Simultaneous Dissolution of Naproxen and Flurbiprofen from a Novel Ternary γ-Cyclodextrin Complex

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A crystalline ternary complex was prepared by sealed-heating of naproxen (NPX) with a flurbiprofen (FBP)/γ-cyclodextrin (γ-CD) inclusion complex. The dissolution rates of NPX and FBP in the ternary complex were almost the same, indicating that FBP and NPX from the complex dissolved simultaneously. The ternary CD complex showing a fascinating dissolution property could be a new formulation for combination therapies.

Key words cyclodextrin; dissolution; powder X-ray diffraction; sealed-heating; inclusion complex

Many drug candidates usually show poor water solubility. Various pharmaceutical techniques such as polymorphic control, co-crystal formation, and nanoparticle preparation are available to improve the physicochemical properties of drugs.[1—4] Cyclodextrin (CD) inclusion complex formation is also a useful technique for improving the dissolution property and enhancing the bioavailability of drugs.[5—7] Co-precipitation, freeze-drying, evaporation, co-grinding, and processing with supercritical CO_2 are used for the preparation of solid CD inclusion complexes.[8—10] We have prepared various types of solid CD inclusion complexes by using the sealed-heating method wherein a mixture of guest and CD molecules is simply heated within an enclosed space.[11—13] In the sealed-heating method, vaporized guest molecules are assumed to be included in the cavity of CDs through the gas phase. Crystalline inclusion complexes of poorly water-soluble drugs can be obtained through the sealed-heating method without using solvents. Specific inclusion complexes that are difficult to prepare can also be obtained through this method. In our recent study, it was found that the sealed-heating of salicylic acid (SA) with polyethylene glycol (PEG)/γ-CD-polypseudorotaxane induced complex formation.[14] In this complex, two SA molecules for each γ-CD molecule were incorporated into the intermolecular spaces between the columns of γ-CD stacks, while double-stranded PEG chains were included in the γ-CD cavity. Although a large number of studies have been carried out on the inclusion complexes of CDs, there are only a few reports on the existence of guest molecules outside the CD cavity.[15—18] New CD complexes with desired properties can be designed if the interstitial spaces outside the CD cavity are utilized for the incorporation of guest drugs. In this study, a ternary γ-CD complex containing two active pharmaceutical ingredients (APIs) was prepared by the sealed-heating method. Using this method, guest drugs could be incorporated into the intermolecular spaces between γ-CD columns. The dissolution property of the obtained complex was studied in order to assess its potential for pharmaceutical applications.

Flurbiprofen (FBP) and naproxen (NPX) are anti-inflammatory drugs with poor water solubility (Fig. 1). In this study, each drug was used as a model guest molecule either for inclusion in the γ-CD cavity or incorporation into the intermolecular spaces. The preparation process of the ternary complex consisted of two steps. First, a conventional FBP/γ-CD inclusion complex was prepared by the co-precipitation method. Second, after drying the complex at 100 °C for 3 h *in vacuo*, NPX was added to prepare a physical mixture (PM). A sealed-heated sample (SH) was obtained by heating the PM in a glass ampoule at 150 °C for 2 h. The structure of the prepared ternary complex was investigated by powder X-ray diffraction (PXRD) analysis. The dissolution test of the ternary complex was performed by the paddle method using acetate buffer (pH=4.0) as a dissolution medium. The sample solution was withdrawn at a specified time, and the drug content was determined by HPLC. Detailed experimental conditions are described in Experimental.

Figure 2 shows the changes in PXRD patterns through the ternary complex formation of NPX, FBP, and γ-CD. The speculated structural changes in γ-CD packing through the complex formation are summarized in Chart 1. In the diffraction pattern of the FBP-γ-CD co-precipitate (Fig. 2c), no peak of FBP (Fig. 2a) and γ-CD heptahydrate (Fig. 2b) was observed. The diffraction pattern of the co-precipitate corresponded to that of the tetragonal columnar form of γ-CD.[19—22] This indicated the complex formation of FBP with γ-CD. Quantitative analysis by spectrophotometry revealed that the molar ratio of the FBP/γ-CD co-precipitate was 1/1. The diffraction pattern of the tetragonal columnar form changed to that of hexagonal columnar form (Fig. 2d) after the co-precipitate was dried at 100 °C for 3 h under reduced pressure.[23,24] This result is consistent with the result of a previous study in which the solid transition from the tetragonal columnar form to a more tightly packed hexagonal-columnar form occurred upon the dehydration of γ-CD inclusion complexes with aromatic guests such as styrene, benzene, and ethylbenzene.[24] Although the removal of adsorbed water from the intermolecular spaces between the γ-CD columns induced the structural transition, the column packing of the γ-CD stacks still remained by the presence of guest molecules inside the cavity. The observed transition in the co-precipitate upon dehydration indicated that FBP was not outside of the γ-CD cavity, but included in the γ-CD cavity.

The PXRD pattern of the PM (Fig. 2f) showed a superposition of the peaks originating from the NPX crystal (Fig. 2e) and the hexagonal columnar form of γ-CD. In contrast, the

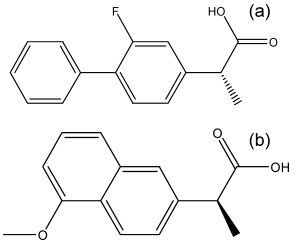

Fig. 1 Chemical Structures of (a) Flurbiprofen (Molecular Weight: 244.26, Water Solubility at 37 °C: 38 μg/ml) and (b) Naproxen (Molecular Weight: 230.26, Water Solubility at 37 °C: 38 μg/ml)

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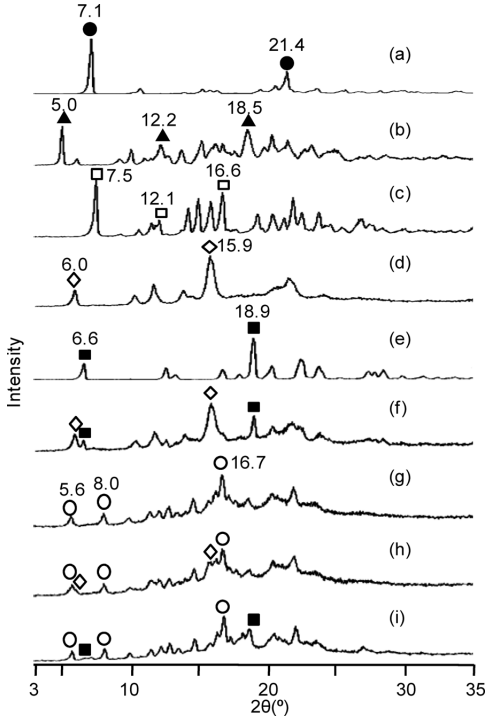

Fig. 2. Changes of Powder X-Ray Diffraction Patterns upon the Ternary Complex Formation of Naproxen, Flurbiprofen and γ-Cyclodextrin

(a) Flurbiprofen, (b) γ-cyclodextrin heptahydrate, (c) flurbiprofen-γ-cyclodextrin co-precipitate, (d) the co-precipitate after drying at 100 °C for 3 h *in vacuo*, (e) naproxen, (f) naproxen/(flurbiprofen/γ-cyclodextrin)=1/(1/1) physical mixture, (g) naproxen/(flurbiprofen/γ-cyclodextrin)=1/(1/1) sealed-heated sample, (h) naproxen/(flurbiprofen/γ-cyclodextrin)=0.5/(1/1) sealed-heated sample and (i) naproxen/(flurbiprofen/γ-cyclodextrin)=2/(1/1) sealed-heated sample. ●, Flurbiprofen; ▲, γ-cyclodextrin heptahydrate; ■, naproxen; □, tetragonal-columnar form; ◇, hexagonal-columnar form;, ○, monoclinic-columnar form.

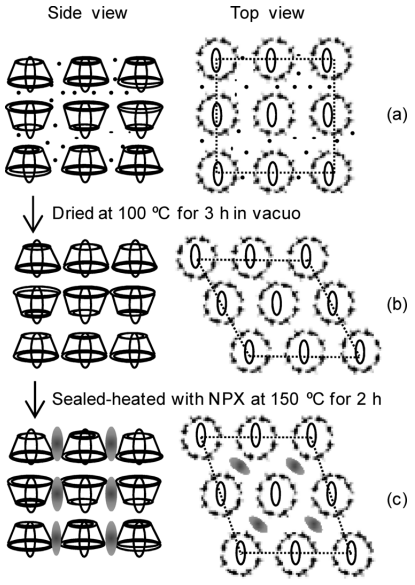

Chart 1. Structural Changes of γ-Cyclodextrin Packing by the Ternary Complex Formation

(a) Flurbiprofen/γ-cyclodextrin=1/1 inclusion complex with tetragonal-columnar form, (b) dried flurbiprofen/γ-cyclodextrin=1/1 inclusion complex with hexagonal-columnar form (c) naproxen/(flurbiprofen/γ-cyclodextrin)=1/(1/1) ternary complex with monoclinic-columnar form.

PXRD pattern of the NPX/(FBP/γ-CD)=1/(1/1) SH (Fig. 2g) showed only new diffraction peaks, indicating the ternary complex formation. New peaks were observed along with the diffraction peaks of the hexagonal columnar form and the NPX crystal in the diffraction patterns of the NPX/(FBP/γ-CD)=0.5/(1/1) (Fig. 2h) and 2/(1/1) (Fig. 2i) SHs. These results showed that the stoichiometry of the ternary NPX/(FBP/γ-CD) complex was 1/(1/1). The diffraction pattern of the SH was similar to that of the monoclinic columnar form reported previously.[14,25] We have proposed that the transformation from the hexagonal columnar form to the monoclinic columnar form takes place upon the complex formation of SA/(PEG/γ-CD-polypseudorotaxane) by the sealed-heating method.[14] The structural change in the molecular arrangement of γ-CD column packing resulted from the increase in the intermolecular spaces between the γ-CD columns by the incorporation of SA into these spaces. Two SA molecules, each having one benzene ring, were incorporated into the intermolecular spaces of the complex, whereas only one NPX molecule with a naphthalene ring could be incorporated into the same spaces. This suggested that the molecular size of guest molecules affected the amount of drug incorporated into the intermolecular spaces. It is worth noting that the crystalline ternary CD complex is unique because it contains two APIs in its structure: FBP in the γ-CD cavity and NPX in the intermolecular spaces.

The dissolution property of the ternary complex in a medium was evaluated for each drug (Fig. 3). Changes in the dissolution profile of FBP by the ternary complex formation are shown in Fig. 3a. The dissolution amount of FBP intact was approximately 30% even after 30 min. This could be attributed to the low wettability and low dissolution rate of FBP itself. The PM showed rapid dissolution from the beginning itself and the dissolution amount reached around 100% in 10 min. Since the PM consisted of NPX intact and the FBP/γ-CD inclusion complex, the rapid dissolution of FBP from the PM could be explained by the inclusion complex formation of FBP with γ-CD. On the other hand, FBP from the ternary complex showed gradual dissolution as compared to the PM. This shows that the complex formation with NPX affected the dissolution property of the FBP/γ-CD inclusion complex. It was assumed that the dissolution of FBP from the ternary complex was suppressed because of the hydrophobicity of the incorporated NPX.

The effect of the ternary complex formation on the NPX dissolution was also evaluated (Fig. 3b). The low dissolution rate of NPX intact can be attributed to its hydrophobic characteristics. The dissolution profile of the PM shows that the dissolution rate of NPX increased fairly. It suggested that coexistence of FBP/γ-CD inclusion complex improved the wettability of NPX intact. In contrast, NPX in the ternary complex exhibited faster dissolution than NPX intact and the PM. These results showed that the ternary complex formation influenced the dissolution rate of NPX as well as that of FBP. It was predicted that the crystal packing of NPX was disturbed during the formation of the ternary complex and subsequently NPX was monodispersed in the intermolecular spaces between γ-CD columns. This might result in the faster dissolution of NPX than the crystalline NPX.[14,26—28]

The dissolution profiles of NPX and FBP from the PM and ternary complex were compared (Fig. 3c). The dissolution rates of NPX and FBP from the PM were found to be different from each other. FBP dissolved immediately due to the inclusion complex formation with γ-CD, whereas the disso-

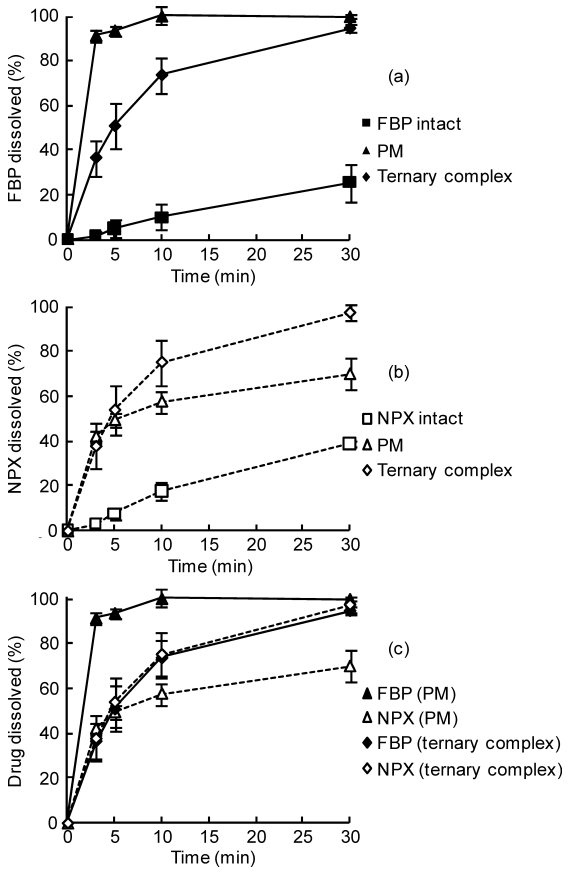

Fig. 3. Dissolution Profiles of Flurbiprofen and Naproxen from Intact, Physical Mixture (Crystalline Naproxen+Flurbiprofen/γ-Cyclodextrin Inclusion Complex), and Ternary Complex in Acetate Buffer (pH=4.0; n=3, Mean±Standard Deviation)

(a) Flurbiprofen dissolution, (b) naproxen dissolution, and (c) comparison of physical mixture and ternary complex.

lution rate of NPX was comparatively low because NPX in the PM was in the crystalline state. On the other hand, the dissolution rates of NPX and FBP in ternary complex were almost the same, indicating that FBP and NPX from the complex dissolved simultaneously. The dissolution study clearly confirmed that the releases of both FBP and NPX were influenced by the ternary complex formation. We speculated that the dissolution rate of the NPX (FBP/γ-CD) ternary complex depended on the release of the incorporated NPX. FBP present in the γ-CD cavity dissolved concurrently after the rapid release of NPX from the intermolecular spaces between γ-CD columns. The dissolution property of the inclusion complex was expected to be controlled by the guest drug incorporated into the intermolecular spaces.

In conclusion, a solid ternary complex of NPX, FBP, and γ-CD was successfully prepared by the sealed-heating method. In the crystalline ternary complex, one FBP molecule was included in the γ-CD cavity and one NPX molecule was incorporated into the intermolecular spaces between γ-CD columns. The dissolution profiles of FBP and NPX from the ternary complex were almost similar. The ternary CD complex, from which two drugs dissolve simultaneously, could be a candidate for new formulations that can be used in combination therapies.[29—31] Further experiments, *e.g.* investigation of the molecular interaction between guest and host molecules, the effect of guest drugs and dissolution media on the dissolution rate, are in progress.

Experimental

Materials Flurbiprofen (FBP) and naproxen (NPX) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan) and Wako Pure Chemical Industries, Ltd. (Japan), respectively. γ-Cyclodextrin (γ-CD) heptahydrate was kindly provided by Cyclochem Co., Ltd. (Japan). All materials were utilized without further purification.

Preparation of FBP/γ-CD Inclusion Complex by Co-precipitaion Method FBP (0.15 g) and γ-CD hyptahydrate (2.6 g) were suspended in distilled water (100 ml). The suspension was stirred at 25 °C for 2 d and then stored for 1 d at 25 °C. The precipitate was filtrated and dried for 1 d at 25 °C. Molar ratio of FBP to γ-CD was spectrophotometrically determined. The inclusion complex was suspended into ethanol, and the suspension was filtrated with 0.45 μm cellulose nitrate membrane filter. FBP concentration in the obtained solution was determined at λ=245.0 nm by UV-160 spectrophotometer (Shimadzu, Japan).

Preparation of Physical Mixture (PM) FBP/γ-CD inclusion complex after drying at 100 °C for 3 h was mixed with NPX at a definite molar ratio in a glass vial for 3 min.

Preparation of Sealed-Heated Sample (SH) Physical mixture (*ca.* 200 mg) was sealed in a glass ampoule (2 ml) and then heated at 150 °C for 2 h.

Powder X-Ray Diffractometry Powder X-ray diffraction (PXRD) measurements were performed using a Miniflex diffractometer (Rigaku, Japan) with the temperature at 25 °C, voltage at 30 kV, current at 15 mA, scanning speed at 4 °C/min, and Cu$K\alpha$ radiation source with a Ni filter.

Dissolution Test The dissolution test was carried out by using a dissolution test apparatus (Toyama Sangyo, Japan) by following the JP apparatus II (paddle) method. The dissolution profile of FBP and NPX was studied in 900 ml of acetate buffer (pH 4.0) at a paddle rotation speed of 100 rpm in a constant temperature bath maintained at 37.0±0.5 °C. The particle size of the powder sample was controlled below 150 μm by sieving as a pretreatment procedure. The dissolution experiment was initiated by taking 9.0 mg and 8.5 mg of the FBP and NPX sample in a dissolution vessel. A 5 ml aliquot of the sample was withdrawn at specific intervals with dissolution medium replacement, and these samples were filtered through a 0.45 μm cellulose nitrate membrane. The solution was diluted with methanol (50/50% (v/v)). Each drug solution was determined by HPLC measurement.

HPLC Measurement HPLC analyses were performed with a Shimadzu HPLC system equipped with a computer interface and software for the integration and analysis of the peaks in the chromatogram. A SUPERIOREX ODS (150×4.6 mm, 5 μm particle size; Shiseido, Japan) column was used for separation in a columnar oven with the temperature at 40 °C. Mobile phase consisted of acetonitrile and phosphate solution (pH adjusted to 2.2 with phosphoric acid) in a ratio of 50/50 (v/v) and flow rate was maintained at 1 ml/min. The detector was operated at 245 nm. FBP and NPX were detected at retention time of 8.1 and 5.0 min, respectively.

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