



Research Article

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Solid State Characterization of Ceftiofur Sodium Polymorph

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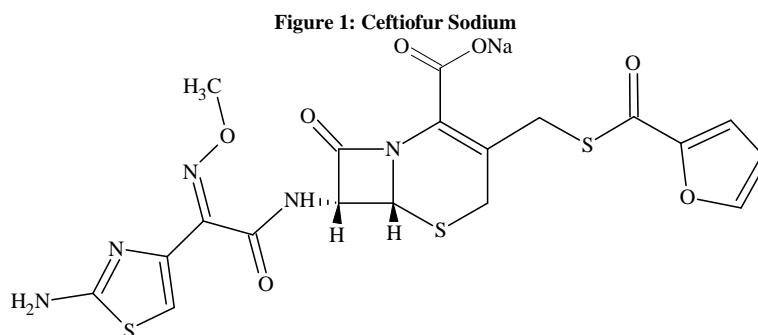
ABSTRACT

The present study deals with the solid state characterization of novel Ceftiofur Sodium having moisture content in the range of 5.0-8.0%. Here the analytical challenge is to determine the exact hydration state, i.e. Dihydrate or Hemipentahydrate or Trihydrate. Based on the results of vacuum assisted PXRD, DSC, TGA, DVS and HSM the hydration state was confirmed as trihydrate wherein one mole in the lattice, forms channel hydrate and two moles are possibly present as ion-associated..

Keywords: Ceftifour Sodium, PXRD, DSC/TGA, DVD, HSM, Hydrates

INTRODUCTION

Ceftiofur Sodium (**Figure 1**), a third-generation cephalosporin antibiotic [1], is administered as an intramuscular injection for treatment of some respiratory diseases in beef cattle, diary cattle, swine and day-old chickens, and to treat interdigital dermatitis in cattle [2]. It has also been assessed for treatment of mastitis and other septic conditions in cattle [3]. The chemical designation is [6r-[6a,7b(z)]]-7-[[[(2-amino-4-thiazolyl)(methoxyimino) acetyl] amino]-3-[[2-furanylcarbonylthio]methyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2- carboxylic acid. The sodium and hydrochloride salts are administered intramuscularly and intravenously.



Molecular Formula : $C_{19}H_{16}N_5NaO_7S_3$
Molecular Weight : 545.54

The amorphous form of Ceftiofur Sodium salts such as sodium salt and amine salt and ester forms of this cephalosporin antibiotic are somewhat unstable chemically and are difficult to purify and are less desirable to work with in manufacturing pharmaceutical formulations containing them. The amorphous salts are difficult solids to isolate in pure form and handle in pharmaceutical manufacturing plants. Hence there is a need to prepared Ceftiofur Sodium in a crystalline form. In our continued research we have identified a process for the preparation of Ceftiofur Sodium as a crystalline product, the crystalline Ceftiofur sodium prepared according to our invention is novel and having good stability over conventional amorphous product [4-6].

The present study deals with the determination of hydration state of a novel polymorph of Ceftiofur sodium using various analytical techniques like vacuum assisted PXRD, DSC & TGA, DVS (Dynamic Vapor Sorption) and Hot Stage Microscopy (HSM).

EXPERIMENTAL SECTION

Material

Ceftiofur sodium was used "as supplied" by Orchid Chemicals and Pharmaceuticals Limited, Chennai, India.

Differential Scanning Calorimetry (DSC)

Calorimetric response of the samples was measured using DSC (Perkin Elmer, Diamond DSC, USA), operating with Pyris[®] software (version 7). Prior to analysis, calibration of the instrument was performed using indium (In). Powder sample (3-5 mg) was weighed into an aluminum pan and sealed with pin-holed lid. Heating rates of 10°C/min was employed under nitrogen purge at 40 ml/min.

Thermogravimetric Analysis (TGA)

TGA was performed using Mettler Toledo 851° TGA/SDTA in pin-holed aluminium crucibles at 10 °C/min under nitrogen purging (20 mL/min). Analysis was performed using Mettler Toledo Star^e software ver. 5.1.

Microscopy

Particle characteristics of solids were assessed by optical/polarized microscopy using a Leica DMLP polarized light microscope (Leica Microsystems, Germany). Photomicrographs were acquired using RICOH XR-X3000D camera (RICOH, Japan). For hot stage microscopy (HSM), samples were mounted in silicone oil on the hot stage (Leica LMW 50, Germany) and heated from 25-350 °C. Data was analyzed using Linksys[®] software.

Powder X-Ray Diffraction (PXRD)

PXRD studies were conducted using Bruker-D8 Advance diffractometer (Bruker, The Netherlands) equipped with a 2 θ compensating slit, CuK α radiation (1.54Å) at 40 kV, 40 mA passing through Ni filter with a wavelength of 0.154 nm at 20 mA and 35 kV. Instrument was calibrated for accuracy of peak positions using corundum. Samples (100-500 mg) were placed into the sample holder and analyzed with rotation to nullify the orientation effect. Data was collected in a continuous scan mode with a step size of 0.01° and step time of 1 sec over a of 2 θ range of 3° to 40°. Analysis was performed with DIFFRAC plus EVA (version 9.0) diffraction software. For variable vacuum assisted PXRD study, sample was analyzed in a vacuum holder, supplied with the equipment. A vacuum equivalent to 10 torr was applied to the sample for 1 hour duration, followed by determination of the diffraction pattern, in the presence of vacuum.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry:

DSC observation of Ceftiofur sodium (**Figure.2**) showed two endotherms in the region of 30-70 °C, followed by a second endotherm in the region of 80-120 °C (heating rate 10 °C/min).

Thermogravimetric Analysis:

TGA observation of Ceftiofur sodium (**Figure.3**) showed an initial weight loss of 3.4%, followed by a small weight loss of 0.45%, and a subsequent weight loss of 5.1%. Calculation in terms of the molar amount of water showed that the first loss of 3.4% corresponds to loss of 1 mole of water, while the second and third step loss together (0.45% and 5.1% respectively) contribute to about 2 moles of water.

Figure 2. DSC Thermogram of Cefitour Sodium

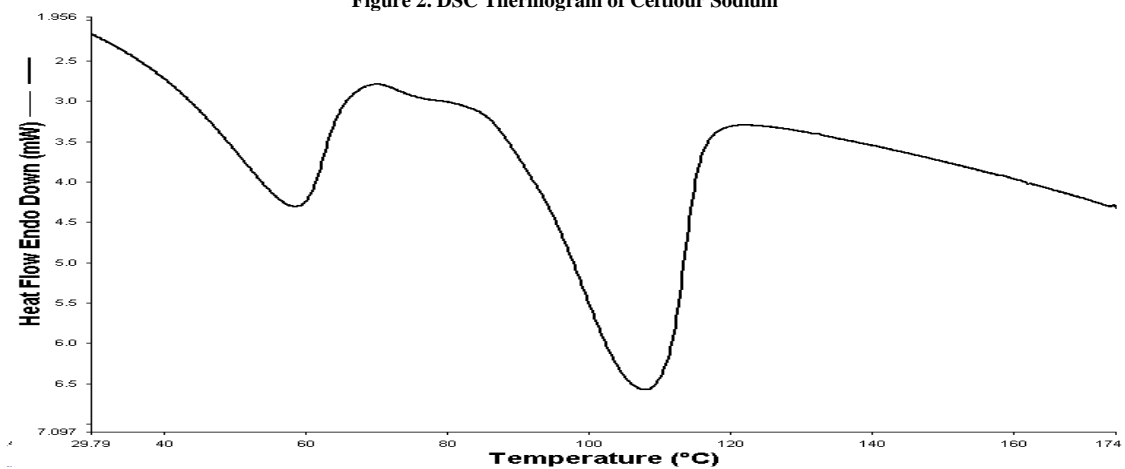
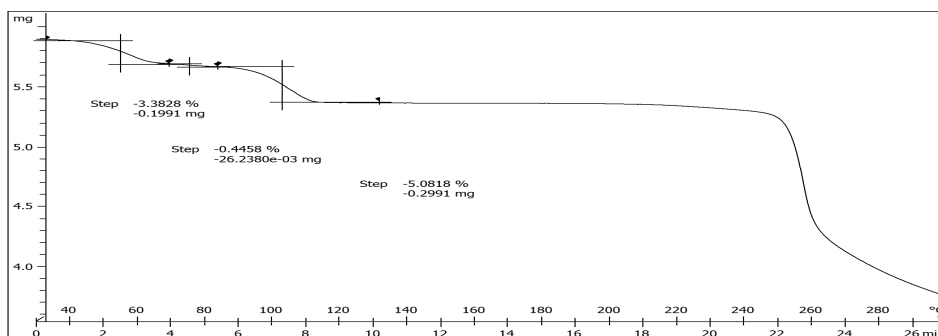


Figure 3: TGA Thermogram of Cefitour sodium



Microscopy:

Microscopic observation of Cefitour sodium showed predominantly lath shaped crystals, with characteristic birefringence (Figure.4). Birefringence confirms the crystalline nature of Cefitour sodium.

Figure 4: Optical and Polarized Light photomicrographs



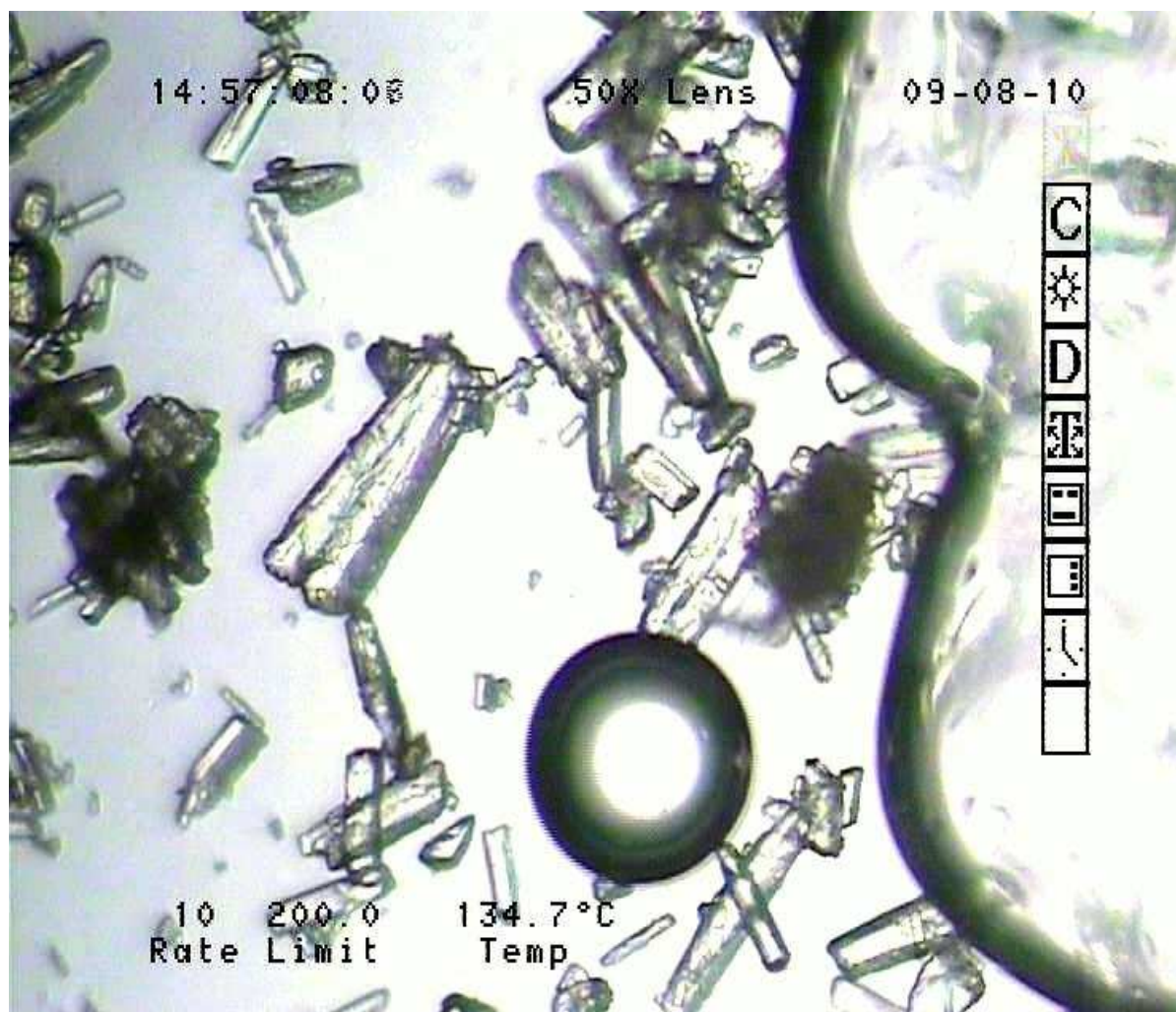
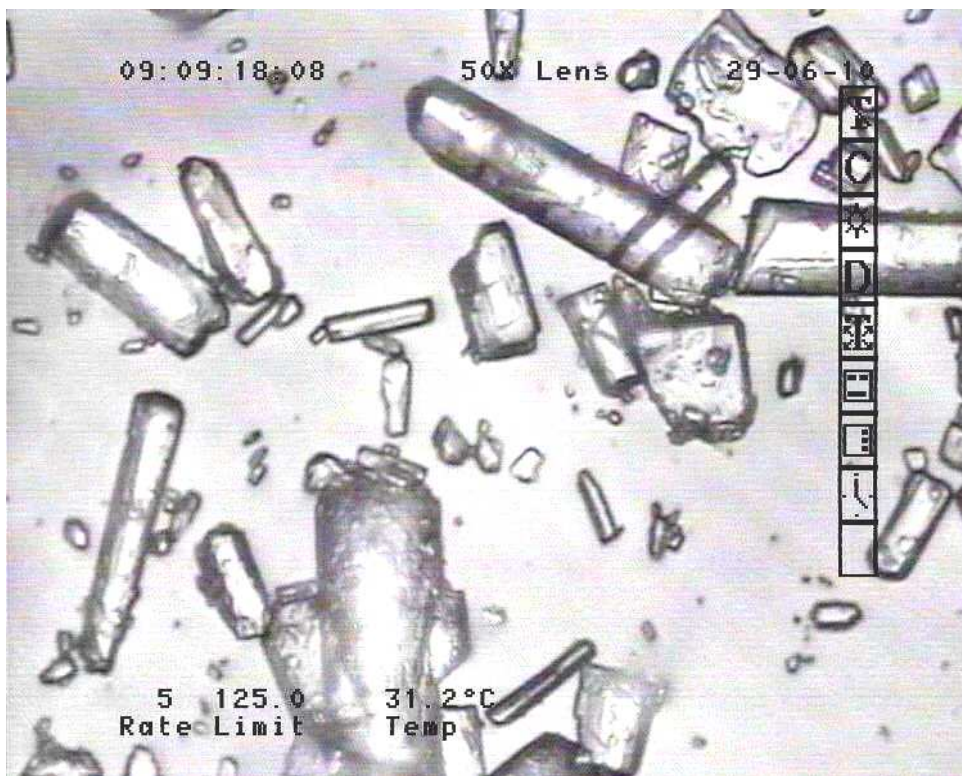


Figure 5: Optical photomicrograph showing the loss of water as bubbles from the crystal lattice at around 135 °C. After loss of water corresponding to the second endotherm observed in DSC, an amorphous solid was obtained.

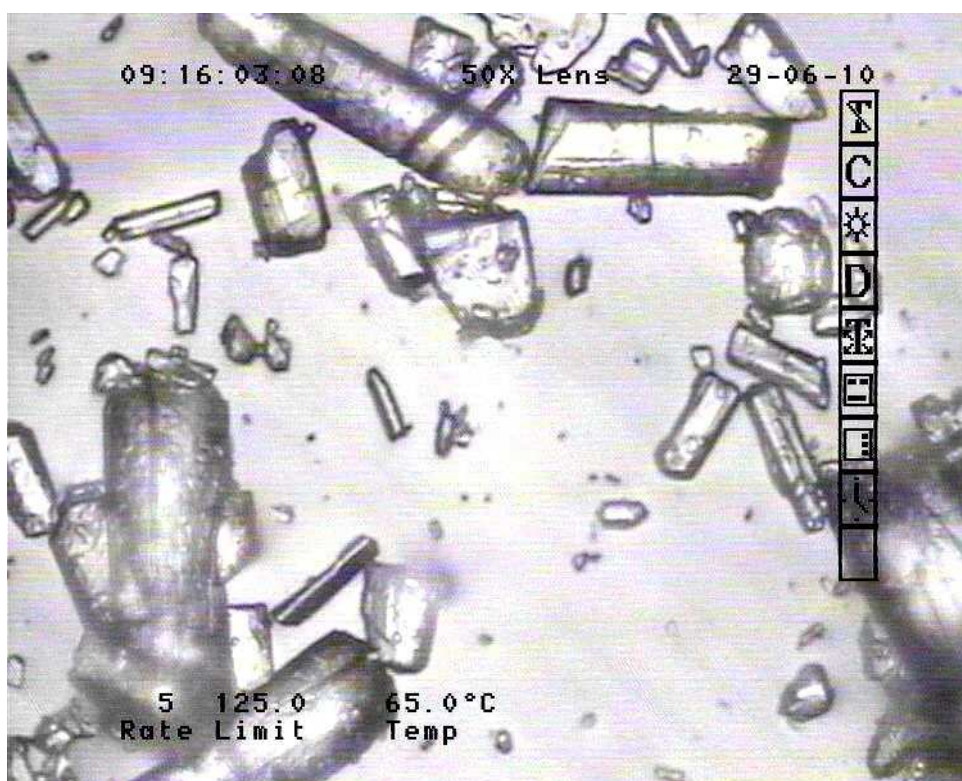
Hot Stage microscopy:

Hot stage microscopic analysis of Ceftriaxone sodium immersed in silicone oil showed no water loss corresponding to the first step loss in TGA.

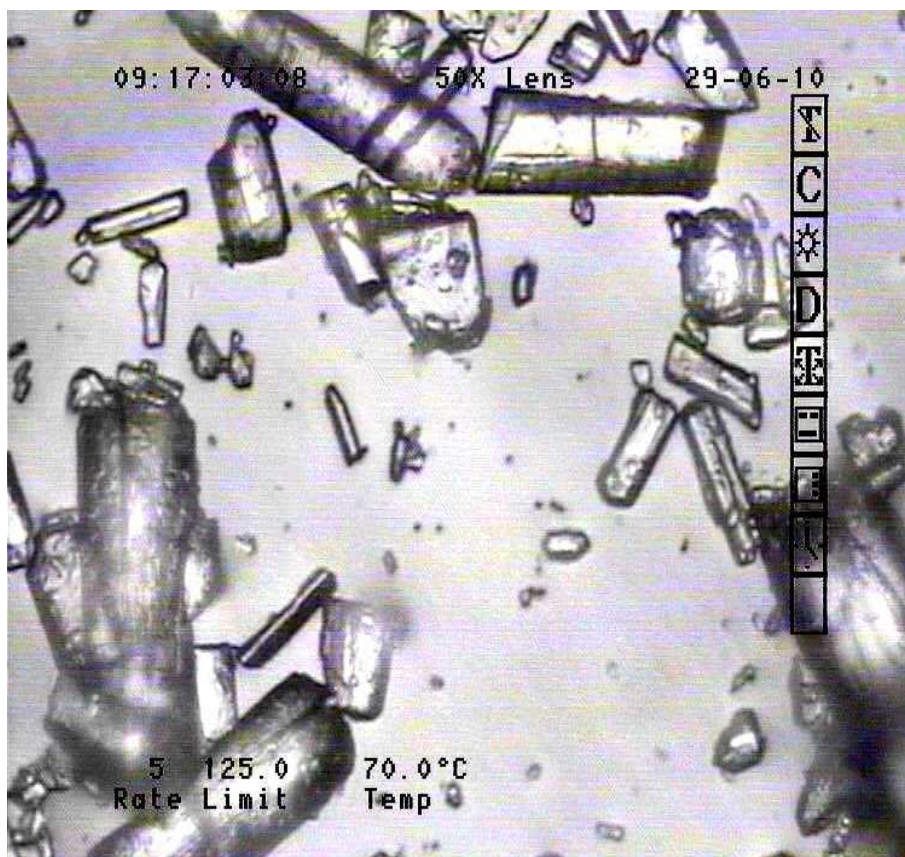
A peculiar observation during HSM was that, during heating from ambient temperature till about 80 °C, the boundaries of the crystal showed progressive darkening, towards the centre of the crystals (**Figure.5 & 6**). The temperature range of progressive darkening correlates with the first water loss observed in the DSC. Such phenomenon of progressive darkening has been reported for a class of hydrates, known as the channel hydrates. Channel hydrates show dehydration beginning at the “ends” of the crystal and continuing toward the centre along the channels. Loss of first water molecules on the surface of channel create a channel for the loss of subsequent water molecules, and set up a thermodynamic gradient in the same direction. Such kind of observation has been reported for a number of molecules, including ampicillin and theophylline [7]. This observation can be easily seen in the hot stage microscopy, wherein, the dehydration appears as a progressive darkening (increasing opacity) from the ends of the crystal toward the centre. However, water loss was observed from the crystals at a temperature of 100-140 °C corresponding to the second DSC endotherm, confirming the second step water loss to be the water of hydration. Optical photomicrograph showing the loss of water as bubbles from the crystal lattice at around 135 °C (**Figure 5**). After loss of water corresponding to the second endotherm observed in DSC, an amorphous solid was obtained.



6a



6b



6c

Figure 6: Optical photomicrographs of, at (a) 31, (b) 65 and (c) 70 °C; showing progressive darkening of crystals towards the centre (indicated by arrows). The temperature range corresponds to the first step water loss observed in the DSC thermogram.

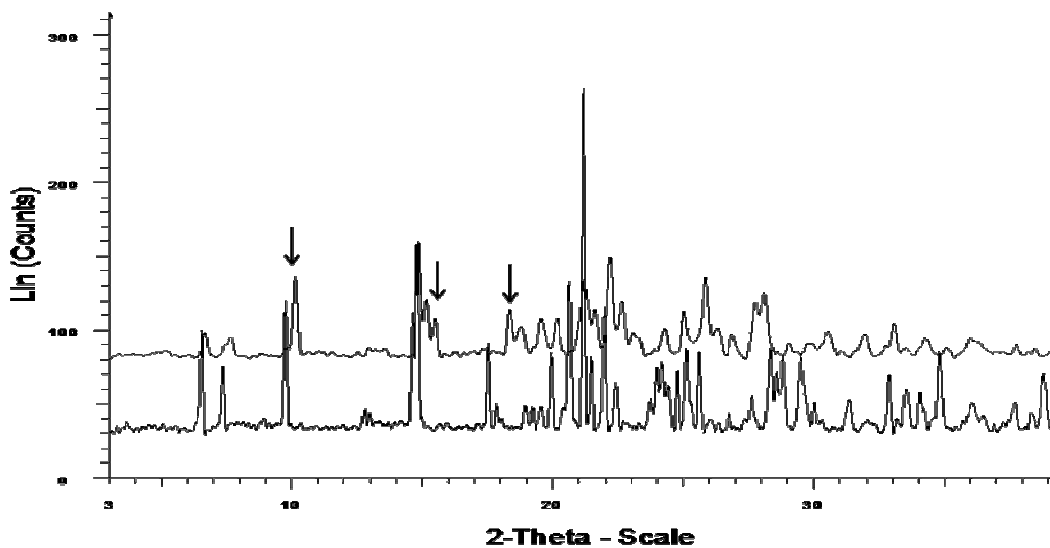


Figure 7: Vaccum assisted PXRD

After loss of water, at around 150 °C, material converted to amorphous form, devoid of any birefringence. Further heating led to charring of the crystal (decomposition) at a temperature of around 240 °C. Therefore, the first endotherm observed in DSC might be due to the loss of water present in the channels, thus indicating it to be the water of hydration.

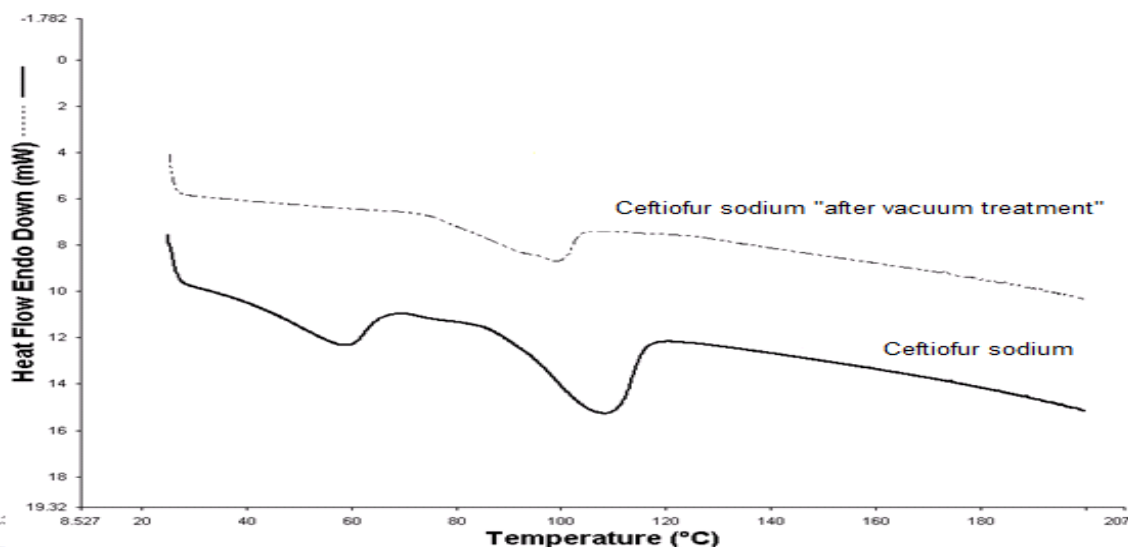


Figure 8: DSC before and after vacuum treatment

Powder X-ray diffraction (PXRD)

PXRD pattern of ceftiofur sodium, before and after application of vacuum, showed significant differences. This proves that application of vacuum led to changes in the crystal lattice of ceftiofur sodium. **Figure. 7** shows the diffractogram of ceftiofur sodium, before and after vacuum application.

Additionally, analysis of the vacuum treated sample by DSC revealed abolition of the first step water loss (**Figure. 8**). This established that first step of water loss was due to water of hydration, present in the channels. Removal of latter by vacuum treatment led to significant changes in the X-ray diffraction pattern.

Crystalline hydrates have been divided into three classes - Isolated site hydrates, channel hydrates, and the ion-associated hydrates.

Isolated site hydrates represent a class of hydrates which have water molecules in their lattice arrangement and the contact between two water molecules in the lattice is intervened by drug molecules. Cephadrine dihydrate belongs to this category of hydrate.

In **channel hydrates**, water molecules present in the lattice lie next to other water molecules, thus forming channels through the crystal. Channel hydrates are subdivided into following classes:

Expanded Channel hydrates are a subclass of channel hydrates, which can absorb or desorb moisture when exposed to higher or lower humidity respectively. This subclass can be identified by shift in the PXRD peaks. Absorption of water lead to increase in the lattice parameters, which can be visualized in shifting all or few PXRD peaks to the smaller 2θ and *vice-versa*. Drugs like Cromolyn sodium belong to this category.

A **planar hydrate** is another subclass of channel hydrates, in which the water in the lattice is arranged in a two-dimensional order or plane. Ibuprofen sodium belongs to the classes of planar hydrates.

In **Dehydrated hydrates** subclass, water is present in the lattice only when they are in the mother liquor. When removed from the mother liquor, the water is lost, but the lattice arrangement is retained.

Ion-associated hydrates [8] contain metal ion-coordinated water. The metal-water interaction can be very strong relative to the other bonding in the molecular crystal. Due to the presence of strong metal-water interaction, dehydration in this class of hydrates takes place only at very high temperatures. Drugs like Calteridol tetra-decahydrate contain hydrate water belonging to this category.

The above mentioned literature support and evaluation of the experimental data indicate that the initial water loss of Ceftiofur sodium, is possibly a channel water. Similarly, the experimental data, coupled with the fact that the compound is a sodium salt, suggest that the water lost at higher temperature is possibly the ion-associated water.

CONCLUSION

Based on the observation in the TGA, HSM (Hot Stage Microscopy - progressive darkening), DSC and vacuum assisted PXRD, it could be concluded that, the novel crystalline Ceftiofur sodium is a trihydrate, wherein one mole in the lattice, forms channel hydrate and two moles are possibly present as ion-associated.

Acknowledgements

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REFERENCES

- [1] Cephalosporin Derivatives, Process for Preparation thereof and Drugs Containing Said Derivatives Usable as Antibiotics. U.S. Pat. No. 4,464,367.
- [2] S A Salmon; J L Watts; C A Case; L J Hoffman; H C Wegener; and R J Yancey, *J Clin Microbiol* **1987**, 33, 2435-2444.
- [3] Erskine RJ; Wilson RC; Tyler JW; McClure KA; Nelson RS; Spears HJ, *Am J Vet Res* **1995**, 56, 481-485.
- [4] Crystalline Sodium Salt of Cephalosporin Antibiotic, WO **2007**/042917 A1.
- [5] Crystalline Sodium Salt of Cephalosporin Antibiotic, WO **2010**/020871 A2.
- [6] Crystalline Sodium Salt of Cephalosporin Antibiotic, WO **2012**/049576 A1.
- [7] K. R. Morris, N. Rodriguez-Hornedo, Encyclopedia of Pharmaceutical Technology, Volume 7, Marcel Dekker, New York, **1993**, pp. 393-440.
- [8] S.R. Byrn. Solid-state chemistry of drugs, Academic Press, New York, **1982**.