

FDA Workshop on „Current State and Future Perspectives of Translational Modeling
Strategies to Support Drug Product Development, Manufacturing Changes and Controls“
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***In vitro* approaches to understanding supersaturation and precipitation of weak bases and enabling formulations**

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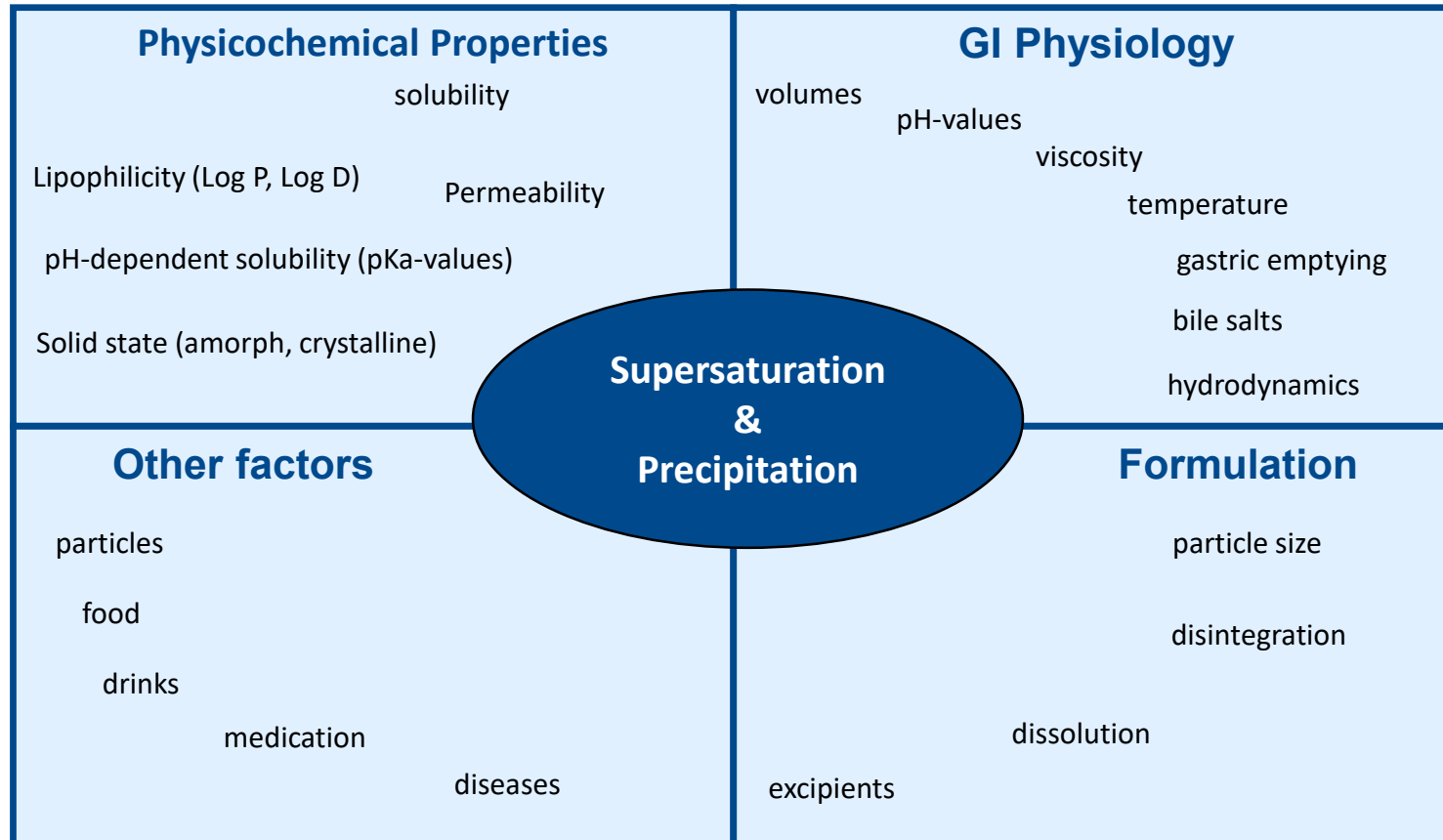
Overview

- Why do we need to consider the supersaturation and precipitation characteristics for enabling formulations and weak bases
- Which experimental parameters are needed in an *in vitro* model.
- Presentation of different small and full scale *in vitro* models
- Limitation of the *in vitro* models and why PBPK model can improve translating the *in vitro* findings to an *in vivo* setting
- Guidance for the use of the different models:
 - OrBiTo decision tree (IR formulations)

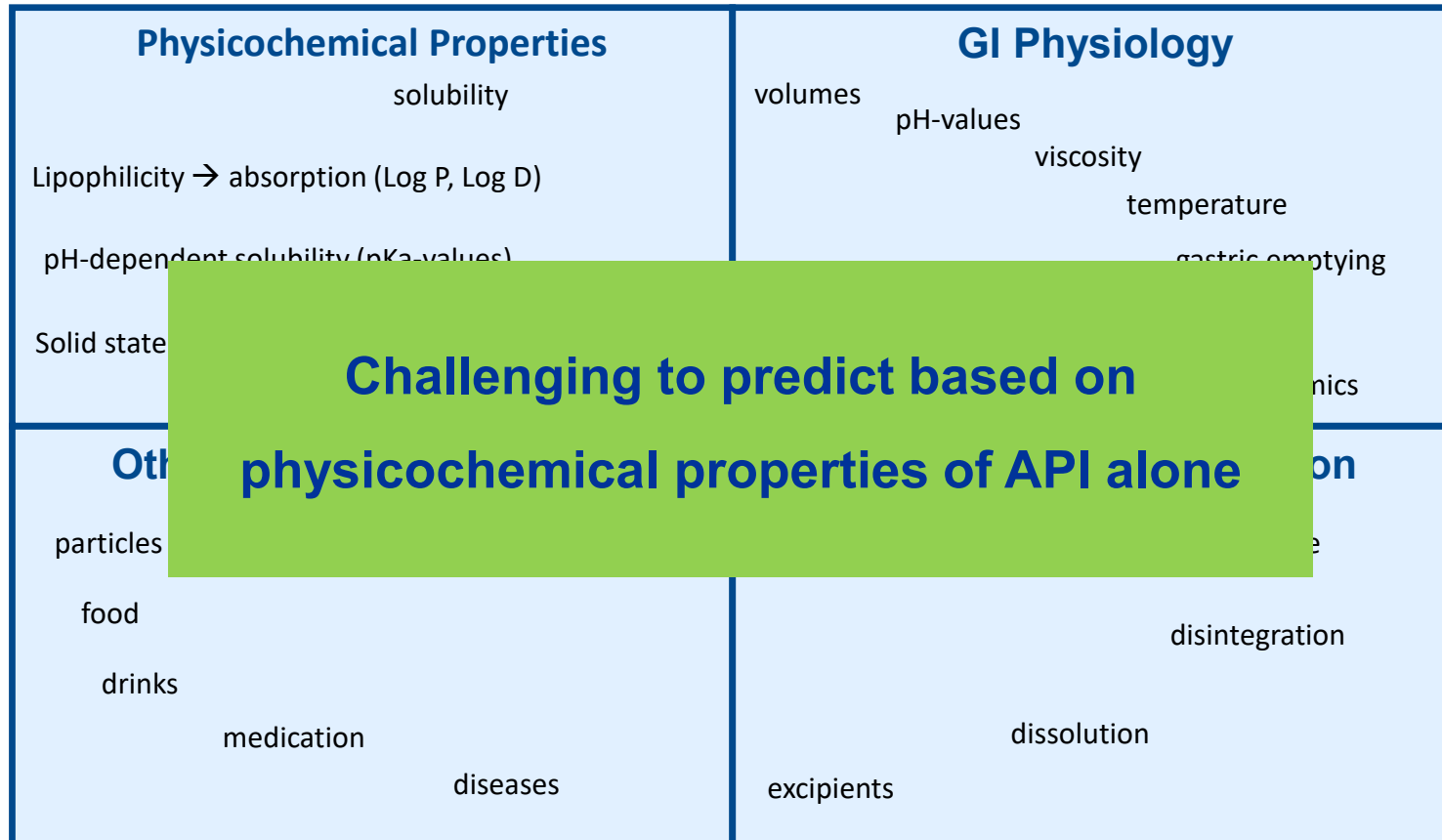
Considerations for poorly soluble drugs

- Many drug candidates are poorly soluble which can result in low and variable absorption
- Supersaturation of the drug in the GI tract is an option to overcome solubility limitations to improve bioavailability
 - salts, co-crystals, solid dispersions etc....
 - pH gradient between stomach and duodenum may induce supersaturation for basic drugs
- Drug in supersaturated state is thermodynamically unstable leading to precipitation in the GI tract
 - different excipients can have a significant effect on the degree of supersaturation and also how long precipitation can be prolonged
- The supersaturation and precipitation behaviour of the drug/formulation needs to be evaluated

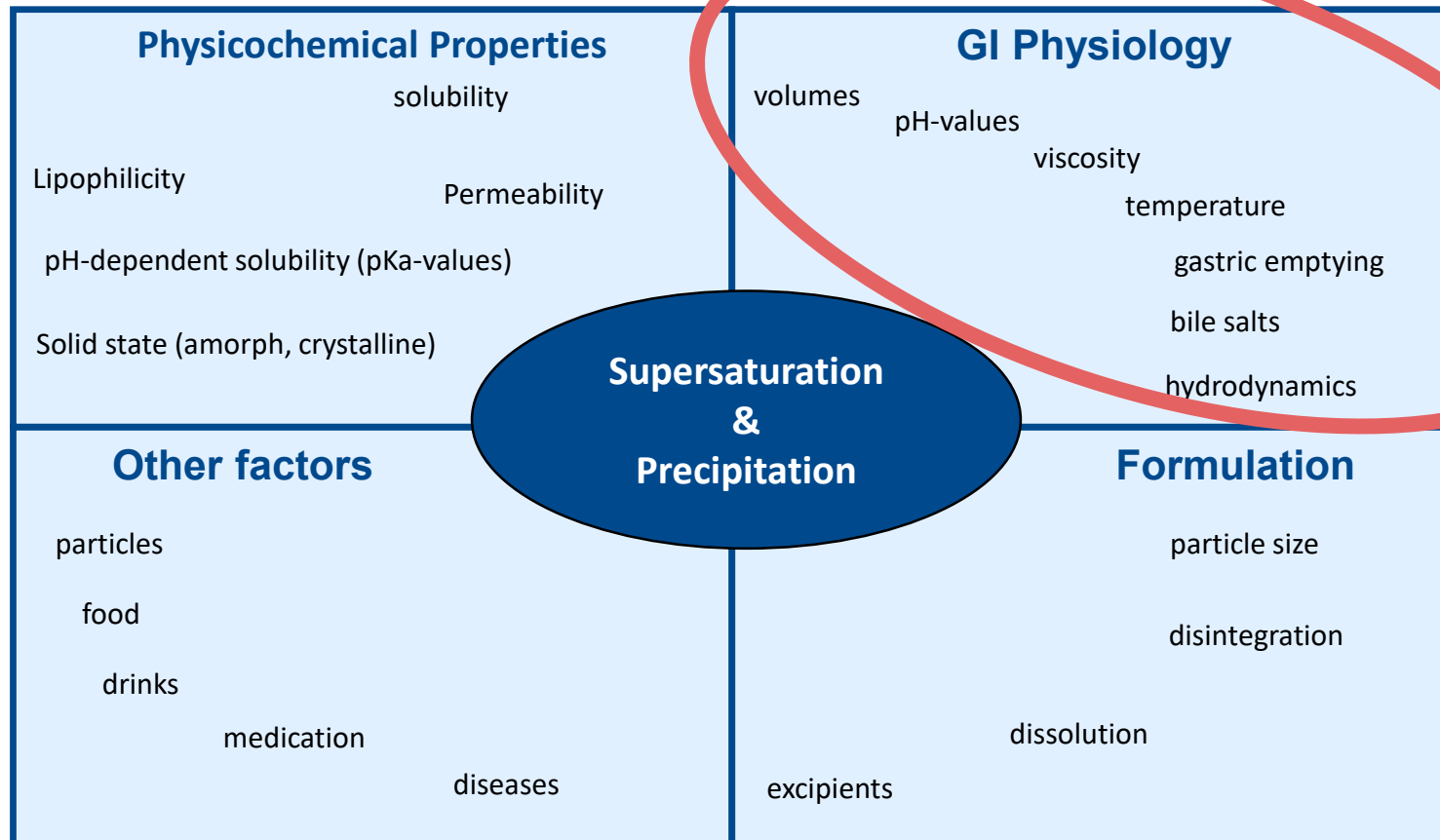
Parameters influencing supersaturation and precipitation



Parameters influencing supersaturation and precipitation

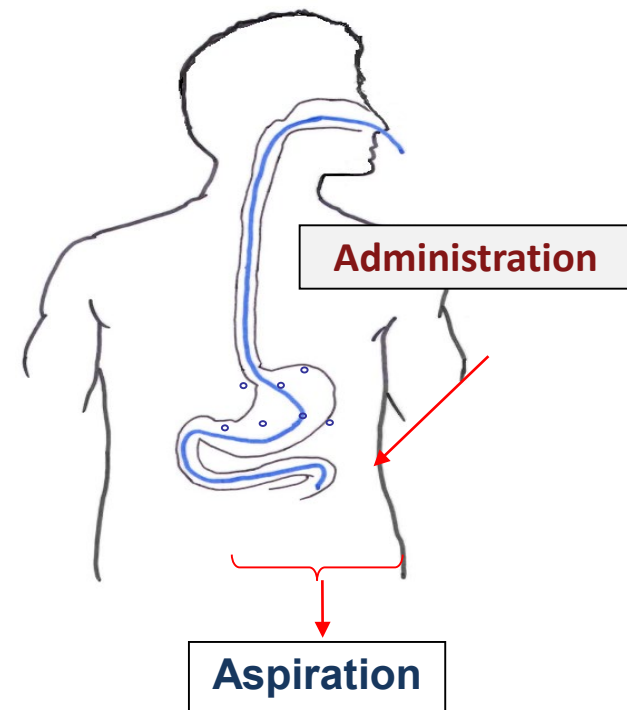


Parameters influencing supersaturation and precipitation



In vivo assessment of GI supersaturation and precipitation

- Limited data available evaluating GI luminal supersaturation and precipitation and its impact on oral absorption
 - Indirect assessment using plasma profiles
 - Gastrointestinal concentration profiling – “Intubation method”
 - introduction of catheter and positioning along proximal intestine to allow drug administration and concurrent collection of intestinal fluids
 - provides insight into luminal supersaturation and precipitation assessment
 - not a viable option for routine use during formulation development
- requirement for predictive tool



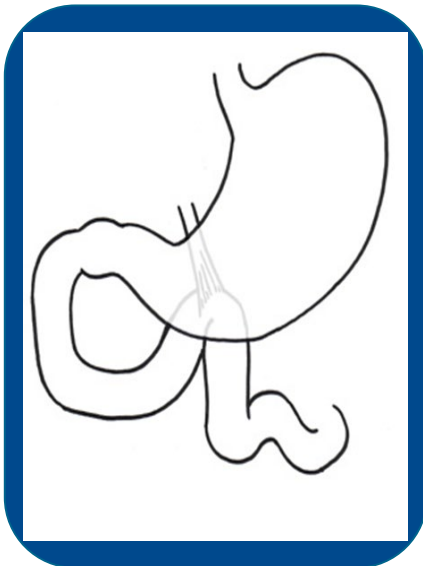
Psachoulias, Pharm Res, 2012, 29: 3486

Psachoulias et al. (2012)

In vitro evaluation of GI supersaturation and precipitation



- Important to assess supersaturation and precipitation not only during **early development** stages, but also before **first in human** studies and in **later stages in formulation development**
- For reliable predicting, *in vitro* tools need to capture the **relevant GI physiological** parameters
- A range of *in vitro* tools are currently available to assess supersaturation and precipitation



Small Scale

Large Scale

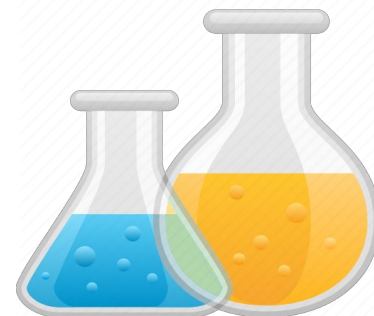
Small scale methods

Facilitate use of small quantities of API during early stages of development with limited material and for the evaluation of prototype formulations

1. Single media tests
2. Models with medium/pH shift
3. Two-stage tests
4. Methods addressing intestinal absorption

Small scale methods – single media tests

- Potential drug precipitation can be inferred from comparing **solubility** in simulated gastric with intestinal media (e.g. dose/solubility ratio)
- Comparison of ketoconazole solubility in biorelevant media
 - SGF (418 mg/ml)² (D/S 0.72 ml) versus FaSSIF (0.017 mg/ml)¹ (D/S 17.6 L)
 - *In vivo* finding suggest precipitation (up to 16% at 300 mg)³
- Several high-throughput methods available utilizing 96 well plate with either optical imaging or turbidimetric spectroscopic methods
 - sample applied by solvent casting (removal of solvent) or as stock solution (e.g.. DMSO, PEG400) with potential addition of a particular medium
- Chandran utilized high-throughput spectrophotometric approach for evaluating precipitation inhibition for solubilized formulations⁴
 - 17 β -estradiol dissolved in various excipients (PEG400, PVP, TPGS) and aliquot added to 96-well together with deionized water and absorbance at 500 nm
 - Absorbance increase as a function of time to reveal precipitation



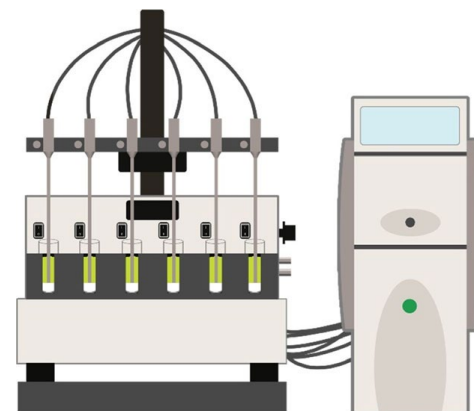
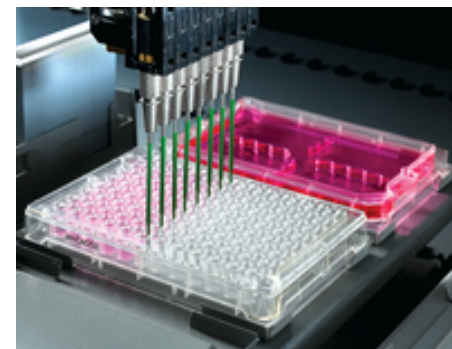
¹Markopoulos, EJPB, 2015,93:173; ²Alsenz, JPS, 2007, 96:1748

³Psachoulias, Pharm Res, 2011, 28: 3145; ⁴Chandran, J Pharm Biomed Anal, 2011, 56:698

Small scale methods – Medium (solvent) shift

Use of solvent shift (DMSO stock to a medium) to investigate supersaturation and precipitation

- Yamashita¹ prepared concentrated drug (ITZ) in DMSO solutions, diluted with FaSSIF in 96 well tray and used HPLC/UV to evaluate precipitation and precipitation inhibitors
- Petrusevska² utilized light scattering (nephelometer) and or turbidity (UV plate reader at 500 nm) in 96 well plate to evaluate potential of precipitation inhibitors (eg. for dipyridamole and fenofibrate from DMSO stocks in buffer containing various excipient concentrations)
- μ Diss Profiler™ (Pion) commercially available automated device using UV fibre optics to obtain real-time information on solubility and dissolution (including supersaturation and precipitation)
 - Palmlund³ setup standardised method where 200 μ L DMSO stock added to 10 ml FaSSIF³ in mini-vessels with micro-paddles and in situ baseline UV spectrum for 60 min
 - Shift in baseline UV spectrum inferring precipitation (e.g.. Albendazole, aprepitant, fenofibrate..)



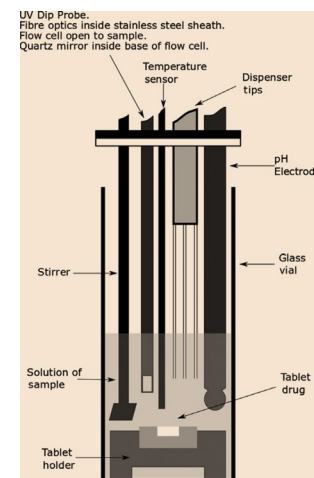
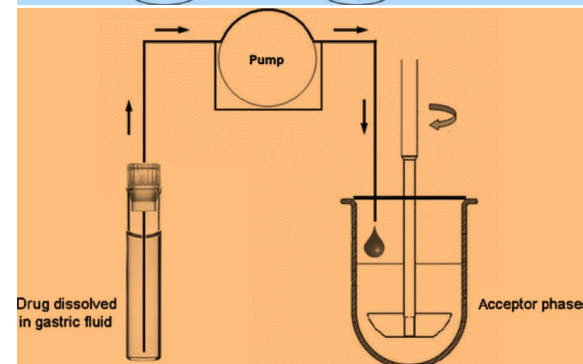
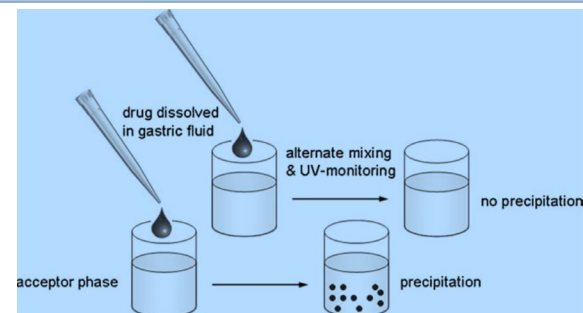
Small scale methods – Medium and pH shifts

Experimental methods enabling pH to be altered or the transfer of drug from the stomach to intestine to be simulated

- Klein¹ evaluated the transfer of SFG to FaSSIF/FeSSIF using either a 96-well plate system (30 μ l \rightarrow 170 μ l) or a mini-paddle device (10 ml \rightarrow 40 ml) for both itraconazole and tamoxifen
 - both methods showed consistent results for no precipitation for tamoxifen and significant precipitation (90%) for itraconazole
- Sirius T3 instrument (Pion Inc.) is an automated titration system utilizing UV fibre optic probe to obtain real-time drug concentration measurements
 - drug concentration profile as a function of pH² (e.g. 1.2 up to 7.2) or through the addition of intestinal media to stomach media³ to show precipitation or lack thereof for different poorly soluble weak bases (e.g. DPM)

¹Klein, AAPS PharmSciTech 2012; 13:1230; ²Gravestock, Anal Methods 2011; 3:560

³Jakubiak, Mol Pharm 2016; 13:586



Evaluate impact of intestinal absorption

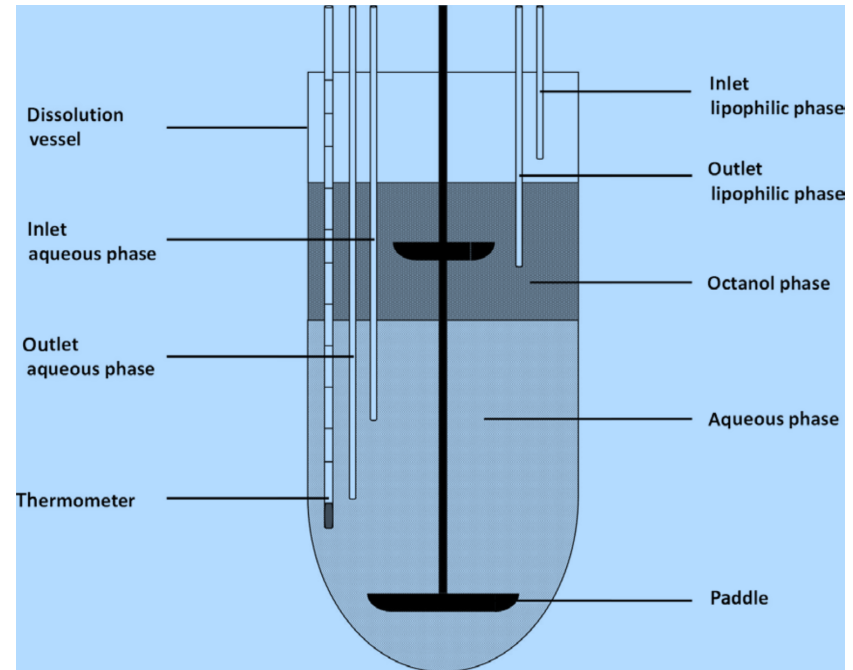
– „Biphasic“ system

- Given the impact of absorption on resulting luminal drug concentrations, methods have been used to include an “absorption” step to facilitate drug partitioning from aqueous donor to organic layer (i.e. sink conditions)
 - two immiscible phases comprising of a lower aqueous (dissolution) and upper organic phase (drug partitioning)
- Solvents used in the past include octanol, cyclohexane/octanol, chloroform, nonanol, nonanol/cyclohexane, hexane¹
 - octanol used most often given its more suitable characteristics
 - completely immiscible and less dense than water, reduced volatility at 37°C

¹Pestieau, EJPS, 2017 10: 203

Biphasic“ system – miniscale biphasic dissolution model with pH shift (miBldi-pH)

- small scale biphasic dissolution test utilizing a pH shift to evaluate drug release and precipitation
- Frank¹ used the method to predict *in vivo* precipitation for different weak bases (e.g. DPM).
 - miniaturized USP II apparatus (50 ml media covered with 15 ml octanol), pH elevated in media, analysis using online UV spectroscopy
 - precipitation extent (35%) closer to *in vivo* (7%) than single phase (90%)
- Absorptive sink dependant upon the drugs partition coefficient between dissolution medium and organic solvent, and what is the influence of drug contact with solvent on dissolution
 - whilst absorption captured, need to consider drugs *in vivo* absorption behaviour for interpretation of findings

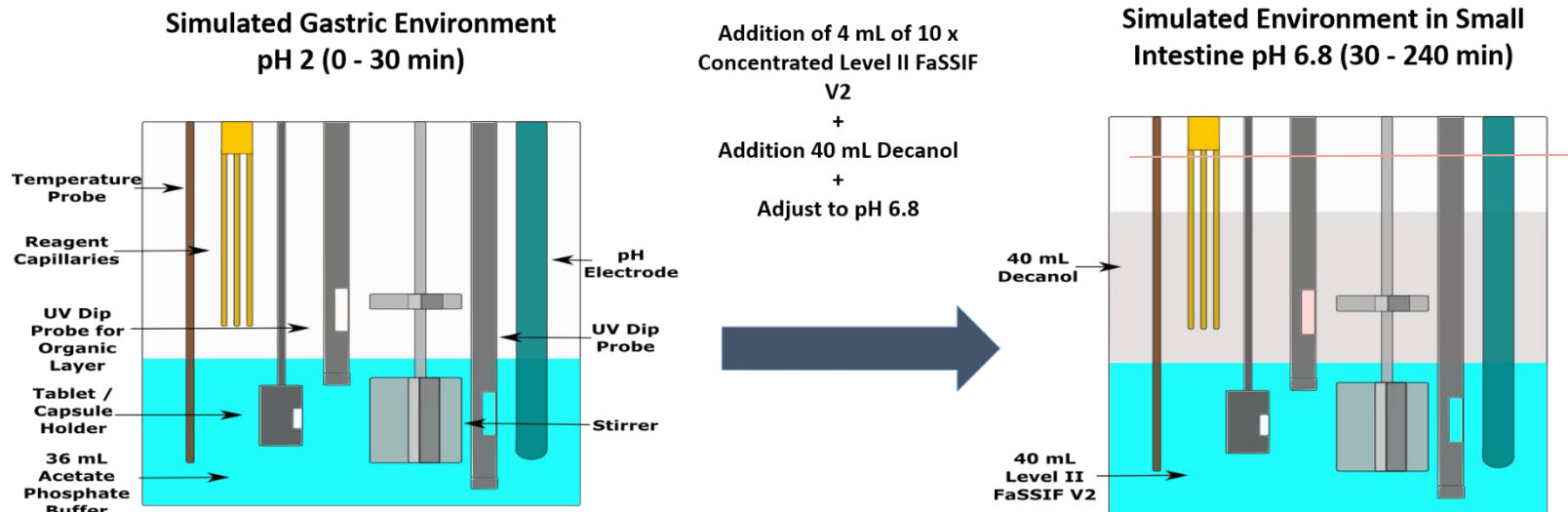


¹Frank EJPS, 2014; 61:32

Evaluate impact of intestinal absorption

– „Biphasic“ system

- InForm (Pion) is a commercially available apparatus for conducting biphasic dissolution experiments
 - fibre optic UV probe to measure “real-life” concentration and potentiometric pH probe to facilitate pH control
 - O’Dwyer recently utilized this method to evaluate supersaturation and precipitation for DPM and KTZ by beginning experiment in gastric media followed by subsequent addition of conc. FaSSIF-V2 and decanol to act as absorption sink
 - experimental precipitation rates and critical supersaturation concentration data for dipyridamole and ketoconazole provided good *in vivo* simulation using Simcyp



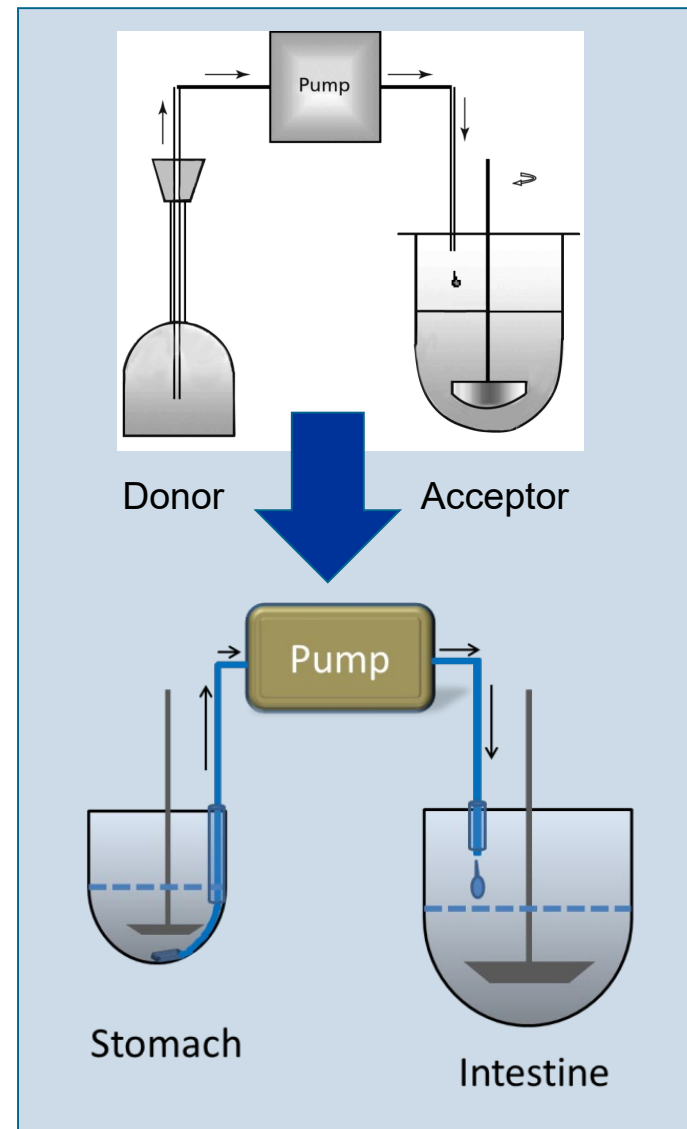
O’Dwyer, APS PharmSci 2019

Full-scale methods to evaluate supersaturation and precipitation

- These methods take into account relevant GI fluid volumes which enable the evaluation of the drug product/formulation at clinically relevant doses after oral administration
- A range of equipment/models available based on compendial UPS dissolution and non-compendial apparatuses
 1. Transfer model
 2. Artificial Stomach Duodenal
 3. Gastrointestinal simulator
 4. BioGIT
 5. Artificial membrane insert system
 6. Simplified “two-stage” method

Closed system “Transfer model”

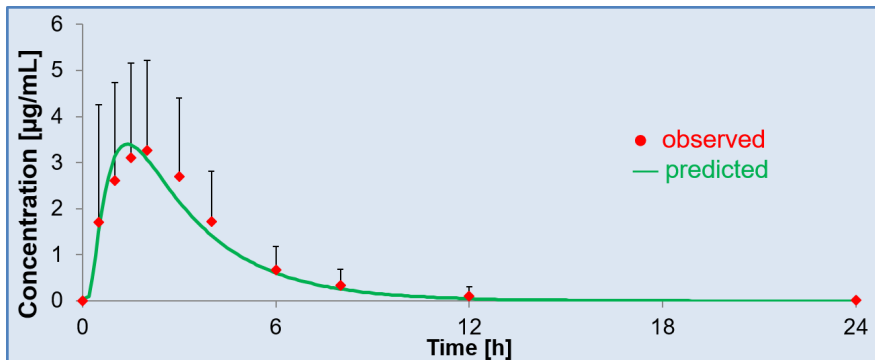
- One of the first attempts simulating the transfer of drug from the stomach to intestine using standard dissolution equipment¹
 - importance of hydrodynamics, transfer rates and media composition (fasting and fed on supersaturation and precipitation)
- Updated by Ruff² in 2017 to include newer media composition, physiologically relevant GI volumes and 1st order gastric emptying rates, disintegration and dissolution
- Concentration time profiles in the acceptor compartment enable precipitation rates to be established and comparison with solubility to establish degree of supersaturation
 - HPLC, variation with inline Raman spectroscopy to monitor precipitation³
- Since a closed system, precipitation overestimated overcome by coupling to PBPK modelling



¹Kostewicz JPP 2004; 56:43, ²Ruff EJPS 2017; 100:42, ³Arnold JPP, 2004; 63:333

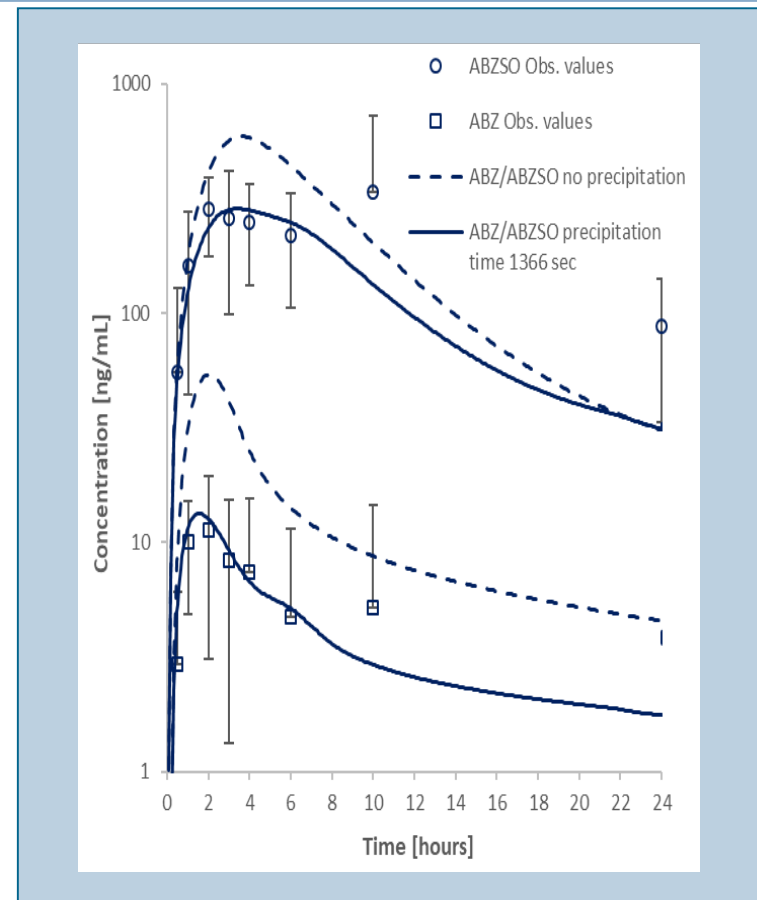
Transfer model – coupling to PBPK modeling

To circumnavigate lack of absorption compartment in TM model and to take into account other physiological variables, *in vitro* data coupled to a PBPK model



Ruff et al. coupled *in vitro* data for Nizoral® (200mg ketoconazole) with Stella® model, plasma KTZ profile was accurately simulated¹

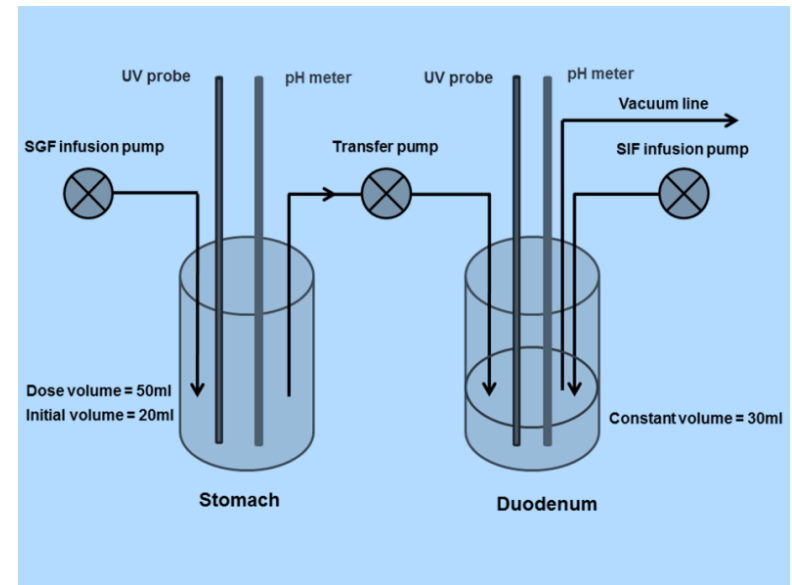
- whilst precipitation *in vitro*, given good permeability, CSC not achieved and hence no precipitation



In vitro transfer model data for Eskazole® (400 mg albendazole) simulated plasma profile better than just using dissolution behaviour using GastroPlus²

Open system – Artificial Stomach Duodenal (ASD)

- Transfer from stomach to duodenum chamber with additional input of stomach and intestinal “secretions” and out-flow from the intestinal chambers
- Continuous evaluation of concentration in both chambers and an investigation of the % solid fraction in duodenal chamber
 - continuous real-time monitoring by pH and UV probe
- Enables dissolution and precipitation to be evaluated
 - success in simulating the relative bioavailability of several drugs (e.g. Carbamazepine, ketoconazole & posaconazole)^{1,2}

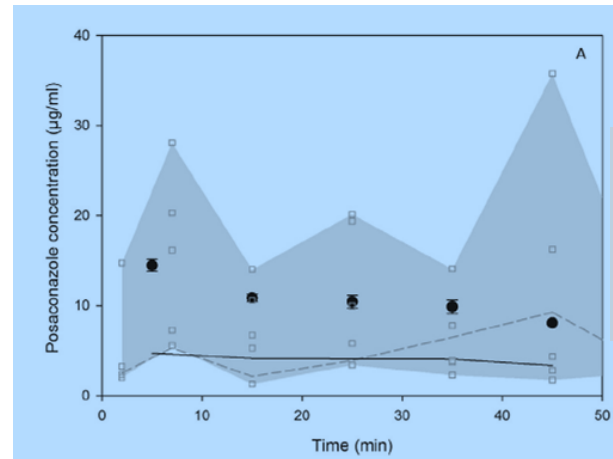
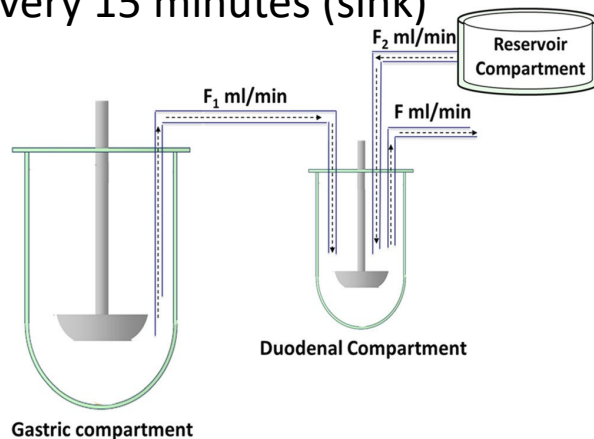


¹Carino JPS 2006; 95:116; ²Butler EJPB 2019; 136:70

Open system – Biorelevant GI Transfer (BioGIT)

- Given impact on intestinal absorption on *in vivo* behaviour, sink conditions can be achieved *in vitro* by altering flow rates in the intestinal compartment
- BioGIT¹ comprises of gastric, duodenal and reservoir compartment containing biorelevant media

- First-order gastric emptying, duodenal compartment renewed with fresh medium every 15 minutes (sink)



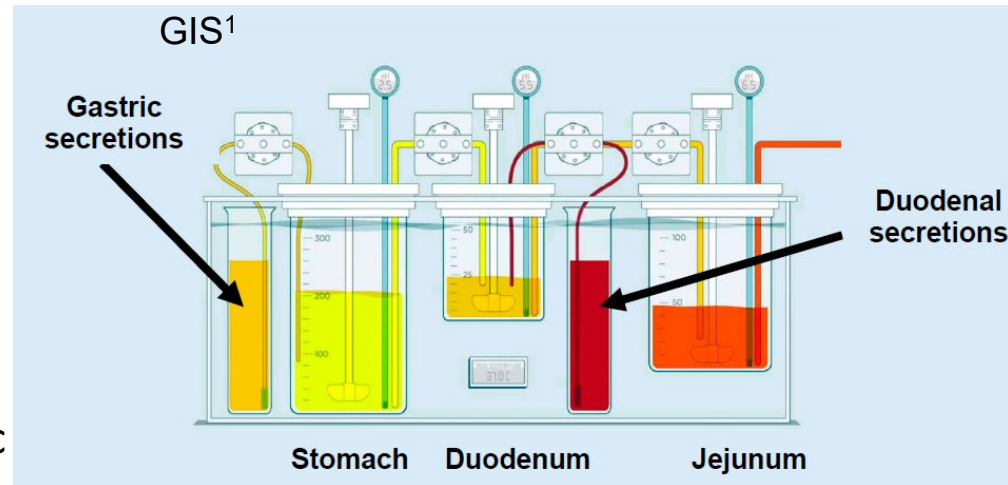
Mean BioGIT (black circles) versus *in vivo* measured posaconazole upper small intestine concentrations¹

- Enables the prediction of intraluminal drug concentrations and fraction precipitated which have shown a high degree of similarity to *in vivo* luminal concentrations
 - Good correlation for itraconazole, posaconazole, ketoconazole, albendazole, nifedipine^{1,2} for predicting fraction precipitated and luminal concentrations without the need for PBPK modeling

¹Kourentas IJP 2016; 515: 352, ²Butler EJPB 2019; 136:70

Open system – Gastrointestinal Simulator (GIS)

- Consists of three chambers simulating the stomach, duodenum and jejunum each containing biorelevant media reflecting the luminal conditions in each
- Relevant flow rates between the compartments (e.g. first order), appropriate volumes and pH with elevated paddle speed to simulate gastric contractions



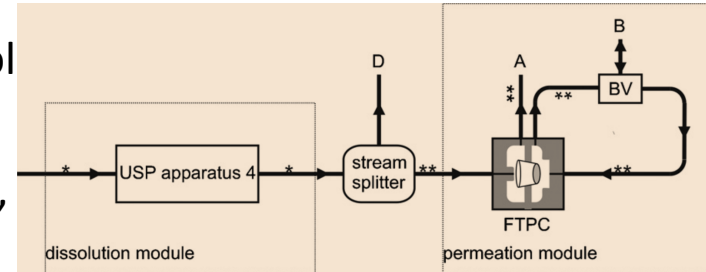
- Dissolution and precipitation behaviour evaluated in a number of studies
 - Matsui et al. demonstrated success in predicting supersaturation and precipitation for dipyridamol & fluconazole as function of pH and consistent with *vivo* DDI study with fluconazol)²
 - Tsume et al.³ used GIS to investigate both dipyridamol and ketoconazole, precipitation observed *in vitro* but given limited *in vivo* precipitation, over estimated

¹Bermejo *Pharmacuetics* 2019 11:122; ²Matsui *Mol Pharm* 2015 12:2418; ³Tsume *EJPS* 2017 102: 126

Integration of „absorption“ barrier

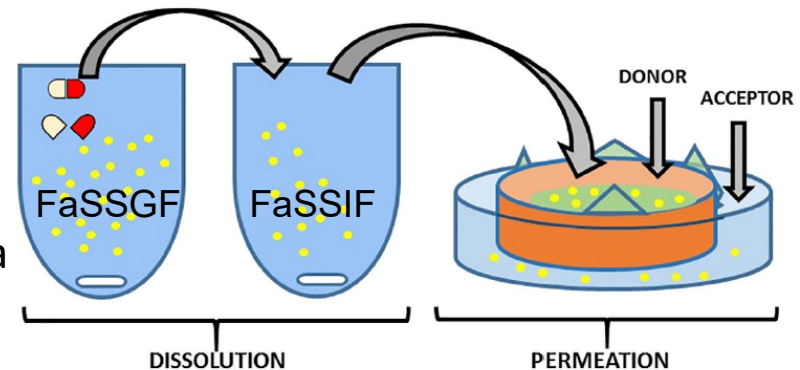
1. Motz¹ utilization a **Caco-2 monolayer** to act as absorption „sink“ combined with an **USP IV apparatus**

- Different dose and release characteristics of propranolol using combined dissolution and permeability
- Influence of absorption on dissolution could be shown, linear dependency of absorption with dose
- Experimental design biased towards maintenance of cell viability/integrity (Krebs buffer, sensitive to flow rates)
 - limitations with using cell cultures: viability, variability, lower surface area



2. **Artificial membrane insert system (AMI)** proposed by Berben², simulates passive absorption of drugs in intestine using a regenerated cellulose membrane

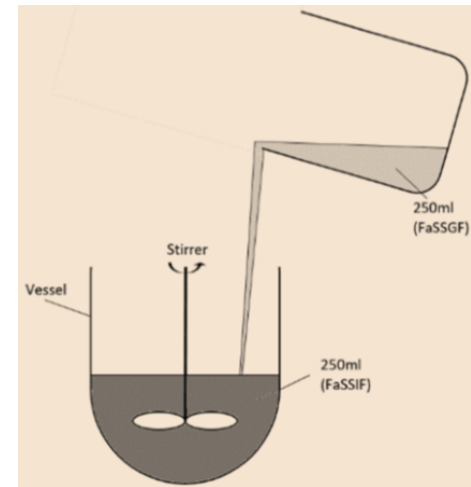
- Interplay between absorption, supersaturation and precipitation examined for loviride, posaconazole, itraconazole and fenofibrate as acidified suspension and enabling formulation demonstrated good correlation with luminal data



¹Motz EJPB 2007; 66:286, ²Berben EJPS 2018;119: 219

Simple “two-stage” method

- Simplified transfer model experimental approach
 - **First stage:** drug placed into compartment simulating gastric conditions
 - **Second stage:** drug solution/dispersion is combined with intestinal media by addition of intestinal media to gastric media (or vice versa...)
- Various examples:
 - **USP QC** method for enteric dosage forms (USP Dissolution 711 – Method A & B)
 - **“Dumping”** test: predissolved drug in 250 ml FaSSGF and poured directly into 350/500 ml FaSSIF various non-supersaturating drugs (e.g. posaconazole) and supersaturating drugs (e.g. ketoconazole)
 - for non-supersaturating drugs, two-stage method gave similar results to transfer model whilst for supersaturating drugs results were different¹
 - **“Dumping”** test: Hansmann² showed no difference in prediction success when coupling in vitro data from “dumping” versus transfer model in Simcyp® for non-supersaturating ciprofloxacin tablets



¹Ruff AAPS Orlando Florida 2015; ²Hansmann 2018 EJPB 122:186

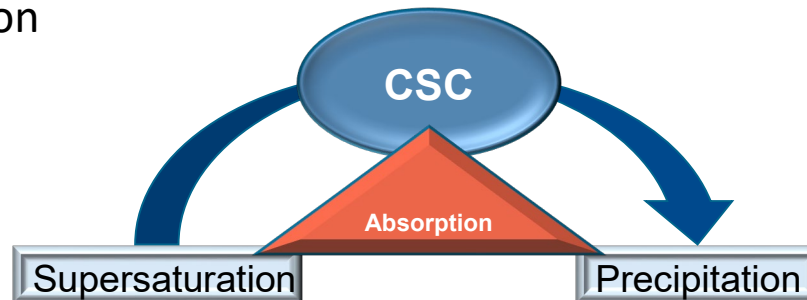
Simple “two-stage” method

- Kambayashi¹ showed “dumping” method (50 ml solution in 0.02N HCl “dumped” into 450 ml FaSSIF-V2)
 - able to predict duodenal precipitation for supersaturating DPM/KTZ when coupled with PBPK Stella[®] model
- Several examples showing usefulness of this simpler experimental setup for evaluating supersaturation and precipitation
 - API alone or in combination with various excipients, fast releasing formulations, and possibly limited to APIs showing a low degree supersaturation

¹Kambayashi 2016; EJPB, 103:95

Coupling of *in vitro* data to PBPK modeling

- No single *in vitro* model considers all of the potential *in vivo* parameters that can influence supersaturation and precipitation
 - experimentally challenging to consider *in vivo* regional differences and variability in GI physiology (fluid volumes and composition, pH, transit times, hydrodynamics) and absorption

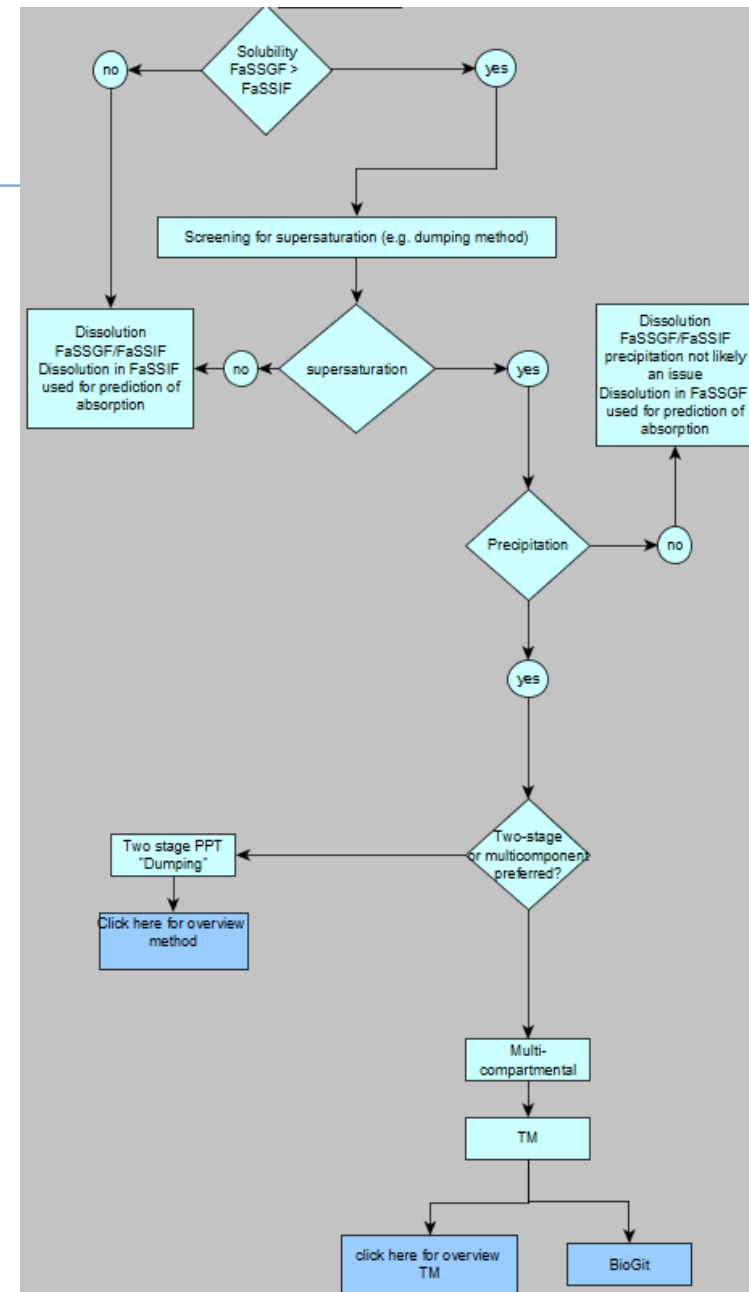


- Range of commercially available, open system and „build-your own“ platforms available
 - Simcyp[®] simulator, GastroPlus[®], PK-Sim[®], Stella[®]
- Commercial programs have evolved to enable an improved integration of experimental data collect from *in vitro* different models
 - SIVA toolkit in Simcyp facilitates the *in vitro* experimental data to be modeled and estimate the input parameters for *in vivo* simulations,
 - physiological system parameters to predict the changes along the GI tract

OrBiTo's Decision Tree

- As part of the OrBiTo project, an experimental approach to evaluate the supersaturation and precipitation behaviour of different formulation types (IR, ER, DR) was generated (Andreas, 2018)
- Example of decision tree in case for IR, weak base, fasting state, dose/solubility in FaSSIF >250 ml
 - combination of solubility, decision for either dissolution or supersaturation screening and in case of precipitation, two stage method is given

¹Andreas EJPB 2018; 130:207



- A range of small scale and large scale models to evaluate supersaturation and precipitation at different stages of drug development are available
- Significant progress has been made in making these tools more physiologically relevant, however, not all aspects having an influence on supersaturation and precipitation can be captured by the model
 - early stages “less critical” rather screening to evaluate the predisposition to precipitation and investigate formulation strategies
 - later stages, PBPK modelling is becoming more important for reliable *in vivo* prediction for absorption (physiological parameters, absorption)
- Models have been validated on a limited number of examples, further validation with a wider range of drug compounds with *in vivo* data, continued collaborative nature between different stakeholders (*in vitro* & *in silico*) and to provide a consensus for standardization of the *in vitro/in silico* methods

Any questions about....

***In vitro* approaches to understanding supersaturation and precipitation of weak bases and enabling formulations?**

