

Thermal Behavior of Ground Mixtures of Heptakis (2,6-di-*O*-methyl)- β -cyclodextrin and Benzoic Acid

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Abstract. Characterization of an inclusion complex prepared from amorphous ground mixtures of heptakis (2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD) and benzoic acid was carried out by a sealed heating method. The formation of the crystalline inclusion complex and the variation in physico-chemical properties of the ground mixture were investigated as a function of heating temperature. On the basis of X-ray diffractometry and Fourier transform infrared spectroscopy, the crystallization of the ground mixture was confirmed to take place at about 135°C. The crystallization temperature was shifted to lower temperatures and the bound molar ratio of benzoic acid to DM β CD increased as the mixing molar ratio of benzoic acid to DM β CD was raised.

Key words: Heptakis (2,6-di-*O*-methyl)- β -cyclodextrin, ground mixture, inclusion complex, thermal analysis, benzoic acid, crystallization.

1. Introduction

There are several methods available for the preparation of inclusion complexes of cyclodextrin, such as coprecipitation [1], kneading [2], spray drying [3], and freeze drying [4]. In these methods, however, solvents are used for the preparation of the complex. Nakai *et al.* [5] demonstrated that the co-grinding method is practical and useful for obtaining an amorphous molecular dispersion system. Inclusion complex formation was observed when cyclodextrins were ground with a guest compound. We have previously reported that the inclusion complex of heptakis (2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD) with benzoic acid was prepared by heating their non-ground and ground mixtures placed in a sealed container (a sealed heating method) [6,7]. The heated, ground mixture was found to have a greater molar ratio of benzoic acid incorporated into DM β CD than the heated non-ground mixture. The grinding time also affected properties of the inclusion complex, such as a crystallization temperature and a molar ratio for inclusion. In addition, we demonstrated a characteristic thermal behavior of the ground mixture by differential scanning calorimetry (DSC), significantly different from the DSC

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behavior of ground DM β CD [7].

In this study we have investigated the thermal behavior of the ground mixture by means of DSC, Fourier transform infrared spectroscopy (FTIR), and X-ray diffractometry.

2. Experimental

2.1. MATERIALS

Heptakis (2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD; lot No. 20) was purchased from Toshin Chemical Co. (Tokyo, Japan) and used without further purification. The purity was ascertained by a single spot on TLC. Benzoic acid was of reagent grade.

2.2. PREPARATION OF GROUND MIXTURES

Ground mixtures of DM β CD and benzoic acid were prepared by adding various amounts of benzoic acid to DM β CD (benzoic acid/DM β CD in the molar ratio 0.25–2.00), followed by grinding for 5 min. An equimolar mixture of both compounds was used for the preparation unless otherwise stated. A Heiko Seisakusho model TI-200 vibrational mill (Tokyo, Japan) made of tungsten carbide was used for grinding.

2.3. THERMAL ANALYSIS

DSC measurements of the ground samples were carried out on a Du Pont TA9900 thermal analysis system under a stream of nitrogen gas by using about 2.0 mg of sample at a heating rate of 8°C/min. A solids sample pan was used without sealing. Thermogravimetry (TG) measurements were carried out at a heating rate of 8°C/min over a temperature range of 40–320°C under nitrogen gas flow on a Du Pont TA9900 apparatus.

2.4. X-RAY DIFFRACTION

X-Ray diffraction patterns for powdered samples were obtained on a Rigaku Denki 2027 diffractometer (Tokyo, Japan) under the following conditions; target Cu, filter Ni, voltage 30 kV, current 5 mA, count range 2000 cps, and scanning speed 4°/min.

2.5. INFRARED (IR) ABSORPTION SPECTROSCOPY

The measurements were carried out on a Nicolet Fourier transform infrared spectrophotometer 5ZDX (WI, USA) using a diffuse reflectance method.

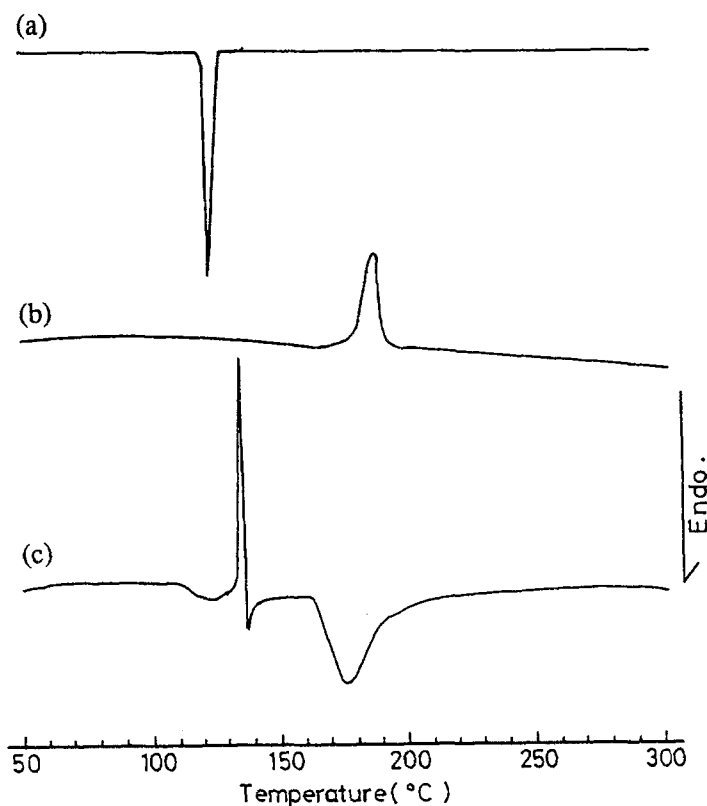


Fig. 1. DSC thermograms of DM β CD–benzoic acid systems: (a) benzoic acid crystals; (b) DM β CD ground for 5 min; (c) DM β CD–benzoic acid mixture ground for 5 min (a molar ratio of 1 : 1).

2.6. DETERMINATION OF INCLUDED AMOUNT OF BENZOIC ACID

The amounts of benzoic acid included in DM β CD were determined spectrophotometrically. Each heated sample was washed with diethyl ether to remove the free benzoic acid. The resulting sample was dried and dissolved in an appropriate amount of 0.1 mol dm^{-3} HCl to determine the amount of benzoic acid included in the host. Although DM β CD is very slightly soluble in diethyl ether (ca. 90 mg in 100 ml of Et₂O at 25°C), the determination was carried out without consideration of the dissolved DM β CD.

3. Results and Discussion

3.1. FORMATION OF CRYSTALLINE INCLUSION COMPLEX BY HEATING

Figure 1 shows the DSC curves of benzoic acid crystals, ground DM β CD and the ground mixture of DM β CD and benzoic acid. Benzoic acid crystals melted at

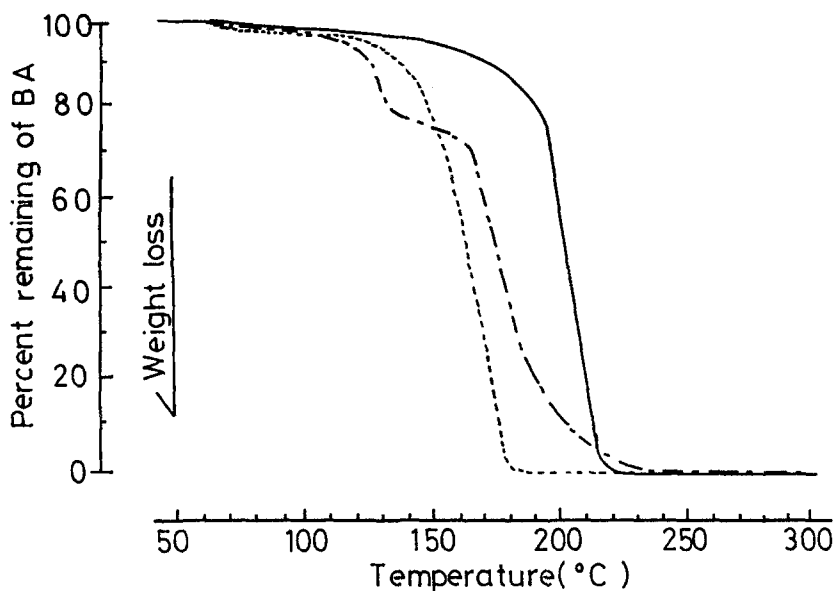


Fig. 2. TG thermograms of DM β CD-benzoic acid systems: —, inclusion complex; - · -, 5 min ground mixture (molar ratio 1 : 1); - - - -; benzoic acid crystals.

122°C. An exothermic peak was observed for the amorphous DM β CD at 187°C, caused by its crystallization. This was confirmed by comparison of X-ray diffraction patterns of the heat-treated samples prepared at temperatures just before and after the exothermic peak. In contrast, one exothermic peak at 135°C and three endothermic peaks at 128, 137, and 170°C were observed for the ground mixture. The exothermic peak seems to be due to the crystallization of the inclusion complex. However, the endothermic peaks are particularly interesting in view of their physical nature.

Figure 2 shows TG curves of the solid inclusion complex of DM β CD with benzoic acid, the ground mixture, and benzoic acid crystals. The TG curve of benzoic acid crystals indicates that the sublimation starts at 80°C. On the other hand, it is apparent that the inclusion complex begins to release benzoic acid at about 150°C, while the ground mixture liberates benzoic acid in two steps that begin at 100 and 165°C. After the TG measurements were completed, both inclusion compound and ground mixture contained no benzoic acid and their X-ray diffraction patterns were identical with that of the crystalline DM β CD. On the basis of comparison between DSC and TG curves for the ground mixture, it becomes apparent that the characteristic DSC peak in the range of 128 to 135°C corresponds to the first weight loss and the endothermic peak at 170°C is referred to the second weight loss observed in the TG curve.

In order to investigate changes in the crystalline state for the ground mixture during the heating process, X-ray diffraction patterns were measured at different

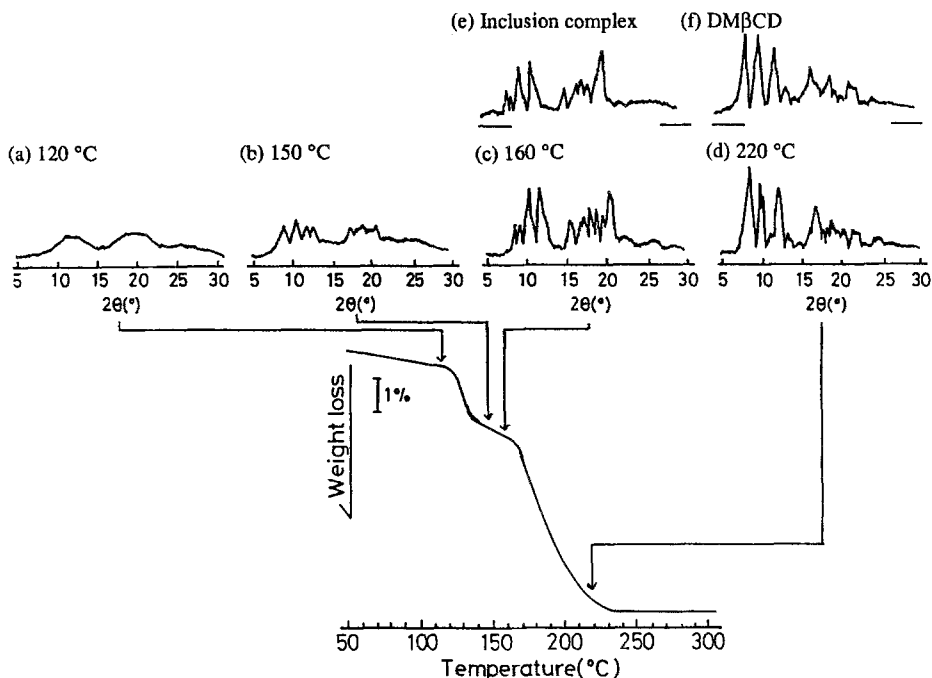


Fig. 3. X-ray diffraction patterns of DM β CD–benzoic acid mixture after heating to various temperatures; heating to (a) 120°C, (b) 150°C, (c) 160°C, (d) 220°C, (e) inclusion complex, (f) DM β CD crystals.

heating stages. Figure 3 shows changes of the X-ray diffraction pattern for the ground mixture upon heating. The sample heated up to 120°C still showed a halo pattern identical with that for the non-heated ground mixture. When the sample was heated up to 150°C, at which the first weight loss was almost completed, a progress of the crystallization was observed. The X-ray diffraction pattern for the sample heated up to 160°C was in agreement with that of the inclusion complex prepared by a coprecipitation method.

On the basis of these results, the first weight loss observed in the TG curve seems to be closely associated with formation of the crystalline inclusion complex. Furthermore, the X-ray diffraction pattern of the sample heated up to 220°C was identical with that of the crystalline DM β CD. This apparently indicates that the second weight loss is caused by release of benzoic acid from the inclusion complex. As regards the ground mixture of DM β CD and naphthalene, Nakai *et al.* already reported similar guest-liberation phenomena during heating [8], while the crystalline DM β CD was obtained without apparent formation of the crystalline inclusion complex composed of DM β CD and naphthalene. To summarize this study, the ground mixture first liberates the unincorporated guest to give the pure complex

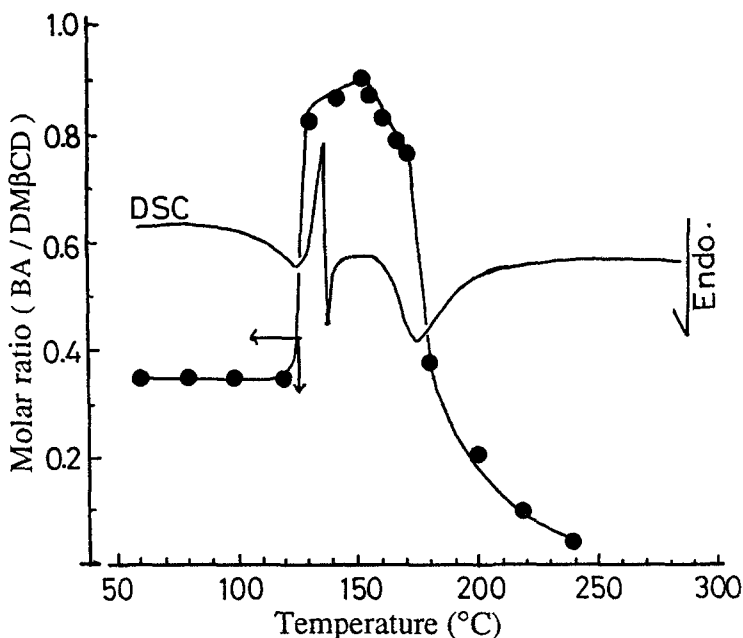


Fig. 4. Variation of bound molar ratio (benzoic acid/DM β CD) after heating: —●—, bound molar ratio (benzoic acid/DM β CD); initial molar ratio, 1.0.

which then loses the included guest at higher temperatures to leave the crystalline host.

3.2. CHANGES IN BOUND MOLAR RATIO OF GUEST TO HOST BY HEATING

Figure 4 shows the variation of the bound molar ratio of benzoic acid to DM β CD as a function of heating temperature. The bound molar ratio is strongly dependent on heating temperature. As shown in Figure 3, the crystalline inclusion complex did not form at 120°C, and the bound molar ratio was as low as 0.33 (benzoic acid/DM β CD). The value of 0.33 was explained by presuming the existence of an incomplete inclusion of benzoic acid in the ground mixture [7]. When a ground sample was heated up to 128°C, the bound molar ratio increased drastically to 0.82, and this increase is closely related to the fraction of the crystalline inclusion complex. The high molar ratio was detected constantly by heat treatment up to 170°C. Further increase of heating temperature over the temperature range of the third DSC endothermic peak resulted in a sudden decrease of the bound ratio. From these results it was rationalized that the endothermic peak at 128°C was caused by sublimation of benzoic acid from the ground mixture and the remaining benzoic acid was completely included in the DM β CD cavity. The exothermic peak at 135°C must be due to crystallization of the inclusion complex which seemed to

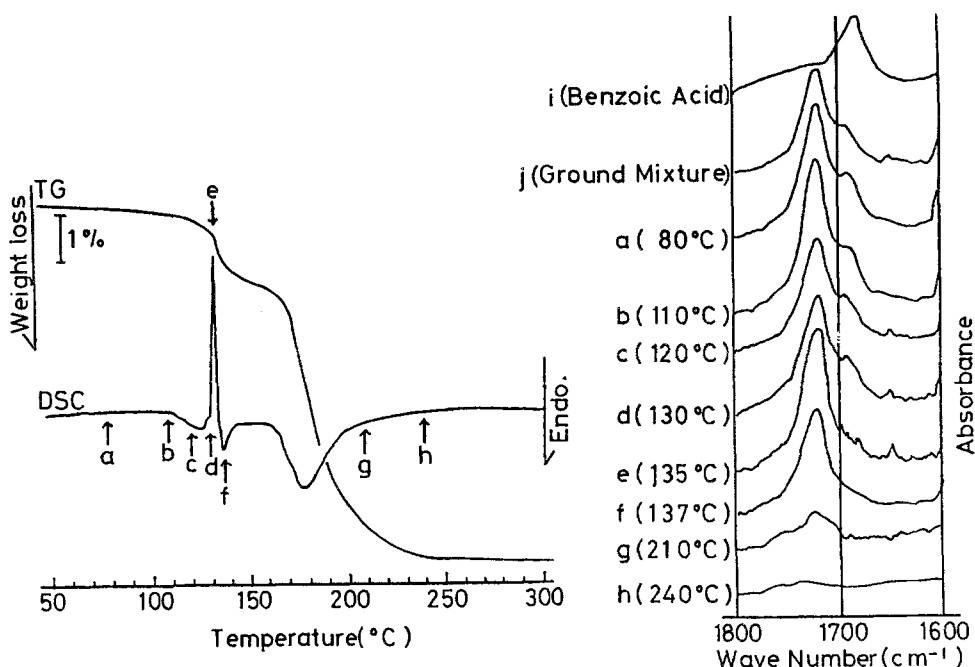


Fig. 5. Correlations of TG and DSC thermograms with FTIR spectra of DM β CD-benzoic acid ground mixture (a molar ratio, 1 : 1): heated up to (a) 80°C, (b) 110°C, (c) 120°C, (d) 130°C, (e) 135°C, (f) 137°C, (g) 210°C, (h) 240°C; (i) benzoic acid without heating, (j) ground mixture without heating.

be induced by the preceding sublimation of benzoic acid. The endothermic peak at about 137°C can be attributed to sublimation of benzoic acid which was not included in the DM β CD cavity. Furthermore, the endothermic peak at 170°C was undoubtedly caused by release of benzoic acid from the inclusion complex.

3.3. IR STUDY OF BENZOIC ACID IN VARIOUS SOLID STATES

To investigate the effect of heating on the molecular states of benzoic acid in the ground mixture, IR measurements were performed on the samples obtained under different heating conditions by a diffuse reflectance method. The IR spectra of the ground mixture composed of DM β CD and benzoic acid are given in Figure 5 after it was heated up to various temperatures. The carbonyl stretching vibration band was observed at 1683 cm^{-1} for the benzoic acid crystals as well as the physical mixture and shifted to a higher frequency at 1720 cm^{-1} for the ground mixture. As the inclusion complex shows the carbonyl band at 1720 cm^{-1} , benzoic acid molecules are separated from each other in the ground mixture. This IR band shift indicates the change of molecular state of benzoic acid from the dimeric hydrogen bonding state in the crystal to the included state in the DM β CD cavity [7]. The ground mixture,

however, showed an additional carbonyl band at 1695 cm^{-1} , benzoic acid still remains as microscopic crystals in the mixture. The peak intensity at 1695 cm^{-1} was weakened as the heating temperature was raised. When the mixture was heated at 137°C , the 1695 cm^{-1} band was not observed at all; consistent with the fact that an endothermic DSC peak, due to the sublimation of benzoic acid, was observed at 137°C . The IR band at 1720 cm^{-1} became progressively weaker as the heating temperature was raised above 200°C . This indicates that benzoic acid was liberated from the inclusion complex. In the light of the above observation, the ground mixture undergoes the following thermal changes as the temperature was raised; sublimation of benzoic acid, crystallization of the inclusion complex, continuous sublimation of benzoic acid and release of benzoic acid from the inclusion complex to leave the crystalline host, in this sequence.

3.4. EFFECT OF MIXING MOLAR RATIO ON THE THERMAL BEHAVIOR OF GROUND MIXTURES

Ground mixtures were prepared at various mixing molar ratios of benzoic acid to $\text{DM}\beta\text{CD}$ (benzoic acid/ $\text{DM}\beta\text{CD}$ in molar ratio, 0.25–2.00). Figure 6 shows the DSC thermograms of the ground mixtures at various mixing molar ratios. Crystallization of the inclusion complex was observed at 178°C as an exothermic peak at a mixing molar ratio of 0.25. As the mixing molar ratio increased, the exothermic peak shifted to a lower temperature range. A ground mixture at a mixing molar ratio of 2.0 crystallized at 106°C , 70°C lower than the temperature observed for a ground mixture at a mixing molar ratio of 0.25. The decrease of the exothermic peak with an increase of the mixing molar ratio could be closely correlated with the foregoing endothermic peak due to the sublimation of benzoic acid. When a ground mixture of 0.25 molar ratio was used, the majority of $\text{DM}\beta\text{CD}$ existed alone, without including the guest. It seems reasonable to judge that the crystallization of this system is dominated by the crystallization behavior of $\text{DM}\beta\text{CD}$ itself rather than that of inclusion complex. At mixing molar ratios of 0.50 and 0.67, sharp endothermic peaks were observed after the exothermic peak appeared (curves (b) and (c), respectively). This means that sublimation of the remaining benzoic acid and the release of benzoic acid from the $\text{DM}\beta\text{CD}$ cavity occurs simultaneously just after the crystallization takes place. At high mixing molar ratios, the crystallization temperature decreased and the third endothermic peak temperature increased as shown in curves (e) and (f).

In order to investigate the inclusion behavior of $\text{DM}\beta\text{CD}$ toward benzoic acid at the crystallization stage, the bound molar ratio was determined before and after the crystallization as a function of the initial mixing molar ratio. Figure 7 illustrates relationships between the initial mixing molar ratio and the bound molar ratio before and after the crystallization, together with the crystallization temperature. At mixing molar ratios of 0.25 and 0.50, no difference in the bound molar ratio was observed between them before and after the crystallization. As for these systems,

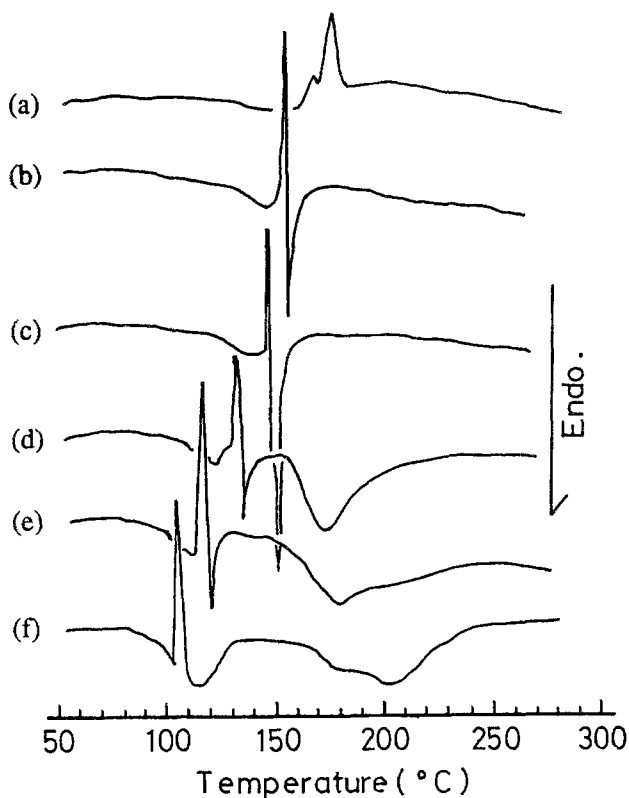


Fig. 6. DSC thermograms for ground mixtures of DM β CD–benzoic acid prepared at various molar ratios; mixing molar ratio (benzoic acid/DM β CD) (a) 0.25, (b) 0.50, (c) 0.67, (d) 1.00, (e) 1.50, (f) 2.00.

the crystallization temperature was high enough to induce benzoic acid release from the systems, except for the benzoic acid tightly included in the DM β CD cavity, so that the inclusion complex crystals could be formed only with the included guest. On the other hand, a ground mixture at an initial mixing molar ratio of 0.67 gave a bound molar ratio as high as 0.41 after the crystallization took place, about 1.8 times greater than that observed before the crystallization stage. Furthermore, when the samples were prepared at higher mixing molar ratios, all the host molecules undergo inclusion of the guest after the crystallization.

In conclusion, the crystalline inclusion complex of DM β CD with benzoic acid was obtained by heating the ground mixture. Sublimation of benzoic acid appeared to be a trigger for the crystallization of inclusion complex from the ground mixture. The temperature at which the sublimation began was dependent on the mixing molar ratio, as well as the grinding time related to the crystallinity of DM β CD as reported previously [7]. The initial fraction of benzoic acid in the ground mixture

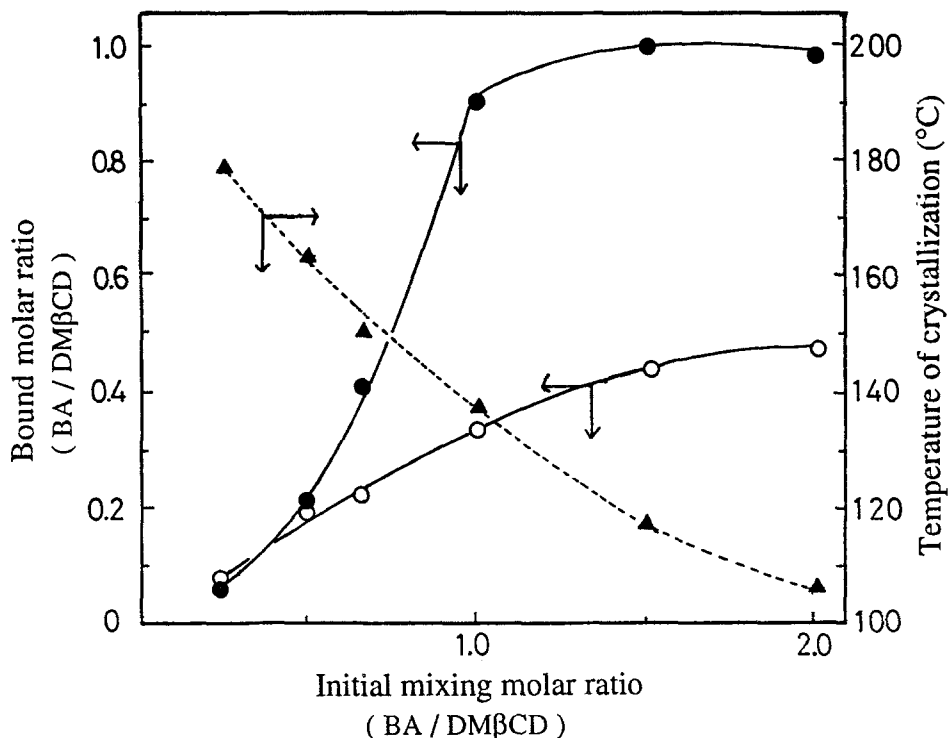


Fig. 7. Comparison of bound molar ratio of benzoic acid to DM β CD before and after crystallization: —○—, bound molar ratio in the ground mixtures before crystallization; —●—, bound molar ratio after crystallization; —▲—, temperature of crystallization determined by DSC.

was found to control the bound molar ratio of the guest to DM β CD as well as the crystallization temperature.

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