Pharmaceutical Research, Vol. 5, No. 7, 1988

Research Article

Hygroscopicity of Cefazolin Sodium: Application to Evaluate the Crystallinity of Freeze-Dried Products

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Received November 11, 1987; accepted February 4, 1988

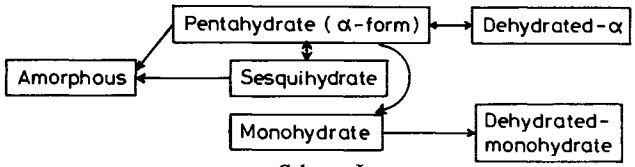
The hygroscopicity behavior of the pentahydrate, monohydrate, amorphous, and dehydrated forms of solid cefazolin sodium (CEZ) was studied under different relative humidity (RH) conditions. Between 42 and 86% RH, the pentahydrate (α form), the monohydrate, and their dehydrated forms absorbed atmospheric moisture equivalent to their hydrate numbers. The pentahydrate demonstrated a hysteresis effect at 15 and 31% RH. On the other hand, the water content of the amorphous form increased linearly with increases in RH. The noncrystalline state was maintained below 56% RH. For the dehydrated α form there was a distinct birefringence when viewed under polarizing light, the X-ray diffraction pattern was weak and diffuse, and the infrared (IR) spectra were discernibly different from that of the amorphous form. The freeze-dried CEZ showed hygroscopic behavior almost similar to that of the dehydrated α form. Two-component mixtures of various CEZ forms showed a linear relationship between the water content and the mixing ratio when stored at 31, 42, and 56% RH. From the hygroscopicity data, the crystallinity of freeze-dried CEZ could be estimated as the percentage of the dehydrated α form.

KEY WORDS: cefazolin sodium; hygroscopicity; pseudopolymorphs; crystalline transformation; freeze-drying; crystallinity.

INTRODUCTION

Cefazolin sodium (CEZ) has been reported to crystallize in several forms according to the preparation conditions (1–4). In freeze-drying, the less stable amorphous form of CEZ occurs when aqueous solutions are frozen and persists following drying (5), but crystalline transformation has been shown to occur by thermal treatment prior to drying and/or the addition of cosolvent (4–6). For various antibiotics, there have been several reports of attempts to prepare freeze-dried products in the crystalline state, improve physical appearance, increase chemical stability, and/or reduce processing time (4,6–10).

The pseudopolymorphism of CEZ consists of three hydrated crystalline forms, namely, pentahydrate, sesquihydrate, and monohydrate, and two dehydrated forms as well as the amorphous form (1,11,12). From the literature reports, the following scheme can be constructed.



Scheme I

According to this scheme, the pentahydrate can give up molecular water and convert to four forms, the dehydrated, monohydrate, sesquihydrate, and amorphous forms. The sesquihydrate can be converted to the pentahydrate with the adsorption of water and to the amorphous form by the removal of water. The monohydrate converts only to the dehydrated form as a result of desorption of water.

Several forms of CEZ can be distinguished and characterized by the combination of the following methods: infrared (IR) spectra (1,13), X-ray diffractometry (11), solution calorimetry (11), solid-state nuclear magnetic resonance (NMR) (12), and water vapor sorption isotherm (14). To determine the degree of crystallinity of solid-state drug, X-ray diffractometry has generally been used, but the value of the crystallinity depends on the choice of amorphous and crystalline standards (11). To avoid this problem, it is important to measure the crystalline form present in the freeze-dried product and to calculate the degree of crystallinity using amorphous and appropriate crystalline standards.

The objectives of this study were (a) to evaluate the hygroscopicity of CEZ—the pentahydrate, the monohydrate, their dehydrated forms, and the amorphous form; (b) determine the crystalline transformation of the various CEZ forms by dehydration and hydration; and (c) determine the crystalline form and estimate the degree of crystallinity of freeze-dried CEZ by its hygroscopic behavior.

MATERIALS AND METHODS

Sample Preparation

Various cefazolin sodium forms were prepared from ce-

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fazolin acid, obtained from SK&F (Philadelphia, Pa.). The crystalline *pentahydrate* (α *form*) was prepared by recrystallization from aqueous ethanol (1). The *monohydrate* was prepared via the addition of a solution of cefazolin acid in dimethylacetamide to an enthanolic solution of sodium acetate (3). The *dehydrated* α *form* and *dehydrated monohydrate* were prepared by vacuum-drying the pentahydrate and monohydrate forms (0.08 Torr, 23°C, 20 hr). The *amorphous form* was prepared by rapid-freezing the CEZ solution in Freon 12 (15) and subsequently freeze-drying. To avoid partial crystallization the CEZ aqueous solution was sprayed rapidly via a hypodermic needle into liquid Freon 12 (< −30°C) and the resultant frozen beads were freeze-dried (15). The freeze-dried samples were stored in sealed glass vials with butyl rubber stoppers to prevent the transfer of moisture and the change of water content (13). In the case of the dehydrated and amorphous forms, the gas phase in the glass vial was purged with dry N_2 gas to maintain a moisture-free environment. The physical mixtures of two components of CEZ were mixed uniformly using a mortar and pestle, with care to avoid any grinding action.

Freeze-dried products were prepared by freeze-drying 18.9% (w/w) of CEZ in water or isopropyl alcohol–water solutions (5%, w/w, alcohol). The vials filled with 5.1 ml of the CEZ solution were placed in a freeze-dryer (Hull, Model X8F12) and the shelf temperature was first lowered to −40°C and then increased to −10 to −15°C and held for 1 hr (thermal treatment). After cooling to below −30°C, the drying cycle was initiated at a shelf temperature of 30 or 45°C. All forms of CEZ were above 98% pure as determined by high-performance liquid chromatography (HPLC).

Evaluation Methods

Hygroscopicity. One-gram samples were accurately weighed in a weighing bottle and exposed to the desired relative humidity (RH) in desiccators containing saturated solutions of inorganic salts (0 to 93 ± 1% RH) at 23 ± 1°C. The sample weight was measured at few-day intervals, and after the sample weight reached a plateau the water content was determined by Karl Fischer titration.

Polarizing Microscopy. The samples were mounted in mineral oil and examined by means of a research polarizing microscope (Reichert Zetopan, Nr 338610). The birefringence was determined for the hydrated, dehydrated, and amorphous samples.

X-Ray Diffraction. X-Ray diffraction patterns were recorded with a Rigaku Denki X-ray diffractometer (Ni filter, CuK_α radiation; voltage, 40 kV; current, 20 mA; time constant, 1 sec; scanning rate, 4°/min).

Infrared (IR) Spectra. IR spectra were recorded as mulls in Nujol using an infrared spectrophotometer (Perkin Elmer 1430). The mulls contained 10 mg of CEZ and 2 drops of Nujol.

RESULTS AND DISCUSSION

Characteristics of Various Forms

Table I shows the properties of various forms of CEZ: the pentahydrate, monohydrate, amorphous, and dehydrated forms. The definition of the terms "crystalline" and "amorphous" were based on X-ray and polarizing microscopy (11,16). The dehydrated and amorphous forms easily acquired a static charge because of the low moisture content. There was no decomposition during the preparation of the various forms, as confirmed by HPLC.

The X-ray powder diffractions in Fig. 1 illustrate characteristic patterns for each form. The dehydrated α form and dehydrated monohydrate exhibited weak and diffuse X-ray patterns (11), while the pentahydrate and monohydrate exhibited sharp X-ray bands.

No distinct peaks were apparent in the X-ray diffraction pattern of the amorphous form, and it also showed nonbirefringence, suggesting a noncrystalline state. On the other hand, the two dehydrated forms showed birefringence similar to their hydrates, suggesting retention of the crystalline state (11).

Infrared spectra of the various CEZ forms are shown in Fig. 2. The spectra for the pentahydrate and monohydrate were similar to those reported previously (1,13). It was possible to characterize the pentahydrate, monohydrate, and amorphous forms by IR but not possible to distinguish between the hydrate and the corresponding dehydrate.

The pentahydrate showed a broad absorption band due to water at 3350 to 3250 cm^{-1} and an amide carbonyl band at 1665 cm^{-1}. The monohydrate showed bands in the OH-stretching region at 3430 and 3560 cm^{-1} and an amide carbonyl band at 1685 cm^{-1}. The major difference between the crystalline and the amorphous forms appeared to be the absence of a spectral band at 1542 cm^{-1} in the latter. Each form showed absorption bands due to a β-lactam carbonyl band at 1760 cm^{-1} and a carboxylate carbonyl band at 1590 to 1600 cm^{-1} (1,13,17).

Table I. Properties of Crystalline and Amorphous CEZ

Physical form	Water content (%, w/w)	X-Ray pattern[a]	Polarizing microscopy[b]	Crystallinity[c]
Pentahydrate	15.9	++	B	C
Dehydrated α	0.1	+	B	W
Amorphous	0.6	−	N	A
Monohydrate	3.7	++	B	C
Dehydrated monohydrate	0.4	+	B	W

[a] (++) sharp; (+) weak; (−) no distinct peaks.
[b] B, birefringent; N, nonbirefringent.
[c] C, crystalline; W, weak crystalline; A, amorphous.

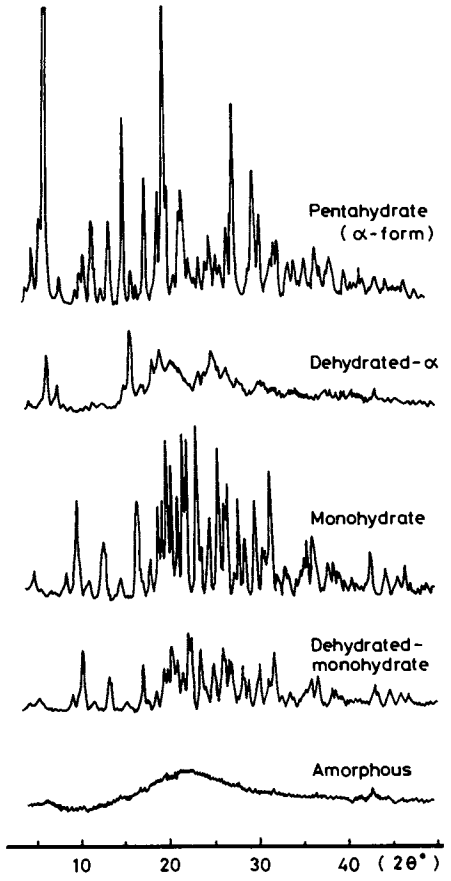


Fig. 1. X-Ray diffraction of various CEZ forms.

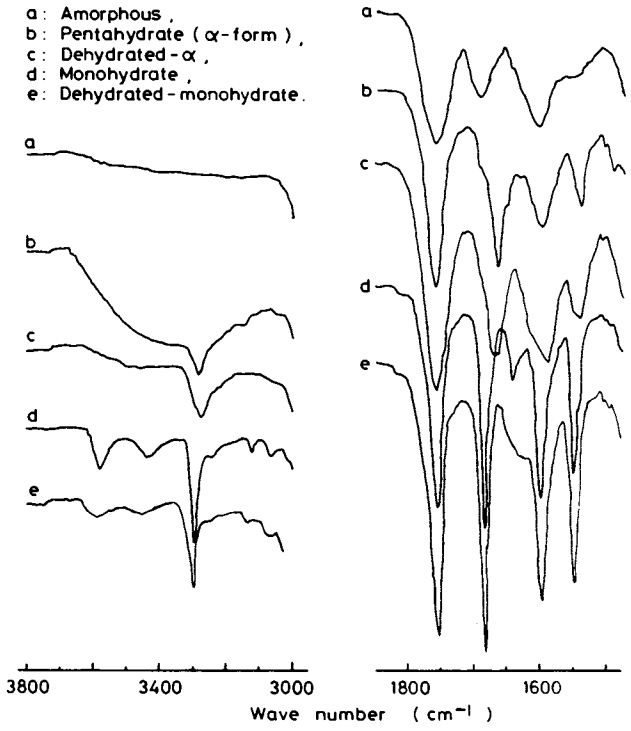


Fig. 2. Infrared spectra of various CEZ forms.

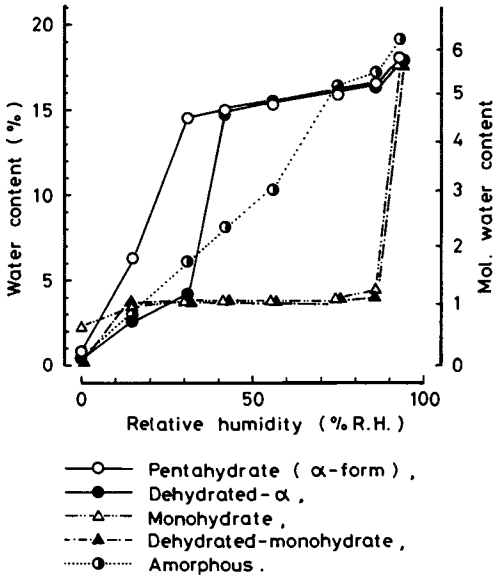


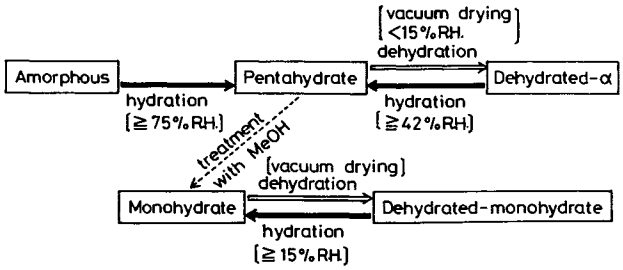
Fig. 3. Water sorption isotherms of CEZ forms obtained by storage under various relative humidity conditions at 23°C. The pentahydrate and monohydrate isotherms were obtained by desorption; the others, by adsorption.

Hygroscopicity of Various Forms

In general, curves for equilibrium water content versus relative humidity (RH) can be produced in either of two ways: the material can be dried under vacuum and then subjected to increased RH (adsorption method) or the material can be saturated at high RH and then subjected to decreasing humidities (desorption method) (18).

Figure 3 shows effect of RH on the equilibrium water content of the CEZ forms after storage at various RH values for 10 days at 23°C. The dehydrated α form, dehydrated monohydrate, and amorphous form tended to take up water by adsorption and therefore the equilibrium water content was obtained by the adsorption method. On the other hand, in the case of pentahydrate and monohydrate, water was lost by desorption under low RH, and then the equilibrium water content was obtained by the desorption method. Between 42 and 86% RH, the equilibrium water content of the pentahydrate, dehydrated α form, monohydrate, and dehydrated monohydrate were almost equivalent to their hydrate numbers, namely, the pentahydrate and dehydrated α form, which converted to pentahydrate, contained about 5 mol of water and the monohydrate and dehydrated monohydrate contained 1 mol of water. Pentahydrate showed a different series of values when adsorbing water than when desorbing water; the equilibrium water content of pentahydrate at 15 and 31% RH was larger than that of the dehydrated α-form, suggesting a hysteresis effect at 15 and 31% RH. With the amorphous form, the equilibrium water content increased linearly with increases in RH.

According to X-ray diffraction and IR spectra of the aged samples shown in Fig. 3, the following diagram of the crystalline transformation of CEZ by dehydration and hydration can be proposed.

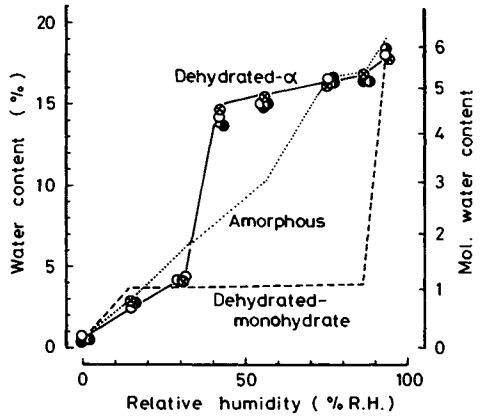


Scheme II

The amorphous form retained the noncrystalline state at below 56% RH and converted completely to the pentahydrate above 75% RH. The pentahydrate and monohydrate did not transform to the noncrystalline state but, rather, to their dehydrated forms by the removal of crystalline water with P_2O_5 or vacuum-drying. Hydration of the dehydrated α form and dehydrated monohydrate restored the strong X-ray diffraction pattern and induced the transformation to their hydrated forms. For these hydrated forms, the structure change by dehydration and rehydration was reversible. By suspending the pentahydrate in anhydrous methanol, the pentahydrate did not convert to the sesquihydrate but, rather, to the monohydrate, as identified from a water vapor sorption isotherm, X-ray diffraction, and IR spectra.

Hygroscopicity of Freeze-Dried Products

In order to determine the crystalline forms present in freeze-dried products, the water vapor sorption isotherms of the products were compared with those of standard forms. Figure 4 shows that the water sorption isotherms of the freeze-dried products were comparable to those of the two dehydrated forms and the amorphous form. The water content of the freeze-dried products was comparable to that of the two dehydrated and amorphous forms, i.e., below 1% (w/w). The freeze-dried products of CEZ showed hygroscopicity behavior almost similar to that of the dehydrated α-form. Further, the X-ray diffraction and IR spectra of these freeze-dried products were almost the same as those of the dehydrated α form and obviously different from those of the dehydrated monohydrate. The equilibrium water contents of the freeze-dried products were between the values of the pentahydrate and the amorphous at 42 and 56% RH. Under these relative humidity conditions, the difference in the equilibrium water content between the pentahydrate and the amorphous forms was maximum at 42% RH. Consequently, these freeze-dried products contained the pentahydrate as a main component and a small amount of the amorphous form.



symbol	solvent	T.T.	water content
●	water	−	0.27 %
○	ipa-water	+	0.55 %
⊗	ipa-water	+	0.41 %
◑	water	−	0.22 %

T.T.= thermal treatment, ipa= isopropyl alcohol.

Fig. 4. Water adsorption isotherms of freeze-dried CEZ samples obtained by storage under various relative humidity conditions at 23°C. The lines represent the standard curves from Fig. 3.

Water Adsorption of Two-Component Mixtures

In order to clarify the possibility to evaluate the crystallinity of freeze-dried product quantitatively using hygroscopicity data, the water adsorption behavior of two-component mixtures was observed under fixed-RH conditions. The physical mixture of the dehydrated α form and the amorphous form showed a linear relationship between the equilibrium water content and the mixing ratio when stored at 31, 42, and 56% RH (Fig. 5). The same tendency as shown in Fig. 5 (a good linear relationship) was also observed in each case of the following two-component physical mixtures: pentahydrate and amorphous, monohydrate and amorphous; and pentahydrate and monohydrate. The results suggest that each component in the physical mixture did not interact with each other and transformed independently. At 31% RH, the amorphous component adsorbed more water than the dehydrated α form, while at 42 and 56% RH the dehydrated α form in the mixture adsorbed more water than the amorphous component. This was believed to be due to the conversion of the dehydrated α form to pentahydrate. In the case of the amorphous form, such conversion did not occur and the noncrystalline state having a lower water content than pentahydrate was retained. The results also suggest that it is possible to estimate the crystallinity of CEZ freeze-dried products from hygroscopicity data using the regression lines shown in Fig. 5 as standard curves.

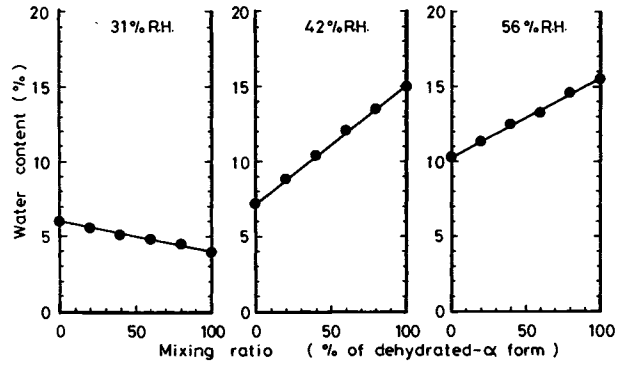


Fig. 5. Water adsorption of physical mixture of the dehydrated α and amorphous forms at 31, 42, and 56% RH for 10 days.

Table II. Equilibrium Water Content and Estimated Crystallinity of Freeze-Dried Products

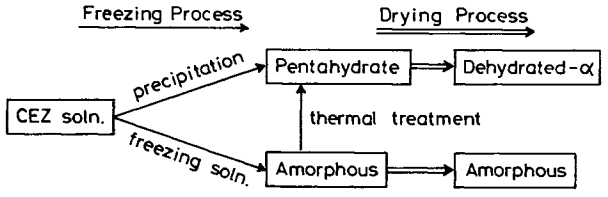
	31% RH		42% RH		56% RH	
Sample[a]	% H_2O	C[b]	% H_2O	C	% H_2O	C
●	4.34	86.4	13.65	82.6	14.88	86.4
○	4.37	84.9	14.19	89.5	15.01	88.9
⊗	4.24	91.5	14.71	96.3	15.45	97.2
◑	4.15	96.0	13.99	87.0	14.96	87.9
SD	±0.2[c]	±10.0	±0.2[c]	±2.5	±0.2[c]	±3.8

[a] Symbols are the same as in Fig. 4.
[b] Percentage crystallinity, represented as percentage of dehydrated α form.
[c] Standard deviation of Karl Fischer method.

Estimation of Crystallinity of Freeze-Dried Products from Hygroscopicity Data

The crystallinity, represented as the percentage of the dehydrated α-form, of freeze-dried products was estimated from the equilibrium water content at 31, 42, or 56% RH shown in Fig. 4 and the corresponding standard curve shown in Fig. 5. Table II shows that for the four freeze-dried products a rather high crystallinity resulted, especially for that prepared using both isopropyl alcohol as a cosolvent and thermal treatment (−10°C for 1 hr).

From these results and an earlier study (6), the following scheme can be suggested for the phase transition during freeze-drying.



Scheme III

The addition of isopropyl alcohol accelerated the precipitation of pentahydrate from CEZ solution during the freezing process and the conversion from amorphous to pentahydrate during thermal treatment.

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