

CEPHALOSPORIN SALTS AND INJECTABLE COMPOSITIONS

Technical Field

This invention is directed to temperature stable semi-synthetic cephalosporin salts whose preparation has not been described in the literature, to the preparation of such salts, and to admixtures containing these salts.

Brief Description of the Drawing

5 Figure 1 is a graphical representation of the infra red absorption spectrum of crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate sulfate salt measured on a KBr dilution thereof.

10 Figure 2 is a graphical representation of the infra red absorption spectrum of the crystalline sesquiphosphate salt of 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate measured on a KBr dilution thereof.

15 Figure 3 is a graphical representation of the infra red absorption spectrum of the crystalline diphosphate salt of 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate measured on a KBr dilution thereof.

20 Figure 4 is a graphical representation of the infra-red absorption spectrum of the crystalline dihydrochloride monohydrate of 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate.

Background Of The Invention

Aburaki et al. U.S. Patent No. 4,406,899 discloses 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoximinoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate in the zwitterion form and mentions corresponding acid addition salts (which are present in the zwitterion form in injectable compositions) and shows that the zwitterion form has broader spectrum activity than ceftazidime and cefotaxime.

However, the aforementioned Aburaki et al. cephalosporins are stable only for a few hours as injectable compositions and the zwitterion form even as a dry powder is unstable at room temperature and loses 30% or more of its activity on storage at elevated temperatures (e.g. 45 deg. C and above) for even one week and therefore requires special insulated packaging and/or refrigeration and is at a packaging and storage disadvantage compared to ceftazidime and cefotaxime.

While Aburaki et al. mentions acid addition salts, the patent does not state how to make these or state which if any of these salts have good stability in dry powder form. Kessler et al., "Comparison of a New Cephalosporin, BMV 28142, with Other Broad-Spectrum β -Lactam Antibiotics", Antimicrobial Agents and Chemotherapy, Vol. 27, No. 2, pp. 207-216, February 1985 mentions the sulfate salt, but does not disclose how to prepare such or that this salt has room temperature stability and good elevated temperature stability in dry powder form.

Summary Of The Invention

It has been discovered herein that certain crystalline acid addition salts of 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate in dry powder form have excellent room temperature stability and have superior elevated temperature stability compared to the zwitterion form. The term "dry powder form" as used herein means a moisture content of less than 5% by weight when measured by loss in weight on drying at atmospheric pressure and a temperature of less than 70°C.

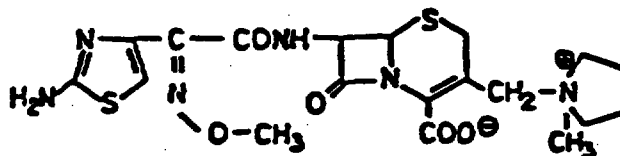
These addition salts are the crystalline dihydrochloride hydrates of 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate. In particular, these acid addition salts are the crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methylpyrrolidinio)methyl]-3-cephem-4-carboxylate dihydrochloride hydrate containing from 2.5% to 7.0% by weight of water; and the crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methylpyrrolidinio)methyl]-3-cephem-4-carboxylate dihydrochloride monohydrate containing from 2.5% to 4.1% by weight of water. Also included is the crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methylpyrrolidinio)methyl]-3-cephem-4-carboxylate dihydrochloride monohydrate having the following X-ray powder diffraction pattern :

X-RAY POWDER DIFFRACTION
Dihydrochloride Monohydrate

	d	I/I₀ (%)
5	10.21	100
	8.62	13
	6.78	23
	6.28	9
	5.84	9
	5.12	4
	5.01	9
10	4.93	5
	4.74	38
	4.62	4
	4.50	4
	4.44	4
	4.26	32
	4.10	4
	3.95	33
15	3.90	28
	3.78	39
	3.64	5
	3.59	13
	3.48	10
	3.39	15
	3.32	10
20	3.21	10
	3.11	10
	3.04	5
	2.99	13
	2.93	15
	2.76	5
	2.63	10
	2.51	10
25	2.43	5
	2.38	7

The term "crystalline" is used herein to mean at least some characterizing arrangement of molecules.

The acid addition salts herein when formed into aqueous injectable compositions and adjusted to pH 6.0 provide the zwitterion in solution. The zwitterion has the structure



5 The broad spectrum utility against various organisms of the zwitterion form, and thus of aqueous compositions made up from the salts herein, is shown by the data in Aburaki et al. U.S. 4,406,899.

10 Aqueous compositions made up from the acid addition salts herein simply by the addition of sterile water provide acidic solutions which provoke unacceptable irritation on intravenous
15 administration to rabbits and unacceptably painful sensation on intramuscular administration to rabbits. The sulfuric acid and di-nitric acid addition salts have reduced solubilities which are insufficient for typical injectable compositions. It has been
20 found herein that these objectionable characteristics are overcome by utilizing the salts herein in physical admixture (that is as an admixture of solids) with a pharmaceutically acceptable non-toxic organic or inorganic base in proportions to provide a pH of about 3.5 to about 7 on dilution with water to a
25 zwitterion activity of from 1 mg/ml to 400 mg/ml, normally 250 mg/ml (as determined by high performance liquid chromatography, hereinafter HPLC).

Detailed Description

- The crystalline salts herein (hereinafter referred to simply as the salts herein) have excellent stability at room temperature and have a potency loss (as determined by HPLC), of less than 1% on storage for a month at room temperature. These salts also have excellent stability at elevated temperatures and have a potency loss (as determined by HPLC) of less than 15% on storage for a month at 45-56 deg. C.
- 10 The di-hydrochloric addition salts have water solubilities greater than 200 mg/ml., and therefore are preferably crystallized from organic solvents, rather than from water, in order to obtain good yields.

5

The salts herein are formed into injectable compositions by diluting with sterile water and buffering to a pH of 3.5-7 to form an injectable concentration of 1 mg/ml up to 400 mg/ml of zwitterion. Suitable buffering agents include, for example, 10 trisodium orthophosphate, sodium bicarbonate, sodium citrate, N-methylglucamine, L(+) lysine and L(+) arginine. For intramuscular or intravenous administration to an adult human, a total dosage of from about 750 to about 3000 mg per day in divided doses is normally sufficient.

15

The salts herein are not desirably formed into injectable compositions simply by the addition of sterile water

20

because the salts herein when dissolved provide very low pH compositions (1.8-2.5) which provide painful sensation on injection. As indicated above, it has been found herein that these shortcomings are overcome by forming the salts herein into a physical, i.e. solid, admixture with pharmaceutically acceptable, normally solid non-toxic organic or inorganic bases 25 in proportions to provide a pH ranging from about 3.5 to about 7, preferably from about 4 to about 6, on dilution of the admixture with water to injectable concentration of 1 mg/ml up to 400 mg/ml of zwitterion, e.g. zwitterion activity of 250 mg/ml as determined by HPLC assay.

30

The exact proportions of ingredients in the physical admixture vary from lot to lot of the salt since the purity of the salt varies from lot to lot. The proportions of ingredients

are established for a particular lot by pretitrating in respect to a sample to obtain a selected pH within the aforementioned range.

5 The physical admixture is readily stored and shipped in solid form thereby taking advantage of the stability of the salts herein and is readily converted into an injectable composition simply by addition of water, e.g. by a nurse or doctor just prior to use.

10 The physical admixture is prepared by blending the salt and the base into a uniform blend, e.g. utilizing a standard blender in a dry atmosphere, and is then preferably filled into a vial or other container, all under aseptic conditions.

15 The bases for use in the admixture include, for example, trisodium orthophosphate, sodium bicarbonate, sodium citrate, N-methylglucamine, L(+) lysine and L(+) arginine. L(+) lysine and L(+) arginine are preferred since admixtures containing these are reconstituted to provide injectable compositions which on injection provide less pain in animals than compositions derived from admixtures containing other bases. The L(+) arginine is
20 very preferably utilized in a proportion to provide a pH of 3.5-6 on dilution of the admixture with water to provide a composition with a zwitterion activity of 250 mg/ml (as determined by HPLC assay).

25 The salts herein and substantially dry physical admixtures containing them can be stored without refrigeration or insulated packaging and still retain high potency.

In several of the preparations herein the unstable zwitterion is used as the starting material. The preparation of this is described in Examples 1-3 of Aburaki et al. U.S.
30 4,406,899. The zwitterion is referred to in Aburaki et al. as

7-[(2)-2-methoxyimino-2-(2-aminothiazol-4-yl)acetamido-3-[(1-methyl-1-pyrrolidinium)methyl]-3-cephem-4-carboxylate.

The invention is illustrated in the following working examples.

Comparative Example I

Preparation of the Sulfuric Acid Addition Salt

1.5 g of zwitterion are added slowly to 10 ml of rapidly stirred 1N H_2SO_4 (1.59 molar equivalents) at 20-26 deg. C. A solution is obtained. Crystallization is then induced by seeding with crystalline sulfuric acid addition salt and the crystalline mass is slurried for 0.5 hours. The crystals are then separated by vacuum filtration, washed with 3 ml of 50% acetone/water (V/V) and with two 5 ml portions of acetone, and vacuum dried at 40-50 deg. C overnight.

A typical yield is 1.3 g of sulfuric acid addition salt. Analysis: Calculated for $C_{19}H_{24}N_6O_5S_2 \cdot H_2SO_4$: %C, 39.44; %H, 4.53; %N, 14.52; %S, 16.62; H_2O , none. Found: %C, 38.91; %H, 4.57; %N, 14.64; %S, 16.71; H_2O , 1.42.

Comparative Example II

Preparation of the Sulfuric Acid Addition Salt

1.5 g of zwitterion are dissolved in 5 ml of water. 5 ml of 1M H_2SO_4 are slowly added to this solution with stirring. Crystallization is then induced by seeding with crystalline acid addition salt and the crystalline mass is slurried for 0.5 hours. The crystals are then separated by vacuum filtration, washed with 3 ml of 50% acetone/water (V/V) and with two 5 ml portions of acetone, and vacuum dried at 40-50 deg. C overnight.

The typical yield is 1.3 g of sulfuric acid addition salt.

Comparative Example III

Preparation of the $(\text{HNO}_3)_2$ Acid Addition Salt

300 mg of zwitterion are dissolved in 2N nitric acid (0.5 ml). The solution is rubbed with a glass rod, diluted with 2-propanol (0.4 ml) and cooled. The crystalline title compound is collected and is sequentially washed with 0.4 ml of 2-propanol H_2O (1:1), 2-propanol and then ether to afford 127 mgs of the dinitrate salt.

Analysis: Calculated for $\text{C}_{19}\text{H}_{24}\text{N}_6\text{O}_5\text{S}_2 \cdot 2\text{HNO}_3$: ZC, 37.62; ZH, 4.32; ZN, 18.47; ZS, 10.57. Found: ZC, 36.92; ZH, 4.10; ZN, 18.08; ZS, 10.67; (H_2O content 0.90%).

Comparative Example IV

Preparation of the Monohydrochloride Acid Addition Salt

1 g of zwitterion is dissolved in 2.08 ml of 1N HCl (1 molar equivalent) at 20-25 deg. C. Thirty ml of acetone are added with rapid stirring over a 15 minute period whereby crystals form. Stirring is continued for 1 hour. The crystals are isolated by vacuum filtration, washed with 10 ml of acetone and vacuum dried at 50 deg. C for 2 hours.

A typical yield is 0.9 g of crystalline monohydrochloride salt. Analysis: Calculated for $C_{19}H_{24}N_6O_5S_2 \cdot HCl$: %C, 41.37; %H, 4.75; %N, 15.2; %S, 11.63; %Cl, 12.86. Found: %C, 39.32; %H, 4.88; %N, 13.95; %S, 11.28; %Cl, 12.44; %H₂O, 4.5. (Corrected for H₂O: %C, 41.17; %N, 14.61; %S, 11.82; %Cl, 13.03).

Comparative Example V

Preparation of the Dihydrochloride Acid Addition Salt
and Preparation of the Monohydrochloride Acid
Addition Salt From It

350 mg of zwitterion are dissolved in 2 ml of 1N-HCl. 10 ml of acetone are added to the resultant solution, with rapid stirring and over a 5 minute interval, whereby crystals form. Stirring is continued for 5 additional minutes. Then 10 additional ml of acetone are added and stirring is carried out for 0.5 hours. The crystals are removed by vacuum filtration, washed with two 5 ml portions of acetone and vacuum dried at 40-45 deg. C for 24 hours.

A typical yield is 300 mg of crystalline dihydrochloride acid addition salt. Analysis Calculated for

$C_{19}H_{24}N_6O_5S_2 \cdot 2HCl$: %C, 41.38; %H, 4.75; %N, 15.2; %S, 11.62; %Cl, 12.8. Found: %C, 40.78; %H, 4.98; %N, 14.7; %S, 11.25; %H₂O, 1.25. (Corrected for H₂O: %C, 41.1; %N, 14.88; %S, 11.39; %Cl, 11.94).

- 5 1 g of dihydrochloride salt prepared as above is slurried in 20 ml of methylene chloride at 20-25 deg. C in a sealed flask and 0.28 ml of triethylamine is added over a 15 minute interval. The crystalline mass is then slurried for 5 hours. The resultant monohydrochloride crystals are then isolated by vacuum
10 filtration, washed with two 5 ml portions of methylene chloride and vacuum dried at 50 deg. C for 2 hours. A typical yield is 800 mg.

Comparative Example VI

Preparation of the Di-orthophosphoric Acid Addition Salt

15

- 1 g of zwitterion is dissolved in 3.4 ml of 144 mg/ml H₃PO₄ (2.2 moles) at 15 deg. C. The resulting solution is suitably filtered to clarify it. 12 ml of acetone are added to the clarified solution, with rapid stirring and over a
20 10 minute period whereby crystals form. Stirring is continued for 10 minutes. Then 30 ml of acetone are added over a 10 minute period, and stirring is continued for an additional 15 minutes. The crystals are collected by vacuum filtration, washed with two 5 ml portions of acetone and two 5 ml portions of ether and dried
25 under high vacuum for 16 hours.

- A typical yield for this type of preparation was 1.1 g of crystalline di-orthophosphoric acid addition salt. Analysis: Calculated for $C_{19}H_{24}N_6O_5S_2 \cdot 2H_3PO_4$: %C, 33.72; %H, 4.47; %N, 12.42. Found: %C, 33.43; %H, 4.65; %N, 12.02; %H₂O, 1.82.
30 (Corrected for H₂O: %C, 34.0; %N, 12.2).

The sesqui-orthophosphoric acid addition salt is formed as described above except that methanol is preferably used in place of all other solvents. This is a desirable salt for pharmaceutical dosage form use.

5

Comparative Example VII

Stabilities at Elevated Temperatures

10

Elevated temperature stabilities were determined by storing the preparations in dry containers at temperatures and for time periods as denoted below and potency losses or gains were determined by HPLC. A % potency gain is indicated by a plus sign in front of a figure. A less than 10% potency loss over a 2 to 4 week period at 45-56 deg. C is usually indicative of less than 10% potency loss over a 2-3 year period at room temperature.

Form	PERCENT LOSS							
	45 Deg. C				56 Deg. C			
	(Weeks)				(Weeks)			
	1	2	4	6	1	2	4	100 Deg. C (Days)
Zwitterion	37	51	71	-	57	-	-	100
H ₂ SO ₄ Salt	2.4 to +5	3	+5	1.4	5 to +6	+3	0 to +6	0-10
(HNO ₃) ₂ Salt	8.8	3.4	0.68	10.3	3.7	2.4	-	-
HCl Salt	4.8	2.3	6.0	6.4	6.4	-	-	-
(HCl) ₂ Salt	0	-	7.4	-	0	-	7.2	12.4
(H ₃ PO ₄) ₂ Salt	0	3.0	1.0	-	2.7	5.0	-	-

Comparative Example VIII

Testing of Physical Admixtures

Physical admixtures were made up of crystalline sulfuric acid salt with (a) trisodium orthophosphate, (b) sodium bicarbonate, (c) L(+) lysine, and (d) L(+) arginine. The bases were added in proportions to provide pH's on dilution of the admixture with water to a zwitterion activity of 250 mg/ml (as determined by HPLC assay) as follows: trisodium orthophosphate (to provide a pH of 6.0); sodium bicarbonate (to provide a pH of 6.0); L(+) lysine (to provide a pH of 6.0); L(+) arginine (to

provide a pH of 6.0). Injectable compositions were made up by reconstituting with sterile water to a zwitterion activity of 250 mg/ml as determined by HPLC assay. There were no solubility problems. Injections (100 mg/kg) were carried out intramuscularly on rabbits with pain within acceptable thresholds. The least pain was with the arginine containing composition.

Similar results of good solubility and acceptable pain on intramuscular injection are obtained on use of the other salts herein in the physical admixtures with the above bases.

Figure 1 is the infra red absorption spectrum of the crystalline sulfate salt prepared as described in Comparative Example I or II pelletized in the crystalline form with potassium bromide.

The X-ray powder diffraction pattern of the crystalline sulfate salt of 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyimino-acetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]-3-cephem-4-carboxylate prepared as described in Comparative Example I or II was determined with a Rigaku Powder Diffractometer using a copper target X-ray tube, a nickel filter, and the sample contained in a glass dish. The scan rate was 2 deg./min. over the range from 5 deg. to 40 deg. and a chart was mechanically recorded to show the angles of maximum diffraction. From this the (d) spacings and relative intensities (I/I_0) were calculated. They are listed below.

d spacing (Å)

I/I₀ (%)

9.20

100

6.80

50

5.50

28

5

5.09

22

4.50

38

4.41

44

4.19

63

3.78

38

10

3.64

44

3.39

25

3.31

31

3.15

47

15

20

25

Example I

Preparation of Dihydrochloride Monohydrate
from Sulfate - Amine Process

(a) Conversion of Sulfate to Zwitterion

5 The sulfate salt prepared as described in Comparative Example 1, 300 g (0.581 mole), is added to an agitated mixture of Amberlite LA-2 (600.5 mL, 1.332 mole), Freon TF (4.5 L) and Water for Injection, USP (900 mL) and the mixture is stirred 1 h at 23°C. After phase separation, the organic layer is washed with water (225 mL) and
10 the combined aqueous phases are stirred with a solution of Amberlite LA-2 (421 mL, 0.933 mole) in Freon TF (3.6 L) for 1 h at 23°C. The aqueous phase is extracted with Freon TF (2 x 375 mL) and the residual solvent removed by vacuum. The solution is treated with decolorizing carbon (30 g) and diatomaceous filter
15 aid (24 g), and the solid removed by vacuum filtration and washed with water (420 mL).

(b) Conversion of Zwitterion to Dihydrochloride Monohydrate

20 The filtrate from part (a) above is cooled to 5°C. Aqueous hydrochloric acid (6 N; 302.4 mL, 1.82 mole) followed by acetone (3.61 L) are then added while maintaining a temperature of 5-9°C. After crystallization commences (various means for inducing crystallization may be desirable at this point such as seeding) the mixture is treated with aqueous hydrochloric acid (6 N; 216 mL, 1.3 mole). The mixture is then stirred for 10 min, and
25 treated with a further quantity of acetone (9.02 L) by addition during 1 h at 5-8°C. The slurry is stirred for 1 h at 0-5°C and the product collected by filtration, washed with acetone (2 x 750 mL), and vacuum dried to constant weight at 45 °C (20 h). The dihydrochloride monohydrate is obtained as a white crystalline
30 solid (259.7 g, 88.6% activity yield). The NMR and IR spectra

confirmed the assigned structure; purity by HPLC analysis was 100%.

Anal. Calcd for $C_{19}H_{26}N_6O_5S_2Cl_2 \cdot H_2O$: C, 39.93; H, 4.94;
N, 14.70; S, 11.22
Cl, 12.41; H_2O , 3.15
Found : C, 39.70; H, 4.80;
N, 14.64; S, 11.12;
Cl, 12.44; H_2O , 3.34

X-Ray Diffraction Pattern

The X-ray diffraction pattern of the crystalline dihydrochloride monohydrate prepared as described in the preceding Example I was determined with a Rigaku Powder Diffractometer using a copper target X-ray tube, a nickel filter and the sample contained in a glass dish. The scan rate was $2^\circ/\text{min}$ over the range from $5-40^\circ$ and a chart was mechanically recorded to show the angles of maximum diffraction. From this the (d) spacings and relative intensities (I/I_0) were calculated and are listed below:

X-RAY POWDER DIFFRACTION
Dihydrochloride Monohydrate

d	I/I₀ (%)
10.21	100
8.62	13
6.78	23
6.28	9
5.84	9
5.12	4
5.01	9
4.95	5
4.74	38
4.62	4
4.50	4
4.44	4
4.26	32
4.10	4
3.95	33
3.90	28
3.78	39
3.64	5
3.59	13
3.48	10
3.39	15
3.32	10
3.21	10
3.11	10
3.04	5
2.99	13
2.93	15
2.76	5
2.63	10
2.51	10
2.43	5
2.38	7

Example II

Preparation of Dihydrochloride Monohydrate from Sulfate - Resin Process

(a) Conversion of Sulfate to Zwitterion

5 The sulfate salt prepared as described in Comparative Example 1, (300 g, 0.518 mole), is added to a mechanically-stirred suspension of Dowex WGR resin (568.6 g, 1.762 mole) in Water for Injection, USP (690 mL) and the mixture stirred for 1 h at 20°C. (The word 'Dowex' is a registered Trade Mark). The resin is
10 a weak base epoxyamine polymer. After the mixing period, the resin is removed by vacuum filtration and washed with water (2 x 288 mL). The filtrate is treated with decolorizing carbon (15 g) and diatomaceous filter aid (7.5 g), and the solids then removed by filtration and washed with water (2 x 144 mL).

15 (b) Conversion of Zwitterion to Dihydrochloride Monohydrate

 The filtrate from (a) above is converted in the same fashion as is described in Example I (b) above. Other weak base water insoluble particulate ion exchange resins may be substituted for Dowex WGR in Example II (a). In this
20 example is Bio-Rad AG3-X4A which is a polystyrene divinylbenzene weak base resin. (The word 'Bio-Rad' is a registered Trade Mark).

 The foregoing procedures of Examples I and II routinely produce monohydrate having a water content in the range of 2.46% to 3.70% with an average value of 3.31%. The
25 value calculated from the stoichiometric formula is 3.15%. Drying at 57°C in a desiccator at reduced pressure (0.001 mm Hg) over P₂O₅ for 5 days or at reduced pressure (10 mm Hg) at 45°C for 2 days results in no loss in weight. Storage stability at 56°C for 3 weeks produced a potency loss of
30 0.6%, and was substantially improved, therefore, as compared to the anhydrate (1.25% H₂O) described in Comparative Example V hereof

(7.2% loss in 4 weeks at 56°C).

5 The dihydrochloride monohydrate of Examples I and II was further characterized by thermogravimetric analysis (TGA) and by differential scanning calorimetry (DSC). The DSC curve is characterized by an onset peak temperature (exotherm) of 196.8°C. The TGA curve is characterized by a loss in weight (water of hydration) of 3.17% over the temperature range of 40.5° to 153.3°C. The theoretical weight loss is 3.15%. The latter behavior is typical of
10 hydrates of known cephalosporin antibiotics.

15 The dihydrate of 7-[α -(2-aminothiazol-4-yl)- α -(2)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)methyl]--3-cephem-4-carboxylate dihydrochloride is prepared by the hydration of the monohydrate described in Examples I and II by exposure to air having a relative humidity in excess of 70%. While the second molecule of water of hydration is not as tenaciously held in the dihydrate as is the case of the monohydrate, a consistent composition as determined by elemental analysis is uniformly obtained, and the dihydrate
20 can be further distinguished from the monohydrate by DSC, TGA, and X-ray powder diffraction pattern.

Example III

Preparation of Dihydrochloride Dihydrate

The dihydrochloride monohydrate prepared as described in Example II is placed in a controlled humidity chamber at 80-93% relative humidity and 25-37°C for from 2 to 7 days. In each instance formation of the dihydrochloride dihydrate was established by analysis. Four samples prepared as described above revealed the following analytical results.

Analytical Results for Dihydrochloride Dihydrate

10	Analyte	Theoretical Value	Sample Number			
			8-1B	12-2	28-1	28-2
	Carbon	38.71	38.76	38.64	38.74	38.53
	Hydrogen	5.13	5.03	4.96	4.79	4.77
	Nitrogen	14.26	14.22	14.19	14.24	14.18
	Sulfur	10.88			10.66	10.22
15	Chlorine	12.03	11.90	11.84	12.12	11.90
	Water %	6.11	6.16	6.99	6.34	6.11
	DSC	endotherm°C	81.6	84.6	85.6	84.6
		exotherm°C	187.8	188.5	187.8	185.7
	TGA (% loss in weight)					
		25-68°C	2.1	2.3	2.9	3.2
		68-180°C	3.4	3.5	3.1	3.1
		Total	5.5	5.8	6.0	6.3

*Karl Fisher method

The dihydrochloride dihydrate described in Example III can be easily dried to give the same dihydrochloride monohydrate described in Examples I and II. This can be accomplished by drying in vacuo or over a desiccant such as P_2O_5 . The storage stability at elevated temperatures as measured by chemical and biological potency of the sample was similar for the monohydrate and dihydrate, but the formation of trace amounts of insoluble particles was observed with the dihydrate. Accordingly, the monohydrate carrying up to about 1% by weight of adventitious water is the preferred form (total water content ca. 2.5-4.1%). Such material when stored at 56°C for 3 weeks exhibits at least a 96% retention of potency.

CLAIMS:

1. The crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methylpyrrolidinio)methyl]-3-cephem-4-carboxylate dihydrochloride hydrate
5 containing from 2.5% to 7.0% by weight of water.
2. The crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methylpyrrolidinio)methyl]-3-cephem-4-carboxylate dihydrochloride monohydrate
10 containing from 2.5% to 4.1% by weight of water.
3. The crystalline 7-[α -(2-aminothiazol-4-yl)- α -(Z)-methoxyiminoacetamido]-3-[(1-methylpyrrolidinio)methyl]-3-cephem-4-carboxylate dihydrochloride monohydrate of Claim 1
15 having the following X-ray powder diffraction pattern:

20

25

30

35



* C L M S *

X-RAY POWDER DIFFRACTION
Dihydrochloride Monohydrate

d	I/I₀ (%)
10.21	100
8.62	13
6.78	23
6.28	9
5.84	9
5.12	4
5.01	9
4.95	5
4.74	38
4.62	4
4.50	4
4.44	4
4.26	32
4.10	4
3.95	33
3.90	28
3.78	39
3.64	5
3.59	13
3.48	10
3.39	15
3.32	10
3.21	10
3.11	10
3.04	5
2.99	13
2.93	15
2.76	5
2.63	10
2.51	10
2.43	5
2.38	7



* C L H S *

4. A physical admixture of the salt according to either of claims 1 and 2 with a pharmaceutically acceptable non-toxic organic or inorganic base in proportions to provide a pH of 3.5 to 7 on dilution of the admixture with water to
5 injectable concentration.

5. A physical admixture according to claim 4 wherein the salt and the base are present in proportions to provide a pH of 4 to 6 on dilution of the admixture with water to
10 injectable concentration.

6. A physical admixture according to claims 4 or 5 wherein the base is L(+) lysine.

15 7. A physical admixture according to claims 4 or 5 wherein the base is L(+) arginine.

8. A physical mixture of the compound claimed in claim 3 and L(+) lysine.
20

9. A physical mixture of the compound claimed in claim 3 and L(+) arginine.

25

30

10. A method for making an acid addition salt according to any one of claims 1 to 3, substantially as described in respect of any of the foregoing Examples I to III.

