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Dehydration effect on the stability of cefixime trihydrate

Satoshi Kitamura, Shigetaka Koda, Akira Miyamae, Tsutomu Yasuda and Yukiyoshi Morimoto

Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Kashima, Yodogawa-ku, Osaka 532 (Japan)

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Summary

Partially dehydrated cefixime trihydrate was found to be unstable due to a highly disordered crystal structure caused by loss of its water of crystallization. It was also confirmed that cefixime trihydrate stored at a relative humidity below its critical value was less stable than the trihydrate stored under moist conditions. On the other hand, completely dehydrated cefixime trihydrate was relatively stable since it underwent transformation to a new anhydrous crystal form which did not contain water capable of participating in the hydrolytic reaction. It was suggested that the degradation mechanism under conditions of dryness differed from that under conditions of humidity, since not only the appearance but also the particular species of degradation products were completely different under the two sets of conditions.

Introduction

Many medicaments are known to crystallize with water molecules as an integral part of their crystal structure. It is a matter of great importance that one should bear in mind the fact that the pharmaceutical hydrates display marked alterations in several physico-chemical properties, such as chemical stability or dissolution rate on being processed to formulation or on exposure to relative humidities of varying degrees (Fukumori et al., 1983; Osawa et al., 1988). Many investigators have described the influence of sorbed moisture on the solid-state decomposition as well as on the kinetics of hygroscopic and moisture adsorption (Umprayn and Mendes, 1987; Carstensen et al., 1988). In the preceding paper, we reported the effect of grinding on the physico-chemical properties of cefixime trihydrate and our conclusions that the grinding energy destroyed the crystalline structure and that the stability of cefixime trihydrate decreased with increasing duration of grinding (Kitamura et al., 1989). Concerning the effect of dehydration on the solid-state stability of cefixime trihydrate, Mooney et al. (1988) reported that the 'anhydrous' form of cefixime was less stable than the 'trihydrate' at elevated temperatures (56–70 °C). However, they did not discuss details of the crystalline state of dehydrated cefixime trihydrate. In the present work, we have investigated changes in the crystalline state of cefixime trihydrate during the process of dehydra-

Correspondence: S. Kitamura, Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Kashima, Yodogawa-ku, Osaka 532, Japan.

tion and discuss the effect of dehydration on the chemical and color stability of cefixime trihydrate.

Materials and Methods

Materials

Cefixime trihydrate (Fujisawa Pharm. Co., Ltd.) was used without further purification. The water content was determined as 10.7% (theoretical value: 10.6%) by the Karl Fisher method. All other reagents were of reagent grade.

Dehydration of cefixime trihydrate

Cefixime trihydrate (0.4 g) was weighed into 5-ml glass bottles. The bottles were placed in a desiccator and dehydrated under reduced pressure at room temperature for an appropriate time period. Phosphorus pentoxide was used as desiccant.

Effect of humidity on the stability of cefixime trihydrate

Cefixime trihydrate (0.4 g) was weighed into weighing bottles. The weighing bottles were kept in a desiccator in which the relative humidity (R.H.) was adjustable by means of the appropriate saturated salt solutions (NaI, NaCl and NaBr) at 50 or 70 °C in a thermostatted chamber (Mini Jet Oven, Toyama Sangyo). The R.H. values of these saturated salt solutions were selected to be 25, 50 and 75%, respectively, at each temperature (Carr and Harris, 1949). Phosphorus pentoxide was used for 0% R.H.

Effect of dehydration on the stability of cefixime trihydrate

Dehydrated cefixime trihydrate in glass bottles was kept at 70 °C in a thermostatted chamber (Mini Jet Oven) and sampled at appropriate intervals.

Analytical procedure

The remaining potency of cefixime was measured by high-performance liquid chromatography (HPLC) according to our previously described procedure (Namiki et al., 1987).

Isolation and identification of major degradation products

The degradation products of cefixime trihydrate were isolated and purified using HPLC. The NMR and fast atom bombardment (FAB) mass spectra were recorded on a JEOL FX-270 (270 MHz) spectrometer and a Finnigan Mat TSQ-70 mass spectrometer, respectively. The NMR signals and mass spectrum of the degradation products were assigned on the basis of our results reported in the previous article, in which the degradation products observed in acidic or basic solutions were discussed (Namiki et al., 1987).

Determination of color changes (ΔE)

Discoloration of cefixime trihydrate was determined using a color difference meter (SZ-Σ80, Nippon Denshoku), following our procedure in a previous paper (Kitamura et al., 1988).

X-ray diffraction

Dehydrated cefixime trihydrate was placed on a sample holder immediately after removal from the desiccator and X-ray powder diffraction patterns obtained using an X-ray diffractometer (Rigaku Denki) under ambient conditions. The conditions for these measurements were: target, Cu; filter, Ni; voltage, 30 kV; current, 10 mA.

Results and Discussion

Effect of humidity on the stability of cefixime trihydrate

The effects of humidity on the solid-state stability of cefixime trihydrate were studied at 50 and 70 °C. Plots of residual amounts of cefixime vs storage period are shown in Fig. 1.

Generally, the decomposition of drug components and solid dosage forms depends not only on temperature but also on humidity. Many authors reported that physically sorbed moisture affects the solid-state stability, since some portion of the drug may dissolve in the moisture layer and readily undergo decomposition in the saturated aqueous layer (Carstensen, 1988).

However, in our study, loss of drug due to the process of decomposition under conditions of 0%

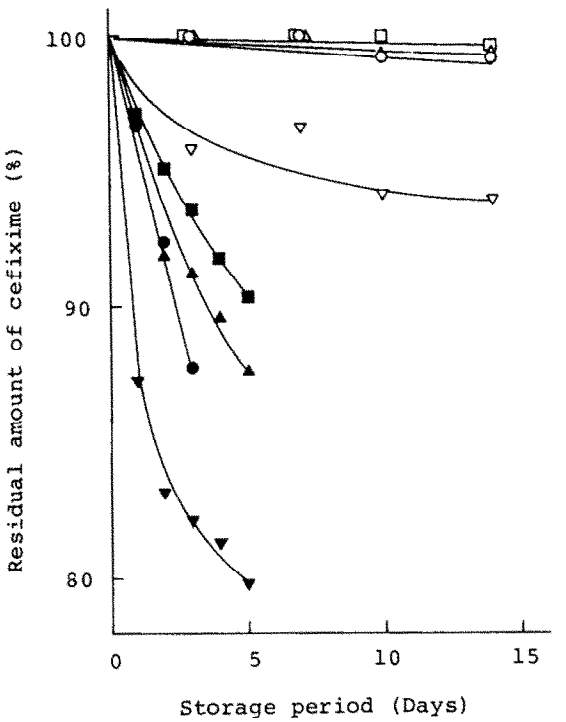


Fig. 1. Effect of humidity on the solid-state stability of cefixime trihydrate. (○) 50 °C, 75% R.H.; (△) 50 °C, 50% R.H.; (□) 50 °C, 25% R.H.; (▽) 50 °C, 0% R.H.; (●) 70 °C, 75% R.H.; (▲) 70 °C, 50% R.H.; (■) 70 °C, 25% R.H.; (▼) 70 °C, 0% R.H.

R.H. was much greater as compared to other conditions of humidity (R.H. 75, 50 and 25%). Mooney et al. (1988) obtained a similar result, reporting that cefixime trihydrate stored in a fiber drum that was incapable of preventing moisture diffusing at elevated temperature decomposed at a more rapid rate than when stored in glass vials. These results were explained by the fact that the relative humidity in the fiber drum fell below the critical value of the relative humidity (estimated to be between 10 and 20% at 25–70 °C), below which cefixime trihydrate undergoes spontaneous dehydration.

Consequently, it was proposed that the humidity within a particular package plays a significant role in the stabilization of hydrated medicaments such as cefixime trihydrate.

Effect of humidity on discoloration of cefixime trihydrate

The effect of humidity upon discoloration of cefixime trihydrate was studied at 50 and 70 °C, yielding the results shown in Fig. 2. Clearly, the discoloration indicated by the color difference (ΔE) for cefixime trihydrate stored at 75% R.H. was greater than those under other conditions of humidity. However, the color change for cefixime trihydrate stored at 0% R.H. differed from those of samples stored under conditions of humidity: the former changed to show a reddish color while the latter samples became yellowish-red. The chromaticity indices (a and b) of the samples were plotted on coordinates a–b by using a color difference meter (Fig. 3).

Fig. 3 demonstrates that the color of cefixime trihydrate stored at 0% R.H. was distinctly different from those at other values of the humidity. The chromaticity index (a) of cefixime trihydrate stored at 0% R.H. increased with increasing duration of storage time, however, the chromaticity index (b) showed a clearly smaller extent of change. In contrast, the corresponding indices (a and b) for samples stored at 25, 50 or 75% R.H. increased with increasing storage time.

Major degradation products in solid-state cefixime trihydrate

The species of degradation products of cefixime

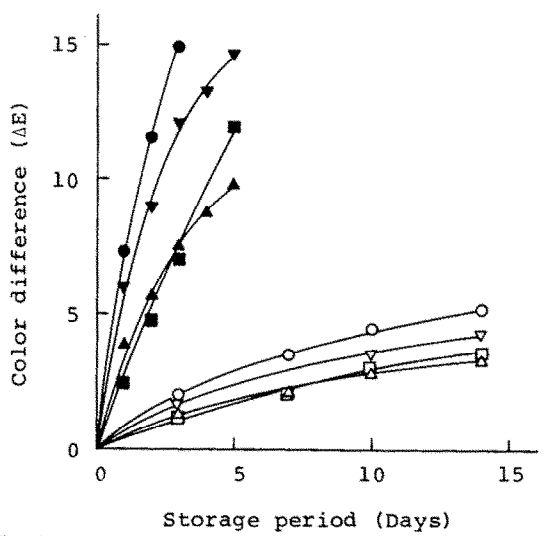


Fig. 2. Effect of humidity on the color stability of cefixime trihydrate. (○) 50 °C, 75% R.H.; (△) 50 °C, 50% R.H.; (□) 50 °C, 25% R.H.; (▽) 50 °C, 0% R.H.; (●) 70 °C, 75% R.H.; (▲) 70 °C, 50% R.H.; (■) 70 °C, 25% R.H.; (▼) 70 °C, 0% R.H.

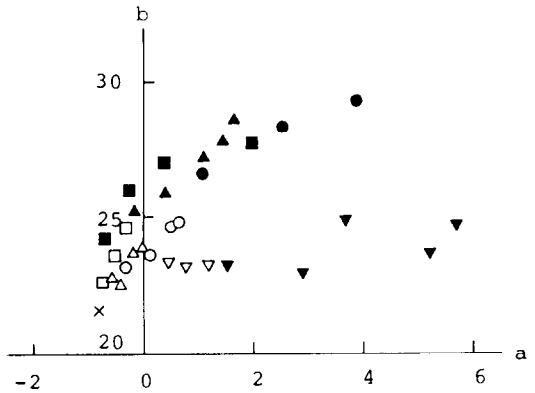


Fig. 3. Values of the chromaticity indices *a* and *b* for cefixime trihydrate under various conditions for storage. (○) 50 °C, 75% R.H.; (△) 50 °C, 50% R.H.; (□) 50 °C, 25% R.H.; (▽) 50 °C, 0% R.H.; (●) 70 °C, 75% R.H.; (▲) 70 °C, 50% R.H.; (■) 70 °C, 25% R.H.; (▼) 70 °C, 0% R.H; (×) intact.

trihydrate stored at 0 and 75% R.H. at 70 °C was investigated by HPLC in order to confirm differences between degradation products under both sets of storage conditions. The liquid chromatograms of cefixime trihydrate stored under the above two conditions are illustrated in Fig. 4. The percentage remaining vs the initial value for cefixime stored under conditions of humidity (3 days at 75% R.H. and at 70 °C) and dryness (2 days at 0% R.H. and at 70 °C) was 87.7 and 83.1%, respectively.

The structures of the degradation products found under both sets of conditions are shown in Scheme 1.

Structures I and II

The degradation products I and II correspond to those reported to be observed in acidic solution in our previous paper, referred to as 4 and 5, respectively (Namiki et al., 1987).

Structure III

Degradation product III showed similar mass and NMR spectra to those of I, except that the proton signal of the 2-aminothiazoyl ring was shifted to lower field (δ 7.85 ppm). Therefore, the structure of III was believed to be that of the *R*-isomeric form at the carboxymethoxyimino group of degradation product I.

Structure IV

The molecular ion $[M + H]^+$ of degradation product IV exhibited a mass of 454, identical to that of cefixime. In the ^{1}H-NMR spectrum, the vinyl proton signals disappeared and two new signals assignable to methylene group were found at δ 4.37 and 2.61 ppm. The other signals remained unchanged. Therefore, the structure of IV was assigned as a lactone form of cefixime as shown in Scheme 1.

Structure V

The molecular ion $[M + H]^+$ of degradation product V also showed a value of 454, equal to that of cefixime. The NMR signals of V were almost the same as those of the degradation product 3 observed in acidic solution in our previous paper (Namiki et al., 1987). Therefore, V was identified as a stereoisomer of the lactone methyl group of degradation product 3 reported by Namiki et al. (1987).

As shown in Fig. 4, the species of degradation products of cefixime trihydrate stored at 0% R.H.

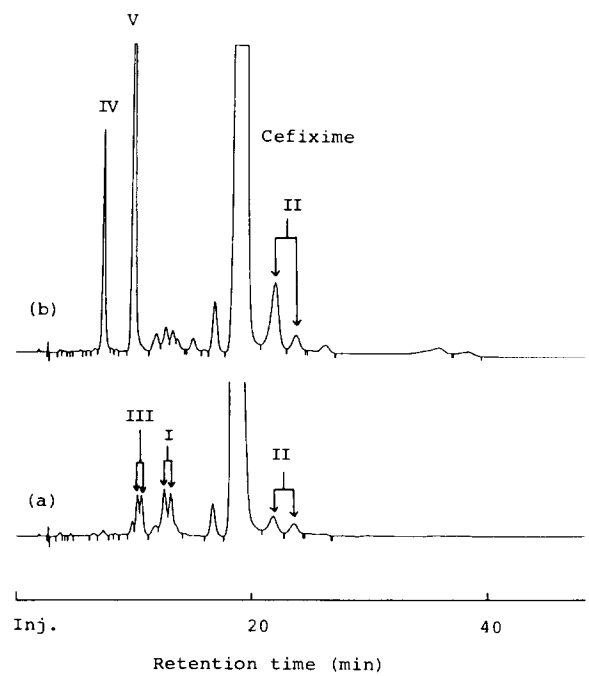
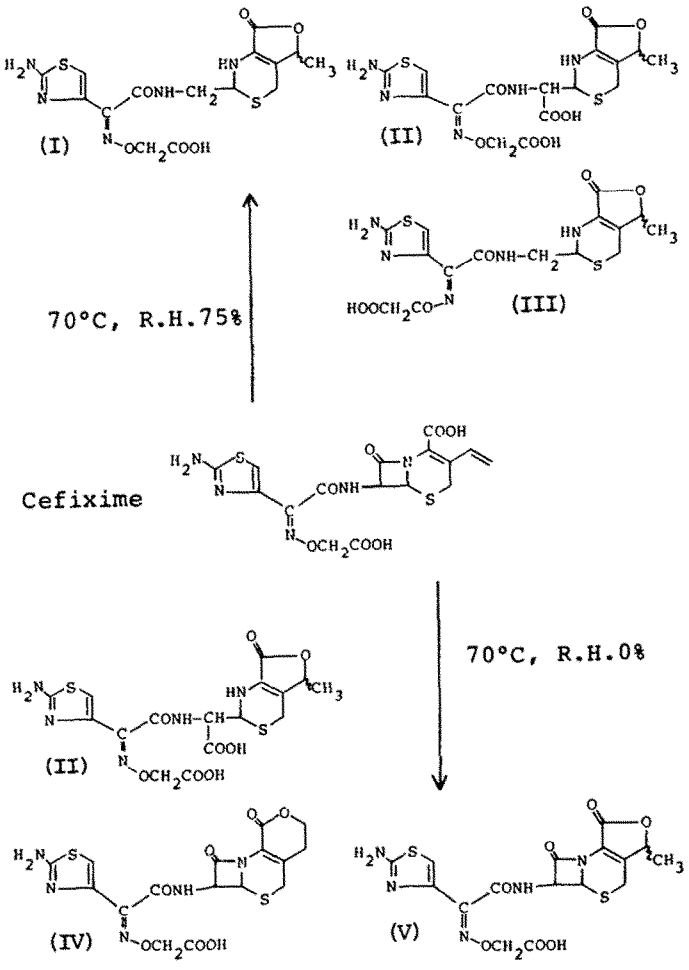


Fig. 4. HPLC of degraded cefixime trihydrate. Storage for (a) 3 days at 70 °C and 75% R.H.; (b) 2 days at 70 °C and 0% R.H.



Scheme 1. Major degradation products of cefixime trihydrate.

were found to differ from those in the case of 75% R.H. Furthermore, it was also confirmed that the forms of degradation products under conditions for storage at 25 or 50% R.H. were almost identical to those observed at 75% R.H. Carstensen et al. (1988) assumed that the sorbed moisture layer or eliminated water of crystallization in drugs behaves like a liquid phase at the interface with the drug substance at elevated temperatures. According to this assumption, the drug substances will form a saturated solution in the aqueous layer and the entire process of degradation is considered to equal the sum of the decomposition in the solid state and that in the drug-saturated aqueous layer. The solid-state degradation of cefixime trihydrate can be explained on the basis of the above assumption, as discussed by Mooney et al. (1988), since the species of degradation products in the solid state were almost identical to those observed

TABLE 1
Water content of dehydrated cefixime trihydrate

Dehydration time (h)	Water content (%)
0	10.7
0.5	8.8
1	6.1
3	3.5
16	0.7
24	0.3

in its aqueous solution. This signifies that in its aqueous solution, cefixime trihydrate adopted the lactone structure (degradation product V) during the first step of degradation. Subsequently, further degradation (hydrolysis and/or decarboxylation) yields degradation products I, II or III via cleavage of the β-lactam ring. In contrast, the degradation products observed at 0% R.H. were mainly IV and V in which the β-lactam ring was not hydrolyzed. Under conditions of dryness water molecules are not considered to participate in the degradation reaction, since the β-lactam ring is well known to undergo hydrolysis readily in acidic aqueous solutions.

These results suggested that the difference in color between samples stored under conditions of dryness and humidity was due to the mechanism of degradation. Despite further efforts aimed at identifying the discolored degradation products, we were unable to isolate or analyze their structures owing to such products being present in insufficient quantities.

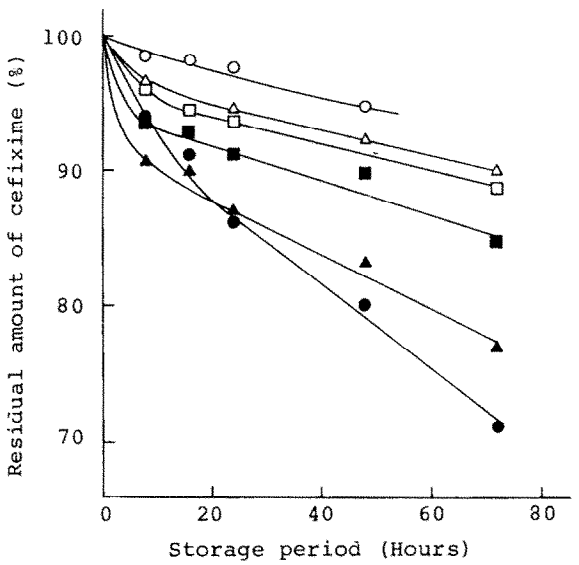


Fig. 5. Effect of dehydration on the solid-state stability of cefixime trihydrate. Samples: (○) intact, (△) dehydrated for 0.5 h, (□) dehydrated for 1 h, (●) dehydrated for 3 h, (▲) dehydrated for 16 h, (■) dehydrated for 24 h.

Effect of dehydration on the stability of cefixime trihydrate

As mentioned previously, it was confirmed that cefixime trihydrate became unstable on storage below its critical relative humidity. We therefore examined the effect of dehydration on the stability of cefixime trihydrate. The moisture content of dehydrated samples is listed together with the corresponding dehydration time in Table 1. The dehydrated samples in which the moisture content was adjusted to yield various levels of water were stored at 70°C in closed glass bottles and the residual amounts of cefixime were evaluated via HPLC, giving the results depicted in Fig. 5.

One readily observes that dehydrated cefixime trihydrate became unstable on increase in the degree of dehydration. However, samples in which the water of crystallization was reduced to lower than 1% were more stable on dehydration than those containing 3–9% of water. Takahashi et al. (1984) reported that dehydration of ampicillin trihydrate resulted in a crystalline state possessing a highly disordered structure and thus partially dehydrated samples became unstable. To ascertain the effect of dehydration on the crystalline state of cefixime trihydrate, X-ray diffraction patterns of cefixime trihydrate dehydrated in such a manner were recorded. The changes in X-ray diffraction profile of cefixime trihydrate during dehydration are shown in Fig. 6.

The peak intensity of the X-ray diffraction pattern of cefixime trihydrate decreased gradually with lowering of the water content; for example, the peak at $2\theta = 9.0°$, characteristic for cefixime trihydrate, disappeared completely following 24 h storage. Although Shefter et al. (1979) reported that the disappearance of ampicillin trihydrate peaks was simultaneously followed as a function of time through the transformation to an amorphous state, for cefixime trihydrate the dehydrated sample showed new diffraction peaks at $2\theta = 9.6$, 16.4, 22.8°, etc., after storage for more than 1 h. The intensities of the new peaks increased with decreasing water content. These results suggest that cefixime trihydrate undergoes transformation not to an amorphous form, but rather, to the anhydrous form with a characteristic X-ray diffraction pattern. However the anhydrous form can readily recover to full moisture content within 4 h under ambient conditions. The rehydrated

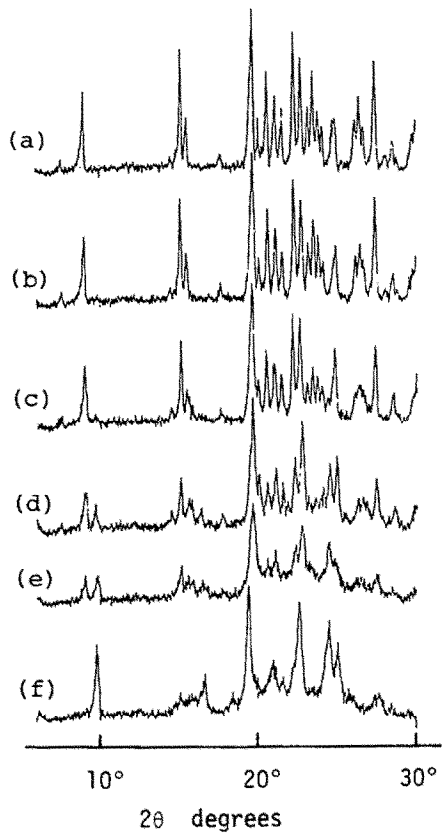


Fig. 6. X-ray diffraction patterns of dehydrated cefixime trihydrate. Samples: (a) intact, (b) dehydrated for 0.5 h, (c) dehydrated for 1 h, (d) dehydrated for 3 h, (e) dehydrated for 16 h, (f) dehydrated for 24 h.

form of anhydrous cefixime yielded the same X-ray diffraction pattern as that of the intact drug.

In a previous paper (Kitamura et al., 1989), we showed that the stability of solid-state cefixime trihydrate decreased with decreasing crystallinity during the process of grinding. In the process of dehydration, the samples dehydrated for 0.5, 1 and 3 h showed behavior similar to that of the ground sample, since the X-ray diffraction intensities of dehydrated samples decreased for increase in the period of dehydration. As a consequence, partially dehydrated cefixime trihydrate (samples dehydrated for 0.5, 1 or 3 h) was unstable due to being present in a highly disordered crystalline state in which the water molecules within the crystal are free to participate in the process of decomposition. However, for samples that were almost fully dehydrated (for 16 or 24 h) this is not the case as they were found to be relatively more stable. An explanation for such characteristic alterations in stability, according to the moisture content, is provided not only by the fact that water molecules participate in the hydrolytic reaction in the solid state, but also by the observation that the fully dehydrated sample has a crystalline structural state in the anhydrous form.

During the investigation of the effect of grinding on the solid-state stability of cefixime trihydrate, the crystalline structure of the ground sample was found to be destroyed, and part of the structure could not be restored via readsorption of water. However, for the dehydration process, dehydrated cefixime trihydrate of decreased crystallinity or as modified to the anhydrous form was suitable for being restored to the intact state immediately upon absorption of water. Our results may suggest that the crystalline state of the ground sample differs from that of the partially dehydrated form, even though the crystallinity of both was weakened by grinding or dehydration.

Conclusion

We have confirmed that cefixime trihydrate becomes unstable on storage below the critical relative humidity. Cefixime trihydrate is transformed into the anhydrous form by removal of its water of crystallization. However, the anhydrous form is considered to be physically unstable, since hydration occurs reversibly on storage under conditions of humidity. Consequently, we suggest that particular attention should be paid to the conditions for storage of pharmaceutical hydrates such as cefixime trihydrate. It is essential that absorption of excessive moisture is avoided at all costs for the case of the product and that the humidity within the package is maintained at an equilibrium level.

Acknowledgments

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References

Carr, D.S. and Hariss, B.L., Solutions for maintaining constant relative humidity. *Ind. Eng. Chem.*, 41 (1949) 2014–2015.

Carstensen, J.T., Effect of moisture on the stability of solid dosage forms. *Drug Dev. Ind. Pharm.*, 14 (1988) 1927–1969.

Fukumori, Y., Fukuda, T., Yamamoto, Y., Shigitani, Y., Hanyu, Y., Takeuchi, Y. and Sato, N., Physical characterization of erythromycin dihydrate, anhydrate and amorphous solid and their dissolution properties. *Chem. Pharm. Bull.*, 31 (1983) 4029–4039.

Kitamura, S., Tanabe, J., Koda, S. and Morimoto, Y., Kinetics and forecasting of discoloration of solid drugs. *Yakuzaigaku*, 48 (1988) 270–276.

Kitamura, S., Miyamae, A., Koda, S. and Morimoto, Y., Effect of grinding on the stability of solid-state cefixime trihydrate. *Int. J. Pharm.*, 56 (1989) 125–134.

Mooney, K.G., Forbes, F.S., Wadgoanker, D., Berger, W., Kulkarni, P., Ku, S., Ferrillo, R., Haseltine, W., Rodriguez-Harnedo, N. and Crnosiya, M., Cefixime: Physical and chemical evaluation of a cephalosporin trihydrate, *University of Wisconsin 7th Update Conference, April* (1988).

Namiki, Y., Tanabe, T., Kobayashi, T., Tanabe, J., Okimura, Y., Koda, S. and Morimoto, Y., Degradation kinetics and mechanism of a new cephalosporin, cefixime, in aqueous solution. *J. Pharm. Sci.*, 76 (1987) 208–214.

Osawa, T., Kamat, M.S. and Deluca, P.P., Hygroscopicity of cefazolin sodium: Application to evaluate crystallinity of freeze-dried products. *Pharm. Res.*, 5 (1988) 421–425.

Shefter, E., Fung, H. and Mok, O., Dehydration of crystalline theophylline monohydrate and ampicillin trihydrate. *J. Pharm. Sci.*, 62 (1973) 791–794.

Takahashi, Y., Nakashima, K., Nakagawa, H. and Sugimoto, I., Effects of grinding and drying on the solid-state stability of ampicillin trihydrate. *Chem. Pharm. Bull.*, 32 (1984) 4963–4970.

Umprayn, K. and Mendes, R.W., Hygroscopicity and moisture adsorption kinetics of pharmaceutical solid: A review. *Drug Dev. Ind. Pharm.*, 13 (1987) 653–693.