

New Methods for Preparing Cyclodextrin Inclusion Compounds. III. Preparation of Heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin-Benzoic Acid Inclusion Compound by Sealed Heating

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The inclusion compound of heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD) and benzoic acid was prepared by heating in a sealed container. Inclusion formation was studied as a function of heating temperature, heating time, mixing molar ratio, and crystallinity of DM β CD measured by X-ray diffractometry and infrared spectrometry. The combining molar ratio of benzoic acid to DM β CD increased with an increase in heating temperature and time. When amorphous DM β CD was used for the inclusion formation, the combining molar ratio of benzoic acid to DM β CD increased to about double the amount of crystalline DM β CD used. It was found that the heating temperature, heating time, mixing molar ratio, and crystallinity of DM β CD affected the formation of inclusion compound.

Keywords heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin; benzoic acid; inclusion compound; sealed heating; combining molar ratio; crystallinity

Solid inclusion compounds of cyclodextrin (CD) with many guest molecules have been prepared by coprecipitation,¹⁾ kneading,²⁾ freeze drying,³⁾ and cogrinding^{4,5)} methods. In previous work, we reported a new method of preparing a solid inclusion compound.^{6,7)} A physical mixture of benzoic acid and either α - or β -CD was sealed in a glass ampule and heated under various conditions. The advantage of the heating method was that it allowed preparation of a large amount of inclusion compound easily without using water as a solvent.

Many derivatives of CD have been synthesized to improve the physicochemical properties of CD.⁸⁾ In particular, selectively methylated CD, heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin (DM β CD) was synthesized by substituting 2- and 6-hydroxyl groups of glucose units with the methyl group. Heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin had a higher water solubility than β -CD (about 30-fold),⁹⁾ and the property of improved the drug solubility was reported.¹⁰⁻¹⁶⁾

In this study, an attempt was made to investigate the inclusion characteristics of DM β CD by a sealed heat method by comparing them with α - and β -CDs.

Experimental

Materials DM β CD was purchased from Toshin Chemical Co. and used without further purification. Benzoic acid was of reagent grade.

Heating of Mixture in a Sealed Container A physical mixture of benzoic acid and DM β CD was prepared at a definite mixing ratio by simple blending with a mortar and pestle. The physical mixture (300 mg) was sealed in a 2 ml glass ampule, then heated at a definite temperature (60–90 °C) and time (0.5–6 h) in an oil bath. The amounts of benzoic acid included to DM β CD were determined spectrophotometrically after washing the heated samples with ethyl ether to remove the excess free benzoic acid.^{3,6,7,17)} The dried sample was dissolved in a definite amount of 0.1 N HCl to determine the included amount.

Solubility Study Excess amount of benzoic acid (90 mg: 7.37×10^{-4} mol) and successively increasing amounts of DM β CD were added and dissolved in 10 ml of 0.1 N HCl. The samples were shaken at 30 °C for 24 h. After equilibration, the samples were filtered with a Milipore filter (0.2 μ m). The amount of benzoic acid dissolved was analyzed spectrophotometrically at 278 nm.

Preparation of Ground DM β CD Ground DM β CD was prepared by using a vibrational mill of Heiko Seisakusho, Model TI-200, which was made of tungsten carbide as reported previously.^{18,19)}

Preparation of Benzoic Acid and DM β CD Inclusion Complex Since the solubility of DM β CD decreased at high temperature, the equimolar mixture of benzoic acid and DM β CD was dissolved in distilled water at room temperature, and then was incubated at 80 °C. Inclusion compound

was obtained as precipitated crystal after the filtration of hot solution.

X-Ray Diffraction Powder X-ray diffraction patterns were measured using a Rigakudenki 2027 diffractometer. Conditions: target Cu, filter Ni, voltage 30 kV, current 5 mA, receiving slit 0.15 mm, count range 2000 cps, scanning speed 4 °/min.

Infrared (IR) Absorption Spectroscopy This was carried out using a Hitachi 295 IR spectrophotometer by the Nujol method.

Results and Discussion

Formation of Benzoic Acid and DM β CD Inclusion Compound In order to determine the interaction between benzoic acid and DM β CD, a phase solubility diagram was prepared. Figure 1 shows the B_s type solubility curve²⁰⁾ obtained for the benzoic acid and DM β CD system at 30 °C. The stoichiometric ratio of solid complex was determined from the plateau region of the solubility diagram, and was estimated to be 1:1. The slope of the initial portion, however, was about 1.14, and the formation of 2:1, 3:1 complexes in solution was assumed.²¹⁾ By the coprecipitation method, the solid complex of 0.997 molar ratio was obtained. From these results, the production of stable 1:1 solid inclusion compound of DM β CD and benzoic acid was confirmed.

Figure 2 shows the changes of X-ray diffraction patterns of the DM β CD-benzoic acid equimolar mixture before and after heating in a glass ampule at 90 °C for 6 h. Figure 2a shows the diffraction pattern of the physical mixture of benzoic acid and DM β CD where the diffraction peaks at

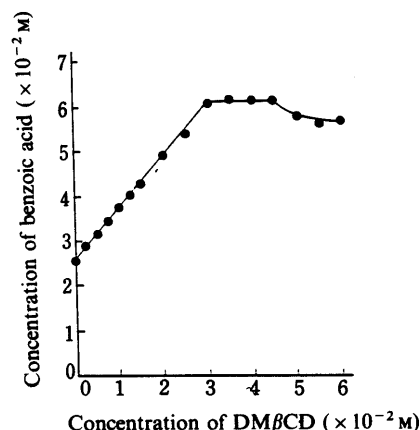


Fig. 1. Phase Solubility Diagram of Benzoic Acid with DM β CD in 0.1 N HCl at 30 °C

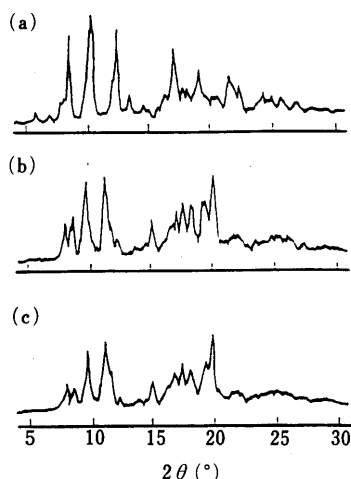


Fig. 2. X-Ray Diffraction Patterns of Benzoic Acid-DM β CD Systems
(a) Physical mixture (mixing molar ratio = 1:1). (b) Sample (a) was heated in an ampule at 90 °C for 6 h. (c) Inclusion compound prepared by coprecipitation method.

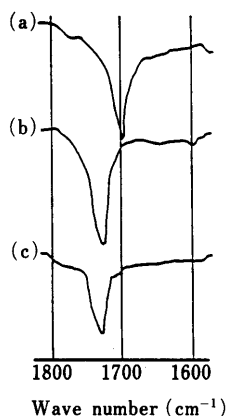


Fig. 3. Infrared Spectra of Benzoic Acid-DM β CD Systems
(a) Physical mixture (mixing molar ratio = 1:1). (b) Sample (a) was heated in an ampule at 90 °C for 6 h. (c) Inclusion compound prepared by coprecipitation method.

$2\theta = 12.3^\circ$ and 19.0° due to DM β CD crystal, and at $2\theta = 8.5^\circ$ due to benzoic acid crystal changed in respective intensity during heating. Figure 2b shows the pattern of the sealed heated sample at 90 °C for 6 h. By heating the sample, the typical diffraction peaks caused by intact crystals disappeared, and new diffraction peaks at $2\theta = 11.5^\circ$, 15.3° , and 19.5° appeared, indicating the formation of a new crystalline compound. The X-ray diffraction pattern of heated sample agreed with that of coprecipitated sample from a comparison of the X-ray diffraction patterns between the sealed heated and the coprecipitated samples (Fig. 2c). Therefore, it was indicated that the new compound formed by the sealed heat method was the inclusion complex of benzoic acid and DM β CD. Using this sealed heat method, however, it was noted that the inclusion formation was not completed.

Figure 3 shows the IR spectra of the benzoic acid and DM β CD system. A carbonyl stretching vibration band was observed at 1700 cm^{-1} in the physical mixture. The sealed heated sample and the coprecipitate showed the same pattern shifting to higher frequency of 1730 cm^{-1} , indicating the break of hydrogen bonding between dimeric benzoic acids in crystal. This result indicated that benzoic acid in the sealed heated sample exists in the same molecular state

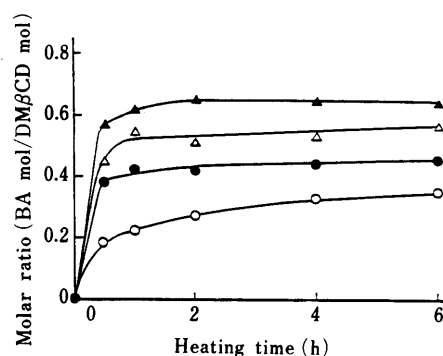


Fig. 4. Combining Molar Ratio of Benzoic Acid to DM β CD after Heating at Various Temperatures

Heating temperature: \circ , 60 °C; \bullet , 70 °C; \triangle , 80 °C; \blacktriangle , 90 °C.

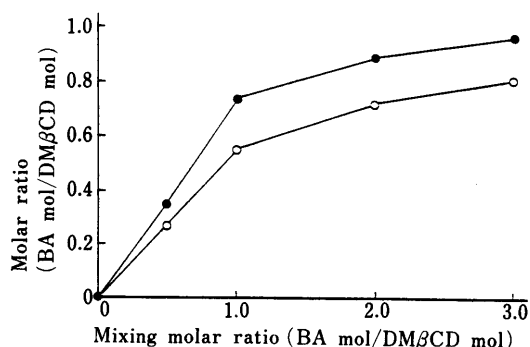


Fig. 5. Combining Molar Ratio of Benzoic Acid to DM β CD as a Function of Mixing Molar Ratio

Heated for 6 h at: \circ , 70 °C; \bullet , 90 °C.

as in the coprecipitated sample. It can thus be said that benzoic acid was included into DM β CD by the sealed heating method.

Inclusion Behavior under Various Conditions

(a) Effects of Heating Temperature and Heating Time on the Inclusion Formation The effects of heating temperature and heating time on the inclusion behavior were investigated. Since the amount of included benzoic acid varied with heating conditions, we defined the combining molar ratio of benzoic acid to DM β CD as the ratio of the number of mol of benzoic acid included to 1 mol of DM β CD. Figure 4 shows the combining molar ratio after heating the equimolar mixture of benzoic acid and DM β CD at temperatures of 60, 70, 80, and 90 °C. The value of combining molar ratio increased with an increase in the heating temperature. This ratio increased significantly at the initial heating period, for example, in the case of the sample heated at 60 °C; the values of the combining molar ratio were 0.22 at 1 h and 0.35 at 6 h. This tendency became more pronounced as the temperatures rose and it was assumed that the inclusion had two processes, a fast process at initial stage and a slow process after that.

When α -CD or β -CD was used as a host molecule, it was reported that the combining molar ratio increased with an increase of heating temperature^{6,7}; in the case of β -CD, the combining molar ratio particularly increased and became 1.0 after heating at 90 °C for 30 min, indicating the complete formation of the inclusion compound of benzoic acid and β -CD by the sealed heat method. In the case of α -CD, the results indicated that the changes in crystal form of α -

CD had an important role in the increase of the combining molar ratio.

As for DM β CD, no change in its crystal form was observed during heating, hence, the changes of physico-chemical state of guest molecules, such as sublimation, appeared to be an important factor inducing the inclusion reaction. The presence of DM β CD molecules which had no participation in the inclusion after heating was unique to the characteristic of the sealed heating of the DM β CD system.

(b) Effects of Mixing Molar Ratio (Benzoic Acid mol/DM β CD mol) on the Inclusion Behavior As the maximum combining molar ratio was observed as 0.62, which was much lower than 1.0 as shown in Fig. 4, it was considered that a large amount of unreacted DM β CD existed in the sealed heat samples. The initial mixing molar ratio of benzoic acid to DM β CD was successively changed. Figure 5 shows the combining molar ratio as a function of the initial mixing molar ratio after heating at 70 and 90 °C for 6 h, respectively. The combining molar ratio increased with an increase of the initial mixing molar ratio of benzoic acid to DM β CD, that is, the amounts of produced inclusion compound depended on the initial amount of benzoic acid. When the initial mixing molar ratio was less than 1.0, the combining molar ratio increased linearly, while for an initial ratio of over 1.0 the relationships showed apparent deviations from linearity. As the vapor pressure of benzoic acid is 0.127 mmHg at 60 °C, and 0.639 mmHg at 70 °C, the frequency of collision of benzoic acid molecules to the surface of DM β CD particles increases with the rise in temperature. Benzoic acid molecules permeate through a DM β CD particle from the surface to react and form the inclusion compound, so the inner part of the DM β CD particles may remain unreacted in the case of a mixing molar ratio of benzoic acid smaller than 1 : 1. This could be the reason for the agreement of the X-ray diffraction patterns of sealed heat sample (Fig. 2b) and of coprecipitated sample (Fig. 2c). When the molar ratio is larger than 1 : 1, which is enough to keep the vapor pressure saturated during the heating, the inclusion reaction can continue it to completion throughout the DM β CD particle, resulting in an increase in the combining molar ratio.

Effects of Crystallinity of DM β CD on the Inclusion Behavior To investigate the effects of crystallinity of DM β CD on the inclusion behavior, an amorphous DM β CD prepared by grinding was used as the host compound. The equimolar mixture of amorphous DM β CD with benzoic acid was heated in an ampule. Figure 6a shows the X-ray diffraction pattern of the physical mixture of benzoic acid and amorphous DM β CD. After heating at 70 °C for 30 min, the physical mixture crystallized into the inclusion compound. Figure 7 shows the changes in combining molar ratio after heating at 60, 70, 80, and 90 °C for 6 h. At the heating of 70, 80, and 90 °C, the combining molar ratio increased with an increase of the heating time, and high values of combining molar ratio were obtained. The ratio increased significantly at the initial heating period in the same manner as when crystalline DM β CD was used as host molecule, while, in the case of the sample heated at 60 °C, this ratio increased gradually during 4 h heating in the same manner as the crystalline DM β CD-benzoic acid physical mixture. After 6 h heating, however, the combin-

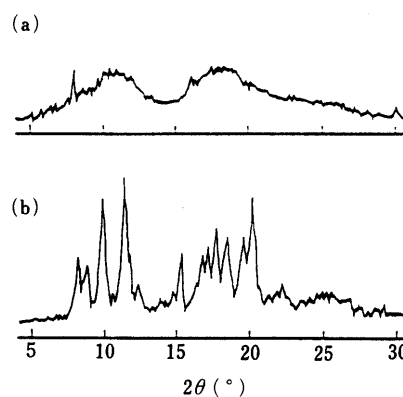


Fig. 6. Changes of X-Ray Diffraction Patterns by Heating in Ampule (a) Physical mixture (mixing molar ratio = 1 : 1). (b) Sample (a) was heated in an ampule at 70 °C for 30 min.

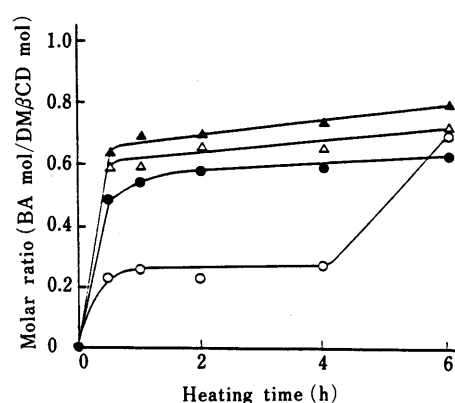


Fig. 7. Combining Molar Ratio of Benzoic Acid to DM β CD after Heating at Various Temperatures (DM β CD was Amorphous before Heating)

Heating temperature: ○, 60 °C; ●, 70 °C; △, 80 °C; ▲, 90 °C.

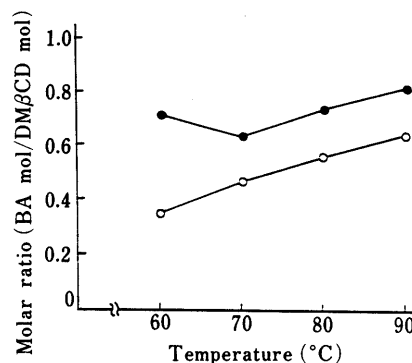


Fig. 8. Effects of DM β CD Crystallinity on Combining Molar Ratio of Benzoic Acid to DM β CD by Heating at Various Temperatures for 6 h

●, amorphous DM β CD + benzoic acid (1 : 1); ○, crystalline DM β CD + benzoic acid (1 : 1).

ing molar ratio increased greatly. In X-ray diffraction experiments, the crystallization of these samples into inclusion crystal was observed after heating at 70, 80, and 90 °C within 30 min.

In the case of sample heated at 60 °C, it was not until 6 h heating that the sample crystallized into the inclusion crystal. In the physical mixture with amorphous DM β CD, the crystallization of inclusion compound appeared to be an important factor in the increase of the combining molar

ratio.

Figure 8 shows a comparison of combining molar ratio between the amorphous DM β CD–benzoic acid equimolar system and the crystalline DM β CD–benzoic acid equimolar system after 6 h heating at various temperatures. Amorphous DM β CD–benzoic acid systems showed higher combining molar ratios at all temperatures than the crystalline DM β CD–benzoic acid systems. It can thus be stated that the disordered state in DM β CD molecular arrangement has high reactivity for the inclusion of benzoic acid with DM β CD, and yields higher values of combining molar ratio than the crystalline DM β CD.

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References

- 1) F. M. Anderson and H. Bundgaard, *Int. J. Pharmaceut.*, **19**, 189 (1984).
- 2) M. Kikuchi, F. Hirayama, and K. Uekama, *Int. J. Pharmaceut.*, **38**, 191 (1987).
- 3) K. Kurozumi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **23**, 3062 (1975).
- 4) Y. Nakai, K. Yamamoto, K. Terada, and K. Akimoto, *Chem. Pharm. Bull.*, **32**, 685 (1984).
- 5) Y. Nakai, S. Nakajima, K. Yamamoto, K. Terada, and T. Konno, *Chem. Pharm. Bull.*, **26**, 3419 (1978).
- 6) Y. Nakai, K. Yamamoto, K. Terada, and D. Watanabe, *Chem. Pharm. Bull.*, **35**, 4609 (1987).
- 7) Y. Nakai, K. Yamamoto, K. Terada, T. Oguchi, D. Watanabe, and H. Saito, *Chem. Pharm. Bull.*, **37**, 1055 (1989).
- 8) R. J. Bergeron, M. P. Meeley, and Y. Machida, *Bioorg. Chem.*, **5**, 121 (1976).
- 9) T. Imai, T. Irie, M. Otagiri, and K. Uekama, *J. Incl. Phenom.*, **2**, 597 (1984).
- 10) B. W. Müller and U. Brauns, *Int. J. Pharmaceut.*, **26**, 77 (1985).
- 11) Y. Nakai, *Yakugaku Zasshi*, **107**, 802 (1987).
- 12) T. Imai, M. Otagiri, H. Saito, and K. Uekama, *Chem. Pharm. Bull.*, **36**, 354 (1988).
- 13) K. Uekama, *Pharm. Int.*, **3**, 61 (1985).
- 14) G. A. El-Gendy, K. Terada, K. Yamamoto, and Y. Nakai, *Int. J. Pharmaceut.*, **31**, 25 (1986).
- 15) H. Okamoto, H. Komatsu, M. Hashida, and H. Sezaki, *Int. J. Pharmaceut.*, **30**, 35 (1988).
- 16) K. Uekama, M. Masaki, K. Arimori, T. Irie, and F. Hirayama, *Yakugaku Zasshi*, **107**, 449 (1987).
- 17) H. P. R. Bootsma, H. W. Frijlink, A. Eissens, J. H. Proost, H. Van Doorne, and C. F. Lerk, *Int. J. Pharmaceut.*, **52**, 213 (1989).
- 18) Y. Nakai, K. Yamamoto, K. Terada, A. Kajiyama, and I. Sasaki, *Chem. Pharm. Bull.*, **34**, 2178 (1986).
- 19) Y. Nakai, K. Yamamoto, K. Terada, and I. Sasaki, *Yakugaku Zasshi*, **106**, 420 (1986).
- 20) T. Higuchi and K. A. Connors, *Adv. Anal. Chem. Instr.*, **4**, 117 (1965).
- 21) K. Tanabe and M. Yamazaki, *Yakuzaigaku*, **46**, 196 (1986).