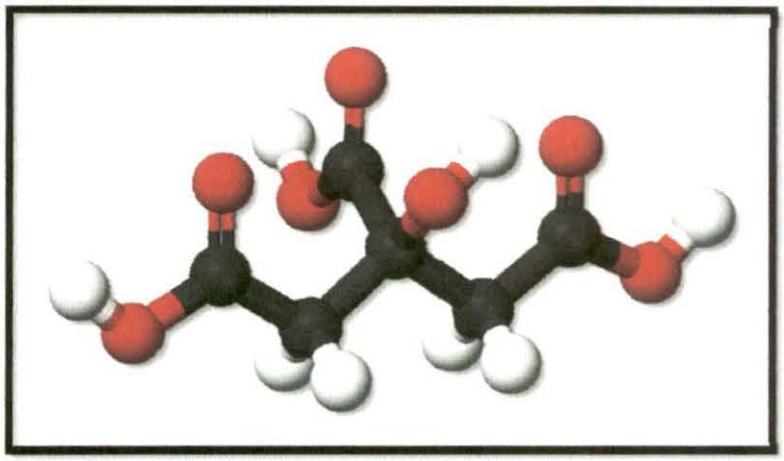
Molecular Formula: $C_{12}H_{22}O_{11}$, **Molecular Weight**: 342.30, **Appearance**: white solid, **Melting Point**: 375.15K

Source: Sigma-Aldrich, Germany.

Purification: Used as purchased as the purity assay of the solute was ≥98%.

Application: It is used to rectify congenital disorder which is most prominent in infancy by human body itself. In humans, maltose is broken down by the enzyme maltase so that there are two glucose molecules from which the glucose metabolism obtains energy. It has specific pharmaceutical and industrial use.

Citric Acid



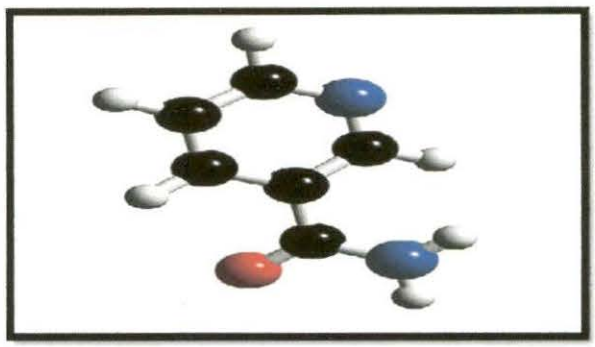
Molecular Formula: $C_6H_8O_7$, **Molecular Weight**: 192.12, **Appearance**: white solid, **Melting Point**: 523.15K

Source: Purchased from Hi Media.

Purification: Its mass purity as supplied is 0.99. The reagent was always placed in the desiccator over P_2O_5 to keep them in dry atmosphere.

Application: The dominant use of citric acid is as a flavouring and preservative in food and beverages, especially soft drinks. It can be used to soften water, which makes it useful in soaps and laundry detergents. Citric acid is widely used as a pH adjusting agent in creams and gels of all kinds. Citric acid is an alpha hydroxy acid and used as an active ingredient in chemical peels. As it occurs in metabolism of almost all living beings, its interactions in an aqueous solution is of great value to the biological scientists. In the pharmaceutical industry, citric acid is used as a stabilizer in various formulations, as a drug component and as an anticoagulant in blood for transfusions and also used as an acidifier in many pharmaceuticals.

Nicotinamide



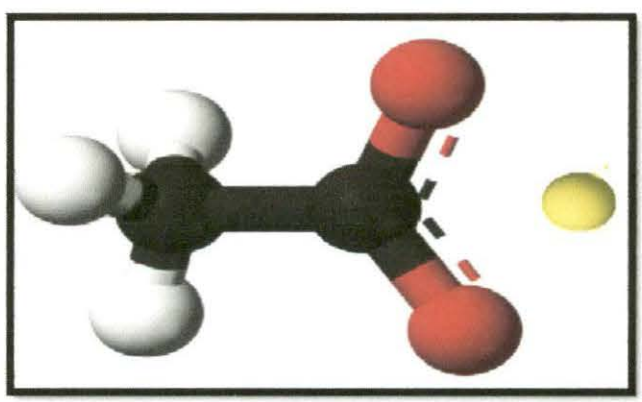
Molecular formula:$C_6H_6N_2O$, **Molecular weight**: 122.12, **Appearance**: white solid, **Melting Point**:401K

Source: Sigma-Aldrich, Germany.

Purification: It was dried from moisture and then cooled and stored in a desiccator prior to use.

Application: Studies show that nicotinamide has anxiolytic (anti-anxiety) properties. A safety study of niacinamide for the treatment of Alzheimer's disease is currently underway. Its anti-inflammatory actions that may be of benefit to patients with inflammatory skin conditions.

Lithium acetate



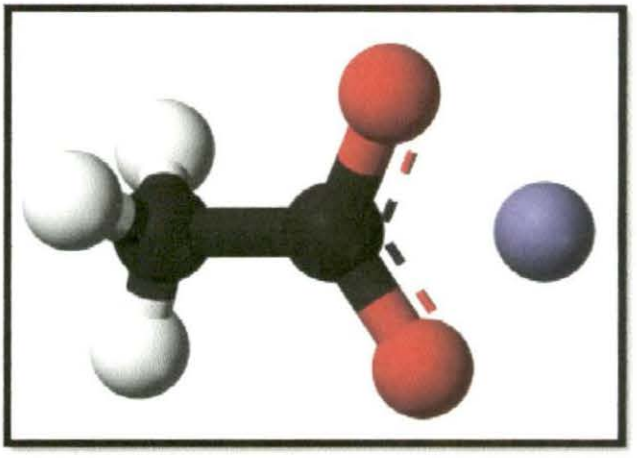
Molecular Formula: $C_3H_3LiO_2$, **Molecular Weight**: 65.99, **Appearance**: white solid, **Melting Point**: 559.15K

Source: Purchased from Merck, India

Purification: Purified by re-crystallization twice from conductivity water. It was dried in vacuum and stored over P_2O_5 under vacuum before use.

Application: Used in the laboratory as buffer for gel electrophoresis of DNA and RNA. This salt has some specific medicinal use.

Sodium acetate



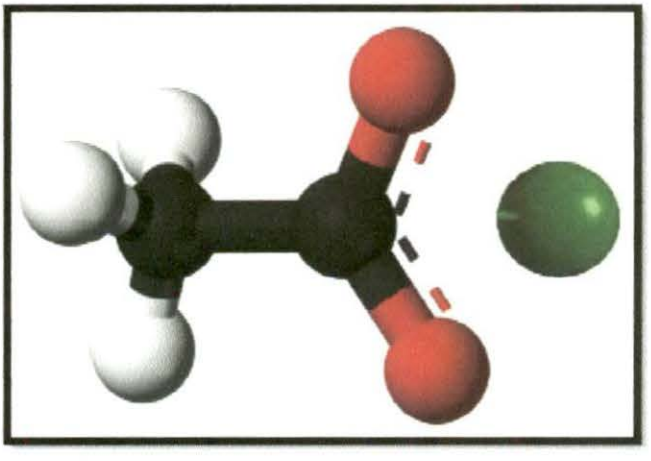
Molecular Formula: $C_3H_3NaO_2$, **Molecular Weight**: 82.03, **Appearance**: white solid, **Melting Point**: 597.15K

Source: Purchased from Merck, India

Purification: Used as parched without further purification.

Application: Sodium acetate may be added to food as a seasoning, helps to impede vulcanization of chloroprene in synthetic rubber production. It is used in the textile industry to neutralize sulphuric acid waste.

Potassium acetate



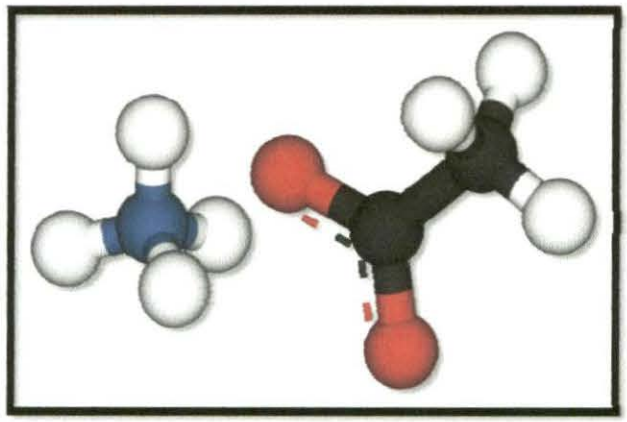
Molecular Formula: $C_3H_3KO_2$, **Molecular Weight**: 98.15, **Appearance**: white solid, **Melting Point**: 565.15K

Source: Purchased from Merck, India

Purification: Used as parched without further purification.

Application: In medicine, potassium acetate is used as part of replacement protocols in the treatment of diabetic ketoacidosis because of its ability to break down into bicarbonate and help neutralize the acidotic state. It is used as a food additive as a preservative and acidity regulator.

Ammonium acetate



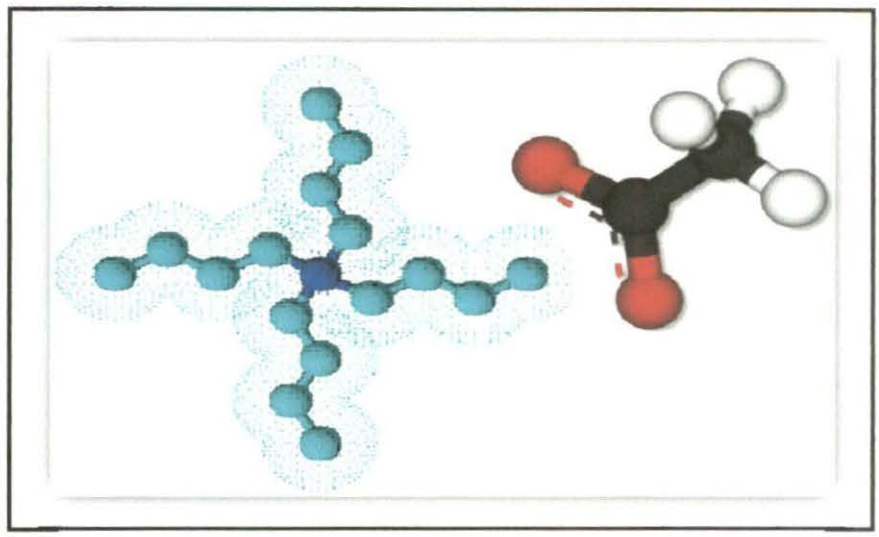
Molecular Formula: $C_3H_7NO_2$, **Molecular Weight**: 77.08, **Appearance**: white solid, **Melting Point**: 387.15K

Source: Purchased from Merck, India

Purification: Purified by re-crystallization twice from conductivity water. It was dried in vacuum and stored over P_2O_5 under vacuum before use.

Application: It is a relatively unusual example of a salt that melts at low temperatures. It is often used as an aqueous buffer for ESI mass spectrometry of proteins and other molecules. It is also used as a food additive as an acidity regulator.

Tetrabutylammonium acetate



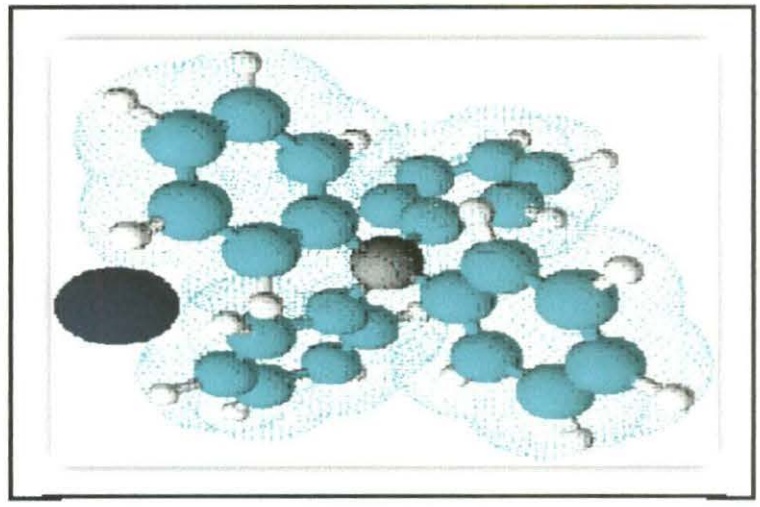
Molecular Formula: $C_{16}H_{36}N.C_2H_3O_2$, **Molecular Weight** : 301.51, **Appearance**: white solid, **Melting Point**: 371.15K

Source: Purchased from Merck, India

Purification: Salt was purified by re-crystallization and the crystallized salt was dried in vacuum for 48 hrs before use.

Application: Used as supporting electrolyte. In organic chemistry it is used as precipitating agent.

Sodiumtetraphenylborate



Molecular Formula: $C_{24}H_{20}BNa$, **Molecular Weight**: 342.22 , **Appearance**: white solid, **Melting Point**: 573.15K

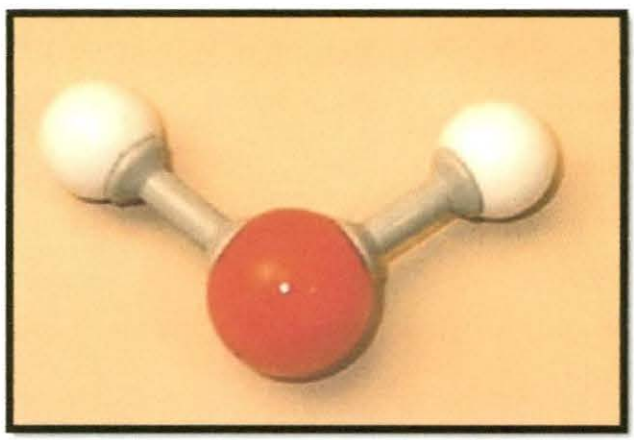
Source: Purchased from Merck, India

Purification: Salt was purified by recrystallization and the crystallized salt was dried in vacuum for 72 hrs before use.

Application: It is used to prepare other tetraphenylborate salts, which are often highly soluble in organic solvents. The compound is used in inorganic and organometallic chemistry as a precipitating agent. It is used as supporting electrolyte.

SOLVENTS

Water



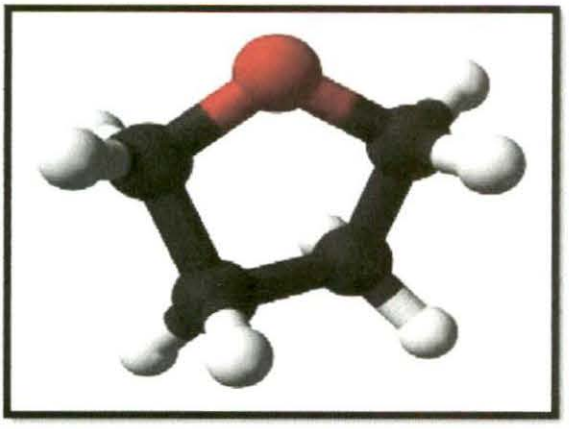
Molecular Formula: H_2O, **Molecular Weight**: 18.02 , **Appearance**: colourless liquid, **Density**: 0.99713 g·cm^3, **Dielectric constant**: 78.35 at 298.15K

Source: Collected by fractional distillation in Laboratory.

Purification: Deionised water was distilled in an all glass distilling set along with alkaline KMnO4 solution to remove organic matter. Precautions were taken to prevent contamination.

Application: Life on earth totally depends on water. It is a superb solvent, generally taken as the universal solvent, due to the marked polarity of the water molecule and its tendency to form hydrogen bonds with other molecules. It is widely used in chemical reactions as a solvent. About 70 to 90 percent of all organic matter is water. The chemical reactions in all plants and animals that support life takes place in a water medium.

Tetrahydrofuran



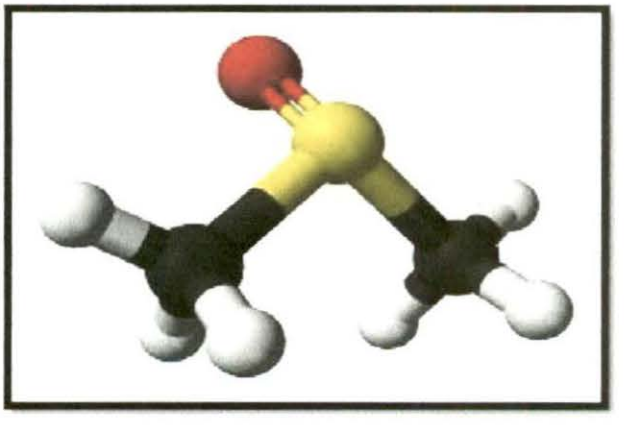
Molecular Formula: C_4H_8O, **Molecular Weight**: 72.11 , **Appearance**: colourless liquid, **Density**: 0.88074 g·cm^3, **Dielectric constant**: 7.58 at 298.15 K

Source: Purchased from Merck, India

Purification: It was kept several days over potassium hydroxide (KOH), refluxed for 24 h and distilled over lithium aluminium hydride (LiAlH4). [5]

Application: THF is often used in polymer science as dissolve polymers prior to determining their molecular mass. The main application of THF is as an industrial solvent for PVC and in varnishes.

Dimethyl sulfoxide



Molecular Formula: $(CH_3)_2SO$, **Molecular Weight**: 78.13 , **Appearance**: Colourless liquid, **Density**: 1.09602 g·cm^3, **Dielectric constant**: 46.7 at 298.15K

Source: Purchased from Thomas Baker, India

Purification: It was purified by passing through Linde 4Å molecular sieves.

Application: Because of its ability to dissolve many kinds of compounds, DMSO plays a role in sample management and high-throughput screening operations in drug design. DMSO increases the rate of absorption of some compounds through organic tissues,
including skin, it can be used as a drug delivery system. It is also extensively used as an extractant in biochemistry and cell biology.

3.2 EXPERIMENTAL METHODS

3.2.1 PREPARATION OF SOLUTIONS

A sock solution for each solute was prepared by mass, and the working solutions were obtained by mass dilution. The uncertainty of molarity of different salt solutions was evaluated to be ± 0.0003 $mol \cdot dm^{-3}$.

3.2.2 MASS MEASUREMENT

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

3.2.3 DENSITY MEASUREMENT

The density was earlier measured by Ostwald- Sprengel type Pycometer having a bulb volume of $25cm^3$ and an internal diameter of the capillary of about

1mm. The pycometer was calibrated at 298.15K with doubly distilled water and THF. The total uncertainity in the density value was ±0.0001gcm^{-3}

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

It can measure mass to a very high precision and accuracy. The weighing pan of a high precision (0.0001g) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation.

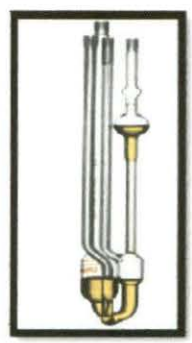
In the digital density meter, the mechanic oscillation of the U-tube is e.g. electromagnetically transformed into an alternating voltage of the same frequency. The period τ can be measured with high resolution and stands in simple relation to the density ρ of the sample in the oscillator[6]:

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

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

3.2.4 VISCOSITY MEASUREMENT

Solvent viscosities were measured using a suspended Ubbelohde-type viscometer,



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

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

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

where, t is the time of flow, ρ is the density and k and l are the characteristic constants of the particular viscometer. The precision of the viscosity measurement was ± 0.003 %. In all cases, the experiments were performed in at least three replicates and the results were averaged.

Relative viscosities (η_r) were obtained using 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 the absolute viscosities, densities and flow times for the solution and solvent respectively.

The viscosity measurements were also done with the help of Brookfield DV-III Ultra Programmable Rheometer fitted to a Brookfield Digital Bath TC-500.

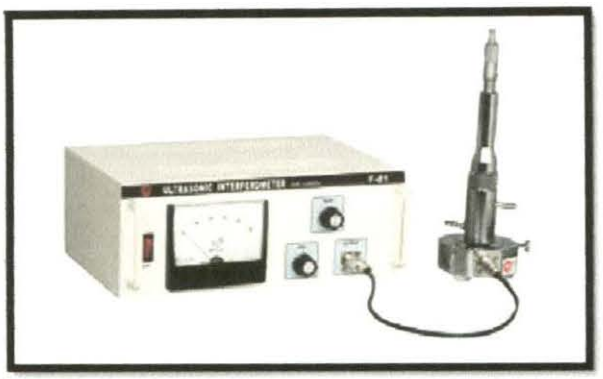
3.2.5 TEMPERATURE CONTROLLER

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

Laboratory water bath is a system in which a 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 different volumes and construction with both digital and analogue controls and greater temperature uniformity, durability, heat retention and recovery. The chambers of water bath lab products are manufactured using rugged, leak proof and highly resistant stainless steel and other lab supplies.

3.2.6 ULTRASONIC SPEED MEASUREMENT

The ultrasonic speed was measured with an accuracy of 0.2% using single-crystal variable-path ultrasonic interferometer (Model M-81 Mittal Enterprises, New Delhi) operating at 4MHz which was calibrated with water, methanol and benzene at required temperature.



The principle used in the measurement of the ultrasonic speed (u) is based on the accurate determination of the wavelength (λ) in the medium. Ultrasonic waves of known frequency (f) are produced by a quartz crystal fixed at the bottom of the cell. These waves are reflected by a movable metallic plate kept parallel to the quartz crystal. If the separation between these two plates is exactly a whole multiple of the sound wavelength, standing waves are formed in the medium. This acoustic resonance gives rise to an electrical reaction on the generator driving the quartz crystal and the anode current of the generator becomes a maximum.

If the distance is now increased or decreased and the variation is exactly one half of wave length (λ /2) or integral multiples of it, anode current becomes maximum. From the knowledge of the wave length (λ), the speed (u) can be obtained by the relation.

$$\text{Ultrasonic speed } (u) = \text{Wave Length } (\lambda) \times \text{Frequency } (f) \qquad (5)$$

The ultrasonic interferometer consists of the following two parts, (i) the high frequency generator, and (ii) the measuring cell. The measuring cell is

connected to the output terminal of the high frequency generator through a shielded cable. The cell is filled with the experimental liquid before switching on the generator. The ultrasonic waves move normal from the quartz crystal till they are reflected back from the movable plate and the standing waves are formed in the liquid in between the reflector plate and the quartz crystal. The micrometer is slowly moved till the anode current on the meter on the high frequency generator shows a maximum. A number of maxima readings of anode current are passed and their number (n) is counted. The total distance (d) thus moved by the micrometer gives the value of the wavelength (λ) with the following relation.

$$d = n \times \lambda/2 \tag{6}$$

Further, the velocity is determined from which the isentropic compressibility (K_S) is calculated by the following formula:

$$K_S = 1 / (u^2 . \rho) \tag{7}$$

where ρ is the density of the experimental liquid.

Figure. 1 shows the Multifrequency Ultrasonic Interferometer i.e. (a) Cross-section of the measuring cell, (b) Position of reflector vs. crystal current (Note : The extra peaks in between minima and maxima occurs due to a number of reasons, but these do not effect the value of $\lambda/2$) and (c) Electronic circuit diagram of the instrument)

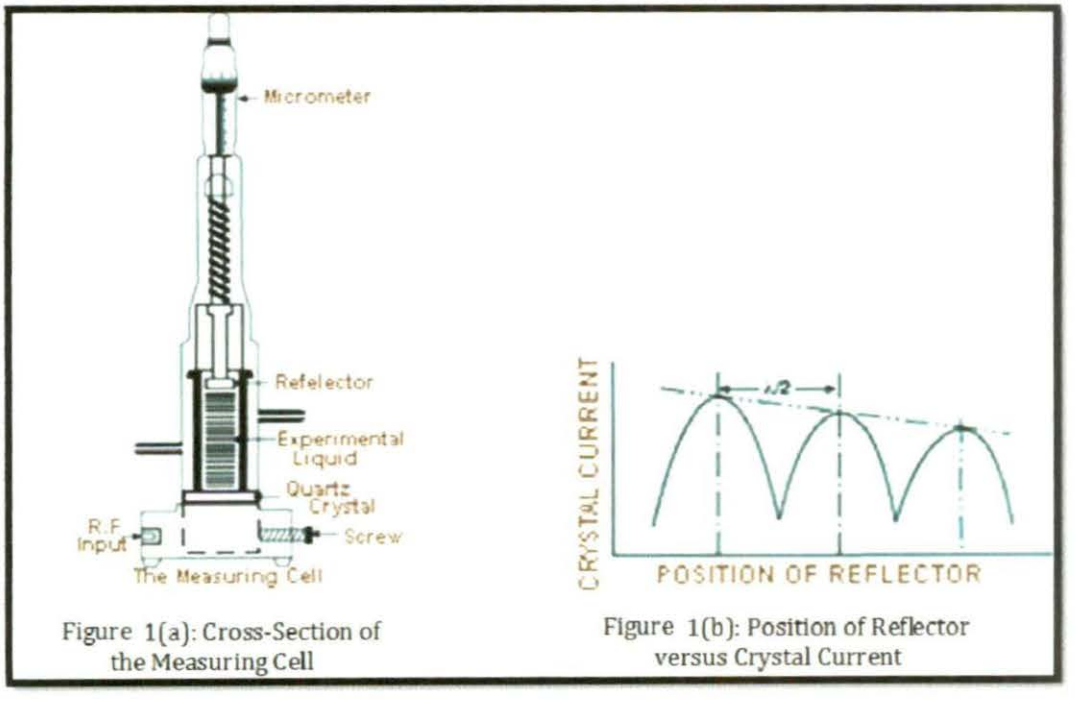


Figure 1(a): Cross-Section of the Measuring Cell

Figure 1(b): Position of Reflector versus Crystal Current

Figure **1: The Multifrequency Ultrasonic Interferometer**

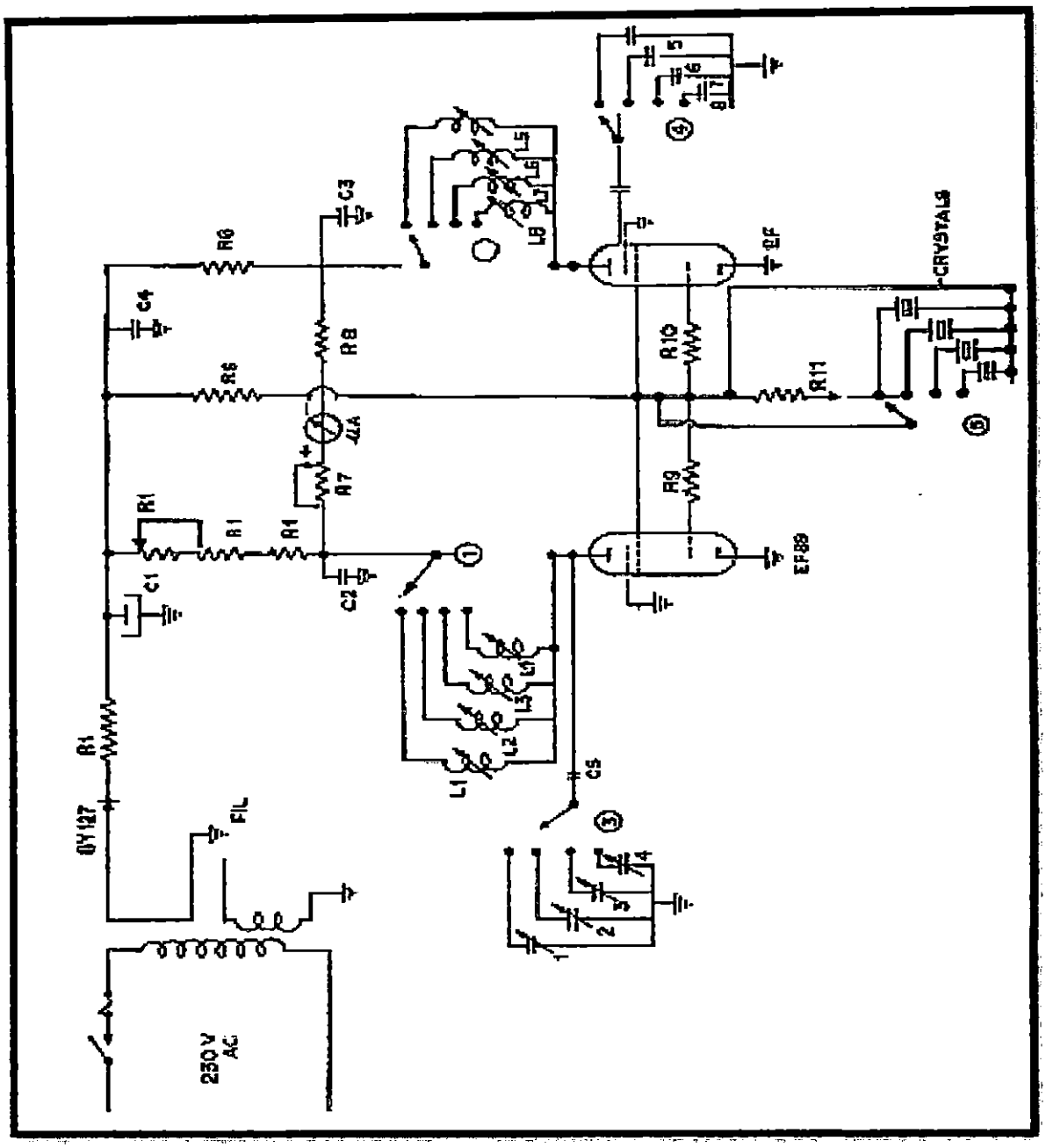
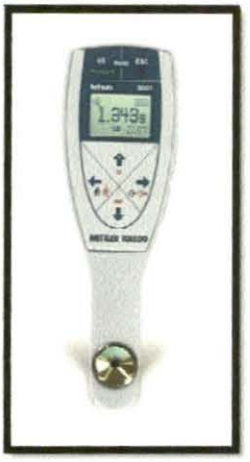


Figure 2: Electronic Circuit Diagram of the Instrument

3.2.7 REFRACTIVE INDEX MEASUREMENT

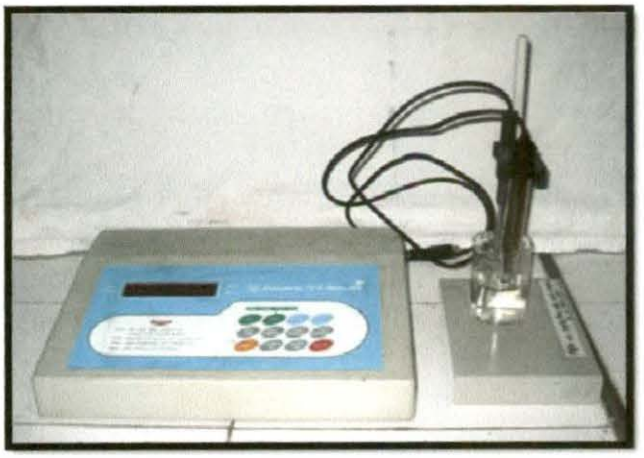
Refractive index was measured with the help of Digital Refractometer (Mettler Toledo 30GS).



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

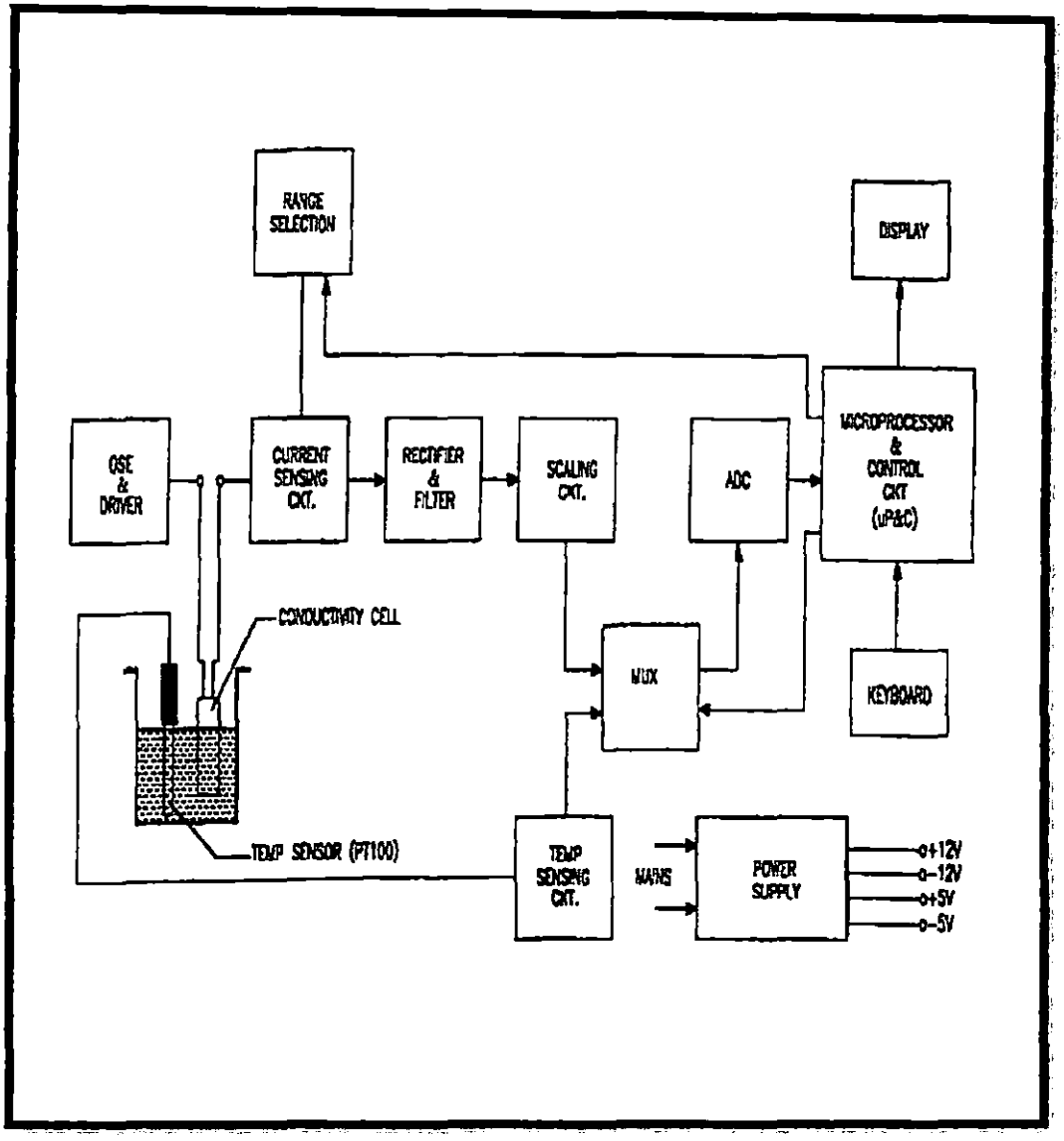
3.2.8 CONDUCTIVITY MEASUREMENT

Conductivity measurement was done using Systronics Conductivity TDS meter-308. It can provide both automatic and manual temperature compensation.



The conductance measurements were carried out on this conductivity bridge using a dip-type immersion conductivity cell of cell constant $1.11 cm^{-1}$. The entire conductance data were reported at 1 KHz and was found to be ±0.3 % precise. The instrument was standardized using 0.1(M) KCl solution. The cell was calibrated by the method of Lind and co-workers [7]. The conductivity cell was sealed to the side of a 500 cm^3 conical flask closed by a ground glass fitted with a side arm through which dry and pure nitrogen gas was passed to prevent admission of air into the cell when solvent or solution was added. The measurements were made in a thermostatic water bath maintained at the required temperature with an accuracy of ± 0.01 K by means of mercury in glass thermoregulator [8].

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



Block Diagram of the Instrument