April 1996 Chem. Pharm. Bull. 44(4) 833—836 (1996) 833

Formation of a Heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin-*p*-nitrophenol Inclusion Compound by Sealed-Heating

Daiichi WATANABE,[1a)] Masato OHTA,[1b)] Zhi Jun YANG, Etsuo YONEMOCHI, Toshio OGUCHI, and Keiji YAMAMOTO*

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan.

Received November 2, 1995; accepted December 19, 1995

The complexes of heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin (DM*β*CD) and *p*-nitrophenol (*p*-NP), which were prepared by sealed-heating and a slow evaporation method, were comparatively evaluated. From the results of differential scanning calorimetry, powder X-ray diffraction, and infrared spectroscopy, the sealed-heated sample was considered as a new complex which was different from the slow evaporation complex. Also, the data from a sublimation experiment indicated that the inhibition of sublimation of *p*-NP was observed only in the sealed-heated sample. These results suggested that *p*-NP molecules were included within the cavity of DM*β*CD molecules, whereas in case of the slow evaporation complex, *p*-NP molecules were located in the intermolecular space among the DM*β*CD molecules.

Key words heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin; *p*-nitrophenol; sealed-heating; inclusion compound; thermal analysis

In the previous paper, we reported that an inclusion compound was obtained by heating the physical mixture of cyclodextrin (CD) and a drug in a sealed container.[2-5] When the physical mixture of heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin (DM*β*CD) and benzoic acid (BA) was heated in a glass ampoule, an inclusion compound was formed, and the combining molar ratio of BA to DM*β*CD increased with an increase in the heating temperature. The mechanism was described as follows: the guest molecules were sublimated then adsorbed to the surface of DM*β*CD particles, followed by inclusion within the cavity of the DM*β*CD crystals. The non-sublimable guest molecules could not be included within the cavity of CDs as the sublimation of the guest molecules was important in the first step.[3] *p*-Nitrophenol (*p*-NP) was included within the cavity of the DM*β*CD in the aqueous solution,[6] whereas in the solid complex prepared by the slow evaporation method, the *p*-NP molecule was not included within the CD cavity, but was located in the intermolecular space among DM*β*CD molecules.[7,8] In this report, we discussed the formation mechanism of an inclusion compound of *p*-NP and DM*β*CD made by a sealed-heating method, as well as the physicochemical properties of the sealed-heated sample.

Experimental

Materials DM*β*CD was purchased from Toshin Chemical Co. and used without further purification. *p*-NP was of special reagent grade purchased from Wako Pure Chemical Industries, Ltd.

Preparation of Sealed-Heated Sample An equimolar physical mixture of DM*β*CD and *p*-NP was prepared by blending them in a vial using a vortex mixer. The mixture (300 mg) was sealed in a 2 ml glass ampoule. Then, the ampoule was heated in an oven at a heating rate of 8 °C/min from 40 °C to 100 °C, and was kept at 100 °C for a definite time. The temperature around the glass ampoule in the oven was monitored by thermocouple.

Preparation of Slow Evaporation Complex 2.857×10^{-3} mol of DM*β*CD was dissolved in 40 ml of distilled water and 2.857×10^{-3} mol of *p*-NP was added, then stirred. The solution was kept at room temperature and was allowed to evaporate slowly.

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

Infrared Absorption Spectroscopy Measurements were carried out by the Nujol method using a Nicolet FT-IR spectrophotometer (5ZDX).

Thermal Analysis DSC measurements 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 10 °C/min.

Heating Experiment in an Aluminum Cell An equimolar physical mixture of DM*β*CD and *p*-NP was filled in a 0.8 ml aluminum cell, and the cell was well closed with aluminum foil as previously described.[9] The well-closed cell was heated at a heating rate of 8 °C/min, and the heated sample was obtained when the sample was heated to a desired temperature.

Sublimation Experiment Sublimation of *p*-NP from various preparations (30 mg) by heat treatment at 100 °C and reduced pressure was determined. The remaining sample was dissolved in a 0.1 N HCl solution, and the quantity of remaining *p*-NP was assayed by the UV absorption method.

Results and Discussion

An equimolar physical mixture of DM*β*CD and *p*-NP was sealed-heated at 100 °C for a definite time. Figure 1 shows the powder X-ray diffraction patterns of the equimolar physical mixture of DM*β*CD and *p*-NP. The

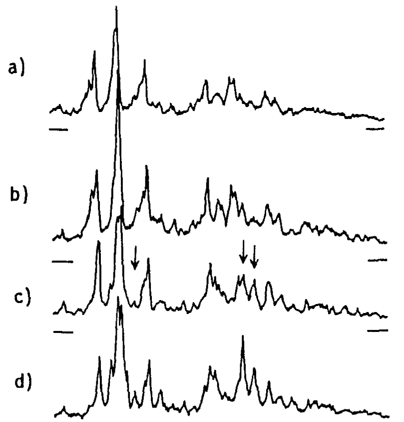

Fig. 1. Powder X-Ray Diffraction Patterns of Sealed-Heated Mixture of *p*-NP and DM*β*CD (Mixing Molar Ratio 1 : 1) at 100 °C

Heating time; a) 0 h, b) 6 h, c) 12 h, d) 24 h.

---

* To whom correspondence should be addressed.

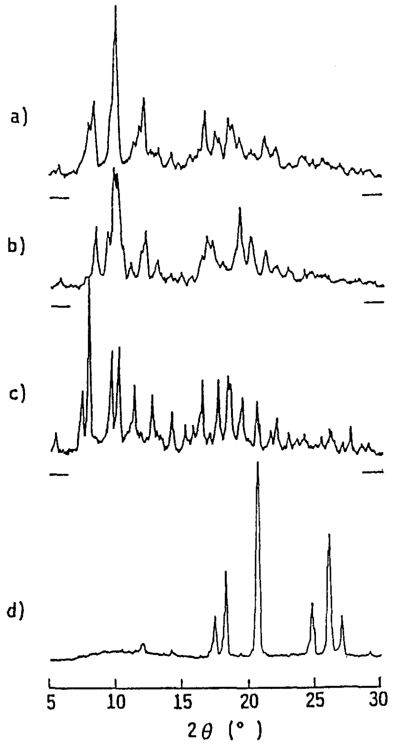

Fig. 2. Powder X-Ray Diffraction Patterns of p-NP-DMβCD Systems

a) Physical mixture (mixing molar ratio 1 : 1), b) sealed-heated sample (heated at 100 °C for 24 h), c) complex prepared by slow evaporation of aqueous solution, d) p-NP.

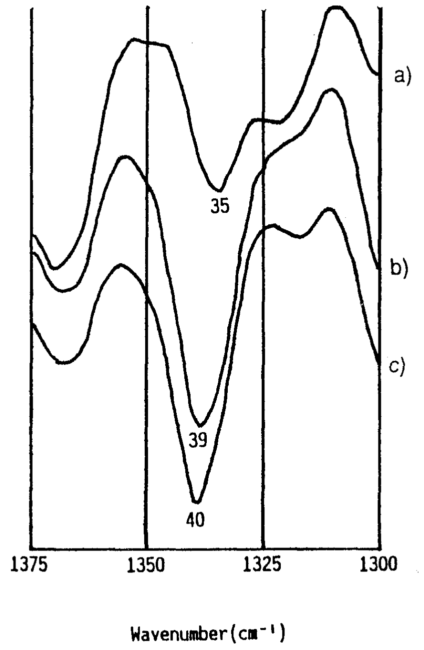

Fig. 3. Infrared Spectra of p-NP-DMβCD Systems

a) Physical mixture (mixing molar ratio 1 : 1), b) sealed-heated sample (heated at 100 °C for 24 h), c) complex prepared by slow evaporation of aqueous solution.

diffraction pattern of the physical mixture (curve a) was simply a superposition of those of two components, *i.e.* the diffraction peaks at $2\theta=9.7°$ and $2\theta=8.1°$ were due to DMβCD crystals, and the peak at $2\theta=18.6°$ was due to p-NP crystal. Curves b, c and d show the powder X-ray diffraction patterns of the physical mixture of DMβCD and p-NP after sealed heating at 100 °C for 6, 12 and 24 h respectively. After 6 h of heating, the diffraction peaks observed in curve a were still the same. However, the diffraction peaks due to DMβCD crystals and p-NP crystals disappeared, and new diffraction peaks appeared at $2\theta=11.0°$, 19.3° and 20.1° when the mixture was heated for 12 h. The intensities of these new diffraction peaks were increased up to 24 h and no change was found after heating for more than 24 h. As no changes were observed in the X-ray diffraction patterns when only DMβCD crystal was sealed-heated for 24 h at 100 °C, the changes observed in the sealed-heated process were presumed to be related to the molecular interaction between DMβCD and p-NP molecules.

Figure 2 shows the powder X-ray diffraction patterns of various p-NP-DMβCD systems. The X-ray diffraction peak positions of the slow evaporation complex (curve c) were identical to the theoretical 2θ values calculated from the unit-cell constants ($a=14.779$ Å, $b=18.965$ Å, $c=28.741$ Å) reported by Harata.[7,8] Thus, it was confirmed that p-NP molecules were located in the intermolecular space among DMβCD molecules. The powder X-ray diffraction pattern of the sealed-heated sample (curve b), however, was different from those of the slow evaporation complex and the physical mixture. These results indicated that the sealed-heated sample formed a new complex which was distinctly different from the slow evaporation complex.

To confirm the results from X-ray diffractometry, IR spectroscopy was employed. Figure 3 shows the IR spectra of various p-NP-DMβCD systems. Curve a shows the IR spectrum of the physical mixture. A stretching vibration band of the NO_2 group was observed at 1335 cm^{-1}. However, the stretching vibration band of the NO_2 group was shifted to a higher wave number (1335—1339 cm^{-1}) in the spectrum of the sealed-heated sample (curve b). The shift of stretching vibration band was considered to be related to the formation of a complex of p-NP and DMβCD by sealed-heating. The sealed-heating process caused the disappearance of intermolecular hydrogen bonding formed in crystals between the nitro group and the hydroxyl group of p-NP molecules.[10,11] Curve c shows the spectrum of the slow evaporation complex showing the peak of the NO_2 stretching vibration band at 1340 cm^{-1}. In the slow evaporation complex, p-NP molecules are not included within the DMβCD cavity, but are located in the intermolecular crevice which had a highly hydrophobic property.[7] A small peak at 1318 cm^{-1} was observed in the IR spectrum of the slow evaporation complex, while in the IR spectrum of the sealed-heated sample, only a small peak was observed at 1318 cm^{-1}. This peak might indicate the difference in molecular state of p-NP between the sealed-heated sample and the slow evaporation complex.

Thermal properties were determind by DSC using a sealed pan which was considered to be similar in condition to the inside of the glass ampoule. Figure 4 shows the DSC curves of various p-NP-DMβCD systems. p-NP (curve a) showed an endothermic peak due to the fusion at 113 °C, whereas no peak was detected in the case of DMβCD. Two endothermic peaks were observed at 135 °C and 195 °C in the physical mixture (curve c) which indicated that some changes took place in the *p*-NP crystals before they reached 113 °C in the DSC measurement. One endothermic peak was observed at 195 °C in the sealed-heated sample (curve d), and this curve was different from the DSC curve of the slow evaporation complex. The slow evaporation complex showed endothermic peaks at 145 °C and 195 °C (curve e).

To clarify about the endothermic peaks which were found in the DSC thermogram of the physical mixture, a heating experiment in an aluminum cell was employed. The results from this experiment were investigated by X-ray diffractometry and IR spectroscopy, and are shown in Figs. 5 and 6, respectively. The physical mixture and the sample heated to 80 °C showed the same X-ray diffraction pattern (Fig. 5, curves a and b) and IR spectra (Fig. 6, curves a and b). These results indicated that no complex formation occured. The sample heated to 190 °C, which was the temperature between the two endothermic peaks, showed some changes both in the powder X-ray diffraction pattern (Fig. 5, curve d) and in the IR spectrum (Fig. 6, curve d). They were exactly identical to those of the sealed-heated sample. In the previous report,[4] two endothermic peaks were also observed in the DSC curve of the physical mixture of DMβCD and BA, and the endothermic peak at a lower temperature originated from the formation of an inclusion compound. Thus, the endothermic peak at 135 °C in this study was considered to correspond to the formation of an inclusion compound as well. The sample which was heated up to 220 °C (Fig. 5, curve e) showed the same powder X-ray diffraction pattern as intact DMβCD crystal (Fig. 5, curve f). The IR spectrum (Fig. 6, curve e) demonstrated that the NO_2 stretching peak intensity was decreased as compared with the sample heated at a lower temperature (Fig. 6, curve d). All of these results indicated that the endothermic peak at 195 °C was considered to represent the elimination of *p*-NP from the inclusion compound. From the DSC curve of the sealed-heated sample (Fig. 4, curve d), an endothermic peak at 135 °C was not observed, while the endothermic peak at 195 °C was clearly observed. This indicated that the sealed-heated sample was a complex in which *p*-NP molecules had already been included within the cavities of the DMβCD molecules. The peak at 195 °C simply explained the elimination of *p*-NP from the inclusion compound.

The endothermic peak which was found in the DSC thermogram of the slow evaporation complex (Fig. 4, curve e) was also investigated by a heating experiment in an aluminum cell (data not shown). The powder X-ray diffraction pattern of the slow evaporation complex heated to 180 °C was identical to that of the sealed-heated sample. The powder X-ray diffraction pattern of the slow

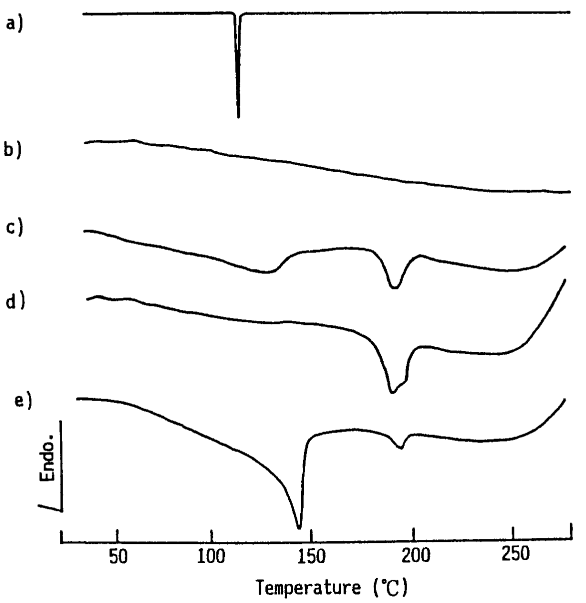

Fig. 4. DSC Curves of *p*-NP-DMβCD Systems

a) *p*-NP, b) DMβCD, c) physical mixture (mixing molar ratio 1 : 1), d) sealed-heated sample (heated at 100 °C for 24 h), e) complex prepared by slow evaporation of aqueous solution.

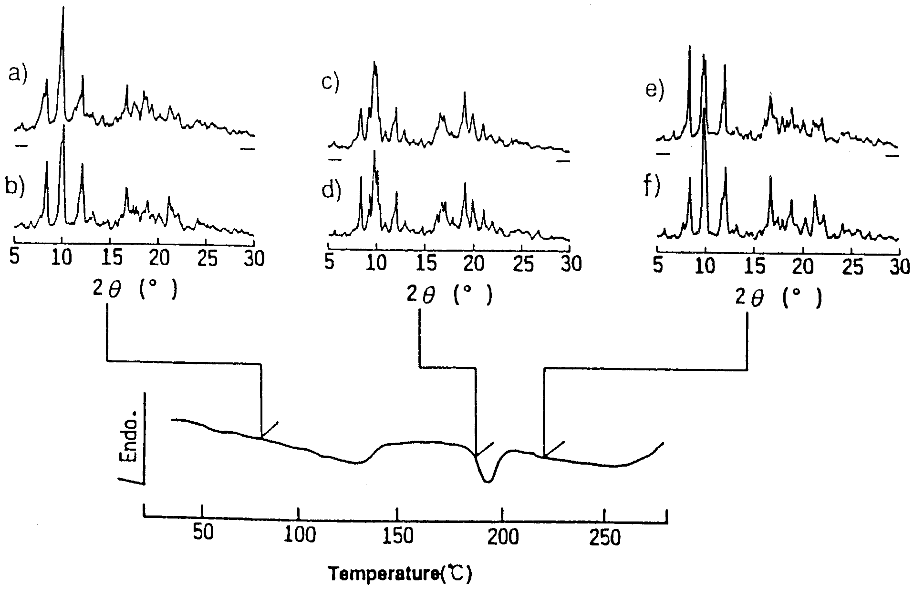

Fig. 5. Powder X-Ray Diffraction Patterns and DSC Curve of the Mixture of *p*-NP and DMβCD

a) Physical mixture (mixing molar ratio 1 : 1), b) heated to 80 °C in aluminum sealed cell, c) sealed-heated sample (heated at 100 °C for 24 h), d) heated to 190 °C in aluminum sealed cell, e) heated to 220 °C in aluminum sealed cell, f) DMβCD.

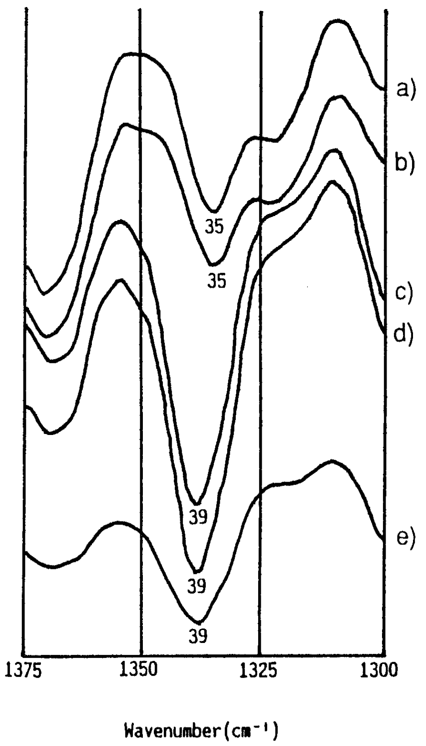

Fig. 6. Infrared Spectra of the Mixture of *p*-NP and DM*β*CD

a) Physical mixture (mixing molar ratio 1 : 1), b) heated to 80 °C in aluminum sealed cell, c) sealed-heated sample (heated at 100 °C for 24 h), d) heated to 190 °C in aluminum sealed cell, e) heated to 220 °C in aluminum sealed cell.

evaporation complex heated to 220 °C was identical to that of DM*β*CD crystals alone. So, it may be concluded that the endothermic peak at 145 °C was considered to be related to the formation of an inclusion compound, and the endothermic peak at 195 °C resulted from the elimination of *p*-NP from the inclusion compound.

Recently, Kawashima *et al.* reported an inclusion compound formation of a new kind between Naphthalene and DM*β*CD by a sealed-heating method,[12] and they also discussed a difference in the drug elimination between inclusion compounds. The sublimation behavior of *p*-NP from the physical mixture, the sealed-heated sample, and the slow evaporation complex under reduced pressure at 100 °C was investigated. Figure 7 shows the changes in *p*-NP contents under reduced pressure at 100 °C. The physical mixture and the slow evaporation complex showed faster sublimation as compared with the sealed-heated sample. After 10 h of sublimation treatment, the physical mixture and the slow evaporation complex showed a lower combining molar ratio of 0.23 and 0.30, respectively, while the sealed-heated sample showed a high combining molar ratio of 0.79. As all samples had similar high crystallinity, the differences in sublimation behavior are likely related to the molecular state of *p*-NP in each preparation. From the slow evaporation complex in which the *p*-NP molecules were located in the intermolecular space of DM*β*CD and the physical mixture in which *p*-NP molecules existed separately from DM*β*CD, sublimation took place more easily than in the sealed-heated sample. Therefore, the sealed-heated sample was considered to be one in which the *p*-NP molecules are located in the cavity of DM*β*CD molecules.

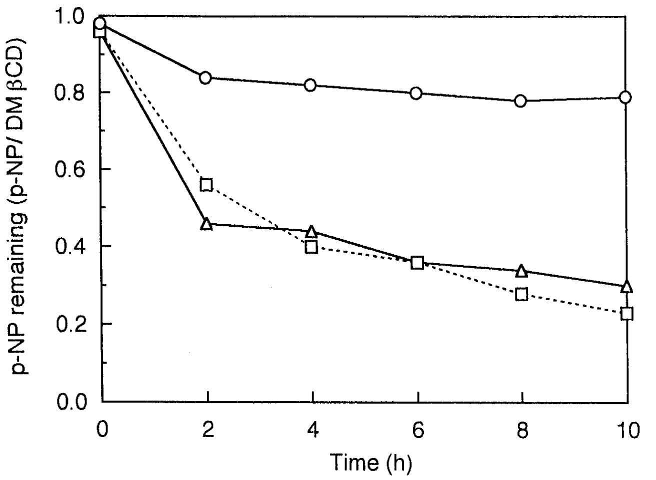

Fig. 7. Sublimation of *p*-NP from Various Preparations by Heat Treatment at 100 °C and Reduced Pressure

○, Sealed-heated sample (heated at 100 °C for 24 h); □, physical mixture (mixing molar ratio 1 : 1); △, complex prepared by slow evaporation of aqueous solution.

From all of the results mentioned above, the sealed-heated sample was estimated to be a different complex from the slow evaporation complex, and the sealed-heated sample might be an inclusion compound in which the *p*-NP molecules are located in the cavity of DM*β*CD molecules.

Acknowledgment This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (07672309).

References

1) Present address: *a) Fujirebio, Inc., 51 Komiya-cho, Hachioji, Tokyo 192, Japan*; *b) Meiji Seika Kaisha, Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama* 222, *Japan.*
2) Nakai Y., Yamamoto K., Terada K., Watanabe D., *Chem. Pharm. Bull.*, **35**, 4609—4615 (1987).
3) Nakai Y., Yamamoto K., Terada K., Oguchi T., Saitou H., Watanabe D., *Chem. Pharm. Bull.*, **37**, 1055—1058 (1989).
4) Nakai Y., Yamamoto K., Oguchi T., Yonemochi E., Hanawa T., *Chem. Pharm. Bull.*, **38**, 1345—1348 (1990).
5) Nakai Y., Yamamoto K., Oguchi T., Yonemochi E., Hanawa T., *Chem. Pharm. Bull.*, **39**, 1532—1535 (1991).
6) Nakai Y., Yamamoto K., Terada K., Oguchi T., Nakamura G., *Yakugaku Zasshi*, **107**, 802—807 (1987).
7) Harata K., *Bull. Chem. Soc. Jpn.*, **61**, 1939—1944 (1988).
8) Harata K., *Chem. Lett.*, 1641—1644 (1984).
9) Hanawa T., Nakai Y., Yonemochi E., Oguchi T., Yamamoto K., *Netsu Sokutei*, **22**, 11—15 (1995).
10) Coppens P., Schmidt G. M. J., *Acta Crystallogr.*, **18**, 62—67 (1965).
11) Coppens P., Schmidt G. M. J., *Acta Crystallogr.*, **18**, 654—663 (1965).
12) Kawashima H., Yonemochi E., Oguchi T., Yamamoto K., *J. Chem. Soc., Faraday Trans.*, **90**, 3117—3119 (1994).