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In Vitro Test Methodologies for Characterizing Bioavailability Enhancing Formulations

Aaron Stewart

Associate Principal Scientist, Global R&D

Talk Outline

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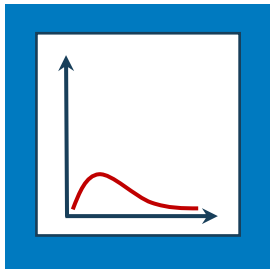
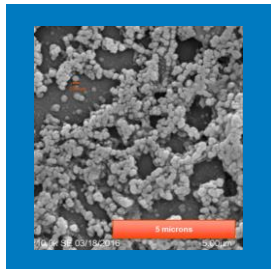


Strategies to Address Low Bioavailability

In Vitro Performance Tools

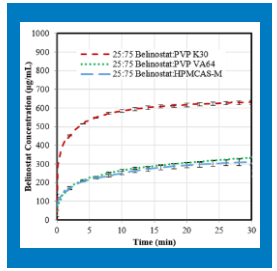
Methodologies

How to select?



Case Study #1

Itraconazole



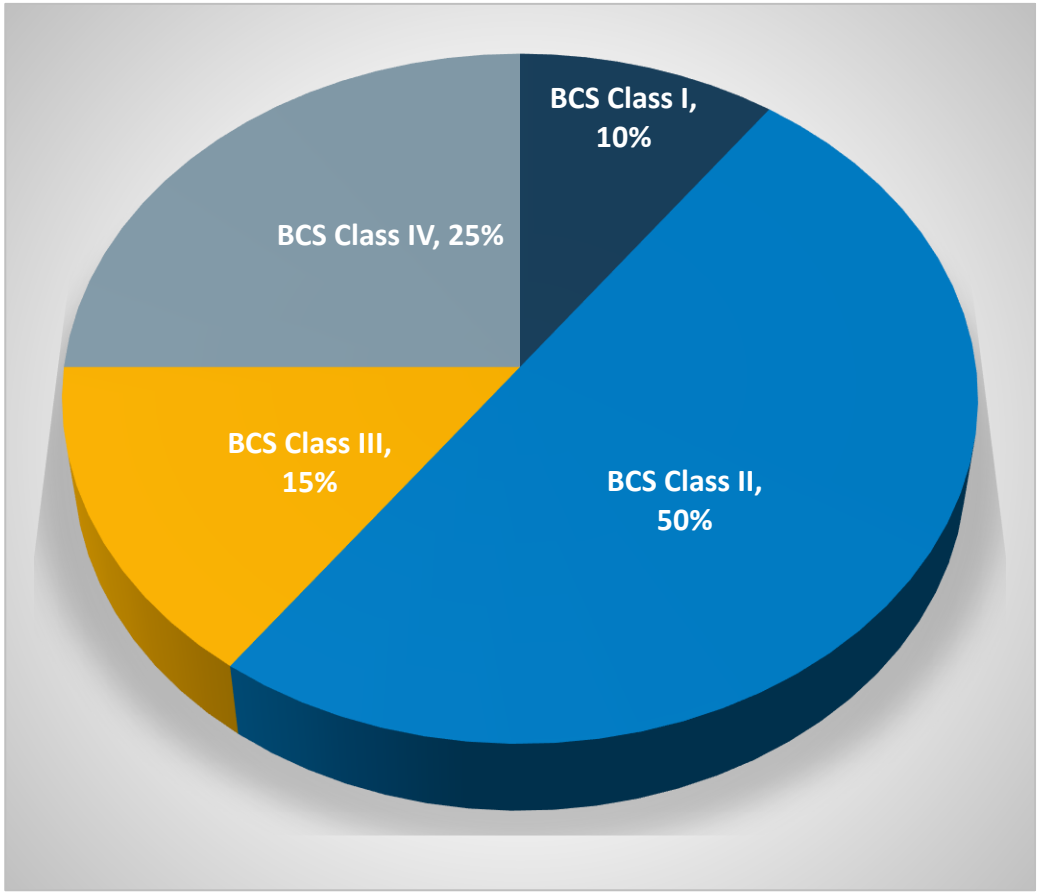
Case Study #2

Belinostat

Summary and Take Away Points

The Ongoing Issue

Low solubility continues to plague development pipelines



Reference:

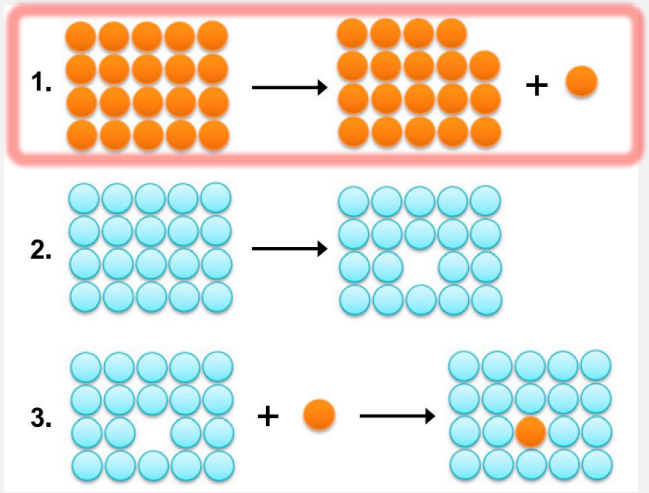
Drug-Like Property Concepts in Pharmaceutical Design, Di, Li; Kerns, Edward H.; Carter, Guy T. In Current Pharmaceutical Design, Volume 15, Number 19, 2009, pp. 2184-2194(11)

Putting Low Drug Solubility in Context

Primary obstacles to low solubility

Solid-state

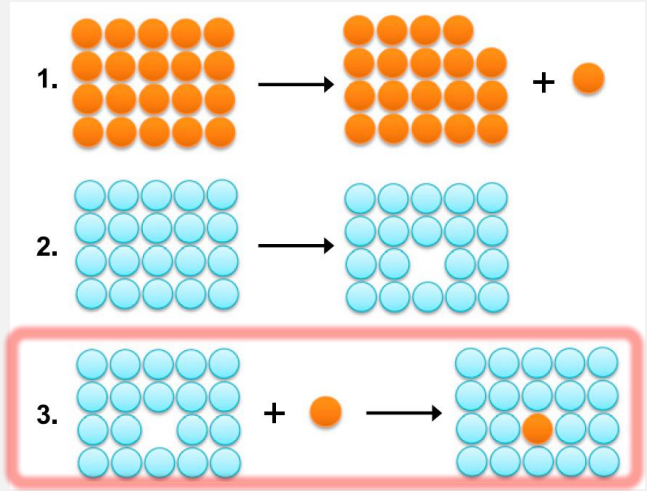
"Brick dust" compounds



- Very strong crystalline lattice energy **(1)** is limiting to solubility
- Address by bypassing crystalline state

Solvation

"Greaseball" compounds



- Very low affinity for water **(3)** is limiting to solubility
- Address by bypassing crystalline state and/or changing the nature of the solvent – "like dissolves like"

It is important to know the solubility performance attributes for your compound as it will aid selecting the right technology

Many Enabling Technologies Are Available

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Solid-State Alteration: Form, Particle Size

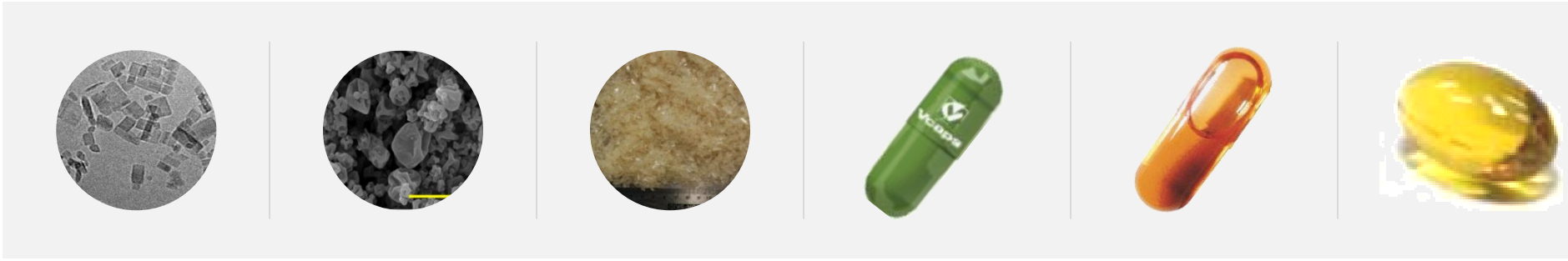
- Polymorphs
- Amorphous Solid dispersions
  - SDD, HME
  - Lyophiles
  - Drug/polymer nanoparticles
  - Mesoporous carriers
- Nanocrystals (200 to 800 nm)
- Nanocrystals (<50 nm)

New crystalline compound

- Cocrystals
- Salts

Solvation, Complexation

- Cosolvents
- Surfactants
- Cyclodextrins
- Lipids:
  - Oils
  - SEDDS/SMEDDS
  - Solid lipid pellets



H.D. Williams et al. "Strategies to Address Low Solubility in Discovery and Development," Pharmacol Rev , 65(2013)315-499

Which Technology To Select for Which Compound?

Why it is an important question….

Matching your compound with the wrong technology

Challenging development

Increase development time and costs

High risk of failure

Exploring multiple technologies in parallel

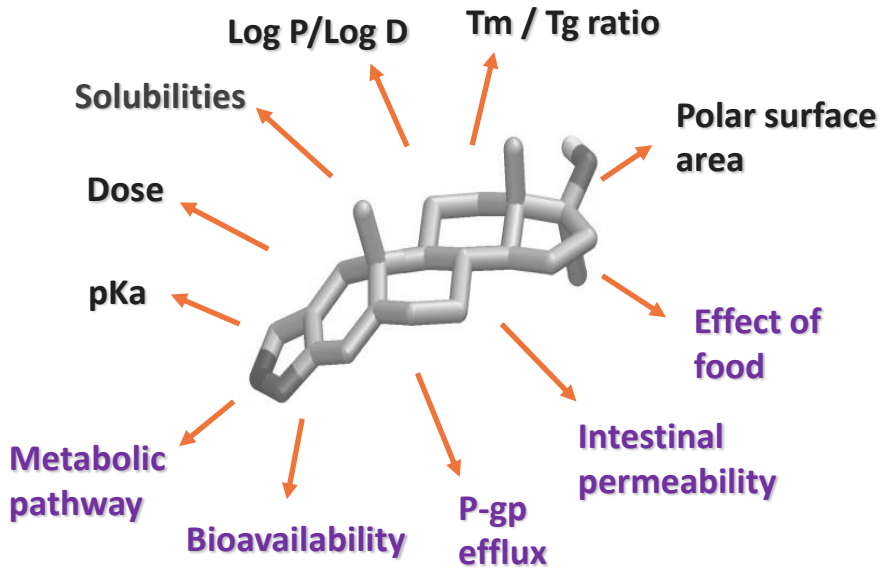
Complex development

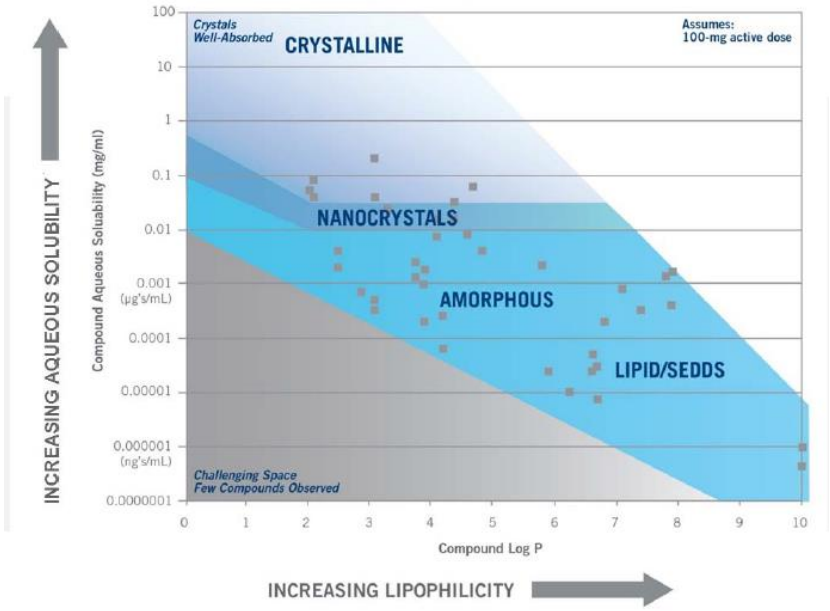
Higher resource requirement (API, costs)

Does not guarantee success

Compound Qualification and Technology Mapping

A science-based approach to selecting a technology for your compound





- Broad, in-depth review considers physical and biological barriers to drug absorption
- Drawbacks: Qualitative assessment. Does not factor in experience and technology precedence

- Leverages large compound *in vivo* datasets and based on data collected during preformulation
- Drawbacks: Data does not include head-to-head comparisons. Would another technology have been better?

Representative Technology Options Relative to Problem Statement

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Technology	Dissolution Rate Limited	Permeability Limited	Solubility and Permeability Limited
Salt, Polymorph, Cocrystal	X		x
Nanocrystals	XX		
Amorphous	XX		X
Lipids	XX	X	XX

H.D. Williams et al. “Strategies to Address Low Solubility in Discovery and Development,” *Pharmacol. Rev.*, 65(2013), 315-499

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In Vitro Bioperformance Tools

Available tools…
How to select?

In vitro & *in silico* tools can be used to assess the rate determining step(s) to absorption

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Basic property prediction

ChemAxon Ltd

- HBD, HBA, PSA
- Acid/base/neutral
- pK_a
- LogP/LogD
- pH-solubility
- Tm
- Provisional BCS

Dimensionless numbers/simple calculations

- Dose Number
- Dissolution Number
- Permeation Number

In vitro assessment

- pH-solubility
- LogP/LogD
- Tm & Tg
- Micelle partition coeff.
- Caco2 permeability
- PAMPA permeability

Dimensionless Numbers

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- **Key Inputs:**
- Crystalline Aqueous Solubility
- Amorphous Aqueous Solubility
- Projected Dose (or range)
- Animal physiology
- Log P/Micelle Partition
- Effective Permeability

Outputs:

Dose Number

$$Do = \frac{Dose/Vol}{C_s}$$

→ How many GI fluid volumes are required to dissolve the dose ?

Permeation Number

$$Pn = k_{abs} \cdot t_{abs}$$

→ How many times can the drug permeate over the course of GI transit ?

Dissolution Number

$$Dn = k_{diss} \cdot t_{abs}$$

→ How many times can the drug dissolve over the course of GI transit ?

Sugano and Terada, Journal of Pharmaceutical Sciences 104:2777-2788, 2015

Fraction Absorbed Classification System (FaCS)

Three Limiting Cases

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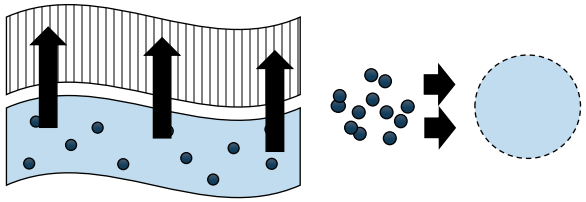
Case 1: Dissolution Rate Limited (DRL)



$$Dn < Pn/Do$$

Cases where this occurs:
- High permeability relative to dose and solubility
- Dissolution rate is slow

Dose Number

$$Do = \frac{Dose/_{Vol}}{C_s}$$

Permeation Number

$$Pn = k_{abs} \cdot t_{abs}$$

Dissolution Number

$$Dn = k_{diss} \cdot t_{abs}$$

Sugano and Terada, Journal of Pharmaceutical Sciences 104:2777-2788, 2015

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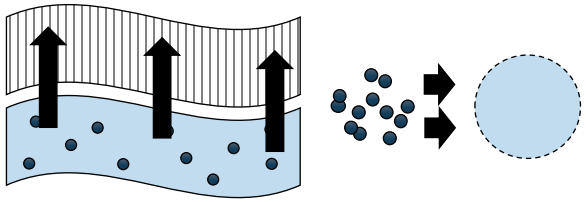
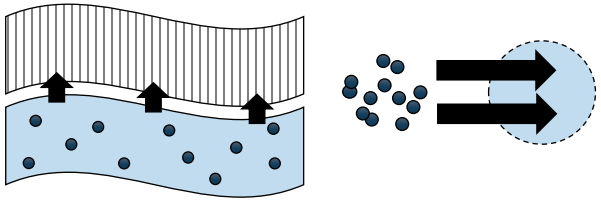


$$Dn < Pn/Do$$

Cases where this occurs:
- High permeability relative to dose and solubility
- Dissolution rate is slow

Case 2: Permeability limited (PL)



$$Pn < Dn \ \& \ Do < 1$$

Cases where this occurs:
- Dissolution rate is fast relative to permeability
- Dose is low relative to solubility

Sugano and Terada, Journal of Pharmaceutical Sciences 104:2777-2788, 2015

Fraction Absorbed Classification System (FaCS)
Three Limiting Cases

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Dose Number

$$Do = \frac{Dose/_{Vol}}{C_s}$$

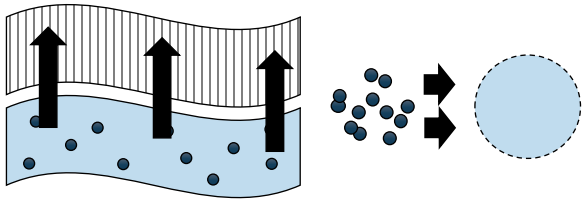
Permeation Number

$$Pn = k_{abs} \cdot t_{abs}$$

Dissolution Number

$$Dn = k_{diss} \cdot t_{abs}$$

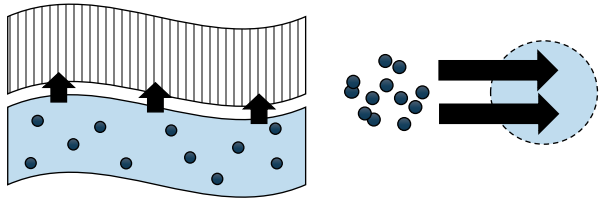
Case 1: Dissolution Rate Limited (DRL)



$$Dn < Pn/Do$$

Cases where this occurs:
- High permeability relative to dose and solubility
- **Dissolution rate is slow**

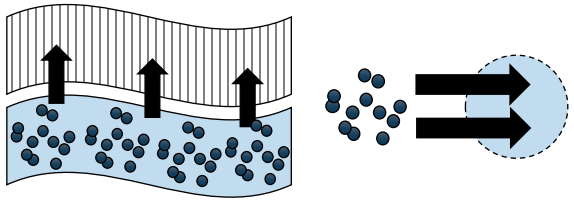
Case 2: Permeability limited (PL)



$$Pn < Dn \ \& \ Do < 1$$

Cases where this occurs:
- Dissolution rate is fast relative to permeability
- **Dose is low relative to solubility**

Case 3: Solubility-permeability limited (SL)



$$Pn/Do < Dn \ \& \ Do > 1$$

Cases where this occurs:
- Low permeability relative to dose and solubility
- **Dose is high relative to solubility**

Sugano and Terada, Journal of Pharmaceutical Sciences 104:2777-2788, 2015

How do we select and design the appropriate *in vitro* test?

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1. Predict *in vivo* problem statement

Problem Statement	Formulation mechanism
Precipitation	Supersaturating formulations
	pH driven supersaturation
Dissolution Rate Limited	Initial particle size
	Dissolution rate changes over time
Solubility-Permeability Limited	ABL limited
	Epithelial limited

2. Select *in vitro* dissolution apparatus

In Vitro toolkit	
Non-sink Single medium	Dissolution – permeation
Non-sink pH shift	Controlled Transfer
Sink single medium	Dissolution - permeation
Sink pH shift	Controlled Transfer
Non-sink Single medium	Dissolution - permeation
Non-sink pH shift	

3. Choose *in vitro* test parameters

In Vitro parameters	
Fluid composition, volume	A/V, volume(s)
	Fluid transfer rates
Fluid composition	A/V, volume(s)
	Fluid transfer rates
Fluid composition, volume	A/V, volume(s)

Multiple Problem Statement-specific Bioperformance *In Vitro* Tools Using Fiber Optics

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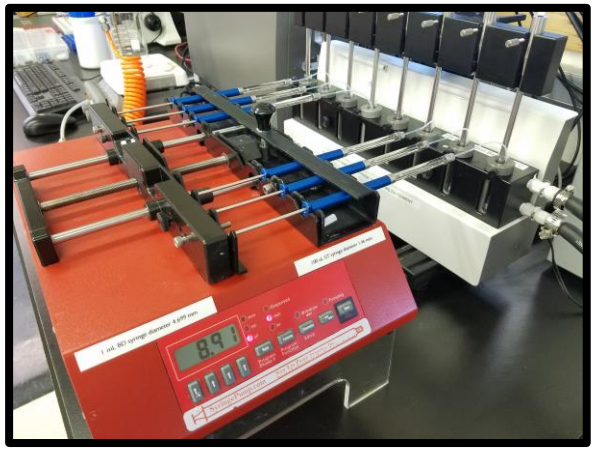
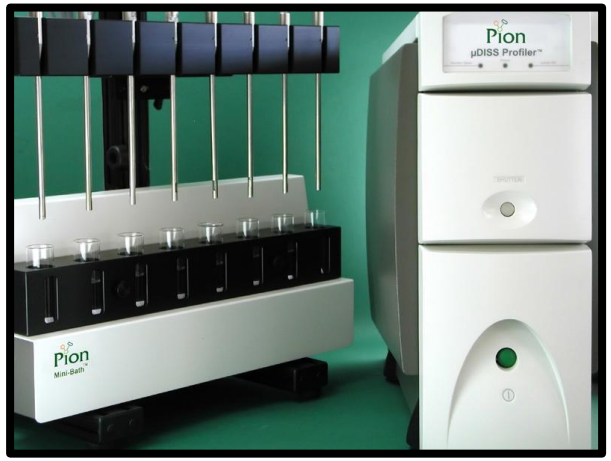
Amorphous Solubility



- Amorphous “solubility”
- Precipitation risk
- Polymer selection
- Drug/polymer interaction

Dissolution



- Dissolution rate
- Precipitation rate
- Maximum apparent concentration
- Speciation

Flux

- Clean measurement of “effective” concentration
- Able to properly account for micelle, colloid, and particle contribution to boundary layer diffusion and dissolution rate

Controlled Transfer

- Dissolution rate
- Precipitation rate vs. emptying rate
- Gastric precipitation
- “Book-end” for formulation performance

Amorphous Solubility and Polymer Screening

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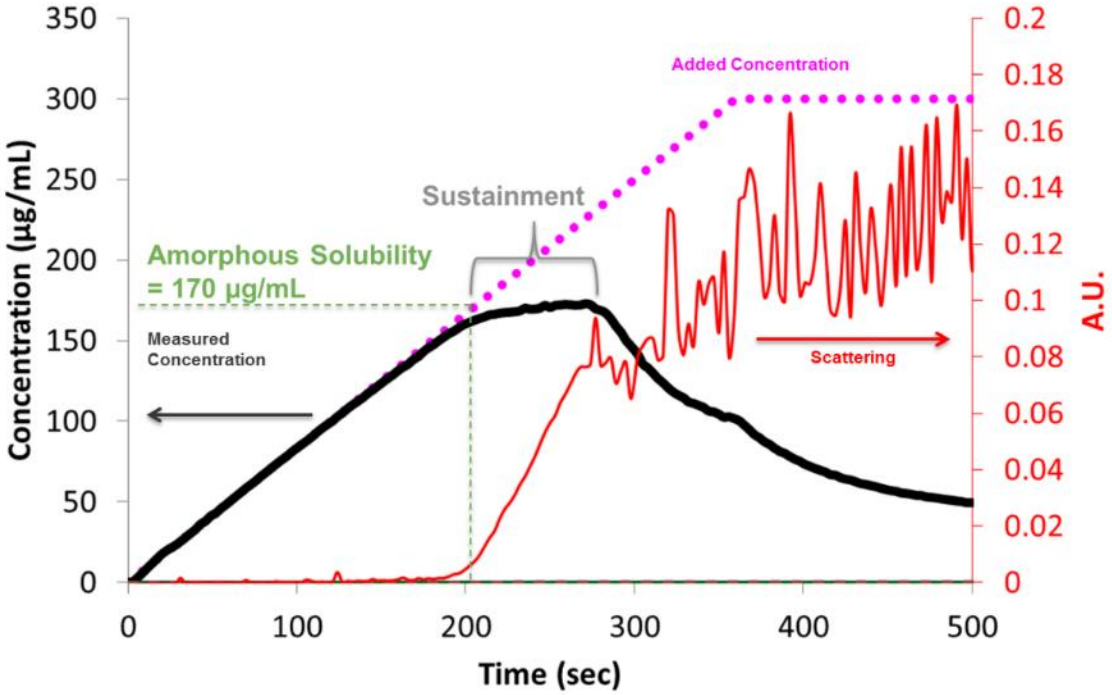
Key Outputs:

- Maximum solubility attainable from amorphous formulation
- Precipitation risk of compound
- Identify lead sustaining polymer(s) for SDD
- Potential to identify strong drug/polymer interaction



Amenable to very low API quantities of 20 mg or less using a small volume setup

Murdande *et al. J Pharm Sci.* 99 (2010), 1254
Murdande *et al. Pharm Res.* 27 (2010), 2704
Almeida e Sousa *et al. Mol. Pharmaceutics*. 12 (2015), 484
Ilevbare and Taylor. *Cryst. Growth Des.* 13 (2013), 1497

Pion UV Probe Dissolution

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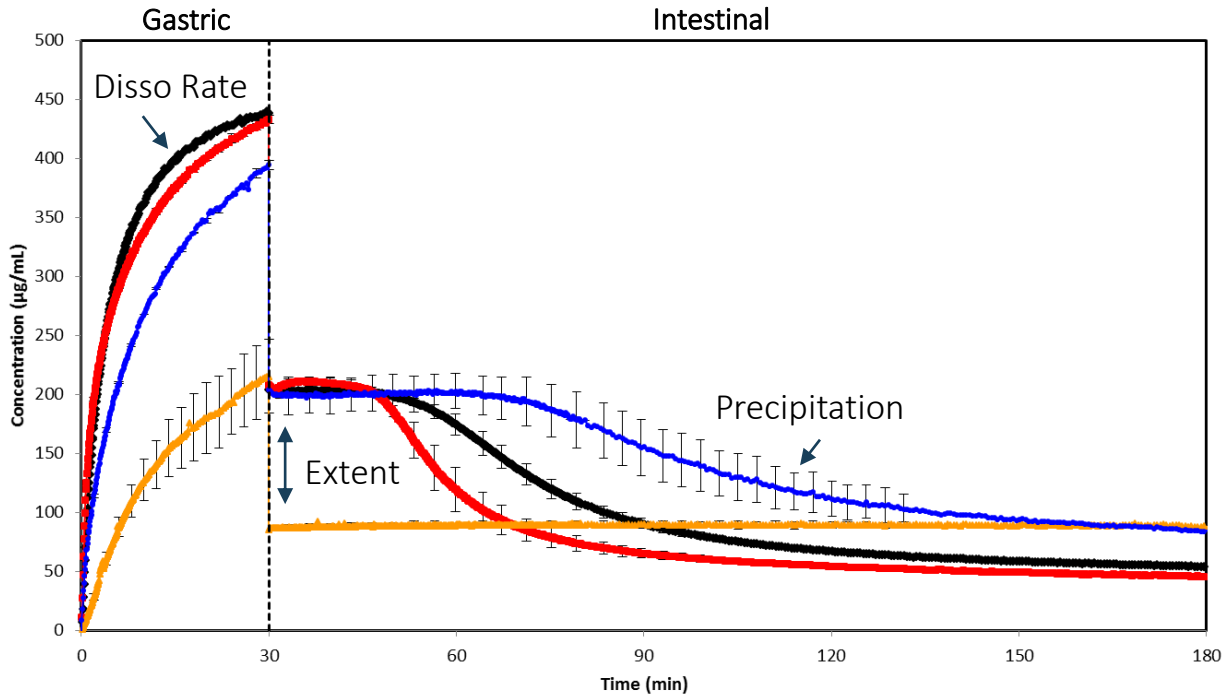
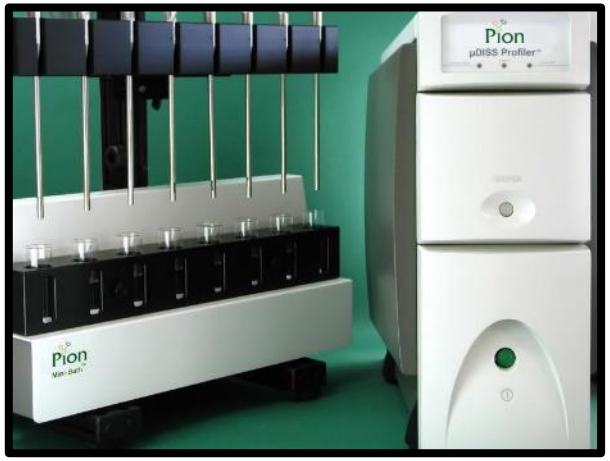
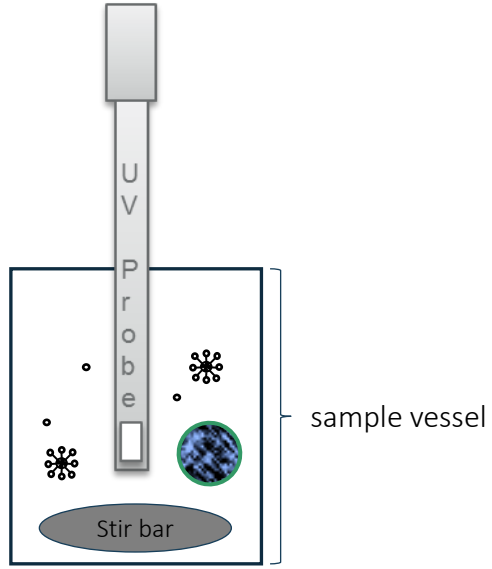
Key Outputs:

- Dissolution rate/extent
- Precipitation (gastric or intestinal)
- Speciation customized for specific API (e.g unbound free drug, micelle bound drug, colloids)

Example gastric to intestinal pH transfer test







Membrane Flux

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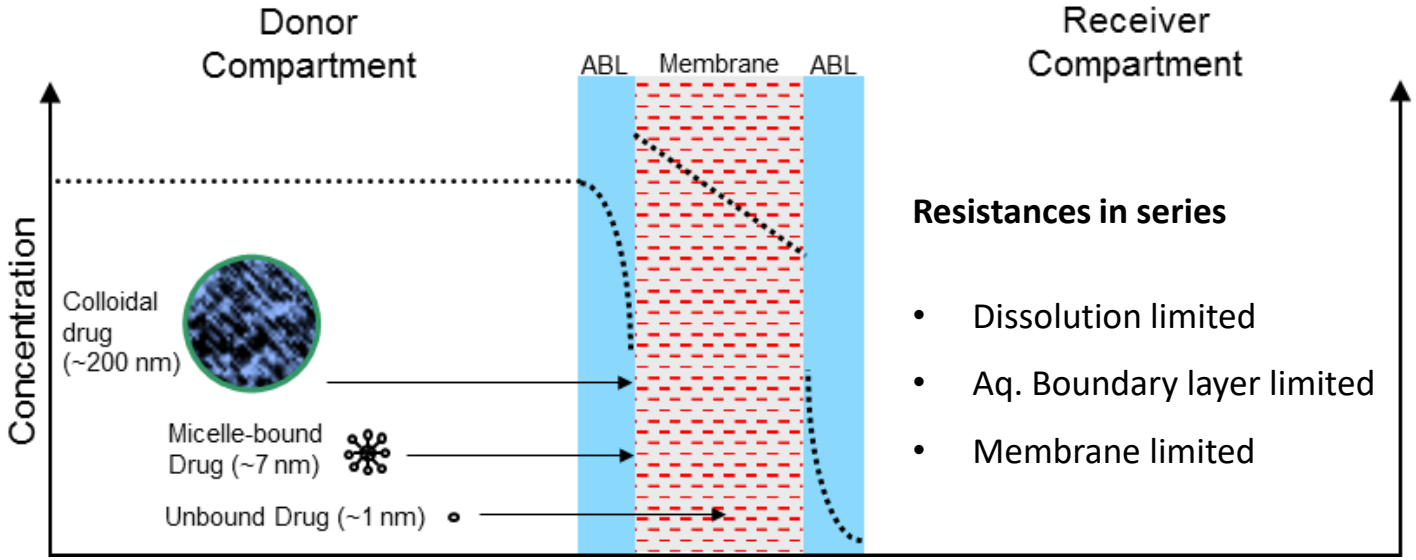
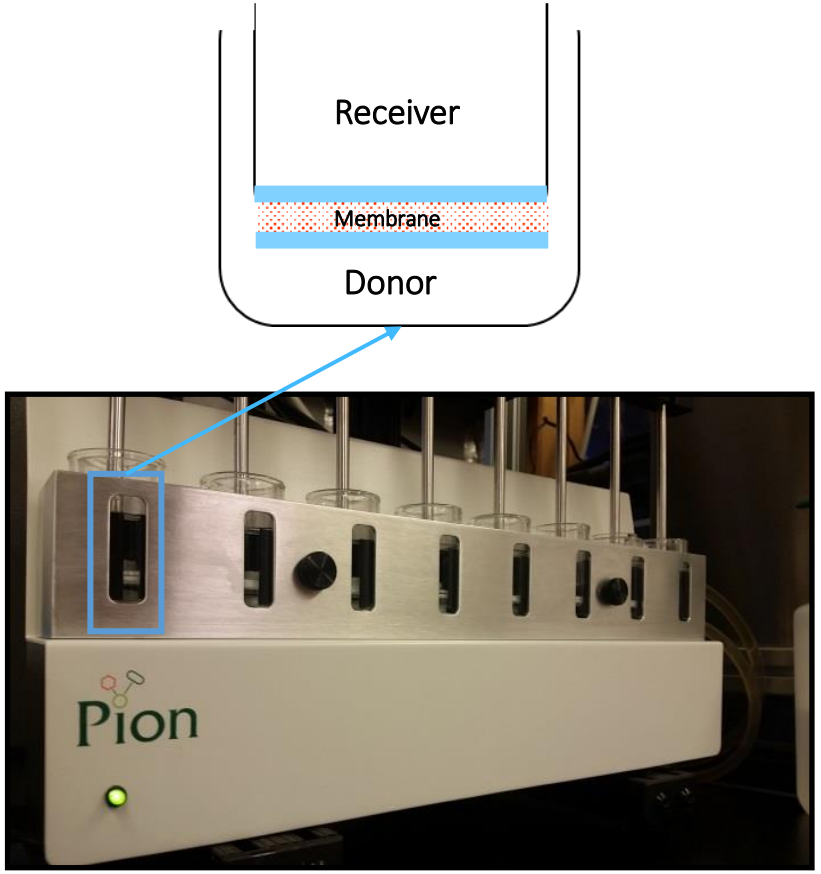
Key Outputs:

- Measures “effective concentration” for species essential for membrane permeation
- Can account for species contribution to boundary layer diffusion and dissolution rate



Concentration gradient example above demonstrates an Aq. boundary layer-limited formulation



Stewart, A. M. *et al.* Development of a Biorelevant, Material-Sparing Membrane Flux Test for Rapid Screening of Bioavailability-Enhancing Drug Product Formulations. *Mol. Pharm.* (2017).

Measured Flux is a Sum of Resistances

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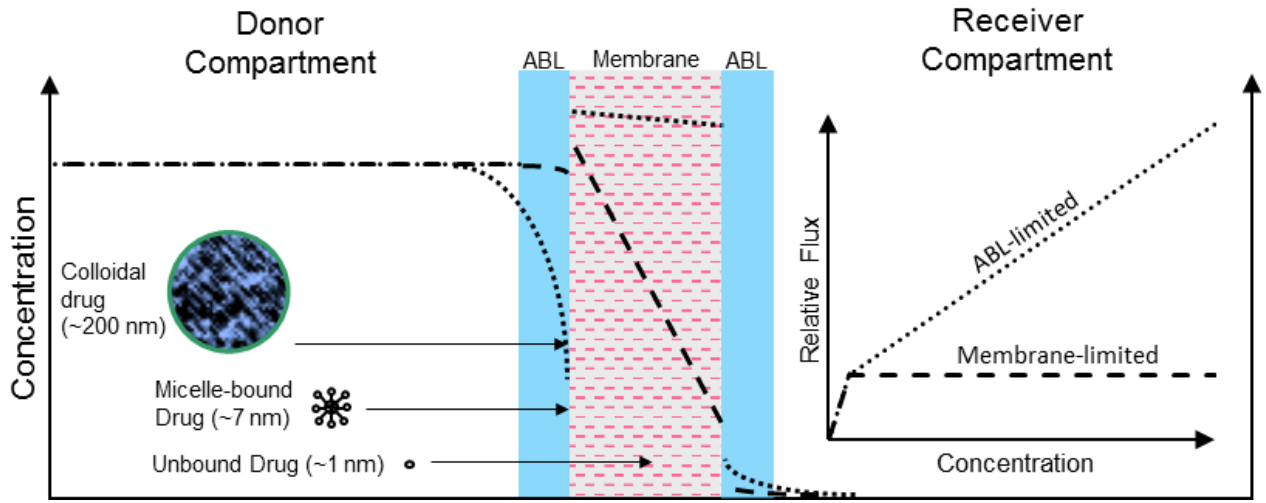


Dissolution Rate

- Dose
- Volume
- Solubility
- Particle size

Boundary Layer Diffusion

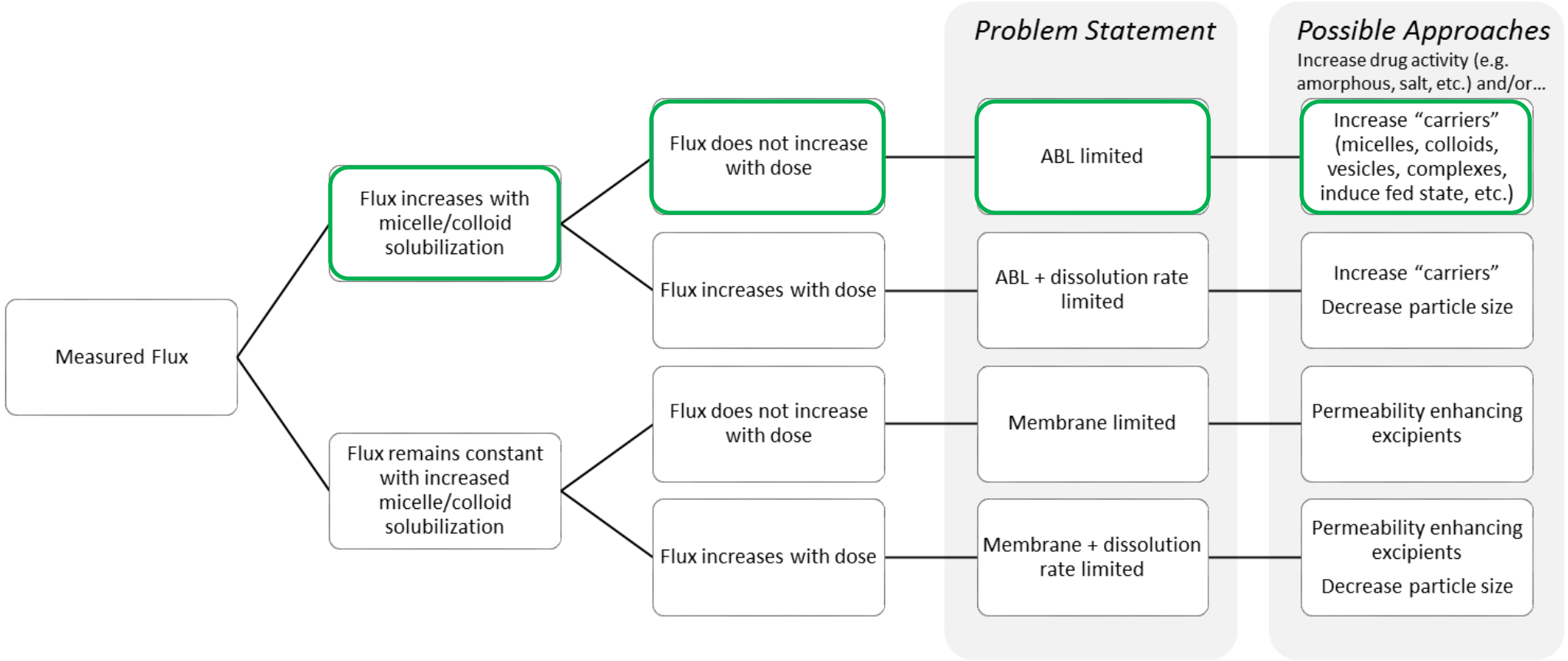
- Unbound drug
- Micelle-bound drug
- Colloidal drug

Membrane Diffusion

- Unbound neutral drug

Membrane Flux

How Data Inform Formulation Decisions



Route highlighted in green typical example of a low aqueous solubility, highly lipophilic BCS Class II compound

Controlled Transfer Dissolution

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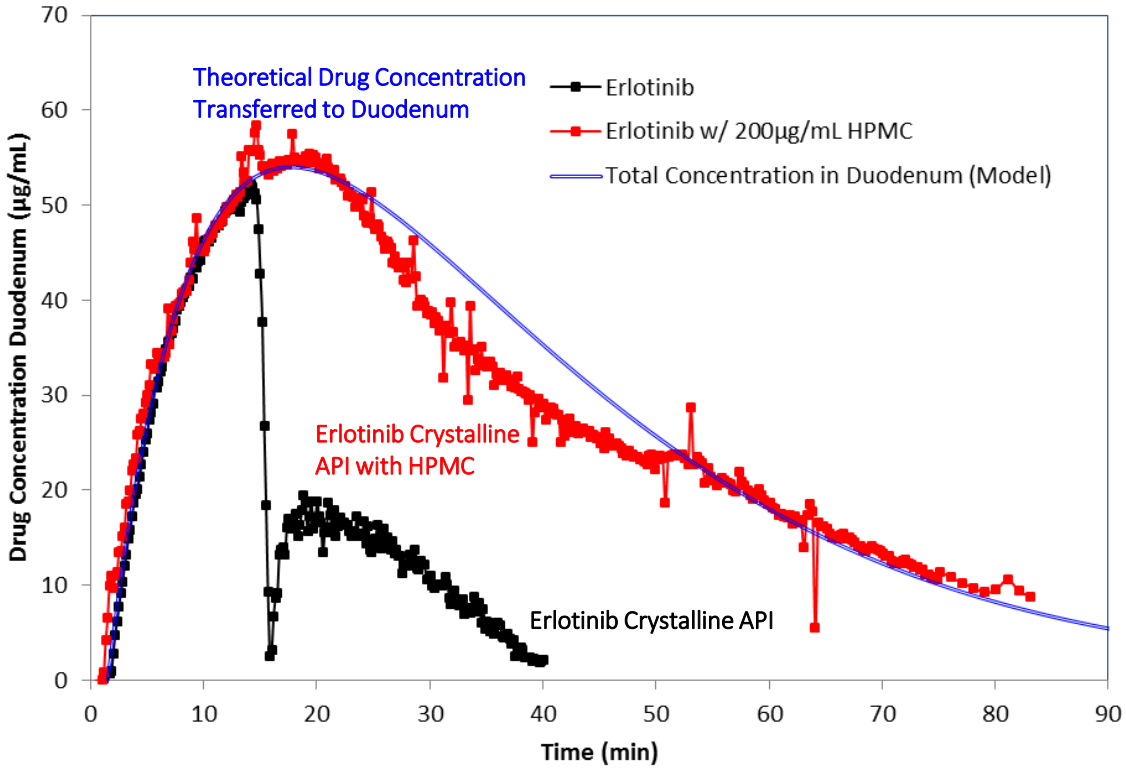
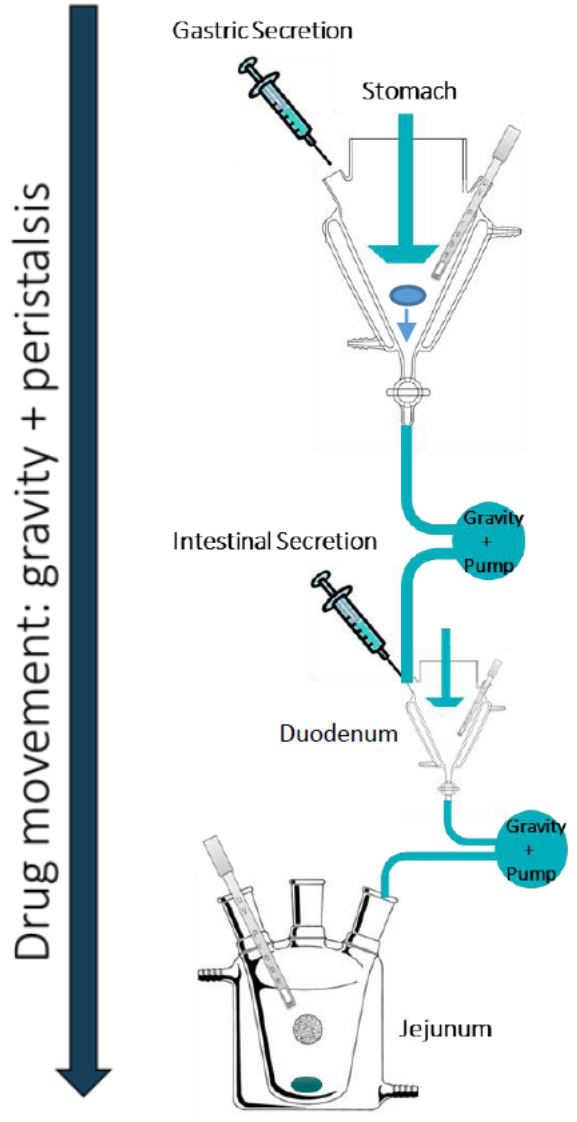
Key Outputs:

- Biorelevant dissolution rate
- Precipitation rate at biorelevant mass/volume transfer
- Gastric precipitation
- Dosage form disintegration



Solid Dosage Form Scale



Intermediate Scale

Digestion test for lipid based formulations

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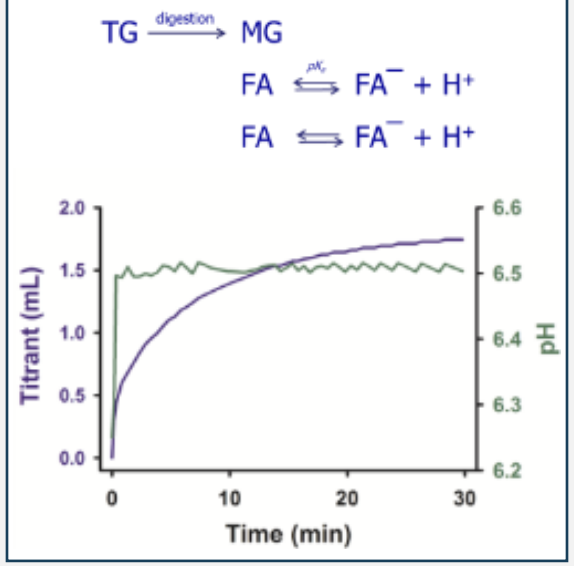
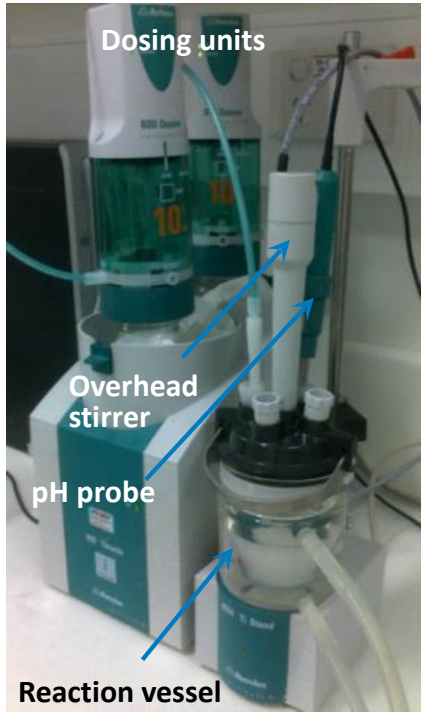
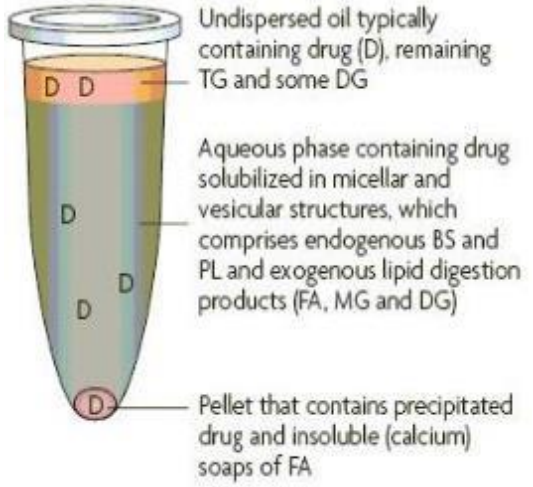
Digestion tests are used to identify those formulations at low risk of showing drug precipitation in the intestine
Provides a performance-based means to select lead formulations

Measure digestion by titration





Sample and measure drug fate by HPLC



Recent success has been demonstrated incorporating an absorption compartment into the digestion test: Alvebratt, C., Keemink, J., Edueng, K., & Cheung, O. (2020). *European Journal of Pharmaceutics and Biopharmaceutics*. https://doi.org/10.1016/j.ejpb.2020.01.010

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Case Study 1: Itraconazole

Case study - Amorphous spray dried dispersions (SDDs) of Itraconazole (ITZ) dosed to rats

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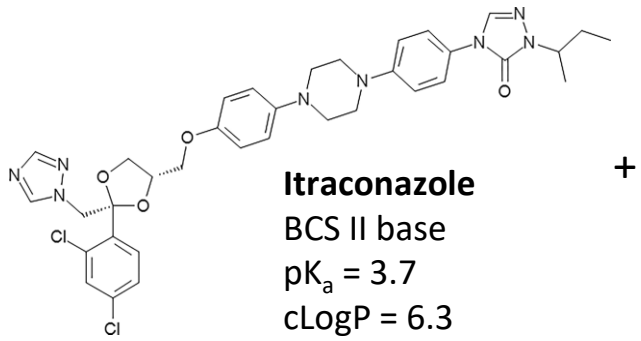
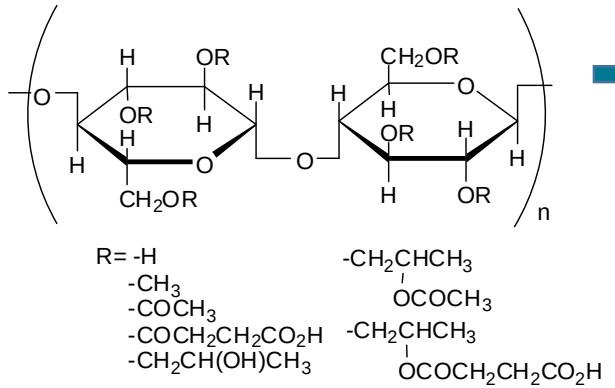


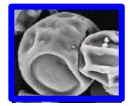
Itraconazole
BCS II base
pK_a = 3.7
cLogP = 6.3

+

Hydroxypropyl Methylcellulose Acetate Succinate (HPMCAS)

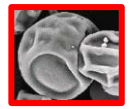


25% active
Hydrophilic SDD
Affinisol 716HP



or

25% active
Hydrophobic SDD
Affinisol 126HP



Formulations dosed to rats
Sprague-Dawley (n=6), fasted
Dose: 50 mg/kg
Dosing vehicle: 0.5% Methocel A4M in H_2O
Dosing route: oral gavage

Key ITZ attributes:

- Itraconazole has exceedingly low aqueous solubility even in the amorphous state (ca. 0.1 µg/mL).
- High lipophilicity and neutral charge state at intestinal pH drives very low solubility but high lipid membrane permeability, resulting in aqueous boundary layer limited flux *in vitro*.

Stewart, A. M. *et al.* Impact of Drug-rich Colloids of Itraconazole and HPMCAS on Membrane Flux In Vitro and Oral Bioavailability in Rats. *Mol. Pharm.* (2017).

Dimensionless numbers can predict impact of solubility, permeability or dissolution rate in vivo for itraconazole

BCS Ref: Amidon, G.L., et al. *Pharm Res.* (1995), 12 (3), 413-420

FaCS Ref: Sugano, K., et al., J Pharm Sci. (2015), 104, 2777-2788

Dose Number

$$Do = \frac{Dose/_{Vol}}{C_s}$$

Dissolution Number

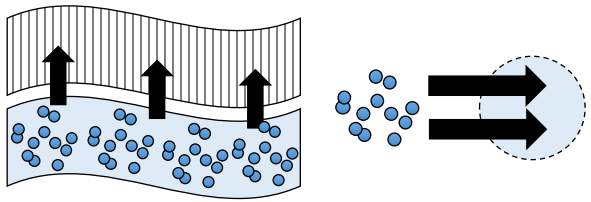
$$Dn = k_{diss} \cdot t_{abs}$$

Permeation Number

$$Pn = k_{abs} \cdot t_{abs}$$

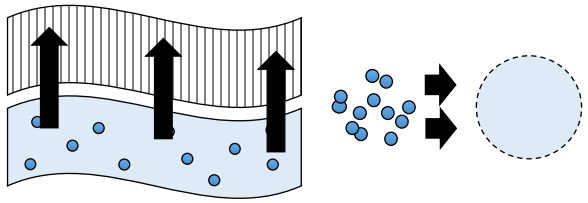
Solubility-permeability limited

ITZ SDDs in rats



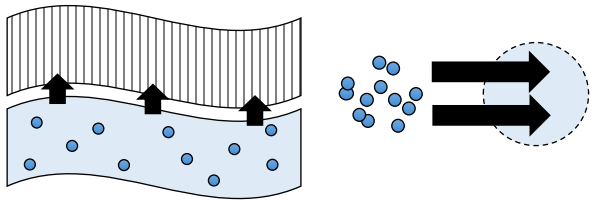
$$Pn/Do < Dn \ \& \ Do > 1$$

Dissolution-limited



$$Dn < Pn/Do$$

Permeability-limited



$$Pn < Dn \ \& Do < 1$$

Itraconazole is highly solubilized in micelles and colloids

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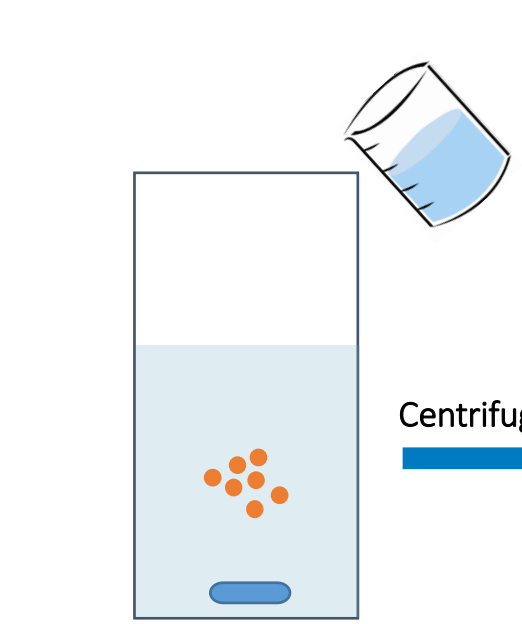
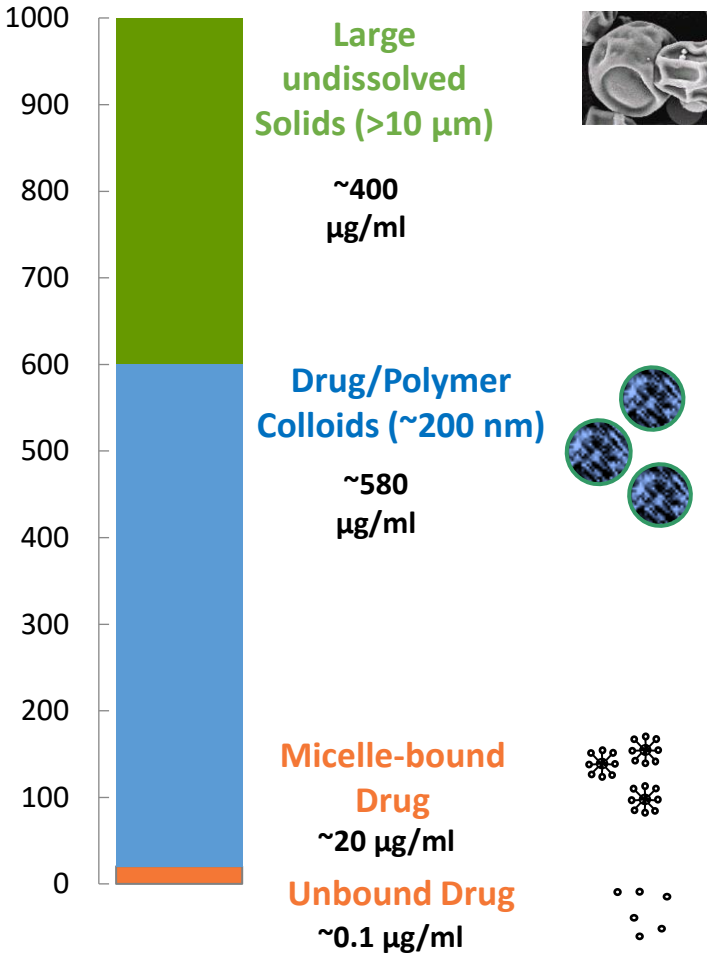
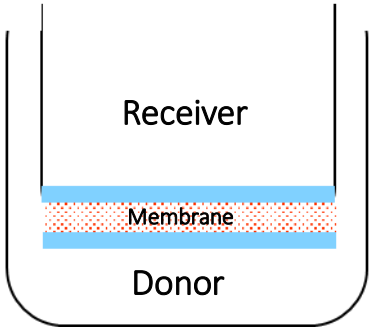


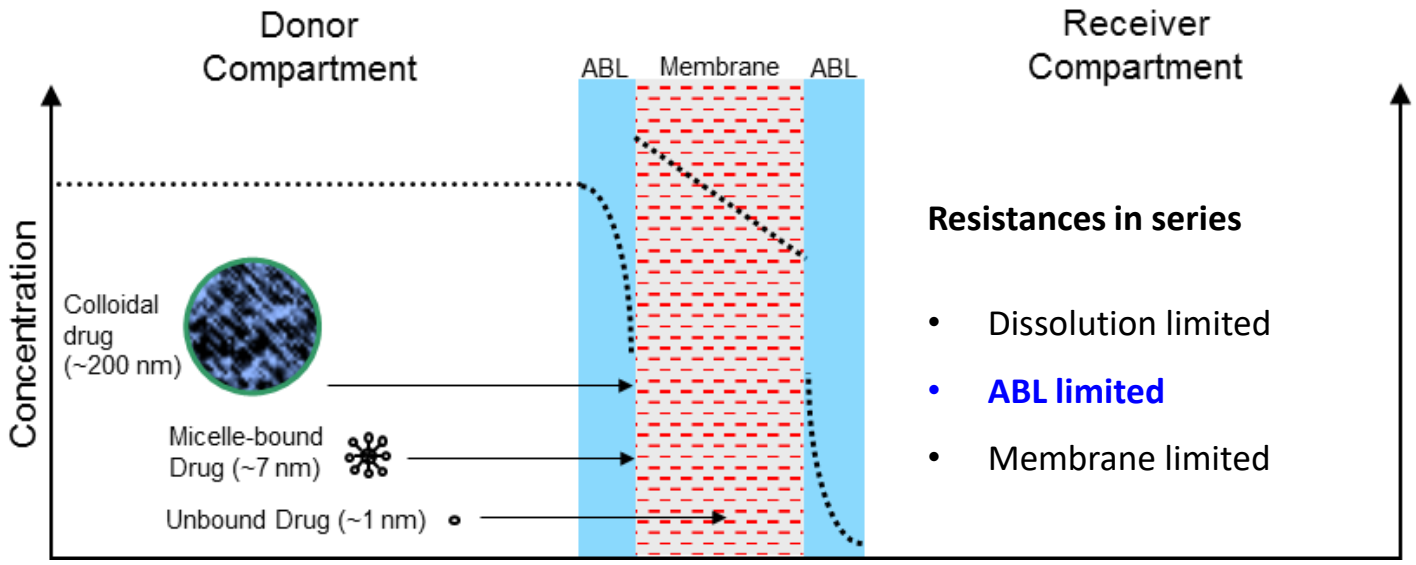
Centrifugation

25% ITZ:Affinisol 716 SDD
pH 6.5, 27 mM bile salts



Material sparing in vitro membrane flux test can assess solubility-permeability limited absorption





Itraconazole = ABL Limited Flux

Hydrophilic SDD has the highest flux *in vitro*

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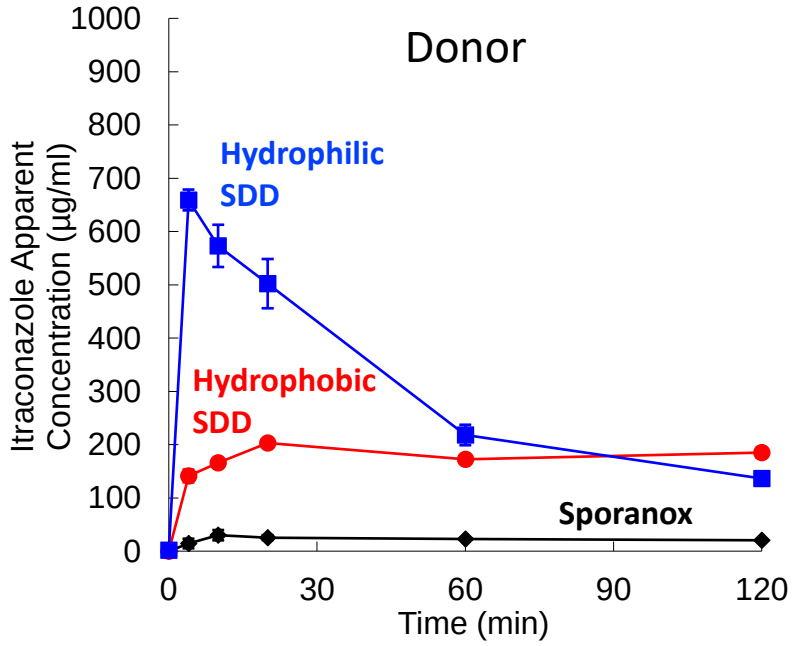
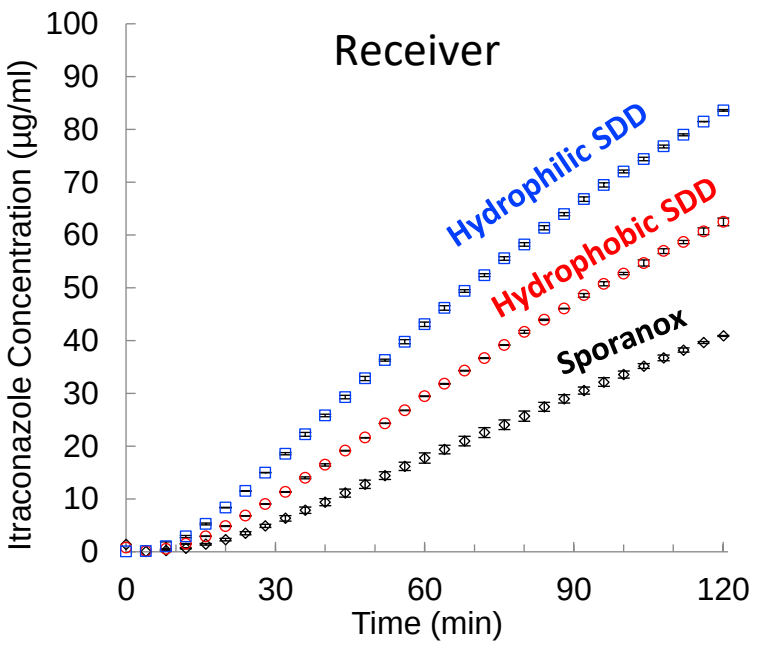




No.	Formulation	Dispersion polymer	Flux (µg/min/cm²)	Colloid (µg/ml)
■	25% ITZ/75% HPMCAS SDD	AFFINISOL 716HP	1.18	602
●	25% ITZ/75% HPMCAS SDD	AFFINISOL 126HP	0.85	150
◆	Sporanox® spray layered dispersion	HPMC	0.53	0

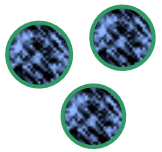
All formulations have the same unbound (0.1 µg/mL) and micelle-bound (20 µg/mL) ITZ concentrations and only differ in the concentration of colloidal drug species. Difference in flux is driven by the nano-sized colloidal species.

ABL limited diffusion in the membrane flux assay can by described by a steady state diffusion model

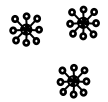
$$j = \frac{D_{eff}}{h_{ABL}}(c_{u,m,c})$$

$$D_{eff} = D_u \cdot f_u + D_m \cdot f_m + D_c \cdot f_c$$

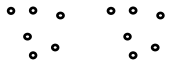
$C_{u,m,c}$ is the sum of all species:



D_c = 4 X 10^{-8} cm^2/s (200 nm)



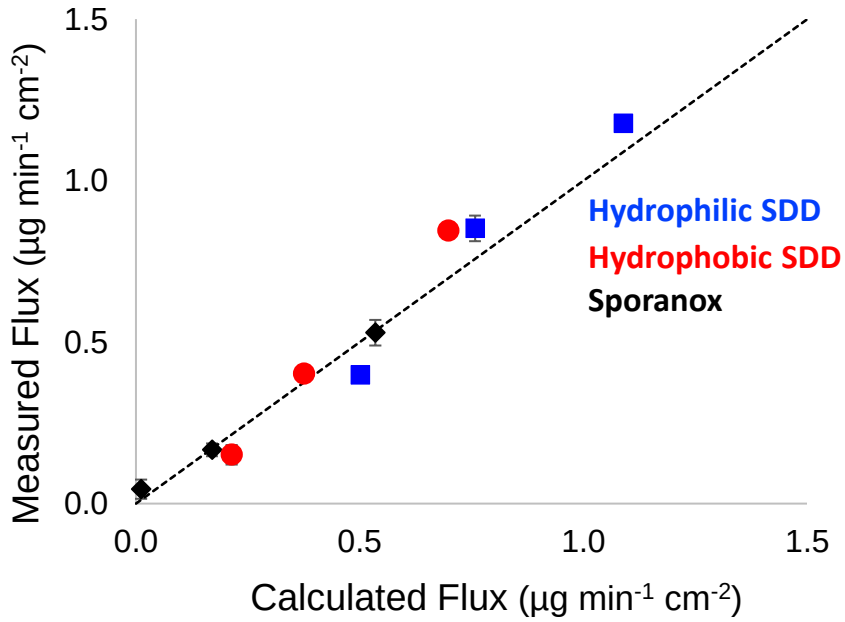
D_m = 1 X 10^{-6} cm^2/s (7 nm)



D_u = 5 X 10^{-6} cm^2/s

Key assumptions

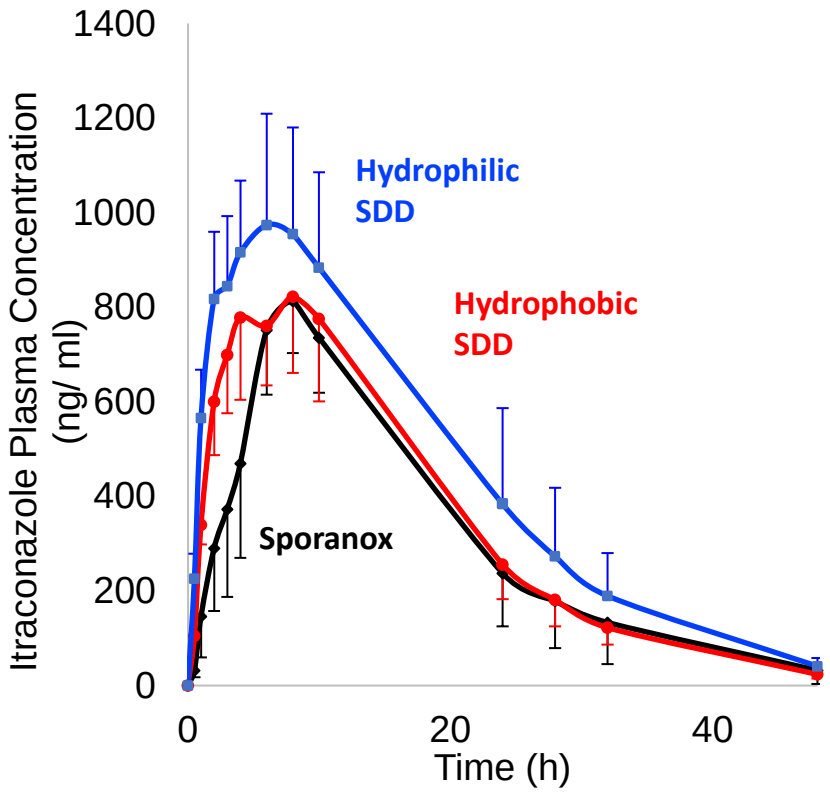
- Psuedo-steady state
- D_u, D_m & D_c concentration independent
- Drug that is unbound, micelle-bound, or in colloids contribute to D_{eff} based on size and abundance
- Minimal transport due to convection
- Well mixed solutions
- Constant ABL thickness

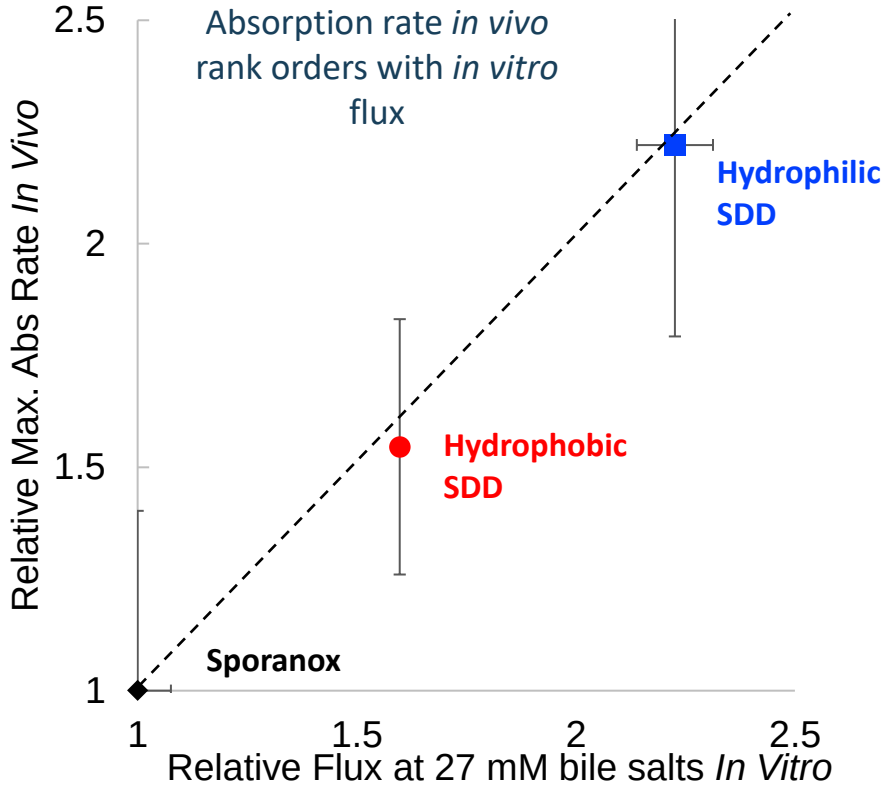


Model supports *in vitro* measurements made in three different media: blank PBS buffer, 6.7 mM SIF, 27 mM SIF.

Dose for all flux measurements was 1000 µg/mL ITZ

Hydrophilic SDD shows the fastest absorption in rats – rank orders with *in vitro* performance





Itraconazole Case Study

Conclusions

- Identified unique drug speciation from ITZ:Affinisol SDDs compared to commercial formulation Sporanox
- Evaluated contributions of these species to in vitro flux based on ABL limited diffusion
- Described contributions of drug species mathematically
- Demonstrated the impact *in vivo*, showing absorption rate trends with *in vitro* flux.

- Key in vitro performance tool: **membrane flux**

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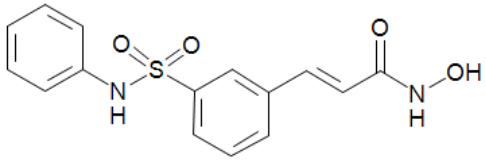
Case Study 2: Belinostat

Case study - SDDs of belinostat dosed to dogs



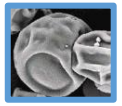
Belinostat

BCS II/IV

pK_a = ≥8 (acidic)

LogP < 2

\+

HPMCAS (weakly acidic) ⇨ **25% active HPMCAS-M SDD** 

Polyvinylpyrrolidone (neutral) ⇨ **25% active PVP K30 SDD** 

Polyvinylpyrrolidone Vinyl Acetate (neutral) ⇨ **25% active PVP VA64 SDD** 

SDDs dosed to beagle dogs
(n=4), fasted
Dose: 50 mg
Dosing vehicle: 0.5% Methocel A4M in H_2O, 15 ml water rinse

Key belinostat attributes:

- High amorphous solubility in biorelevant media (>500 µg/mL).
- Amorphous solubility is impacted by the presence of polymer.
- Dissolution rate is a key driver for absorption and differs depending on SDD formulation and testing method.

Stewart, A. *et al.* Mechanistic Study of Belinostat Oral Absorption from Spray Dried Dispersions. *J. Pharm. Sci.* (2018).

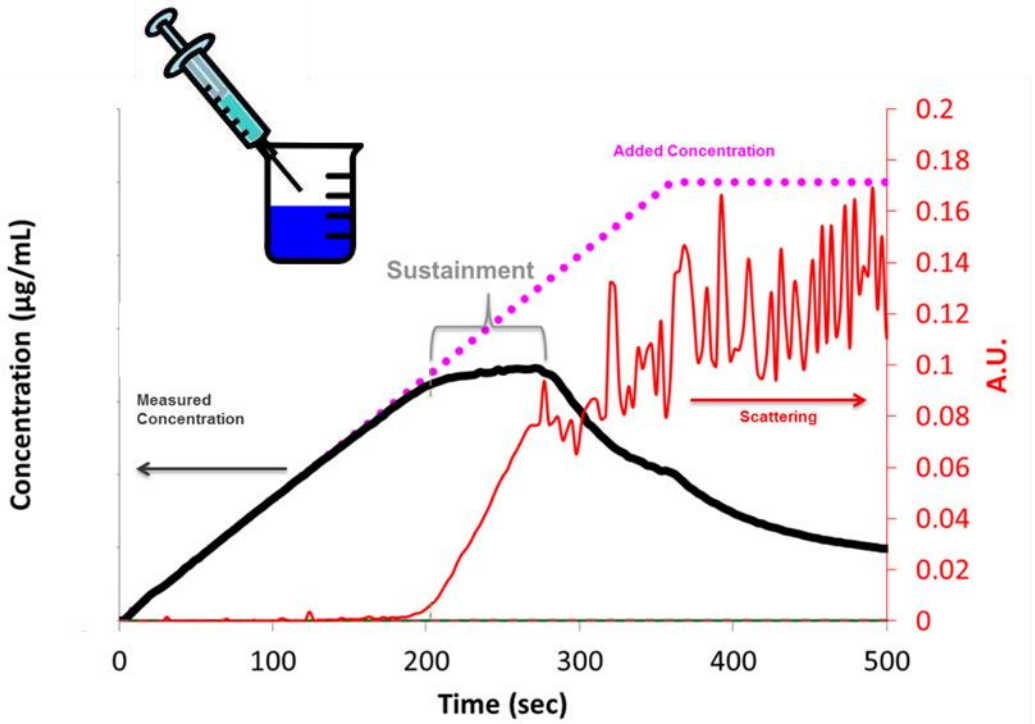
Belinostat apparent amorphous solubility depends upon dispersion polymer type

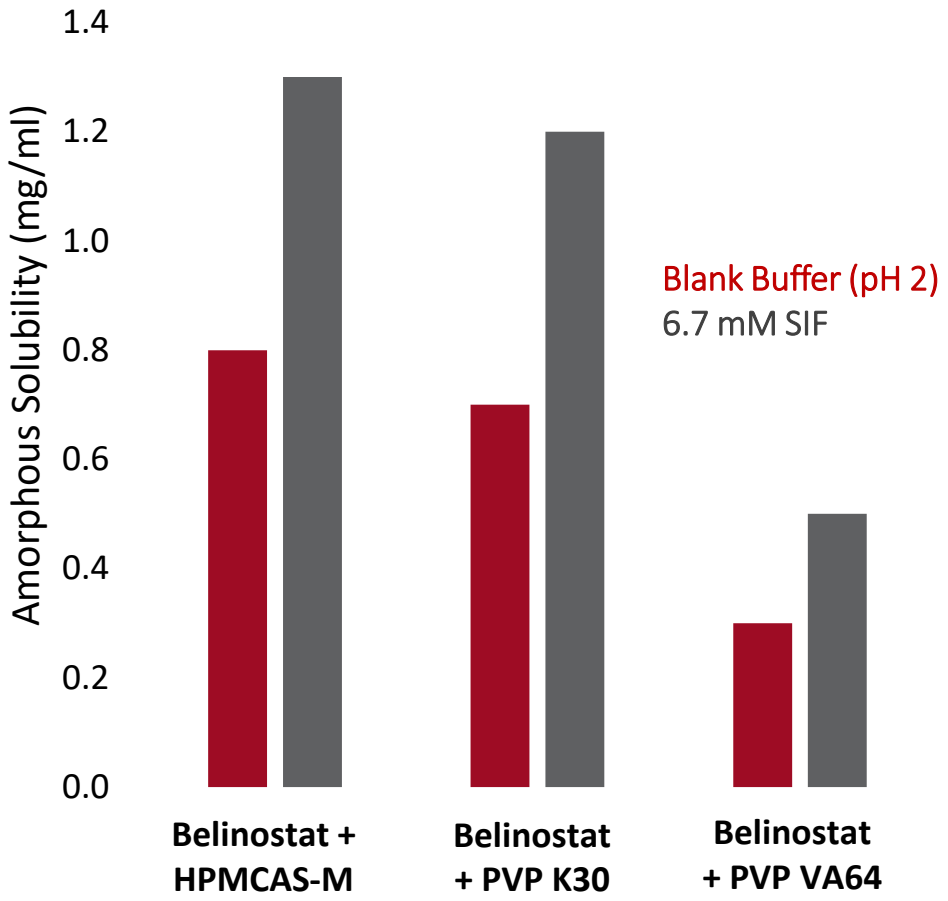
Belinostat

BCS II/IV

pK_a = ≥8 (acidic)

LogP < 2





Amorphous solubility is defined as the onset of amorphous liquid-liquid phase separation. Presence of polymer influences the LLPS concentration.

Dimensionless numbers can predict impact of solubility, permeability or dissolution rate *in vivo* for belinostat

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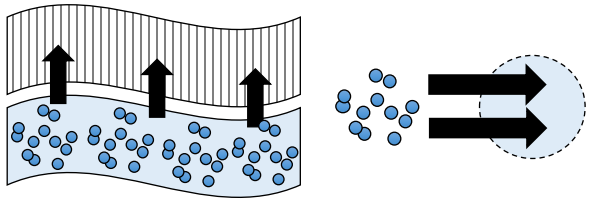
BCS Ref: Amidon, G.L., et al. *Pharm Res.* (1995), 12 (3), 413-420

FaCS Ref: Sugano, K., et al., J Pharm Sci. (2015), 104, 2777-2788

Dose Number

$$Do = \frac{Dose/_{Vol}}{C_s}$$

Solubility-permeability limited

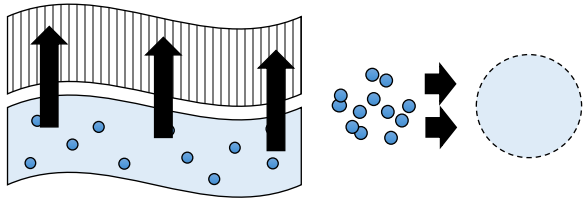


$$Pn/Do < Dn \ \& \ Do > 1$$

Dissolution Number

$$Dn = k_{diss} \cdot t_{abs}$$

Dissolution-limited **Belinostat SDDs in dogs**

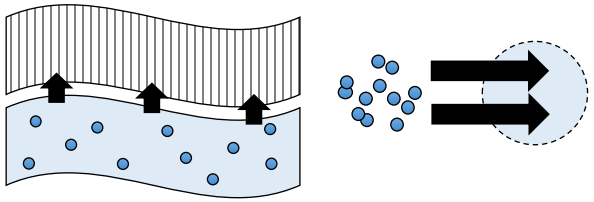


$$Dn < Pn/Do$$

Permeation Number

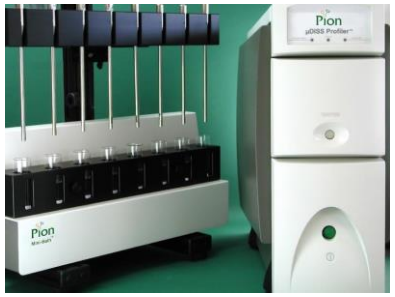
$$Pn = k_{abs} \cdot t_{abs}$$

Permeability-limited



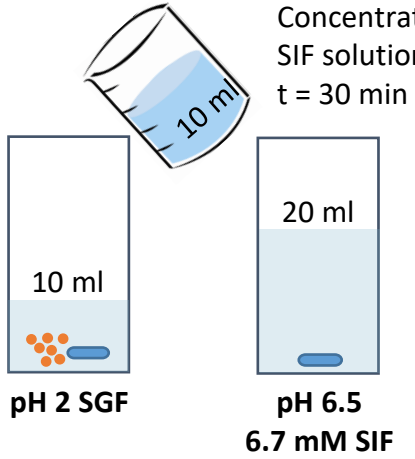
$$Pn < Dn \ \& Do < 1$$

Evaluate belinostat dissolution performance using pH transfer test versus single medium test



In situ fiber optic detection

Gastric transfer test (pH 2 SGF → 6.5, 6.7 mM SIF)

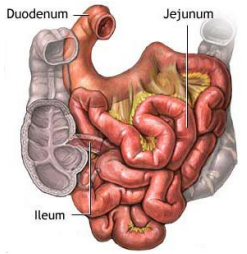


Non-sink Dose: 1000 µg/mL in SGF

Intestinal pH test (pH 6.5, 6.7 mM SIF)

Non-sink Dose: 2000 µg/mL in SIF

In vivo



source: daviddarling.info

Assumes:
- Fasted state
- 50 mL gastric volume
- 50 mL intestinal volume

Dose/Volume/Solubility:	*In vitro* Gastric	*In vitro* Intestinal	*In vitro* Intestinal	*In vivo* Gastric	*In vivo* Intestinal
HPMCAS-M SDD	1.3	0.4	1.5	1.3	0.8
PVP K30 SDD	1.4	0.4	1.7	1.4	0.8
PVP VA64 SDD	3.3	1.0	4.0	3.3	2.0

Test design should be optimized towards the anticipated dose number and conditions *in vivo*.

Relative extents of dissolution between SDDs depends upon dissolution medium composition

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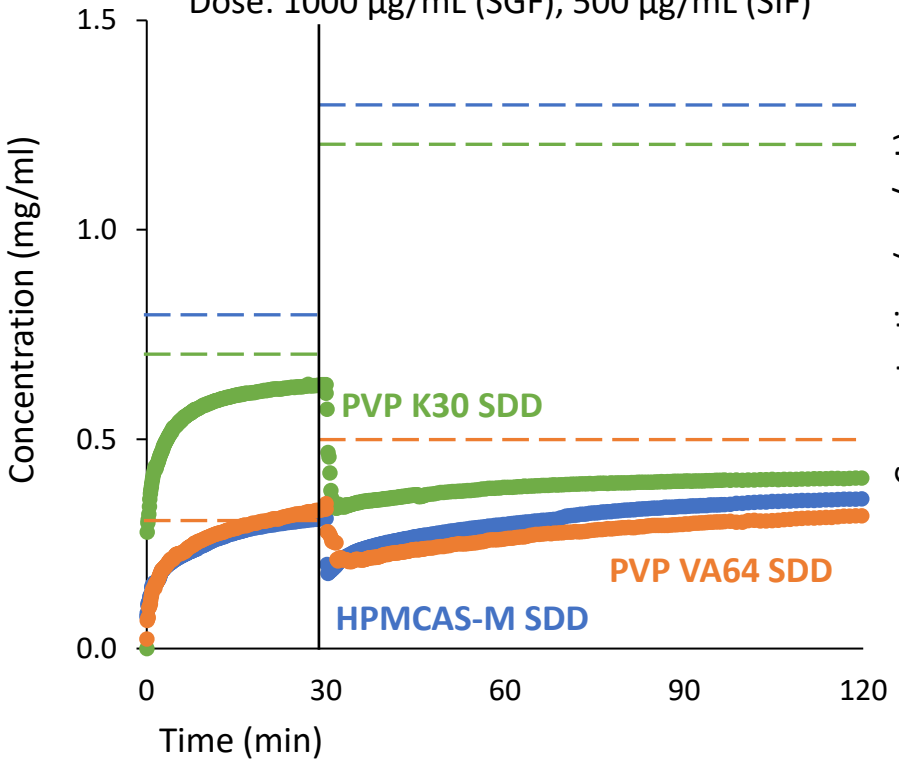
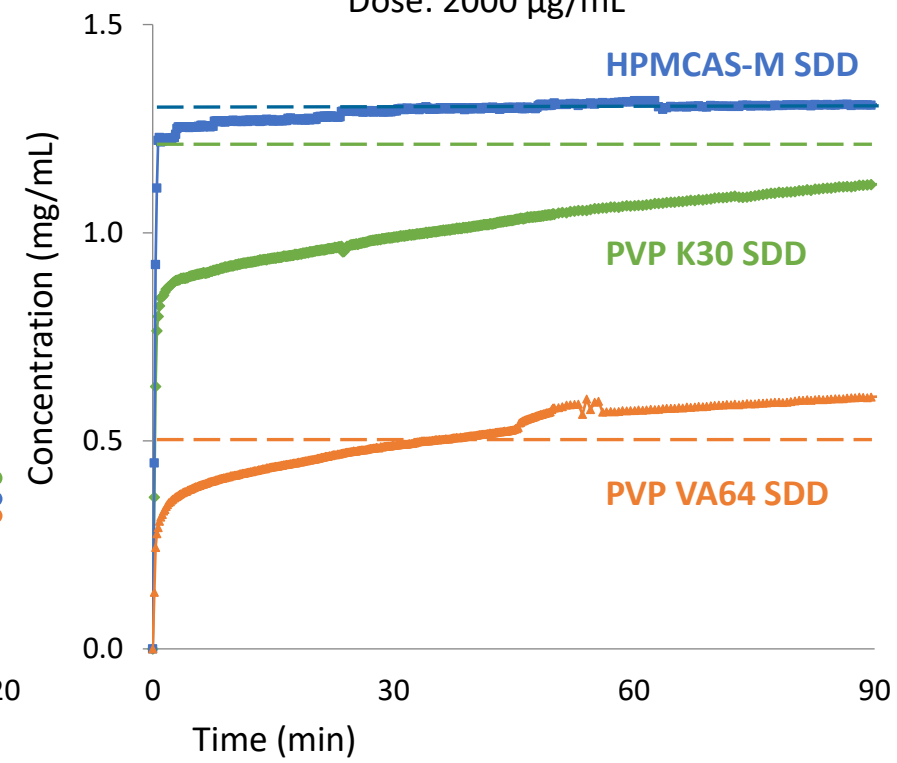
Gastric transfer (pH 2 SGF → 6.5, 7 mM SIF)
K30 SDD > M SDD ≈ VA64 SDD
Dose: 1000 µg/mL (SGF), 500 µg/mL (SIF)



Intestinal pH test (pH 6.5, 7 mM SIF)
M SDD > K30 SDD > VA64 SDD
Dose: 2000 µg/mL



Dashed lines represent the apparent amorphous solubility measured in SGF and SIF from the amorphous solubility assay (slide 30)

Gastric → intestinal transfer test better rank orders SDDs with respect to *in vivo* performance in dogs

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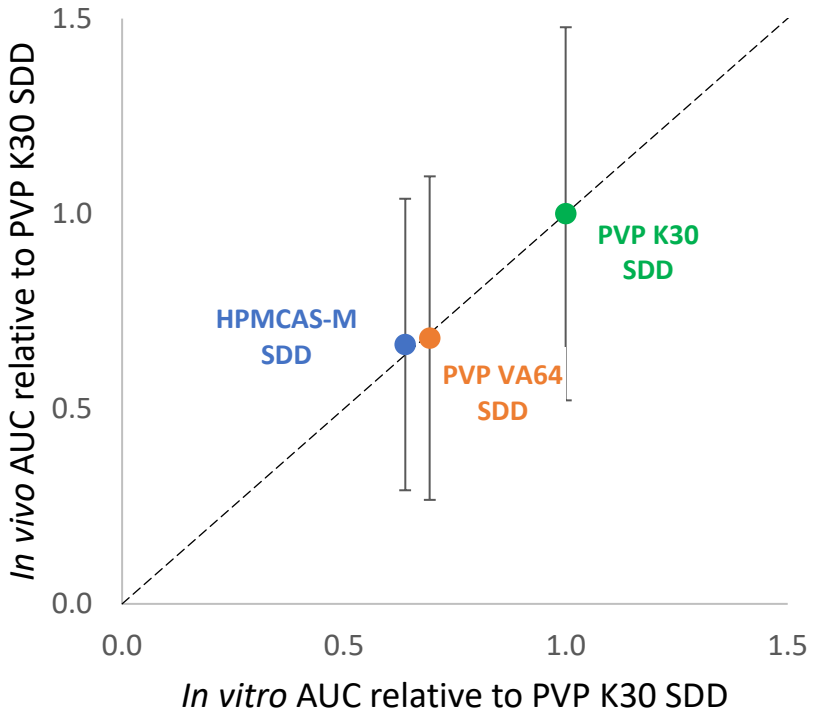
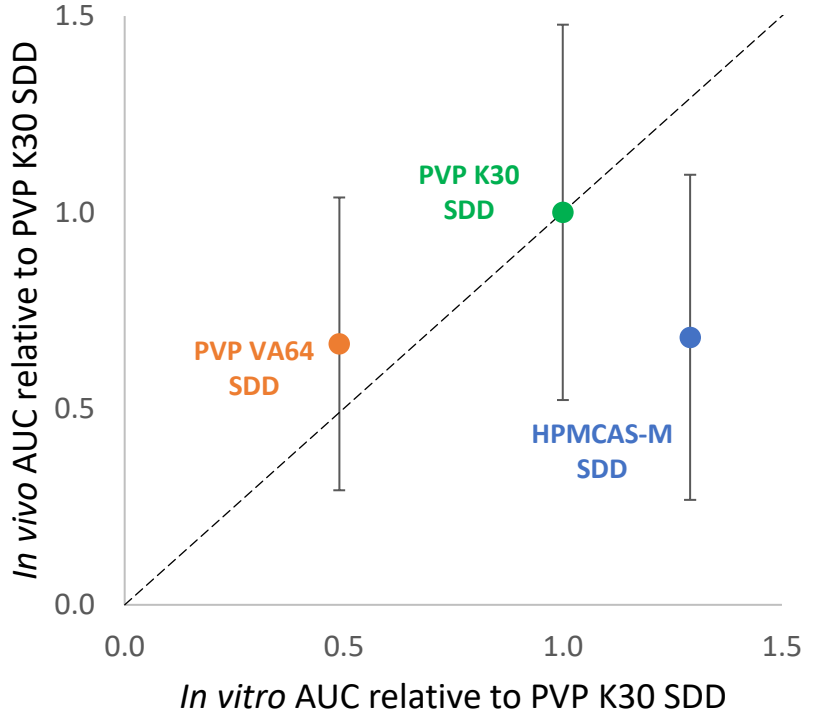
Gastric transfer (pH 2 → pH 6.5, 7 mM SIF)



Intestinal pH test (pH 6.5, 7 mM SIF)



Sequential exposure to SGF and SIF at a more relevant dose/volume/solubility (dose number) is a better indicator for rank-ordering *in vivo* exposure from each SDD.

Using amorphous solubility and dissolution data as key inputs to absorption model supports hypothesis of dissolution rate limited absorption

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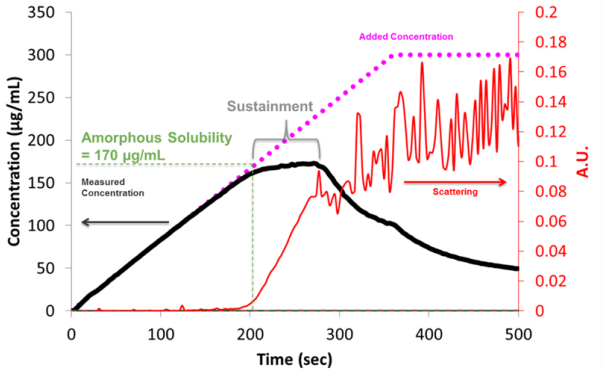
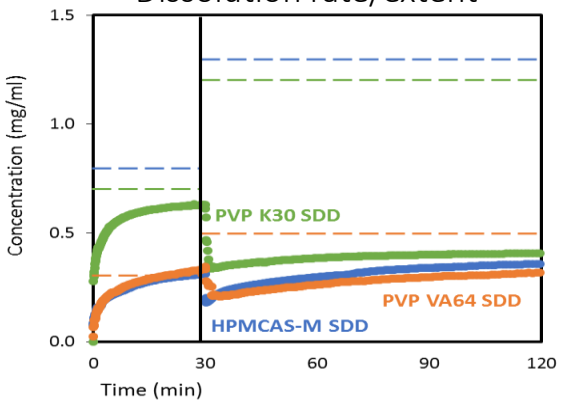
In vitro inputs to model

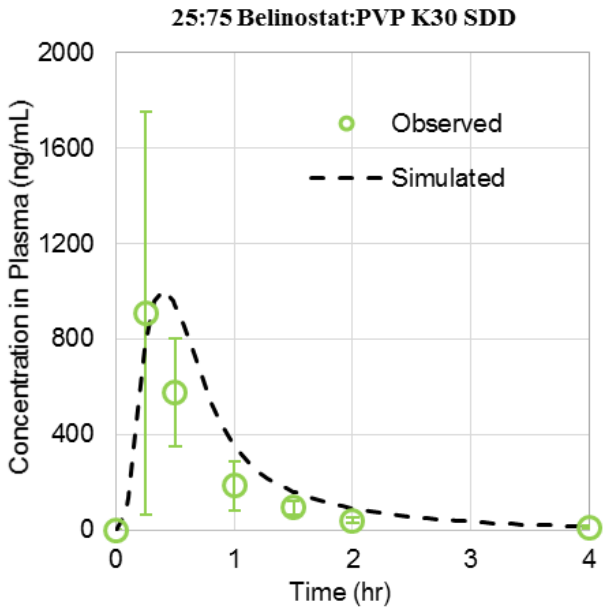
Amorphous solubility

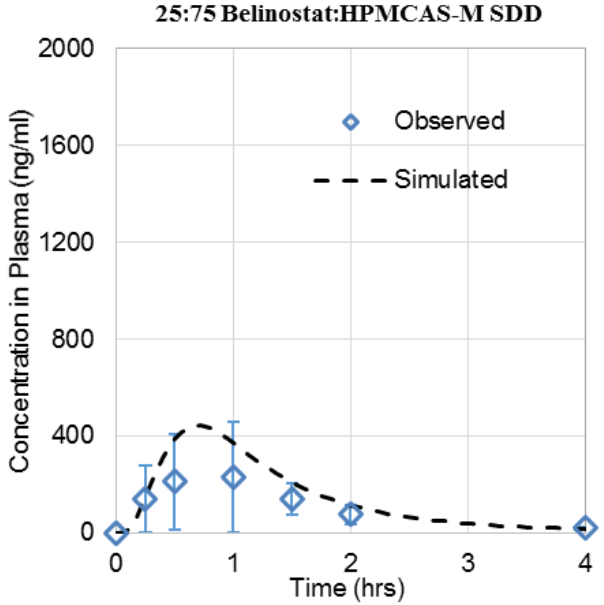


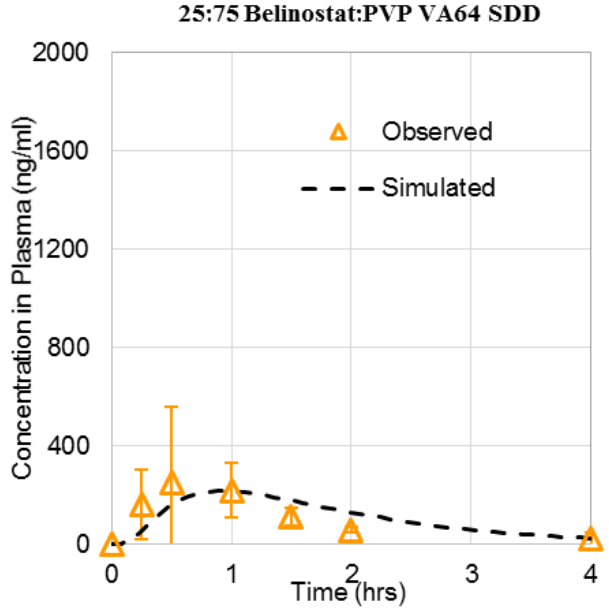
Dissolution rate/extent



In silico predictions







Belinostat Case Study

Conclusions

- Amorphous solubility of belinostat depends on polymer type.
- SGF/SIF transfer test a better indicator of *in vivo* performance.
- Used *in vitro* inputs to describe blood plasma profiles via absorption modeling.
- Rate-determining step to absorption: dissolution rate and extent achieved in the stomach prior to transit down the GI tract.

- Key in vitro performance tool: **solvent shift amorphous solubility + fiber optic probe dissolution**

Summary

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- The need for enabling technologies for improving oral bioavailability is not going away, and is likely going to increase moving forward.
- There is an industry driver to continue to develop an understanding of how to characterize and use in vitro data for supersaturating BAE formulations.
- With careful considerations for in vitro test methodology and design, we can bridge the gap from every compound being a “research project” to a platform-minded in vitro approach for BAE formulations in general.
- This is enabled through mechanistic understanding of bioperformance from BAE formulations independent of platform technology.
    - What is the rate limiting step to absorption?
    - What formulations characteristics will address this rate limiting step?
    - What in vitro tools do we have that can evaluate BAE formulation based characteristics?
    - In vivo relevance?

Acknowledgements

Lonza Pharma & Biotech colleagues

- Michael Grass, PhD
- Deanna Mudie, PhD
- Ian Yates
- Michael Morgen, PhD
- David Vodak, PhD
- David Lyon, PhD

Contact Us