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Effect of Manufacturing Procedures on the Dissolution and Human Bioavailability of Diphenylhydantoin

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The dissolution characteristics of commercial diphenylhydantoin (DPH) crystals, freeze-dried DPH, 20% simple blend DPH powder, and 20% solvent deposition DPH powder were determined by the use of a USP dissolution test apparatus in pH 1.2 and pH 7.5 test media. The solvent deposition method was found to give DPH with the fastest dissolution rate probably due to improvement in the wettability of the crystals as well as decrease in the particle size. Content uniformity was assessed and accelerated testing of the preparations was also performed to explore optimum formulations. No conversion of the crystal form of DPH or freeze-dried DPH was detected through powder X-ray diffractometry and IR spectrometry under the accelerated storage conditions.

Based on the results of the above *in vitro* studies, 20% DPH solvent deposition powder and the DPH crystals (as a reference) were selected for a bioavailability study in humans. A cross over study in 6 healthy male volunteers revealed the existence of a critical particle size for satisfactory bioavailability. Other factors, such as the use of diluents, also substantially affect the bioavailability of a DPH preparation, even though the crystals used to prepare the finished product were all from the same batch. These results have important implications for manufacturing procedures for DPH preparations.

Keywords——dissolution characteristics; diphenylhydantoin; solvent deposition; simple blend; content uniformity; acceleration test; human bioavailability; manufacturing procedures

Diphenylhydantoin (DPH) is scarcely soluble in water and the plasma level varies significantly from brand to brand as well as from dosage form to dosage form[1] following oral administration in humans. The therapeutic effective range of plasma level is reported to be 10—20 μg/ml[2] and the biological half life differs from person to person.[3] It is well estabilished that the rate determining step of DPH absorption is the release process of DPH from its dosage form in the gastrointestinal tract. Various attempts to improve the dissolution behavior of DPH preparations have been made, such as pulverization of DPH crystals,[4] conversion of crystal forms,[5] coprecipitation with polyvinylpyrrolidone,[6] and solubilization with methylcellulose.[7]

However, little has been reported on the effect of manufacturing procedures on the physical and biological characteristics of DPH.

The purpose of the present investigation was to study the effect of manufacturing procedures on the *in vitro* dissolution behavior and human bioavailability of DPH preparations which were prepared by various manufacturing methods.

Experimental

Materials——Diphenylhydantoin (DPH, JP IX) was purchased from Fujinaga Seiyaku Co., Ltd. Lactose and potato starch used were of JP IX grade and all the other chemicals were of reagent grade unless otherwise stated.

Particle Size Distribution of DPH Crystals——The distribution of particle size of DPH crystals was

determined by a conventional sieving method using JIS-grade standard sieves. 10 g of DPH was passed through 42, 80, 100, 150 and 200 mesh sieves and the residue on each sieve was weighed. The weight of the residue was divided by the total weight to give a percentage.

Preparation of Freeze Dried DPH——DPH was dissolved in dioxane, and the solution was freeze-dried in a YAMATO DC-35 freeze-drier.

Preparation of 20% DPH Powder——a) Simple Blend Method: DPH crystals were mixed with excipients (lactose: potato starch=7: 3, w/w) to make a 20% powder in a V-type blender.

b) Solvent Deposition Method: DPH crystals were dissolved to make a 4% solution in an equivolume mixture of methyl alcohol and dichloromethane. The solution was incorporated little by little into the excipient with a motor and pestle to evaporate the solvents. The resultant mixture was dried at 40°C for 4 h. Fig. 1 shows the general procedures for the preparation of 20% DPH powder.

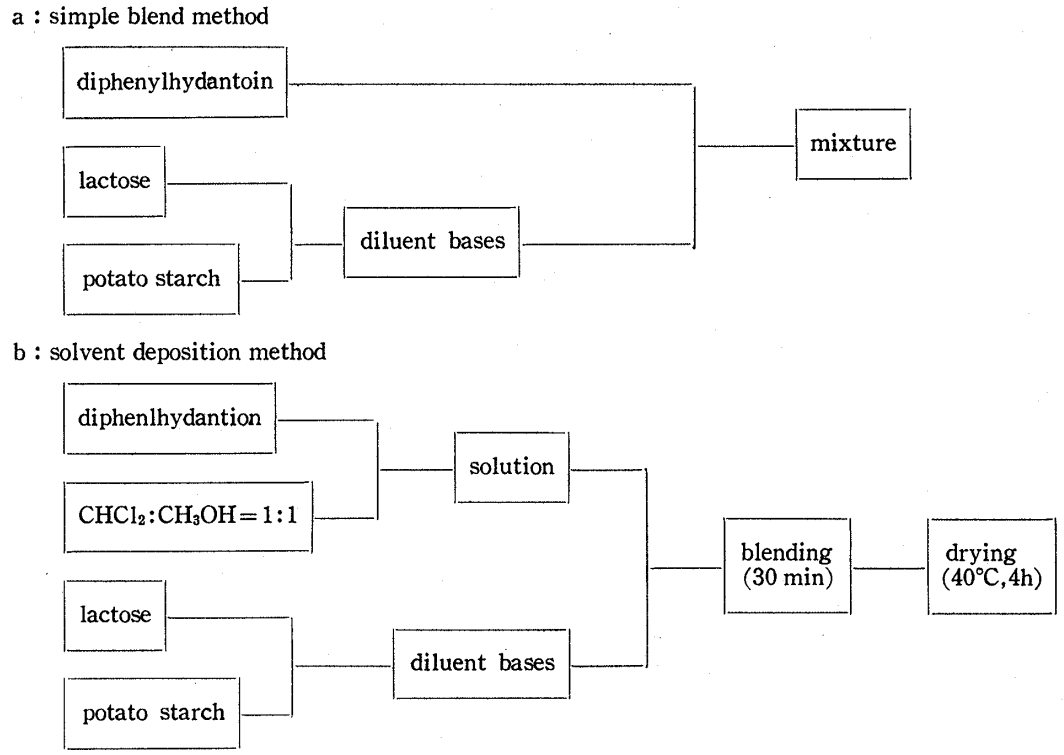

Fig. 1. Preparation of 20% Diphenylhydantoin Powder

Dissolution Study——Dissolution of DPH was tested in a USP dissolution test apparatus, Method II, in JP IX disintegration test medium No. 1 (pH 1.2) or No. 2 (pH 7.5) at an agitation speed of 100 rpm at 37°C. The amount of DPH subjected to the test was 200 mg of DPH crystals, 1000 mg of 20% solvent deposited powder and freeze-dried DPH powder equivalent to 200 mg of DPH crystals. Five ml of sample solution was withdrawn at appropriate intervals through a membrane filter (pore diameter: 0.22 μm) and immediately replaced with an equal volume of the test medium. The absorbance was determined at 235 nm and 260 nm on a spectrophotometer and the DPH concentration was calculated based on the absorption difference, from a previously determined calibration curve.

Determination of Content Uniformity of 20% DPH Powder——a) Twenty Percent (20%) DPH Powder prepared by the Simple Blend Method: Ten samples of adequate weight were withdrawn from random positions at appropriate intervals. Twenty five ml of 0.1 N NaOH was added to 25 mg of each sample in a centrifuge tube. The mixture was shaken for 10 min and centrifuged at 2500 rpm for 10 min. Then 0.01 N NaOH was added to 1 ml of the supernatant to make 10 ml. The absorbance of the solution was determined at 235 nm and 260 nm against distilled water.

b) Twenty Percent (20%) DPH Powder prepared by the Solvent Deposition Method: Ten samples, each 50 mg, were randomly taken and subjected to potency assay according to the assay procedure described above.

Acceleration Test——The 20% DPH powder prepared by the solvent deposition method was stored at 60°C, 75% relative humidity (RH) for 4 weeks. Freeze-dried DPH was stored at 60°C or 60°C, 75% RH for 4 weeks. Dissolution tests for both preparations were performed every week according to the testing procedure described above.

Powder X-ray Diffraction and IR Spectrum of DPH——DPH crystals, freeze-dried DPH and the materials

stored for acceleration testing were subjected to X-ray diffractometry and IR spectrophotometry. The X-ray diffraction pattern was obtained with a Rigakudenki RU-200 diffractometer by using monochromated Cu-*K* radiation. A Hitachi EPI-G3 machine was used for IR spectrophotometry.

Human Bioavailability Study——a) Formulation and Dose: DPH crystals or 20% DPH powder prepared by the solvent deposition method (each equivalent to 300 mg of DPH) was administered orally.

b) Subjects: A cross over study was carried out in 6 healthy male volunteers aged between 24 and 34 (mean 27.6), and weighing between 53 and 79 kg (mean 60.8 kg). The volunteers gave their written consent after the objectives and the procedures of the trial had been explained to them. No abnormalities were found on clinical examination, in the results of hematological and biochemical profiles, or in their electrocardiograms.

c) Trial Design: The 6 volunteers had been fasted overnight and continued fasting for 3 h after the dose was given with 200 ml of water. The study was single-blind and each formulation was dosed at weekly intervals and randomly allocated. Blood samples were taken by venepuncture into plastic centrifuge tubes containing heparin at 2, 4, 6, 8, 12, 24, and 48 h after administration of the drug. The blood was immediately centrifuged and the plasma was removed for analysis and stored at 4°C. Urine was collected at the same intervals as the blood and subjected to analysis.

d) Assay of the Plasma Level: The plasma samples were assayed for DPH by the method of enzyme immunoassay.[8]

e) Assay of the Urinary Level: Urine concentarion of DPH was determined by solvent extraction, formation of trimethylsilyl derivatives, and gas–liquid chromatography of the resulting products. 5-(*p*-Tolyl)-5-phenylhydantoin was selected for use as an internal standard in the assay of DPH.[9]

Results and Discussion

Figure 2 shows the particle size distribution of DPH crystals used in the present study. The size of most crystals was in the range of 177—350 μm. Larger particles than 350 μm amounted to 0.7% and particles smaller than 74 μm accounted for about 10% of the total weight.

X-ray diffractometry of freeze-dried DPH suggested a relative decrease in the size of the crystals. Therefore, reduced crystallinity might contribute to the increase of the dissolution rate. From a practical viewpoint, it is very important to confirm that specified dissolution characteristics are retained even after prolonged storage. The results of dissolution tests of freeze dried DPH stored under accelerated conditions are shown in Fig. 3. Little change in dissolution behavior was detected through dissolution studies after storage of freeze-dried DPH for 4 weeks at 60°C and at 60°C, 75% RH. Therefore, it is concluded that 20% DPH

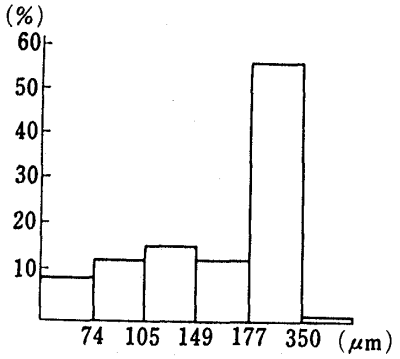

Fig. 2. Particle Size Distribution of Diphehylhydantoin

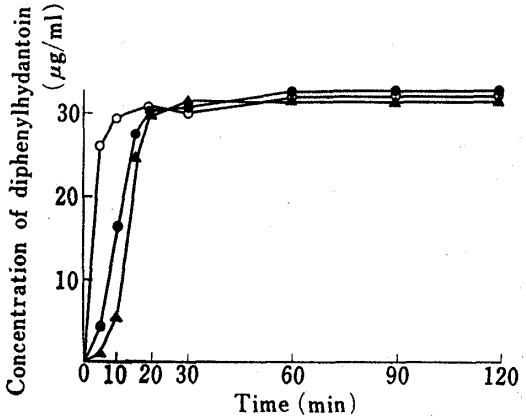

Fig. 3. Dissolution of Accelerated Diphenylhydantoin Preparations

—○—: 20% diphenylhydantion powder prepared by the solvent deposition method and stored at 60°, 75% RH for 4 weeks.
—●—: freeze-dried diphenylhydantion stored at 60° for 4 weeks.
—▲—: freeze-dried diphenylhydantion stored at 60°, 75% RH for 4 weeks.

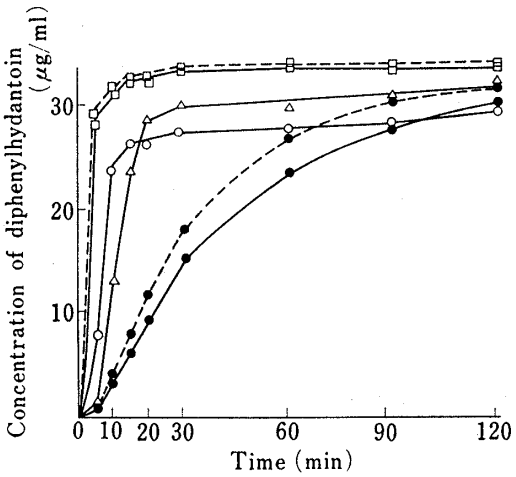

Fig. 4. Dissolution of Diphenylhydantoin Crystals and Various Preparations in Artificial Gastric Juice and Artificial Intestinal Fluid by the USP Paddle Method (500 ml, 100 rpm, 37°C)

In artificial intestinal fluid;
—□—: 20% diphenylhydantoin powder prepared by the solvent deposition method.
—●—: commercial diphenylhydantoin crystals.
In artificial gastric juice;
—□—: 20% diphenylhydantoin powder prepared by the solvent deposition method.
—△—: Freeze-dried diphenylhydantoin.
—○—: 20% diphenylhydantoin powder prepared by the simple blend method.
—●—: commercial diphenylhydantoin crystals.

powder prepared from freeze-dried DPH was acceptable as far as stability of dissolution behavior is concerned. However, the solubility of DPH in dioxane is fairely low and thus lyophilization of DPH requires a large volume of solvent.

Dissolution curves in artificial gastric juice and artificial intestinal fluid of commercial DPH crystals and 20% DPH powder prepared by the solvent deposition method are shown in Fig. 4. Compared with DPH crystals, an increase of dissolution rate was achieved by the simple blend method and a further increase in dissolution rate by the solvent deposition method and by freeze-drying DPH. The 20% powder prepared by the solvent deposition method released 90% of the drug in five minutes. The solubility of DPH at 37°C was determined to be 30.1 μg/ml and 37.1 μg/ml at pH 5.4 and pH 7.4, respectively.[10] The plateau reached by 20% DPH powder prepared by solvent deposition was consistent with the saturated concentration at pH 7.5, and since the apparent pK_a' of DPH is 8.06,[10] the saturated concentration at pH 5.4 might not be significantly different from that at pH 1.2. In these test media no significant change in dissolution was observed for a given formulation, although a considerable difference was found in the dissolution curves of commercial DPH crystals and the 20% powders. The simple blend method probably improved the dissolution of DPH through an increase of hydrophilicity of the crystals due to mixing with lactose. However, even without lactose freeze-dried DPH showed a similar enhancement of dissolution.

Thus, the solvent deposition method was found to be superior to the other manufacturing methods tested in this study. The 20% powder prepared by solvent deposition produced a saturated concentration of DPH in 20 minutes but it took more than 2 h for commercial DPH crystals to reach a plateau.

Dissolution characteristics are largely related to particle size distribution and wettability of DPH crystals. Simple blending of the crystals with diluents improved the wettability and substantially increased the dissolution rate. The solvent deposition method was found to give the fastest dissolution rate probably due to decrease in particle size as well as improvement in wettability. The superiority of solvent deposition as regards increase of the dissolution rate might be due to the formation of fine crystals at the surface of diluents which facilitated dissolution by improving the wettability of the crystals as well as by increasing the effective surface area for dissolution.

However, it is very important to assure continuous release of drug to improve the bioavailability of such a scarcely soluble drug as DPH. Fig. 5 shows the results of a dissolution test in an apparent sink condition. This condition was generated by supplying 500 ml of fresh dissolution medium to the test solution after dissolution for 30 min. The 20% powder restored the plateau concentration almost immediately after addition of 500 ml of fresh test medium, while the dissolution rate of the crystals decreased to approximately half following an addition of 500 ml fresh medium. These results suggest that *in vivo*, where a sink condition may prevail, the 20% powder might give better bioavailability than the crystlas.

The effect of manufacturing procedures on content uniformity is shown in Fig. 6. The therapeutic range of DPH is relatively narrow and clinical efficacy and such side effects as nystagmus, ataxia, and somnolence are plasma concentration dependent. Therefore, content uniformity of DPH is of very great concern to formulation scientists. For the simple blend preparation, considerable variation was observed over a long time period of mixing. Even after blending for 7 min the variation was significantly larger at a probability level of 1% than in the solvent deposition method, as demonstrated in Table I.

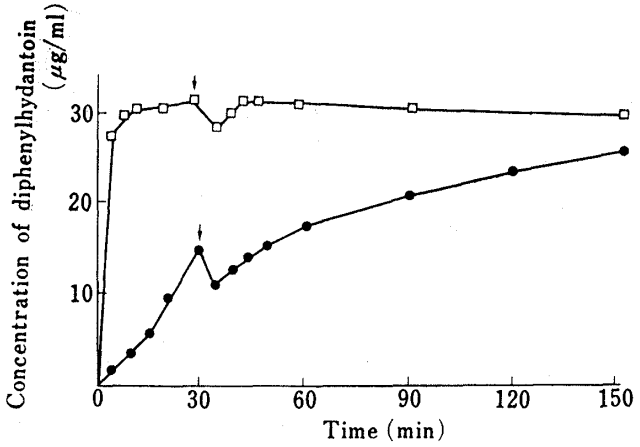

Fig. 5. Dissolution of Diphenylhydantoin Crystals and 20% Diphenylhydantoin Powder prepared by the Solvent Deposition Method in 500 ml of the 1st Disintegration Fluid by the USP Paddle Method (100 rpm, 37°C)

Fresh 500 ml of the test medium was supplied after dissolution for 30 min.
—●—: diphenylhydantoin crystals.
—□—: 20% diphenylhydantoin powder.

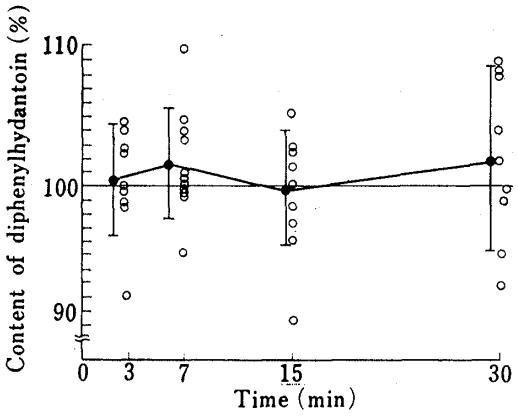

Fig. 6. Content Uniformity of 20% Diphenylhydantoin Powder prepared by the Simple Blend Method

: denotes mean±S.D.

TABLE I. Content Uniformity of 20% Diphenylhydantoin Powder

Sample No.	Simple blend method (7 mins' blending)	Solvent deposition method
1	95.00%	99.49%
2	99.17%	100.24%
3	99.49%	100.30%
4	99.78%	100.64%
5	100.11%	100.65%
6	100.47%	100.76%
7	103.11%	100.91%
8	103.53%	101.13%
9	104.38%	101.19%
10	108.96%	101.50%
$\bar{X}\pm$S.D.	101.40±3.77%	100.68±0.57%

Based on the results of dissolution, stability testing, and content uniformity studies, the solvent disposition method appears to be one of the most promising manufacturing procedures to ensure uniform bioavailability of DPH.

We will now describe a human study in which 20% powder prepared by the solvent deposition method was tested in human volunteers in comparison with the commercial crystals. Figs. 7 and 8 demonstrate the results of the cross over study of DPH in human volunteers.

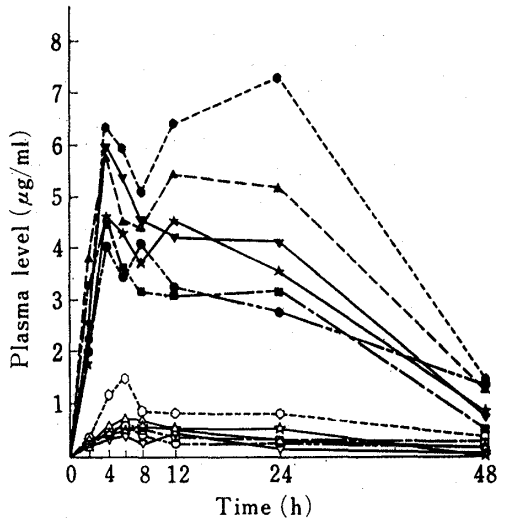

Fig. 7. Plasma Levels of Diphenylhydantoin in a Cross-over Study of Six Human Volunteers following Oral Administration of 300 mg of Commercial Diphenylhydantoin Crystals (Open Symbols) and 1500 mg of 20% Diphenylhydantoin Powder prepared by the Solvent Deposition Method (Closed Symbols)

Each symbol of the same shape corresponds to the same volunteer.

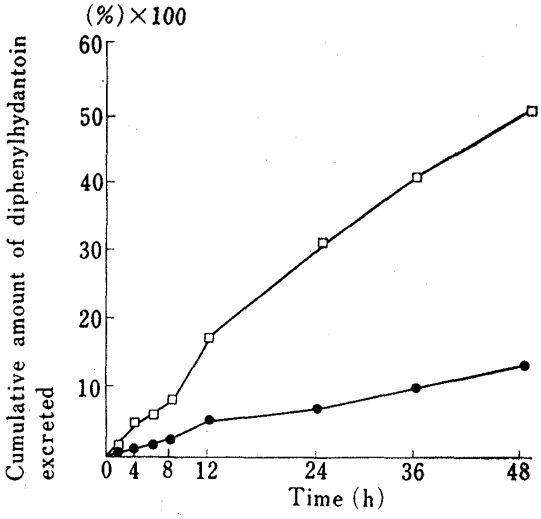

Fig. 8. Cummulative Urinary Excretion of Diphenylhydantoin following Oral Administration of 300 mg of Commercial Crystals (—○—) or 1500 mg of 20% Powder prepared by the Solvent Deposition Method (—□—)

Table II. Mean Plasma Levels compared by the Paired *t*-Test

Time h	Plasma level, μg/ml		Significance level[a]	
	Diphenylhydantoin crystals	20% Diphenylhydantoin powder		
2	0.22±0.05[b]	2.71±0.08[b]	$t=7.763$	$p<0.01$[c]
4	0.63±0.03	5.27±0.97	$t=14.470$	$p<0.01$
6	0.71±0.43	4.57±1.01	$t=11.477$	$p<0.01$
8	0.51±0.22	4.21±0.70	$t=15.345$	$p<0.01$
12	0.48±0.24	4.54±1.30	$t=9.056$	$p<0.01$
24	0.46±0.19	4.37±1.67	$t=6.212$	$p<0.01$
48	0.14±0.01	1.07±0.34	$t=7.008$	$p<0.01$

a) *t*-Test.
b) Mean±S.D.
c) $t(5, 0.01)=4.32$.

The commercial crystals gave a very low plasma level and showed a significant difference from the 20% powder at each sampled time at a significance level of 1% as listed in Table II. The areas under the plasma level-time curve (AUC) are tabulated in Table III. The 20% powder gave 7.63—11.6 times larger AUC than the DPH crystals in individual volunteers and the mean was 8.97 times larger. The urinary excretion study also revealed a lower excretion rate of DPH from the crystals. It is widely accepted that the metabolic rate of DPH is very small in humans. Therefore, it is concluded that the crystals gave significantly lower bioavailability than the 20% powder due to poor dissolution characteristics of the formulation.

The *in vitro* and *in vivo* studies suggest the existence of a critical particle size of DPH for good bioavailability. No correlation between particle size and serum concentration of DPH in different forms below a mean particle diameter range of 17.5—45 μm was found (11),

Table III. Areas under the Plasma Level-Time Curves in Human Volunteers following Oral Administration of 300 mg of Diphenylhydantoin Crystals or 1500 mg of 20% Diphenylhydantoin Powder prepared by the Solvent Deposition Method

Subject	AUC, μg/ml·h		20%/cryst.
	Diphenylhydantoin crystals	20% diphenylhydantoin powder	
S.Y.	11.81	123.48	10.46
S.S.	16.90	195.90	11.59
K.W.	15.11	123.35	8.16
A.T.	18.85	146.85	7.79
S.T.	14.18	158.04	11.15
C.H.	33.34	245.49	7.63
Mean	18.45	165.55	8.97
S.D.	7.72	47.47	
P	<0.01		

but a study on the bioavailability of DPH in dogs at a particle size range of up to 100 μm revealed that the AUC was inversely related to the particle size (12). These results indicate that larger particle size than about 50 μm might only be critical for the dissolution and absorption processes. The DPH crystals used in this study were mostly distributed in the particle size range of 177—350 μm as shown in Fig. 2. When the same batch of crystals was processed by solvent deposition, the bioavailability was increased remarkably. This suggests that the particle size might be reduced in the course of the manufacture to below the critical range of crystal size distribution.

Although intensive studies have been performed in attempts to correlate the bioavailability of DPH and formulation factors, *i.e.*, particle size distribution, excipients, tablet disintegration time, and dissolution rate *in vitro* (13, 14), few studies on manufacturing factors have been reported.

The present study suggests that the manufacturing method affects the bioavailablity of DPH preparations substantially, even when portions of the same batches of DPH crystals and diluents are used in the different manufacturing processes.

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