Flux Measurements using Pion µFLUX
Solubility/Permeability in Absorption
Biopharmaceutics Classification System (BCS)

HIGH SOLUBILITY
CLASS 1 (some uptake?)
diltiazem    antipyrine
labetolol    glucose
captopril    L-dopa
enalapril    metoprolol
propranolol  phenylalanine

LOW SOLUBILITY
CLASS 2 (lipophilic)
flurbiprofen  ketoprofen
naproxen      desipramine
diclofenac    itraconazole
piroxicam     carbamazepine
phenytoin     verapamil

CLASS 3 (hydrophilic)
famotidine    atenolol
cimetidine    acyclovir
ranitidine    nadolol
hydrochlorothiazide

CLASS 4 (some efflux?)
terfenedine   furosemide
cyclosporine

HIGH PERMEABILITY
LOW PERMEABILITY
pH 1 – 7.5

a  RATE OF DISSOLUTION limits in vivo absorption
b  SOLUBILITY limits absorption flux
c  PERMEABILITY is rate determining
d  No IVIV (in vitro - in vivo) correlation expected

www.fda.gov/cder/guidance/2062dft.pdf
Differential equations for oral drug absorption

\[ \frac{dX_{\text{solid}}}{dt} \approx -k_{\text{diss}} \cdot \text{Dose} \left( 1 - \frac{X_{\text{dissolv}}}{S_{\text{dissolv}} \cdot V_{\text{GI}}} \right) \]

\[ \frac{dX_{\text{perm}}}{dt} = k_{\text{perm}} \cdot X_{\text{dissolv}} \]

Sugano, K. Expert Opin Drug Metab Toxicol 2009, 259-293
Absorption Limited Processes

(A) Dissolution Rate Limited
- Dissolution rate limited absorption (Rate of Dissolution < Rate of Permeation)

(B) Permeability Rate Limited
- Permeability limited absorption

(C) Solubility Limited
- Solubility limited absorption (Amount of Available Material Limits Flux)

Courtesy of Kiyohiko Sugano; Sugano et al, DMPK 2007 (4) 225-254
# Absorption Limiting Steps

<table>
<thead>
<tr>
<th>Limiting Step</th>
<th>Conditions</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Dissolution   | $T_{\text{diss}} > 199\ \text{min}$  
                | $P_{\text{eff}} > 2 \times 10^{-4}\ \text{cm/s}$  
                | $D_{\text{abs}} \gg D_{\text{dose}}$  
                | Mainly refers to particle size, absorbed amount non-proportionally increases with dose |
| Permeability  | $T_{\text{diss}} < 50\ \text{min}$  
                | $P_{\text{eff}} < 2 \times 10^{-4}\ \text{cm/s}$  
                | $D_{\text{abs}} \gg D_{\text{dose}}$  
                | Highly soluble drugs or drugs dosed in solution, absorbed amount proportionally increases with dose |
| Solubility    | $T_{\text{diss}} < 50\ \text{min}$  
                | $P_{\text{eff}} > 2 \times 10^{-4}\ \text{cm/s}$  
                | $D_{\text{abs}} < D_{\text{dose}}$  
                | Saturation occurs, absorbed amount does not increase with dose |

L.X. Yu Pharm. Res. 1999, 16 (12) 1883
Absorption Relates to Flux

Flux Through Membranes
the net number of moles of particles crossing unit area per unit time perpendicular to unit area

\[
\text{Flux} = \frac{dm}{A \cdot dt} = \frac{V \cdot dc}{A \cdot dt}
\]
Flux and Permeability

\[ K_P = \frac{C_{m0}}{C_D} \]

\[ dC_m/dx = \frac{(C_{m0} - C_{mh})}{h} \]

**FICK’S FIRST LAW**

\[ \text{flux} = D_m \frac{dC_m}{dx} \]

\[ = D_m \left[ C_{m0} - C_{mh} \right] / h \]

\[ = D_m K_P (C_D - C_A) / h \]

\[ = P_m (C_D - C_A) \]

\[ K_P = \frac{C_{mh}}{C_A} \]
In Vitro Permeability Setup:
no sink conditions, Aqueous Boundary Layer

DONOR

ACCEPTOR

ABL_DON

ABL_ACC

C

h_ABL

h_ABL

Fiber Optics Advanced Training Course | May 24 - 25, 2016 | Pion Inc. Billerica MA, USA
Copyright © Pion Inc. 2015
Diffusional Resistance of Series is Additive

Effective measured permeability

\[ \frac{1}{P_e} = \frac{1}{P_{ABL}} + \frac{1}{P_m} \]

membrane permeability

Depends on stirring or agitation efficiency and size of the molecule
Limiting Cases

\[ \frac{1}{P_e} = \frac{1}{P_{ABL}} + \frac{1}{P_m} \]

1) \( P_m \gg P_{ABL} \) \quad \Rightarrow \quad P_e \approx P_{ABL}

2) \( P_m \ll P_{ABL} \) \quad \Rightarrow \quad P_e \approx P_m
What is $P_{ABL}$ or $P_{UWL}$

\[
P_m = k_m \cdot \frac{D_m}{h_m}
\]

\[
P_{ABL} = \frac{D_{aq}}{h_{ABL}}
\]
Flux:
dissolution/solubility/permeability

Permeability through membranes combines partitioning and diffusion does not depend on concentration

\[ P_m = k_m \frac{D_m}{h_m} \]

\[ P_e = P_m \left( \frac{1}{1 + \frac{P_m}{P_{ABL}}} \right) \]

FLUX
depends on dissolution rate, solubility and permeability

\[ Flux = \frac{V}{A} \frac{dc}{dt} = P_e \cdot c_D(t) \]
Absorption Phase of PK Profile

Determined by FLUX of the API through membranes

\[ Flux = \frac{V}{A} \frac{dc}{dt} = P_e \cdot c_D(t) \]

\[ \frac{1}{P_e} = \frac{1}{P_m} + \frac{1}{P_{ABL}} \]


Fiber Optics Advanced Training Course | May 24 - 25, 2016 | Pion Inc. Billerica MA, USA
Why Dissolution Study May Not Predict In Vivo Data?

What Limits the Absorption
µFLUX™ Apparatus

\[
\text{Flux} \quad J(t) = \frac{dm}{A \cdot dt} = P_e \cdot c(t)
\]

Combining benefits of \textit{in situ} concentration monitoring with dissolution-permeability setup
Number of Permeability Chambers:
4 donor/receiver pairs mounted on MB8 platform

Working Volume:
16 - 22 mL

Area of Membrane Holders (Teflon):
14 mm (1.54 cm²)

Stirring:
Magnetic stir bars, individual computer RPM control for all chambers

Permeation Barriers:
  a) Filter supported artificial membranes (PAMPA)
  b) Filter supported cell monolayers (Caco-2, MDCK, etc.)
  c) Size exclusion membranes (dialysis membranes)
  d) Skin or skin sub-layers layers (same as Franz cell systems)
  e) User designed

Temperature Control:
Connection to external water bath
About Membrane: Double-Sink™ PAMPA correlates with GIT

Prediction of Human Jejunal Permeability

\[
\log P_{\text{eff}} = -0.05 + 0.86 \log P_{\text{eff}} \text{ in combo}
\]

\[
\log P_{\text{eff}}^{\text{in combo}} = -\log \left( \frac{1}{P_{125\mu m}^{\text{ABL}}} + \frac{1}{P_m^{(5.0/7.4)}} + \frac{1}{P_{\text{para}}^{R,\phi,\varepsilon/\delta}} \right)
\]

**µFLUX Permeability Experiment**

**Carbamazepine**

**Donor:**
- Prisma™ HT buffer, pH 6.5
- Initial concentration: 70 µg/mL

**Acceptor:**
- Acceptor Sink Buffer (ASB, pH 7.4)

**Membrane:**
- PVDF support (hydrophobic, 0.45 µm pore size)
- GIT lipid (Pion Double-Sink™ Model)

**Permeability Calculation:**

\[
\text{Slope} \times \frac{\text{Vol}}{\text{Area}} / c_D(0) \\
\sim 84 \times 10^{-6} \text{ cm/s}
\]
Case Study #1: Enhanced FLUX

Formulated API ~ 250 µg/mL after 10 min

Solubility Enhancement ~ 42 times

Unformulated API ~ 6 µg/mL after 60 min

Flux 0.939 µg/(min*cm²)

Flux Enhancement ~ 40 times

Flux 0.023 µg/(min*cm²)
Case Study #2: Suppressed FLUX

**Solubility Enhancement ~ 2.5 times**

**Formulated API ~ 950 µg/mL after 0.1 min**

**Unformulated API ~ 400 µg/mL after 30 min**

**Flux Suppression ~ 1.6 times**

**Flux 0.575 µg/(min*cm²)**

**Flux 0.370 µg/(min*cm²)**
μFLUX Experiment: Studying Food Effect

Research Compound

Donor:
Load 67 µg/mL
SIF: 0.2%
Fast Conditions

Flux: 5.9*10^{-2} µg/(min cm²)

Negative Food Effect Found in Animal Studies

500% Solubility

Donor:
Load 67 µg/mL
SIF: 2%
Fed Conditions

Flux: 6.6*10^{-2} µg/(min cm²)

10% Flux Increase
**μFLUX Experiment: Dose Effect**

**Research Compound**

**FLUX**

---

**Ch1, μg/mL vs. hours, (768)**

- **Donor:**
  - Load 67 μg/mL
  - SIF: 0.2%
  - Fast Conditions

- Flux: 5.9*10^{-2} μg/(min cm^2)

**Ch2, μg/mL vs. hours, (768)**

- Flux: 7.6*10^{-2} μg/(min cm^2)

**Solubility Limited Flux**

---

**Ch3, μg/mL vs. hours, (768)**

- **5x DOSE**

- **Donor:**
  - Load 330 μg/mL
  - SIF: 0.2%
  - Fast Conditions

**Ch4, μg/mL vs. hours, (768)**

- Flux: 7.6*10^{-2} μg/(min cm^2)
μFLUX Experiment
Danazol in Buffer (FeSSIF\textsubscript{Blank} pH 5.0)

Permeability of Danazol
\(~411\times10^{-6}~\text{cm/s}\)

\[
\text{FLUX} = \text{Slope} \times \frac{\text{Volume}}{\text{Area}}
\]

FLUX in FeSSIF\textsubscript{Blank}
\(1.0 \times 10^{-4}~\mu\text{g/(cm}^2\text{ s)}\)

Donor Compartment

Acceptor Compartment

0.32 \mu\text{g/mL}
µFLUX Experiment  
Danazol in FeSSIF (Food effect)

Donor Compartment

13 µg/mL

Solubility Enhancement > 40 fold 
Flux Enhancement ~ 5.4 fold 

Human Food Effect: ~4 fold

FLUX in FeSSIF
5.4*10^{-4} µg/(cm^2 s)

Permeability of Danazol in FeSSIF
~42*10^{-6} cm/s

Acceptor Compartment
Danazol:
Dissolution profiles in the donor chambers in FaSSIF-full & FeSSIF-full media at 0.4mg/mL

- Danazol_FeSSIF Full-Rep1
- Danazol_FeSSIF Full-Rep2

Danazol:
Appearance profiles in the acceptor chambers for corresponding donors in FaSSIF-Full & FeSSIF-Full medium.

- Danazol_FeSSIF Full-Rep1
- Danazol_FeSSIF Full-Rep2
- Danazol_FaSSIF Full-Rep1

μFLUX: Positive Food Effect (~5 fold)
Human Food Effect: ~4 fold

μFLUX Experiment Food Effect
Danazol in FaSSIF vs FeSSIF
µFLUX Experiment Food Effect
Gresiofulvin in FaSSIF vs FeSSIF

µFLUX: No Food Effect
Human: No effect or slightly positive

Griseofulvin:
Dissolution profiles in the donor chambers in FaSSIF-full & FeSSIF-full media at 0.6mg/mL

Griseofulvin:
Appearance profiles in the acceptor chambers for corresponding donors in FaSSIF-Full & FeSSIF-Full medium.
Peculiar Supersaturation of Prazosin.HCl

Load 200 µg/mL
Flux of Prazosin HCl at Different Loads

pH 6.5
Amorphous Solubility Limits Flux

Joint Research of Abbvie and Purdue University Scientists


DOI 10.1002/jps.23826
Dissolution/Flux in FaSSIF

Research Compound SGF - FaSSIF transformation after 30 min of the assay

Winner formulation performed best in dog model
Amorphous Solid Dispersions of Meloxicam: Flux

Untreated Meloxicam API Load 105 μg/mL

Soluplus Formulation API Load 134 μg/mL

VA 64 Formulation API Load 108 μg/mL

VA 64/TPGS Formulation API Load 121 μg/mL
How to make sense of complex supersaturating formulations

Flux is not constant!

Area under Concentration-Time Profile in Receiver as Formulation Ranking Parameter
… “findings [of the study] clearly point out the flaws in using solute concentration in estimating solute activity or supersaturation, and reaffirm the use of flux measurements to understand supersaturated systems.”
Acknowledgements

Lab Team

Oksana Tsinman
Ram Lingamaneni
Janki Patel


µDISS Profiler Publications (incomplete)


