Freeze-Drying of Drug–Additive Binary Systems. I. Effects of Freezing Condition on the Crystallinity

Toshio OGUCHI, Katsuhide TERADA,[1] Keiji YAMAMOTO, and Yoshinobu NAKAI*

Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoicho, Chiba 260, Japan. *Received November 10, 1988*

Aqueous methyl *p*-hydroxybenzoate (MPHB) solutions containing various amounts of α-cyclodextrin (α-CD) were freeze-dried. The crystalline states of MPHB and the freeze-dried products have been investigated. From a comparison of the powder X-ray diffraction patterns, it was found that rapid freezing provided amorphous products, while slow freezing provided crystalline inclusion complexes. To estimate the amorphous MPHB fraction, crystalline MPHB was removed by means of sublimation treatment, and residual MPHB was determined by ultraviolet spectrophotometry. It was found that the amount of amorphous MPHB in a rapidly frozen sample was greater than that in a slowly frozen sample. Amorphous MPHB molecules were considered to exist in two different states, namely the included state in the α-CD cavity and the dispersed state in the intermolecular hydrogen-bonding network of α-CDs. From studies with linear oligosaccharides, it was suggested that the freezing condition influenced the amount of MPHB molecules dispersed in the intermolecular hydrogen-bonding network.

Keywords freeze-dry; cyclodextrin; saccharide; amorphous; crystallinity; sublimation; X-ray diffraction; infrared spectrum

Freeze-drying is one of the most useful and common drying processes in the pharmaceutical and food industries. The freeze-drying technique has become quite important for maintaining drug activity, sterility, and accurate proportions of trace components during the manufacture of injections. Basic studies of freeze-drying include the work of MacKenzie on collapse phenomena[2] in the freeze-drying process and Deluca *et al.* on freezing behavior as determined by electric resistance measurement.[3] Freeze-dried samples are sometimes obtained with low crystallinity or in an amorphous state. With some drugs, a decrease of crystallinity may have adverse effects on many physicochemical properties. Therefore, crystallization techniques during the freeze-drying process have received much attention, and several methods have been reported, such as annealing,[4] seed-crystal addition,[5] humidity control,[6] and organic solvent addition.[7]

In practical freeze-drying, saccharides are sometimes used as additives for the formation of a "freeze-dried cake" and for the stabilization of products. The relationship between collapse temperature and solute composition was reported in binary saccharide and saccharide–inorganic salt systems.[8] For freeze-dried samples of acetylcholine and adenyl triphosphate with some saccharides, the relationship between the stability of these drugs and the hydration properties of the saccharide were also reported.[9]

In the present study, we have investigated the influence of the addition of α-cyclodextrin and the freezing condition on the molecular behavior of methyl *p*-hydroxybenzoate. The dispersion properties of methyl *p*-hydroxybenzoate in freeze-dried α-cyclodextrin have also been investigated.

Experimental

Materials Methyl *p*-hydroxybenzoate (MPHB), salicyclic acid and *p*-hydroxybenzoic acid were of special reagent grade. Aspirin was of JP XI grade. α-Cyclodextrin (α-CD), maltohexaose, maltopentaose and maltotriose (Nakarai Chem. Co., Ltd.) were stored in a desiccator containing P_2O_5 in a vacuum.

Freeze-Drying Procedure Various amounts of additives (0—300 mg) were dissolved in aqueous MPHB solution (5×10^{-3} M, 25 ml), which was then filtered through a membrane filter (pore size: 1.0 μm). These solutions were placed in a freeze-drying flask (volume: 250 ml) and frozen with liquid nitrogen (required about 3 min for freezing) or kept in a bath thermostated at −13 °C for 24 h (required 1—3 h for freezing). Frozen samples were dried in a vacuum using a Neo Cool DC'55-B freeze-dryer (Yamato).

Powder X-Ray Diffraction A Rigaku Denki 2027 diffractometer was used. The measurement conditions were as follows: target Cu, filter Ni, voltage 30 kV, current 5 mA, scintillation counter.

Differential Scanning Calorimetry (DSC) A Perkin Elmer model 1B differential scanning calorimeter was used. Samples were sealed in aluminum pans for solid sample and were measured at the scanning speed of 8 °C/min.

Sublimation Treatment For the purpose of estimating the amorphous fraction of MPHB in freeze-dried samples, the crystalline MPHB was removed by sublimation.[10] The samples were evacuated at 110 °C for 4 h using a vacuum sample drying apparatus (Ishii Shouten Ltd.) equipped with a vacuum pump (160 VP-D, Hitachi). The amount of remaining methyl *p*-hydroxybenzoate was determined by using an ultraviolet spectrophotometer (Shimadzu UV-200S) at 255 nm after dissolution in Clark Lubs' buffer solution (pH = 1.45, $\mu = 0.09$). The water contents of evacuated samples were measured with an AQ-3C AQUACOUNTER (Hiranuma) to calculate the MPHB/α-CD ratio.

Infrared (IR) Spectra A Hitachi 295 IR spectrometer was used. The Nujol method was applied for measurement.

Results and Discussion

First, we investigated the freeze-drying of a one-component system as shown in Fig. 1. Freeze-dried MPHB and freeze-dried α-CD showed different characteristics of crystallinity from each other. The amorphous state of α-CD was obtained by freeze-drying, while MPHB showed no significant difference in X-ray diffraction patterns between freeze-dried and intact samples. Similar results to those with MPHB were observed in freeze-dried samples of

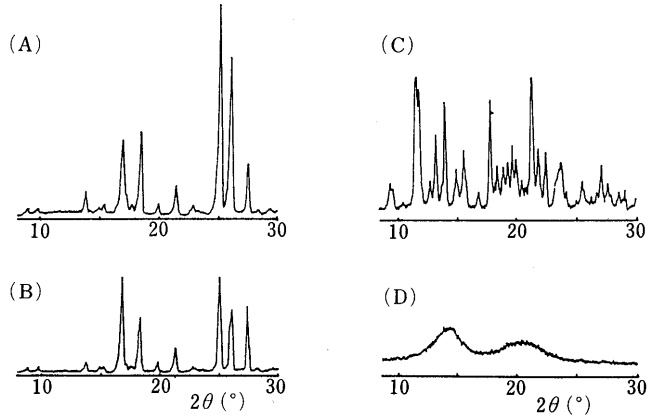

Fig. 1. Powder X-Ray Diffractograms of MPHB and α-CD
(A) intact MPHB, (B) freeze-dried MPHB, (C) intact α-CD, (D) freeze-dried α-CD.

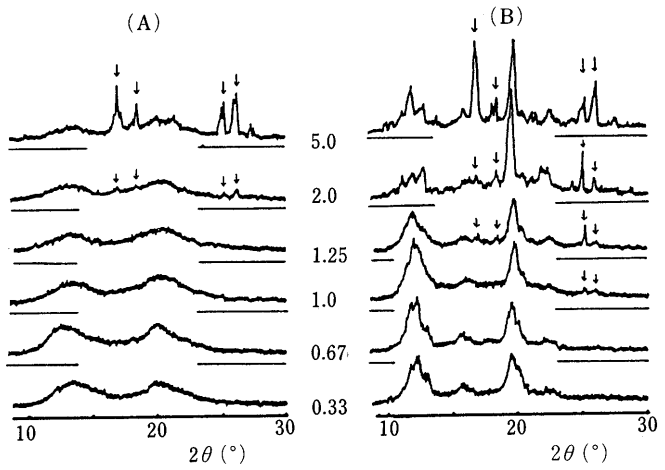

Fig. 2. Powder X-Ray Diffractograms of α-CD–MPHB Binary Freeze-Drying Systems

(A) rapid freezing, (B) slow freezing. Numbers indicate the molar ratio of MPHB/α-CD before freezing. The peaks due to methyl *p*-hydroxybenzoate crystals are indicated by arrows.

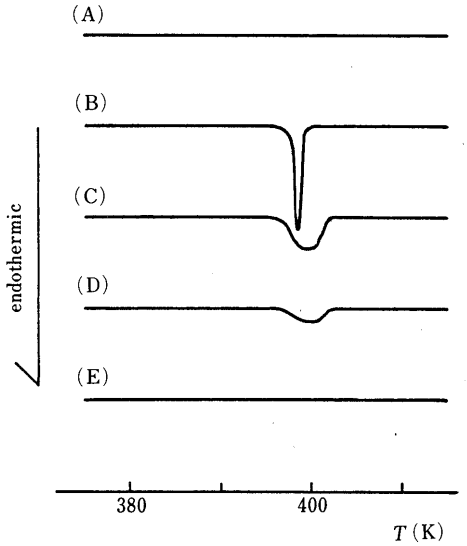

Fig. 3. DSC Curves of α-CD–MPHB Freeze-Dried Samples Obtained by Slow Freezing

(A) intact α-CD, (B) intact MPHB, (C), (D), (E) freeze-dried samples (MPHB/α-CD molar ratios of 5.0, 1.25, 0.67, respectively).

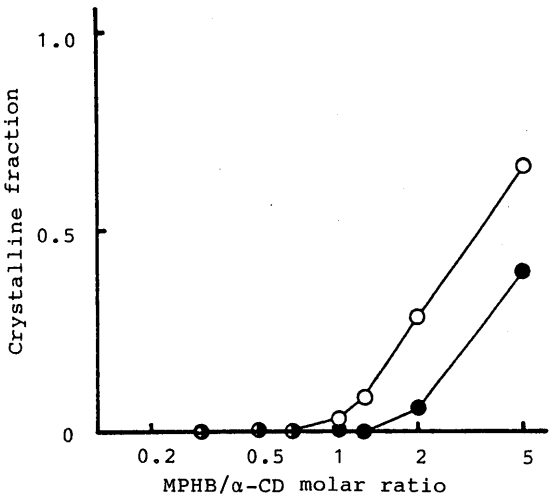

Fig. 4. Crystalline Fraction of MPHB in Freeze-Dried Samples Determined by DSC Measurements

●, amorphous products obtained by rapid freezing; ○, crystalline products obtained by slow freezing.

aspirin, *p*-hydroxybenzoic acid, and salicylic acid. Figure 2 shows the powder X-ray diffraction patterns of binary (MPHB/α-CD) freeze-dried samples of various molar ratios. We used two freezing conditions; namely a rapid decrease of temperature by immersion in liquid nitrogen from 0 °C to −196 °C (rapid freezing) and a gradual decrease of temperature from *ca.* 20 °C to −13 °C (slow freezing). Two typical diffraction patterns were obtained for the products: the halo pattern showing the amorphous state, and the crystalline pattern characteristic of the α-CD inclusion complex. The amorphous state was usually obtained by rapid freezing and the crystalline complex by slow freezing. At high molar ratio of MPHB/α-CD, however, typical diffraction peaks of MPHB crystals were observed (four sharp lines at $2\theta = 17.0$, 18.5, 25.2, and 26.2°) even in the rapidly frozen samples. In freeze-drying of α-CD with salicylic acid or *p*-hydroxybenzoic acid, two types of freeze-dried products were similarly obtained using different freezing conditions.

MacKenzie[2,11] presented the "supplemented phase diagram" by assuming non-equilibrium phase behavior in the freezing process, and interpreted the relationship between the freezing behavior and the crystallinity of freeze-dried products. In cooling of the sample solution, the concentration of the solution gradually increased due to the formation of ice crystals. On further cooling, the viscosity of the solution increased owing to the increasing concentration and lower temperature. When the viscosity reached 10^7 cP, glass transition took place and the solution solidified.[2,11,12] It was supposed that the crystallinity of freeze-dried products is related to whether crystallization of the solute occurred before the glass transition or not. Therefore, it was suggested that in the MPHB–α-CD system, the crystallization of α-CD complex took place during slow freezing, while it did not during rapid freezing.

It was also observed that the intensities of X-ray diffraction peaks due to MPHB crystals reduced with increasing amount of α-CD. At the MPHB/α-CD molar ratio of 1.25, the crystalline MPHB peaks were not detected in the rapidly frozen sample, although in the slowly frozen sample MPHB crystalline peaks were still observed at the molar ratio of 1.0. Crystalline fractions of MPHB were determined from DSC measurements to investigate the influence of the freezing condition on the MPHB crystal growth.

Figure 3 shows the DSC curves of α-CD–MPHB freeze-dried samples. Endothermic peaks were observed at 396 K in the samples which showed X-ray diffraction peaks of MPHB crystals. These endothermic peaks were due to the melting of MPHB existing as crystals in freeze-dried samples. Amounts of crystalline MPHB were determined from the area of these peaks. The crystalline fraction of MPHB, the proportion of MPHB molecules in the crystalline state with respect to the total MPHB molecules in the freeze-dried sample, is plotted against the MPHB/α-CD molar ratio on a logarithmic scale in Fig. 4. The amounts of crystalline MPHB were clearly different between amorphous and crystalline freeze-dried samples. At the same

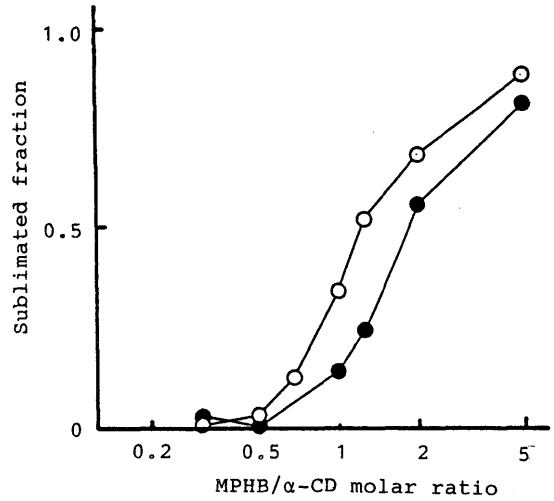

Fig. 5. Percent of MPHB Sublimed from Freeze-Dried Products by Heating at 110 °C in a Vacuum for 4 h

●, amorphous products obtained by rapid freezing; ○, crystalline products obtained by slow freezing.

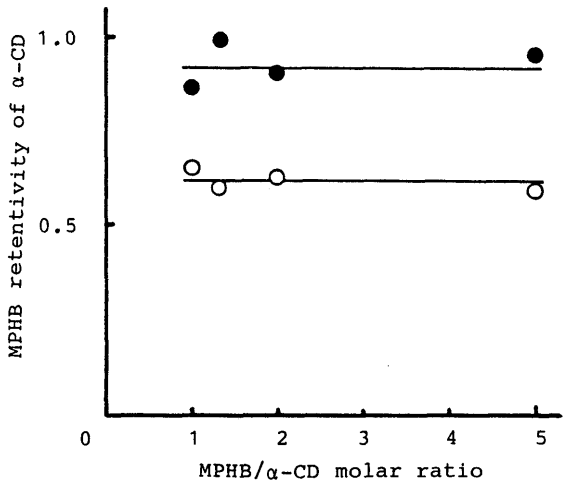

Fig. 6. MPHB Retentivity in α-CD Freeze-Drying Systems

●, amorphous products obtained by rapid freezing; ○, crystalline products obtained by slow freezing. Freeze-dried samples were heated at 110 °C in a vacuum for 4 h before quantitative determination of MPHB.

molar ratio, the amount of crystalline MPHB was much greater in the slowly frozen sample. It was also found that the MPHB/α-CD molar ratios at which the endothermic peaks disappeared were consistent with the molar ratios at which the crystalline MPHB peaks in X-ray diffractograms were no longer detectable. As MPHB crystals are sublimable, the amount of MPHB molecules not present as crystal can be determined by ultraviolet spectrophotometry after the removal of crystalline MPHB by heating in a vacuum.[10] The amount of MPHB removed by sublimation for 4 h, taken as representing the crystalline state, is plotted against the MPHB/α-CD molar ratio on a logarithmic scale in Fig. 5. The results indicate that the amount of MPHB in the crystalline state differed significantly between the amorphous and crystalline samples and that the crystalline fraction of MPHB increased with decreasing amount of α-CD added. Although the amount of MPHB removed by sublimation was more than the crystalline fraction determined by DSC, this might be due to the difference of evaluation of MPHB molecules in the microcrystalline or paracrystalline state between the two methods.

The retentivity was defined as the molar ratio of MPHB to α-CD after the sublimation treatment, as shown in Eq. 1.

$$\text{retentivity} = \frac{\text{number of MPHB molecules remaining after sublimation treatment}}{\text{number of α-CD molecules}} \quad (1)$$

The retentivity of α-CD is plotted against the initial MPHB/α-CD molar ratio in Fig. 6. From this figure, it was recognized that the number of MPHB molecules retained by one molecule of α-CD was independent of the amount of α-CD added in both crystalline and amorphous samples, and that the MPHB retentivity was influenced by the freezing condition. The values of MPHB retentivity were 0.92 (MPHB/α-CD) in an amorphous sample and 0.62 in a crystalline sample. α-Cyclodextrin molecules were considered to have a different ability to hold MPHB molecules between the amorphous state and the crystalline state. Nakai *et al.* reported[13] that α-CD molecules formed an intermolecular hydrogen-bonding network in ground mixtures, and it was considered that drug molecules were dispersed stably in the

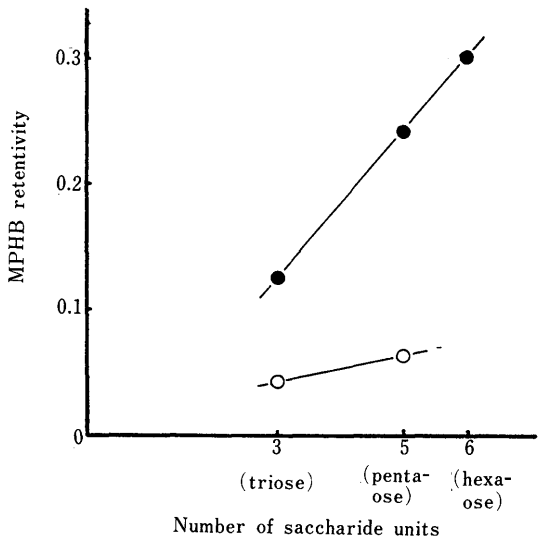

Fig. 7. MPHB Retentivity in Oligosaccharide Freeze-Drying Systems

●, rapid freezing; ○, slow freezing. Freeze-dried samples were heated at 110 °C in a vacuum for 4 h before quantitative determination of MPHB.

network as well as in the cyclodextrin cavity. The stoichiometric ratio of crystalline α-CD inclusion complex prepared by the freeze-drying method has usually been determined as 0.5 (MPHB/α-CD),[14] although Uekama *et al.* reported the stoichiometric ratio of 1.0 for α-CD–MPHB inclusion complex prepared by a coprecipitation method.[15] This difference was ascribed to the difference of crystal structure of the inclusion complexes, that is, a layer-type structure from the coprecipitation method and a channel-type structure from the freeze-drying method. The difference between the retentivity in the freeze-dried samples and the stoichiometric ratio of the channel-type inclusion crystal may correspond to the amount of MPHB present in the hydrogen-bonding network of α-CD molecules in freeze-dried samples.

Freeze-drying using linear oligosaccharides, which have no inclusion ability, was investigated to elucidate the difference of MPHB retentivity.[16] Maltotriose, maltopentaose and maltohexaose, which consisted of 3, 5 and 6

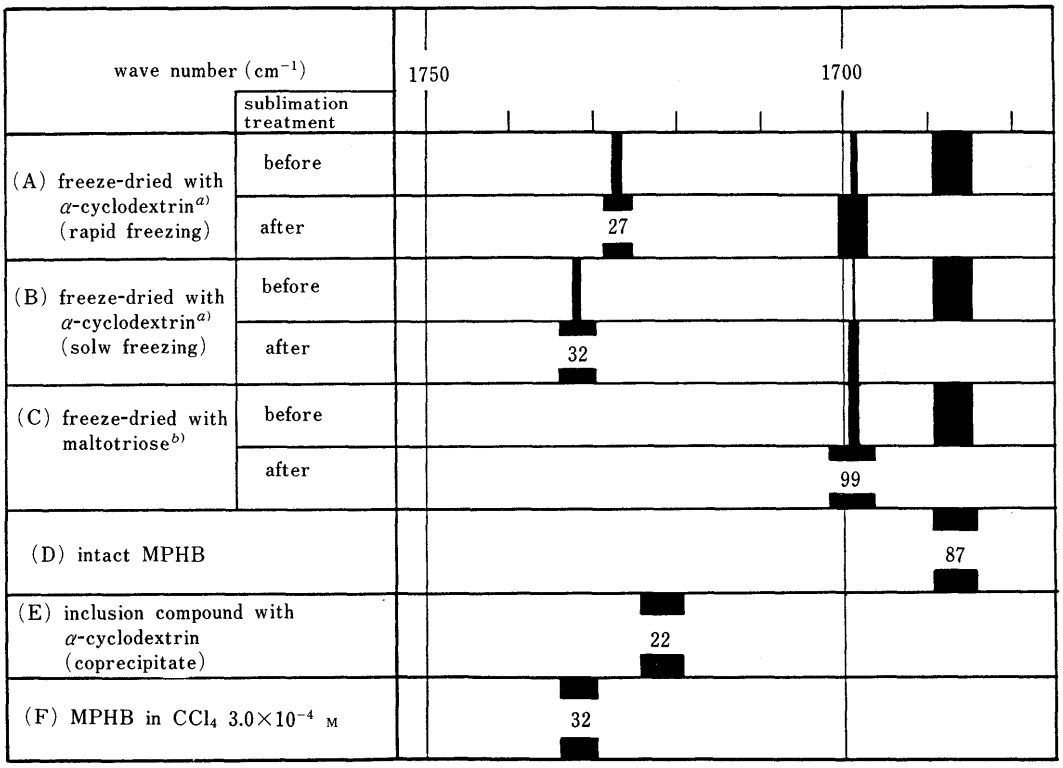

Fig. 8. Comparison of Carbonyl Stretching Vibrations of MPHB in Various States

Freeze-dried samples were measured before and after storage at 110 °C in a vacuum for 4 h. *a*) Initial molar ratio at preparation, 5.0 (MPHB/α-CD); molar ratio after sublimation treatment, (A) 0.92, (B) 0.62. *b*) Initial weight ratio at preparation, 0.19 (MPHB/maltotriose); weight ratio after sublimation, 0.020.

glucose units, respectively, were used. These oligosaccharides were obtained as amorphous materials, and all of the freeze-dried samples were also in the amorphous state. Figure 7 shows the relationship between the MPHB retentivity and the number of glucose residues of each saccharide. The result of slow freezing of the maltohexaose system was not plotted as the sample was too hygroscopic. The value of retentivity was calculated as the ratio of MPHB to six glucose residues of each oligosaccharide for comparison. The oligosaccharides had low ability to hold MPHB molecules in the system compared with α-CD. As the oligosaccharides had no inclusion ability, MPHB molecules could be dispersed only in hydrogen-bonding networks formed between oligosaccharide molecules. It was also found, however, that increase of the chain length of oligosaccharide caused an increase of MPHB retentivity. As the saccharide chain lengthened, the hydrogen-bonding network became more stable[16] and a greater amount of MPHB could exist stably in it. In these systems, the effect of freezing condition was also significant, and rapid freezing resulted in a high MPHB retentivity.

Molecular states of MPHB molecules in freeze-dried samples were investigated by means of IR spectroscopy. The IR spectra of freeze-dried samples were measured before and after heating at 110 °C in a vacuum to remove MPHB crystals from the systems. The observed carbonyl stretching absorption wave numbers are summarized in Fig. 8. Before sublimation treatment, a strong absorption band was observed at 1687 cm^{-1} indicating the presence of MPHB crystals in the freeze-dried samples. Two other carbonyl bands were observed at higher wave number in α-CD systems. After the treatment of the freeze-dried samples containing α-CD, only two carbonyl stretching bands were observed. The bands near 1700 cm^{-1} were considered to reflect hydrogen-bond formation between MPHB and α-CD molecules, as the same shift was observed in freeze-dried sample with maltotriose.[16] From the comparison with the spectrum of MPHB in CCl_4 solution, the absorption bands at 1720—1730 cm^{-1} in freeze-dried samples were estimated to be the stretching absorption of free carbonyl groups. It was confirmed from the IR spectra that there were two different states for the MPHB molecules in the freeze-dried sample, that is, inclusion in α-CD cavity and molecular dispersion in α-CD intermolecular hydrogen-bonding network.

The crystallinity of drugs is closely related to the stability of pharmaceutical products. Crystallinity change of drugs during freeze-drying is an important phenomenon, which must be considered in the evaluation of freeze-dried pharmaceuticals.

References and Notes

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