Solute Ordering Behaviour of L-Alanine in Aqueous Solution

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Abstract**—L-alanine is not particularly hydrophobic and is non-polar. L-alanine is a small, naturally occurring amino acids are chiral and non-reactive hydrophobic methyl group (-CH3) as a side chain. Zwitterionic species of L-Alanine are often closely dependent on the aggregation behavior of the ionic liquids in the aqueous solution is very important from both fundamental and applied aspects. In this work, the purpose of this research is to determine the dissolution temperature of L-alanine in aqueous solution by dissolution solubility experiment method and an assessment of solute ordering behavior of L-alanine in aqueous solution, in terms of critical aggregation concentration (CAC). To determine the critical aggregation concentration, the measurement of conductivity meter have been carried out experimentally. Using conductivity method, CAC values of L-Alanine in aqueous solution were determined at different temperatures. It is known that, for aqueous solution of ionic surfactant, the critical aggregation concentration decreases at certain point and then increase with increasing temperature which creates a similar shape which can be seen from figure 7 that is the curves of conductivity vs concentration. The standard Gibbs energy of aggregation is also obtained by using van’t Hoff’s equation. The gravimetric method for solubility data of L-Alanine is also presented and discussed.**

Keywords**— Ionic Surfactant, Conductivity, Critical aggregation concentration (CAC), Solute clustering.***

I. INTRODUCTION

L-alanine is a small, naturally occurring amino acids are chiral and non-reactive hydrophobic methyl group ($-CH_3$) as a side chain. L-alanine has zwitterionic form ($^+NH_3$-C_2H_4-COO^-) both in crystals and in aqueous solution in a large range of pH (Jalkanen et al., 2007). Figure 1 shows the molecular structure of L-Alanine.

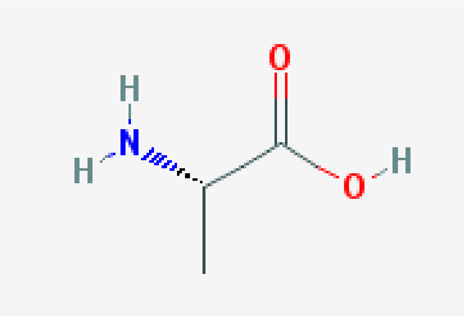

Fig. 1: Molecular structure of L-Alanine

According to Ivan et.al., (2007), the structural properties of the alanine molecules in aqueous solution can be clearly different from the crystalline phase, due to the interaction of hydrogen bonds.Specifically, this researcher found that the biggest difference between the two forms of orientation and alanine can be seen that the bond length carboxylate (COO^-) group: an aqueous C-O looks strong bond length do with the number of water molecules that form hydrogen bonds with the COO^- group. Furthermore, the hydrogen bond length is shorter and the angle is greater hydrogen bond to L-alanine in water than the crystal.

In the crystals of the three groups of protons of the ammonium group (NH_3^+) is used to form N-H···O hydrogen bonds with oxygen atoms of three carboxylate groups (COO^-) contiguous amino acid molecules, thereby linking the molecules together to form a crystal structure with three dimensions. In contrast, the major determinant zwitterionic structure is an aqueous solution of hydrogen bonding interactions with surrounding water molecules. The L-alanine zwitterionic species in water and the crystals depicted in Figures 2 and 3. Figure 4 below shows the structural differences of the L-alanine zwitterion in aqueous and crystalline structures. (Jalkanen et al., 2007).

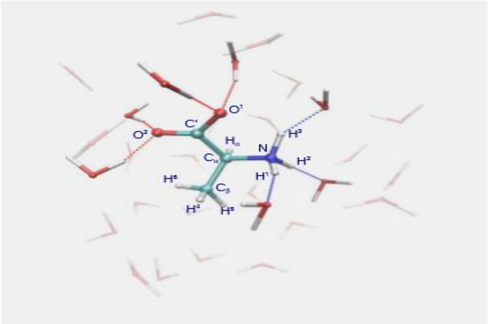

Fig. 2: Ball and stick representation of the amino acid L-alanine in water. Water molecules in the first hydration shell are shown in solid. The dotted lines indicate hydrogen bonding.

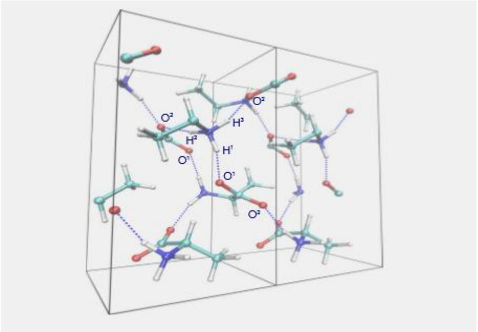

Fig. 3: L-alanine crystalline structure. Two unit cells with species constrained to periodic conditions. The system crystallizers in the $P2_12_12_1$ space group, with four zwitterionic molecules per unit cell. (Adopted from: Lehmann et al., 1972)

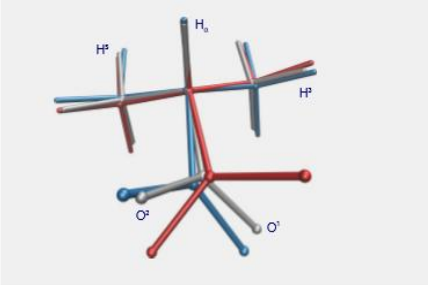

Fig. 4: The structural differences of the L-alanine zwitterion in aqueous and crystalline structures. The calculated (blue), experimental (silver) crystalline and solvated molecule (red) structures. The structures are aligned along C_a-H_a bond. (Source: Jalkanen et al., 2007).

Studies of amino acids have been carried out by many previous researchers, for example is L-isoleucine. Neverthless, there have not been many studies on L-Alanine, for example, dissolution and the critical aggregation concentration. In this work, L-Alanine was chosen as a compound for this fundamental study reflecting its interesting crystal chemistry and its varied surface properties.

Concentration is another parameter which is important to the self-assembly of biomolecules.The concentration dependence of self-assembling in solution is expected to be similar to that of biosurfactants, which have both hydrophobic and hydrophilic parts. By analogy with surfactants, a concentration study may allow one to determine the critical aggregation concentration (CAC).

This paper reports on examination of the solute ordering behavior of L-Alanine in aqueous solution associated with measurement of the influence of dissolution, conductivity for determination of critical aggregation concentration. Many research publications suggest that ionic liquids form aggregates in aqueous solutions. It should be noted that different terminologies have been used in the literature for the aggregate or micelle formation of the ionic liquids. The reason may lie in the fact that, sometimes, aggregates of ionic liquids could be formed, but the attractive interaction between alkyl chains was not strong enough to form micelles, especially for ionic liquids with shorter alkyl chains. Therefore, the terms “aggregate” and “critical aggregation concentration (CAC)” have been frequently used in this paper (J.Wang & H.Wang, 2014). Thermodynamic analysis of the aggregation behavior of L-Alanine by using Gibbs energy of aggregate formation, is important for a deeper understanding of the driving forces and some molecular details involved in the aggregation processes (Bai et al., 2008). The nature of the surfactant molecule, which has both hydrophilic and hydrophobic groups, responsible for their tendency to minimize the free energy of the system by adsorption at various interfaces. However, when all interfaces are saturated, the overall energy reduction may continue through their mechanisms as shown in Figure 5. The physical manifestation of such a mechanism is crystallization or precipitation of the surfactant solution that is bulk phase separation. An alternatives aggregates formation of molecules or micelles that remain in solution as a thermodynamically stable, dispersed species with different characteristics from those monomeric solution (John Wiley & Sons, 1999).

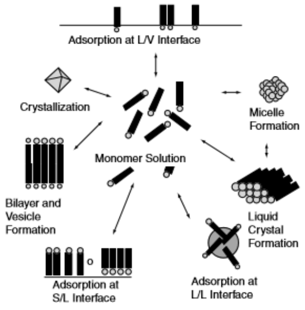

Fig. 5: A surfactant in solution has various options in terms of its surface activity. (Source: John Wiley & Sons, 1999)

II. METHODOLOGY

A. *Materials and Methods*

Material. L-Alanine ($C_3H_7NO_2$, MW of 89.094) was obtained from Merck. Distilled water was used as a solvent.

Determination of Dissolution Temperature. Solubility data was determined by previous researcher over the temperature range between 15ºC to 75ºC by placing the sample in a 100mL distilled water. This method is called as gravimetric method. To achieve the first objective, the dissolution temperature was determined by placing the sample in jacketed reactor. The L-alanine was added to a fixed amount of 50 mL water and the sample was agitated at the desired temperature for 2h with temperature range between 20ºC to 85ºC and rate of 0.5ºC/min. L-alanine was observed until the last crystals were dissolved and the reading of the temperature was noted. Then, the liquid samples were withdrawn from the reactor and to be tested on conductivity meter to achieve the second objective which is determination of critical aggregation concentration.

Determination of Critical Aggregation Concentration. The CAC value was determined by using the conductivity test at different temperatures. For this method, the solution should be in a clear solution to get the correct reading on the conductivity meter. Therefore, to conduct this experiment, the solubility data should be referred to ensure that the concentration of the L-Alanine at particular temperature was dissolved which means the solution are in the clear solution. When the solutions in clear solution or it is fully dissolve, then probe meter will be immersed into the solution, and the reading of the conductivity was noted. The procedure was repeated by increased the amount of L-Alanine to the solution until the CAC values is shown in the trendline in the graph of conductivity vs concentration.

After the CAC value was determined, the Gibbs free energy was calculated by using the van’t Hoff’s equation [15]:

$$\ln(x) = \frac{\Delta S_d}{R} - \frac{\Delta H_d}{RT_{sat}} \qquad (1)$$

Where x is the mole fraction of solute in the solution, ΔS_d and ΔH_d are the enthalpy of dissolution respectively, T_{sat} is saturation temperature. The Gibbs free energy for this process can be calculated by the following equation (Anuar et al., 2009) :

$$\Delta G = \Delta H - T\Delta S \qquad (2)$$

III. RESULTS AND DISCUSSION

A. *Determination of Dissolution Temperature*

The L-Alanine solubility was found from the previous researcher and it is stated in the Table 1. The data was plotted in the Figure 6.

Table 1: Solubility data of L-Alanine

Concentration (g/ 50 mL H_2O)	$T_{soluble}$(ºC)
8	20
9	32
10	39
11	46.5
12	54.5
13	64
14	75

Table 2: Dissolution temperature of L-Alanine

Concentration (g/ 50 mL H_2O)	T_{diss}(ºC)
8	31.47
9	41.67

10	51.92
11	61.89
12	68.71
13	78

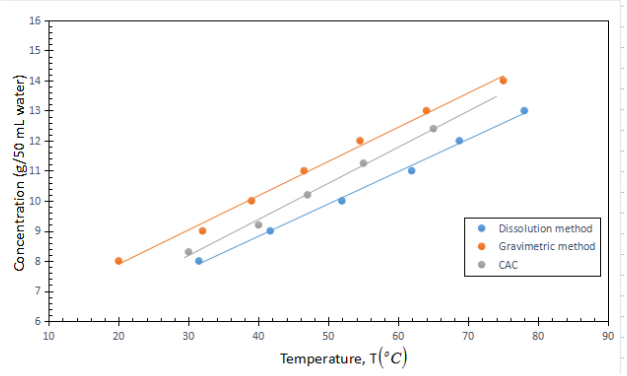

Fig. 6: Plot of Concentration L-Alanine with temperature.

The variation of L-Alanine species with concentration and temperature of dissolution are shown in Figure 6. The trend shows when the concentration of the solution increased, the temperature of dissolution also increased. Dissolution and gravimetric methods shows that have a big different temperature to dissolve the L-Alanine at the fixed amount of solvent. The L-Alanine cannot dissolve at that particular time according to the solubility data when conducting the dissolution solubility experiment. Therefore, the conductivity test was done to the solution to determine what is actually happaned at that time. From the experiment that have been carried out, the aggregate was formed in the solution at the particular concentration.

B. Determination of Critical Aggregation Concentration by Conductivity Test

Figure 7 shows the relationship between the conductivity and concentration, and it is obvious that the relationships for each concentration are made of two straight line with different slopes. The result shows that when the concentration of the L-Alanine increases, the conductivity also increased. But, at certain point after the sudden change point which is the CAC value, the conductivity is most likely keep constant. The trend for every different temperatures increases significantly when the concentration increased, then the trend shows constant direction after the sudden change point. This is because the free ions of L-Alanine compound which are NH_3^+ and COO^- at that particular time start to colloid and form aggregates. For all set of temperatures, below the CAC, the addition of surfactant concentration to an aqueous solution causes an increase in the number of charge carrier, NH_3^+ and COO^- and consequently, an increase in the conductivity. Above the CAC, further addition of surfactant increases the micelle concentration while monomer concentration remains approximately constant (at the level CAC). A plot of conductivity against concentration is, thus expected to show a break at the CAC (Figure 7).

From the Figure 7, the result shows that all temperatures have CAC values which is the point whereas the sudden change occur. The result as shown in Table 3. Once the CAC values was determined, then the result will be plotted on the Figure 6 which is the trend between gravimetric method and dissolution method. When referred to that trend, the CAC value trend is in between the solubility data and dissolution data which means in order to get the dissolution temperature, the aggregate is start to form at that particular temperature and that will make the dissolution temperature higher than the temperature of solubility (gravimetric).

L-Alanine, was ionized in the aqueous solution to form NH_3^+ and COO^- ions in the solution. Self-dissociation of L-Alanine into the aggregate is strongly cooperative and occurs at the defined concentration called critical aggregation concentration. Below the CAC, the amphiphile dissolves as monomers. Once the concentration beyonds CAC, the monomers concentration remains unchanged while the aggregation concentration increased. The CAC can be determined by the conductivity method of L-Alanine solution.NH_3^+ and COO^- ions are known as charge carriers which will increase the conductivity of the solution when ionization takes place.

At the beginning of the experiment, a small amount of L-Alanine is added into the distilled water. In a L-Alanine dilute solution, the concentration of L-Alanine is below its CAC, hence it behaves as normal electrolyte and ionizes to give out NH_3^+ which soluble in the aqueous phase while COO^- ions solubilize its hydrophilic head in the water and hydrophobic tail extent out the water surface. The ions exist as solvated monomer instead of aggregate due to low L-Alanine concentration. The number of monomers was increased as the amount of the L-Alanine was added into the solution. At the same time, the increase of conductivity that have been detected due to the increase of L-Alanine ions carried more charges within the solution. Once the amount of L-Alanine added into the aqueous solution is equals to the CAC, the first aggregate start to form spontaneously in the solution.

Bowers et al. has been investigated the aggregation behavior of 1-butyl-3-methylimidazolium tetrafluoroborate and 1-methyl-3-octylimidazoliumchloride and iodide in aqueous solution by using surface tension, conductivity, and small angle neutron scattering (SANS) measurements. The results showed that these ionic liquids act as short chain cationic surfactants in aqueous solution and form aggregates above their CAC values. The conductivity data for 1-methyl-3-octylimidazoliumchloride and iodide indicated the possible presence of aggregates at concentrations below the CACs.

Moderassi et al. have used several methods such as conductivity, surface tension, and SANS to investigate the aggregation behavior of 1-alkyl-3-methyl-imidazolium bromides in water. From the study, it showed that the ionic liquids with a butyl chain do not favor an aggregate self-assembly.

Thermodynamics analysis on the aggregation behavior of L-Alanine by using Gibbs energy was done. The calculated thermodynamic properties are shown in Figure 8 and 9.

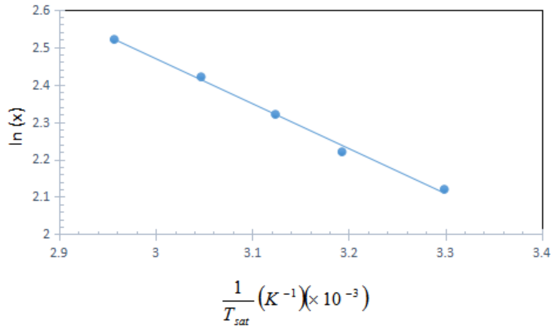

Fig. 8: Van’t Hoff solubility data of L-Alanine, showing negative deviation.

From the Figure 8, the gradient value is -1.994, therefore the ΔH_d=16.578 J/mol. And the y-intercept for the graph data is 6.0668, therefore the value of ΔS_d=50.44 J/mol.K.When inserted the value of ΔH_d and ΔS_d into the equation of Gibbs energy, the graph of Gibbs energy against temperature as shown in Figure 9.

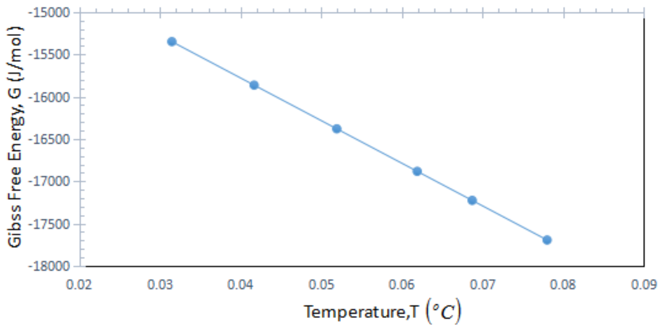

Fig. 9: Plot of Gibbs free energy against temperature.

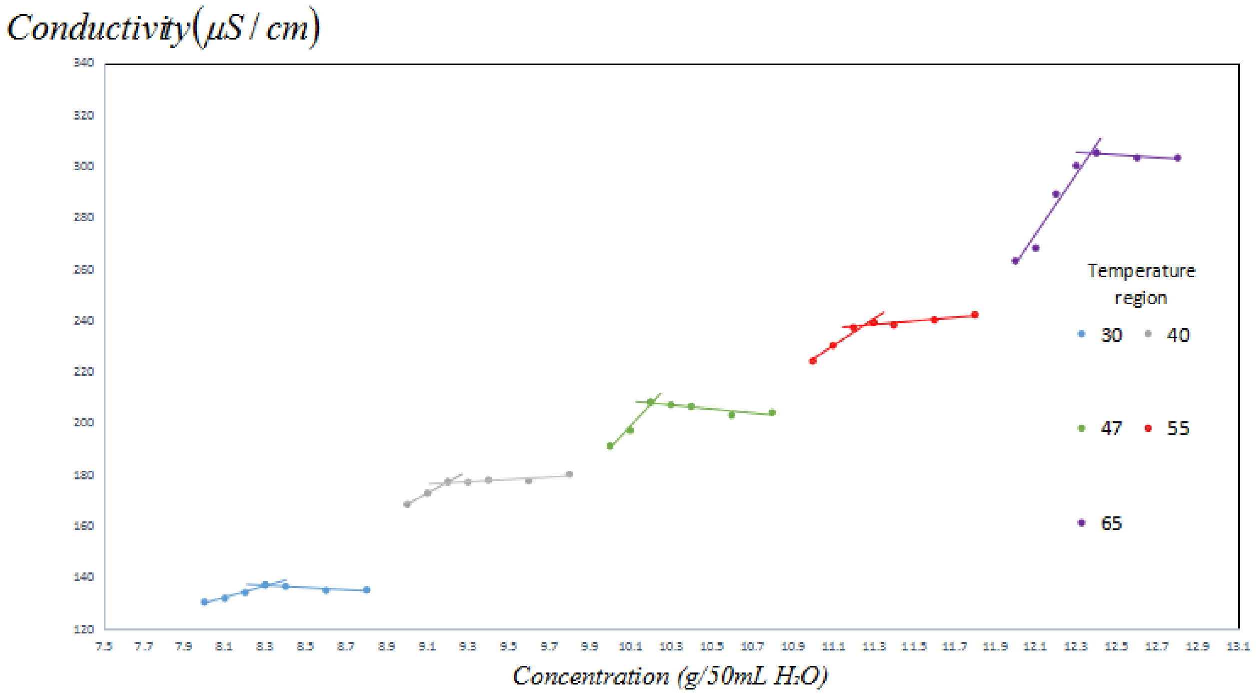

Fig. 7: Relationship between conductivity and concentration with variation of temperature for 30°C, 40°C, 47°C, 55°C and 65°C.

Table 3: CAC values for different temperature

Temperature(°C)	CAC value(g/50mL H_2O)
30	8.3
40	9.2
47	10.2
55	11.25
65	12.4

The enthalpy of dissolution appears to be an endothermic process, while the entropy show positive value when referred to the data on Figure 8 and applying straight line equation. The contribution of positive values of enthalpy come from the endothermic reaction of breaking solute hydrogen bonds to create a cavity for the solutes with water. The negative value of Gibbs energy support the fact suggested by Davey et al. That this system exhibits negative deviation from ideal behavior, indicating strong solute-solvent interaction. Here it is suggested that there are strong interactions between the hydrophilic head of the molecules (consisting of NH_3^+ and COO^-) and the surrounding water molecules, this despite the solute's sparingly soluble behavior in water.

In summary, behavior of aggregation of ionic liquids in the solutions was analyzed and some of it comply with the studies done from over the past decade where researcher has made progress in the application of the study. It can be expected that the aggregation will find more applications in the future. However, research in this field is still in its infancy, and there are a number of issues which require further investigation. In future work, the several studies are suggested such as a deeper study should be carried out on the microstructure of ionic liquids aggregates. Besides, the effect of surface tension, density, viscosity, and light scattering of L-Alanine and addition of an additive on the aggregation behavior in terms of critical aggregation concentration of ionic liquids in solutions should be also investigated.

For the recommendations for future research, the stirring of the solution must controlled not to be too fast during the experiment to avoid the formation of bubbles as bubbles can affect the conductivity. Besides, the substances should added slowly to the water to prevent the formation of bubbles.

IV. CONCLUSION

From the trend line between gravimetric and dissolution method shows that the temperature to dissolve the L-Alanine in water has a big different. Therefore, the conductivity test were determined experimentally for various temperatures and concentrations to analyze why it could happened. The critical aggregation concentration (the line plotted between gravimetric and dissolution method) were determined by estimated at the sudden change point of the plot of conductivity against concentration. The result shows the aggregation formed at particular concentrations and temperatures during dissolving the L-Alanine and water solution. When the concentration of L-Alanine is increased, the conductivity also increased but above the CAC values, the aggregate are formed which means the free ions NH_3^+ and COO^- start to colloid. Also, the values of fundamental thermodynamic properties like Gibbs free energy have been calculated by using van't Hoff equation.

ACKNOWLEDGMENT

Thank you to my supervisor, Dr.Siti Nurul 'Ain Yusop for her guidance towards my reseacrh. Other than that, I would like to express my gratitude to Dr.Nornizar Anuar, Sir Fitri Othman, Dr Nik Salwani Md Azmi and all staff in the Faculty of Chemical Engineering at Universiti Teknologi MARA for funding of this work .

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