Implications of solution-phase phenomena and nanodroplet formation upon dissolution of amorphous dispersions in aspirated human intestinal fluids

Ahmed Elkhabaz
Merck & Co., Inc., Rahway, NJ, USA

Oral Delivery Workshop – 2025 CRS Annual Meeting and Exposition
Philadelphia, PA
Jul 14th, 2025

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Outline & Acknowledgements

- Levels of supersaturation and competition with crystallization
- Liquid-liquid phase separation and the formation of colloidal amorphous nanodroplets
- Nanoparticle drifting mechanism and *in vivo* benefits
- ASD dissolution behavior and impact on diffusive flux rates
- What can go wrong with ASDs besides crystallization

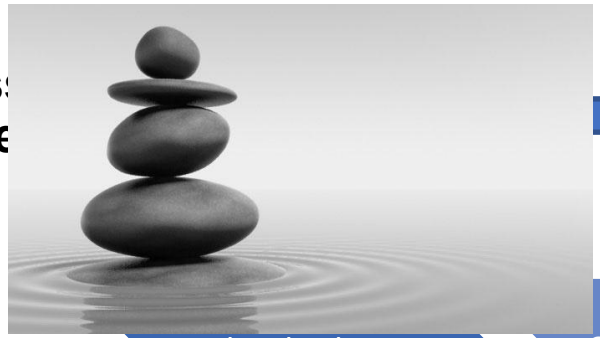
Merck & Co., Inc., Rahway, NJ, USA:

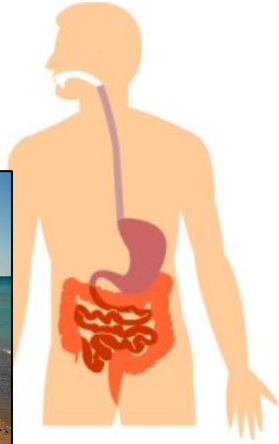
- Andre Hermans
- Matt Crowe
- Sanjaykumar Patel

Purdue & Past Collaborators:

- Lynne Taylor
- Patrick Augustijns
- Garth Simpson
- Dana Moseson
- Joachim Brouwers

Insoluble APIs and the future of supersaturating formulations





Asses
of supe

?

molecules have poor aqueous solubility*

Supersaturating formulations

Formulation strategies and complex phase behavior

Problem 1

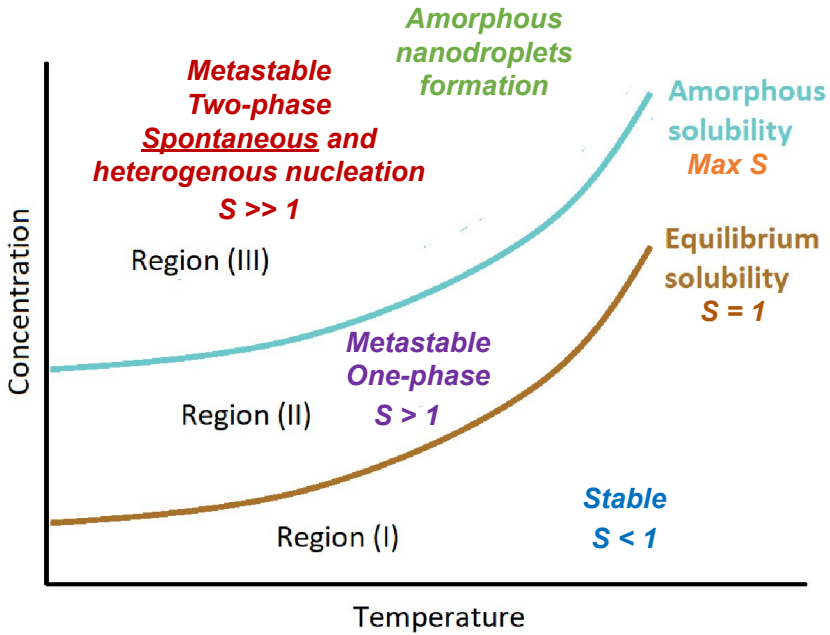
Problem 2

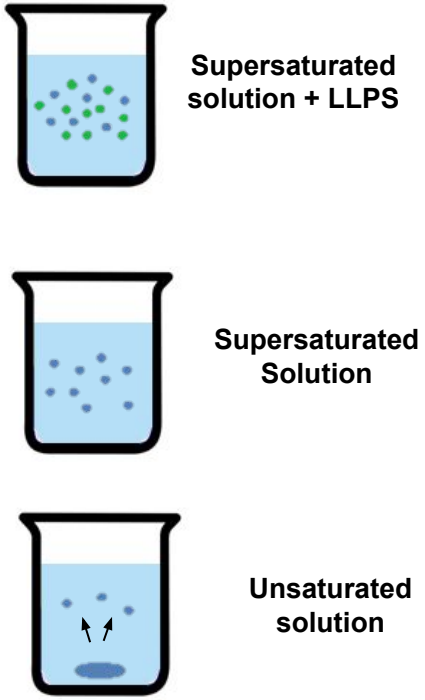
*L. Benet et al. The AAPS Journal, 2011, 13, 4, 519-547 - Pictures from the internet

Supersaturation and liquid-liquid phase separation

$$J = A\,exp\ \left(\frac{-16\pi\ {v_o}^2{\gamma_s}^3}{3(K_BT)^3(LnS)^2}\right)$$

J is nucleation rate
S is supersaturation
T is temperature
γ is interfacial tension
Vo is molecular volume
A is pre-exponential factor





The primary driving force for nucleation/crystallization in solutions is **Supersaturation**

Implications of highly supersaturating solutions and formation of colloidal amorphous nanodroplets

Upon exceeding the **amorphous solubility :**

- Upper limit of molecularly dissolved drug
- Upper limit of supersaturation

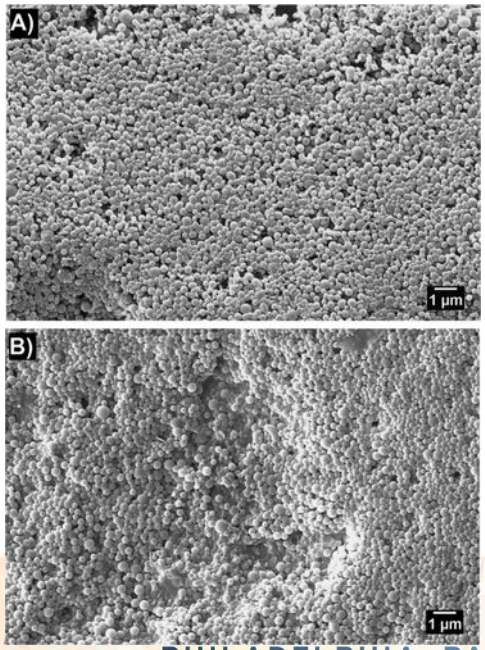
A second phase is formed where the drug is present as drug-rich amorphous nanodroplets/nanoaggregates (LLPS)

Can act as precursor for crystallization

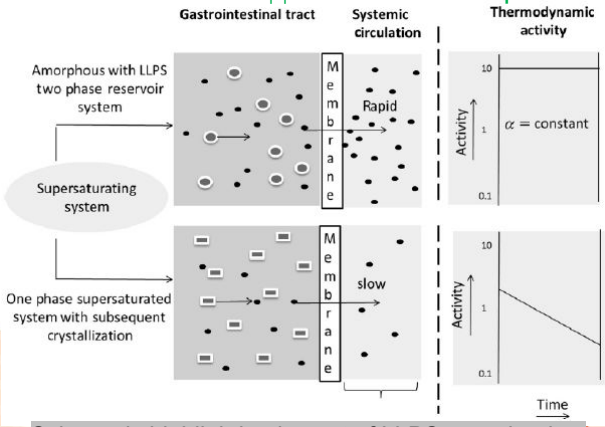
Deplete the supersaturated state

Can be stabilized

Reservoir for replenishment *in vivo* - ↑↑ membrane transport



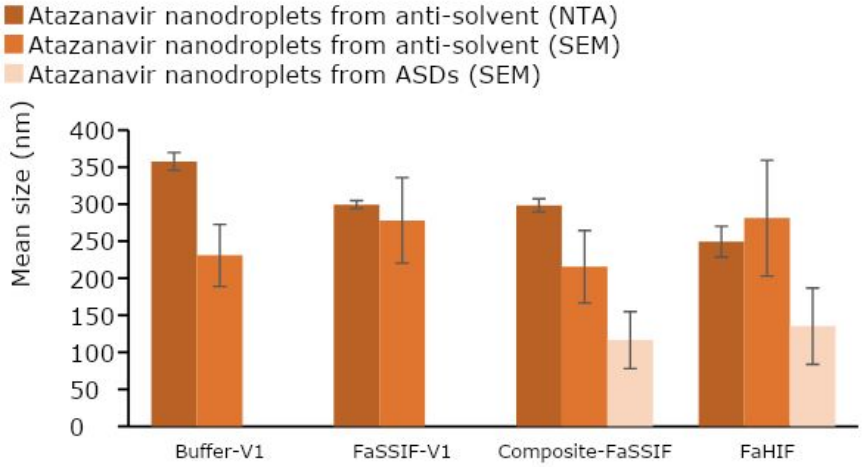
SEM images of **(A) atazanavir** & **(B) posaconazole** amorphous nanodroplets in *ex vivo* FaHIF.

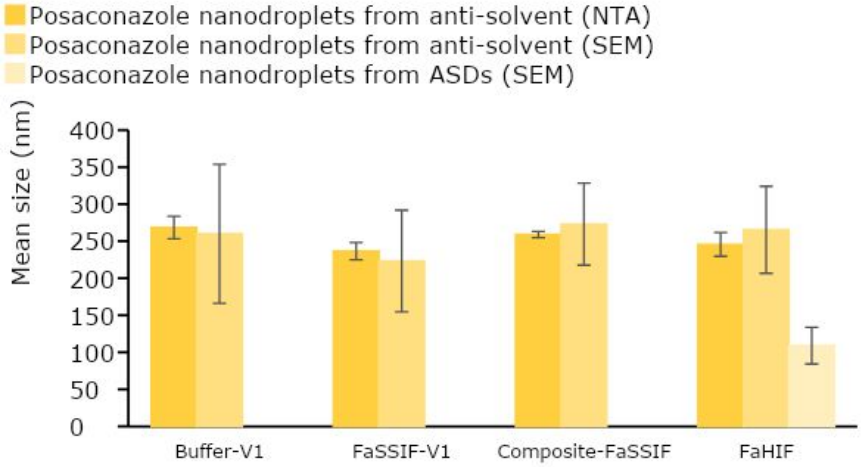


Schematic highlighting impact of LLPS nanodroplets on maximizing membrane transport

Elkhabaz et al., Molecular Pharmaceutics 2019, 16,12, 5042-5053 - Raina et al., Journal of Pharmaceutical Sciences 2014, 103, 9, 2736–2748

Characterization of amorphous nanodroplets formed in simulated and aspirated fluids





Nanodroplets formed from the ASDs were somewhat smaller than those generated using the solvent-shift method

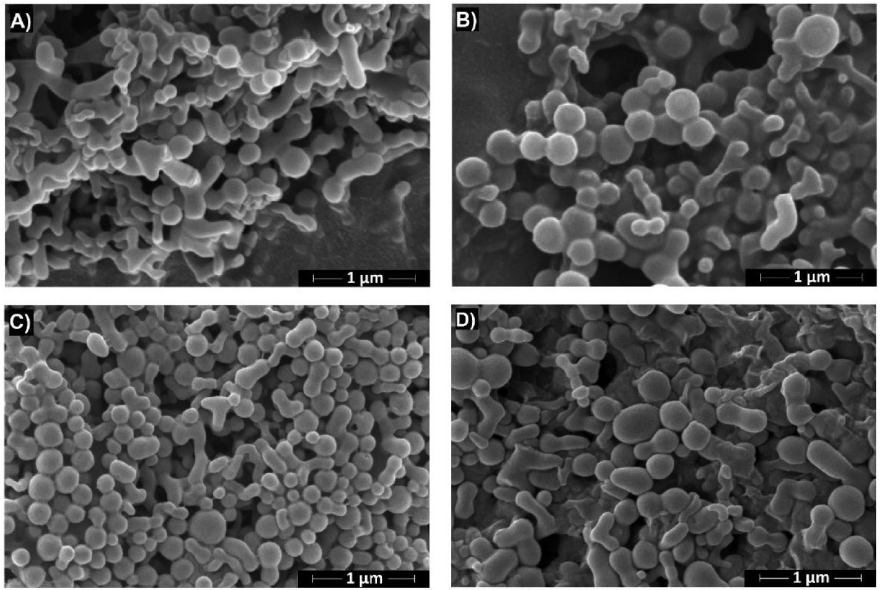
↑ solubilizing species media → increased surface wetting, resulting in smaller contact angle and wider spread of the drop over the surface of the ASD thin films

Contact angle measurements for thin-film ASDs

	Atazanavir (°)	**Posaconazole (°)**
Buffer-V1	62.4 ± 2.4	59.1 ± 0.4
FaSSIF-V1	56.1 ± 1.5	49.7 ± 1.0
Composite-FaSSIF	54.5 ± 3.2	51.8 ± 3.6
FaHIF	52.9 ± 1.7	40.7 ± 2.1

Elkhabaz et. al., Journal of Pharmaceutical Sciences 2025, 114, 1, 336-349

Atazanavir nanodroplets ~200 nm formation in simulated and aspirated intestinal fluids



Drug-rich nanodroplets have been postulated to enhance oral absorption. Mechanisms include:

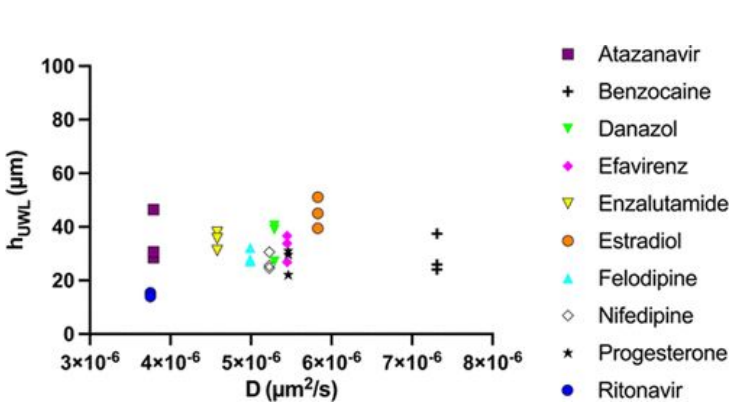
- Rapidly dissolving species that replenish drug absorbed across the membrane.
- Particle drifting effect across the aqueous boundary layer adjacent to the membrane surface improving permeation.

For particle drifting, nanodroplet size is important e.g. anacetrapib nanoparticles ~100 > ~350 nm nanoparticles for absorption advantage in dogs.

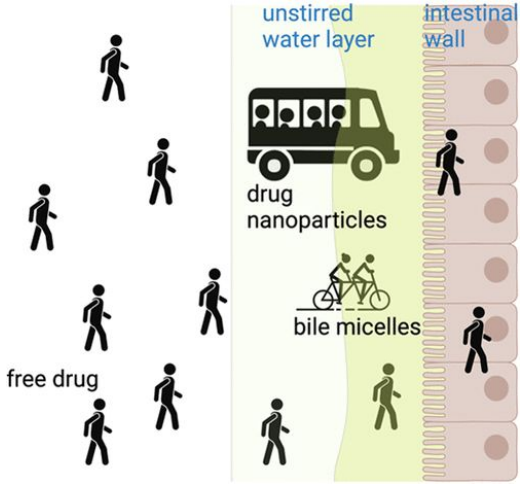
Nanodroplets formation mechanism and size is likely impacted by several factors; Those generated from ASD dissolution were relatively smaller than those generated using a solvent-shift method.

Elkhabaz et. al., Journal of Pharmaceutical Sciences 2025, 114, 1, 336-349

Particle drifting effect for amorphous/colloidal nanoparticles



- Molecular weight had no effect on thickness of unstirred water layer (UWL).
- ↑ thickness of the unstirred water layer ↑ retardation effect for diffusion of polymer (HPMCAS) and endogenous surfactant (sodium taurocholate).
- Two possible mechanisms of mass transfer:
  (1) direct interactions between particle and membrane phase.
  (2) interfacial permeation of free drug only, which is released from nanoparticles.



- Particle drifting effect is a sequential reaction.
- Particle dissolution in the UWL → permeation of free drug across the membrane.
- Particle (re)dissolution rate is dependent on particle size, concentration, and drug solubility.

Yang et. al., Mol. Pharmaceutics 2024, 21, 11, 5510-5528

Amorphous nanoparticles *in vivo* assessment in beagle dogs

- Pharmacokinetic parameters after administration of anacetrapib suspensions to dogs at a dose of 4 mg and 12 mg respectively.
- Using TPGS as a tool to modulate the size of nanoparticles formed, a differential response in bioavailability of anacetrapib was observed with smaller nanoparticles.
- ↓ Nanodroplet size ↑ AUC and Cmax

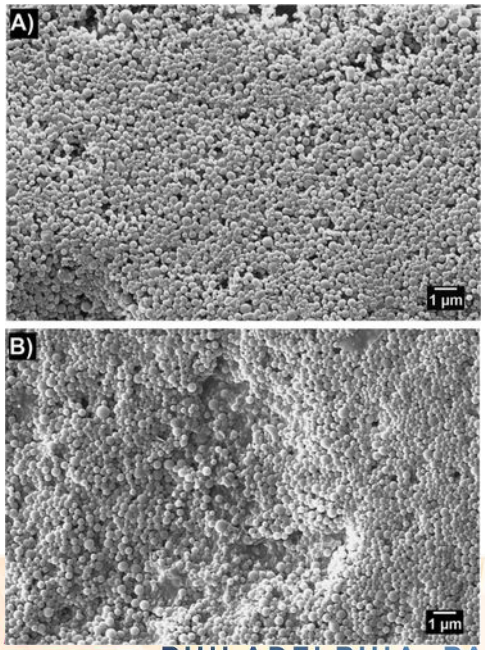
Extrudate Composition and Mean Particle Size	$AUC_{0\text{-}24hr}$ (nM× h)	C_{max} (nM)	T_{max} (h)
10% TPGS (48 nm)	548 ± 276	137 ± 72	1.0 (1.0-2.0)
3.5% TPGS (102 nm)	429 ± 221	101 ± 59	2.0 (1.0-2.0)
2% TPGS (190 nm)	190 ± 88	39 ± 17	2.0 (1.0-2.0)
1% TPGS (347 nm)	167 ± 103	33 ± 22	2.0 (1.0-4.0)
10% TPGS (48 nm) w/high-fat meal	2568 ± 631	388 ± 123	4.0 (4.0-6.0)
3.5% TPGS (102 nm) w/high-fat meal	2850 ± 767	416 ± 91	4.0 (4.0-6.0)
1% TPGS (347 nm) w/high-fat meal (8 μm)	1492 ± 663	230 ± 81	4.0 (2.0-4.0)
Formulation and Mean Particle Size	**$AUC_{0\text{-}24hr}$ (nM× h)**	**C_{max} (nM)**	**T_{max} (h)**
10% TPGS (48 nm)	808 ± 272	172 ± 71	1.5 (1.0-2.0)
3.5% TPGS (102 nm)	790 ± 387	198 ± 124	2.0 (1.0-2.0)
2% TPGS (190 nm)	352 ± 154	69 ± 18	2.0 (1.0-2.0)
1% TPGS (347 nm)	444 ± 398	76 ± 39	1.5 (1.0-4.0)
Micron-sized amorphous API	43 ± 38	8 ± 4	2.0 (1.0-2.0)

Kesisoglou et. al., Journal of Pharmaceutical Sciences 2019, 108, 9, 2917-2925

Implications of highly supersaturating solutions and formation of colloidal amorphous nanodroplets

Upon exceeding the **amorphous solubility :**

- Upper limit of molecularly dissolved drug
- Upper limit of supersaturation



SEM images of **(A) atazanavir** & **(B) posaconazole** amorphous nanodroplets in *ex vivo* FaHIF.

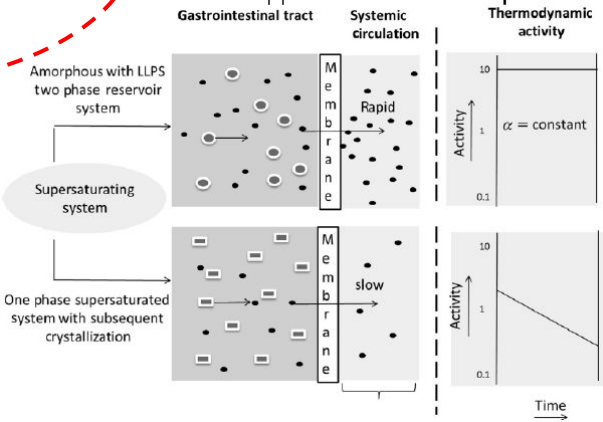
A second phase is formed where the drug is present as drug-rich amorphous nanodroplets/nanoaggregates (LLPS)

Can act as precursor for crystallization

Deplete the supersaturated state

Can be stabilized

Reservoir for replenishment *in vivo* - ↑↑ membrane transport

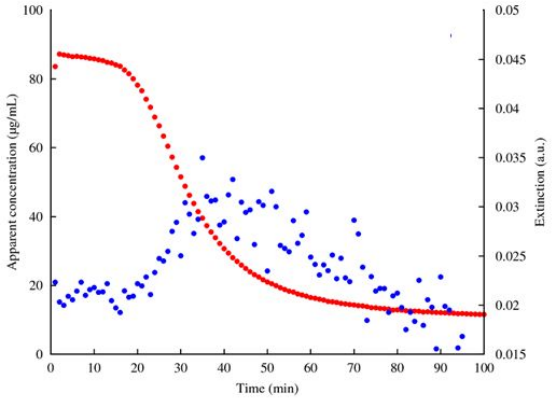


Schematic highlighting impact of LLPS nanodroplets on maximizing membrane transport

Elkhabaz et al., Molecular Pharmaceutics 2019, 16,12, 5042-5053 - Raina et al., Journal of Pharmaceutical Sciences 2014, 103, 9, 2736–2748

Nucleation Induction time measurements of fast and slow crystallizers

Longer induction time = Supersaturation is more stable
Induction times usually inform on ASD performance

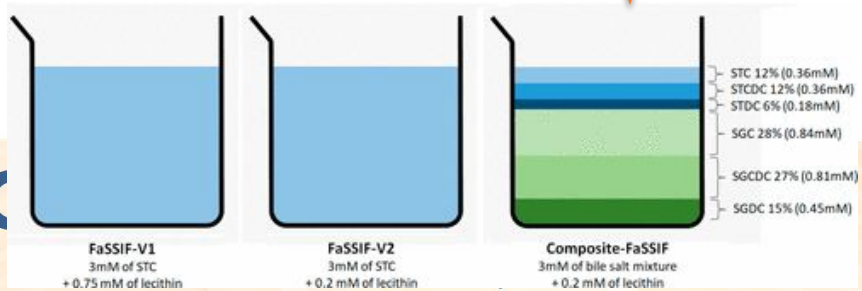


$t_{ind} = t_n + t_g$

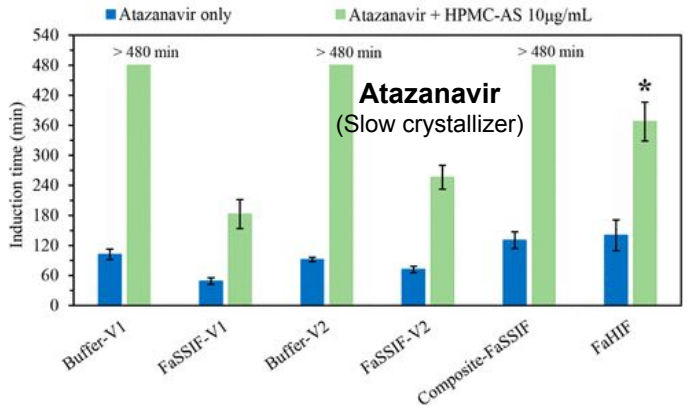
t_n= Time for critical nucleus formation

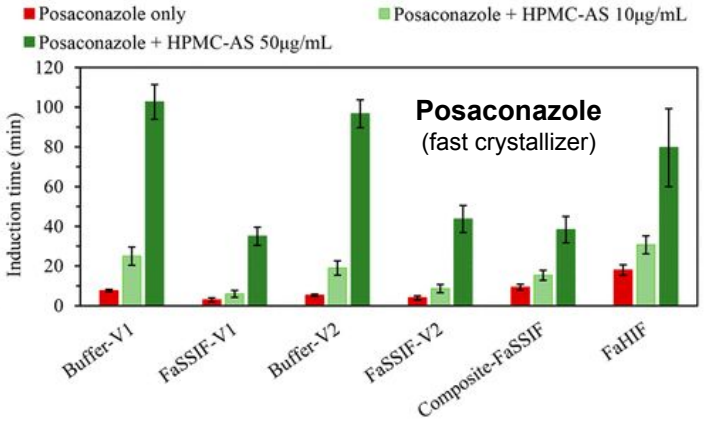
t_g= Time to growth to a detectable size

API spike from organic solvent



Induction times





Elkhabaz et. al., Crystal Growth and Design 2021, 21, 5, 2807-2820

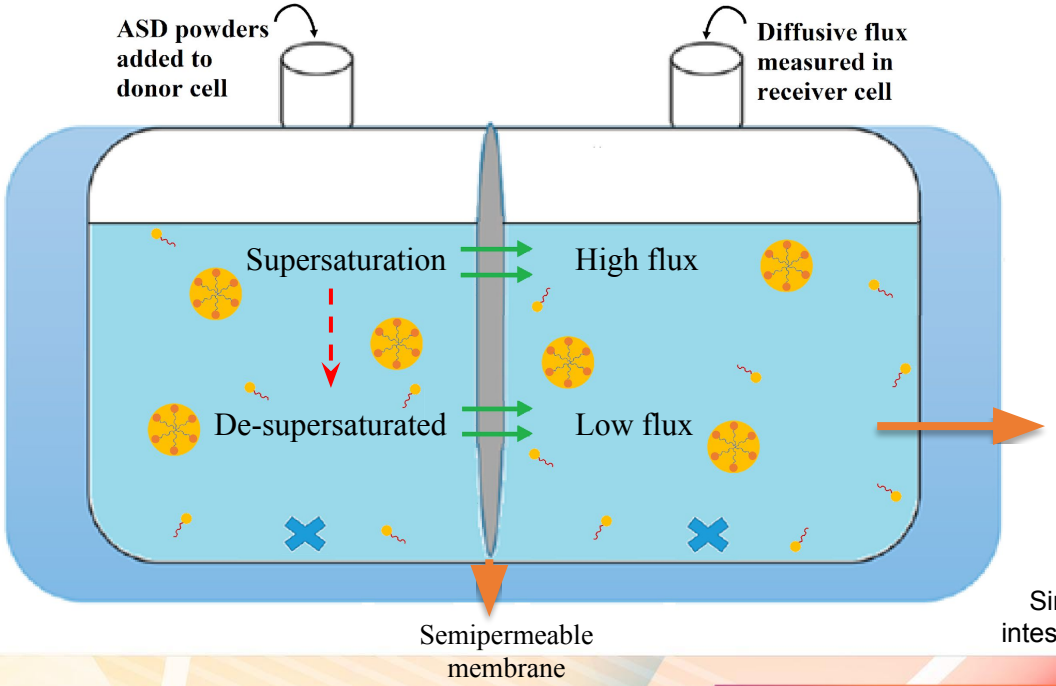
Diffusive flux measurements performed concurrently with ASD dissolution in side-by-side diffusion cells

- $SR = \frac{a}{a_{eq}} = \frac{\gamma C}{\gamma_{eq} C_{eq}}$

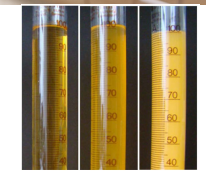
$$J = \frac{Da}{h\gamma_m}$$

$$SR = \frac{J}{J_{eq}}$$

SR is the supersaturation ratio
a is the activity of solute
γ is the activity coefficient
C is the concentration of solute
J is the diffusive flux
D is the diffusion coefficient
h is the thickness of the membrane



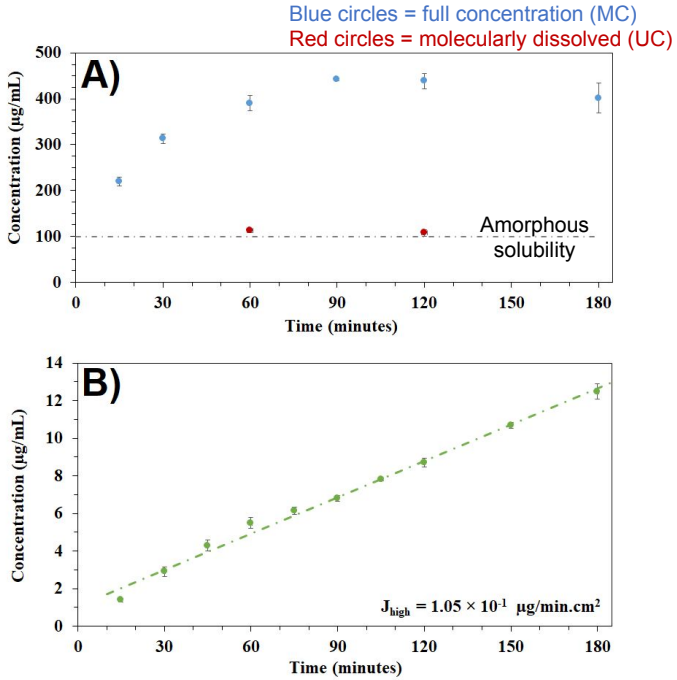
Human Intestinal Fluids (HIF)



Simulated intestinal fluids

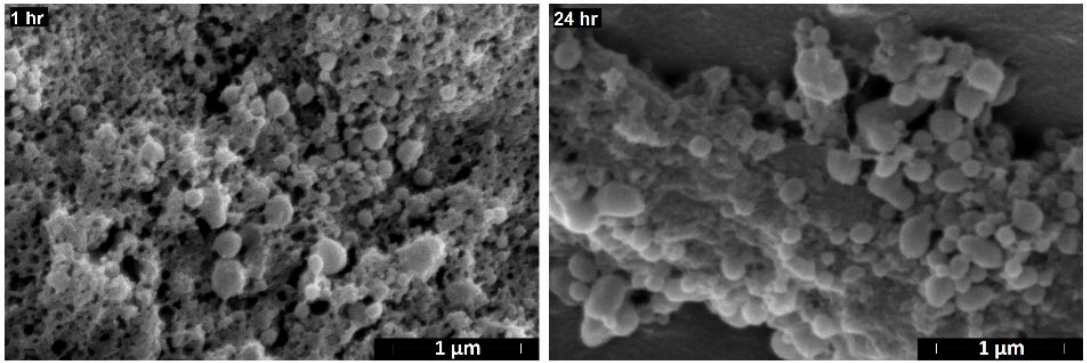
Elkhabaz et al., Molecular Pharmaceutics 2019, 16,12, 5042-5053

Dissolution of atazanavir ASDs in aspirated intestinal media



Dissolution of 10% DL atazanavir ASDs in FaHIF at 37°C
Panel A shows donor cell/Panel B shows receiver cell

Time-lapse SEM imaging of 10% DL atazanavir ASDs in FaHIF



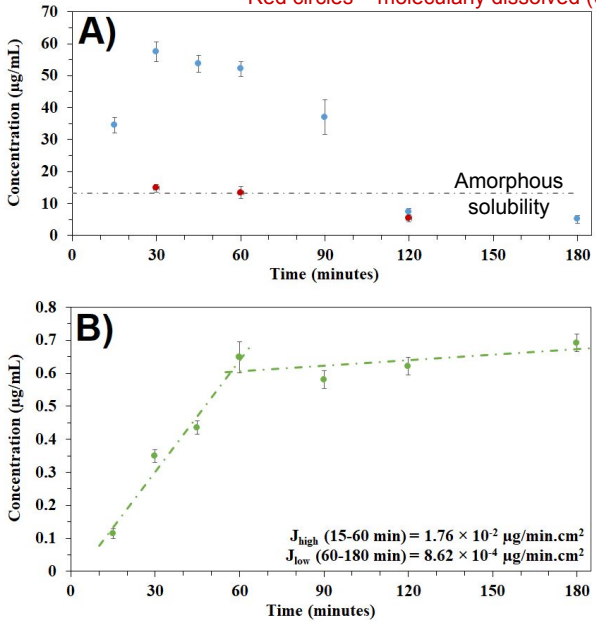
- Full release (polymer-controlled) in ~60 minutes
- Maximum supersaturation → Maximum diffusive flux

Normalized flux measurements for atazanavir ASDs in µg/min.cm²		
	FaHIF	Composite-FaSSIF
Flux (J) from 10% ASD dissolution	$1.05X10^{-1}$	$1.08X10^{-1}$
Flux (J) from 25% ASD dissolution	$0.99X10^{-1}$	$1.03X10^{-1}$
Flux (J) from solution at amorphous solubility	$1.11X10^{-1}$	$1.05X10^{-1}$

Elkhabaz et. al., Journal of Pharmaceutical Sciences 2025, 114, 1, 336-349

Dissolution of posaconazole ASDs in aspirated intestinal media

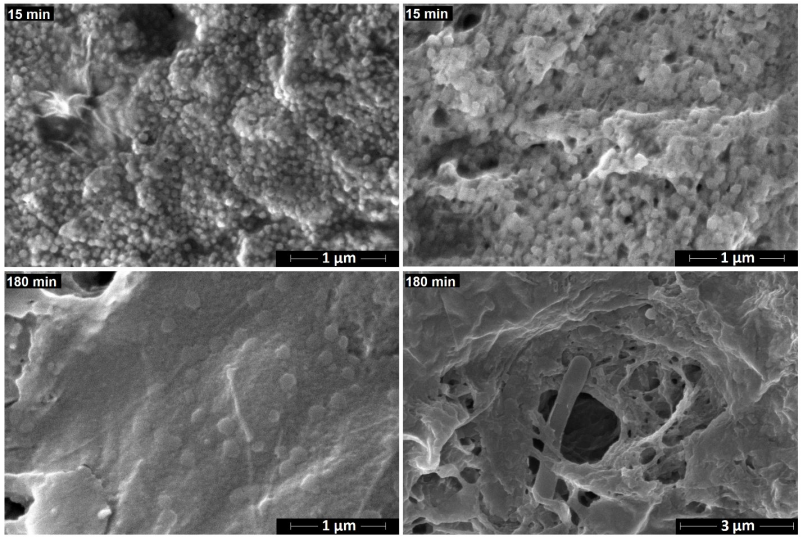
Blue circles = full concentration (MC)
Red circles = molecularly dissolved (UC)



Dissolution of 10% DL posaconazole ASDs in FaHIF at 37°C
Panel A shows donor cell/Panel B shows receiver cell

Transient supersaturation
↓
Crystallization
↓
High to low diffusive flux

Time-lapse SEM imaging of 10% DL posaconazole ASDs in FaHIF



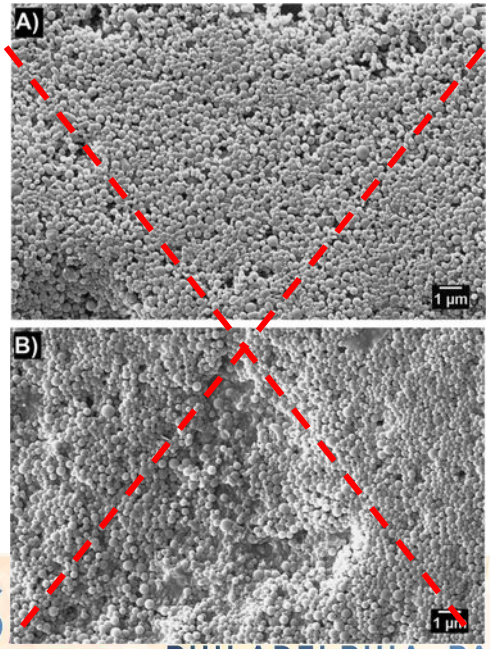
Normalized flux measurements for posaconazole ASDs in µg/min.cm^2		
	FaHIF	Composite-FaSSIF
Flux (J_{high}) from 10% ASD dissolution	1.76X10^{-2}	NA
Flux (J) from solution at amorphous solubility	1.91X10^{-2}	1.92X10^{-2}
Flux (J_{low}) from 10% ASD dissolution	8.62X10^{-4}	2.63X10^{-4}
Flux (J) from solution at crystalline solubility	6.61X10^{-4}	6.32X10^{-4}

Elkhabaz et. al., Journal of Pharmaceutical Sciences 2025, 114, 1, 336-349

Implications of highly supersaturating solutions and formation of colloidal amorphous nanodroplets

Upon exceeding the **amorphous solubility :**

- Upper limit of molecularly dissolved drug
- Upper limit of supersaturation



SEM images of **(A) atazanavir** & **(B) posaconazole** amorphous nanodroplets in *ex vivo* FaHIF.

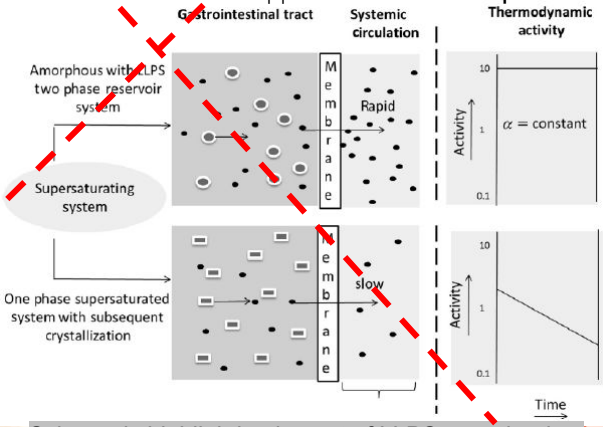
A second phase is formed where the drug is present as drug-rich amorphous nanodroplets/nanoaggregates (LLPS)

Can act as precursor for crystallization

Deplete the supersaturated state

Can be stabilized

Reservoir for replenishment *in vivo* - ↑↑ membrane transport

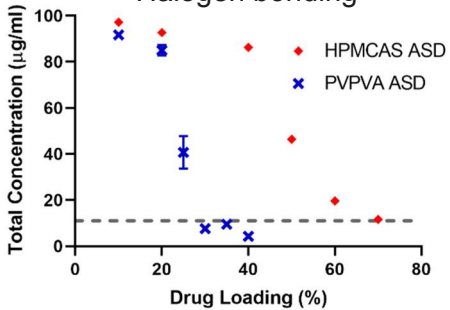


Schematic highlighting impact of LLPS nanodroplets on maximizing membrane transport

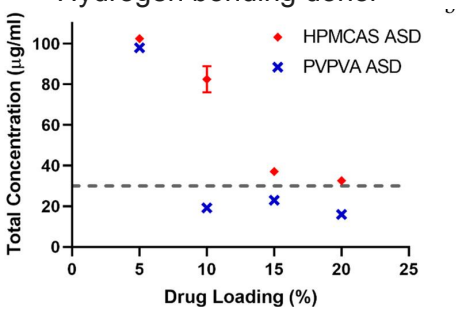
Elkhabaz et al., Molecular Pharmaceutics 2019, 16,12, 5042-5053 - Raina et al., Journal of Pharmaceutical Sciences 2014, 103, 9, 2736–2748

Impact of T_g and H-bonding on release of ASDs and subsequent nanodroplet formation

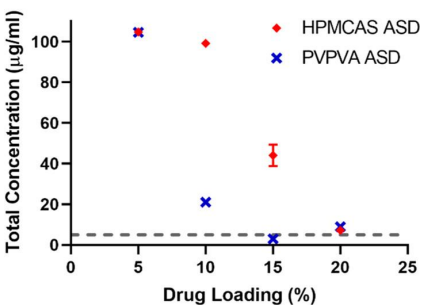
Loratadine ↓ Tg ↓ Log P
Halogen bonding



Paclitaxel ↑ Tg ↓ Log P
Hydrogen bonding donor



Ivacaftor ↑ Tg ↑ Log P
Hydrogen bonding donor



The limit of congruency (LoC) is potentially much lower for PVPVA64 ASD compared to HPMCAS ASD. H-bonding and Tg/wet Tg of the compound play a key factor in ASD congruent release as was illustrated in other literature for various model compounds.

Other mechanisms like amorphous-amorphous phase separation on hydration may be involved.

↑ Tg compounds ↓ LoC
↓ Polymer solvent penetration & chain disentanglement – referred to 'swelling without relaxation' thereby forming a gel or bolus.
Log P appeared to do little to no effect on LoC and nanodroplet formation.

Yang et al., Molecular Pharmaceutics 2021, 18, 5, 2066-2081

Concluding Remarks

- Solution-phase phenomena often observed during ASD release in simulated fluids, including amorphous nanodroplet formation and drug crystallization, also occurs in aspirated intestinal fluids.

- Intraluminal supersaturation and generation of amorphous nanodroplets is highly likely *in vivo* upon ASD release as long as the conditions are non-sink.

- Prolonged maximization of the supersaturated phase, along with a co-existence of a rich nanodroplet phase, was consistent with the maintenance of high membrane flux during ASD dissolution.

- Rapid crystallization of a temporarily-supersaturated ASD, would result in de-supersaturation of the dissolved concentration and depletion of the short-lived drug-rich phase, thereby plummeting the membrane flux to a low value.

- Apart from crystallization, LoC and DL are crucial considerations when formulating an ASD, which would depend on several factors including polymer used, degree of H-bonding and native Tg of the compound.

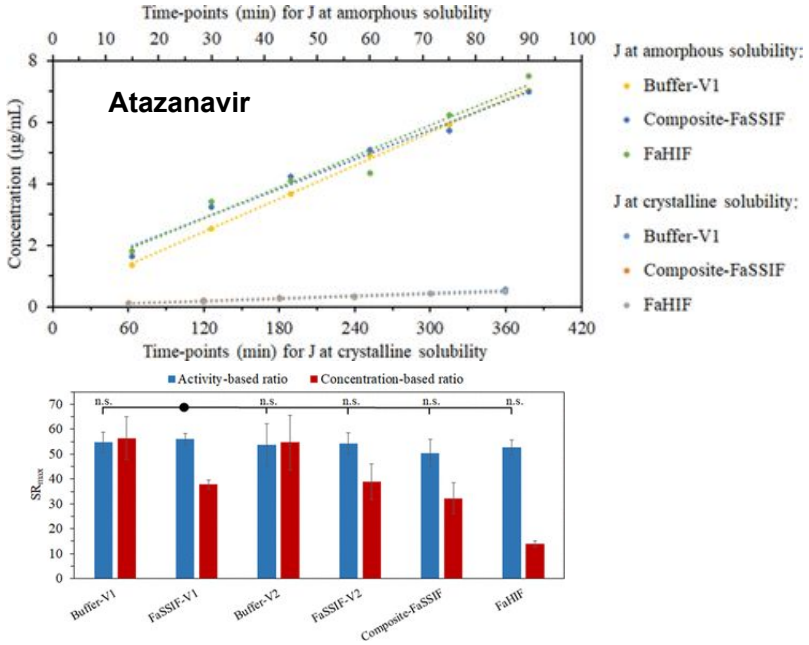
Thank you!

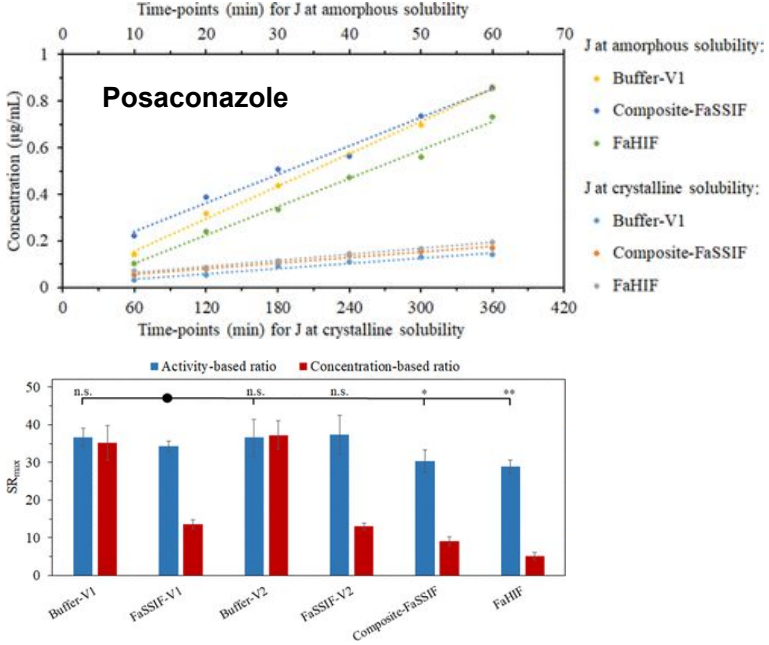
Questions?

Please check out the recent special issue from AAPS Open (Springer Nature): "Innovations in Enabling Formulations and Characterization of Small Molecules for Drug Development" – Open for submissions

Backup Slides

Diffusive flux and supersaturation rates (activity-based vs concentration-based)





Activity-based measurements for supersaturation, conducted by diffusive flux experiments, are largely independent of the medium, i.e. deconvoluted from solubilization effects, leading to better estimates of supersaturation ratios.

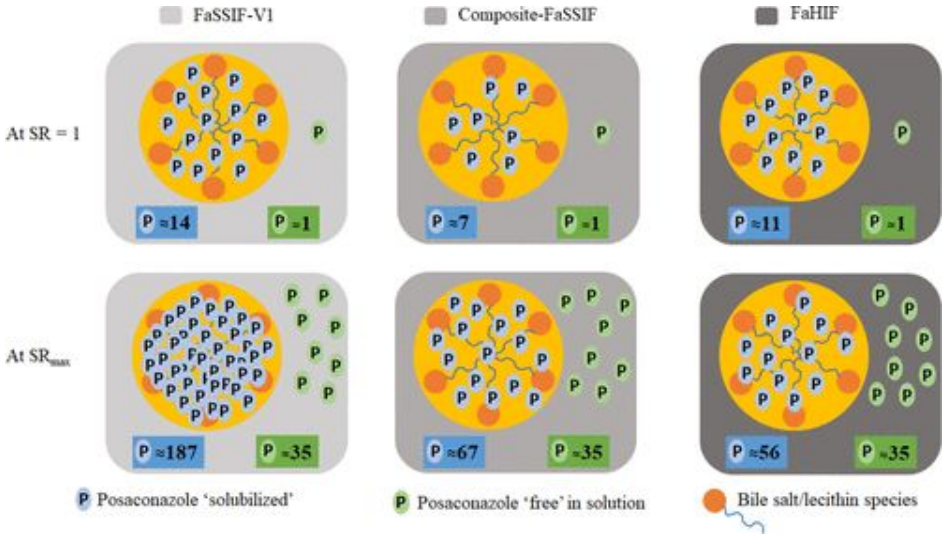
Elkhabaz et al., Molecular Pharmaceutics 2019, 16,12, 5042-5053

Solubilization versus supersaturation

$$K_{m/w} = \frac{C_m}{C_w}$$

medium	$K_{m/w}$ at crystalline solubility		$K_{m/w}$ at amorphous solubility	
	atazanavir	posaconazole	atazanavir	posaconazole
FaSSIF-V1	1.9	13.9	1.3	5.3
composite-FaSSIF	2.2	7.4	1.3	1.9
FaHIF	5.1	11.3	1.3	1.6

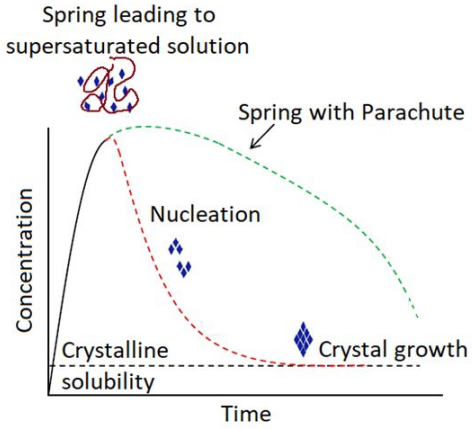
- $K_{m/w}$ was observed to be highly concentration dependent and varies as a function of medium, reflecting a difference in their solubilization capacity.
- $K_{m/w}$ is dependent on the drug, that is, posaconazole had higher solubilization capacities than atazanavir, which explains why the discrepancies in supersaturation ratios were more prominent for posaconazole.



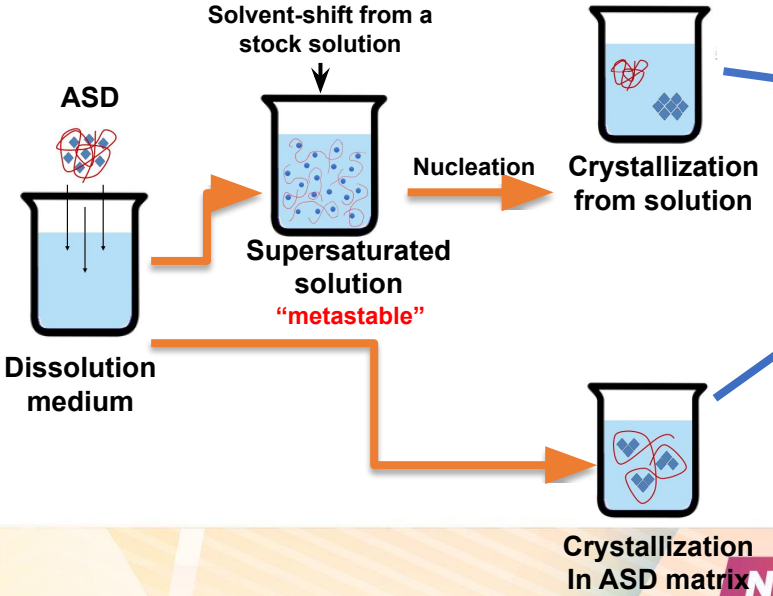
Posaconazole micellar partitioning at saturation (crystalline solubility or SR = 1) and maximum supersaturation (amorphous solubility or SR_{max}) levels.

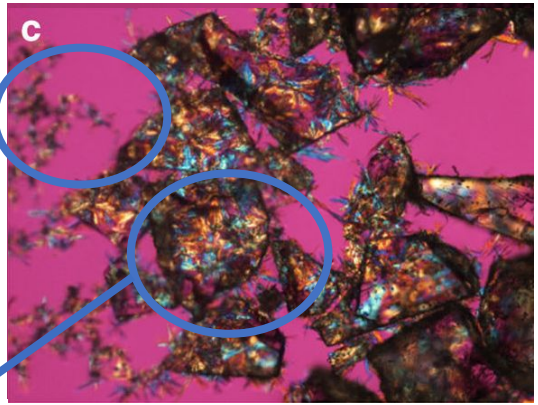
Elkhabaz et al., Molecular Pharmaceutics 2019, 16,12, 5042-5053

Phase transformations and routes of ASD crystallization during dissolution



Is Spring-Parachute still valid?





Amorphous celecoxib (10x)

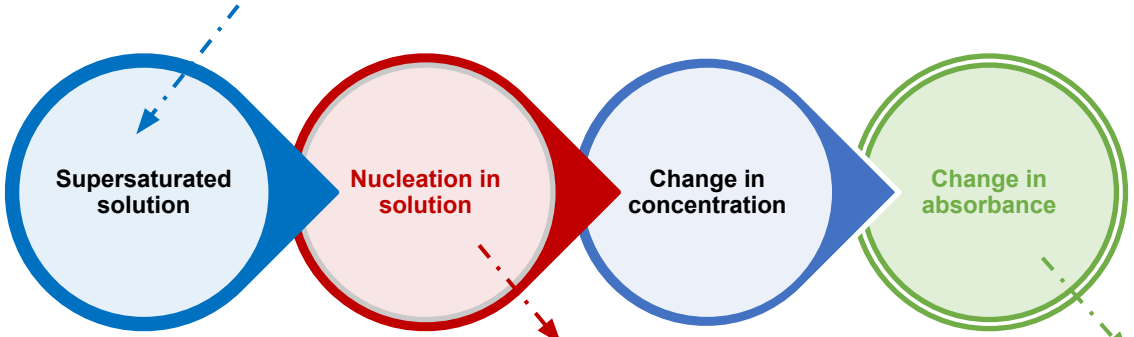
Amorphous celecoxib after 10 min exposure to aqueous phosphate buffer pH 6.8

Spring and parachute figure adapted from Williams et al., Pharmacol. Rev. 2013, 65, 315–499.
Schematic adapted from Alonzo et al., 2010 Pharm. Res. 27(4):608-618.
Xie, T., and Taylor, L. S. (2016). Pharmaceutical Research. 33(3):739-750

Solvent-shift coupled with UV fiber optic probe

What is Solvent-shift?

API in organic solution → API in aqueous supersaturated solution



Induction time

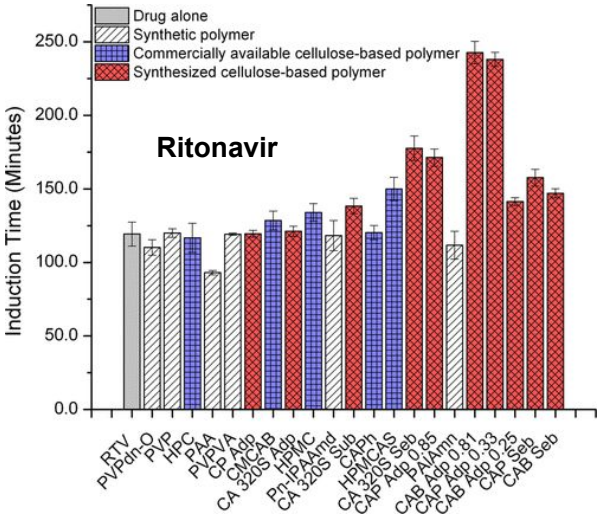
An experimental observation that determines the onset of nucleation in solution.

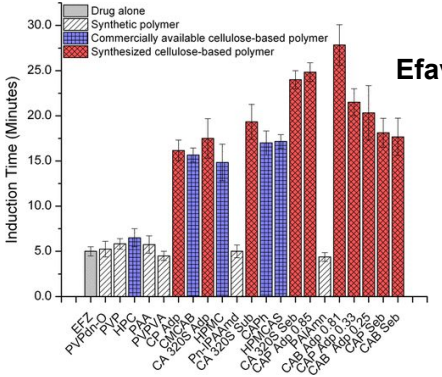
$$t_{ind} = t_c + t_g$$

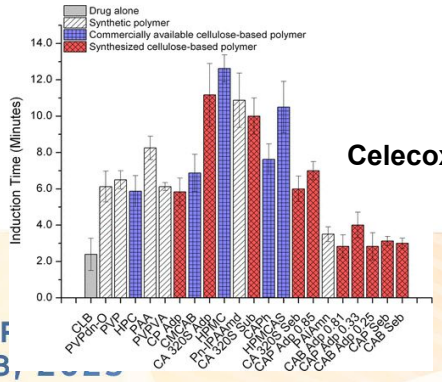
t_c is the time for critical nucleus formation. t_g is the time for nucleus growth to a detectable size.

Examples of nucleation induction times and applications in literature

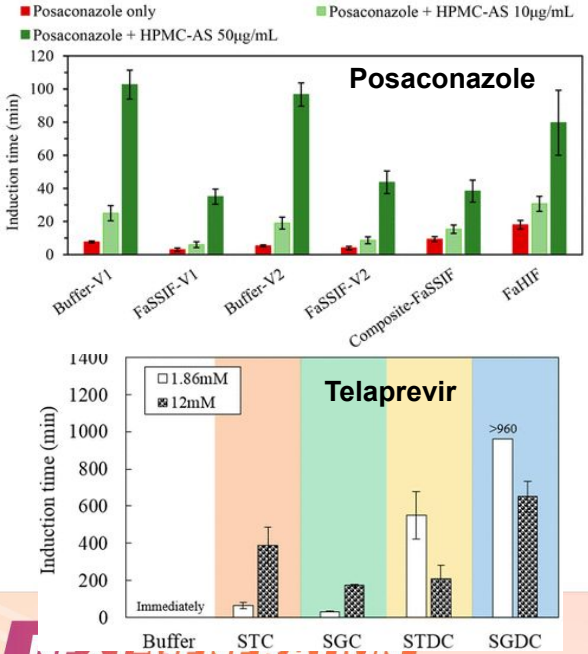
Impact of polymer







Impact of media and its components



Ilevbare et al. (2012), 13 (2): 740−751 - Elkhabaz et al., (2021), 21:2807-2820 - Lu et al., (2017), 17 (5), 2782−2791

Induction times and crystallization window: Fast vs slow

Slow Crystallizers

Maintain supersaturation in the solution phase, for at least a physiologically relevant for absorption.

Nucleation kinetics are relatively slow even without addition of polymers (nucleation suppression with a polymer is usually profound and synergistic).

Exhibit a spring-parachute model during dissolution when formulated as an ASD.

Easily generate amorphous nanoaggregates in solution above the amorphous solubility threshold.

Fast crystallizers

Nucleation kinetics are rapid, and API can crash in solution instantaneously.

Addition of polymers is required to maintain supersaturation. Prone to exhibit suboptimal dissolution performance and lose the amorphous solubility advantage quickly.

Selection of polymer and ASD development is more challenging.

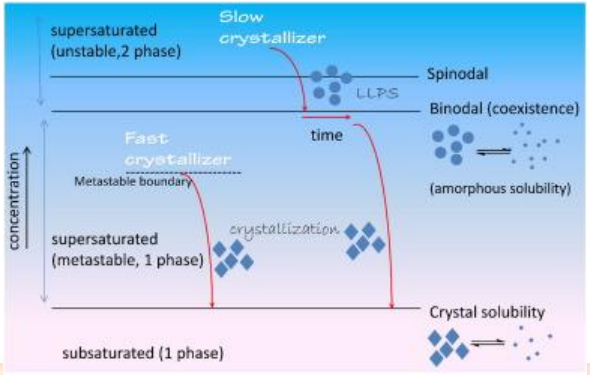


Figure from *Taylor, L.S.. and Zhang, G. G. Z. (2016), 101:122-142.*