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Crystal Modification of Phenytoin with Polyethylene Glycol for Improving Mechanical Strength, Dissolution Rate and Bioavailability by a Spherical Crystallization Technique

YOSHIAKI KAWASHIMA,*,[a] TETUROU HANDA,[a] HIROFUMI TAKEUCHI,[a] MOTONARI OKUMURA,[a] HIDEO KATOU[b] and OSAMU NAGATA[b]

Gifu Pharmaceutical University,[a] 5–6–1, Mitahora-higashi, Gifu 502, Japan and Hokuriku Seiyaku, Co., Ltd.,[b] 37–2, Inokuchi, Katsuyama, Fukui 911, Japan

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Phenytoin crystals with improved mechanical strength, dissolution rate and bioavailability were obtained by using a novel crystallization process termed "spherical crystallization." An alkaline solution of phenytoin held at 40 °C was poured into a well dispersed mixture of isopropyl acetate and hydrochloric acid containing a water-soluble polymer, *e.g.* polyethylene glycol (PEG), at 20 °C. Fine crystals of phenytoin incorporating PEG in proportion to its concentration in the solvent were directly agglomerated. The agglomerates with PEG had a free-flowing property due to their spherical shape and smooth surface. The PEG incorporated in the agglomerate resulted in increased mechanical strength, dissolution rate and bioavailability of the resultant agglomerated crystals of phenytoin.

Keywords——crystal design; phenytoin; spherical crystallization technique; polyethylene glycol; friability; dissolution rate; bioavailability

Introduction

Careful monitoring of the plasma concentration of phenytoin is required to assure its therapeutic effect, because of the difficulty in maintaing an effective blood concentration; differences in the bioavailabilitites of various commercial brands were found,[1,2] due to the poor solubility of the drug. Therefore, various techniques to improve the solubility of phenytoin such as micronization,[3] conversion of crystal form,[4] coprecipitation with polyvinylpyrrolidone (PVP)[5] complex formation with cyclodextrin[6] and solid dispersion with polyethylene glycol (PEG)[7] have been devised. To formuate the products prepared by these techniques into dosage forms, further processings such as mixing with filler, granulation and other powder processing, are required.

The aim of the present study was to obtain agglomerated crystals of phenytoin with improved pharmaceutical properties, such as flowability, mechanical strength, dissolution rate and bioavailability, and to compound them directly into the formulation. To accomplish this, agglomerates of fine crystals of phenytoin incorporating a water-soluble polymer, *e.g.* PEG, were prepared by using the spherical crystallization technique developed by the present authors.[8] By using this technique, microcrystals prepared by various crystallization methods such as neutralization, solvent change and salting-out can be simultaneously agglomerated with a bridging liquid, which preferentially wets the crystals, free from the crystallization solvent. In the present study, the effects of PEG in the agglomerate on the mechanical strength, dissolution rate and bioavailability were investigated.

Experimental

Materials——Phenytoin (JPX grade) was gift from Dainippon Pharmaceutical Co., Osaka (Aleviatin, lot PN076). Gelatin (Koso Chemical Co., Ltd., Tokyo), polyvinylpyrrolidone (PVP K-30, Nakarai Chemicals Ltd., Kyoto), polyethylene glycol (PEG) 4000 (Kishida Chemical Co., Osaka) and all other chemicals were of reagent grade.

Preparation of Spherically Agglomerated Crystals of Phenytoin with Water-Soluble Polymer——Phenytoin (4 g) was dissolved in 20 ml of 1 N sodium hydroxide at 40 °C. This solution was poured into a mixture of isopropyl acetate (13.5 ml) used as a bridging liquid and 0.07 N hydrochloric acid (280 ml), containing a water-soluble polymer, such as gelatin, PVP or PEG 4000. The system was thermally controlled at 20 °C and was agitated at 600 rpm using a turbine type agitator with 6 blades. During the process, fine crystals of phenytoin were produced by neutralization of sodium phenytoin with hydrochloric acid. The precipitated crystals were simultaneously agglomerated with the water-soluble polymer through the bridging action of isopropyl acetate. The amount of PEG included in the agglomerate was determined by measuring the area of the endothermic peak at the melting point of PEG (55—57 °C) on the thermogram obtained with a differential scanning calorimeter (DSC, Rigaku Electric Co., Tokyo).

As a reference, primary crystals without agglomeration were prepared by the same procedure in the absence of the bridging liquid and the water-soluble polymer.

Measurement of the Sizes of Agglomerates and Constituent Crystals of Agglomerate——The average diameter of agglomerates was determined by a sieve analysis. The agglomerate was supersonically disintegrated in 0.1% Tween 80 saturated with phenytoin. The sizes of constituent crystals of the agglomerate were measured by a photographic counting method.

Measurement of Mechanical Strength of the Agglomerates (Friability Test)——The agglomerated crystals fractionated into the range of 12 to 48 mesh (297 to 1410 μm) were placed on a 80 mesh sieve (opening, 177 μm) and impacted with a rotap machine (ES-65, Iida Manufacturing Co., Osaka). At appropriate intervals, the amount of pulverized agglomerate that had passed through the 80 mesh sieve was weighed.

Measurement of Wettability of the Agglomerates——Contact angles of water and aqueous solutions of PEG against the agglomerated crystals and the original crystals of phenytoin were measured to investigate the wettabilities of the crystals. Agglomerated crystals (2 g) were compressed into a tablet (diameter, 20 mm; porosity, 0.1—0.2) and a drop of the liquid to be tested (diameter <0.5 mm) was placed on the surface of the tablet. The contact angle of the drop was measured directly with a contact angle meter (CA-A, Kyowa Kagaku Co., Tokyo). The contact angle varied continuously with penetration of the drop into the tablet. In the present paper, the contact angle was defined as the extrapolated value at time $=0$ as reported by Koishi and Itoh.[9]

Dissolution Test of the Agglomerates——The dissolution tests of the agglomerates (equivalent to 100 mg of phenytoin) were performed by the rotating basket method specified in the JPX in distilled water or an aqueous solution of PEG (900 ml) at 37 °C and 100 rpm rotating speed of the basket. The size ranges of the agglomerates and the reference granules tested were 20 to 28 mesh (590 to 840 μm). The original phenytoin crystals and the primary crystals without agglomeration were dispersed in 1 ml of water with a vortex mixer for 30 s and then poured into the

1 ml of plasma
5 μg of amobarbital (internal standard)
1 ml of phosphate buffer (pH 7.4)
↓
separation with SEP-PAK C_{18} Cartrige®
↓ removal of discarded components
5 ml of water
2 ml × 2 of acetate buffer (pH 4.85)
2 ml of water
0.5 ml of 50% methanol
↓
extraction with 1 ml × 2 of ethanol
↓
evaporation under reduced pressure
↓
dissolved in 500 μl of 0.01 M potassium phosphate monobasic–60% methanol
↓
HPLC

Chart 1. Pretreatment of Plasma Sample for HPLC Analysis of Phenytoin

test solution. An empty basket was rotated during the test so as to keep the same fluid movement in the vessel. Aliquots were withdrawn at appropriate intervals, and filtered through a membrane filter (pore diameter: 0.3 μm). The volume of the test solution was maintained by adding an equal volume of the test medium. The sample was diluted appropriately with ethanol–water mixture (volume ratio = 1 : 9) and assayed spectrophotometrically at 230 nm to determine the concentration of phenytoin dissolved (model 556 spectrophotometer, Hitachi Manufacturing Co., Ltd., Tokyo).

Absorption Test of the Agglomerates——The absorption test of the agglomerates was carried out using male beagle dogs ($n = 4$), weighing 10.3 to 12.0 kg. Each dog was fasted overnight, then a capsule (No. 000) containing the agglomerates or the primary crystals (equivalent to 400 mg of phenytoin) was administered orally with 100 ml of water. The capsules were given according to a cross-over design with an interval of one week between the two administrations. The concentration of phenytoin in plasma was assayed spectrophotometrically at 230 nm with a high-performance liquid chromatograph (Japan Spectroscopic Co., Tokyo), following the procedure illustrated in Chart 1.

Results and Discussion

Effect of Water-Soluble Polymer on Micromeritic Properties of the Agglomerates

The size distributions of the agglomerated crystals produced without and with various water-soluble polymers, *e.g.* gelatin, PVP and PEG 4000, are shown in Fig. 1. Adding the water-soluble polymer to the system decreased the average diameter of the resultant agglomerates (210 to 670 μm) compared to that of the agglomerate without the polymer (840 μm). Polyvinylpyrrolidone most effectively decreased the size of the agglomerate. With 0.001% PVP, the cumulative percent of the agglomerates smaller than 100 μm was 10%. Hirakawa and Harada[3b] reported that during the crystallization process, PVP added to the system was adsorbed on the surfaces of crystals and prevented their growth, resulting in fine crystals (average diameter, 3 μm). In the present study, the PVP adsorbed on the crystals imparted a hydrophilic property to them, making them poorly wettable with isopropyl acetate. Therefore, the crystals were hardly agglomerated with isopropyl acetate. When the

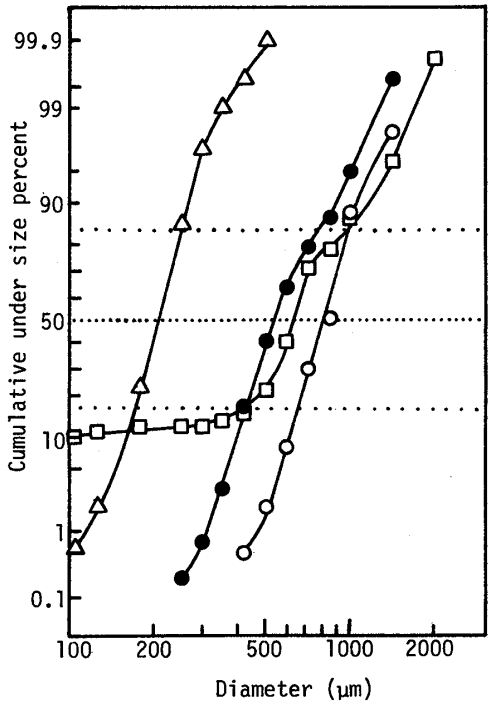

Fig. 1. Effect of Water-Soluble Polymers on the Diameter of Agglomerates

○, no additive; △, gelatin 0.01%; □, PVP 0.001%; ●, PEG 4000 0.01%.

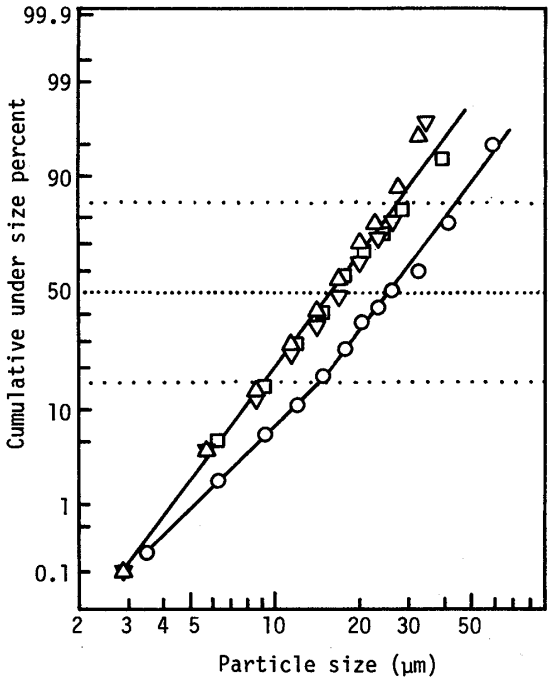

Fig. 2. Particle Size Distribution of Constituent Crystal of Agglomerate

Concentration of PEG in crystallization solvent: ○, 0%; △, 0.5%; ▽, 5%; □, 20%.

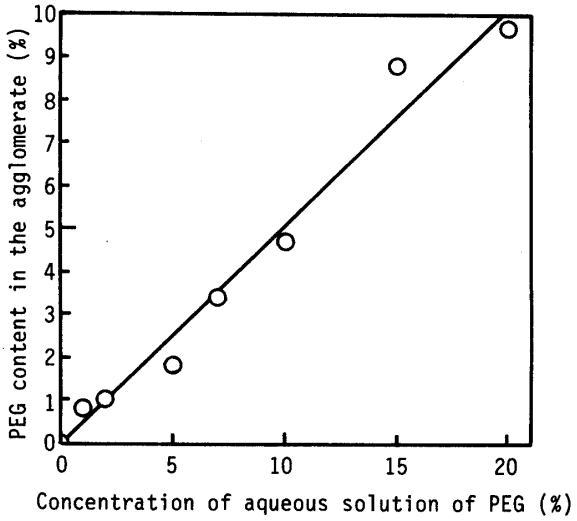

Fig. 3. Polyethylene Glycol Content in Agglomerates as a Function of PEG Concentration in Crystallization Solvent

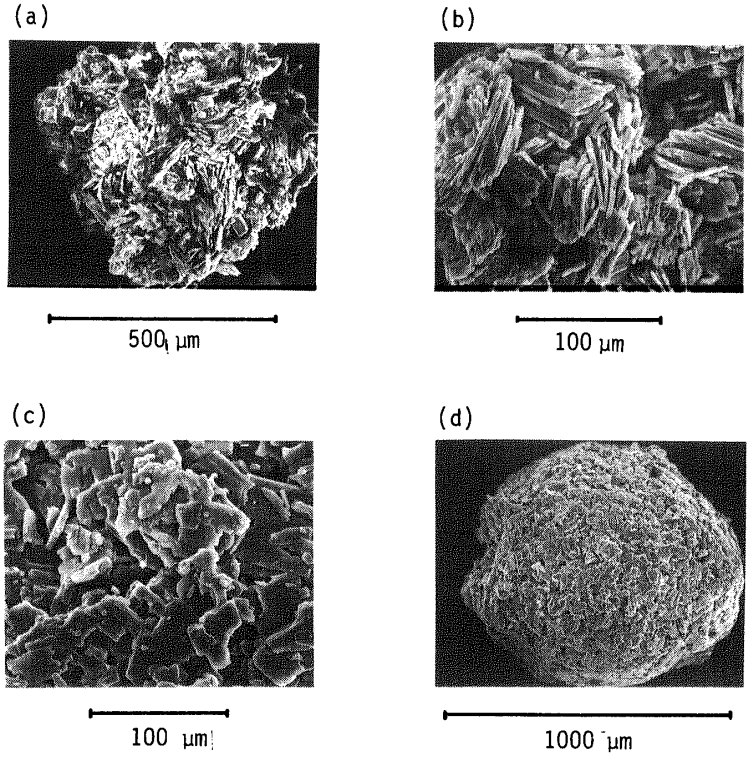

Fig. 4. Scanning Electron Microphotographs of Phenytoin Agglomerates
Concentration of PEG in crystallization solvent: (a) and (b) 0%, (c) and (d) 20%.

concentrations of gelatin and PVP in the system were higher than 0.01 and 0.001%, respectively, the crystals could not be agglomerated, since at higher concentrations than these critical values, the surface of the crystals is presumably virtually covered with the adsorbed polymer.

With PEG 4000, the crystals could be agglomerated even if the concentration of PEG was increased to 20%. The reduction of the sizes of constituent crystals of the agglomerate was small compared to that found with PVP,[3b] as shown in Fig. 2. The findings suggest that PEG is poorly adsorbed on the surface of the crystals. Polyethylene glycol adsorbed at the interface between water and isopropyl acetate reduced the interfacial tension of the bridging liquid between the crystals, and decreased the cohesive force acting to agglomerate the crystals.[10]

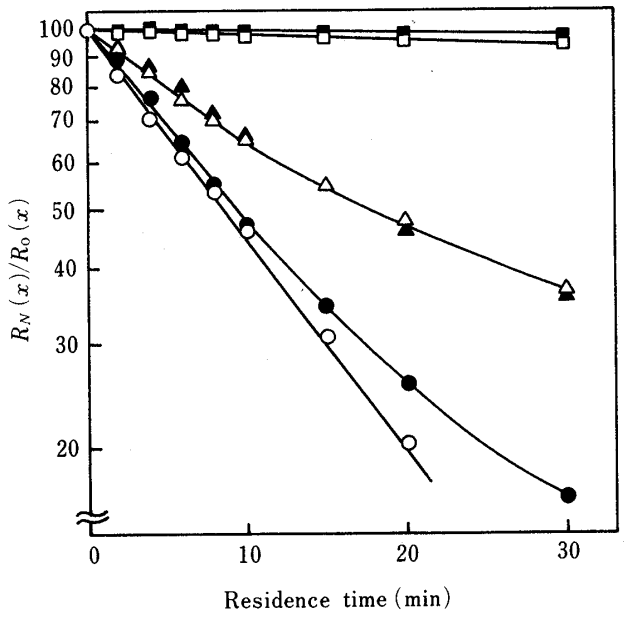

Fig. 5. Friability Test of Agglomerates

Concentration of PEG in crystallization solvent: ○, 0%; ●, 0.05%; △, 0.1%; ▲, 0.5%; □, 15%; ■, 20%.

The PEG adsorbed at the interface reduced the sizes of the agglomerates (Fig. 1) and was assumed to be incorporated in the agglomerate.

The amount of PEG (<10%) included in the agglomerated crystals increased in proportion to that in the crystallization medium (<20%) as shown in Fig. 3. The amount of PEG contained in the powder of primary crystals without agglomeration was higher than that of the agglomerates. This finding indicated that PEG could be incorporated physically into the agglomerate by the adhesion of PEG to the surface or in the voids of the agglomerate. This was confirmed by infrared spectroscopic analysis, differential scanning calorimetry and X-ray analysis, which did not detect any change of the crystalline form of phenytoin, or any interaction with PEG.

Scanning electron microscopic photographs of the agglomerated crystals with and without PEG are shown in Fig. 4. While no difference was found in the sizes and shapes of the constituent crystals of the agglomerate with and without PEG, a marked difference in the surface topography was found. The agglomerate without PEG was composed of small aggregates (40 to 90 μm in diameter) of the crystals and its surface was rough. With increasing concentration of PEG in the crystallization medium, the resultant agglomerate became more spherical and its surface was more closely compacted. The bridging liquid, *i.e.* isopropyl acetate, with reduced cohesion force, loosely agglomerated the crystals, which were re-arranged into a closely compacted structure in the agglomerate by the external force applied by the collisions against the vessel wall and the impeller during the crystallization.

The friability data are shown in Fig. 5, in which the weight % of the agglomerates retained on the 80 mesh sieve after impaction was plotted against the residence (impaction) time, according to Eq. 1.[11]

$$R_N(x) = R_0(x) \cdot (1 - Pr)^N \qquad (1)$$

where Pr is the probability of breakage of the agglomerate per impaction, $R_N(x)$ is the cumulative percent over size x and N is the number of impactions (or residence time). Increase in the PEG content of the agglomerate significantly increased the mechanical strength of the agglomerate. This was due to the decrease of the surface roughness, as shown in Fig. 4, and the solid bridges of PEG formed between the internal crystals in the agglomerate.

Dissolution Behavior of the Agglomerated Crystals

Polyethylene glycol incorporated in the agglomerated crystals improved the wettability of the agglomerated crystals with water, as shown in Fig. 6. The contact angle was reduced to about 50° when PEG was incorporated in the agglomerate, but was independent of PEG content. This could be explained by assuming that the PEG adhered to the surface of the primary crystals or was included in the interstices of primary crystals in the agglomerate, being spread thinly over the surface of the tablet of the agglomerates during the compression.

The improvement of wettability of the agglomerate led to a rapid dissolution of the agglomerate. The dissolution rates of the agglomerated crystals were faster than that of the granules prepared by comminuting a slug, as shown in Fig. 7. With increasing PEG content in the agglomerate, the agglomerated crystals dissolved more rapidly. The dissolution rate of the agglomerate with 9.7% PEG was faster than that of the original (bulk) phenytoin crystals (average size = 17 μm). This finding was due to the fact that the agglomerate disintegrated rapidly into the primary crystals (average size = 7—8 μm). In the case of the primary crystals without agglomeration, the dissolution rate was the fastest among all the samples tested in spite of the poor wettability with water. When the agglomerated crystals were compressed into tablets, no difference in dissolution rates was found irrespective of the PEG content in the tablets, as predicted from the results in Fig. 6.

Reference tests of dissolution of the agglomerated crystals without PEG were carried out in an aqueous solution of PEG. The dissolution rate of the crystals was enhanced by adding PEG to the dissolution medium as can be seen in Fig. 8. The improved wettability of the aqueous solution of PEG can account for the increased dissolution rate of the crystals in Fig. 8. The contact angle of aqueous solutions of PEG against phenytoin crystals decreased with increasing PEG concentration as shown in Fig. 9. The increase in the dissolution rate of crystals in an aqueous solution of PEG was less dependent upon the PEG concentration, as compared to the results in Fig. 7. The interstices or voids in the agglomerate might be filled with a saturated solution of PEG, which could enhance the wetting and disintegration of the

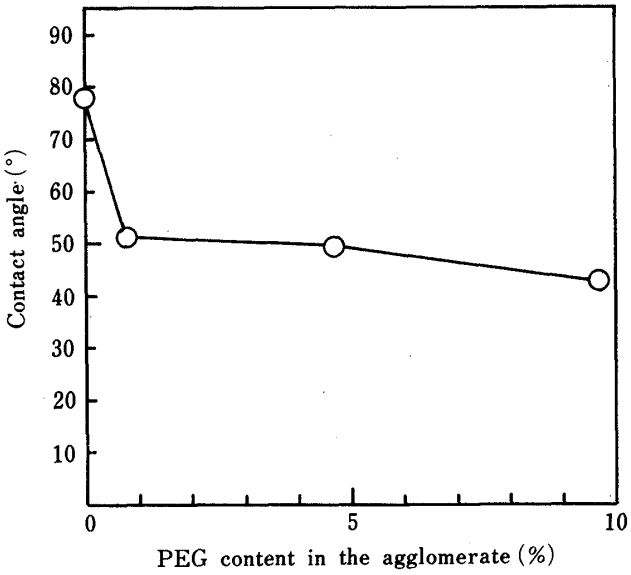

Fig. 6. Effect of PEG Content in Agglomerate on the Wettability

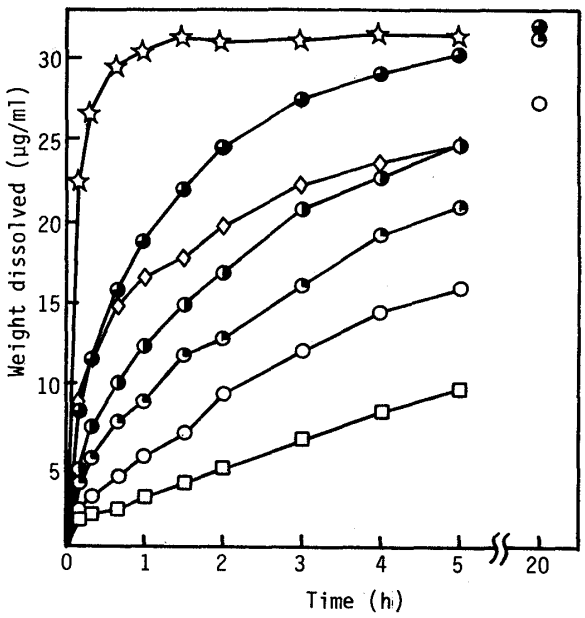

Fig. 7. Dissolution Profiles of Phenytoin Crystals

Agglomerates (PEG content): ○ (0%), ◔ (0.8%), ◑ (4.7%), ◕ (9.7%).☆, recovered crystals without agglomeration; ◇, bulk crystals; □, dry granules prepared from a slug.

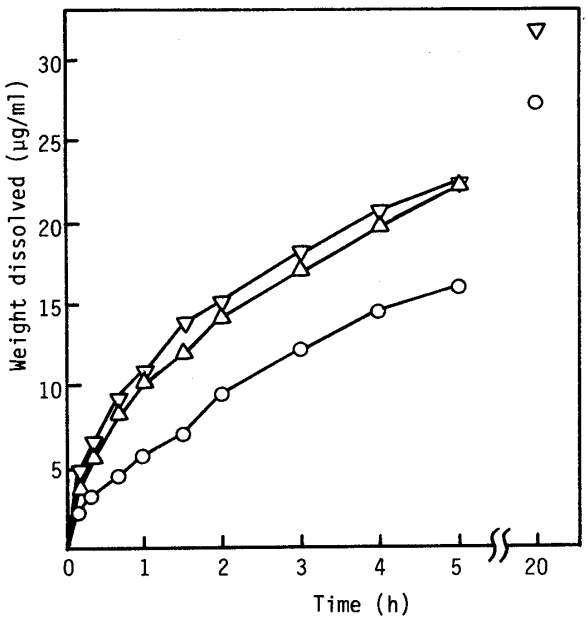

Fig. 8. Effect of PEG in the Dissolution Medium on the Dissolution Rate of Agglomerates

Concentration of PEG in dissolution medium: ○, 0%; △, 0.1%; ▽, 1.0%.

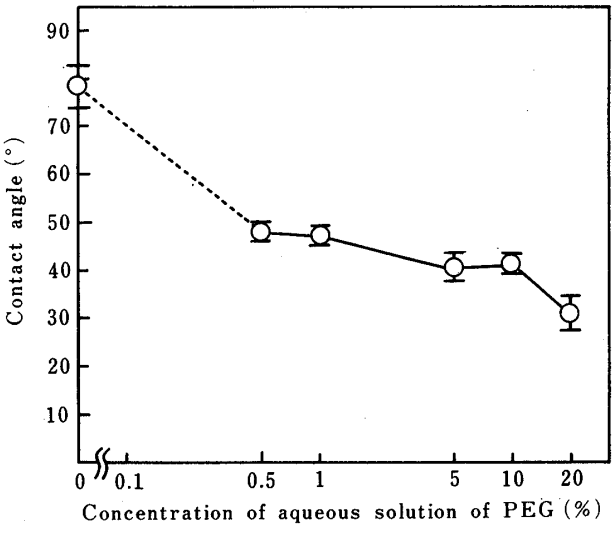

Fig. 9. Contact Angle of Aqueous Solutions of PEG against Bulk Phenytoin Crystals in Air

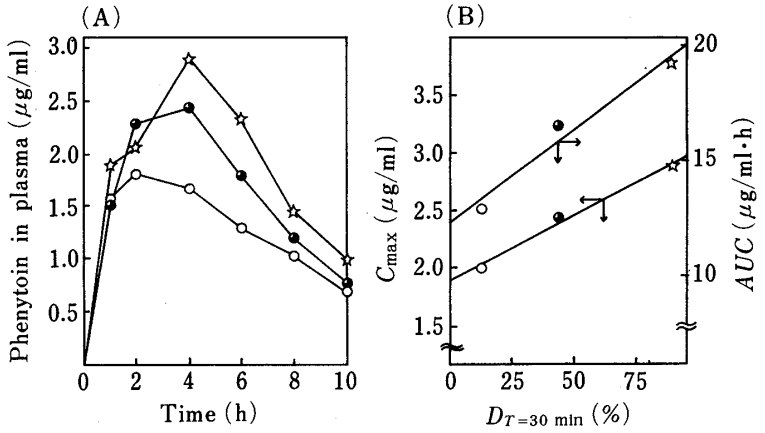

Fig. 10. Plasma Phenytoin Levels as a Function of Time (A), and Relationship between AUC or C_{max} and Dissolved Weight of Phenytoin in Water at 30 min (B)

☆, recovered crystals without agglomeration; ◕, agglomerate with PEG (9.7%), ○, agglomerate without PEG.

agglomerated crystals (see Fig. 9). The increased solubility of phenytoin found in the aqueous solution of PEG can also be interpreted in terms of the rapid dissolution of the agglomerate containing PEG.

Absorption Test of the Agglomerated Crystals

Hard gelatin capsules filled with the agglomerated crystals were orally administered to beagle dogs and the plasma concentration of phenytoin was monitored, as represented in Fig. 10-A. The agglomerated crystals incorporating PEG increased the area under the blood concentration–time curve (AUC) and the C_{max} of phenytoin, as predicted from the dissolution rates in Fig. 7. The highest plasma concentration (2.9 μg/ml) was obtained with the primary crystals.

Figure 10-B shows a linear correlation between the pharmacokinetic parameters, *i.e.* AUC and C_{max}, and the dissolution rate parameter, $D_{T=30\,min}$, as also found in the previous paper.[12] $D_{T=30\,min}$ is the percent of the drug dissolved within 30 min after starting the dissolution test. This finding indicated that the bioavailability of phenytoin depended on the initial dissolution rate *in vitro*. The values of AUC divided by the specific surface area of the primary crystals and the agglomerated crystals without and with PEG were 2.6×10^{-3}, 1.55×10^{-1} and 1.98×10^{-1} [$\mu g \cdot ml^{-1} \cdot h/cm^2 \cdot cm^{-3}$], respectively. This finding indicated that the agglomerated crystals with PEG were the most effective in terms of availability, due to improved wettability.

In conclusion, it is considered that incorporating PEG into agglomerates of poorly soluble crystals by using the spherical crystallization technique improved the dissolution rate and bioavailability. Further, the incorporation of PEG into the agglomerates increased their mechanical strength.

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