Study of Complex Formation of Carbamazepine with Thiourea

Yutaka Inoue,*[,a] Sayuri Sato,[a] Chisa Yamamoto,[a] Mikio Yamasaki,[b] and Ikuo Kanamoto[a]

[a] *Department of Drug Safety Management, Faculty of Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan: and* [b] *Rigaku Corporation; 3–9–12 Matsubara-cho, Akishima, Tokyo 196–8666, Japan.*

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The aim of this study, we evaluated a complex between thiourea (TU) and carbamazepine (CBZ) of a poorly soluble drug by using powder X-ray diffraction (PXRD), Fourier transform infrared (FT-IR) spectroscopy, X-ray crystallography and the solubility test. PXRD of TU/CBZ=2/1, 1/1, and 1/2 prepared by solvent evaporation (EVP) revealed characteristic diffraction peaks at 2θ=6.7°, 8.8°, 13.5°, and 20.4°, therefore molecular interaction between TU and CBZ presumably occurred. Results of the FT-IR spectroscopy, asymmetric and symmetric NH stretching vibration of TU were shifted to high region by TU/CBZ=2/1, 1/1, and 1/2 EVP. TU/CBZ=2/1 and 1/1 EVP had absorption derived from TU. It was considered that complex were formed by TU/CBZ=1/2. X-Ray crystallography of TU and CBZ revealed a crystal structure with one TU molecule arranged near two CBZ molecules. Molecules of the same type overlap in this layer. When doing a solubility test by using CBZ and samples of EVP, physical mixture and crystals in TU/CBZ=1/2 to confirm the solubility in water of TU/CBZ complex, there is no difference with the CBZ. It considered that the structure of a complex differs from the tunnel structure of inclusion complexes that has been previously reported contribute to result it.

Key words thiourea; carbamazepine; complex; X-ray crystallography; hydrogen bond

Over the past few years, many poorly soluble drugs have been discovered. Improving their solubility is key to development of oral preparations. Various preparation techniques have been researched and developed to improve drug solubility in water. One such technique is the formation of cocrystals. Cocrystals are crystals that are regularly arranged as a result of molecular interaction between molecules of multiple constituents in a crystal structure; this interaction may be the result of hydrogen bonds, van der Waals force, or some other mechanism. Unlike salt formation, cocrystal formation does not involve converting constituents into an ionic form. The formation of cocrystals of a pharmaceutical involves forming crystalline complexes consisting of a drug and various excipients. An advantage of this process is that the drug need not be chemically modified to improve its physical properties. Various methods such as solvent evaporation,[1] grinding,[2,3] and slurrying[4] are used to prepare cocrystals, and changes in solubility[5,6] and improvement in physical stability[7] and bioavailability[8] as a result of the formation of cocrystals have been studied.

Thiourea (TU) forms inclusion complexes with guest molecules in a tunnel structure,[9] and TU is used to isolate and purify substances. Like TU, urea forms inclusion complexes with a tunnel structure,[9] and a study has reported improving its solubility of guest molecules and improving its stability to light.[10] One difference between TU and urea is that TU inclusion complexes have a tunnel structure with a larger inner diameter than that of urea inclusion complexes.[11] As a result, TU is better at forming adducts with branched and cyclic compounds while urea is better at forming adducts with molecules in straight chains. When TU and urea form adducts with the same substance, TU and urea are reported to include the guest molecule at a different molar ratio.[12] TU has been found to form complexes in shapes besides a tunnel structure. A study coground TU and ethenzamide; this resulted in formation of a new complex at a molar ratio of 1/1 and improved the dissolution of ethenzamide.[13]

Carbamazepine (CBZ) is an antiepileptic and is also used as an analgesic to specifically treat pain associated with trigeminal neuralgia.[14] CBZ is a poorly soluble drug and is known to have four anhydrous polymorphs.[15] CBZ is known to form complexes with various molecules, and studies have described improvement of its solubility and bioavailability.[16] A study has reported that formation of HP-βCD and CBZ complexes resulted in improved solubility and rapid anticonvulsant action when administered parentally to mice.[17]

The current study used powder X-ray diffraction (PXRD), Fourier transform infrared (FT-IR) spectroscopy, and X-ray crystallography to examine the crystal structure of complexes of TU and CBZ formed by solvent evaporation and cogrinding. In addition, this study featured an assessment of the solubility of TU/CBZ complexes.

Experimental

Materials The CBZ used (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was a commercial reagent. The TU used (Wako Pure Chemical Industries, Ltd.) was ground for 30 s with a mortar and pestle to indicate its orientation. The structural formulae of these reagents are shown in Fig. 1. Other reagents were of special reagent grade (Wako Pure Chemical Industries, Ltd.).

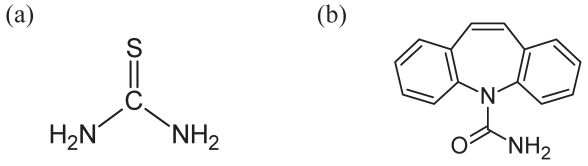

Fig. 1. Chemical Structure of (a) Thiourea (TU) and (b) Carbamazepine (CBZ)

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: yinoue@josai.ac.jp

Methods: Preparation of a Concentrated Sample by Solvent Evaporation (EVP) The EVP of TU and CBZ was prepared by weighing 0.2 g of TU and CBZ at a molar ratio of 2/1, 1/1, and 1/2, placing the mixture in a pear-shaped flask, and then dissolving it in 60 mL of ethanol. The solvent was evaporated from this solution using a rotary evaporator (R-215 Rotavapor, Nihon Buchi) to yield the EVP.

Preparation of a Physical Mixture (PM) A PM of TU and CBZ was prepared by placing TU and CBZ in a vial at a molar ratio of 1/2 and then mixing the two for 3 min with a Vortex Mixer.

Preparation of Single Crystals TU and CBZ crystals were prepared by heating 0.2 g of the PM in a water bath at 40°C and then completely dissolving the PM in 50 mL of ethanol. The solution was left to stand at 4°C for 48 h, resulting in gradual evaporation of the solvent.

PXRD Measurement PXRD patterns were measured at room temperature with a powder X-ray diffractometer (model MiniFlex II, Rigaku, Tokyo, Japan) using Cu$K\alpha$ radiation. Measurement conditions were a voltage of 30 kV, a current of 15 mA, a scanning speed of 4°C/min, and a measurement range of 2θ=3–35°.

FT-IR Spectroscopy A FT-IR spectrophotometer (FT/IR-410, JASCO) was used for IR spectroscopy of samples tableted with potassium bromide (KBr). Samples were prepared by adding KBr at a mass ratio of 1/10 (sample/KBr). Background correction was done using KBr disks. Scanning was done 16 times from 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

X-Ray Crystallography X-Ray crystallography was performed at −180°C (using a spray cooler) using an X-ray crystallography system (VariMax-DW with RAPID, Rigaku) with Cu$K\alpha$ radiation. Crystallography conditions were a voltage of 40 kV, a current of 30 mA, an oscillation angle of 2 deg, and an exposure time of 48 s/deg. Data were processed at $2\theta_{max} \leq 136.3°$. Data were corrected for Lorentz and polarization factors.

Dissolution Profile Dissolution testing of samples was conducted using dissolution apparatus (NTR-593, Toyama Sangyo Co., Ltd., Japan) at 37±0.5°C with 900 mL of distilled water, which was stirred at 50 rpm using the paddle method. Each sample made the sieve 75–500 μm, and performed the diameter of a particle using the 200 mg. 0.45 μm membrane filters, 10 mL dissolution samples were collected at 5, 10, 15, 30, and 60 min. Using distilled water, 5 mL of each filtered sample was diluted to 50 mL. Samples of the diluted solutions were analyzed for CBZ concentrations using ultraviolet-visible spectrophotometer (UV-2500PC, Shimadzu, Kyoto, Japan) at 284 nm.

Solubility Test For solubility testing, samples were weighed accurately for the equivalent of 200 mg of CBZ. These samples were placed in a test solution (50 mL of distilled water) and then shaken in a shaking incubator (Bio Shaker BR-42FL, TAITEC) at 37°C for 6 h and 24 h. Ten milliliter of each sample was measured and filtered with a 0.45 μm membrane filter. Distilled water was added to 5 mL of filtrate to dilute the sample solution to 50 mL, and then absorbance was measured with an ultraviolet-visible spectrophotometer (UV-2500PC, Shimadzu) at a wavelength of 284 nm. Solubility was then calculated.

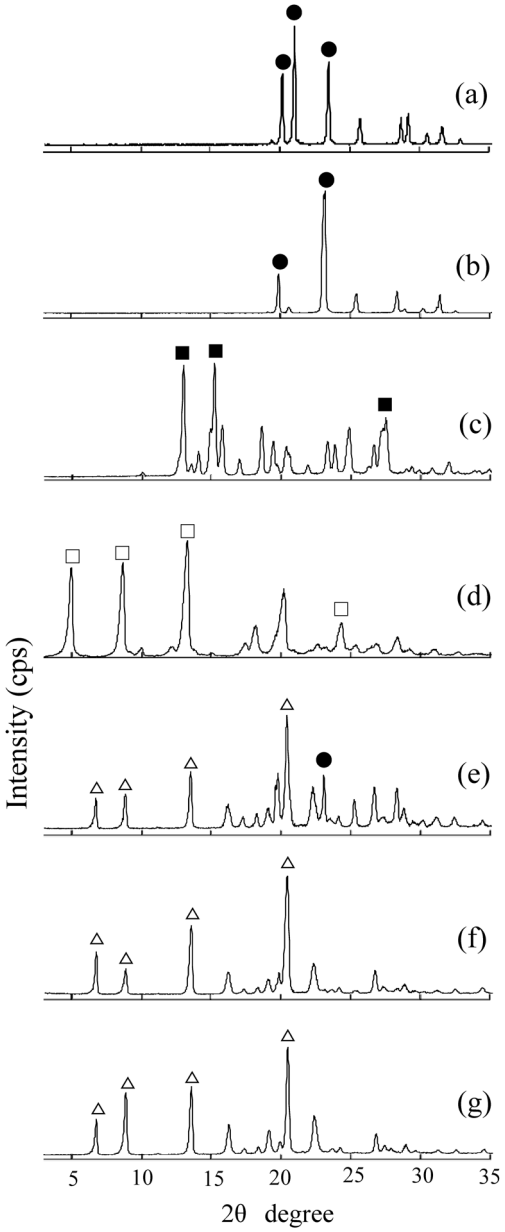

Fig. 2. PXRD Patterns of TU/CBZ Systems

(a) TU, (b) TU-EVP, (c) CBZ, (d) CBZ-EVP, (e) EVP (molar ratio of TU/CBZ=1/2), (f) EVP (TU/CBZ=1/1), and (g) EVP (TU/CBZ=1/2). The diffraction peaks due to TU, CBZ, CBZ-EVP and complex were indicated by ●, ■, □, and △.

Results and Discussion

Examination of Molecular Interaction of TU and CBZ PXRD was performed to examine the crystalline state of concentrated samples of TU and CBZ resulting from solvent evaporation (Fig. 2). TU was found to produce diffraction peaks at 2θ=20.0° and 23.0° and concentrated TU resulting from solvent evaporation (TU-EVP) was found to produce diffraction peaks at 2θ=19.6° and 23.1°, so TU and TU-EVP had the same crystal structure. Diffraction peaks due to CBZ were produced at 2θ=12.9°, 15.1°, and 27.4°. Concentrated CBZ resulting from solvent evaporation (CBZ-EVP) produced peaks at 2θ=8.7°, 13.3°, and 24.5°. CBZ is reported to have four anhydrous polymorphs, and crystallization of CBZ by ethanol is known to result in Form II.[15] Characteristic peaks of CBZ Form II were produced at 2θ=8.7°, 13.3°, 18.6°, and 24.5°, indicating the same diffraction pattern as that of CBZ-EVP.

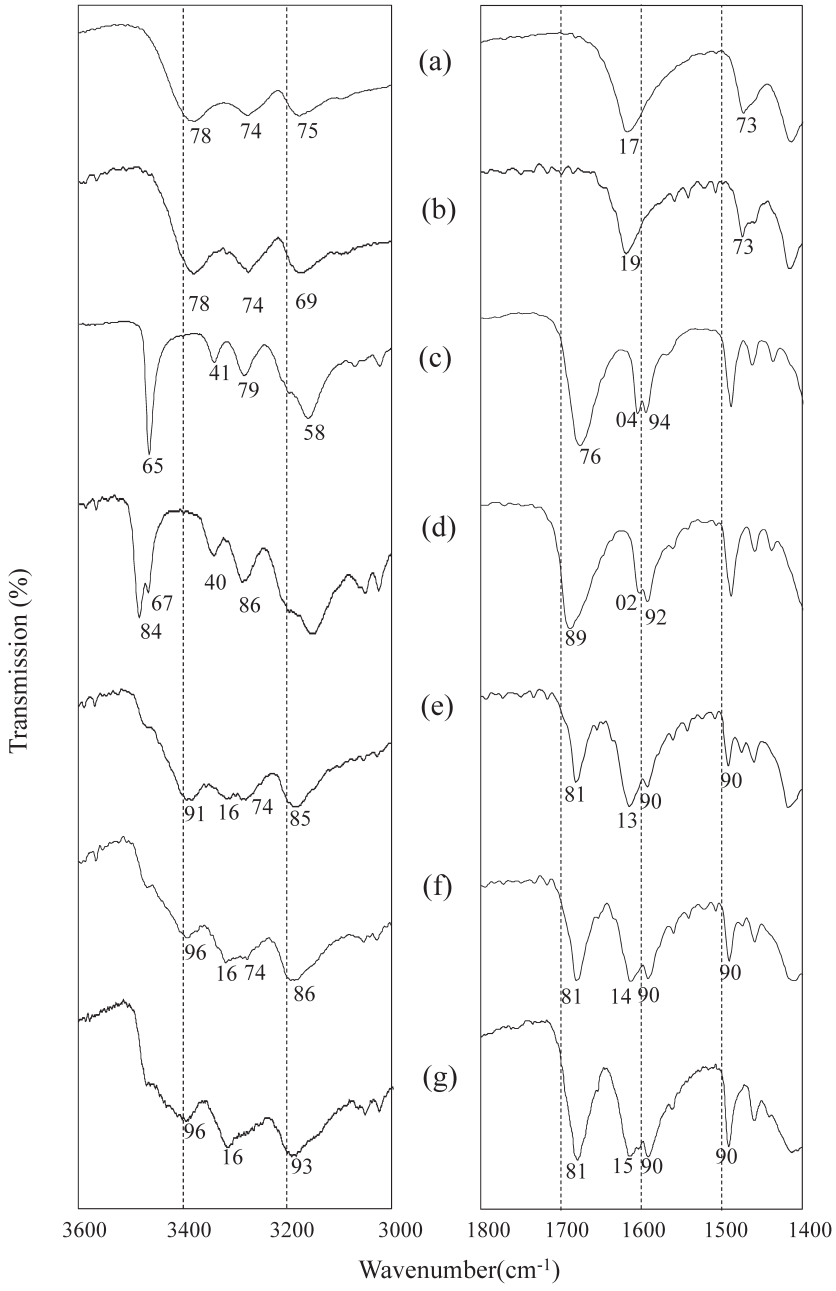

Fig. 3. FT-IR Spectra of TU/CBZ Systems

(a) TU, (b) TU-EVP, (c) CBZ, (d) CBZ-EVP, (e) EVP (molar ratio of TU/CBZ=2/1), (f) EVP (TU/CBZ=1/1), and (g) EVP (TU/CBZ=1/2).

This indicates that solvent evaporation resulted in CBZ Form II. In systems with TU/CBZ=2/1, 1/1, and 1/2 EVP, characteristic diffraction peaks were produced at 2θ=6.7°, 8.8°, 13.5°, and 20.4°, so molecular interaction between TU and CBZ presumably occurred. However, TU/CBZ=2/1 EVP was found to produce diffraction peaks due to TU at 2θ=23.1°, suggesting that TU remained.

FT-IR absorption spectroscopy was performed to examine molecules in a solid state. The FT-IR spectra for the prepared samples are shown in Fig. 3. TU was found to have absorption due to N–H stretching vibration at around 3378 cm^{-1}, 3274 cm^{-1}, and 3175 cm^{-1} and absorption due to N–H bending vibration at around 1617 cm^{-1}. CBZ had an absorption peak due to carbonyl (C=O) stretching vibration in its primary amide at around 1676 cm^{-1}, while CBZ-EVP had a peak at around 1689 cm^{-1}. CBZ had absorption at around 3341 cm^{-1} and 3279 cm^{-1} due to symmetric and asymmetric NH stretching in its primary amide while CBZ-EVP was found to have absorption at around 3340 cm^{-1} and 3286 cm^{-1}. CBZ was found to have transformed into CBZ Form II as a result of evaporation of the ethanol solution. CBZ-EVP produced absorption at 1689 cm^{-1} due to carbonyl stretching in the primary amide of CBZ. For TU/CBZ=2/1, 1/1, and 1/2 EVP, absorption shifted to 1681 cm^{-1}. This presumably indicates that carbonyl group of CBZ carbonyl group interacted with TU. TU-EVP produced absorption at 3378 cm^{-1}, 3274 cm^{-1}, and 3169 cm^{-1} due to asymmetric and symmetric NH stretching in TU. All 3 of the EVP samples (TU/CBZ=2/1, 1/1, and 1/2) had a high level of absorption, indicating that the amino group (NH) of TU is involved in complex formation. TU/CBZ=2/1

and 1/1 produced absorption at 3274 cm^{-1}, indicating that TU remained. These findings presumably indicate that complexes were formed by TU/CBZ=1/2.

X-Ray Crystallography of Complexes The structure of complexes was examined further *via* X-ray crystallography of the crystals prepared TU and CBZ. Figure 4 shows the diffraction pattern for TU/CBZ=1/2 EVP according to X-ray crystallography. The two diffraction patterns match, so EVP has the same crystal structure as single crystals.

X-Ray crystallography revealed that TU and CBZ form complexes at a molar ratio of 1/2 (Fig. 5). Structural parameters and lengths of hydrogen bonds are shown in Tables 1 and 2. In complexes, TU and CBZ form a layer with a TU molecule arranged near two CBZ molecules. Molecules of the same type overlap in this layer (Fig. 5a). NH…O hydrogen bonds are formed between the two CBZ molecules. This dimer configuration has been described as a crystal structure of a typical anhydrous polymorph of CBZ.[18] As examples of molecular interaction in CBZ cocrystals, benzoquinone and terephthalaldehyde are reported to form cocrystals with CBZ at a ratio of 1 : 2. In such an event, all of the NH groups of the primary amide of CBZ form hydrogen bonds. Symmetric NH groups are reported to be involved in bonding between CBZ dimers while asymmetric NH groups are reported to be involved in bonding with partners.[19] In the crystals obtained in the current study, however, amino group of CBZ was not

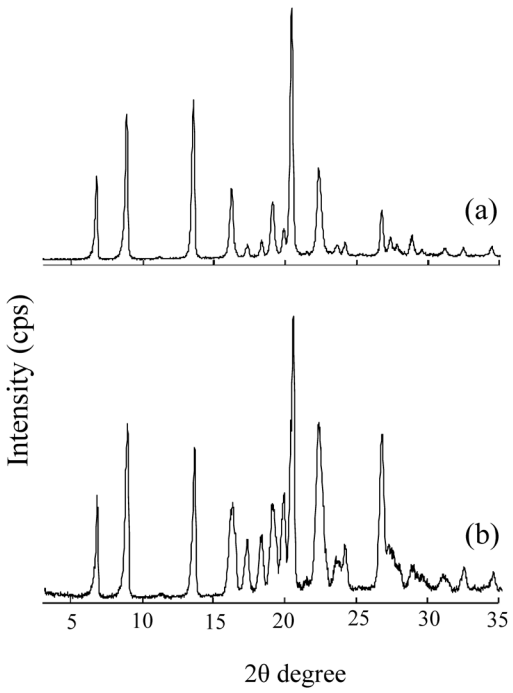

Fig. 4. PXRD Patterns of TU/CBZ (Molar Ratio of TU/CBZ=1/2)
(a) EVP. (b) Single crystal.

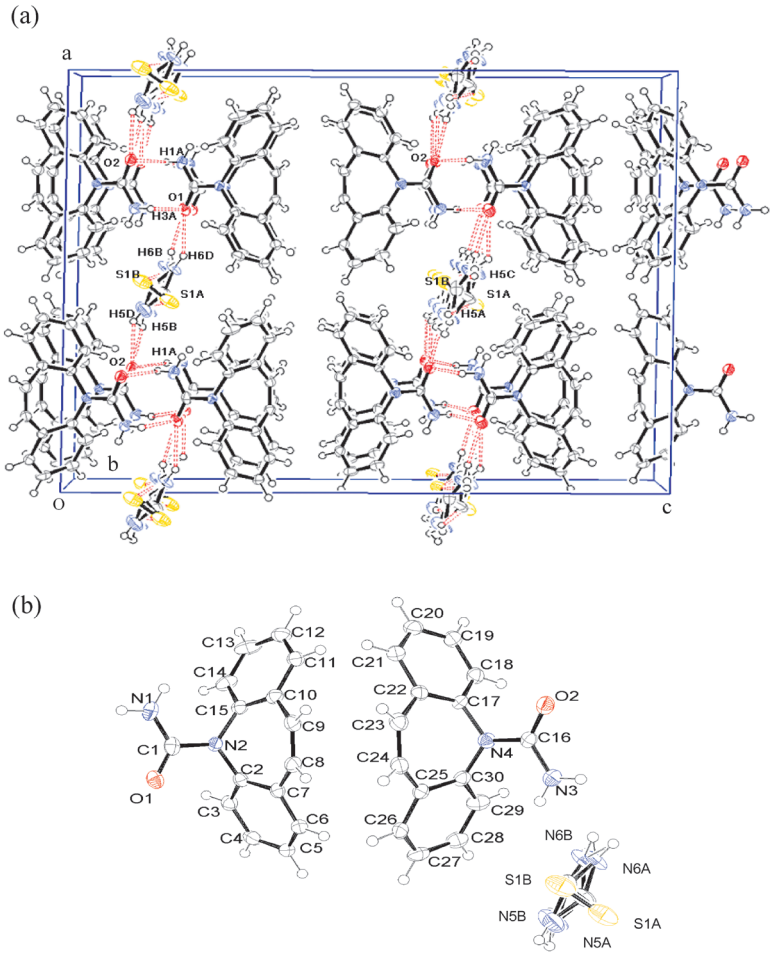

Fig. 5. Structure and Packing of TU/CBZ Complex in the Crystal Lattice
(a) Packing of the complex in a crystal lattice. (b) Structure of the complex.

involved in interaction with TU. TU formed NH…O hydrogen bonds with the carbonyl group of the CBZ dimer. TU facilitated interaction *via* N–H…S hydrogen bonds in a molecule.

In the tunnel structure of TU inclusion complexes, the N–H…S bond length is reported to be about 3.4 Å at room temperature.[20] In the complexes obtained in the current study, the minimum distance between TU molecules was 10.8 Å, so molecules were spread far apart. Because of this, there was no interaction between TU molecules in complexes. In TU inclusion complexes, TU molecules form a tunnel structure as a result of hydrogen bonding.[21] Hydrogen bonds between TU molecules were not noted in the crystals obtained in the current study, so complexes that were not inclusion complex were formed.

Evaluation of the Solubility of CBZ as a Result of Complexation A CBZ dissolution and solubility test were performed with CBZ, PM, EVP, and crystals to study changes in the solubility of CBZ as a result of complexation of CBZ and TU. The results are shown in Fig. 6 and Table 3. Since urea is hydrotropic, a study reported using urea to improve the dissolution of a poorly soluble drug.[22] Formation of complexes with TU, an analog of urea, should improve the solubility of CBZ. Results of the dissolution test, initial dissolution rate of crystals was improved than the other samples. CBZ, PM, and EVP had slower dissolution behavior than single crystals presumably because CBZ caused aggregation and/or agglomeration in the solvent. In addition, PM and EVP showed elution be different from the CBZ. This result was presumed to be a property of hydrotropic thiourea. The next solubility examina-

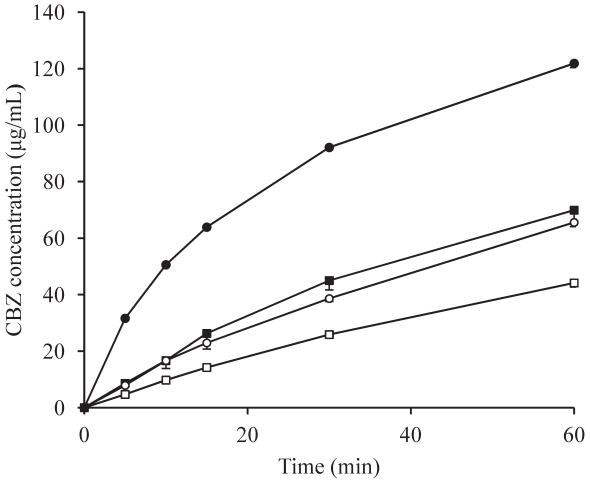

Fig. 6. Dissolution Profiles of TU/CBZ Systems

□: CBZ, ■: PM, ○: EVP, ●: single crystal. Results were expressed as mean±S.D. (n=3).

Table 1. Crystallographic Parameters of TU/CBZ Complexes

Empirical formula	$C_{31}H_{28}N_6O_2S$
Formula weight	548.66
Crystal color, habit	colorless, platelet
Crystal dimensions	0.240×0.070×0.030 mm
Crystal system	monoclinic
Lattice type	C-centered
Lattice parameters	a=19.8041(4) Å
	b=5.26250(10) Å
	c=25.7811(5) Å
	β=90.858(6)°
	V=2686.57(9) Å^3
Space group	Cc (#9)
Z	4
D_{calc}	1.356 g/cm^3
μ (Cu$K\alpha$)	14.029 cm^{-1}
Radiation	Cu$K\alpha$ (λ=1.54187 Å)
Monochromator	multi-layer mirror
Temperature	−180.0°C
No. observations (all reflections)	4667
No. variables	399
Reflection/parameter ratio	11.70
Residuals: R1 ($I>2.00\sigma(I)$)	0.0460
Residuals: R (all reflections)	0.0486
Goodness of fit indicator	1.043
Maximum peak in final diff. map	0.59 e-/Å^3
Minimum peak in final diff. map	−0.34 e-/Å^3
Structure solution:	Direct methods

Table 2. Hydrogen Bond Interactions (Å) of TU/CBZ Complexes

Donor	H	Acceptor	D…A (Å)	D-H (Å)	H…A (Å)	D-H…A (°)
N1	H1A	O2	2.871 (4)	0.880	2.022	161.9
N3	H3A	O1	2.898 (4)	0.880	2.043	162.3
N5A	H5A	S1A	3.090 (8)	0.880	2.381	137.47
N5A	H5B	O2	2.904 (12)	0.880	2.039	167.4
N5B	H5D	O2	2.861 (10)	0.880	2.246	126.7
N6A	H6B	O1	2.873 (10)	0.880	2.308	122.0
N6B	H6C	S1B	3.122 (17)	0.880	2.355	145.8
N6B	H6D	O1	2.930 (11)	0.880	2.143	148.4

D: Donor. A: Acceptor.

Table 3. Solubility Test in Water at 37°C for CBZ in TU/CBZ Systems

	Solubility of CBZ (μg/mL)			
Time (h)	CBZ	PM (1/2)	EVP (1/2)	Single crystal (1/2)
6	180±1.0	193±1.3	217±0.7	207±0.7
24	183±0.7	197±1.3	213±0.7	203±1.7

Results were expressed as mean±S.D. (n=3).

tion failed to improve the solubility of CBZ. Because the complexes formed in this study had a structure unlike the tunnel structure of inclusion complexes.

Conclusion

TU and CBZ were found to form complexes at a molar ratio of 1/2. Although it is known that TU will form clathration union of tunnel structure by a hydrogen bond, X-ray crystallography indicated that TU molecules in complex did not form hydrogen bonds. TU forms a cocrystal structure unlike the typical tunnel structure of inclusion complexes. When TU is added to solid pharmaceutical preparations such as tablets and granules to enhance solubility and stability, it presumably forms a crystal structure at the molecular level rather than simply combining with drug particles. In this study, solubility failed to improve, but evidence of cocrystal formation by an analog of urea is crucial to areas like pharmaceutical additives and cosmetics. This finding should lead to future research and development.

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