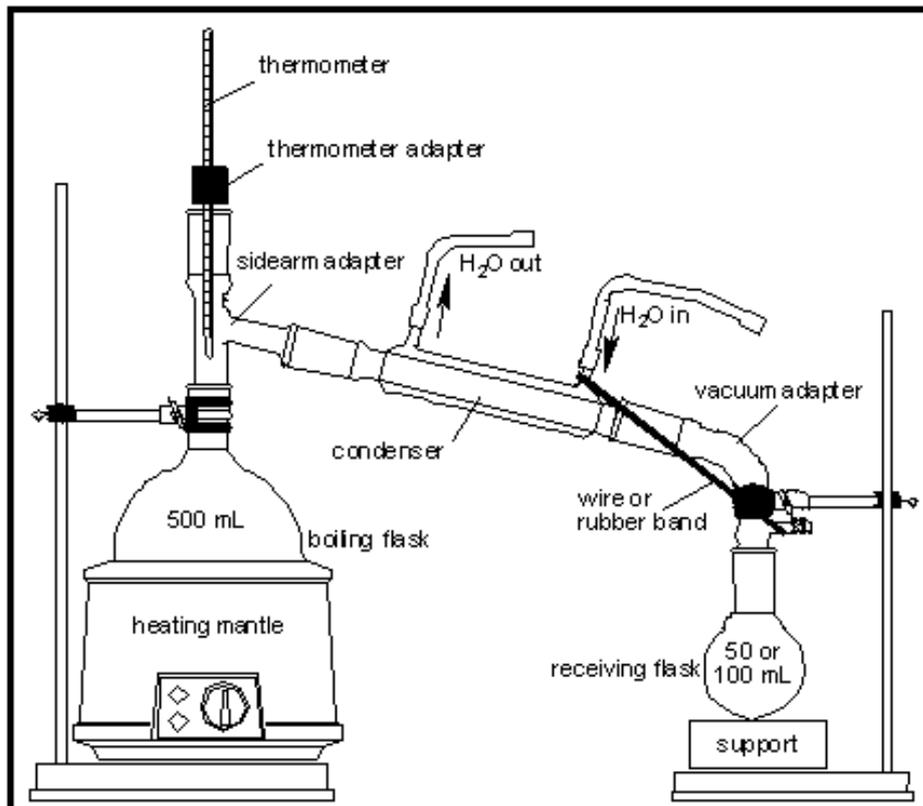


## CHAPTER III

### 3.1. Details of the Chemicals Used

#### 3.1.1. Solvents

In this research work numerous aqueous solutions were employed as solvents. For preparing those various aqueous solutions de-ionized water was employed which is collected from the de-ionization plant in laboratory of University of North Bengal. Latter on de-ionized water was doubly distilled by an all glass distilling chamber (as shown in Figure 3.1) with little amount of alkaline  $\text{KMnO}_4$  solution. The conductivity of the doubly distilled water then measured with a Systronic Conductivity meter- 308 was found to be  $<10^{-6} \text{ S.cm}^{-1}$  at  $298.15 \text{ K}$ .<sup>1</sup> Adequate mass of solid and liquid components were mixed with required adjustments to have an exact mass fraction of the solid component in the aqueous solvent systems.<sup>2</sup> Precautions must be taken while to keep away contamination by  $\text{CO}_2$ , moisture and other impurities. Hence the comapritive error in solvent composition was try to be managed within 1% of the expected mass fraction. Various physico-chemical properties of these respective aqueous solvent systems were expressed in respective next chapters.



**Fig 3.1.** Schematic representation of the water distillation set.

**Experimental Section**

**Table 3.1.** Details (Purity and provenance, *etc.*) of the cosolutes used.

| <b>Chemical</b>                                     | <b>Source</b>            | <b>Purification</b> | <b>Mass Fraction purity</b> | <b>CAS No</b> |
|---|--------------------------|---------------------|-----------------------------|---------------|
| $\beta$ cyclodextrin                                | Sigma-Aldrich, Germany   | -                   | >0.990                      | 7585-39-9     |
| HP cyclodextrin                                     | Sigma-Aldrich, Germany   | -                   | >0.980                      | 128446-35-5   |
| $\alpha$ cyclodextrin                               | Sigma-Aldrich, Germany   | -                   | $\geq 0.980$ (HPLC)         | 10016-20-3    |
| Uracil  | Sigma-Aldrich, Germany   | Recrystallization   | >0.990                      | 66-22-8       |
| Tetrabutyl Phosphonium p-Toluene Sulphonate(TBPPTS) | Sigma-Aldrich, Germany   | -                   | $\geq 0.950$ (NT)           | 116237-97-9   |
| N,N –Dimethyl Formamide                             | Sd fine Chemicals, India | Non                 | >0.998                      | 68-12-2       |
| Dimethyl Sulphoxide                                 | Sd fine Chemicals, India | Non                 | >0.998                      | 67-68-5       |
| Aniline   | Sd fine Chemicals, India | Non                 | >0.995                      | 62-53-3       |
| Salicylaldehyde                                     | Sd fine Chemicals, India | Non                 | >0.995                      | 90-02-8       |
| Zinc acetate dehydrate                              | Sd fine Chemicals, India | Non                 | >0.995                      | 5970-45-6     |

Numerous chemicals may employed as cosolutes to prepare the the aqueous solvent systems. Details of these cosolutes are given in Table 3.1. Glycine and L-alanine were purified by recrystallised from warm distilled water at 90-95 °C. After filtration, the

## Experimental Section

residues were placed in a *vacuo* for dehydration for several hours. The melting points of glycine and L-alanine were measured by open capillary method and found to be 233 and 258 °C,<sup>3,4</sup> respectively. Although, uracil was used as found from the commercial sources but before use it was thoroughly dried over anhydrous CaCl<sub>2</sub> in *vacuo* for many hours.

### 3.1.2. Solutes

A number of biologically active compounds were employed here as solutes for the present research works. Stock solutions prepared of these solutes in different aqueous solvent systems were made by mass and the other various working solutions for the ensuing physico-chemical studies were prepared by mass dilution. Molalities (*m*) of solutes were changed into corresponding molarities (*c*) by using of the experimental densities. All solutions have to be prepared afresh with required precautions to nullified any type contamination and then degassed with the help of dry nitrogen. The uncertainty of molality (*c*) of the solutes in solutions was found as  $\pm 0.001 \text{ mol.kg}^{-1}$ . Details of these solutes are given in Table 3.2.

**Table 3.2.** Details (Purity and provenance, *etc.*) of the solutes used.

| Chemical*        | Purification      | Mass fraction<br>purity | CAS No   |
|------------------|-------------------|-------------------------|----------|
| Paracetamol      | None              | >0.990                  | 103-90-2 |
| L-Proline        | Recrystallization | >0.990                  | 147-85-3 |
| L-Leucine        | Recrystallization | >0.980                  | 61-90-5  |
| Alanine          | Recrystallization | $\geq 0.98$             | 56-41-7  |
| L-phenyl alanine | Recrystallization | $\geq 0.98$             | 63-91-2  |
| Glycine          | Recrystallization | $\geq 0.985$            | 56-40-6  |
| N-Acetyl Glycine | Recrystallization | $\geq 0.985$            | 543-2-48 |
| Caffeine         | None              | >0.990                  | 58-08-2  |
| Allopurinol      | Recrystallization | >0.980                  | 315-30-0 |

\*Source: Sigma-Aldrich, Germany.

paracetamol, caffeine were not purified further but they were dried over and placed in chamber and *vacuo* through anhydrous CaCl<sub>2</sub> for several hours before use.

### 3.2. Experimental Methods

#### 3.2.1. Mass measurement

All Mass of the solutes and cosolutes were measured in a digital analytical electronic balance (Mettler Toledo, Switzerland, AG 285) depicted in Figure 3.2. In this balance the weighing pan is present inside a clear enclosure with doors to operate to avoid any type of dust particles gathering and misbalanced from any air currents. It determine the masses with very high precision and accuracy (mass measurements are found to be accurate by  $\pm 0.01$  mg).



**Fig 3.2.** Mettler Toledo digital balance, Switzerland, Model-AG 285.

#### 3.2.2. Density measurement

Since the density is the important parameter for calculation of the volumetric and viscometric properties so it measured in digital density meter where different experimental aqueous solvent systems and the solutions of different concentration are measured at the different experimental temperatures with the aid of a digital density meter (Anton Paar, DMA-4500M). Figures 3.3-3.6 shows the density meter, its display, sample filling and cell drying, respectively.



Fig 3.3. Anton Paar density meter (DMA-4500M).

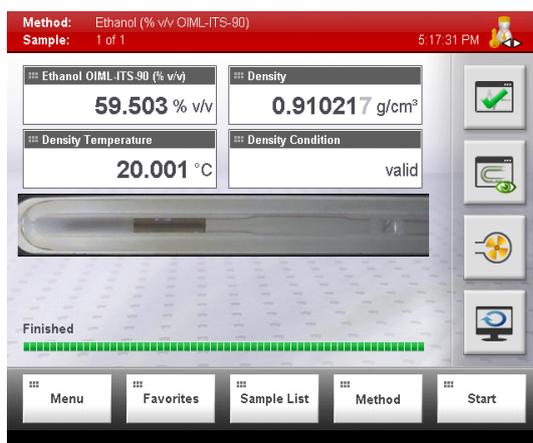


Fig 3.4. Display of Anton Paar density meter.



Fig 3.5. Filling of sample with a syringe.



**Fig 3.6.** Drying the measuring cell.

The mechanical oscillation of the U-tube of this density meter is electromagnetically turned into an alternating voltage having the same frequency. The oscillation period ( $\tau_0$ ) is accurately measured with high resolution and there is a simple relation found between the oscillation period ( $\tau_0$ ) and the density ( $\rho$ ) of the examined sample is given by the following equation:<sup>6</sup>

$$\rho = A\tau_0^2 - B \quad (1)$$

A and B stand for the instrument constants that can be obtained by calibration with two liquids of accurately known densities. The densities of these two liquids at least made a difference of  $\pm 0.01 \text{ g} \cdot \text{cm}^{-3}$  and values of  $\tau_0$  of the adjustment media found to change by at least 0.0001 units. Modern tech and modified instruments can measure and store the A and B constants after calibration is done. In an average it is so done with water and air. For the numerous experiments, though, the density meter was calibrated before using it by doubly distilled de-ionized degassed water and hot dry air at the various experimental temperatures under atmospheric pressure. In this instrument the temperature is kept at the experimental temperatures with an accuracy of  $\pm 1 \times 10^{-2} \text{ K}$  with help of an automatic built-in Peltier technique. The stated repeatability and accuracy of the densities are found  $\pm 1 \times 10^{-5} \text{ g} \cdot \text{cm}^{-3}$  and  $\pm 5 \times 10^{-5} \text{ g} \cdot \text{cm}^{-3}$ , respectively. But if the instrumental accuracy of the densities of the experimental solutions was compared to the densities of a known molal aqueous NaCl solution by using the given by Pitzer,<sup>7</sup> the calculated uncertainty of the densities for maximum of the solutions was found to be better than  $\pm 2 \times 10^{-5} \text{ g cm}^{-3}$ .

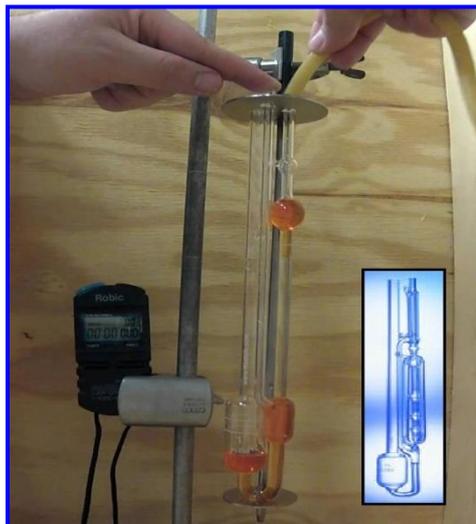
**3.2.3. Viscosity measurement**

The kinematic viscosities were measured from suspended-level Cannon type Ubbelohde viscometer (capillary type). The time of efflux of a constant volume liquid sample through the capillary was measured with the help of digital stopwatch with a time accuracy of  $\pm 0.01$ s. The viscometer was always placed vertically submerged in the thermostatic bath that maintained at the experimental temperature with accuracy of  $\pm 0.01$  K. After a while the thermal equilibrium was established, the flow times of the respective solution were measured thrice and the mean of all these measurements were considered for the calculation of viscosity. During the measurements required precautions were taken to keep away from evaporation losses and any contamination. The efflux time for water at temperature 298.15 K was measured as 428.9 s. The kinematic viscosity ( $\nu$ ) and the absolute viscosity ( $\eta$ ) are calculated by using the following equation:<sup>8</sup>

$$\nu = kt - \frac{L}{t} \quad (2)$$

$$\eta = \nu\rho \quad (3)$$

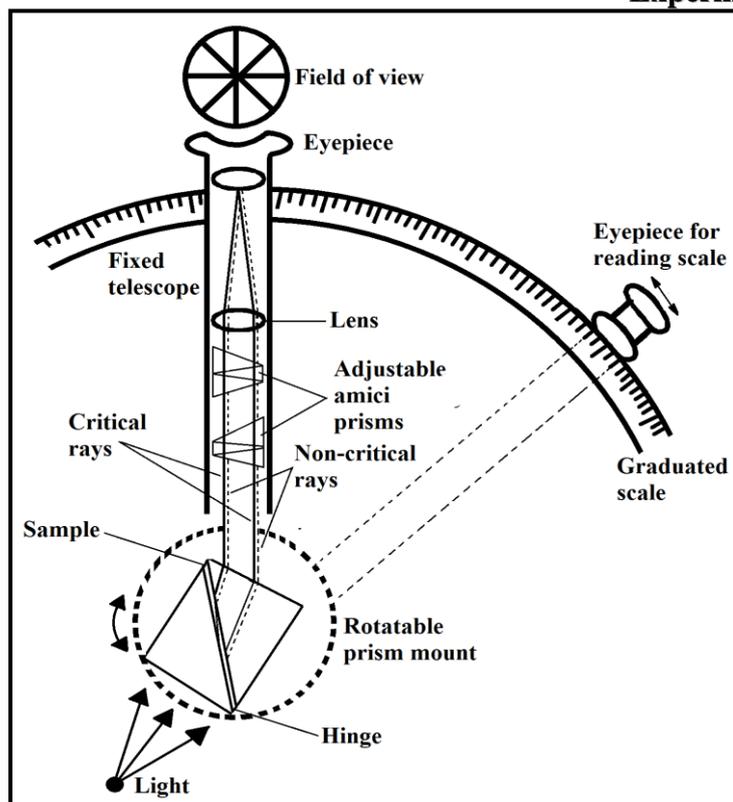
k and L are known to be the characteristic viscometer constants;  $t$  and  $\rho$  stand for the efflux time of flow in seconds and sample density, respectively. The calibration constants (k and L) were determined with purified demonized double distilled water and methanol and they are found to be  $4.23 \times 10^{-3}$  and 4.000, respectively. Considering out of the record, the fact that the correct kinetic energy of the ions were found to be almost negligible and the uncertainty of viscosities was within  $\pm 4 \times 10^{-4}$  mPa · s based on our newest study on different pure liquids. Figure 3.7 depicts the suspended-level Cannon type Ubbelohde viscometer (capillary type) used.



**Fig 3.7.** A suspended-level Cannon type Ubbelohde viscometer (capillary type).

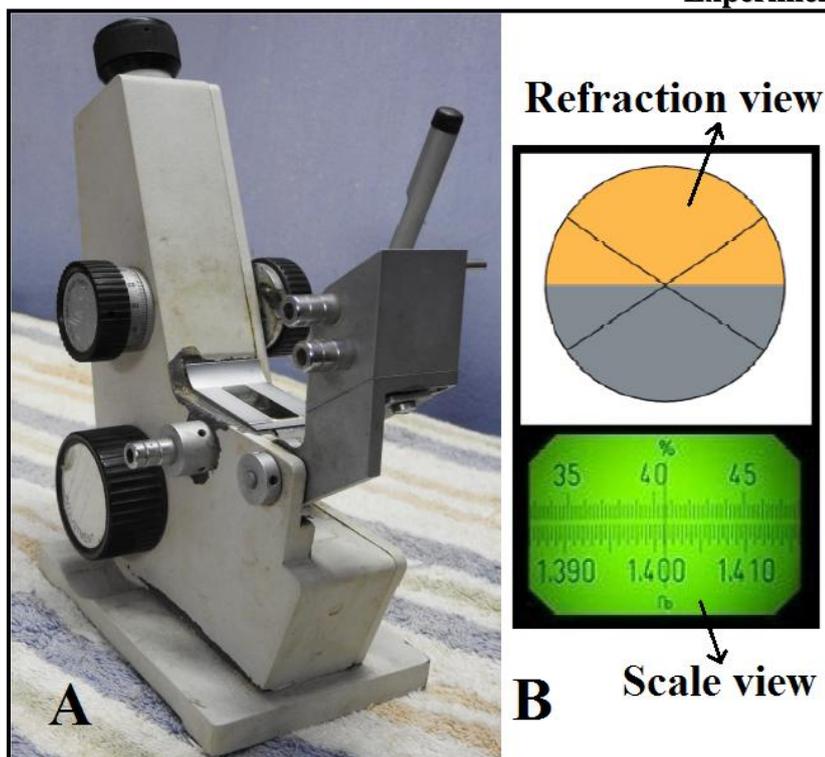
### 3.2.4. Refractive index measurement

Refractive indices of the experimental liquid samples were measured with the help of a Abbe-refractometer (Cyberlab, MA01527, USA) by using sodium D-line light ( $\lambda=589.3$  nm, an mean of the two emission lines at 589.0 nm and 589.6 nm) at 298.15 K. The Abbe-refractometer is one of the most reliable and widely used refractometer in various laboratory and it has the range within  $n_D = 1.3$  to 1.7. Figure 3.8 schematically represents this optical system. The experiment liquid is directly put in the prism assembly of the instrument using an airtight hypodermic syringe and is sandwiched like a skinny film ( $\sim 0.1$ mm) between the two prisms. The upper prism is solidly framed on a bearing that allow its rotation through the side arm presented by dotted lines. The lower prism is hanged to the upper prism to permit the separation for washing and for the introduction of the particular sample. When light reflects into the prism, the lower surface being rough is transformed into origin for the endless number of rays that passage along the sample at all angles.



**Fig 3.8.** Schematic illustration of the Abbe-refractometer optical system.

The light rays are get refracted on the smooth polished-ground face and interface of the upper prism and sample, respectively. Then it travel along a fixed telescope. Divergent rays of various colors are combined into a single white beam by two triangular prisms in contact (Amici prisms). The beam verify almost exactly in the path to that of sodium D-ray. The eyepiece is marked with crosshairs in the telescope shown in the (Figure 3.8). At the time of measurement of the refractive index the angle of the prism is changed until the light-dark interface just coexists with the crosshairs. After that the prism position is read from the locked scale. Mean of three numbers of measurements was taken for each solution mixture. The Abbe-refractometer is represented in Figure 3.9. During the determination, water from a thermostatic bath keep at  $298.15 \pm 0.01$  K was convey along the refractometer and it was calibrated by calculating the refractive indices of doubly distilled de-ionized degassed water at temperature 298.15 K. The uncertainty in refractive indices was found within  $\pm 0.0002$ .



**Fig 3.9.** A: Abbe-refractometer (Cyberlab, MA01527, USA); B: View of the refractometer through the eyepiece.

### 3.2.5. Spectrophotometric measurements

Absorption spectra of biologically active compounds or solutes in various aqueous media were measured on a Jasco V-530 double beam UV-VIS spectrophotometer at 298.15 K. Figure 3.10 represents the UV-VIS Spectrophotometer. It was connected with a thermostatic array to control a temperature of  $298.15 \pm 0.01$  K. Quartz cells of 1 cm path length were employed to keep the samples and the reference solvents at the time of spectral determination. A stock solution of biologically active samples was prepared in the aqueous solvent systems and 2 mL of it was poured into the quartz cell and study of absorption was done against a selected reference solvent system. Then solution of co-solute (of definite concentration) in the reference solvent or in an aqueous solvent was added stepwise by utilizing pre-calibrated Hamilton syringe. After 30 seconds the absorbance of the resulting solution was calculated at each step.



**Fig 3.10.** Double beam UV-VIS Spectrophotometer (Jasco V-530) and the thermostatic bath.

### 3.2.6. Ultrasonic Velocity Measurements

There are three types of experimental techniques are employed to determine the ultrasonic sound velocities in liquid mixtures and pure liquids. They are: (i) Pulse method, (ii) Continuous wave method and (iii) Interferometer technique. By comparing of the relative merits of the various methods, interferometer method is considered as the most accurate method acceptable for speed measurements. Hunter and Dardy,<sup>9</sup> Dobbs and Fine gold,<sup>10</sup> Fort and Moore<sup>11</sup> measured the speed of sound for liquids and liquid mixtures by using interferometric technique with  $\pm 0.15\%$  uncertainty. In the present study ultrasonic speeds of the experimental liquid samples were measured with an exactness of 0.3% utilizing a multi-frequency ultrasonic interferometer (F-05, Mittal Enterprises; New Delhi, India) operating at 2 MHz. It was calibrated with pure benzene, doubly distilled de-ionized degassed water maintained at  $298.15 \pm 0.01$  K by paasing thermostatic water surrounding the jacketed cell (of 2 MHz) keeping the liquid sample with the help of circulating pump. The uncertainty in ultrasonic speeds was found around  $\pm 0.2 \text{ m s}^{-1}$ .

The determination of ultrasonic speed ( $u$ ) by ultrasonic interferometer is dependent on the exact calculation of wavelength ( $\lambda$ ) in the medium. In this process ultrasonic waves of frequency ( $f$ ) are created by a crystal of quartz hanged at the lower part of the cell. These ultrasonic waves are get reflected by a transportable plates of metal (keeping parallel to the quartz crystal). While the interspace between these two plates becomes a whole multiple of the wavelength of ultrasonic sound, the standing waves are created in that medium. In this situation, acoustic resonance is

### Experimental Section

generated. The acoustic resonance produce an electrical reaction in the generator that shows the quartz crystal and as a result of the current of anode in the generator approached the maximum level. When the distance is enlarged or diminished by exactly one half of the wavelength ( $\lambda/2$ ) or an integer multiple of the wavelength, the current of anode again reached to the maximum level. If  $d$  represents the separation between consecutive adjoining maxima of current of anode and the full number of oscillation (usually  $n = 20$ ) counted. Then the total distance moved by the micrometer in  $n$  oscillations is given by:

$$d = n \times \frac{\lambda}{2} \quad (4)$$

The speed ( $u$ ) of the wave and frequency ( $f$ ) of the cell are found to be related with its wavelength ( $\lambda$ ) by the relation,

$$u = \lambda \times f \quad (5)$$

$$\text{Or } u = \lambda \times f = \frac{2d}{n} \times f \quad (6)$$

So with a known cell, the frequency of the ultrasonic speed ( $u$ ) can be measured. The ultrasonic interferometer has the three main portions: (i) The high frequency generator (single and multi-frequency) is specially outlined to excite the crystal of quartz hang at the lower part of the measuring cell. Its resonant frequency is provided for the production of the ultrasonic wave in the experimental liquid present in the measuring cell, (ii) shielded cable and (iii) The measuring cell (1, 2, 3 and 4 MHz) is specially drafted with double walled cell which controls the temperature of the sample liquid constant through out the whole process. To increase or minimise the reflector plate in the liquid a good micrometer has been employed. It is organised at the top of the cell and works from a known gap.

The total assembly of the instrument is represented on Figure 3.11 in which the output terminal of that high frequency creator is hanged with the measuring cell by a shielded cable. In the beginning the cell is filled up with the study solution and then keep the switch of the generator is on. Genarally it was found that the ultrasonic waves generally move normal from the crystal of quartz crystal until they are reflected back by the movable plate and the standing waves are created in liquid in between the quartz crystal and the reflector plate. After that the micrometer forwarded very slowly till the anode current exhibits a maximum deflection on the display meter

### Experimental Section

of the high frequency generator. Different no. of maxima of current of anode are observed and the total number of oscillation ( $n$ ) is computed. The total space ( $d$ ) thus moved by the micrometer provides the wavelength ( $\lambda$ ) by using the Eq. (4).

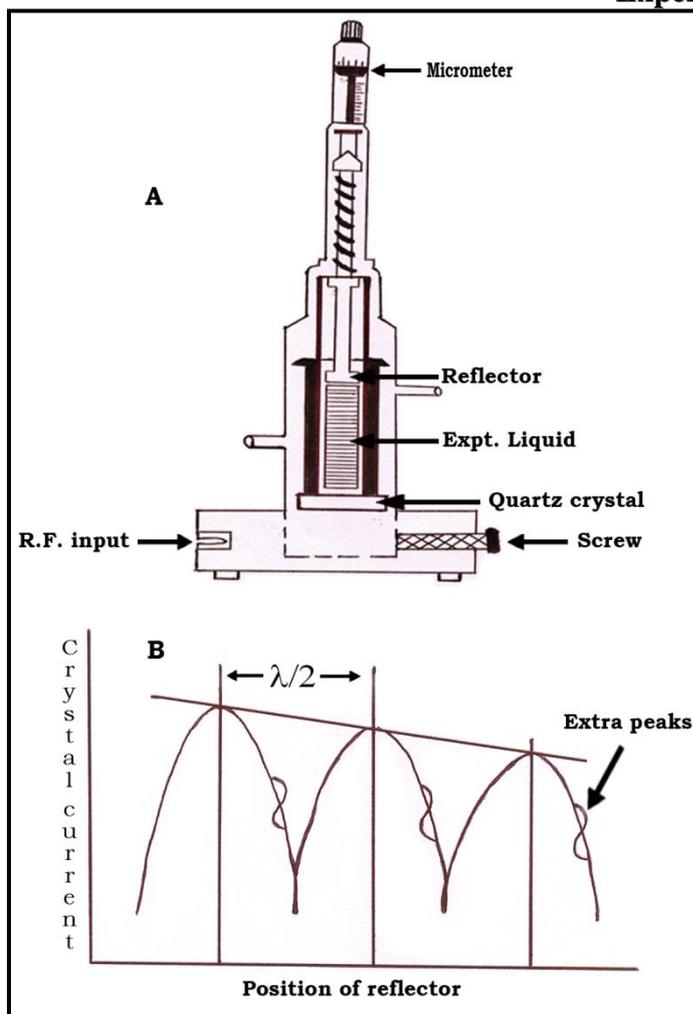


**Fig 3.11.** Ultrasonic interferometer (F-05, Mittal Enterprises, India).

In Fig. 3.12 shows a cross-section view of the measuring cell of ultrasonic interferometer having multi-frequency. The position of reflector *versus* crystal current are also shown in the fig 3.12. The additional peaks [appearing in Figure 3.12 B] in between minima and maxima found due to a various reasons but these are not affecting the  $\lambda/2$  values.

#### 3.2.7. pH Measurements

A Systronics digital pH meter employed in the present study to record some the pH's while required for the experimental study solutions. It was calibrated before use at pH = 4.00 using a buffer capsule of pH = 4.00 (purchased from Sigma-Aldrich, Germany). Figure 3.13 shows the Systronics digital pH meter which is utilized during the present study.



**Fig 3.12.** (A) A cross-section of the measuring cell of a multi-frequency ultrasonic interferometer; (B) position of reflector *versus* crystal current.



**Fig 3.13.** Systronics digital pH meter.

### References:

- [1] A. Sarkar, B. K. Pandit, B. Sinha, *J. Chem. Thermodyn.* 98 (2016) 118-125.
- [2] D. Brahman, B. Sinha, *J. Chem. Eng. Data* 56 (2011) 3073-3082.
- [3] B. Sinha, A. Sarkar, P. K. Roy, D. Brahman, *Int. J. Thermophys.* 32 (2011) 2062-2078.
- [4] A. Sarkar, B. Sinha, *J. Serb. Chem. Soc.* 78 (8) (2013) 1225-1240.
- [5] I. J. Warke, K. J. Patil, S. S. Terdale, *J. Chem. Thermodyn.* 93 (2016) 101-114.
- [6] Oscillating U-tube. Electronic document,  
[http://en.m.wikipedia.org/wiki/Oscillating\\_U-tube](http://en.m.wikipedia.org/wiki/Oscillating_U-tube), Oct 12, 2013.
- [7] K. S. Pitzer, J. C. Peiper, R. H. Busey, *J. Phys. Chem. Ref. Data* 13 (1984) 1-102.
- [8] T. S. Banipal, et.al , *Thermochimica Acta.* 553 (2013) 31-39.
- [9] Y. L. Hunter, et.al. *Soc. Amer.* 36 (1964) 1914.
- [10] E. R. Dobbs, L. Finegold, *Ibid*, 32 (1960) 1215.
- [11] R. J. Fort, W. R. Moore, *Trans Faraday Soc.* 61 (1965) 2102.