Experimental
Material and Sample Preparation

The two amino acids, aspartic acid and glutamic acid of highest purity for biochemistry (chromatographically homogeneous) were obtained from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and were used without further purification. However, before use they were dried over P₂O₅ in a vacuum desiccator. Analytical reagent grade anhydrous sodium and potassium acetates and urea crystal extra pure were obtained from Merck Limited Worli, Mumbai. Sodium and potassium acetates were further purified by recrystallising from double distilled water. After recrystallisation sodium and potassium acetates were dried under vacuum at room temperature. Lysozyme obtained from SIGMA-ALDRICH CHEMIE Gmbh Steimhein, Germany, was used for sample preparation. Sugars viz. D-glucose and maltose were obtained from Qualigans fine chemicals (a division of Glaxo Smith Kline Pharmaceuticals Limited, Mumbai). The solutions were prepared by weight with laboratory double distilled water and all weights were corrected to value in vacuo (Leader balance Works, Varanasi, U.P).

The densities, viscosities and ultrasonic velocities were determined for the following solutions:

1. Aspartic acid and glutamic acid in CH₃COONa solutions of different concentrations (1M and 2M).
2. Aspartic acid and glutamic acid in 1M CH₃COOK solutions.
3. D-glucose in aqueous lysozyme solution.
4. Maltose in aqueous lysozyme solution
5. Urea in aqueous lysozyme solution.
Temperature Control

For the measurements of density and viscosity, a thermostated paraffin bath was used to maintain a uniform temperature. The paraffin bath was of about 5 litres' capacity in which an immersion heater (1.0 KW), an electric stirrer (Remi made), a check thermometer, a contact thermometer were immersed. A relay [Jumo type NT 15.00, 220V ≈ 6A (GDR)] was used to control the variation in temperature. The thermal stability was found to be within ± 0.1°C.

Calibration of pyknometer

Pyknometer is an apparatus used for measuring the density of a liquid. It consists of a small bulb with a flat bottom (of about 9 ml capacity) and a graduated stem for measuring the density of the experimental liquid. It is etched with very fine marks. Each mark on the stem of the pyknometer was calibrated using double distilled water. The clean and dried pyknometer was weighed and filled with double distilled water. Filled pyknometer was weighed again. The mass of the distilled water was determined by the difference in these two masses. Then the pyknometer was immersed in the paraffin bath maintained at the required temperature, and volume changes were recorded as a function of temperature, and thus each mark of the stem was calibrated. The density of distilled water at different temperatures required for calibration was given by the standard equation:

\[ d = A_0 + A_1t + A_2t^2 + A_3t^3 \]
where $A_0$, $A_1$, $A_2$ & $A_3$ are constants and the values of $A_0$, $A_1$, $A_2$ and $A_3$ are $1.0004238$, $-3.6067599 \times 10^{-6}$, $-5.6632867 \times 10^{-6}$ and $1.5613054 \times 10^{-8}$, respectively, while $t$ is the temperature in °C.

From the known values of mass and density of water, the volume corresponding to each mark of the pyknometer was determined. Reproducibility of calibration was checked by repeating the above procedure with different weights of distilled water. Using the known values of mass and volume, the densities at the required temperature were determined. The values of the observed densities were compared with those of the reported ones. It was found that the accuracy of the measurement was within ±0.1% accuracy.

**Calibration of Viscometer**

Cannon-Ubbelohde Viscometer [1] was used for the determination of viscosities of solutions.

The viscometer consists of three parallel arms viz., receiving, measuring and auxiliary, for forming the suspended level arrangement in a triangular fashion. The measuring arm has a fine capillary tube with two bulbs A and B. It forms a ‘U’ with the receiving arm. The measuring arm is etched with two marks (a & b), one above the bulb B and the other below the bulb B. The two fudicial marks ‘a’ and ‘b’ were used for recording the time of fall of the test solution. The viscometer was designed in a manner so that (1) the center of gravity of the three bulbs was aligned vertically to reduce the effect of acceleration due to gravity and (2) the resulting efflux time for water was set close to 80 seconds at room temperature (depending upon the dimensions of
viscometer). In order to minimize the experimental errors, capillary effects of the two liquid surfaces were neutralized by each other, so that the surface tension correction for the apparatus was negligible and the transport of material was carried out freely under the weight of the total volume of the test liquid.

The calibration of viscometer was done by using the distilled water. A sufficient amount of distilled water was filled into the bulb A to avoid any air bubble being introduced into the capillary arm while the bulb B was filled. Now the viscometer was clamped in a thermostat keeping the measuring arm perfectly vertical. The viscometer was allowed to stand in the thermostat for half an hour to minimize thermal fluctuations.

Then the distilled water was sucked into the measuring bulb with the help of vacuum pump. The time of fall of the distilled water from the upper mark 'a' to lower mark 'b' was recorded several times and the mean of very close readings was determined at each required temperature. A stop-watch (accuracy: 0.1 second) was used for measuring time.

Viscosities (η) were calculated using Poiseuille's equation:

$$\eta = \frac{\pi h \rho g r^4 t}{8 L V}$$

where

- \( h \) = height of the liquid column in the viscometer
- \( \rho \) = density of the liquid
- \( g \) = acceleration due to gravity
\( r \) = radius of the capillary of the viscometer

\( L \) = length of the capillaries

\( t \) = time of fall of the test liquid of volume \( V \) to fall through capillary.

The expression can be written in this way also

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

where \( \beta = \frac{\pi r^4 g}{8 L V} \).

\( \beta \) is a constant quantity and it is the characteristic of the viscometer. Its value has been calculated by making use of the reported values of viscosities of distilled water at several temperatures.

The accuracy of the calibrated viscometer was checked by measuring the viscosities of distilled water at various temperatures and then comparing the experimental value with the reported ones. Reproducibility was found to be within \( \pm 0.2\% \).

**Measurements**

1. **Density:** A known amount of test sample was transferred to the calibrated pyknometer. The pyknometer was then immersed in the thermostated bath. The volume corresponding to each of the marks was recorded as a function of temperature.

2. **Viscosity:** The test solution was transferred to the viscometer. The viscometer was then placed in the thermostat and time of fall of the test solution was recorded.
Ultrasonic Velocity

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 measurement of velocity \( (v) \) is based on the accurate determination of the wavelength \( (\lambda) \) in the medium. Ultrasonic waves of known frequency \( (f) \) are produced by a quartz plate fixed at the bottom of the cell. The waves are reflected by a movable metallic plate kept parallel to the quartz plate. If the separation between these two plates is exactly a whole multiple of the ultrasound wavelength, standing waves are formed in the medium. The 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 \( (\lambda/2) \) or a multiple of it, anode current again becomes maximum. From the knowledge of wavelength \( (\lambda) \), the velocity \( (v) \) can be obtained by the relation:

\[ v = \lambda \times f \]

Description

The ultrasonic interferometer consists of the following two parts:

i. The high frequency generator

ii. The measuring cell

The high frequency generator is designed to excite the quartz plate fixed at the bottom of the measuring cell at its
resonant frequency to generate ultrasonic waves in the experimental liquid in the "Measuring Cell". A microammeter to observe the changes in current and two controls for the purpose of sensitivity regulation and initial adjustment of micro ammeter are provided on the high frequency generator.

The Measuring Cell is a specially designed double walled cell for maintaining the temperature of the liquid constant during the experiment. A fine micrometer screw has been provided at the top, which can lower or raise the reflector plate in the cell through a known distance. It has a quartz plate fixed at its bottom.

**Adjustment of Ultrasonic Interferometer**

The instrument was adjusted in the following manner:

1. The Cell was inserted in the square-base socket and was clamped to it by a screw provided on one of its sides.
2. The curled cap of the cell was unscrewed and removed, then the test solution was filled in it and the cap was screwed.
3. Water was circulated through the two tubes in the double walled cell in order to maintain the desired temperature during the experiment.
4. The Cell was connected with a high frequency generator by a coaxial cable provided with the instrument.
5. The generator was given 15 seconds warming up time before recording readings.
6. The sudden rise or fall in temperature of the circulated liquid was avoided to prevent the thermal shock to the quartz crystal.

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

**Measurement**

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 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 high frequency generator shows a maximum. A number of maximum readings of anode current are passed on and their 'n' is counted. The total distance (d) thus moved by the micrometer gives the value of wavelength (λ) with the help of the following relation,

\[ d = n \times \lambda / 2. \]

Once the wavelength (λ) is known, the velocity (v) in the liquid can be calculated with the help of following relation:

\[ v = \lambda \times f \]

**Study With Variation in Temperature**

If the variation in the velocity with temperature is to be studied, water at various desired constant temperatures is made to circulate through the double walled jacket of the cell. The
ripples are provided at the lower cylindrical portion of the cell for circulating water around the experiment liquid.

Ultrasonic velocity in solutions was measured by determining the wavelength of sound in these media using a multi-frequency ultrasonic interferometer model M-82 (Mittal Enterprises, India) working at 2MHz. The temperature of the solution was controlled by circulating water through the jacket of a double walled cell from a constant temperature controlled bath of thermal stability: ± 0.03K.

Reference