Regular Article

Stability Order of Caffeine Co-crystals Determined by Co-crystal Former Exchange Reaction and Its Application for the Validation of *in Silico* Models

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The purpose of the present study was to determine the thermodynamic stability orders of co-crystals by co-crystal former (CCF) exchange reactions. Caffeine (CA) was employed as a model drug. The CCF exchange reaction was performed by liquid-assisted grinding using ethanol. When oxalic acid (OX) was added to CA–citric acid co-crystal (CA–CI), CA–CI converted to CA–OX, suggesting that CA–OX is more stable than CA–CI. The stability orders of other co-crystals were determined in the same manner. The stability order of CA co-crystals was determined as CA–OX≈CA–*p*-hydroxybenzoic acid (HY)>CA–CI>CA–malonic acid>CA–maleic acid. The stability order correlated with the difference in hydrogen bond energy estimated *in silico*, except for CA–HY. The *π*–*π* stacking in CA–HY was suggested as a reason for this discrepancy. The CCF exchange reaction was demonstrated as a useful method to determine the stability order of co-crystals, which can be used for the validation of *in silico* parameters to predict co-crystal formation.

Key words co-crystal; co-crystal former; exchange reaction; caffeine; hydrogen bond energy

Co-crystals have recently received much attention as a means of improving physicochemical properties of active pharmaceutical ingredients.[1–10] The number of potential co-crystal formers (CCFs) can reach up to several hundred; therefore, an efficient strategy for CCF screening in drug discovery and development is required. High throughput screening (HTS) technologies have been employed for CCF screening.[10–15] However, CCF selection by HTS remains a time- and resource-consuming task. Therefore, an effective pre-screening method for CCF selection would be valuable for drug discovery and development. In the case of salt selection, the difference in pK_a values between a drug and a counter-ion (ΔpK_a) has been used as a criterion for the selection of potential counter-ion candidates.[16] Similarly, some *in silico* physicochemical parameters for CCF selection were proposed by several research groups.[17–20] For example, the difference in hydrogen bond energy between a co-crystal and each of the sole components (ΔE) was proposed by Musumeci *et al.*, based on the hypothesis that the formation of a co-crystal becomes more probable as ΔE becomes larger.[21–23] However, ΔE has not been rigorously validated due to the lack of information about the stability order of co-crystals. The stability order of co-crystals can be determined by CCF exchange reactions. In the literature, there were a few studies on the CCF exchange reactions (a sulfonamide derivative[24] and carbamazepine[25]). However, in these studies, only a few CCFs were employed so that the data is insufficient for validating *in silico* models.

The purpose of the present study was to determine the stability orders of co-crystals by CCF exchange reaction. Caffeine (CA) was employed as a model drug. The stability order of five CA co-crystals was determined using the CCF exchange reaction. The stability order was then compared with the ΔE estimated *in silico*.

Experimental

Materials *p*-Hydroxybenzoic acid was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Other agents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of CA Co-crystals CA–*p*-hydroxybenzoic acid co-crystal (CA–HY), CA–oxalic acid co-crystal (CA–OX), CA–citric acid co-crystal (CA–CI), CA–malonic acid co-crystal (CA–MO), and CA–maleic acid co-crystal (CA–MA) were prepared by liquid-assisted grinding. CA (25 mg, 0.13 mmol) was mixed with each CCF and chloroform (25 μL) in a 1-mL glass vial with 2.4-mm tungsten balls. The mixture was shaken in a BMS-TMS 200 shaker at 1800 rpm at ambient temperature. Chloroform was then evaporated under nitrogen gas flow for more than 1 h. The amount of CCF, the molar ratio of the co-crystal components, and the shaking duration are shown in Table 1.

CCF Exchange Reaction Equimolar amounts of CCF (1–7 mg) were added to each co-crystal in a 1-mL glass vial with 2.4-mm tungsten balls. Ethanol (5 μL) was then added to the vial and the vial was shaken for 2–48 h (in most cases 22 h). Ethanol was then evaporated under nitrogen gas flow for more than 1 h.

Table 1. Preparation Conditions for Each Co-crystal

Co-crystal	Amount of CCF (mg)	Molar ratio of the components (CA : CCF)	Reaction time (h)
CA–HY	35	1 : 2	44
CA–OX	6	2 : 1	22
CA–CI	25	1 : 1	22
CA–MO	7	2 : 1	6
CA–MA	15	1 : 1	26

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Powder X-Ray Diffraction (PXRD) PXRD patterns of all samples were collected in the reflectance mode using a Rigaku SmartLab diffractometer (Cu$K\alpha$ radiation (40 kV and 30 mA), experimental 2θ range (5° to 40°), scan rate (10°/min), step size (0.02°), Rigaku D/teX ultra-high-speed position-sensitive detector). All samples were prepared on silicon single crystal sample holders.

Results and Discussion

Preparation of CA Co-crystals In this study, we focused on the organic acids as CCFs. The five co-crystals, *i.e.*, CA co-crystals of *p*-hydroxybenzoic acid (HY), oxalic acid (OX), citric acid (CI), malonic acid (MO), and maleic acid (MA) were prepared by liquid-assisted grinding. These CCFs cover a wide range of ΔE from -3 to -10 kJ mol^{-1}.[23] The PXRD patterns of CA, the CCFs, and the prepared co-crystals are shown in Fig. 1. The PXRD patterns of the co-crystals obtained in this study were identical to those reported previously.[26–29] It was previously reported that CA–MA existed as 1 : 1 and 2 : 1 co-crystallization systems.[28,29] However, we found that CA–MA (2 : 1) changed to CA–MA (1 : 1) upon the presence of equimolar MA, suggesting that CA–MA (1 : 1) is more stable than CA–MA (2 : 1). Therefore, CA–MA (1 : 1) was used in this study. It was also known that CA–HY existed as 1 : 2 and 2 : 1 co-crystallization systems.[29] According to the previous report, the lattice energy of CA–HY (1 : 2) was lower than that of CA–HY (2 : 1),[30] suggesting that CA–HY (1 : 2) was more stable than CA–HY (2 : 1). Therefore, CA–HY (1 : 2) was used in the following study.

Determination of the Stability Order among CA Co-crystals Using the CCF Exchange Reaction The CCF exchange reactions were performed to determine the stability order among the five CA co-crystals studied herein. Instead of chloroform, ethanol was used as a solvent since the solid mixture aggregated when stirred with chloroform. The reaction time was firstly set to 2 h. However, some reactions did not complete within 2 h (data not shown). Therefore, the reaction time was set to 22 h (overnight) considering the convenience of experimental operation. CCF exchange reactions almost completed after 22 h except for the reaction between CA–MA and MO.

The PXRD patterns before and after CCF exchange reactions are shown in Figs. 2–11 and Table 2, respectively. When HY was added to CA–OX or when OX was added to CA–HY

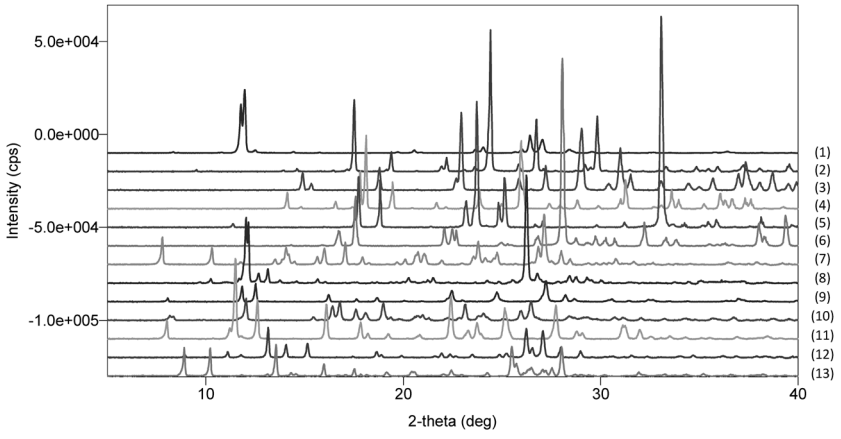

Fig. 1. PXRD Patterns of Caffeine, CCFs, and Co-crystals

From top to bottom: (1) caffeine (CA), (2) *p*-hydroxybenzoic acid (HY), (3) oxalic acid (OX), (4) citric acid (CI), (5) malonic acid (MO), (6) maleic acid (MA), (7) caffeine–*p*-hydroxybenzoic acid co-crystal (CA–HY) (1 : 2), (8) caffeine–*p*-hydroxybenzoic acid co-crystal (CA–HY) (2 : 1), (9) caffeine–oxalic acid co-crystal (CA–OX), (10) caffeine–citric acid co-crystal (CA–CI), (11) caffeine–malonic acid co-crystal (CA–MO), (12) caffeine–maleic acid co-crystal (CA–MA) (1 : 1), and (13) caffeine–maleic acid co-crystal (CA–MA) (2 : 1).

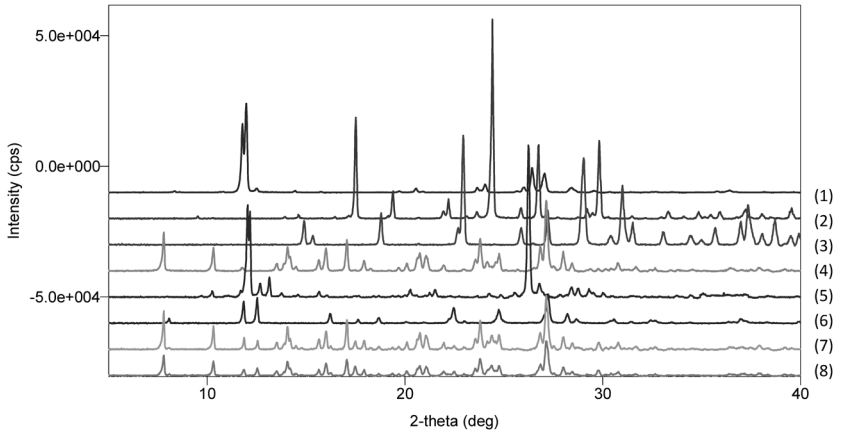

Fig. 2. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–HY (1 : 2) and CA–OX

From top to bottom: (1) CA, (2) HY, (3) OX, (4) CA–HY (1 : 2), (5) CA–HY (2 : 1), (6) CA–OX, (7) CA–HY (1 : 2) mixed with OX, and (8) CA–OX mixed with HY.

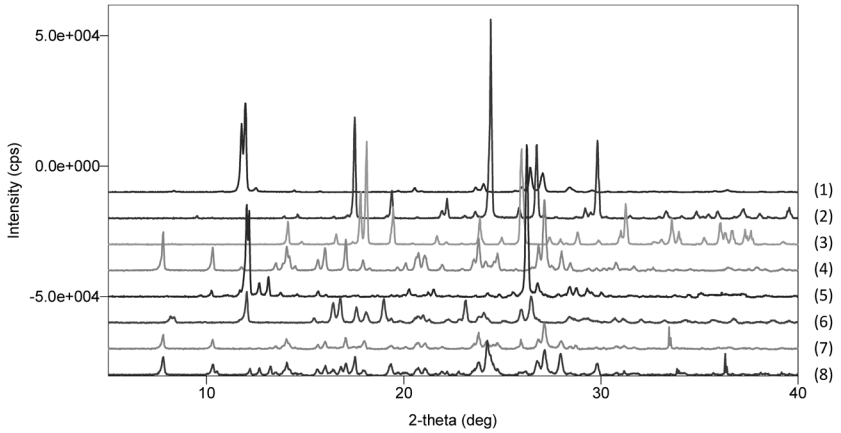

Fig. 3. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–HY (1 : 2) and CA–CI

From top to bottom: (1) CA, (2) HY, (3) CI, (4) CA–HY (1 : 2), (5) CA–HY (2 : 1), (6) CA–CI, (7) CA–HY (1 : 2) mixed with CI, and (8) CA–CI mixed with HY.

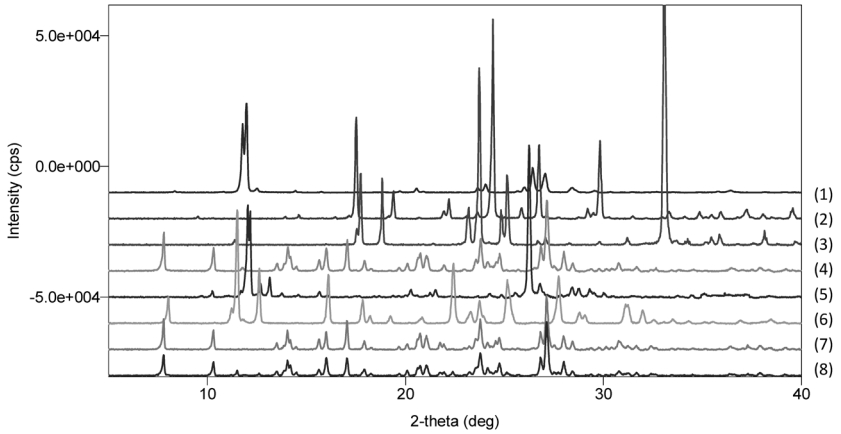

Fig. 4. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–HY (1 : 2) and CA–MO

From top to bottom: (1) CA, (2) HY, (3) MO, (4) CA–HY (1 : 2), (5) CA–HY (2 : 1), (6) CA–MO, (7) CA–HY (1 : 2) mixed with MO, and (8) CA–MO mixed with HY.

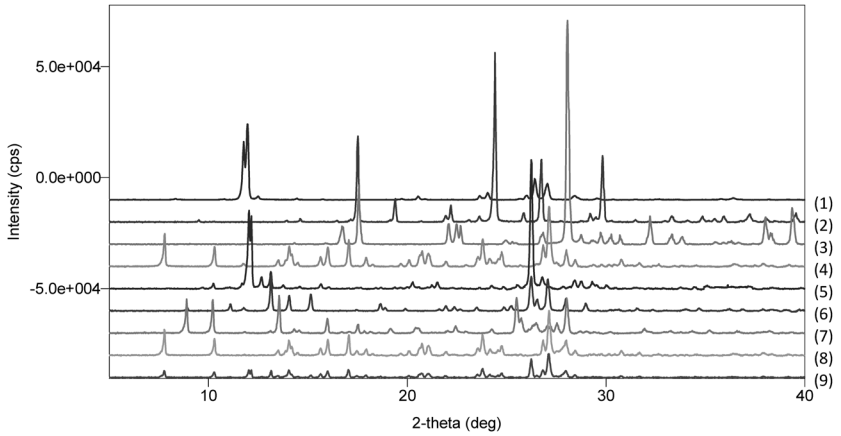

Fig. 5. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–HY (1 : 2) and CA–MA (1 : 1)

From top to bottom: (1) CA, (2) HY, (3) MA, (4) CA–HY (1 : 2), (5) CA–HY (2 : 1), (6) CA–MA (1 : 1), (7) CA–MA (2 : 1), (8) CA–HY (1 : 2) mixed with MA, and (9) CA–MA (1 : 1) mixed with HY.

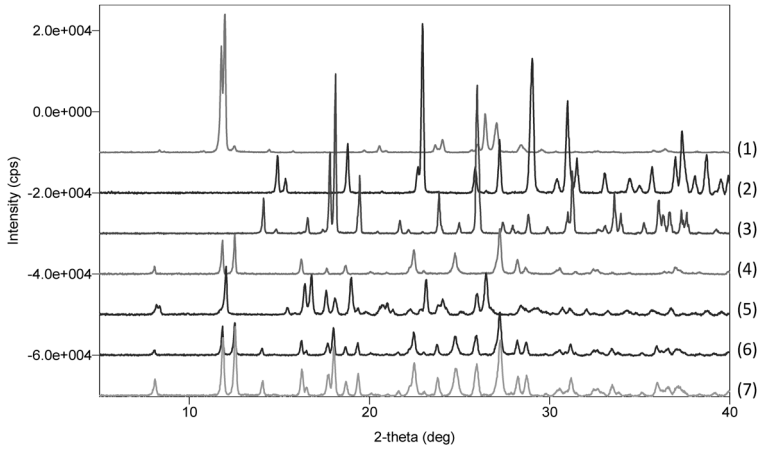

Fig. 6. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–OX and CA–CI

From top to bottom: (1) CA, (2) OX, (3) CI, (4) CA–OX, (5) CA–CI, (6) CA–OX mixed with CI, and (7) CA–CI mixed with OX.

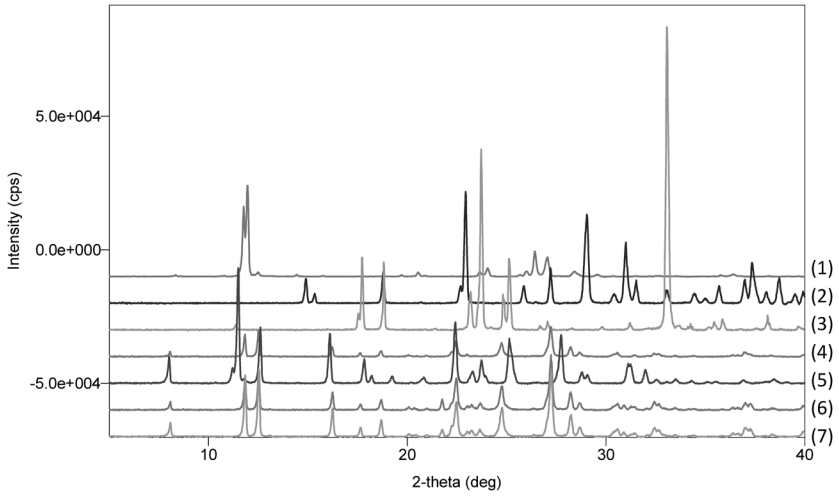

Fig. 7. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–OX and CA–MO

From top to bottom: (1) CA, (2) OX, (3) MO, (4) CA–OX, (5) CA–MO, (6) CA–OX mixed with MO, and (7) CA–MO mixed with OX.

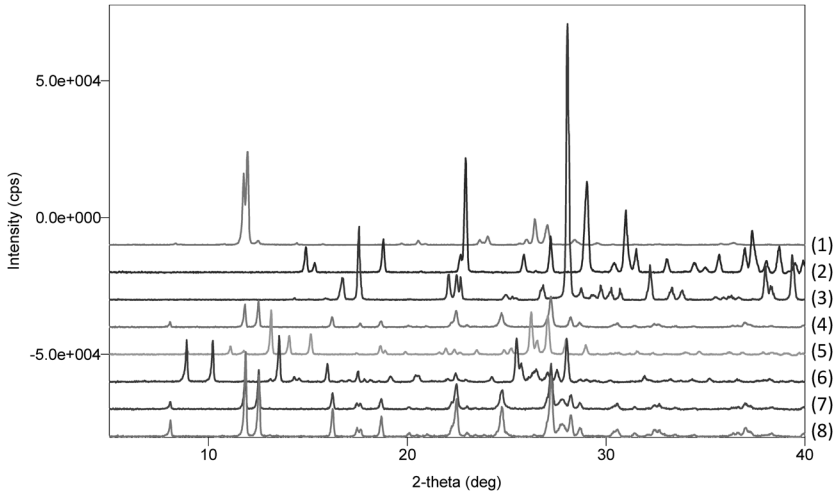

Fig. 8. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–OX and CA–MA (1 : 1)

From top to bottom: (1) CA, (2) OX, (3) MA, (4) CA–OX, (5) CA–MA (1 : 1), (6) CA–MA (2 : 1), (7) CA–OX mixed with MA, and (8) CA–MA (1 : 1) mixed with OX.

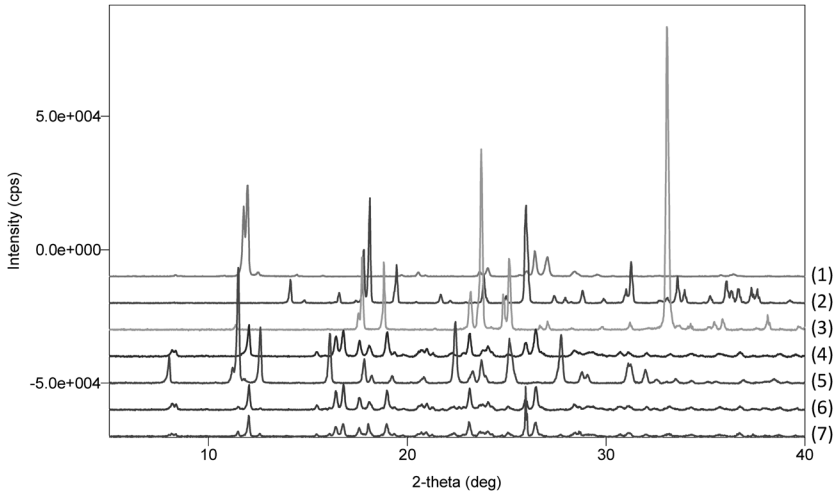

Fig. 9. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–CI and CA–MO

From top to bottom: (1) CA, (2) CI, (3) MO, (4) CA–CI, (5) CA–MO, (6) CA–CI mixed with MO, and (7) CA–MO mixed with CI.

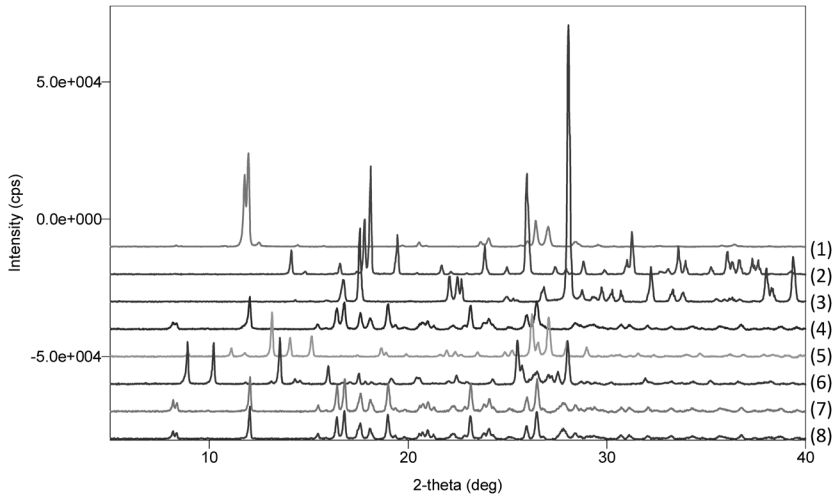

Fig. 10. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–CI and CA–MA (1 : 1)

From top to bottom: (1) CA, (2) CI, (3) MA, (4) CA–CI, (5) CA–MA (1 : 1), (6) CA–MA (2 : 1), (7) CA–CI mixed with MA, and (8) CA–MA (1 : 1) mixed with CI.

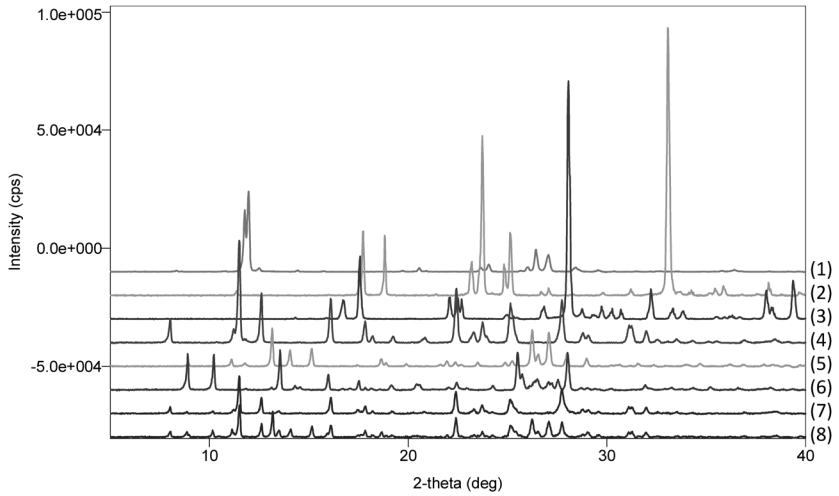

Fig. 11. PXRD Patterns Obtained for the CCF Exchange Reaction of CA–MO and CA–MA (1 : 1)

From top to bottom: (1) CA, (2) MO, (3) MA, (4) CA–MO, (5) CA–MA (1 : 1), (6) CA–MA (2 : 1) (7) CA–MO mixed with MA, and (8) CA–MA (1 : 1) mixed with MO.

Table 2. Summary of CCF Exchange Reactions Using Five CA Co-crystals

Original co-crystal	CCF added					$-\Delta E^{a)}$ (kJ mol^{-1})	$-E_{latt}{}^{b)}$ (kJ mol^{-1})
	p-Hydroxy benzoic acid	Oxalic acid	Citric acid	Malonic acid	Maleic acid		
CA–HY	—	CA–OX and CA–HY[c)]	N.R.[d)]	N.R.[d)]	N.R.[d)]	6	353.19
CA–OX	CA–OX and CA–HY[c)]	—	N.R.[d)]	N.R.[d)]	N.R.[d)]	10	340.14
CA–CI	CA–HY	CA–OX	—	N.R.[d)]	N.R.[d)]	8	—
CA–MO	CA–HY	CA–OX	CA–CI	—	N.R.[d)]	8	324.66
CA–MA	CA–HY	CA–OX	CA–CI	CA–MO[e)]	—	3	202.81

a) Previously reported values.[23)] *b*) Previously reported values.[19,30)] *c*) Mixture of CA–OX and CA–HY. *d*) N.R.: No reaction occurred. *e*) The reaction was not completed within 22 h.

(1 : 2), these co-crystals partially converted to CA–HY (1 : 2) or CA–OX, respectively (Fig. 2). In both reactions, a mixture of CA–HY (1 : 2) and CA–OX was obtained. The PXRD patterns did not change after 22 h and 44 h. Therefore, a mixture of CA–HY (1 : 2) and CA–OX was probably existed in equilibrium. When HY or OX was added to CA–CI, CA–MO, and CA–MA, these co-crystals converted to CA–HY or CA–OX, respectively (Figs. 3–8). These results suggest that CA–HY and CA–OX are more stable than the other three co-crystals. When CI was added, CA–MO and CA–MA converted to CA–CI whereas CA–HY and CA–OX did not (Figs. 3, 6, 9, 10). When MO was added, only CA–MA converted to CA–MO (Figs. 4, 7, 9, 11); however, the reaction between CA–MA and MO was incomplete after 22 h. When MA was added, no co-crystal converted to CA–MA (Figs. 5, 8, 10, 11). Therefore, the thermodynamic stability order of CA co-crystals was determined as:

$$\text{CA}-\text{OX} \approx \text{CA}-\text{HY} > \text{CA}-\text{CI} > \text{CA}-\text{MO} > \text{CA}-\text{MA}$$

Validation of *in Silico* Prediction Methods In the previous literature, the validations of predictive parameters for co-crystal formation were performed based on the probability of finding a co-crystal in a database such as the Cambridge Structural Database.[17,23)] However, there is potential risk of error through using this validation method. As the existence of co-crystals depends on the nucleation process as well as the thermodynamic stability of the products, some co-crystals may be incorrectly judged as unstable even though they may form once nucleation has occurred. Furthermore, lack of data in the crystallographic database may be a consequence of a particular co-crystal synthesis having not yet been attempted or reported. For example, CA-adipic acid co-crystal had been explored over the years and finally crystallized (nucleated) in 2007.[31)] This type of error can be avoided by using the stability order of co-crystals determined by the CCF exchange reaction for validating *in silico* predictions.

As shown in Table 2, the stability order of CA co-crystals correlated with the ΔE for these systems except for HY. The π–π stacking in the CA–HY co-crystal system,[29)] which is not accounted in ΔE, could be a possible reason for this discrepancy. Previously, Moribe *et al.* reported the stability order of carbamazepine co-crystals as glutaric acid>succinic acid>malonic acid,[25)] whereas the order of the ΔE is opposite, *i.e.*, malonic acid (-12 kJ mol^{-1})>succinic acid (-11 kJ mol^{-1})>glutaric acid (-7 kJ mol^{-1}).[23)] The π–π stacking mode in each of these carbamazepine co-crystals are also different.[32)] These results would suggest that the stability order of co-crystals does not simply correlate with ΔE. In the literature, based on the *ab initio* lattice energy calculation with the 6–31 G basis set (Table 2), CA–HY was calculated to be more stable than CA–MO,[19,30)] suggesting that the energies of molecular interactions other than the hydrogen bonds also contribute to the stability of co-crystals. To achieve more concrete conclusion, expansion of the model co-crystals to the other type of CCF is currently under investigation.

In conclusion, the CCF exchange reaction can be a useful method for determining the stability order among co-crystals, which can then be used for validation of *in silico* predictions. However, this method has some drawbacks. For example, a pairwise comparison of the stability order consumes lots of time and resources for experiment. In addition, the increase of the number of CCFs also requires much more time and resources for data analysis. These problems would be solved by a better algorithm for sorting the stability order. Further investigation into the correlation between co-crystal stability and an *in silico* prediction parameter such as ΔE is under way for other model drugs.

Conflict of Interest The authors declare no conflict of interest.

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