

Effect of sodium chloride on the solubility and transformation behavior of L-glutamic acid

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The effect of NaCl concentration on the kinetics and thermodynamics of L-glutamic acid have been studied. The solubility of each glutamic acid polymorph in the NaCl solution was determined by the gravimetric method; the quantity of each polymorph was determined by the spectral method. The behavior of the polymorphic transformation has also been investigated using Raman and imaging techniques. The particle size of L-glutamic acid during the polymorphic transformation process was measured using a laser particle size analyzer. Experimental data show that NaCl affects the polymorphism of L-glutamic acid. In aqueous solutions without additives at temperatures below 30°C, the alpha form of glutamic acid is spontaneously generated, while in the presence of NaCl, the alpha form is rapidly converted to the beta form.

Keywords: L-Glutamic acid, sodium chloride, polymorphism, solubility, Raman spectra.

Вплив хлориду натрію на розчинність і трансформацію L-глутамінової кислоти. *Fei Lu, Yuan-Sheng Ding*

Вивчено вплив концентрації NaCl на кінетику і термодинаміку L-глутамінової кислоти. Розчинність кожного поліморфу глутамінової кислоти у розчині NaCl визначали гравіметричним методом; кількість кожного поліморфу визначено спектральним методом. Поведінку поліморфної трансформації досліджено за допомогою комбінаційного розсіювання світла і методів візуалізації. Розмір частинок L-глутамінової кислоти під час процесу поліморфного перетворення вимірювали з використанням лазерного аналізатора розміру частинок. Експериментальні дані показують, що NaCl впливає на поліморфізм L-глутамінової кислоти. У водних розчинах без добавок при температурі нижче 30°C альфа-форма глутамінової кислоти утворюється спонтанно, в той час як у присутності NaCl альфа-форма швидко перетворюється у бета-форму.

Исследовано влияние концентрации NaCl на кинетику и термодинамику L-глутаминовой кислоты. Растворимость каждого полиморфа глутаминовой кислоты в растворе NaCl определяли гравиметрическим методом, количество каждого полиморфа определяли спектральным методом. Поведение полиморфного преобразования исследовано с помощью рамановской техники и техники изображений. Размер частиц L-глутаминовой кислоты во время процесса полиморфного превращения измеряли с использованием лазерного анализатора размера частиц. Экспериментальные данные показывают, что NaCl влияет на полиморфизм L-глутаминовой кислоты. В водных растворах без добавок при температуре ниже 30°C альфа-форма глутаминовой кислоты образуется спонтанно, в то время как в присутствии NaCl альфа-форма быстро превращается в бета-форму.

1. Introduction

Though two polymorphs of benzamides were discovered by Uller and Lebeek in 1832, the polymorphism has been studied since the 1960s, especially in drug research

[1]. Polymorphism is the phenomenon in which a solid chemical compound exists in more than one crystalline form. Different polymorphs have different physical, chemical properties, such as melting point, hard-

ness difference, stability, dissolution rate, etc. [2, 3]. Consequently, the control of polymorphism is of great interest in biological science, chemistry, pharmacology and other fields [4, 5].

In the field of polymorphism, L-glutamic acid is a typical compound, which has two polymorphic forms: metastable α and stable β . Both crystal polymorphs have orthogonal lattices with space group P212121, but their lattice parameters are different, and their morphology is also different. Crystals of the α -form have a compact prismatic shape, and the β -form is lamellar [6–8].

During the preparation process, the influences of solvents, temperature, additives and other factors on polymorphism has been well studied. Among them, it was proved that the temperature factor can deeply affect the transition from the metastable α -form to the stable β -form: at crystallization above 45°C, the transformation rate is so high that the β -form is mainly obtained in solutions; during the crystallization process of the same solution below 30°C, only the α -form is nucleated and grows due to the very low rate of the phase transition [9, 10].

The influence of additives is another important issue in the control of the crystallization process [11]. Firstly, additives can change the thermodynamic behavior of solutes, for example, increase or decrease the solubility of solutes, entropy and enthalpy of the solution system [12]. Secondly, additives may change the dynamic behavior of the solutes by increasing or decreasing the growth rate of the polymorphs to promote or delay the transformation behavior [13].

In this work, the effect of sodium chloride (NaCl) on the crystallization of polymorphs of L-glutamic acid crystals during spontaneous nucleation below 30°C was studied. The kinetics and thermodynamics performance of two polymorphic forms were analyzed using Raman data obtained at various concentrations of NaCl.

2. Experimental

2.1 Chemicals

L-glutamic acid is obtained from Beijing Aoboxing Biotechnology Co., Ltd. and Sodium chloride is purchased from Tianjin Northern Tianyi Chemical Reagent Factory.

2.2 Sample preparation

Preparation of beta crystals: Supersaturated to a certain extent L-glutamic acid was completely dissolved at 80°C, then rapidly cooled to 50°C with continued stirring

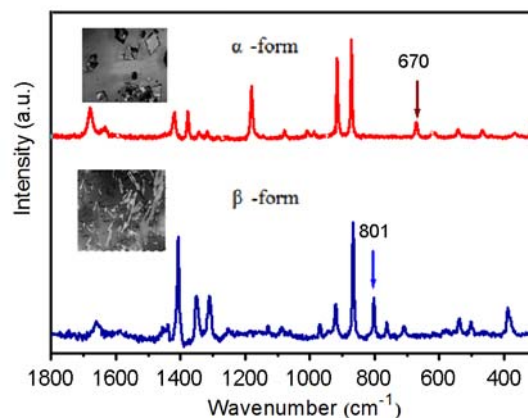


Fig. 1. Raman spectra of pure α - and β -form L-glutamic acid.

at a constant temperature for more than 6 h until the precipitated crystals completely took the β -form; then the crystals were filtered off and the samples were dried in an oven at 60°C.

Preparation of α crystal: L-glutamic acid with a certain degree of saturation is completely dissolved at 80°C, and then rapidly cooled to 20°C. Stirring is continued. After 10 min of exposure, the crystals are filtered and washed with anhydrous ethanol. The crystals obtained are dried in an oven at 60°C.

2.3. Characterization of L-glutamic acid-crystals

The Raman test uses the Xplora laser Raman spectrometer manufactured by our laboratory, HORIBA Jobin Yvon, France. The laser wavelength is 633 nm and the spectral range is 200 cm^{-1} – 1800 cm^{-1} .

The crystal morphology was observed using an Olympus BX51 microscope with an attached CCD video camera (Olympus, Japan). The detailed image was obtained using a field-emission scanning electron microscope (JEOL JSM-6700F). Particle size distribution was determined by nanolaser particle size analyzer. As Fig. 1 shows, the two polymorphs of L-glutamic acid have different crystallization habits. The crystal shape of α -form is granular morphology, and the crystal shape of β -form crystal is narrow flake morphology. It is suitable to distinguish the two crystal forms according to the crystal shape.

Solid-state Raman spectroscopy usually quantifies polymorphic components according to the proportion of the height or area of the selected characteristic peaks [14–16]. In this work, the identity of the α - and β -constituents of L-glutamic acid was con-

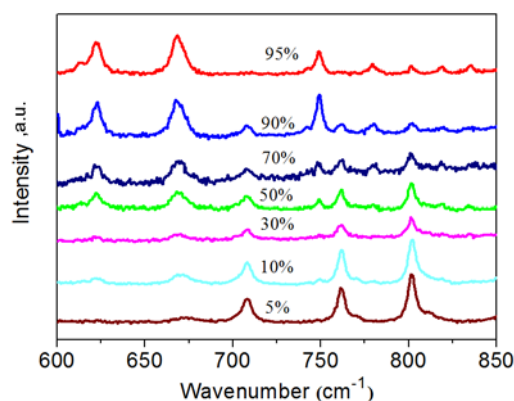


Fig. 2. Raman spectrum of a powder mixture of L-glutamic acid polymorphs.

firmed using Raman spectroscopy and the corresponding individual spectra are presented in Fig. 1. The main α -peaks occur at 433 cm^{-1} , 670 cm^{-1} , 987 cm^{-1} and 1006 cm^{-1} , 1181 cm^{-1} and the β -peaks, respectively, at 709 cm^{-1} , 801 cm^{-1} , 942 cm^{-1} and 1127 cm^{-1} . The peak 670 cm^{-1} of the α -form and the peak 801 cm^{-1} of the β -form were used as references to make quantitative calculations. It can be seen from Fig. 2 that the intensity of the β -form characteristic peak increases with the α -form content in the binary mixtures. Table 1 shows the calculation results and indicates the value of $T_\alpha: I_{670}/(I_{670}+I_{801})$ and the content of α polymorph X_α for linear regression to obtain the regression curve (Fig. 3) and equations $T_\alpha = 0.654X_\alpha + 0.0857$, $R^2 = 0.993$, $\text{RSD} = 0.0039$.

2.4. Determination of solubility

A supersaturated solution of α -form crystals or β -form crystals was prepared and stirred at a certain temperature until the solution was equilibrated. A certain amount of supernatant was removed and filtered. The filtrate was placed in a weighed glass container and weighed again. After being dried in a 60°C oven, the solubility was determined by the mass of the dried solid and the mass of evaporated water. In this experiment, the effect of supersaturation and sodium chloride on the conversion of the L-glutamic acid crystal form was examined by Raman spectroscopy.

Table 1. The T_α values for powder mixtures with different contents of α polymorph, X_α

The content of α polymorph., X_α	0.05	0.10	0.30	0.50	0.70	0.90	0.95
T_α	0.1270	0.1566	0.2819	0.4127	0.5359	0.6707	0.7019

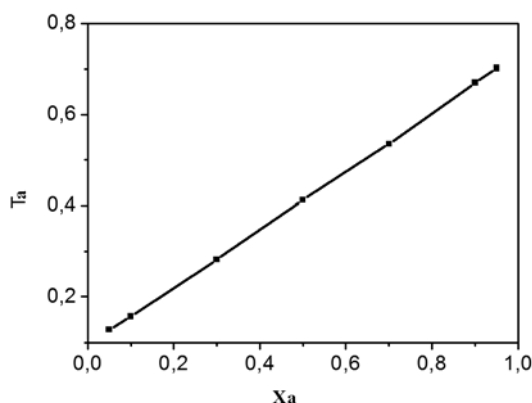


Fig.3. The standard curve of FT-Raman analysis.

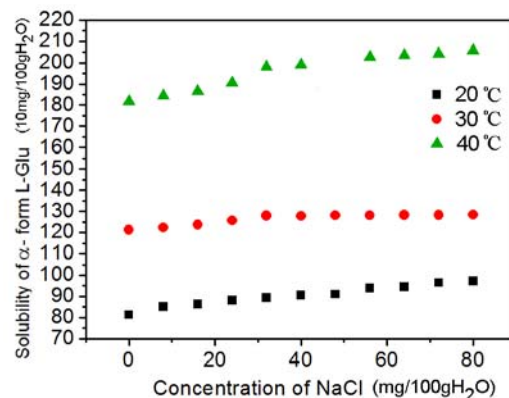


Fig. 4. Effect of NaCl on the solubility of α -form L-glutamic acid in water.

2.5 Transformation experiments

The α -form saturated solutions were prepared at 20°C with or without different concentrations of NaCl. Experiments on the transformation of the α -form doped with a NaCl solution into β -form crystals were carried out as follows: a saturated solution prepared by adding a sufficient amount of NaCl (suspension density approximately 20–30 wt. %) to the α -form of crystals was continuously stirred and suspended (stirring speed 350 RPM). A portion of the suspension was taken at regular intervals, immediately filtered and dried. The polymorphic composition of solids was determined by Raman analysis as described above.

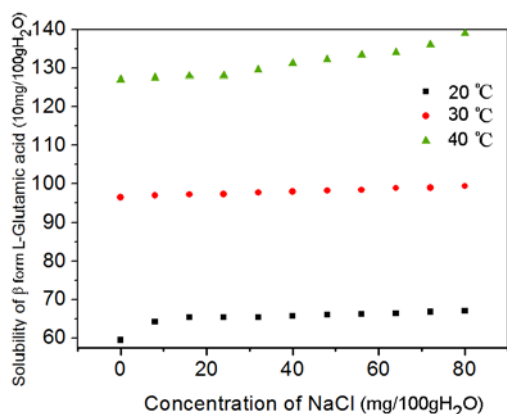


Fig. 5. Effect of NaCl on the solubility of β -form L-glutamic acid in water.

3. Result and discussion

As shown in Fig. 4 and Fig. 5, the solubility of L-glutamic acid in water was determined by the above experimental method. The solubility of both polymorphs increases with increasing temperature. The solubility of the α -polymorph is higher than that of the β -form, indicating that the α -form is metastable. The degree of solubility determined by our analysis is in good agreement with the data obtained by other ways [17], which indicates that the method has relatively high reliability. Fig. 4 and Fig. 5 also show the dependency of solubility of L-glutamic acid on temperature and NaCl concentration. A general trend is that the solubility of both polymorphs increase with increasing temperature for all used NaCl concentrations. At a certain temperature, the solubility increases with an increase in the NaCl concentration. When the concentration of NaCl was higher than 80 mg/100gH₂O, the solubility changed little. The addition of NaCl had a significant effect on the conductivity of L-glutamic acid; the higher the conductivity, the higher the solubility. Our result is consistent with the Bromley-Zemaitis model of activity coefficient [18], according to which the higher the concentration of electrolytes, the higher the solubility of solutes. According to M. Pudipeddi et al. [19], the ratio of the solubility of different crystal forms of polymorphic systems always approaches a constant of less than 2 at any temperature and solvent. Therefore, the solubility ratios of the two crystal forms of L-glutamic acid in different solvents and temperatures were calculated. As shown in Table 2, all the ratios are less than 2, and most of them are between 1.2 and 1.6, i.e. the measured solu-

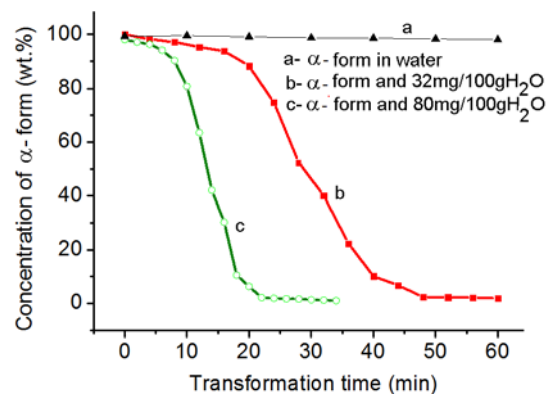


Fig. 6. Effect of NaCl concentration on the transformation from the α -form to the β -form in NaCl aqueous solutions at 20°C.

Table 2. Comparison of Solubility of L-glutamic acid of α - and β -forms

Temperature, °C	C_{NaCl} , g/100gH ₂ O	S_{β}/S_{α}
20	0.0000	1.37
	0.0080	1.33
	0.0160	1.32
	0.0320	1.37
	0.0800	1.45
30	0.0000	1.26
	0.0080	1.26
	0.0160	1.27
	0.0320	1.31
	0.0800	1.29
40	0.0000	1.43
	0.0080	1.45
	0.0160	1.46
	0.0320	1.53
	0.0800	1.48

bility of polymorphs of L-glutamic acid conforms to this rule; that indicates the accuracy of the measured solubility values, as shown in the table above.

In order to study the effect of NaCl on the crystallization of L-glutamic acid, polymorphic transformation experiments were carried out in aqueous solutions with and without NaCl. The conditions and results of the polymorphic transition from the α - to β -form are shown in Fig. 6. It is clear that the pure α -form very slow changes in water. After crystallization, most of the α -crystals can survive for more than 30 days in a stationary solution at 20°C. This means that

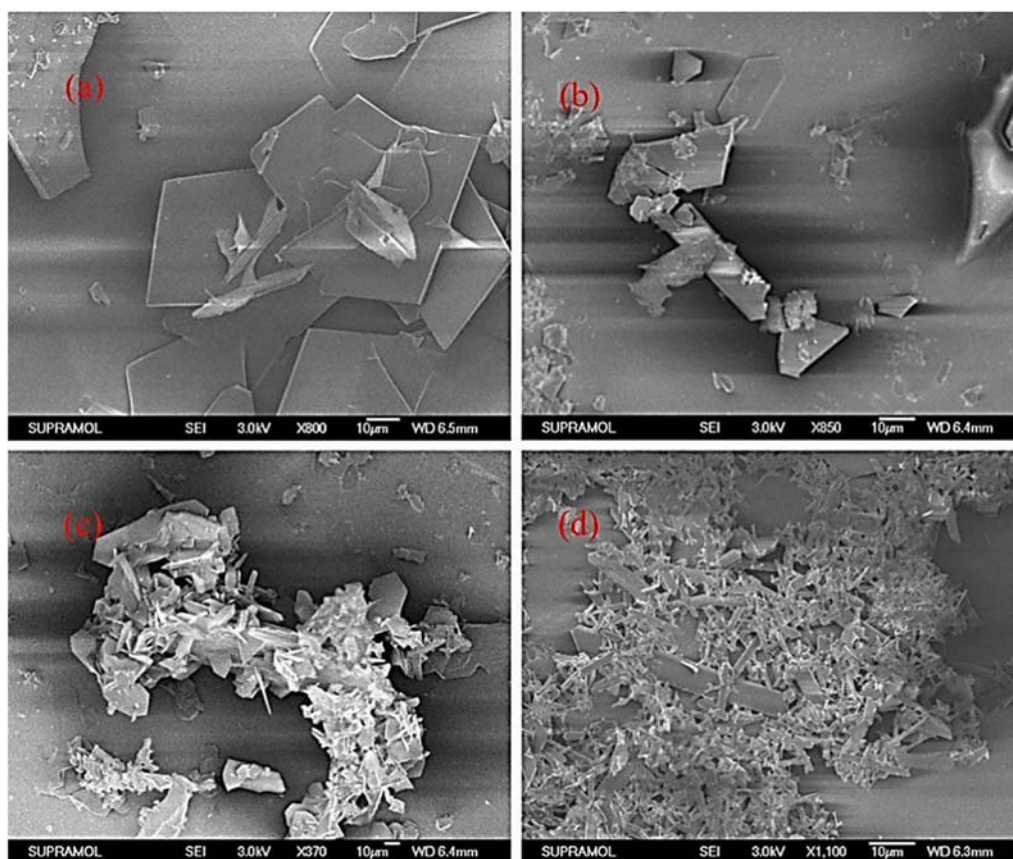


Fig. 7. SEM Images of transformation from the α -form to β -form (a: 10 min; b: 30 min; c: 60 min; d: 100 min) ($C_{\text{NaCl}} = 16\text{mg}/100\text{gH}_2\text{O}$).

the phase transition rate is very slow at 20°C . The experimental results also showed that the rate of transition from the α - to β -form in the presence of NaCl was much faster than that in the absence of NaCl, and the completion time was much shorter. With an increase in the NaCl concentration, the phase transformation rate is obviously accelerated. When the concentrations of NaCl were $80\text{ mg}/100\text{gH}_2\text{O}$ and $32\text{ mg}/100\text{gH}_2\text{O}$, the pure β -form could be obtained within 30 min and 1h, respectively. In other words, when the degree of supersaturation is determined, the greater the amount of NaCl added, the faster the β -form crystal growth rate, the higher the conversion rate, and the shorter the time required for complete conversion of the crystal form.

Fig. 7 shows the transform morphology of glutamic acid over time with the addition of NaCl. It can be seen that the α -form gradually dissolves, the β -form begins to nucleate on the surface of the α -form and grow, and then L-glutamic acid is com-

pletely converted to the β -form at the end of the polymorphic transformation. The results show that secondary nucleation is the main nucleation mode of the β -form in the presence of sodium chloride [20].

The average particle size of the obtained crystal is $85\text{ }\mu\text{m}$ at the beginning of the experiment (Fig. 8). After 10 min, the average grain size was measured to be $56\text{ }\mu\text{m}$. According to the results, β -form crystals are gradually formed as the reaction proceeds, and the average particle size of the crystal grains in the system gradually decreases. Further, β -form crystals continue to grow after the α -form crystals are mainly transformed to the β -form. In this case, the average particle size of crystals increases as shown in Fig. 8d-8e.

The regulation effect of additives on crystal polymorphism is manifested in the following: if the additive is strongly adsorbed on the main crystalline surface of the polymorph, it inhibits the growth of the crystalline polymorph and promotes the conversion of solute molecules into another

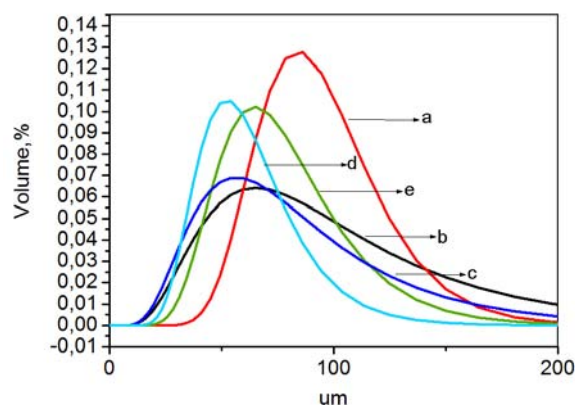


Fig. 8, Particle size data during transformation from the α -form to β -form (a: 10 min; b: 30 min; c: 60 min; d: 100 min; e: 120 min) (C_{NaCl} = 16mg/100gH₂O).

crystalline polymorph. As shown in Fig. 9 the α -polymorph of L-glutamic acid consists of a series of crystal planes. According to crystallization kinetics, the (020) plane is the main growth plane of the α -L-glutamic acid which contain a lot of carboxyl groups and amino-groups [21]. NaCl can be adsorbed on the (020) surface by electrostatic action, thus inhibiting the growth of the α -polymorph and enhancing the rate of transforming to the β -form.

4. Conclusions

In this paper, the effects of additives (NaCl) on the solubility and solution-mediated transformation of two forms of L-glutamic acid were revealed. The competitive nucleation of these two forms is related to the concentration of NaCl. For a constant degree of supersaturation, the greater the amount of added sodium chloride, the greater the proportion of the rhombohedral α -form L-glutamic acid is converted to the needle-shaped β -form L-glutamic acid during the same reaction time. Sodium chloride has a significant effect on the solubility of L-glutamic acid. According to the experimental results, our proposed mechanism is that the electrolytes present can be absorbed on the main surface of the α -crystal as a result of Coulomb interactions between ions and glutamic acid molecules, so the α -form stops growing and turns into the β -form.

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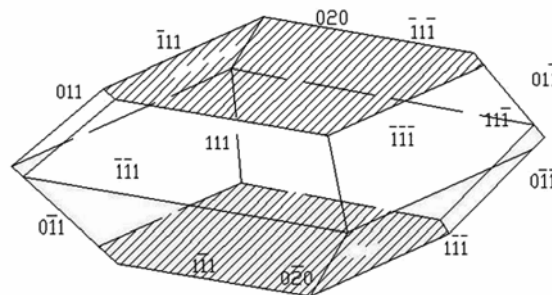


Fig. 9. Structure schematic of the α -form L-glutamic acid polymorph.

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