DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(8), 1453-1470 (1994)

PHYSICOCHEMICAL CHARACTERIZATION OF PHENOBARBITAL POLYMORPHS AND THEIR PHARMACEUTICAL PROPERTIES

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ABSTRACT

Simple and reproducible methods of preparing six modifications (forms A, B, C (monohydrate), D (dioxane solvate), E (hemihydrate) and F of phenobarbital were established from those previously reported. The physicochemical properties of these modifications were measured using X-ray diffractometry, Fourier-transformed infrared spectrophotometry, differential scanning calorimetry, thermogravimetry, scanning electron microscopy, solubility and others. The dissolution properties of all modifications in JP XI, 1st fluid (pH 1.2) were evaluated at 37°C containing 0.5% gelatin to prevent polymorphic transformation during the test. Forms A and D showed comparatively higher solubilities among all modifications. The tapping rate constants (k) of all crystal forms were estimated based on Kuno's equation, the k value for form F being the largest, whereas that of form D, the smallest, and the order of k of magnitude of the constant was F > B > E > C > A > D. The tablet hardness after compression at 1000 kg/cm^2 depended on the polymorphic form. Form D was the hardest, while forms B and F were the softest, in the order D > A > C > E > B = F.

INTRODUCTION

The physicochemical properties of bulk powders of drugs affect their bioavailability of the preparations through effects on the dissolution rate. Thus, the pharmaceutical design of drugs, specially those of polymorphic forms which are practically insoluble in water is important.[1-3]

Phenobarbital is widely used as a hypnotic or sedative drug, of which there have been many reports concerning its polymorphic modifications,[4-9] dissolution rates[10] and bioavailability.[11] The stability of polymorphic forms under compression[12] and the mechanical strength of tablets[13,14] were also investigated as formulation studies. However, the preparation methods and the physicochemical properties differed and were not always clear. In this study, we thoroughly reconsidered and summarized most of the preparation methods of polymorphs reported , together with some of our original methods. The physicochemical properties of these forms were investigated, and their pharmaceutical properties were evaluated.

MATERIALS AND METHODS

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

Preparation of polymorphs Six modifications of the drug were prepared using various organic solvents and the preparation methods as described in Results and Discussion. The procedures were as follows:

1) Recrystallization I: A hot saturated 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 h.

2) Recrystallization II: A hot saturated solution of the drug was allowed to stand at 5°C. The separated crystals were then filtered and dried in vacuo at room temperature for 24 h.

3) Recrystallization III: A saturated solution of the drug was dropped on distilled water at room temperature. The separated

crystals were then filtered and dried in vacuo at room temperature for 24 h.

4) Recrystallization IV: A saturated solution of the drug was poured into distilled water, and stirred for 1 h at room temperature. The separated crystals were then filtered and dried in vacuo at room temperature for 24 h.

5) Evaporation I: Solutions of several concentrations of the drug were evaporated using a rotary evaporator at a constant temperature.

6) Evaporation II: Solutions of several concentrations of the drug were evaporated in a beaker at room temperature.

7) Evaporation III: Solutions of several concentrations of the drug were evaporated in a beaker at a constant temperature.

8) Spray drying: A solution of the drug (1w/v%) in various solvent mixtures were fed into a mini-spray drier (model Mini-spray GB-21, Yamato Kagaku, Co., Tokyo, Japan) through 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 or 100°C.

9) Freeze drying: Dioxane solution of the drug (1 w/v%) was lyophilized using an Eyela freeze dryer (model FD-5, Tokyo Rikakikai Co., Tokyo, Japan).

10) Suspension: Powder (10 g) was added to 100 ml of distilled water, stirred for 1 h, then the suspension was allowed to stand at 5°C. The separated crystals were filtered and dried in vacuo at room temperature for 24 h.

11) Heating: The bulk powder was heated at 175°C for 2 h.

Micrometric characterization The true density of the crystals was determined using an air comparison pycnometer (model 930; Beckman-Toshiba Co., Tokyo, Japan). The specific surface area (Sw) of the powder was measured by the air permeability method (SS-100; Shimadzu Co., Kyoto, Japan), assuming the particles to be spherelical. The specific surface area diameter was calculated from the value of Sw. The contact angle of crystals to distilled water was measured using a contact anglemeter (Type CA-D, Kyowa Kagaku Co., Tokyo, Japan) after the compressed

pellets modifications were prepared by a compression/tension tester (Autograph, model IS-5000, Shimadzu Co., Kyoto, Japan) at 1000 kg/cm^2 for 5 min.

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 s; counting range, 1 kcps; scanning speed 4^o 2θ/min.

Fourier transformed infrared (FT-IR) spectroscopy The sample powder was dispersed in KBr powder (sample concentration 5%) and analyzed. FT-IR spectra were obtained by powder diffuse reflectance on a FT-infrared spectrophotometer (type FT-IR 1600, Perkin Elmer Co., Yokahama, Japan) and modified using the Kubelka-Munk equation.

Thermal analysis Differential thermal analysis (DTA) and thermogravimetry (TG) were performed using type DTG-30 and TG-30 instruments (Shimadzu Co., Kyoto, Japan). Differential scanning calorimetry (DSC) was performed with a type 3100 instrument (Mac Science Co., Tokyo, Japan). 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^oC/min; N_2 gas flow rate, 30 ml/min.

Scanning electron microscopy (SEM) SEM photographs of samples were taken with a scanning electron microscope (model JSM-T20, Jeol Datum Co., Tokyo, Japan) at a magnification of x 200 or 1500.

Dissolution study The dissolution profiles of samples were investigated in 1st fluid (JP XII; pH 1.2) or in 1st fluid containing 0.5 w/v% gelatin using a type NTR-VS 3 dissolution tester (Toyama Sangyo, Osaka, Japan). An excess of sample (1.0 g) was introduced into 300 ml of dissolution medium in a 1000-ml round-bottomed flask with a plastic cover. The flask was fixed onto the sample holder in a thermostatically regulated water bath maintained at 37 ± 0.5^oC, and stirred with a paddle at 150 rpm. Aliquots (2 ml) of the solution were withdrawn at various times with a syringe through a 0.8-μm membrane filter and

suitably diluted with dissolution medium. The drug concentration was measured spectrophotometerically (model UV-160A, Shimadzu Co., Kyoto, Japan) at 240 nm.

Measurement of tapping rate constant The tapping rate constants of the powders were measured as follows: Sample powder (5 g) was placed in a graduated cylinder (1 cm in diameter and 20 ml in volume) and the apparent volume was measured during tapping (RHK-type tapping instrument, Konishi Co., Osaka, Japan) The tapping rate constants were estimated by the least-squares method based on Kuno's equation (eq. 1).

$$\rho_f - \rho_n = (\rho_f - \rho_o) \exp(-kn) \qquad \text{eq. 1}$$

ρ_f is the bulk density of the sample powder at an infinite tapping number, ρ_n is the bulk density at tapping number n, ρ_o is the bulk density at the initial packing, k is the tapping rate constant and n is number of taps.

Tablet hardness Tablets (200 mg) were compressed at1.5 cm/min using a 0.8-cm diameter punch and die by a compression/tension tester (Autograph, model IS-5000, Shimadzu Co., Kyoto, Japan) at 1000 kg/cm^2 for 5 min. The hardness of the tablet was measured 3 times using a hardness tester (Erweka Co.).

RESULTS AND DISCUSSION

Preparation of phenobarbital modifications

Various phenobarbital modifications have been prepared by several different means, which were often somewhat vague. We reriewed them all and propose the simple methods as described under "Experimental". The results are shown in Table 1. We rearranged all the modification data and identified 6 reproducible modifications. The relationships between the reported modifications and those obtained in the present study are shown in Table 2.

Micromeritic characterization of modifications

The micromeritic properties of all modifications are shown in Table 3. The true densities were slightly different, reflecting the molecular packing in the crystal lattice; that of form C was the largest and that of form D was the smallest. The specific surface

Table I. Methods of Recrystallization and Resultant Crystal Forms

Methods and solvents	Crystal form
Recrystallization I	
acetonitrile	form A
methanol, ethanol, iso-propanol, n-butanol, n-propanol	form B
iso-butanol, n-amylalcohol, ethyl acetate	form B
chloroform, methylene chloride, acetone, ethyl ether	form B
Recrystallization II	
chloroform, methylene chloride, acetonitrile	form A
ethanol, iso-propanol, ethyl acetate	form B
Recrystallization III	
methanol	form B
dioxane	form E
Recrystallization IV	
methanol, acetonitrile	form A
dioxane	form E
Evaporation I	
acetone at 0, 40 or 60°C, methanol at 40 or 60°C	form B
Evaporation II	
methanol, acetone, ethyl acetate	form B
chloroform, ethyl ether	form C
Evaporation III	
methanol, ethanol, iso-propanol, n-propanol	form B
ethyl acetate, chloroform	form B
methylene chloride, acetone	form B
methyl ethyl ketone, ethyl ether	form B
acetone/water (8:2), acetone/water (9:1)	form B
Spray drying	
chloroform/acetone (1:1) at 50 or 100°C	form A
methylene chloride/acetone (1:1) at 50 or 100°C	form A
chloroform/methanol (1:1) at 50 or 100°C	form A
chloroform at 50 or 100°C, methylene chloride at 100°C	form A
methylene chloride/methanol (1:1) at 100°C	form A
methlene chloride/methanol (1:1) at 50°C	form C
Freezed drying	
dioxane	form D
Suspending in distilled water	
dried in vacuo	form E
dried at room temperature	form C
Heating	
heated at 175oC for 2 h	form F

Table 2. Reported Phenobarbital Modifications

Present study	form A	form B	form C[a]	form D[b]	form E[c]	form F
m.p.	166°C	175°C	-	-	-	180°C
Mesley et al.[6]	III	II	XIII	-	XII	I
	167°C	174°C	-	-	-	175°C
Huang et al.[4]	III	II	-	-	-	I
	166-7°C	174°C	-	-	-	177°C
El-Banna et al.[7]	III	II	XIII	-	XII	I
Cleverley et al.[5]	III	II	V	-	-	I
Nogami et al.[10]	-	anhydrate	hydrate	-	-	-
Kato et al.[11]	III	II	hydrate	-	-	I
	-	173°C	-	-	-	177°C

a, monohydrate; b, dioxane solvate; c, hemihydrate.

Table 3. Micromeritic Characteristics of Phenobarbital Modifications

Modifications	Density±S.D.[a] (g/cm^3)	Sw^b±S.D. (cm^2/g)	d^c±S.D. (μm)	CA^d±S.D. (degree)
Form A	1.45 ± 0.03	2160 ± 15	10.4 ± 0.0	73 ± 6
Form B	1.39 ± 0.01	1330 ± 7	32.5 ± 0.2	49 ± 2
Form C	1.47 ± 0.08	2330 ± 30	17.7± 0.2	54 ± 1
Form D	1.28 ± 0.08	1890 ± 5	24.9 ± 0.1	41 ± 2
Form E	1.37 ± 0.02	2040 ± 8	21.4 ± 0.1	48 ± 3
Form F	1.37 ± 0.02	1170 ± 8	37.5 ± 0.3	49 ± 3

a, standard deviation (n=5); b, specific surface area (n=3); c, mean particle size (n=5); d, contact angle (n=4).

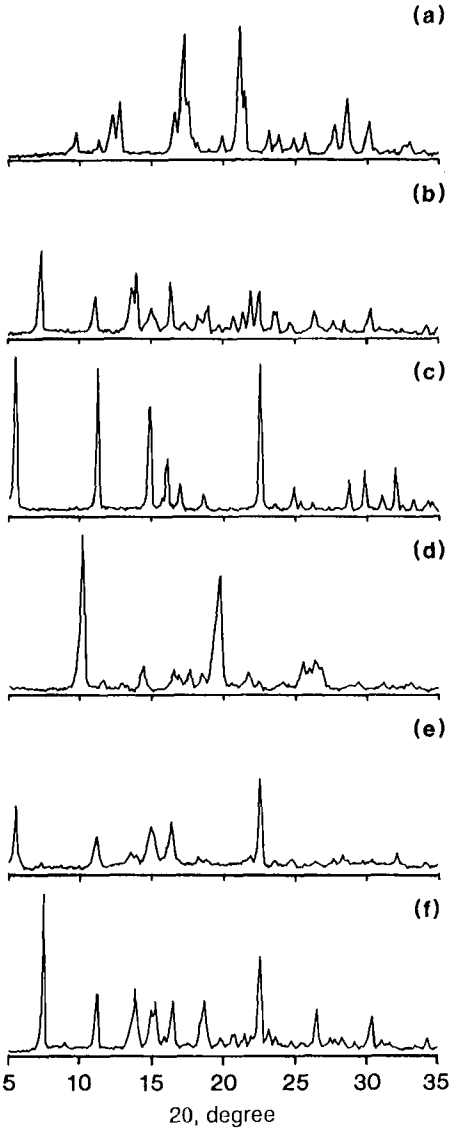


Fig. 1 Powder X-ray Diffraction Profiles of Six Kinds of Phenobarbital Modifications
(a), form A; (b), form B; (c), form C (monohydrate); (d), D (dioxane solvate); (e), form E (hemihydrate) (f), form F.

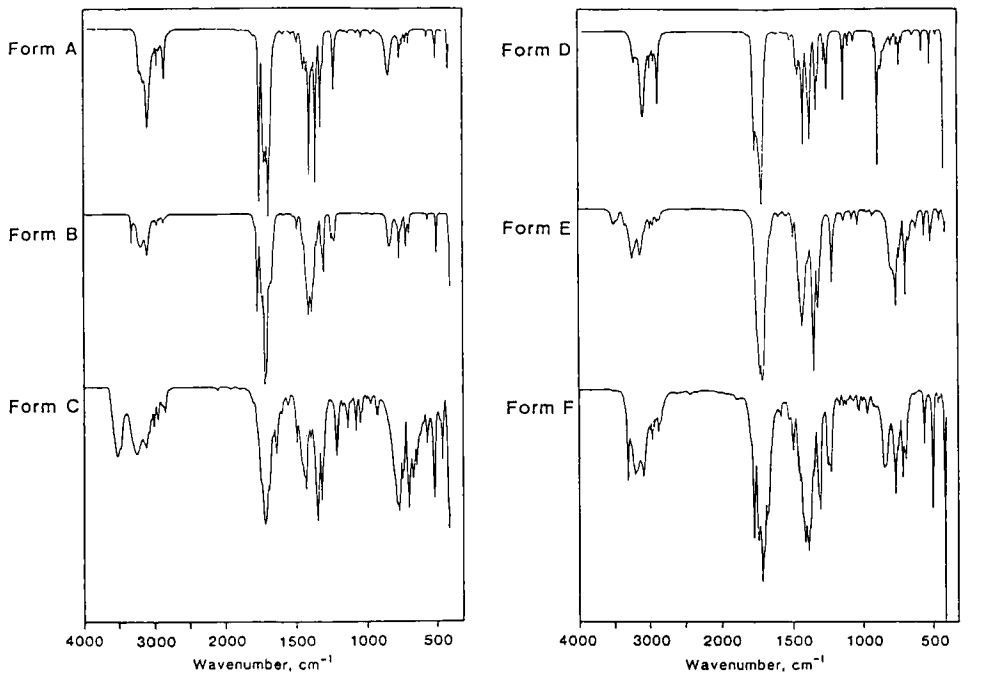


Fig. 2 FT-IR Spectra of Phenobarbital Modifications

areas (Sw) and the equivalent diameters were also different; form F was the largest and form A was the smallest. The hydrates C and E, and dioxane solvate D showed lower contact angles. On the contrary, form A exhibited the largest contact angle, suggesting poor wettabilitiy.

Physicochemical characterization of modifications

Figure 1 shows the powder X-ray diffraction profiles of six modifications. Their characteristic diffraction peaks were as follows: Form A, 17.1, 21.1 and 28.6°; form B, 7.4, 14.1,17.3 and 22.5°; form C, 5.6, 11.2 and 22.6°; form D, 10.2 and 19.5°; form E, 5.6 and 22.5° and form F, 7.4, 15.8 and 22.5° (2θ).

Figure 2 shows the FT-IR spectra of these modifications. Mesley et al. (6) have described the IR spectra of the modifications as follows: The band at 3400-3000 cm^{-1} is attributable to the N-H stretch vibration of a secondary amine. It is therefore considered that the bands at 1580-1490 cm^{-1} and

840 cm^{-1} are attributable to N-H stretch and bending vibrations. The bands at 1800-1600 cm^{-1} are attributable to the NH-CO-NH stretch vibration of the barbital ring. Forms A, B and F were significantly different at 3400-3000 cm^{-1}, with two peaks at 1770 and 1730-1680 cm^{-1} and the finger regions, but detailed crystal structures remain unclear. However, forms C and E had a peak at 3500 cm^{-1}, attributable to the O-H stretch vibration and peaks at 1730-1680 cm^{-1} were attributable to the C=O in the barbital ring, indicating that there was no peak at 1770 cm^{-1} on the spectra of the hydrates. We therefore considered that the O-H group attributable to crystal water interacted with the C=O group in the barbital ring. On the other hand, form D had a significantly different spectrum from those of the other forms, and with a band at 1116 cm^{-1} attributable to dioxane and particular peaks at 869 cm^{-1}.

Thermal behavior of modifications

Figures 3 and 4 show the DSC and TG curves respectively, of the modifications. Form F had an endothermic peak at 179.8°C due to melting accompanying sublimation, suggesting a stable form at high temperature. Form A showed an endo-exothermic phenomenon at 166.0°C, an endothermic peak at 179.2°C with a shoulder at 175.3°C and no weight loss on the TG curve. Form B showed an endothermic peak at 179.2°C with a shoulder at 175.3°C. The X-ray diffraction profile of form A after heating at 170°C for 5 min was identical to that of form B. Forms C and E showed an endothermic peak at 50-70°C with a 7.0 and 3.2% weight loss due to the dehydration of one and half moles of water per mole of the drug, respectively, and a melting peak at 179°C. The X-ray diffraction profile after heating forms C and E at 130°C for 10 min were identical to that of form B. On the other hand, form D showed an endothermic peak at 60-90°C with 25.4% weight loss due to the desolvation of one mole of dioxane and two endothermic peaks 168°C and 178°C, due to transformation and melting, respectively. After heating form D at 130°C for 10 min, the X-ray diffraction profile was identical to that of form A.

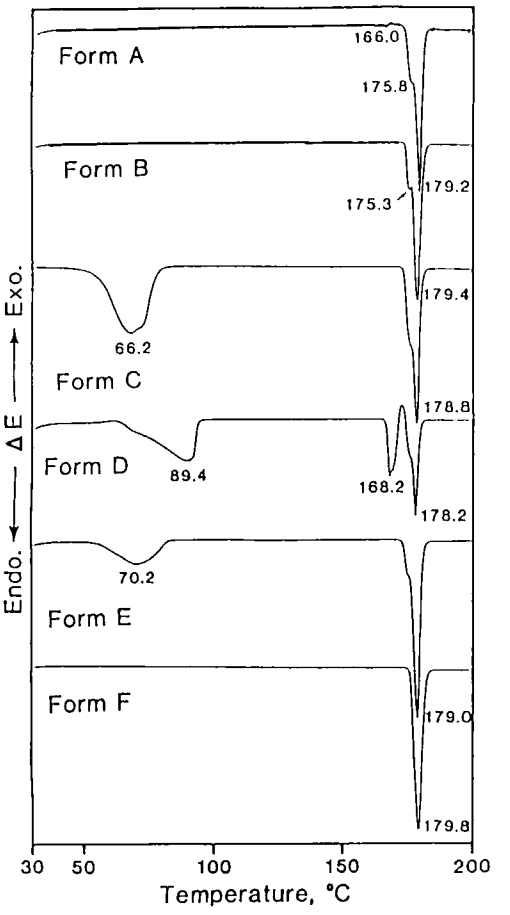


Fig. 3 Differential Scanning Calorimetry Curves of Phenobarbital Modifications

Morphological characterization

Figure 5 shows SEM photographs of the modifications. Distinct morphological differences were evident among these samples. Form A consisted of aggregated particles with the primary particles being less than 3 μm in diameter. Form B particles gave rise to larger crystals of about 300 μm in length, which were platy crystals. Form C was hexagonal tabular crystals with a cracked surface, since dehydration occurred during SEM observation in vacuo. Form D consisted of large aggregated

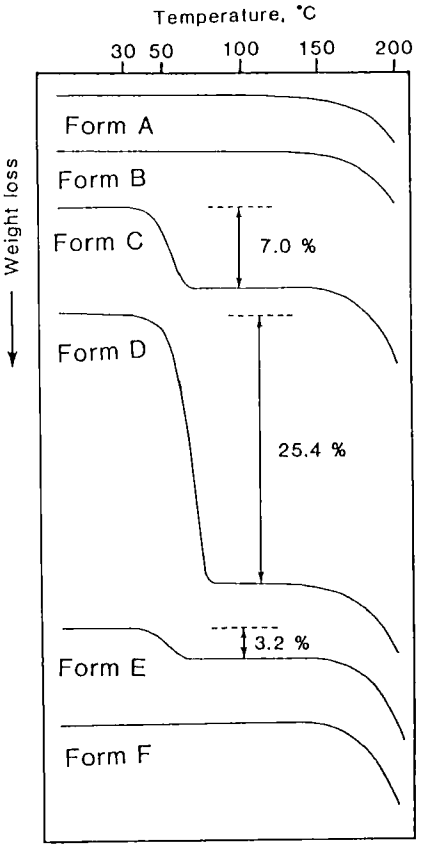
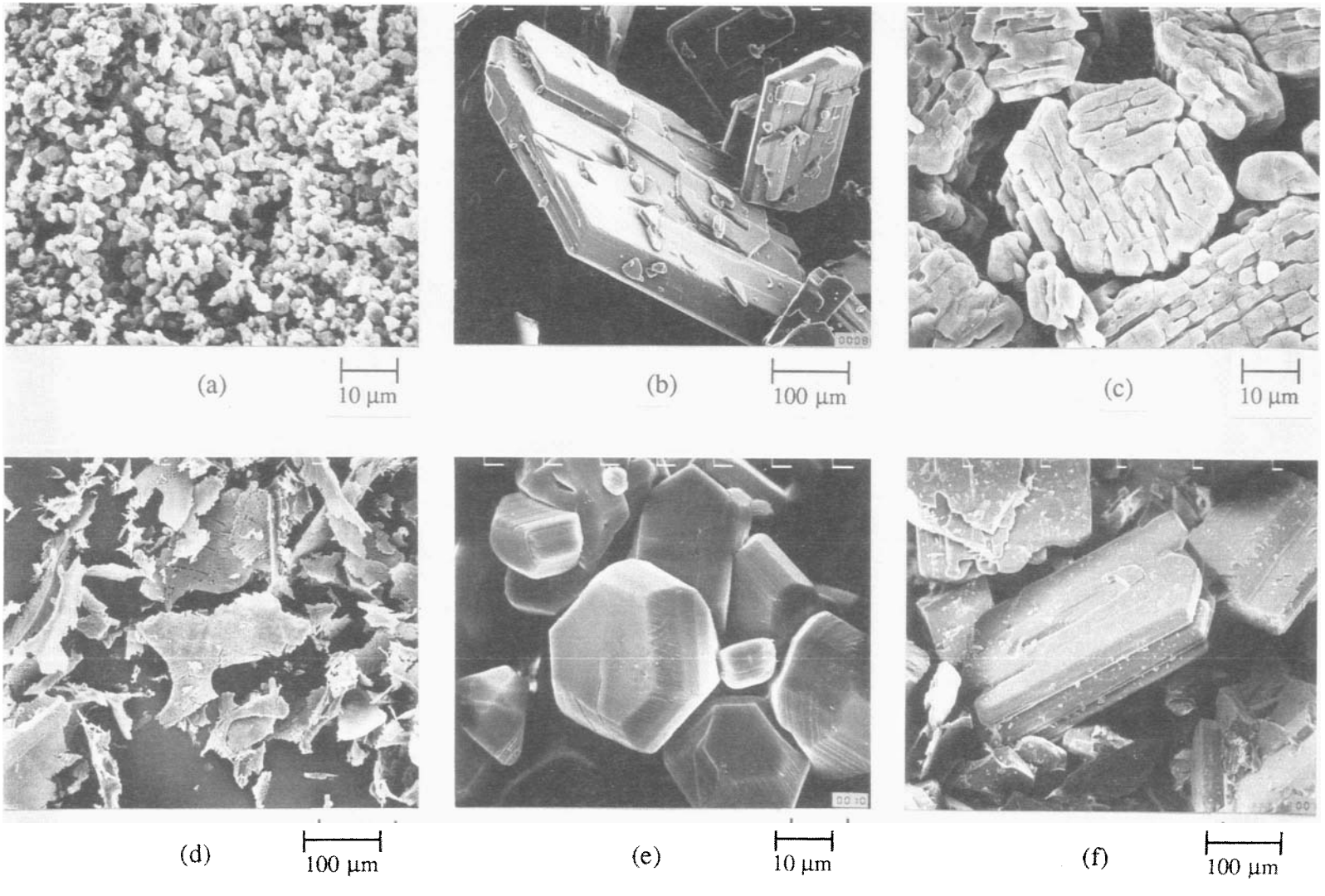


Fig. 4 Thermogravimetry Curves of Phenobarbital Modifications

particles with highly porous crystals, also indicating desolvation during SEM observation. Form E was hexagonal crystals with a smooth surface. Form F was platy crystals accompanying fine particles and had the largest mean diameter.

Dissolution behavior in 1st fluid (JPXI) of the modifications

Figure 6 shows the dissolution profiles of all modifications in 1st fluid (pH 1.2) at 37°C. Since the modifications transformed to a stable form under the dissolution test conditions as shown in Fig 6-(a), the solubilities were not estimated by conventional means. Therefore, to estimate the solubility 1st fluid containing 0.5% of gelatin was used to prevent polymorphic transformation



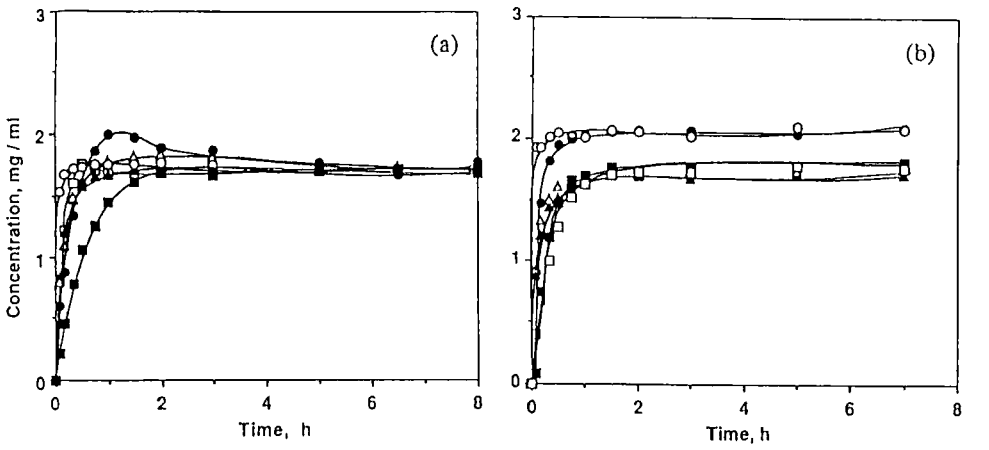


Fig. 6 Dissolution Profiles of Phenobarbital Modifications in 1st Fluid (pH 1.2) at 37°C.
(a), 1st fluid; (b), 1st fluid containing 0.5% of gelatine
(●), Form A; (△), form B; (■), form C;
(○), form D; (□), form E; (▲), form F.

during the dissolution test. The solubility (Table 4) of form A was 2.07 mg/ml and was14% higher than that of form C (Fig. 6-(b)). Since form D transformed to form A during the dissolution test, the solubility was the same as that of form A. The solubilities of forms B and F were almost the same as that of form C. These results indicated that forms A and D had comparatively higher solubilities among these modifications.

Effect of polymorphic forms on the tapping rate

Figure 7 shows Kuno's plots of the powders of various polymorphic forms. They were all straight lines. The tapping rate constants (k) were are estimated from the plots by the least-squrares method, and summarized in Table 5. The results suggested that the k of form F was the largest, that of form D was the smallest and the order of was F > B > E > C > A > D. Since form D was the dioxane solvate and forms C and E were hydrates, the order of k of the polymorphic forms was F > B > A, which was the same as

Table 4. Solubility of Modifications in 1st Fluid Containing 0.5% of Gelatin at 37°C

Intact modifications	Resultant form[a]	Cs[b] + S.D.[c] (mg/ml)
Form A	Form A	2.07 ± 0.01
Form B	Form B	1.74 ± 0.02
Form C	Form C	1.77 ± 0.01
Form D	Form A	2.07 ± 0.03
Form E	Form C	1.76 ± 0.03
Form F	Form F	1.71 ± 0.02

a, after 7-h dissolution test, sample was dried at room temperature; b, solubility; c, standard deviation (n=4).

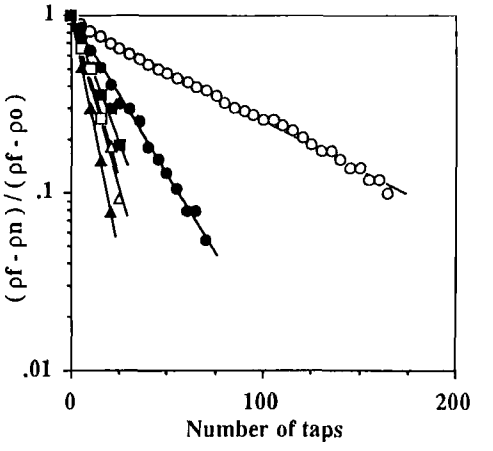


Fig. 7 Kuno's Plot for the Powders of Various Polymorphic Forms (●), Form A; (△), form B; (■), form C; (○), form D; (□), form E; (▲), form F.

Table 5. Tapping Rate Constant of the Powder of Polymorphic Forms

Forms	k^a (1/n)	r^b
Form A	4.03×10^{-2}	0.997
Form B	8.72×10^{-2}	0.989
Form C	6.57×10^{-2}	0.995
Form D	1.28×10^{-2}	0.998
Form E	8.54×10^{-2}	0.985
Form F	12.63×10^{-2}	0.999

a, tapping rate constant; b, correlation coefficient constant.

that of the average diameter. It seemed that there were a relationship between the lowest contact angle of form D and the lowest k value. These results indirectly suggested the powder flowability of polymorphic forms.

Effect of polymorphic forms on the tablet hardness

Tablet 6 shows the results of tablet hardness after compression at 1000 kg/cm^2. Form D was the hardest, and the order was D > A > C > E > B = F. Since the crystal shapes of forms B and F were plate (Fig. 5), tablets of them showed a capping tendency, and it seemed that the mechanical strength was lower than that of the other forms. The order of tablet hardness was reversed in that of the tapping rate constant (Table 5). This suggested that the tablet hardness and the tapping rate constant depended on the surface characteristics, which were the specific surface area and adhesion of the powder particles.

Table 6. Tablet Hardness of Polymorphic Forms

Forms	Tablet hardness (kg ± S.D.[a])
Form A	4.2 ± 0.5
Form B	0.9 ± 0.2
Form C	2.9 ± 0.5
Form D	8.7 ± 0.3
Form E	1.4 ± 0.2
Form F	0.8 ± 0.1

a, standard deviation (n=4).

CONCLUSION

The physicochemical properties of most of the phenobarbital modifications and the reproducibility of the preparation methods were reviewed. Form A was more soluble, and it can be prepared by simple recrystallization using ethanol or iso-propanol which are non toxic organic solvents. Tablets of forms A and D were harder, but those of forms B and F had a capping tendency. It was therefore considered that the selection of polymorphic form for the active bulk powder is significant for high quality pharmaceutical preparation.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Miss Seiko Nagahama and Miss Hisami Kema for their technical assistance.

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