Application of drug physicochemical characterisation in drug discovery

A Biopharmaceutical perspective

Dr. Anita Nair
Solubility and Phys. Chem Properties
Site Operation - Analytics Healthcare
01 Introduction

02 Physicochemical aspects in different phases of R&D
   a) Lipophilicity
   b) Solubility

03 Biorelevant dissolution

04 Product design and Manufacturability

05 Summary
Introduction
Introduction to Pharmaceutical Research and Development

The Formulations...

**Drug Substance**
(Active Pharmaceutical Ingredient, API)

► **NCEs:**
'New Chemical Entities'  
- Small Molecules (MW till ~600 g/mol)  
- Usually Synthetically manufactured

► **NBES:**
'New Biological Entities'  
- z.B. Antibodies, proteins  
- Biological Macromolecules  
- Typically manufactured by Biotechnology

**Drug Product**

► **Mostly for oral Application:**  
- Solid: Tablet, Capsule  
- Liquid: Solutions, Suspensions

► **Almost always Injections:**  
- Intravenous  
- Subcutaneous
Introduction to Pharmaceutical Research and Development

The Formulations...

FDA* Approval small molecules (‘NCEs’) vs Biomolecules (‘NBEs’):

NMEs: new molecular entities
BLAs: biologics licence applications
*FDA: U.S. Food and Drug Administration
Drug Discovery Phases

- **Medicinal Chemistry Research**
  - Target selection
  - Compound discovery

- **Drug Development**
  - Preclinical study
  - Clinical study (Phases I, II & III)

- **Marketing/Drug Fostering and Evolution**
  - Application/Approval
  - Marketing
  - Postmarketing Study/Re-examination

- **Key Concepts**
  - Discovery of New Compound
  - Optimization for Commercial Production

Number of compounds screened in drug discovery phases

Amount of compound available for studies
Drug Discovery Phases

Medicinal Chemistry Research → Drug Development → Marketing/Drug Fostering and Evolution

- Target selection
- Compound discovery
- Preclinical study
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Pharmaceutical R&D process chain

- DPLD: Target identification / validation
- DPLO: Lead Discovery hit and lead identification
- DPED: Lead optimisation
- DP0: Exploratory development
- DP1: Pre-clinical development phase 0
- DP2: Clinical development phase 1
- DP3: Clinical development phase 2
- DP4: Clinical development phase 3
- Launch to market "phase 4"

Research → Development
Introduction
The active: pharmacodynamics

Way of the solid drug at oral application

**Formulation properties:**
- excipients, coating
- "immediate release" vs "extended release"

⇒ **Clinical Development**

**Solid state properties** (but also formulation options):
- lattice energy
- wettability
- dissolution kinetics

⇒ **Exploratory Development**

**Intrinsic molecular properties:**
- lipophilicity
- solubility in GI fluids and plasma pH

⇒ **Lead Optimization**
Introduction
The active: pharmacodynamics

Physiological aspects

<table>
<thead>
<tr>
<th>GI tract</th>
<th>Fasted pH</th>
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<tbody>
<tr>
<td>Stomach</td>
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<td>5.0 – 6.5</td>
<td>4.0 – 6.0</td>
</tr>
<tr>
<td>Jejunum</td>
<td>6.5 – 7.0</td>
<td>5.5 – 6.5</td>
</tr>
<tr>
<td>Ileum</td>
<td>7.0 – 7.5</td>
<td>6.8 – 7.5</td>
</tr>
<tr>
<td>Colon</td>
<td>5.5 (proximal) – 7.0 (distal)</td>
<td></td>
</tr>
</tbody>
</table>

Requirement to the API:
Solubility in the GI tract
Permeability

Molecular physicochemical properties:
Solubility
Balance between hydrophilicity / lipophilicity
Ionisation

Only the dissolved API will be absorbed
For absorption API must be permeable
Drug Absorption Process: LADME

**Distribution**

**Elimination**

**Bioavailability**

**Liver**

**Tablet Disintegration**

**Solubility & Dissolution rate**

**Permeability**

**Liberation**

**Absorption**

**Intestine**

**Stomach**

**Elimination**

**Portal vein**

**Gall bladder**

Only dissolved drug is absorbed
The Biopharmaceutics Classification System

Highly Soluble
- Dose to solubility ratio < 250mL.

Highly Permeable
- Permeability ≥85%
Physicochemical aspects in different phases of R&D
Permeability & Permeation Mechanisms

The ability of a drug to pass across biological membrane is defined as drug permeability. Passive diffusion is the major absorption pathway.
Measurement of Permeability

Methods suggested in Biowaiver Guidance*

- In vivo intestinal perfusion studies in human or suitable animal models
- In vitro studies using excised human or animal intestinal tissues
- In vitro permeation across a monolayer or cultured epithelial cells e.g. CaCo-2 cells

NCE Optimization

- Lipophilicity of a molecule relates to intestinal absorption, membrane permeability, protein binding and distribution and can be measured by log P and log D
- Permeability can be improved either by incorporating permeation enhancers in formulations or by optimizing the molecule stucture.
Permeability measurement by in vitro methods

CaCo2 Assay

By measuring the transport rate of a compound across a Caco-2 cell monolayer membrane, the \textit{in vivo} absorption of the compound across the gut wall can be well predicted.

Each well contains an offset apical channel, the apical assist, to guide manual pipette tips. The apical assist channel ends just short of the membrane surface to eliminate the chance of membrane or monolayer disruption while pipetting.
Physicochemical aspects in different R&D phases

- Lipophilicity: partition coefficients log P / log D
Physicochemical aspects in different phases of R&D
Characterisation for Lead optimisation

Lipophilicity
• Partition coefficients \( \log P / \log D \)

<table>
<thead>
<tr>
<th>( \log P )</th>
<th>( \log D_{7,4} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log P = \log \left( \frac{C_{S}^{\text{Octanol}}}{C_{S}^{\text{Water*}}} \right) )</td>
<td>( \log D = \log \left( \frac{C_{S}^{\text{Octanol}}}{C_{S}^{\text{Buffer pH7.4}}} \right) )</td>
</tr>
</tbody>
</table>

- for non-ionisable substances or
- at the isoelectrical point (*): using a buffer based on pKa, in that the compound is not ionised

- (partial) ionisation considered for ionisable substances
- buffer (usually): pH 7.4 (pH in blood)

For ionisable compounds:
Dissociation depending on pK_a-value

\[
\log D_{7,4} = \log P + \log \left( \frac{1}{1+10^{pK_{a}-7.4}} \right)
\]
Physicochemical aspects in different phases of R&D
Partition Coefficient measurement

Shake flask method is a “gold standard” for estimation of partition coefficient

For High Throughput Screening (HTS) HPLC based retention time method is used for lipophilicity estimations

Increased lipophilicity translates to poor solubility
Physicochemical aspects in different R&D phases

- Solubility
important molecular property that influences the intestinal absorption → determines bioavailability
- useful during lead selection and optimization and serves as a screening parameter
- required for biopharmaceutical classification (BCS)
- necessary for salt selection and optimization of formulation
Physicochemical aspects in different phases of R&D
Characterisation for Lead optimisation

**Solubility**

- Solubility product

\[ K_S = \frac{c_{dissolved}}{c_{solid}} = c_{API_{dissolved}} \cdot c_{counter-\text{ion}_{dissolved}} \]

- Energetic contribution

For API salts:
- influence of counterions on solubility
- common-ion effect: presence of ions of the same species can lead to decreased solubility (e.g. Chloride + HCl-salts)

Crystalline API:
- Lattice energy must be overcome
- Generally, higher, the worse the solubility

Amorphous API:
- No (significant) lattice energy
- Solubility dependent on hydration

Polar API:
- Strong hydration (e.g. protonation in pH range of buffer)

Non-polar API:
- Weak hydration

Amphiphilic API:
- Formation of mesophases (micelles)
Physicochemical aspects in different phases of R&D Characterisation for Lead optimisation

**Solubility**
- Determination by shake flask method

Suspension of API in shaking incubator at 37 °C for 24 h

Solubility [µg/mL]

- e.g.: pH-dependent solubility
  - Acid
  - Base

Solubility vs pH graph
Factors affecting Solubility

**Starting material**
Solid (thermodynamic) / Predissolved (kinetic)

**Solid state form**
Crystalline/Amorphous
(Pseudo)Polymorph- solvates, hydrates, salts, co-crystals

**pH of buffer system**
Especially important for answering the question “does it precipitate in vivo?”

**Co-solvent**
not applicable for thermodynamic solubility, DMSO for kinetic solubility

**Temperature**

**Impurities**

**Bile Salts/ Surfactants**
Physicochemical aspects in different R&D phases

- Solubility
- Kinetic v/s Thermodynamic
Physicochemical aspects in different phases of R&D

**Solubility - Starting Material**

**Kinetic Solubility**
- Estimation of solubility of API- DMSO stock solution in pH 7.4 buffer.
- Advantages include use of minimum amount of compound, ensuring quick results with sufficient throughput.

**Thermodynamic solubility**
- Equilibrium solubility estimation on solid compound in a medium.
- It takes comparatively longer.
Physicochemical aspects in different phases of R&D

Why should kinetic solubility not be used for compound optimization???

Generally: kinetic solubility ≥ thermodynamic solubility
Different approaches of solubility measurements can yield different results and has to be interpreted accordingly

<table>
<thead>
<tr>
<th>Batches</th>
<th>Kinetic solubility (pH 7.4) mg/mL</th>
<th>Thermodynamic solubility (pH 7.4) mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.055</td>
<td>0.01 (impurities)</td>
</tr>
<tr>
<td>2</td>
<td>0.045</td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>0.053</td>
<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>0.053</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>0.051</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>0.052</td>
<td>0.002</td>
</tr>
<tr>
<td>7</td>
<td>NT</td>
<td>0.007</td>
</tr>
<tr>
<td>9</td>
<td>NT</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**Batch 1, crystalline**

**Batch 6, partly crystalline**

**Batch 7, partly crystalline**

**Batch 9, crystalline**
Physicochemical aspects in different phases of R&D

Kinetic v/s Thermodynamic solubility

- Does it precipitate? (relevant for assays)
- Starting from predissolved compound (DMSO stock solution)
- Medium: Gen pH 7.4 buffer

- Relevant for
  - Biochemical/Cellular assays, MXS stability, CaCo2
  - Protein-crystal structure

- Does it dissolve?
- Medium: Buffers pH 1-9 or biorelevant media (FaSSIF /FeSSIF)

- Relevant for
  - Bioavailability
  - Feasibility of solid formulations

- Linked to dissolution
  - How fast does it dissolve?
Physicochemical aspects in different R&D phases

- Solubility
- Solid state form
Physicochemical aspects in different phases of R&D

Solid state characterisation

What types of solid state forms exist?

- **Amorphous**
  - missing long range order

- **Polymorphic forms**
  - Different arrangement of one component in the crystal lattice, e.g. due to different conformations

- **Pseudo-polymorphic forms**
  - Assembly of solvents in the crystal lattice (hydrates, solvates)
    - stoichiometric or non-stoichiometric

- **Salts**
  - Assembly of counter-ions in the crystal lattice
    - proton transfer
    - stoichiometric

- **Co-crystals**
  - Assembly of non-ionised co-formers (solids) in the crystal lattice
    - stoichiometric

Physicochemical aspects in different phases of R&D
Solid state characterisation

Solid state selection: Opportunities and risks

Risk in stability: “fall down” in the energy landscape

Beside solubility and stability other points to be considered in solid state selection:
- solid state properties
- robust manufacturability
- achieved purity, residual solvent content
- ...

Physicochemical aspects in different R&D phases

- Solubility
- Bile Salts / surfactants
- pH
**Physicochemical aspects in different phases of R&D**

**Biorelevant Solubility**

**Need:** Solubility in aqueous buffers are not representative of the solubility of API *in vivo*.

**Application:**
- In vivo relevant solubility.
- Useful for NCE optimisation.
- Helps to study food effects
Physicochemical aspects in different phases of R&D
Effect of pka (pKs)

Ionisability

Acid dissociation constant:

\[ K_s = \frac{c^{H^+} \cdot c^{A^-}}{c^{HA}} \quad \text{pK}_s = -\log(K_s) \]

Base dissociation constant:

\[ K_B = \frac{c^{OH^-} \cdot c^{BH^+}}{c^B} \quad \text{pK}_B = -\log(K_B) \]

Henderson-Hasselbalch: ('Buffer equation')

\[ \text{pH} = \text{pK}_s + \log \frac{c^{A^-}}{c^{HA}} \]

**Half of equivalence:** at pH = pKs are 50% of the molecule ionised

Determination by potentiometric titration:
- pH-titration pure buffer und API solution
- increased consumption (= volume titrant) in API solution ~ amount ionised species

**Procedure:**
- Range pH 2-11
- Amount API ≈5 mg
- Using of co-solvent (Methanol) for weakly soluble compounds → extrapolation on 0 % co-solvent

<table>
<thead>
<tr>
<th>pKa₁</th>
<th>pKa₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>8.5</td>
</tr>
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</table>
Physicochemical aspects in different phases of R&D

Effect of pH & solid state form

Why solid state characterisation?
- Absorption of an API is also influenced by solid state properties

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Stability range of salt
- Solution in equilibrium with salt form as solid state
- Solubility limited by solubility product of salt

\[ K_s = [BH^+] \cdot [\text{counterion}^-] \]

Stability range of free base
- Solution in equilibrium with free base as solid state
- Increasing solubility with gradual ionisation

\[ S = S_0 \left(1 + \frac{[H^+]}{K_a}\right) \]

\[ \mu_{\text{salt}} = \mu_{\text{base}} \]
Physicochemical aspects in different R&D phases

- Solubility & Dissolution
Physicochemical aspects in different phases of R&D

**Solid state characterisation**

**Why solid state characterisation?**
- Absorption of an API is also influenced by solid state properties

**Dissolution**
- kinetically controlled
  \[ \frac{dc}{dt} = A \cdot D \cdot \frac{c_s - c}{d} \]
  - specific surface area
  - concentration gradient

**Solubility**
- thermodynamically controlled
  \[ \mu_{\text{solid}} = \mu_{\text{dissolved}} \]
  - chemical potential:
    - lattice energy
    - hydration energy

- Example:
  - e.g. \( \sim 1 \) h
  - e.g. \( \sim 24 \) h
Physicochemical aspects in different phases of R&D

Dissolution and methods of determination

*Dissolution* is the rate at which the solid substance dissolves in a medium.

Compendial dissolution apparatus namely basket, paddle and flow through apparatus are commonly used to evaluate the dissolution rate of drug substances and formulations.

**Salient features**
- Working volume ranges from 100-1000 ml (min vol. 250mL)
- Sample size 50 – 1000 mg or depending on the dose
Applications of Dissolution

- **Quality Control tool**
  Evaluate Batch to Batch variability

- **Input data for PBPK in silico models** *(Transfer model)*

- **In vitro in vivo correlation (IVIVC)*

- **Surrogate tool for BE testing**
  Biowaivers or SUPAC/Variations

- **Identify critical manufacturing variables**
  - Effect of excipients and process.

- **Tool to evaluate formulation strategy**
  Enabling formulation design
  Screening tool for salts / formulations
Potential scenarios of precipitation

- Immediate Precipitation
- 1st order Precipitation
- No Precipitation

% Dissolved vs. Time in min

- Stomach
- Intestine

supersaturation
Potential factors affecting supersaturation and precipitation along GI Tract

<table>
<thead>
<tr>
<th>Gastric Emptying</th>
<th>GI Volumes</th>
<th>GI pH Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile salts and other surfactants</td>
<td>Permeability</td>
<td>Precipitate characteristics</td>
</tr>
<tr>
<td>Solubility</td>
<td>Formulation design</td>
<td>GI Hydrodynamics</td>
</tr>
<tr>
<td>Disease states</td>
<td>Drug interaction</td>
<td></td>
</tr>
</tbody>
</table>
The transfer model* is a physiologically relevant two stage modified USP dissolution method for drug substances and formulations.

This can be used to examine the GI supersaturation and precipitation of compounds.


In vitro considerations:

- Gastric Emptying
- GI pH Profile
- GI Volumes
- Formulation design
- Bile salts and other surfactants
- GI Hydrodynamics
## Physiologically relevant dissolution parameters

<table>
<thead>
<tr>
<th>In vivo parameter</th>
<th>In vitro parameter</th>
<th>Set up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastric emptying</strong></td>
<td>Transfer rate</td>
<td>peristaltic pump, Zero order (2.5mL/min - 9mL/min) or First order transfer rate (9min⁻¹)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>pH</td>
<td>Stomach: Simulated gastric fluid (pH 2.0)</td>
</tr>
<tr>
<td>stomach: 1.6; intestine: 6.5 (fasted)</td>
<td>Buffers to reflect in vivo conditions</td>
<td>Intestine: Maleate/ Phosphate buffer (pH 6.5)</td>
</tr>
<tr>
<td><strong>Bile salts</strong></td>
<td>Bile salts</td>
<td>Biorelevant media*</td>
</tr>
<tr>
<td>HGF, HIF (Taurocholate, Lecithin)</td>
<td>Biorelevant media to reflect in vivo conditions</td>
<td>FaSSIF (pH 6.5)</td>
</tr>
<tr>
<td><strong>Volume of fluids</strong></td>
<td>Volume of fluids</td>
<td>Donor: 250 mL (50mL + 1 glass of water)</td>
</tr>
<tr>
<td>Stomach: 50 mL Intestine: 100-500 mL</td>
<td>Volumes of biorelevant media to reflect physiological conditions and to maintain sink conditions</td>
<td>Acceptor: 350 / 500 mL</td>
</tr>
<tr>
<td><strong>Hydrodynamics</strong></td>
<td>Hydrodynamics</td>
<td>Rpm: 75 rpm / 100 rpm</td>
</tr>
<tr>
<td>Gastric / intestinal peristaltic</td>
<td>Paddle speed</td>
<td></td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>Temperature of media</td>
<td>37°C ± 0.5°C</td>
</tr>
<tr>
<td>37°C</td>
<td></td>
<td></td>
</tr>
</tbody>
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**IVISIVC: In vitro-in silico-in vivo Correlation**

In Drug Discovery several *in vitro* and *in silico* tools are used to understand the *in vivo* behavior of the compounds and formulations.

**Dissolution & Solubility**

- **In vitro**
- **In Silico**
- **In vivo**

**Prediction models are as good as the input data**

- GastroPlus®
- PK Sym®
- Sym Cyp®
- Stella® ...
Applications
Better PBPK input parameter than dissolution in single media

Fasted state plasma profile predictions of Arlevert® (A) and Stugeron® (B) tablets using the dissolution-only and supersaturation and precipitation (obtained from transfer experiments at a rate of 3 h−1) PBPK models.**

Applications

Comparison of Formulations

Plasma concentration time profiles for the active metabolite of Albendazole (Albendazole-sulphoxide) in rats dosed as: a) 45 mg/kg b) 4.5 mg/kg lipid type IIIa-solution and d) 45 mg/kg HPβCD-solution. Data are given as mean ± SEM (n = 6).§

Transfer profiles presented as the percent of dose in solution versus time for 50 mg of Albendazole formulated as b) lipid type IIIa-suspension and d) HPβCD-solution given as mean profiles ± SD (n = 3). The dashed line represents the equilibrium solubility for each figure. §
What kind of information can a transfer experiment provide?

**Discovery**
API behavior with respect to supersaturation and precipitation. Absorption model input data for Gastroplus

**Development**
Influence of supersaturation, precipitation and precipitate, formulation screening

**Clinical trials**
*In vitro in vivo* correlation and PBPK modeling

Complexity of the *in silico* model
Product Design and Manufacturability
Selection of process depends on the product design and properties of material used. Biopharmaceutical assessment provides the information needed to select an appropriate solid form, excipients and process.

Summary

- Dissolution and permeability of a drug substance are the two rate determining steps for drug absorption.
- Thermodynamic solubility assessment across the GI pH and in biorelevant media provides relevant input data for bioavailability predictions.
- Solubility of a compound is dependent on several factors like crystallinity, polymorphism, presence of solvents, temperature, etc. Hence, calculated solubility values cannot completely capture the solubility behavior of a compound.
- Formulation strategies that enhance dissolution behavior of API can improve the fraction of drug absorbed on oral administration.
Thank you for your attention

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