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ORIGINAL ARTICLE

Solubility-dependent complexation of active pharmaceutical ingredients with trimethyl-β-cyclodextrin under supercritical fluid condition

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Abstract The effect of the solubility of active pharmaceutical ingredients (APIs) in supercritical carbon dioxide (SC-CO_2) on their complexation behavior with trimethyl-β-cyclodextrin (TM-β-CD) has been investigated. Flurbiprofen or naproxen, the solubility of which is lower than that of ibuprofen, was mixed with TM-β-CD and the complexation phenomena on SC-CO_2 processing was evaluated using powder X-ray diffraction, differential scanning calorimetry and IR measurement. Drug complexation depended both on SC-CO_2 treatment time and on drug solubility in CO_2. The inclusion complex formation of flurbiprofen with TM-β-CD proceeded slowly compared with the case of ibuprofen. The slower complexation behavior was also observed when naproxen was used as the guest molecule. These results indicate that dissolution of drug molecules in SC-CO_2 is a rate-determining step for the inclusion complex formation with TM-β-CD and that complexation proceeds after dissolving the both components in SC-CO_2.

Keywords Supercritical carbon dioxide · Inclusion complex · Active pharmaceutical ingredient · Cyclodextrin

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Introduction

Extraction using supercritical fluids has been used practically in the food industry and in analytical chemistry [1, 2]. As carbon dioxide shows a relatively low critical temperature and pressure and is also non-toxic, several trials of pharmaceutical application, such as the extraction of active pharmaceutical ingredients (APIs) [3], the size reduction of drug particles [4], and the preparation of polymorphs [5] have been demonstrated using this solvent. The supercritical fluid technique has also been employed to prepare solid dispersions [6]. One of the advantages of using supercritical carbon dioxide (SC-CO_2) is the ability to prepare solid dispersions without using organic solvents. However, the intrinsic poor solubility of the API in SC-CO_2 often makes it difficult to prepare the desired products.

Cyclodextrin (CD), which is composed of cyclic oligosaccharides, has been used as a host molecule to form an inclusion complex with organic compounds in order to enhance solubility and stability [7–9]. The structure and stoichiometry of the inclusion complexes depends on the specific structure of the guest molecules and the preparation method used for the complexation [9]. Conventional methods used for the preparation of the CD inclusion complex are coprecipitation, kneading and grinding [10–12]. Co-grinding of drug with cyclodextrins sometimes induces the nanoparticle formation in addition to the inclusion complex formation [13]

The preparation of solid dispersions or inclusion complexes of API and host additive under the supercritical fluid condition is one of the more promising approaches to improving its solubility and stability.

We have previously reported on the formation of a complex between ibuprofen and cyclodextrin using supercritical carbon dioxide [14]. Ibuprofen was used for the model API because of its relatively high solubility in SC-CO_2 compared to other medicines [15]. Dissolution of both drug and cyclodextrin in SC-CO_2 appeared to be important to the inclusion complex formation. However, the effect of drug solubility in SC-CO_2 on the inclusion behavior was not investigated. In this study, we prepared trimethyl-β-CD (TM-β-CD) inclusion complex with APIs using SC-CO_2 processing. Non-steroidal anti-inflammatory drugs, ibuprofen, flurbiprofen and naproxen were used as the model drugs. The effect of drug solubility on the complexation was evaluated.

Experimental

Materials

Flurbiprofen was kindly provided by Kaken Pharmaceutical Co. Ltd., Japan. Ibuprofen and naproxen were purchased from Tokyo Kasei Kogyo Co., Ltd. Japan and Wako Pure Chemical Co. Ltd., Japan, respectively. Heptakis (2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) was obtained from Tohshin Chemical Co. Ltd., Japan.

Estimation of the amount of dissolved drug in supercritical carbon dioxide

The amount of API dissolved in SC-CO_2 was estimated by the method previously reported [5]. The drug (0.2 g) was put into a glass vial and placed in the reaction vessel. The API was kept under supercritical conditions at a pressure of 26 MPa and a temperature of 60 °C for 1 h. Sample/supercritical CO_2 mixtures (0.2 mol CO_2 equivalent) were sprayed into ethanol, which was used as a solvent for the assay. The amount of dissolved API in supercritical CO_2 was determined spectrophotometrically using a UV-160 spectrophotometer (Shimadzu, Japan) and was recorded as the molar ratio of the drug to CO_2.

Preparation of drug-CD complex by supercritical fluid processing

Processing using supercritical carbon dioxide was performed with the experimental apparatus reported previously (SC sprayer®, Nikkiso, Co. Ltd., Japan) [14]. The physical mixture of drug and TM-β-CD at a molar ratio of 1:1 was placed in a glass container (30 mL) with a magnetic stirrer. The glass container, covered with a polytetrafluoroethylene membrane filter (0.45 μm), was put into a temperature-controlled extraction vessel (35 °C, internal volume: 90 mL). Carbon dioxide was introduced into the vessel by a pump NP-AX-403 (Nihon Seimitsu Kagaku Co., Ltd., Japan) up to the desired pressure (12 MPa) and the supercritical condition was maintained for a definite interval (5, 15 and 30 min).

Preparation of flurbiprofen-TM-β-CD co-precipitate

Flurbiprofen ethanolic solution (0.03 M, 50 mL) and TM-β-CD aqueous solution (0.03 M, 50 mL) were mixed at 25 °C for 12 h. The solution was cooled and stored at 4 °C to promote the co-precipitatation. The resulting powder was filtered and put in a desiccator under reduced pressure to remove the residual solvent. Stoichiometry of the obtained flurbiprofen-TM-β-CD inclusion complex was determined as 1:1

Powder X-ray diffraction (PXRD) measurement

Powder X-ray diffraction measurement was carried out on a Rigaku Miniflex diffractometer (Rigaku Corporation, Japan) under the following conditions: 30 kV voltage, 15 mA current, a scanning speed of 4° min^{-1}, a Ni filter and a radiation source of CuKα.

Differential scanning calorimetry (DSC)

The EXSTAR6000 DSC6200 (Seiko Instruments, Japan) differential scanning calorimeter was used for

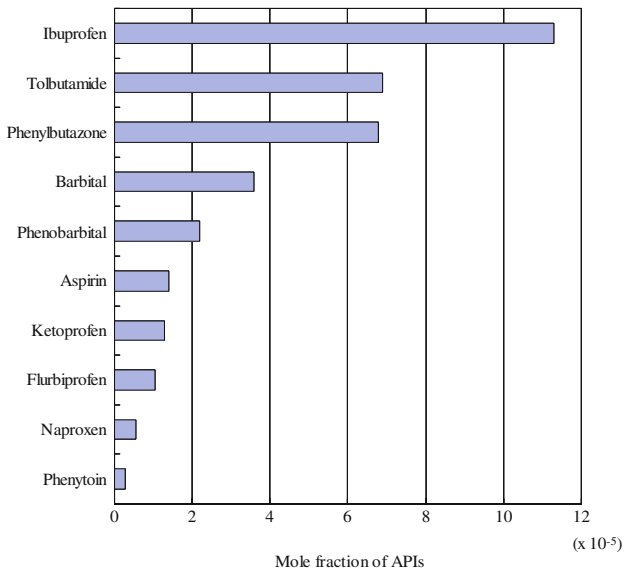

Fig. 1 Mole fraction of active pharmaceutical ingredients (APIs) dissolved in SC-CO_2 at 26 MPa and 60 °C

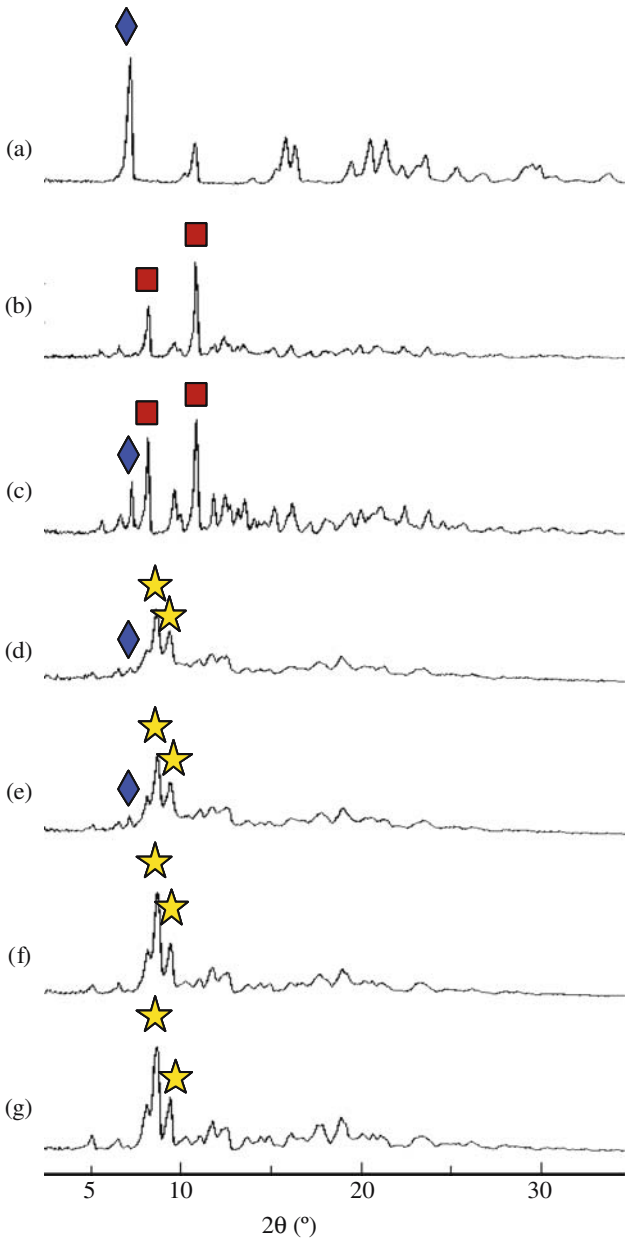

Fig. 2 Powder X-ray diffraction patterns of flurbiprofen–TM-β-CD mixture. (**a**) Flurbiprofen, (**b**) TM-β-CD, (**c**) physical mixture (molar ratio of 1:1), SC-CO_2 processing for (**d**) 5 min, (**e**) 15 min, (**f**) 30 min, (**g**) co-precipitate

thermal analysis. The measurement was carried out over a temperature range of 50–200 °C at a heating rate of 5 °C/min under nitrogen gas flow of 60 mL/min using a crimp-aluminum pan.

Fourier-transformed infrared (FT-IR) spectroscopy

The fourier-transformed infrared spectra were measured using a JASCO FT/IR-230 spectrophotometer (Japan Spectroscopy Co., Ltd., Japan) by the KBr disc method with a resolution of 4 cm^{-1} and the accumulation of 32 scans.

Results and discussion

Solubility difference of APIs in SC-CO_2

Gravimetric [16] and spectroscopic [17] techniques have been used previously to precisely determine drug solubility in SC-CO_2. Both methods require special equipment and long operating times. In this study, the amount of dissolved API in SC-CO_2 was simply estimated by spraying the API/SC-CO_2 mixture into ethanol. Figure 1 shows the amount of dissolved APIs in SC-CO_2 after storing at 26 MPa and 60 °C for 1 h. It can be seen that the mole fractions of dissolved ibuprofen (11.3×10^{-5}), tolbutamide (6.9×10^{-5}) and phenylbutazone (6.8×10^{-5}) were relatively high. The

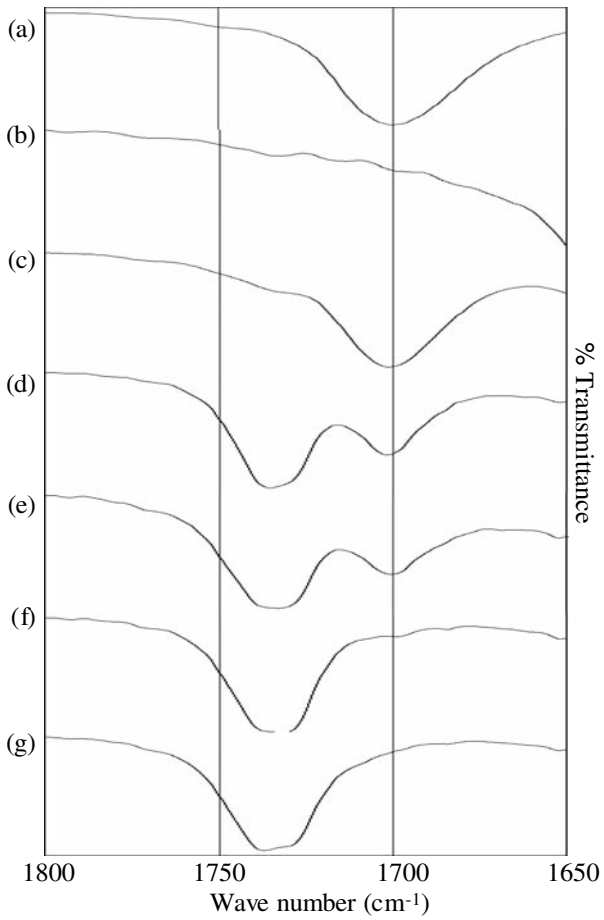

Fig. 3 Change in FT-IR spectra of flurbiprofen–TM-β-CD mixture. (**a**) Flurbiprofen, (**b**) TM-β-CD, (**c**) physical mixture (molar ratio of 1:1), SC-CO_2 processing for (**d**) 5 min, (**e**) 15 min, (**f**) 30 min, (**g**) co-precipitate

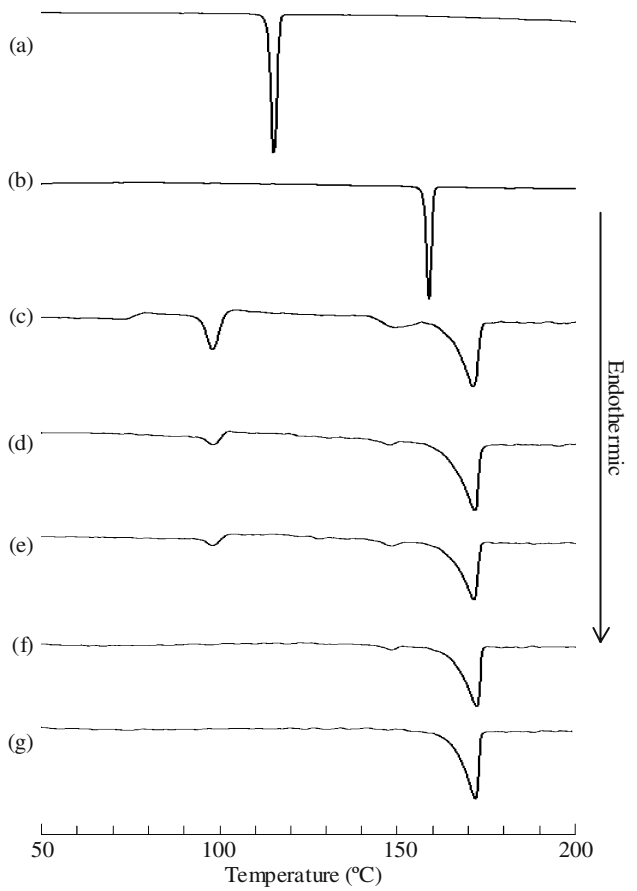

Fig. 4 Change in DSC curves of flurbiprofen–TM-β-CD mixture. (**a**) Flurbiprofen, (**b**) TM-β-CD, (**c**) physical mixture (molar ratio of 1:1), SC-CO_2 processing for (**d**) 5 min, (**e**) 15 min, (**f**) 30 min, (**g**) co-precipitate

mole fractions of dissolved flurbiprofen (1.0×10^{-5}) and naproxen (0.5×10^{-5}) were almost one tenth of that of ibuprofen. Since in a previous work [5], the dissolved amount of phenylbutazone in SC-CO_2 obtained with this method was correlated with the drug solubility measured by conventional methods, the dissolved amount of APIs seems to reflect their solubility in SC-CO_2.

API-TM-β-CD inclusion complex formation by SC-CO_2 processing

Changes in the powder X-ray diffraction (PXRD) patterns of the physical mixture of flurbiprofen and TM-β-CD after the SC-CO_2 processing are shown in Fig. 2. The diffraction peaks arising from flurbiprofen and TM-β-CD crystals disappear and new diffraction peaks appear after SC-CO_2 processing. The X-ray diffraction pattern of the flurbiprofen–TM-β-CD 30 min SC-CO_2-processed sample is almost same as that of the co-precipitate and the same as that of the ibuprofen-TM-β-CD inclusion complex reported previously [15]. These results indicate that flurbiprofen and TM-β-CD formed an inclusion complex after SC-CO_2 processing and that the crystal structure was almost same as that of the ibuprofen–TM-β-CD

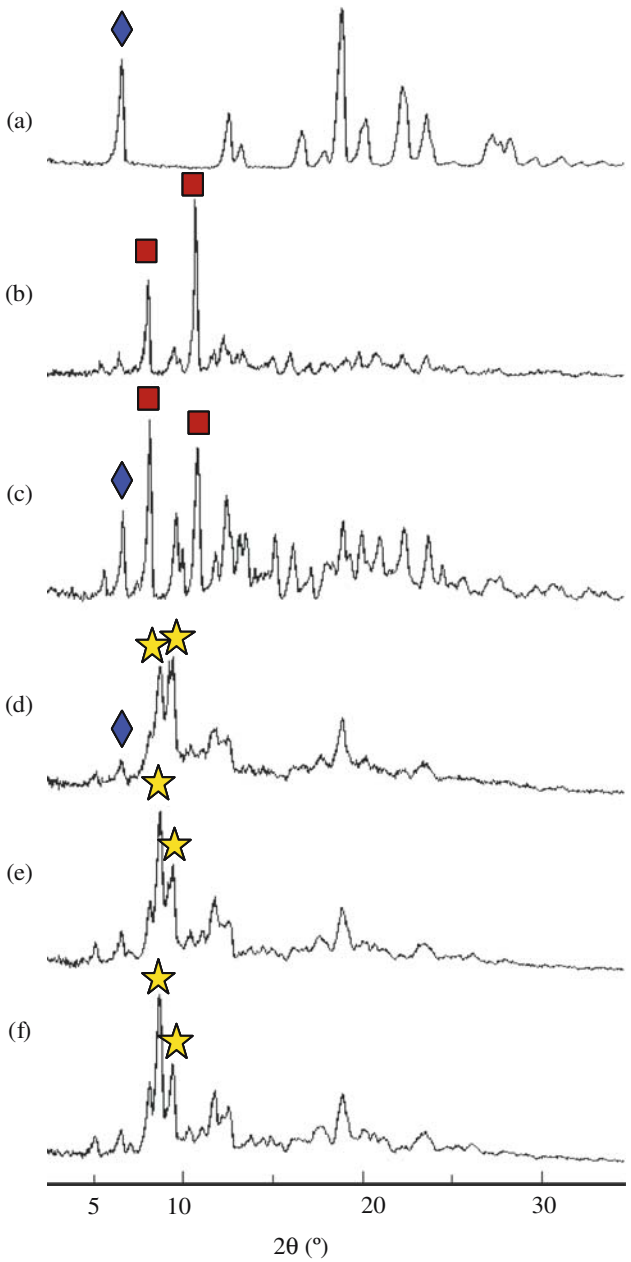

Fig. 5 Powder X-ray diffraction patterns of naproxen–TM-β-CD mixture. (**a**) Flurbiprofen, (**b**) TM-β-CD, (**c**) physical mixture (molar ratio of 1:1), SC-CO_2 processing for (**d**) 15 min, (**e**) 30 min, (**f**) 1 h

inclusion complex. Structural changes from the physical mixture to the inclusion complex occurred as a function of the SC-CO_2 processing time. Finally, the complexation appeared to terminate after SC-CO_2 processing for 30 min resulting in the disappearance of the diffraction peaks of flurbiprofen.

The fourier-transformed infrared spectra and DSC results also support the conclusion that the complex formation between flurbiprofen and TM-β-CD depends upon the processing time. In the FT-IR spectra of the physical mixture, the carbonyl stretching vibration band of flurbiprofen at 1,700 cm^{-1} in the crystals remained unchanged but shifted to the higher frequency of 1,737 cm^{-1} after SC-CO_2 processing with TM-β-CD as shown in Fig. 3. The observed IR peak shift was attributed to the breakdown of the intermolecular hydrogen bonds present in flurbiprofen crystals, followed by the formation of an inclusion complex of flurbiprofen through the interaction with TM-β-CD. The spectral changes seemed to be almost complete after processing for 30 min. The effect of processing time on the thermal properties of the SC-CO_2-processed mixture is shown in Fig. 4. The physical mixture shows endothermic peaks at around 98 °C and 150 °C due to the fusion of flurbiprofen crystals and TM-β-CD, respectively. In addition, a new melting peak at 173 °C appeared, suggesting that the complexation between flurbiprofen and TM-β-CD might be promoted by heating. In the SC-CO_2-processed samples, an increase in processing time induced a decrease of the fusion enthalpies of both flurbiprofen and TM-β-CD, respectively, accompanied by an a new endothermic peak at 173 °C. As flurbiprofen and TM-β-CD co-precipitate also showed an endothermic peak at 173 °C, the new endothermic peak observed in SC-CO_2-processed samples was attributed to the heat of fusion of the inclusion complex induced by the SC-CO_2 processing. Disappearance of the melting peak of flurbiprofen crystals indicates that the complexation completed after 30 min of SC-CO_2 processing.

Naproxen, which has a lower solubility than flurbiprofen, was used to investigate the formation of an inclusion complex with TM-β-CD by SC-CO_2 processing. The effect of processing time on the PXRD patterns is shown in Fig. 5. It was observed that the changes in the PXRD pattern depend on the

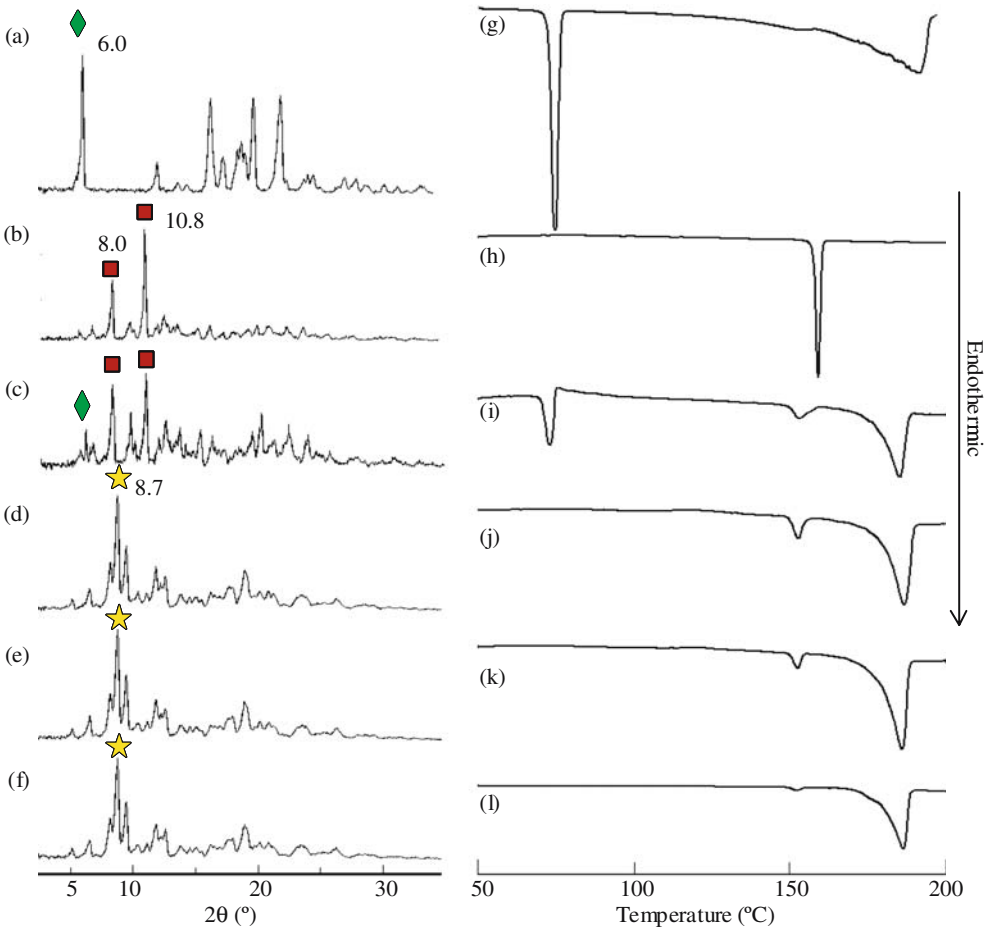

Fig. 6 Effect of SC-CO_2 processing time on ibuprofen–TM-β-CD inclusion complex formation. PXRD patterns of (**a**) ibuprofen, (**b**) TM-β-CD, (**c**) physical mixture (molar ratio of 1:1), SC-CO_2 processed sample for (**d**) 5 min, (**e**) 15 min and (**f**) 30 min. DSC curves of (**g**) ibuprofen, (**h**) TM-β-CD, (**i**) physical mixture (molar ratio of 1:1), SC-CO_2 processed sample for (**j**) 5 min, (**k**) 15 min and (l) 30 min

processing time. No further changes were observed after processing for more than 30 min, though it was difficult to differentiate the diffraction peaks of naproxen from those of the SC-CO_2-processed samples. The powder X-ray diffraction pattern of the SC-CO_2-processed sample was almost same as that of flurbiprofen–TM-β-CD, indicating an inclusion complex with a similar host-guest structure.

The effect of API solubility in SC-CO_2 on the complexation with TM-β-CD

Figure 6 shows the changes in PXRD patterns and DSC curves of the ibuprofen–TM-β-CD physical mixture on SC-CO_2 processing. When ibuprofen was used as a guest molecule, the changes in the PXRD pattern from the physical mixture to the inclusion complex occurred very rapidly. A processing time of 5 min was enough to form ibuprofen–TM-β-CD complex by the supercritical processing. These findings suggest that the formation rate of TM-β-CD inclusion complex with ibuprofen, flurbiprofen or naproxen depend on the solubility of the API in SC-CO_2.

When the solubility of the API-TM-β-CD inclusion complex in SC-CO_2 is lower than that of the API or TM-β-CD, the inclusion complex is precipitated from SC-CO_2 fluid. We reported that TM-β-CD was soluble in SC-CO_2. Actually, 6.5% of the initial amount (430 mg) was dissolved by SC-CO_2 after processing for 3 h [15]. Though it was difficult to determine the solubility of the inclusion complex as well as that of APIs and CDs in supercritical condition, direct solubility measurement of the API in SC-CO_2 could provide information, which would help predict the formation of an inclusion complex.

It took a long time to prepare the API-TM-β-CD inclusion complex when the solubility of API in SC-CO_2 was lower. However, once the API dissolved in SC-CO_2, the inclusion complex formed. The operating conditions, such as pressure, temperature and processing time, should also contribute to the complex formation rate.

Conclusions

The inclusion complexation behavior of TM-β-CD and API by SC-CO_2 processing has been demonstrated. The dissolution of both API and TM-β-CD in SC-CO_2 was shown to be a rate-determining step for the formation of the inclusion complex. It was shown that the formation of a complex depends on the solubility of the API in the SC-CO_2 and that the rate of formation of the complex decreased with decreasing solubility of API.

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