Physicochemical Stability of Phenobarbital Polymorphs at Various Levels of Humidity and Temperature

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The physicochemical stability of six phenobarbital modifications [forms A, B, C (monohydrate), D (dioxane solvate), E (hemihydrate), and F] at various levels of humidity and temperature were measured using X-ray diffractometry and differential scanning calorimetry. Form D was identified as a new crystalline form (dioxane solvate). Polymorphic transformations of the modifications were investigated by the Kissinger method under nonisothermal conditions. Change of polymorphic content of phenobarbital modifications under various humidity levels at 45°C was evaluated by X-ray powder diffraction. The polymorphic stability under isothermal conditions was estimated kinetically, based upon the Jander equation. Forms A, B, and F were stable at 0 and 75% RH and 45°C for 3 months. On the contrary, forms C, D, and E transformed during storage. The transformation rates of form D were larger than that of forms C and E.

KEY WORDS: phenobarbital; polymorphism; physicochemical stability; X-ray diffractometry; humidity.

INTRODUCTION

The dissolution behavior of polymorphic forms of drugs that are practically insoluble in water is important in the pharmaceutical design of preparations, because it affects their dissolution rate and, hence, bioavailability (1,2). The physicochemical stability of polymorphic drug forms is also affected during storage by environmental factors, i.e., moisture, temperature, light, and excipients, which can cause the dissolution rate and bioavailability to fluctuate (3).

Phenobarbital is widely used as a hypnotic and sedative. Its polymorphic modifications (4–14), dissolution rates (15), and bioavailability (16) have been studied. The stability of polymorphic forms under compression (17), the mechanical strength of tablets (18,19), and the effects of high temperatures (20,21) were also investigated during formulation studies. However, there have been no publications concerning the physicochemical stability of solid phenobarbital forms. In the present study, we investigated the physicochemical properties and stability of these forms kinetically.

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MATERIALS AND METHODS

Materials

Bulk phenobarbital powder JP XI (lot No. T46513) was obtained from Maruishi Pharm. Co., Osaka, Japan. The 13 organic solvents and all other chemicals used were of analytical grade.

Preparation of Polymorphs

Six modifications of the drug were prepared using the various organic solvents and preparation methods as follows.

Form A. Form A was obtained by spray drying. A suspension of 1% drug in chloroform/acetone (1:1) was fed into a mini-spray drier (Model Mini-spray h_o, Yamato Kagaku, Co., Tokyo) using a peristaltic pump at a flow rate of 10 mL/min. The temperature at the inlet of the drying chamber of the apparatus was maintained at 50°C.

Form B. A hot saturated methanolic solution of the drug was allowed to stand at room temperature. The separated crystals were then filtered and dried *in vacuo* at room temperature for 24 hr.

Form C. Ten grams of drug was added to 100 mL of distilled water, then stirred for 1 hr, and the suspension was allowed to stand at 5°C. The separated crystals were filtered and dried *in vacuo* at room temperature for 24 hr.

Form D. A saturated dioxane solution of the drug was lyophilized using an Eyela freeze dryer (Model FD-5, Tokyo Rikakikai Co., Tokyo.

Form E. Ten grams of form C was added to 100 mL of distilled water, then stirred for 1 hr, and the suspension was allowed to stand at 5°C. The separated crystals were filtered and dried *in vacuo* at room temperature for 1 hr.

Form F. The bulk powder was heated at 175°C for 2 hr.

X-Ray Powder Diffraction Analysis

Diffractograms were taken at room temperature with an X-ray diffractometer (XD-3A, Shimadzu Co., Kyoto, Japan). The operating conditions were as follows: target, Cu; filter, Ni; voltage 25 kV; current, 10 mA; receiving slit, 0.1 mm; time constant 1 sec; counting range, 1 kcps; and scanning speed, 1° 2θ/min.

Thermal Analysis

Differential thermal analysis (DTA) and thermogravimetry (TG) were performed using Type DTG-30 and TG-30 instruments (Shimadzu Co.). Differential scanning calorimetry (DSC) was performed with a Type 3100 instrument (Mac Science Co., Tokyo). The operating conditions in an open-pan system were as follows: sample weight, 5 mg; heating rate, 1, 2.5, 5, 10, 15, 20, and 30°C/min; and N_2 gas flow rate, 50 mL/min.

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Measurement of the Crystal Content

Known amounts of standard mixtures were obtained by physically mixing form B, C, or E with the amorphous form or forms A and D in a mortar with a spatula. The calibration curves for measuring the crystal content of forms B, C, D and E were obtained based upon the area intensity of the X-ray diffraction peaks at 13.6, 5.6, 21.2, and 22.5° (2θ) attributable to each form, respectively. The plots showed a good linear correlation and were always reproducible over 10 standard samples.

Physicochemical Stability of Modifications at Various Humidity Levels at 45°C

Samples (50 mg) were placed in glass containers, then stored at 0, 6, 17, 31, 51, 75, 79, 87, 96, and 100% (various saturated salt solutions) relative humidity (RH) at 45°C in a desiccator. The samples were removed from the desiccator at various times for X-ray powder diffraction analysis and TG. The amount of polymorphic form transformed was determined by X-ray powder diffraction described above, and the levels of the hydrate were determined from the weight loss determined from the TG data.

RESULTS AND DISCUSSION

Powder X-Ray Diffraction Profiles of Modifications

The powder X-ray diffraction profiles of six modifications had significant profiles and were independent from each other as shown in Figs. 3 and 4. These modifications showed the specific X-ray diffraction peaks as follows: form A, 17.1, 21.1, and 28.6°; form B, 7.4, 17.3, and 22.5°; form C, 5.6 and 11.2°; form D, 10.2 and 19.5°; form E, 5.6, 11.2 and 22.5°; and form F, 7.4, 15.8, and 22.5° (2θ). Forms A, B, C, E, and F had already been identified with the reported crystalline forms in the literature (4–16), but form D is a new modification.

Thermal Behavior of Modifications

Figure 1 shows the DSC curves of the modifications. Form A showed an endo-exothermic phenomenon at 166.0°C, an endothermic peak at 179.2°C with a shoulder at 175.8°C, and no weight loss on the TG curve. Form B showed an endothermic peak at 179.4°C with a shoulder at 175.3°C. Form F showed an endothermic peak at 179.8°C due to the melting accompanying sublimation, suggesting a stable form at high temperature. Forms C and E showed an endothermic peak at 50–70°C with a 7.0 and 3.2% of weight loss due to the dehydration of 1.0 mol and .5 mol of water per mol of the drug, respectively, and a melting peak at 179°C. On the other hand, form D showed an endothermic peak at 60–90°C with 25.4% weight loss due to the desolvation of 1 mol of dioxane and two endothermic peaks at 168 and 178°C, due to transformation and melting, respectively.

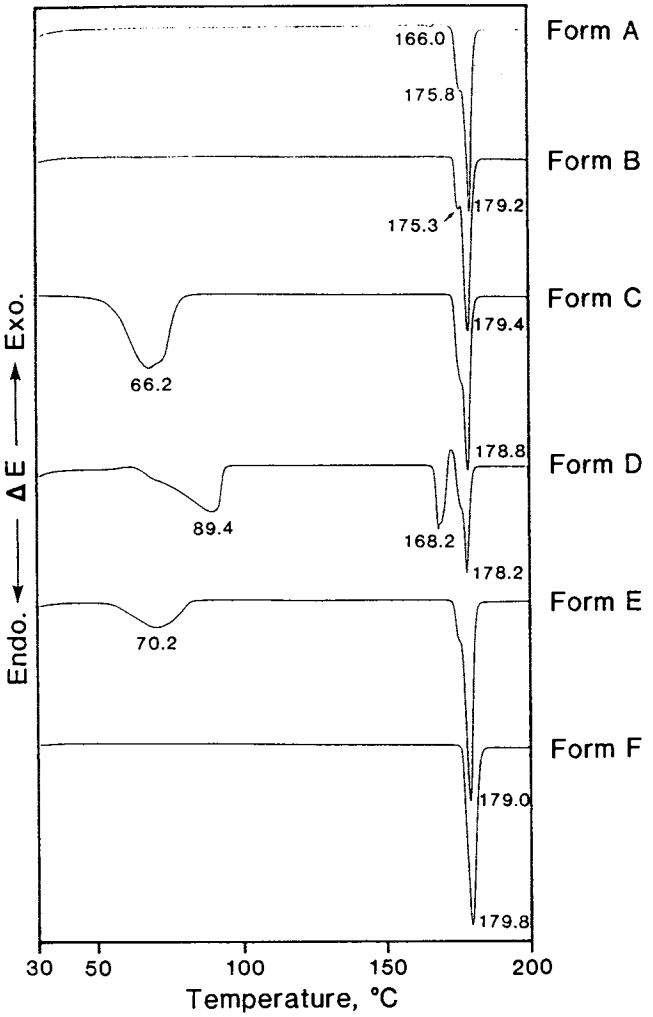

Fig. 1. Differential scanning calorimetry (DSC) curves of phenobarbital modifications (heating rate, 10°C/min).

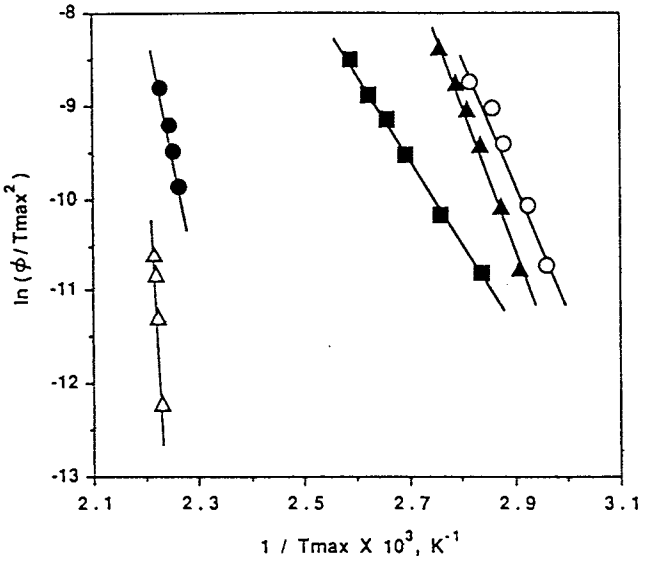

Fig. 2. Kissinger plots of phenobarbital modifications: (●), form A; (△) form B; (○) form C; (■) form D; (▲) form E.

Table I. Activation Energies of Transformation of Phenobarbital Modifications from Kissinger Plots

Transformation system	H^a (kJ/mol)	E^b (kJ/mol)	r^c
Form A → form B	3.72×10^{-2}	257	0.998
Form B → form F	—	892	0.991
Form C → form B (dehydration)	6.89	144	0.993
Form D → form A (desolvate)	4.41	77.6	0.999
Form E → form B (dehydration)	2.51	131	0.996

[a] Latent heat.
[b] Activation energy.
[c] Correlation coefficient constant ($n = 6$).

The results suggested that forms A, B, and F are polymorphs (8–16), form C is a monohydrate (14–16), and form E is the hemihydrate (6), as reported previously. Form D is a new crystalline dioxane solvate form.

Figure 2 shows the Kissinger plots of modifications, which are shown in Eq. 1 (22).

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

where ϕ is the heating rate, T_{max} is the absolute temperature at the maximum peak, E is the activation energy, and R is the gas constant. All plots are a straight line, so the activation energies for the transformations were calculated from the slope and are summarized in Table I. Since all the transformations of forms C and E to B were caused by dehydration, the activation energies were almost the same. On the contrary, the activation energy of form D was almost half that of form C, suggesting that the interaction of water with the drug was much stronger than that of dioxane. The activation energy for the polymorphic transformation of form B to F was three times larger than that of form A to B, which was two or three times larger than that of dehydration or desolvation. Matsuda and Tatsumi (20) reported that the activation energy for polymorphic transformation of furosemide forms I, II, and III to form IV were 969, 2340, and 246 kJ/mol, respectively, by means of the Kissinger method. Otsuka and Kaneniwa (21) reported that the activation energy for dehydration of cephalexin monohydrate and dihydrate was 71.3 and 49.2 J/mol, respectively, by means of the Kissinger method. These results showed that the activation energy for polymorphic transformation was larger than that for dehydration or desolvation.

Physicochemical Stability of Modifications at Various Relative Humidities

Figure 3 shows the powder X-ray diffraction profiles of polymorphic forms A, B, and F after storage at 75% RH, 45°C, for 3 months. The diffraction profiles of forms A, B, and F did not change at all at 0 and 75% RH at 45°C, which suggests that these forms are stable under practical use conditions as a commercial pharmaceutical preparation.

Figure 4 shows the X-ray diffraction profiles of pseudopolymorphic forms C, D, and E. Appreciable changes in the diffraction profiles of forms C, D and E were observed after 3 months in storage. New diffraction peaks at 7.4, 22.5, and 21.1° appeared in the diffraction profiles of form C after storage at 0% RH, suggesting that form C transformed into a mixture of forms A and B. However, new peaks at 7.4, 17.3, and 22.5° appeared in the profiles of form C after storage at 75% RH, suggesting that it had transformed into form B.

In the diffraction profiles of form D, the peaks at 10.2 and 19.5° disappeared and new peaks at 17.1, 21.1, and 28.6° appeared after storage at 0 and 75% RH, suggesting that form D transforms into form A at any level of humidity.

On the other hand, in the diffraction profiles of form E new diffraction peaks at 7.4, 22.5, 21.1, and 28.6° appeared

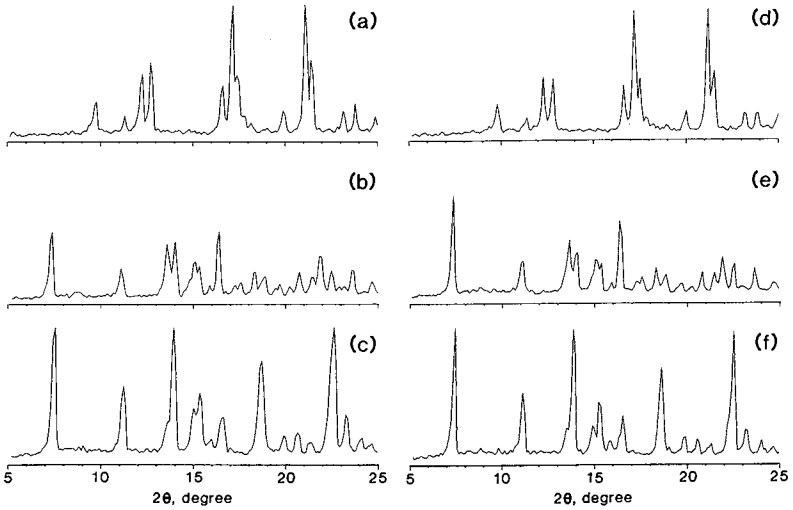

Fig. 3. Effect of storage conditions on the X-ray diffraction profiles of phenobarbital forms A, B, and F. (a, d) Form A; (b, e) form B; (c, f) form F. (a–c) Intact samples; (d–f) after storage at 75% RH and 45°C for 3 months.

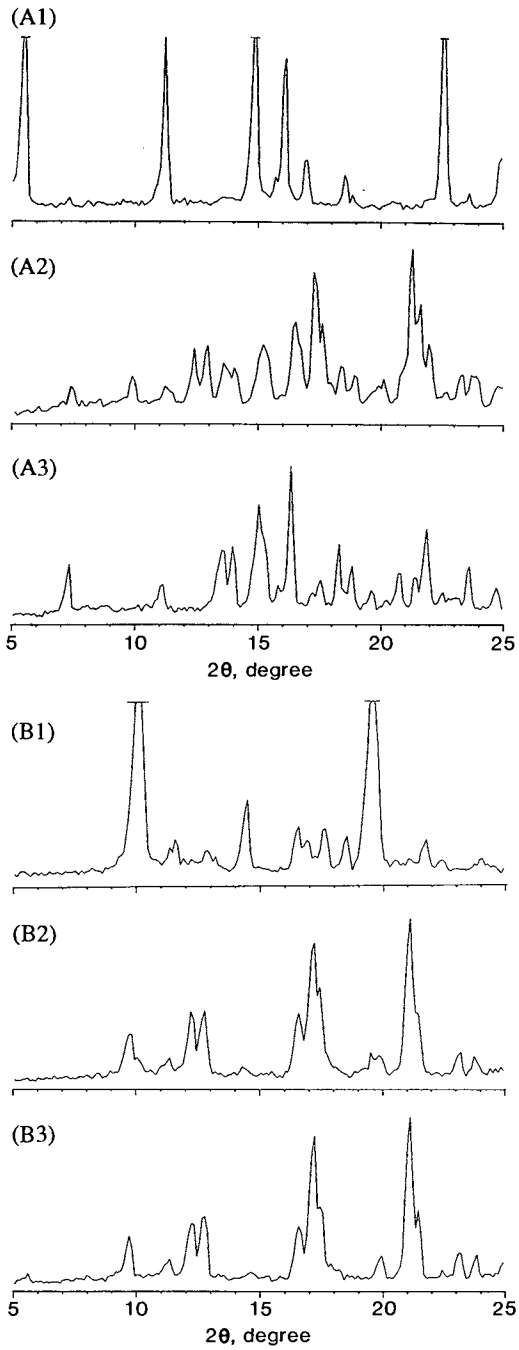

Fig. 4. Effect of storage conditions on the X-ray diffraction profiles of phenobarbital forms C, D, and E. (A) Form C (monohydrate); (B) form D (dioxane solvate); (C) form E (hemihydrate). (A1, B1, C1) Intact; (A2, B2, C2) stored at 0% RH at 45°C; (A3, B3, C3) stored at 75% RH at 45°C. (A2) After 54 days (forms A and B); (A3) after 11 days (form B); (B2) after 2.5 hr (form A); (B3) after 1 hr (form A); (C2) after 55 days (forms A and B); (C3) after 11 days (form B).

after storage at 0% RH, but new peaks at 7.4, 17.3, and 22.5° appeared after storage at 75% RH, suggesting that form E transformed to form B at 75% RH and to a mixture of forms A and B at 0% RH. These results suggested that the hydrates of forms C and E (monohydrate and hemihydrate) transformed to B through A under isothermal condition.

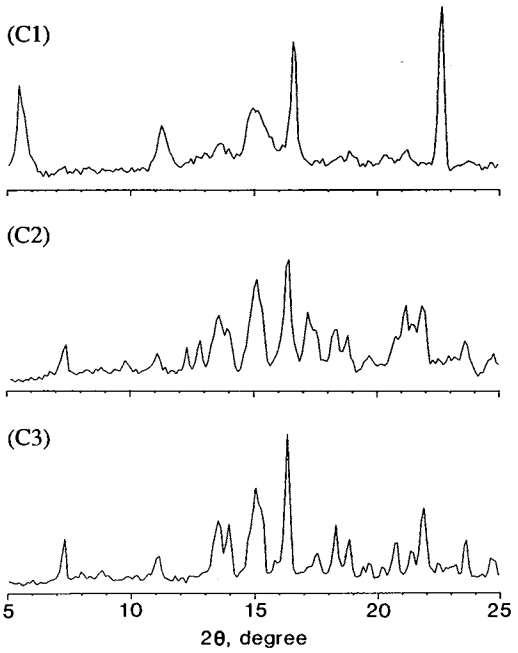

Fig. 4. Continued.

To clarify the stability of the hydrates, the water contents of forms C and E were determined by the TG method after storage at various RH levels at 45°C for 2 weeks. Figure 5 shows the relationship between the amount desorbed for these forms and the RH. The results suggested that both forms were unstable at less than 75% RH. Form C (monohydrate) transformed to form E (hemihydrate) at 100% RH, and both hydrates gradually transformed to the anhydrate (forms A or B) even at 100% RH at 45°C.

Polymorphic Transformation Kinetics under Various Levels of Relative Humidity

The transformation kinetics of the stability of forms C, D, and E were estimated by means of X-ray diffraction. The transformation processes of forms C, D, and E at 0 and 75%

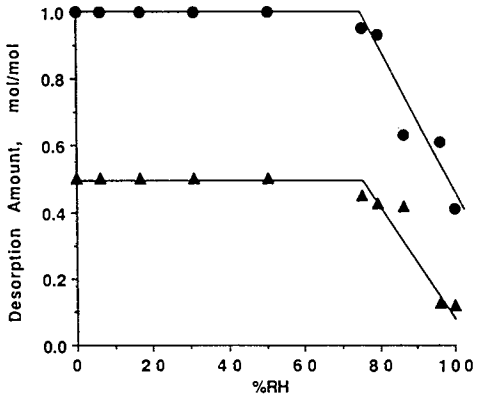

Fig. 5. Relationship between the amount of water desorbed from phenobarbital forms C and E and the relative humidity. (●) Form C; (▲) form E.

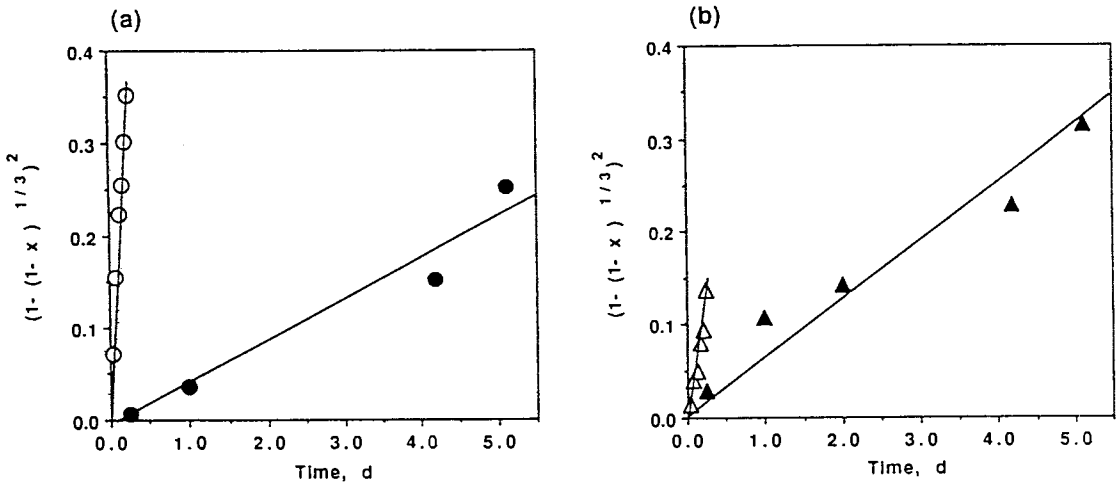

Fig. 6. Jander plots of phenobarbital forms C and E at 0 and 75% RH at 45°C. (○) Form C at 0% RH; (●) form C at 75% RH; (△) form E at 0% RH; (▲) form E at 75% RH.

RH at 45°C were estimated by X-ray diffraction. Although the surface morphology of the phenobarbital modifications differed, it was assumed that the transformation kinetic mechanisms differed depending on the modification, because the surface area and the particle shape affected the polymorphic transformation rate. This study also assumed that the transformation followed the three-dimensional diffusion mechanism [Jander equation; Eq. (2)] (23), such that the transformation rates of all modifications were comparable.

$$[1-(1-x)^{1/3}]^2 = kt + C \qquad (2)$$

where x is the conversion of the crystal form at time t, k is the transformation rate constant, and C is a constant.

The transformation rates of forms C, D, and E were estimated based on the Jander equation, and the plots gave straight lines, as shown in Figs. 6 and 7. The calculated transformation rate constants are summarized in Table II. The transformation kinetics suggested that form D had an induction period in the earlier reaction stage, whereas forms C and E did not. The transformation rate constant of form D at 75% RH was 65% larger than that at 0% RH and the induction period at 75% RH was shorter than at 0% RH, indicating that the transformation to form A was slightly accelerated by the water vapor. On the contrary, the transformation rates of forms C and E at 0% RH were much faster than those at 75% RH, and both forms had no induction period. These results suggested that form D was a dioxane solvate, whereas forms C and E were hydrates, since the latter were more affected by humidity than the former (form D). These results also suggested that forms A, B, and F were stable under ordinary conditions but that forms C, D, and E were very unstable.

In conclusion, the physicochemical stability of the phenobarbital modifications was investigated and the following conclusions were made: forms A, B, and F were stable at 0 and 75% RH, 45°C, for 3 months. Forms C and E (monohydrate and hemihydrate) were transformed to anhydrate at lower humidity and the polymorphic transformation followed the Jander equation. Form D was identified as a new crystalline form.

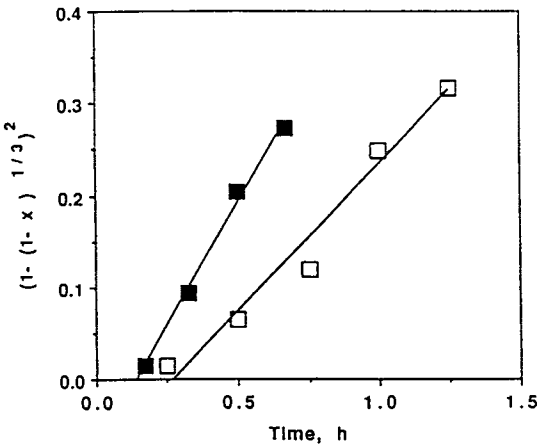

Fig. 7. Jander plots of phenobarbital form D at 0 and 75% RH at 45°C. (□) Form D at 0% RH; (■) at 75% RH.

Table II. Transformation Rate Constants of Modifications at 0 and 75% RH at 45°C

Modifications, form	0% RH		75% RH	
	k^a (hr^{-1})	r^b	k^a (hr^{-1})	r^b
A	0.00	—	0.00	—
B	0.00	—	0.00	—
C	5.13×10^{-2}	0.978	8.21×10^{-5}	0.990
D	3.16×10^{-1}	0.984	5.30×10^{-1}	0.996
E	2.30×10^{-2}	0.968	8.83×10^{-5}	0.988
F	0.00	—	0.00	—

[a] Transformation rate constant.
[b] Correlation coefficient constant.

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