[Chem. Pharm. Bull. 33(8)3422—3428(1985)]

Physico-chemical Properties and Isothermal Transition of Acetazolamide Polymorphs[1)]

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(Received November 6, 1984)

Acetazolamide has two polymorphic forms (forms A and B), which were characterized by X-ray powder diffractometry, infrared spectrophotometry, differential scanning calorimetry and thermogravimetry. The solubility and dissolution rate of form B were about 1.1 times higher than those of form A. The transition temperature obtained by solubility measurement was 78 °C, and the heats of transition (ΔH_{trans}) calculated by solubility measurement and by differential scanning calorimetry were 2.6 and 1.7 kJ·mol^{-1}, respectively. The free energy change ($\Delta G_{25\,C}$) between the two polymorphic forms was calculated to be 357 J·mol^{-1}. In addition, the kinetics of isothermal transition of form A to form B at high temperature was investigated by means of differential scanning calorimetry; this transition appeared to follow the mechanism of random nucleation with first-order kinetics. The activation energy for this transition was calculated to be 246 kJ·mol^{-1} from the Arrhenius plots.

Keywords——acetazolamide; polymorphism; solubility; dissolution rate; thermodynamic parameter; differential scanning calorimetry; isothermal transition; kinetic analysis

Acetazolamide is well known as an inhibitor of carbonic anhydrase and is used as a diuretic drug. Kuhnert-Brandstätter[2)] suggested the existence of two polymorphic forms of acetazolamide because he observed different melting points, but further detailed study on its polymorphism has not been reported so far.

In this work, we investigated the physico-chemical properties, thermodynamic parameters and kinetics of isothermal transition of acetazolamide polymorphic forms.

Experimental

Materials——Acetazolamide was a commercial product of JP X grade (Lederle Co., Ltd.). All other chemicals were reagent-grade commercial products.

Preparation of Polymorphic Forms——Form A: Acetazolamide (5 g) was dissolved in 700 ml of methanol at 64—65 °C. The solution was cooled slowly and maintained at 5 °C overnight. The resulting crystals were collected by filtration and dried at 80 °C in a vacuum.

Form B: Form A (5 g) was heated at 205 °C for 5 h. The particle sizes of the two polymorphic forms thus prepared were in the range of 62—74 μm.

Characterization of Polymorphic Forms——The two polymorphic forms were characterized by X-ray powder diffractometry (Rigaku Denki, Geigerflex 2011, Cu-K_α radiation, 40 kV, 10 mA, Ni-filter, 120000 cpm), infrared (IR) spectrophotometry (Hitachi, model 215, in Nujol), differential scanning calorimetry (DSC, Perkin-Elmer, model DSC-2C) and thermogravimetry (TG, Perkin-Elmer, model TGS-2).

Dissolution Rate Measurement——The dissolution rate was determined according to the disk method with a slight modification.[3)] A disk (diameter: 1.3 cm) was prepared by compressing 200 mg of the sample under a pressure of 250 kg·cm^{-2} in a hydraulic press for the preparation of KBr tablets for IR spectrophotometry. It was confirmed by X-ray diffractometry that no polymorphic transition took place during the compression. The compressed disk was

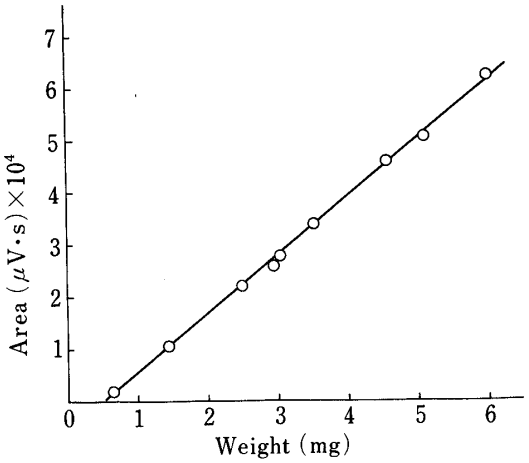


Fig. 1. Calibration Curve for Acetazolamide Form A by DSC

Sensitivity range, 8.4 mJ·s^{-1}; heating rate, 40 °C·min^{-1}; r=0.999.

stuck to the bottom of a rotary basket (JP X) with double-sided tape and rotated at 100 rpm in the test solution (500 ml) at 30 °C. The test solution was 0.1 M phosphate buffer solution (pH 7.0). The concentration of dissolved drug was measured at 270 nm with a spectrophotometer (Hitachi, model 200-20) by circulating the solution through the flow cell continuously.

Solubility Measurement——Each polymorphic form in an amount in excess of its solubility was added to 100 ml of 0.1 M phosphate buffer solution (pH 7.0) in a 500 ml flask maintained at various temperatures (20, 25, 30 and 35 °C), and it was immediately shaken at 80 strokes·min^{-1}. Aliquots (2 ml) of the sample solution were taken at appropriate times and filtered through a Millipore filter (pore size: 0.45 μm), then diluted and assayed spectrophotometrically at 270 nm. During the solubility measurements, no polymorphic transition was observed.

Determination of Transition Temperature and Heat of Transition by DSC——The apparatus was the same as that used for characterization of polymorphic forms.

(1) Transition Temperature Measurement: The transition temperature was determined by plotting the temperature of transition against heating rate, and then extrapolating the curve to zero heating rate. The conditions were as follows: sample weight, 2.2—15.4 mg; sensitivity range, 20.9 mJ·s^{-1}; heating rate, 0.625—20 °C·min^{-1}.

(2) Heat of Transition (ΔH_{trans}) Measurement: ΔH_{trans} was determined by measuring the area under the transition peak with a Chromatopac (Shimadzu, C-R1A). The conditions were as follows: sample weight, 8.3—13.6 mg; sensitivity range, 41.8 mJ·s^{-1}; heating rate, 10 °C·min^{-1}; standard sample, indium (ΔH_f=3.3 kJ·mol^{-1}).

Kinetic Analysis of Isothermal Transition——The kinetics of the isothermal transition of form A to form B was investigated by means of differential scanning calorimetry as described in the previous paper.[1] The amount of form A in the presence of form B was determined by measuring the area under the transition peak of form A. The conditions for measurement were as follows: sample weight, 5±0.5 mg; sensitivity range, 8.4 mJ·s^{-1}; heating rate, 40 °C·min^{-1}. The calibration curve showed good linearity (r=0.999) as can be seen in Fig. 1. In order to verify the utility of this calibration curve, several mixtures of polymorphic forms were prepared, and the amount of form A was determined from the calibration curve. The experimental and theoretical values were in good agreement.

Scanning Electron Microscopy——Changes of crystal shapes during isothermal transition of polymorphic forms were observed with a scanning electron microscope (Nihondenshi, JSM-T20).

Results and Discussion

Characterization of Acetazolamide Polymorphic Forms

The X-ray powder diffraction patterns of the two polymorphic forms are shown in Fig. 2. In the diffraction pattern of form A, very high peaks were observed at 9.9°, 24.8° and 29.4° (2θ), which were not found in the diffraction pattern of form B. On the other hand, form B gave the highest diffraction peak at 13.7° (2θ) and characteristic peaks at 19.6°, 22.3°, 26.0°, 26.9° (2θ) and elsewhere. Thus, the diffraction pattern of form A was clearly different from that of form B.

The IR spectra of the two polymorphic forms are shown in Fig. 3. The spectrum of form A was different from that of form B. In particular, form A showed characteristic absorption peaks in the region of 1100—900 cm^{-1}, and form B gave a specific peak at about 940 cm^{-1}.

As shown in Fig. 4, the DSC curve of form A exhibited two endothermic peaks: one at

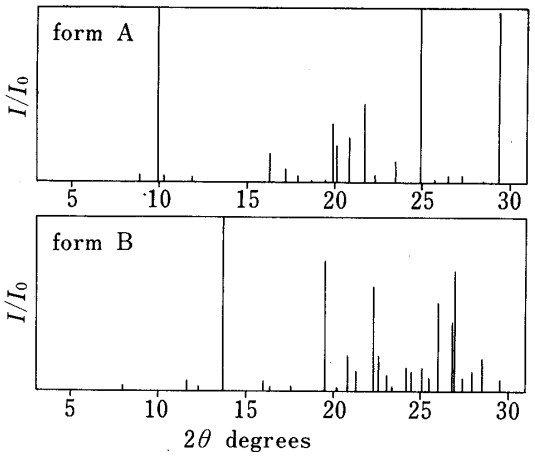


Fig. 2. X-Ray Powder Diffraction Patterns of Acetazolamide Polymorphic Forms

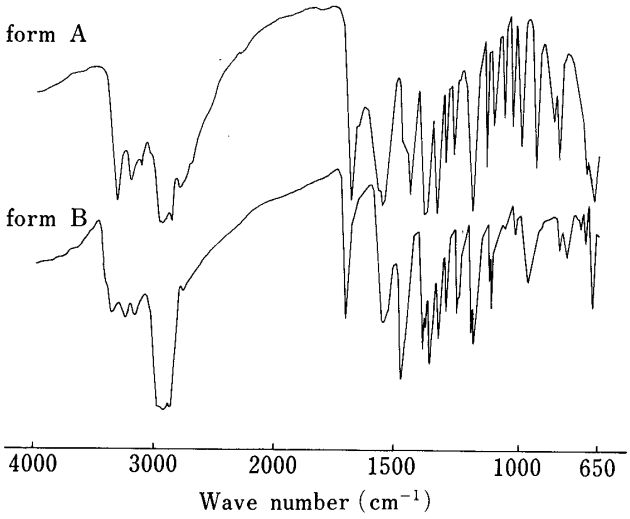


Fig. 3. IR Spectra of Acetazolamide Polymorphic Forms (in Nujol)

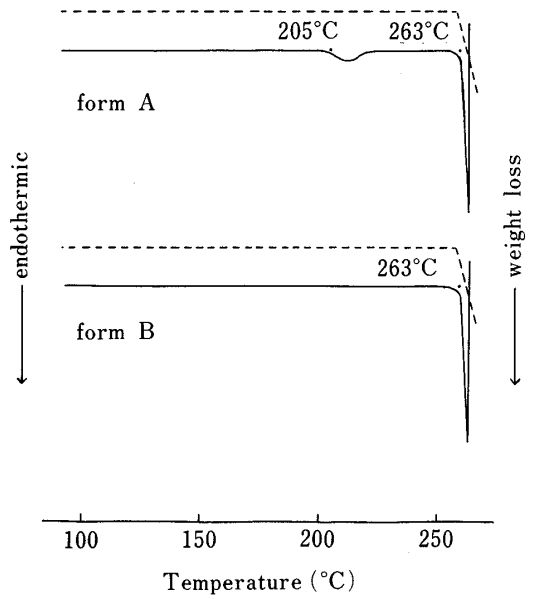


Fig. 4. DSC–TG Curves of Acetazolamide Polymorphic Forms

——, DSC curves; – – – –, TG curves; sensitivity range, 41.8 mJ·s^{-1}; heating rate, 5 °C·min^{-1}.

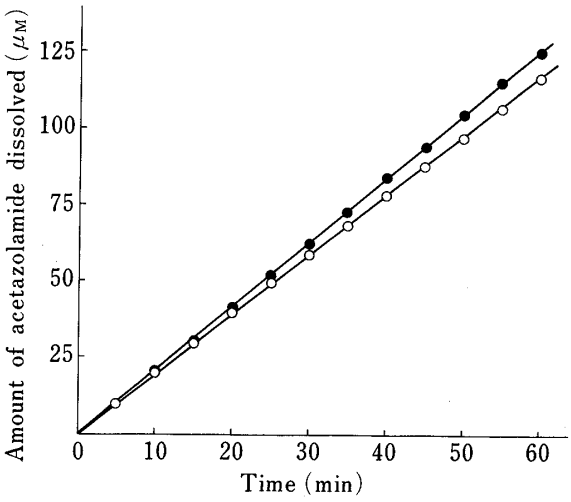


Fig. 5. Dissolution Curves of Acetazolamide Polymorphic Forms in 0.1 M Phosphate Buffer Solution (pH 7.0) at 30 °C

○, form A (r=1.000); ●, form B (r=1.000).

205 °C corresponded to the transition of form A to form B, and the other at 263 °C was attributable to the melting point accompanied with decomposition of form B. Form B gave only one endothermic peak at 263 °C corresponding to the melting point accompanied with decomposition.

In the TG curves of forms A and B, no change in weight was observed until the melting point (Fig. 4).

Dissolution Rate Determination

The dissolution curves of forms A and B are shown in Fig. 5. Each point represents the average of three experiments. Form B dissolved slightly faster than form A; the dissolution rates of forms A and B were 1.94 and 2.11 μM·min^{-1}, respectively.

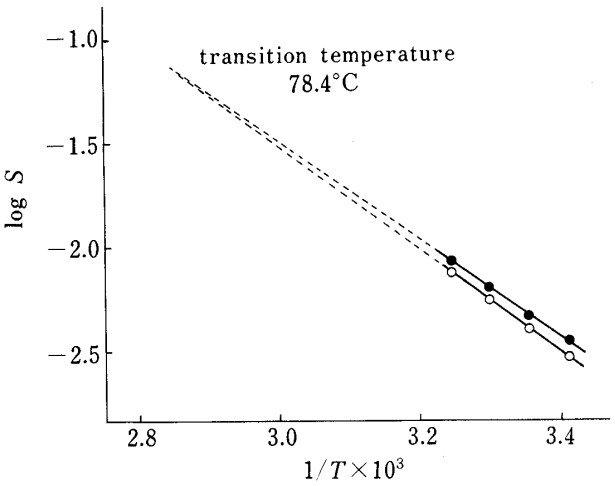


Fig. 6. The van't Hoff Type Plots for Acetazolamide Polymorphic Forms in 0.1 M Phosphate Buffer Solution (pH 7.0)

S, solubility (μM); ○, form A ($r=0.996$); ●, form B ($r=0.996$).

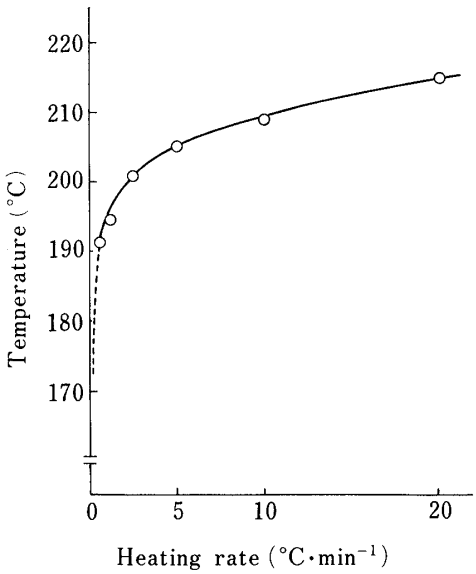


Fig. 7. Effect of Heating Rate of DSC on Apparent Transition Temperature of Acetazolamide (Form A→Form B)

TABLE I. Thermodynamic Parameters for Acetazolamide Polymorphic Forms Obtained by Solubility Measurement

	Transition temperature (°C)	ΔH_{soln} (kJ·mol^{-1})	ΔH_{trans} (kJ·mol^{-1})	$\Delta G_{25\,°C}$ (J·mol^{-1})	ΔS_{trans} [a] (J·K^{-1}·mol^{-1})
Form A	78.4	47.3	2.6	357	7.2
Form B		44.7			

a) ΔS_{trans}: entropy change.

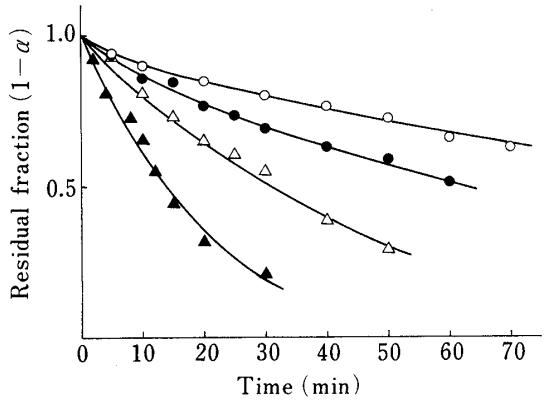


Fig. 8. Residual Fraction of Form A during Isothermal Transition to Form B of Acetazolamide

○, 180 °C; ●, 185 °C; △, 190 °C; ▲, 195 °C.

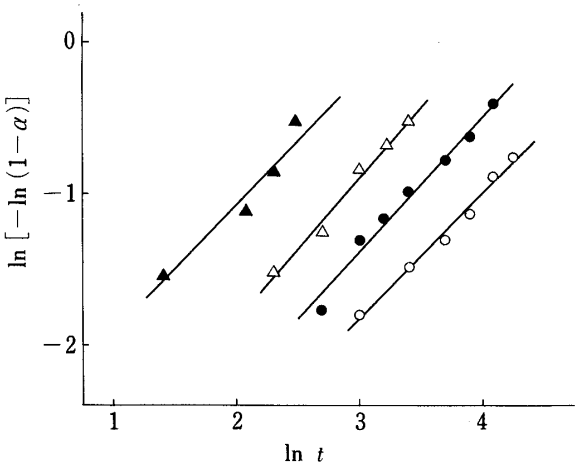


Fig. 9. Plots of $\ln[-\ln(1-\alpha)]$ *versus* $\ln t$ for Isothermal Transition of Form A to Form B of Acetazolamide ($\alpha=0.15$—0.5)

○, 180 °C ($m=0.82$, $r=0.993$); ●, 185 °C ($m=0.91$, $r=0.990$); △, 190 °C ($m=0.95$, $r=0.992$); ▲, 195 °C ($m=0.87$, $r=0.964$).

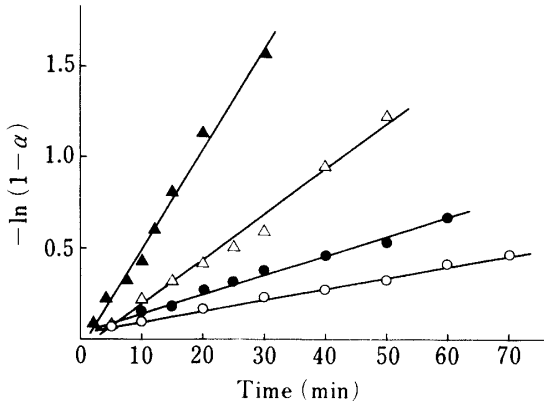


Fig. 10. Plots of $-\ln(1-\alpha)$ *versus* t for Isothermal Transition of Form A to Form B of Acetazolamide

○, 180 °C ($r=0.997$); ●, 185 °C ($r=0.995$); △, 190 °C ($r=0.993$); ▲, 195 °C ($r=0.994$).

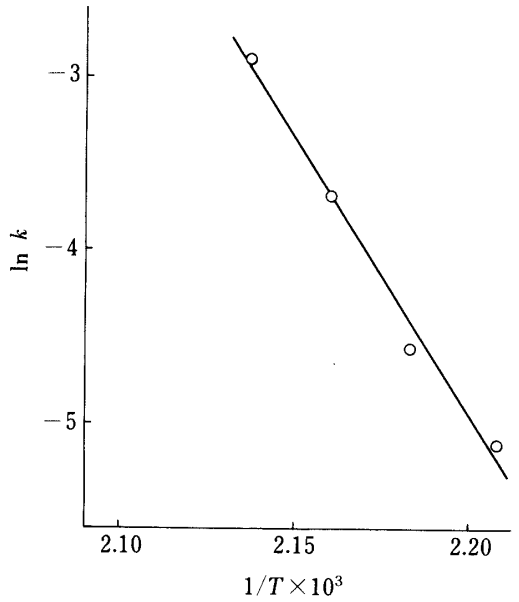
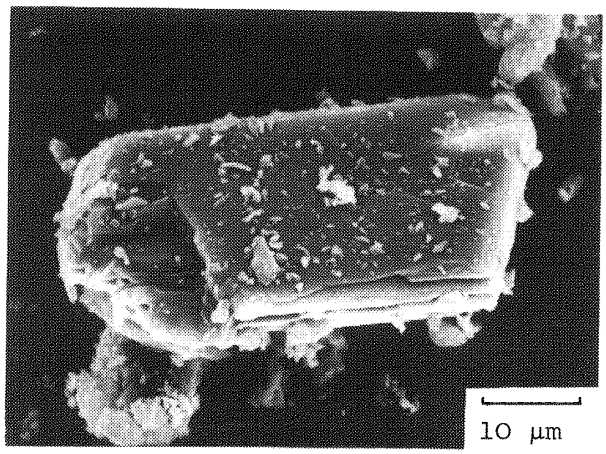
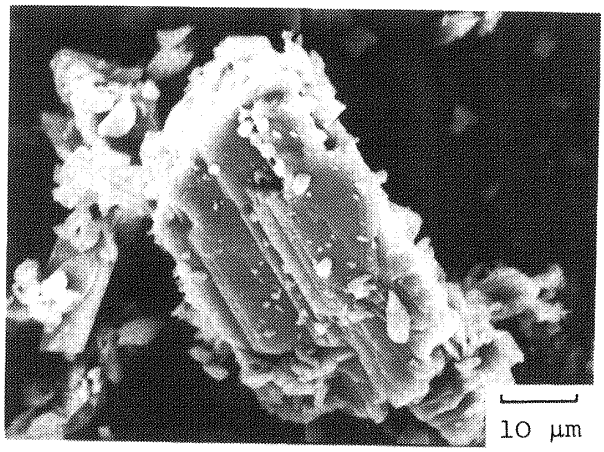


Fig. 12. Arrhenius Plots for Isothermal Transition of Acetazolamide Polymorphic Forms (Form A→Form B, $r=0.994$)



(a)



(b)

Fig. 11. Scanning Electron Microphotographs of Acetazolamide Crystals during Isothermal Transition of Form A to Form B at 195 °C

(a) Form A before heating (×2000); (b) after 30 min (×1500).

Solubility Determination and Thermodynamic Parameters

The difference of solubility between forms A and B was very small; the solubility of form B was about 1.1 times higher than that of form A. The saturated concentrations for each polymorphic form were plotted according to a van't Hoff type equation as shown in Fig. 6. The transition temperature estimated from the intersection of the two straight lines was 78.4 °C. The transition temperature was also obtained by DSC measurement. In this case, however, it was difficult to determine the transition temperature accurately by extrapolation, as in the case of sulfathiazole described by Kanke and Sekiguchi[4] (Fig. 7). The heats of solution (ΔH_{soln}) of forms A and B were 47.3 and 44.7 kJ·mol^{-1}, respectively, and ΔH_{trans} was 2.6 kJ·mol^{-1} (Table I). On the other hand, ΔH_{trans} determined by the DSC measurement was 1.7 kJ·mol^{-1}, which is in reasonable agreement with the value obtained from the van't Hoff type plots. The free energy change ($\Delta G_{25\,C}$) for acetazolamide polymorphic forms was 357 J·mol^{-1}, which is a relatively small value. Therefore, it is presumed, following Aguiar and

TABLE II. Values of m[a] for Solid-State Reaction Rate Equations[10]

Equation	m	Mechanism
$\alpha=kt$	1.24	Zero-order mechanism (Polany–Winger equation)
$1-(1-\alpha)^{1/2}=kt$	1.11	Phase boundary reaction, cylindrical symmetry
$1-(1-\alpha)^{1/3}=kt$	1.07	Phase boundary reaction, spherical symmetry
$-\ln(1-\alpha)=kt$	1.00	Random nucleation, first-order mechanism
$[-\ln(1-\alpha)]^{1/2}=kt$	2.00	Random nucleation, two-dimensional growth of nuclei (Avrami–Erofeev equation)
$[-\ln(1-\alpha)]^{1/3}=kt$	3.00	Random nucleation, three-dimensional growth of nuclei (Avrami–Erofeev equation)
$\alpha^2=kt$	0.62	One-dimensional diffusion
$(1-\alpha)\ln(1-\alpha)+\alpha=kt$	0.57	Two-dimensional diffusion
$[1-(1-\alpha)^{1/3}]^2=kt$	0.54	Three-dimensional diffusion (Jander equation)
$(1-2\alpha/3)-(1-\alpha)^{2/3}=kt$	0.57	Three-dimensional diffusion (Ginstling–Brounshtein equation)

a) $\ln[-\ln(1-\alpha)]=\ln B+m\cdot\ln t$ ($\alpha=0.15$—0.50).

Zelmar,[5] that acetazolamide polymorphic forms would not significantly affect the bioavailability, just as in the case of mefenamic acid,[5] acetohexamide,[6] tolbutamide,[7] indomethacin[8] and benoxaprofen.[9]

Kinetics and Mechanism of Isothermal Transition

The isothermal transition curves of form A to form B at 180, 185, 190 and 195 °C are shown in Fig. 8.

The kinetic analysis of this transition was carried out according to the method of Hancock and Sharp[10] (see also the previous paper[1]). In this method, the slope (m) is estimated by plotting $\ln[-\ln(1-\alpha)]$ against $\ln t$, based on Eq. 1:

$$\ln[-\ln(1-\alpha)]=\ln B+m\cdot\ln t \qquad (\alpha=0.15\text{—}0.5) \tag{1}$$

where α is the fraction of transition and B is a constant. The values of m for the various theoretical equations of solid-state decomposition are listed in Table II.[10]

The value of m for the transition of form A to form B was calculated to be 0.89 ± 0.06 (mean $\pm$ S.D., $n=4$) as shown in Fig. 9. Accordingly, this transition appears to follow the mechanism of random nucleation with first-order kinetics.[11,12] It is considered that this transition takes place in a homogeneous system of fine particles and that the transition rate is proportional to the number of residual particles of form A. The plots of $-\ln(1-\alpha)$ against time at four temperatures gave straight lines (Fig. 10). The transition rate constant at 195 °C was about 9 times as high as that at 180 °C.

From the results of observation with a scanning electron microscope, it was concluded that the crystal shapes during isothermal transition of form A to form B did not significantly change, as shown in Fig. 11.

In addition, the activation energy for this transition calculated from the slope of the Arrhenius plots was 264 kJ·mol^{-1} (Fig. 12).

References and Notes

1) This paper forms Part XV of "Studies on Drug Nonequivalence," The preceding paper, Part XIV: T. Umeda, N. Ohnishi, T. Yokoyama, T. Kuroda, Y. Kita, K. Kuroda, E. Tatsumi, and Y. Matsuda, *Chem. Pharm. Bull.*, **33**, 2073 (1985).
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