

Report

Estimation of the Degree of Crystallinity of Cefazolin Sodium by X-Ray and Infrared Methods

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The pentahydrate (α form) of cefazolin sodium (CEZ) exhibited sharp X-ray diffraction peaks, while the dehydrated α form showed weak but distinct diffraction peaks. As expected the amorphous form exhibited a diffuse and halo diffraction pattern. The X-ray procedure to estimate the degree of crystallinity of CEZ was based upon the measurement of the total scattering and the scattering from the crystalline region of the drug. The major difference in the infrared (IR) spectra among the three forms of CEZ was the absence of a spectral band at 1542 cm^{-1} in the amorphous form. The IR procedure was based upon the measurement of the peak percentage area ratio between the bands at 1542 and 1760 cm^{-1} , where the latter was used as a normalizing peak. The degree of crystallinity of CEZ samples, obtained by either freeze-drying aqueous CEZ solutions or storing the crystalline forms under different humidity conditions, was determined by these two methods. Although the correlation of results by the two methods was good, the X-ray procedure appears to be superior since it can differentiate among the three solid CEZ forms, whereas IR could distinguish between only crystalline and amorphous CEZ, reproducibly.

KEY WORDS: cefazolin; crystallinity; X-ray diffractometry; infrared (IR); freeze-drying.

INTRODUCTION

Hygroscopicity behavior of hydrates of drugs and other organic compounds has been reported in the literature (1,2). The environmental humidity has been shown to be an important determinant in dehydration and rehydration of such compounds. Pikal *et al.* (3), using solution calorimetry, reported that the desolvation of cephalosporin hydrates in either amorphous or solvated forms results in a high-energy amorphous state. They also reported, using X-ray diffractometry, a loss of crystallinity in cefamandole sodium monohydrate and cefazolin sodium pentahydrate. In the case of cefazolin sodium (CEZ), the loss was reversible, since structural changes occurring on dehydration were completely recovered after rehydration.

CEZ can be prepared as different crystalline hydrates, viz., the pentahydrate (α form), the monohydrate, the ses-

quihydrate, and the amorphous form (4–6). The hydrates can be desolvated under low relative humidity conditions to yield crystalline dehydrated forms (7). Gatlin *et al.* reported on the phase transitions in frozen CEZ solutions (6) and Koyama *et al.* showed that this solid-state transition during freeze-drying could be enhanced by thermal treatment and the use of isopropyl alcohol as a cosolvent (8).

During secondary drying the relative humidity in a freeze-dryer is extremely low, and hence it is possible that a drug hydrate may lose water of crystallization. In an earlier study it was shown that both freeze-drying and subjecting CEZ to varying relative humidity conditions resulted in transition to either the α form, the dehydrated α , or the amorphous form (7). The purpose of this study was to assess the use of X-ray diffractometry and infrared (IR) spectrophotometry in quantitating the various forms of CEZ which result during freeze-drying or storage under various relative humidities. Because of the ease at which CEZ undergoes dehydration at low relative humidities, it is important to examine the effect of the removal of water of crystallization on the overall crystallinity of freeze-dried CEZ.

The methods used to quantitate or characterize the crystallinity of solid drugs include infrared spectrophotometry (9,10), solution calorimetry (3), suspension density (11), and X-ray diffractometry (10,12). The X-ray and IR methods have been used to distinguish solvates and anhydrous forms of compounds because of the differences in crystal lattice structures which give rise to characteristic X-ray reflections

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and also due to the addition of new stretching frequencies in the infrared spectra resulting from solvation (13).

MATERIALS AND METHODS

Materials

CEZ Pentahydrate (α Form). The α form was prepared by crystallization of CEZ, obtained from Smith Kline and French Laboratories (Philadelphia, Pa.), by the method of Kariyone, *et al.* (4). The water content, determined by Karl Fischer titration, was $15.9 \pm 1\%$ (w/w). Distinct birefringence was observed when viewed under polarized light with an optical microscope (Zetopan, Reichert).

Dehydrated α Form. The dehydrated form was prepared by drying the α form at 23°C under vacuum ($80 \mu\text{Hg}$). The water content was about $0.5 \pm 0.1\%$ (w/w). This form showed birefringence when viewed microscopically under polarized light.

Amorphous CEZ. The amorphous form was prepared by rapidly freezing a solution of CEZ in Freon 12 (Racon Co., Kansas) and subsequently freeze-drying the frozen CEZ spheres. The water content of the dried amorphous form was about $0.5 \pm 0.1\%$ (w/w). This form was completely nonbirefringent when viewed under polarized light. Amorphous CEZ was also obtained when crystalline CEZ was manually ground vigorously in a ceramic mortar for 5 min.

Crystallinity Standards. Crystalline and amorphous CEZ, as described above, was mixed in glass vials to obtain standards of different crystallinity (0, 20, 40, 60, 80, and 100%, w/w).

Preparation of Samples

Controlled Humidity Samples. The CEZ α form and dehydrated α form were stored in closed desiccators containing saturated salt solutions at 15, 31, 42, and 75% relative humidity (RH) and 23°C for 10 days. Equilibrium water content was measured by Karl Fischer titration.

Freeze-Dried Samples. Solutions containing 18.9% (w/w) of CEZ were prepared by the method of Koyama *et al.* (8) and a volume corresponding to 1 g of cefazolin free acid was filled into 10-ml glass vials. The vials were transferred to the freeze-dryer (Model X8F12, Hull Corp.). The shelf temperature was reduced to -40°C to freeze the solution. For those batches in which thermal treatment was included, the shelf was warmed to -10°C and held at this temperature for 1 hr. Drying was carried out at a shelf temperature of either 30 or 45°C and was terminated after the product had equilibrated with the shelf temperature and held there for 3–4 hr to ensure a moisture content of about $0.5 \pm 0.1\%$ (w/w).

Evaluation Techniques

X-Ray Diffractometry. About 400 mg of CEZ sample was pressed into a concave metallic disk, 15 mm in diameter, and irradiated with CuK_α radiation using a Rigaku Denki (Rigaku, Japan) X-ray powder diffractometer. The following conditions were employed: Cu target, Ni filter, 40-kV voltage, 20-mA current, receiving slit of 0.15° , GM counter as a detector, 1-sec time constant, and $2.5^\circ/\text{min}$

scanning speed. The data were computed by a PDP-11 computer connected to the X-ray unit.

IR Spectrometry. IR spectra were recorded on a 1430-ratio recording IR spectrophotometer (Perkin Elmer, Connecticut). About 10 mg of CEZ sample was lightly triturated with mineral oil. A thin film of the triturate was applied to the NaCl cells and the IR spectra were recorded between 4000 and 200 cm^{-1} . Before each scan, the baseline was adjusted at 55% transmittance.

RESULTS AND DISCUSSION

Estimation of the Degree of Crystallinity by the X-Ray Method

Figure 1 shows the X-ray diffraction spectra of CEZ pseudopolymorphs. The intensity of the X-ray diffraction peaks was very strong for the pentahydrate obtained by recrystallization from ethanol, whereas the samples obtained by either rapid freeze-drying or manually grinding in a mortar showed diffuse and halo diffraction patterns, indicating the presence of the amorphous state. The dehydrated α form also showed very weak diffraction peaks compared to the hydrated form. However, the dehydrated form differed from the amorphous form in its birefringence behavior under polarized light.

X-Ray procedures for the estimation of the degree of crystallinity were based upon the measurement of the X-ray scattering from the entire sample and from the crystalline region of the sample. The intensity of the X-ray scattering from the crystalline region of the sample is proportional to the area under the sharp peaks above the background scat-

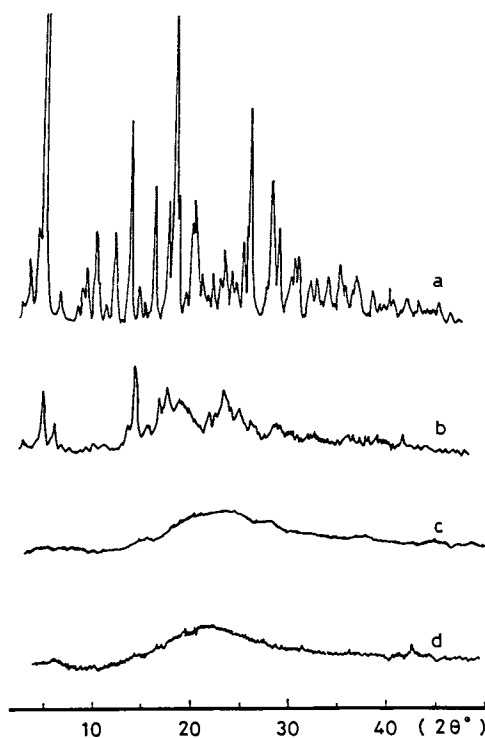


Fig. 1. X-Ray diffraction spectra of CEZ standards: pentahydrate (α form) (a); dehydrated α form (b); amorphous forms by grinding (c) and freeze-drying (d).

tering, while the intensity of the X-ray scattered from the entire sample is proportional to the total area of the X-ray pattern (10).

In this study, the diffraction peaks of the samples due to the crystalline region were separated from the diffraction scattering due to the amorphous scattering using a computer program. The X-ray diffraction due to the background air was very small and hence was neglected. The percentage ratios of the area due to the crystalline peaks above the background and total area due to the entire sample were plotted against the percentage crystallinity of standard samples. Figure 2 shows the regression lines of such plots for standard physical mixtures of the pentahydrate and amorphous forms (curve a) and dehydrated and amorphous forms (curve b). The correlation coefficients were 0.993 and 0.999, respectively. The percentage crystallinity of unknown samples obtained by freeze-drying and different storage conditions was calculated based upon these plots.

Estimation of the Degree of Crystallinity by the IR Method

The determination of crystallinity by IR spectrophotometry was based upon the measurement of intensity of a peak which is characteristic of the crystalline state with reference to a peak which is independent of the crystal state of the substance. Such procedures have been used for digoxin and chloramphenicol palmitate (10,14). Figure 3 shows the IR spectra of CEZ pseudopolymorphs. The region between 1500 and 1800 cm^{-1} was chosen for discrimination between the crystalline and the amorphous forms. The pentahydrate showed a distinct peak at 1542 cm^{-1} (amide II band) which was absent in the case of the amorphous form. The band at 1660 cm^{-1} (amide I band) for the pentahydrate was shifted to 1690 cm^{-1} in the amorphous form. Also, the overall peak sharpness was reduced in the amorphous form. The intensity of the band at 1760 cm^{-1} (β -lactam carbonyl), however, remains invariant in the crystalline and amorphous forms.

The characteristic band at 1542 cm^{-1} was found only with the crystalline forms and therefore was selected as the

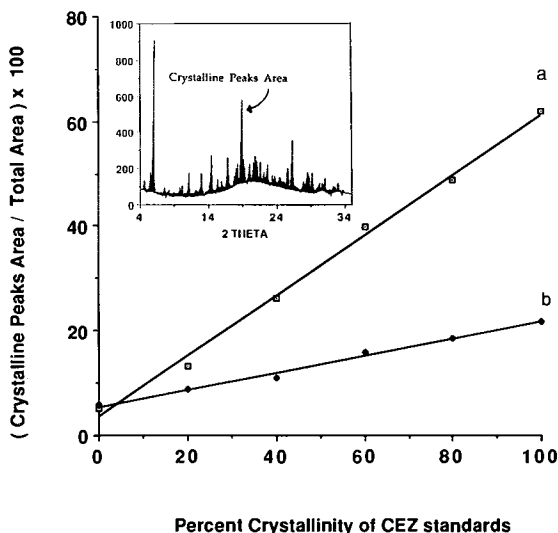


Fig. 2. Regression lines for CEZ standards as a function of percentage crystallinity using X-ray diffractometry: CEZ α form (a); dehydrated α form (b).

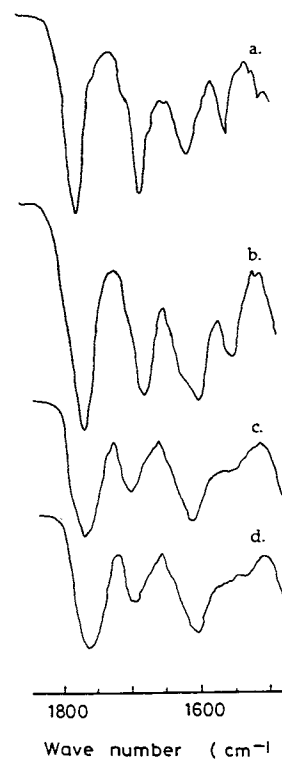


Fig. 3. Infrared spectra of CEZ standards: pentahydrate (α form) (a); dehydrated α form (b); amorphous forms by grinding (c); and freeze-drying (d).

analytical band. The intensity of this band, as reflected by the area under the peak, decreased as the percentage of the amorphous form increased. The area of this peak was normalized by taking a percentage ratio of the area of this peak to that of the peak at 1760 cm^{-1} . (The band at 1760 cm^{-1} does not change with the nature of the solid state.) The percentage ratios calculated for standard samples of physical mixtures are plotted against the percentage crystallinity in Fig. 4. A good correlation was observed, with correlation

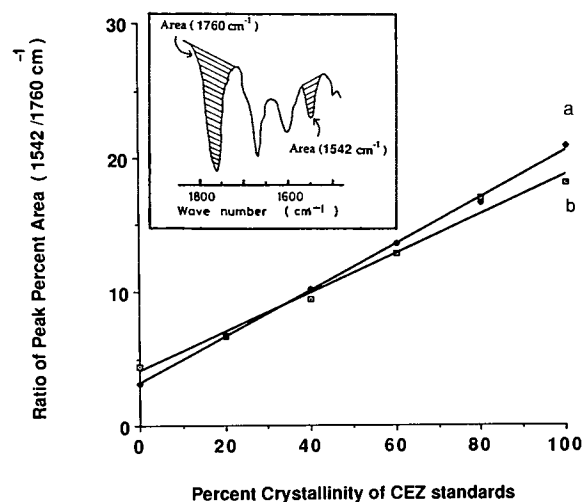


Fig. 4. Regression lines for CEZ standards as a function of crystallinity using IR spectrophotometry. Dehydrated α form (a); pentahydrate (α form) (b).

coefficients of 0.993 and 0.999, respectively, for the mixtures containing the pentahydrate and amorphous forms (curve b) and dehydrated and amorphous forms (curve a).

Degree of Crystallinity of Controlled Humidity Samples

Table I shows the degree of crystallinity of the pentahydrate and dehydrated forms when stored under different relative humidity conditions. When stored at 15% RH, the dehydration of the pentahydrate resulted in a reduction in the degree of crystallinity as reflected by weak crystalline X-ray diffractions. Based upon the intact pentahydrate as 100%, this sample was calculated as being 37.8% crystalline. However, birefringence was still observed, and although the IR peaks between 1500 and 1800 cm^{-1} became broader, the relative peak positions did not change. This suggests that even though a partial loss of crystal lattice structure and increased disorder (as seen from the X-ray pattern) were observed as the water molecules were removed during dehydration, the sample did not convert completely to the amorphous state during dehydration. Under higher relative humidity conditions (42 and 75%) the water content of the pentahydrate remained unchanged; the degree of crystallinity was calculated to be 92 and 96%.

The dehydrated form remained weakly crystalline when stored at 15% RH and became increasingly crystalline at 31, 42, and 75% RH. Under these RH conditions, the water content increased to 15.9% (w/w) at 75% RH; this is equivalent to 5 mol of water for the pentahydrate. By the X-ray method the degree of crystallinity approached 90%.

Since the IR method was based upon the relative changes in peak intensity at 1542 cm^{-1} and since this peak apparently was not affected by dehydration or rehydration of the crystalline forms, the changes in the degree of crystallinity based upon IR observations were not as sensitive as those observed by X-ray diffraction.

Degree of Crystallinity of Freeze-Dried Samples

Table II shows the results of the determination of crystallinity of CEZ when CEZ solutions (18.9%, w/w) were dried under various freeze-drying conditions. The dried samples contained less than 0.55% (w/w) water and the

Table I. The Degree of Crystallinity of CEZ Stored Under Various Relative Humidity Conditions

Sample	RH (%)	% H ₂ O ^a	X-Ray pattern	% crystallinity ^b	
				IR	X-Ray
Dehydrated α	15	3.0	Weak	84.6	21.4
	31	11.0	Medium	85.0	67.2
	42	14.5	Sharp	83.6	75.0
	75	15.9	Sharp	92.0	86.0
Pentahydrate	15	6.3	Weak	103.0	37.8
	31	14.5	Sharp	102.0	87.0
	42	15.7	Sharp	109.0	92.0
	75	15.9	Sharp	103.0	96.2

^a Karl Fischer method.

^b Based upon pentahydrate as a standard.

Table II. Estimation of the Degree of Crystallinity of Freeze-Dried Products

Batch No.	Freeze-drying		Shelf temp. (°C)	% H ₂ O ^b	% crystallinity ^c	
	Solvent ^a	TT ^a			IR	X-Ray
1	Water	—	45	0.27	87.4	79.0
2	IPA-water	+	45	0.55	105.0	106.0
3	IPA-water	+(N ₂)	45	0.41	104.0	104.0
4	Water	—	30	0.22	95.4	95.0

^a IPA, 5% (w/w) isopropyl alcohol; TT, thermal treatment for 1 hr at -10°C.

^b Karl Fischer method.

^c Based upon intact dehydrated CEZ as 100%. Correlation coefficient between the two methods = 0.990.

X-ray patterns showed the presence of the dehydrated form. IR spectra showed the band at 1542 cm^{-1} and the birefringence observations confirmed the presence of the crystalline state. The percentage crystallinity values determined by IR and X-ray methods were in good agreement.

From these results it appears that quantitative analysis of two-component physical mixtures of crystalline and amorphous forms of CEZ can be performed by X-ray and IR methods. In the case of samples stored under different humidity conditions the X-ray method was suitable to determine the percentage crystallinity, whereas the IR method did not differentiate between the intermediate dehydrated and the hydrated forms of CEZ. Nevertheless, a good correlation was observed between the IR and the X-ray methods in determining the degree of crystallinity of freeze-dried CEZ samples when the dehydrated α form was considered as the standard crystalline form.

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