Studies on Methods of Particle Size Reduction of Medicinal Compounds. VIII.[1] Size Reduction by Freeze-Drying and the Influence of Pharmaceutical Adjuvants on the Micromeritic Properties of Freeze-Dried Powders[2]

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The size reduction of solid drugs was attempted utilizing the freeze-drying of a two-component system comprising a solute and a solvent. It was found that the concentration of the drug to be frozen greatly influenced the average particle size of the resulting powder. The component of a system which forms a hydrate or a solvate produced more finely divided particles than did that of a simple eutectic system. This can be attributed to the effect of the peritectic reaction in the process. As the griseofulvin and benzene system gives a phase diagram with an incongruent melting point and forms a 1:2 solvate, the average particle size of the freeze-dried powder could be reduced to the submicron level. On the addition of surfactants to this system, micronized particles were obtained and the dissolution rate of griseofulvin was improved considerably.

Keywords——freeze-drying; particle size reduction; potassium chloride; sodium chloride; griseofulvin-benzene solvate; nonionic surface active agent; dissolution rate of griseofulvin

According to the Noyes-Whitney equation, dissolution rate is directly proportional to the effective surface area of the dissolving solid. The surface area term varies inversely with the diameter. Therefore, the particle size of a medicinal compound may affect the dissolution rate, absorption rate, and bioavailability. Micronization of solid drugs has been performed by several mechanical techniques, but these procedures may affect the physico-chemical stability of drugs owing to the long period of milling. Moreover, foreign substances may contaminate the bulk contents in manufacturing processes. Hence, many kinds of fluid-energy mills, which can be operated at low temperature and under sterile conditions,[4] have recently been employed for heat-labile medicinal compounds such as antibiotics, but it is not easy to prepare drug powders with a narrow size distribution range. For these reasons, methods other than mechanical milling for size reduction have been developed. Previous reports[5] from this laboratory have indicated that the formation of solvates with solvents[5] or of eutectic mixtures with readily soluble compounds[6] is applicable for size reduction or improvement of the dissolution behavior of drugs.

In this communication the authors describe particle size reduction by freeze-drying, utilizing the phase transition. As model systems, the water-potassium chloride system and the water-sodium chloride system were selected. As an organic pharmaceutical, griseofulvin

1) Part VII: K. Sekiguchi, Y. Tsuda, M. Kanke, E. Suzuki, and M. Iwatsuru, Chem. Pharm. Bull. (Tokyo), 26, 1279 (1978).
2) Presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1977.
3) Location: 9–1, Shirokane 5-chome, Minato-ku, Tokyo.
4) E.L. Parrott, J. Pharm. Sci., 63, 813 (1974).
5) K. Sekiguchi, K. Ito, E. Owada, and K. Ueno, Chem. Pharm. Bull. (Tokyo), 12, 1192 (1964); K. Sekiguchi, I. Horikoshi, and I. Himuro, ibid., 16, 2495 (1968); I. Himuro, Y. Tsuda, K. Sekiguchi, I. Horikoshi, and M. Kanke, ibid., 19, 1034 (1971).
6) K. Sekiguchi and N. Obi, Chem. Pharm. Bull. (Tokyo), 9, 866 (1961); K. Sekiguchi and N. Obi, and Y. Ueda, ibid., 12, 134 (1964).

was freeze-dried from a benzene solution and the influence of adjuvants, *i.e.*, nonionic surface active agents, on the micromeritic properties was investigated.

Experimental

Materials——Potassium Chloride and sodium chloride were of reagent grade. Griseofulvin was twice recrystallized from aceton (mp 220°). Analytical-grade benzene was purified by fractional distillation. Sorbitan monostearate and polyoxyethylene monostearate were of commercial quality.

Procedure for Freeze-Drying——An aqueous solution of potassium chloride and sodium chloride, and a benzene solution of griseofulvin in the presence or absence of nonionic surfactants were poured into a round-bottomed flask previously chilled in a refrigerant and then rapidly frozen to form a thin–layer on the bottom of the vessel. The refrigerant employed was acetone–dry ice mixture. After the solution had frozen completely, the flask was fitted with a freeze-drying apparatus (model D-3, Ishii Co.) and sublimation of the solvent was initiated using a vacuum pump (type 4VP-C$_3$, Hitachi). The pressure in the apparatus was usually 0.05 mmHg. Approximately ten hours were required to dry 30 ml of the frozen aqueous solution.

Measurements of Specific Surface Area——A BET gas adsorption apparatus (model 600-p, Shibata Chemical Apparatus Co.) was used. The sample weight was 0.5—3 g and the gas used for adsorption was N$_2$.

Scanning Electron Microscopy——The surface structure of freeze-dried particles was surveyed using a scanning electron microscopy (MINI-SEM model MSM-4, Hitachi-Akashi Co.).

X-Ray Powder Diffractometry——An X-ray diffraction analyzer type-7F from Japan Electron Optics Laboratory Co. was used (Ni filter, Cu-Kα radiation, $\lambda = 1.542$ Å).

Determination of Dissolution Rate——Procedure A: A quantity of griseofulvin powder in excess of its solubility was weighed and rapidly introduced to a 200 ml water-jacketed cell containing exactly 100 ml of distilled water maintained at $37 \pm 0.2°$. The solution was stirred with a Teflon-covered magnetic stirring bar at a constant rate of about 200 rpm. At suitable intervals, aliquots of the solution were withdrawn, filtered through Sartorius membrane filter papers (pore size 0.45 μ), and immediately diluted with an appropriate amount of distilled water.

Procedure B: The automatic recording system described by Sekiguchi *et al.* was used.[7] The absorbance of dissolved griseofulvin was monitored at 295 nm with a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer.

Determination of Griseofulvin——Griseofulvin in the filtrate of Procedure A in the dissolution experiments was analyzed at 295 nm with shimadzu UV-200 double-beam spectrophotometer. Since the adsorption of griseofulvin on the membrane filters could not be ignored,[8] standard solutions previously filtered through membrane filters were used to determine griseofulvin concentrations in the dissolution mediums. The absorbance due to adjuvants was corrected for.

Results and Discussion

Effect of Concentration on the Properties of Freeze-Dried Products

As a simple eutectic system, the water-potassium chloride system was selected. The phase diagram is schematically represented in Fig. 1, where the eutectic point is −10.7°, and its salt concentration is 19.7 g in 100 g of solution.[9] Since it is thought that the salt concentration in an aqueous solution subject to freeze-drying influences the average particle size and the micromeritic properties of the resulting powder, solutions prepared at various salt concentrations were freeze-dried. The specific surface areas of potassium chloride obtained are shown in Table I. When a liquid system with a lower salt composition than the eutectic
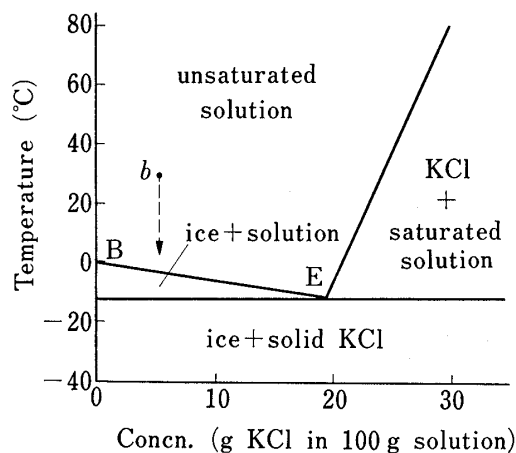

Fig. 1. Phase Diagram of the Potassium Chloride and Water System

7) K. Sekiguchi, K. Shirotani, and M. Kanke, *Yakugaku Zasshi*, **95**, 195 (1975).
8) W.L. Chiou and L.D. Smith, *J. Pharm. Sci.*, **59**, 843 (1970).
9) E.W. Washburn (ed.), "International Critical Tables of Numerical Data, Physics, Chemistry and Technology," Vol. 4, McGraw-Hill Book Company, Inc., New York, 1928, p. 254.

point E, for example system b, is cooled, ice first freezes out on the curve BE. As cooling continues, more ice freezes out until point E; at this point, the eutectic mixture freezes out. Accordingly, the frozen mass is composed of large ice crystals and the mixture of very fine ice and salt crystals, designated cryohydrate. Through the freeze-drying of this mass, micronized salt powder is produced. On the other hand, in the case of a higher salt composition than that at point E, potassium chloride initially crystallizes out. Therefore the specific surface area of freeze-dried products may be much smaller than in the former case. The results of Table I indicate that this assumption is correct. Assuming that the particles

TABLE I. Specific Surface Area and Particle Size of Potassium Chloride
obtained by Freeze-Drying an Aqueous Solution

Concn. of solution (g/100 g)	Specific surface area (m²/g)	Average diameter (μ)[a]
5.03	1.4	2.2
10.01	2.1	1.5
20.06	2.2	1.4
25.02	0.3	9.8
30.05	0.4	7.8

a) $d=6/\rho \cdot S$. d: average diameter, $\rho=1.98$, S: specific surface area.

are spherical, the calculated average particle size is 1—2 μ at lower concentrations than the eutectic composition, but 10 μ at higher concentrations.

Electron microphotographs of freeze-dried particles are shown in Fig. 2. Particles obtained from a 5.03 g/100 g solution are finely divided, but particles from a 30.05 g/100 g solution can be as large as 30 μ.

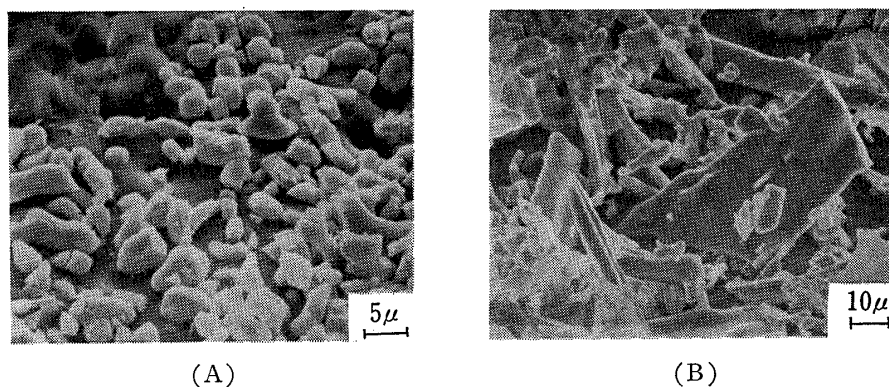(A)   (B)

Fig. 2. Electron Micrographs of Potassium Chloride Particles

The concentration of KCl was 5.03 g/100 g in (A) and 30.05 g/100 g in (B).
(A) 2000×, (B) 1000×.

As a system with an incongruent melting point, the water and sodium chloride system was employed. As shown in Fig. 3, the phase diagram has one intersection at point C, which corresponds to the transition point of dihydrate to anhydrous salt.[9,10]

The X-ray powder diffraction patterns of sodium chloride and its dihydrate are shown in Fig. 4. Sodium chloride dihydrate was prepared following the method of Adams and Gibson.[10] Measurements of the dihydrate were performed by cooling the sample powder with dry ice. Twenty minutes after removing the dry ice, the X-ray diffraction patterns of the dihydrate changed to those of sodium chloride.

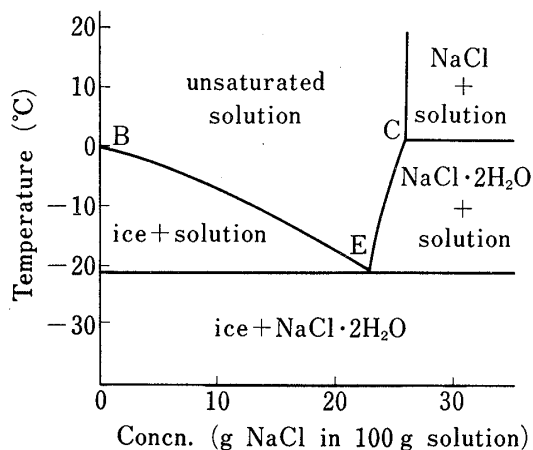10) L.H. Adams and R.E. Gibson, *J. Am. Chem. Soc.*, **52**, 4252 (1930).

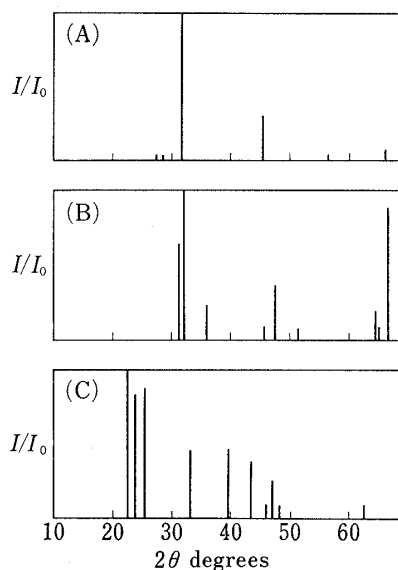Fig. 3. Phase Diagram of the Sodium
Chloride and Water System

Fig. 4. X-Ray Powder Diffraction Patterns
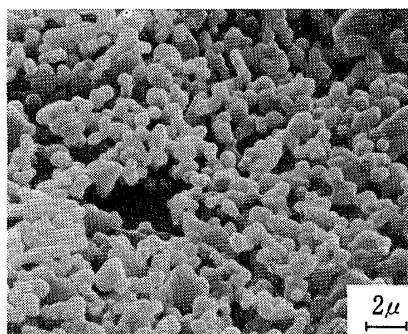of Sodium Chloride, Its Dihydrate, and Ice

(A) sodium chloride.
(B) sodium chloride dihydrate cooled with dry ice.
(C) ice prepared from atmospheric moisture, cooled
with dry ice.

The specific surface areas of products freeze-dried from solutions of various salt concentra-
tions are shown in Table II. The values for freeze-dried powders obtained from solutions
prepared at lower concentrations than the eutectic composition are 2—3 m²/g. The success
in size reduction may be attributed to the fact that ice crystallizes initially in the freezing
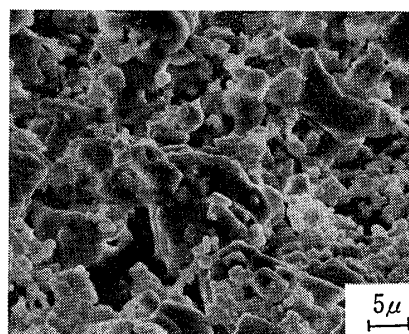
TABLE II. Specific Surface Area and Particle Size of Sodium Chloride
obtained by Freeze-Drying an Aqueous Solution

Concn. of solution (g/100 g)	Specific surface area (m²/g)	Average diameter $(\mu)^{a)}$
5.01	2.3	1.2
10.05	3.3	0.84
19.93	2.5	1.1
24.97	1.2	2.2
26.30	1.1	2.5

a) $d=6/\rho \cdot S$ $(\rho=2.17)$.

(A)   (B)

Fig. 5. Electron Micrographs of Sodium Chloride Particles

The concentration of NaCl was 10.05 g/100 g in (A) and 24.97 g/100 g in (B).
(A) 5000×, (B) 2000×.

process and the dehydration of dihydrate operates favorably in the sublimation process. The values obtained from solutions of slightly higher concentrations than the eutectic composition are about 1 m²/g and are considerably larger than those for potassium chloride; this increase may be brought by the dehydration reaction during the removal of water vapor from the frozen mass. The calculated average particle sizes of sodium chloride are 1 μ in the former and 2 μ in the latter case.

Microphotographs of the freeze-dried powders are shown in Fig. 5. Particles obtained from a 10.05 g/100 g solution are uniform and spherical, whereas particles obtained from a 24.97 g/100 g solution are a mixture of crystals of various sizes.
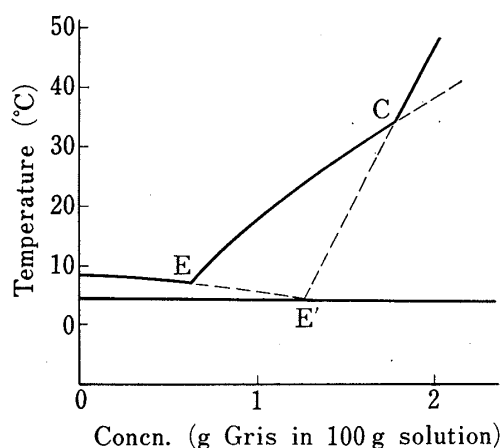

Fig. 6. Phase Diagram of the Griseo-fulvin (Gris) and Benzene System

E′: metastable eutectic point.

As an organic pharmaceutical, griseofulvin was chosen. Preliminary experiments indicated that benzene was the most suitable solvent with regard to the solubility of griseofulvin and the ease of sublimation. The phase diagram of the benzene and griseofulvin system suggested by Sekiguchi et al.[11] is schematically represented in Fig. 6. As illustrated, the solvate, which is composed of one molecule of griseofulvin and two molecules of benzene, decomposes at near 35°. Table III indicates that the powders obtained by freeze-drying of solutions at concentrations between 0.2 g/100 g and 1.324 g/100 g have very large specific surface areas and the average particle size was 0.5 μ. The production of these very fine particles may be attributed to the desolvation in the solid state. These results

TABLE III. Specific Surface Area and Particle Size of Griseofulvin obtained by Freeze-Drying a Benzene Solution

Concn. of solution (g/100 g)	Specific surface area (m²/g)	Average diameter (μ)[a]
0.200	7.2	0.57
0.400	9.3	0.44
0.586	10.3	0.40
0.800	11.2	0.36
0.999	9.7	0.42
1.324	8.4	0.49

a) $d=6/\rho \cdot S$　$(\rho=1.47)$.

showed that size reduction of griseofulvin is attainable efficiently to a submicron level without any mechanical milling procedures.

Some antibiotics or enzymes are frequently freeze-dried as amorphos forms.[12] Although they are superior to the crystal form as regards solubility, their higher hygroscopisity and reduced stability may be disadvantageous in practical use. Therefore X-ray diffractometry of freeze-dried griseofulvin was carried out. It was found that most samples (only one exception) had good crystallinity, as shown in Fig. 7.

11) K. Sekiguchi, K. Shirotani, M. Kanke, H. Furukawa, and M. Iwatsuru, Chem. Pharm. Bull. (Tokyo), 24, 1621 (1976).

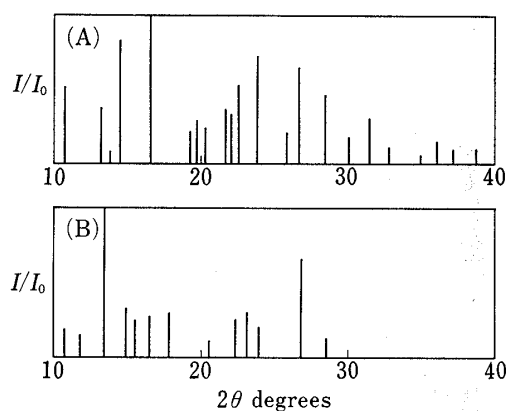12) M. Murakami, K. Aoki, and Y. Matsukura, Yakuzaigaku, 29, 115 (1969).

Fig. 7. X-ray Powder Diffraction Patterns of Griseofulvin prepared by Freeze-Drying

(A) griseofulvin having good crystallinity.
(B) griseofulvin having poor crystallinity.
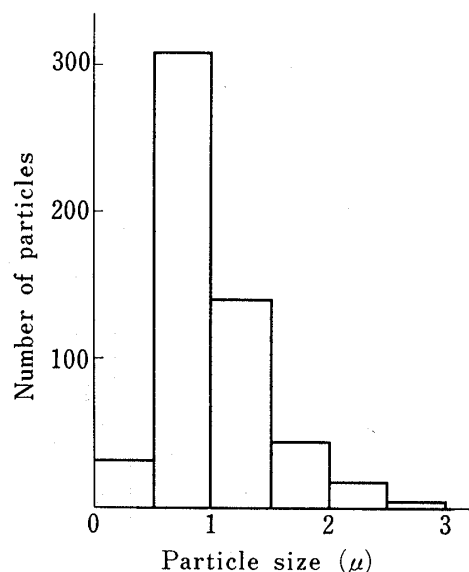(Peaks showed the relative intensity less than 5% were neglected.)

Fig. 8. Particle Size Distribution of Griseofulvin obtained by Freeze-Drying

Average particle size: 0.96 μ.

The particle size distribution of freeze-dried griseofulvin was measured as follows: a small amount of powder was placed on a slide grass, and made to disperse into primary particles as far as possible. Microphotographs were magnified and the Green's diameters of approximately five hundred particles were measured. As described in Fig. 8, the particle size distribution range is narrow; this result is beyond the capability of current mechanical milling procedures. The mean diameter is 0.96 μ, somewhat larger than the values obtained from N_2 gas adsorption studies, but this may be caused by the partial agglomeration of primary particles.

Effect of Surfactants on the Properties of Freeze-Dried Products

Since the crystals of griseofulvin are strong and water-insoluble, the authors attempted to improve these properties by the addition of nonionic surfactants. Using the ordinary methods, a benzene solution of griseofulvin in the presence of a surfactant was freeze-dried and the specific surface area of the product was measured. As shown in Table IV, size reduction was accomplished efficiently. When the ratio of sorbitan monostearate to griseofulvin was varied, the specific surface areas showed a tendency to decrease as the amount of surfactants added increased. On electron microscopy, the presence of large or agglomerated particles was observed. These results may be attributed to the comparatively low melting points of the surfactants used in the experiments.

TABLE IV. Effect of Nonionic Surface-Active Agents on the Freeze-Drying of Griseofulvin

Surfactant	Concn. of solution (g/100 g)		Specific surface area (m²/g)
	Griseofulvin	Surfactant	
Sorbitan monostearate	0.586	0.293	6.3
Sorbitan monostearate	0.586	0.147	10.4
Sorbitan monostearate	0.586	0.059	14.0
Polyoxyethylene monostearate	0.586	0.147	6.7

Figure 9 shows electron micrographs of freeze-dried griseofulvin and griseofulvin with polyoxyethylene monostearate. The particle diameter of griseofulvin is about 1 μ, whereas in the presence of the surfactant large or irregular agglomerates are seen. The X-ray diffraction patterns of griseofulvin with surfactants were the same as those of griseofulvin alone, and on the whole the crystallinity was good.

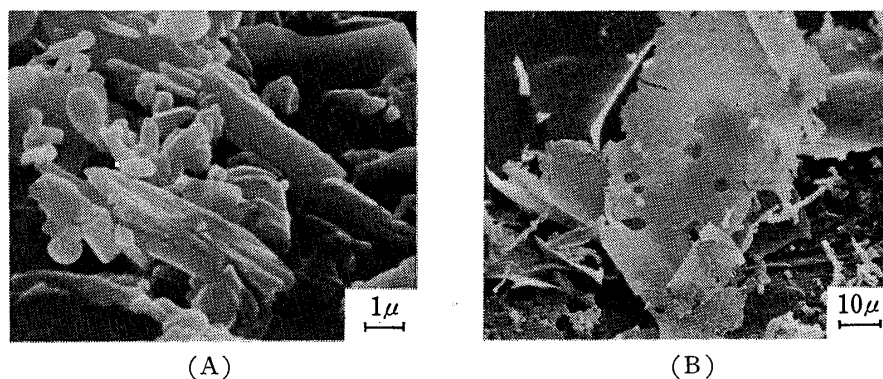(A)   (B)

Fig. 9.   Electron Micrographs of Griseofulvin Particles

(A) shows griseofulvin alone and (B) shows griseofulvin with polyoxyethylene monostearate.
The concentration of griseofulvin was 0.586 g/100 g.
(A) 10000×,   (B) 1000×.

Effect of Aging

The specific surface areas of freeze-dried powders were measured after ten weeks storage at room temperature. As shown in Table V, the changes were hardly perceptible. It appeared that crystal growth had not occurred and that the solvents had sublimed completely.

TABLE V.   Effect of Aging on the Specific Surface Areas of Compounds
obtained by Freeze-Drying

System	Concn. of solution (g/100 g)	Specific surface area (m²/g)	
		Freshly prepared	After 10 weeks
KCl–H₂O	10.01	2.1	1.2
NaCl–H₂O	10.05	3.3	2.7
Griseofulvin–Benzene	0.586	10.3	9.2
Griseofulvin–Sorbitan monostearate–Benzene	0.586 0.147	10.4	11.7
Griseofulvin–Polyoxyethylene monostearate–Benzene	0.586 0.147	6.7	4.8

Dissolution of Griseofulvin

The effects of particle size and surfactants on the dissolution rate of griseofulvin were investigated. The samples were 100—200 mesh griseofulvin, freeze-dried griseofulvin, and freeze-dried griseofulvin with sorbitan monostearate or polyoxyethylene monostearate. As shown in Fig. 10, the dissolution rate of freeze-dried griseofulvin is faster than that of 100—200 mesh powder. There are a number of reports referring to the effect of the particle size of water-insoluble drugs on their dissolution rate.[13] The variables introduced in the dissolution studies are the particle size, the rate of agitation, pH of the dissolution medium, the

13) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **55,** 1323 (1966); P. Finholt and S. Solvang, *ibid.*, **57,** 1322 (1968); S. Lin, J. Menig, and L. Lachman, *ibid.*, **57,** 2143 (1968); W.L. Chiou and S. Riegelman, *ibid.*, **58,** 1505 (1969).
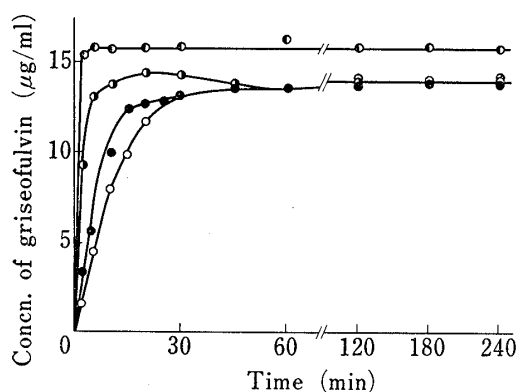
Fig. 10. Effect of Particle Size and Surface-Active Agents on Griseofulvin (Gris) Dissolution

Key: ○ 100—200 mesh Gris,
● Gris prepared by freeze-drying,
◑ Gris-Sorbitan monostearate prepared by freeze-drying,
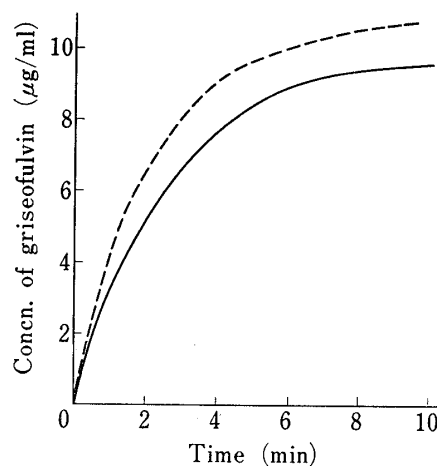◐ Gris-Polyoxyethylene monostearate Prepared by freeze-drying.

Fig. 11. Dissolution Profiles of Micronized and 100—200 Mesh Griseofulvin (Gris) Powders using an Automatic Recording System

Key: ——— 100—200 mesh Gris,
------ Gris micronized by freeze-drying.

presence or absence of adjuvants, and others. As many of these factors produce complicated effects, the net effect on the dissolution rate depends on the relative magnitudes of the variables. Thus, if the experimental conditions are altered, unexpected results are occasionally obtained. Therefore, in order to determine the dissolution rate more precisely, an automatic recording system was employed and the dissolution behavior of griseofulvin for the initial ten minutes was measured. As shown in Fig. 11, the results resembled the preceding ones. Although the hydrophobic properties of the powder may increase with the degree of subdivision because of the adsorption of air and the electrostatic charge, the increase of the specific surface area may compensate for these hydrophobic properties.

In the presence of polyoxyethylene monostearate, the dissolution rate and the solubility of griseofulvin were enhanced. Since the HLB of polyoxyethylene monostearate is 18.2, the wetting effect and the solubilization by this surfactant appear to be favorable. In contrast to this case, the addition of sorbitan monostearate brings about an increase of the dissolution rate, but the solubility after about sixty minutes is similar to the control value. The HLB of this surfactant is 4.7 but its absence of wetting and solubilizing effect, may be counterbalanced by an ability to inhibit agglomeration and also to disperse the griseofulvin particles. In addition, the salting-out effect may affect the solubility of griseofulvin. These results indicate that the addition of suitable materials can improve the physico-chemical characteristics of pharmaceuticals prepared by the freeze-drying technique.

Conclusion

If the system comprizing a solid drug and a solvent is a eutectic, it is evident according to phase conversion theory that a micronized powder will be obtained by the freeze-drying. Therefore the rapid freeze-sublimation techniques of Lemberger et al.,[14] are not always required. Since the cooling rate of a drug solution greatly influences the crystal growth, as pointed out by DeLuca and other investigators,[15] a fairly rapid freezing rate is required to ensure effective size reduction to a submicron level. As supercooling usually occurs, a solu-

14) A.P. Lemberger, T. Higuchi, L.W. Busse, J.V. Swintosky, and D.W. Wurster, J. Am. Pharm. Assoc., 43, 342 (1954).
15) P.P. DeLuca, J. Vac. Sci. Technol., 14, 620 (1977); L.-R. Rey, Dev. Biol. Stand., 36, 19 (1977); Von Dipl-Ing. P. Stricker, Pharm. Ind., 32, 291 (1970).

tion to be frozen must be cooled to a considerably lower temperature than the eutectic temperature. By employing these conditions and freeze-drying a drug solution prepared at a lower concentration than the eutectic composition, a micronized fine powder can be obtained successfully. When a drug solution prepared at a higher concentration than the eutectic composition is freeze-dried, the specific surface area of the resulting powder is reduced as the concentration increases in the case of a simple eutectic system, whereas in the case of a system with an incongruent melting point the decrease is comparatively small because of the peritectic reaction. If the physicochemical stability of the products is confirmed, size reduction of pharmaceuticals by freeze-drying may prove to be a valuable technique.