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## New Methods for Preparing Cyclodextrin Inclusion Compounds. I. Heating in a Sealed Container

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A new method for preparing a solid inclusion compound was developed. A physical mixture of benzoic acid and  $\alpha$ - or  $\beta$ -cyclodextrin (CD) or the ground mixture was sealed in a container after adsorbing a definite amount of water vapor, then heated to a temperature ranging from 43 to 142°C. The results of powder X-ray diffraction and infrared spectroscopy showed that the crystalline inclusion compound was produced when the container was heated at over 70°C. The combining molar ratio of benzoic acid to  $\alpha$ -CD in the inclusion compound prepared by this method was higher than that obtained by the coprecipitation method. Physical mixtures of benzoic acid and  $\alpha$ - or  $\beta$ -CD were also sealed in a stainless steel vessel under nitrogen gas pressure, then heated to 127°C. The pressurized samples had a higher combination ratio than the non-pressurized samples.

**Keywords**—cyclodextrin; inclusion complex formation; heating; X-ray diffraction; combining ratio; infrared spectrum

It is well known that cyclodextrins (CDs) form inclusion compounds with many kinds of drugs and improve the physical and chemical properties of the drugs.<sup>1)</sup> Solid inclusion compounds have been prepared by coprecipitation,<sup>2)</sup> kneading,<sup>3)</sup> freeze-drying,<sup>2)</sup> and co-grinding.<sup>4)</sup> In the present study, we attempted to obtain inclusion compounds by heating mixtures of  $\alpha$ - or  $\beta$ -cyclodextrin and a drug. As inclusion compounds were obtained by this method, the physicochemical properties were compared with those of inclusion compounds by the coprecipitation method in terms of powder X-ray diffraction and infrared (IR) spectroscopy.

### Experimental

**Materials**— $\alpha$ -CD and  $\beta$ -CD were purchased from Nakarai Chemicals Ltd. and Andou Kasei Co., respectively. The CD hydrates were obtained by recrystallization from distilled water. The dried  $\alpha$ - and  $\beta$ -CDs were prepared by heating the CD hydrates at 110°C for 3 h *in vacuo* for grinding. Benzoic acid was of JPX grade.

**Preparation of Physical Mixture and Ground Mixture**—The physical mixture was prepared by simple blending of benzoic acid and  $\alpha$ - or  $\beta$ -CD with a mortar and pestle. The molar mixing ratio of benzoic acid to CD was 1 : 1 or 1 : 2. A ground mixture was obtained by grinding the physical mixture for 5 min using a vibrational mill as described previously.<sup>5)</sup> The ground mixtures were stored in an atmosphere of 48.3% relative humidity at 40°C, and allowed to adsorb water vapor until adsorption equilibrium was attained.<sup>6)</sup> A Hiranuma AQ-3C aquacounter was used to determine the water content.

**Heating in a Sealed Container**—Two methods were used. In one, about 1 g of the mixture was sealed in a glass ampule (the volume was 2.25 ml), which was heated at a definite temperature (43–142°C). In the other, about 1 g of the physical mixture of benzoic acid with  $\alpha$ - or  $\beta$ -CD hydrate was sealed in a stainless steel vessel (the volume was 27.5 ml), which was heated at 127°C for 10–90 min with or without pressurization by N<sub>2</sub> gas. The N<sub>2</sub> gas pressure was measured with a pressure gauge (Nagano, SUS 316). The amount of benzoic acid combining with CD was determined spectrophotometrically after washing the contents of the vessel with ethyl ether to remove the excess benzoic acid.

**Preparation of Inclusion Compounds by the Coprecipitation Method**—An aqueous solution containing benzoic acid and  $\alpha$ -CD hydrate or benzoic acid and  $\beta$ -CD hydrate was agitated vigorously for 5 h at 60 °C. The solution was filtered and the filtrate was allowed to stand for 2 d at room temperature to allow the crystallization of the inclusion compound. After filtration, the coprecipitate was dried for 24 h at room temperature and washed with ethyl ether, then dried again for 24 h at room temperature. The amount of benzoic acid in the inclusion compounds was determined spectrophotometrically.

**Powder X-Ray Diffraction Measurement**—A Rigaku Denki 2027 diffractometer was used. The measurement conditions were the same as those reported in the previous paper.<sup>5)</sup>

**Thermal Analysis**—A Shimadzu DT-20B unit was used for thermogravimetry (TG). The heating rate was 1 K/min. A Perkin-Elmer DSC-1B was used for differential scanning calorimetry (DSC) at a scanning speed of 4 K/min using a liquid cell.

**IR Absorption Spectroscopy**—A Hitachi 295 infrared spectrophotometer was used. The measurements were made by the nujol method.

## Results and Discussion

### Effects of Heating on the Crystal Properties of $\alpha$ - and $\beta$ -CD

The  $\alpha$ -CD hydrate contained 6 water molecules as water of crystallization (9.99% water content).<sup>7)</sup> The amorphous  $\alpha$ -CD hydrate (10.2% water content) was prepared by grinding of dried  $\alpha$ -CD and water vapor adsorption. Figure 1 shows the X-ray diffraction patterns of various  $\alpha$ - and  $\beta$ -CDs. Two kinds of amorphous  $\alpha$ -CDs, namely dried and H<sub>2</sub>O-adsorbed,

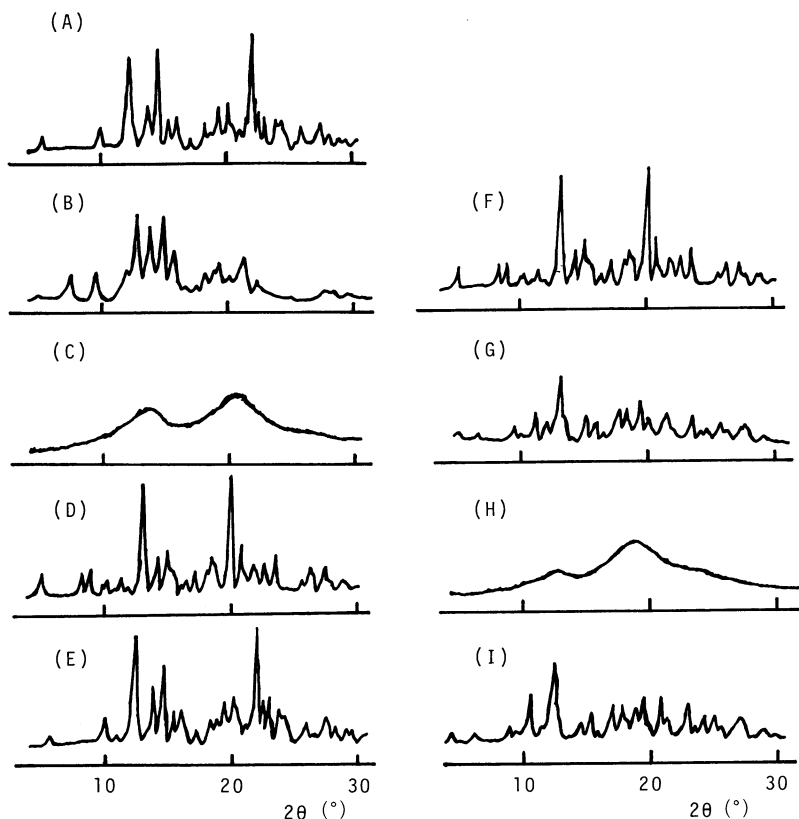


Fig. 1. Powder X-Ray Diffraction Patterns of Various CDs

(A),  $\alpha$ -CD hydrate (form A); (B), dried  $\alpha$ -CD; (C), ground amorphous  $\alpha$ -CD (H<sub>2</sub>O adsorbed); (D), heated A at 90 °C for 1 h; (E), heated C at 70 °C for 1 h; (F), heated C at 90 °C for 1 h; (G),  $\beta$ -CD hydrate; (H), ground amorphous  $\beta$ -CD (H<sub>2</sub>O adsorbed); (I), heated H at 90 °C for 1 h.

have the same halo patterns (curve C), while the crystalline  $\alpha$ -CD hydrate and the dried  $\alpha$ -CD showed different X-ray diffraction patterns from each other (curves A and B). The X-ray diffraction patterns of  $\alpha$ -CDs after heating in glass ampules are also shown in Fig. 1 (curves D to F). The hydrated samples of the crystalline and amorphous  $\alpha$ -CD showed changes in X-ray diffraction patterns on heating, while no change was observed for the dried crystalline and the dried amorphous  $\alpha$ -CD. The polymorphic transition of the crystalline  $\alpha$ -CD hydrate from form A to B on heating was ascertained from the facts that the endothermic peak appeared at 78 °C on the DSC curve, and that the water content did not change before and after the heating. It was also found that the amorphous  $\alpha$ -CD hydrate recrystallized to form A of  $\alpha$ -CD hydrate on heating at below 78 °C (curve E), and to form B of the hydrate on heating at over 78 °C (curve F). On the other hand,  $\beta$ -CD hydrate showed no polymorphic transition. Only the crystallization of amorphous  $\beta$ -CD hydrate to crystalline  $\beta$ -CD hydrate was observed, as shown in Fig. 1 (curves G-I).

### Formation of Inclusion Compound by Heating of Physical Mixture of Benzoic Acid with $\alpha$ - or $\beta$ -CD Hydrate in Ampules

Figure 2 shows the powder X-ray diffraction patterns of the mixtures of benzoic acid and either  $\alpha$ - or  $\beta$ -CD hydrate before and after heating in glass ampules. The diffraction patterns after heating were consistent with the patterns of the respective inclusion compounds obtained by the coprecipitation method. Irrespective of the kind of CDs and the initial mixing ratio of benzoic acid to CDs, it was found that the inclusion compounds were formed by heating in the

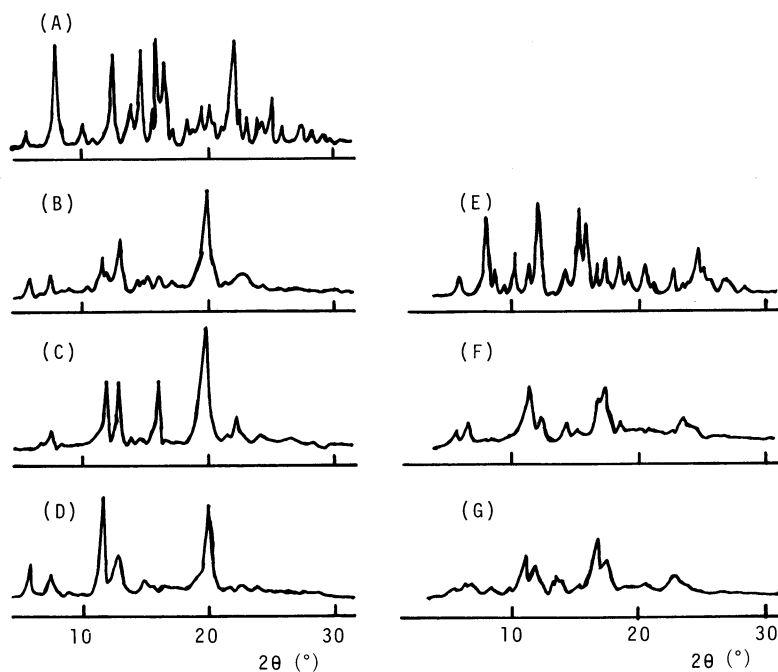


Fig. 2. Powder X-Ray Diffraction Patterns of Benzoic Acid (BA)-CD Hydrate System

(A), physical mixture of BA and  $\alpha$ -CD hydrate (initial mixing molar ratio (IMR) of BA to CD = 1 : 1); (B), mixture of BA and  $\alpha$ -CD hydrate heated at 90 °C for 30 min (IMR = 1 : 2); (C), mixture of BA and  $\alpha$ -CD hydrate heated at 90 °C for 30 min (IMR = 1 : 1); (D), BA- $\alpha$ -CD inclusion compound prepared by the coprecipitation method; (E), physical mixture of BA and  $\beta$ -CD hydrate (IMR = 1 : 1); (F), mixture of BA and  $\beta$ -CD hydrate heated at 90 °C for 30 min (IMR = 1 : 1); (G), BA- $\beta$ -CD inclusion compound prepared by the coprecipitation method.

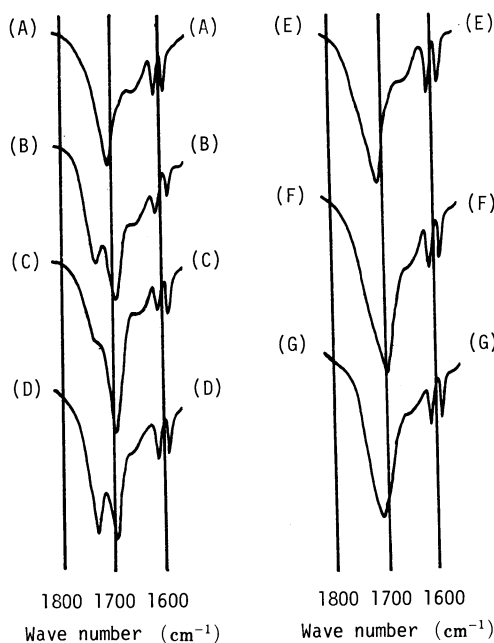


Fig. 3. IR Spectra of BA-CD Hydrate Systems  
(A)-(G), same as in Fig. 2.

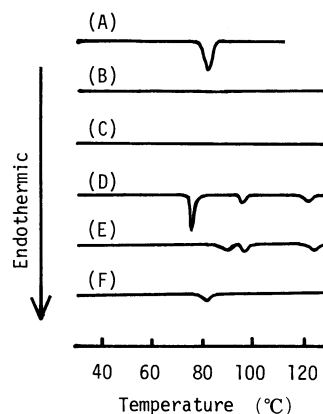


Fig. 4. DSC Curves of CD and BA-CD Hydrate Systems

(A),  $\alpha$ -CD hydrate (form A); (B),  $\alpha$ -CD hydrate (form B); (C),  $\beta$ -CD hydrate; (D), physical mixture of BA and  $\alpha$ -CD hydrate (form A) (IMR = 1:1); (E), physical mixture of BA and  $\alpha$ -CD hydrate (form B) (IMR = 1:1); (F), physical mixture of BA and  $\beta$ -CD hydrate (IMR = 1:1).

ampules.

For  $\beta$ -CD, the combining ratio of benzoic acid to  $\beta$ -CD was 1:1 in both the coprecipitation complex and the complex obtained by heating in ampules. For  $\alpha$ -CD, the combining ratio of benzoic acid to  $\alpha$ -CD was 1:2 in the coprecipitation complex. In the heating method, however, the combining ratio varied with the experimental conditions; increasing initial mixing ratio and increasing heating temperature caused an increase in the combining ratio of the inclusion compound, and finally the combining ratio approached about 4:5. As the combining ratio increased, the water content of the inclusion compound decreased.

IR spectra of the various complexes are shown in Fig. 3. As the benzoic acid molecules are dimerized by strong hydrogen bonding in the crystal, the carbonyl stretching vibration band appeared at  $1704\text{ cm}^{-1}$ , as reported previously.<sup>8)</sup> The carbonyl bands of benzoic acid in the  $\alpha$ -CD inclusion compound prepared by the coprecipitation method appeared at  $1740$  and  $1698\text{ cm}^{-1}$ . These bands are considered to be due to free and hydrogen-bonded carbonyls, respectively.<sup>9)</sup> The  $\alpha$ -CD inclusion compound of 1:2 ratio obtained by the heating method showed nearly the same IR spectrum to that obtained by the coprecipitation method except for minor intensity differences. However, benzoic acid in the  $\alpha$ -CD inclusion compound having a higher combining ratio showed a strong carbonyl absorption band at  $1698\text{ cm}^{-1}$  and a shoulder at  $1740\text{ cm}^{-1}$ . It was suggested that the hydrogen-bonded dimer was dominant for benzoic acid in the  $\alpha$ -CD inclusion compound of high combining ratio, and the molecular interaction mode was somewhat different from that of the 1:2 inclusion compound.

The IR spectra of  $\beta$ -CD systems were in good accordance with those of the coprecipitate and the heated complexes, indicating the practical usefulness of the heating method for preparing inclusion compounds similar to those obtained by the coprecipitation method.

Figure 4 shows the DSC curves of  $\alpha$ - and  $\beta$ -CD hydrate and the physical mixtures of benzoic acid with  $\alpha$ - or  $\beta$ -CD hydrate. The endothermic peaks at  $78^\circ\text{C}$  in the  $\alpha$ -CD hydrate

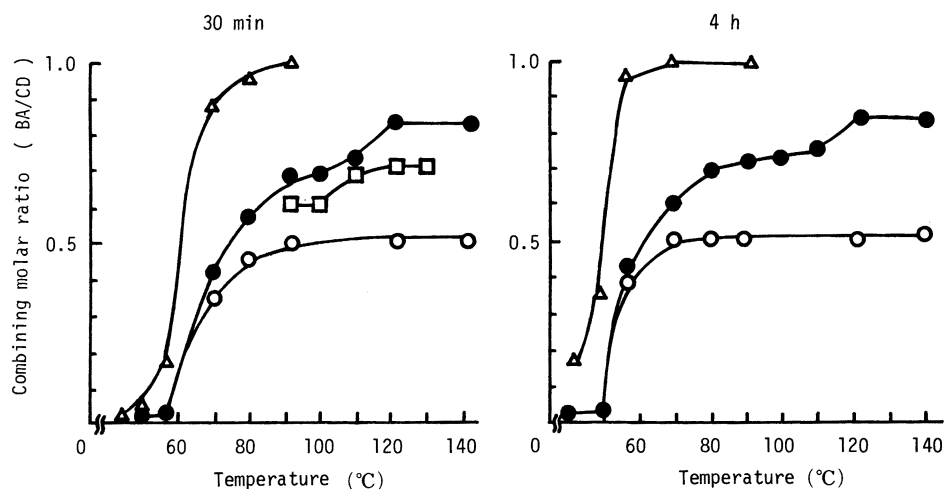


Fig. 5. Combining Molar Ratio of BA to CDs after Heating in Ampules for 30 min or 4 h at Various Temperatures

○, BA- $\alpha$ -CD hydrate (form A) (IMR=1:2); ●, BA- $\alpha$ -CD hydrate (form A) (IMR=1:1); □, BA- $\alpha$ -CD hydrate (form B) (IMR=1:1); △, BA- $\beta$ -CD hydrate (IMR=1:1).

(form A) and in the  $\alpha$ -CD hydrate (form A)–benzoic acid system were due to the transformation into polymorphous  $\alpha$ -CD (form B). The  $\beta$ -CD hydrate did not show any peaks below 130 °C. The small endothermic peaks, at 93 °C in curve D, at 87 and 93 °C in curve E and at 82 °C in curve F could be attributed to the inclusion of benzoic acid in CD molecules. The endothermic peaks at 121 °C in curves D and E were due to the melting of excess benzoic acid crystals.

Figure 5 shows the combining ratios of benzoic acid with  $\alpha$ -CD or  $\beta$ -CD in various mixtures after heating in ampules at a definite temperature (43–142 °C) for 30 min or 4 h. The physical mixture of benzoic acid with  $\beta$ -CD hydrate changed to the inclusion compound of 1:1 combining ratio after heating at 70 °C for 4 h or at 90 °C for 30 min. The mixture of benzoic acid with  $\alpha$ -CD hydrate (form A) formed the inclusion compound of 1:2 combining ratio after heating at 70 °C for 4 h or at 90 °C for 30 min, when the initial mixing ratio of benzoic acid to  $\alpha$ -CD was 1:2. In the case of the mixtures of benzoic acid with  $\alpha$ -CD hydrate, where the initial mixing ratio was 1:1, the higher combining ratio inclusion compounds were obtained, while only the 1:2 inclusion compound was obtained by the coprecipitation method. In the  $\alpha$ -CD–benzoic acid complex crystal, it was suggested that the  $\alpha$ -CD molecules had a channel structure and that the benzoic acid molecules existed in that structure.<sup>10)</sup> In the inclusion complex obtained by the heating method, the benzoic acid appeared to exist in a more than stoichiometric ratio with respect to the channel structure.

#### Heating of Physical Mixtures of Benzoic Acid with Amorphous CDs and Heating of Ground Mixtures

Figure 6 shows the effects of heating in ampules on the powder X-ray diffraction patterns of physical mixtures of benzoic acid with amorphous  $\alpha$ -CD hydrate and on those of ground mixtures of benzoic acid with  $\alpha$ -CD stored in an atmosphere of 55% relative humidity after grinding. After heating at 60 °C for 30 min, the ground mixture crystallized into the inclusion compound (curves E–H). From the changes in the X-ray pattern of the physical mixture, however, both the transformation to the inclusion compound and the crystallization of  $\alpha$ -CD (form B) were observed. As the heating temperature was increased, the X-ray diffraction patterns came to coincide with that of the inclusion compound even in the case of the physical

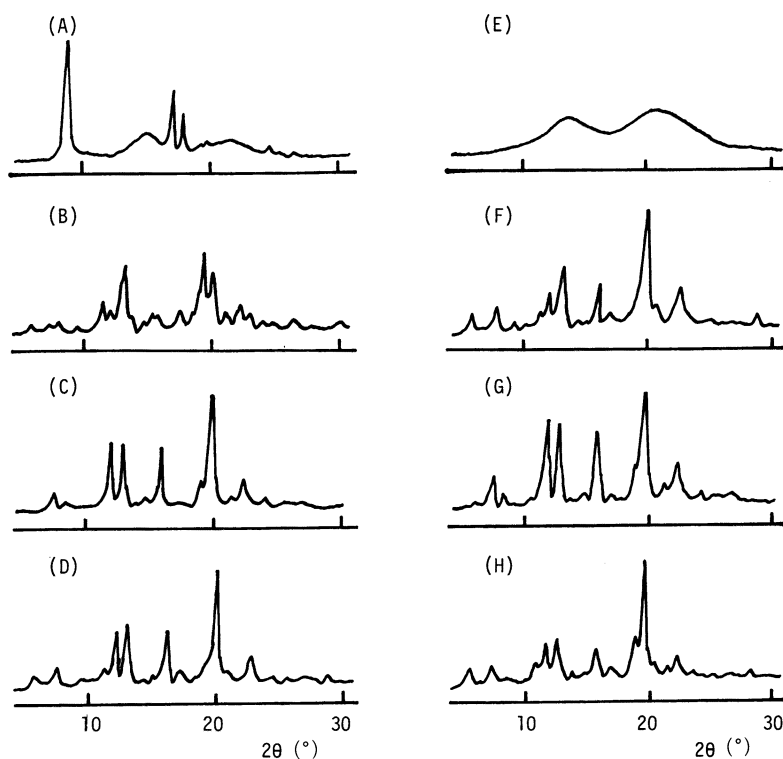


Fig. 6. Powder X-Ray Diffraction Patterns of BA-Amorphous CD Systems

(A), physical mixture of BA and amorphous  $\alpha$ -CD (IMR = 1 : 1); (B), heated A at 60 °C for 30 min; (C), heated A at 90 °C for 30 min; (D), heated A at 125 °C for 30 min; (E), ground mixture of BA and  $\alpha$ -CD (IMR = 1 : 1); (F), heated E at 60 °C for 30 min; (G), heated E at 90 °C for 30 min; (H), heated E at 125 °C for 30 min.

TABLE I. Combining Molar Ratio of Benzoic Acid to Cyclodextrins after Heating in Ampules at Various Temperatures for 30 min  
(mol of benzoic acid/mol of CD)

	Heated for 30 min at		
	60 °C	90 °C	125 °C
Physical mixture with amorphous $\alpha$ -CD <sup>a)</sup>	0.57	0.72	0.81
Ground mixture with $\alpha$ -CD <sup>a)</sup>	0.86	0.70	0.80
Physical mixture with amorphous $\beta$ -CD <sup>a)</sup>	0.90	1.00	
Ground mixture with $\beta$ -CD <sup>a)</sup>	1.00	1.00	

a) IMR = 1 : 1.

mixture. When  $\beta$ -CD was used instead of  $\alpha$ -CD, similar results were obtained, *i.e.*, the formation of the  $\beta$ -CD benzoic acid inclusion compound by heating in ampules was observed.

Table I shows the combining ratio of benzoic acid to  $\alpha$ -CD or  $\beta$ -CD after the heating of various mixtures. In the physical mixture with amorphous  $\alpha$ -CD hydrate, increasing heating temperature caused an increase of the combining ratio, as observed in the physical mixture with  $\alpha$ -CD hydrate. In the ground mixture with  $\alpha$ -CD, the combining ratio did not depend on the heating temperature. This result is ascribed to the occurrence of molecular interaction

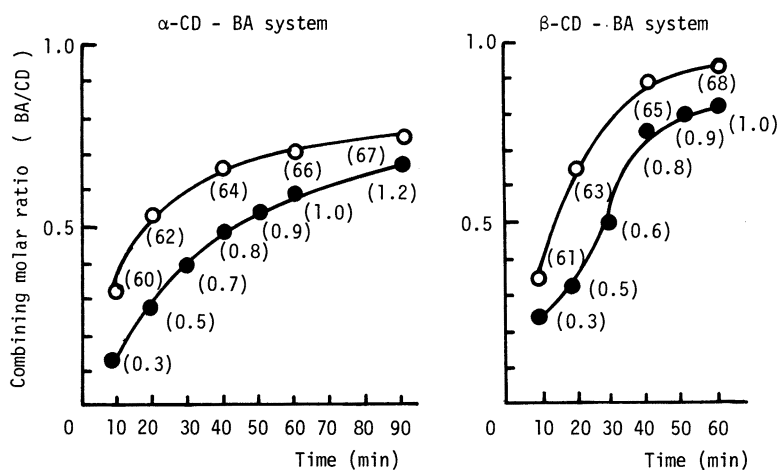


Fig. 7. Effect of  $N_2$  Pressure on the Combining Molar Ratio of BA to CD after Heating at  $127^\circ C$  in a Stainless Steel Vessel

○, under pressure; ●, without pressure. Numbers in parentheses indicate gas pressure ( $kg \cdot cm^{-2}$ ).

between benzoic acid and CD molecules during the grinding. In the case of  $\beta$ -CD mixtures, a constant ratio of 1:1 was observed, regardless of the heating temperature and the dispersed state.

#### Effects of $N_2$ Gas Pressure on the Inclusion Formation by Heating

Figure 7 shows the relationship between the combining ratio and the heating time with or without  $N_2$  gas pressure. The mixtures used were the benzoic acid- $\alpha$ -CD hydrate physical mixture (initial mixing molar ratio = 1:1) and the benzoic acid- $\beta$ -CD hydrate physical mixture (initial mixing molar ratio = 1:1). A high  $N_2$  gas pressure caused an increase in the combining ratio of the inclusion complex, although the complex formation rates were different from the cases when the samples were heated in ampoules. The data in Fig. 7 also indicate an important role of the water vapor pressure on the inclusion formation. Finally, when physical mixtures of benzoic acid with dried CDs were heated in a stainless steel vessel, the pressure change was very small and the inclusion complex was not obtained.

In conclusion, the sealed heating method is a new and effective method to prepare inclusion compounds of organic molecules with CDs, as it is very simple and does not require any dissolution process. Further investigations are in progress to extend this method to other organic molecules and to elucidate the mechanism of the inclusion phenomenon.

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