EXPERIMENTAL
MATERIALS AND SAMPLE PREPARATION

The amino acids: L-leucine, and L-asparagine hydrate and di-peptide: glycylglycine used in this work were obtained from SRL (Mumbai). The salts namely, sodium chloride, sodium nitrate and potassium nitrate were purchased from E. Merck (India). All the chemicals were of ≥99% purity. The amino acids and di-peptide were used as such without further purification. They were dried at ~110°C and kept in vacuum desiccator over P₂O₅ for several hours before use. The salts were recrystallized twice in triply distilled water, dried in a vacuum oven and then kept over P₂O₅ in a vacuum desiccator at room temperature for a minimum of 24 hours. All the solutions were made by weight using a balance having an accuracy of ±0.1 mg. Stock solutions of 1.5 M concentration of NaCl, NaNO₃ and KNO₃ prepared in triply distilled water and used as solvents for the preparation of solutions. Various molal solutions of amino acids and di-peptide were prepared in 1.5 M aqueous solutions of NaCl, NaNO₃ and KNO₃.

TEMPERATURE CONTROL

A thermostated paraffin bath was used to maintain the desired temperature during the measurements of density and viscosity. The bath was made up of an immersion heater (1.5 KW), a stirrer, a check thermometer (Labotherm-N., German make), a contact thermometer and a relay [Jumo type, NT 15.0, 220 V = 10A (German make)]. Thermal stability of the thermostat was found to be within ± 0.1°.

DENSITY MEASUREMENT

Pyknometer consisting of a small bulb with flat bottom of approximately 8.5 ml capacity having a graduated stem was used for the density measurements. The volume at each mark of the pyknometer was
calibrated with the triple distilled water. The densities of pure water at various required temperatures were taken from literature for calibration purpose (286). In order to check the reproducibility of calibration, the same process was repeated a number of times with different amounts of water. The reproducibility of density values was found to be within ±0.0002 gm/cm³. The test solution was introduced into the calibrated pyknometer, weighed and then it was immersed in the paraffin bath. By recording the volume changes as a function of temperature, the densities of solutions were determined at required temperatures.

**VISCOSITY MEASUREMENT**

Cannon-Fenske viscometer was used for the viscosity measurement of various solutions under study. The viscometer consists of three parallel arms with a common base. The viscometer was calibrated with the triple distilled water. The viscosity coefficient values of water at different temperatures were taken from literature (287). The clean and dry viscometer was filled with test solution and was clamped in the thermostated bath in a vertical position. In order to avoid the absorption of moisture by solution, the open ends of the three arms of viscometer were attached with the anhydrous calcium chloride glass tubes through rubber tubes. The solution was sucked into the measuring bulb and was allowed to stand there for about two minutes by closing the calcium chloride tubes with rubber corks and then the corks were removed for recording the time of fall of solution from the upper to lower end of the bulb. After taking several readings at the desired temperature the average of the very close values of time of fall was taken.

The viscosity coefficient (η) was calculated employing the following Poiseuille’s equation,
\[ \eta = \pi gh \rho t r^4 / 8vl \]  

where \( g, h, \rho, r, l \) and \( t \) are acceleration due to gravity, height of the column in the viscometer, density of the liquid, radius of the viscometer's capillary, length and time of fall for the liquid of volume \( v \) through the capillary, respectively. The above equation can also be written as

\[ \eta = \rho \beta t \]  

where \( \beta = \pi g h r^4 / 8vl \) is a constant for a given viscometer. The viscosity value of the test solution was calculated using the reported viscosity values of pure water at various temperatures. Equation [3] was employed for the calculation of viscosity values of solutions.

\[ \eta_1 = (\rho_1 t_1 / \rho_2 t_2) \times \eta_2 \]  

where \( \eta_1 \) and \( \eta_2 \) are viscosity values of solution and solvent, respectively; \( \rho_1 \) and \( \rho_2 \) are density values of solution and solvent, respectively; and \( t_1 \) and \( t_2 \) are the time of fall of the solution and solvent, respectively. The reproducibility in viscosity measurements was found to be within \( \pm 0.003 \times 10^{-4} \) Nm\(^2\)s.

ULTRASONIC VELOCITY MEASUREMENT

An ultrasonic interferometer (Mittal's model: M-77, India) was used for the measurement of ultrasound velocity at a frequency of 4 MHz in the temperature range: 298.15 – 323.15 K. Water from ultra-thermostat (Type U-10) was circulated through the brass jacket surrounding the cell and the quartz crystal. The jacket was well insulated and the temperature of the solution under study was maintained to an accuracy of \( \pm 0.1^\circ \). The instrument was calibrated with the triple distilled water. The ultrasonic velocity values of water at different temperatures were taken from literature for calibration purpose (288).
WORKING PRINCIPLE

An ultrasonic interferometer is a simple and direct device to determine the ultrasonic velocity in liquids with a high degree of accuracy. The principle used in the measurement of velocity (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 plate fixed at the bottom of the cell. A movable metallic plate kept parallel to the quartz plate reflects these waves. If the separation between these two plates is exactly a whole multiple of the ultrasound wavelength, standing waves are formed in the medium. This acoustic resonance gives rise to an electrical reaction on the generator driving the quartz plate and the anode current of the generator becomes maximum. If the distance is now increased or decreased and the variation is exactly one half wavelength (λ/2) or multiple of it, the anode current again becomes maximum. The velocity can be obtained by using the relation,

\[ u = \frac{\lambda \times f}{4} \]  \[4\]

DESCRIPTION

The ultrasonic interferometer consists of two parts (i) the high frequency generator and (ii) the measuring cell. The “high frequency generator” is designed to excite the quartz plate fixed at the bottom of the measuring cell and its resonant frequency to generate ultrasonic waves in the liquid filled in the “measuring cell”. A micrometer to observe the change in current and two controls for the purpose of sensitivity regulation and initial adjustments of micrometer are provided on the panel of the high frequency generator. The “measuring cell” is a specially designed double walled cell for maintaining a constant temperature of the liquid during experiment. A fine micrometer screw has been provided at
the top, which can lower or raise the reflector plate in the liquid in the cell through a known distance. It has quartz plate fixed at its bottom.

Instrument was adjusted in the following manner:

(i) The cell was inserted in the square base socket and clamped to it with the help of a screw provided on one of its sides.

(ii) The curled cap of the cell was unscrewed and removed from the double walled construction of the cell. In the middle portion of it the experimental liquid was poured and screwed the curled cap.

(iii) Water was circulated through the two chutes in the double wall construction in order to maintain the desired temperature.

(iv) The cell was connected with the high frequency generator by a co-axial cable provided with the instrument.

For the initial adjustment, two knobs are provided on high frequency generator, one is marked with ‘Adj’ and the other with ‘Gain’. With knob marked ‘Adj’ the position of needle on the ammeter was adjusted and the knob marked ‘Gain’ was used to increase the sensitivity of the instrument for greater deflection. The ammeter was used to record the maximum deflections by adjusting the micrometer.

MEASUREMENTS

The measuring cell was connected to the output terminal of the high frequency generator through a shielded cable. The cell was filled with the liquid before switching on the generator. The ultrasonic waves of 4 MHz frequency produced by a gold plated quartz crystal fixed at the bottom of a cell are passed through the medium. A movable plate reflects the waves and the standing waves are formed in the liquid in between the reflector plate and the quartz crystal. Acoustic resonance due to these standing
waves gives rise to an electrical reaction to the generator driving the quartz plate and the anode current of the generator becomes maximum. The micrometer screw was raised slowly to record the maximum anode current. The wavelength was determined with the help of a total distance moved by the micrometer for twenty maximum readings of the anode current. The total distance \( d \) gives the value of wavelength with the help of the relation, \( d = n \times \lambda / 2 \), where \( n \) is the maximum number of readings. Using the wavelength, the ultrasound velocity in the liquid was obtained with the help of Equation [4]. The reproducibility in velocity measurement was found to be within \( \pm 0.5 \text{ ms}^{-1} \).

**PRECAUTIONS**

1. The generator was switched on after filling the cell by the experimental liquid.

2. The experimental liquid was removed from the cell after use.

3. The micrometer was kept open at 25 mm after use.

4. The sudden rise or fall in the temperature of circulated liquid was avoided to prevent thermal shock to the quartz crystal.

5. While cleaning the cell, care was taken not to spoil or scratch the gold plating on the quartz crystal.

6. The generator was given 15 minutes warming up time before observation.