Host-guest chemistry of antibacterial molecular crystal in supercritical CO_2 with solvent

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Abstract

There are several techniques to improve the properties of a medicine, such as polymorphs of crystals, amorphization, salts, and cocrystals [1]. In particular, host-guest chemistry, which means fixing carbon dioxide (CO_2) molecular inside of drug molecular crystal, is expected to enhance drug solubility and improve drug dissolve behavior, because released CO_2 in water can lower pH locally. In addition, this technique would achieve the advantages of easy handling, safety in terms of less harmful solvents, and high yields compared to the other methods.

In this work, we focus on the mechanism of host-guest chemistry for drug molecular crystal with CO_2 by conducting both experimental and computational approaches on "how much the host-guest chemistry accelerated by a cosolvent in supercritical CO_2".

We choose Enoxacin as a target medicine, which has low solubility (< 0.1 mg ml $^{-1}$ in water at 25 ℃) [2]. For the experiment, Enoxacin was placed in a container and contacted with supercritical CO_2 ($scCO_2$) at 80 ℃ and 20.0 MPa for 2 h in the presence of some solvents (EtOH, 1PrOH, 1BtOH, Acetone, Ethyl acetate). As a result, the solvents with hydroxy groups accelerates host-guest chemistry, which means Enoxacin can capture more CO_2 inside of its crystal.

For the computational calculation, using quantum chemistry and thermodynamic relationships, we constructed a model to describe the host-guest chemistry for drug molecular crystal with CO_2, which has 2 steps: (1) relaxation of crystal structure pairing with CO_2, (2) recrystallization while retaining CO_2 inside. As a result, the experimental result is well explained by enthalpy change in the process (1) in the model. That is the solid dissolution of $scCO_2$ at the interface is an important process on the formation of host-guest drug molecular crystal with CO_2.

Keywords
host-guest chemistry, supercritical CO_2, COSMO-SAC

1. Introduction

Enoxacin, a type of quinolone antibiotic, has an antibacterial effect on Staphylococcus, Escherichia coli, Pseudomonas aeruginosa, Influenza and Neisseria gonorrhoeae [3]. However, it is an insoluble drug classified as class II of Biopharmaceutics Classification System (BCS) [4], and its solubility needs to be improved.

There are several attempts for quinolones including Enoxacin to increase solubility: cocrystals [5, 6], salts [7, 8], polymorphs [9], amorphous, and liposomes. For the method of formulation, for example, cocrystals can be prepared by solvent evaporation from chloroform solution [8], by liquid assisted grinding using acetonitrile [10], and by supercritical antisolvent (SAS) method, which means acetone solution sprayed onto poor solvent $scCO_2$ [11]. These conventional methods, however, have low safety due to the use of large amounts of harmful solvents, low productivity due to multiple unit operations, and poor yields.

Our research group focused on host-guest chemistry with CO_2. We have previously confirmed that CO_2 inclusion crystals increase the solubility of medicines. Contacting the drug powder with $scCO_2$, which has high diffusivity and high CO_2 density, enables CO_2 enter into the organic crystal lattice of the drug. This technique has the advantages of safety, easy handling, and high yields.

The object of this study is to consider the mechanism of CO_2 inclusion crystals by clarifying the effects of solvent addition and molecular type, both experimentally and computationally. In the experiment, we investigated how 5 types of solvents promote the host-guest chemistry of Enoxacin and CO_2. In the calculations, we tried to describe the experiment using molecular information obtained by quantum chemical calculations based on the COSMO method [15] to explain the effects of solvent addition and molecular type.

2. Experimental

2.1. Materials

Enoxacin Sesquihydrate (> 98.0 %) was purchased from TOKYO CHEMICAL INDUSTRY CO., LTM. As pretreatment, raw Enoxacin was heated to be anhydrate form at 120 °C for 2 hours under Ar flow (40 ml min^{-1}) removing hydrated water.

Ethanol (EtOH, > 99.5 %), Ethyl Acetate (EA, > 99.5 %), Acetone (AC, > 99.5 %), 1-Butanol (1BtOH, > 99.0 %), 1-Propanol (1PrOH, > 99.5 %) were purchased from FUJIFILM Wako Pure Chemical Corporation. Carbon dioxide (> 99.99 %) was from Fujii Bussan Co., Ltd.

2.2. Experimental Apparatus and Procedure

The preparation of the host-guest crystals was performed by contacting the drug with $scCO_2$. Figure 1 shows the apparatus diagram. First, we placed 60 mg of Enoxacin anhydrate powder in the upper of the hyper-pressure cell (70mL) and 2 mL (molar fraction $\simeq 2\times10^{-2}$) of solvent in the lower. We used Ethanol (EtOH), Ethyl Acetate (EA), Acetone (AC), 1-Butanol (1BtOH), 1-Propanol (1PrOH) for solvent. The inlet valve was opened to allow CO_2 to flow in, and the system was kept at a temperature of 40 °C and a pressure of 20.0 MPa for 2 hours. After that, the pressure was depressurized at a depressurization rate 0.1 MPa min^{-1}, and then the powder in hyper-pressure cell will be collected for the following evaluation.

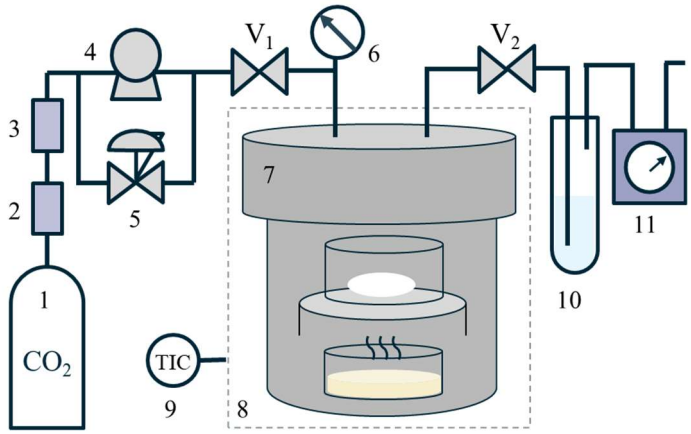


Fig. 1. Schematic diagram of apparatus for Enoxacin / CO_2 inclusion crystal: (1) CO_2 cylinder, (2) drier, (3) cooler, (4) pump, (5) back pressure, (6) pressure gauge, (7) high-pressure vessel, (8) oven, (9) temperature indicator controller, (10) glass trap, and (11) gas flow meter.

Two evaluation methods were used to determine the progress of the host-guest chemistry.

Powder X-ray diffraction (PXRD), which can distinguish crystal structures, is performed on a Rigaku Miniflex600 with a Cu Kα radiation (1.5418 Å). Each sample was scanned from 2 to 50° in 2θ with a step size of 0.02° and scan rate at 2° min^{-1} in 2θ. We calculated the progress of crystal phase transition by a semi-quantification based on a modified RIR method [12] as the following equation:

$$\frac{w_{with\ CO_2}}{w_{anhydrate}} = K \times \frac{I_{wi\quad CO_2}}{I_{anhydrate}} \tag{1}$$

where w is the weight of specific crystal phase; I is the intensity of specific peak for specific crystal phase; and K is a constant determined by crystal phase. The relationship of cocrystal and remained Enoxacin is estimated by using the fact that the ratio of characteristic peak heights in PXRD is proportional to the ratio of components in the mixture. The characteristic peak in this study is at 2θ=15.2° for with CO_2 phase and at 2θ = 14.7° for Enoxacin anhydrate.

Thermogravimetric analysis (TGA), which is used for checking the mass change of a sample with temperature, is performed on a thermogravimetric analyzer SHIMADZU TGA-50. About 3 mg of each powder sample is placed in an aluminum cell and scanned at 5 °C min^{-1} from 30 °C to 300 °C with nitrogen follow at 50 ml min^{-1}. The mass loss observed in TGA is caused by the decomposition and release of CO_2 taken into the crystal by heating. Therefore, the amount of contained CO_2 was calculated from the amount of mass loss, which is at 220 °C in this study.

3. Modeling of host-guest chemistry on crystal formation of drugs with CO_2 molecule

The host-guest phenomenon in this study is explained in two steps as shown in Figure 2: (1) a process in which ENX slightly dissolves in CO_2 and coexists with CO_2, and (2) a process in which ENX recrystallizes while keeping CO_2 inside the crystal.

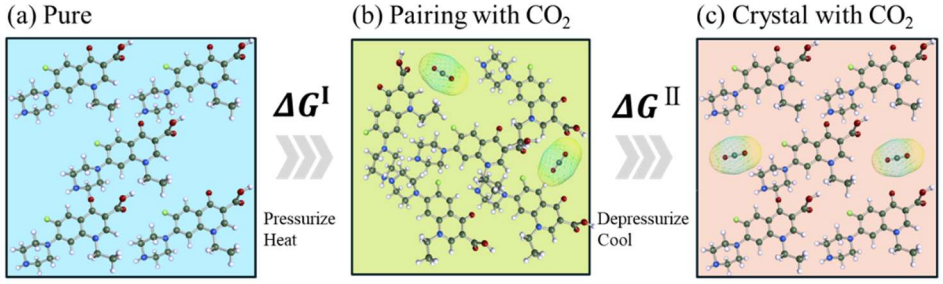


Fig. 2. Modeled phase of the host-guest phenomenon: (a) Pure Enoxacin is pressurized and heated to (b) Paired with CO_2. After that, it is depressurized and cooled to (c) crystal with CO_2.

In particular, we focus on the process (1), which is divided into ΔG_s^{fus} of melting and ΔG_s^{pair} of stabilization by the fused phase with Enoxacin, CO_2 and solvent.

$$\Delta G^I = \Delta G_s^{fus} + \Delta G_s^{pair} \tag{2}$$

ΔG_s^{fus} was calculated using the following equation [13], which represents the temperature dependence of the solubility of a solid in solution:

$$\Delta G_s^{fus} = \Delta H_s^{fus}\left(\frac{T}{T_s^{fus}} - 1\right) \tag{3}$$

where ΔG_s^{fus} is Gibbs free energy change by fusion; ΔH_s^{fus} is fusion enthalpy of Enoxacin; T_s^{fus} is melting temperature of Enoxacin; and T is the targeted temperature.

ΔG_s^{pair} is expressed as the change in Gibbs free energy when changing from the "melted state" to the "coexisting state with Enoxacin, CO_2, and solvent".

Considering that the reference fugacity is the same value in the before and after states, and that the multiplication of the activity coefficient and mole fraction is unity in the fused phase, the equation can be transformed as follows:

$$\Delta G_s^{pair} = \mu_s^{pair} - \mu_s^{fus} = RT\,ln\left(\gamma_s^{pair} x_s^{pair}\right) \tag{4}$$

where γ_s^{pair} is activity coefficient of Enoxacin paired with CO_2; and x_s^{pair} is molar fraction of Enoxacin paired with CO_2. To calculate γ_s^{pair}, we used the COSMO-SAC method [14], which uses molecular information obtained by quantum chemical calculations based on the COSMO calculation [15]. In this study, COSMO calculation was conducted by a software TURBOMOLE 6.5 [16]. From the molecular surface charge distribution $p(\sigma)$, the output of the COSMO calculation, we can obtain the segment activity coefficient $\Gamma(\sigma_m)$:

$$\ln \Gamma(\sigma_m) = -\ln\left\{\sum_{\sigma_n} p(\sigma_n)\Gamma(\sigma_n)\exp\left(\frac{-\Delta W(\sigma_m,\sigma_n)}{RT}\right)\right\} \quad (5)$$

which $\Gamma(\sigma_m)$ is segment activity coefficient; $\Delta W(\sigma_m,\sigma_n)$ is the exchange energy required to form the segment pair of σ_m and σ_n from a neutral pair; and $p(\sigma_n)$ is the probability of finding the segment σ_m in mixture. Finally, the activity coefficient of Enoxacin paired with CO_2 γ_s^{pair} is obtained by the following equation and that enables to calculate ΔG^I:

$$ln\,\gamma_s^{pair} = \frac{A_s}{a_{eff}}\sum_{\sigma_m} p_s(\sigma_m)[ln\,\Gamma^{pair}(\sigma_m) - ln\,\Gamma^s(\sigma_m)] \quad (6)$$

where A_s is surface area of Enoxacin ; and a_{eff} is surface area of surface charge segment. In this work, we could assume the ΔG^{I} is much larger than ΔG^{II}, $\Delta G^I \gg \Delta G^{II}$.

4. Result and discussion

4.1. Experiment

In this study, we investigated how much the progression of host-guest chemistry is accelerated by solvents: Ethanol (EtOH), Ethyl Acetate (EA), Acetone (AC), 1-Butanol (1BtOH), 1-Propanol (1PrOH).

The PXRD patterns in Figure 3 shows the change in diffraction pattern before and after $scCO_2$ treatment. There are still some peaks of Enoxacin anhydrate are remained, which indicate some Enoxacin anhydrate are still remained even after $ScCO_2$ treatment. This means the powder collected after treatment is a mixture of new formed crystal and Enoxacin anhydrate. Furthermore, the diffraction pattern after treatment is the same in all solvent types. In other words, the crystal structure changes after the treatment and the same CO_2 inclusion crystals are formed regardless of the solvent type.

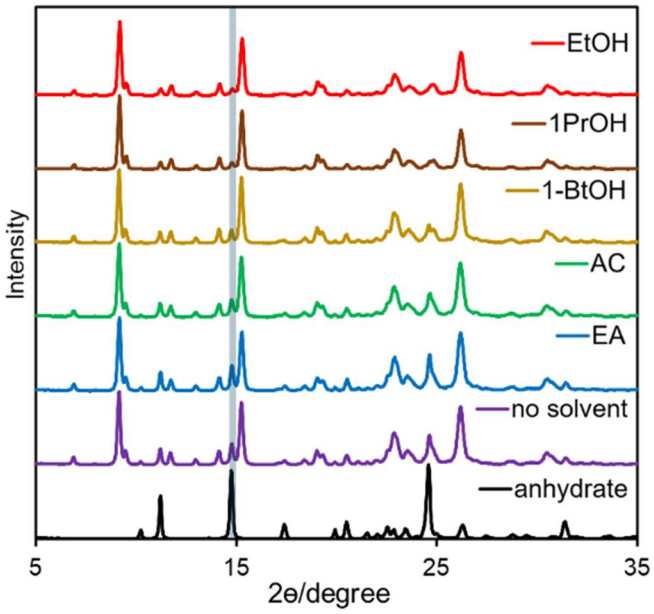


Fig. 3. PXRD results for different solvent types.

Figure 4 shows the results of TGA for different solvent types. All the results have a mass loss starting from 239°C due to carboxyl, carbonyl of naphthalene, and piperazine being shucked off [17]. On the other hand, in addition to this, the treated results have a mass loss of 4.4~6.4% starting at around 158°C. This seems to be due to the release of captured CO_2 by heating. Stoichiometrically, when Enoxacin : CO_2 = 2 : 1, the mass fraction of CO_2 is 6.4%.

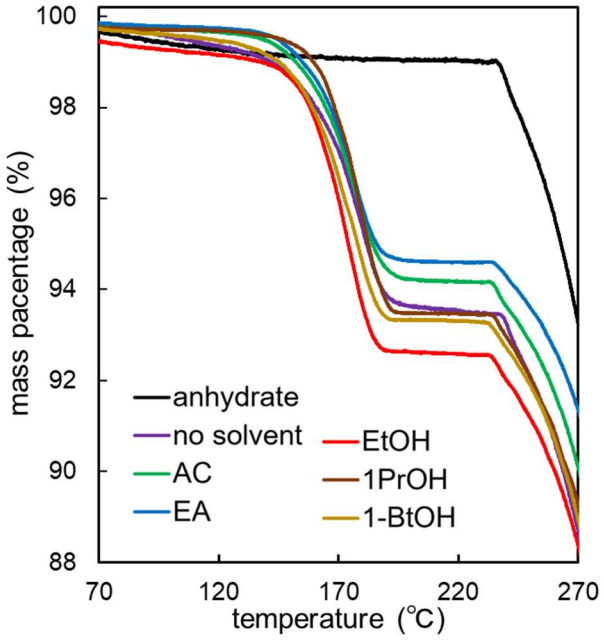


Fig. 4. TGA results for different solvent types.

The comparison of the ability of each solvent type to enhance CO_2 uptake is shown in Figure 5. The semi-quantitative analysis of PXRD showed that the effect of solvent on the crystal formation with CO_2, EA < AC < 1BtOH < 1PrOH < EtOH, in that order, especially the protic solvents alcohols had a higher effect in promoting the crystal transition. CO_2 content from TGA result showed a similar trend, with EtOH maximum and EA minimum CO_2 uptake.

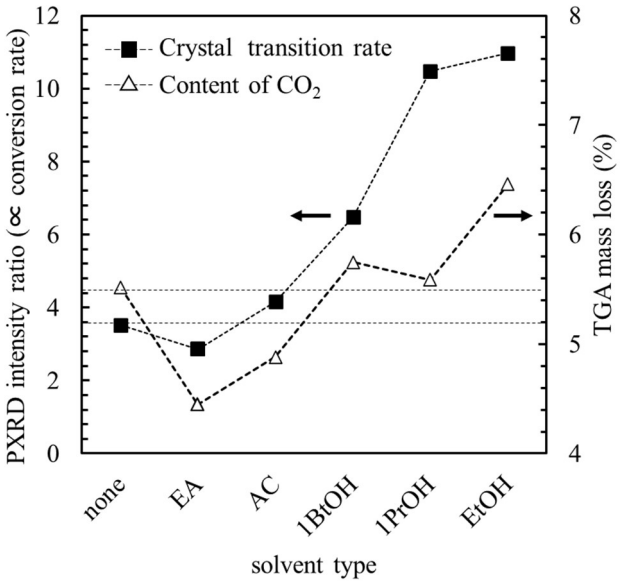


Fig. 5. PXRD intensity ratio in equation (1) and mass loss of TGA at 220 ℃ for different solvent types.

4.2. Modeling of host-guest chemistry on crystal formation of drugs with CO_2 molecule

The results of the calculations for the effect of changing solvent type and molecule type are shown in Figure 6. Gibbs free energy change of process (1) ΔG^I can separate into an entropy term ΔH representing interaction and an entropy term $T\Delta S$ representing configuration such as size and shape. In this study, we focus on ΔH:

$$\Delta G^I = \Delta H - T\Delta S \quad (7)$$

Figure 6 (a) shows enthalpy change from the "melted state" to the "coexisting state with Enoxacin, CO_2, and solvent" ΔH_s^{pair} for each solvent type. The values and slopes of ΔH_s^{pair} of EtOH, 1PrOH, and 1BtOH are negative, which indicates that they have thermodynamic stability and strong interactions. This calculated result is consistent with the experimental result mentioned above. Furthermore, the smaller ΔH_s^{pair} in the CO_2 rich composition with alcohol solvent suggests that the solvent plays a role in helping the Enoxacin solid dissolve into scCO_2 at the interface.

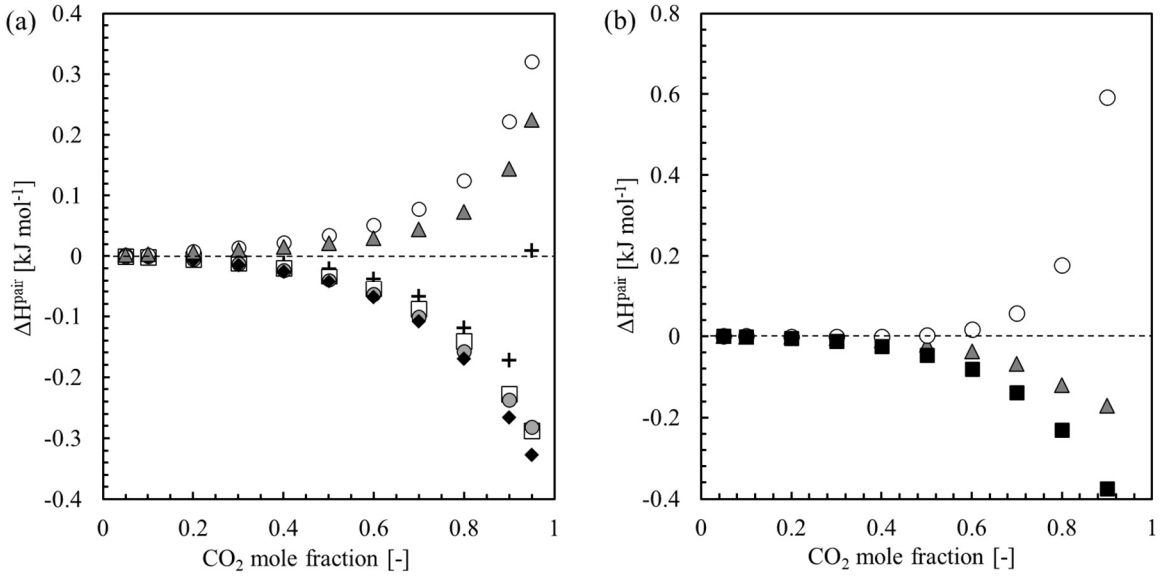
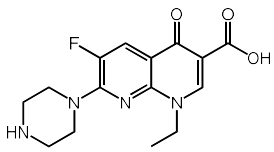
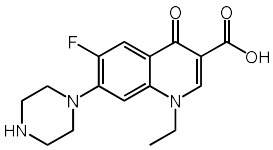
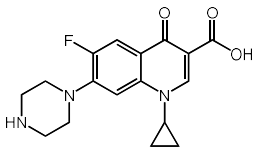


Fig. 6. Calculated enthalpy changes ΔH_s^{pair} for the effect of (a) solvent type: +, no solvent; ♦, 1PrOH; ●, 1BtOH; □, EtOH; ▲, EA; ○, AC, and (b) molecule type: ■, Norfloxacin; ▲, Enoxacin; ○, Ciprofloxacin.

Next, we discuss the effect of changing the molecular type on the enthalpy. We have already investigated that among the three quinolones, only Enoxacin and Norfloxacin form CO_2 inclusion crystals in experiments, while Ciprofloxacin does not (Table.1). The calculation in Figure 6 (b) also indicates that only Enoxacin and Norfloxacin have $\Delta H_s^{pair} < 0$, that is, the reaction is exothermic and proceeds easily. Thus, the above calculations are a good representation of the experimental results for the effect of changing solvent type and molecule type.

Table. 1.

Experimental results of CO_2 host-guest chemistry of quinolones

Component	Enoxacin	Norfloxacin	Ciprofloxacin
Structure			
Host-guest formation	Yes	Yes	No

Finally, we will discuss the mechanism by considering the phenomenon from both experimental and computational perspectives. From the calculation results, it was found that the formation of host-guest chemistry with CO_2 depends on whether ΔH_s^{pair} becomes smaller

at high CO_2 mole fractions. This indicates the requirement for improved stability of Enoxacin at the interface, which is a CO_2-rich phase. Based on these facts, we assumed that the CO_2-driven crystal phase transition may be caused by the following mechanism. First, dissolution of solid Enoxacin occurs at the interface upon contact with $scCO_2$. After that, CO_2 diffuses in the relaxed solid and forms a new crystal structure in which CO_2 is retained in the crystal.

5. Conclusion

In this work, we have considered how much the host-guest chemistry accelerated by a cosolvent in $scCO_2$ by conducting both experimental and computational approaches.

In the experiment, CO_2 inclusion crystals were prepared by only contacting Enoxacin with $scCO_2$ in the presence of solvents. As a result, both crystal transition rate and CO_2 content increased in the order of EA < AC < 1BtOH < 1PrOH < EtOH, which confirms that the solvent has the effect of promoting the host-guest chemistry.

We constructed the computational model that describes the experiment in the two steps: (1) a process in which ENX slightly dissolves in CO_2 and coexists with CO_2, and (2) a process in which ENX recrystallizes while keeping CO_2 inside the crystal. This calculation result is consistent with the experimental results on the effect of different solvent types and molecule types. In particular, the small ΔH_S^{pair} at high CO_2 mole fraction, in other words, the improvement of stability by coexistence with CO_2, was found to be important. This suggests that the dissolution process of medicine into $scCO_2$ at the interface is an important process that determines whether CO_2 host-guest crystal is formed or not.

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