CHAPTER-II

Material and Methods

This chapter describes the material and methods or experimental techniques used for the present study of various physico-chemical properties of non-ionic surfactant-amino acid mixture as well as pure surfactant.

2.1 Materials:

The non-ionic surfactants used for the present study were polyoxyethylene (10) cetylether (Brij-56) (M.Wt.683) and polyoxyethylene (20) (Brij-58) (M.Wt.1122) product of Sigma Aldrich USA. These were used as received. The additives amino acids viz. Alanine (M.Wt. 89.09), Phenylalanine (M.Wt. 165.19), Glycine (M.Wt. 75.07), Serine (M.Wt.109.09) and Methionine (M.Wt. 149.21) were the product of Thomas Baker (99%purity) and also be used as received.

The molecular Structures of non-ionic surfactants are given as follows.

\[
\begin{align*}
&H_{33} C_{16} O \quad n=10 \\
&\text{Structure of (Brij-56)} \\
&H_{33} C_{16} O \quad n=20 \\
&\text{Structure of (Brij-58)}
\end{align*}
\]
The molecular Structures of amino acids are given as follows.

(a) Zwitterionic Str. of Alanine  
(b) Zwitterionic Str. of Phenylalanine

(c) Zwitterionic Str. of Glycine  
(d) Zwitterionic Str. of Serine

(e) Zwitterionic Structure of Methionine

Doubly distilled water with specific conductance 2-4 µ S cm⁻¹ at 303.15 K is used in the preparation of all solutions of different concentrations.
2.2 Experimental methods:

2.2.1 Cloud Point Determination

The cloud point of surfactants is an important and fruitful physicochemical exercise for judging the quality and characteristics of surfactant alone or in a mixture possible use in process especially where elevated temperature prevails. The effect of additives on the cloud point (CP) of non-ionic surfactant has been studied for more than six decades. A cloudy dispersion appears upon heating of aqueous solution of non-ionic surfactant. The threshold temperature at which clouding is observed is known as cloud point (CP). The cloud point is an important property which can be used in diverse application like foam controlling in an industrial processes where surfactant may cause instability. The foam stability drops at a temperature near the cloud point (CP).

At elevated up to certain temperature narrow range, the aqueous solution of non-ionic surfactant suddenly becomes turbid so that phase separation occurs is also known as cloud point (CP). The phase separation is observed as a cloud point is accompanied by rapid increase in size of micelle at elevated temperature or aggregation of relatively small micelles by decrease in intermicellar repulsion. Therefore interpretation of cloud point as a critical phenomenon in micellar solution implies that a critical point is approached when micelles comes together and above the critical point, they separate out as second phase i.e. below cloud point a single phase of micellar solution exist while above cloud point the water solubility of surfactant is reduce which result into cloudy dispersion due to aggregation of micelle.

Clouding for non ionic surfactant reflects change from water soluble to oil soluble when temperature raised. The cloud point values for a mixture of polymer surfactant can be correlated to hydrophilic or hydrophobic character of the mixture i.e. cloud point of non ionic
surfactant decreases with increasing the length of the hydrocarbon chain and decreasing the length of oxyethylene chain\textsuperscript{18-19}. The structural changes in surfactant molecule on the cloud point \textsuperscript{20} indicate that, at constant oxyethylene percentage the cloud point is lower by decrease molecular weight of surfactant, broader distribution of polyoxyethylene chain length, branching of hydrophobic group in surfactant molecule, replacement of the terminal hydroxyl group of hydrophilic group by a methoxyl and replacement of the ether linkage between the hydrophobic and hydrophilic group by an ester linkage. The cloud point of non ionic surfactant can also be increase by the addition of polyelectrolyte’s or ionic surfactants that interacts with them and causes them to acquire a change \textsuperscript{21-22}. Theories to explain phase separation have been proposed by Krafft and Wiglow \textsuperscript{23} and later by Murray and Hartely \textsuperscript{24}.

**Experimental details:**

The cloud point (CP) was determined by controlled heating of the sample solution of non ionic surfactant in absence and presence of additives amino acids- (viz. Alanine (Ala), Phenylalanine (Phe), Glycine (Gly), Methionine (Met) and Serine (Ser) in thin glass tube immersed in beaker containing water until the solution got turbid. The sample solution was then allowed to cool slowly under the stirring condition. The temperature at which disappearance of turbidity (clear solution) should considered as the cloud point. Heating and cooling was regulated to about 1\textdegree C/min. near the cloud point. The reproducibility of the measurement was found to be within \(\pm 0.2 \textdegree C\).

The phenomenon of cloud point is reversible process. The CP values of surfactant-amino acid mixture may guide to its hydrophobic or hydrophilic character. This information is useful in pharmaceutical preparation, drug delivery, and biochemical formulation, enhance oil recovery process etc.
2.2.2 Ultrasonic Velocity Measurement

The measurement ultrasonic velocity is most innovative and an important technique for investigation of nature of intermolecular and intermolecular interaction between the solute-solute, solute-solvent and solvent-solvent molecules in pure solution and solution mixture\textsuperscript{25-26}. These interactions might be in the form of structure making or structure braking phenomenon occurring in the solutions. Therefore this technique is widely used in finding the interaction in the solution mixtures which includes polymer, soap, milks, drugs, amino acids and peptides, oils and fats…etc. In order to get more information, an attempt has been made to correlate the experimental findings with those predicated theoretically on the basis of molecular models\textsuperscript{27-30}. The nature and degree of molecular interactions in different solutions depends upon the nature of solvent, the structure of solute molecule and the extent of solvation taking place in the solution\textsuperscript{31}.

From the ultrasonic velocity measurement, different acoustic parameters such as acoustic impedance (Z), adiabatic compressibility ($\beta_{ad}$), intermolecular free length ($L_f$), molar volume ($V_m$), molar sound velocity ($R_m$) were determine to investigate the structural and physicochemical behavior of solution mixture. The ultrasonic waves have been used by many workers\textsuperscript{32-42} for the investigation of molecular interactions and complex formation in the systems. Thus ultrasonic velocity\textsuperscript{43-45} measurement absorption is a reliable and popular method for determining nature of molecular interactions and thermodynamic properties of solutions.
In present investigation single frequency (2MHz) ultrasonic interferometer (Photograph-1) supplied by Mittal Enterprises, New Delhi was used. This manual instrument was then converted into a fully automatic, software controlled instrument having the facility to record digital reading of maxima or minima on digital panel as well as in computer. The accuracy in ultrasound velocity measurement was checked by comparing the observed velocity values of water (1496.4 m/s) and acetone (1164.2 m/s) with those of their literature values (1496 m/s)$^{46}$ and (1165 m/s)$^{47}$ respectively.

The ultrasonic interferometer consists of the following parts-
(a) The High Frequency Generator: 2 MHz
(b) The Measuring Cell: 2 MHz

**a) The High Frequency Generator:**

Model F-81 (Photograph-1) is designed to excite the quartz crystal fixed at the bottom of the measuring cell at its resonant frequency to generate ultrasonic waves in the experimental liquid filled in the “Measuring Cell”. A micrometer to observe the changes in current and two controls for the purpose of sensitivity regulation and initial adjustments of the micrometer are provided on the panel of the high frequency generator.

**b) The Measuring Cell:**

It is specially designed double walled cell (Photograph-2) 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 liquid in the cell through a known distance. It has a quartz crystal fixed at its bottom. Least count of micrometer is 0.001 mm and maximum displacement of reflector is 20 mm. The maximum capacity of cell is 10 cm$^3$. 
Photograph-2 The Measuring Cell
The instrument was adjusted in the following manner:

1. The measuring cell was placed in the round base socket and clamped to it with the help of screw.
2. The knurled cap of cell was unscrewed and lifted away from double walled construction of the cell. In the middle portion of it experimental liquid was poured and then the knurled cap was screwed perfectly. Excess liquid overflowing from the cell was wiped out.
3. Water at desired temperature from a thermostat having accuracy of 0.1°C was circulated through the two chutes in double wall of the cell.
4. The High Frequency Generator was connected with cell by co-axial cable provided with the instrument.
5. The micrometer was moved slowly in either clockwise or anticlockwise direction till the anode current on the ammeter on the High Frequency Generator shows a maximum or minimum.
6. The readings maxima and minima are recorded with the help of microprocessor operated computerized system graphically.

**c) Working Principle of Ultrasonic Interferometer:**

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 \( \lambda \) 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 wavelength \((\lambda/2)\) or multiple of it the anode current becomes maximum. From the knowledge of wavelength \((\lambda)\) the velocity \((U)\) can be obtained by the relation:

\[
\text{Velocity} = \text{Wavelength} \times \text{Frequency} \\
U = \lambda \times f
\]

**d) Experimental Details:**

The interferometer was initially adjusted with the help of two knobs provided on high frequency generator, one marked with “Adj” and 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 grater deflection if desired. The interferometer cell was filled with the experimental solution and connected to output terminals of the high frequency generator through the shielded cable. Water was circulated around the measuring cell from a thermostat maintained at required temperature. When the solution in cell attains the temperature of bath, the micrometer screw was slowly moved till the anode current meter showed a maximum. The frequency ‘F’ of the crystal is accurately known. Here the frequency of the crystal is 2 MHz, the sound velocity \(U\) in the solution was calculated with the help of equation.

\[
U = \lambda \times f
\]

The speed of sound is measured with an uncertainty of \(\pm 0.2\%\) using a single crystal variable path ultrasonic interferometer F-81 (Mittal Enterprises, New Delhi) operating at 2MHz which is calculated with
water and acetone as said earlier. In order to maintain the uncertainty of the measurement several maxima and minima are allowed to pass and their number \( n \) is counted. All maxima and minima are recorded with the help of microprocessor operated computerized system graphically (Graph-I). Experimental setup used for ultrasonic velocity measurement was as shown in Photograph-1.

Mark\(^{48} \) showed that, the thermodynamic properties such as adiabatic compressibility \((\beta_{\text{ad}})\), Intermolecular free length \((L_i)\), Molar Sound Velocity \((R_m)\), Specific Acoustic Impedance \((Z)\), Molar Volume \((V_m)\) and Surface tension \((\gamma)\) are calculated from the observed density \((\rho)\) and ultrasonic velocity \((U)\). With the help of following equations –

\[
    (\beta_{\text{ad}}) = \frac{1}{\rho U^2}
\]

\[
    (L_i) = k(\beta_{\text{ad}})^{1/2}
\]

\[
    (R_m) = \left(\frac{\bar{M}}{\rho}\right)U^{1/3}
\]

\[
    (Z) = \rho U
\]

\[
    (V_m) = \frac{\bar{M}}{\rho}
\]

\[
    (\gamma) = (U^{3/2})(6.3 \times 10^{-4})\rho
\]

Where \( U \) is the ultrasound velocity, \( \rho \) is the density, \( K \) is the Jacobson’s temperature depended constant, \( \bar{M} \) is the effective molecular weight and can be calculated using relation.

\[
    \bar{M} = X_1M_1 + X_2M_2
\]

\( M_1 \) and \( M_2 \) are molecular weights and \( X_1 \) and \( X_2 \) are the mole fractions of component-1 additive and component-2 surfactant solution as solvent.

The ultrasonic method fails completely, if the velocity of sound in the liquid exceeds 1600 m/s and it is also inferior to thermodynamic method\(^1\).
Graph I- Computerized Ultrasonic Velocity measurement of at 30 °c
2.2.3 Density Measurement

One of the most important physical properties of hydrocarbon or hydrocarbon mixture is its density. Density ( ) is defined as the mass of the fluid per unit volume. In general, it varies with pressure, temperature and is expressed as gm.cm\(^{-3}\) in CGS, Kgm\(^{-3}\) in SI or /bft\(^{-3}\) in the English system. It is one of the most important physical properties for calculating properties like specific dispersion, refractivity intercept, and specific refraction and in calculation of kinematic and absolute viscosities from each other.

Fig.1: Bicapillary Pycnometer
There various methods used for determining density ($\rho$) or specific gravity of a liquid are –

1. Westphal balance
2. Specific gravity balance (Chain-o-matic)
3. API hydrometer
4. Pycnometer
5. Bicapillary pycnometer.

The first two methods are based on the principle of Archimedes: A body immersed in a liquid is buoyed up by a force equal to the weight of the liquid it displaces. The API hydrometer is usually used for determining oil gravity in the oil field. When a hydrometer is placed in oil, it will float with its axis vertical after it has displaced a mass of oil equal to the mass of hydrometer. The pycnometer is an accurately made flask, which can be filled with a known volume of solution. The bicapillary pycnometer method for density measurement was developed by Lipkin et al having a precision of $\approx 0.00001$ gram per ml and a probable accuracy of the same magnitude. Open-arm bicapillary pycnometer used for density determination in this method is as shown in Fig.1. When less quantities of solution is available and high accuracy is required then bicapillary pycnometer method is probably best known method is used for determination of densities of solutions.

**Experimental Details:**

The pycnometer was thoroughly washed with hot chromic acid followed by washing with distilled water and acetone. Finally pycnometer was dried with a stream of hot air from hot air blower. This gives a smooth flow of liquid without drop sticking behind. After complete washing, cleaning and drying it was weighed accurately on a Shimadzu digital balance having sensitivity 0.001 g. The pycnometer was filled with air bubble free triply distilled water by dipping the end of ‘A’ limb into the water taken in beaker of 15-20 ml volume. Water
filled up to mark ‘D’ in limb ‘B’ by capillary action. The pycnometer was weighed again to obtain the mass of water taken in pycnometer.

Then the pycnometer was mounted accurately vertical in a transparent glass sided thermostat for 25-30 minutes to attend thermal equilibrium. The temperature of the thermostat was controlled within ±0.1 K. The height of water in limb ‘A’ and ‘B’ (say $h_1$ and $h_2$) were noted at various temperatures, with the help of magnifying lens. From the known densities of water at various temperatures and the weight of water taken in pycnometer, corresponding volumes of water were calculated. By using this data characteristic constant of pycnometer ‘m’ and ‘c’ values of are obtained by standard calculation method.

Pycnometer was then removed from thermostat and cleaned again as mentioned above. It was then filled with experimental solution and mounted in the thermostat vertically. The procedure was repeated to find the total height ($h_1+h_2$) for experimental solutions at various temperatures. From their total heights the corresponding volumes of solutions under investigation were obtained from ‘m’ and ‘c’ values. From this the corresponding densities are determined by the standard procedures49-53.
References:


