THE MODE OF INCLUSION COMPLEXATION OF BROMAZEPAM WITH DIMETHYL β -CYCLODEXTRIN.

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ABSTRACT

Inclusion complex formation of bromazepam with heptakis-(2,6-di-O-methyl)- β -cyclodextrin (DM- β -CD) in aqueous solution was confirmed by the solubility method. A₁-type phase solubility diagram was obtained with 50 folds increase in the solubility of bromazepam. Crystalline inclusion complexes of bromazepam with DM- β -CD in 1 : 1 and 1 : 2 molar ratios were obtained by the co-precipitation method as indicated by the X-ray diffraction analysis. Infrared (IR) spectra of the inclusion complex of bromazepam and DM- β -CD was different depending on the molar ratio, whether it is 1 : 1 or 1 : 2. In the same time, the differential scanning calorimetry (DSC) analysis showed different thermal behaviour depending on the molar ratio of the inclusion complex. These results revealed that the mode of molecular interaction of 1 : 1 inclusion complex of bromazepam with DM- β -CD was different from that of 1 : 2.

INTRODUCTION

Bromazepam is one of the 1, 4-benzodiazepines usually used as minor tranquilizers. They have poor water solubility and this could impair their biological availability. Inclusion complexation with cyclodextrins has been extensively applied for the improvement of solubility, dissolution, bioavailability and stability of many drugs¹. Coprecipitation is one of the methods used for the preparation of the cyclodextrin guest complexes depending on the properties of the guest

components². In case of water soluble drugs, equimolar quantities or a ten-fold excess of drugs are dissolved directly in concentrated hot or cold aqueous solutions of the cyclodextrins. The inclusion compounds crystallize out immediately or upon slow cooling or evaporation. On the other hand, complexes of cyclodextrins with poor water-soluble drugs could be prepared by dissolving the two components in alcohol or any other solvent followed by evaporation of the solvent at suitable temperature³⁻⁵. The mode of interaction between the host (cyclodextrin) and the guest (drug) was found to be different according to the type of cyclodextrin used^{6,7}. Moreover, the mode of inclusion complexation between a steroidal drug and β -cyclodextrin was different for different molar ratios⁸.

The aim of the present work is to prepare inclusion compounds of bromazepam with DM- β -CD in two molar ratios applying the coprecipitation method. IR, X-ray and DSC techniques were utilized to gain insight into the mode of interaction of the components of the inclusion complexes.

EXPERIMENTAL

Materials:

Bromazepam (7-bromo-1,3-dihydro-5-(2-pyridinyl)-2H-1,4-benzodiazepin-2-one) was kindly donated by Roche

Co. Ltd., Japan and was used without further purification. Heptakis (2, 6-di-O-methyl)- β -cyclodextrin (DM- β -CD) was purchased from Toshin Chemicals Co., Japan and was used without further purification also. All other chemicals and organic solvents were of analytical grade. Deionized double distilled water was used throughout this study.

Methods:

Solubility studies

Solubility measurements were carried out according to the method of Higuchi and Connors⁹. An excess amount of bromazepam (50 mg) was added to 10 ml of aqueous Clark and Lubs's buffer solution of pH 9 containing various concentrations of DM- β -CD and the solutions were shaken in a water bath (Taiyo Incubator Personal) at $30 \pm 0.5^\circ\text{C}$. The solubilized bromazepam was analyzed spectrophotometrically using Shimadzu double beam UV-VIS spectrophotometer at 233 nm. The apparent 1:1 stability constant (K) was calculated from the linear part of the phase solubility diagram.

Preparation of the inclusion complex of bromazepam with DM- β -CD using the coprecipitation method:

Suitable amounts of bromazepam and DM- β -CD were dissolved in acetone 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. Two different molar ratios (1:1 and 1:2) of bromazepam with DM- β -CD were investigated.

X-ray diffractometric investigation:

A Rigakudenki 2204 diffractometer was used in the study of the x-ray diffraction patterns of the coprecipitates prepared. Measure-

ment conditions were as follows^{4,5}: target Cu, filter Ni, voltage 30 Kv, current 5 mA, time constant 0.5 second, scanning speed $4^\circ/\text{min.}$, chart speed 40 mm/min. and count range 1000 or 2000 cps.

Differential scanning calorimetric investigation:

Perkin Elmer Model DSC-2 was used and operated at scanning speed of 10 K/min., a range of 5 mcal/s, and under nitrogen stream using a sample pan for the liquid. The sample weight was about 3-5 mg. The DSC scans of the physical mixtures as well as the coprecipitates in 1:1 and 1:2 molar ratios were carried out.

Infrared (IR) absorption spectroscopy:

A Hitachi 295 IR spectrophotometer was used and the measurements were carried out according to the KBr disk method.

RESULTS AND DISCUSSION

Complexation in aqueous solution:

The phase solubility diagram obtained for bromazepam with DM- β -CD in solution is shown in Fig. 1. The solubility of bromazepam increased linearly as a function of concentration of DM- β -CD and thus the solubility diagram could be classified as A_L -type⁹. The apparent 1:1 stability constant (K) for bromazepam with DM- β -CD was found to be 227 M^{-1} .

Coprecipitates of bromazepam with DM- β -CD:

Fig. 2 shows the powder X-ray diffraction patterns of bromazepam with DM- β -CD systems in 1:1 and 1:2 molar ratios. The diffraction pattern of the physical mixture in either 1:1 or 1:2 molar ratio is simply a superposition of those of the two components. Coprecipitates of

bromazepam with DM- β -CD in 1:1 molar ratio showed new crystalline diffraction peaks (Curve B) at $2\theta = 8.2^\circ, 8.9^\circ, 11.2^\circ, 18.2^\circ$ and 19.2° which are quite different from the diffraction peaks of bromazepam and DM- β -CD physical mixture. This could indicate the formation of a crystalline inclusion compound. After heating of the coprecipitate at 495 K for 2 minutes (Curve C), the diffraction peaks of DM- β -CD and bromazepam appeared and increased in intensity compared to the peaks of the inclusion compound. This may be a result of the dissociation of the inclusion complex by heating. The coprecipitate of bromazepam with DM- β -CD in 1:2 molar ratio (curve E) showed a crystalline diffraction pattern with new peaks at $2\theta = 8.2^\circ, 8.9^\circ, 9.6^\circ, 11.2^\circ, 18.3^\circ$ and 19.3° which are quite different from the diffraction peaks of bromazepam and DM- β -CD physical mixture. This could indicate the formation of a crystalline inclusion compound. The diffractogram of the coprecipitate 1:2 has a diffused diffraction part beyond $2\theta = 20.5^\circ$ as compared to that of the coprecipitate 1:1 molar ratio. On heating the coprecipitate of 1:2 molar ratio at 373 K for 4 minutes (Curve F), the diffused diffraction part beyond $2\theta = 20.5^\circ$ became crystalline in addition to the presence of new crystalline diffraction peaks at $2\theta = 7.9^\circ, 9.6^\circ, 15.9^\circ, 18.1^\circ$ and 19.4° . After heating the coprecipitate of bromazepam with DM- β -CD in 1:2 molar ratio at 495 K for 2 minutes, crystalline diffraction patterns appeared with the crystalline peaks of the inclusion compound at $2\theta = 8.2^\circ, 8.9^\circ$ and 9.6° and 11.2° (curve G). From X-ray diffraction data, the crystalline inclusion compound of bromazepam with DM- β -CD either in 1:1 or 1:2 molar ratio was assumed to be formed by the coprecipitation method.

Fig. 3 shows the DSC curves of bromazepam with DM- β -CD coprecipitation systems in 1:1 and 1:2 molar ratios. Physical mixture of bromazepam with DM- β -CD in 1:1 molar ratio (curve A) shows an endothermic peak at 505 K due to the fusion of bromazepam. DSC curve of the coprecipitate in 1:1 molar ratio shows two broad endothermic peaks at onset temperature 474 K and another endothermic one at 506 K. After heating the coprecipitate at 495 K for 2 minutes, it shows only one endothermic peak at 500 K. Therefore, the broad endothermic peak of the coprecipitate before heating at 474 K may be due to phase transition or dissociation of the inclusion compound as seen from the change of the X-ray diffraction pattern of the coprecipitate after heating as compared to that before heating. The endothermic peak at 506 K or 500 K of the coprecipitate before and after heating, respectively, may be due to the fusion of excess bromazepam or as a result of dissociation of the inclusion compound.

The DSC curve of the physical mixture of bromazepam with DM- β -CD in 1:2 molar ratio is shown in Fig. 3 (curve D). An endothermic peak at 496 K is observed due to the fusion of bromazepam. On the other hand, the coprecipitate in the same molar ratio (Curve E) shows two peaks; one exothermic at 357 K and another broad endothermic with two consequent tops at onset temperature of 464 K, and no endothermic peak in the melting region of bromazepam is observed. Upon heating the coprecipitate at 373 K for 4 minutes (curve F), the exothermic peak disappeared and two endothermic peaks were observed at 470 K and 508 K, respectively. Hence, the exothermic peak of the coprecipitate before heating might be due to the recrystallization or crystalline

phase change as confirmed by the X-ray diffraction pattern of the coprecipitate before and after heating. Upon heating the coprecipitate at 495 K for 2 minutes (curve G), the exothermic and the endothermic peaks shown on the DSC curve of the coprecipitate before heating disappeared. Therefore, the broad endothermic peak of the coprecipitate before heating may be due to crystalline phase transition as predicted from X-ray diffraction pattern of the coprecipitate before and after heating at 495 K.

Fig. 4 shows the IR spectra of bromazepam with DM- β -CD systems in 1:1 and 1:2 molar ratios. Bromazepam crystals (curve A) have one carbonyl stretching band at 1702 cm^{-1} . The IR spectrum of the coprecipitate of bromazepam with DM- β -CD in 1:1 molar ratio (curve B) shows higher and lower frequency shifts for the carbonyl band elicited by the appearance of two bands at 1712 and 1688 cm^{-1} . The change of the carbonyl band to become forked is typical to the Fermi resonance¹⁰. In addition, the skeletal stretching of the aromatic ring at 1614 cm^{-1} became a forked band at 1621 and 1615 cm^{-1} , which may be due to the change of the environment around bromazepam molecules by DM- β -CD molecules. These IR spectral changes of molecular vibrations of bromazepam in carbonyl and skeletal ring region could be due to the interaction between bromazepam and DM- β -CD molecules through inclusion complexation. Upon heating the coprecipitate of 1:1 molar ratio at 495 K for 2 minutes (curve C), the higher and the lower frequency shifts for the carbonyl band of bromazepam still existed at 1708 and 1688 cm^{-1} but the band size and intensity decreased than before heating.

The IR spectrum of the coprecipitate of bromazepam with DM- β -CD in 1:2 molar ratio (curve D) shows higher frequency shift with a distinct band at 1712 cm^{-1} and lower frequency shift with a shoulder at 1695 cm^{-1} . These features are quite different from those of the coprecipitate in 1:1 molar ratio. Upon heating the coprecipitate of 1:2 molar ratio at 373 K for 4 minutes (curve E), higher and lower frequency shifts with two large distinct bands at 1712 and 1688 cm^{-1} , respectively, were observed. These features are similar to those of the coprecipitate in 1:1 molar ratio before heating. This indicates that some sort of molecular reorientation occurred upon heating the coprecipitate in 1:2 molar ratio. This result could be confirmed by the disappearance of the exothermic peak on the DSC thermogram as mentioned previously (Fig. 3, curve F). After heating the coprecipitate of 1:2 molar ratio at 495 K for 2 minutes, the IR spectrum (curve F) showed higher and lower frequency shifts with the appearance of a large and intense band at 1689 cm^{-1} . This again confirms the molecular reorientation by heating the coprecipitate at 495 K passing by 373 K.

Fig. 5 shows the IR spectra of bromazepam with DM- β -CD systems in 1:1 and 1:2 molar ratios in the 900 to 700 cm^{-1} region using the KBr disk method. Curve A showed the out of plane C-H bending band of bromazepam at 820 cm^{-1} while curve B of DM- β -CD did not show this band. The coprecipitate of bromazepam with DM- β -CD in 1:1 molar ratio showed higher and lower frequency shifts for out of plane C-H bending from 820 cm^{-1} to 836 and 809 cm^{-1} (curve C). This change in the C-H out of plane bending was accompanied by the change in the carbonyl stretching of

bromazepam as seen previously, which may be due to the molecular interaction between bromazepam and DM- β -CD through inclusion complexation process. Upon heating the coprecipitate at 495 K for 2 minutes (curve D), the shift of C-H out of plane bending at 820 cm^{-1} observed at 835 and 809 cm^{-1} but with decreased band size and intensity. In addition, small shoulder at 820 cm^{-1} as in intact bromazepam arises beside the two new bands; which may be due to dissociation of the inclusion compound by heating as mentioned previously in the X-ray diffraction and the DSC results. On the other hand, in case of the coprecipitate in 1:2 molar ratio, the band of the C-H out of plane bending at 820 cm^{-1} disappeared without the appearance of new bands instead. These IR results reveal, once again, that the mode of interaction of bromazepam with DM- β -CD differs according to the molar ratio studied. Upon heating the coprecipitate of 1:2 molar at 373 K for 4 minutes (curve F), the new bands of C-H out of plane bending at 835 and 809 cm^{-1} appeared instead of the band at 820 cm^{-1} which is similar to the IR spectrum of the 1:1 coprecipitate before heating. This confirms the occurrence of molecular reorientation upon heating the coprecipitate in 1:2 molar ratio. After heating the coprecipitate of 1:2 molar ratio at 495 K for 2 minutes (curve G), the new bands instead of the band of the C-H out of plane bending at 820 cm^{-1} disappeared again in contrast to the case after heating at 373 K confirming the occurrence of molecular reorientation by heating of the coprecipitate.

In summary, bromazepam in the inclusion compound with DM- β -CD and in a higher combining molar ratio (1:1) showed a strong carbonyl absorption band at 1688 cm^{-1} and a small forked one at 1712 cm^{-1} accompanied by appearance of two new

bands instead of the band of the C-H out of plane bending. On the other hand, the lower combining molar ratio (1:2), showed a strong carbonyl absorption band at 1712 cm^{-1} and a shoulder at 1695 cm^{-1} accompanied by disappearance of the band of C-H out of plane bending.

Conclusion

The different behaviours of the inclusion compounds in the two molar ratios studied could lead to the conclusion that the mode of interaction of bromazepam with DM- β -CD in 1:1 molar ratio is different from that in 1:2 molar ratio.

Fig. 1 : Phase Solubility Diagram of Bromazepam with DM- β -CyD in Aqueous Buffer Solution (pH 9.0) at 30°C.

Fig. 2 : Powder X-ray Diffraction Patterns of Bromazepam 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- Coprecipitate 1:1 after heating at 495 K for 2 min
- D- Physical Mixture 1:2
- E- Coprecipitate 1:2
- F- Coprecipitate 1:2 after Heating at 373 K for 4 min
- G- Coprecipitate 1:2 after heating at 495 K for 2 min

Fig. 3 : DSC curves of Bromazepam with DM- β -CyD Physical Mixtures and Coprecipitates in 1:1 and 1:2 Molar Ratios.

Key : the same as in Fig. 2.

Fig. 4 : IR Spectra of Bromazepam with DM-SCVD systems in 1:1 and 1:2 molar Ratios. ($1800-1600 \text{ cm}^{-1}$)

A- Bromazepam Crystals
 B- Coprecipitate 1:1
 C- Coprecipitate 1:1 after Heating at 495 K for 2 min
 D- Coprecipitate 1:2
 E- Coprecipitate 1:2 after Heating at 373 K for 4 min
 F- Coprecipitate 1:2 after Heating at 495 K for 2 min

Fig. 5 : IR spectra of Bromazepam with DM-B-CyD Systems in 1:1 and 1:2 Molar Ratios Using KBr Disk ($900-700 \text{ cm}^{-1}$)

A- Bromazepam crystals
 B- DM-B-CyD crystals
 C- Coprecipitate 1:1
 D- Coprecipitate 1:1 after Heating at 495 K for 2 min
 E- Coprecipitate 1:2
 F- Coprecipitate 1:2 after Heating at 373 K for 4 min
 G- Coprecipitate 1:2 after Heating at 495 K for 2 min

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كيفية تكوين المتراكب المنغمس بين البرومازيام

وداي ميشيل البيتا سيكلوديكترين

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تم في هذا البحث التأكد من تكوين المتراكب المنغمس بين البرومازيام وداي ميشيل البيتا سيكلوديكترين في الحالة السائلة وذلك باستخدام طريقة الاذابة.

وقد وجد ان نمط الاذابة يتبع نظام AL مع زيادة قدرها ٥ مرة في اذابة البرومازيام. كذلك تم الحصول على المتراكب المنغمس في صورة بلورية باستعمال طريقة الترسيب المتزامن للبرومازيام مع داي ميشيل البيتا سيكلوديكترين بنسبة جزيئية 1:1 ، ٢:1 على الترتيب وقد أثبت ذلك باستخدام طريقة تحليل تشتت أشعة اكس.

وقد وجد أن مطياف المتراكب المنغمس بالأشعة تحت الحمراء يختلف طبقاً للنسب الجزيئية للمتراكب وقد وجدت هذه الظاهرة مرة أخرى عند إجراء المسح السعري التفاضلي للمتراكبات المنغمة المتكونة.

وقد استخلص من ذلك أن كيفية تكوين المتراكب المنغمس بين البرومازيام وداي ميشيل بيتا سيكلوديكترين تختلف باختلاف النسب الجزيئية المستخدمة.