In Vitro Test Methodologies for Characterizing Bioavailability Enhancing Formulations

Aaron Stewart
Associate Principal Scientist, Global R&D
Talk Outline

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:
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

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

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?
<table>
<thead>
<tr>
<th>Technology</th>
<th>Dissolution Rate Limited</th>
<th>Permeability Limited</th>
<th>Solubility and Permeability Limited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt, Polymorph, Cocrystal</td>
<td>X</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Nanocrystals</td>
<td>XX</td>
<td></td>
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<tr>
<td>Amorphous</td>
<td>XX</td>
<td>X</td>
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</tr>
<tr>
<td>Lipids</td>
<td>XX</td>
<td>X</td>
<td>XX</td>
</tr>
</tbody>
</table>

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**

**Basic property prediction**
- 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

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Dimensionless Numbers

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

- **Outputs:**

  - **Dose Number**
    \[ Do = \frac{Dose}{Vol} \cdot C_s \]
    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?

Fraction Absorbed Classification System (FaCS)

Three Limiting Cases

**Dose Number**
\[
D_o = \frac{Dose}{Vol} \cdot C_s
\]

**Permeation Number**
\[
P_n = k_{abs} \cdot t_{abs}
\]

**Dissolution Number**
\[
D_n = k_{diss} \cdot t_{abs}
\]

Case 1: Dissolution Rate Limited (DRL)

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

Fraction Absorbed Classification System (FaCS)  
Three Limiting Cases

Dose Number

\[ Do = \frac{Dose}{Vol} \cdot C_s \]

Permeation Number

\[ Pn = k_{abs} \cdot t_{abs} \]

Dissolution Number

\[ Dn = k_{diss} \cdot t_{abs} \]

Case 1: Dissolution Rate Limited (DRL)

\[ Dn < Pn/Do \]

Case 2: Permeability limited (PL)

\[ Pn < Dn \quad \text{and} \quad Do < 1 \]

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

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

Fraction Absorbed Classification System (FaCS)
Three Limiting Cases

Dose Number
\[ Do = \frac{Dose}{Vol} \]
\[ D_n = k_{diss} \cdot t_{abs} \]

Permeation Number
\[ P_n = k_{abs} \cdot t_{abs} \]

Dissolution Number

Case 1: Dissolution Rate Limited (DRL)
\[ D_n < P_n/Do \]
Cases where this occurs:
• High permeability relative to dose and solubility
• Dissolution rate is slow

Case 2: Permeability limited (PL)
\[ P_n < D_n & Do < 1 \]
Cases where this occurs:
• Dissolution rate is fast relative to permeability
• Dose is low relative to solubility

Case 3: Solubility-permeability limited (SL)
\[ P_n/Do < D_n & Do > 1 \]
Cases where this occurs:
• Low permeability relative to dose and solubility
• Dose is high relative to solubility

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

<table>
<thead>
<tr>
<th>Problem Statement</th>
<th>Formulation mechanism</th>
<th>In Vitro toolkit</th>
<th>In Vitro parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation</td>
<td>Supersaturating formulations</td>
<td>Dissolution – permeation</td>
<td>Fluid composition, volume</td>
</tr>
<tr>
<td></td>
<td>pH driven supersaturation</td>
<td>Controlled Transfer</td>
<td>A/V, volume(s)</td>
</tr>
<tr>
<td>Dissolution Rate Limited</td>
<td>Initial particle size</td>
<td></td>
<td>Fluid transfer rates</td>
</tr>
<tr>
<td></td>
<td>Dissolution rate changes over time</td>
<td></td>
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</tr>
<tr>
<td>Solubility-Permeability Limited</td>
<td>ABL limited</td>
<td></td>
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<td></td>
<td>Epithelial limited</td>
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<tr>
<td></td>
<td></td>
<td>2. Select <em>in vitro</em> dissolution apparatus</td>
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<td></td>
<td></td>
<td>3. Choose <em>in vitro</em> test parameters</td>
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</tbody>
</table>
Multiple Problem Statement-specific Bioperformance In Vitro Tools Using Fiber Optics

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

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

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

- Dissolution rate
- Precipitation rate vs. emptying rate
- Gastric precipitation
- “Book-end” for formulation performance
Amorphous Solubility and Polymer Screening

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
Pion UV Probe Dissolution

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

![Disso Rate](image1)

![Precipitation](image2)

![Extent](image3)

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Membrane Flux

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

Donor Compartment

Receptor Compartment

Concentration

Dissolution limited
Aq. Boundary layer limited
Membrane limited

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Measured Flux is a Sum of Resistances

**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**

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

![Theoretical Drug Concentration Transferred to Duodenum](image)

- Erlotinib
- Erlotinib w/ 200μg/mL HPMC
- Total Concentration in Duodenum (Model)

**Solid Dosage Form Scale**

**Intermediate Scale**

Drug movement: gravity + peristalsis

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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

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
Case Study 1: Itraconazole
Case study - Amorphous spray dried dispersions (SDDs) of Itraconazole (ITZ) dosed to rats

Itraconazole
BCS II base
\( pK_a = 3.7 \)
\( c\text{LogP} = 6.3 \)

Hydroxypropyl Methylcellulose Acetate Succinate (HPMCAS)

Formulations dosed to rats
Sprague-Dawley (n=6), fasted
Dose: 50 mg/kg
Dosing vehicle: 0.5% Methocel A4M in H\(_2\)O
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 \textit{in vitro}.

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

**BCS**  

**FaCS**  

**Dose Number**  
\[ D_0 = \frac{Dose}{Vol} \times C_s \]

**Dissolution Number**  
\[ D_n = k_{diss} \times t_{abs} \]

**Permeation Number**  
\[ P_n = k_{abs} \times t_{abs} \]

**Solubility-permeability limited**  
\[ P_n/Do < D_n \& Do > 1 \]

**Dissolution-limited**  
\[ D_n < P_n/Do \]

**Permeability-limited**  
\[ P_n < D_n \& Do < 1 \]
Itraconazole is highly solubilized in micelles and colloids.

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

- Large undissolved Solids (>10 µm) ~400 µg/ml
- Drug/Polymer Colloids (~200 nm) ~580 µg/ml
- Micelle-bound Drug ~20 µg/ml
- Unbound Drug ~0.1 µg/ml

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

- Dissolution limited
- ABL limited
- Membrane limited

Itraconazole = ABL Limited Flux
Hydrophilic SDD has the highest flux *in vitro*

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

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 be described by a steady state diffusion model

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

\[ D_{\text{eff}} = D_u \cdot f_u + D_m \cdot f_m + D_c \cdot f_c \]

\( C_{u,m,c} \) is the sum of all species:

- **Dc** = \( 4 \times 10^{-8} \) cm\(^2\)/s (200 nm)
- **Dm** = \( 1 \times 10^{-6} \) cm\(^2\)/s (7 nm)
- **Du** = \( 5 \times 10^{-6} \) 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 \( D_{\text{eff}} \) 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
Case Study 2: Belinostat
**Case study - SDDs of belinostat dosed to dogs**

**Belinostat**

- BCS II/IV
- $pK_a = \geq 8$ (acidic)
- LogP $< 2$

**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.

Belinostat apparent amorphous solubility depends upon dispersion polymer type

Belinostat
BCS II/IV
pKₐ = ≥8 (acidic)
LogP < 2

Blank Buffer (pH 2)
6.7 mM SIF

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

**BCS**

**FaCS**

---

**Dose Number**

\[
Do = \frac{Dose}{Vol} \div C_s
\]

**Dissolution Number**

\[
Dn = k_{diss} \cdot t_{abs}
\]

**Permeation Number**

\[
Pn = k_{abs} \cdot t_{abs}
\]

**Solubility-permeability limited**

\[Pn/Do < Dn \& Do > 1\]

**Dissolution-limited**

\[Dn < Pn/Do\]

**Permeability-limited**

\[Pn < Dn \& Do < 1\]
Evaluate belinostat dissolution performance using pH transfer test versus single medium test

In vitro fiber optic detection

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

- Add Concentrated SIF solution at t = 30 min

<table>
<thead>
<tr>
<th>Dose/Volume/Solubility</th>
<th>In vitro Gastric</th>
<th>In vitro Intestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMCAS-M SDD</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>PVP K30 SDD</td>
<td>1.4</td>
<td>0.4</td>
</tr>
<tr>
<td>PVP VA64 SDD</td>
<td>3.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Non-sink Dose:** 1000 µg/mL in SGF

### Intestinal pH test
(pH 6.5, 6.7 mM SIF)

<table>
<thead>
<tr>
<th>In vitro Intestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
</tr>
<tr>
<td>1.7</td>
</tr>
<tr>
<td>4.0</td>
</tr>
</tbody>
</table>

**Non-sink Dose:** 2000 µg/mL in SIF

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

### In vivo

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

<table>
<thead>
<tr>
<th>In vivo Gastric</th>
<th>In vivo Intestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>3.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*source: daviddarling.info*
Relative extents of dissolution between SDDs depends upon dissolution medium composition

Gastric transfer (pH 2 SGF $\rightarrow$ 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

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.

**In vitro** inputs to model

**In silico** predictions

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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

• 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?
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