“Dissolved” Species:
Biopharmaceutical Roles & Ways to Measure

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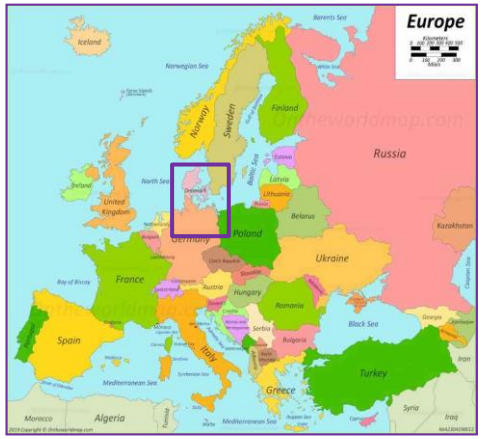
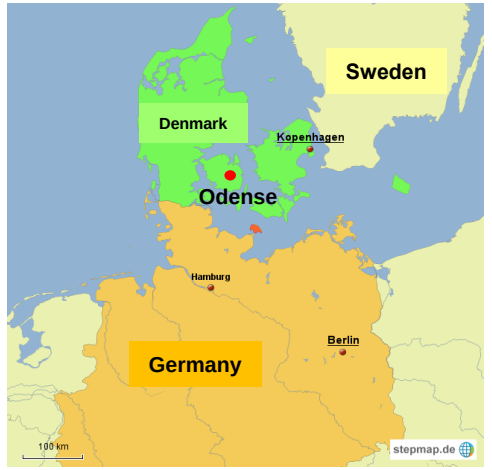
FDA-MCERSI Workshop
on Drug Dissolution in Oral Drug Absorption

May 23-24, 2023

SDU
UNIVERSITY OF SOUTHERN DENMARK





Disposition

- Which “dissolved” species are there?
- What is their role for oral drug absorption?
- How to quantify drug in different dissolved states during dissolution?

Disposition

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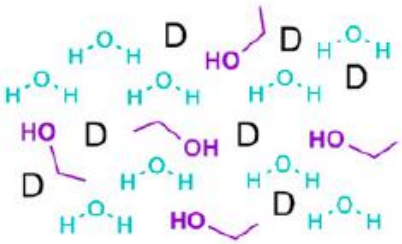
Common strategies to address low drug solubility

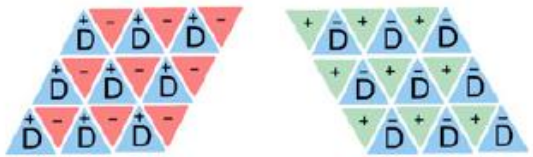
cosolvents

e.g. Prograf®, Valium®



salts

e.g. ziprasidone HCl, diclofenac sodium

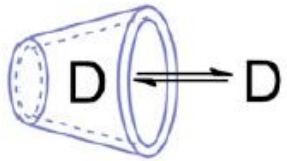


surfactants

e.g. Taxol®, Prograf®

cyclodextrins

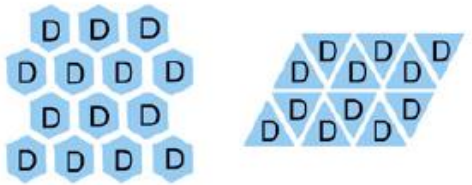
e.g. Vfend®, Sporanox® IV



particle size reduction

e.g. Emend®, Rapamune®

polymorphs



lipid-based systems

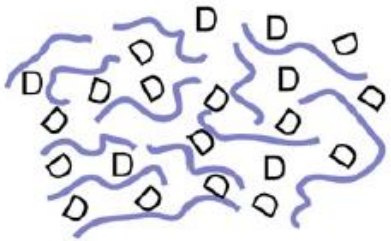
e.g. Neoral®, Agenerase®

co-crystals

e.g. AMG 517

amorphous solid dispersions

e.g. Kaletra®, Sporanox®



From Williams et al. 2013

Journal of Pharmaceutical Sciences 105 (2016) 2832–2839

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Bile Salt Micelles and Phospholipid Vesicles Present in Simulated and Human Intestinal Fluids: Structural Analysis by Flow Field–Flow Fractionation/Multiangle Laser Light Scattering

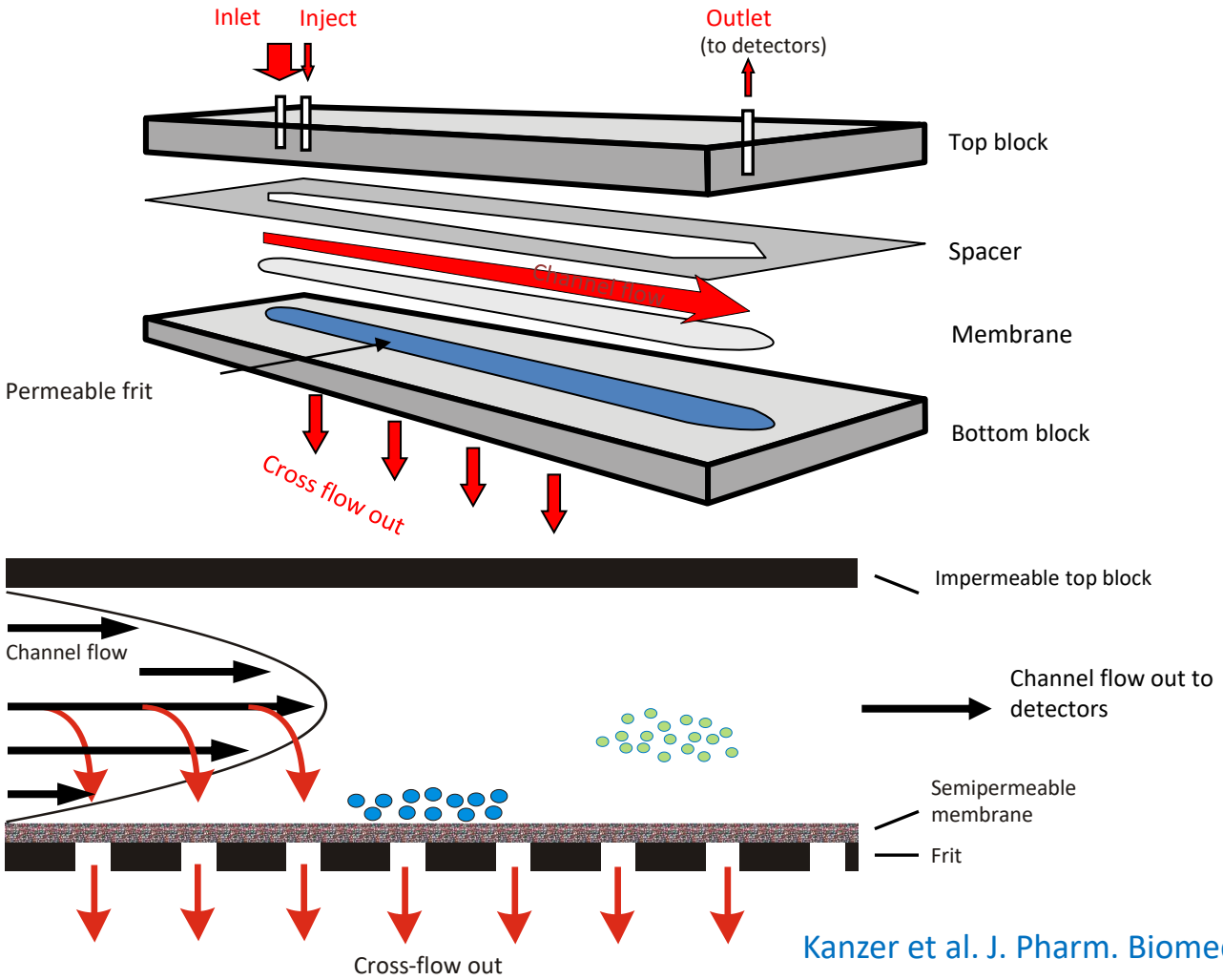
Philipp A. Elvang [1], Askell H. Hinna [1], Joachim Brouwers [2], Bart Hens [2], Patrick Augustijns [2], Martin Brandl [1,*]

Various ”dissolved” species arising in human intestinal & biomimetic media

Asymmetric flow field-flow fractionation

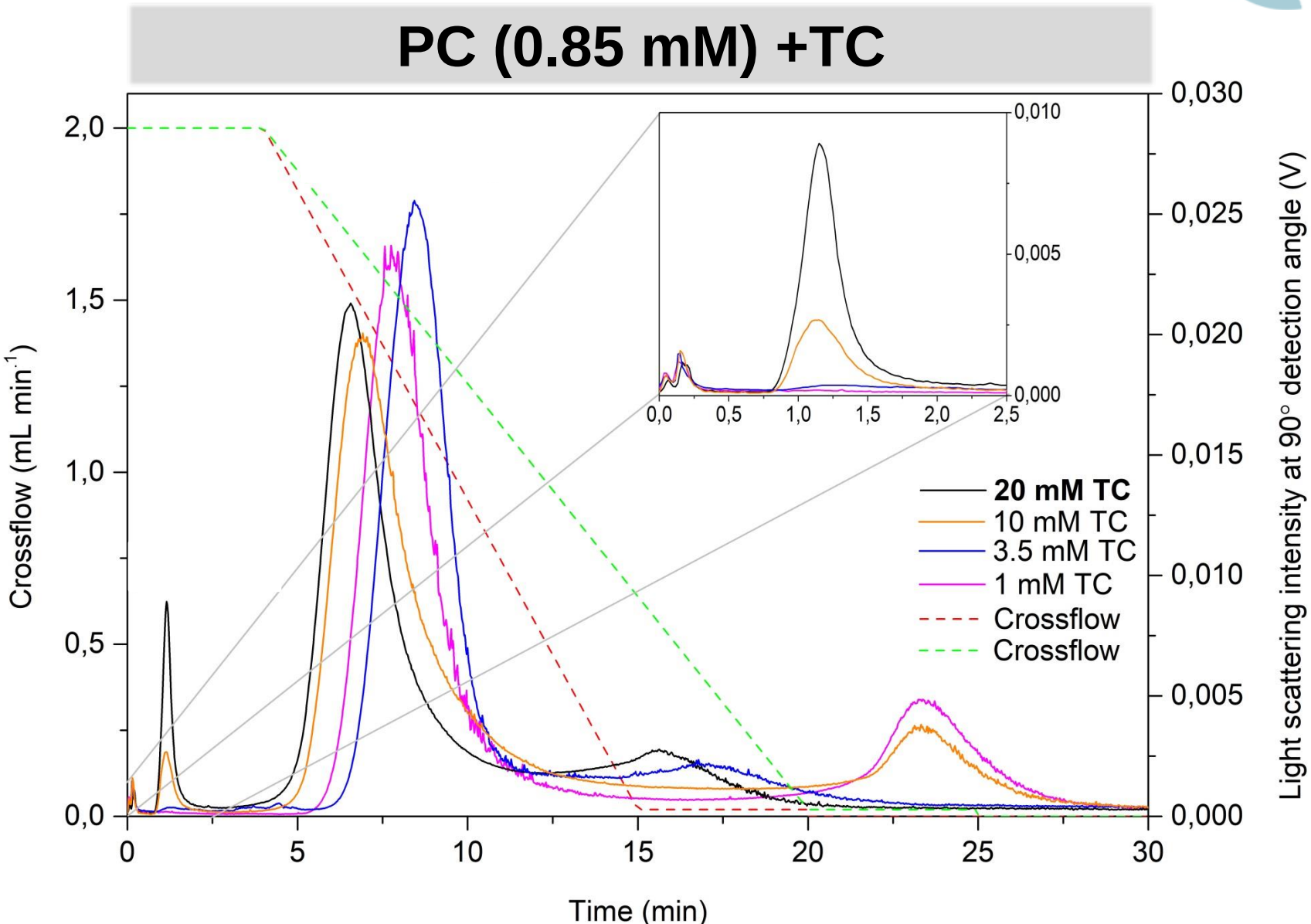
Analysis of the colloidal associates arising in aqueous medium

Flow field-flow fractionation



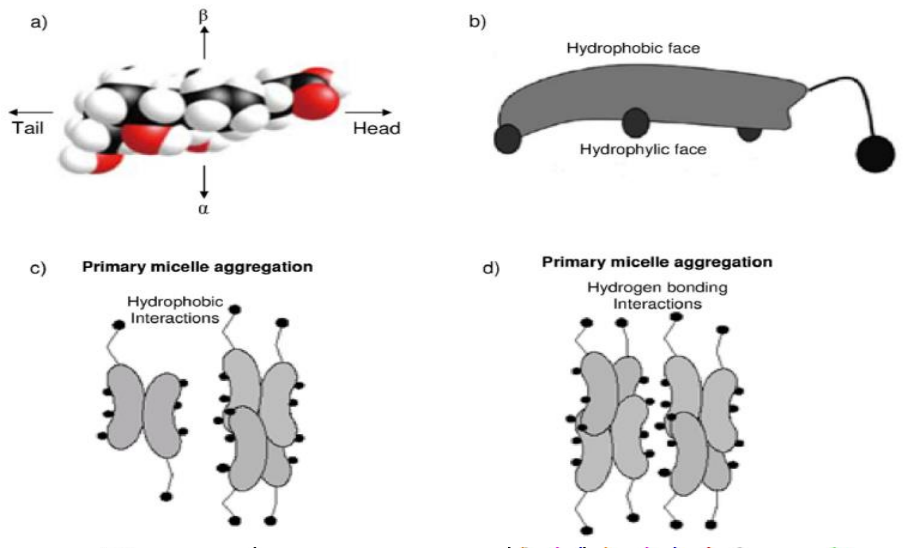
Kanzer et al. J. Pharm. Biomed. Analysis (2010)

Colloidal structures in fasted state artificial intestinal fluids



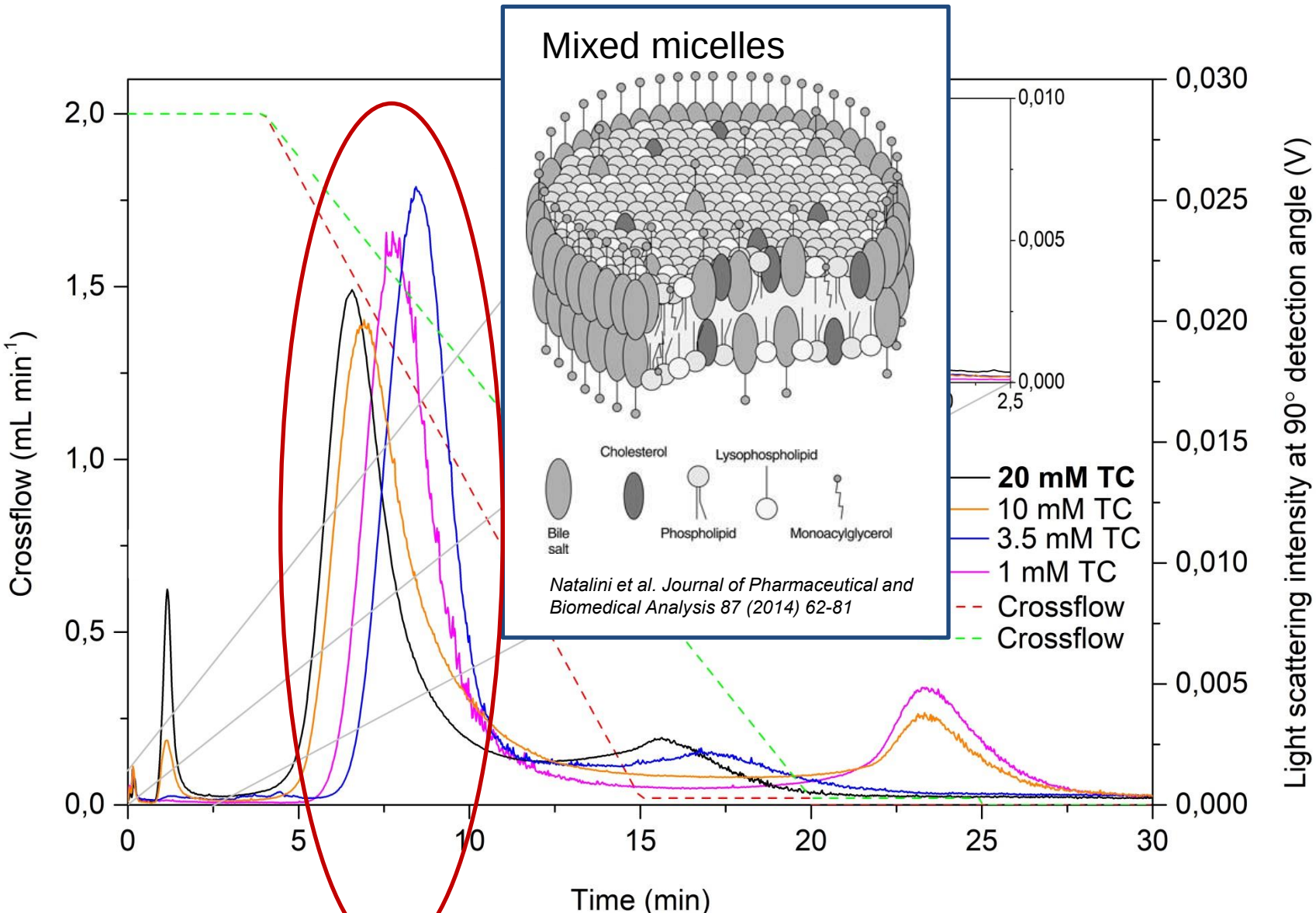
Colloidal structures in fasted state artificial intestinal fluids

Taurocholate micelles

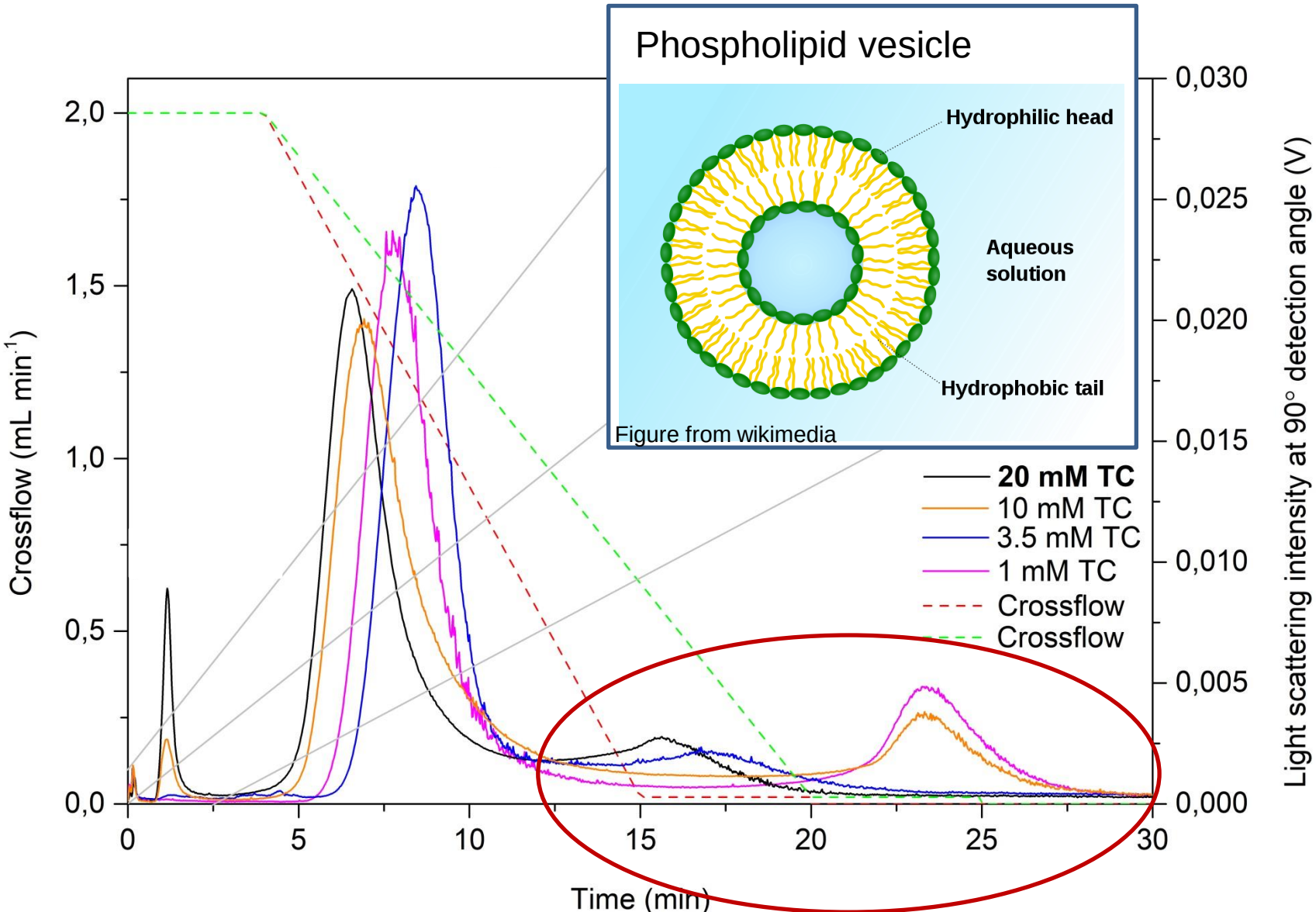


Natalini et al. Journal of Pharmaceutical and Biomedical Analysis 87 (2014) 62-81

Colloidal structures in fasted state artificial intestinal fluids



Colloidal structures in fasted state artificial intestinal fluids



Colloidal associates in human aspirates

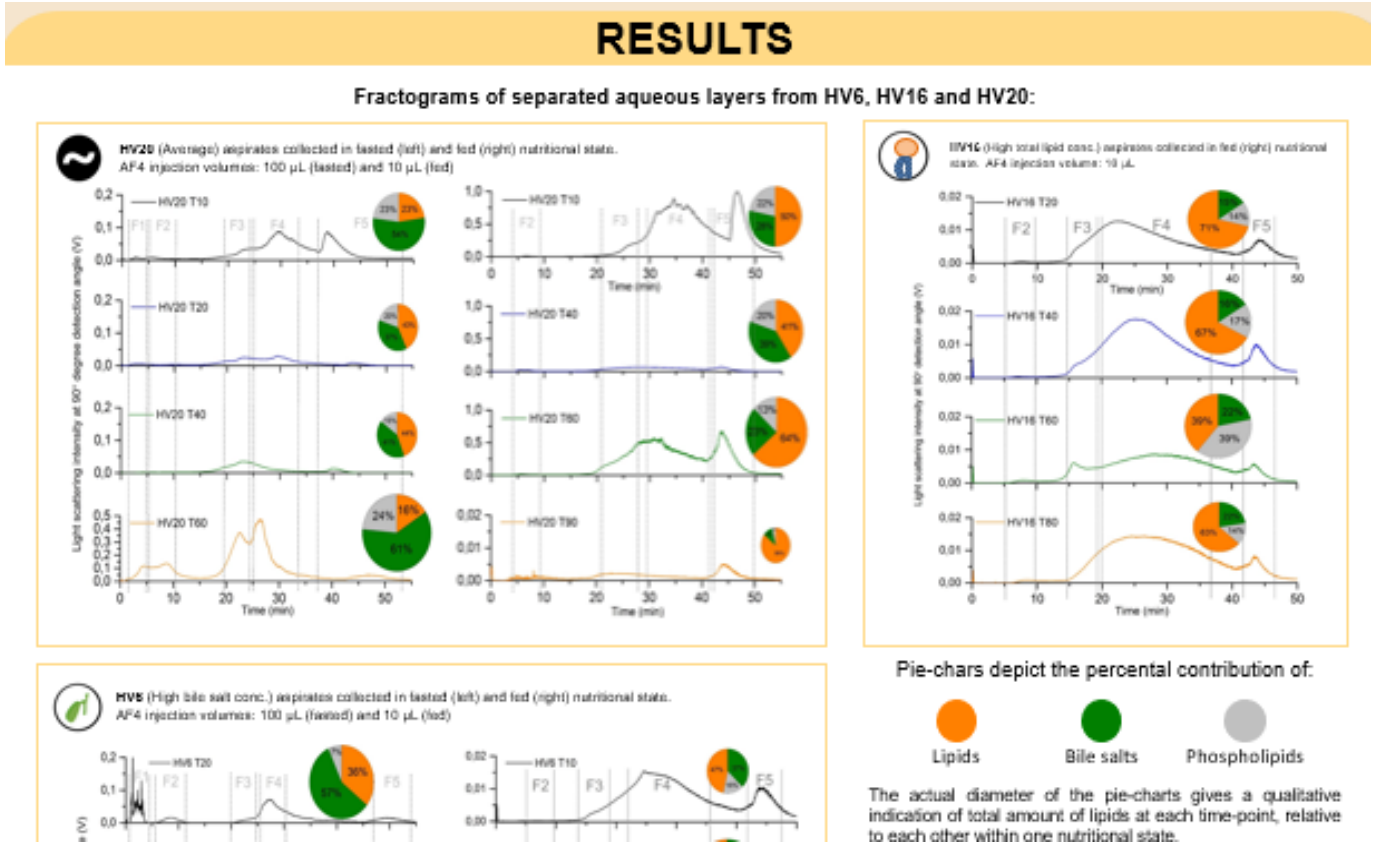
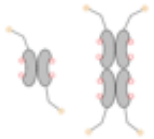
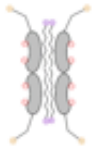
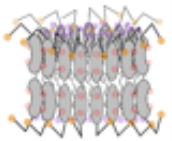


Table 1

Fraction no.	Illustration	Description
F1		Bile salt-rich (mixed-)micelles. Sizes below MALLS limit (<20 nm)
F2		Mixed micelles consisting of bile salts and lipids. Diameters between 20-40 nm.
F3-4		Mixture of swollen mixed micelles and liposomes. Other in-vivo strucuturs (e.g. proteines) could be present. Diameters: <200 nm
F5		Large strucutures that only elutes when there is no retention in AF4. Likely being vesicle structures and aggregates Diameters: >200 nm

Case 1

Various ”dissolved” species arising during dissolution

ASDs of poorly soluble drugs

spontaneous formation of colloidal associates

European Journal of Pharmaceutical Sciences 40 (2010) 25–32

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Formation of nano/micro-dispersions with improved dissolution properties upon dispersion of ritonavir melt extrudate in aqueous media

Ingunn Tho[a,b,*], Bernd Liepold[c], Joerg Rosenberg[c], Markus Maegerlein[c], Martin Brandl[a,d], Gert Fricker[b]

Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 359–365

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

In situ formation of nanoparticles upon dispersion of melt extrudate formulations in aqueous medium assessed by asymmetrical flow field-flow fractionation

Johanna Kanzer[a,b], Stefan Hupfeld[a], Terje Vasskog[c], Ingunn Tho[a], Peter Hölig[d], Markus Mägerlein[d], Gert Fricker[b], Martin Brandl[a,e,*]

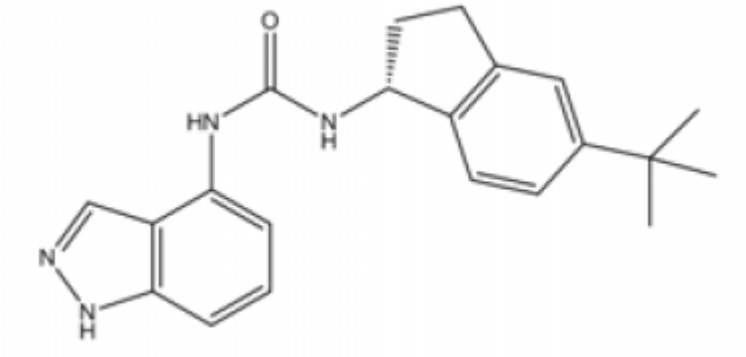
Kerstin J Frank[1,3]
Ulrich Westedt[2]
Karin M Rosenblatt[2]
Peter Hölig[2]
Jörg Rosenberg[2]
Markus Mägerlein[2]
Gert Fricker[3]
Martin Brandl[1]

International Journal of Nanomedicine
International Journal of Nanomedicine 2012:7 5757–5768
Dovepress
ORIGINAL RESEARCH

The amorphous solid dispersion of the poorly soluble ABT-102 forms nano/microparticulate structures in aqueous medium: impact on solubility

Amorphous solid dispersion of ABT-102

ABT-102
TRPV1 (transient receptor potential vanilloid type 1)-receptor antagonist



solubility in buffer: c = 0.05 µg/ml

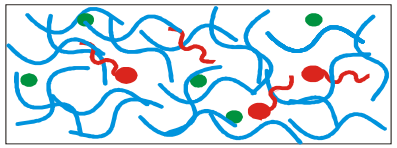
Frank et al. Int. J. Nanomedicine, (2012)

Composition of melt extrudate

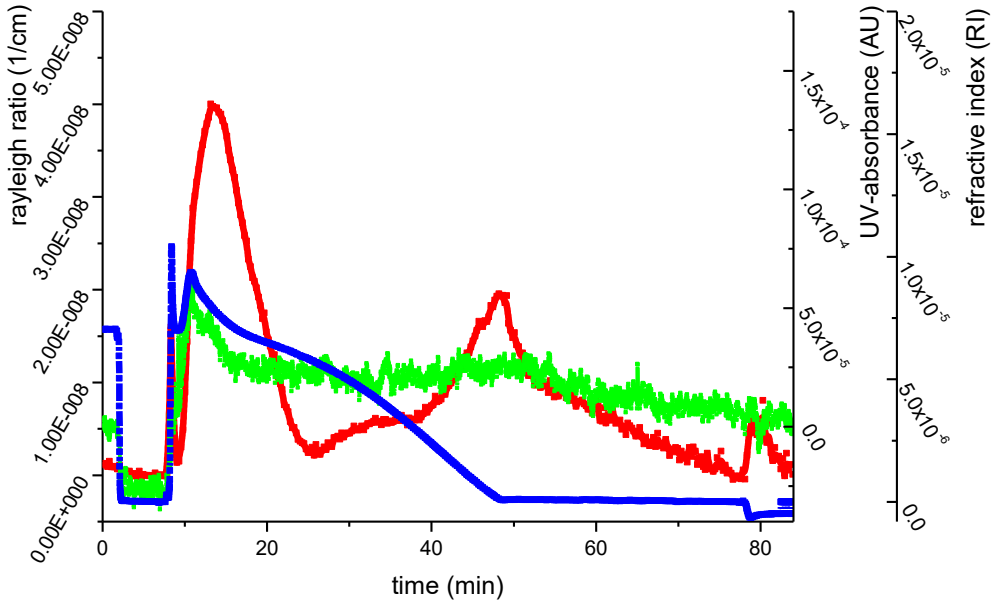
Ingredients	F1 percentage [%]
ABT-102	5
Copovidon Typ K28 (Kollidon® VA 64)	81.5
Sucrose palmitate (Surfhope® D-1615)	1.5
Poloxamer 188 (Pluronic® F68)	6.0
Polysorbate 80 (Tween 80®)	5.0
Fumed silica (Aerosil 200®)	1.0

From Frank et al. Int. J. Pharm., (2012)

Schematic representation of melt extrudate

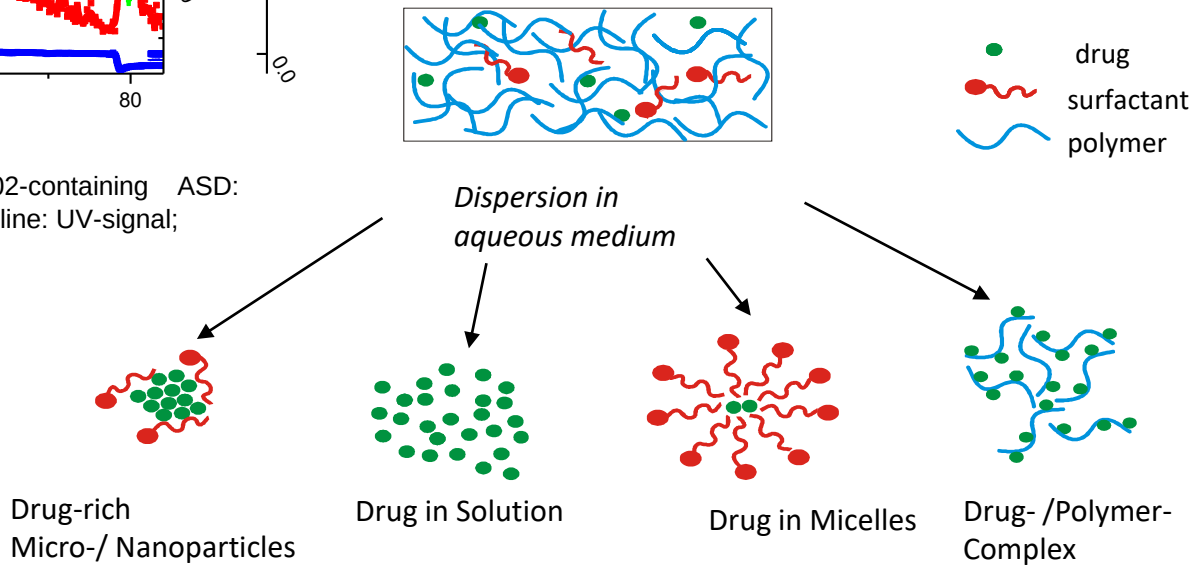


ASD: in-situ formation of supramolecular assemblies in aqueous medium



Fractogram of aqueous dispersion of ABT-102-containing ASD: red line: rayleigh ratio (light scattering at 90°), green line: UV-signal; blue line: differential refractive index.

From Frank et al. Int. J. Nanomedicine, (2012)



Brandl et al. Abbott Symposium, Ludwigshafen, (2010)

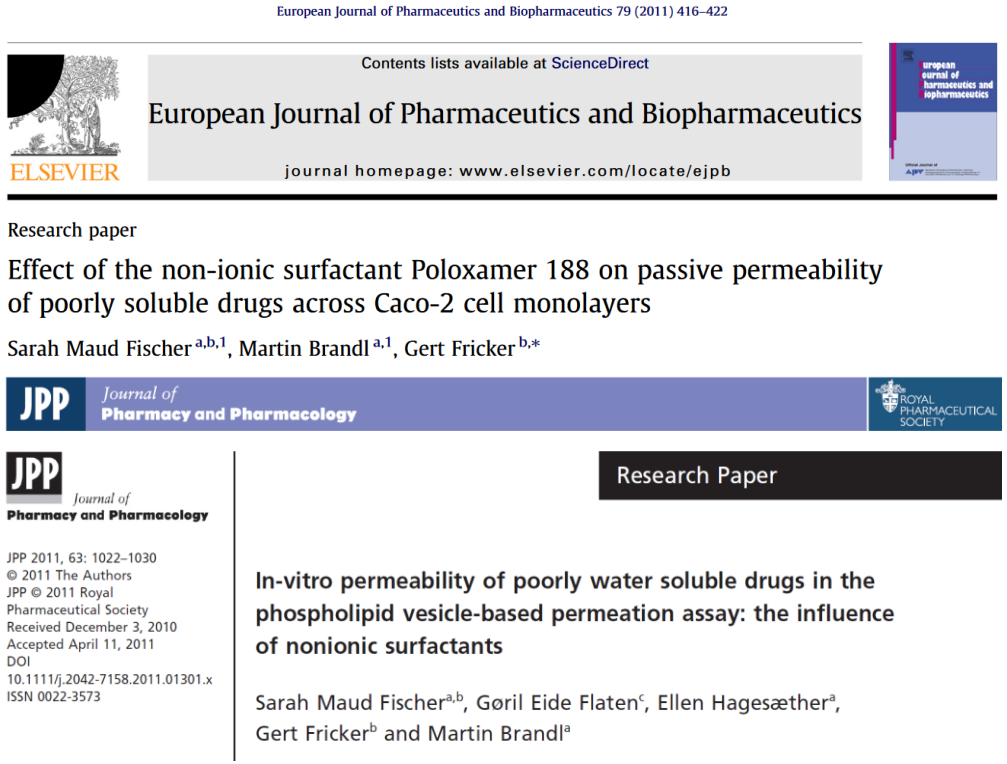
Disposition

- Which dissolved species are there?
- What is their role for oral drug absorption?
- How to quantify drug in different dissolved states during dissolution?

Case 2

Impact of micellar solubilization

Model Ketoprofen: non-ionic surfactants



European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 416–422

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Effect of the non-ionic surfactant Poloxamer 188 on passive permeability of poorly soluble drugs across Caco-2 cell monolayers

Sarah Maud Fischer [a,b,1], Martin Brandl [a,1], Gert Fricker [b,*]

JPP Journal of Pharmacy and Pharmacology

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 1022–1030

Received December 3, 2010
Accepted April 11, 2011
DOI
10.1111/j.2042-7158.2011.01301.x
ISSN 0022-3573

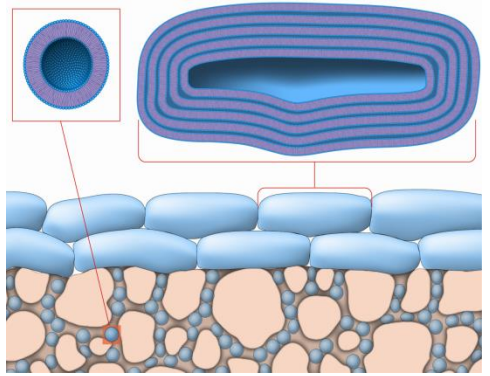
Research Paper

In-vitro permeability of poorly water soluble drugs in the phospholipid vesicle-based permeation assay: the influence of nonionic surfactants

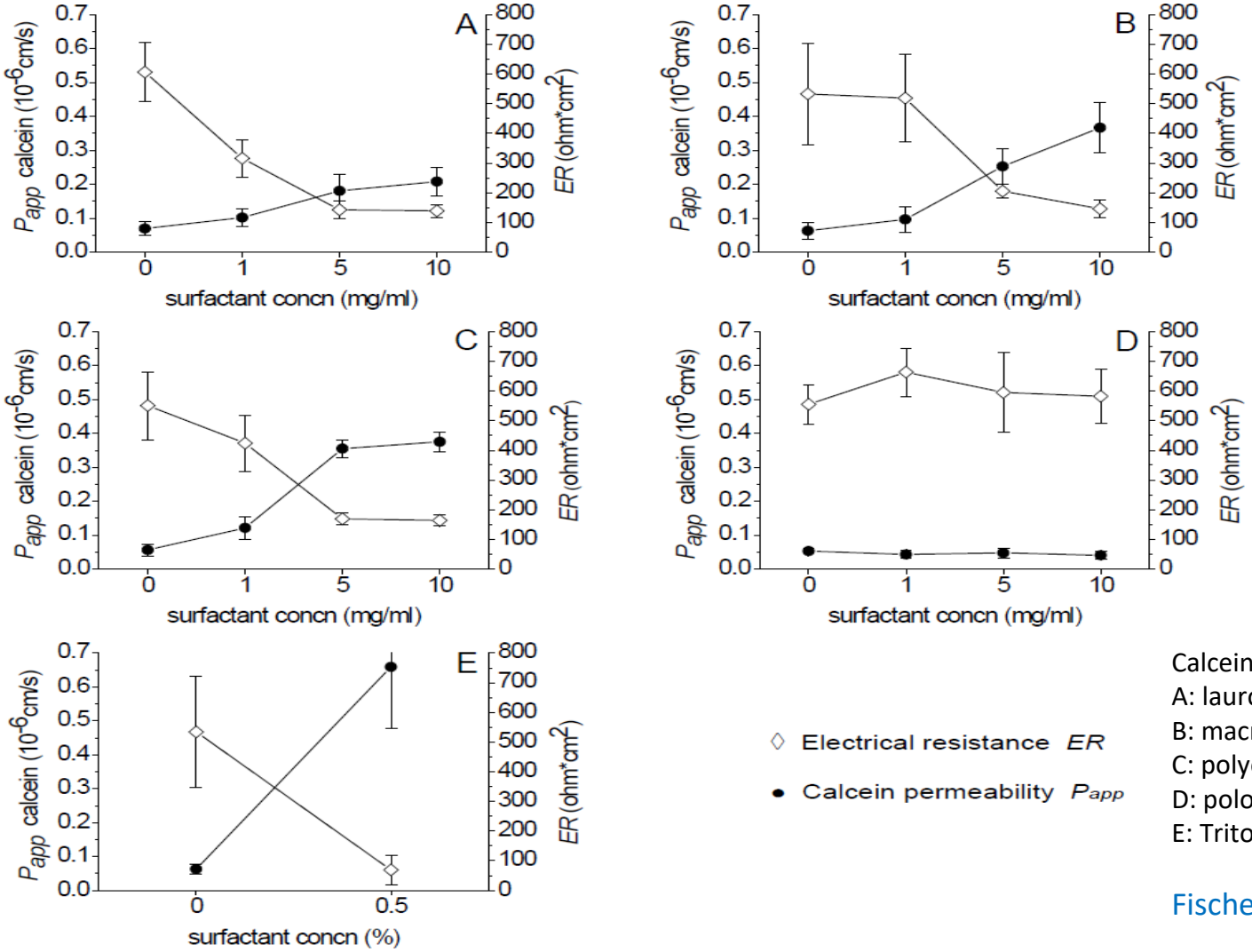
Sarah Maud Fischer[a,b], Gøril Eide Flaten[c], Ellen Hagesæther[a], Gert Fricker[b] and Martin Brandl[a]

Integrity of the permeation barrier

impact of various surfactants



Phospholipid Vesicle-based Permeation: PVPA-model

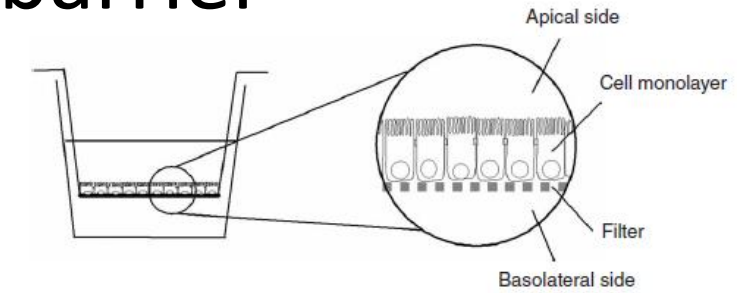


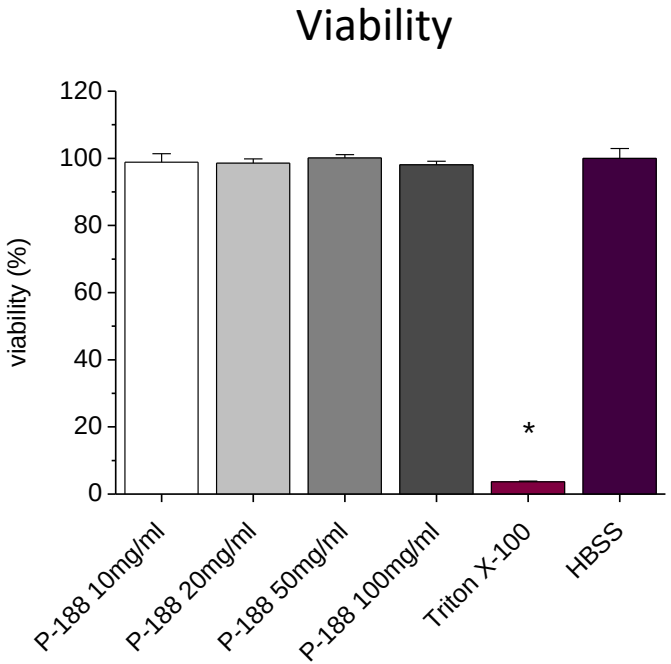
Calcein permeability and ER
A: lauroyl macrogol-32 glycerides,
B: macrogol 15 hydroxystearate,
C: polyoxyl 40 hydrogenated castor oil,
D: poloxamer 188,
E: Triton-X 100,

Fischer PhD-thesis SDU 2012

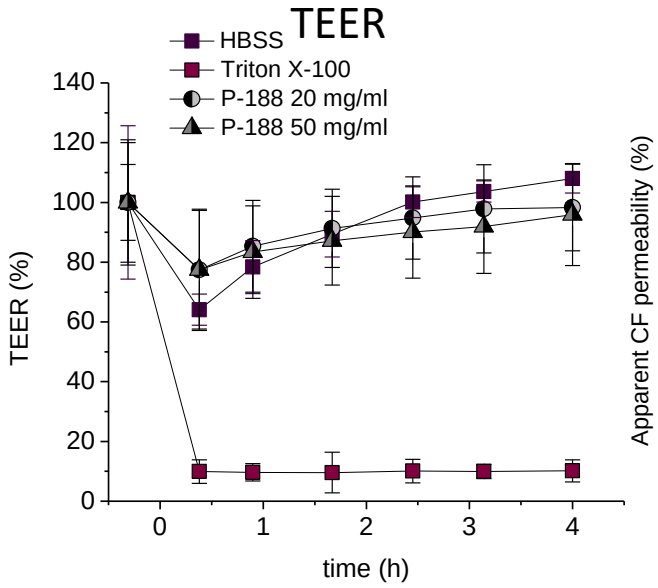
Integrity of the permeation barrier

impact of various surfactants

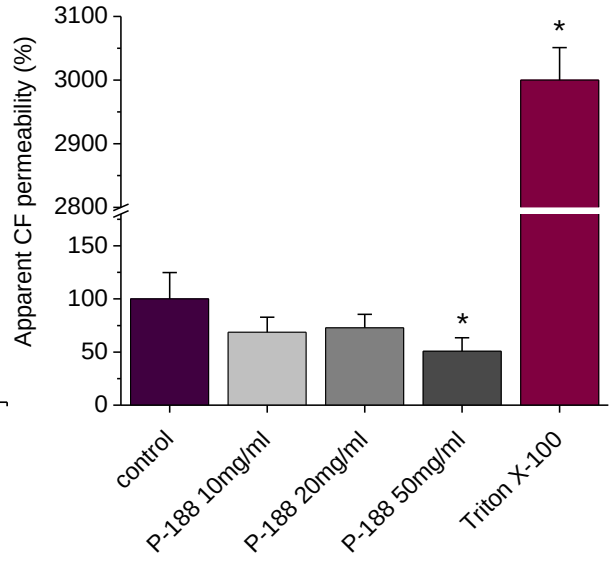




alamarBlue® cytotoxicity assay
after 6 h incubation with P-188, Triton-X 100 (1 % v/v), and HBSS (negative control).
Data are shown as percentage of the negative control (= 100 %), (mean ± SD, n=8)



TEER over time
Pre-incubation with HBSS, incubation with P-188, Triton X-100 and HBSS (control), (mean ± SD, n≥8; n=5 for Triton X-100).

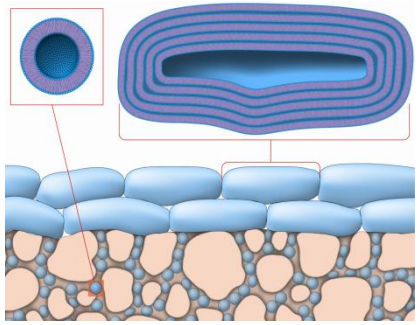


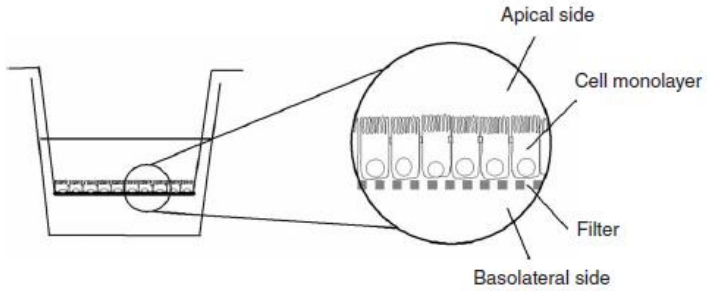
Apparent CF permeability
In absence /presence of P-188 and Triton-X 100 (1 %). Data are given as percentage of the control value (HBSS), (mean ± SD, n≥8; n=5 for Triton X-100).

Fischer PhD-thesis SDU 2012

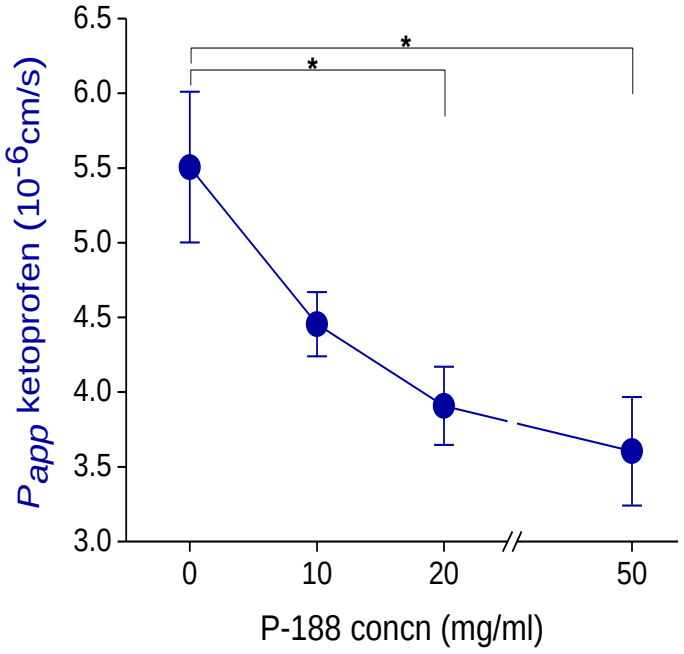
Ketoprofen in-vitro permeation:

impact of P-188



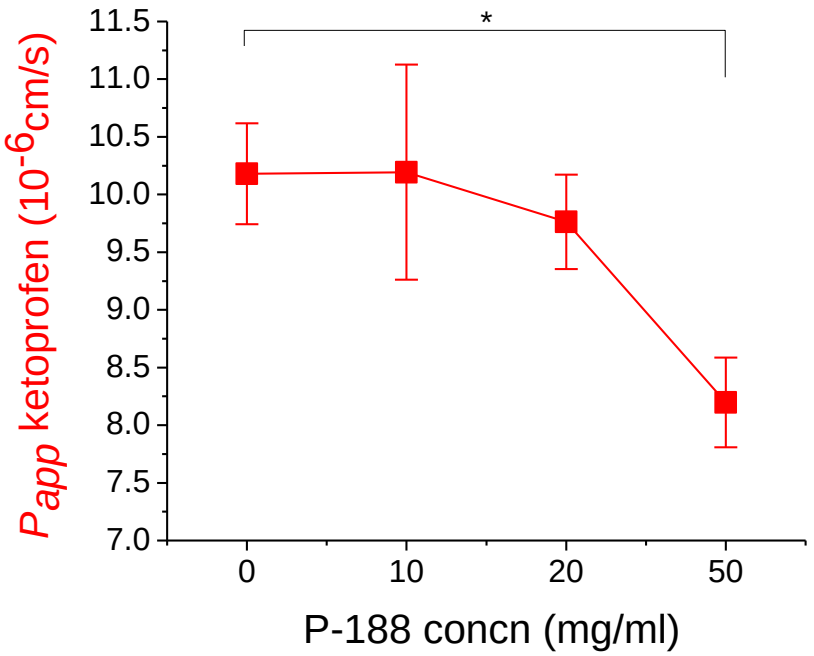


PVPA
Permeation Assay



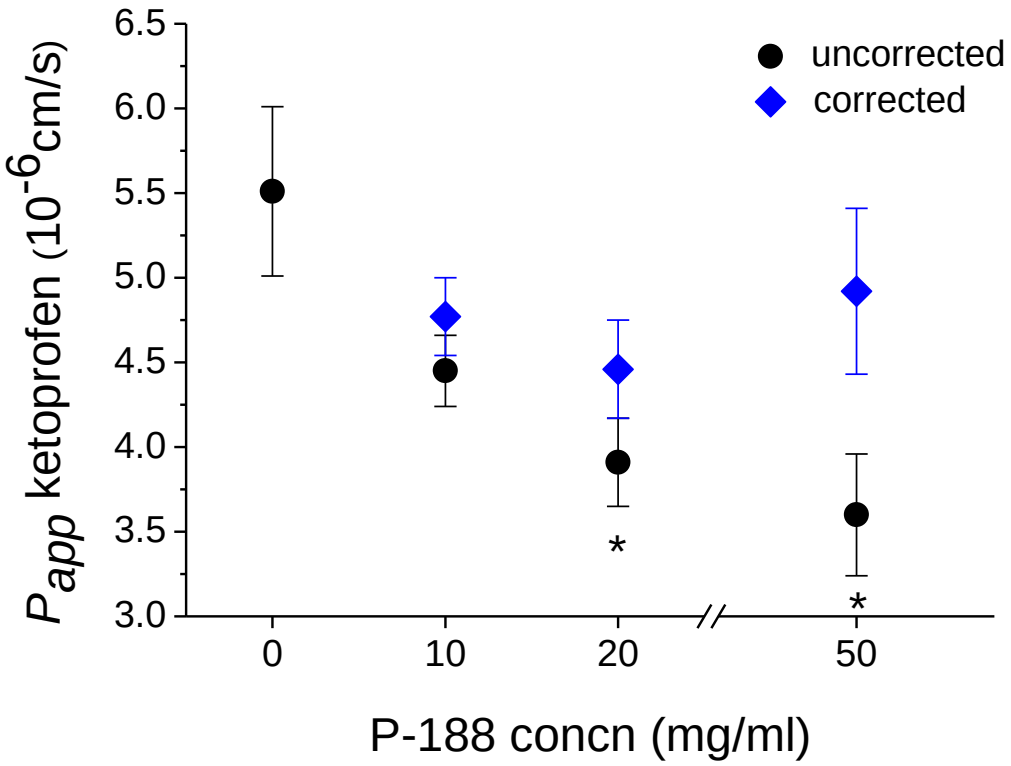
Fischer et al., J Pharm Pharmacol, 63, 1022-1030 (2011)

Caco-2
Permeation Assay



Fischer et al Eur. J. Pharm. Biopharm. (2011)

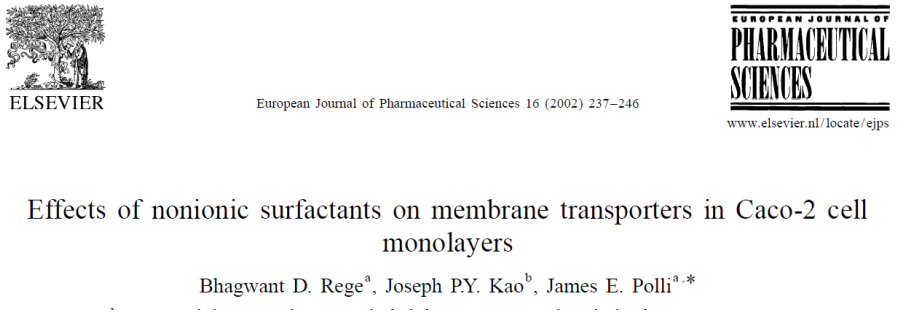
in vitro permeability



Fischer et al., *Int J Pharm*. 2012; Fischer et al., *J Pharm Pharmacol.* 2012; Fischer et al., *J Pharm Pharmacol*, 2011

Key insights of the Ketoprofen study

- *At a fixed concentration of "dissolved" drug*
  - The drug's ability to overcome a barrier is reduced in presence of a solubilizing surfactant (poloxamer P-188).
  - Irrespective, whether it's a biological or biomimetic barrier (Caco-2 monolayer or PVPA).
  - This effect correlates with the affinity of the drug to the surfactant micelles
- Cave!
  - Not true if transporters are involved



Journal of Pharmaceutical Sciences
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American Pharmacists Association
APhA

RESEARCH ARTICLE

What Is the Mechanism Behind Increased Permeation Rate of a Poorly Soluble Drug from Aqueous Dispersions of an Amorphous Solid Dispersion?

Kerstin J. Frank, Ulrich Westedt, Karin M. Rosenblatt, Peter Hölig, Jörg Rosenberg, Markus Mägerlein, Gert Fricker, Martin Brandl

First published: 24 April 2014 | https://doi.org/10.1002/jps.23979 | Citations: 4

Case 1 (revisited)

Impact of the various ”dissolved” species on in vitro permeation

ASD of poorly soluble ABT-102: solubility & in-vitro permeation

Impact surfactants on solubility & permeation

Solubility of ABT-102. Apparent solubility: Concentrations of ABT-102 in the supernatant after centrifugation of the sample dispersions ($n = 6–7$, mean ± SD). Molecular solubility: Concentrations of *molecularly dissolved ABT-102* in the sample dispersions, assessed by inverse dialysis ($n = 4–6$, mean ± SD).

	Apparent solubility [μg/ml]	Molecular solubility [μg/ml]
F1 in HBSS++	0.58 ± 0.08	0.15 ± 0.01
F1 in FaSSIF	5.43 ± 0.41	0.16 ± 0.01
ABT-102 in HBSS++	0.05 ± 0.01	0.09 ± 0.01
ABT-102 in FaSSIF++	2.11 ± 0.28	0.08 ± 0.01
ABT-102 + placebo-extrudate in HBSS++	0.06 ± 0.01	0.08 ± 0.01

Frank et al. Int. J. Pharm. (2012)
Frank et al. J. Pharm. Sci. (2014)

Impact surfactants on solubility & permeation

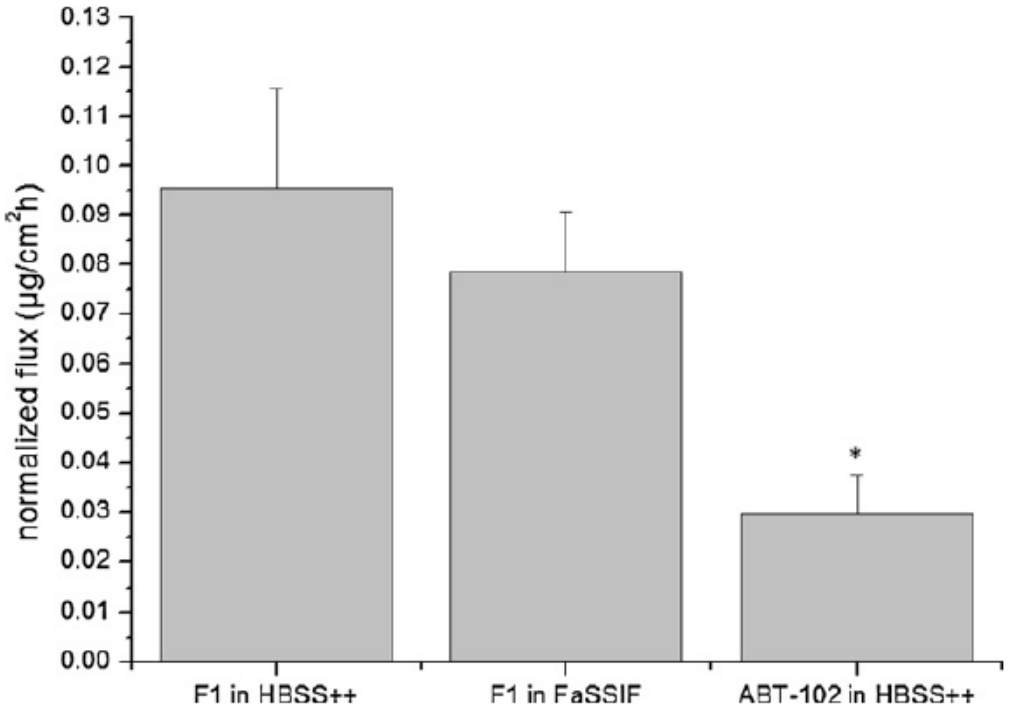


Fig. 1. Caco-2 permeation rates: Normalized flux (divided by area of filter surface) of dispersions of ABT-102 crystals in HBSS++ ($n = 5$; mean ± SD) and of dispersions of the ASD F1 ($n = 8$; mean ± SD) in HBSS++ and FaSSIF. * Significance calculated by unpaired Student's t-test ($p \leq 0.05$).

Frank et al. Int. J. Pharm. (2012)
Frank et al. J. Pharm. Sci. (2014)

Solubility of ABT-102. Apparent solubility: Concentrations of ABT-102 in the super-

Table 2. Solubility of ABT-102

	Apparent Solubility (μg/mL)	Molecular Solubility (μg/mL)
ASD (HC)	12.31 ± 1.52	0.16 ± 0.01
ASD (LC)	0.58 ± 0.08	0.15 ± 0.01
ABT-102 crystals	0.05 ± 0.01	0.09 ± 0.01

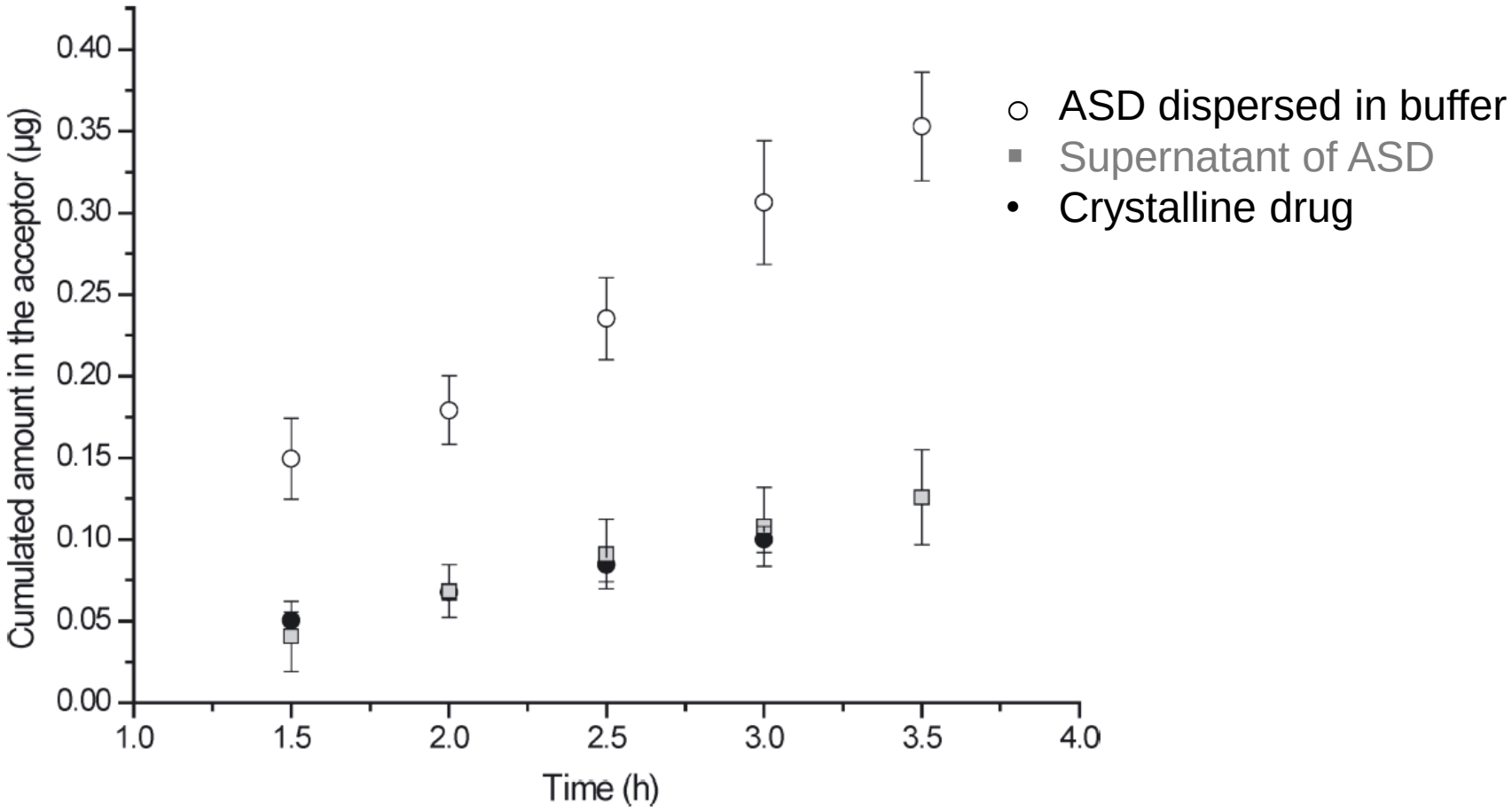
Apparent solubility: concentration in the supernatant after centrifugation. Molecular solubility: assessed by inverse equilibrium dialysis. Mean ± SD, n = 4–6.

Table 3. Permeation Rate of ABT-102 from Dispersions of the ASD and from Crystalline ABT-102

	Permeation Rate (μg/cm^2 h)	
Sample	Mean Values	SD
ASD (HC)	0.128	0.024
ASD (LC)[a]	0.095	0.026
ABT-102 crystals[a]	0.03	0.008

n = 5–8, T = 37°C; HBSS^{++} buffer. HC = 3.2 mg (ASD)/mL, LC = 0.16 mg (ASD)/mL.

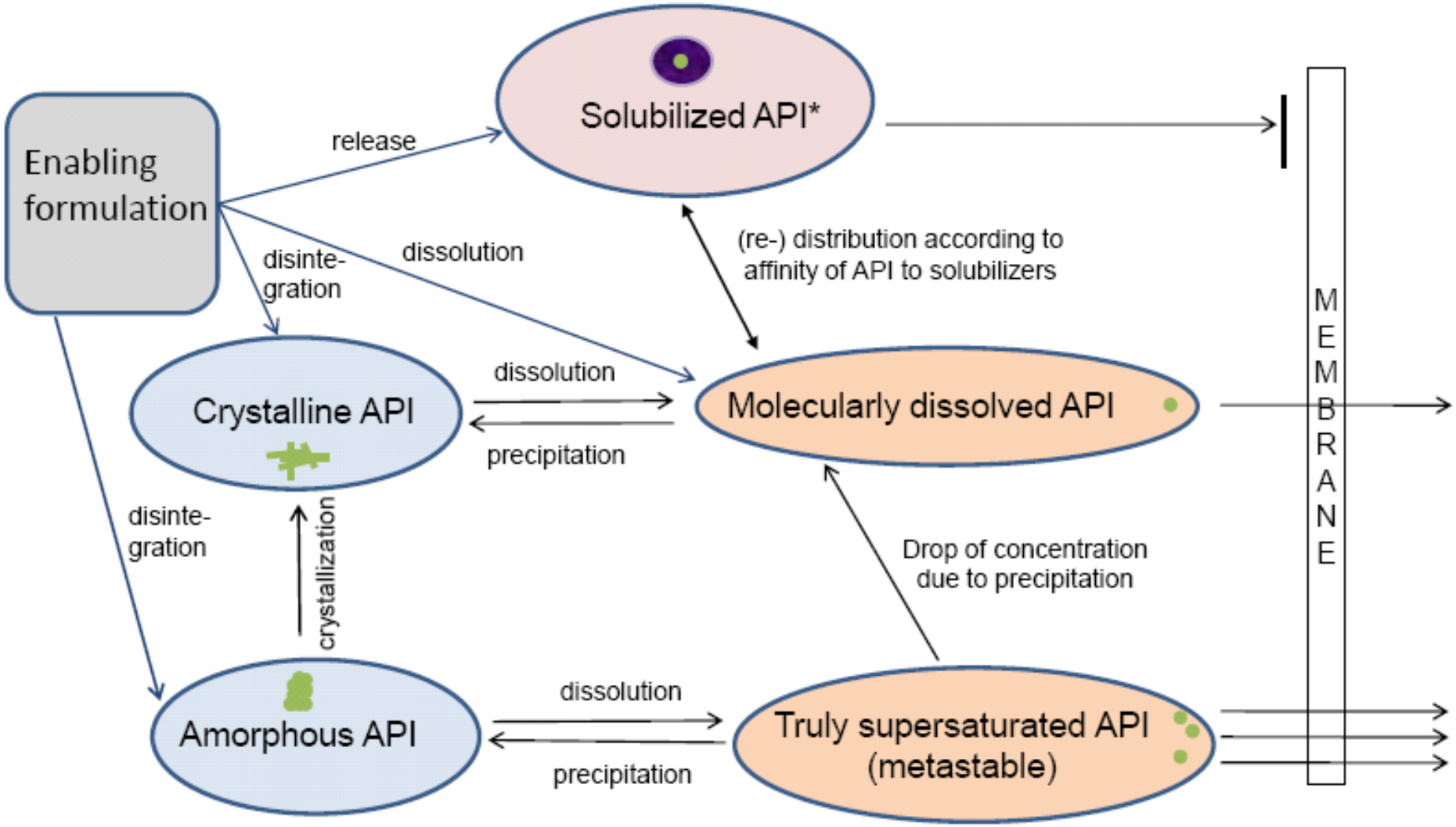
Impact of drug-rich micro-/ nanoparticles on permeation



Cumulative amounts of ABT-102 in the acceptor of the Caco-2 experiments versus time.
All samples were dispersed in HBSS++. All mean ± SD. Error bars depict SD.

Frank et al. J. Pharm. Sci. (2014)

Key findings => Hypothetic model



Buckley et al., Eur. J. Pharm. Sci 2013

Disposition

- Which dissolved species are there?
- What is their role for oral drug absorption?
- How to quantify drug in different dissolved states during dissolution?

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Microdialysis and nanofiltration allow to distinguish molecularly dissolved from colloid-associated drug concentrations during biomimetic dissolution testing of supersaturating formulations

Florentin Lukas Holzem [a], Jeannine Petrig Schaffland [c], Martin Brandl [a,*], Annette Bauer-Brandl [a], Cordula Stillhart [b]

Case 3

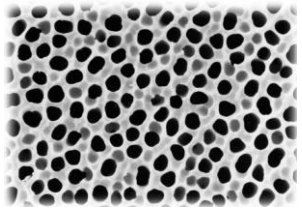
Impact of sampling approach on dissolution outcome

Model Posaconazol ASD

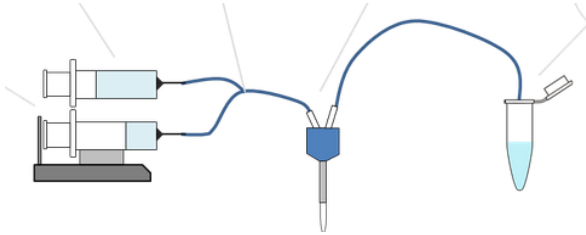
centrifugation vs nanofiltration vs microdialysis

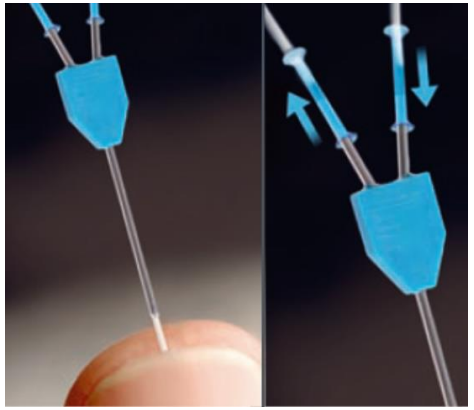
Sampling methods

Centrifugation at 13,000 rpm (corresponding to 17,949 rcf) and 37 ◦C for 2 min



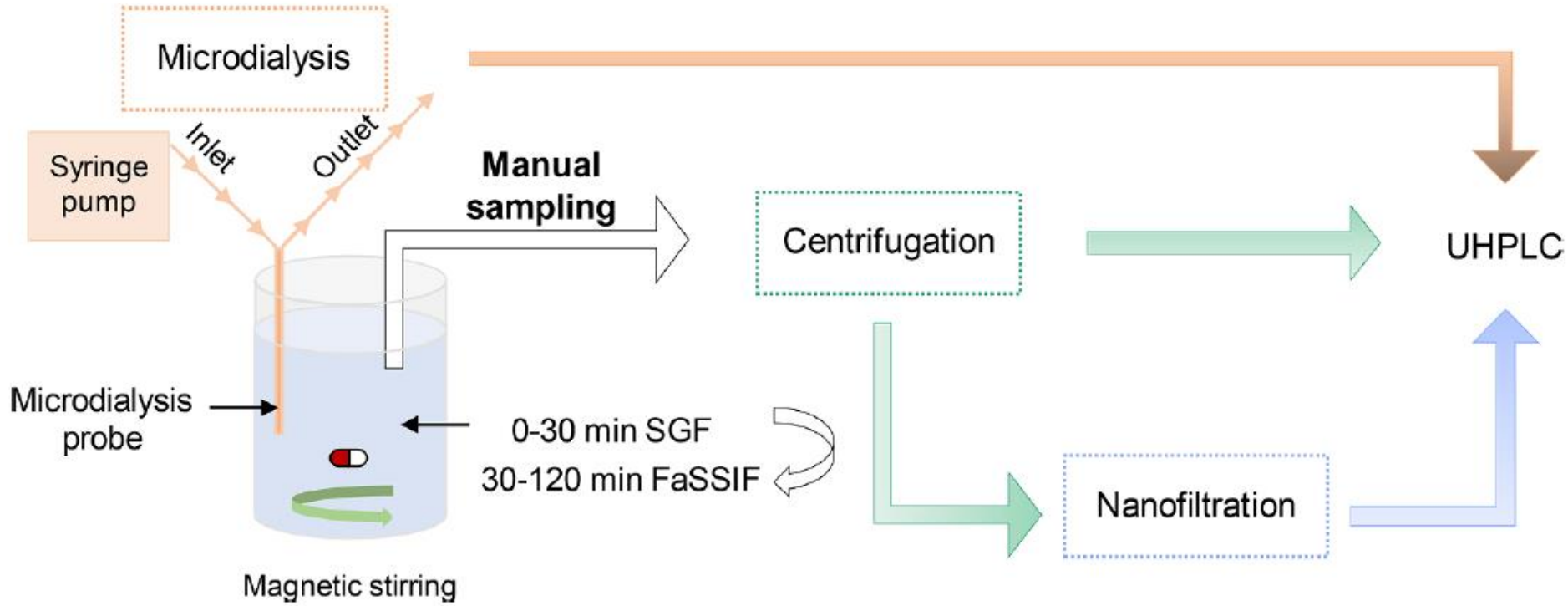
filtration through an Anotop® syringe filter (Whatman®, Maidstone, UK) with a nominal pore size of 20 nm



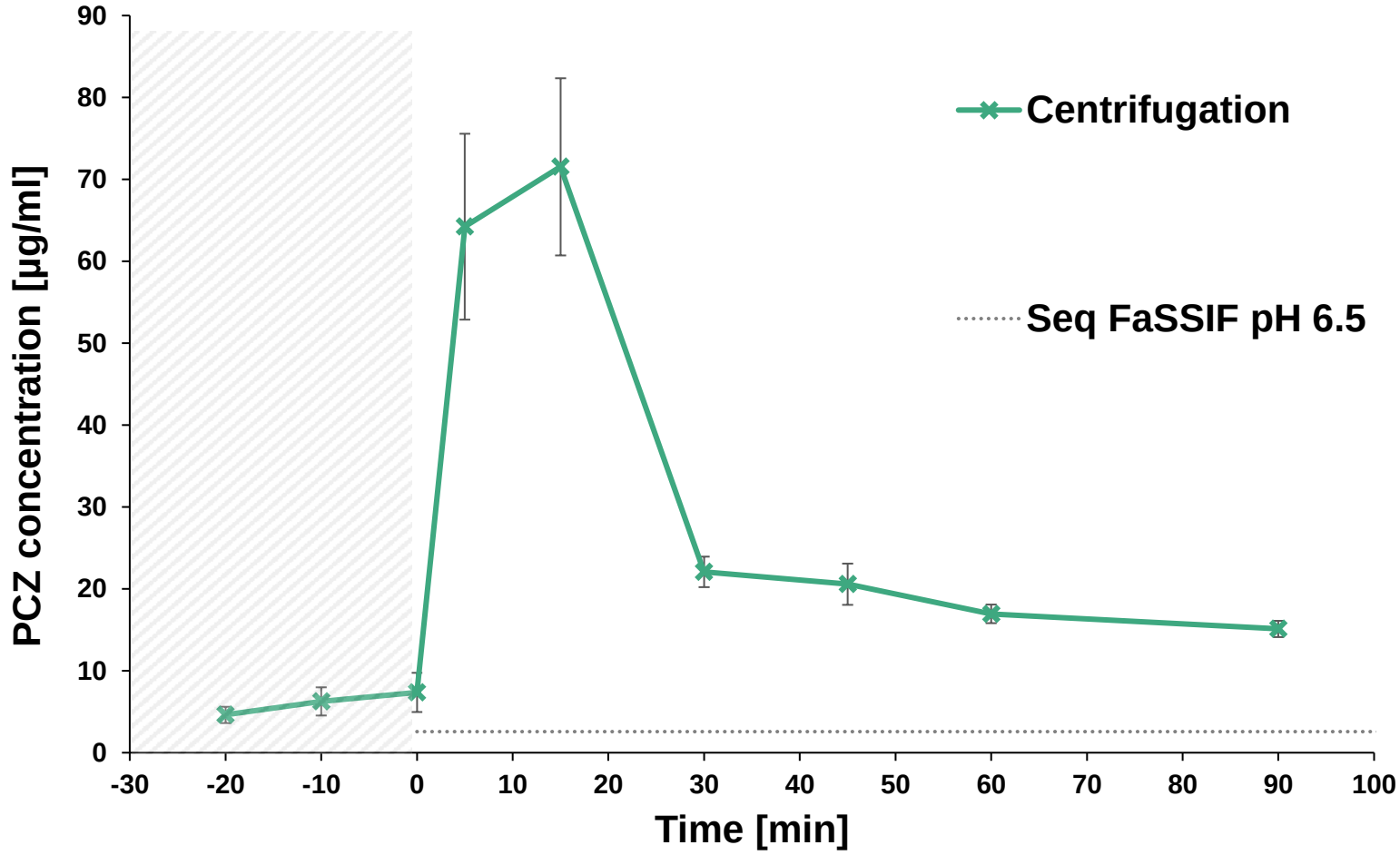


CMA/20 microdialysis concentric probes:
10 mm effective membrane length,
20 kDa molecular weight cut-off (~ 4 nm) polyarylethersulfone membrane;
2.5 mL syringes, SMA/4004 syringe pump

Two-stage dissolution with simultaneous sampling

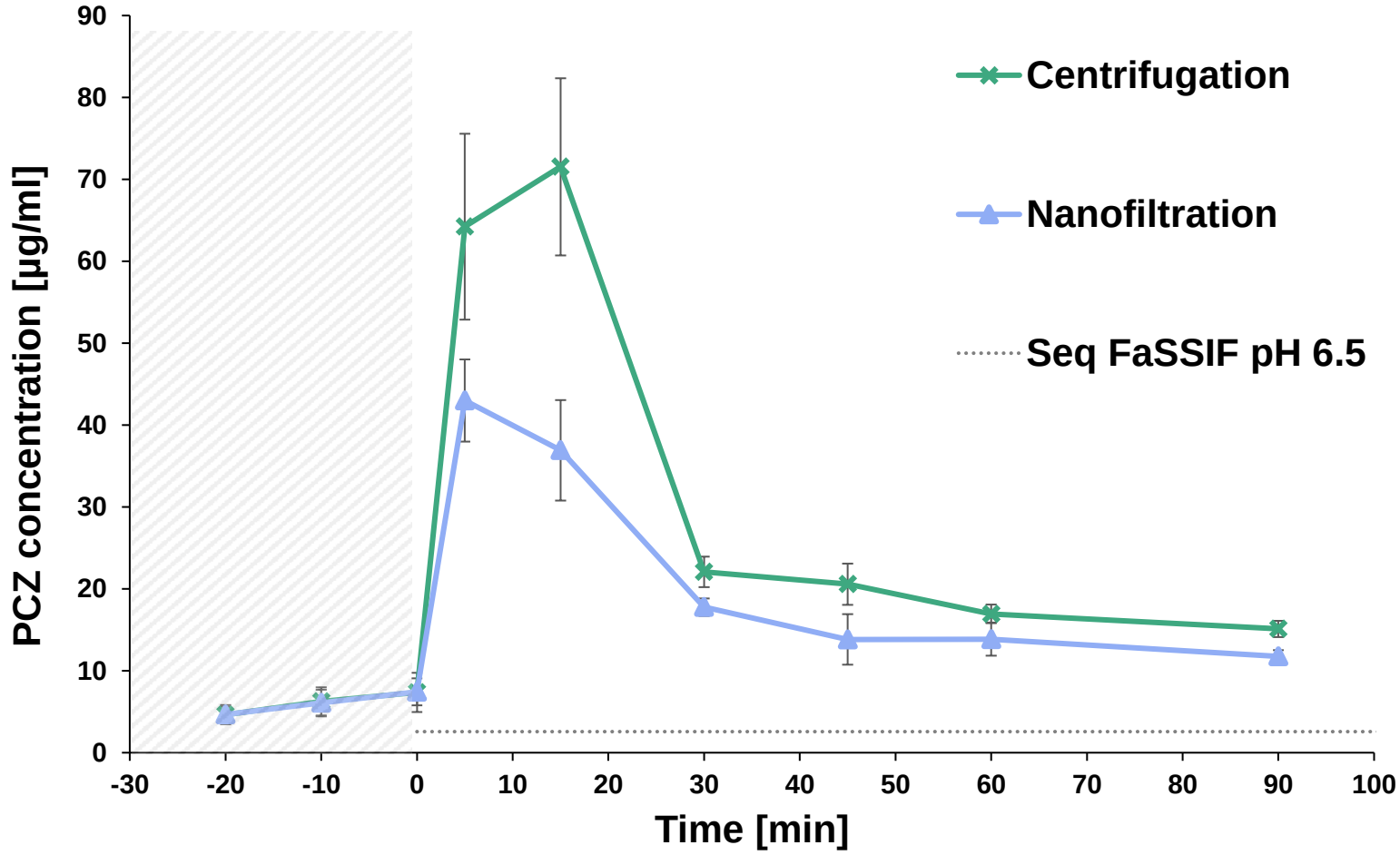


Two-Stage Dissolution of Posaconazole ASD



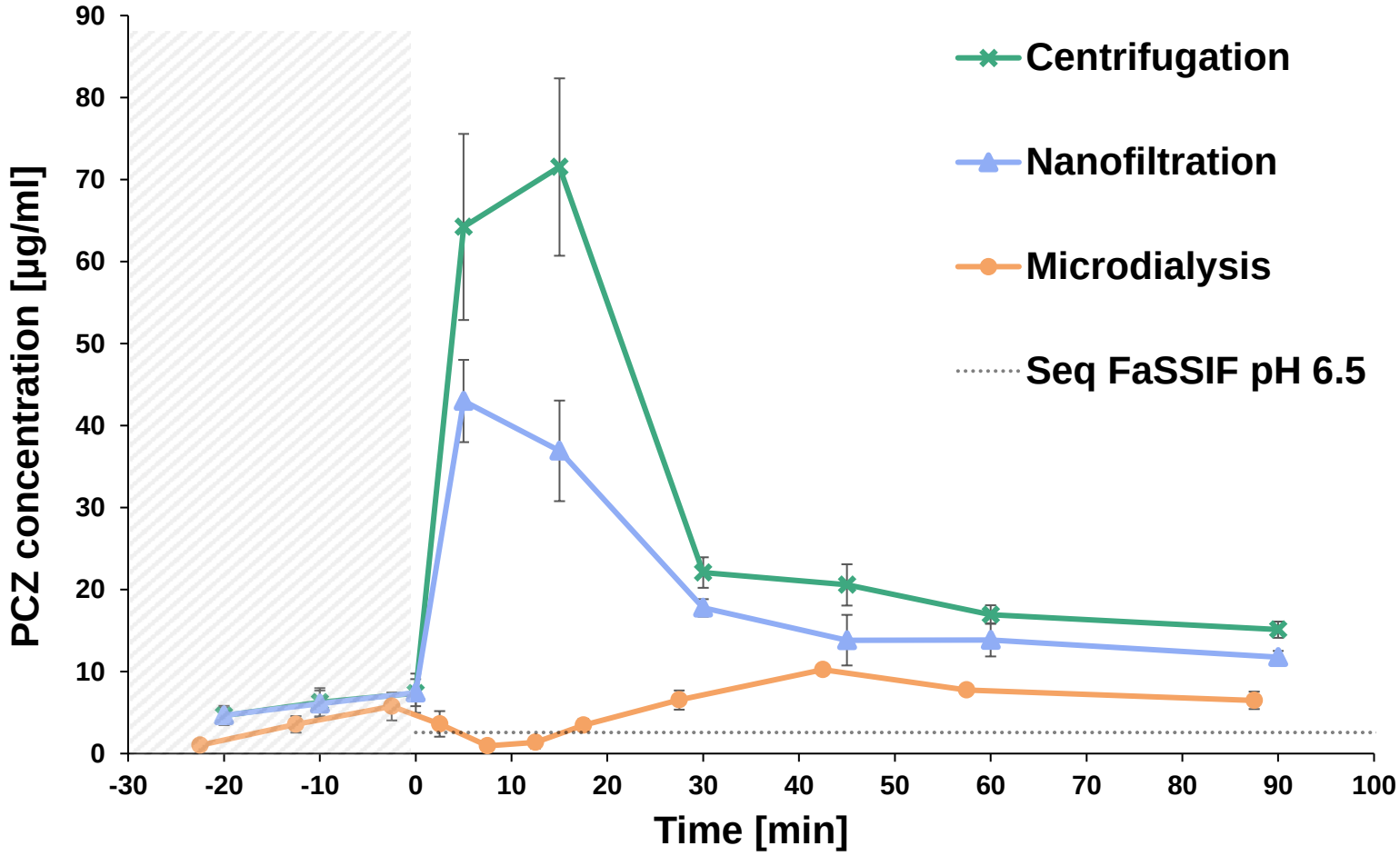
HPMCAS-containing ASD (Noxafil® Tablet, ground aliquot)

Two-Stage Dissolution of Posaconazole ASD



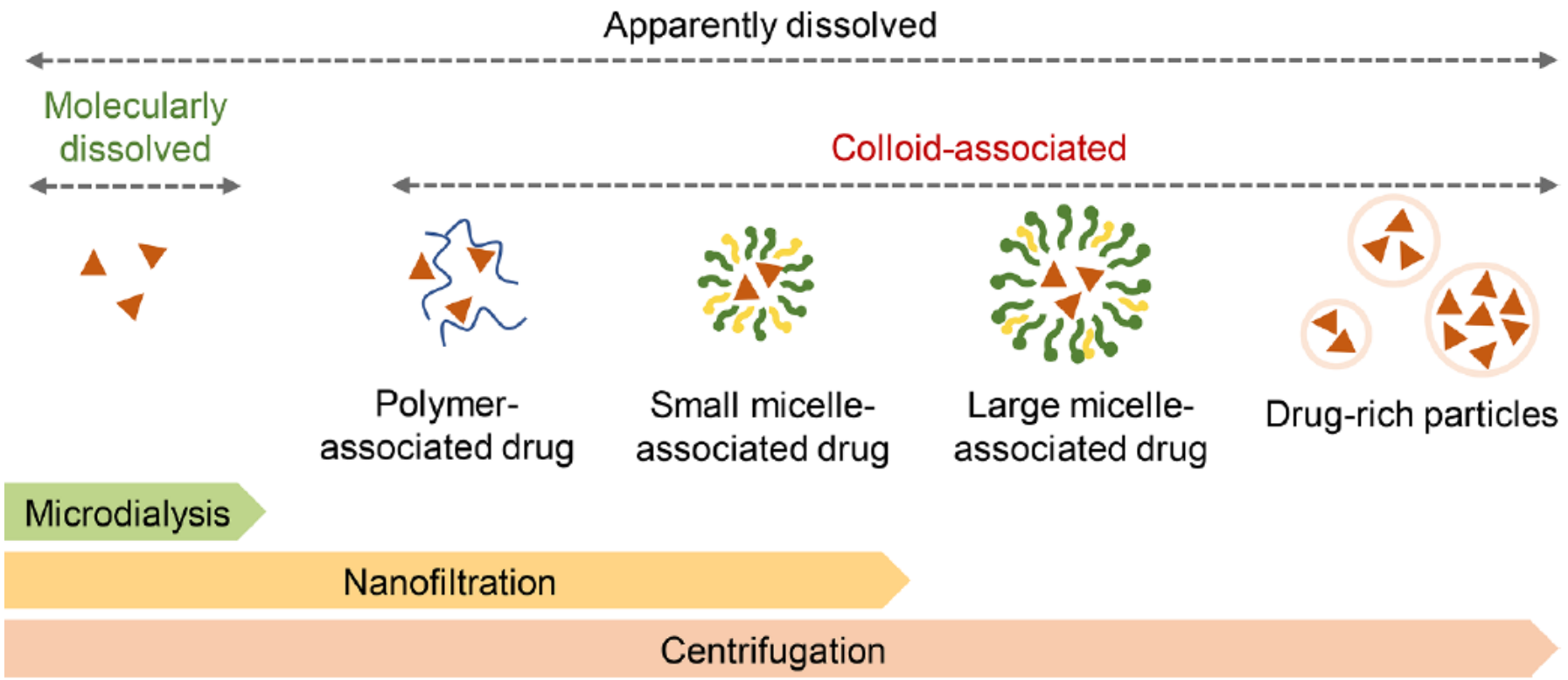
HPMCAS-containing ASD (Noxafil® Tablet, ground aliquot)

Two-Stage Dissolution of Posaconazole ASD



HPMCAS-containing ASD (Noxafil® Tablet, ground aliquot)

Differentiating between various “dissolved” species



in a close-to-realtime manner suited for dissolution studies

Take home messages

- With enabling formulations and/or use of biomimetic media, there may during dissolution arise drug
  - associated with various colloidal species,
    - which may enhance solubility & dissolution
    - but not necessarily permeation
  - in form of drug-rich amorphous or crystalline submicron particles
    - which may trigger (molecular) supersaturation
- Differentiation between the species
  - is feasible, yet challenging especially in time-resolved studies
  - but appears useful for mechanistic studies

Thank you for your attention!

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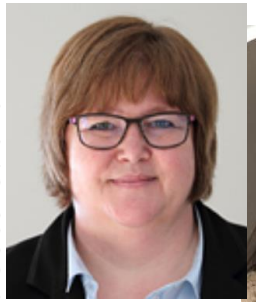
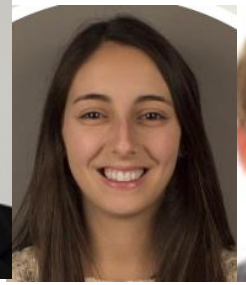
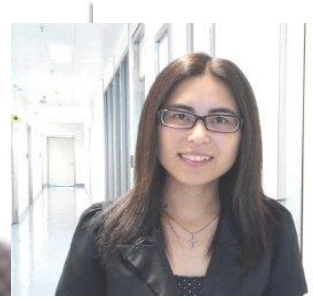

I. Tho


P. Nunes


P Stein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