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Influence of amino acid additives on solution behaviour of L-alanine

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Abstract: The solubility experiment of L-alanine solution was performed in a 250ml jacketed glass crystallizer without and with amino acid additives at temperature from 15°C to 75°C by means of gravimetric method. On the whole, L-leucine additive significantly altered the solubility of L-alanine and Glycine additive caused an erratic pattern on the solubility data of L-alanine. The hydrophobic methyl side chain of L-leucine additives is believed to contribute to the formation of water clathrate in the solution which affected the interaction of L-alanine molecules in water solvent and thus modified the solubility of L-alanine. Finally, thermodynamic data analysis of L-alanine solution was extensively assessed. The negative deviation of L-alanine from the ideal solution is as a result of high solute-solvent interaction, which is due to the hydrophobicity and clathrate phenomenon of the water molecules in the solution.

Keywords: *Amino acid, L-alanine, Crystallization, Solubility.*

Introduction

Understanding the basic properties of molecules in a solution is crucial for a better design and optimized condition of crystallization process. The solubility data is an important basic property which directly impact on the crystal nucleation, growth[1] and polymorphism[2] and hence has garnered attention in the fundamental studies of the solubility of biomolecules in solution. Recently, there are many researchesinvestigating strategies on improving the solubility of crystal compound such as additives effect[1,3], cocrystal formation[4], solvent effect[5] and thermal effect[6].Amino acids are one of simple biomolecules and are an important building block of native protein. Due to their importance in many industrial applications such as food and pharmaceutical sectors, many researchers are interested on the solubility enhancement[7,8] as well as the thermodynamic properties of amino acids[9].

Therefore, in this work, L-alanine was selected as the model compound, in which it is classified as a non-essential aliphatic amino acid which contains a methyl group[10]. Meanwhile an additive compound from different amino acid types including L-leucine and Glycine were used in an attempt to study their effect on L-alanine solubility behaviour. Thermodynamic data assessment of pure L-alanine in aqueous solution was also conducted in this work.

Solubility and Thermodynamic Properties Theory

The solubility of the non-ideal solution can be expressed using van't Hoff equation[11] as follow to:

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

Where x is the solute mole fraction, T_{sat} is the saturation temperature, R is the gas constant (8.314 J/mol.K) and ΔH_d and ΔS_d are the enthalpy and entropy of the dissolution process, respectively.

An ideal solution exists when the interaction between solute and solvent molecules is identical with the interaction between solute and solvent molecules themselves. The solubility for an ideal solution can be expressed using an ideal van't Hoff equation as follows:

$$\ln(x) = \frac{\Delta H_f}{R}\left[\frac{1}{T_f} - \frac{1}{T_{sat}}\right] \qquad (2)$$

The ideal van't Hoff equation can also be written as follow:

$$\ln(x) = -\frac{\Delta H_f}{RT_{sat}} + \frac{\Delta S_f}{R} \qquad (3)$$

Where ΔH_f is enthalpy of fusion, ΔS_f is entropy of fusion and $T_f$is melting temperature. All the other parameters are identical as in equation 1. Most in the previous literature focus on the comparison between the ideal solubility and non-ideal solubility data which provide useful information in determination of the strength interaction between solute and solvent molecules[12,13].

In addition, the change in Gibbs free energy change, ΔG approach can be used to describe the behaviour and ideality of solutions. The ΔG for dissolution

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process can be expressed in terms of enthalpy and entropy change associated with the mixing (dissolution) process as shown below:

$$\Delta G = \Delta H - T\Delta S \qquad (4)$$

The deviation of the real solution from ideality can be measured using excess Gibbs free energy change, ΔG_{excess} and activity coefficient, γ_a[14]. The excess Gibbs free energy is defined as the difference between Gibbs free energy of non-ideal (ΔG) and ideal system (ΔG_{ideal}) as shown in equation 5:

$$\Delta G_{excess} = \Delta G - \Delta G_{ideal} \qquad (5)$$

For an ideal system, Gibbs free energy can also be expressed as[15]:

$$\Delta G_{ideal} = -RT\ln(x) \qquad (6)$$

Where x is the mol fraction of one of the components in the solution and the entropy change associated with this dissolution process, ΔS can be expressed by using equation 4. By taking the enthalpy of the mixing process as zero for ideal system, then

$$\Delta S = -R\ln(x) \qquad (7)$$

The overall Gibbs free energy can be correlated with activity (a), of one of the components in solution:

$$\Delta G = RT\ln(a) \qquad (8)$$

The term activity (a), is used to explain the deviation of behaviour of a solution from ideality. In any non-ideal solution, interactions occur between the components which reduce the effective concentration of the solution. The activity is a way of describing this effective concentration. In an ideal solution at infinite dilution, there are no interactions between components and the activity (a) equals the concentration. Non-ideality in real solutions at higher concentrations causes a divergence between the values of activity and concentration[16]. For nonideal solution, the activity, a is unequal with mol fraction (x) and they can be correlated with the activity coefficient (γ_a) as shown below:

$$a = \gamma_a x \qquad (9)$$

Similarly, equation 9 can be expressed as:

$$x\gamma_a = \exp\frac{\Delta H_f}{R}\left[\frac{1}{T_f} - \frac{1}{T}\right] \qquad (10)$$

Materials

L-alanine (≥99.0% pure), L-leucine (98.0% pure) and Glycine (≥98.0% pure) were purchased from MERCK SDN. BHD. and were used without further purification. Distilled water was used in preparing L-alanine solution.

Solubility Experiment

Binary system (L-alanine + Water)

Isothermal method was used to determine the solubility of L-alanine + water system. The binary system solution was prepared by dissolving an excess amount (total mass added was recorded) of L-alanine in a fixed volume of distilled water. The solution was placed in a 250 ml glass jacketed batch crystallizer, equipped with temperature probe and glass stirrer, controlled by a digital speed regulator. Meanwhile, the water that flowed in and out from the crystallizer glass jacket was programmed using a programmable refrigerated bath. The solution was agitated at 350 rpm and heated 10 °C above the desired equilibrium temperature until the solution was homogenously mixed.

Next the temperature of the solution was reduced to the desired temperature (75, 60, 45, 30 and 15) °C and held constant for about 24 hrs to achieve equilibrium condition. The temperature readings in the crystallizer were monitored using a LabVIEW Signal Express v8.5 software. At equilibrium solution, liquid samples were withdrawn from the liquid phase using a 10 ml-plastic syringe.

Approximately 5 ml of solution was filtered from the plastic syringe through 0.20 μm syringe filter and transferred to empty vial. The vial containing solution was weighed and dried in the oven at temperature 40 °C[12]. Finally, the vial containing the solute was weighed and the dissolved amount of L-alanine in vial was subtracted with the initial weight of vial. The amount of water evaporated was determined by the different value between the vial filled with solution and the same vial containing only dissolved solute. Three samples were taken from each experiment at different saturation temperature for reproducibility of the result. Gravimetric method was used to analyze the amount of solute in the solution.

Ternary system (L-alanine + additive + Water)

The ternary solutions were prepared by adding a known amount of additive in the L-alanine solutions. The solubility experiments of ternary system were performed by using the same method and equipment used in the solubility experiments of the binary system. In this experiment, the additive (L-leucine and Glycine) were added at different percentages of 3wt%, 5wt% and 10wt% (solvent free basis) of L-alanine. The ternary system in the presence of L-leucine additive can be identified as Ala-Leu system and the ternary system containing of Glycine additive is denoted as Ala-Gly system.

Results and Discussion

Solid-liquid equilibrium of L-alanine

Binary system: L-alanine + Water

Figure 1 demonstrates the solubility of amino acids; i.e. glycine, L-alanine and L-leucine. The solubility was reported in terms of gram of L-alanine/100 g of water. Overall, the solubility of amino acids increased with the increase of temperature. The solubility of glycine is higher than L-alanine and L-leucine has the lowest solubility than these two components. Note that the solubility obtained from this work is slightly higher at temperature of 40 °C than reported by Raghavalu et al.[17], but almost the same as the result presented by Daldrup et al.[18].

The evaporation of water and solute in small quantities may occur during the experiment as the crystallization experiment setup was not properly sealed. However, the top of the crystallizer was sealed by using tape as a preventive measure to minimize the evaporation of solute and water.

Thus, the evaporation effect was negligible in the calculation of solubility data. Messer et al.[19] in their work also suggested that the evaporation effect at a higher temperature could lead to water loss which causes inconsistency between the actual amount of water calculated by mass balance, hence results in an inconsistency of solubility reading.

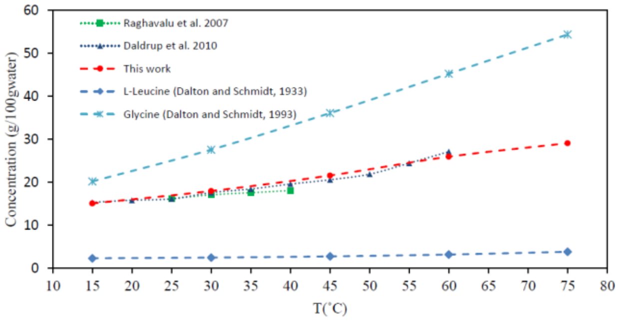

Figure 1: Solubility Diagram of amino acids in Water, showing Glycine has the highest solubility, followed by L-alanine and L-leucine.

Ternary system: L-alanine + Additive + Water

The presence of one amino acid in the solution has been reported to affect the solubility of another amino acid[20,21]. This effect was also observed in the present work as demonstrated in Figure 2 and Figure 3. Overall, the solubility of L-alanine is significantly affected by the presence of L-leucine.

From Figure 2, the solubility of the ternary system increases with L-leucine content in the solution. The solubility of the ternary system in the presence of 3wt% L-leucine shows a slightly lower solubility than the solubility of the binary system. However, the presence of 5wt% and 10wt% L-leucine results in a significantly higher solubility than the solubility of the binary system.

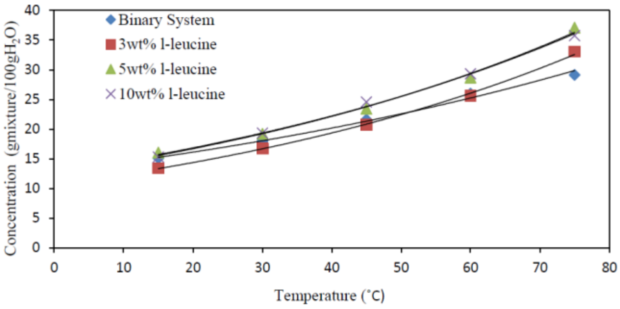

Figure 2: Comparison of L-alanine Solubility Plot between the Binary System and Ternary System for L-leucine Additive.

The presence of glycine causes an erratic pattern in the solubility of the solution. Figure 3 demonstrates the solubility of binary system and L-alanine with glycine added in different percentages. Overall, the solubility of the ternary system in the presence of 3wt% glycine lowers the solubility of the system as compared to the solubility of the binary system. Nevertheless, the solubility of the ternary system with 5wt% and 10wt% glycine differs slightly than the solubility of the binary system. Hence, it can be concluded that the addition of glycine as additive does not produce specific patterns to the solubility of the ternary system.

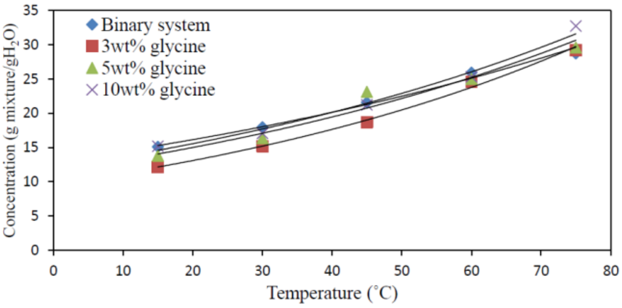

Figure 3: Comparison of L-alanine Solubility Plot between the Binary System and Ternary System for Glycine Additive.

Additive molecules may either increase or decrease the solubility of a solute in a given solvent. The effect that they have will depend on two factors: a. The interaction of the additive molecule with the solute molecule; b. The effect substituent additive molecules have on the structure of water[16]. In neutral solutions, amino acids (L-alanine, glycine and L-leucine) present as zwitterionic species contain proton donor group (–COO-) and proton acceptor group (– NH_3+). Both charged groups play an important role in the formation of a non-covalent bond (i.e: electrostatic bond, hydrogen bond) between amino acids' molecules. In the mixture of two amino acids, for example ternary system in this work (Ala-Leu and Ala-Gly) the formation of complex amino acids may occur. The side chain of additive molecules play an important rule in affecting the solubility of L-alanine solution. L-leucine additives have the longest hydrophobic side chain of CH_2–$CHCH_3CH_3$ as compared to glycine and L-alanine which indicate that L-leucine molecules is the strongest hydrophobic character as compared with glycine and L-alanine. Nevertheless, Glycine additive have an –H side chain which is a lesser hydrophobic character as compared with L-leucine and L-alanine. The presence of the hydrophobic side chain of L-leucine in the Ala-Leu mixture contributes to the formation of water clathrate. The water molecules tend to be isolate along with the hydrophobic groups and the motion of water molecules is slowed down the vicinity of hydrophobic side chain of L-leucine[16]. This phenomenon interrupts the interactions of L-alanine-water. The modification of this interaction impacts the actual solubility of the L-alanine in the aqueous solution containing L-leucine additive molecules. Therefore, it is suggested that at a particular temperature the components of Ala-Leu mixture are more stable in liquid form rather than transforming into solid phase. Thus, the results in the solution mixture contain more component concentration (L-leucine and L-alanine) than the concentration of the pure component (L-alanine) in the binary system.

Van't Hoff Solubility Plot and Thermodynamic Properties Data

The van't Hoff solubility plot of the binary system and ideal system of L-alanine solutions were calculated using equation 1 and 2 respectively, as shown in Figure 4. The Ideal system is defined as the prefect mixing between solute and solvent. The values of enthalpy of fusion, ΔH_f, and the fusion temperature (also termed as melting temperature), $T_f$used in equation 2 for an ideal system measured by differential scanning calorimetry (DSC) were found to be 105.11 kJ/mol and 283.61 °C, respectively.

In Figure 4, the van't Hoff solubility plot for the binary system was found to be significantly higher than the ideal solubility plot. The negative deviation of this behaviour indicates that there are interactions between hydrophilic side chains ($-COO^-$ and $-NH_3^+$) of the L-alanine and water molecules which are defined as hydrogen bond interactions[12,13]. In addition, there are interactions between water molecules and the hydrophobic ($-CH_3$) side chain of L-alanine and this interaction is known as hydrophobic interactions[15,16]. Both interactions play a major factor affecting the solubility of non-polar molecules such as L-alanine in water solution and these factors are discussed further in the next paragraph.

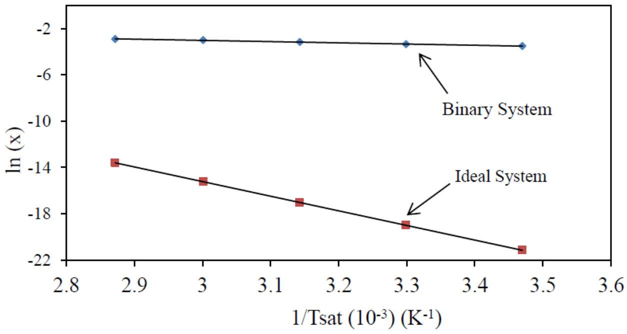

Figure 4: Van't Hoff Solubility plot of L-alanine Solution in Ideal and Binary System.

The negative gradient for both binary and ideal system graphs indicate a positive value of enthalpy for the binary and ideal systems[15] as shown in Table 1. From Table 1, the value of enthalpy for the ideal system (105,113.7 J/mol) is significantly higher than that of the binary system (8,717.6 J/mol) and consequently, the slope of the van't Hoff plot of ideal system is steeper than that of binary system.

The positive values of overall enthalpy change calculated from the binary and ideal systems indicate that the dissolution process is an endothermic process. The endothermic process involved during the dissolution process in the binary system can be divided into two stages; a) the energies required to break the solute-solute intermolecular bond (in crystal) to form an isolated solute (L-alanine), b) the energies required for breaking the solvent (water) hydrogen bond to create a cavity which is large enough for an isolated solute being accommodated in the cavity of water. The formation of hydrogen bond between L-alanine molecules and water molecules results in an exothermic reaction[12,16].

Table 1: Calculated Thermodynamic Properties from Solubility Data.

System	ΔH_d (J/mol)	ΔS_d (J/mol.K)
Binary	8717.54	1.10
Ideal	105113.73	175.87

Nevertheless, the overall endothermic reaction shown by the result in Table 1 might indicate that the endothermic reaction during the dissolution process outweigh the exothermic process from the hydrogen bond formation[12,16]. The same phenomenon were also reported by previous authors[22,23] dealing with the thermodynamic behaviour of dissolution of non-polar amino acids in water solvent.

The calculated entropy, ΔS for the binary system was found to be less than the ideal system. The decreasing value of ΔS from the ideal system to the binary system is presumably due to the nature of the hydrophobic effect of L-alanine molecules in the solution[15,23]. L-alanine molecule consists of a hydrocarbon side chain ($-CH_3$) which is hydrophobic in nature and induces clathrate formation in the L-alanine solution. Clathrate formation is the result of a water ordering effect, in which the water molecules have a propensity to form a cage-like structure covering of the solute molecules (L-alanine) resulting in a well-ordered arrangement of hydrophobic side chain ($–CH_3$) of L-alanine surrounded by water clathrate and consequently decreases in entropy of the binary system[12,24,25]. Therefore, the unfavourable change in entropy and enthalpy indicates the solution of L-alanine and water is not a favourable process and thus deviated from ideality.

Figure 5 represents the plot of calculated ΔG for the binary system and the ideal system by using equation 4 and 6, respectively. From Figure 5, it can be seen that ΔG_{ideal} value is positive within the range of 50,716.5 J/mol $<\Delta G_{ideal}<$ 39,365.5 J/mol. In the meantime, the values of ΔG for the binary system were recorded in the range of 8,400.9 J/mol < ΔG < 8,334.9 J/mol.

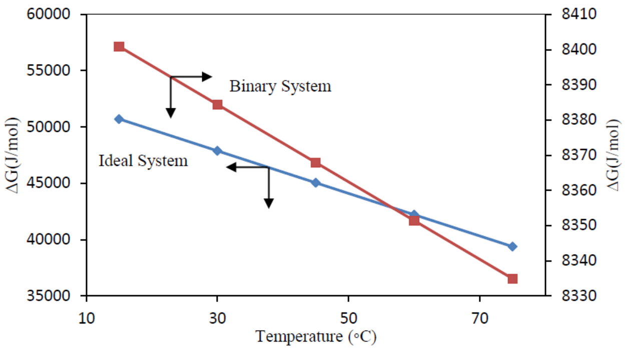

Figure 5: Gibbs Free Energy Change, ΔG; A) Ideal System, B) Binary System.

The trend shows the decrease in ΔG values of both systems with the increase of solubility temperature. In the case of the ideal system, the increase of entropy term (TΔS) in equation 4 with the increasing of temperature caused the value of ΔG to also increase. Meanwhile, in the case of the binary system, the decreased of ΔG indicates that the water clathrate formation in the L-alanine solution decreasing as the temperature increased and changed the thermodynamics properties. As the water clathrate decreased, the entropy of the solution gained and thus the Gibbs free energy change ΔG decreased[16,26].

The difference between the free Gibbs energy change of the binary and ideal system is termed as, ΔG_{excess}. The calculated values are plotted in Figure. 6 and it can be seen that the plot of excess free Gibbs energy decreased proportionally with the increase in solubility temperature. Nevertheless, the values of ΔG_{excess} found in this work were very big, and thus reflecting a large deviation of this system from ideality.

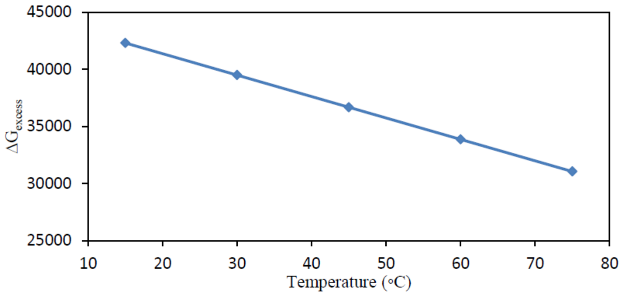

Figure 6: Excess Gibbs free energy, ΔG_{excess} at different Solubility Temperature.

The thermodynamic property of the binary system obtained from this research was compared to the previous authors and presented in Table 2. It can be seen that the calculated ΔGin the present work is in good agreement with the experimental value reported by Fasman[27] as well as the predicted value obtained from previous authors[22,28,29] with a maximum deviation of 7.88%. The possible reason for this significant deviation is that the value of ΔH_d and ΔS_d were evaluated at different temperature range where the values obtained in this work were evaluated at a temperature range of 288.15K – 348.15K. Whilst, the values obtained by Fasman[27] was at a temperature of 298.15K and the predicted values of previous authors were evaluated at a large temperature range of 273.15K – 373.15K.

Table 2: Calculated and Experimental Values ΔG,(T= 298.15K).

Author (s)	ΔG (j/mol)	%error
Present work	8939.34	-
Fasman, (1976)	8532.45	4.56
Park et al. (2003)	8316.44	6.97
Lee and Kim, (2010)	8234.64	7.88
Pazuki et al. (2009)	8445.34	5.53

Figure 7 depicts the activity coefficient plot, γ_a of L-alanine at different points of solubility temperatures. The values of the activity coefficient obtained are in the range of (1.32 x $10^{-7}<\gamma_a<$ 755.64 x 10^{-7}) for temp range from 10 – 70°C. The plot reveals that the values of γ_a increased with the increase of solubility temperature. However, the overall values of γ_a is far from unity ($\gamma_a = 1$), which indicates that the binary system experienced negative deviation from ideality as the calculated values of γ_a were less than 1[13].

This behaviour is supported by the conclusion made on the value of ΔG_{excess} indicating that there is an intermolecular interaction between strong hydrophilic head of L-alanine molecules (consisting of $-NH_3^+$ and $-COO$) and the surrounding water molecules. In addition, the hydrophobic interaction between water molecules and hydrophobic side chain group ($-CH_3$) of L-alanine which led to the water clathrate formation also contributed to the negative deviation of the binary solution from ideality.

It is anticipated that the activity coefficient, γ_a is influenced by the value of enthalpy of fusion, ΔH_f and melting temperature, T_f. The high value of ΔH_f resulted in a very small value of activity coefficient, which signifies negative deviation from ideal system. Thus, it is believed that the value of ΔH_f (105,113.7 J/mol) measured in the present study by DSC may be somewhat too high. In other words, the ΔH_f value could be overestimated and resulted in large deviations between the ideal and binary solubility[13].

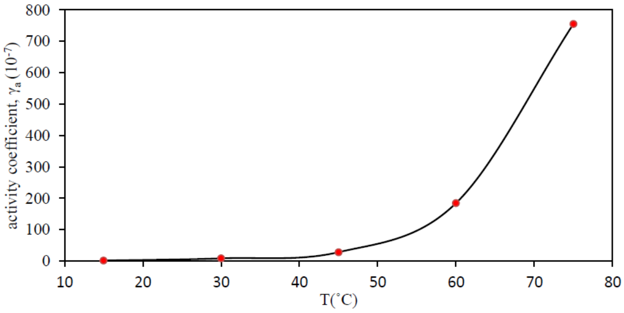

Figure 7: Activity Coefficient, γ_a Calculated for L-alanine in Water Solvent.

Conclusion

Based on the findings, the solubility of L-alanine + water (binary system) increased with the increase of solution temperature. Meanwhile, the presence of L-leucine additive caused a significant deviation of L-alanine solubility which is suspected to be due to the formation of complex amino acids that modified the hydrophobicity of L-alanine molecules in solution. The van't Hoff plot and activity coefficient result suggested that L-alanine deviates from ideal solution by negative deviation representing high solute-solvent interaction, which is expected due to the hydrophobicity and clathrate phenomenon of the solutes. Important thermodynamic properties of L-alanine in water solvent such as enthalpy, entropy, Gibbs free energy and activity coefficient were also successfully calculated.

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