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Thermal, Physicochemical, and Micromeritic Properties of Freeze-dried Chloramphenicol Palmitate[1)]

ETSUKO SUZUKI, KAORU CHIHARA, and KEIJI SEKIGUCHI*

School of Pharmaceutical Sciences, Kitasato University, 9-1, Shirokane 5-chome, Minato-ku, Tokyo 108, Japan

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The size reduction of chloramphenicol palmitate to a submicron level was accomplished by the freeze-drying of a solution composed of chloramphenicol palmitate and benzene. The crystal form of the resulting powder was characterized by using X-ray diffractometry, infrared spectroscopy, and differential scanning calorimetry (DSC). The first two methods showed the powder to be polymorph B, but DSC measurements indicated that it contains a trace (or seeds) of polymorph A. This was confirmed by hot-stage microscopy. The dissolution rates and solubilities of freeze-dried powders and polymorph A were determined.

Keywords——chloramphenicol palmitate; polymorph; freeze-drying; particle size reduction; differential scanning calorimetry; dissolution; nonionic surfactant

As described in a previous paper,[2)] we obtained very fine powders of medicinal compounds employing the freeze-drying technique and found that their dissolution was excellent. In this report, we describe the freeze-drying of chloramphenicol palmitate (CPP) and the micromeritic properties of the products.

Experimental

Materials——Highly purified polymorphs A and B of CPP were supplied by Sankyo Co., Ltd. Benzene, sorbitan monostearate, and polyoxyethylene monostearate were of the same grade as in the previous report.[2)] Isopropyl alcohol for dissolution experiments was of analytical grade.

Procedure for Freeze-drying——A benzene solution of CPP in the absence or in the presence of a surfactant was treated according to the method described earlier.[2)]

Measurements of Specific Surface Area——A BET gas adsorption apparatus (model P-600, Shibata Chemical Apparatus Mfg. Co., Ltd.) was used. A sample weight of 0.5—7 g was taken and the gas used for adsorption was N_2.

Scanning Electron Microscopy——The surface appearance of CPP was observed by scanning electron microscopy (MINI-SEM model MSM-4, Hitachi-Akashi Co., Ltd.).

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

Infrared (IR) Spectroscopy——A sample was mulled in Nujol, the mull was placed between sodium chloride plates, and the spectrum was taken on a Jasco IRA-1 grating infrared spectrophotometer.

Differential Scanning Calorimetry (DSC)——A Perkin-Elmer DSC-1B differential scanning calorimeter was used. All measurements were carried out under semi-closed conditions.

Thermomicroscopy——The thermal behavior of CPP polymorphs was observed microscopically with Mettler FP 5 and FP 52 apparatus (Mettler Instrument Co., Ltd.).

Determination of Dissolution Rate——A quantity of CPP powder in excess of its solubility was weighed and rapidly introduced into a 200 ml water-jacketed cell containing exactly 100 g of 50% aqueous isopropyl alcohol maintained at 25±0.1°C. The solution was stirred with a Teflon-covered magnetic stirring bar at a constant rate of 300 rpm. At suitable intervals, aliquots of the solution were withdrawn, filtered through Sartorius membrane filters (pore size 0.2 μm), and immediately diluted with appropriate amounts of 50% aqueous isopropyl alcohol. CPP in the diluted filtrate was analyzed at 275 nm with a Shimadzu UV-200 double-beam spectrophotometer.

Determination of Solubility——A quantity of CPP powder in excess of its solubility was weighed and added to an ampule containing 50% aqueous isopropyl alcohol. The ampule was maintained at 25±0.1°C with continuous shaking. After 190 h, an aliquot of the solution was withdrawn and the concentration of CPP was determined as mentioned above.

Results and Discussion

Size Reduction of CPP by Freeze-drying

A benzene solution of CPP was rapidly frozen using an acetone-dry ice mixture as a refrigerant, and benzene was immediately sublimed off under reduced pressure (< 0.01 mmHg). Electron micrographs of the resulting powder and polymorph A of CPP are shown in Fig. 1. The freeze-dried powder appears to be bulky and fluffy. The values of specific surface area are shown in Table I. The surface area of the freeze-dried powder is very large (average value of 4 samples, 15.6 m^2/g; range, 13.6—16.9 m^2/g) and the calculated average particle diameter is about 0.3 μm, assuming that the particles are spherical (density of polymorph B = 1.273[3b]).

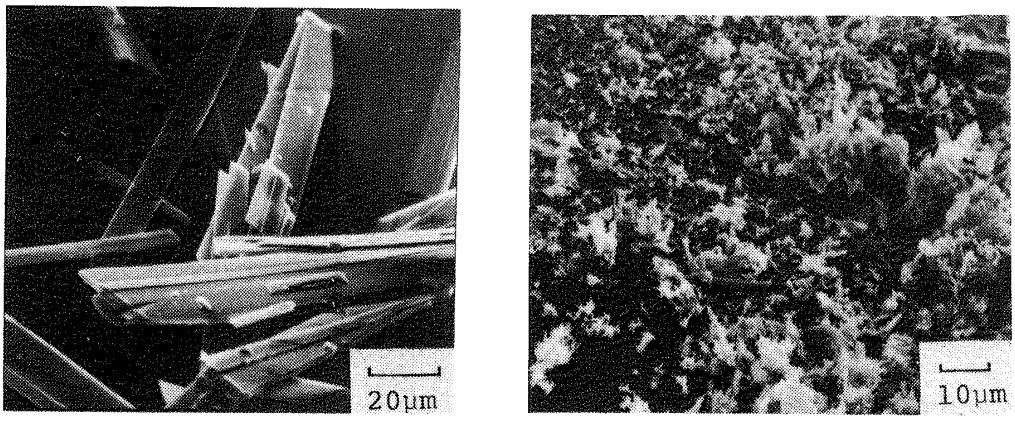

Fig. 1. Scanning Electron Micrographs of CPP
(a) polymorph A, 700×.
(b) freeze-dried product, 1000×.

TABLE I. Specific Surface Area of CPP

Sample	Specific surface area (m^2/g)
Polymorph A	1.4
Freeze-dried product[a]	
Surfactant	
None	15.6
Sorbitan monostearate	9.6
Polyoxyethylene monostearate	10.8

a) The concentrations of CPP in benzene solutions are in the range of 0.496—0.507 w/w %. The amount of surfactant is a quarter of CPP by weight.

It is said that factors affecting the biological efficacy of CPP include polymorphism and particle size. Among the polymorphs of CPP, the therapeutically active one is the metastable B form. In the United States Pharmacopeia (1980) and the British Pharmacopoeia 1980, it is required that the content of polymorph A should be not more than 10%. The particle size is another important factor affecting the bioavailability of CPP. Kelbaek *et al.* established that in the best oral suspension, almost all of the CPP particles were 1 μm or smaller in diameter and the particle size should be within 50 μm for complete hydrolysis and effective absorption.[4]

Therefore, the polymorphic form of CPP obtained by the freeze-drying was investigated by IR spectroscopy and X-ray powder diffractometry. The results for polymorphs A and B are

shown in Figs. 2 and 3, together with those for polymorph C which was prepared according to the method of Aguiar and Zelmer.[5]

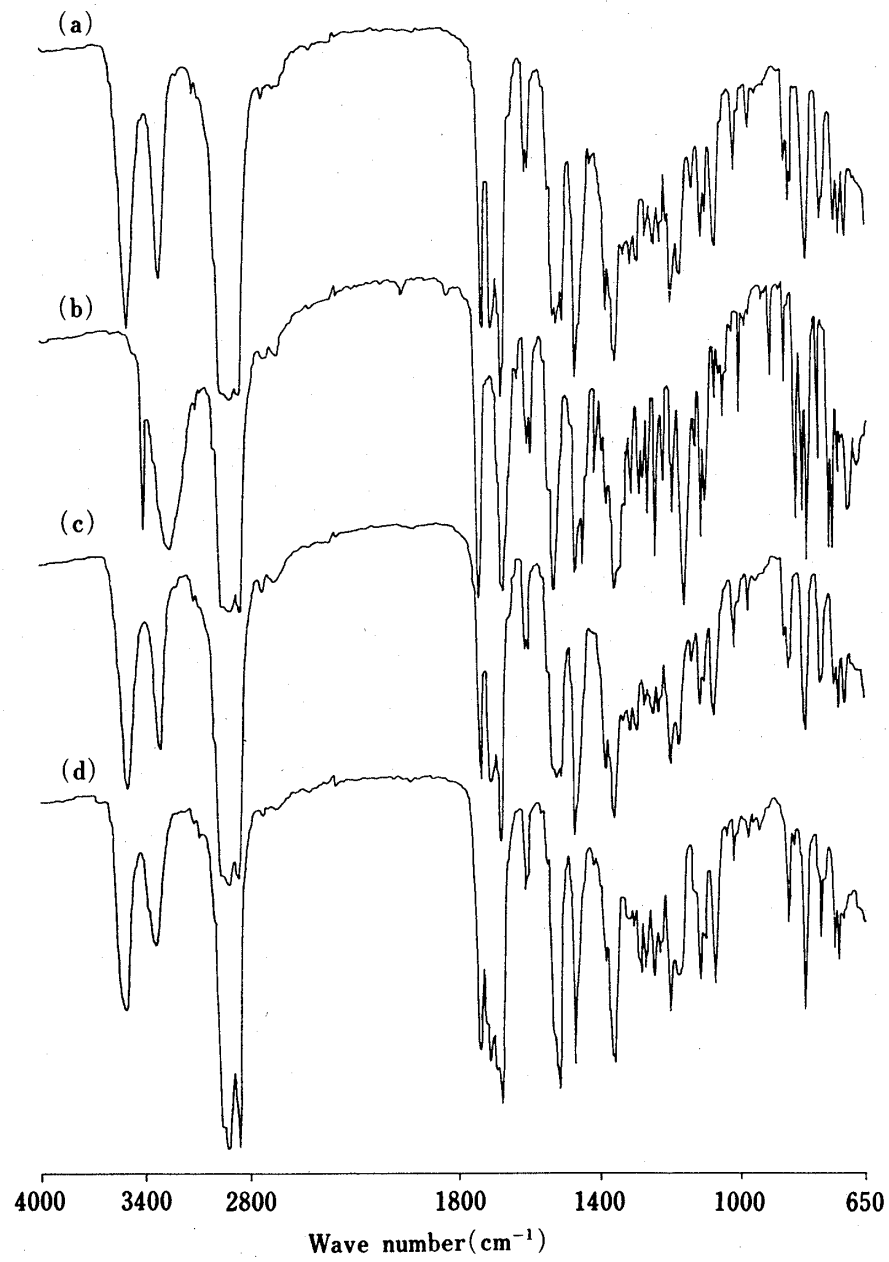

Fig. 2. Infrared Spectra of CPP (Nujol Mull)

(a) freeze-dried product, (b) polymorph A, (c) polymorph B, (d) polymorph C.

1) Infrared Spectroscopy——The spectra of polymorphs A and B coincided with the reported spectra.[6] The crystal structure of polymorph A has been determined by Eguchi and Iitaka.[7] They suggested the existence of an intramolecular hydrogen bond between the NH and OH groups, and an intermolecular hydrogen bond between the OH and C=O groups from the values of the bond lengths and angles. Borka and Backe-Hansen[6b] analyzed the IR spectra of CPP, and concluded that a sharp peak near 3410 cm^{-1} and a broad peak near 3270 cm^{-1} of polymorph A are due to the stretching vibration of free or weakly bonded NH and strongly hydrogen-bonded OH, respectively, while two peaks of polymorph B near 3490 cm^{-1} and 3325 cm^{-1} are due to intermolecularly hydrogen-bonded OH and hydrogen-bonded NH, respectively. The validity of these assignments was supported by the rates of exchange of CPP to deuterated CPP in a deuterated solvent and the position of the new ND or OD band.

In the case of polymorph C, the spectrum was appreciably different from that of polymorph B. For example, the stretching vibrations due to NH and OH groups are shifted slightly to higher frequency as compared with those of polymorph B, and therefore, the hydrogen bonds of these groups in polymorph C are considered to be relatively weaker than those in polymorph B. From the above, it is certain that the polymorphic form of the freeze-dried powder is polymorph B. Although it was reported that the IR spectrum of the amorphous CPP is identical with that of polymorph B,[6b] its content should be negligible because the alteration from amorphous to B form is fairly rapid.[3a]

2) X-Ray Powder Diffractometry——As shown in Fig. 3, the X-ray diffraction patterns of polymorphs A, B, and C evidently differ and the pattern of the freeze-dried powder is identical with that of polymorph B.

3) Thermal Analysis———The thermal behavior of polymorphs A, B, and C, and the freeze-dried powder was examined and their thermograms are depicted in Figs. 4 and 5. Polymorph A showed a melting peak at

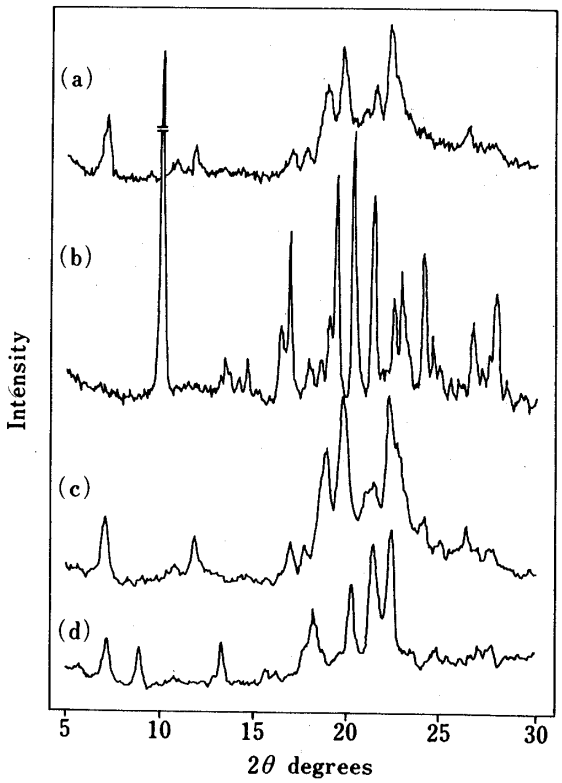

Fig. 3. X-Ray Powder Diffraction Patterns of CPP

(a) freeze-dried product, (b) polymorph A, (c) polymorph B, (d) polymorph C.

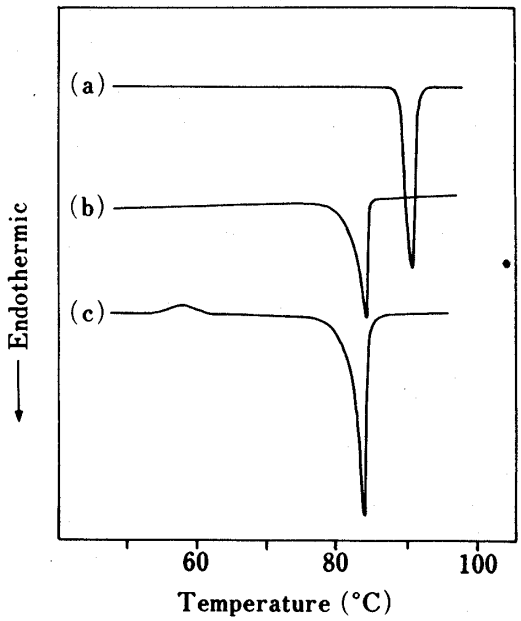

Fig. 4. DSC Curves of CPP Polymorphs under Semi-closed Conditions

(a) polymorph A: heating rate, 8°C/min; sample weight, 3.953 mg.
(b) polymorph B: heating rate, 2°C/min; sample weight, 8.568 mg.
(c) polymorph C: heating rate, 8°C/min; sample weight, 4.355 mg.

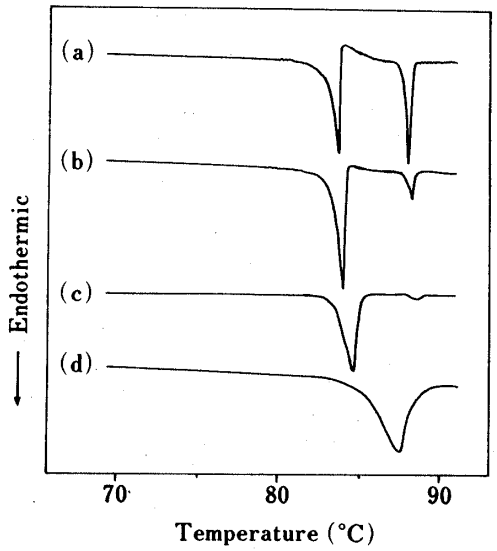

Fig. 5. DSC Curves of Freeze-dried CPP under Semi-closed Conditions

(a) heating rate, 0.5°C/min; sample weight, 5.550 mg.
(b) heating rate, 1°C/min; sample weight, 3.203 mg.
(c) heating rate, 4°C/min; sample weight, 2.762 mg.
(d) heating rate, 16°C/min; sample weight, 2.556 mg.

88—89°C while polymorph B showed one at 82—83°C. From the areas of these peaks, the heats of fusion of polymorphs A and B were calculated to be 16.0±0.2 kcal/mol and 11.1±0.3 kcal/mol, respectively. For both polymorphs, a single endothermic peak appeared independent of heating rate. This result supports the conclusion that polymorph A of CPP is more stable than polymorph B above ordinary temperature under atmospheric pressure.[8] When polymorph C was heated rapidly, a shallow exothermic peak at about 56°C and then a sharp endothermic peak at 82—83°C appeared, but the exothermic peak became almost undetectable at slower heating rates, *e.g.* 1°C/min. To elucidate the thermogram of polymorph C, the X-ray diffraction patterns were measured while polymorph C was being heated slowly. At first, the pattern was C type, then became a mixture of C and B types, and finally changed to pure B type. On the other hand, the DSC curve of the sample which showed the X-ray pattern characteristic of polymorph B exhibited a single endothermic peak at 82°C. From these results, it is suggested that the exothermic peak around 56°C was caused by the alteration of the crystal structure to that of polymorph B.

As shown in Fig. 5, the thermograms of freeze-dried CPP were modified by changing the heating rate. At a slow rate, an endothermic peak is immediately followed by an exothermic peak. With further heating, the second endothermic peak appeared at 88°C. These patterns were examined by utilizing a hot-stage microscope. When freeze-dried CPP was heated at a rate of 1°C/min, the melting of polymorph B around 83—84°C was immediately accompanied by the appearance of tabular single crystals in the droplets of the melt (Fig.

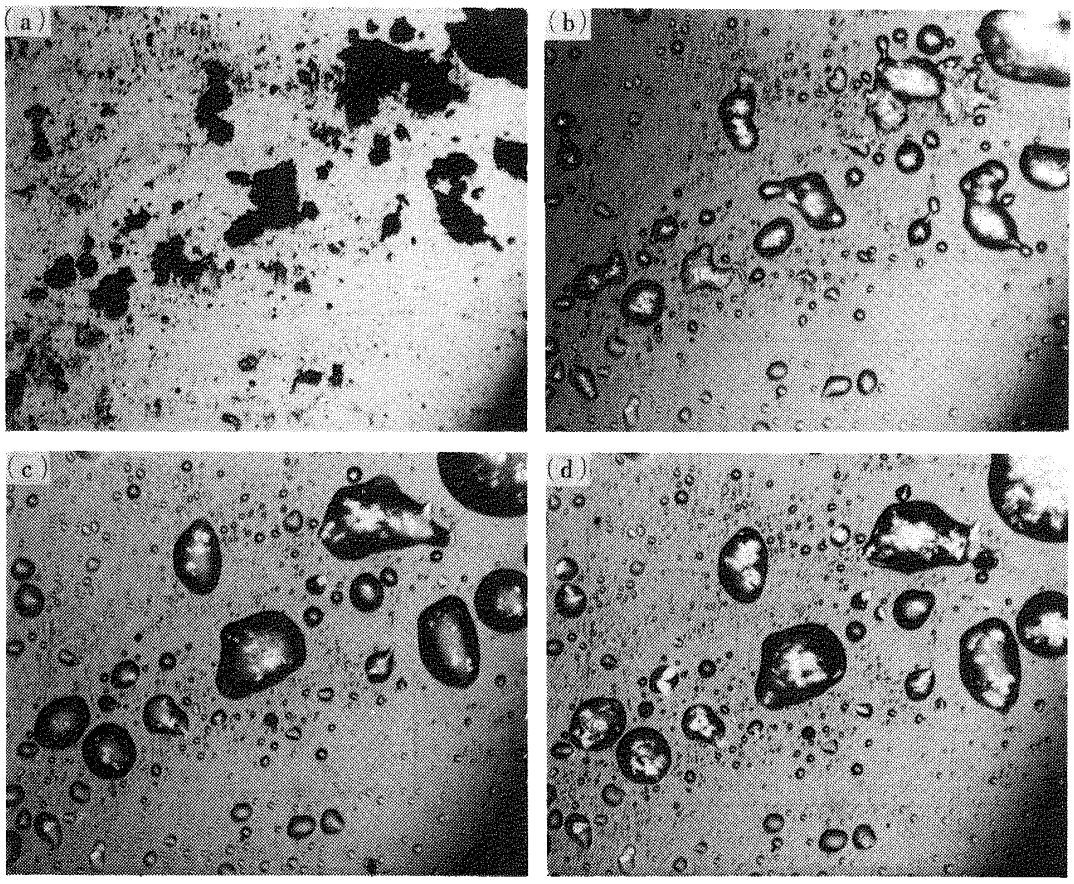

Fig. 6. Photomicrographs in Polarized Light of Freeze-dried CPP during Heating

(a) 25°C.
(b) 84.5°C; melting of polymorph B and beginning of crystallization of polymorph A.
(c) 85.2°C; growth of polymorph A in droplets of melt.
The tabular bright crystals in the droplets are polymorph A.
(d) 87.0°C; polymorph A just short of melting in droplets of melt.
The crystals of polymorph A increase in number and size relative to photograph (c).
The photographs were taken in the same field at 60×.

6). With further heating, these crystals melted entirely at 88—89°C. When the heating was stopped the moment polymorph B melted completely and the tabular crystals appeared, and then this sample was cooled to room temperature, the growth of the tabular crystals occurred instantaneously and soon after, the crystallization was accomplished. This solid was identified as polymorph A from its IR spectrum. From these observations, it is suggested that the first endothermic peak, the subsequent exothermic peak, and the second endothermic peak correspond to the melting of polymorph B, the crystallization to polymorph A, and the melting of polymorph A, respectively. In contrast with freeze-dried CPP, the crystallization of polymorph A during heating of polymorph B did not occur. This may be due to some or all of the following reasons: (1) the melting points of the two polymorphs are so close that the amount of supercooling is insufficient; (2) the heating rate is relatively rapid as compared with the speed of crystallization of polymorph A; (3) the freeze-dried powder is contaminated by polymorph A. Among these three, (1) and (2) are common to freeze-dried powder and polymorph B. Therefore, it is reasonable to suppose that (3) is responsible for the large difference in the DSC patterns. As the heating rate in DSC measurements became faster, the area of the second endothermic peak became smaller and finally a single broad endothermic peak was observed (Fig. 5(d)). This may be because there is insufficient time for the crystallization of polymorph A, leading to overlapping of the exothermic and endothermic peaks.

To estimate the content of polymorph A in freeze-dried CPP, quantitative IR analysis was performed, that is, the relative absorbance ratio at 858 cm^{-1} (characteristic of polymorph B) to 843 cm^{-1} (characteristic of polymorph A) was calculated. The IR spectra of a series of mixtures with known amounts of polymorphs A and B were measured by the Nujol mull technique. Figure 7 shows the curve obtained when the relative absorbance ratios were plotted against the A content (per cent). The amounts of polymorph A contained in freeze-

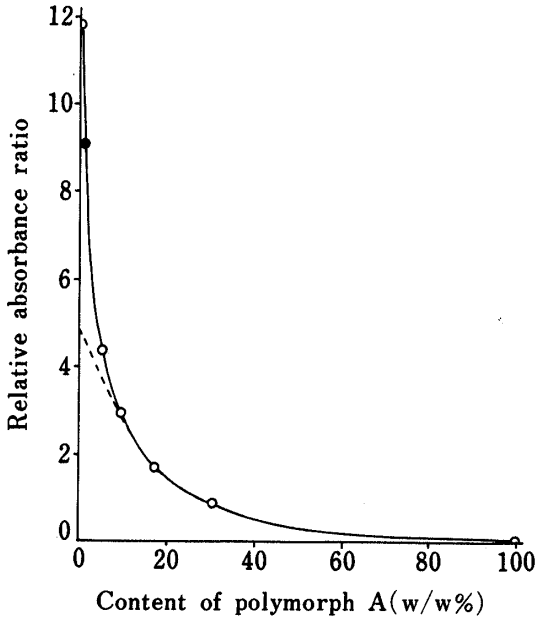

Fig. 7. Relative Absorbance Ratio *versus* Content of Polymorph A in Mixtures of Polymorphs A and B of CPP

The ordinate is the relative absorbance ratio at 858 cm^{-1} (characteristic of polymorph B) to 843 cm^{-1} (characteristic of polymorph A).
○, mixtures with known amounts of polymorphs A and B; ●, freeze-dried products (average value of 16 samples); ----, data from reference.[6a]

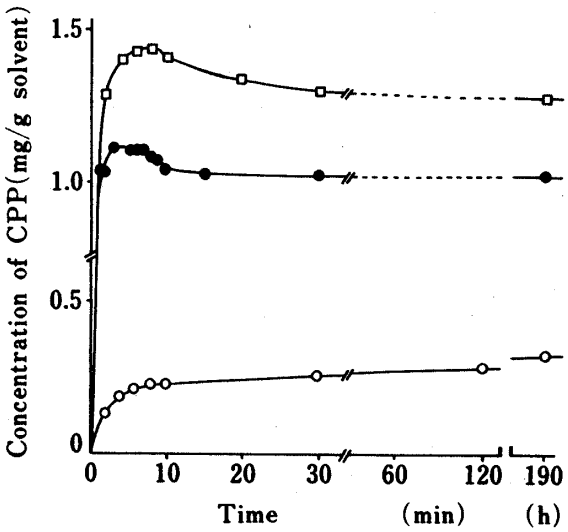

Fig. 8. Dissolution Curves for CPP in 50% Aqueous Isopropyl Alcohol

○, polymorph A; ●, freeze-dried CPP; □, freeze-dried CPP-sorbitan monostearate.

dried powders (16 samples) were estimated based on this standard curve, but the values were not significantly different from that for pure polymorph B ($0.3<p<0.2$). From the area of the higher endothermic peak in the DSC curve of the freeze-dried powder which contains a small amount of polymorph A (below the detection limit in the quantitative IR analysis), the amount of CPP recrystallized as polymorph A by the melt-mediated alteration was calculated. In Fig. 5(a) and 5(b), approximately 30 and 10% of the sample powders had transformed to polymorph A. From these calculations, it is suggested that a trace of polymorph A contained in freeze-dried CPP accelerates the alteration from polymorph B to polymorph A during the heating process.

In conclusion, CPP prepared by the freeze-drying consists mainly of polymorph B, but contains seeds of polymorph A in very small amounts, which can only be detected by DSC measurements or hot-stage microscopy.

Effect of Surfactants on Freeze-drying of CPP

The freeze-drying of a benzene solution of CPP was performed in the presence of a surfactant, namely, sorbitan monostearate (HLB=4.7) or polyoxyethylene monostearate (HLB=16.9). The amount of surfactant was a quarter of CPP by weight. The specific surface areas are shown in Table I. Although CPP is micronized effectively, the specific surface areas are somewhat smaller than that of CPP alone. On electron microscopy, the presence of large or agglomerated particles was observed. This may be attributable to the comparatively low melting points of the surfactants employed in the experiments (in DSC measurements, both of surfactants showed melting peaks at about 40—50°C). The X-ray diffraction patterns were identical with that of polymorph B.

Dissolution of CPP

The dissolution rates were measured for the following samples: polymorph A, freeze-dried CPP, freeze-dried CPP-sorbitan monostearate, and freeze-dried CPP-polyoxyethylene monostearate. As a dissolution medium, 50% aqueous isopropyl alcohol was used because of the relative ease of obtaining measurable concentrations of CPP in the medium. Two kinds of freeze-dried products (shown in Fig. 8) dissolved faster than polymorph A. Although it is not shown in Fig. 8, polyoxyethylene monostearate did not affect the dissolution of CPP. The discrepancy in the effect on the dissolution behavior of CPP may be caused by the difference in HLB of these two surfactants. The solubility values at 25±0.1°C were 32, 101, and 126 mg/100g solvent for polymorph A, freeze-dried CPP, and freeze-dried CPP-sorbitan monostearate, respectively. The free energy difference between polymorph A and freeze-dried powder (polymorph B) was 680 cal/mol. The value is somewhat smaller than those found in the literature,[5,9] and this may be due to the contamination of the powder with polymorph A.

References and Notes

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