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Representation of Solid−Liquid Equilibrium of L-Ornithine−L-Aspartate + Water + Methanol System Using the Chen Model for Mixed-Solvent Electrolyte Solution

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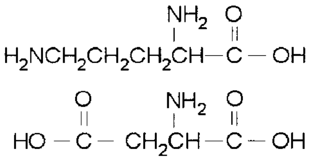
Solubility measurements were performed in the temperature range of 298−333 K, for a methanol content varying between 0 mass % and 90 mass %. The phase equilibrium condition of L-ornithine−L-aspartate (LOLA) + water + methanol and the mean ionic activity coefficients of LOLA in a ternary system were calculated by using the Chen model, allowing the description of the mixed-solvent electrolyte systems. The mean ionic activity coefficients were calculated with nine binary parameters: six parameters for the binaries LOLA + water (taken from conductance measurements) and water + methanol (taken from the literature) and three parameters for the binary LOLA + methanol (obtained by regression of solubility data). Using binary adjustable parameters and solubility data, the model satisfactorily correlates the ternary (LOLA + water + methanol) system over the entire range of temperature and concentrations.

Introduction

Recent advances in the biochemical industry have drawn much attention to the development of efficient processes for the separation, concentration, and purification of valuable bioproducts. To design a rational process, it is necessary to have a fundamental understanding of biochemical systems. The precipitation of a solid solute dissolved in a pure or mixed solvent is possible if supersaturation was previously created in the solution. Therefore, to define the conditions for precipitation, a knowledge of the solid−liquid equilibrium is required. For the successful representation of the solubilities of L-ornithine−L-aspartate (LOLA) in the mixed-solvent systems and the dependence on temperature and methanol content, it is necessary to be able to represent accurately the activity coefficients in the solution.

The chemical structure of the hydrocarbon backbone of the amino acid plays an important role in its interactions with water molecules and with different amino acids.[1] In aqueous solutions the carboxyl group of the amino acid loses a proton and becomes negatively charged, whereas the amino group gains a proton and becomes positively charged. This results in the formation of a strong electrostatic field around the amino acid molecules, which gives rise to important amino acid−amino acid interactions. LOLA can be prepared by reacting L-ornithine with L-aspartic acid in the presence of water and has been used for pharmaceutical use.[2] Figure 1 depicts the chemical structure of LOLA. Because a LOLA solution is an electrolytic solution, its measurable physicochemical properties can be treated using thermodynamic methods. Chen et al.[3] used their version of the electrolyte NRTL model[4] to model the solubilities of electrolyte in solution. Their model contains a long-range interaction term, represented by a Pizer−Debye−Hückel form,[5] and a short-range interaction term given by a modified form of the NRTL equation.[6]



$$H_2NCH_2CH_2CH_2\overset{NH_2}{\overset{|}{C}H}-\overset{O}{\overset{\|}{C}}-OH$$
$$HO-\overset{O}{\overset{\|}{C}}-CH_2\overset{NH_2}{\overset{|}{C}H}-\overset{O}{\overset{\|}{C}}-OH$$

Figure 1. Chemical structure of L-ornithine−L-aspartate.

The purpose of this study is to investigate the effects of temperature and methanol on the solubility of LOLA. The solubilities of LOLA in water and in aqueous solutions of methanol were measured over a wide temperature range. In addition, a thermodynamic description of the ternary solubilities of LOLA was also developed. From the models based on the local composition concept, the Chen model was used to describe the activity coefficients in the ternary systems LOLA + water + methanol, mainly for the following reasons: (i) it accounts for the interactions of all species in solution (ion−ion, molecule−ion, and molecule−molecule); (ii) the short-range contributions are accounted for by the NRTL expressions, which are adequate for electrolyte solutions; (iii) it is based on mole fractions, and no specific area or volume data are needed; (iv) only binary parameters are needed, and generally they can be easily obtained from the literature.

Experimental Section

Materials and Procedure. Solubilities of LOLA in water and in aqueous solutions of methanol were determined according to the isothermal method. The apparatus used for the solubility determinations was a 50 mL glass

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vessel fitted with a magnetic stirrer. Either heated or cooled medium from a thermostated bath (polyScience, model 9510, controlled to ±0.05 K) could be circulated through the jacket of the cell. Mixtures were prepared by mass using a balance (Ohaus, analytical). The balance had a range of measurement up to 100 g, with an uncertainty of ±0.0001 g. Deionized water (conductance of <4 μs/cm) was used. Methanol (Merck, ±99.8 mol %) was also used without any additional purification, and LOLA was supplied by Sigma Chemical Co. and dried in a vacuum oven at 50 °C before being used. Weighed quantities of LOLA, methanol, and water were charged to the cell and agitated for 1 h. At the end of this time, only a small amount of crystal was left undissolved. Small quantities of solvent mixture (starting with 1 mL and reducing) were then added to the mixture at hourly intervals until all traces of crystalline material had disappeared. The conductance was measured with a Metrohm 660 conductivimeter. Solubility measurements were carried out for a methanol content between 0 mass % and 90 mass %. All solubility data obtained in this study represented the average values of at least two measurements. The inaccuracy of solubility measurements was ±0.5%.

Prediction Model of the Solubility of Ternary Systems. The thermodynamic description of solid−liquid equilibrium is an important step in precipitation studies, which requires the knowledge of the activity coefficients of the electrolyte species in the temperature and the concentration ranges studied. Electrolyte thermodynamics enables the calculation of the activity coefficients, and thus the description of the nonideality of the system is possible. For both ternary and binary organic solvent free systems, the standard state adopted for each ion is the hypothetical ideal dilute solution at unit concentration in water at system temperature and pressure. The equation relating the molality (m) of a strong electrolyte in the binary and ternary systems can be derived as[7]

$$m_{\mathrm{T}} = (\gamma_{m,\pm,\mathrm{B}}/\gamma_{m,\pm,\mathrm{T}})m_{\mathrm{B}} \tag{1}$$

where subscript T is the ternary system, subscript B is the binary system, and $\gamma_{m,\pm}$ is the mean ionic activity coefficient based on molality. The ternary solubilities are calculated by eq 1 from the solubility in water and the activity coefficients in both systems, thus providing the description of ternary solubilities as a function of temperature and methanol concentration in the mixed solvent. As previously mentioned, the mean ionic activity coefficients in binary and ternary systems can be described by the Chen model.[4,8] In this model the mean ionic activity coefficient is expressed as the sum of two terms: one is related to the ion−ion long-range electrostatic interactions, and the other is due to a short-range contribution, which accounts for the interactions between all species:

$$\ln \gamma_{\pm} = \ln \gamma_{\pm}^{\mathrm{lr}} + \ln \gamma_{\pm}^{\mathrm{st}} \tag{2}$$

The extended Debye−Hückel equation proposed by Pitzer[5] is used to represent the long-range ion−ion interactions, being the mean ionic activity coefficient contribution defined as

$$\ln \gamma_{\pm}^{\mathrm{pdh}} = -\left(\frac{1000}{M_{\mathrm{s}}}\right)^{1}{/}^{2}A_{\phi}\left[\frac{2{Z_i}^2}{\rho}\ln(1+\rho {I_x}^{1/2}) + \frac{{Z_i}^2{I_x}^{1/2} - 2{I_x}^{3/2}}{1+\rho {I_x}^{1/2}}\right] \tag{3}$$

where ρ is the closest approach parameter, set equal to 14.9 as suggested by Pitzer. M_{s} is the solvent molecular weight, Z_i is the charge of ion i, and I_x is the ionic strength on a mole fraction basis

$$I_x = (1/2)\sum {Z_i}^2 x_i \tag{4}$$

in which x_i is the molal fraction. A_ϕ is the Debye−Hückel constant, defined as

$$A_{\phi} = \frac{1}{3}\left(\frac{2\pi N_{\mathrm{A}} d_0}{1000}\right)^{1/2}\left(\frac{e^2}{\epsilon k_{\mathrm{B}} T}\right)^{3/2} \tag{5}$$

where N_{A} is Avogadro's number, d_0 is the solvent density, e is the electron charge, k_{B} is Bolzmann's constant, ϵ is the solvent dielectric constant, and T is the absolute temperature.

The short-range interaction contribution was accounted for by the use of a local composition concept. To develop the local composition concept, the NRTL model was adopted by Chen et al.;[8] the corresponding expressions for the mean ionic activity coefficient of LOLA in binary and ternary systems is given by

$$(\ln \gamma_{\pm}^{\mathrm{lc}})_{\mathrm{B}} = \frac{x_w^2 G_{ca,w}\tau_{ca,w}}{(2x_{ca}G_{ca,w} + x_w)^2} + \frac{x_w G_{w,ca}\tau_{w,ca}}{x_{ca} + x_w G_{w,ca}}\left(1 - \frac{x_{ca}}{x_{ca} + x_w G_{w,ca}}\right) - G_{ca,w}\tau_{ca,w} - \tau_{w,ca} \tag{6}$$

$$(\ln \gamma_{\pm}^{\mathrm{lc}})_{\mathrm{T}} = \frac{\sum_m x_m G_{ca,m}}{\sum_j x_j G_{j,m}}\left(\tau_{ca,m} - \frac{\sum_j x_j G_{j,m}\tau_{j,m}}{\sum_j x_j G_{j,m}}\right) + \frac{\sum_j x_j G_{j,ca}\tau_{j,ca}}{\sum_j x_j G_{j,ca}}\left(1 - \frac{x_{ca}}{\sum_j x_j G_{j,ca}}\right) - G_{ca,w}\tau_{ca,w} - \tau_{w,ca} \tag{7}$$

where

$$G_{i,j} = \exp(-a_{i,j}t_{i,j}) \qquad \text{and} \qquad t_{i,j} = \Delta g_{i,j}/RT$$

The solubility description of the ternary system LOLA + water + methanol by eq 1 with the mean ionic activity coefficients calculated as indicated before involves the knowledge of nine binary parameters: τ_{ij}, τ_{ji}, and α_{ij} for the binary systems LOLA + water, water + methanol, and LOLA + methanol. The activity coefficients calculated by these equations are mole fraction based, and they are related to the molal activity coefficients by the relationship

$$\ln \gamma_{m,\pm} = \ln \gamma_{\pm} - \ln[1 + 0.001M_{\mathrm{s}}(\upsilon_{\mathrm{c}} + \upsilon_{\mathrm{a}})m] \tag{8}$$

where υ_{c} and υ_{a} are the electrolyte stoichiometric coefficients of the cation and anion, respectively.

Results and Discussion

Parameters for Calculation. An advantage of the models based on the local composition concept is that they require only the parameters of its constituent binaries. The solubility of the ternary system LOLA + water + methanol is described by eq 1, where the binary and ternary mean ionic activity coefficients were calculated according to the Chen model, with nine binary parameters: six binary

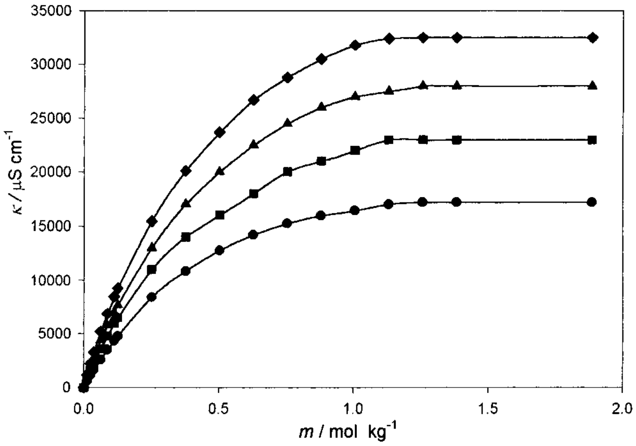

Figure 2. Conductivity as a function of molality: ●, 298.15 K; ■, 313.15 K; ▲, 323.15 K; ◆, 333.15 K.

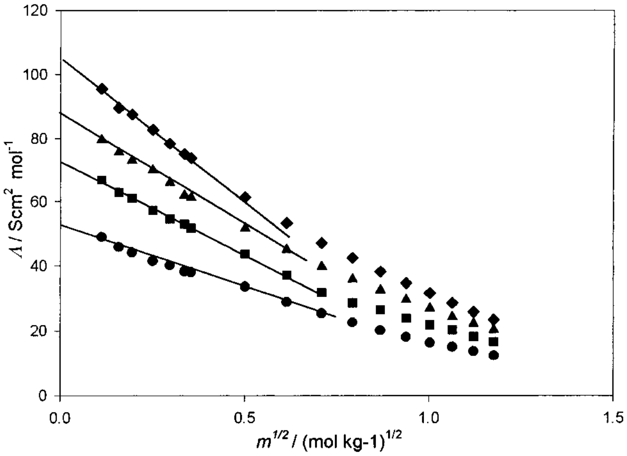

Figure 3. Molal conductivity of LOLA: ●, 298.15 K; ■, 313.15 K; ▲, 323.15 K; ◆, 333.15 K.

interaction energy parameters τ_{ij} for the pairs LOLA + water, water + LOLA, LOLA + methanol, methanol + LOLA, water + methanol, and methanol + water and three nonrandomness factors for the binaries LOLA + water, LOLA + methanol, and water methanol ($\alpha_{ij} = \alpha_{ji}$). For pure molecular systems, the Chen model is basically the NRTL model and the binary water + methanol parameters are the NRTL parameters, which could be determined from experimental vapor−liquid equilibrium data[9] as a function of temperature.

For a LOLA + water system, the binary parameters were obtained from data correlation using the Chen model and experimental data of the molal ionic activity coefficient. The conductivity of strong electrolytes at low concentration is found to be accurately a linear function of the square root of concentration, decreasing as the concentration increases. The conductivity data obtained (Figure 2) were analyzed using the Kohlrausch equation.[10]

$$\Lambda = \Lambda_0 - bm^{1/2} \tag{9}$$

In this equation, Λ_0 is the limiting molal conductivity. From the experimental molal conductivity, Λ is plotted as a function of the square root of the molality in Figure 3. Extrapolation to zero concentration yields the limiting molal conductivity Λ_0. The activity coefficient is given by[11]

$$-\log_{10} \gamma_{\pm}^2 = 2\beta m^{1/2}\theta^{1/2}/(1 + \theta m^{1/2}) \tag{10}$$

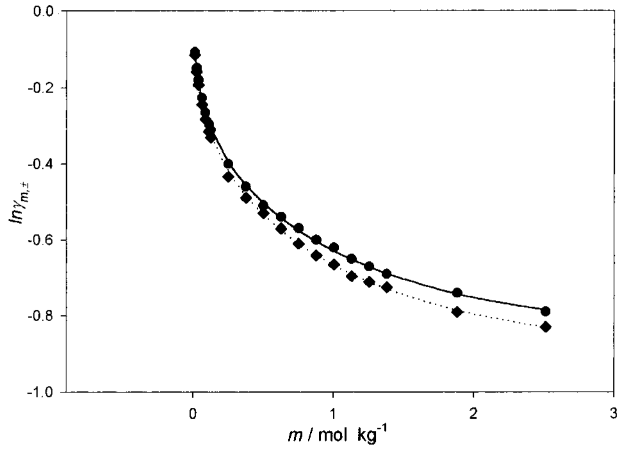

Figure 4. Comparison of experimental (●, 298.15 K; ◆, 333.15 K) and calculated molality mean ionic activity coefficients of aqueous LOLA (—, 298.15 K; ···, 333.15 K).

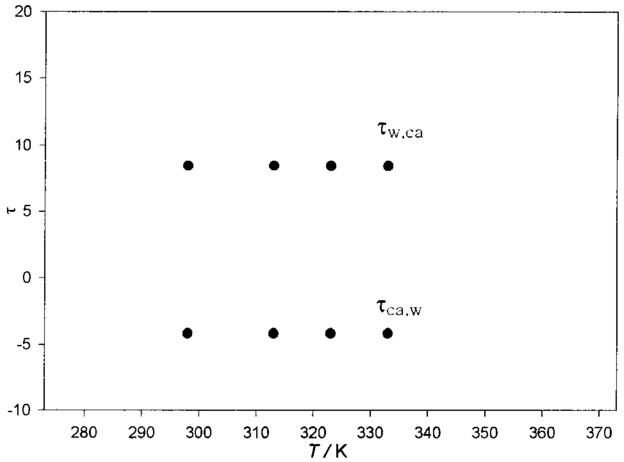

Figure 5. Binary parameters at various temperatures.

Table 1. Limiting Molal Conductivity

T/K	298.15	313.15	323.15	333.15
Λ_0/Scm^2·mol^{-1}	52	74	90	105

Table 2. NRTL Binary Interaction Energy Parameters (τ_{ij}) and the Nonrandomness Factor (α)

	τ_{12}	τ_{21}	α
water + methanol[a]	845.2062/RT	−253.8802/RT	0.2994
LOLA + water	−4.15	8.45	0.2
LOLA + methanol[b]	11	−3.2	0.145

[a] Reference 9. [b] Optimized parameters from measurements.

In this equation, θ is the dissociation degree of an electrolyte (= Λ/Λ_0) and β is the constant for the conductivity equation. Table 1 shows the values of the limiting molal conductivity of LOLA. Even though the values of the limiting molal conductivity decrease with decreasing temperature, the dissociation degree (θ) of LOLA is not changed by temperature variance. Therefore, the activity coefficients are weakly dependent on temperature (Figure 4). For a LOLA + water system, the binary parameters were determined at temperatures between 298.15 K and 333.15 K. In Figure 5, the binary parameters were observed to have a weak temperature dependence. The weak temperature dependence of the binary parameters is an important and desirable property of the local composition model.[8] Table 2 shows the four binary interaction energy parameters and two nonrandomness factors. Because the data for the LOLA + methanol system were not available

Table 3. Values of the Dielectric Constant (ϵ), Density (d_0), and the Parameter of Pitzer's Equation from Methanol + Water (A_ϕ)

methanol mass %	d_0/g·cm^{-3}	ϵ	A_ϕ	d_0/g·cm^{-3}	ϵ	A_ϕ
	T = 298.15 K			T = 313.15 K		
0	0.997	78.5	0.39058	0.997	73.1	0.40363
10	0.983	74.1	0.42292	0.979	68.9	0.43727
30	0.951	64.3	0.51467	0.942	59.5	0.53408
50	0.916	55.0	0.63822	0.901	50.4	0.67013
70	0.872	45.0	0.84135	0.856	41.0	0.88943
90	0.820	35.7	1.15524	0.803	32.6	1.21904
	T = 323.15 K			T = 333.15 K		
0	0.995	69.9	0.41197	0.983	66.6	0.42002
10	0.978	65.7	0.44815	0.977	62.8	0.45780
30	0.938	56.6	0.54853	0.935	53.9	0.56220
50	0.897	47.8	0.69054	0.892	45.3	0.71316
70	0.848	41.0	0.84449	0.841	36.7	0.95028
90	0.791	32.6	1.15344	0.783	29.9	1.24359

Table 4. Binary Solubility of LOLA + Water: Experimental Results and Mean Ionic Activity Coefficients Calculated by Using the Chen Model

T/K	x/mol·kg of H$_2$O^{-1}	$\gamma_{m,\pm}$
298.15	3.580	0.4398
303.15	3.864	0.4350
308.15	3.958	0.4315
313.15	4.190	0.4273
318.15	4.383	0.4234
323.15	4.546	0.4196
328.15	4.701	0.4159
333.15	4.862	0.4121

in the literature, the LOLA + methanol binary parameters should be obtained through the regression of the experimental ternary solubility values for LOLA + water + methanol. Table 2 shows the optimized binary LOLA + methanol energy parameters and nonrandomness factor.

The calculation of the Debye–Hückel constant by eq 4 demands dielectric constant knowledge. The value of this property would be a function of the solute content in the solution. However, in this work, the experimental determinations have a concentration lower than 5 molal and, thus, a considerable amount of solvent molecules remains free of the ionic species influence,[12] which leads us to disregard the influence of the electrolyte in the environment dielectric constant. Table 3 shows the dielectric constant of the water + methanol mixture over the entire range of concentration and for temperatures between 298.15 K and 333.15 K. After the values of the fundamental physical constants are introduced to eq 4, it becomes

$$A_\phi = 1.4006 \times 10^6 d_0^{\ 1/2}/(\epsilon T)^{3/2} \qquad (11)$$

The A$_\phi$ values in the different solvent compositions are listed in Table 3.

Solubility. Experimental results of LOLA solubility in water and the mean ionic activity coefficients estimated by using the Chen model are given in Table 4 , and the ternary solubility data in aqueous methanol mixtures are presented in Table 5, where the effect of the methanol content can be inferred. The results using eq 1 are also listed in Table 5.

The average relative deviation was 0.9%, which was obtained by comparing the calculated solubilities with experimental values. From Table 5 the calculated solubilities show good agreement with the experimental values.

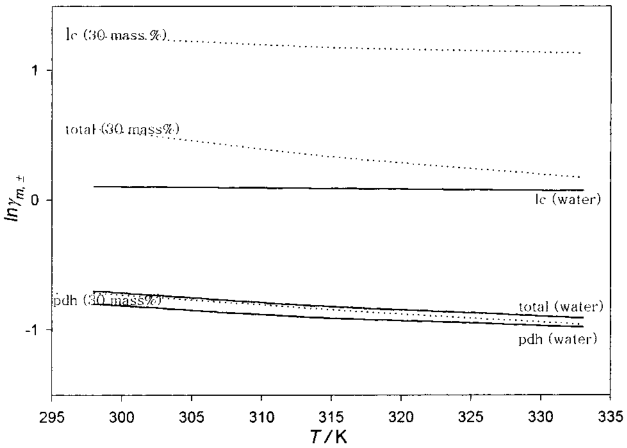

Figure 6. Long-range (pdh) and short-range (lc) contributions of the mean ionic activity coefficient calculated by using the Chen model, in the binary system LOLA + water (solid lines) and in the ternary system LOLA + water + methanol at 30 mass % (dotted lines).

Table 5. Ternary Solubility of LOLA in Aqueous Solutions of Methanol

methanol mass %	x	x_c	x	x_c
	T = 298.15 K		T = 313.15 K	
0	3.5625	3.5549	4.1683	4.1361
10	2.7188	2.7242	3.3075	3.2960
30	1.0375	1.0310	1.4900	1.4833
50	0.2063	0.1984	0.3013	0.3127
70	0.0269	0.0259	0.0425	0.0426
90	0.0022	0.0027	0.0025	0.0031
	T = 323.15 K		T = 333.15 K	
0	4.5027	4.4920	4.8475	4.8543
10	3.6738	3.6608	4.0313	4.0205
30	1.8050	1.8084	2.1500	2.1471
50	0.4050	0.4170	0.5425	0.5388
70	0.0563	0.0534	0.0838	0.0703
90	0.0032	0.0034	0.0035	0.0037

The mean ionic activity coefficients obtained for the ternary system LOLA + water + methanol increase with the methanol content. At constant methanol concentration the increase in temperature results in a decrease in the activity coefficient values, this variation being strongly marked for high concentrations. The long-range and short-range interaction contributions for the mean ionic activity coefficient are presented in Figure 6. Two limiting situations were analyzed: the binary system LOLA + water and the ternary system LOLA + water + methanol at 30 mass %. The long-range contribution is weakly dependent on the methanol content. Therefore, the influence of the methanol content on the activity coefficient is mainly due to the short-range contribution. The long-range contribution is due to the ion–ion interactions, which are slightly dependent on the molecular solution concentration, whereas the ion–molecule and the molecule–molecule interactions are accounted for in the short-range contribution, increasing with the methanol content. The long-range contribution is also weakly dependent on temperature, but the short-range contribution decreases with temperature, being more pronounced at high methanol content. These considerations enable us to conclude that the long-range contribution may be set as a constant factor, the variation of the activity coefficient being mainly due to the short-range contribution. As a result, it can be concluded that the solubility of LOLA is more sensitive to methanol content than to temperature.

Conclusion

The solid–liquid equilibrium of LOLA was represented over the entire range of temperature and concentrations using the Chen model for mixed-solvent electrolyte systems. The solubility of LOLA in aqueous methanol mixtures was described with ionic mean activity coefficients calculated by using the Chen model. It describes satisfactorily the experimental solubility data of LOLA in water + methanol mixtures. Because the long-range contribution is due to the ion−ion interactions, which are slightly dependent on the molecular solution concentration, whereas the ion−molecule and molecule−molecule interactions are accounted for in the short-range contribution, the influence of the methanol content on the activity coefficient is mainly due to the short-range contribution. Notably, over the whole range of methanol content studied, the LOLA solution has a drowning-out effect by methanol content. It can also be seen that the effect of temperature is smaller than the effect of the methanol content. Finally, the experimental solubility and parameters obtained in this work can be used as essential data and models for the crystallization of LOLA.

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