Nucleation of the Theophylline:Salicylic Acid 1:1 Cocrystal – ESI

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Solubility determination

The solubility was determined for SA, THP II and THP:SA in chloroform at the nucleation temperature 10 °C by gravimetric method. The structure of the solid forms for each used were obtained prior to solubility experiments by PXRD. THP II and SA were used as received from Sigma-Aldrich where THP II was identified as the solid form. THP:SA was used directly after synthesis in a chloroform slurry and identified by PXRD. Solutions containing each component were allowed to equilibrate with excess solid in a sealed 20 mL vial for 72 h. The 20 mL vials were stirred with small PTFE magnetic stir bars at 400 rpm. A reference vial containing just chloroform solvent was placed in the bath besides the samples and checked by digital thermometer to ensure the temperature in the vials remained at the nucleation temperature. Samples were stopped stirring and allowed to settle for 1 hour. 5 mL or 10 mL samples were then taken of the liquid and filtered by 0.2 µm PTFE filters into pre weighed vials with lids. The lids were then removed and total evaporation of the chloroform ensued. After 72 hours the vials containing dried solid are weighed once and then again 2 hours later. If there is no change in weight the chloroform has completely evaporated. Repeat experiments were taken for each the SA, THP II and cocrystal under each set of conditions. The PXRD pattern of the excess solid from the settled solution was recorded to ensure that the solubility of the desired polymorph was determined.

Solid phase characterisation

PXRD data were collected in reflection mode with an Empyrean diffractometer (PANalytical, Phillips) equipped with CuK\(_{α1,2}\) radiation (\(γ = 1.5406 \text{ Å}\)) operating at 40 kV and 40 mA at room temperature. Samples were scanned between 2θ values of 5 and 40° at a step size of 0.01313° 2θ/s, 73 s per step.

Differential scanning calorimetry (DSC) was performed on a Netsch Polyma 214 DSC. Samples were analysed in a nitrogenous environment with a temperature ramp rate of 10 °C min\(^{-1}\) over a temperature scan range from 20 and 310 °C. Crystals were isolated from solvent with Whatman filter paper. 5-7 mg of crystals were added to concave aluminium pans which were sealed using a crimping press and then the lid was pierced. The instrument was calibrated using samples of indium and lead.

Thermogravimetric analysis (TGA) was carried out under nitrogen using TGA instrument TA Q50 V20.13 Build 39. Samples were placed on platinum pans and heated up to 500 °C at a ramp rate of 20 °C min\(^{-1}\).
For single crystal x-ray diffraction (SC-XRD) analysis cocrystal material was dissolved in chloroform to create a saturated solution. A crystal of suitable quality was obtained by slow evaporation crystallisation and analysed by SC-XRD. The unit cell parameters were consistent with that on record in the CSD.¹

For Scanning Electron microscopy (SEM), following visible nucleation of vials of all three systems; TPH:SA, THP II and SA over a variety of conditions the slurry was isolated immediately. Analysis of the solid isolated from the slurry was carried out using SEM on the Jeol CARRYSCOPE, coated in gold by a 30 second sputter to ensure a fine coating and minimise ionization. Samples isolated from the same slurries were analysed also by PXRD to identify solid forms.

The THP:SA cocrystal was successfully synthesized as shown by subsequent physical characterisation including PXRD and DSC. The THP:SA cocrystal diffractogram was identical to a previously reported powder pattern for the cocrystal entered to the Cambridge structural database (CSD) under the reference code KIGLES01 (Figure 1). The PXRD pattern of the cocrystal is distinctly different to diffractograms of pure SA and the two low-temperature forms of pure THP.¹ (Figure 2). The diffractogram patterns of the samples taken from the bulk powder following synthesis revealed peaks only corresponding to THP:SA cocrystal, proving that conversion is completed within 72 h.

![Figure 1](image.png)

Figure 1 Diffractogram of previously reported THP:SA cocrystal pattern in CSD (Ref. Code: KIGLES01) (red), synthesized THP:SA cocrystal (blue) and THP:SA isolated from vial following induction time experiments in chloroform at 10 °C.
Figure 2 THPSA cocrystal diffractogram versus the two pure coformers THP II (CSD Ref. code BAPLOT01) and SA (CSD Ref. code SALIAC). The THP II powder was analysed as received and identified as polymorphic form II (CSD Ref. code BAPLOT01). THP II solid isolated following induction time experiments was also identified as form II as per the diffractogram in Figure 3.

Figure 3 Diffractogram of THP form II powder pattern from CSD (Ref. code BAPLOT01) compared with THP II as received and THP II isolated following induction times in chloroform at 10°C.
Figure 4 DSC curves obtained for THP:SA, THP II and SA using a ramp rate of 10°C min⁻¹. The temperature corresponding to the onset of each endotherm peak is labelled.

The DSC curve in Figure 4 shows the melting point of the cocrystal is between that of the API and coformer. The melting point of THP:SA is 186.2 °C, SA is 158.8 °C and THP II is 272.0 °C. A DSC curve previously reported in the literature of THP:SA shows the onset of melting as 188.5 °C. One main difference between the experiments is the different heating rates used as the work reported previously operated at 5 °C min⁻¹ whereas the data in the current work was collected at 10 °C min⁻¹, sample mass also effects detection of melting peaks along with different instruments. The same cocrystal sample for which we ran DSC was also analysed by PXRD and matched the PXRD pattern reported by the authors of the previous work outlined above. The THP form II melting point is 273 °C which matches the data from the same paper. The same previous work also reports a melting point onset for SA of 159 °C, PXRD patterns supported this identification of the SA samples.

Table 1 DSC data calculated by Netsch Proteus software

| No. Samples | Onset of Endotherm (°C) (Average) | Enthalpy of Fusion (J g⁻¹) | Molar Heat of Fusion (KJ mol⁻¹) | Melting point from Zhang et. al. °C |
|-------------|----------------------------------|---------------------------|-------------------------------|----------------------------------|
| THP:SA      | 4                                | 186.2                     | 145.1                         | 46.142                           | 188.5                           |
| SA          | 1                                | 158.8                     | 178.5                         | 24.633                           | 159.0                           |
| THP II      | 3                                | 272.0                     | 167.6                         | 30.168                           | 273.0                           |

THP:SA presents the lowest enthalpy of fusion of 145.1 J g⁻¹. This value is lower than the enthalpy of fusion of both THP II and SA. SA has the highest enthalpy of fusion at 178.5 J g⁻¹.
The cocrystal is in the form of needles (Fig. 5(a)), SA is also in the form of needles (Fig. 5(b)) and THP II is plate-like (Fig. 5(c) and (d)).

**Fig. 5** (a) THP:SA crystals obtained by evaporation from chloroform. (b) Crystals of SA obtained by evaporation from chloroform (c) and (d) THP II crystals obtained by evaporation from chloroform.
For a spherical nucleus of critical size, the nucleation free energy ($\Delta G_{\text{crit}}$) per mole of nuclei (J mol$^{-1}$), also called the nucleation work, in ESI Eq [1] is given as

$$\Delta G_{\text{crit}} = \frac{16\pi \gamma^3 v_o^2 N_A}{3(kT \ln S)^2} \quad [1]$$

In Figure 5 it is shown that the nucleation work, $\Delta G_{\text{crit}}$, is the highest for THP:SA closely followed by that of SA. The nucleation work is much lower for THP II. However, notably at higher supersaturation the nucleation work is even less than the kinetic energy of the system: 3/2RT and should proceed spontaneously. However, it doesn’t showing one of the known weaknesses of the CNT.

![ΔGcrit vs. RLnS](image)

**Figure 5** $\Delta G_{\text{crit}}$ versus RLnS for all three solid phases.

The critical radius size for THP:SA, THP and SA under different driving forces has been calculated according to ESI Eq [2] and presented in Figure 6.

$$r_{\text{crit}} = \frac{2v_o \gamma N_A}{RT \ln S} \quad [2]$$

From ESI Eq [2] the number of molecules per critical nucleus can be estimated$^2$:

$$N_{\text{crit}} = \frac{4\pi r_{\text{crit}}^3}{3v_o} \quad [3]$$

Where $r_{\text{crit}}$ is critical radius (m), $v_o$ is molecular volume (m$^3$) as shown in Table 4 (main text), $\gamma$ is interfacial energy (J m$^{-2}$) and $N_A$ is Avogadro’s number and $N_{\text{crit}}$ is the number of molecules per critical nucleus. At comparable driving force the THP:SA critical nucleus has a larger radius than SA and THP has the smallest $r_{\text{crit}}$. THP:SA requires a slightly lower number of molecules than SA to make up the nucleus. As seen in Figure 6 at comparable driving force, 724 J mol$^{-1}$ ($S=1.36$), the least number of molecules to form a nucleus is required for the THP system (1 molecule), SA requires 15 molecules and the most required by THP:SA (16 THP:SA heterodimers which includes 16 THP and 16 SA molecules).
Figure 6 (a) Critical nucleus size ($r_{\text{crit}}$) in nm versus supersaturation for THP:SA cocrystal, SA and THP II systems. The largest critical nucleus size for nucleation is exhibited by THP:SA followed by SA whereas THP displays the smallest $r_{\text{crit}}$. (b) $N_{\text{crit}}$, the number of molecules per critical nucleus versus supersaturation. For the cocrystal molecules means heterodimers.

Figure 7 Volume of the critical nucleus ($v_n$) versus driving force $RT\ln S$ (J mol$^{-1}$) for the three systems.
Figure 8 (a) (b) and (c) Shown respectively are $N_{\text{crit}}$, $r_{\text{crit}}$, and $v_n$ for THP:SA, SA and THP at equal driving force of 724 J mol$^{-1}$. (d) The volume of the critical nucleus, $v_n$ is compared for the three systems at the same nucleation time of ~1750 seconds.
Table 2 $\tau_g$ from the Poisson distribution fit and the $\tau_g$ defined as the first experimentally observed induction time point on that distribution.

| THP:SA | SA | THP |
|--------|----|-----|
| $S$    | $\tau_g$ (Poisson) | $\tau_g$ (first point) | $S$    | $\tau_g$ (Poisson) | $\tau_g$ (first point) | $S$    | $\tau_g$ (Poisson) | $\tau_g$ (first point) |
| 1.46   | 2959 | 3205 | 1.30   | 681   | 338 | 1.13 | 1732 | 1980 |
| 1.57   | 1889 | 1497 | 1.35   | 59    | 205 | 1.18 | 1733 | 1471 |
| 1.77   | 1611 | 1216 | 1.38   | -934  | 195 | 1.22 | 920  | 710  |
| 2.16   | 827  | 735  | 1.40   | 29    | 188 | 1.27 | 1126 | 870  |
| 2.25   | 1199 | 992  | 1.45   | 298   | 210 | 1.31 | 531  | 530  |
| 2.56   | 777  | 612  | -      | -     | -   | 1.36 | 357  | 445  |
| 2.89   | 534  | 530  | -      | -     | -   | -    | -    | -    |

Table 3 presents estimation of preexponential factors using an approach where the diffusivity of the cocrystal is taken as the geometric mean of THP and SA diffusivities:

$$D_{AB} = \sqrt{D_A \cdot D_B}$$  \[4\]

In this case it would be assumed that there are cocrystal assemblies diffusing throughout the liquid and therefore the $C_e$ term used in this calculation would be the concentration of the cocrystal in equilibrium with the solid cocrystal phase and the value of $v_o$ used would be the molecular volume of the cocrystal.

Table 3 $A$ calculated from Eq [13] and Eq [14] from the main text for THP:SA using the cocrystal diffusivity as a geometric average (ESI Eq [4]) and the molecular volume of the cocrystal. The bottom row represents the new values calculated when the diffusivity of the rate limiting step – THP is used in both cases and the molecular volume of THP is used in the surface integration version.

| THP:SA cocystal | A. [13] (volume diffusion) | A. [14] (interface transfer) |
|-----------------|---------------------------|-------------------------------|
| $D$ from ESI Eq [4] and $v_o$ of cocrystal | $(x10^{10})$ (m$^3$ s$^{-1}$) | $(x10^{09})$ (m$^3$ s$^{-1}$) |
| Manuscript estimations assuming THP to be the limiting component ($D$ of THP for [13] $D$ and $v_o$ of THP in [14]) | 1.67 | 2.13 |
| | 1.56 | 2.38 |
Methods for calculating $J$

Below are 4 variations of methods used to calculate the nucleation parameters $A$, and $\gamma$ form the nucleation rate parameter $J$. Method 1 is the same as what is presented in the main body of work and the others are for comparison.

Method 1:

$$J = \frac{1}{\tau_{\text{nuc}} V}$$

Where $\tau_{\text{nuc}} = \tau_{50} \cdot \tau_g$ and $\tau_g$ is the first point.

Method 2:

$$P(\tau) = 1 - e^{(-JV(\tau-\tau_g))}$$

Where $\tau$, $\tau_g$ and $J$ are estimated from the best fit of the Poisson distribution to the experimental distribution.

Method 3:

$$P(\tau) = 1 - e^{(-JV(\tau-\tau_g(f\text{irst point})))}$$

Where $\tau$, and $J$ are estimated from the best fit of the Poisson distribution to the experimental distribution but $\tau_g$ is defined as the first point.

Method 4:

$$J = \frac{1}{\tau_{50} V}$$

Where no growth time is accounted for and $J$ is estimated from the experimental induction times $\tau_{50}$.

The values of the parameters used for each method are presented in Table 5–8.
Figure 9 CNT plots for Methods 1-4.
Figure 10 Pre-exponential factor calculated for the 3 systems by the 4 different methods outlined above.

Figure 11 Interfacial energy calculated for the 3 systems by the 4 different methods demonstrated little change between methods.
The comparison reveals that in the SA system the Poisson distribution overestimates \( \tau_{50} \) which, when accompanied by small \( \tau_g \) values, results in lower than expected \( J \) values with respect to \( J \) estimated from the experimental \( \tau_{50} \) values when a growth time is not considered. This led to a lower pre-exponential factor for SA by the method using Poisson distributions than expected. The same effect was not seen for THP:SA or THP as the Poisson distribution is a better fit to those experimental data. As the Poisson distribution does not represent the experimental data well in the SA system, the nucleation rates (\( J \)) have been estimated from the inverse of \( \tau_{\text{nuc}} \) and \( V \) (eq. 6). This method was also utilised in the other systems for consistency. At the same driving force THP:SA has the lowest nucleation rate followed by SA and then THP which has the highest nucleation rate.

Table 4

| S   | Method 1 | Method 2 |
|-----|----------|----------|
|     | \( \tau_{\text{nuc}} \) (s) | \( \tau_{50} \) (s) | \( \tau_g \) | \( J \) (m\(^3\)s\(^{-1}\)) | \( \tau_{\text{nuc}} \) (s) | \( \tau_{50} \) (s) | \( \tau_g \) | \( J \) (m\(^3\)s\(^{-1}\)) |
| THPSA | 1.46 | 4121 | 7326 | 3205 | 12.13 |
|       | 1.57 | 1783 | 3280 | 1497 | 28.04 |
|       | 1.77 | 1289 | 2505 | 1216 | 38.79 |
|       | 2.16 | 1000 | 1735 | 735  | 50.00 |
|       | 2.25 | 755  | 1747 | 992  | 66.23 |
|       | 2.56 | 633  | 1245 | 612  | 78.99 |
|       | 2.89 | 286  | 816  | 530  | 174.83 |
| THP   | 1.13 | 2905 | 4885 | 1980 | 17.21 |
|       | 1.18 | 2095 | 3566 | 1471 | 23.87 |
|       | 1.22 | 1707 | 2417 | 710  | 29.29 |
|       | 1.27 | 1230 | 2100 | 870  | 40.65 |
|       | 1.31 | 1430 | 1960 | 530  | 34.97 |
|       | 1.36 | 593  | 1160 | 445  | 84.32 |
| SA    | 1.30 | 9848 | 10186| 338  | 5.08 |
|       | 1.35 | 7041 | 7246 | 205  | 7.10 |
|       | 1.38 | 2579 | 2774 | 195  | 19.39 |
|       | 1.40 | 2563 | 2751 | 188  | 19.51 |
|       | 1.45 | 1810 | 2020 | 210  | 27.62 |

Table 5

| S   | Method 2 |
|-----|----------|
|     | \( \tau_{\text{nuc}} \) (s) | \( \tau_{50} \) (s) | \( \tau_g \) | \( J \) (m\(^3\)s\(^{-1}\)) |
| THPSA | 1.46 | 3112 | 6071 | 2960 | 11.14 |
|       | 1.57 | 1408 | 3297 | 1889 | 24.62 |
|       | 1.77 | 879  | 2490 | 1611 | 39.43 |
|       | 2.16 | 869  | 1696 | 827  | 39.88 |
|       | 2.25 | 469  | 1669 | 1200 | 73.88 |
|       | 2.56 | 441  | 1219 | 778  | 78.6 |
|       | 2.89 | 231  | 765  | 534  | 150.02 |
| Method 3 | THPSA | $\tau_{90}$ (s) | $\tau_{50}$ (s) | $\tau_{95}$ (s) | J (m³ s⁻¹) |
|----------|-------|----------------|----------------|----------------|------------|
| THP      | 1.13  | 3123           | 4855           | 1732.43        | 11.1       |
|          | 1.18  | 1515           | 3247           | 1732.57        | 22.88      |
|          | 1.22  | 1206           | 2126           | 920.13         | 28.73      |
|          | 1.27  | 889            | 1932           | 1042.96        | 39.64      |
|          | 1.31  | 1445           | 1976           | 531.32         | 23.98      |
|          | 1.36  | 660            | 1017           | 357.12         | 52.5       |
| SA       | 1.30  | 9312           | 9993           | 681.44         | 3.72       |
|          | 1.35  | 7471           | 7530           | 59.37          | 4.64       |
|          | 1.38  | 3733           | 2798           | -934.40        | 9.28       |
|          | 1.40  | 2716           | 2745           | 29.21          | 12.76      |
|          | 1.45  | 1833           | 2043           | 210.00         | 18.91      |

Table 6

| S | Method 4 |
|---|----------|
| THPSA | $\tau_{90}$ (s) | J (m³ s⁻¹) |
| 1.46  | 7326     | 6.83       |
| 1.57  | 3280     | 15.24      |
| 1.77  | 2505     | 19.96      |
| 2.16  | 1735     | 28.82      |
| 2.25  | 1747     | 28.62      |
| 2.56  | 1245     | 40.16      |
| 2.89  | 816      | 61.27      |

Table 7

| S | Method 4 |
|---|----------|
| THPSA | $\tau_{90}$ (s) | J (m³ s⁻¹) |
| 1.30  | 9627     | 338        |
| 1.35  | 7316     | 205        |
| 1.38  | 2401     | 195        |
| 1.40  | 2555     | 188        |
| 1.45  | 1924     | 210        |
|    | THP   |         |     |
|----|-------|---------|-----|
| 1.13| 4885  | 10.24   |
| 1.18| 3566  | 14.02   |
| 1.22| 2417  | 20.69   |
| 1.27| 2100  | 23.81   |
| 1.31| 1960  | 25.51   |
| 1.36| 1160  | 43.10   |
|    | SA    |         |     |
| 1.30| 10186 | 4.91    |
| 1.35| 7246  | 6.90    |
| 1.38| 2774  | 18.02   |
| 1.40| 2751  | 18.18   |
| 1.45| 2020  | 24.75   |
References

(1) Zhang, S.; Chen, H.; Rasmuson, Å. C. Thermodynamics and Crystallization of a Theophylline-Salicylic Acid Cocrystal. *CrystEngComm* **2015**, *17* (22), 4125–4135. https://doi.org/10.1039/c5ce00240k.

(2) Yang, H.; Sva, M.; Zeglinski, J.; Rasmuson, Å. C. Influence of Solvent and Solid-State Structure on Nucleation of Parabens. *2014*. https://doi.org/10.1021/cg500449d.

(3) Rehfeldt, S.; Stichlmair, J. Measurement and Calculation of Multicomponent Diffusion Coefficients in Liquids. *Fluid Phase Equilib.* **2007**, *256* (1–2), 99–104. https://doi.org/10.1016/J.FLUID.2006.10.008.

(4) Kashchiev, D.; van Rosmalen, G. M. Review: Nucleation in Solutions Revisited. *Cryst. Res. Technol.* **2003**, *38* (78), 555–574. https://doi.org/10.1002/crat.200310070.

(5) Jiang, S.; Ter Horst, J. H. Crystal Nucleation Rates from Probability Distributions of Induction Times. *Cryst. Growth Des.* **2011**, *11* (1), 256–261. https://doi.org/10.1021/cg101213q.