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STABILITY OF THE MONOHYDRATE CRYSTAL FORM OF CEFAZOLIN SODIUM AS A FUNCTION OF MOISTURE

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ABSTRACT

Moisture effects on the stability of the monohydrate crystal form of cefazolin sodium were investigated. Results show that increased moisture content of this compound adversely affects the microbiological and polarographic stability at elevated temperatures. In addition, butyl rubber stoppers provided more protection against moisture than natural rubber stoppers at higher humidity conditions.

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INTRODUCTION

Several publications have appeared in the literature on the stability of cefazolin sodium[1] (sodium salt of 3-[[(5-methyl-1,3,4-thiadiazol-2yl) thio] methyl] -7-[2-(1H-tetrazol-1-yl)-acetamido] -3-cephem-4-carboxylic acid) in solution (1-3). Only one study, however, dealt with the effects of moisture on several cefazolin sodium crystal forms (3).

The object of this study was to determine the effects of moisture on the stability of the monohydrate crystal form of cefazolin sodium. Other cefazolin sodium crystal forms have been described by Kariyone et al. (3). Various moisture levels of the monohydrate polymorph were obtained by vacuum drying. Additional stability information was obtained with butyl and natural rubber vial stoppers. These have different moisture vapor transmission properties.

MATERIALS AND METHODS

The crystal form of the cefazolin sodium used was determined to be the monohydrate by X-ray powder diffraction[2] and by infrared spectroscopy[3] (Fig. 1).

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[1] KEFZOL®, Eli Lilly and Company, Indianapolis, IN. 46206

[2] Norelco X-ray Diffraction-Unit, North American Phillips, Mount Vernon, New York 10550

[3] Beckman Model IR 12, Beckman Instruments, Inc., Fullerton, Calif. 92634

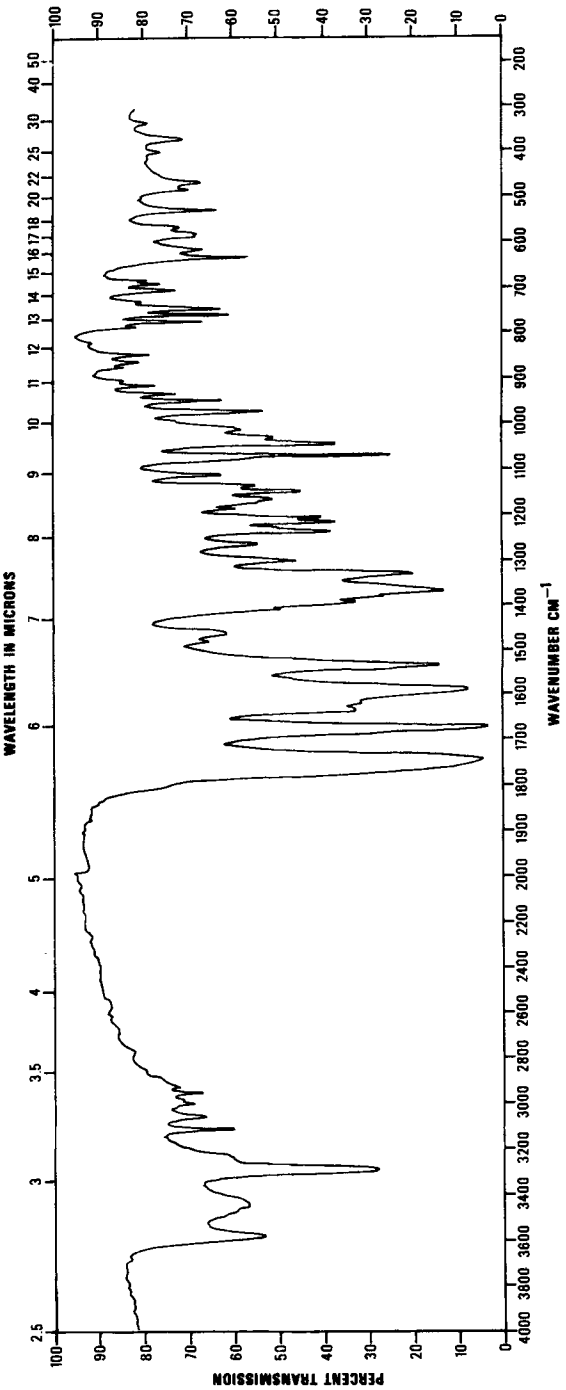

FIG. 1 Infrared spectrum of cefazolin sodium(monohydrate). (The pentahydrate form shows a broad absorption band due to water at 3350 3250 cm^{-1} and an amide carbonyl band at 1665 cm^{-1}. The monohydrate form shows simple bands due to water at 3430 and 3560 cm^{-1} and an amide carbonyl band at 1685 cm^{-1}.)

Treatments of the sections investigated are presented in Table 1. Dried samples of cefazolin sodium were prepared by vacuum drying in a Virtis[4] Model 10-100 dryer below 100 microns mercury.

One gram of cefazolin activity was stored in each Type III(4) glass vial. The vials were stoppered with either butyl or natural rubber stoppers purchased from the West Company.[5]

Samples were assayed initially and stored at 5°, 25°, 37°, 50°, and 37°-75% relative humidity (R.H.). Potency was determined microbiologically (1,2,5) and polarographically (1,6) at various time intervals (Tables 2 and 3). Microbiological assays measure the activity of the compound versus the conventional Bacillus

TABLE 1

OUTLINE OF CEFAZOLIN SODIUM TREATMENTS

Section	A	B	C
Cefazolin Activity per Vial	1 g.	1 g.	1 g.
Samples Vacuum Dried		X	
Rubber Stopper Compound	Butyl	Natural	Natural

[4]The Virtis Company, Gardiner, New York 12525

[5]The West Company, Phoenixville, Pa. 19460

TABLE 2

CEFAZOLIN SODIUM MICROBIOLOGICAL STABILITY

Storage	Age	% INITIAL CEFAZOLIN ACTIVITY		
		A	B	C
5°	24 Mo.	101	99	99
25°	3 Mo.	93	97	98
	6 Mo.	98	99	97
	12 Mo.	100	99	98
	18 Mo.	100	99	98
	24 Mo.	99	100	97
37°	3 Mo.	95	99	96
	6 Mo.	96	100	95
	12 Mo.	95	99	94
50°	3 Mo.	89	100	87
	6 Mo.	80	100	77
	12 Mo.	54	87	51
37°-75% R.H.	3 Mo.	95	98	93
	6 Mo.	94	99	87
	12 Mo.	94	89	77

TABLE 3

CEFAZOLIN SODIUM POLAROGRAPHIC STABILITY

Storage	Age	% INITIAL CEFAZOLIN ACTIVITY		
		A	B	C
5°	12 Mo.	105	101	98
	24 Mo.	106	102	96
25°	6 Mo.	105	100	97
	12 Mo.	97	98	96
	24 Mo.	104	101	96
37°	12 Mo.	95	99	92
50°	12 Mo.	51	88	48

subtilis (ATCC 6633) test organism. Polarographic assays measure the reduction of the thiadiazole group at the 3 - position. The method is based on measurement of the reduction wave generated at a half-wave potential of -0.63 volts. This method therefore measures the integrity of the thiadiazole side chain (Fig. 2).

Six replicates were obtained for initial microbiological assays with two to four replicates performed at subsequent assay intervals. The percent relative standard deviation for the cylinder plate assay as performed in this study is approximately ± 4.8% for 95% confidence limits about the mean. Polarographic assays consisted of three replicates on initial samples with one replicate determination for all following assays. The percent relative standard deviation for the polarographic assay as performed in this study is approximately ± 2.0% for 95% confidence limits about the mean. Moisture content of intact vials was obtained by the Karl Fischer (4) titrimetric method (Table 4). The pH was determined on 10% w/v cefazolin sodium

CHEMICAL STRUCTURE OF CEFAZOLIN SODIUM

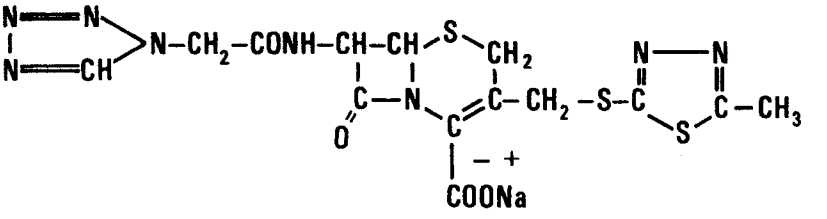

FIG. 2 Chemical structure of cefazolin sodium

TABLE 4

CEFAZOLIN SODIUM KARL FISCHER MOISTURE DATA

Storage	Age	% WATER		
		A	B	C
	Initial	3.90	1.57	4.30
5°	24 Mo.	4.52	3.65	5.07
25°	12 Mo.	3.96	2.75	4.44
	24 Mo.	4.40	3.91	4.92
37°-75% R.H.	3 Mo.	4.78	3.88	4.87
	6 Mo.	3.94	4.57	4.63
	12 Mo.	3.95	4.32	5.56

solutions using a Corning pH meter[6] (Table 5). Spectrophotometric[7] color determinations at 410, 450, and 490 nm., nephelometric[8] clarity readings, thin layer chromatograms[9] (TLC) (7), X-ray diffractograms (8), and infrared spectra (3) were obtained in addition to the potency data.

RESULTS AND DISCUSSION

Cefazolin sodium is adequately stable for two years[10] when stored in suitable containers at room temperature (25°). This

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[6]Corning Scientific Instruments, Model 7 pH Meter, Medfield, Mass. 02052

[7]Hitachi Perkin-Elmer, 139 UV-Vis Spectrophotometer, Arthur H. Thomas Company Scientific Apparatus, Philadelphia, Pa. 19105

[8]Coleman Photo-Nephelometer, Model 7, Coleman Instruments, Div. Perkin-Elmer Corp., Oakbrook, Ill. 60521

[9]Drummond 10 Ml. disposable pipettes for spotting Brinkman Silica Gel F254 Plates. Solvent System - 50 (ethylacetate): 20 (acetone): 10 (glacial acetic acid): 10 (purified water). Sample and standard spotted at 25 mg./ml. level.

[10]Unpublished data submitted to the Food and Drug Administration.

TABLE 5

CEFAZOLIN SODIUM pH STABILITY

Storage	Age	pH		
		A	B	C
	Initial	5.50	5.70	5.49
5°	12 Mo.	5.39	5.56	5.27
	24 Mo.	5.11	5.30	4.69
25°	3 Mo.	5.38	5.60	5.20
	6 Mo.	4.76	5.00	4.70
	12 Mo.	5.27	5.53	5.11
	18 Mo.	5.05	5.36	4.80
	24 Mo.	4.81	4.96	4.67
37°-75% R.H.	12 Mo.	4.75	4.86	5.05

study evaluates stability of cefazolin sodium in one crystal form as a function of moisture.

Drying (Table 1) did not result in a change in the monohydrate crystal structure when evaluated by X-ray powder diffraction.

Table 1 presents the treatments performed on cefazolin sodium. Data for 24 month 5° and 25° samples and 12 month 37° samples show good microbiological and polarographic stability in all sections tested (Tables 2 and 3). The vacuum dried Section B, which contained less than 2% moisture initially, retains approximately 87% of initial potency when stored at 50° for 12 months. In contrast, non-dried samples containing approximately 4% water (Sections A and C) stored

at identical conditions retained only about 50% of their initial potency.

The stability effects of stopper-related moisture vapor transmission (MVT) was investigated by stoppering vials in Section A with butyl rubber stoppers and by using natural rubber closures for vials in Section C. The MVT of natural rubber is generally much greater than the MVT of butyl rubber (9). The storage condition that gave evidence of decreased potency for vials stoppered with natural stoppers as compared to butyl stoppers was 37° - 75% R.H. A 1.26% increase in moisture at 12 months for samples stoppered with natural stoppers under this condition led to a 23% loss of microbiological potency. A negligible increase in moisture for butyl stoppered vials resulted in only a 6% loss in potency at 12 months (Table 2 and 4, Sections A and C).

All 25° stability samples tested remained acceptable with respect to color, clarity, TLC, X-ray, and infrared spectroscopy.

CONCLUSIONS

Moisture effects on the stability of the monohydrate crystal form of cefazolin sodium were investigated. Results show that increased moisture content of this crystal form affects the microbiological and polarographic stability at elevated temperatures.

In addition, butyl rubber stoppers provided more protection against moisture than rubber stoppers at higher humidity conditions.

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