[Chem. Pharm. Bull. 21(7)1592—1600(1973)]

UDC 547.854.5.03 : 615.011.3.014

Dissolution Behavior of Solid Drugs. III.[1] Determination of the Transition Temperature between the Hydrate and Anhydrous Forms of Phenobarbital by Measuring Their Dissolution Rates[2]

Keiji Sekiguchi, Motoko Kanke, Yasuyuki Tsuda,[3a]
Katsushi Ishida,[3b] and Yoji Tsuda[3c]

School of Pharmaceutical Sciences, Kitasato University,[3a] Teikoku Hormone Mfg. Co., Ltd.,[3b] and Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.[3c]

(Received March 28, 1973)

The transition temperature and the heat of transition between the hydrate and the anhydrous forms of phenobarbital were determined to be 36.4° and 1.87 kcal/mole, respectively by the initial dissolution rate measurements. These values were in good agreement with values obtained by the conventional solubility equilibrium method and by the differential scanning calorimetry (DSC), as well as with transition temperature estimated by the electrical conductivity and vapour pressure measurements. Furthermore, metastable solubility values calculated from experimentally determined stable solubility values and ratios of dissolution rates for both forms agree closely with values obtained by the conventional solubility method.

The assumption that the dissolution processes for both forms of phenobarbital are diffusion controlled was further supported in this study by the equality of dissolution activation energy for both forms, as well as by the magnitude of this activation energy, and by the diffusion layer thickness.

Many pharmaceutical substances are often capable of existing in different crystalline forms, including polymorphs and hydrates. Changes in physicochemical properties and differences in biological availability caused by different crystalline forms are often noticed. For example, it is said that the solubilities and the relative rates of dissolution in distilled water of anhydrous ampicillin and its trihydrate form, as well as the relative *in vivo* availabilities of the two forms differ significantly.[4] Thus, even if one assumes constant conditions for solute transport away from the crystal surface, a constant drug effectiveness cannot always be expected, since transition between alternative crystal forms may or may not occur depending on various environmental factors. In such a case, a knowledge of transition temperature and of heat of transition between polymorphs or between hydrate and anhydrous forms will be useful in the formulation and quality control of pharmaceutical products.

In a previous paper[1] dissolution rates of sulfathiazole polymorphs were measured, and good results were obtained in the application of such measurements to determination of the transition temperature, the heat of transition, and metastable solubility.

In the present study, phenobarbital was chosen as a model compound forming a hydrate and the use of dissolution rate measurements in the determination of transition temperature, transition energy, and metastable solubility was further tested. Initial dissolution rates were measured for both the anhydrous form, or Eriksson's[5] form I, and the hydrate form, or Eriksson's form II, and were used in the calculation of the various pertinent thermodynamic

---

1) Part II: M. Kanke and K. Sekiguchi, *Chem. Pharm. Bull.* (Tokyo), 21, 878 (1973).

2) Presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April, 1971.

3) Location: *a*) *9–1, Shirokane 5 chome, Minato-ku, Tokyo*; *b*) *5–1, Akasaka 2 chome, Minato-ku, Tokyo*; *c*) *1 Kashima-cho, Higashiyodogawa-ku, Osaka.*

4) J.W. Poole and C.K. Bahal, *J. Pharm. Sci.*, **57**, 1945 (1968); J.W. Poole, G. Owen, J. Silverio, J.N. Freyhof, and S.B. Rosenman, *Current Therap. Res.*, **10**, 292 (1968); D.A. Wadke and G.E. Reier, *J. Pharm. Sci.*, **61**, 868 (1972).

5) S.O. Eriksson, *Svensk Farm. Tidskr.*, **65**, 353 (1961).

parameters in the same manner as described in the previous work.[1] The results are discussed and compared with values obtained by four alternate methods, including the conventional solubility method, differential scanning calorimetry (DSC), electrical conductivity measurement during heating, and vapour pressure technique.

Theoretical

As in the case of dissolution rate measurements for sulfathiazole polymorphs reported previously,[1] in the analysis of the experimental dissolution rate data for the hydrate and anhydrous forms of phenobarbital an assumption was made that dissolution rates are controlled by diffusion between a thin saturated film at the interface (saturated layer) and the bulk solution, as expressed by

$$\frac{dC}{dt} = K(C_s - C) \tag{1}$$

where dC/dt is the dissolution rate, Cs is the saturated concentration (solubility) of the drug in the solvent, C is the concentration of the drug in the bulk solution at time t, and K is an apparent rate constant. When a pure solvent is used for the dissolution medium, the relationship between drug concentration of bulk solution and optical absorbance during the initial stage of dissolution satisfies the Beer's law, as expressed by

$$\frac{dE}{dt} = \varepsilon \frac{dC}{dt} \tag{2}$$

where E is the absorbance of the solution and ε is the molar extinction coefficient. Substituting equation (1) into equation (2),

$$\frac{dE}{dt} = \varepsilon K(C_s - C) \tag{3}$$

Under experimental condition where $Cs \gg C$, equation (3) may be reduced to

$$\frac{dE}{dt} = \varepsilon K C_s \tag{4}$$

and,

$$\log\left(\frac{dE}{dt}\right) = \log K + \log C_s + \log \varepsilon \tag{5}$$

The Arrhenius equation is applicable to the description of the temperature dependency of the dissolution rate constant K, and may be written as,

$$\log K = -\frac{Ea}{2.303R}\cdot\frac{1}{T} + \log A \tag{6}$$

where Ea is the activation energy for dissolution, R is the gas constant, and A is a frequency factor. Similarly, for the temperature dependency of solubility,

$$\log C_s = -\frac{\Delta H}{2.303R}\cdot\frac{1}{T} + \text{const.} \tag{7}$$

where ΔH is the heat of solution. Substituting equation (6) and (7) into (5),

$$\log\left(\frac{dE}{dt}\right) = -\frac{(\Delta H + Ea)}{2.303R}\cdot\frac{1}{T} + \text{const.} \tag{8}$$

thus predicting a linear relationship between log (dE/dt) and $1/T$. This relationship was validated experimentally, thus allowing the quantity $(\Delta H + Ea)$ for the hydrate and anhydrous forms to be calculated from the slope of a plot of log (dE/dt) versus $1/T$. The heat of transition, or the difference in ΔH between the hydrate and anhydrous forms can be readily determined, since dissolution processes are assumed to be diffusion controlled, and Ea values for both hydrate and anhydrous forms are thought to be the same.

Although equilibrium solubility measurements have been conventionally utilized for the determination of solubilities, an accurate solubility of metastable form cannot always be expected, since conversion from metastable to stable forms can often occur due to a relatively long experimental time required for such measurements. However, the solubility of a metastable form (Cs') can be reasonably estimated from the dissolution rates for stable and metastable forms (dE/dt and dE'/dt, respectively) as obtained in the present study, and the solubility value of the stable form (Cs) which is determinable by conventional solubility measurements. That is, since equation (4) and the same apparent rate constant can be applied to both stable and metastable forms, metastable solubility will be calculated by the following equation,

$$C_s' = C_s \times \frac{dE'/dt}{dE/dt} \tag{9}$$

Experimental

Material——The anhydrous form of phenobarbital ($C_{12}H_{12}O_3N_2$): commercial product of JP grade was dissolved in hot distilled water and recrystallized at about 60°, mp 174—177°. The hydrate form of phenobarbital ($C_{12}H_{12}O_3N_2 \cdot H_2O$): obtained by saturating distilled water at room temperature, filtering, and allowing it to recrystallize in a refrigerator; however, this method was rather time-consuming and yielded poorly, the following recrystallization method was adopted throughout the present study. Commercial products of JP grade was dissolved in NaOH solution, to which HCl solution was added at room temperature. The precipitated phenobarbital was carefully washed with distilled water and air dried at room temperature.[5]

Preparation of Sample Disks——Sample disks were prepared in the same manner as described in a previous paper,[6] *i.e.*, about 0.5 g of sample powder in an evacuable die were compressed into disks of 20 mm in diameter by hydraulic press applying a compression force of 5 tons per disk for 5 minutes. Under the experimental conditions of compression used, transition between the anhydrous and hydrate forms was not observed.

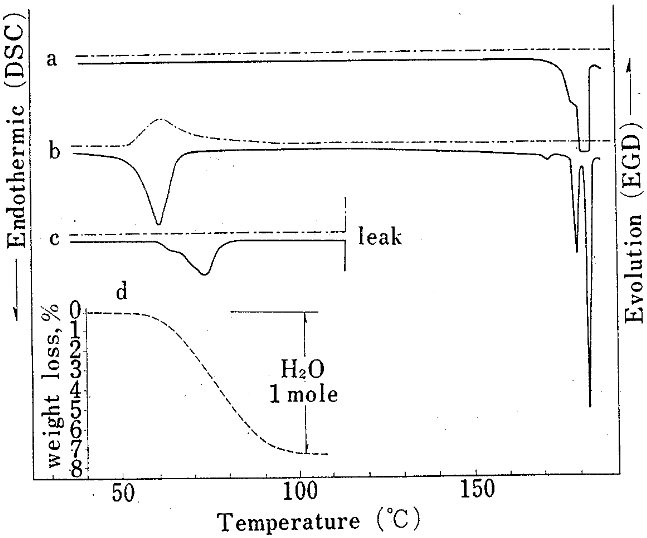

Fig. 1. Thermal Behavior of Anhydrous Phenobarbital and its Hydrate

-·-·-: EGD curves, ——: DSC curves, ------: TG curve
a : anhydrous (open system) 5.1 mg, 8°/min
b : hydrate (open system) 6.3 mg, 4°/min
c : hydrate (closed system) 7.7 mg, 4°/min
d : hydrate 4.913 mg, 8°/min

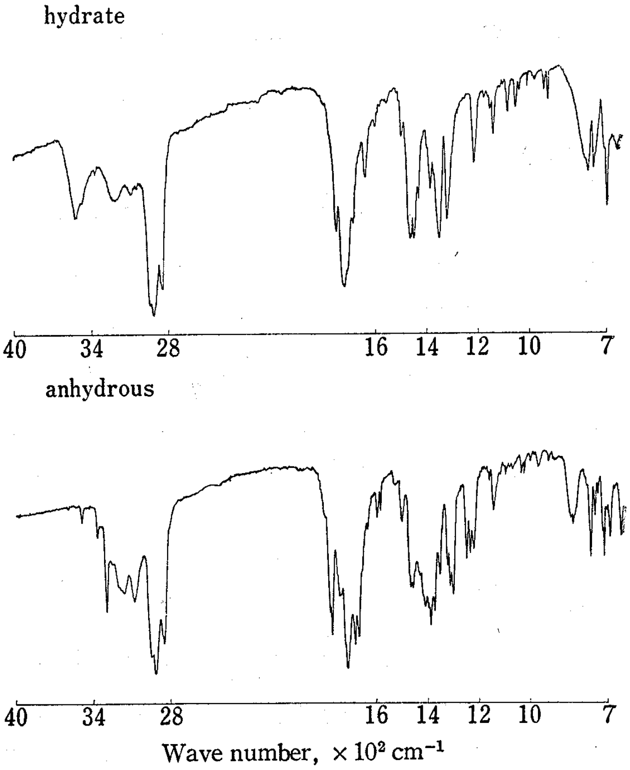

Fig. 2. Infrared Absorption Spectra of Phenobarbital (nujol mull)

---

[6] M. Kanke and K. Sekiguchi, *Chem. Pharm. Bull.* (Tokyo), **21**, 871 (1973).

Characterization of Crystal Forms——Crystal forms of sample powder or disks prepared as above were characterized by DSC, thermogravimetry (TG), and infrared spectrophotometry (IR) before and after dissolution rate measurements and solubility equilibrium determinations. The DSC apparatus used was a Perkin-Elmer DSC-1B Differential Scanning Calorimeter, the TG apparatus used was a Perkin-Elmer TGS-1 Thermogravimeter, and IR absorption was measured by a Jasco IRA-1 Grating Infrared Spectrophotometer. Typical patterns of DSC, TG, and IR for the anhydrous and hydrate forms of phenobarbital are shown in Fig. 1 and 2. The DSC curves near the melting point indicate the existence of anhydrous polymorphs of phenobarbital.

Dissolution Rate Measurements——The apparatus and procedure used were essentially similar to those described previously.[1] A disk prepared as described above was mounted in a stainless steel holder specially made to expose only one side of the disk to the dissolution medium, and was then at zero time placed into a water jacketed measurement cell containing 400.0 g of water, which was maintained at the desired temperature by circulating water from a thermostated bath through the jacket of the cell. The dissolution medium was circulated by pump at a constant flow rate of 440 ml/min through the flow cell apparatus of a spectrophotometer (Type: Hitachi 101). Absorbance at a wave length of 235 mμ was recorded automatically by a recorder (TOA EPR-3T), giving a continuous record of absorbance versus time. The dissolution medium was agitated by magnetic stirrer at a speed of 600 rpm.

Equilibrium Solubility Method——An excess amount (two times that required to reach the saturated concentration) of sample powder was placed in 50 ml of distilled water in the same type of cell used for the dissolution rate measurements, and agitated at 600 rpm at controlled temperatures of 15°, 20°, 25°, 35°, 45°, 50°, and 55°. At appropriate time intervals, exact amounts of sample solution were transferred by a cotton-filtered pipette into weighing bottles, which were immediately weighed. The solvent was then evaporated off in a dryer at 60° until constant weights of phenobarbital (anhydrous residue) were attained.

Differential Scanning Calorimetry (DSC)——When the solid sample pan is used for phenobarbital hydrate, the endothermic peak begins to appear at about 50°. By simultaneous use of an evolved gas detector (EGD) with the DSC measurements, it was determined that the endothermic peak was assigned to both the transition from the hydrate to the anhydrous forms and the evaporation of water under the semi-closed conditions (in the solid sample pan). However, under fully closed conditions obtained by using the liquid sample pan the endothermic peak is considered to be practically due to the heat of transition, although the peak occurred at somewhat higher temperature by suspended transition. Therefore, solid sample pans were used for measurements of transition temperature, and liquid sample pans were used for measurements of heat of transition.

(1) Transition Temperature Measurements (Fig. 8): sample: phenobarbital hydrate; sample weight: 3.9—4.6 mg; sensitivity: 8—16 mcal/sec; heating rate: variable, from 2° to 64°/min; chart speed: variable, from 5 to 160 mm/min. Temperature corrections were made by Indium, employing a sample kit provided by the manufacturer.

(2) Heat of transition Measurements: sample: phenobarbital hydrate; sample weight: 6.6—6.9 mg; sensitivity: 4 mcal/sec; heating rate: 8°/min; chart speed: 20 mm/min. The areas under the transition peaks were measured by weighing the peak area cut from photocopies of the recorder charts.

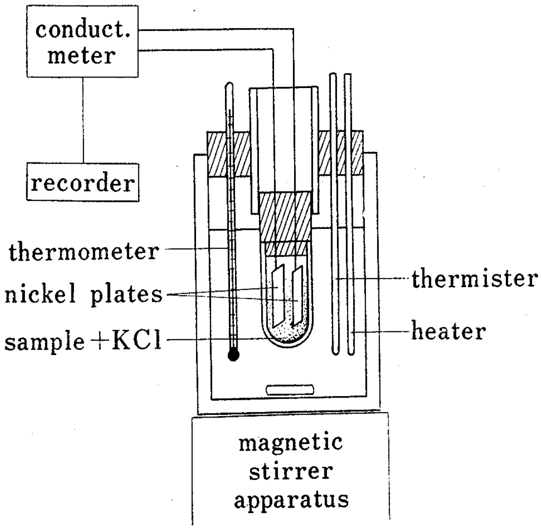

Fig. 3. Schematic Diagram of the Apparatus used for Electrical Conductivity Measurements

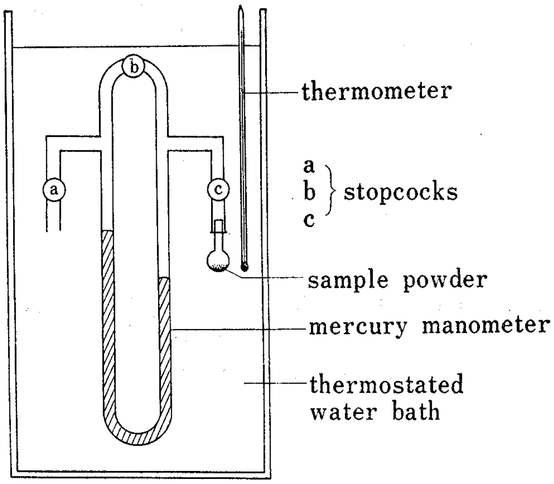

Fig. 4. Schematic Diagram of the Apparatus used for determining the Vapour Pressure

Thermogravimetry (TG)——Sample amount of about 4—5 mg, and heating rates of 4° and 8°/min were employed. The weight loss indicates that one mole of crystal water was contained in hydrate form of phenobarbital (Fig. 1).

Electrical Conductivity Measurement——The apparatus used is shown in Fig. 3. To magnify conductivity change by the peritectic transition, powder of hydrate and KCl were mixed in a ratio of approximately 3: 1[7], and packed tightly in a test tube. Two 5×20 mm nickel electrodes spaced 0.5 mm apart were inserted into the packed tube, and the protruding ends of the electrodes connected to an electrical conductivity meter (TOA Model CM 1CB). The test tube was then placed in a water bath and conductivities at a heating rate of about 0.2°/min were recorded. A significant conductivity change indicates transition from the hydrate to the anhydrous forms (Fig. 9). In addition, measurements were also carried out at constant temperatures near the estimated transition point.

Vapour Pressure Method——The apparatus used is shown in Fig. 4. About 800 mg of the hydrate were placed in the sample bulb. After evacuating the system by a vacuum pump, it was placed in a thermostated water bath for 5 hours. The differences in the level of the mercury in the manometer resulting from gradual stepped temperature increases were observed and recorded.

Result and Discussion

Determination of Transition Temperature and Heat of Transition

I) By Dissolution Rate Measurements——The initial dissolution rates for the hydrate and anhydrous forms of phenobarbital at various temperatures were measured, plots were made of log (dE/dt) versus $1/T$, and the best straightline fits for the two data sets were determined by the method of least squares, as shown in Fig. 5. The transition temperature, indicated by the intersection of the two curves, is 36.4°. The transition energy was calculated to be 1.87 kcal/mole by taking the difference between the heats of dissolution ($\Delta H+Ea$) for the two forms calculated from the slopes of the curves in accordance with equation (8).

II) By Solubility Measurements——Concentration-time curves for the hydrate and anhydrous forms were obtained at various temperatures, as shown in Fig. 6. The solubility values determined from these data are given in Table I. The van't Hoff plots for these data

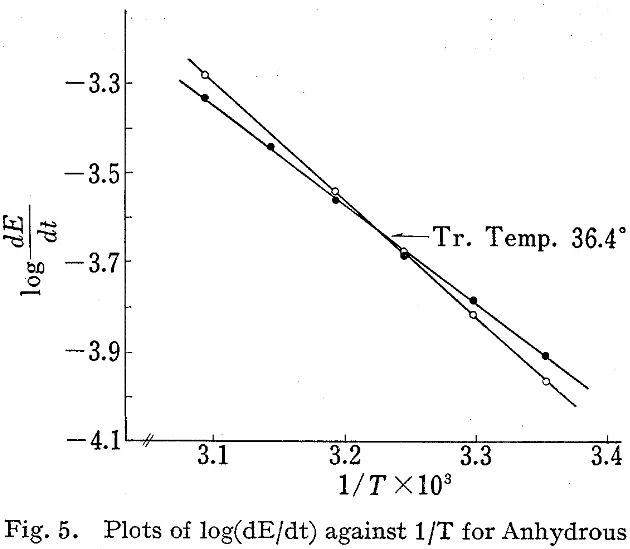

Fig. 5. Plots of log(dE/dt) against 1/T for Anhydrous and Hydrate Forms of Phenobarbital in Water

●: anhydrous form ○: hydrate form

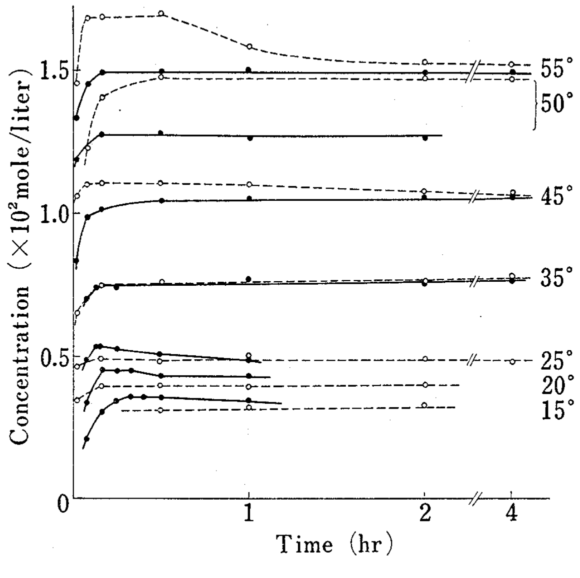

Fig. 6. Concentration-Time Curves for Determining Solubilities of Anhydrous and Hydrate Forms of Phenobarbital at Various Temperatures

●: anhydrous form ○: hydrate form

---

[7] The DSC curve of the KCl-phenobarbital hydrate mixture was the same as that of the hydrate itself. The fact shows that no interaction occurs between KCl and the hydrate.

are shown in Fig. 7. The fitted curves indicate a transition temperature of 35.1° and a transition energy of 1.48 kcal/mole. Before and after each solubility measurement, the solid sample phase was examined by DSC and IR. Conversion from the anhydrous to the hydrate forms was observed during solubility measurements at temperatures below 35°, and from the hydrate to the anhydrous forms above 35°, except in the case of the 50° run.

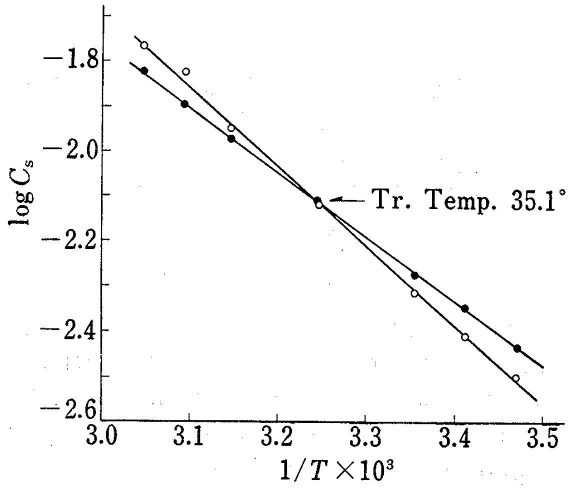

Fig. 7. Plots of Solubility Values for Anhydrous and Hydrate Forms of Phenobarbital in Water

●: anhydrous form ○: hydrate form

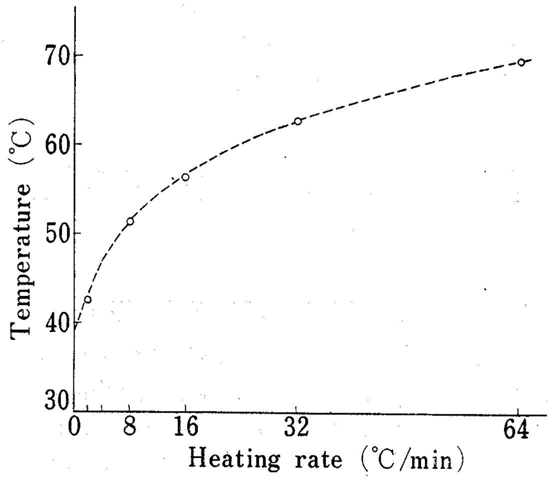

Fig. 8. Effect of Heating Rate of DSC on Transition Temperature of Phenobarbital (Hydrate→Anhydrous Forms)

sample weight: 3.9—4.6 mg
sensitivity 8—16mcal/sec
heating rate: 2—64°/min

III) By DSC——The relationship between transition initiation temperature and heating rate for a series of heating rates is shown in Fig. 8. The transition temperature should, in principle, be obtained by extrapolating the curve of Fig. 8 to a heating rate of 0°/min, but it is difficult to accurately extrapolate the curve, since the shape of the curve in this region is not precisely known. However, the transition temperature can be estimated to be about 38° from Fig. 8. Calculation of the average heat of transition, based on transition peak area measurements from 9 runs, indicated a transition energy of 1.78±0.05 kcal/mole.

IV) By Electrical Conductivity Measurements——As shown in Fig. 9, a change in conductivity was observed at about 50° when a heating rate of 1° per 5 minutes was employed. Somewhat higher transition temperature obtained by this measurement will be partly due to the construction of the apparatus in which sample temperature was not directly measured, since a gradual change in conductivity was noted when holding the temperature constant at 40.5°. So far as the results we obtained, an accurate transition temperature determination by this measurement is thought to be difficult and time-consuming. Transition energy could not be determined by this technique.

V) By Vapour Pressure Method——The logarithm of vapour pressure is plotted as a function of the reciprocal of absolute temperature in Fig. 10, for which two straight lines were determined by the method of least squares. The transition temperature was found to be about 47° from the intersection of two curves, which are interpreted as representing the vapour pressure of the hydrate–anhydrous solid–vapour system in the experimental temperature range of approximately 25—47°, and of the anhydrous solid-solution-vapour system above about 47°. However, the determination of transition temperature by this technique is not considered to be practical, because the measurement of vapour pressure at a fixed temperature requires at least several days to attain the true equilibrium.

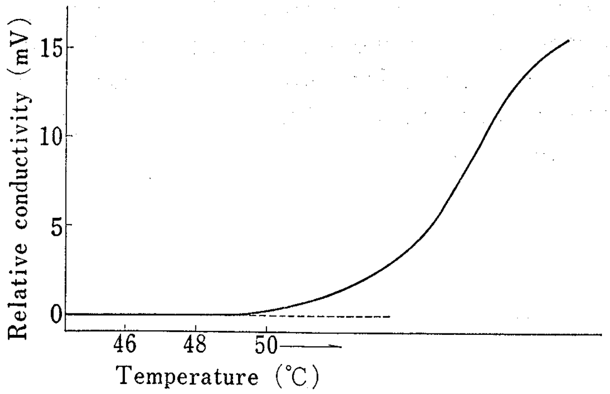

Fig. 9. Change of Conductivity induced by Transition from the Hydrate to Anhydrous Forms of Phenobarbital (Hydrate powder was mixed with KCl)

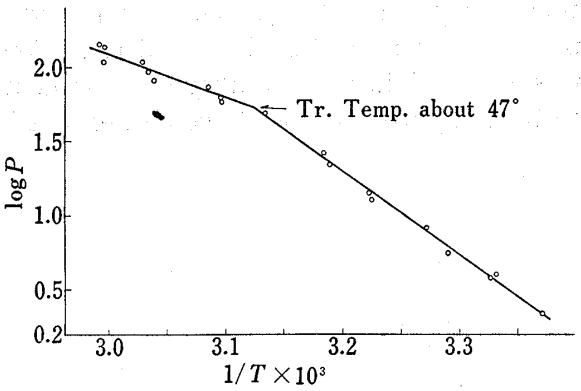

Fig. 10. The Logarithm of Vapour Pressure of the Hydrate versus the Reciprocal of Absolute Temperature

Determination of Metastable Solubility

Solubilities of the metastable form can be calculated from equation (9) by utilizing the experimentally determined solubilities for the stable form and the ratios of dissolution rates determined by the straight lines obtained by the method of least squares for both forms. Solubilities calculated in this manner are given in Table I. From this table, it is seen that the calculated metastable solubility values are in good agreement with those actually determined by conventional solubility measurements. Therefore, the validity of the assumption that the apparent dissolution rate constant for the stable form (K) is equal to that for the metastable form (K') in developing equation (9) is supported experimentally.

Table I. Solubility Data for Anhydrous and Hydrate Forms of Phenobarbital in Water at Various Temperatures

Temp. (°C)	Exptl. Soly., $\text{M} \times 10^2$		Calcd. Metastable Soly., $\text{M} \times 10^2$	
	Anhydrous	Hydrate	Anhydrous	Hydrate
15	0.368	0.318	0.398	
20	0.451	0.392	0.465	
25	0.532	0.483	0.542	
35	0.775	0.770	0.781	
45	1.055	1.108		1.145
50	1.266	1.506		1.439
55	1.499	1.698		1.781

Activation Energy for Dissolution

The activation energies of dissolution (Ea) for both the hydrate and anhydrous forms were calculated to be 3.98 kcal/mole and 3.60 kcal/mole, respectively by taking the difference between the heat of dissolution and the heat of solution calculated from the van't Hoff plot of log Cs versus $1/T$. Judging from the magnitude and the apparent equality of activation energy of dissolution for both forms, the assumption that dissolution processes for hydrate and anhydrous forms of phenobarbital are diffusion controlled appears to be valid.

Thickness of Diffusion Layer

Thickness of the diffusion layer δ was calculated by the following equations, as previously reported.[1] It is assumed that the experimentally determined apparent dissolution rate constant, K in equation (1), may be represented

$$K = \frac{SD}{V\delta} \tag{10}$$

where S is the surface area of the disk exposed to dissolution medium, V is the volume of dissolution medium, and D is the diffusion constant. Further, D may be estimated by the Stokes-Einstein equation,

$$D = \frac{kT}{6\pi\mu r} \tag{11}$$

where k is the Boltzmann constant, T is the absolute temperature, μ is the dissolution medium viscosity (viscosity of water in this case), and r is the radius of a solute molecule calculated from the molecular weight of phenobarbital, and the weight and volume of the disk. In the calculation of molecular radius, the molecules of the hydrate and anhydrous forms of phenobarbital are assumed to be closely packed spheres in the sample disk, and the molecular weight of anhydrous form was utilized in the calculation of r. Substituting this value of r, together with the appropriate values of μ and T into equation (11), D was calculated to be $5.70 \times 10^{-6}\text{cm}^2/\text{sec}$ at 25°. It is assumed that the r value calculated is an adequate estimate of the radius of a molecule of phenobarbital in solution. Substituting the value of D with values of S, V, and K or K' determined experimentally into equation (10), the thickness of the diffusion layer δ was calculated to be 3.82×10^{-3}cm and 3.63×10^{-3}cm for hydrate and anhydrous forms, respectively at 25°. The close values of diffusion layer thickness for both forms indicate that solute species in the saturated layer is the same for both the hydrate and anhydrous forms. It appears also reasonable from the value of δ to assume the dissolution processes for the hydrate and anhydrous forms of phenobarbital to be diffusion controlled.[8]

Conclusion

The transition temperature and the heat of transition between the hydrate and the anhydrous forms of phenobarbital obtained by dissolution rate measurements are in good agreement with values obtained by the conventional solubility equilibrium method and by DSC, as well as with transition temperature estimated by the electrical conductivity and vapour pressure measurements, as listed in Table II. Furthermore, metastable solubility values calculated from experimentally determined stable solubility values and the ratios of dissolution rates for both forms agree closely with values obtained experimentally.

The determination of transition temperature and the heat of transition between hydrate and anhydrous forms have hitherto been carried out by various methods of thermal analysis or by the solubility equilibrium method. In the thermal analysis, however, it is difficult to determine the accurate transition temperature, because the transition is always delayed by molecular hysteresis. On the other hand, it takes rather long experimental times to attain

Table II. Transition Temperature and Heat of Transition between Hydrate and Anhydrous Forms of Phenobarbital obtained by Various Methods

Method	Transition temp. (°C)	Heat of transition (kcal/mole)
Dissolution rate	36.4	1.87
Solubility	35.1	1.48
DSC	about 38	1.78±0.05
Conductivity	about 50	—
Vapour pressure	about 47	—

---

[8] K. Hirota, "Han'no-sokudo (Kyoritsu Zensho)," Kyoritsu Shuppan, Tokyo, 1957, p. 149.

a concentration equilibrium by the conventional solubility method, and a metastable form often converts rapidly to a stable form when exposed to the solvent. Transition temperature determination by the vapour pressure method takes a minimum of several days to attain true vapour pressure equilibrium at each temperature, and so is not particularly practical. However, the dissolution rate measurements presented here require so short an exposure time that even the dissolution rates of metastable forms can be measured before conversion has occurred.

Nogami, *et al.*[9] discussed the dissolution phenomena involving a phase change from metastable to stable forms observed in the cases of phenobarbital and *p*-hydroxybenzoic acid. They based their data analysis on the assumption that the processes of dissolution of the metastable form and crystallization of the stable form are both diffusion controlled, if crystallization is not delayed under the conditions of their experiments. However, as Fig. 6 shows, the retard of crystallization of the stable form is rather common with metastable form; therefore, in the present work, we assumed that the dissolution processes for both forms of phenobarbital are diffusion controlled, and that deposition of stable form is retarded at least at the initial stages of dissolution, and then analyzed the data obtained in accordance with assumption, as described above. Support for the validity of these assumptions is provided by the good agreement between transition temperature, heat of transition, and metastable solubility determined by the initial dissolution rates and results by other methods. The assumption is further supported by the apparent equality of dissolution activation energy for both forms, as well as by the magnitude of this activation energy, and by the diffusion layer thickness.

Accordingly, it is thought that the initial dissolution rate measurement presented in this study is simple and less time-consuming, and that the method is applicable to a wide range of drugs exhibiting both stable and metastable forms.

---

9) H. Nogami, T. Nagai, and T. Yotsuyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 499 (1969).