Co-crystal phase diagram determination by solution addition method

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Abstract

Multicomponent crystals such as co-crystals, salts and solid solutions can be used to modify physical properties of active pharmaceutical ingredients. Phase diagrams of such multicomponent crystals are essential for crystallization process development, especially in the case where multiple solid phases may coexist. However, additional components and solid phases make phase diagrams more complex and their determination more time consuming. We propose to accelerate this process by identifying the eutectic points and constructing the rest of the phase diagram using thermodynamic models, informed by further measurements, if necessary. To achieve this acceleration, in this work, a novel method is proposed for determining the eutectic points in a co-crystal system. This solution addition method implements gradual compositional changes to traverse various regions of the phase diagram. Phase boundaries are determined by monitoring changes in liquid phase (UV-vis) and solid phase (Raman), and eutectic points are obtained from intersects of phase boundaries. The results from solution addition are compared to an equilibration method which combines gravimetry, XRPD and NMR to identify the eutectic solution composition starting from a composition in the three-phase region of a co-crystal phase diagram. Both methods were able to locate all eutectic points, allowing construction of the ternary phase diagrams of benzoic acid and isonicotinamide in ethanol.
Introduction

Multicomponent crystals such as co-crystals, salts or solvates via the introduction of a second component in the crystal lattice are a way of modifying the physical properties from those of single component solids.1-4 Such solid forms with multiple components can be useful within the pharmaceutical industry as these new crystalline solids may have suitable solubility/dissolution, morphology, stability, flow properties or other manufacturability parameters.5-8 Many co-crystals of active pharmaceutical ingredients have been reported with various advantages over pure compounds.9 Accurate knowledge of the phase diagram for such complex multicomponent systems is essential for robust crystallization process development. However, with additional phases and components, obtaining this becomes a complicated and time-consuming task when screening multiple solvents and co-formers.

Figure 1 shows an example ternary phase diagram of components A and B and a solvent. In this case, A and B can exist as individual solid phases as well as a 1:1 co-crystal. The liquid region at solvent rich corner shows a region where all components exist as a solution. Each of the three blue regions show a single solid phase A, B or AB in equilibrium with the liquid phase. Phase boundaries between the liquid region and the single solid phase regions are outlined by the liquidus lines. Eutectic points exist where two liquidus lines intercept. The regions shaded red are composed of two solid phases and a liquid phase with composition of the eutectic point. Examples of two conformer and solvent ternary phase diagrams for small organic molecules have been widely reported for co-crystals of various stoichiometries.10-12

Common approaches to obtain phase diagrams by equilibration involve creating a set of suspensions, allowing them to equilibrate and analyzing the solution phase or solid phase compositions, seeding with all potential solid forms when necessary to ensure that equilibrium solid phases are present under given conditions. These suspensions are represented by green squares in Figure 1. After equilibration, equilibrium solid phases can be identified (e.g., by XRPD) while liquid phase composition can be determined (e.g., by HPLC, NMR or via a mass balance).11-16 The resulting phase compositions can then be used to draw tie-lines and phase boundaries. With these established methods, obtaining a reliable phase diagram is time consuming, especially with more complex phase diagrams at multiple solvent compositions as a large number of measurements are required. If too few points across the phase diagram are chosen, a region may be missed, resulting in an inaccurate phase diagram which in turn may lead to unwanted surprises in crystallization process development.
Figure 1. Traditional approach to determining co-crystal phase diagrams. Green squares represent selected compositions to sweep across the phase diagram. Analysis of solid and liquid phases can be used to construct tie-lines and subsequently phase boundaries on a typical 1:1 co-crystal ternary plot.

In this work we propose a new calibration-free solution addition method which focuses on directly obtaining the eutectic points of a multicomponent phase diagram rather than the solid-liquid line using Process Analytical Technology (PAT) without the need to isolate samples. Phase boundaries are obtained by changing composition via solution addition, analogous to measuring solubility by solvent addition method or discontinuous isoperibolic thermal analysis. By focusing on the eutectic points, locating phase diagram regions can be accelerated with a minimum number of experiments. The liquidus line can then be estimated using thermodynamic models from the data measured using constant solubility product or activity as well as pure component solubility data. Solubility product is related to the equilibrium constant which describes the composition of multicomponent systems such as salts or co-crystals where activity of the co-crystal solid is close to 1. This extrapolation is also applied to the more traditional equilibration method. We demonstrated and verified the solution addition method by measuring the phase diagram of benzoic acid (BZA) and isonicotinamide (INA) in ethanol. Benzoic acid and isonicotinamide has been reported to form co-crystals in 2:1 and 1:1 stoichiometries. This system has been previously studied but there are discrepancies regarding the presence of the 2:1 co-crystal region between those studies and this work.

Methods

Materials. Benzoic acid (≥99.5%) and isonicotinamide (99%) were supplied by Sigma-Aldrich (Gillingham, UK). Ethanol (99.96%) was supplied by VWR Chemicals (Fontenay-sous-Bois, France). Isonicotinamide was supplied as the stable form II.
**Solubility.** Pure component solubility of benzoic acid in ethanol at 25 °C was available in literature. However, for isonicotinamide it was measured by suspending excess solid in ethanol in a 20 mL vial under agitation with temperature control at 25 °C using a Polar Bear Plus (Cambridge Reactor Design). The suspension was filtered, and the composition of the saturated liquid was determined gravimetrically.

**Solution addition method.** This method relies on using PAT tools for monitoring liquid and solid phases to detect phase boundaries while gradually adding a solution and moving across a phase diagram. The experiments were carried out in a 100 ml EasyMax 102 glass vessel (Mettler Toledo), using an experimental setup shown in Figure 2. The trajectory starting point composition was weighed out and allowed to equilibrate for several hours. Benzoic acid and isonicotinamide co-crystals form rapidly, however, for a co-crystal phase diagram with long nucleation time, the mixture can be seeded with small amounts of multiple solid phases to ensure equilibrium solid phases are present. Reactor temperature was controlled at 25°C and agitation set to 300 RPM. Depending on the direction of the trajectory, a concentrated solution of isonicotinamide or benzoic acid was prepared in ethanol. The solution was loaded into a syringe and the initial mass was recorded. Solution was pumped into the vessel via a PHD Ultra (Harvard Apparatus) syringe pump at a rate of 1.5-10.0 ml/hr for 4-15 hours. The syringe was weighed again at the end to obtain mass change over time.

![Figure 2. Solution addition method setup.](image)

Liquid phase composition was monitored by UV-vis, 6mm Hellma ATR probe with Carl Zeiss MCS600 Spectrometer. UV-vis peak for benzoic acid appears at around 230 nm, for
isonicotinamide at around 270nm. The raw spectrum was processed using first derivatives and the edges of the relevant peaks were used to plot concentration trends. The solid phase was monitored by Raman using a Kaiser Optical Systems RXN2 with the PhAT probe with a 250 mm spacer and 6mm optic. The raw data was processed using 2nd derivative for baseline correction. Savitzky-Golay smoothing over 10pts and SNV offset correction were also applied. The resulting trends were also smoothed using LOWESS. Both UV-vis and Raman signals were used calibration-free as they were used to detect change rather than absolute quantities. In the setup used, the composition was changed by adding a solution to ensure uniform addition, however, if a suitable solid or slurry dosage system is available that could be used instead.

Seven trajectories were carried out with starting points in various 2-phase and 3-phase regions of the ternary plot. One could traverse the entire phase diagram in one trajectory, however, the distance of the trajectory depends on the volume change possible in chosen vessel. With this setup, starting volume was around 50-60 ml with 30-45 ml added via the pump.

The rate of addition influences how close a system is to equilibrium during the experiment. If the addition rate is too fast, the growth and dissolution of solids will not be able to keep up with the change in composition leading to large errors. When crossing boundaries where a new solid phase must nucleate in order to stay close to equilibrium, pumping too fast will increase the lag time for nucleation and phase boundary detection. Several addition rates were investigated for trajectories which involve going from a two-phase region to three-phase and again to two-phase. The nucleation is caused by the build-up of supersaturation for the co-crystal. In the absence of a stable solid phase, the liquid solubility follows a pseudo-equilibrium line until nucleation. If two addition rates produce different results, the pumping rate needs to be reduced. If they give similar results, the system is likely to be close to equilibrium independent of kinetics. Some variation is expected in the two to three-phase transition due to the stochastic nature of nucleation.

**Equilibration method.** Various compositions of benzoic acid, isonicotinamide and ethanol were prepared in 8ml vials with a magnetic stirrer bar. The solute composition was chosen to be in high excess to increase the probability of hitting the three-phase region while retaining sufficient mixing. The vials did not have to be seeded with the co-crystal for this system as both 2:1 and 1:1 co-crystals nucleate easily, however, for some co-crystal systems, seeding with all potential co-crystal forms is advisable. The vials were sealed and held at 25°C and 1000 RPM for two weeks using a Polar Bear Plus. Vials were weighed before and after the two-week period to ensure no evaporation of solvent occurred. After the equilibration period,
solids were left to settle, and liquid was siphoned from the top and filtered through a 0.2µm syringe filter into a clean vial. The remaining slurry was filtered using a Buchner funnel to recover solids without washing for XRPD analysis. The combined concentration of both co-formers of the liquid phase was determined gravimetrically after allowing all ethanol to evaporate. The solid residue was redissolved in deuterated methanol and analyzed by ¹H NMR to determine the solute ratio.

**Figure 3. Equilibration method.**

XRPD measurements were carried out from 4 to 35° 2θ with a 0.015° step size and 1 s step. Samples were placed on a 28-well plate supported by 7.5 µm Kapton film. Data was collected on a Bruker D8 Advance II diffractometer, source radiation Cu Kα1, λ = 1.540596 Å. ¹H NMR was carried out using Bruker Advance 3 at 400 MHz with 4 scans on samples dissolved in deuterated methanol in 5 ml tubes.

Liquid phase composition in equilibrium with the equilibrium solid phase(s) present were determined by combining the NMR and gravimetry results. XRPD results then show which solid phase was in equilibrium with the determined liquid composition. If two solid phases were found in XRPD, the liquid phase composition corresponds to the eutectic point in the phase diagram. The phase diagram can be completed if all three-phase regions were successfully targeted and all eutectic points determined. All additional data corresponding to liquid phase compositions in equilibrium with a single solid phase in the two-phase regions can be used to plot the solid-liquid line of the given region.

After determining the location of the eutectic points, any missing gaps in the liquidus line were filled in by additional measurements for comparison to constant solubility product estimation.
Results and Discussion

Solution addition. The PAT employed in the equipment setup is used for monitoring of solid and liquid phases while traversing a ternary phase diagram by changing the composition. Raman spectroscopy can identify solid forms present and therefore can determine the time point when certain solid phases disappear and when new forms crystallize. UV-Vis spectroscopy monitors the concentration of species in the liquid phase on a relative scale and was used calibration-free. The liquid phase concentration is expected to follow the shape of the liquidus line when moving sufficiently slowly through a two-phase region. However, when moving through a three-phase region, liquid composition will remain constant at the eutectic as the dissolution of one solid is balanced by the growth of another. The starting point of a trajectory can be anywhere in the phase diagram if the initial position is in equilibrium with all stable phases present. If the starting point is equilibrated in the three-phase region, no nucleation of new phases is necessary during translation across the phase diagram into neighboring regions. However, if the starting point is in a two-phase region, there will be a lag as the supersaturation builds before nucleation of the third stable phase takes place. The starting point location can be selected using knowledge of solubility of pure components and possible solid phases if known but starting in three-phase region will require some experimentation. The methodology is presented here for a co-crystal system of benzoic acid and isonicotinamide in ethanol. The 1:1 and 2:1 co-crystals nucleated very quickly without the need to seed.

Figure 4 shows the compositional change during trajectory 1 on a ternary phase diagram as well as the UV-vis and Raman trends over the course of solution addition as it shows both 2 to 3-phase and 3 to 2-phase region transitions. The starting point in this trajectory is a suspension of isonicotinamide solid in saturated ethanol, to which a concentrated solution of benzoic acid in ethanol is added depicted by the black line. The UV signal at 222nm corresponds to a peak from the first derivative of the benzoic acid spectrum, 284 nm corresponds to the first derivative of isonicotinamide peak. The Raman signal at 1616 cm\(^{-1}\) corresponds to the 1:1 co-crystal solid and that at 1085 cm\(^{-1}\) corresponds to the isonicotinamide solid based on measured reference spectra.
Figure 4. For the trajectory shown on the left ternary phase diagram (mol fraction) an example normalized UV-vis and Raman trends during solution addition are shown on the right. Boundaries detected on the UV-vis trajectory are represented by yellow diamonds with corresponding number. Boundaries determined by Raman are represented by blue squares. The first Raman phase boundary can be identified either by the appearance of 1616 cm⁻¹ signal (1:1 co-crystal) or reduction in 1080 cm⁻¹ (isonicotinamide). UV 284 nm corresponds to isonicotinamide and 222 nm to benzoic acid.

As can be seen by the Raman intensity in Figure 4, with zero intensity at 1616 cm⁻¹ and high intensity at 1085 cm⁻¹, in the initial slurry only isonicotinamide solid is present. At this stage, as benzoic acid is added to the solution, the UV-vis signal measures the solubility line of isonicotinamide in isonicotinamide-benzoic acid solutions. The first phase diagram boundary is detected when the 1:1 co-crystal crystallizes as the trajectory enters the three-phase region. This is shown by the sudden rise in the Raman intensity at 1616 cm⁻¹ as well as by the decline in Raman 1085 cm⁻¹ indicating a dissolution of isonicotinamide solid. A constant signal from both UV wavelengths around similar time shows also that the liquid phase composition is at the eutectic between 1:1 co-crystal and isonicotinamide liquidus lines. The solution composition at the eutectic is constant as the additional benzoic acid is added because the changing system composition only changes the ratio of the two solid phases, isonicotinamide and 1:1 co-crystal. These boundaries are marked by yellow squares (UV-vis) and blue squares (Raman) on the ternary phase diagram on the left of Figure 4.

The second domain boundary we encountered during this trajectory is a transition from a three-phase region where 1:1 co-crystal, isonicotinamide and solution coexist to a two-phase region where the 1:1 co-crystal exists in equilibrium with solution. This transition is marked by the disappearance of isonicotinamide solid in the Raman signal at 1085 cm⁻¹ in Figure 4. Raman 1616 cm⁻¹ signal does not decisively plateau, therefore, 1085 cm⁻¹ signal was used to determine the location of the boundary. Furthermore, the UV signals show a transition from constant to increasing intensity at approximately 20:00, which indicates that the liquid phase is no longer at the eutectic and instead follows the 1:1 co-crystal solid-liquid line. The eutectic
point where isonicotinamide and 1:1 co-crystal liquidus lines intersect can be triangulated from this one trajectory. This was determined to be at the following mol fractions: \( x_{\text{BZA}} = 0.00664 \) and \( x_{\text{INA}} = 0.0375 \).

The process is then repeated with a new trajectory until all the boundaries of interest have been crossed. After a boundary is detected the direction of the trajectory can be switched by adding a solution of different composition. This can be useful in precisely narrowing down the location of a domain boundary, particularly when nucleation of new phase is required to maintain equilibrium. The rate at which composition can be changed while remaining near equilibrium will depend on growth and dissolution kinetics of the system. Therefore, multiple rates of addition need to be tested and compared to ensure the result is not significantly influenced by the kinetics of crystallization.

The first experiments to see the effect of rate of addition were carried out at 10 ml/hr. However, the dissolution and especially the growth of solid phases in the three-phase region limited the ability of the liquid phase to stay in equilibrium. The addition rate adequate for keeping near equilibrium to obtain the domain boundary is therefore related to the growth and dissolution kinetics of the solid phases present. Addition rates of 1.5-3 ml/hr were shown to produce similar results and therefore allowed to work close to equilibrium. Resulting phase boundaries at three different solution addition rates are shown in Figure 5. The trajectories include a two to three-phase boundary as well as a three to two-phase boundary.

Figure 5. Addition rate comparison on the location of detected domain boundary due to kinetic effects. Phase boundaries detected by monitoring the liquid phase using UV-vis are shown on the (left) and from solid phase using Raman on the right. There are two sets of phase boundaries, going from two-phase to three-phase region and back to two-phase region of 2:1 co-crystal and solution.
Using suitable addition rates and starting points, the trajectories 1-7 shown in Figure 6 were carried out to determine all phase boundaries. The composition change inside the 100 ml vessel is shown by the black trajectory lines. Phase boundaries detected by Raman by the appearance or disappearance of certain solid phases are shown by blue squares. Similarly, phase boundaries from solute concentration changes detected by UV-vis are illustrated by yellow squares. In most cases, the phase boundary detection points are very close for UV-vis and Raman such that the Raman blue squares and the UV-vis boundary yellow squares overlap. Repeats were carried out for most trajectories at slightly different starting positions to improve accuracy for the triangulation of eutectic points.

From the detected phase transitions and domain boundaries, eutectic points can be triangulated by connecting the boundary points to composition of pure solids. The eutectic point location is derived from the intersection point of two lines from adjacent phase boundaries. This is further visualized in the supplementary information where Figure S1 shows an illustrative example of this methodology on a simple 1:1 co-crystal system between A and B. The domain boundary is the straight line connecting a pure solid composition point and intersecting the detected boundary point. A eutectic point is positioned at the intersection of two domain boundaries. If multiple boundary transition points are measured per phase boundary, the co-crystal composition along with the eutectic may also be determined via triangulation if unknown.
Phase boundaries were drawn by connecting solid phase compositions with the domain boundary transition points and extending them until they meet at the eutectic point as described in the methods section. The phase boundaries detected by both solution addition and equilibration methods coincide well with each other in all cases. In cases where boundaries were identified from multiple trajectories, consistency is also achieved. The eutectic point between 2:1 and 1:1 co-crystal solubility lines lies so close to the benzoic acid – ethanol line that when using a best fit from boundaries detected from trajectories 5, 6 and 7 the INA concentration is lower than can be accurately measured via triangulation. This indicates that only a very small amount of isonicotinamide in solution is needed to result in more stability for the 1:1 co-crystal. The compositions of eutectic points as determined by solution addition method are presented in Table 1.
Table 1. Eutectic points determined using solution addition method given in mole fractions.

<table>
<thead>
<tr>
<th>Eutectic point</th>
<th>X_{BZA}</th>
<th>X_{INA}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid – 2:1 co-crystal</td>
<td>0.179</td>
<td>1.61x10^{-3}</td>
</tr>
<tr>
<td>2:1 co-crystal – 1:1 co-crystal</td>
<td>68.9x10^{-3}</td>
<td>0</td>
</tr>
<tr>
<td>1:1 co-crystal - isonicotinamide</td>
<td>6.64x10^{-3}</td>
<td>37.5x10^{-3}</td>
</tr>
</tbody>
</table>

Accuracy of this method depends on PAT detection limits, rate of addition and the proximity of the phase boundary detection point to the eutectic. If the phase boundary is crossed far away from the eutectic, any error in detection limits is amplified by the triangulation method. On the other hand, sufficient slurry density is required for Raman signal and high surface area for fast growth and dissolution. Solid-liquid lines were drawn assuming a constant solubility product which was averaged form the eutectic points, just as was done in the equilibration method. Using online monitoring and pumps, the solution addition method can be automated and miniaturized to allow for rapid screening of potential solvent systems for co-crystallization, accelerating process development. The method can also be used to determine unknown co-crystal composition via triangulation if the solid landscape is unknown.

Early experiments identified issues with reaching far sides of the phase diagram, for example going from benzoic acid, 2:1 co-crystal and liquid three-phase region to the two-phase benzoic acid solid-liquid region. The volume change required to traverse to the left while already on the very left side of the diagram increases dramatically. Similar issue occurs on the isonicotinamide side of the phase diagram. Even though the isonicotinamide solid-liquid region is wider than for benzoic acid, the lower solubility of isonicotinamide means composition change requires larger volume change if only solution is to be added. The vessel composition could be changed by adding both solid and liquid, however, by adding solution, composition uniformity is guaranteed, and automation becomes easier. The solution for determining these phase boundaries was to start with slurries of single component solids and add the co-former solution at very slow rate until nucleation of co-crystal phase is detected.

This approach simplifies finding the starting points for the four outer phase boundaries as each trajectory can pass through two-phase boundaries: first the nucleation of the co-crystal and the second after leaving the three-phase region going into co-crystal solid-liquid region. For the middle two-phase boundaries, the starting point may require some trial and error as the phase diagram may be asymmetrical.
**Equilibration.** Figure 7 shows a ternary phase diagram with the initial vial slurry compositions as circles with the corresponding solid phases present as determined by XRPD. The resulting liquid compositions are also displayed as squares as determined by the combination of NMR and gravimetric analysis. The initial compositions were chosen in three separate runs starting with the highest concentration to ensure that in the first run all regions of the phase diagram are covered. The following two runs at lower concentrations were targeted to fill in the gaps on the solid liquid lines to compare data to solid-liquid line estimation from eutectic points. These initial compositions equilibrate into a liquid and one or more solids. For instance, point A splits up in a solution with composition highlighted A_L and a solid that was identified by XRPD to be (within the detection limit) a single co-crystal phase. This initial composition therefore equilibrated by splitting into a co-crystal solid and a solution over a dashed tie line shown below. The solution composition is therefore a point on the solid-liquid line of the 1:1 co-crystal.

Point B on the other hand splits up in a solution with composition B_L and a solid sample that consists of both the 1:1 co-crystal and isonicotinamide. Other initial points in close proximity but with lower concentration and different component ratios equilibrates to a solution composition with the same composition and also a solid sample that consists of both the 1:1 co-crystal and isonicotinamide. These results indicate that both initial compositions lie in the three-phase region and the measured solution composition is the eutectic composition of the eutectic point where the 1:1 co-crystal and isonicotinamide solubility intercept.

The measurements resulted in the identification of all three eutectic points: between solubility line of benzoic acid and 2:1 co-crystal, between 2:1 co-crystal and 1:1 co-crystal solubility lines, and 1:1 co-crystal and isonicotinamide solubility lines. The eutectic points from three phase regions are generally closely clustered together showing good agreement.
Figure 7. Equilibration results and starting composition. All overall vial compositions equilibrated are shown as circles. The solid phase as identified by XRPD is depicted by shading of the circle. In the cases where multiple solid phases were present in the sample, circle was shaded with both colors irrespectively of the ratio of the two phases. Liquid points along the two-phase boundary are red. Liquid composition points from vials with multiple solid phases present as determined by XRPD are classed as eutectic points, shaded blue. The liquid phase composition as measured by gravimetry and NMR are depicted as squares. Phase boundaries were plotted to connect solid phase composition to the averaged eutectic points.

The solid-liquid boundary was estimated using constant solubility product. The solubility product was taken from the two eutectic points surrounding a solid-liquid boundary and averaged. Eutectic point compositions, the standard deviations and solubility product for each solid phase are given in Table 2. The two-phase regions for solid benzoic acid and for solid isonicotinamide in solution along the sides of the phase diagram are narrow due to the relatively high stability of the co-crystals and the low concentration of the other co-former at the eutectic.
The solid-liquid line estimated from the constant solubility product is very close to the actual solid-liquid line measured by this method. Phase diagram measurements using this equilibration method will most likely result in few points in the two-phase solid-liquid region despite aiming for the three-phase region, which will assist in drawing the co-crystal solubility line. A comparison with ideal the solubility product is shown in Figure 8.

There are some unexpected results from solid phase verification. For example, two points in the 1:1 region show traces of benzoic acid or 2:1 co-crystal. Similarly, all points in the 2:1 region show traces of benzoic acid. This is most likely due to a combination of the absence of a washing step of solid samples after filtration and the high solubility of benzoic acid in ethanol. Washing was avoided to prevent phase transformation, but new solid phases could have formed during drying as solute in residual saturated solution crystallizes. PXRD analysis on slurry is a solution to this if available.

In the 1:1 co-crystal solid-liquid region, the tie-lines pass through or very close to the overall composition satisfying the mass balance, assuming solid phase is pure. However, in the 2:1 region the two tie-lines both pass on the left side of the overall composition. This does not mean that the overall composition lies in the three-phase region though. To be in the BZA-2:1 co-crystal three-phase region, the tie-lines would have to be on the right of the overall composition. Also, the liquid phase composition does not converge to a eutectic for all points with both 2:1 co-crystal and benzoic acid identified in solid phase. The liquid composition relies on NMR to determine the ratio of the two solutes. As the solid-liquid line of the 2:1 co-crystal is extremely close to the benzoic acid – ethanol axis, the concentration of isonicotinamide is very low. The relative error from NMR measurement is high at this very low ratio of isonicotinamide to benzoic acid leading to a higher error in liquid phase composition points along the 2:1 co-crystal solubility curve compared the 1:1 co-crystal. In order to achieve higher accuracy under such circumstances, alternative analytical methods such as HPLC may be used.

The solid-liquid lines can be either measured experimentally using the equilibration method or approximated from the obtained eutectic points, assuming the activity coefficient is independent of composition. The solid-liquid lines for pure solids can be approximated by straight lines from pure component solubility points to the eutectics and the solid-liquid line of the co-crystal can be approximated using a constant solubility product. The solubility product of A and B at the eutectics can be calculated using:
\[ K_{sp} = x_A x_B \quad (K_{sp} = x_A^2 x_B \text{ for 2:1 co-crystal}) \quad (1) \]

where \( x_A \) and \( x_B \) are mole fractions in equilibrium.

The solid-liquid line can be calculated using constant solubility product calculated as an average of the two solubility products from the two adjacent eutectic points. The ideal solubility product of a co-crystal can be calculated based on the commensurate melting temperature \( T_m \) and the associated heat of melting \( \Delta H_{fus} \) using:

\[ K_{sp} = x_A x_B \approx \frac{1}{4} \exp \left[ \frac{- \Delta H_{fus}(T_m)}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) \right] \quad (2) \]

Equation 2 assumes ideal mixing of A and B and the change in heat capacity on melting is neglected. The thermodynamic properties of the 1:1 co-crystal measured by DSC are \( T_m = 438.6 \) K and \( \Delta H_{fus} = 54.6 \) kJ/mol.

Table 2. Eutectic points and standard deviations determined using equilibration method given in mole fractions.

<table>
<thead>
<tr>
<th>Eutectic point</th>
<th>( x_{BZA} )</th>
<th>Std. dev.</th>
<th>( x_{INA} )</th>
<th>Std. dev.</th>
<th>( K_{sp} \text{ at eutectic} )</th>
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<tr>
<td>Benzoic acid – 2:1 co-crystal</td>
<td>0.165</td>
<td>8.63x10^{-3}</td>
<td>0.454x10^{-3}</td>
<td>6.47x10^{-6}</td>
<td>2:1 co-crystal 12.4x10^{-6}</td>
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<td>2:1 co-crystal – 1:1 co-crystal</td>
<td>79.5x10^{-3}</td>
<td>2.82x10^{-3}</td>
<td>1.37x10^{-3}</td>
<td>21.1x10^{-3}</td>
<td>2:1 co-crystal 8.70x10^{-6}</td>
</tr>
<tr>
<td>1:1 co-crystal - isonicotinamide</td>
<td>3.210x10^{-3}</td>
<td>0.316x10^{-3}</td>
<td>34.6x10^{-3}</td>
<td>2.87x10^{-3}</td>
<td>1:1 co-crystal 0.112x10^{-3}</td>
</tr>
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</table>
The heat of fusion and melting temperature have been used to calculate the ideal solubility product of the co-crystal at 25 °C. The value of the ideal solubility product was calculated to be $K_{sp}^{ideal} = 2.17 \times 10^{-4}$. Constant solubility product was calculated from the arithmetic mean of two 1:1 co-crystal eutectic points from the equilibration method to be $K_{sp} (1) = 1.06 \times 10^{-4}$. Finally, a constant solubility product from all measured points along the 1:1 co-crystal solid-liquid curve was averaged leading to a solubility product of $K_{sp} (2) = 8.81 \times 10^{-3}$. The resulting solid-liquid curves for the 1:1 co-crystal are shown along with measured points and boundaries from equilibration method in Figure 8.

The curve from the ideal solubility product severely overpredicts the solubility of the co-crystal. However, this is to be expected as the activities of components in the liquid phase are not accounted for in the calculation. The constant solubility product calculated from the eutectic points provides a significant improvement over ideal solubility. Calculating an average solubility product from all measured liquid phase compositions provides negligible
improvement, showing that the estimation of the solubility line just from eutectic points has been sufficient in this case. In cases where constant solubility product does not provide sufficient accuracy for process development, eutectic points determined via solution addition method can be used to parametrize an activity coefficient model (e.g. UNIFAC, NRTL or other) to account for non-ideal behavior.

Figure 9. Comparison between equilibration method (black) and solution addition method (red)

There is a good agreement between the two methods. The phase boundaries and eutectic points are shown in Figure 9. There is a slightly larger deviation in the position of the domain boundaries between 2:1 and 1:1 co-crystal regions. The deviation is caused by later phase boundary detection in trajectories 6 and 7. The low solubility of 1:1 co-crystal and hence slower transformation may explain this. Literature sources with this phase diagram report a similar location of the 1:1 co-crystal region, however, the 2:1 region is either very narrow or missing. This may be due to slightly different solvent purity used or the omission of starting compositions in the 2:1 co-crystal region in literature. This may be caused by a composition sweep in the measurement plan passing below or just at the 2:1-1:1 eutectic.

Conclusions

The solution addition method for obtaining the eutectic points of the co-crystal phase diagram has been demonstrated on benzoic acid and isonicotinamide in ethanol and verified using and equilibration based method. The location of the biphasic 1:1 co-crystal region in equilibrium with the solution is consistent with literature, while this work shows that the 2:1 region is present and wide, albeit at a high concentration of benzoic acid. The solid-liquid line estimation
between two eutectic points using a constant solubility product coincides well with the measured data in this case, allowing the construction of the solid-liquid lines. The solution addition method employs a novel approach to phase diagram determination that is easy to automate for rapid screening. The focus on determining eutectic points rather than solid-liquid lines greatly reduces the number of experiments needed to obtain the phase diagram and may for instance accelerate selecting the best solvent in process development for a particular multicomponent crystal. It also has been shown that both Raman and UV can be utilized independently for boundary detection, therefore, a smaller setup requiring less material could be used than the 100 mL setup used in this study. This method can easily be extended to other multicomponent systems including solvates, solid solutions, salts and chiral molecules.

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**Supporting Information**

Graphical illustration of triangulation of eutectic points from detected phase boundaries.

**References**


Co-crystal phase diagram determination by solution addition method

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Synopsis:

Solution addition method developed to determine ternary phase diagrams of co-crystals using an automated lab reactor and calibration-free PAT. With focus on eutectic points method demonstrated and validated using benzoic acid and isonicotinamide in ethanol.