

Effect of Water Content on the Solid-State Stability in Two Isomorphous Clathrates of Cephalosporin: Cefazolin Sodium Pentahydrate (α Form) and FK041 Hydrate

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This study clearly demonstrates that clathrated water molecules can contribute to both chemical stabilization and destabilization of clathrates. The solid-state stabilities for two isomorphous clathrates of cephalosporin, cefazolin sodium and FK041, were investigated in terms of the effects of water content. The isomorphous ranges of water content were estimated to be 3.5—5 mol/mol for α -form cefazolin sodium and 2—4 mol/mol for FK041 hydrate. Upon the isomorphous dehydration, α -form cefazolin sodium was destabilized as the water content decreased below 4.25 mol/mol owing to the disruption of hydrogen bonding network in lattice channels. In this case, the hydration of clathrated water up to 4.25 mol/mol contributed to the physical and chemical stability of the crystals. On the contrary, the isomorphous hydration in FK041 hydrate contributed to the chemical destabilization owing to the high water activity. The difference in water activity between α -form cefazolin sodium and FK041 hydrate could be attributed to the size of water channels.

Key words clathrate; solid-state stability; cephalosporin; cefazolin sodium; FK041; water activity

Cefazolin sodium (CEZ·Na, Fig. 1a) is an antibiotic for injection, and is known to crystallize in several hydrate forms. Besides several reports on the pseudopolymorphism,¹⁻⁴ the crystal structure of the pentahydrate (α CEZ·Na) has been also reported by some pharmaceutical scientists.^{5,6} In these papers, it was indicated that a part of crystalline water included in α CEZ·Na located in lattice channels and could be easily moved out under low humidity conditions without affecting the lattice.⁶ In general, crystals which include other molecular species in the crystal lattice are called clathrates, and sometimes appear as non-stoichiometric molecular adducts at a given conditions due to relatively weak interactions.⁷ α CEZ·Na is also classified to clathrates, and the isomorphous hydration and dehydration are considered to take place by water diffusion through lattice channels. On the other hand, we have reported that FK041 (Fig. 1b),⁸ which is an orally administered cephalosporin, can also appear as isomorphous clathrates with water and some organic solvents.⁹

The objective of this study was to evaluate the effect of hydration state on the solid-state stabilities of these two isomorphous clathrates. Zografi has mentioned that it is important to know how these clathrated water molecules affect the chemical stability of the host pharmaceutical molecules because a large amount of water per unit mass of crystal have the potential to be released and associated as moisture.¹⁰ But the structural specificity in degradation mechanism has not been demonstrated by actual examples, as far as we know. Because cephalosporins tend to show moisture sensitive stabilities, it was expected that these two clathrates could provide appropriate examples to demonstrate the characteristic decomposition mechanisms.

Experimental

Materials CEZ·Na and FK041 were manufactured by the Chemical Development Labs. of Fujisawa Pharmaceutical Co., Ltd. All other chemicals used were of reagent grade.

Karl Fischer Titrimetry Water content, described as % or mol ratio, was determined by Karl Fischer analysis (Model MK-AII, Kyoto Electron-

ics, Kyoto, Japan). About 200 mg of each sample was accurately weighed and quickly transferred to the titration vessel containing a mixture of formamide and methanol (2 : 1) prior to titration.

Powder X-Ray Diffractometry (PXRD) The diffractometer (MPD-1880, Phillips, Netherlands) consisted of 4 kW generator (voltage 40 kV and current 30 mA) with a graphite-monochromated $\text{CuK}\alpha$ radiation source ($\lambda=1.5418 \text{ \AA}$) was used to obtain powder X-ray diffraction patterns. Changes in the X-ray diffraction pattern upon heating were examined using the same system equipped with an Anton Paar TTK temperature attachment and controller.

Water Sorption/Desorption Study and Measurement of Water Activity Water sorption/desorption studies and measurements of water activity were carried out gravimetrically on a microbalance system (VTI MB-300W, VTI Corporation, FL, U.S.A.) equipped a Cahn Digital Recording Balance. The resolution of the microbalance is 1 μg . Experiments were carried out on about 10 mg of sample under stepwise increasing or decreasing relative humidities at 10, 25 and 40 °C. The equilibrium criterion for sample weight was less than 0.01% for three successive 5 min intervals.

High-Performance Liquid Chromatography (HPLC) The equipment included: auto injector, Shimadzu SIL-9A; pumps, Shimadzu LC-9A and Hitachi L-6000; detectors, Shimadzu SPD-2A and Hitachi L-4000; and an automatic data processor, Shimadzu C-R5A.

Each α CEZ·Na sample (equivalent to 100 mg anhydride α CEZ·Na) was dissolved in the internal standard solution to make exactly 100 ml. The internal standard solution was a solution of *p*-acetanisidide in 0.1 mol/l phosphate buffer (pH 7.0). The injection volume was 5 μl . $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (2.27 g) and citric acid monohydrate (0.47 g) were dissolved in 935 ml water

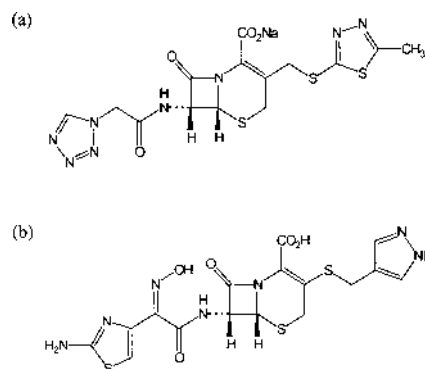


Fig. 1. Chemical Structures of Cephalosporins of (a) CEZ·Na and (b) FK041

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and 65 ml of acetonitrile was added, and this solution was used as the mobile phase. Other pertinent HPLC conditions were as follows: detection wavelength, 254 nm; column, octadecylsilanized silica gel for liquid chromatography (10 μ m): Nucleosil 10C₁₈ (M. Nagel, Germany); column dimensions, 150 mm \times 4 i.d.; column temperature, 25 $^{\circ}$ C; the flow rate was adjusted so that the retention time for CEZ was about 8 min.

Each FK041 hydrate sample (25 mg) was dissolved in pH 6.0 phosphate buffer to make exactly 50 ml. The injection volume was 5 μ l. A mixture of pH 2.4 perchlorate buffer and acetonitrile (5:1) was used as the mobile phase. Other pertinent HPLC conditions were as follows: detection wavelength, 254 nm; column, octadecylsilanized silica gel for liquid chromatography (10 μ m): Inertsil ODS-2 (GL Science Inc.); column dimensions, 250 mm \times 4.6 i.d.; column temperature, 40 $^{\circ}$ C; the flow rate was adjusted so that the retention time for FK041 was about 5.5 min.

2-Propanol Sorption Study About 200 mg of each sample was stored over 2-propanol in a desiccator for 24 h. The amount of 2-propanol was determined by gas chromatography (Model GC-14A, Shimadzu Scientific Instruments, Inc.). The experimental conditions were as follows: detector, FID (150 $^{\circ}$ C); column, G-300 (2 μ m) 1.2 mm \times 40 m; column temperature, 60 $^{\circ}$ C; injection temperature, 120 $^{\circ}$ C; carrier gas, helium (the flow rate: approx. 25 cm per second). Each sample was quickly weighed to minimize the influence of the atmosphere on water content.

pH Measurement Equivalent to 0.5 g anhydride of α CEZ \cdot Na was dissolved in water and diluted with water to make exactly 5 ml. pH measurements were carried out using a pH meter (F-16, HORIBA, Kyoto, Japan).

Results and Discussion

Isomorphic Hydration and Dehydration Behaviors in α CEZ \cdot Na and FK041 Hydrate Prior to the stability tests for α CEZ \cdot Na and FK041 hydrate, the isomorphic hydration and dehydration behaviors were investigated.

Powder X-ray diffractometry was performed first. Although α CEZ \cdot Na is known to show isomorphic desolvation, the range of water content for isomorphic dehydration has not been clear. Thus, powder X-ray diffraction patterns were measured for α CEZ \cdot Na samples of varying water contents: 5.3, 4.5, 4.0, 3.5, 3.0 mol/mol. The results showed that, in dehydration down to approximately 3.5 mol/mol, α CEZ \cdot Na was isomorphic and further dehydration disrupted the crystal lattice, as shown in Fig. 2.

Figure 3a shows the moisture sorption and desorption isotherms of α CEZ \cdot Na at 25 $^{\circ}$ C as measured by the microbalance method. In a stepwise dehydration process with a humidity interval of 2% relative humidity (RH) (Fig 3a, curve A–B–C), a large inflection point was observed at 16.0% RH and 4.25 mol/mol (point B). Further, in a stepwise hydration process (curve B–D–E), a small inflection point was observed at around 30% RH and 4.50 mol/mol (point D).

In a subsequent stepwise dehydration process after the hydration up to 50% RH and 5 mol/mol, the desorption isotherm gave a different curve from the hydration curve (B–D–E), and showed a large inflection point at 16% RH and 4.5 mol/mol (point F). The observed open hysteresis loop (B–D–E–F) and inflection points (B, D, and F) reveal that α CEZ \cdot Na can take intermediate metastable hydration states at 4.25 and 4.5 mol/mol in the isomorphic hydration and dehydration between 3.5–5 mol/mol.

As for FK041 hydrate, continuous hydration and dehydration were observed between 2 and 4 mol/mol, as shown in Fig. 3b. Contrary to the sorption and desorption isotherms of α CEZ \cdot Na, no hysteresis was observed. And an intermediate

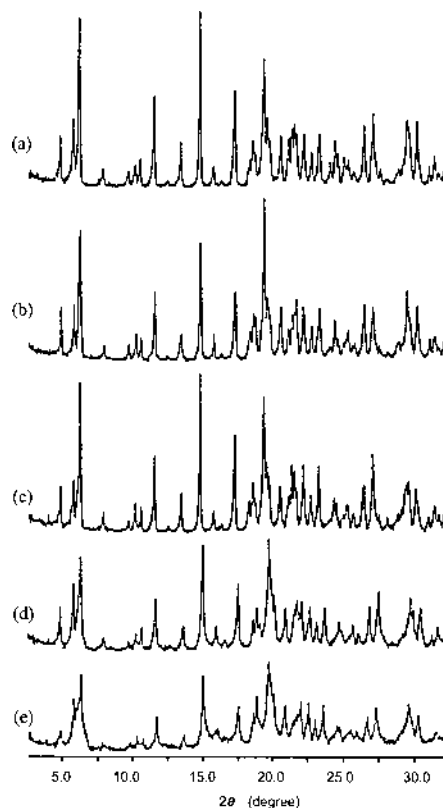


Fig. 2. Powder X-Ray Diffraction Patterns for α CEZ \cdot Na Samples of Several Water Contents
(a) 5.0 mol/mol, (b) 4.5 mol/mol, (c) 4.0 mol/mol, (d) 3.5 mol/mol, (e) 3.0 mol/mol.

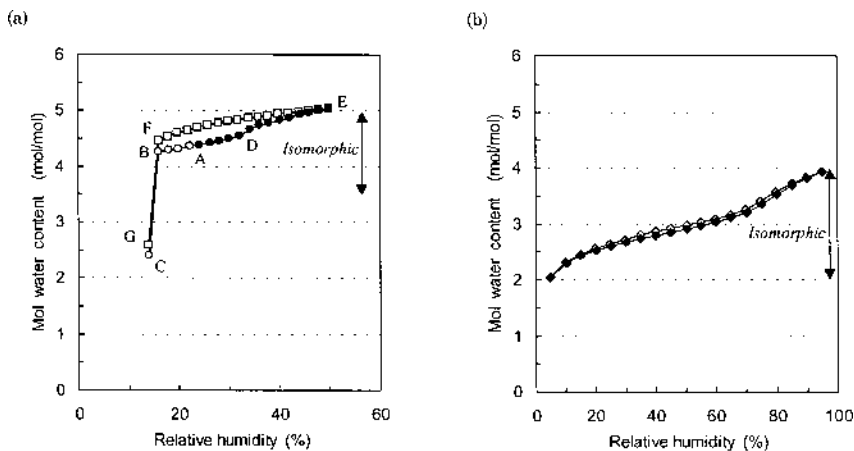


Fig. 3. Water Sorption and Desorption Isotherms for (a) α CEZ \cdot Na and (b) FK041 Hydrate at 25 $^{\circ}$ C

metastable hydration state was observed at 3 mol/mol. As shown in Fig. 4, the powder X-ray diffraction patterns for FK041 samples stored over silica gel (almost 0% RH) and water (100% RH) at room temperature were identical within the limits of the experiment. Thus, the hydration and dehydration of FK041 hydrate between 2 and 4 mol/mol were confirmed to be isomorphic.

Effect of Isomorphic Variation in Water Content on the Solid-State Stability in α CEZ·Na α CEZ·Na samples including various mol water contents between 3.79 and 5.24 mol/mol were stored in closed glass vials at 40 °C. The residual percentages of these samples were measured by the HPLC method after storage for 1, 3, and 6 months. Figure 5a shows the residual percentages as a function of mol water content in these test conditions.

The obtained stability profile indicated that α CEZ·Na was stable when the water content was controlled between 4.25 and 5 mol/mol, while dehydration below 4.25 mol/mol and hydration over 5 mol/mol caused acceleration of the degradation.

The hydration to α CEZ·Na over 5 mol/mol was considered to take place on the surface of the crystals and to form a sorbed moisture layer¹¹⁾ where the decomposition of CEZ mainly proceeded. As the decomposition in the sorbed moisture layer proceeded, additional solution of a portion of the crystal surface might take place with the consequent release of 5 equivalent moles of water which had been crystalline

water molecules. Thus, the degradation was remarkably accelerated as the storage period was extended, as shown in Fig. 6 (open circles).

On the contrary, the α CEZ·Na samples with water content below 4.25 mol/mol decomposed in a different way, giving different degradation curves, as indicated in Fig. 6 (closed circles and closed triangles). The critical hydration state of 4.25 mol/mol corresponded to the intermediate metastable hydration state observed in the water sorption and desorption study described above. Therefore, dehydration below 4.25 mol/mol was believed to disrupt the original hydrogen network in the lattice channels. And the disruption of the hydrogen network accelerated the thermal decomposition of CEZ molecules due to the physical destabilization of the crystal lattice.

Effect of Isomorphic Variation in Water Content on the Solid-State Stability in FK041 Hydrate FK041 hydrate samples of varying water content between 2.45 and 3.36 mol/mol were stored in closed glass vials at 40 °C for 3 months or 70 °C for 9 d. Figure 5b shows the residual percentages measured by the HPLC method as a function of mol water content. It was noteworthy that the residual percentage of FK041 decreased as the hydration proceeded, contrary to the degradation profile of α CEZ·Na. Furthermore, the effect of hydration on the stability was confirmed to become larger as the storage temperature increased. These results suggested that the water activity of FK041 hydrate was large so that some of the water molecules included in the lattice channels could easily move out depending on the storage temperature.

To confirm this hypothesis, the water activities for α CEZ·Na and FK041 hydrate were measured at several temperatures using a microbalance. As shown in Fig. 7, the water activity for FK041 hydrate was highly dependent on the hydration state and temperature compared to that for α CEZ·Na. Thus, the water molecules included in FK041 hydrate could affect the FK041 degradation as reactants by directly contacting to FK041 molecules or as mediators by forming a sorbed moisture layer on the surface of the crystals.

Explanation of the Difference in Water Activity between α CEZ·Na and FK041 Hydrate in Terms of Inclusion Environment We reported in our previous paper that water molecules, which adsorbed into the lattice channels of FK041 hydrate above 50% relative humidity, could be re-

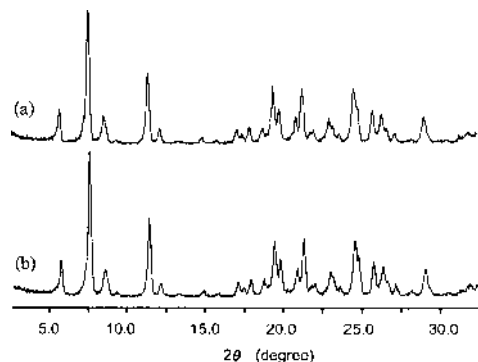


Fig. 4. Powder X-Ray Diffraction Patterns for FK041 Hydrate Samples (a) Stored over silica gel at room temperature for 24 h, (b) stored over water at room temperature for 24 h.

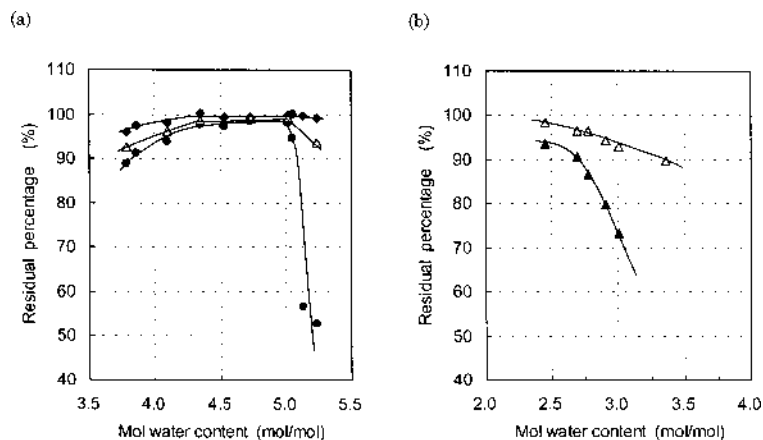


Fig. 5. Correlation between Mol Water Content and Residual Percentage of (a) α CEZ·Na and (b) FK041 Hydrate (◆) 40 °C, 1 month; (△) 40 °C, 3 months; (●) 40 °C, 6 months; (▲) 70 °C, 9 d.

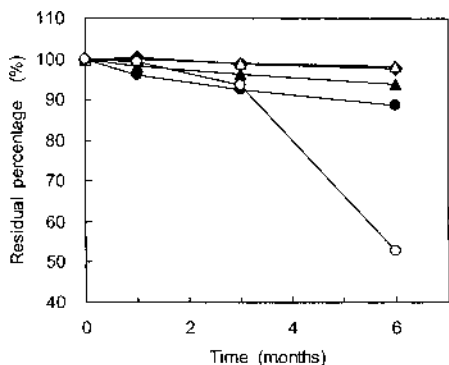


Fig. 6. Degradation Curves at 40°C for α CEZ·Na Samples of Several Mol Water Contents

Initial mol water content: (●) 3.79 mol/mol, (▲) 4.09 mol/mol, (◆) 4.34 mol/mol, (△) 5.02 mol/mol, (○) 5.24 mol/mol.

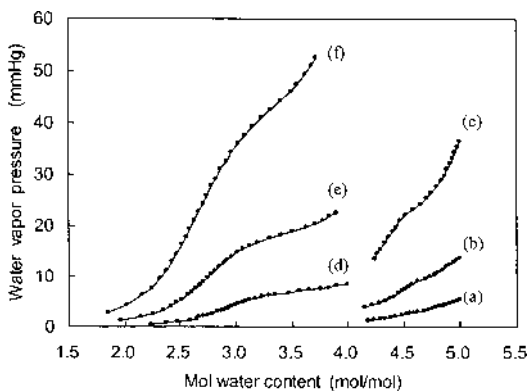


Fig. 7. Water Activities for α CEZ·Na and FK041 Hydrate as a Function of Mol Water Content

(a) α CEZ·Na, 10°C, (b) α CEZ·Na, 25°C, (c) α CEZ·Na, 40°C, (d) FK041 hydrate, 10°C, (e) FK041 hydrate, 25°C (f) FK041 hydrate, 40°C.

placed with 1/3 equivalent mol of 2-propanol without changing the crystal lattice. Comparing the molecular size of 2-propanol with that of 3 mol of water, the water molecules originally present were thought to locate in the relatively wide space in the lattice channels. To get information about the channel size in α CEZ·Na, α CEZ·Na including 4.25 mol/mol of water was also exposed to 2-propanol vapor pressure in a desiccator for 1 d, and inclusion of 2-propanol into the crystals was investigated by the GC method. No 2-propanol was detected, indicating that the vacant space in the lattice channels did not match with 2-propanol in terms of size and shape.

On the other hand, pH measurements for solutions of aged α CEZ·Na samples, which had varying amounts of water and which were stored in tightly closed amber glass bottles with an inner polyethylene stopper, provided unexpected information about the channel size. The closed circles in Fig. 8 show the correlation between the initial water content of the tested α CEZ·Na samples and the solution pH after storage at 5°C for 20 months. The correlation indicated that the solution pH after storage decreased as the initial water content decreased. Furthermore, a subsequent 3 months storage after once opening the bottle for pH measurement at month 20 promoted further decreases in pH (see open circles in Fig. 8). Considering that no obvious degradation occurred during the storage, the result could be interpreted that α CEZ·Na took in a small

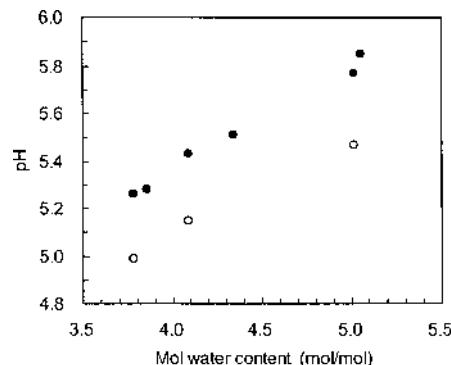


Fig. 8. Correlation between Initial Mol Water Content and the Solution pH for α CEZ·Na

(●) after the storage at 5°C for 20 months, (○) after the storage at 5°C for 23 months. (The glass bottles were once opened at month 20.)

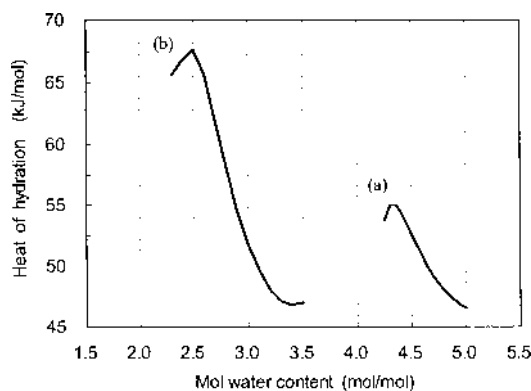


Fig. 9. Heat of Hydration as a Function of Mol Water Content for (a) α CEZ·Na and (b) FK041 Hydrate

amount of carbon dioxide from head space of the glass bottle into the vacant spaces in the lattice channels. Therefore, the vacant space where the removed water molecules were originally located was considered to be more suitable for carbon dioxide than 2-propanol in terms of molecular size and shape.

Subsequently, hydrogen bonding environments for water molecules, which could hydrate and dehydrate depending on relative humidity, were compared between α CEZ·Na and FK041 hydrate in terms of heat of hydration. Heat of hydration as a function of water content was calculated according to the Clausius-Clapeyron equation¹²⁾:

$$d \ln P/d(1/T) = -\Delta H_h/R$$

where ΔH_h is the heat of hydration and R is the gas constant. P is the water vapor pressure at absolute temperature, T , and the data shown in Fig. 7 were used for the calculation. The results showed that the heat of hydration for both α CEZ·Na and FK041 hydrate became close to the heat of water condensation (45—43 kJ/mol in the range 0—50°C¹³⁾), as shown in Fig. 9.

Thus, clathrated water molecules included in both α CEZ·Na and FK041 hydrate were confirmed to be trapped in lattice channels with weak hydrogen bonding interactions. Therefore, the difference in water activity between α CEZ·Na and FK041 hydrate could be attributed to the difference in channel size.

Conclusions

α CEZ·Na showed isomorphic hydration and dehydration between 3.5—5 mol/mol, and was destabilized as the isomorphic dehydration below 4.25 mol/mol proceeded due to the disruption of hydrogen bonding network in lattice channels. In this case, the hydration of clathrated water up to 4.25 mol/mol contributed to the physical and chemical stabilization of the crystals. The water molecules, which hydrate and dehydrate between 4.25—5 mol/mol, were supposed to be included in the tight lattice channels which corresponded to carbon dioxide in size, and did not affect the chemical stability owing to the less water activity. On the contrary, the isomorphic hydration between 2—4 mol/mol in FK041 hydrate contributed to the chemical destabilization owing to the high water activity. These results clearly demonstrated that clathrated water molecules could contribute to both chemical stabilization and destabilization of clathrates. And the obtained information in this study should enable us to interpret complex stabilities in other pharmaceutical clathrates.

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