Chem. Pharm. Bull.
16(12)2495—2502(1968)

UDC 615.453.2.014

Studies on the Method of Size Reduction of Medicinal Compounds. III.[1,2] Size Reduction of Griseofulvin by Solvation and Desolvation Method using Chloroform. (3)

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(Received July 19, 1968)

In order to find the limit of size reduction by the method of solvation and desolvation, the effect of repeated solvation and desolvation of $CHCl_3$ on the specific surface area of griseofulvin particles was investigated by the BET gas adsorption method. It was found that the antibiotics was effectively pulverized by a single application of the method. The applicability was also tested with several substances forming solvates and hydrates and the specific surface areas were all found to be 2—3 m^2/g, unless a solution was formed during the desolvation process. The heat of solvation of griseofulvin with $CHCl_3$ and the heat of activation of the desolvation reaction were estimated to be 6.4 ± 0.5 and 20 ± 1 kcal/mole by the differential scanning calorimetry and gas evolution analysis.

Previously, the authors showed that size reduction of griseofulvin was accomplished by forming its one-to-one molecular compound with $CHCl_3$ and then removing the solvent from the solvate.[1] Since the "solvation and desolvation" method was thought to be practically applicable to other drug substances, investigations have been continued further. In the present paper, the effect of repeated solvation and desolvation of $CHCl_3$ upon the specific surface area of griseofulvin and the applicability of the method to substances which formed solvates or hydrates were investigated. Also, the heat of formation of griseofulvin chloroformate, the heat of activation of the desolvation reaction and the adsorption isotherm of $CHCl_3$ vapor on griseofulvin were determined.

Experimentals

Materials——1. Griseofulvin: Recrystallized from benzene. mp 220°. 2. Chloroform: The S.C. chemical was distilled and the fraction between 61—62° was used. 3. Griseofulvin monochloroformate: Prepared by crystallizing from $CHCl_3$. 4. Glucuronamide monohydrate: Commercial product was recrystallized from H_2O and was air-dried. 5. Potassium glucuronate dihydrate: Recrystallized from CH_3OH–H_2O mixture and was air-dried. 6. Sodium glucuronate isoniazone dihydrate: Prepared by crystallization from CH_3OH–H_2O mixture. The crystals were made free from the adhering water by refluxing in CH_3OH for 1/2 hour. 7. Citric acid monohydrate: The J.P. product was recrystallized from H_2O. 8. Picric acid monodioxanate: Crystallized from dioxane. The filtered crystal was immediately stored in an ampoule. 9. Sulfathiazole monoacetonate: Prepared by crystallizing the J.P. product from acetone.

The stoichiometric ratios of the solvates were determined by loss on drying or by elemental analysis.

Measurement of Specific Surface Area——The apparatus for the BET gas adsorption method was constructed and used. The sample weight was 10—20 g. The gas for adsorption was nitrogen and the measurement was done at $-195.8°$ by cooling the sample with liquid nitrogen.

Measurement of Adsorption Isotherm——A static method was employed. Greaseless bulbs of special type were used in the apparatus, because usual vacuum cocks were not fit for the vapor of organic solvent.

Differential Scanning Calorimetry (DSC) and Gas Evolution Analysis (GEA)——Simultaneous measurement of DSC and GEA were done with the Perkin-Elmer DSC-1B Differential Scanning Calorimeter. The sample weight was 10—20 mg. The gas introduced was nitrogen or helium. A suitable scan speed was selected for each measurement.

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1) Part II: K. Sekiguchi, K. Ito, E. Owada, and K. Ueno, *Chem. Pharm. Bull.* (Tokyo), **12**, 1192 (1964).
2) Presented at the 87th Annual Meeting of the Pharmaceutical Society of Japan at Kyoto, Apr. 1967.
3) Location: *Shirokane, Shiba, Minato-ku, Tokyo.*

Differential Thermal Analysis (DTA)——The apparatus reported in the previous paper was used.[4] Since experimental conditions in DTA differs from those in DSC, desolvation occured at temperatures somewhat higher than that by DSC.

Results and Discussion

Effect of Repeated Solvation and Desolvation upon Specific Surface Area

In order to find the limit and mechanism of the size reduction, the effect of repeated solvation and desolvation on the specific surface area of griseofulvin and its chloroformate particles was examined by the BET method using nitrogen as adsorbing gas. It was observed that when ordinary crystals are sorbed with $CHCl_3$ vapor under vacuum, the apparent volume of the crystal mass was increased by about 50%; however, further change in volume was not observed during subsequent desorption and sorption. As shown in Fig. 1, a zigzag curve, not a continuous one is obtained and the size reduction is practically finished by one cycle of solvation and desolvation. The specific surface area after each desolvation reaches over 2 m^2/g, whereas that of the solvated crystals is found to be about 1 m^2/g after the second solvation. Although no liquid was observed visually at the end of sorption of vaporous $CHCl_3$, the fact will suggest that a minute amount of liquid is formed between particles by such as capillary condensation. Thus, the primary particles are bridged, so that the surface area is decreased considerably. It is therefore concluded that repeated applications of the method afford little influence on the specific surface area. However, when a solid substance forms a molecular compound with a gas, its surface area will increase continuously by repetition of sorption and desorption of the gas.

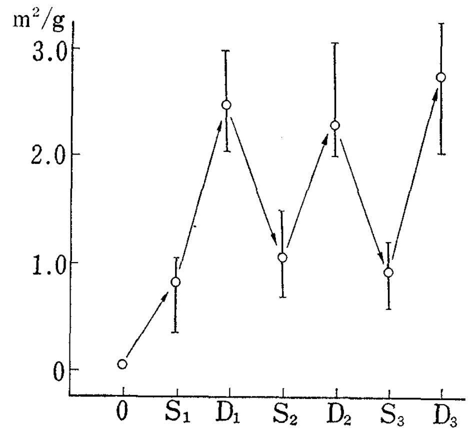

Fig. 1. The Effect of Repeated Solvation and Desolvation upon Specific Surface Area of Griseofulvin and Its Chloroformate

O: ordinally crystal S_1: solvated crystal
D_2: desolvated crystal

Table I. Specific Surface Areas of Various Solvates prepared by Crystallization in Solutions and of the Desolvated Compounds by Heating under Reduced Pressure

Solvated compounds	Solvated form specific surface area[a] (m^2/g)	Desolvated form	
		Desolvation temp. (°C)	Specific surface area[a,b] (m^2/g)
Glucuronamide monohydrate	0.27	70	2.71
Potassium glucuronate dihydrate	0.24	120	2.57
Sodium glucuronate isoniazone dihydrate	1.30	120	2.20
Citric acid monohydrate	0.01	100	0.89[c]
Picric acid monodioxanate	0.01	60	2.10
Sulfathiazole monoacetonate	0.11	70	3.53
Griseofulvin monochloroformate	0.05	150	2.05

a) The specific surface area was measured by BET method at room temperature.
b) Except that of the dehydrated citric acid, the mean diameter of each desolvated compound is calculated at 1—2 μ.
c) Liquid formation was noticed during dehydration.

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[4] K. Sekiguchi, T. Yotsuyanagi, and S. Mikami, *Chem. Pharm. Bull.* (Tokyo), **12**, 994 (1964).

Size Reduction of Other Substances by the Solvation and Desolvation Method

Substances which form solvates will behave similarly as griseofulvin during solvation and desolvation. Specific surface areas of several solvates isolated from their solutions and those of the desolvated compounds are given in Table 1. Except that of the dehydrated citric acid, all the areas fall in a range of 2—3.5 m^2/g after desolvation. The general applicability of the method is thus demonstrated. In the case of citric acid hydrate, liquid formation was noticed during dehydration and the specific surface area of the dehydrated compound amounted to only 0.89 m^2/g.

Adsorption Isotherm of Chloroform Vapor on Griseofulvin

The adsorption and desorption behaviors were investigated by a volumetric method at 30°. In the adsorption experiment as shown in Fig. 2, the amount of adsorbed $CHCl_3$ is small until the relative vapor pressure P/P_0 reaches at about 0.5; then, it increases rapidly and the one-to-one combination is nearly completed at about 0.6. The ratio of the adsorbate to the adsorbent remains constant at higher relative pressure, though a small increase is observed when the external pressure is almost saturated. On the other hand, desorption does not take place unless the P/P_0 value reaches nearly to 0. Even in such a condition, complete desorption was impossible.

Although the difficulty of desorption may be attributed to the fact that the induction period for decomposition of the chloroformate is too long for the practical measurement, such a distinct hysteresis effect is rare in literature, except that reported on the isotherm for the sodium salt of sulfamethomidine and its hydrate.[5]

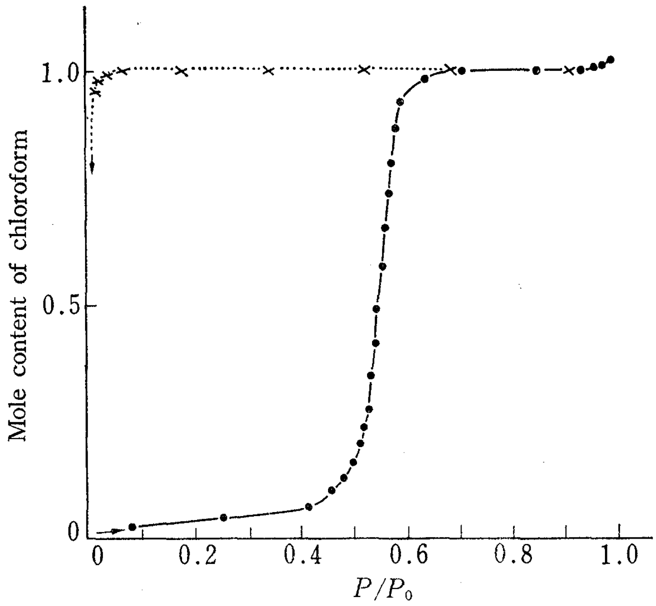

Fig. 2. Adsorption Isotherm of Chloroform at Room Temperature (30°)

——— adsorption curve ······· desorption curve

DSC and GEA Curves of Griseofulvin Chloroformate

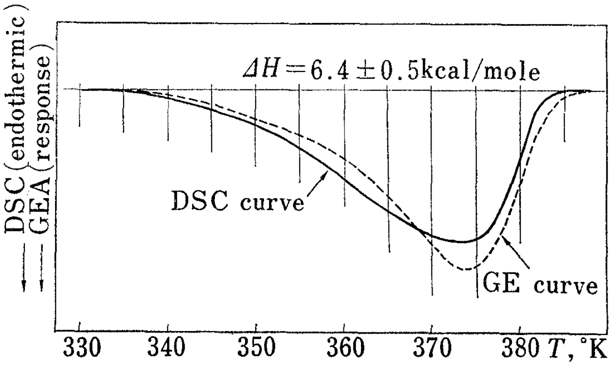

Fig. 3. Simultaneous DSC–GEA Curves of Griseofulvinmonochloroformate

sample weight: 17.0 mg, scan speed: 4°/min

In order to make clear the process of desolvation during heating, simultaneous measurements of DSC and GEA were done under various conditions. The solid and the dotted curve in Fig. 3 are typical DSC and GEA curves, respectively. The fact that the beginning and the ending temperature of desolvation in the DSC curve is recorded a few degrees lower than those in the GEA curve is attributed to the presence of dead space in the DSC furnace, since the discrepancy of the two curves becomes smaller by

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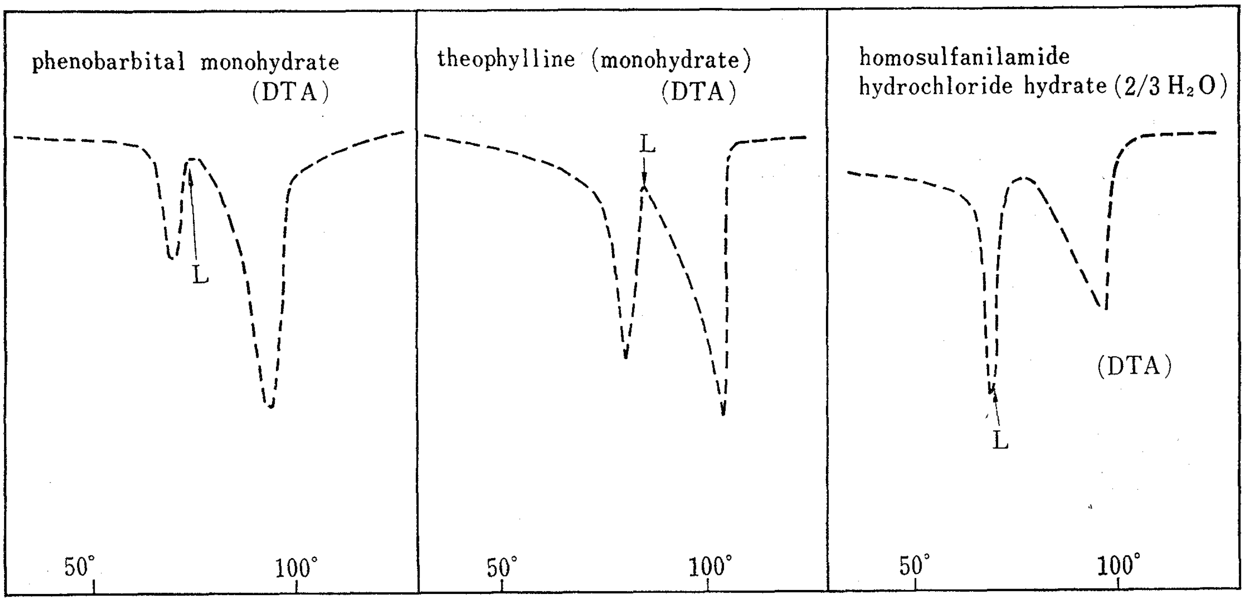
[5] T. Takahashi and Y. Sato, *Yakugaku Kenkyu*, **32**, 811 (1960).

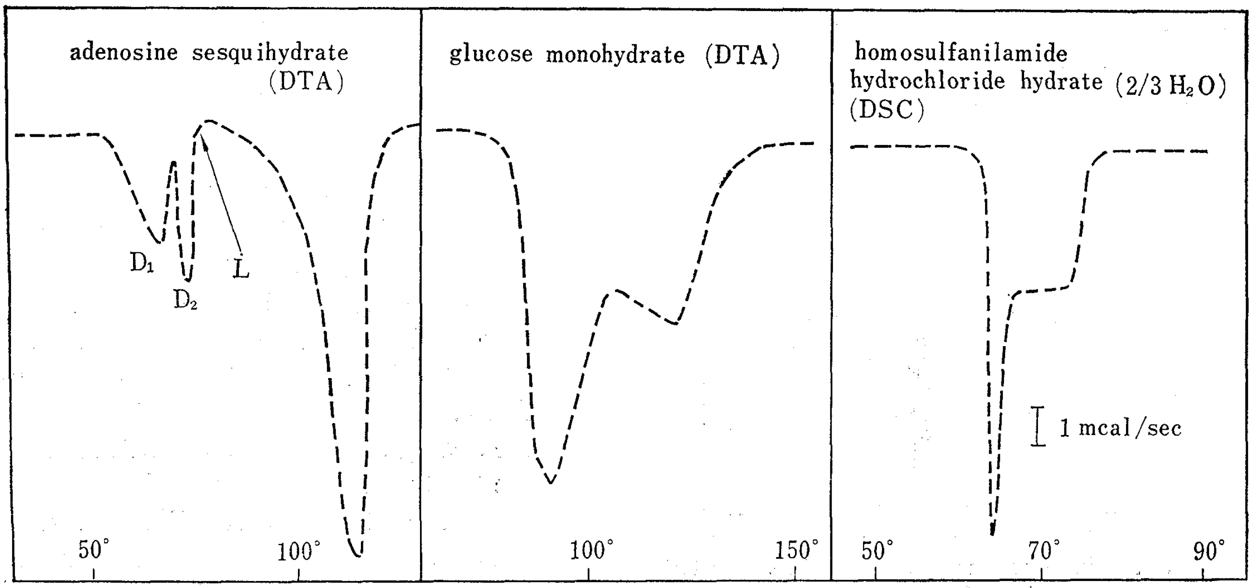
slowing the scan speed. It is to be noted that under any experimental conditions, the change in the DSC curve due to the over-all desolvation process is shown by a single peak. As has been reported in the previous paper,[1)] the similar type of thermogram was obtained by DTA irrespective of the amount of the chloroformate and the rate of heating. From data in the authors' laboratory, when a hydrate or a solvate produces a liquid phase during dehydration or desolvation by heating, corresponding DTA and DSC curves give two peaks at least under appropriate conditions. The first peak is thought to be attributable to the peritectic decomposition of the hydrate or the solvate and the second to the evaporation of water or solvent. On the other hand, the over-all dehydration or desolvation process is shown not by two peaks but by one peak, if no liquefaction occurs during

(A) Curves with Two or More Peaks (Decomposition with Liquid Formation)



SW=74.7 mg; HR=1.3°/min SW=78.6 mg; HR=1.7°/min SW=65.2 mg; HR=1.7°/min

Above L, transparent crystals of the hydrate turn white by decomposition.

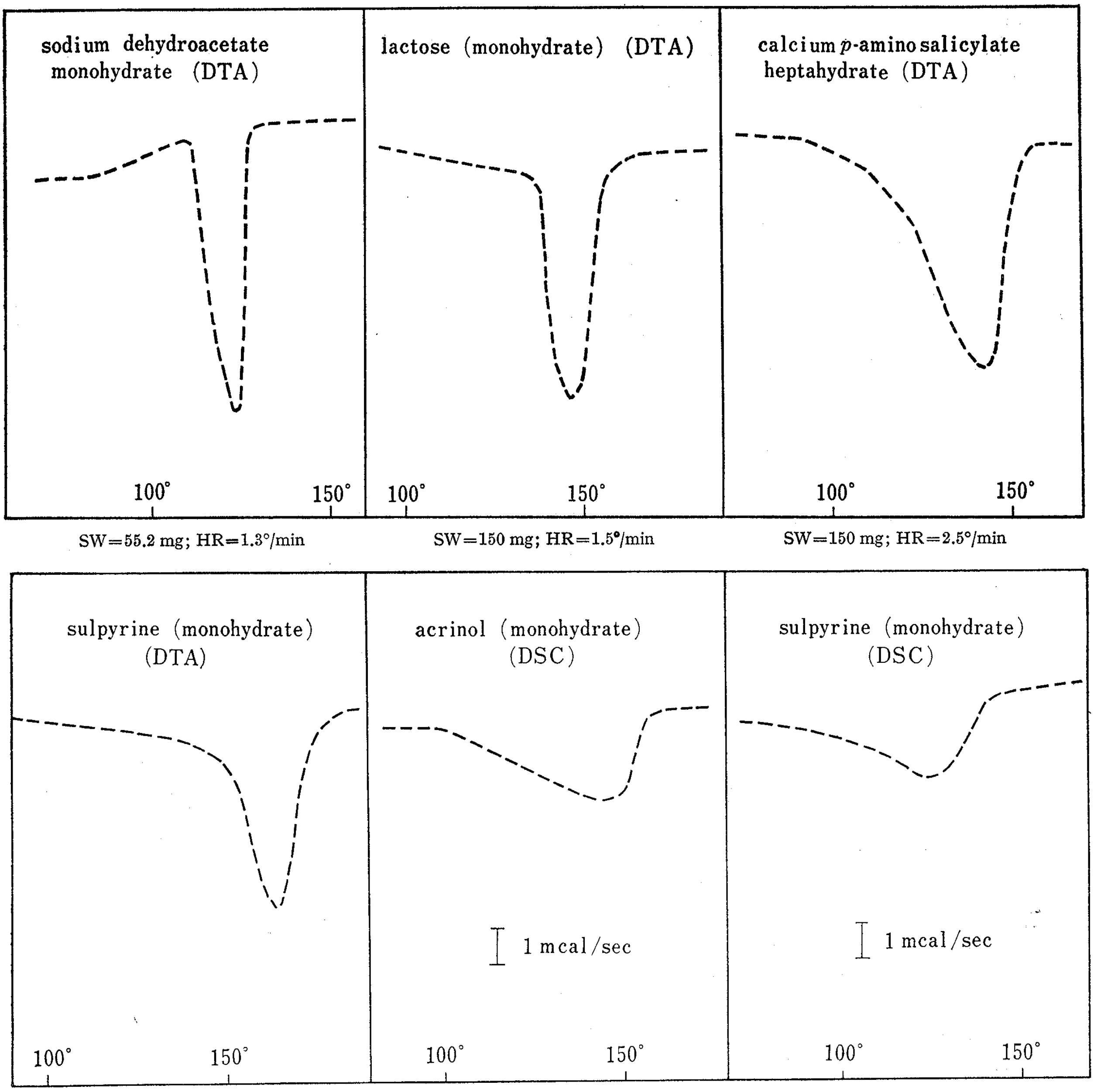


SW=95.7 mg; HR=2.3°/min SW=150 mg; HR=3.5°/min SW=12.1 mg; S=8°/min; R=16

D_1 and D_2 show stepwise decomposition of the hydrate.

Fig. 4. (continued)

(B) Curves with One Peak (Decomposition without Liquid Formation)



SW=55.2 mg; HR=1.3°/min SW=150 mg; HR=1.5°/min SW=150 mg; HR=2.5°/min

SW=140 mg; HR=2.0°/min SW=10.7 mg; S=8°/min; R=16 SW=10.2 mg; S=16°/min; R=16

Fig. 4. DTA and DSC Curves for Dehydration of Various Hydrates with or without Liquid Formation

SW, sample weight; HR, heating rate; R, range; L, the temperature at which liquid formation is first observed with eye

heating. Curves in Fig. 4 illustrate the two kinds of thermograms under semi-open environmental condition. From these findings, it is clear that the decomposition reaction of griseofulvin chloroformate under atomospheric pressure takes place at temperatures much below the supposed peritectic temperature for the system of griseofulvin and $CHCl_3$ and no liquid is formed. From areas under DSC peaks, the heat of desolvation of the chloroformate and the heat of fusion of griseofulvin are calculated at 6.4±0.5 and 10.0±0.2 kcal/mole, respectively.

Kinetic Parameters from GEA Curves

Although a number of non-isothermal methods for obtaining kinetic parameters were reported in the literature, those that involve an assumption as to the order of reaction are not proper for the analysis of solvate decomposition because the value of the order of such a reaction is difficult to predict. Therefore, methods without presumption of the reaction order should be used. Among them, the one by Ozawa[6] is thought to be applicable for estimating activation energy by GEA, since precise control of the heating rate is possible by the apparatus used.

The kinetic equation for the nth order reaction is generally expressed by

$$-\frac{dW}{dt}=A\exp\left(-\frac{\varDelta E}{RT}\right)\cdot W^n \qquad (1)$$

in which W is the fractional residual amount of the solvate; T, the absolute temperature; R, the gas constant; t, the time and A, $\varDelta E$ and n, the frequency factor, the activation energy and the order of the reaction, respectively. According to Ozawa, the above equation can be converted into equation (2) by several transformations;

$$\frac{A\varDelta E}{a_1R}P\left(\frac{\varDelta E}{RT_1}\right)=\frac{A\varDelta E}{a_2R}P\left(\frac{\varDelta E}{RT_2}\right)=\cdots\cdots=\frac{A\varDelta E}{a_iR}P\left(\frac{\varDelta E}{RT_i}\right)=\cdots\cdots \qquad (2)$$

where T_i is the temperature at which a given fraction of a solvate changes under a certain constant heating rate a_i and P is the function introduced by Doyle.[7] From the above, the equation (3) for obtaining the activation energy, is derived by approximation.

$$-\log a_1-0.4567\frac{\varDelta E}{RT_1}=-\log a_2-0.4567\frac{\varDelta E}{RT_2}=\cdots\cdots$$
$$=-\log a_i-0.4567\frac{\varDelta E}{RT_i}=\cdots\cdots \qquad (3)$$

The temperature T_i for each scan speed a_i can be detremined graphically from the corresponding cumulative decomposition curve in Fig. 5. The plots of the logarithms of the scan speeds versus the reciprocals of the temperatures give straight lines as seen in Fig. 6, and from the slopes, the activation energy is calculated at 20±1 kcal/mole within the range of residual fraction from 10 to 90%. Using this value, the residual fraction is plotted against log $(\varDelta E/aR)$ $P(\varDelta E/RT)$ and a dotted curve in Fig. 7 is obtained. When this is compared by lateral shift with the theoretical curves for the reactions having indicated orders, the best superposition is found with the one for the zero order reaction. Thus, the reaction order for the decomposition of griseofulvin chloroformate will be nearly equal to 0, and the frequency factor can be calculated at 10.5 from the length of the shift.

Making use of this knowledge about the order, a trial and error method proposed by Kofstad[8] was applied to GEA date. In his treatment, equation (4) which is derived from equation (1) is used.

$$(1-n)\cdot\ln W+\ln\frac{d\ln W}{dT}=\ln A-\frac{\varDelta E}{RT} \qquad (4)$$

By giving a proper value as the reaction order n, the reciprocal temperature is plotted against the left side of the equation and the linearity of the curve is examined. The plots in Fig. 8 show that wide linear relations are held for the zero and 1/3 rd order reactions and the activation energies are calculated at 25 and 15 kcal/mole, respectively. Therefore, the values of the kinetic parameters for the decomposition reaction of griseofulvin chloroformate will be reasonablly accepted since analyses by the two methods give similar results.

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6) T. Ozawa, *Bull. Chem. Soc. Japan*, **38**, 1881 (1965).
7) C.D. Doyle, *J. Appl. Polymer Sci.*, **5**, 285 (1961).
8) P. Kofstad, *Nature*, **179**, 1362 (1957).

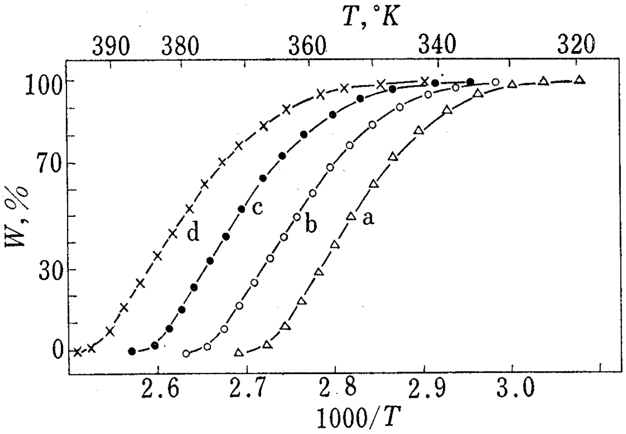

Fig. 5. The Cumulative GEA Curves of Desolvation of Griseofulvin–chloroformate plotted against the Reciprocal of Absolute Temperature

scan speed: a, 1°/min; b, 2°/min; c, 4°/min; d, 8°/min

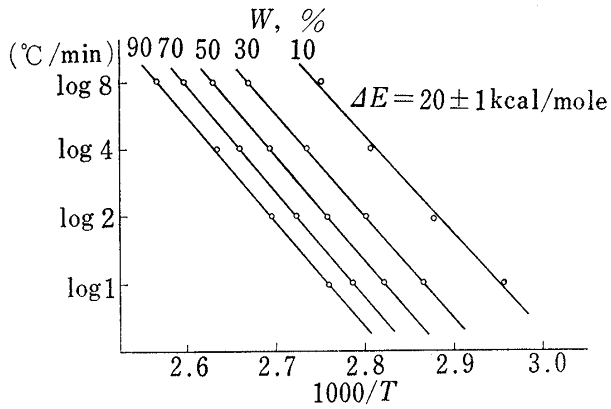

Fig. 6. The Plots of Logarithms of Scan Speed Versus the Reciprocal of Absolute Temperature for Indicated Conversions of the Desolvation of Griseofulvin–chloroformate

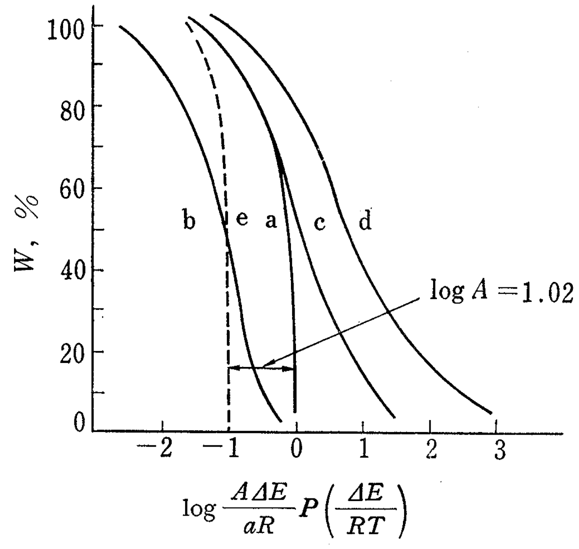

Fig. 7. The Theoretical GEA Curves

a, zero order reaction; b, 1st order reaction; c, 2nd order reaction; d, 3rd order reaction; e, experimental curve

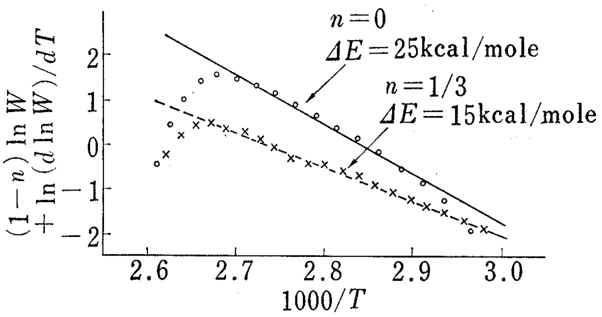

Fig. 8. Kofstad's Equation

Conclusion

The results above show that the method of size reduction by solvation and desolvation is applicable to not only griseofulvin but also to other compounds forming solvates or hydrates, and the specific surface areas of them reach usually to 2—3 m^2/g. The products are weak agglomerates of minute primary crystal particles. Also, it becomes clear that size reduction is accomplished by one cycle of solvation and desolvation, and further application is unnecessary.

The decrease in specific surface area after solvation of the once desolvated compound is supposed to be due to capillary condensation at points of contact between primary particles. However, more effective size reduction will probably be attained by repetition of the method, if a gaseous substance which forms a weak compound with the substance to be pulverized can be used in place of a liquid solvent. Even in such a case, there will be a limit because the increased vapor pressure of extremely minute particles promotes growth of crystals.

In the application of the method, the conditions of desolvation is thought to affect the result considerably. For example, when the temperature is too high, growth of particles may

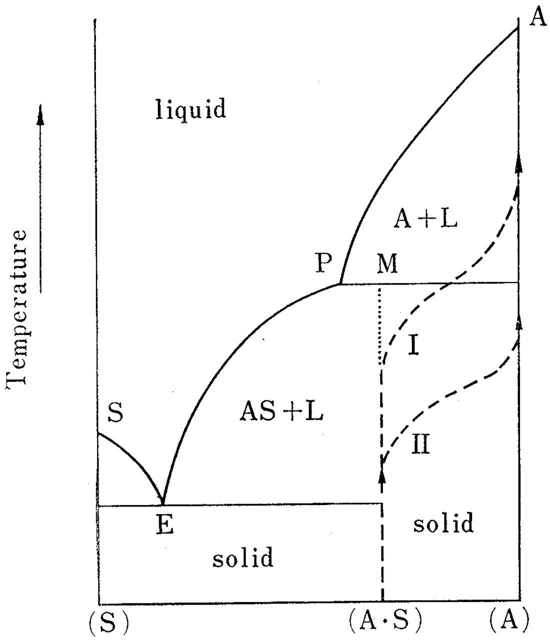

Fig. 9. Schematic Phase Diagram of the System of a Solid Substance (A) and a Solvent (S) between which a Solvate is formed

occur by sublimation. However, the most important one is that the operation must be carried out without forming solution. The fact will be understood by the schematic diagrams in Fig. 9. As is observed with the hydrate of citric acid, if the solvate is rapidly heated and the solvent vapor is not effectively exhausted, desolvation will take place in a manner represented by path I. In such a case, a solution of which composition is given by P will be produced at the transition point by the following peritectic reaction,

$$\text{A}\cdot\text{S} \longrightarrow \text{A}+\text{Soln. of A in S}$$

where A·S, A and S are the solvate, the substance to be pulverized and the solvent, respectively. As the result, crystallization in liquid occurs and larger particles are mixed in the desolvation product. On the other hand, when desolvation proceed along the path II, the substance will be effectively and uniformly pulverized.

In the previous papers,[1,9,10,] the authors suggested that a finely divided powder will be formed by freeze-drying of a dilute solution of a substance in water or in organic solvent. However, the method by solvation and desolvation is superior to the one by freeze-drying in that the operating temperature is not too low and the amount of substance treated in one batch operation is larger.

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9) K. Sekiguchi, N. Obi, and Y. Ueda, *Chem. Pharm. Bull.* (Tokyo), **12**, 134 (1963).
10) K. Sekiguchi, *Yakkyoku*, **16**, 9 (1965).