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Effects of Grinding and Drying on the Solid-State Stability of Ampicillin Trihydrate

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The effects of grinding and drying (removal of water of crystallization) processes on the solid-state stability of ampicillin trihydrate were studied. The ground samples became more unstable as the grinding time increased. This phenomenon is considered to be due to a greater freedom of movement of water molecules, which are sufficiently free to participate in a solid-state hydrolytic reaction in the impaired lattice induced by grinding. A stabilization effect was observed when the ground sample was stored at high humidity, although ampicillin was susceptible to hydrolysis. This effect was interpreted in terms of a decrease of the ability of water molecules to move within the crystal.

The dehydration of ampicillin trihydrate resulted in a higher disorder of the crystal structure. Partially dehydrated samples were unstable. However, an almost completely dehydrated sample was relatively stable because of the virtual absence of water able to participate in the hydrolytic reaction

Keywords——ampicillin trihydrate; solid state stability; grinding; drying; crystal water

A number of drugs crystallize with water of crystallization. In general, water molecules in these hydrate crystals are almost always involved in hydrogen bonds, and these hydrogen bonds often contribute to the coherence of the crystal structure. In the process of pharmaceutical operations, such as milling, blending, drying, compression, and so on, most drugs are exposed to many mechanical stresses. Such stresses may affect the bonding strength of hydrogen bonds within the hydrate crystal. Thus, it is important to study the effect of these stresses on the physico-chemical properties and the stability of drugs in the preformulation study. In some hydrates which are labile to hydrolysis, it is expected that water molecules may participate in the hydrolytic reaction when the bonding force between water molecules and drug molecules is weakened. In the previous paper, we reported on the properties of water of crystallization of sodium prasterone sulfate[1] and the effects of grinding and drying on the solid-state stability.[2] Although sodium parasterone sulfate was relatively labile to hydrolysis, the ground sodium prasterone sulfate was more stable under highly humid conditions than at low humidity. We concluded that this interesting phenomenon was due to strengthening of the bonds between water molecules and prasterone sulfate molecules under highly humid conditions. In this work, we studied the solid-state stability of ampicillin trihydrate and the effects of grinding and dehydration on the stability. Grant *et al.*[3] reported on the solid-state stability of ampicillin anhydrate and monohydrate, and stated that the monohydrate was less stable than the anhydrate because the water molecules within the hydrate crystal were sufficiently free to participate in a solid-state hydrolytic reaction. Austin *et al.*[4] reported that the stability was decreased, possibly due to a greater freedom of movement of water molecules in the impaired lattice, when part of the water of crystallization was removed, and that the trihydrate was less stable above 70 °C, probably due to the breakdown of the crystal lattice

and rupture of the sensitive β-lactam by the liberated water. Lo[5] also studied the solid-state stability of ampicillin trihydrate at high temperature and reported the same results. We studied the effects of grinding and drying (dehydration) on the solid-state stability of ampicillin trihydrate at relatively low temperature, below 40 °C, and also studied the effect of humidity and the behavior of water of crystallization.

Experimental

Materials——Ampicillin trihydrate was purchased from Yunjin Pharm. Ind. Co., Ltd. (Korea). The trihydrate sample had a measured potency of 851 μg/mg by biological assay, and 849 μg/mg by iodometric assay. The water content was found to be 13.6% (calcd; 13.4%).

Grinding——Effect of Grinding on the Stability of Ampicillin Trihydrate: Ampicillin trihydrate (20 g) was ground in an automated mortar (Type No. 16, Ishikawa Kojyo Co., Ltd.) for an appropriate period (15, 30 min, 1, 2, or 3 h).

Effect of Humidity on the Stability of Ground Ampicillin Trihydrate: Ampicillin trihydrate (60 g) were ground in an automated mortar (Type No. 18, Ishikawa Kojyo Co., Ltd.) for 3 h. The ground sample was passed through a 32 mesh sieve.

Dehydration——Ampicillin trihydrate (0.4 g) was weighed into 10 ml ampules. The ampules were placed in a drying oven (Hivac Oven HV-3, Tabai Mfg. Co., Ltd.) and dehydrated under reduced pressure at room temperature for an appropriate period (100 min, 3, 3.5, 4 h, or overnight). Phosphorus pentoxide was used as a desiccant. After dehydration, the ampules were sealed immediately.

Kinetic Procedure——Effect of Grinding on the Stability of Ampicillin Trihydrate: The ground sample (0.5 g) was weighed into 10 ml ampules and sealed. The ampules were kept at 40 °C in a thermostated chamber (Type PR3A, Tabai Mfg. Co., Ltd.), and sampled at appropriate intervals.

Effect of Humidity on the Stability of Ground and Intact Ampicillin Trihydrate: The sample (0.4 g) was weighed into weighing bottles. The weighing bottles were kept in a desiccator adjusted to the appropriate relative humidity (R.H.) by means of a saturated salt solution at 40 or 20 °C in a thermostated chamber (Type PR3A, Tabai Mfg. Co., Ltd.). Phosphorus pentoxide was used for 0% R.H.

Effect of Dehydration on the Stability of Ampicillin Trihydrate: The ampules containing dehydrated samples were kept at 40 °C in a thermostated chamber (Type PR3A, Tabai Mfg. Co., Ltd.).

Analytical Procedure——An iodometric method was used for determination of the residual ampicillin. Weighed samples were dissolved in a mixture of *n*-propanol–water.[6] The procedure reported by Finholt *et al.*[7] was followed except that 5 ml of pH 4 citrate buffer solution was used instead of phthalate buffer solution.

Water Content——Water content assay was performed by the Karl–Fischer method (Type MK-AII, Kyoto Densi Kogyo Ltd.).

X-Ray Diffraction——X-Ray diffraction patterns were obtained with an X-ray diffractometer (Geigerflex 2027, Rigaku Denki Co., Ltd.). The measurement conditions were as follows; target Cu, filter Ni, voltage 25 kV, and current 5 mA.

Determination of Degree of Crystallinity: The degree of crystallinity was determined by the method of Black *et al.*[8] The intact sample was defined as having 100% crystallinity and the amorphous form, which was prepared by dehydration of ampicillin trihydrate under reduced pressure at room temperature followed by rehydration at 5 °C and 81% R.H. for 2 d, was defined as having 0% crystallinity. The degree of crystallinity of the sample was determined from the calibration curve shown in Fig. 1. The effect of water content on the total X-ray reflection intensity was investigated with a dehydrated sample whose water content and degree of crystallinity were 3.5 and 21.5%, respectively. The X-ray diffraction patterns of the sample and its rehydrated products, whose water contents were 7.5 and 13.0%, were measured. No effect of the water content was observed. The same results were also obtained with the amorphous form.

Thermal Measurement——Thermal behavior of the water of crystallization was measured by differential thermal analysis (DTA) and thermogravimetry (TG) (model DT-20B and TG-20, Shimadzu Seisakusho Ltd.) at a scanning rate of 10 °C/min.

Specific Surface Area——Specific surface areas of powders were determined by the BET method, using a specific suface area meter (model P-700, Shibata Chemical Apparatus Mfg. Co., Ltd.) and nitrogen gas.

Scanning Electron Microscopy——A scanning electron microscope (model S-450, Hitachi Ltd.) was used.

Results and Discussion

Effect of Grinding on the Solid-State Stability of Ampicillin Trihydrate

The X-ray diffraction patterns of the intact and 3 h ground samples are shown in Fig. 2.

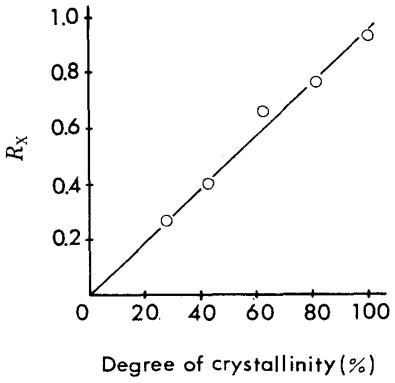

Fig. 1. Degree of Crystallinity (%) *vs.* R_X, the Ratio of the Area under the Diffraction Peaks to the Total Area

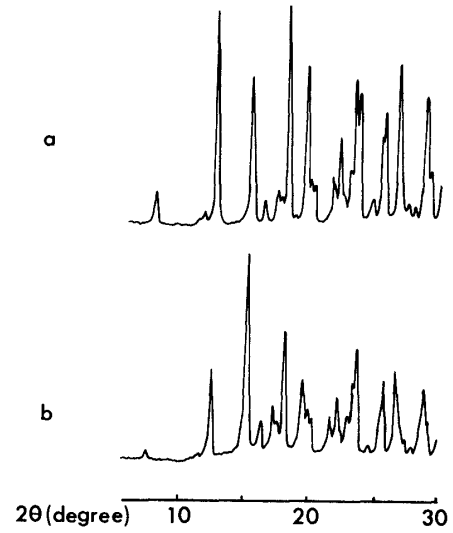

Fig. 2. X-Ray Diffraction Patterns of Intact and Ground Ampicillin Trihydrate

a, intact; b, ground for 3 h.

TABLE I. Specific Surface Area (S_w), Degree of Crystallinity, and Water Content of Ground Ampicillin Trihydrate

Grinding time	S_w (m^2/g)	Degree of crystallinity (%)	Water content (%)
Intact	1.82	100.0	13.6
15 min	5.02	93.7	12.8
30 min	—	90.0	12.4
1 h	3.35	88.5	12.2
2 h	—	78.0	11.2
3 h	3.60	77.0	11.1

No changes of the peak positions were observed, but the area under the broad, diffuse peak which underlies the crystalline peaks increased slightly. This result indicates that the crystalline form was transformed to the amorphous form by grinding. The degree of crystallinity was determined from the X-ray diffraction patterns. The determined degrees of crystallinity, specific surface areas, and water contents of the ground samples are listed in Table I. The degree of crystallinity decreased with increasing grinding time to 77.0% for the 3 h ground sample. On the other hand, the specific surface area increased at the initial stage of grinding. However, as the grinding process proceeded, agglomeration occurred, leading to a decrease in specific surface area. Drug loss during grinding for 3 h was as little as 1.0%.

DTA curves of the ground samples are depicted in Fig. 3. The endothermic peak due to dehydration of the intact sample was divided into two peaks by grinding, and the area of the peak at lower temperature (below 80 °C) increased with grinding time. This result suggests that the bonding force (strength of the hydrogen bond) between water molecules and ampicillin molecules is weakened by grinding, and the amount of water molecules having a greater freedom of movement in the impaired lattice is increased.

As shown above, the grinding process decreased the degree of crystallinity of ampicillin trihydrate and increased the freedom of movement of water molecules in the impaired lattice. Next, the effect of these factors on the stability of the ground ampicillin trihydrate was studied. The solid-state stability testing of the ground sample was carried out at 40 °C and the

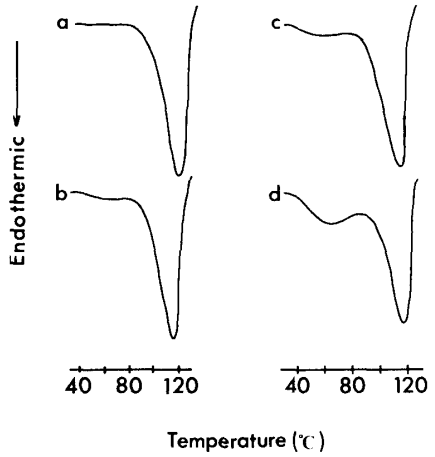

Fig. 3. DTA Curves of Intact and Ground Ampicillin Trihydrate

Grinding time: a, intact; b, 30 min; c, 1 h; d, 3 h.

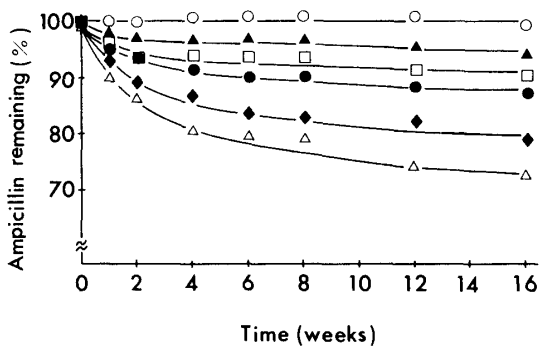

Fig. 4. Effect of Grinding on the Stability of Ampicillin Trihydrate

Grinding time: ○, intact; ▲, 15 min; □, 30 min; ●, 1 h; ◆, 2 h; △, 3 h.

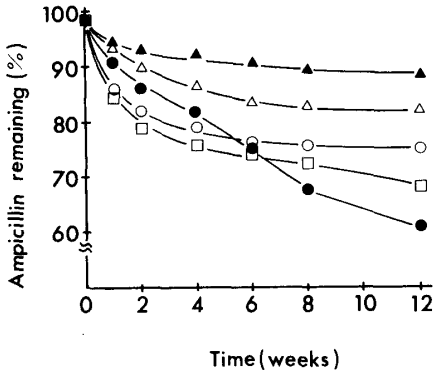

Fig. 5. Effect of Humidity on the Stability of Ground Ampicillin Trihydrate at 40 °C

R.H.: □, 0%; ▲, 11%; △, 40%; ○, 80%; ●, 96%.

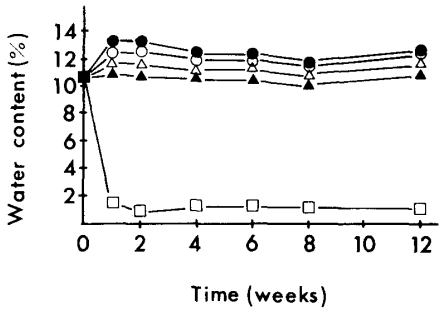

Fig. 6. Water Content of Ground Ampicillin Trihydrate Stored at Various Humidities and 40 °C

R.H.: □, 0%; ▲, 11%; △, 40%; ○, 80%; ●, 96%.

results are shown in Fig. 4. The samples became more unstable as the grinding time was increased. This phenomenon is considered to be due to a greater freedom of movement of water molecules, which become sufficiently free to participate in the solid-state hydrolytic reaction in the impaired lattice induced by grinding.

Effect of Humidity on the Solid-State Stability of the Ground Ampicillin Trihydrate

The effects of humidity on the solid-state stability of the ground ampicillin trihydrate were studied at 40 °C. The results are shown in Fig. 5. At 0% R.H., drug loss in the initial phase of decomposition was the largest. It is thought that the breakdown of the crystal lattice by dehydration occurred as shown in Fig. 6 and hydrolysis by the liberated water proceeded. However, the amount of liberated water became too small for the reaction to continue in the later decomposition phase. At levels of humidity other than 96% R.H., drug loss due to decomposition was larger at higher humidity than at lower humidity. This is probably due to the increase of absorbed free water as discussed later. When the ground sample was stored at 96% R.H., however, the decomposition rate in the initial phase was depressed compared with that at 80% R.H., although the amount of absorbed water of the sample stored at 96% R.H.

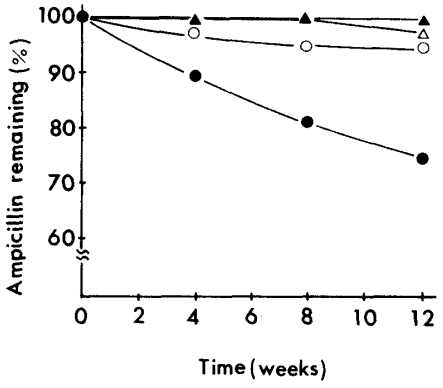

Fig. 7. Effect of Humidity on the Stability of Intact Ampicillin Trihydrate at 40 °C
R.H.: ▲, 11%; △, 40%; ○, 80%; ●, 96%.

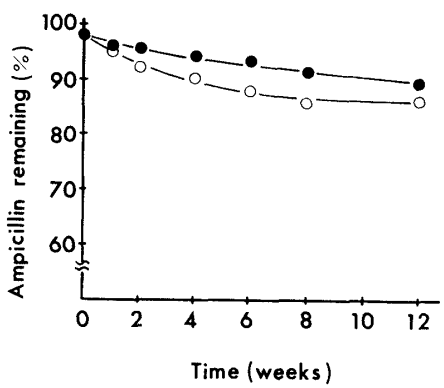

Fig. 8. Effect of Humidity on the Stability of Ground Ampicillin Trihydrate at 20 °C
R.H.: ○, 79%; ●, 97%.

Table II. Water Content, Residual Percentage of Intact Drug, and Degree of Crystallinity of Ground Ampicillin Trihydrate after Storage for 1 Week under Various Conditions

Storage conditions	Water content (%)	Residual percentage	Degree of crystallinity (%)
Initial	10.6	98.2	76.6
20 °C, 79% R.H.	12.9	95.2	76.3
97% R.H.	13.6	96.1	88.7
40 °C, 0% R.H.	1.5	84.6	0.0
11% R.H.	11.0	94.2	75.0
40% R.H.	11.7	93.8	76.2
80% R.H.	12.4	85.9	76.2
96% R.H.	13.2	90.6	84.7

was larger than under other conditions of humidity. However, the decomposition rate in the later phase was faster than under other conditions of humidity.

Figure 7 shows the time course of decomposition of the intact ampicillin trihydrate at various levels of humidity at 40 °C. In the case of the intact sample, the decomposition rate increased with increasing relative humidity from 11 and 96% R.H. On the basis of this result, it was suggested that the decomposition of the intact sample occurred in the adsorbed moisture layer.[9] The pseudo zero-order decomposition constant in the adsorbed moisture layer was determined by adding 2 ml of water to 100 mg of ampicillin trihydrate at 40 °C. The percentage decomposition after storage at 96% R.H. for 4 weeks was calculated from the determined pseudo zero-order rate constant (40.8 mg/week), the adsorbed water content (0.7%), and the solubility of ampicillin trihydrate at 40 °C (9.17 mg/ml). The calculated value and the observed value were 0.06 and 10.4%, respectively. This disagreement between the two values suggests that ampicillin is hydrolyzed in the solid state by water molecules within the crystal rather than in the adsorbed moisture layer, and further suggests that the state of water molecules within the crystal is an important factor in the solid-state decomposition of ampicillin trihydrate. From these considerations, it is suggested that the decomposition of intact ampicillin trihydrate occurred in the adsorbed water layer at first, followed by decomposition within the crystal. Therefore, the decomposition rate increased with increasing humidity. In the case of ground ampicillin trihydrate, the fast decomposition rate in the initial phase was probably due to hydrolysis by water molecules absorbed in the crystal defects

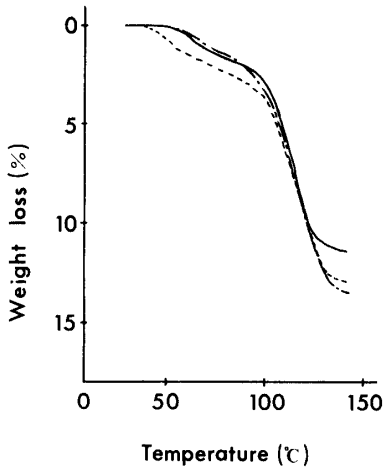

Fig. 9. Effect of Storage at High Humidity on TG Curves of Ground Ampicillin Trihydrate

Storage conditions: ——, initial; ------, 20 °C and 79% R.H.; — - —, 20 °C and 97% R.H.

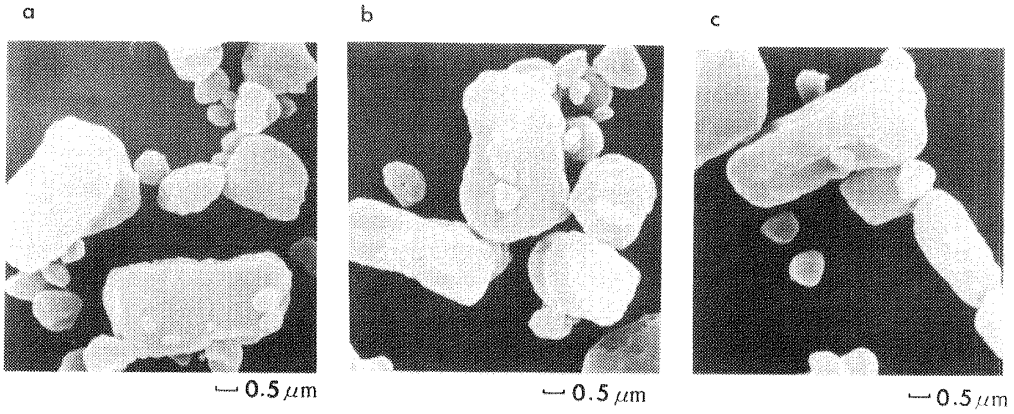

Fig. 10. Scanning Electron Micrographs of Ground Ampicillin Trihydrate before and after Storage at High Humidity

Storage conditions: a, initial; b, 20 °C and 79% R.H.; c, 20 °C and 97% R.H.

induced by grinding. The stabilization at 96% R.H. was probably due to depression of the movement of water molecules within the crystal resulting from the increase of the degree of crystallinity, as discussed later. However, in the later decomposition phase, the amount of decomposition was the largest at 96% R.H., since the decomposition initiated in the adsorbed moisture layer became large. As shown in Fig. 8, stabilization effects at high humidity (above 95% R.H.) were also observed at 20 °C.

In order to investigate the depression of the movement of water molecules within the crystal, the X-ray diffraction patterns of the ground sample stored at various levels of humidity for 1 week were measured. In Table II, the degrees of crystallinity determined from the X-ray diffraction patterns, the residual percentages of ampicillin, and the water contents of the stored samples are shown. Only the degree of crystallinity of the sample stored at above 95% R.H. increased, although decomposition occurred. Figure 9 shows TG curves of the ground ampicillin trihydrate and a sample stored at high humidity and 20 °C for 1 week. A part of the water of crystallization of the ground ampicillin trihydrate was lost quite easily; some weight loss was seen at below 80 °C in the TG curve. When this ground sample was stored at 79% R.H., the absorbed water was mostly lost at lower temperature, while in the case of the sample stored at 97% R.H., the absorbed water was not lost at lower temperature. Thus, it was shown that the crystal lattice damage induced by grinding was repaired during storage at above 95% R.H., and most of the absorbed water molecules became tightly bound to ampicillin molecules. At low humidity, the absorbed water molecules still had a greater

freedom of movement. Figure 10 shows the scanning electron micrographs of the ground samples before and after storage at various levels of humidity at 20 °C for 1 week. No difference in external appearance was seen between the ground samples stored at 97 and 79% R.H. for 1 week. The differences of specific surface areas between the ground sample at initial time (3.07 m^2/g), that after 1 week at 97% R.H. (2.84 m^2/g), and that after 1 week at 79% R.H. (2.69 m^2/g) were small. From these results, it seems unlikely that ampicillin was dissolved in the adsorbed water layer, then recrystallized on the crystal surface at high levels of humidity.

We reported previously on the stability of sodium prasterone sulfate and stated that ground sodium prasterone sulfate was stabilized above 40% R.H. However, ground ampicillin trihydrate was stabilized only at above 95% R.H. In general, dehydration results in two types of crystallographic behavior.[10] The first kind of behavior occurs when dehydration results in a change in crystal structure. The second kind of behavior occurs when dehydration does not result in a change in crystal structure, but produces a crystal with voids and cavities. James *et al.*[11] and Boles *et al.*[12] reported on the crystal structure of ampicillin trihydrate in detail, and described an intricate network of hydrogen bonding which involves all three water molecules. When ampicillin trihydrate was dehydrated, the amorphous form was obtained as reported by Shefter *et al.*[13] Ampicillin trihydrate is included in the first group. However, sodium prasterone sulfate may be classified into the second group. In the case of the second group, absorbed water molecules would be incorporated into the voids in the dehydrated crystal directly, while in the case of the first group, direct incorporation of water molecules into the hydrate crystal would be difficult. That is to say, the impaired crystal lattice of the ground sodium prasterone sulfate would be easily repaired by the direct incorporation of water molecules. However, in the case of ampicillin trihydrate, this would be difficult. The difference of solid-state stability between sodium prasterone sulfate and ampicillin trihydrate is probably due to this difference between the properties of the two groups.

Effect of Dehydration on the Stability of Ampicillin Trihydrate

As mentioned before, when ampicillin trihydrate was dehydrated, the amorphous anhydrous form was obtained, and the sample became unstable on storage at 0% R.H. Thus, the effect of dehydration on the solid-state stability of ampicillin trihydrate was studied. The

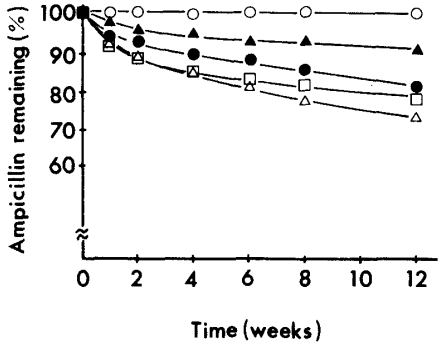

Fig. 11. Effect of Dehydration on the Stability of Ampicillin Trihydrate

Initial water content: ○, 12.0%; ▲, 10.0%; ●, 6.8%; △, 3.4%; □, 0.6%.

TABLE III. Water Content and Degree of Crystallinity of the Dehydrated Ampicillin Trihydrate

Water content (%)	Degree of crystallinity (%)
9.3	62.6
5.0	35.6
3.6	26.8
1.5	14.7

water contents and the degrees of crystallinity of dehydrated samples are listed in Table III. The degree of crystallinity decreased with the progress of dehydration, reflecting the breakdown of the crystal lattice. The solid-state stabilities of these dehydrated samples were studied at 40 °C. The results are shown in Fig. 11. The sample became unstable as a result of dehydration. It is suggested that the dehydration of ampicillin trihydrate resulted in greater disorder of the crystal structure and that the water molecules within the hydrate crystal became sufficiently free to participate in a solid-state hydrolytic reaction. However, the sample which was almost fully dehydrated was relatively stable. This was probably due to the virtual absence of water molecules able to participate in the hydrolytic reaction in the solid state.

Conclusions

Some drugs are stabilized by the formation of hydrate crystals in the solid state, even though they are labile to hydrolysis. If mechanical treatments, such as grinding and compression, which induce disorder of the crystal structure are applied to these hydrates, it is expected that hydrolysis by the water molecules present within the hydrate crystal will occur. Ampicillin trihydrate became unstable during grinding. However, the ground sample was more stable when it was stored at high humidity. This stabilization effect was thought to be due to a decrease of the ability of water molecules to move within the crystal due to repair of the damaged crystal lattice. Similar phenomena will probably occur in other hydrate crystals. Therefore, it is important to study the effects of mechanical operations on the physical or chemical properties of drugs in the preformulation study.

References and Notes

1) H. Nakagawa, Y. Takahashi, Y. Fujimoto, S. Maeda, and I. Sugimoto, *Chem. Pharm. Bull.*, **29**, 1466 (1981).
2) H. Nakagawa, Y. Takahashi, and I. Sugimoto, *Chem. Pharm. Bull.*, **30**, 242 (1982).
3) N. H. Grant and H. E. Alburn, *Nature* (London), **207**, 645 (1965).
4) K. W. B. Austin, A. C. Marshall, and H. Smith, *Nature* (London), **208**, 999 (1965).
5) P. K. A. Lo, Ph. D. Dissertation, State University of New York, 1976.
6) E. Pawelczyk, T. Hermann, M. Zajac, B. Knitter, and B. Smilowski, *Pol. J. Pharmacol. Pharm.*, **32**, 47 (1980).
7) P. Finholt, G. Jürgensen, and H. Kristiansen, *J. Pharm. Sci.*, **54**, 387 (1965).
8) D. B. Black and E. G. Lovering, *J. Pharm. Pharmacol.*, **29**, 684 (1977).
9) L. J. Leeson and A. M. Mattocks, *J. Am. Pharm. Assoc.*, **47**, 329 (1958); J. T. Carstensen, "Pharmaceutics of Solids and Solid Dosage Forms," Wiley, New York, 1977, p. 196.
10) S. R. Byrn, "Solid-State Chemistry of Drugs," Academic Press, New York, 1982, p. 171.
11) M. N. G. James, D. Hall, and D. C. Hodgkin, *Nature* (London), **220**, 168 (1968).
12) M. O. Boles and R. J. Girven, *Acta Crystallogr., Sect. B*, **32**, 2279 (1976).
13) E. Shefter, H.-L. Fung, and O. Mok, *J. Pharm. Sci.*, **62**, 791 (1973).