

Jumping into Metastable 1:1 Urea-Succinic Acid Cocrystal Zone by Freeze-Drying

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ABSTRACT: Aqueous solutions with molar ratios between urea and succinic acid from 0.3:1 to 3:1 were evaporated at room temperature, products were pure or mixtures of stable 2:1 urea-succinic acid cocrystals, urea or succinic acid. By freeze-drying, metastable 1:1 urea-succinic acid cocrystal formed. The different mixtures of the 1:1 cocrystals reveal several 'hidden' metastable zones in a ternary phase diagram of the 2:1 cocrystal. The formation of the 1:1 cocrystal indicated that the solution composition points in the phase diagram "jump" over the stable zone into the metastable zones.

Cocrystals¹ formed by an active pharmaceutical ingredient (API) with a co-former^{2,3} can improve solubility, dissolution rate, stability etc.^{4,5} The pharmaceutical industrials become more and more interested in cocrystallisation⁶, because it is a versatile approach to discover new solid forms of API.⁷ Urea and succinic acid (SA) can form two kinds of cocrystals. 2:1 urea and succinic acid (U-SA) cocrystal is thermodynamic stable with the hydrogen bonding of a complex 3D structure, while 1:1 U-SA cocrystal has layer structure. The 2:1 urea - succinic acid (U-SA) cocrystals were easily obtained by cooling crystallization or evaporation from various solvents.⁸ As shown in Figure 1, in 2:1 U-SA cocrystals⁹, each SA molecule is connected to four different urea molecules, and two of urea molecules are in the same layer. Each urea molecule is connected to another urea and two SA molecules, and one urea and one SA molecule are in the same layer. Compared with 2:1 cocrystal with a complex 3D structure, the 1:1 cocrystal¹⁰ has a layer structure. Each urea / SA molecule is connected to four different SA / urea molecules, respectively, and all the molecules connected to each other are in the same layer. The characterized diffraction peaks are at 18.2 ° and 21.1 ° for 2:1 cocrystal, while at 9.4 °, 19.6 ° and 27.3 ° for 1:1 cocrystals, which are easy to be distinguished from powder XRD of pure SA or urea crystal. The 1:1 U-SA cocrystal, which is metastable compared with 2:1 cocrystal, hardly crystallised by slurry grinding, cooling crystallisation or evaporation^{9,11}. Alhalaweh

has successfully obtained 1:1 U-SA cocrystal by spray drying⁹, and, as we know, no other methods were reported to obtain 1:1 cocrystal with pure water as solvent. However, the mechanism is unclear, which may be due to the kinetics of cocrystallisation⁹. Therefore, it is important to understand cocrystallisation in metastable phase/zone where the crystallisation processes are highly influenced by kinetics of crystallisation¹².

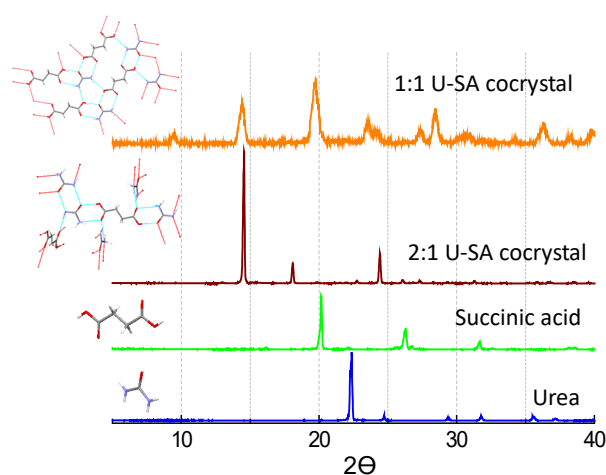


Figure 1. PXRD (top to bottom) of pure 1:1 U-SA cocrystal, 2:1 -SA cocrystal, SA, Urea from freeze drying of the frozen solution and the structures of their molecules⁹.

In this work, shown as Figure 2, the solutions (S1- S8 in Table 1), with molar ratios between succinic acid and urea from 0.3:1 to 3:1, were prepared by dissolving 0.4 g succinic acid and corresponding amount of urea in 10 mL water to form undersaturated solution at room temperature. 2 ml of the solutions were evaporated at room temperature (S1-S8), were freeze dried after freezing the solution at -20 °C for 12 h (S1-S8) or sprayed into liquid nitrogen (S4, S7) to form frozen particles before freeze-drying (see ESI† for details)

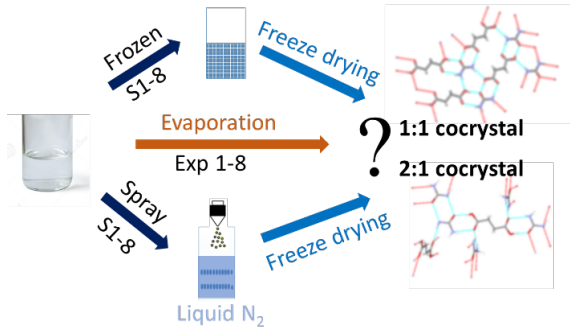


Figure 2. The product obtained by evaporation drying, freeze-drying of the frozen solution and freeze-drying of the frozen particles by spraying the solution into liquid N₂.

Table 1. The molar ratio of urea to SA in the solutions (S1-S8), and the final dried products, from evaporation and freeze-drying of the frozen solution, determined by PXRD

Solution No.	Molar ratio, $r_{U:SA}$ (urea : SA)	Dried product			
		Evaporation drying		Freeze drying after frozen	
S1	0.30, 0.40, 0.50	2:1 CO, SA	2:1 CO, SA	2:1 CO, SA	SA
S2	0.55, 0.60, 0.75	2:1 CO, SA	2:1 CO, SA	2:1 CO, 1:1 CO, SA	SA
S3	0.80, 0.85, 0.90	2:1 CO, SA	2:1 CO, SA	1:1 CO, SA	SA
S4*	1.00	2:1 CO, SA	2:1 CO, SA	1:1 CO, SA	SA
S5	1.20, 1.30, 1.40	2:1 CO, SA	2:1 CO, SA	2:1 CO, 1:1 CO, SA	SA
S6	1.60, 1.70, 1.80	2:1 CO, SA	2:1 CO, SA	2:1 CO, SA	SA
S7*	2.00	2:1 CO, SA	2:1 CO, SA	2:1 CO, SA	SA
S8	2.20, 2.50, 3.00	2:1 CO, urea	2:1 CO, urea	2:1 CO, urea	urea

CO: cocrystal. *extra experiment: same solution was sprayed in to liquid N₂ before freeze-drying.

Figure 3 (a) shows the schematic phase diagram of cocrystal of succinic acid and urea, which is consistent with the reported phase equilibrium of urea, SA and water⁸. In the evaporation experiments with S1 to S8, the molar ratios of urea to SA in the solution ranged from 0.30 to 3.00. When the $r_{U:SA}$ equals to 2 (S7), pure 2:1 cocrystal formed during the evaporating and after dried. It indicates the congruent dissolution for 2:1 cocrystal and, therefore, 2:1 cocrystal is congruent system in water^{10,13}. While the $r_{U:SA}$ is below 2 (S1-S6) or above 2 (S8), mixture of 2:1 cocrystal with SA and mixture of 2:1 cocrystal with urea were obtained in evaporation, respectively. Three different kinds of products crystallised, which were dependent on the molar ratios in the solution (Table 1), and these were in agreements with the three phase zones, locating on the bottom of the ternary phase diagram in Figure 3 (a).

With the solutions of S1 to S8, seven different kinds of products were obtained by freeze-drying. As expected, when the $r_{U:SA}$ equals to 2 (S7) and above 2 (S8), pure 2:1 cocrystal (PXRD in Figure 1) and mixture of 2:1 cocrystal and urea were obtained, respectively. At $r_{U:SA} < 0.50$ (S1) and $1.60 < r_{U:SA} < 1.80$ (S6), mixture of 2:1 cocrystal and SA formed. The products obtained by

freeze-drying of frozen solution in each of these four experiments (S1, S6, S7, S8) were same as those obtained by the evaporation, indicating these phase boundaries, solid curves in Figure 3 (b), are consistent with those in the ternary phase diagram in Figure 3 (a).

However, at $0.55 < r_{U:SA} < 1.40$ (S2-S5), at least a few of 1:1 cocrystals formed in each product, and the single zone (2:1 cocrystal + SA) in Figure 3 (a) was split up four new zones, where the boundaries were shown as dashed curves in Figure 3 (b). At $r_{U:SA} = 1$ (S4), pure 1:1 cocrystal crystallised (PXRD shown in Figure 1), indicating a metastable 1:1 cocrystal zone. At $1 < r_{U:SA} < 1.4$ (S5), the mixture of 1:1 cocrystal and 2:1 cocrystal was obtained, and this metastable zone seems to follow the (thermodynamic stable) phase law, the products obtained in this zone are the mixture of two pure solid phase (1:1 and 2:1 cocrystal) besides. This also applies to the metastable zone that at the ratio below 1 but above 0.8, the mixture of 1:1 cocrystal and SA was obtained. But surprisingly, at $0.55 < r_{U:SA} < 0.75$, the product was mixture of 1:1 cocrystal, 2:1 cocrystal and SA, indicating a stronger influence of SA when the composition point is close to SA point. The crystals obtained from S4 to S1, pure 1:1 cocrystal (S4), mixtures of 1:1 cocrystal (S3 and S2) and no 1:1 cocrystal (S1), indicate that the father distance of the composition point locates to 1:1 cocrystal zone, the weaker influence of 1:1 cocrystal zone becomes. The pure 1:1 cocrystals and 1:1 cocrystals in different mixtures, obtained by freeze-drying, were stable at room temperature for three months, and no transformation from 1:1 cocrystal to 2:1 cocrystal was observed.

After spraying the solution with $r_{U:SA} = 2.00$ (S7*) into liquid nitrogen, the frozen particles were dried by freeze-drying, and 2:1 cocrystals were obtained as expected. By the same method on the solution of S4* with $r_{U:SA} = 1.00$, some 1:1 cocrystals can be obtained, however, mixed with 2:1 cocrystals. Figure 4(a) and (b) show the SEM images of the product's surfaces (S4) after freeze-drying of the frozen solution and porous spherical particle's surface (S4*) after freeze-drying of the frozen particles. In Figure 4(a), some relatively large plate-shape crystals (about 100 μm length) with many small plate-shape crystals randomly locate on the surface, and, therefore, the surface looks very rough. In Figure 4(b), the spherical particles formed after spraying and freeze-drying have porous structure on the surface and inside, where the pores were previously filled with water/ice before freeze-drying.

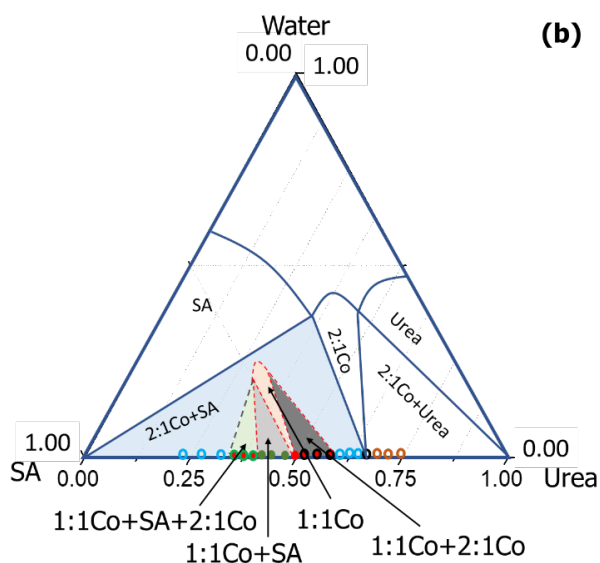
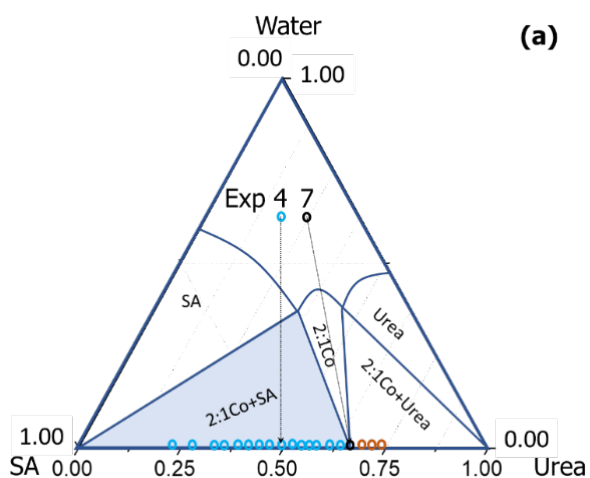


Figure 3. Schematic phase diagram of U-SA cocrystal. (a) Crystals obtained in experiments of evaporation at room temperature with the evaporation routes for S4 and S7 (dotted lines) in 2:1 cocrystal phase diagram (solid curves). (b) Crystals obtained in experiments of freeze-drying of frozen solutions, where several metastable phases/zones related to 1:1 U-SA cocrystal (dashed curves) appear.

During the evaporation of the solutions S2 to S5, the solutions went into stable zone (SA, 2:1 cocrystal phase/zone), stable 2:1 cocrystal and SA crystals formed until the end of the evaporation, and no 1:1 cocrystal appeared due to its instability in solution. However, 1:1 cocrystal or the mixtures with it were obtained by the freeze-drying with the solution of S2 to S5. Comparing Figure 3 (a) with (b), the boundaries (dashed curves) between five different crystal products reveal four “hidden” metastable phases, i)

pure 1:1 cocrystal, ii) 1:1 cocrystal + SA, iii) 1:1 cocrystal + SA + 2:1 cocrystal, iv) 1:1 cocrystal + 2:1 cocrystal, inside the stable phase zone of 2:1 cocrystal + SA. By freeze-drying of the frozen solutions, the composition points of the solution did not follow the slow evaporation routes shown in Figure 3 (a), but seem to “jump” over the stable zone and into the metastable phase/zone related to 1:1 cocrystal.

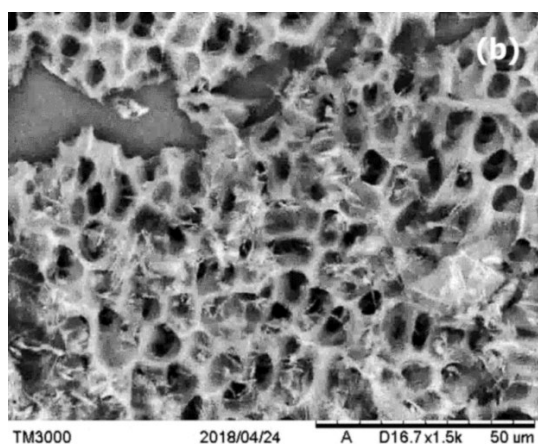
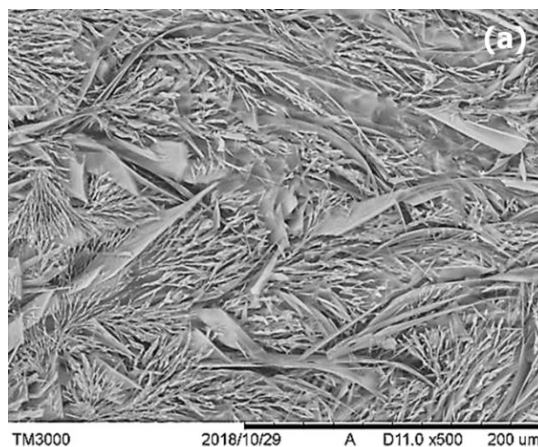


Figure 4. Surface images of the freeze-drying products. (a) freeze-drying of the frozen solution (b) freeze-drying of the particles by spraying into liquid N₂.

The final products were also dependent on the freezing speed of the solution, i.e. the different final crystal products from S4 and S4*. There might be several possible reasons for the formation of mixture of 1:1 and 2:1 cocrystal in S4* by spraying into liquid N₂ and freeze-drying afterwards. During the spraying of the solution into liquid N₂, the SA and urea molecules were trapped in solutions of very small volume, which were separated by the water/ice. The water/ice freeze dried to form the pores shown in Figure 4 (b). In each small volume solution, the molar ratio of urea and SA might be not 1:1 as it uniformly was in bulk solution.

Therefore, the mixtures formed instead of pure 1:1 cocrystal. But this hypothesis is not consistent with the experimental results of Exp 7*. The product, in Exp 7* at $r_{U:SA} = 2.00$, was pure 2:1 cocrystal by the same crystallisation process. The mixture products obtained in Exp 4* may be due to the kinetics of the cocrystallisation. With faster freezing rate by spraying the solution into liquid N₂, the crystallisation process seems to “jump” through the 2:1 cocrystal + SA phase/zone but “fall” in the 1:1 cocrystal and 2:1 cocrystal phase/zone. The composition points did not successfully approach the bottom line before completing the cocrystallisation, due to the “short jump” in the phase diagram driven by too high supersaturation during the spraying into liquid N₂. This is consistent that 1:1 cocrystal may be incongruent system, due to the solubility difference between urea and SA in water is larger than 10 times^{8,14,15}. The spraying drying also helped the solution “jump” in the phase diagram to obtain 1:1 cocrystal as reported⁹, and, however, the high supersaturation during the spray-drying did not result in “short jump”. It is probably due to the much (orders of magnitude) faster molecular movement in very high temperature during the spray-drying compared with the molecular movement after spraying into the liquid N₂.

Nevertheless, the mechanisms behind the “jump” are not fully understood, the cocrystallisation processes during the frozen, spraying and freeze-drying need to be further systematically investigated. The influence of microscale porous structure¹⁶, as well as the amorphous formed¹⁷ in freeze-drying, on the cocrystallisation, is also need to be investigated. The better understanding of the phase diagram is not only necessary to control the crystallisation process^{18,19}, but also potential to develop new methods to approach new cocrystals.

In conclusion, the freeze-drying of urea and succinic acid solution can produce metastable 1:1 U-SA cocrystal which cannot be obtained by evaporation, due to the instability of 1:1 cocrystal in solution. The products of 1:1 cocrystal and mixtures with it obtained in freeze-drying reveal metastable zones related to 1:1 cocrystal, which split the 2:1 cocrystal + SA phase/zone up to five zones, where the new metastable zones are 1:1 cocrystal and 1:1 cocrystal with 2:1 cocrystal, with 2:1 cocrystal and succinic acid, and with succinic acid. The pure 1:1 cocrystal was obtained by freeze-drying of the frozen solution with molar ratio 1:1 of urea and succinic acid, however, with same solution by freeze-drying after spraying the same solution into liquid nitrogen, the mixtures

of 1:1 and 2:1 cocrystals formed. By both freeze-drying methods, the solution seems to “jump”, over the stable zone, into the metastable zones. This work shows a new understanding of (metastable) cocrystal phase diagram, which is useful to control the cocrystallisation process and obtain the desired cocrystals by freeze-drying. However, the mechanisms on the nucleation and crystallisation of cocrystal during the frozen and freeze-drying still need to be further investigated.

SUPPORT INFORMATION

ESI† Materials and detailed experimental method. The Supporting Information is available free of charge on the ACS Publications website.

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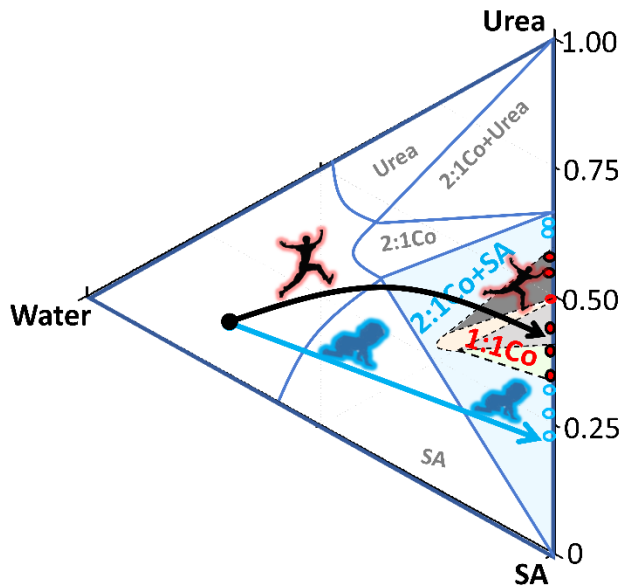
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Evaporation at room temperature → stable 2:1 urea-succinic acid cocrystals or mixtures

Freeze drying → metastable 1:1 cocrystals or mixtures

The solution composition points “jump” over stable zone (2:1 cocrystal + succinic acid) into metastable zones (1:1 cocrystal, or mixtures with 2:1 cocrystal and succinic acid) in phase diagram.

New method and designable approach to produce metastable cocrystal