Complex Formation of Nitrazepam in Coprecipitating and in Co-grinding with Methylated β-Cyclodextrins

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Complex formation of nitrazepam with heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CyD) together with α-, β-, and γ-cyclodextrins (CyDs) in aqueous solution was confirmed by the solubility method. A_L type phase solubility diagrams were obtained in all cases. The highest stability constant and the highest solubilized amount of nitrazepam was obtained with DM-β-CyD, in the order DM-β-CyD > β-CyD > TM-β-CyD > γ-CyD > α-CyD. Complex formation of nitrazepam with DM-β-CyD and TM-β-CyD in the solid state was assessed by differential scanning calorimetry (DSC), infrared (IR) spectroscopy and X-ray diffractometry. Crystalline inclusion complex of nitrazepam with DM-β-CyD in 1 : 2 molar ratio was obtained by the coprecipitation method. X-Ray diffraction analyses explained that nitrazepam was transformed from the crystalline to non-crystalline (amorphous) state by grinding with methylated β-CyDs. DSC analyses revealed that the heat of fusion due to nitrazepam disappeared in the ground mixture and coprecipitate of nitrazepam with DM-β-CyD in 1 : 2 molar ratio, which demonstrated the optimum content of dispersal nitrazepam with DM-β-CyD in the solid state. IR spectra showed that there were higher frequency shifts for the carbonyl stretching band of nitrazepam in the ground mixtures and coprecipitates of DM-β-CyD and TM-β-CyD similar to the case of nitrazepam in $CHCl_3$ solution, which were considered due to the monomolecular dispersion of nitrazepam in a hydrophobic environment.

Keywords nitrazepam; cyclodextrin; dimethyl-β-cyclodextrin; trimethyl-β-cyclodextrin; ground mixture; inclusion complex; interaction; coprecipitation

Nitrazepam belongs to the 1,4-benzodiazepine class of tranquilizing agents and is used principally as a hypnotic[1] and an anticonvulsant agent.[2] The complexation between benzodiazepines and cyclodextrins (CyDs) has been investigated in a few reports during the past decade.[3–7] We reported in a previous paper the effect of grinding on the dispersed state of clobazam with CyDs.[7] We inferred from infrared (IR) spectra data that clobazam molecules were dispersed monomolecularly under the hydrophobic circumstances of a ground mixture of dimethyl-β-cyclodextrin (DM-β-CyD) and trimethyl-β-cyclodextrin (TM-β-CyD).

The present study was carried out as an extension of the previous work to evaluate the interaction mode of 1,4-benzodiazepine (nitrazepam) with methylated β-CyDs, and to gain insight into the behavior of complex formation in aqueous solution and in solid state. The objective was also to evaluate the dispersion state of nitrazepam in the ground mixture of methylated β-CyDs.

Experimental

Materials 1,3-Dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (nitrazepam) was kindly donated by Sankyo Co., Ltd. and was used without further purification. α-, β-, and γ-Cyclodextrins were purchased from Nakarai Chemicals, Ltd. and were used as previously described.[7] Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD), and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CyD) were purchased from Toshin Chemicals Co. and were used without further purification. All other chemicals and organic solvents were of analytical reagent grade, and deionized double-distilled water used throughout the study.

Solubility Studies Solubility measurements were carried out according to the method of Higuchi and Connors.[8] The procedures were the same as previously,[7] an excess amount of nitrazepam (50.0 mg) was added to 10 ml water containing CyDs at various concentrations and the solutions were shaken in a water bath (Taiyo Incubator Personal) at 30 ± 0.5 °C. The solubilized nitrazepam was analyzed spectrophotometrically (Shimadzu double beam UV-VIS spectrophotometer UV-160) at the ultraviolet (UV) maximum 258 nm of nitrazepam. The apparent 1 : 1 stability constant (K) was calculated from the initial linear portion of the phase solubility diagram.

Preparation of Ground Mixtures A vibrational mill (Heiko Seisakusho model TI-200) made of tungsten carbide was used. The total weight of specimen was 2.0 g. Ground mixture of nitrazepam with DM-β-CyD was prepared in 1 : 1 and 1 : 2 molar ratios and the grinding time was 10 min. Ground mixture of nitrazepam with TM-β-CyD was also prepared in 1 : 1 molar ratio.

Preparation of Coprecipitated Complex of Nitrazepam with TM-β-CyD Coprecipitate of nitrazepam with TM-β-CyD was prepared by the coprecipitation method (solvent method).[9] Suitable amounts of nitrazepam and TM-β-CyD were dissolved in methanol and the solvent was evaporated at 40 °C in a water bath. The coprecipitate was dried *in vacuo* at room temperature until a constant weight was obtained, and was then stored in a desiccator. Total removal of the solvent from the coprecipitate was confirmed by thermogravimetry.

Preparation of Coprecipitated Complex of Nitrazepam with DM-β-CyD Coprecipitate of nitrazepam with DM-β-CyD was prepared by the coprecipitation method as with TM-β-CyD, but using acetone as a solvent. When acetone was used instead of methanol, high crystallinity coprecipitate was obtained. Coprecipitates of nitrazepam with DM-β-CyD were prepared in 1 : 1 and 1 : 2 molar ratios.

X-Ray Diffractometry (Powder Method) A Rigakudenki 2204 diffractometer was used. Measurements conditions were the same as those reported earlier.[7]

Differential Scanning Calorimetry (DSC) Perkin Elmer Model DSC-2 used was operated at a scanning speed of 10 K/min, a range of 5 mcal/s, and under a nitrogen stream using a sample pan for the liquid. The sample weight was about 3—5 mg.

IR Absorption Spectroscopy A Hitachi 295 IR spectrophotometer was used and measurements were made according to the KBr disk method.

Results and Discussion

Complexation in Aqueous Solution Complex formation of nitrazepam with DM-β-, and TM-β-CyDs together with α-, β-, and γ-CyDs in aqueous solution at 30 ± 0.5 °C was studied by the solubility method. The phase solubility diagram obtained for nitrazepam with DM-β-CyD is shown in Fig. 1. The solubility of nitrazepam increased linearly as a function of concentration of DM-β-CyD, and thus

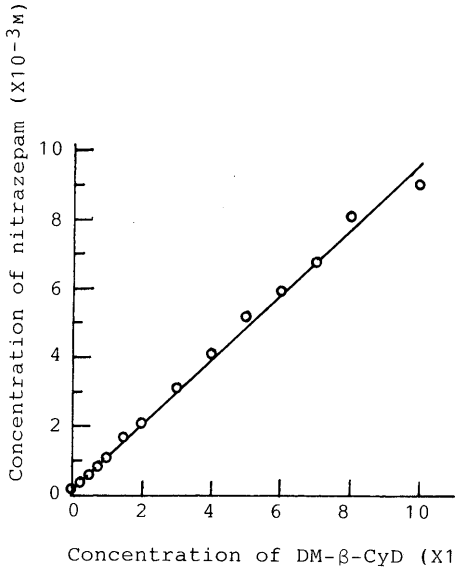

Fig. 1. Phase Solubility Diagram of Nitrazepam with DM-β-Cyd in Water at 30 °C

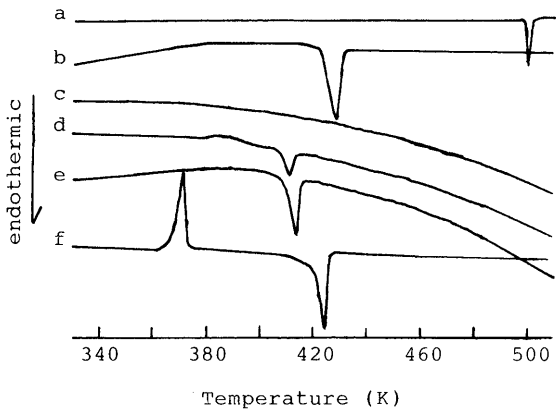

Fig. 2. DSC Thermograms of Nitrazepam with TM-β-CyD Systems in 1 : 1 Molar Ratio

a, nitrazepam crystals; b, TM-β-CyD crystals; c, coprecipitate; d, ground mixture; e, ground mixture after heating at 398 K for 4 min; f, ground TM-β-CyD.

the solubility diagram can be classified as A_L-type.[8] The phase solubility diagrams of nitrazepam with α-, β-, γ-, and TM-β-CyDs also showed A_L type. The apparent 1 : 1 stability constants (K) for nitrazepam with α-, β-, γ-, DM-β-, and TM-β-CyDs were calculated as 22, 131, 45, 494, and 64 M^{-1}, respectively. Our data on α-, β-, and γ-CyD were in good agreement with the results of Anderson and Bundgaard,[3] Uekama *et al.*[4] and Okada *et al.*[10] We observed that DM-β-CyD with nitrazepam gave the highest stability constant and the highest solubilized amount among the CyDs. The magnitude of increase is due to the high aqueous solubility and adequate cavity size of DM-β-CyD.[11]

Coprecipitate and Ground Mixture of Nitrazepam with TM-β-CyD The coprecipitate and ground mixture of nitrazepam with TM-β-CyD in 1 : 1 molar ratio were prepared and their interactions were investigated by DSC, IR spectroscopy, and X-ray diffractometry.

Figure 2 shows the DSC curves of nitrazepam with TM-β-CyD systems. In nitrazepam crystal (curve a), an endothermic peak was observed at 499 K due to the melting of

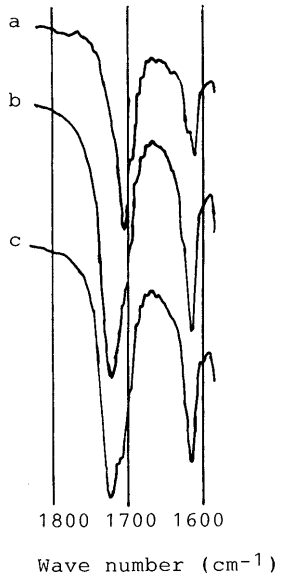

Fig. 3. IR Spectra of Nitrazepam with TM-β-CyD Systems in 1 : 1 Molar Ratio Using KBr Disk

a, physical mixture; b, coprecipitate; c, ground mixture.

nitrazepam. TM-β-CyD (curve b) showed an endothermic peak at 423 K due to the melting of TM-β-CyD, while coprecipitate of nitrazepam with TM-β-CyD (curve c) did not show any peak in the melting region of nitrazepam and TM-β-CyD.

The ground mixture of nitrazepam with TM-β-CyD (curve d) showed a small exothermic peak at 385 K and an endothermic peak at 408 K. There are three possible reasons for exothermic change of ground mixture: crystallization of nitrazepam, that of TM-β-CyD or a complex formation including the complex crystallization. The third possibility seems the most probable from the following considerations. As the subsequent endothermic temperature is 91 K lower than the nitrazepam melting point, the exothermic peak cannot be due to the crystallization of nitrazepam. Assuming the crystallization of TM-β-CyD, it is not reasonable to suppose that TM-β-CyD independently crystallized, leaving nitrazepam amorphous in the ground mixture in the heating process. Nitrazepam would crystallize and show the melting heat in this case. Therefore, complex formation including the complex crystallization seems the most probable and this consideration is supported by the DSC curve e, where only an endothermic change was observed at 408 K (curve e) after heating of the ground mixture at 398 K for 4 min. The ground TM-β-CyD alone (curve f) showed two peaks: an exothermic peak at 367 K due to crystallization and an endothermic peak at 423 K due to the fusion of the crystallized TM-β-CyD.

We noted from DSC analyses that a coprecipitate of nitrazepam with TM-β-CyD showed no peak on the DSC curve, while the ground mixture showed both exo- and endothermic peaks, although both gave halo diffraction patterns on their X-ray diffractograms (Fig. 4, curves b and c). This indicates the existence of a structural difference between these two amorphous preparations.

Figure 3 shows the IR spectra of nitrazepam with TM-β-CyD systems. Nitrazepam had a carbonyl stretching

band at 1704 cm^{-1} in crystalline state. The coprecipitate (curve b) and ground mixture (curve c) showed similar higher frequency shifts to 1721 cm^{-1} as compared to the physical mixture. In previous papers, Nakai *et al.* reported similar results with clobazam in ground mixture with TM-β-CyD and DM-β-CyD[7] and with *p*-acetoxydiphenyl in ground mixture of TM-β-CyD.[12] From these IR spectra data, we can assume the free state of the carbonyl group of nitrazepam under hydrophobic circumstances in dispersion with TM-β-CyD.

Figure 4 shows X-ray diffraction patterns of nitrazepam with TM-β-CyD systems. The diffraction pattern of the physical mixture of nitrazepam with TM-β-CyD (curve a) was simply a superposition of those of the two components,

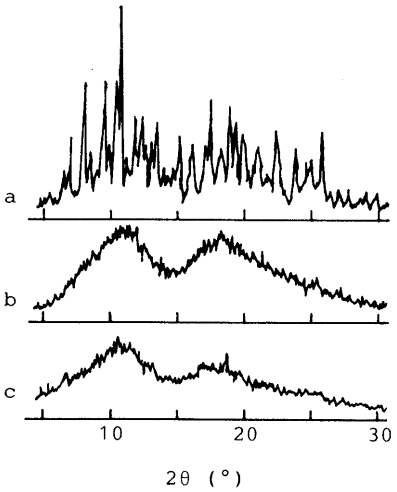

Fig. 4. Powder X-Ray Diffraction Patterns of Nitrazepam with TM-β-CyD Systems in 1 : 1 Molar Ratio

Keys, as in Fig. 3.

while that of the coprecipitate (curve b) and the ground mixture (curve c) showed halo patterns indicating production of the amorphous state of nitrazepam with TM-β-CyD by coprecipitation method as well as by grinding method. Those results showed the structural difference between these two amorphous preparations which is detectable only by DSC measurements.

Coprecipitates of Nitrazepam with DM-β-CyD Coprecipitates of nitrazepam with DM-β-CyD in 1 : 1 and 1 : 2 molar ratios were examined by DSC, IR spectroscopy, and X-ray diffractometry.

Figure 5 shows the powder X-ray diffraction patterns of coprecipitates of nitrazepam with DM-β-CyD in 1 : 1 and 1 : 2 molar ratios. The coprecipitate of 1 : 1 showed a crystalline diffraction pattern (curve c) different from each component but with a characteristic peak of nitrazepam at $2\theta = 7.0°$, which indicated the presence of excess nitrazepam. The coprecipitate of nitrazepam with DM-β-CyD in 1 : 2 molar ratio showed a new crystalline pattern without the characteristic peak of nitrazepam at $2\theta = 7.0°$ (curve d). There were new diffraction peaks at $2\theta = 7.7°$, 8.2°, 11.2°, and 18.5° which were quite different from the diffraction peaks of nitrazepam and DM-β-CyD, and this could indicate the formation of the crystalline inclusion complex.

Figure 6 shows DSC thermograms of coprecipitates of nitrazepam with DM-β-CyD systems. The physical mixture of nitrazepam with DM-β-CyD in 1 : 1 molar ratio (curve a) showed an endothermic peak at 490 K due to the fusion of nitrazepam, while coprecipitate in the same molar ratio (curve b) showed two peaks: one exothermic peak at 393 K and one endothermic peak at 482 K, the latter was due to the fusion of excess nitrazepam. The exothermic peak at 393 K of 1 : 1 coprecipitate may be due to the recrystallization process because its X-ray diffractogram (Fig. 5c)

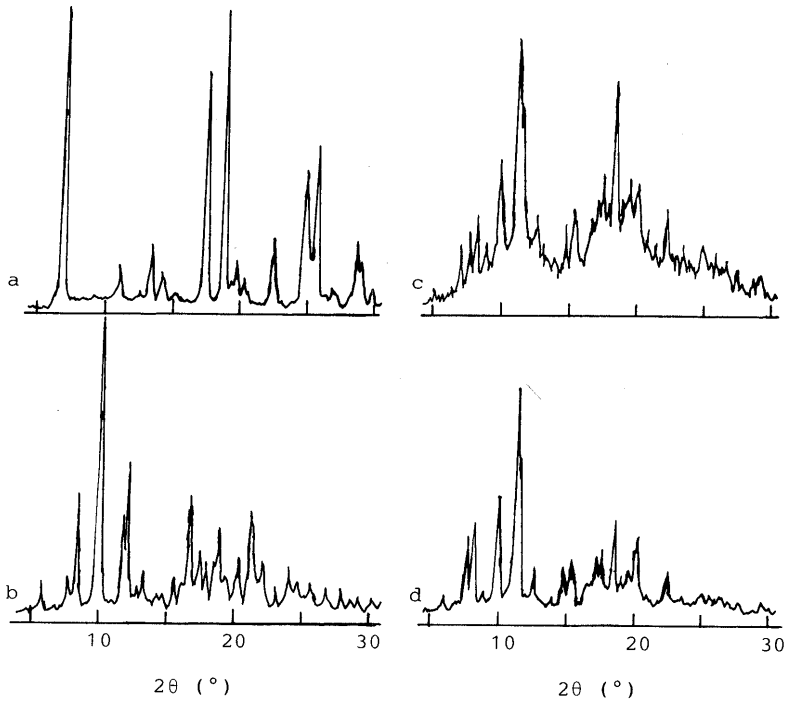

Fig. 5. Powder X-Ray Diffraction Patterns of Coprecipitates of Nitrazepam with DM-β-CyD in 1 : 1 and 1 : 2 Molar Ratios

a, nitrazepam crystals; b, DM-β-CyD crystals; c, coprecipitate 1 : 1; d, coprecipitate 1 : 2.

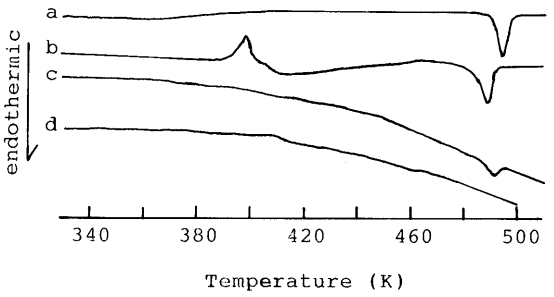

Fig. 6. DSC Curves of Nitrazepam with DM-β-CyD Physical Mixtures and Coprecipitates in 1 : 1 and 1 : 2 Molar Ratios

a, physical mixture 1 : 1, b, coprecipitate 1 : 1; c, physical mixture 1 : 2; d, coprecipitate 1 : 2.

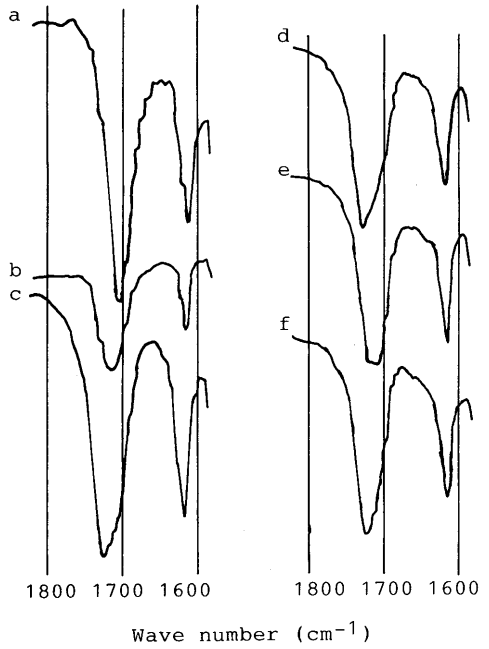

Fig. 7. IR Spectra of Nitrazepam in Coprecipitates and Ground Mixtures with DM-β-CyD (KBr Disk) and Nitrazepam in $CHCl_3$ Solution (5.0×10^{-3} M) Using 0.2 mm CaF_2 Cell

a, nitrazepam crystals; b, nitrazepam in $CHCl_3$ solution; c, coprecipitate 1 : 1; d, coprecipitate 1 : 2; e, ground mixture 1 : 1; f, ground mixture 1 : 2.

showed a more diffused background than that of the 1 : 2 coprecipitate. The physical mixture of nitrazepam with DM-β-CyD in 1 : 2 molar ratio (curve c) showed an endothermic peak at 488 K due to the fusion of nitrazepam, while the DSC curve of coprecipitate in the same molar ratio (curve d) showed a small exothermic peak at 408 K and no appreciable endothermic peak in the melting region of nitrazepam. The disappearance of melting heat of nitrazepam might be due to the inclusion complex formation, as the coprecipitate showed a new crystalline X-ray diffraction pattern.

Figure 7 shows the IR spectra of the carbonyl stretching region of nitrazepam in different systems with DM-β-CyD and in $CHCl_3$ solution. Nitrazepam had one carbonyl stretching band at 1704 cm^{-1} in the crystalline state (curve a). IR spectrum of nitrazepam in $CHCl_3$ solution (curve b) had a higher frequency at 1720—1710 cm^{-1} (broad band) than in solid state. IR spectrum of the coprecipitate in 1 : 2 molar ratio (curve d) showed a higher frequency

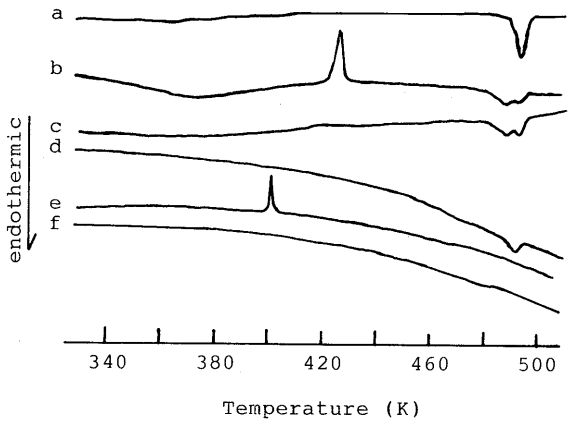

Fig. 8. DSC Curves of Physical and Ground Mixtures of Nitrazepam with DM-β-CyD in 1 : 1 and 1 : 2 Molar Ratios

a, physical mixture 1 : 1; b, ground mixture 1 : 1; c, ground mixture 1 : 1 after heating at 423 K for 1 h; d, physical mixture 1 : 2; e, ground mixture 1 : 2; f, ground mixture 1 : 2 after heating at 423 K for 2.5 h.

shift with a distinct band at 1726 cm^{-1} than in 1 : 1 molar ratio. These IR spectra data suggest the free state of the carbonyl group of nitrazepam in the hydrophobic environment of DM-β-CyD.

As described above, the coprecipitate of nitrazepam with DM-β-CyD in 1 : 2 molar ratio showed the characteristic properties: the new crystalline pattern of X-ray diffraction, no heat of fusion and the higher frequency shift in IR spectrum. It was concluded that, by the coprecipitation method, the crystalline inclusion complex of nitrazepam with DM-β-CyD in 1 : 2 molar ratio would be obtained.

Ground Mixture of Nitrazepam with DM-β-CyD The ground mixtures of nitrazepam with DM-β-CyD in 1 : 1 and 1 : 2 molar ratios were studied for comparison with their corresponding physical mixtures. Their physicochemical properties were investigated by DSC and IR spectroscopy.

Figure 8 shows the DSC curves of physical and ground mixtures of nitrazepam with DM-β-CyD in 1 : 1 and 1 : 2 molar ratios. The physical mixture, curve a, showed an endothermic peak at 490 K due to nitrazepam melting, while the ground mixture in the same molar ratio (curve b) had three peaks: one exothermic peak at 420 K which might be due to the crystallization of both DM-β-CyD and DM-β-CyD with nitrazepam complex, and two other endothermic peaks at 480 and 490 K. After heating the ground mixture *in vacuo* at 423 K, just at the exothermic temperature, for 1 h (curve c), the DSC curve showed no exothermic peak, but only the two endothermic peaks at 480 and 490 K which could be due to the fusion of excess nitrazepam. As reported by Nakai *et al.*,[13] there was a critical amount above which a crystalline portion remained in the ground mixture.

The ground mixture of nitrazepam with DM-β-CyD in 1 : 2 molar ratio (curve e) showed one exothermic peak at 400 K and no appreciable endothermic peak in the melting region of nitrazepam. Ground DM-β-CyD showed an exothermic peak at 438 K on the DSC curve (not shown) due to the crystallization. The temperature of the exothermic peak of 1 : 2 ground mixture decreased to 400 K.

The exothermic change of the ground mixtures may be explained by the crystallization of complex with the same consideration as described in the ground mixture with TM-

β-CyD. The ground mixture of 1:1 molar ratio showed the melting of nitrazepam, while 1:2 molar ratio did not show the melting heat: these results suggest that nitrazepam interacted with DM-β-CyD forming the 1:2 complex. DM-β-CyD has different properties of complex formation from TM-β-CyD: a large K value and 1:2 complex formation.

IR spectrum of the ground mixture of nitrazepam with DM-β-CyD in 1:1 molar ratio (Fig. 7, curve e) showed higher frequency shift with a broad band at 1720—1705 cm^{-1}. As this ground mixture showed endothermic peaks on the DSC thermogram due to the fusion of excess nitrazepam, the IR absorption band seemed to consist of the sum of the overlapping spectral lines of excess and dispersed nitrazepam. Curve f in Fig. 7 shows IR spectrum of the ground mixture of nitrazepam with DM-β-CyD in 1:2 molar ratio; a higher frequency shift with a distinct band at 1721 cm^{-1} was observed. This distinct higher frequency shift indicated the completion of molecular dispersion of nitrazepam into the hydrophobic matrix of DM-β-CyD.[14] The state of nitrazepam in the ground mixtures was the same as that of nitrazepam in $CHCl_3$ solution, from the observation of IR spectra.

As shown in Fig. 1, the formation of soluble inclusion complex of nitrazepam with DM-β-CyD in aqueous solution was confirmed by the formation of A_L type phase solubility diagram. DSC measurements, IR spectroscopy and X-ray diffractometry results suggested the formation of a 1:2 molar ratio inclusion complex of nitrazepam with DM-β-CyD in the solid state. These results are reasonable because Yoshida *et al.*[15] reported the formation of A_L type phase solubility diagram of nimodipine with 2-hydroxypropyl-β-CyD in aqueous solution, and the formation of 1:3 (guest:host) complex of nimodipine with 2-hydroxypropyl-β-CyD in the solid state.

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References

1) I. Haider, *Br. J. Psychiatry*, **114**, 337 (1968).
2) J. W. Lance, *Med. J. Aust.*, **1**, 113 (1968).
3) F. M. Anderson and H. Bundgaard, *Arch. Pharm. Chemi. Sci. Ed.*, **10**, 80 (1982).
4) K. Uekama, S. Narisawa, F. Hirayama, and M. Otagiri, *Int. J. Pharmaceut.*, **16**, 327 (1983).
5) H. P. R. Bootsma, H. W. Frijlink, A. Eissens, J. H. Proost, H. Van Doorne, and C. F. Lerk, *Int. J. Pharmaceut.*, **51**, 213 (1989).
6) H. W. Frijlink, A. J. M. Schoonen, and C. F. Lerk, *Int. J. Pharmaceut.*, **49**, 91 (1989).
7) Y. Nakai, A. E. Aboutaleb, K. Yamamoto, S. I. Saleh, and M. O. Ahmed, *Chem. Pharm. Bull.*, **38**, 728 (1990).
8) T. Higuchi and K. A. Connors, *Anal. Chem. Instr.*, **4**, 117 (1965).
9) W. L. Chiou and S. Riegelmans, *J. Pharm. Sci.*, **60**, 1281 (1971).
10) Y. Okada, Y. Kubota, K. Koizumi, S. Hizukuri, T. Ohfuji, and K. Ogata, *Chem. Pharm. Bull.*, **36**, 2176 (1988).
11) K. Uekama, *Pharm. Int.*, **6**, 61 (1985).
12) Y. Nakai, S. Nakajima, K. Yamamoto, K. Terada, and T. Konno, *Chem. Pharm. Bull.*, **28**, 1552 (1980).
13) Y. Nakai, E. Fukuoka, S. Nakajima, and K. Yamamoto, *Chem. Pharm. Bull.*, **25**, 3340 (1977).
14) Y. Nakai, S. Nakajima, K. Yamamoto, K. Terada, and T. Konno, *Chem. Pharm. Bull.*, **26**, 3419 (1978).
15) A. Yoshida, M. Yamamoto, T. Itoh, T. Irie, F. Hirayama, and K. Uekama, *Chem. Pharm. Bull.*, **38**, 176 (1990).