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# Effect of grinding on the solid-state stability of cefixime trihydrate

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## Summary

The effect of grinding on the physicochemical properties of cefixime trihydrate was studied by means of X-ray diffraction analysis, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), equilibrium water amounts and color difference measurement ( $\Delta E$ ). Crystalline cefixime trihydrate was confirmed to change to a non-crystalline solid after 4 h of grinding in a ball mill, since X-ray diffraction peak intensities decreased with increasing grinding time. Dehydration temperature of ground cefixime trihydrate also lowered with increasing grinding time, and the activation energy for dehydration of intact cefixime trihydrate and the samples ground 4 h (amorphous form) were calculated by Kissinger's method to be 72.4 kcal/mol and 67.5 kcal/mol, respectively. The decreased crystallinity with grinding is considered to be due to an increase of water molecules having greater freedom of movement in the crystal lattice. The overall decomposition of solid-state cefixime trihydrate could be expressed by pseudo first-order reaction, and the crystallinity of the ground sample was estimated by an equation expressing the overall decomposition rate constant; which is the sum of the decomposition in 100% crystalline and in 0% crystalline (amorphous) states. Kinetic studies of discoloration of ground cefixime trihydrate showed an increase in the apparent rate constant for discoloration with the increase in the grinding time.

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## Introduction

There are a number of hydrated drug substances in which water molecules generally play an essential role in crystal formation. Crystallinity of such compounds can easily be degraded by mechanical forces, with a resulting decrease of chemical stability of drug substances. Since milling, blending, drying or compressing are common processes in pharmaceutical operations, investigating the ef-

fect of such forces upon the chemical stability of drug substances is very important (Florence et al., 1974; Kaneniwa et al., 1975; Pikal et al., 1977). Grinding generally affects the crystallinity of a compound, so the interaction between water and drug molecules in crystals subjected to such forces has been extensively studied. Investigators mention the relationship between chemical stability and the crystallinity of the hydrate of some antibiotics (Pikal et al., 1978; Takahashi et al., 1984), and crystal deterioration has been explained by the weakened interaction between water and drug molecules in the crystal lattice (Otsuka et al., 1984; Kaneniwa et al., 1984). However, the discoloration phenomenon of pharmaceutical medica-

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ments has never been discussed quantitatively even though the appearance of a drug substance is one of the most important factors in estimating its stability during the formulation process or storage. Thus, if the influence of grinding on the physicochemical properties of drug substances can be obtained, it could be of great importance in developing solid dosage forms. In this paper, we report the grinding effects on the physicochemical properties such as crystallinity as well as chemical and color stability of cefixime trihydrate.

## Materials and Methods

### Materials

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

### Methods

**Grinding.** Cefixime trihydrate (10 g) was ground in an automated mortar (Mixer/Mill, SPEX Industries, Inc.) for appropriate periods (5, 10, 15, 30 min, 1 h, 2.5 h and 4 h).

**Assay method.** High-performance liquid chromatography reported by Namiki et al. (1987) was used for the determination of cefixime potency.

**Determination of color change ( $\Delta E$ ).** The color of the samples stored at elevated temperature was determined with a color difference meter (SZ-Σ80, Nippon Denshoku) in order to obtain lightness and chromaticity coordinates in the *L-a-b* system (Hunter, 1958). From these values, Hunter's color difference,  $\Delta E$ , was calculated by the following equation.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \quad (1)$$

where the *L-a-b* coordinate system is classed as an opponent color system in which a lightness coordinate (*L*) runs from 0 for black to 100 for white: a red-green coordinate (*a*) is positive for red colors and negative for green and a yellow-blue coordinate (*b*) is positive for yellow colors and negative

for blue. And  $\Delta L$ ,  $\Delta a$ , and  $\Delta b$  indicate the deviation of *L*, *a*, and *b* values obtained from the heat-deteriorated samples from those of the initial samples.

**Measurement of water content.** Samples were stored in desiccators with saturated salt solutions of various relative humidity (R.H.) values (0–83% R.H.) at 25°C. When the sample reached a constant weight, the water content was determined as sample weight loss or gain. The initial water content of the sample was determined by the Karl Fischer method (type MK-II, Kyoto Denshi Kogyo).

**Measurement of X-ray diffraction.** The X-ray diffraction pattern was measured by an X-ray diffractometer (Rigaku Denki). The measuring conditions were as follows; target Cu, filter Ni, voltage 25 kV, and current 5 mA.

**Thermal analysis.** A thermogravimetric analyzer (model TG/DTA 200, Seiko Denshi Kogyo) was used to confirm the dehydration of cefixime trihydrate by heating 5 mg of the sample at a heating rate of 10°C/min in an open pan under the flow of nitrogen. Thermal behavior of the water of crystallization was examined in closed aluminum pans using a differential scanning calorimeter (DSC) (model DSC 200, Seiko Denshi Kogyo). Scanning rates of 5, 10, 15 or 20°C/min were chosen.

**Scanning electron microscopy.** A scanning electron microscope (model S-450, Hitachi) was used to observe the surface state of the ground samples.

**Measurement of crystallinity.** Crystallinity was determined by applying the method of Otsuka et al. (1983). The intact sample was defined as having 100% crystallinity; an amorphous form prepared by grinding for 4 h and dehydrating under reduced pressure at room temperature followed by rehydration at ambient conditions was defined as having 0% crystallinity.

The degree of crystallinity of a sample was determined by mixing it with 20% of lithium fluoride (LiF) as an internal standard. About 150 mg of a mixed sample was loaded into a stainless holder, and X-ray diffraction patterns were measured using the conditions mentioned above.

**Storage at elevated temperatures.** The intact and ground samples were stored in glass bottles

under a closed system at 50, 60, 65, or 70 °C in a drying oven (Mini Jet Oven, Toyama Sangyo).

## Results and Discussion

### Effect of grinding on the crystallinity of cefixime trihydrate

The X-ray diffraction patterns of the intact cefixime trihydrate and 1 h and 4 h ground samples are shown in Fig. 1.

The positions of the ground sample peaks were almost the same, but the peak heights decreased with increasing grinding time, indicating that the crystalline state became amorphous with grinding. To estimate the crystallinity of the ground sample,

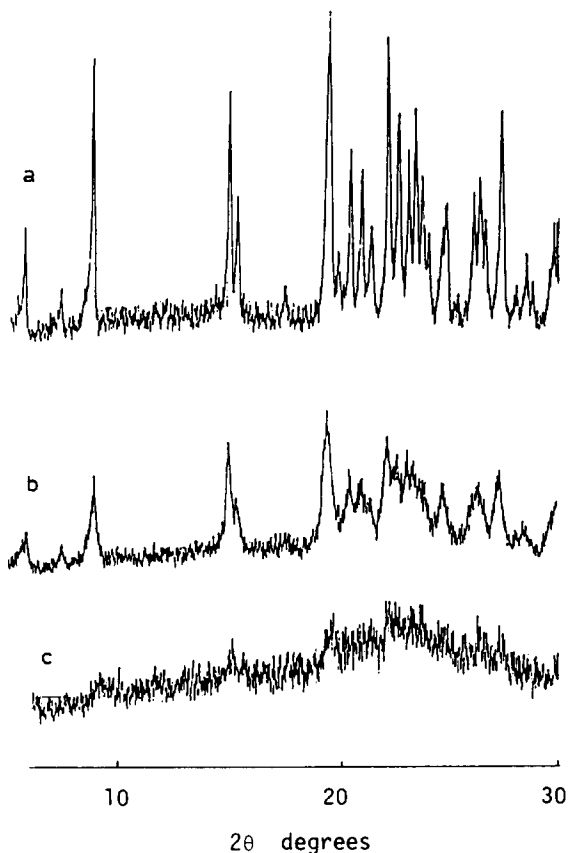


Fig. 1. Effect of grinding on X-ray diffraction profile of cefixime trihydrate. a: intact phase. b: 1 h in a ball mill. c: 4 h in a ball mill.

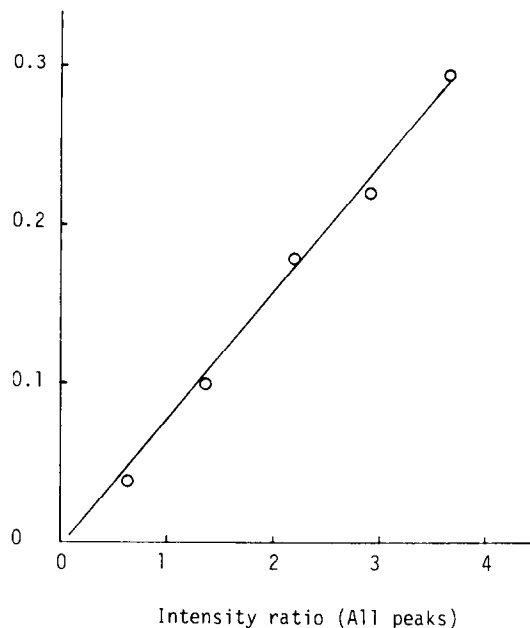


Fig. 2. Relationship between the intensity ratio at  $2\theta = 9.0^\circ$  and that for the sum of the 14 major X-ray diffraction peaks of cefixime trihydrate ( $2\theta = 5.0^\circ \sim 30.0^\circ$ ) with respect to internal standard (LiF,  $2\theta = 45.0^\circ$ ).

the relationship between  $I(9.0)$  vs  $I(\text{all})$  was examined where  $I(9.0)$  is the ratio of the X-ray diffraction intensity of cefixime trihydrate at  $2\theta = 9.0^\circ$  to that of LiF at  $2\theta = 45.0^\circ$ , and  $I(\text{all})$  is the ratio of intensity of the major 14 X-ray diffraction peaks of cefixime trihydrate from  $2\theta = 5.0^\circ$  to  $30.0^\circ$  to that of LiF at  $2\theta = 45.0^\circ$ . As the correlation coefficient of  $I(9.0)$  versus  $I(\text{all})$  gave a straight line ( $R = 0.998$ ) (see Fig. 2),  $I(9.0)$  was found to be useful to estimate the crystallinity of ground samples.

In order to obtain the relationship between  $I(9.0)$  and the degree of crystallinity, the intact sample (100% crystallinity) and 4 h ground sample (0% crystallinity) were mixed in appropriate weight ratios to make standard samples having 0%, 20%, 40%, 60%, 80% and 100% crystallinity. A plot of  $I(9.0)$  versus the degree of crystallinity of the standard samples is shown in Fig. 3.

The plot gave a straight line with a slope of  $k = 2.96 \times 10^{-3}$  and a correlation coefficient of  $r = 0.996$  by a least squares fit of the data. The

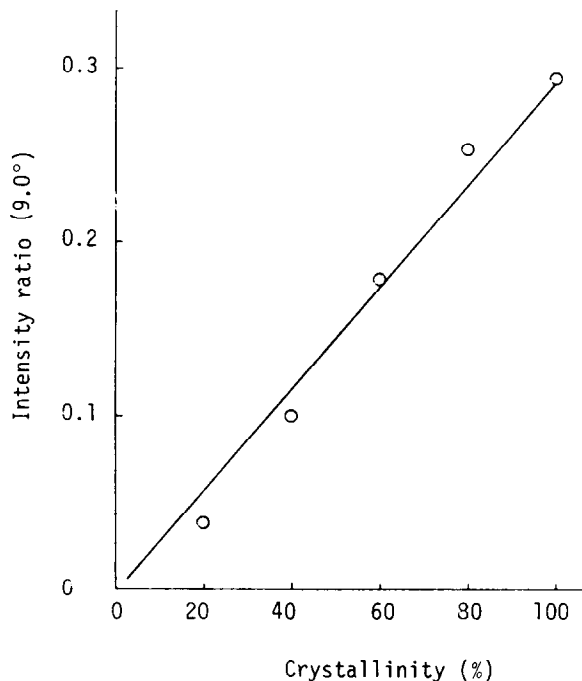


Fig. 3. Relationship between the degree of crystallinity and the X-ray diffraction intensity ratio of cefixime trihydrate ( $2\theta = 9.0^\circ$ ) with respect to internal standard (LiF,  $2\theta = 45.0^\circ$ ).

degree of crystallinity ( $X$ ) of a sample was thus obtained by Eqn. 2:

$$X = \frac{I(9.0)}{2.96 \times 10^{-3}} \quad (2)$$

Fig. 4 shows the effect of grinding on the degree of crystallinity (average of 3 determinations) as determined by the method mentioned above.

The degree of crystallinity decreased with increasing grinding time.

Scanning electron micrographs (SEM) were taken and the surface characteristics of ground cefixime trihydrate were examined.

These SEM photographs shown in Fig. 5 indicate that the particles seemed to form aggregates

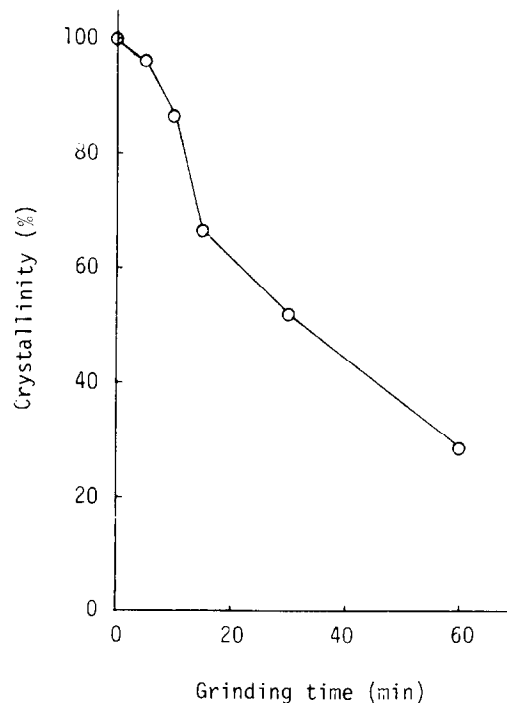


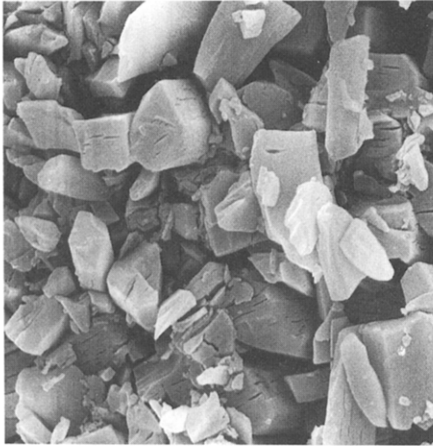
Fig. 4. Effect of grinding on crystallinity.

when the grinding time exceeded 10 min, and the amounts of agglomerated parts increased as the grinding process proceeded.

#### *Physicochemical properties of water molecules in cefixime trihydrate*

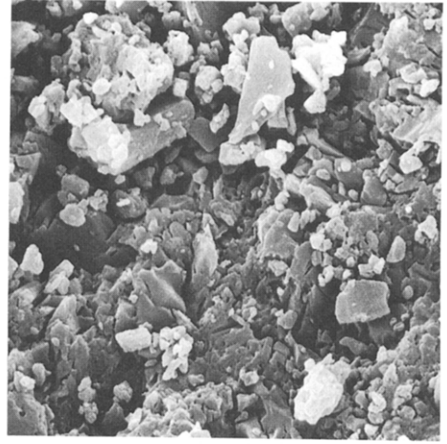
Thermogravimetric analysis (TGA) curve of intact cefixime trihydrate is shown in Fig. 6.

The weight loss occurring over a temperature range from about 50 to 120°C is 10.1%. And this weight loss is nearly equal to the stoichiometric value of 10.6% calculated for a trihydrate of cefixime. Therefore endothermic peaks observed around 110°C in the DSC curves of the ground samples (Fig. 7) were confirmed to be due to loss of water molecules in the crystal lattice. The relationship between the dehydration temperature and the crystallinity of ground cefixime trihydrate is shown in Fig. 8.



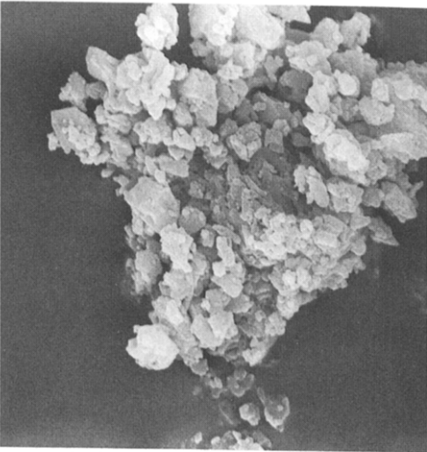
**a**

3 $\mu$ m



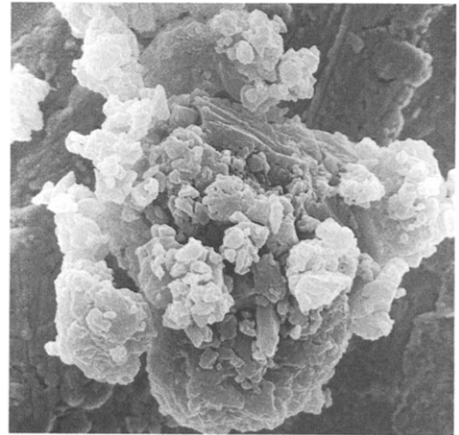
**b**

3 $\mu$ m



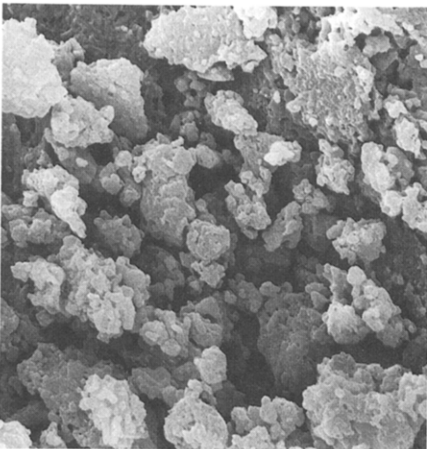
**d**

3 $\mu$ m



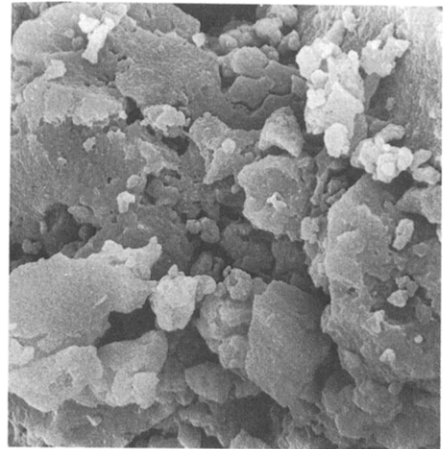
**c**

3 $\mu$ m



**e**

3 $\mu$ m



**f**

3 $\mu$ m

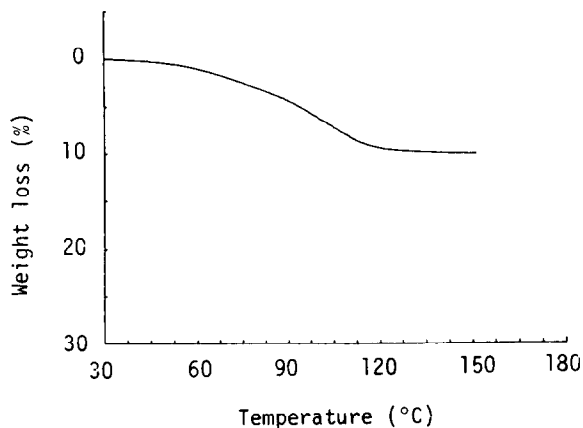


Fig. 6. TGA thermogram of intact cefixime trihydrate.

It is apparent that the dehydration temperature of the ground sample decreased linearly with decreasing crystallinity. Thus, a thermal kinetic study of an activation energy for dehydration was examined to clarify the grinding effect on the physical properties of water molecules in the crystal lattice. For the dehydration kinetics, Kis-

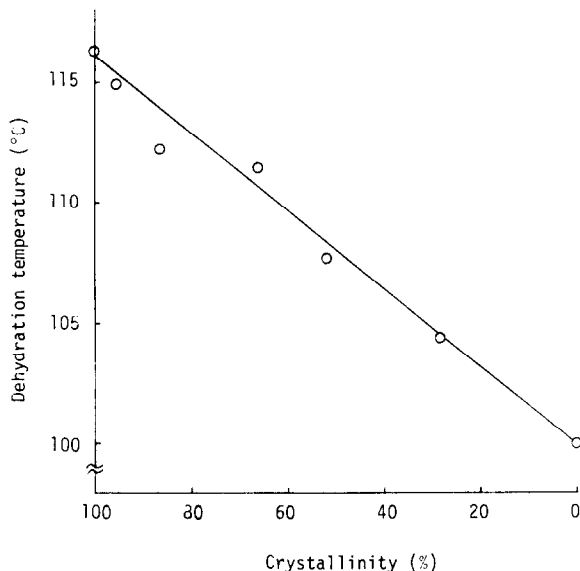


Fig. 8. Relationship between the degree of crystallinity and the dehydration point of ground cefixime trihydrate.

singer's method (Kissinger, 1957) was applied with the following equation:

$$\frac{d[\ln(\phi/T_m^2)]}{d[1/T_m]} = -\frac{E}{R} \quad (3)$$

where  $T_m$  is the temperature of the endothermic peak maximum,  $\phi$  is a heating rate,  $R$  is the gas constant, and  $E$  is the activation energy. Kissinger plots for the intact cefixime trihydrate and 4 h ground sample obtained by the least-squares method are shown in Fig. 9.

The activation energy for dehydration of intact cefixime trihydrate and that of 4 h ground sample were 72.4 and 67.5 kcal/mol, respectively. These calculated energies for dehydration clearly show that the grinding process affects the bonding force between water molecules and cefixime molecules.

Mooney et al. (1988) reported that the chemical stability of cefixime trihydrate strongly decreased when it was stored below the critical humidity for dehydration. They reported that the dihydrate and anhydrous forms obtained by the dehydration process were less stable than the trihydrate form. That is, the water molecules in the crystal lattice were considered to have a significant role in the

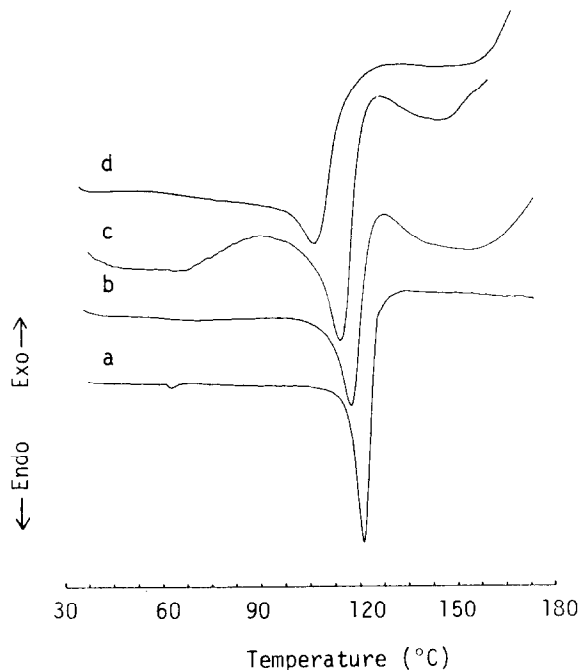


Fig. 7. Effect of grinding on the DSC curves of cefixime trihydrate. a: intact phase. b: 15 min in a ball mill. c: 1 h in a ball mill. d: 4 h in a ball mill.

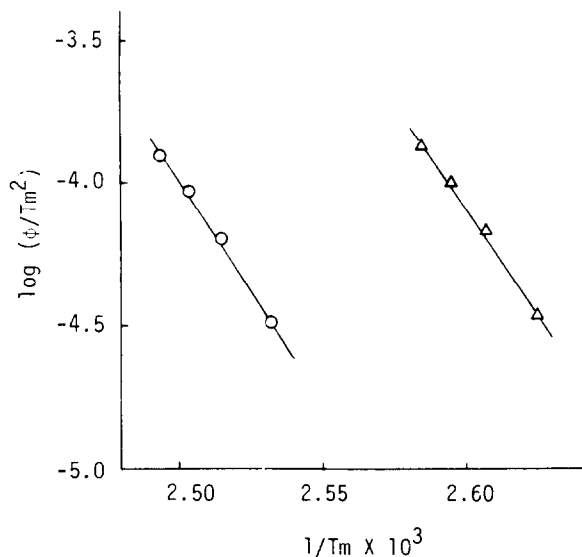


Fig. 9. Kissinger's plots for the thermal dehydration of cefixime trihydrate.  $\circ$ , Intact phase;  $\triangle$ , 4 h in a ball mill.

chemical stability of cefixime trihydrate. Therefore, the moisture adsorption isotherm at 25°C for the ground samples was studied. Fig. 10 shows the water content vs various relative humidity (R.H.) diagram of the intact and 1 h, 2.5 h and 4 h ground samples after storage at 25°C for 2 weeks.

The molar water content of the intact cefixime trihydrate remained constant at 3 mol/mol between 23 and 83% R.H., but it lost almost all water molecules between 0 and 11% R.H. Although the ground samples showed a similar behavior to the intact sample between 0 and 11% R.H., the water content of ground samples stored between 23 and 83% R.H. was lower than that of the intact sample. As the grinding time increased, the ground samples tended to lose water molecules at a lower R.H. Furthermore, the samples ground for 2.5 and 4 h did not absorb the same amount of water as the intact sample, even if they were stored under highly humid conditions. Based on these results, it was considered that the particles of cefixime trihydrate were agglomerated during the grinding procedure and a part of the crystalline lattice of the ground sample was destroyed by the grinding force. Therefore, the crystal lattice damage induced by grinding may not be repaired during storage under humid conditions. These re-

sults suggested that the bonding force between water molecules and cefixime molecules was weakened by grinding through crystal lattice disruption, and the number of water molecules having a greater freedom of movement in the impaired lattice was increased (Austin, 1965).

#### *Effect of grinding on the solid-state stability of cefixime trihydrate*

The effect of grinding on the chemical stability of cefixime trihydrate was studied. The remaining potencies of the ground cefixime trihydrate stored at 70°C for 2 days in closed bottles were measured. Although solid-state decomposition kinetics of some drugs have been discussed (Carstensen, 1988), theoretical treatment of the effect of grinding on the chemical stability has not been proposed yet. Then Eqn. 4 is proposed to clear the relationship between crystallinity and degradation rate of the ground cefixime trihydrate, if the overall decomposition rate of the ground sample is expressed by the sum of the decomposition rate

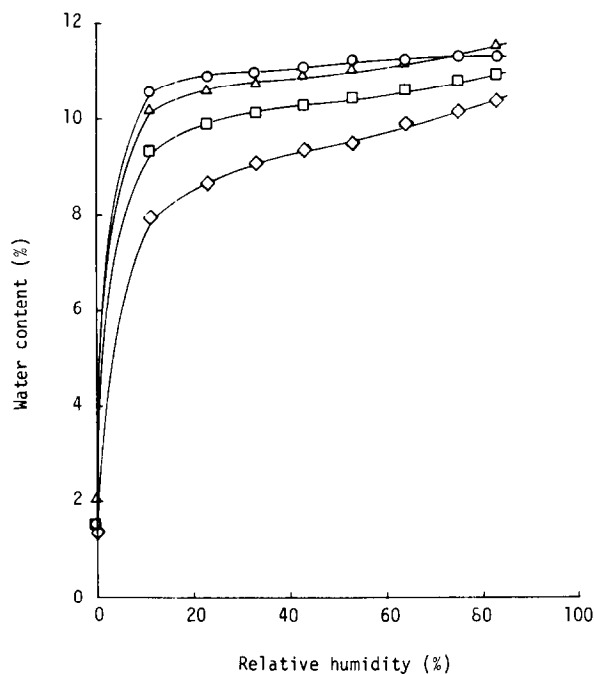


Fig. 10. Changes of water content of ground cefixime trihydrate at various R.H.s at 25°C.  $\circ$ , Intact phase;  $\triangle$ , 1 h in a ball mill;  $\square$ , 2.5 h in a ball mill;  $\diamond$ , 4 h in a ball mill.

constants in the amorphous and 100% crystalline states.

$$-\frac{dA'}{dt} = \{k_1X + k_2(1 - X)\}A' \quad (4)$$

In Eqn. 4,  $k_1$  and  $k_2$  are the decomposition rate constants in the 100% crystalline state (intact) and in the 0% crystalline state (4 h ground sample), respectively.  $A'$  is the remaining potency of cefixime at time  $t$ . Eqn. 4 is integrated to:

$$\ln A' = \{(k_2 - k_1)X - k_2\}t + \ln A'_0 \quad (5)$$

In Eqn. 5,  $A'_0$  is the  $A'$  value at  $t = 0$ . It is clear that a plot of  $\ln A'$  vs  $t$  should give a straight line. The data of the heat-deteriorated ground cefixime trihydrate are plotted according to Eqn. 5 in Fig. 11. The linearity of  $\ln A'$  against  $t$  was good in all cases. In this case,  $k_1$  and  $k_2$  could be expressed by the decomposition rate constant of intact and 4 h ground samples. Therefore, the crystallinity of the ground sample can be calculated by using Eqn. 5. Each crystallinity of the ground cefixime trihydrate for 5, 10, 15 and 30 min is shown in Table 1. The crystallinity of these ground samples estimated by using the decomposition rate constant was similar to that obtained by the X-ray method.

Thus, it is confirmed that crystallinity affects the chemical stability of cefixime trihydrate, and that the decomposition kinetics make it possible to discuss the quantitative effect of grinding.

#### *Effect of grinding on color stability of cefixime trihydrate*

As we have already reported the kinetics of discoloration of cefixime trihydrate in solid state (Kitamura et al., 1988), it is possible to obtain an apparent order of reaction ( $n$ ) and rate constant ( $k$ ) for discoloration. The values of  $\Delta E$  in Eqn. 1 of the aged samples were determined, and the apparent rate constant and the apparent reaction order for the color change were estimated using Eqns. 6 and 7 (Matsuda, 1980):

$$d\Delta E/dt = k(\Delta E)^n \quad (6)$$

$$\log \Delta E = \frac{1}{1-n} \log(1-n)k + \frac{1}{1-n} \log t \quad (7)$$

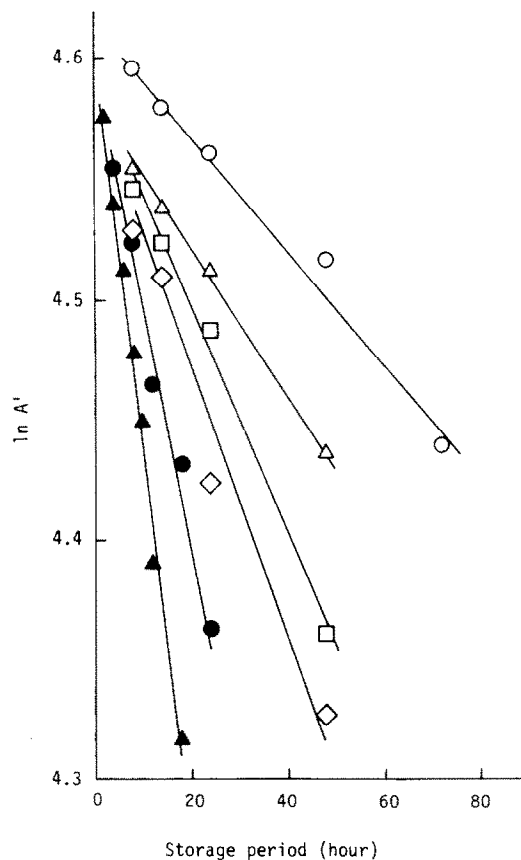


Fig. 11. Effect of grinding on the stability of cefixime trihydrate grinding time at 70°C. ○, Intact phase; △, 5 min in a ball mill; □, 10 min in a ball mill; ◇, 15 min in a ball mill; ●, 30 min in a ball mill; ▲, 4 h in a ball mill.

where  $t$  denotes time,  $k$  is the apparent rate constant, and  $n (< 1)$  is the apparent reaction order.

TABLE 1

*Crystallinity of ground cefixime trihydrate*

Grinding time (min)	Crystallinity	
	Decomposition rate constant method (%)	X-ray method (%)
5	96	96
10	84	87
15	80	66
30	50	52



TABLE 2

Grinding effect on reaction parameters of cefixime trihydrate for discoloration at 60°C

Grinding time (min)	Degree of crystallinity (%)	Rate constant (h <sup>-1</sup> )	Reaction order
Intact	100	$1.3 \times 10^{-1}$	$-6.0 \times 10^{-1}$
15	66	$1.9 \times 10^{-1}$	$-5.8 \times 10^{-1}$
30	52	$4.6 \times 10^{-1}$	$-5.6 \times 10^{-1}$

For practical reasons, intact, 15 min and 30 min ground cefixime trihydrate samples were employed for discoloration kinetic study, because the appearance of samples ground for more than 30 min was distinctly different from that of the intact sample. The apparent order of reaction and the rate constant at 60°C of the intact, 15 and 30 min ground cefixime trihydrate samples are shown in Table 2.

In this study, it is possible to compare the apparent rate constants of these samples because these samples showed almost the same apparent reaction order (-0.6). The obtained apparent rate constants for 15 and 30 min ground samples were 1.5 and 3.5 times larger than that of the intact sample. These results led us to the conclusion that the color changes in cefixime trihydrate were caused by grinding procedures. The relationship between  $\log t$  and  $\log \Delta E$  of cefixime trihydrate samples ground 15 min which were stored at 50, 60, 65 and 70°C is shown in Fig. 12.

The good linearity permitted an Arrhenius plot examination to discuss the activation energy and frequency factor for discoloration of the sample ground 15 min.

Plots of  $\ln k$  against the reciprocal of the absolute temperature ( $1/T$ ) also give a straight line

TABLE 3

Activation energy and frequency factor for discoloration of ground cefixime trihydrate

Grinding time (min)	Frequency factor (h <sup>-1</sup> )	Activation energy (kcal/mol)
Intact	$1.23 \times 10^{23}$	36.3
15	$1.52 \times 10^{23}$	36.3

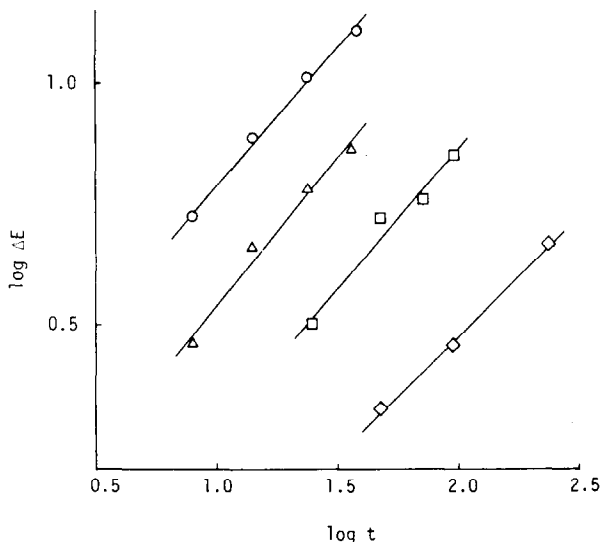


Fig. 12. Time courses for discoloration of 15 min ground cefixime trihydrate at various temperatures. ○, 70°C; △, 65°C; □, 60°C; ◇, 50°C.

as shown in Fig. 13. Activation energy and the frequency factor were estimated by the method of least squares. The results including the intact cefixime trihydrate are summarized in Table 3.

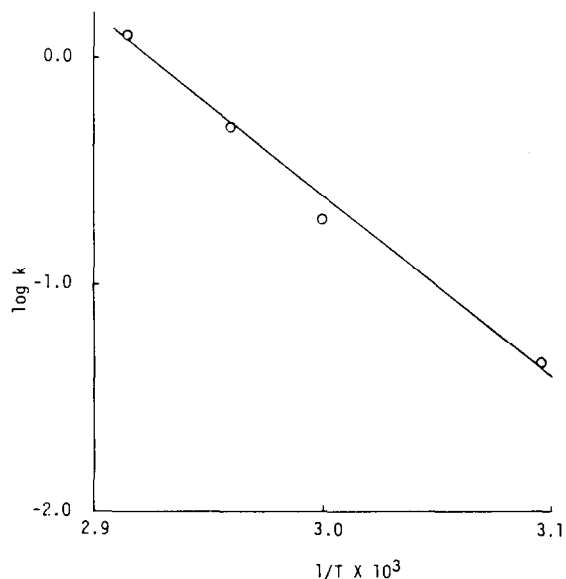


Fig. 13. Arrhenius plot for discoloration process of 15 min ground cefixime trihydrate.

Although the activation energy of the sample ground 15 min and the intact sample (Kitamura et al., 1988) showed almost the same value (36 kcal/mol), the frequency factor of the ground sample was 1.2 times larger than that of the intact one. These results confirm that the discoloration of cefixime trihydrate in solid-state is greatly affected by its crystallinity.

## Conclusion

It was confirmed that the interaction between water molecules and cefixime molecules was weakened by grinding. The crystal lattice damage induced by grinding significantly affected the chemical stability as well as the discoloration. Our results suggest that the kinetic study of decomposition and discoloration is very useful in estimating the effect of mechanical stresses such as grinding on drug substances.

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