[Chem. Pharm. Bull. 26(11)3457—3462(1978)]

UDC 615.453.2.011.3.073 : 548.734.3

On the Hydration States of 3-(4-Carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfophenylacetoamido)-ceph-3-em-4-carboxylate Monosodium Salt (Cefsulodin Sodium, SCE-129)

Hideo Nakamachi, Isamu Aoki, and Hiroyuki Asakawa

Chemical Research Laboratories, Central Research Division,
Takeda Chemical Industries, Ltd.[1]

(Received May 29, 1978)

3-(4-Carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium (cefsulodin sodium) was found to exist in crystals in three states of hydration. The hexahydrate was obtained by crystallization from aqueous ethanol and was dehydrated to the dihydrate and anhydrate. The properties of the crystal water in cefsulodin sodium were investigated.

Keywords——crystalline cefsulodin sodium; hydration state; interstitial property of crystal water; thermogravimetric analysis; X-ray powder diffraction analysis

3-(4-Carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium salt (cefsulodin sodium, SCE-129) is a new semisynthetic cephalosporin, which shows potent antipseudomonal activity almost comparable to that of gentamicin.[2]

Crystalline cefsulodin sodium was obtained as a hexahydrate by crystallization from aqueous ethanol. The dihydrate and anhydrate could be obtained by dehydration. The purpose of this study was to clarify the properties of the crystal water in cefsulodin sodium. Crystals with less than 6% water content were so hygroscopic that they were difficult to handle under usual atmospheric humidity. In the present paper, the weight change of cefsulodin sodium was measured with a thermogravimeter under controlled relative humidity (RH), in parallel with X-ray powder diffraction analysis with an X-ray diffractometer equipped with a high-temperature specimen holder.

Experimental

Preparation of Crystals——a) Hexahydrate: To a solution of SCE-129 (10 g) in water (40 ml) was added EtOH (40 ml) portionwise at room temperature (25°), then the mixture was allowed to stand for 30 min. Next, EtOH (20 ml) was added again portionwise and this mixture was allowed to stand for 30 min. The crystalline precipitates were collected by filtration, washed with H_2O–EtOH (1: 5) and immediately stored under 85% RH affording slightly yellowish crystals of SCE-129 hexahydrate. *Anal.* Calcd. for $C_{22}H_{19}N_4NaO_8S_2 \cdot 6H_2O$; C, 39.9; H, 4.72; N, 8.46. Found: C, 39.8; H, 4.48; N, 8.53. Water content, Calcd.: 16.3%. Found: 16.8% (determined by the Karl–Fischer method).

b) Dihydrate: The dihydrate was prepared by drying the hexahydrate in a desiccator over anhydrous $CaCl_2$ for more than 24 hr. *Anal.* Calcd. for $C_{22}H_{19}N_4NaO_8S_2 \cdot 2H_2O$; C, 44.74; H, 3.92; N, 9.49. Found: C, 44.35; H, 3.98; N, 9.37. Water content, Calcd.: 6.1%. Found: 6.8% (determined by the Karl–Fischer method). $[\alpha]_D^{20}$ +18.2° ($c=1.0$ based on anhydrate, H_2O). UV $\nu_{max}^{H_2O}$ nm ($E_{1cm}^{1\%}$): 262 (274 based on anhydrate). pK_a (COOH) 2.6.

c) Amorphous: An aqueous solution of cefsulodin sodium (200 mg/20 ml) was lyophylized to afford amorphous cefsulodin sodium.

Instrumentation——X-Ray powder diffraction patterns under atmospheric and controlled conditions were recorded with Rigakudenki X-ray diffractometers, Rotaflex (RU-3) and Geigerflex (D-3F), respectively. A commercial high-temperature specimen holder (Rigakudenki) attached to the latter diffractometer was

---

1) Location: *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.*

2) *a*) H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Morimoto, and T. Masuda, *J. Med. Chem.*, **17**, 1312 (1974); *b*) H. Nomura, I. Minami, T. Hitaka, and T. Fugono, *J. Antibiot.* (Tokyo), **29**, 928 (1976).

used to carry out measurements at various stages of heating and decreasing or increasing RH. An Omron solid-state temperature relay with a chromel–alumel thermocouple was used to control the temperature of the holder, in which the inlet voltage of the heater was set as desired using an autotransformer. The device shown in Fig. 1 was designed to control the RH of the experimental conditions at 25°. Nitrogen gas adjusted to the appropriate RH by being passed over a saturated salt solution (Table I) was introduced into the sample chamber of the holder. The RH was monitored with an Ace AY-11 hygrometer and the windows of the sample chamber were covered with Mylar film and sealed. Before measurement under each different RH condition, the specimen was stored under the desired RH until no further change took place in the X-ray diffraction pattern.

Infrared (IR) (nujol), nuclear magnetic resonance (NMR) (D_2O) and ultraviolet (UV) (H_2O) spectra were recorded on a Hitachi 215, Varian EM-390 and Perkin-Elmer 450 spectrometer, respectively.

Thermogravimetric analysis was performed with a Perkin-Elmer TGS-1 thermobalance[3] at a scanning rate of 2.5°/min or 1.25°/min under an atmosphere of dry nitrogen. The weight change under various RH stages was determined by a method similar to that described for the X-ray diffraction measurements. The RH and weight change were simultaneously charted with a 2-pen recorder.[4]

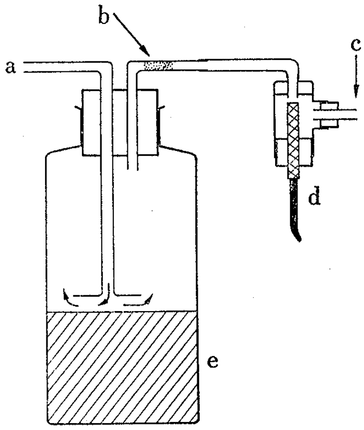


Fig. 1. Experimental Device for Humidity Control

a) Gas inlet.
b) Glass wool.
c) Gas outlet to high-temperature specimen holder.
d) Sensor of hygrometer.
e) Saturated salt solution (Table I).

Table I. Salt Solutions Used for Humidity Control

Salt	RH, %	Observed RH, %
NaOH	5	—
LiCl	13	—
KF	27	27
$CaCl_2$	31	31
K_2CO_3	41	41
$Ca(NO_3)_2$	46	44
NaBr	57	53
NaCl	75	71
KBr	85	75

RH 0%: dry N_2 gas from steel gas cylinder.

Water Content-RH Diagram——The water content of each specimen was determined by measuring the weight loss using a thermogravimeter, after storing cefsulodin sodium hydrate under the conditions shown in Fig. 8b.

Results and Discussion

Cefsulodin Sodium was found to exist as crystals in three states of hydration. The hexahydrate was obtained by crystallization from aqueous ethanol and was dehydrated to the dihydrate over anhydrous calcium chloride at room temperature. NMR and UV spectra of the dihydrate are shown in Fig. 2 and 3, respectively. Anhydrous cefsulodin sodium crystals were obtained by dehydrating of the dihydrate by gradual heating up to *ca.* 120° under dry nitrogen gas atmosphere. The crystallinity of the hexahydrate, dihydrate and anhydrate was confirmed by their X-ray diffraction patterns (Fig. 4a—c). Amorphous

---

[3] To stabilize the temperature control circuit, germanium transistors Q7 (2N1304) and Q9 (2N1302), on the integrated circuit board (219-0154) were both replaced with silicone transistors A-512, and Q8 (2N1304) with C-507, respectively. A compensate resistor of 200 Ω was connected in parallel with the resistor R11.

[4] Resistors R101 and R102 of the zero position of the recorder, Solvo/riter II, were replaced with resistors of 68 Ω.

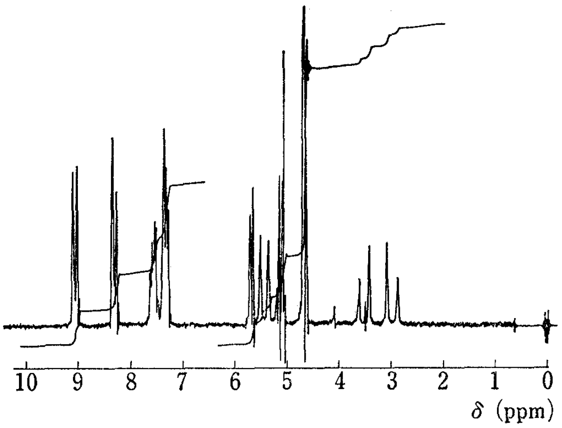


Fig. 2. Proton Magnetic Resonance Spectrum (90 MHz) of Cefsulodin Sodium Dihydrate in D_2O

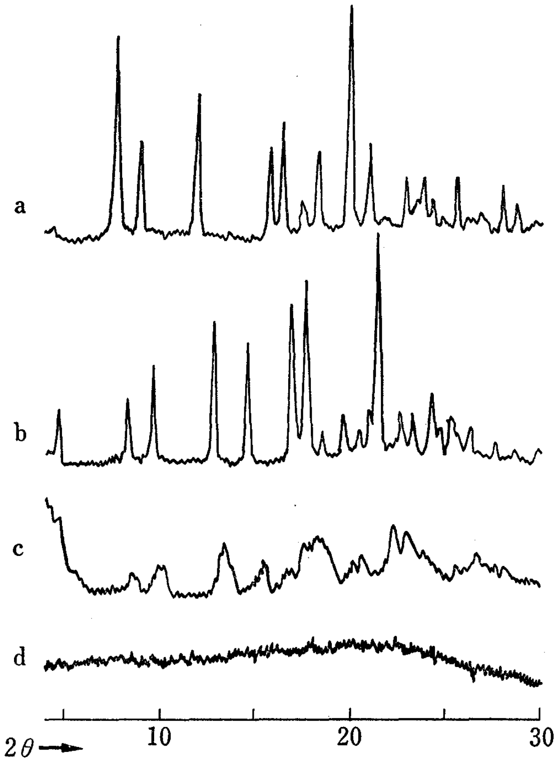
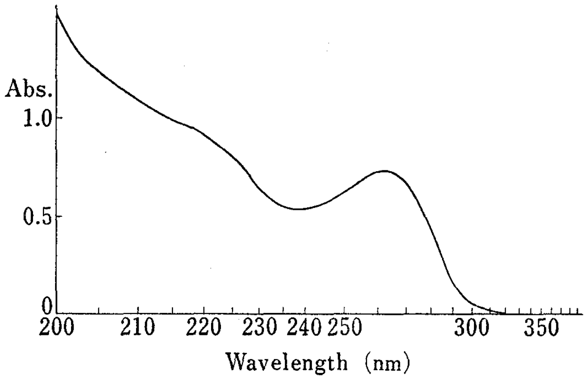


Fig. 4. X-Ray Powder Diffraction Patterns of Various Hydration States of Cefsulodin Sodium

Hexahydrate (a), dihydrate (b) and amorphous (d) cefsulodin sodium patterns were recorded using a Rigakudenki RU-3 X-ray generator with CuKα radiation (40 kV, 60 mA). The anhydrate (c) pattern was recorded using a Rigakudenki D-3F X-ray generator with CuK radiation (40 kV, 30 mA).



Fgi. 3. Ultraviolet Absorption Spectrum of Cefsulodin Sodium Dihydrate in H_2O

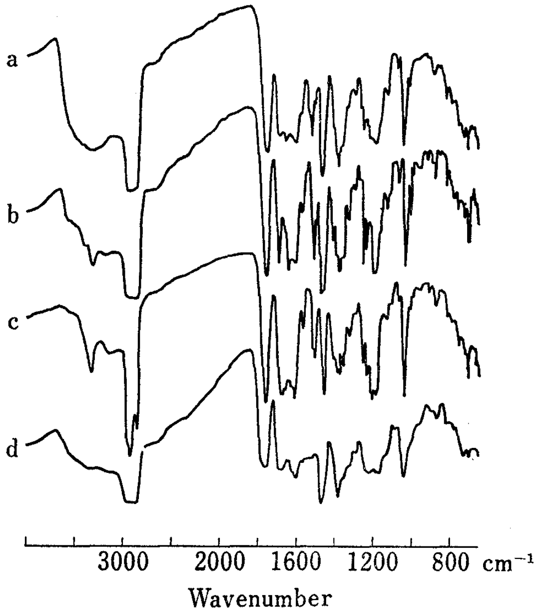


Fig. 5. Infrared Spectra of Various Hydration States of Cefsulodin Sodium

a) Hexahydrate, b) dihydrate, c) anhydrate, d) amorphous.

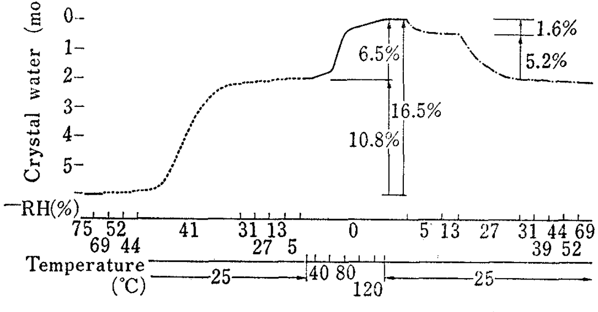


Fig. 6. Weight-change Profile of Cefsulodin Sodium Crystals during Dehydration and Hydration

cefsulodin sodium was obtained by lyophilization of the aqueous solution. Its diffraction pattern (Fig. 4d) showed only diffuse scattering. The IR spectra (Fig. 5a—d) of these crystalline and amorphous cefsulodin sodium showed apparent differences. At about 3300 $\mathrm{cm^{-1}}$ the shapes of the absorption spectra due to νNH and νOH were different. In the IR spectrum of the hexahydrate or dihydrate, absorption at 1660 $\mathrm{cm^{-1}}$ or 1640 $\mathrm{cm^{-1}}$, respectively, was observed between the amide and carboxylate absorption bands. The anhydrous and amorphous cefsulodin sodium lacked in the corresponding absorption. Differences were also observed in the absorption bands of amide (1600—1680 $\mathrm{cm^{-1}}$) and sulfonic acid (1200 $\mathrm{cm^{-1}}$), which are susceptible to the influence of crystal water.

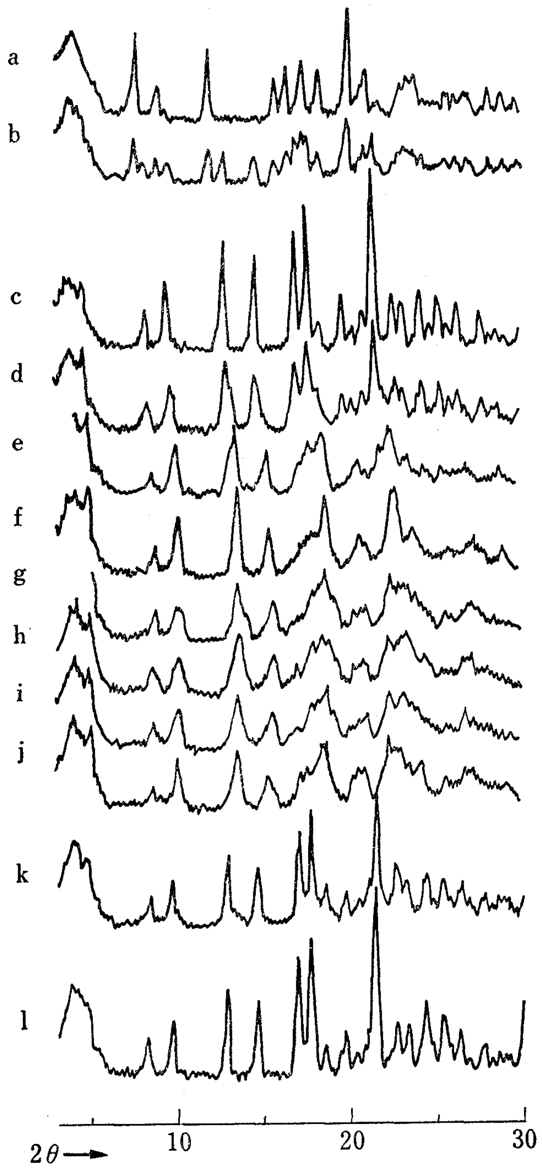


Fig. 7. Change of X-Ray Powder Diffraction Patterns of Cefsulodin Sodium during Dehydration (a—h) and hydration (i—l)

X-Ray diffraction patterns were recorned under the following conditions.

	a	b	c	d	e	f	g	h	i	j	k	l
RH (%)	75	41	0	0	0	0	0	0	0	5	27	44
T (°C)	25	25	40	45	50	80	110	130	25	25	25	25
min		30		15	20	20	20	20		10	90	

Figure 6 shows the weight change of the hexahydrate with changing RH and/or temperature. The dotted line shows the weight change at 25° during stepwise decrease of RH starting from 75%. At RH of 41%, it lost 4 mol equivalents of crystal water and was transformed into the dihydrate, whose crystal form was confirmed by X-ray diffraction. The dihydrate was stable until almost 0% RH at 25°. The solid line section indicates the weight loss of the dihydrate on heating under RH of 0%. From about 40° to 80°, it rapidly lost about 1.5 mol equivalents of crystal water and approximately 0.5 mol of water which remained was slowly lost from 80° to 120°. On cooling this specimen to 25° under RH of 0%, no weight change occurred, but as RH was increased stepwise, the weight increased in two steps as shown by the dot-dashed line in Fig. 6. Thus a weight gain of about 0.5 mol equivalent of water was observed under RH of 5% and further 1.5 mol equivalents under RH of 27% within 90 min. The X-ray diffraction pattern and IR and NMR spectra of the resulting substance confirmed that the specimen had reverted to the dihydrate form at this stage. Under 31—69% RH, this state of hydration was maintained. However, when the dihydrate was stored under RH of more than 70%, for example, at 85% RH for 40 hr, it gained about 4 mol equivalents of water and was transformed into the hexahydrate.

The change in X-ray diffraction patterns accompanying the dehydration or hydration of cefsulodin sodium crystals was recorded using a high-temperature specimen holder and is shown in Fig. 7. Table II summarizes the results of these two parallel experiments. During the dehydration of the hexahydrate to the dihydrate the specimen became a mixture of the two hydrates (Fig. 7a—c). In contrast, during the dehydration of the dihydrate (Fig. 7c—j),

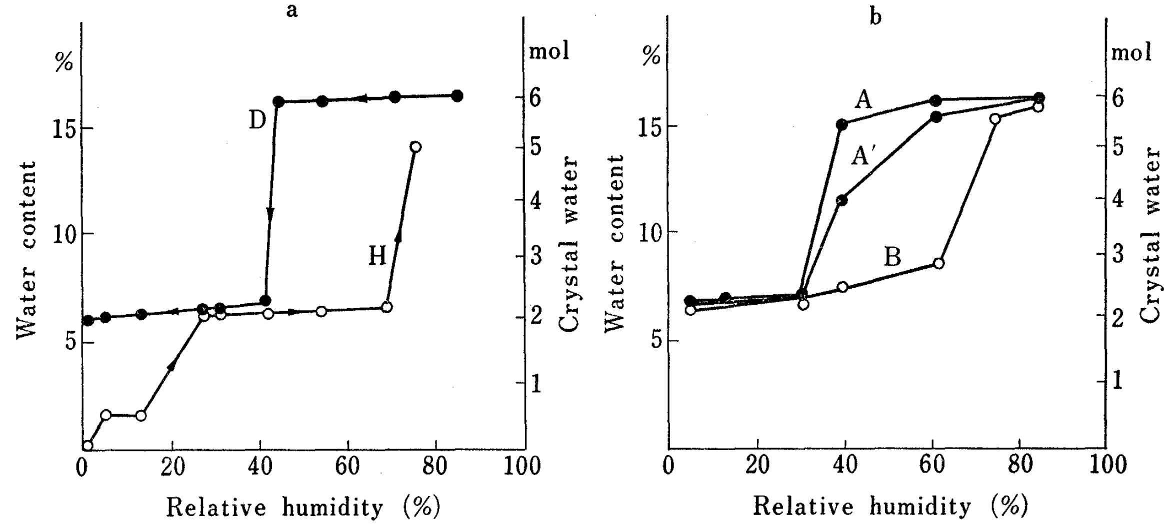


Fig. 8. Comparison of Water Content-relative Humidity Diagrams of Cefsulodin Sodium obtained by Two Methods

a) Weight change was measured with a thermogravimeter during decreasing and increasing RH. D: dehydration, H: hydration.

b) Water content was measured after storage under each RH. A: hexahynrate after storage for 40 hr, A′: hexahydrate after storage for 120 hr, B: dihydrate after storage for 120 hr.

Table II. Phase Transition during Dehydration and Hydration of Cefsulodin Sodium (SCE-129) Crystals

Stage	Conditions		Gravimetric analysis					X-ray diffraction method	Observation
	T (°C)	RH (%)	Weight change (%) obsd.	Weight change (%) calcd.[a]	Weight change (%) mol	Crystal water	Crystal form[b]	Crystal form[b]	
Dehydration of hexahydrate	25	85—44				6	A	A	X-Ray diffraction peaks of the dihydrate appeared in the X-ray diffraction pattern of the hexahydrate
		38	−10.8	−10.88	−4			A + B	
		38—0				2	B	B	
	25—45	0	−6.5	−6.10	−2	(0.5)		B	X-ray diffraction lines of the dihydrate shifted gradually toward shorter spacing
	45—80							B → C′	
	80							C′ → C	
	80—130							C	
Hydration of anhydrate	25	0—5				0		C	Interconversion between the dihydrate and the anhydrate was reversible
		5	+1.6	+1.60	+0.5			C → C′	
		5—13				0.5		C′	
		27	+5.2	+4.58	+1.5			C → B	
		27—75				2	B	B	
		85 (40 hr)	+11	+10.88	+4	6	A		

a) $4H_2O/SCE\text{-}129\cdot 6H_2O = 0.1088$, $2H_2O/SCE\text{-}129\cdot 2H_2O = 0.0610$, $1.5H_2O/SCE\text{-}129\cdot 2H_2O = 0.0458$, $0.5H_2O/SCE\text{-}129\cdot 0.5H_2O = 0.0160$.

b) Determined by the X-ray diffraction method. A, B and C mean crystal forms of the hexahydrate, dihydrate and anhydrate, respectively. C′ represents the stage of about 0.5 mol crystal water.

the diffraction lines gradually shifted toword shorter spacing with line broadening and at the last stage of the dehydration, a definite change to the X-ray pattern of anhydrous cefsulodin sodium could be seen. This anhydrous cefsulodin sodium was hydrated (Fig. 7i—l), and the sequence of changes in the X-ray diffraction patterns was the reverse of the above. These results indicate that conversion of anhydrous cefsuldoin sodium to the dihydrate and *vice versa* causes reversible expansion or contraction of the crystal lattice and no significant change in molecular arrangement. The strong hygroscopicity of cefsulodin sodium having less than 2 mol (6%) of crystal water can be attributed to the interstitial property of the water in cefsulodin sodium crystals.

In the present study, the relationship between the water content of cefsulodin sodium and RH at 25° was determined by measuring the weight change with changing RH of the atmosphere using a thermogravimeter. The water content–RH diagram obtained by this method is shown in Fig. 8a and compared with that (Fig. 8b) obtained by the measurement of water content after storage of each hydrate for several days in desiccators controlled at various RH conditions, which is the method generally used to determine the diagram. No substantial difference between Fig. 8a and Fig. 8b was found except in the dehydration process of the hexahydrate or the hydration process of the dihydrate which required a long time to reach an equilibrium. The present method is useful when the specimen is too hygroscopic to be handled under ordinary atmospheric humidity even for a short period of time. The measurement also needs only a small amount of specimen and can be completed in a short time.

Acknowledgement The authors wish to express their gratitude to Drs. E. Ohmura and M. Nishikawa for their helpful advice and encouragement.