with tyrosine, as seen in Fig. 1 for X and XIV. Whether this effect is exerted on either tetrahydrofolate or tetrahydropteridine (18) must await further study. All active compounds were assayed for inhibition of dopamine-β-hydroxylase (Table IV). From the data, X was shown to be the most active overall in both systems and was selected for the *in vivo* assay.

In vivo results of X (Table V) are explained on the basis of the assays performed. Assays I and III (35) are paired and show no inhibition. Assay I was repeated due to a high norepinephrine level after α-methyltyrosine treatment. Assay II results indicate significant inhibition; however, the correction for the recovery of norepinephrine from alumina is not included in assay II, while I and III allow for this error[13]. Thus, while X inhibited both tyrosine hydroxylase and dopamine-β-hydroxylase *in vitro*, it was not active *in vivo*. Whether X is metabolized too rapidly and fails to attain the required tissue concentration is not known.

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Direct Preparation of Spherically Agglomerated Salicylic Acid Crystals During Crystallization

Y. KAWASHIMA *[x], M. OKUMURA *, H. TAKENAKA *, and A. KOJIMA[‡]

Received August 17, 1983 from the **Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan* and the [‡]*Sanwa Chemical Research Laboratory, 1212, Shimojyo, Kasugai 486, Japan.* Accepted for publication October 11, 1983.

Abstract □ Needle-like salicylic acid crystals were transformed into a spherically shaped dense form during crystallization by the spherical crystallization technique. Agitation of a mixture of ethanol–water–chloroform containing salicylic acid yielded spherically agglomerated salicylic acid crystals. The crystallinity of the agglomerated salicylic acid decreased when the amount of ethanol in the solvent mixture was decreased. The wettability of the agglomerated crystals increased when the amount of ethanol in the solvent mixture was decreased, and this enhanced the dissolution rate of the crystals. The remarkable improvements in the flow and packing of the agglomerated crystals enabled the direct compression of the crystals.

Keyphrases □ Salicylic acid—crystallization of spherically agglomerated crystals □ Crystallization—agglomeration of salicylic acid crystals

A novel agglomeration technique to transform a microcrystalline drug into an agglomerated form during the crystallization process was previously described (1). This technique could enable subsequent processes, such as separation, filtration, drying, etc. to be carried out more efficiently. Furthermore, the resultant agglomerated crystals could be easily

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compounded with other pharmaceutical powders due to their spherical form. We refer to this technique as "spherical crystallization."

Spherically agglomerated crystals of aminophylline were prepared directly by agitating a mixture of chloroform, ethanol, and water which contained ethylenediamine and theophylline (2). It was found that sodium theophylline monohydrate crystals, produced by salting out, were simultaneously agglomerated into spherical shapes in a mixture of an ethylenediamine solution of theophylline, an aqueous solution of sodium chloride, ethanol, and chloroform (3).

In the present study, the needle-like salicylic acid crystals simultaneously formed and agglomerated into a spherical shape in a mixture of water, ethanol, and chloroform. The aim of the study was to elucidate the effects of the composition of crystallization solvent on the physicochemical properties of the resultant agglomerated crystals, *e.g.*, crystalline form, crystallinity, wettability, dissolution rate, and micromeritic properties. The agglomeration behavior of the crystals was also investigated by measuring the sedimentation volume of the resultant crystals in liquid.

EXPERIMENTAL SECTION

Spherical Crystallization Process—*Method 1*—Salicylic acid[1,2] (0.5 g) was dissolved in ethanol (1–5 mL) in a test tube thermally controlled at 60°C. After cooling the ethanol solution to room temperature, 10 mL of water was added to the solution to yield the needle-like salicylic acid crystals. The mixture was allowed to stand for 1 h, then chloroform (0.3–0.5 mL) was added and the mixture was shaken horizontally at 200–400 cpm, using a shaker[2], for 10 min. The mixture was transferred to a measuring cylinder (25 mL) to measure the equilibrium sedimentation volume of the agglomerated crystals. The agglomerated crystals were separated and dried.

Method 2—Fifty milliliters of the ethanol solution (containing 12.5 g of salicylic acid maintained at 40°C) was poured into a mixture of chloroform (9 mL) and water (250 mL). The system was thermally controlled at 5°C and was agitated at 600 rpm by a turbine agitator with 6 blades for 1 h. The resultant crystals were separated and dried and their micromeritic properties were measured.

Measurement of Physicochemical Properties—The crystalline form of the agglomerated salicylic acid was determined using an X-ray diffractometer[3] with 0.15418 nm radiation. The relative diffraction intensity of salicylic acid (2θ = 17.1°) to that of sodium chloride (2θ = 31.6°), used as an internal standard, was taken as a measure of the crystallinity of salicylic acid. Sodium chloride particles fractionated into 150–200 mesh were incorporated into the diffraction samples with an agate mortar and pestle. The coefficient of variation of the relative intensities of diffraction peaks, due to the preferred orientation of particles in a powder, was 0.24. Wettability of the agglomerated crystals for water was determined by the contact angle of water to the agglomerated crystals and measured by the method of Lerk *et al.* (4). The agglomerated crystals weighing 2 g were compressed into a tablet (19.9 mm diameter, 5.3–5.8 mm thickness) at 50–60 kg/cm^2. The pressure was applied for 10–15 min to obtain an acceptable tablet without disintegration during the measurement. A large drop of saturated solution of salicylic acid was placed on the tablet surface, and the height of the drop was measured by a cathetometer. The contant angle (θ) was determined by using the following equations:

$$\theta > 90° \qquad \cos\theta = -1 + \sqrt{\frac{2}{3(1-\epsilon)}\left(\frac{2}{Bh^2} - 1\right)} \qquad \text{(Eq. 1)}$$

$$\theta > 90° \qquad \cos\theta = 1 - \sqrt{\frac{Bh^2}{3(1-\epsilon)(1 - Bh^2/2)}} \qquad \text{(Eq. 2)}$$

$$B = \frac{\rho g}{2\gamma} \qquad \text{(Eq. 3)}$$

where h is the height of the drop, ϵ is the porosity of the tablet, and ρ and γ are the density and the surface tension of the liquid drop. The porosity of the tablet was determined by measuring the apparent density of the tablet and the true density of the agglomerated crystals using a helium-air comparison pycnometer[4]. The surface tension of the salicylic acid saturated solution was measured by a surface tensiometer[5]. The dissolution test of the agglomerated crystals was carried out by the rotating basket method specified in USP at 100 rpm and 37°C (5). One hundred milligrams of the agglomerated crystals were dissolved in deaerated distilled water. Two-milliliter aliquots were withdrawn at appropriate intervals and an equal volume of water was replaced to the vessel. Salicylic acid was assayed spectrophotometrically[6] at 530 nm after adding 1% ferric nitrate to the aliquot.

Measurement of Micromeritic Properties of the Agglomerated Crystals—As a measure of the flow of the crystals, the angle of repose of the agglomerated crystals was measured by pouring powder on a plate (10 cm diameter)[7]. The packing ability of the agglomerated crystals was investigated by tapping them into a 50-mL measuring cylinder using a tapping machine[7]. The ability of the crystals to be formed into tablets was tested by using a single punch machine[8]. The average weight and dimension of the tablets were: 0.382 g, 10.05 mm diameter, and 4.14 mm thickness, respectively. The hardness was measured by a moving plate hardness tester[9].

Table I—Solubility and Degree of Supersaturation

	Ethanol Fraction in the Solvent, %	Conc., μg/mL	Degree of Supersaturation
With Agglomeration	8.8	2.39	19.02
	16.0	3.16	13.19
	22.4	4.65	8.27
	28.0	9.05	3.95
	32.7	16.39	2.03
Without Agglomeration	8.8	2.22	20.48
	16.0	2.82	14.78
	22.4	4.00	9.62
	28.0	6.87	5.20
	32.7	14.28	2.33

RESULTS AND DISCUSSION

Agglomeration Behavior of Salicylic Acid Crystals in Liquid—The recovery of the crystals agglomerated by spherical crystallization (method 1) sharply decreased with >25–30% ethanol in the solvent mixture (Fig. 1). With 33% ethanol in the solvent mixture, 28% of the loading was recovered as agglomerated crystals. Without agglomeration, when no chloroform was added to

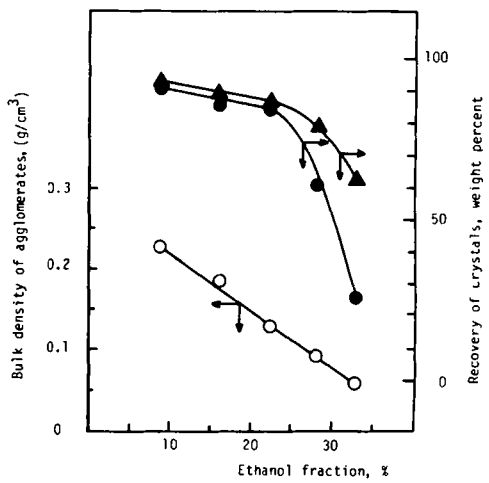

Figure 1—*Recovery and bulk density of agglomerated crystals. Key: (●) recovery of the agglomerated crystals, (▲) recovery of the primary crystals, and (○) bulk density of the agglomerated crystals.*

[1] Nakarai Chemicals, Ltd., Japan.
[2] V-S type; Iwaki Co., Japan.
[3] JDX type; Nihon Denshi, Co., Japan.
[4] Type 1302; Shimadzu Co., Japan.
[5] CBVP-A3 type; Kyowa Co., Japan.
[6] 100-60 type; Hitachi, Ltd., Japan.
[7] Konishi Seisakusho Co., Japan.
[8] Erweka-GmbH, Germany.
[9] Kyowa Seiko Co., Japan.

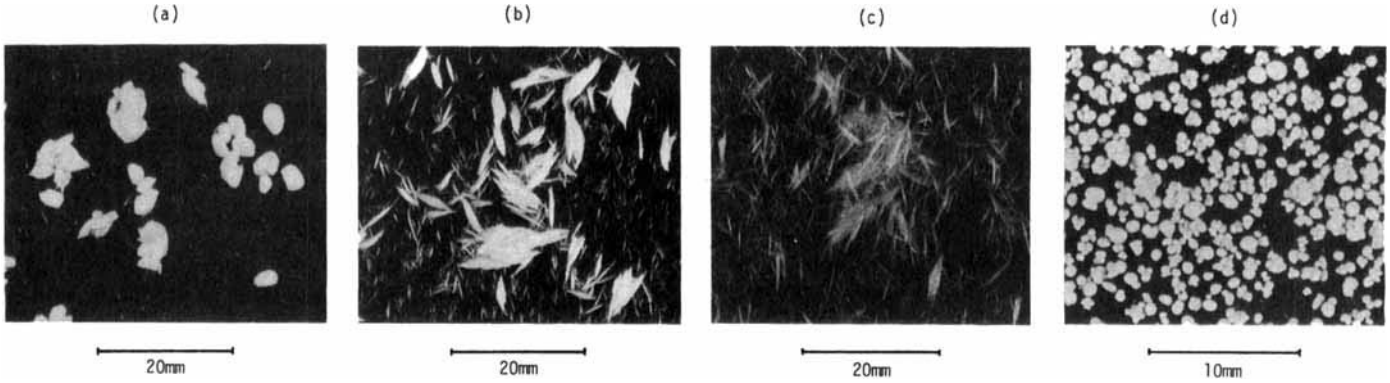

Figure 2—*Photographs of agglomerated crystals prepared with various ethanol fractions by methods 1 and 2: (a), (b), and (c) agglomerated crystals prepared by method 1 (the ethanol fractions in the solvent are 8.8, 22.4, and 28.0%, respectively); (d) spherically agglomerated crystals prepared by method 2.*

the system, the recovery of the crystals was 63%. This finding indicated that the solubility of salicyclic acid in the solvent for spherical crystallization, *i.e.*, ethanol–water–chloroform, was higher than in the ethanol–water mixture without agglomeration, as suggested in Table I.

The sedimentation volume of the agglomerated crystals prepared by spherical crystallization (method 1) was measured to investigate the effect of the composition of the solvent mixture on the agglomeration behavior. Bulk density of the agglomerated crystals, defined as the weight of the crystals recovered divided by the equilibrium sedimentation volume, was plotted against the ethanol fraction of the solvent. The bulk density decreased linearly when the amount of ethanol in the solvent mixture was increased (see Fig. 1). The size of the particles composing the agglomerated crystals decreased with a decrease in the amount of ethanol in the solvent mixture as shown in Fig. 2. Elworthy and Worthington (6) reported that finer sulfadiazine crystals were produced in more highly supersaturated solvents. The degree of supersaturation (Sd) was defined by:

$$Sd = \frac{Co}{Ce} \qquad \text{(Eq. 4)}$$

where Co and Ce are the initial loading and the remaining drug concentrations after crystallization in the solvent, respectively.

As shown in Table I, decreasing the amount of ethanol increased the degree of supersaturation, resulting in fine crystals. The larger agglomerated crystals were loosely compacted and elongated as shown in Fig. 2, resulting in a higher sedimentation volume, *i.e.*, a lower bulk density. The strength of the agglomerated crystals prepared in a solvent mixture with 28.0% ethanol was not enough to hold an agglomerated form after drying. In this study, the solvent mixture with small amount of ethanol afforded the dense, spherically agglomerated crystals.

Physicochemical Properties of the Resultant Crystals—The X-ray diffraction patterns of the agglomerated crystals are shown in Fig. 3; all the patterns coincided with that of the original salicylic acid, and no change in the crystalline form of the basic crystals was found, even after agglomeration. The diffraction intensity varied with the amount of ethanol in the solvent mixture, and may be dependent on the basic crystal size. Increasing the amount of ethanol in the solvent mixture caused the diffraction peaks to increase. The relative diffraction intensities of salicylic acid (17.1°) to that of sodium chloride (31.6°) are shown in Fig. 4 as a function of the amount of ethanol in the solvent mixture. The relative diffraction intensities were assumed as the crystallinity of the elementary crystals composing the agglomerate. As expected, the crystallinity increased when the amount of ethanol in the solvent mixture was increased. The crystallinity of the agglomerated crystals were influenced more strongly by the amount of ethanol in the solvent mixture than the crystals that were not agglomerated. Recrystallization of salicylic acid from chloroform occluded in the agglomerated crystals during drying may have increased the crystallinity of the dried agglomerated crystals, when compared with those that were not agglomerated.

Wettability of the agglomerated crystals by water was investigated by measuring the contact angle of water to the compressed crystals. It was expected that the contact angle would vary with the amount of ethanol in the solvent mixture, since the crystallinity and the elementary crystal size of the agglomerated crystals varied with the amount of ethanol. Gulinkina *et al.* (7) found that a dye crystal with a low crystallinity was more wettable than a dye crystal with a high crystallinity. The contact angles of the agglomerated crystals produced in the solvent with >25% ethanol were higher than those of the crystals that were not agglomerated, as shown in Fig. 5, due to their higher crystallinities. When the amount of ethanol was <25%, the contact angles of the agglomerated crystals became smaller than those of the primary crystals that were not agglomerated. This may be due to the fact that the basic crystal size of the agglomerated material produced in the solvent mixture with small amounts of ethanol was larger than that of the crystals that were not agglomerated.

The differences in the wettability of the crystals suggested that the dissolution behavior of the crystals might vary. The time required for 50% of solute dissolution of the agglomerated crystals prepared from the solvent with 22.4% ethanol (T_{50} = 7 min) was significantly shorter than the others (T_{50} = 20–22 min) at the 1% level, since the crystals were easily wetted, as shown in Fig. 5. Furthermore, their specific surface areas were fairly large, compared with

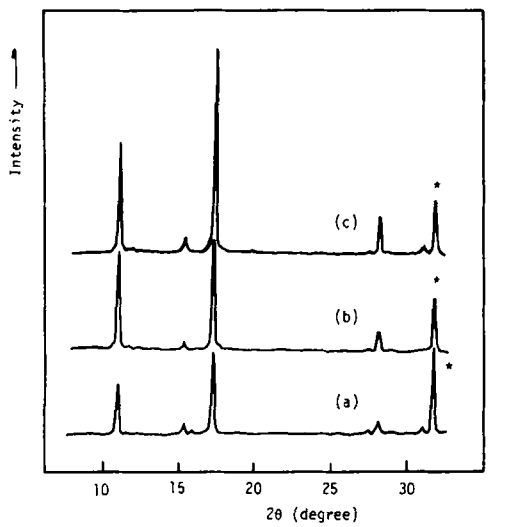

Figure 3—*X-ray powder diffraction patterns of agglomerated salicylic acid crystals. The ethanol fractions for (a), (b), and (c) are 8.8, 22.4, and 28.0%, respectively; (*) denotes the diffraction peak of sodium chloride.*

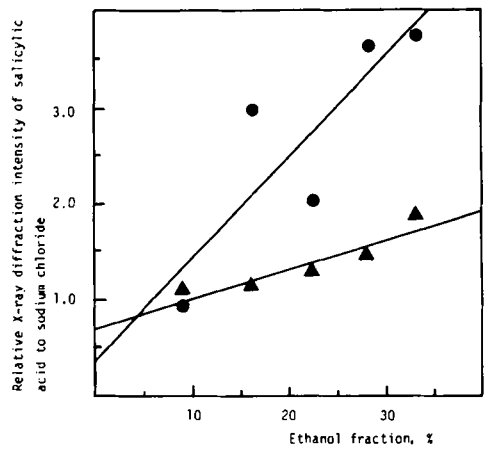

Figure 4—*Effect of ethanol fraction in the solvent on the crystallinity of salicylic acid. Key: (●) agglomerated crystals and (▲) primary crystals.*

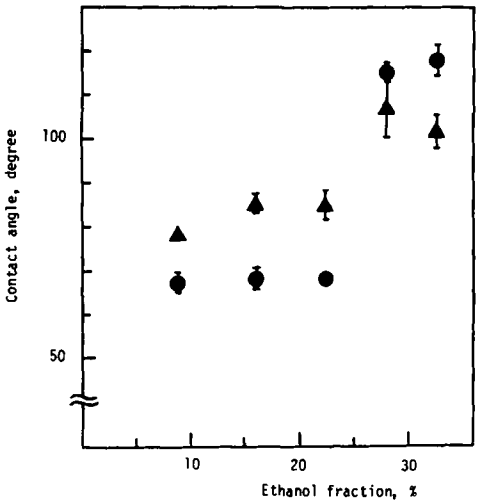

Figure 5—*Contact angles of agglomerated and primary crystals as a function of the ethanol fraction in the solvent. Key: (●) agglomerated crystals and (▲) primary crystals.*

those of the agglomerated crystals prepared with 8.8% ethanol, as suggested in Fig. 2. Although the agglomerated crystals prepared with 28.0% ethanol had large specific surface areas, their poor wettability resulted in a slower dissolution rate (Fig. 6). The dissolution rate of the spherically agglomerated crystals in Fig. 2d did not decrease as much as predicted from their low specific surface areas because of their improved wettability (Fig. 5).

Micromeritic Properties of Agglomerated Salicylic Acid Crystals Prepared by method 2—The agglomerated crystals prepared by method 2 were more compact and more spherical than those prepared by method 1, as shown in Fig. 2. The average diameter of the agglomerated crystals was 930 μm, with a size range from 460 to 1210 μm. The micromeritic properties of the agglomerated crystals were significantly improved compared with those of the crystals that were not agglomerated. The angle of repose of the agglomerated crystal was 36°, while that of the crystals that were not agglomerated was 51°. The packing ability was investigated by tapping the crystals in a measuring cylinder. The data were analyzed by Kawakita and Lüdde (8) and Kuno (9) equations, represented by Eqs. 5 and 6, respectively:

$$\frac{n}{c} = \frac{1}{ab} + \frac{n}{a}$$

$$a = \frac{V_0 - V_\infty}{V_0}, \quad c = \frac{V_0 - V_n}{V_0} \qquad \text{(Eq. 5)}$$

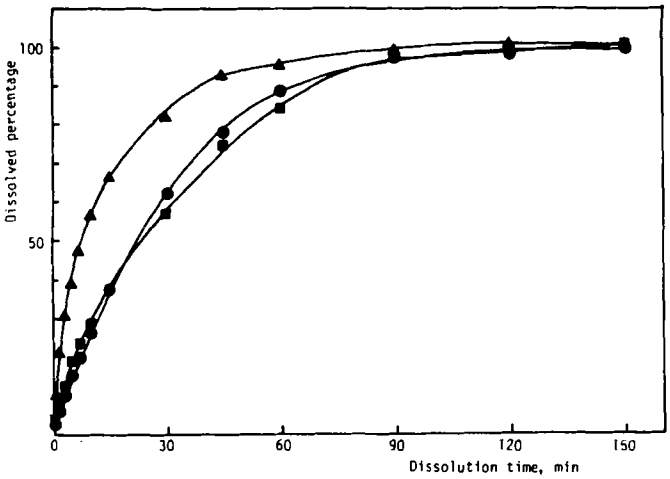

Figure 6—*Dissolution patterns of agglomerated crystals. Key: ethanol fractions of (●) 8.8, (▲) 22.4, and (■) 28.0%.*

Table II—Micromeritic Properties of Primary Crystals and Agglomerates

Micromeritic Properties	Agglomerates	Primary Crystals
Angle of repose	36°	51°
a^a	0.0955	0.3397
b^a	0.0466	0.0295
k^b	0.0049	0.0092
Closest packing density	0.488 g/cm³	0.160 g/cm³
Tablet	Compressible[c]	Not Compressible

[a] Parameter in Eq. 5. [b] Parameter in Eq. 6. [c] Tablet properties: diameter, 10.05 mm; thickness, 4.14 mm; average weight, 0.382 g; weight variation, 2.56% (the maximum percentage difference from the mean weight).

where a and b are the constants, n is the tap number, and V_0, V_n, and V_∞ are the powder bed volumes at initial, nth tapped, and equilibrium state, respectively.

$$\rho_f - \rho_n = (\rho_f - \rho_0) \exp(-kn) \qquad \text{(Eq. 6)}$$

where ρ_f, ρ_n, and ρ_0 are the apparent densities at equilibrium, nth tapped, and initial state, respectively, and k is the constant. All the data satisfied the relationships represented by Eqs. 5 and 6. The packing ability was assessed by comparing the constants a, b, and k in Eqs. 5 and 6, respectively, as shown in Table II. The constant a represents the proportion of consolidation at the closest packing attained. The reciprocals of b and k represent the packing velocity. The constant a for the agglomerated crystals was smaller than that for the crystals that were not agglomerated. This indicated that the agglomerated crystals were easily packed, even without tapping. The larger b value for the agglomerated crystals proved that the packing velocity of the agglomerated crystals by tapping was slower than that of the crystals that were not agglomerated. The smaller k in Eq. 6 for the agglomerated crystals coincided with the above finding. The slow packing velocity corresponded with the proportion of the consolidation of powder bed per tap, which was low. The closest packing density of the agglomerated crystals was larger than that of the crystals that were not agglomerated. Due to the improved flow properties and packing ability of the agglomerated crystals, they were directly compressible; whereas, the crystals that were not agglomerated were not compressible due to their poor flow properties.

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