Study of the Interaction of Clobazam with Cyclodextrins in Solution and in the Solid State

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Inclusion complexes of clobazam with α-, β-, γ-cyclodextrins (CyDs) and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) in aqueous solution and in the solid phase were studied by the solubility method, infrared (IR) spectroscopy, differential scanning calorimetry (DSC), and X-ray diffractometry. In addition, inclusion complex of clobazam with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin and the solid dispersion of clobazam with methyl cellulose (MC) in a ground mixture were investigated by IR, DSC and X-ray diffractometry. It was observed that DM-β-CyD had the highest stability constant among the four CyDs in solution. Thermal and X-ray diffraction analyses showed that clobazam molecules existed in a molecularly dispersed state in the ground mixture of CyDs. Infrared spectra showed lower frequency shifts in the case of the ground mixtures of clobazam with natural CyDs, which can be attributed to the formation of hydrogen bonds between the two carbonyl groups of clobazam and hydroxyl groups of natural CyDs. In contrast, higher frequency shifts were observed in the case of the ground mixtures of clobazam with methylated CyDs and MC and these were considered to be due to the monomolecular dispersion of clobazam in a hydrophobic environment. The mode of interaction of clobazam with DM-β-CyD was different from that with natural CyDs in the ground mixtures. Furthermore, the crystalline inclusion complex of clobazam with DM-β-CyD was obtained by heating of the coprecipitate *in vacuo* at 120 °C for 1 h.

Keywords clobazam; cyclodextrin; dimethyl-β-cyclodextrin; methyl cellulose; ground mixture; inclusion compound; interaction

Clobazam is widely used as an antianxiety agent and it has anticonvulsant effects in psychotherapy.[1–4] It has 1,5-nitrogen atoms in contrast to the widely prescribed 1,4-benzodiazepines. Cyclodextrins (CyDs) have been extensively applied to improve the solubility, dissolution, stability, and bioavailability of various poorly soluble drugs.[5–8] Recently, chemically modified cyclodextrins have received considerable attention in many fields, because their physicochemical properties are different from those of natural CyDs, *e.g.* heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and heptakis(2,3,6-tri-*O*-methyl)-β-CyD (TM-β-CyD) are much more soluble in both water and organic solvents as compared with the parent CyD.[9–11] In addition, the trimethylated CyD ring is remarkably distorted from the regular symmetry of CyD owing to steric hindrance involving the methyl groups.[12]

The present study is mainly concerned with the inclusion complex formation of clobazam with α-, β-, γ-, DM-β, and TM-β-CyDs, to gain insight into the modes of interaction in aqueous solution and in the solid state. The infrared (IR) spectra of ground mixtures with CyDs in the solid state and the IR spectra of clobazam in CCl_4 solutions with some additives were compared. In order to determine the role of methoxyl groups of DM-β-CyD and TM-β-CyD in the shift of the carbonyl stretching vibration of clobazam to higher frequency, methyl cellulose (MC) in a ground mixture with clobazam was studied. The physicochemical properties of inclusion complexes were also examined in the solid state.

Experimental

Materials 7-Chloro-1-methyl-5-phenyl-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (clobazam) was kindly supplied by Hoechst Pharmaceutical Co., Ltd. (Egypt & Japan), and was used without further purification. α-, β-, and γ-Cyclodextrins were purchased from Nakarai Chemicals, Ltd., Japan. α-Cyclodextrin was dried *in vacuo* at 80 °C for 3 h and stored in a vacuum desiccator. β-, and γ-Cyclodextrins were dried *in vacuo* at 110 °C for 3 h and stored in a vacuum desiccator over phosphorus pentoxide. Dimethyl-β-, and trimethyl-β-CyDs were purchased from Toshin Chemicals Co., Japan, and were used without further purification. Methyl cellulose 400 cP (MC) was purchased from Wako Pure Chemical Industries Ltd., Japan, and was used without further purification. All other materials and organic solvents were of analytical reagent grade.

Preparation of Ground Mixtures A vibrational mill (Heiko Seisakusho model TI-200) was used. The total specimen weight was about 2 g. The molar ratio of clobazam to CyDs was 1 : 2 and the grinding time was 5 min. A ground mixture of 10% clobazam with 90% MC was also prepared.

Solubility Studies Solubility measurements were carried out according to the method of Higuchi and Connors.[13] An excess amount of clobazam (50.0 mg) was added to 10 ml of aqueous Clark & Lubs's buffer solution (16.6 mM, $\mu = 0.721$, pH 9.0) containing various concentrations of CyDs. The solutions were shaken in a water bath at 30 ± 0.5 °C. The pH 9.0 buffer solution was used as clobazam was almost unionized at this pH and decomposition was negligible. After equilibrium was attained, an aliquot was filtered using a membrane filter of pore size 0.2 μm (Toyo Roshi Kaisha, Ltd., Japan). Then 0.5 ml of the sample was appropriately diluted with the same buffer solution and analyzed spectrophotometrically (Shimadzu double beam spectrophotometer UV 200S) at the UV maximum (229 nm) of clobazam. The 1 : 1 stability constant (K) was calculated from the linear part of the phase solubility diagram.

Preparation of Inclusion Complex of Clobazam with DM-β-CyD Inclusion complex of clobazam with DM-β-CyD was prepared by the coprecipitation method (solvent method).[14] Suitable amounts of clobazam and DM-β-CyD were dissolved in methanol and the solvent was evaporated slowly at 40 °C in a water bath. The coprecipitate was dried *in vacuo* at room temperature for 24 h until constant weight was obtained, and then stored in a desiccator. Coprecipitates at various molar ratios of clobazam with DM-β-CyD were prepared. The coprecipitate at 1 : 2 molar ratio was heated in a vacuum dryer over phosphorus pentoxide at various temperatures.

Infrared Spectroscopy A Hitachi 295 IR spectrophotometer was used to obtain the spectra of the ground mixtures (KBr disk method). We also used an FT-IR spectrophotometer (5-ZDX, Nicolet Analytical Instrument, U.S.A.) to measure the spectra of inclusion complexes by the Nujol film method.

Powder X-Ray Diffraction The powder X-ray diffractometer (Rigaku Geigerflex 2204; Rigaku Denki Co., Ltd., Japan) was operated under the following conditions; target Cu, filter Ni, voltage 30 kV, current 5 mA, time constant 0.5 s, scanning speed 4°/min, chart speed 40 mm/min and count range 1000 or 2000 cps.

Differential Scanning Calorimetry (DSC) A Perkin Elmer model DSC-2 was operated at a scanning speed of 10 °C/min, range 5 mcal/s, under a nitrogen stream, using a sample pan for liquids. The sample weight was about 4—5 mg. Peak position was determined by extrapolation of the base line and the declining line to the point of intersection.

Results and Discussion

Complexation in Aqueous Solution The complexing behavior of clobazam with α-, β-, γ- and DM-β-CyDs in aqueous buffer solution (pH 9.0) at 30±0.5 °C was studied by the solubility method. Figure 1 shows the phase solubility diagrams. The solubility of clobazam increased with increasing concentration of CyDs, showing A_L-type phase solubility diagrams. The 1:1 stability constants (K) for clobazam with α-, β-, γ-, and DM-β-CyDs were calculated as 12, 58, 36, and 306 M^{-1}, respectively. Uekama *et al.*[5] studied the solubility of clobazam with α-, β-, and γ-CyDs in water at 25 °C, and obtained A_L-type phase solubility diagrams with α- and β-CyD (K values were 10 and 49 M^{-1}, respectively) and B_s-type phase diagram ($K=36\ M^{-1}$) with γ-CyD. Our data were in agreement with those of Uekama *et al.* in the cases of α-, and β-CyDs, but in the case of γ-CyD only agreement of the K value was obtained. The experimental conditions, such as solvent, temperature, the range of CyD concentration and the total amount of clobazam used, may affect the type of phase solubility diagram obtained with γ-CyD. We noted from the phase solubility diagrams that DM-β-CyD with clobazam gave the highest stability constant and the highest solubilized amount among the CyDs, which may be attributed to the different physicochemical properties of DM-β-CyD as compared with natural CyDs.

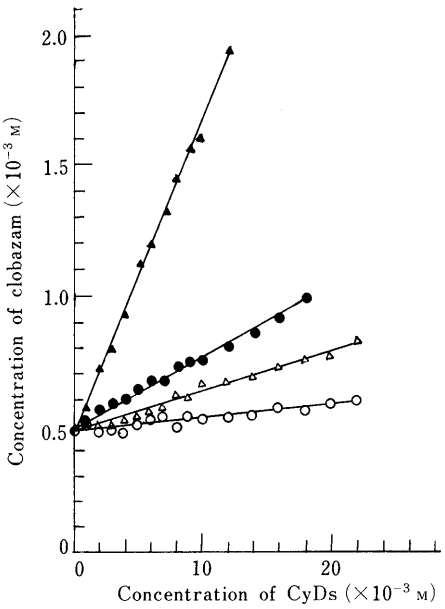

Fig. 1. Phase Solubility Diagrams of Clobazam with CyDs in Aqueous Buffer Solution (pH 9.0) at 30 °C

○, clobazam with α-CyD; ●, clobazam with β-CyD; △, clobazam with γ-CyD; ▲, clobazam with DM-β-CyD.

Ground Mixtures of Clobazam with CyDs The ground mixtures of clobazam with CyDs in 1:2 molar ratio rather than 1:1 were studied, because the 1:2 ground mixtures gave significant shifts in IR spectra from 1:1 mixtures, and their X-ray diffraction patterns and DSC curves were examined for comparison with the corresponding physical mixtures at 1:2 molar ratio. Figure 2 shows the IR spectra of the carbonyl stretching region of clobazam in the solid state. Clobazam had two carbonyl bands at 1695 and 1675 cm^{-1} in the crystalline state. The physical mixtures of clobazam with CyDs showed no appreciable spectral changes compared to crystalline clobazam. Curves B, C, and D in Fig. 2 show the IR spectra of ground mixtures of clobazam with α-, β- and γ-CyDs, respectively. Lower frequency shifts for both carbonyl groups of clobazam to 1692 and 1660 cm^{-1} were observed. Uekama *et al.*[5] reported similar results, that is, the lower frequency shift of

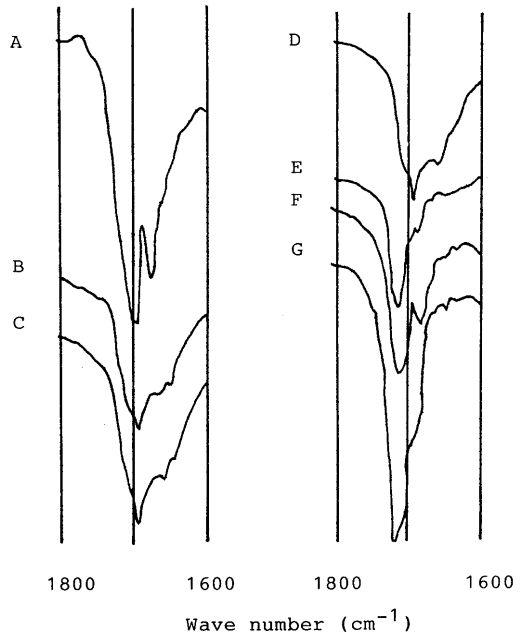

Fig. 2. IR Spectra of Clobazam with CyDs and MC in Ground Mixtures Using KBr Disk

A, clobazam crystals; B, ground mixture of clobazam with α-CyD; C, ground mixture of clobazam with β-CyD; D, ground mixture of clobazam with γ-CyD; E, ground mixture of clobazam with DM-β-CyD; F, ground mixture of 10% clobazam with 90% methyl cellulose; G, ground mixture of clobazam with TM-β-CyD.

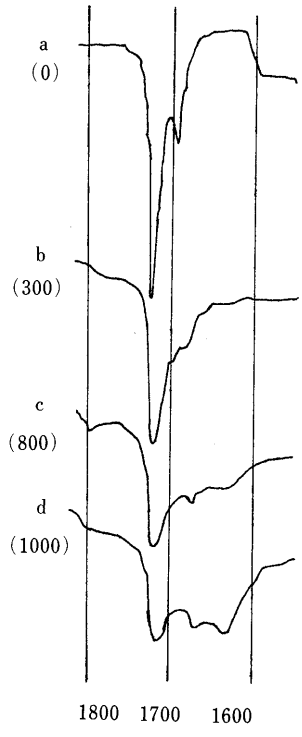

Fig. 3. IR Spectra of Clobazam in CCl_4 Solution Containing Various Amounts of *n*-Butanol

Each spectrum was measured using a 0.2 mm CaF_2 cell. The concentration of clobazam was 5×10^{-3} M. Numbers in parentheses indicate the concentration ratio of clobazam to *n*-butanol.

the carbonyl band of diazepam in the solid complex with γ-CyD.

The curves a, b, c, and d in Fig. 3 show IR spectra of clobazam in CCl_4 solution and clobazam in CCl_4 solution containing various concentrations of *n*-butanol, respectively. There were lower frequency shifts for both carbonyl groups of clobazam in curves b, c and d from 1724 and 1695 cm^{-1} to 1720—1710 (broad band) and 1670 cm^{-1}, respectively. This should be due to the formation of hydrogen bonding between both carbonyl groups of clobazam and hydroxyl groups of *n*-butanol. These IR spectra in CCl_4 solution were considered as evidence for the formation of hydrogen bonding between clobazam and natural CyDs in the ground mixture. Studies of the IR spectra of medicinals in the solid state (ground mixtures) and in solution were reported by Nakai *et al.*[15,16]

The curves E, F and G in Fig. 2 show IR spectra of ground mixtures of clobazam with DM-β-CyD, MC and TM-β-CyD, respectively. Higher frequency shifts were observed for both carbonyl groups of clobazam to 1710 and 1680 cm^{-1} in the case of DM-β-CyD and MC, and to 1720 and 1680 (small shoulder) cm^{-1} in the case of TM-β-CyD. The mode of interaction between clobazam and methylated CyDs was different from that with natural CyDs, even with straight chain MC. The presence of methoxyl groups prevented hydrogen bond formation with clobazam. In a previous paper, Nakai *et al.* reported lower and higher frequency shifts for the carbonyl stretching band of *p*-acetoxydiphenyl in ground mixture.[16] From a comparison of the IR spectra of β-CyD and TM-β-CyD ground mixtures, it was considered that the formation of hydrogen bonding between *p*-acetoxydiphenyl and β-CyD caused a lower frequency shift of the carbonyl band, while the inclusion of *p*-acetoxydiphenyl into the CyD cavities caused a higher frequency shift. The free state of carbonyl groups of clobazam in a hydrophobic environment was assumed in these dispersion systems. This state of clobazam in the ground mixtures was of interest in connection with the molecular state of clobazam in CCl_4, as the IR spectra showed similar patterns.

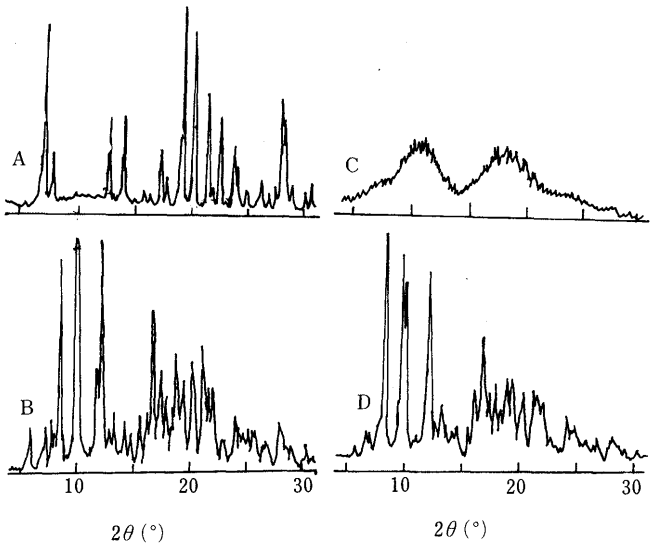

Fig. 4. Powder X-Ray Diffraction Patterns of the Physical and the Ground Mixtures of Clobazam with DM-β-CyD

A, clobazam crystals; B, physical mixture; C, ground mixture (ground for 5 min); D, ground mixture after heating at 150 °C for 1.5 h.

Figure 4 shows the powder X-ray diffraction patterns of physical and ground mixtures of clobazam with DM-β-CyD. The diffraction pattern of the physical mixture of clobazam with DM-β-CyD was simply a superposition of those of the two components, while that of the ground mixture showed a halo pattern indicating the production of an amorphous state of clobazam with DM-β-CyD by grinding. The ground mixtures of clobazam with α-, β-, and γ-CyDs also showed halo patterns like that of DM-β-CyD on their X-ray diffractograms (not shown). These results indicate that clobazam molecules were present in a molecularly dispersed state with CyDs. After heating of the ground mixture of clobazam with α-, β-, and γ-CyDs *in vacuo* at 150 °C for 1.5 h, no change in their X-ray diffraction patterns was observed, while after heating of the DM-β-CyD ground mixture under the same conditions, a crystalline pattern was observed similar to that of the each components (Fig. 4D). These data indicated that the mode of interaction of DM-β-CyD with clobazam was different from that of natural CyDs in the ground mixtures, and this agreed with the IR results. A ground mixture of clobazam with MC showed a halo diffraction pattern, suggesting a monomolecular dispersion or micro-assembly of molecules of clobazam in the ground mixture of MC.

Figure 5 shows the DSC curves of clobazam with CyDs and MC. In the case of clobazam crystal and the physical mixtures, an endothermic peak was observed at 185 °C due to the melting of clobazam. Ground mixtures of clobazam with α-, β- and γ-CyDs showed no appreciable endothermic peak in the melting region of clobazam, and only a broad endothermic peak was observed at about 147 °C due to the evaporation of the adsorbed water. These thermal behavior changes could be explained in terms of monomolecular dispersion of clobazam molecules in the ground mixtures.

Ground DM-β-CyD showed an exothermic peak at 165 °C due to crystallization (curve i), and the ground mixture of clobazam with DM-β-CyD (curve e) showed two peaks; an exothermic peak at 124 °C and an endothermic peak at 177 °C. The exothermic peak could be due to the crystallization of the amorphous ground sample and the endothermic peak could be due to fusion of clobazam or the fusion of the crystallized inclusion complex. Ground mixture of aspirin with DM-β-CyD was studied by Nakai *et*

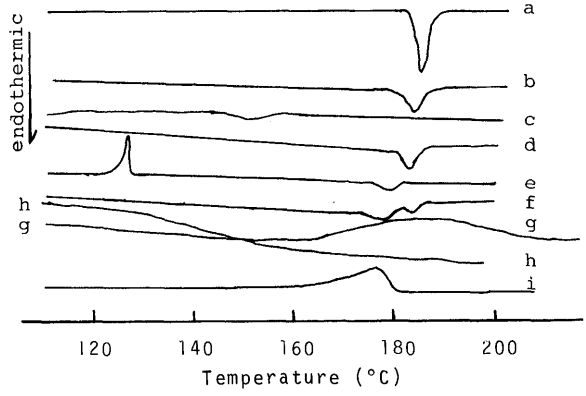

Fig. 5. DSC Curves of Clobazam with CyDs and MC

a, clobazam crystals; b, physical mixture of clobazam with β-CyD; c, ground mixture of clobazam with β-CyD; d, physical mixture of clobazam with DM-β-CyD; e, ground mixture of clobazam with DM-β-CyD; f, ground mixture of clobazam with DM-β-CyD after heating at 150 °C for 1.5 h; g, ground mixture of clobazam with TM-β-CyD; h, ground mixture of clobazam with MC; i, ground DM-β-CyD.

al.[17] and they observed two peaks in the DSC curve; one exothermic peak and an endothermic peak at 187 °C due to the melting of the inclusion complex. We can conclude from the DSC data that the mode of interaction of DM-β-CyD with clobazam was different from the mode of interaction of natural CyDs with clobazam in the ground mixtures, in agreement with the X-ray diffraction and IR spectral data.

The ground mixture of clobazam with TM-β-CyD (curve g) showed only a broad exothermic peak at 163 °C which was due to the crystallization of amorphous ground mixture, while the ground TM-β-CyD showed two peaks on the DSC curve (not shown): one exothermic peak at 94 °C and an endothermic peak at 150 °C due to the fusion of TM-β-CyD. As the crystallized compound had no endothermic peak around 163 °C, or until 217 °C, it is reasonable to consider that the inclusion compound was formed by the heating of the ground mixture of clobazam with TM-β-CyD.

The ground mixture of clobazam with MC (curve h) showed only a broad endothermic peak which was due to the evaporation of the adsorbed water. When the 2nd run was done on the MC ground mixture, no heat of fusion was recorded on the thermogram. The ground mixture of 10% diazepam with 90% microcrystalline cellulose (CC) showed an amorphous X-ray pattern and no heat of fusion for diazepam on the DSC thermogram.[18] Our results with the ground mixture of MC were similar to those of the ground mixture of CC, so that the clobazam molecules were apparently dispersed mono-molecularly in the MC matrix.

Coprecipitates of Clobazam with DM-β-CyD Coprecipitates of clobazam with DM-β-CyD were prepared by the coprecipitation method and their properties were examined by FT-IR spectroscopy, X-ray diffractometry and DSC.

Figure 6 shows FT-IR spectra of clobazam with DM-β-CyD coprecipitates at various molar ratios. FT-IR spectra showed that clobazam crystals exhibit two carbonyl stretching bands at 1692 and 1668 cm^{-1}. Increase of the molar ratio of DM-β-CyD caused an increase in the higher frequency shift of both carbonyl stretching vibrations up to 1706 and 1677 cm^{-1}. The increase of the molar ratio of DM-β-CyD over 1 : 2, however, caused no further significant change in the spectra. As the shifts were maximum at 1 : 2 molar ratio, we focussed our study on the coprecipitate of 1 : 2 molar ratio (clobazam/DM-β-CyD).

Figure 7 shows FT-IR spectra of the coprecipitate of clobazam with DM-β-CyD in 1 : 2 molar ratio and the effect of heating at various temperatures on the carbonyl stretching vibration of clobazam. Curve C in Fig. 7 shows the FT-IR spectrum of the coprecipitate before heating and exhibits a higher frequency shift for both carbonyl groups of clobazam from 1692 and 1668 cm^{-1} to 1706 and 1677 cm^{-1}, respectively. As mentioned previously, the ground mixture of clobazam with DM-β-CyD in 1 : 2 molar ratio gave the same shift to higher wave number, so that the molecular states of clobazam were considered to be similar in the preparations obtained by the grinding and coprecipitation methods. By heating the coprecipitate at various temperatures, the higher shift started to decrease gradually to 1692 and 1669 cm^{-1}, respectively, the same as in the crystals. The decrease may be due to a crystallization process in the coprecipitate (as will be described in connection with the X-ray diffraction data).

Figure 8 shows the variation of the powder X-ray diffraction patterns of clobazam with DM-β-CyD coprecipitate in 1 : 2 molar ratio by heating. The diffraction pattern of the physical mixture (Fig. 4B) was simply a superposition of those of the two components, while the coprecipitate gave a somewhat different diffraction pattern from the crystalline pattern. On heating the coprecipitate, it began to crystallize at 110 °C (curve C) with the appearance of new crystalline peaks at $2\theta = 7.0°$, 8.0°, 9.5° and 10.5° and the disappearance of small crystalline peaks of DM-β-CyD. Disappearance of the crystalline peaks of DM-β-CyD may be due to the rearrangement of DM-β-CyD molecules to

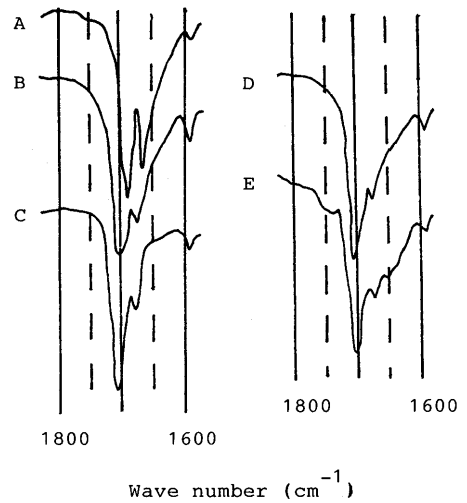

Fig. 6. FT-IR Spectra of Coprecipitates of Clobazam with DM-β-CyD at Various Molar Ratios (Clobazam: DM-β-CyD)

A, clobazam crystals; B, coprecipitate 1 : 1; C, coprecipitate 1 : 2; D, coprecipitate 1 : 3; E, coprecipitate 1 : 4.

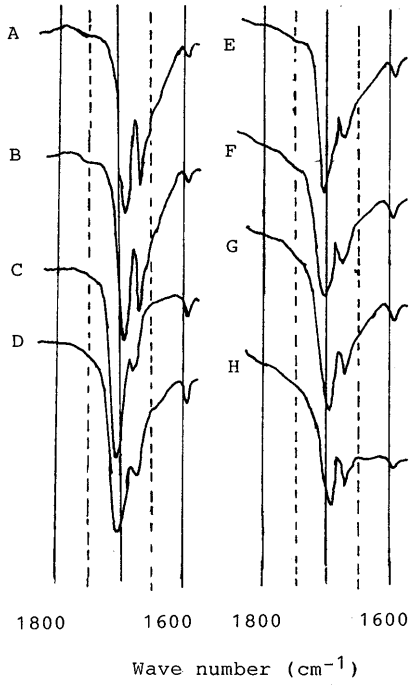

Fig. 7. FT-IR Spectra of Coprecipitate of Clobazam with DM-β-CyD in 1 : 2 Molar Ratio after Heating at Various Temperatures

A, clobazam crystals; B, physical mixture; C, coprecipitate before heating; D, after heating at 60 °C for 1 h; E, at 100 °C for 1 h; F, at 120 °C for 1 h; G, at 140 °C for 30 min; H, at 150 °C for 30 min.

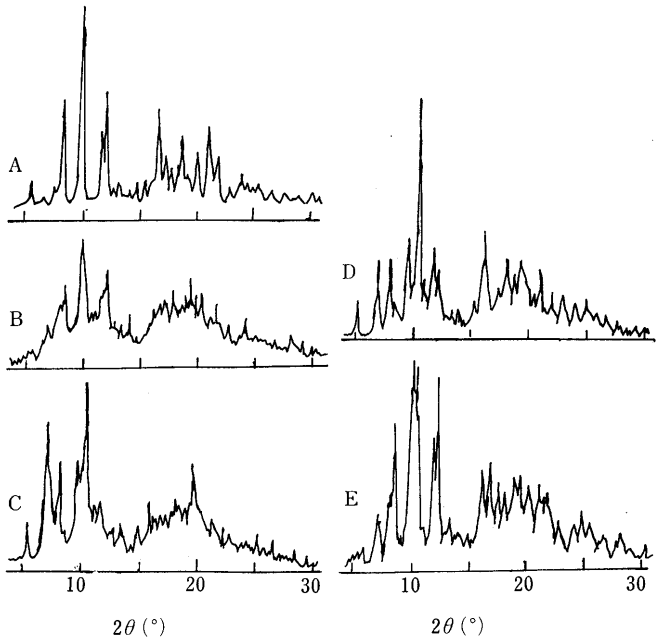

Fig. 8. Powder X-Ray Diffraction Patterns of Clobazam with DM-β-CyD 1:2 Molar Ratio Systems and Effect of Temperature on Crystallization

A, DM-β-CyD crystals; B, coprecipitate before heating; C, after heating at 110 °C for 1 h; D, at 120 °C for 1 h; E, at 150 °C for 30 min.

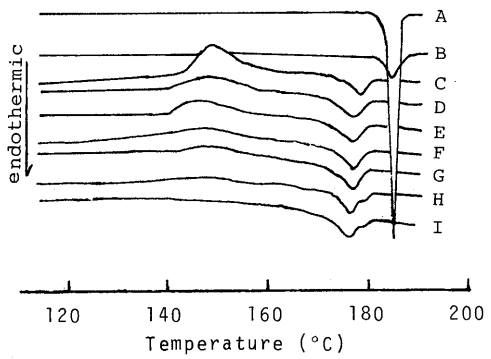

Fig. 9. DSC Curves of Clobazam with DM-β-CyD Systems in 1:2 Molar Ratio

A, clobazam crystals; B, physical mixture; C, coprecipitate before heating; D, after heating at 60 °C for 1 h; E, at 80 °C for 1 h; F, at 100 °C for 1 h; G, at 120 °C for 1 h; H, at 140 °C for 30 min; I, at 150 °C for 30 min.

form the inclusion compound. After the heating of the coprecipitate at 120 °C for 1 h (curve D), the diffraction peaks at $2\theta = 7.0°$, 8.0°, 9.5°, 10.5°, and 16.0° were quite different from the diffraction peaks of clobazam and DM-β-CyD, suggesting the formation of the crystalline inclusion compound. After the heating of the coprecipitate at 140 °C for 30 min (not shown), it started to dissociate into each component because some crystalline peaks of DM-β-CyD and clobazam appeared beside the diffraction peaks of the inclusion compound. After heating at 150 °C for 30 min (curve E), the diffraction peaks of DM-β-CyD and clobazam increased in intensity compared to the peaks of the inclusion compound, and after heating for 1 h it gave a diffraction pattern similar to that of the physical mixture. The ground mixture of clobazam with DM-β-CyD after heating at 150 °C for 1.5 h also gave a diffraction pattern similar to that of the physical mixture; this indicated similar behavior of both the ground mixture and the coprecipitate of clobazam with DM-β-CyD. From the X-ray diffraction data, we can conclude that the heating of the partially crystalline coprecipitate of clobazam with DM-β-CyD resulted in formation of the crystalline inclusion compound.

Figure 9 shows DSC thermograms of clobazam with DM-β-CyD systems. Curve C shows the DSC curve of the coprecipitate before heating with two peaks; one was an exothermic peak at 141 °C which was due to crystallization of the inclusion compound and the other was an endothermic peak at 175 °C, which may be due to fusion of the crystallized inclusion compound. On heating the coprecipitate, the exothermic peak on the DSC curve became smaller until it nearly disappeared. After the heating at 120 °C (curve G), the curve showed only the endothermic peak at 175 °C, where it gave a crystalline X-ray diffraction pattern of the inclusion compound as mentioned previously. Curve H shows the DSC curve of the coprecipitate heated at 140 °C for 30 min, in which two endothermic peaks at 176 and 181 °C were observed; the peak at 181 °C might be due to the fusion of clobazam as a result of the dissociation of the inclusion compound. Curve I shows the DSC curve of coprecipitate after heating at 150 °C for 30 min, in which two endothermic peaks at 175 and 181 °C were observed. It should be noted that the ground mixture of clobazam with DM-β-CyD after the heating at 150 °C for 1.5 h gave a similar DSC curve (Fig. 5f) which suggest that similar interactions exist in both the ground mixture and the coprecipitate.

In conclusion, the crystalline inclusion compound of clobazam with DM-β-CyD in 1:2 molar ratio was obtained by the coprecipitation method after heating *in vacuo* at 120 °C for one hour.

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