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Solubility Study of Electrocrystallized L-isoleucine in Aqueous Phase

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Abstract: An examination of electrocrystallized L-Isoleucine solubility and the resulting crystals produced following the solubility pattern is presented in this study. The data in this study is compared with previous study without the influence of electric potential and it is revealed that under the influence of electric potential, the solubility of L-Isoleucine decreases as the electric potential increases. The solubility also is proven to be negatively deviated from ideal system. The plot of van't Hoff solubility data suggested decreasing of transition temperature of L-isoleucine polymorph with increasing electric potential applied to the solution. The polymorphs produced are found to be enantiotropically related even when electric potentials were applied to the solution. It is also suggested the L-isoleucine molecules arrangement in solution has become more ordered as the electric potentials were increased.

Key words: Solubility, L-isoluecine, electrocrystallization, van't Hoff solubility

INTRODUCTION

Solubility is one of the important parameter in crystallization where it is often used in occurrence related to biological, pharmaceutical, environmental, physical and analytical chemistry and also engineering sciences. Solubility is the amount of a compound dissolved in a solution at a certain temperature and pressure (Bouillot *et al.*, 2011). It is a key property for a process design of crystallizing products as it determines the amount of cooling required to get a certain amount of product. Solubility information in crystallization process is very important as it provides understanding on crystallization behaviour and hence allowing a better control in the process. It is also a well known fact that solubility is basic to the design and optimisation of crystallization process as well as a prerequisite to many crystallization characteristics. Study of material solubility is important as it reflects molecular ordering in the precursor solution.

Figure 1 shows a schematic solubility diagram depicting the undersaturated region, metastable zone width (MSZW) and supersaturated region. In the undersaturated region, crystallization could not occur but crystal dissolution takes place. In the saturated region, crystallization does not occur spontaneously as the nucleation is kinetically inactive. However, other external factors can be introduced to encourage the crystallization
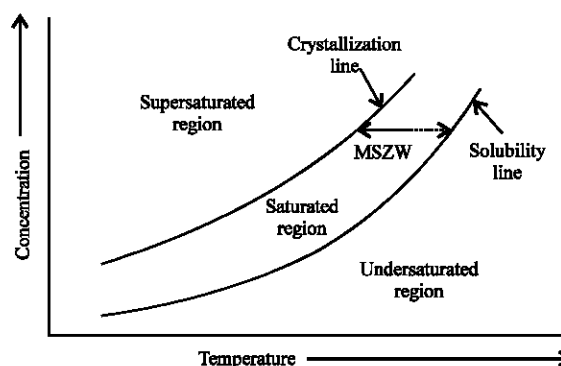

Fig. 1: A schematic solubility diagram

in this region such as electrical charge or seeding. Meanwhile in supersaturated region, nucleation could occur spontaneously (Hammadi and Veesler, 2009).

Electrocrystallization is the process of crystallization under the effect of electric field (Walsh and Herron, 1991). In this process, an electronic conductor is needed for the charge transfer to take place in the ionic solutions. In order for the crystallization to occur, the crystallizing compound moves toward the interface by means of convective diffusion followed by surface diffusion of atoms where it will nucleate and grow to form larger crystal.

Many studies have been conducted by the previous researchers to determine the factors affecting the

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solubility of high value added compounds such as amino acids. Among them are; the effect of pH of the solution by Pradhan and Vera (1998), temperature by Ferreira *et al.* (2009), pressure by Furuta *et al.* (1995), presence of other amino acids by Grosse Daldrup *et al.* (2010) and the presence of cation and anion by Soto *et al.* (1998).

Transformations from solution to solute molecules are dominated by intermolecular coordination with solvent molecules to the solid-solid state. The strong solute-solute interactions lead to molecular self-assembly and a three-dimensional crystallographic structure. Dissolved solute in solution at equilibrium also provides the interactions between the solute and solvent in a process, in which the thermodynamics properties can be calculated. To determine the thermodynamics properties of dissolved solute in solution, van't Hoff equation is applied and plotted. The van't Hoff plot of an ideal system was derived from Eq. 1 as described by Mullin (2001):

$$\ln x_{ideal} = \frac{\Delta H_f}{R}\left[\frac{1}{T_f} - \frac{1}{T}\right] \qquad (1)$$

Whereas, in real system, the van't Hoff was described as Eq. 2:

$$\ln x = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (2)$$

where, x is the mole fraction of solute in solution, T_f is the melting temperature, T is the temperature of the solution, ΔH_f is the molal enthalpy, ΔH and ΔS are the enthalpy and entropy of dissolution, respectively and R is the gas constant (8.314 J mol^{-1} K). Ideal Gibbs free energy, ΔG_{ideal} was calculated from Eq. 3. The known values of ΔH and ΔS from van't Hoff plot were used to calculate Gibbs free energy, ΔG using Eq. 4 and excess Gibbs free energy, ΔG_{excess} using Eq. 5:

$$\Delta g_{ideal} = RT\ln(x)_{ideal} \qquad (3)$$

$$\Delta G_d = \Delta H_d - T\Delta S_d \qquad (4)$$

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

In this study, L-isoleucine, ($C_6H_{13}O_2N$, MW = 131.2, purity of 99.6%) was purchased from MERCK. For solubility study, distilled water was used to make up the solution. The carbon plate with purity more than 99% was cut into small plate size with dimensions of 2 cm width and 10 cm length.

MATERIALS AND METHODS

Solubility of L-isoleucine: The solubility experiment of L-Isoleucine was carried out in 250 mL jacketed reactor and operated at 350 rpm through constant mixing provided by retreat curve impeller. The data was determined over the temperature ranging from 15-75°C. A small amount of L-isoleucine powder was weighed and added to the system containing distilled water at a certain temperature. Carbon electrode was dipped into the solution and power supply was turned on. The immersed surface area of electrodes in the solution was 7.27 cm^2. The solution was stirred and the L-isoleucine powder was added (in a small amount at a time) into the solution. The powder was weight prior to addition to the solution. The solution containing L-isoleucine powder was allowed to equilibrate for 4 h to ensure that all solid powder dissolves. L-isoleucine was continued added to the solution until no further L-isoleucine could dissolve before the temperature of the solution was increased. The steps were repeated for the above-mentioned temperature range. Figure 2 shows the schematic diagram of solubility experimental set-up.
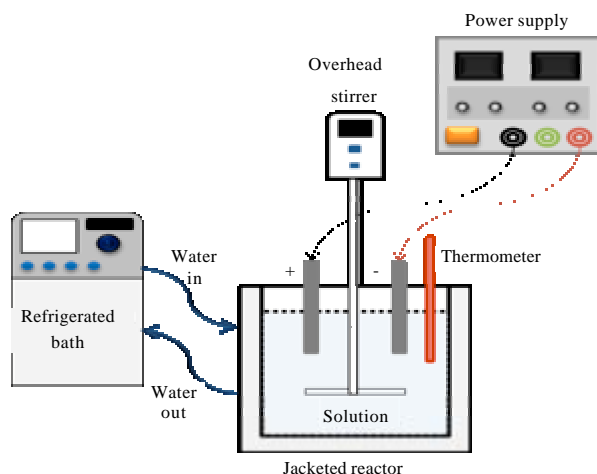

Fig. 2: Schematic diagram of experimental set-up

Characterization of L-isoleucine crystal: L-isoleucine crystals were characterized to investigate the type of polymorph present in the solution at temperature of 25 and 55°C with the presence of 3 and 20 v of electric potential. The experimental procedures carried out to obtain the crystals were as follows: L-isoleucine powder was added into a jacketed reactor containing 200 mL distilled water at temperature 80°C. The solution was stirred at 350 rpm with the presence of electric potential. After all the powder was dissolved, the temperature was decreased and allowed to equilibrate for 4 h before the crystal was collected and dried in the oven. The crystal collected was characterized by using X-Ray Powder Diffraction (XRPD) (Rigaku) with Cu Kα radiation at 40 mA, 40 kV, 2θ between 3 and 40 and rate of 0.01° sec⁻¹.

RESULTS

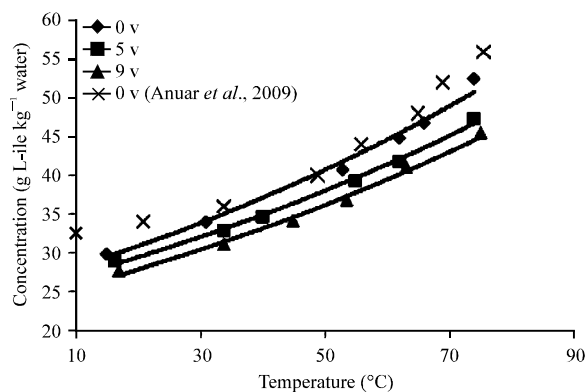Results of this study are shown in Fig. 3-7 and Table 1 and 2.

Table 1: Types of polymorph present in solution at different electric potential

Voltage (V)	25°C	55°C
*0	A	B
3	A+B	A
20	A+B	B

*Data obtained from Anuar *et al.* (2009)

Table 2: Calculated thermodynamics properties from solubility data

Voltage (V)	T_r (°C)	Polymorph form	ΔH (J mol⁻¹)	ΔS (J mol⁻¹ K)
*Ideal	-	-	89783	160
*0	45.0	A	4071	-31
		B	10916	-9
0	58.0	A	6329	-24
		B	12704	-5
5	49.5	A	5661	-27
		B	9403	-15
9	39.0	A	4924	-30
		B	8946	-17

*Data obtained from Anuar *et al.* (2009)

Fig. 3: Solubility data with and without the influence of direct current
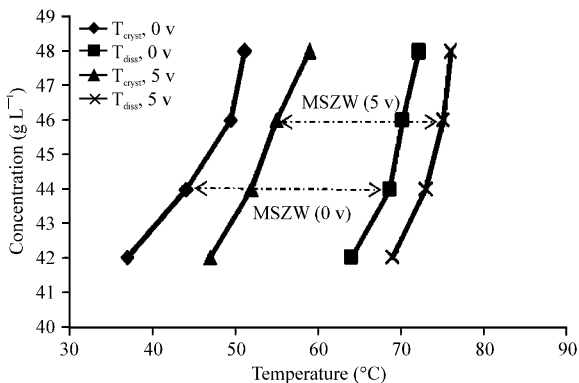
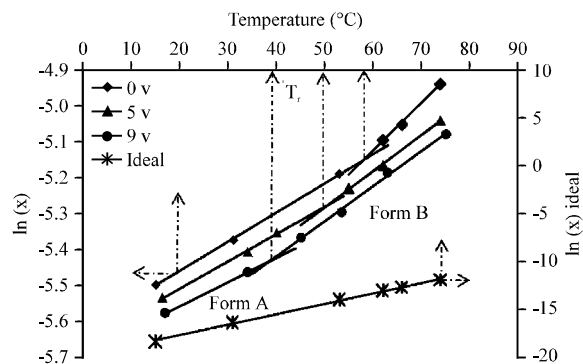Fig. 4: Metastable zone width of L-isoleucine crystallization

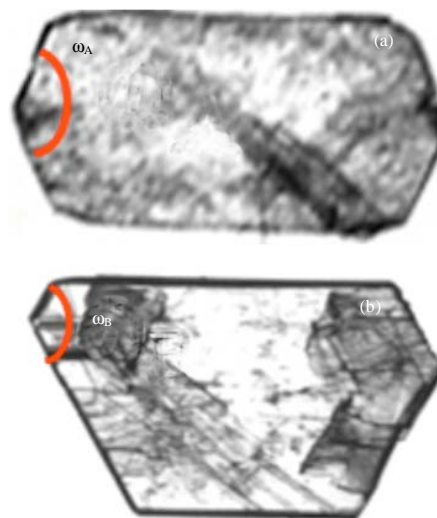Fig. 5: van't Hoff solubility data for ideal system and with and without direct current

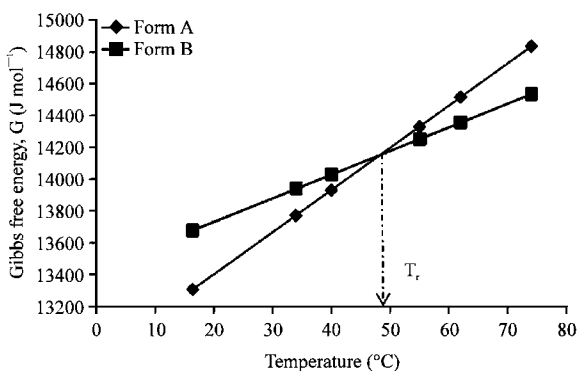Fig. 6(a-b): Polymorphic forms of L-isoleucine

Fig. 7: Gibbs free energy plot of form A and B for 5 v system

DISCUSSION

In these solubility experiments, L-isoleucine was dissolved in 200 mL of distilled water without and in the presence of 5 and 9 v of electric potentials. It was found that the solubility of L-isoleucine in water without the effect of electric potential (0V) is higher than solubility of L-isoleucine with presence of 5 and 9 v of electric potential (Fig. 3). From the result, it can be seen that the solubility of L-isoleucine decreases when the electric potential applied increases. The decreasing of solubility of L-isoleucine in the presence of electric potential could be because electric field has substantial effect on crystallite conformation. Electric potential induces the preferential conformation of L-isoleucine cluster crystal formation and causes the number of the crystallites growth increases (Nanev and Penkova, 2001). Somehow, the electric potential promotes the onset of crystallization and thus causes the L-Isoleucine crystal to nucleate faster, hence lowers the solubility of the solution. Lower solubility also means that the upper boundary for the metastable zone width (MSZW) is shifted to a higher temperature as shown in Fig. 4. Crystallization experiment carried out in solution with the presence of electric potential also shows that the crystallization temperature, T_{cryst} increases compared to T_{cryst} without the electric potential (denoted as 0V in Fig. 4). The crystallization temperature, T_{cryst} and dissolution temperature, T_{diss} at 0 v were obtained from Anuar et al. (2009).

The van't Hoff solubility data of L-isoleucine in the presence of 5 and 9 v of electric potential were found to have higher solubility than ideal system (Fig. 5). This is due to the strong interactions between L-isoleucine with water molecules but considerably lower than the 0 v and shows negative deviation from ideality. The deviation from ideality become lesser as the electric potential applied is increases which indicates that the electric

potential reduces the strong interaction between L-isoleucine with water molecules. It can be concluded that the van't Hoff solubility data approaches ideal system as the electric potential increases.

Figure 5 also shows the existence of two straight breaking line of the van't Hoff solubility plot for 0, 5 and 9 v data. These breaking lines are intersecting at temperatures which might be regarded as a transition temperature, T_r. The transition temperature obtained from this experiment for 0, 5 and 9 v is 58, 49.5 and 39°C, respectively. Anuar et al. (2009) suggested that this behavior is due to the existence of two polymorphic forms (form A and B) of L-isoleucine and it was suggested that the transition temperature, T_r for their system at 45°C.

Figure 6 shows the morphology of L-isoleucine which is generally with plate-like hexagonal shape. Nevertheless, the morphology of the two polymorphic forms of L-Isoleucine, form A and form B are almost identical, but with different apex angle, ω where the apex angle for form A, ω_A is in the range of 116-120° while the apex angle for form B, ω_B is in the range of 111-115° (Anuar et al., 2009).

By looking at the trend of transition temperature in Fig. 5, increasing the electric potential reduces the transition temperature, T_r. Thus, it is postulated that increasing the electric potential to 20 v could result in the decrease of T_r to less than 39°C and decreasing the electric potential to 3 v could increase the T_r to a temperature higher than 49.5°C. The result shown in Fig. 5 indicates that the crystals collected at temperature less than 39 and 49.5°C yield form A and B polymorph, respectively. Thus, the X-RD analysis for crystals recovered from solution cooled to temperature 55 and 25°C for electric potentials of 3 and 20 v were carried out and are shown in Table 1. The result shows that the polymorphs produced at temperatures below and above the T_r, with the effect of electric potential produces different polymorph than expected (see result for 0 v). Solution crystallized at 25°C and with electric potentials of 3 and 20 v produces mixtures of form A and B. Meanwhile, for solution crrystallized at temperature 55°C, electrical potential of 3 and 20 v produces form A and B polymorph, respectively.

Table 2 shows the calculated thermodynamic properties of solubility solution state. For comparison purposes with the solubility without the electric potential, it is assumed that the crystals recovered from solution with solubility below and above the T_r produces form A and B polymorph, respectively. The enthalpies of dissolution, ΔH shows an endothermic process for both polymorphs for 0, 5 and 9 v data while the entropies for both polymorphs for 0, 5 and 9 v data show negative

value. Major contribution to the large positive values of overall ΔH of L-isoleucine is because of the unusually strong interactions hydrogen bond between the solute molecules (Grant *et al.*, 1984), overweigh the exothermic reaction during the formation of hydrogen bond between water molecules with carboxyl and amino group of L-isoleucine. While for enthalpy of dissolution with positive values implies that less energy is needed to break the bond in order to create the cavity between the water molecules and solutes as the electric potential increases, as it decreases with increasing electric potential. From Privalov and Gill (1989), decrease in entropy is caused by the water ordering effect (clathrate) where the water molecules surround the solutes molecules to form an ordered arrangement. When the arrangements of the molecules are more ordered, the predictability of the molecules position is higher, hence decreasing the entropy since entropy is a measure of molecular disorder or randomness (Cengel and Boles, 2007). Quantity of clathrate forms depends on composition as well as temperature. The entropy for polymorph B is higher than polymorph A for 0, 5 and 9 v systems because the formation of clathrate is hard to occur as the temperature of the solution increases (Lizhuang *et al.*, 2002). Thus, polymorph B has more free molecules and more disorder arrangement of crystal than polymorph A. The entropy for polymorph A and B is decreasing as the voltage supply is increased; which could indicate that the increment of electric field causes the arrangement of the molecules becomes more ordered.

The plot of Gibbs free energy-temperature for 5 v data (Fig. 7) proves that the polymorphs are enantiotropically related, where the transition temperature (T_r) is 49~49.5°C. For an enantiotropic system, it is possible for a transition to occur reversibly between the polymorph exists by applying heating and cooling condition to the system (Purohit and Venugopalan, 2009; Katrincic *et al.*, 2009). The energy-temperature diagram for both 0 and 9 v also show the same trend as 5 v. According to Purohit and Venugopalan (2009), the polymorph with lower free energy contents and solubility is more stable compared to polymorph with higher free energy content and solubility. Figure 7 revealed that Form B polymorph is more stable compared to form A polymorph above the transition temperature which is 49.5°C as the free energy for form B is lower than form A. However, Form A polymorph is more stable compared to form B polymorph below the transition temperature. This condition is also applied for 0 and 9 v above and below their respective transition temperature which is 58 and 39°C. The transition temperature, T_r shows the discrepancies behaviour as the electric potential increases as shown in Table 2.

CONCLUSION

The effects of solubility of L-isoleucine in binary system without and with the influence of 5 and 9V electric potential have been studied. The solubility of L-isoleucine in water was found to decrease as the electric potential increases. The solubility data of L-isoleucine with the effect of electric potential was compared to the solubilty data of L-isoleucine without the effect of electric potential by Anuar *et al.* (2009) and it shows that the solubility of L-isoleucine with electric potential is less than solubility of L-isoleucine without the influence of electric potential. The thermodynamic properties of L-isoleucine under the influence of electric potential were also calculated. This L-isoleucine/water system show negative deviation from ideal system but the deviation decreases as the voltage increases which indicate solute-solvent interactions become weaker as the electric potential was applied. Crystals produced at temperature below T_r were mixtures of both form A and B while above the T_r produces form A for solution with applied electric potential 3 v and form B for 20 v. The enthalpies of dissolution, ΔH was found to decrease as the electric potential increases, indicating that less energy is needed to create cavity between water and solute molecules. Meanwhile the entropy decreases as the electric potential increases which might indicate that L-isoleucine arrangement has become more ordered. The plot of Gibbs free energy-temperature revealed that the polymorphs produced remain as enantiotropically related even when electric potentials were applied to the solution.

ACKNOWLEDGMENT

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