

Figure S1: A simple model for diffusion from a droplet of BnOH/TFT is used to describe saturation as a function of time for various conditions of solvent extraction. A) concentration in the continuous phase outside the droplet boundary for slow (75% BnOH), medium (45% BnOH), and fast (0% BnOH) extraction. B) droplet volume, C) concentration and solubility, and D) saturation $(C_{API} - C_{API}^*) / C_{API}^*$ as a function of time for an extracting droplet decreasing in size and increasing in %TFT for three extraction conditions.

To demonstrate how solvent extraction rate is altered in this work and how it relates to API saturation within a droplet, the driving force for crystal nucleation and growth, we introduce a simple model of diffusion. Several assumptions are made in order to arrive at the equations which solve Fick's 2nd law (Eq. S1). First, assume the monolayer of droplets at the bottle of the Petri dish, composed of both solvent and antisolvent, are a semi-infinite plane of both solvents. Second, assume the partition of the solvent (BnOH) between the droplet phase and the aqueous phase significantly favors the droplet phase, such that the concentration at $z = 0$ and $t > 0$ is equal to the solubility limit of BnOH in H₂O (3.5 mL/100 mL) where z is the distance BnOH diffuses from the droplet monolayer into the aqueous phase. In this case, our model represents a semi-infinite plane with constant uniform surface ($z = 0$) concentration^{1,2} where we augment C_{inf} (Eq. S2-S4) in order to change ΔC , impacting the rate of flux at $z = 0$ (Eq. S3). The mass lost from a defined area, A , can then be calculated from Equation S4 as a function of time. The loss of BnOH through A contributes to a loss in droplet volume and increase in antisolvent fraction within the droplet. Thus, this approximation estimates how droplet volume, API concentration, solubility limit, and saturation change as a function of time with varying solvent extraction rates (i.e. ΔC). The calculation below (Eq. S4-S10, S12) is applied for an area of droplets corresponding to one magnified image (200x) for which 25 droplets are observed and assuming an initial droplet volume of 2 nL.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (S1)$$

$$\frac{C(z) - C_{inf}}{C_0 - C_{inf}} = 1 - \operatorname{erf}\left(\frac{z}{2\sqrt{Dt}}\right) \quad (S2)$$

$$j(t)|_{z=0} = \sqrt{\frac{D}{\pi t}} (C_0 - C_{inf}) \quad (S3)$$

$$m(t) = 2A \sqrt{\frac{Dt}{\pi}} (C_0 - C_{inf}) \quad (S4)$$

$$V_d(t) = \frac{1}{d(t)} \left(V_s d_s - \frac{1}{n} m(t) \right) \quad (S5)$$

$$d(t) = \left(\frac{n_s(t)}{n_t(t)} \right) d_s + \left(\frac{n_{as}}{n_t(t)} \right) d_{as} \quad (S6)$$

$$n_s(t) = d_s \frac{\frac{1}{2} V_s}{M_s} - \frac{m(t)}{M_s} \quad (S7)$$

$$n_t(t) = n_s(t) + n_{as} \quad (S8)$$

$$C_{API}(t) = C_{API}^S \frac{V_d(t)}{V_s} \quad (S9)$$

$$\%AS(t) = \frac{1 \text{ nL}}{V_d(t)} \quad (S10)$$

$$C^*(\%AS) = -1 \times 10^{-6} (\%AS)^4 + 2 \times 10^{-4} (\%AS)^3 - 0.133 \times 10^{-2} (\%AS)^2 - 0.2006 (\%AS) + 29.747 \quad (S11)$$

$$S(t) = \frac{C_{API}(t) - C^*(t)}{C_{API}^S} \quad (S12)$$

The saturation can be augmented by changing C_{inf} by first selection of a solvent with solubility in the continuous phase and second, by partially presaturating the aqueous continuous phase with that solvent. In this study, nucleation and growth rates are controlled by this simple adjustment and performed to achieve crystallization of GSK1 with low aspect ratios desirable for manufacturability.

Table S1: Definition of parameters used to generate Figure S1 which are variable with time or distance.

Parameter	Definition
$C(z)$	Concentration of BnOH as function of distance from droplet monolayer in g/100 mL
t	Time in seconds
z	Distance from droplet monolayer in centimeters
$j(t)$	Flux of BnOH in g/cm ²
$m(t)$	Mass diffused through area A
$V_d(t)$	Volume of droplet as function of time
$d(t)$	Density of droplet calculates as molar fraction of solvent and antisolvent densities
$C_{API}(t)$	Concentration of API within droplet in mg/mL
%AS(t)	% TFT (antisolvent) as function of time (v/v)
$n_t(t)$	Total moles of BnOH and TFT inside a droplet
$n_s(t)$	Total moles of BnOH inside a droplet
$S(t)$	Saturation inside droplet as function of time
C^* (%AS)	Solubility as function of % antisolvent, generated from best fit from Figure S2

Table S2: Definition of constant parameters used to generate Figure S1.

Parameter	Value	Definition
C_0	3.5 g/100 mL	Concentration of BnOH at $z = 0$
C_{inf}	0%, 45%, or 75% of C_0	Concentration of BnOH in the aqueous continuous phase in g/100mL
D	8.2×10^{-6} cm ² /s	Diffusion coefficient for BnOH in H ₂ O
A	4.82×10^{-3} cm ²	Area of one image containing 25 droplets
C_{API}^S	13.3 mg/mL	Initial concentration of API inside droplet
n	25 droplets	Number of droplets inside defined area A
V_s	2 nL	Initial droplet volume
n_{as}	8.14×10^{-9} moles	Moles of TFT in 1 nL droplet
d_s	1.06 g/mL	Density of BnOH
d_{as}	1.19 g/mL	Density of TFT
M_{as}	146.11 g/mol	Molar mass of TFT
M_s	108.14 g/mol	Molar mass of BnOH

Table S3: Elapsed time from droplet generation until first observed crystal nuclei and estimated saturation measure from Figure S1 model.

C_{inf} (% BnOH)	Time to First Crystal (sec)	S(t) from Figure S1D
0	938	5.0
45	1545	2.7
75	3507	1.7

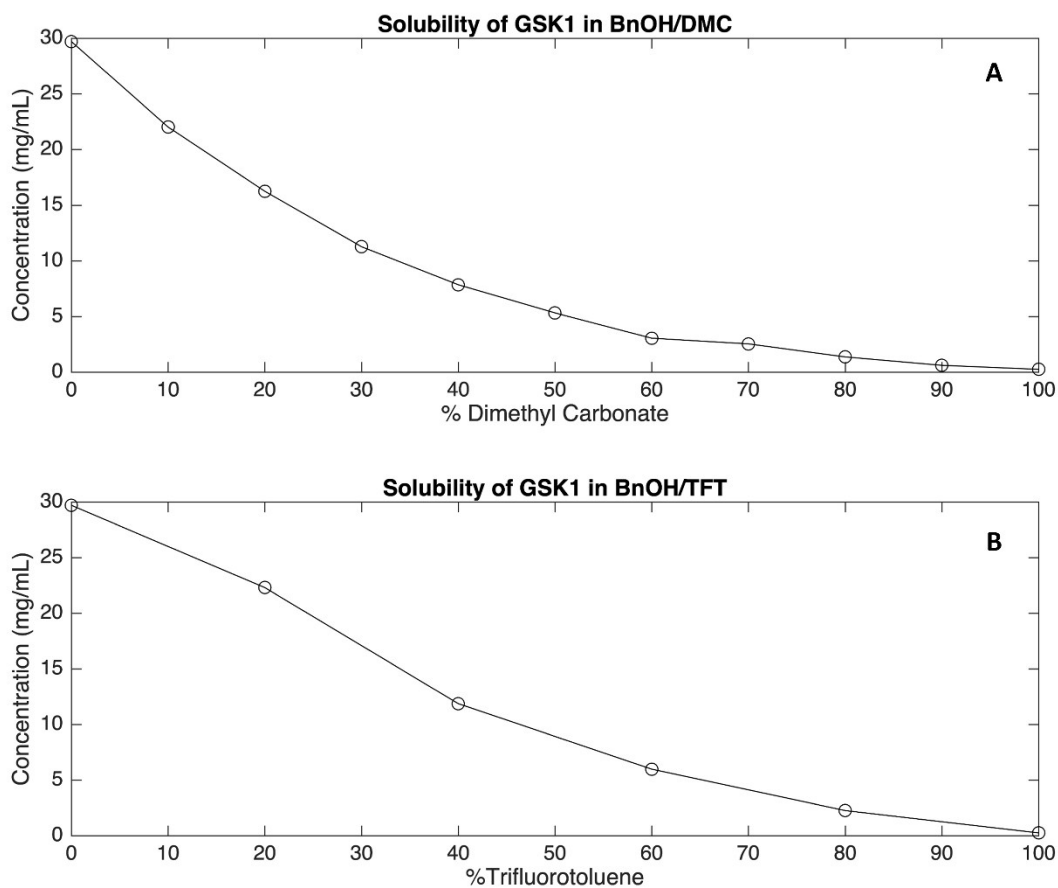


Figure S2: Solubility of GSK1 in solvent/antisolvent mixtures, A) BnOH/DMC and B) BnOH/TFT. Solubility decreases with increasing antisolvent for both conditions.

References

1. E. L. Cussler, *Diffusion: mass transfer in fluid systems*, Cambridge university press, 2009.
2. J. Crank, *The mathematics of diffusion*, Oxford university press, 1979.