New Methods for Preparing Cyclodextrin Inclusion Compounds. II.[1] Effects of Heating Temperature, Water Content and Drug Properties on the Inclusion Formation

Yoshinobu NAKAI,* Keiji YAMAMOTO, Katsuhide TERADA,[2a] Toshio OGUCHI, Hironobu SAITO[2b] and Daiichi WATANABE[2c]

Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoicho, Chiba 260, Japan. *Received September 24, 1988*

Inclusion compounds were prepared by heating mixtures of a drug and α-cyclodextrin (α-CD) hydrate in a sealed container. Inclusion formation was studied as a function of heating temperature, water content of α-CD and drug properties, using powder X-ray diffraction, infrared spectroscopy, and differential scanning calorimetry. Drug molecules were included in α-CD molecules by heating above 80 °C in ampules. Inclusion compounds were prepared with six different drugs. The rates of inclusion formation were well correlated with the vapor pressures of the drugs, that is, higher vapor pressure of drugs resulted in faster inclusion compound formation. It was revealed that the heating temperature, water content of α-CD, and vapor pressure of the drug are important parameters affecting the formation of inclusion compounds by the heating method.

Keywords cyclodextrin; inclusion complex formation; heating; X-ray diffraction; differential scanning calorimetry; combining ratio

Cyclodextrins (CDs) are known to form inclusion compounds and are used in various research fields.[3] In the pharmaceutical field, they are applicable to the enhancement of solubility and to the stabilization of drugs.[4] There are various methods for preparing solid inclusion compounds, such as coprecipitation and freeze-drying.[5] In the previous paper, we reported that inclusion compounds were obtained by the heating of a drug–CD mixture in a sealed container.[1]

In the present study, the effects of temperature, water content of α-CD and drug properties on inclusion compound formation by the heating method were investigated using powder X-ray diffraction, infrared (IR) spectroscopy and differential scanning calorimetry (DSC).

Experimental

Materials α-CD was purchased from Nakarai Chemicals Ltd. α-CD hydrate was prepared by keeping the original α-CD in a desiccator at a relative humidity (RH) of 79.4% at 30 C. Benzoic acid, salicylic acid, and methyl *p*-hydroxybenzoate were of JP X grade. *p*-Hydroxybenzoic acid, *m*-hydroxybenzoic acid, and *p*-nitroaniline were of special reagent grade.

Preparation of Inclusion Compound (1) Coprecipitation Method: As all the drugs used in this study showed B_s-type phase solubility diagrams with α-CD,[6a] the inclusion compounds were prepared as described previously.[6b]

(2) Heating of Mixture in a Sealed Container: Each drug was mixed with α-CD hydrate at the molar ratio of 1 : 1. About 400 mg of the mixture was enclosed in a 2 or 50 ml glass ampule and was heated at the desired temperature for the specified time. Then the sample was washed with ethyl ether to remove the uncomplexed drug.

Measurement of Water Content Water contents were varied by drying α-CD hydrate or by adding water to α-CD hydrate. The Karl Fischer method was used to determine water content.

Powder X-Ray Diffraction A Rigaku Denki 2027 powder X-ray diffractometer was used. Powder X-ray diffraction patterns were measured as described previously.[7]

Differential Scanning Calorimetry (DSC) A Perkin Elmer DSC-1B was used. The measurement conditions were as follows: heating rate, 2 K/min; range, 2 mcal/s. A liquid pan was used.

Determination of the Combining Ratio The combining ratio of drug to α-CD was determined by the ultraviolet (UV) absorption method using a Shimadzu UV-200S spectrophotometer after dissolving the complex in 1 : 1 ethanol water mixture (methyl *p*-hydroxybenzoate) or 0.1 N HCl aqueous solution (other drugs).

Results and Discussion

Inclusion Compound Formation by Heating of a Mixture in Ampules It has already been reported that *p*-hydroxybenzoic acid and methyl *p*-hydroxybenzoate form inclusion compounds with α-CD by the coprecipitation method.[8,9] Therefore, these two drugs were selected as model compounds for the investigation of the new method of inclusion compound preparation. Figure 1 shows the powder X-ray diffraction patterns of the inclusion compounds obtained by the coprecipitation method (A) and of the complexes obtained by the heating method (B). The diffraction patterns of inclusion compounds obtained by the coprecipitation method (A) agreed well with the results reported by Uekama *et al.*[9] Diffraction patterns of the complexes obtained by the heating method were the same as those of the respective inclusion compounds obtained by the coprecipitation method. The data in Fig. 1 confirm that the crystalline inclusion compounds were obtained by the heating method (B).

IR spectra of the obtained complexes of *p*-hydroxybenzoic acid and methyl *p*-hydroxybenzoate with α-CD were measured for both preparations, as shown in Fig. 2. IR spectra of the inclusion compounds obtained by the heating method coincided with those of the respective coprecipitated inclusion compounds. The results of powder X-ray diffraction and IR spectroscopy revealed that the inclusion compounds obtained by the heating method had

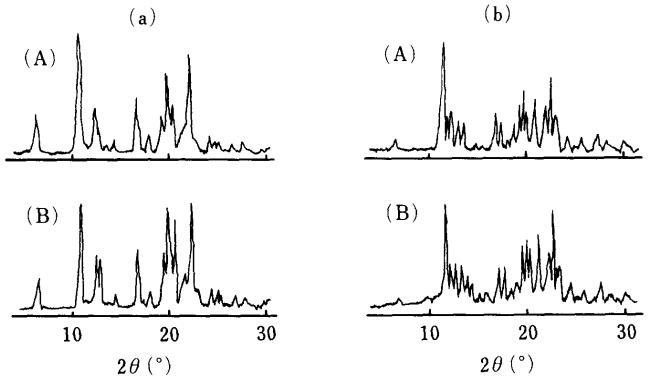

Fig. 1. Powder X-Ray Diffraction Patterns of Drug–α-CD Complexes

(a) Methyl *p*-hydroxybenzoate. (b) *p*-Hydroxybenzoic acid. A: Inclusion compound prepared by the coprecipitation method. B: Complex obtained by the sealed heating method.

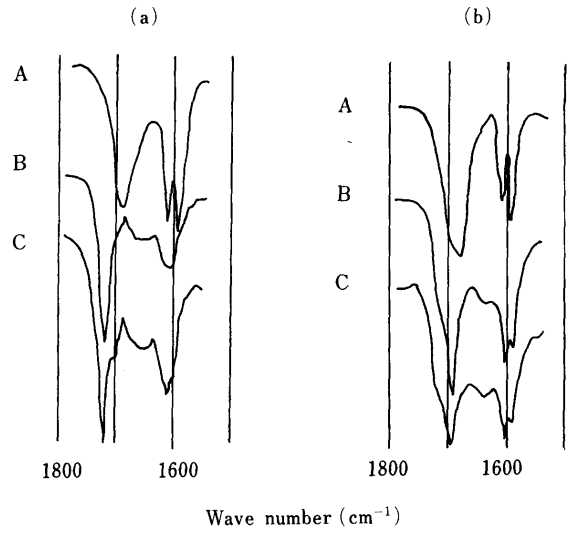

Fig. 2. IR Spectra of Drug–α-CD Complexes

(a) Methyl *p*-hydroxybenzoate. (b) *p*-Hydroxybenzoic acid. A: Drug alone. B: Inclusion compound prepared by the coprecipitation method. C: Complex obtained by the sealed heating method.

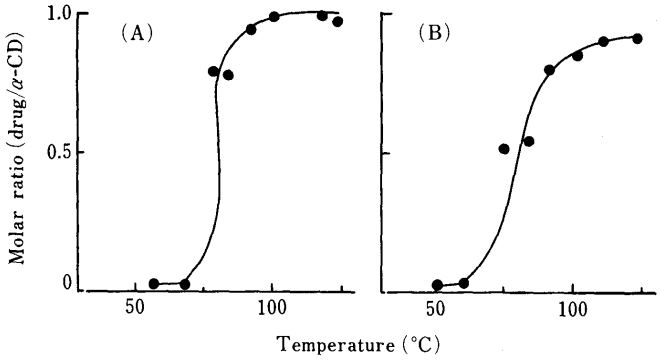

Fig. 3. Effects of Heating Temperature on the Combining Ratio of Drug to α-CD after Heating for 2 h in Ampules

A: Methyl *p*-hydroxybenzoate. B: *p*-Hydroxybenzoic acid.

the same physical properties as the coprecipitated inclusion compounds.

Effects of Temperature and Water Content on the Inclusion Compound Formation The effects of temperature on the inclusion compound formation of drugs to α-CD were studied for the *p*-hydroxybenzoic acid–α-CD and methyl *p*-hydroxybenzoate–α-CD systems. Figure 3 shows the combining molar ratio of drug to α-CD hydrate after storage at various temperatures for 2 h in ampules. For the methyl *p*-hydroxybenzoate–α-CD system, the combining ratio increased sharply at about 80 °C and then reached 1.0 at above 100 °C. On the other hand, the *p*-hydroxybenzoic acid system showed a slightly different pattern; the inclusion formation was less complete at about 80 °C and the combining ratio increased quite gradually compared with that in the methyl *p*-hydroxybenzoate–α-CD system. It was concluded that inclusion formation generally begins above 80 °C, but each drug would require a different heating temperature and storage time to complete the inclusion formation by this method.

In the above experiments, we used α-CD hydrate in which the water content was about 10%. The effects of water content of α-CD on the inclusion compound formation reaction were studied in the methyl *p*-hydroxybenzoate–α-CD system. The water content of α-CD was varied from 2 to 20% as shown in Fig. 4. Figure 4 also illustrates the influence of ampule volume, 2 or 50 ml, on the inclusion formation. At low water contents, no inclusion formation of methyl *p*-hydroxybenzoate with α-CD was observed at 100 °C for 2 h. At more than 8% water content in the 2 ml ampule and 16% in the 50 ml ampule, however, complete formation of the 1 : 1 inclusion compound was observed. The effects of water content could be explained by the behavior of water of crystallization. Namely, at less than 7% water content of α-CD before heating, α-CD was dehydrated by heating as reported previously[10] and this made it impossible to form the inclusion compound with methyl *p*-hydroxybenzoate. The increase in the ampule volume caused an increase in the water content required to start the inclusion reaction. This was considered to be due to the difference of free space in ampules, that is, much more water existed as water vapor at 100 °C in the 50 ml ampule compared to the 2 ml ampule.

Effects of Drug Properties on the Inclusion Compound Formation Takeo and Kuge reported that α-CD formed solid inclusion compounds with many organic compounds, such as benzoic acid and salicylic acid.[11] Inclusion compounds were prepared with six drugs (benzoic acid, salicylic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, methyl *p*-hydroxybenzoate and *p*-nitroaniline) by the heating

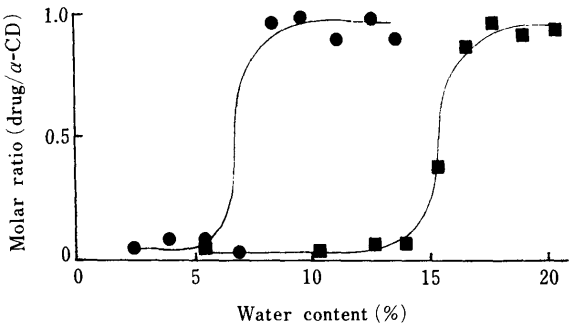

Fig. 4. Effects of Water Content of α-CD on the Combining Ratio of Methyl *p*-Hydroxybenzoate to α-CD after Heating at 100 °C for 2 h in Ampules

●: Ampule of 2 ml volume. ■: Ampule of 50 ml volume.

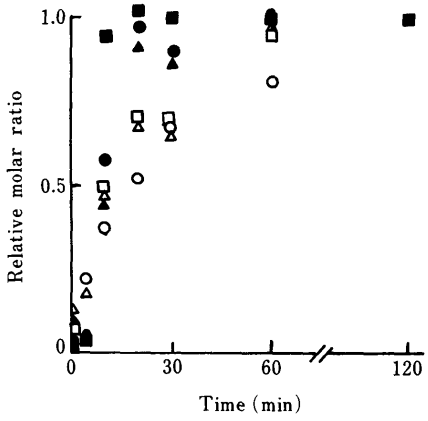

Fig. 5. Relative Molar Ratio of Drug to α-CD as a Function of Heating Time at 110 °C in Ampules

●: Methyl *p*-hydroxybenzoate. ■: Benzoic acid. ▲: Salicylic acid. ○: *p*-Hydroxybenzoic acid. □: *m*-Hydroxybenzoic acid. △: *p*-Nitroaniline.

TABLE I. Vapor Pressure[12] and Solubility in Water

Drug	Vapor pressure at 100 °C (mmHg)	Solubility in water at 25 °C ($\times 10^{-2}$ mol·l^{-1})
Benzoic acid	1.26	2.8
Salicylic acid	0.397	1.7
Methyl *p*-hydroxybenzoate	—	1.5
p-Hydroxybenzoic acid	0.00030	4.2
m-Hydroxybenzoic acid	0.00149	5.5
p-Nitroaniline	0.0136	0.2

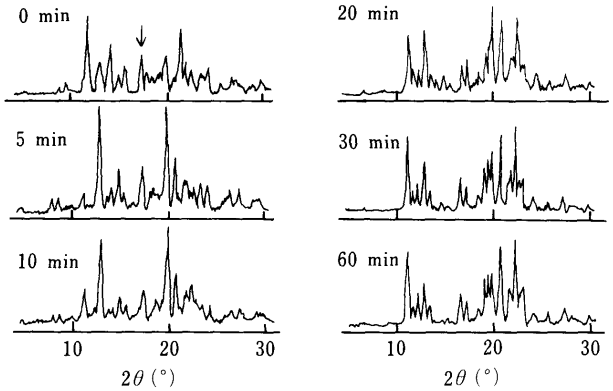

Fig. 6. Powder X-Ray Diffraction Patterns of a Mixture of *p*-Hydroxybenzoic Acid and α-CD as a Function of Heating Time at 110 °C in Ampules

The arrow shows an X-ray diffraction peak of crystalline *p*-hydroxybenzoic acid crystals.

method, and the effects of drug properties on the inclusion compound formation were investigated. Figure 5 shows the relative molar ratio of drugs to α-CD as a function of heating time at 110 °C, where relative molar ratio means the relative value of the drug–α-CD molar ratio in the inclusion compound at a given time to that at 120 min. Methyl *p*-hydroxybenzoate, benzoic acid, and salicylic acid formed inclusion compounds with α-CD rapidly as compared with *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, and *p*-nitroaniline. Table I lists the vapor pressures[12] at 100 °C and solubilities in water at 25 °C of the drugs used in this study. The rates of inclusion compound formation were well correlated with the vapor pressures of drugs rather than the solubilities, that is, greater vapor pressures of drugs resulted in faster inclusion compound formation. Hence, the sublimation of the drug, together with heating temperature and water content, is an important factor for inclusion compound formation by this method.

Drug Inclusion Process into α-CD on Heating the Mixture in a Sealed Container Figure 6 shows the changes of powder X-ray diffraction patterns of the physical mixtures of *p*-hydroxybenzoic acid–α-CD hydrate as a function of heating time at 110 °C in ampules. From the time course of the X-ray diffraction patterns, it can be seen that each characteristic diffraction peak was changed with heating time. As we reported previously, α-CD has two polymorphic forms; form A is stable below 78 °C and form B is stable above 78 °C. The X-ray diffraction peaks of crystalline *p*-hydroxybenzoic acid and of form A α-CD were observed in the patterns of the physical mixture as well as of the 3 min heated sample. After 5 min of heating, new

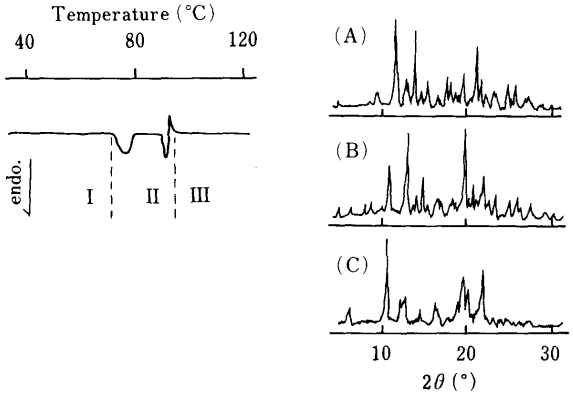

Fig. 7. DSC Curve and Powder X-Ray Diffraction Patterns of a Mixture of Methyl *p*-Hydroxybenzoate and α-CD

A: Heated to 70 °C. B: Heated to 85 °C. C: Heated to 90 °C.

diffraction peaks due to form B α-CD appeared. After that, the characteristic peaks due to form B α-CD ($2\theta = 13.0$, 20.0°) decreased. At the same time, the peaks due to the inclusion compound ($2\theta = 11.5$, 20.5, 22.0°) increased. Finally, only the peaks due to the inclusion compound were apparent after 30 min. These results show that the polymorphic transition from form A α-CD to form B α-CD took place before the inclusion compound formation.

Figure 7 shows the powder X-ray diffraction patterns and the DSC curve of the methyl *p*-hydroxybenzoate–α-CD hydrate system. Each X-ray diffraction pattern was measured after heating in ampules up to 70, 85 and 95 °C at a heating rate of 2 K/min, respectively. Below the temperature of the first endothermic peak (state I in DSC), the X-ray diffraction pattern showed the superimposed peaks of form A α-CD and crystalline methyl *p*-hydroxybenzoate. After the first endothermic peak (state II in DSC), the pattern showed X-ray diffraction peaks of form B α-CD and crystalline methyl *p*-hydroxybenzoate. When the mixture was heated over 93 °C (state III in DSC), the characteristic powder pattern of the inclusion compound of methyl *p*-hydroxybenzoate and α-CD was observed. These results indicate that the first endothermic peak was due to the transition of α-CD from form A to form B and the second endothermic peak was due to the inclusion compound formation between form B α-CD and methyl *p*-hydroxybenzoate. The second peak was followed by the exothermic peak that was due to the crystallization of the inclusion compound. Thus, it was suggested that polymorphic transition of α-CD from form A to form B has an important role in the inclusion compound formation of the drug with α-CD.

From the above results, the following factors are concluded to influence inclusion compound formation, (1) heating temperature, (2) water content and crystal form of α-CD, and (3) sublimation of the drug.

References and Notes

1) Part I: Y. Nakai, K. Yamamoto, K. Terada and D. Watanabe, *Chem. Pharm. Bull.*, **35**, 4609 (1987).
2) Present address: *a*) *Faculty of Pharmaceutical Sciences, Toho University, 2–2–1 Miyama, Funabashi, Chiba 274*; *b*) *Sankyo Co., Ltd., 2–58, Hiromachi 1-chome Shinagawaku, Tokyo 140*; *c*) *Fujirebio Co., Ltd., 51, Komiyacho Hachioji Tokyo, 192, Japan.*
3) H. Taguchi, N. Kunieda and M. Kinoshita, *Makromol. Chem.*, **184**,

925 (1983).
4) *a*) K. Uekama, T. Fujinaga, M. Otani and H. Seo, *J. Pharmacobio-Dyn.*, **4**, 726 (1981); *b*) M. Otagiri, T. Miyaji, K. Uekama and K. Kinoshita, *J. Pharm. Sci.*, **61**, 1841 (1972).
5) M. Kurozumi, N. Nambu and T. Nagai, *Chem. Pharm. Bull.*, **23**, 3062 (1975).
6) *a*) T. Higuchi and K. A. Conners, *Adv. Anal. Chem. Instr.*, **4**, 117 (1966); *b*) Y. Nakai, K. Yamamoto, K. Terada and K. Akimoto, *Chem. Pharm. Bull.*, **32**, 685 (1984).
7) Y. Nakai, S. Nakajima, K. Yamamoto, K. Terada and T. Konno, *Chem. Pharm. Bull.*, **26**, 3419 (1978).
8) K. Harata, *Bull. Chem. Soc. Jpn.*, **50**, 1416 (1977).
9) K.Uekama, Y. Ikeda, F. Hirayama, M. Otagiri and M. Shibata, *Yakugaku Zasshi*, **100**, 994 (1980).
10) Y. Nakai, K. Yamamoto, K. Terada, A. Kajiyama and I. Sasaki, *Chem. Pharm. Bull.*, **34**, 2178 (1986).
11) K. Takeo and T. Kuge, *Agric. Biol. Chem.*, **34**, 1787 (1970).
12) *a*) N. Y. Sidgwick, *J. Chem. Soc.* (London), **117**, 396 (1920); *b*) N. Y. Sidgwick and H. E. Rubie, *ibid.*, **119**, 1013 (1921).