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# Freeze-drying of drug-additive binary systems III. Crystallization of α-cyclodextrin inclusion complex in freezing process

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## Summary

The crystallization of α-cyclodextrin (α-CD) inclusion complexes during the process of freeze-drying has been studied. Benzoic acid, salicylic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid and methyl *p*-hydroxybenzoate were used as guest molecules. Various amounts of guest compound were dissolved in aqueous α-CD solution. By annealing the binary solutions at −13 to −18°C, the inclusion complex crystallized readily at a molar ratio of 2 : 1 (α-CD : guest). All inclusion crystals obtained via freeze-drying were of a channel-type structure, while in the coprecipitation method *p*-hydroxybenzoic acid and methyl *p*-hydroxybenzoate formed a layer-type inclusion crystal with α-CD. Further, an aqueous binary solution of α-CD and aspirin (2 : 1 molar ratio) was slowly frozen at −14°C, and then lyophilized. The infrared spectra and the powder X-ray diffractograms indicated that the channel-type inclusion compound of aspirin with α-CD was formed by freeze-drying, although the coprecipitation method did not provide the inclusion compound crystal. It was assumed that the mechanism of solid-phase formation differed between the freezing and coprecipitation processes and that the channel-type structure of α-CD inclusion crystal was preferentially formed during the freezing process.

## Introduction

In aqueous solution, cyclodextrin (CD) usually forms a 1 : 1 inclusion complex with a guest molecule (Bender and Komiyama, 1978; Szejtli, 1982). It is known, however, that crystals of inclusion compounds do not necessarily have a stoichiometry of 1 : 1 (Bender and Komiyama, 1978). In crystals of the heptakis(2,6-di-*O*-methyl)-β-CD (DMβCD) complex with *p*-nitrophenol, the *p*-nitrophenol molecules were not included within the cavity but located in the intermolecular space between DMβCD molecules (Harata, 1988), although 1 : 1 inclusion complexation was observed in aqueous solution (Nakai et al., 1987). The crystal structure of α-CD–*m*-nitrophenol (1 : 2) has also been reported, in which half of the *m*-nitrophenol moiety is located externally to the α-CD ring (Harata et al., 1978). These results suggest that the structure of inclusion complexes in the solid phase is not correlated with the mode of inclusion in solution.

The crystal structures of α-CD inclusion compounds have been grouped into three categories

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according to the particular mode of packing of α-CD molecules, i.e., cage,- channel- and layer-type structures (Harata, 1979). It is considered that the thermodynamic stability of the 'crystalline complex' with the individual guest may determine its own crystal structure. In previous papers, the molecular interactions between α-CD and drugs have been investigated in the freeze-dried samples (Oguchi et al., 1989a,b). The present paper describes the crystallization behavior of inclusion complexes during the process of freezing in connection with their molecular interactions and a comparison of the crystal structures of α-CD inclusion compounds obtained by freeze-drying with those for coprecipitation.

## Materials and Methods

*Materials*

Benzoic acid (BA; Koso Chemical Co., Ltd.), salicylic acid (SA; Wako Pure Chemical Ind., Ltd.), *m*-hydroxybenzoic acid (mHBA; Nakarai Chemicals Ltd.), *p*-hydroxybenzoic acid (pHBA; Wako) and methyl *p*-hydroxybenzoate (MPHB; Wako) were of special reagent grade. Aspirin (Iwaki Seiyaku Co., Ltd.) was of JP XI grade. α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD) and tri-*O*-methyl-β-cyclodextrin were purchased from Nakarai, Andou Kasei Co. and Toshin Chemicals Co., respectively, and stored in a desiccator containing $P_2O_5$ in a vacuum. Microcrystalline cellulose (Avicel PH-101, Asahikasei) was used after heating in a vacuum at 110 °C for 3 h.

*Freeze-drying process*

(a) α-Cyclodextrin-aspirin system: This was carried out according to our previous procedure (Oguchi et al., 1989a). An aqueous solution (25 ml) containing aspirin ($5 \times 10^{-3}$ M) and α-CD ($5 \times 10^{-3}$, $1 \times 10^{-2}$ M) was kept at −14 °C for 20 h for freezing. After immersion in liquid nitrogen for complete freezing, the frozen solution was lyophilized. (b) α-Cyclodextrin-other guest molecule systems: Various amounts of a guest compound were dissolved in aqueous α-CD solutions, ranging in molar ratio (guest/α-CD) from 0.2 to 1.0. The concentrations of α-CD were fixed according to the water solubility of each guest compound as follows: 8 w/v% for BA, 4 w/v% for SA and pHBA, and 1 w/v% for pHBA. The sample solutions (2 ml in 30 ml vial tube) were kept in liquid nitrogen for 5 min or in a bath thermostated at −10 to −21 °C beyond 16 h (followed by immersion in liquid nitrogen), and then lyophilized.

*Coprecipitation method*

Coprecipitation was applied to obtain the inclusion compound crystal of α-CD and a guest molecule. The procedure was the same as that described in the previous paper (Oguchi et al., 1989a).

*Preparation of α-cyclodextrin-epichlorohydrin polymer*

α-Cyclodextrin-epichlorohydrin polymer was prepared by a method similar to the synthesis of β-CD-epichlorohydrin polymer (Harada et al., 1981). α-Cyclodextrin (0.0105 mol) was dissolved in 150 ml of water, and then 40 ml of 20% NaOH solution was added. Epichlorohydrin (0.127 mol) was added dropwise to the α-CD solution at 60 °C and the mixture was kept at 65 °C for 24 h. After neutralization with 2 N HCl, the solution was dialyzed with distilled water for several days, and freeze-dried.

*Circular dichroism spectroscopy*

Cyclodextrin ($1.0 \times 10^{-2}$ M) and a guest compound ($5.0 \times 10^{-4}$ M) were dissolved in Clark Lubs' buffer solution (pH 1.45). Circular dichroism spectra were obtained on a JEOL J-500A spectrometer at ambient temperature (about 25 °C).

*Powder X-ray diffractometry*

A Rigaku Denki 2027 diffractometer was used. Measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 5 mA; scintillation counter.

*Infrared spectroscopy*

A Hitachi 295 infrared spectrometer was used. The nujol method was applied for measurement.

*Differential scanning calorimetry (DSC)*

The thermal behavior of the specimen at low temperature was measured by a Perkin Elmer 1B differential scanning calorimeter. Sample solutions (10–15 μl) were hermetically sealed in aluminum pans and cooled to −80 °C. The measurement was carried out during rewarming at a scanning speed of 4 or 8 °C/min.

*Electrical resistance measurement*

Electrical resistance of the aqueous α-CD-BA binary solution was measured during cooling and rewarming using a Freezing Analyzer (Edwards, Kiese & Co.).

## Results and Discussion

*Interaction between α-cyclodextrin and aspirin in aqueous solution*

The interaction of α-cyclodextrin (α-CD) and aspirin in aqueous solution has been investigated. In the circular dichroism spectra, no Cotton effect was observed in the α-CD-aspirin system, whereas in the β-CD-aspirin system, a Cotton effect was induced by inclusion of a guest molecule in the CD cavity. Nakai et al. (1984) reported that in the $^1$H-NMR spectra, the addition of aspirin had no effect on $^1$H-NMR spectrum of α-CD. Further, the phase solubility diagram of the α-CD-aspirin system was of the $A_L$ type according to the classification of Higuchi and Connors (1965). These results indicated that inclusion of the aspirin molecule in the α-CD cavity was not complete in solution, and that the inclusion compound crystal of α-CD and aspirin could not be obtained by the coprecipitation method.

*Freeze-drying of α-cyclodextrin-aspirin binary system*

Aqueous α-CD-aspirin (1 : 1 and 2 : 1 molar ratios) solutions were frozen at −14 °C, and then lyophilized. The α-CD-aspirin (2 : 1) freeze-dried sample was obtained in the crystalline state, however, it showed a different powder X-ray diffraction pattern from the α-CD crystals as shown in Fig. 1. The X-ray diffraction pattern of the freeze-dried sample as shown in Fig. 1a, in which

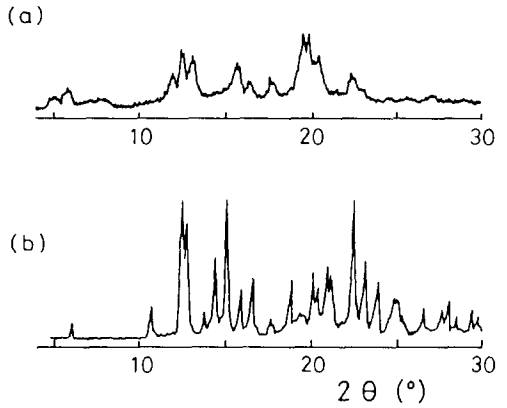


Fig. 1. Powder X-ray diffraction patterns of the freeze-dried α-CD-aspirin (2 : 1) sample (a) in comparison with the α-CD crystal (b).

diffraction peaks appeared at $2\theta = 12.0$ and 20.0 °, was remarkably similar to the diffractogram characteristic of the α-CD inclusion compound of a channel-type structure.

Molecular states of aspirin molecules in the freeze-dried sample were studied by means of infrared spectroscopy. Fig. 2 shows the infrared (IR) spectra of aspirin in the range of 1500–1800 $cm^{-1}$. The spectrum of aspirin crystals (Fig. 2a) was characterized by the acetoxyl and carboxyl carbonyl stretching bands at 1757 and 1700 $cm^{-1}$, respectively. The corresponding carbonyl stretch-

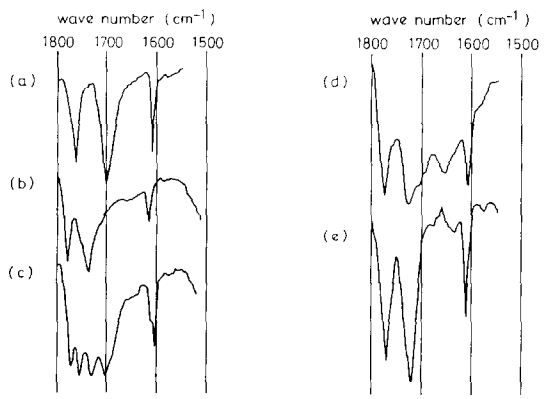


Fig. 2. Infrared spectra of aspirin in various preparations. (a) Aspirin crystal, (b) freeze-dried sample of α-CD-aspirin (2 : 1), (c) freeze-dried sample of α-CD-aspirin (1 : 1), (d) inclusion compound of β-CD-aspirin, (e) inclusion compound of tri-*O*-methyl-β-CD-aspirin.

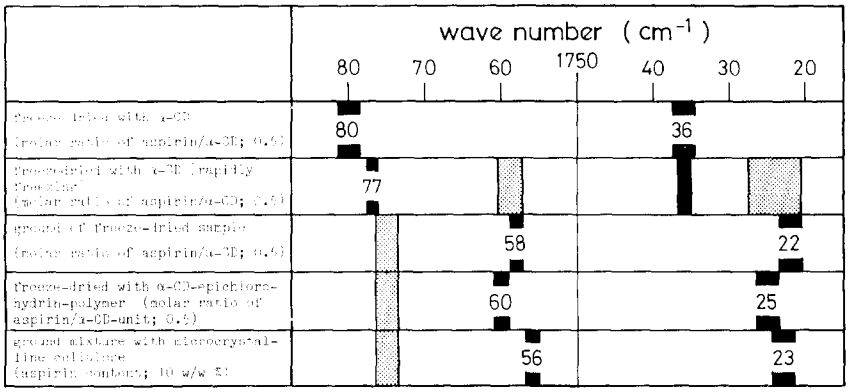


Fig. 3. Comparison of carbonyl stretching vibrations of aspirin in various preparations.

ing bands of the 2 : 1 (α-CD : aspirin) freeze-dried sample were observed at 1780 and 1736 $cm^{-1}$, respectively, indicating that the aspirin molecules were in a different state from the crystals. The IR spectrum of the 2 : 1 freeze-dried sample was found to be similar to that of the inclusion compound crystals of aspirin with β-CD or tri-*O*-methyl-β-CD prepared by coprecipitation. Nakai et al. (1980) considered that the IR peak shifts of aspirin due to the inclusion in the β-CD cavity were attributable to the change of the dimerized aspirin molecules to the monomeric state. The results indicated that aspirin molecules in the freeze-dried sample existed in a state closely similar to the included state within the α-CD cavity. In the 1 : 1 freeze-dried sample, the appearance of four carbonyl absorption peaks suggested the coexistence of the aspirin crystals and included aspirin molecules.

The α-CD-aspirin (2 : 1) freeze-dried sample was ground using an agate mortar and pestle in order to reduce the crystallinity. The IR spectrum of aspirin in this ground sample resembled that of aspirin ground mixture with microcrystalline cellulose (MCC) as shown in Fig. 3. It was reported that the aspirin molecules existed in the amorphous state and were located in the intermolecular hydrogen-bond network of MCC in the ground mixture with MCC (Nakai et al., 1978). It was suggested that the grinding of the freeze-dried sample transformed the aspirin molecules from the included state in the α-CD cavity to the dispersed state in the intermolecular hydrogen-bond network of α-CDs. To obtain the α-CD-aspirin freeze-dried sample of low crystallinity, freeze-drying was carried out under rapid freezing conditions using liquid nitrogen. α-Cyclodextrin-epichlorohydrin polymer, which is an α-CD derivative randomly cross-linked with epichlorohydrin, was also used for freeze-drying. Both of the low-crystallinity samples showed obvious differences in the IR spectra from the crystalline freeze-dried sample, indicating that the aspirin molecules were dispersed in the hydrogen-bond network of α-CD or α-CD-epichlorohydrin polymer. It was confirmed that the crystalline state of the host α-CD molecules was required for the existence of included aspirin.

Consequently, although the aspirin molecule could not ordinarily be included within the α-CD cavity, the formation of channel-type inclusion crystals was possible through the freeze-drying of aspirin and α-CD binary system. In the channel-type structure, the α-CD molecules stacked coaxially along the *c* axis to form continuous hydrophobic channels (Harata, 1979). The inclusion formation of aspirin in the α-CD freeze-dried sample might be permitted by the relatively large hydrophobic spaces formed by two vis-a-vis α-CD molecules. This inclusion mode was recognized as being reasonable from considerations of CPK atomic models.

*Crystal structures of α-cyclodextrin inclusion compound prepared by coprecipitation*

The crystal structures of α-CD inclusion com-

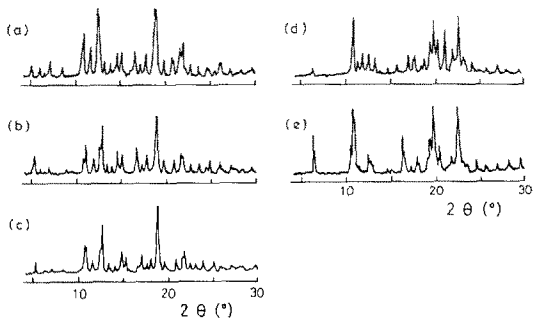


Fig. 4. Powder X-ray diffraction patterns of α-CD inclusion compounds with: (a) BA, (b) SA, (c) mHBA, (d) pHBA, (e) MPHB.

plexes have been investigated using BA, SA, mHBA, pHBA and MPHB as guest compounds. Fig. 4 shows the powder X-ray diffraction patterns of α-CD inclusion compounds prepared by coprecipitation with each of the five guest compounds. The X-ray diffraction patterns were characterized by a hexagonal channel-type structure for the inclusion compound with BA, SA and mHBA and by an orthorhombic layer-type structure for the inclusion compound with pHBA and MPHB (Takeo and Kuge, 1970; Harata, 1977; Uekama, 1980). Further, the stoichiometry of α-CD : guest was determined as 2 : 1 and 1 : 1 for the channel- and layer-type structures, respectively.

*Crystallization of α-cyclodextrin inclusion complex in the freezing process*

The binary solutions containing α-CD and benzoic acid derivatives were freeze-dried. The crystallization behavior of the freeze-dried samples has been investigated.

To investigate the relationship between the crystallinity of freeze-dried samples and freezing conditions, the α-CD-BA (2 : 1) solutions frozen with liquid nitrogen were annealed at various temperatures, and followed by lyophilization. Fig. 5 shows the effects of annealing temperature on the powder X-ray diffractograms of the freeze-dried samples. The crystal growth of the inclusion compound was observed by the annealing at −13 and −16 °C, whereas no annealing or annealing at −21 °C provided the amorphous freeze-dried samples. This result indicated that the channel-type inclusion compound could crystallize by means of annealing above a certain temperature. Binary freeze-dried samples of various α-CD-BA molar ratios were prepared. The freezing temperatures were varied from −10 to −21 °C. It was confirmed that the freezing procedure (without pre-freezing with liquid nitrogen) had almost the same effects as those of annealing on the crystallinity of freeze-dried sample. The relationship between the crystallinity of freeze-dried samples and the freezing temperature is shown in Fig. 6. At molar ratios around 0.5 (BA/α-CD), the crystalline samples were readily obtained even at low freezing temperature. Remarkably, the molar ratio 0.5, at which the crystalline sample was liable to form during freeze-drying, agreed well with the binding ratio of the inclusion compound prepared by coprecipitation.

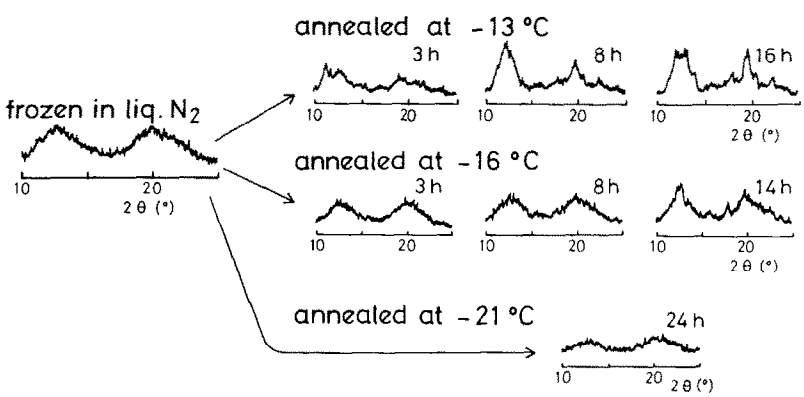


Fig. 5. Effect of annealing temperature on the crystallinity of a freeze-dried sample of the α-CD-BA (2 : 1) system.

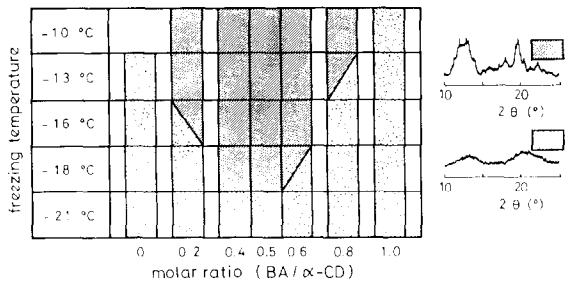


Fig. 6. Crystallinity characteristics of the binary freeze-dried samples at various α-CD-BA molar ratios.

The thermal behavior of the frozen α-CD-BA solution during rewarming was investigated by DSC. The DSC curves of the α-CD-BA binary solutions are shown in Fig. 7 as a function of molar ratios (BA/α-CD). The characteristic thermal behavior was observed before the melting of ice crystals, i.e., an appreciable exothermic peak appeared at a molar ratio of 0.5.

It is known that the loss of ionic mobility accompanied by freezing causes a decrease in the electrical conductivity of an aqueous solution. Electrical conductivity measurements (DeLuca et al., 1965a,b) were employed for evaluating the solidification mode of solute during the freezing

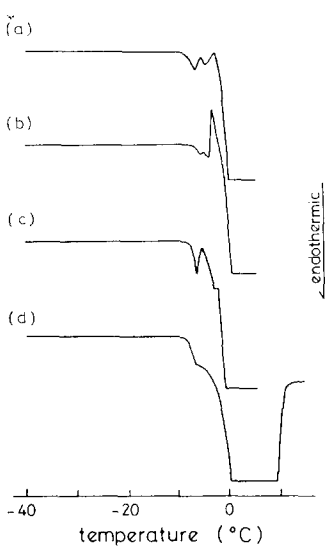


Fig. 7. DSC curves of aqueous α-CD-BA solutions with molar ratios (BA/α-CD) of: (a) 0.2, (b) 0.5, (c) 0.8, (d) 1.0 (heating rate: 4°C/min).

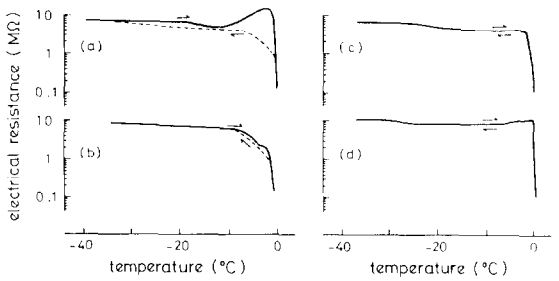


Fig. 8. Electrical resistance of aqueous α-CD (8 w/v%) solution with or without BA during cooling and rewarming. (a) α-CD-BA (2:1) solution, (b) α-CD-BA (1:1) solution, (c) α-CD solution, (d) $H_2O$.

process. Fig. 8 shows the relative electrical resistance vs temperature curves during cooling and rewarming processes. It was noteworthy that the solution of molar ratio 0.5 (BA/α-CD) exhibited evident hysteresis before a sudden decrease in resistance due to ice melting. Such hysteresis could generally be observed when a solute crystallized during rewarming (Kobayashi, 1981). Therefore, the DSC exothermic peak in Fig. 7 was attributed to the crystallization of the inclusion compound of α-CD and BA.

The effects of annealing were investigated by DSC measurements of the α-CD-BA system of molar ratio 0.5 (BA/α-CD). An aluminum DSC pan, in which the sample solution was sealed, was immersed in liquid nitrogen for complete freezing, then kept in a bath thermostated at −16 or −21°C for 20 h. After reimmersion in liquid nitrogen, the DSC measurement was carried out. No exothermic peak was observed after annealing at −16°C, whereas after annealing at −21°C the exothermic peak was observed at −4°C (Fig. 9). The crystallization of the inclusion complex should occur during the −16°C annealing, whereas for the −21°C annealing, the solute remained in the amorphous state. These results were considered to agree with the above results (shown in Fig. 6) pertaining to the relationship between the crystallinity of freeze-dried samples and the annealing conditions.

Salicylic acid, as well as BA, formed a channel-type inclusion compound with α-CD having a stoichiometry of 2:1 (α-CD:SA) on

coprecipitation. The crystalline features of the freeze-dried samples were studied using SA as a guest compound. In the same manner as the α-CD-BA system shown in Fig. 6, the crystalline sample of the α-CD-SA inclusion compound was readily obtained at a molar ratio of 0.5 (SA/α-CD). Similar results were also obtained in the α-CD-mHBA system.

In the systems of α-CD-pHBA and α-CD-MPHB, layer-type (1 : 1) inclusion compound crystals were obtained by coprecipitation, differing from the systems containing BA, SA or mHBA. We also used pHBA and MPHB as guest compounds to investigate the relationship between the crystallization behavior of each inclusion complex and the molar ratio of guest/α-CD. Similar to the results for BA, SA and mHBA, the crystalline freeze-dried sample was obtained at a molar ratio of 0.5 (guest/α-CD) even by freezing at lower temperature. Particular interest was in the crystal structure of the inclusion compound obtained by freeze-drying at a molar ratio of 0.5 (guest/α-CD). Fig. 10 shows the powder X-ray diffraction pattern of the α-CD-MPHB (2 : 1) freeze-dried sample prepared by freezing at −13°C in comparison with the typical X-ray diffraction patterns of the α-CD inclusion crystals obtained by coprecipitation. It was noteworthy that the powder X-ray diffraction pattern of the α-CD-MPHB (2 : 1) freeze-dried sample was evidently different from that of the layer-type inclusion crystal of α-CD and MPHB, but similar to that of the channel-type inclusion crystal of α-CD-BA. In the α-CD and MPHB system, the crystal structure of the inclusion compound prepared by freeze-drying was different from that obtained by the coprecipitation of the α-CD and MPHB system. Similar results were also obtained in the α-CD-pHBA system.

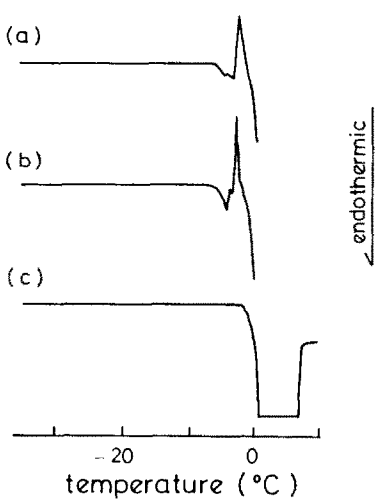


Fig. 9. Effect of annealing temperature on the DSC curve of BA/α-CD (0.5) system. (a) No annealing, (b) annealed at −21°C for 20 h, (c) annealed at −16°C for 20 h (heating rate: 4°C/min).

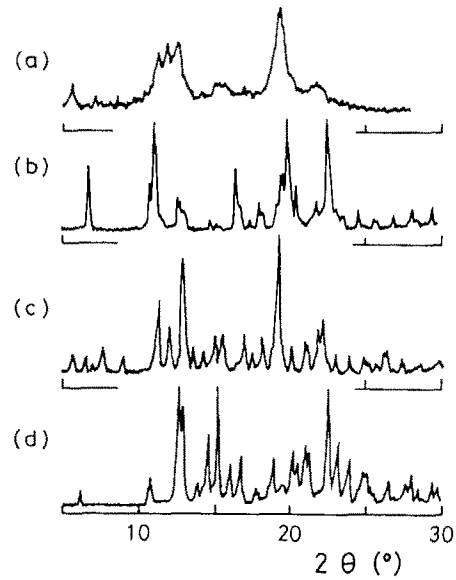


Fig. 10. Comparison of powder X-ray diffraction patterns. (a) Freeze-dried sample of α-CD-MPHB (2 : 1), (b) layer-type inclusion crystal of α-CD-MPHB (1 : 1), (c) channel-type inclusion crystal of α-CD-BA (2 : 1), (d) α-CD crystal.

## Conclusion

In the present study, the α-CD inclusion compound was found to crystallize easily at a molar ratio of 0.5 (guest/α-CD) during the freezing process, and the host α-CD molecules were arranged in the channel-type stacking regardless of the guest compounds. Crystallization during the freezing process took place in a non-equilibrium manner (to some extent) at low temperature, whereas during coprecipitation the crystallization proceeded in

an equilibrium state. Such different conditions of solid-phase formation might exert an influence on the crystal structure of the inclusion compound.

In the α-CD-aspirin system, freeze-drying provided the inclusion compound, which could not be obtained by coprecipitation. The resulting inclusion compound crystal had a channel-type structure, similar to the inclusion compounds of α-CD-benzoic acid derivatives, in which the aspirin molecule was considered to be included in the hydrophobic space formed by two α-CD molecules. It was suggested that the possible formation of the α-CD-aspirin inclusion compound was attributable to the difference in crystallization mechanism between the freezing and coprecipitation processes. Namely, the preferential arrangement of the host α-CD molecules in the channel-type structure could contribute to the formation of large hydrophobic spaces by vis-a-vis α-CD molecules, permitting the inclusion complex formation of aspirin and α-CD.

## Acknowledgement

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