Phospholipids used for in vitro biopharmaceutical characterization of oral drugs

PRC Webinar

14th of January 2026

Ann-Christin Jacobsen, PhD

In vitro biopharmaceutical characterization

- **Biopharmaceutics:** Predicting if and to what extent a drug is absorbed

In vitro methods

Dissolution testing

Permeation studies

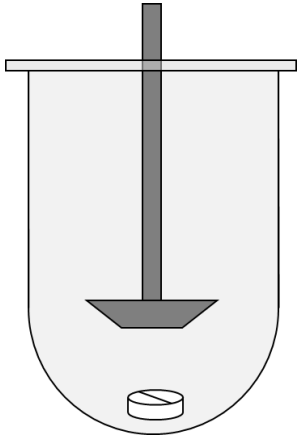
Phospholipids???

Combined dissolution/permeation studies

Phospholipids in dissolution testing

Dissolution testing

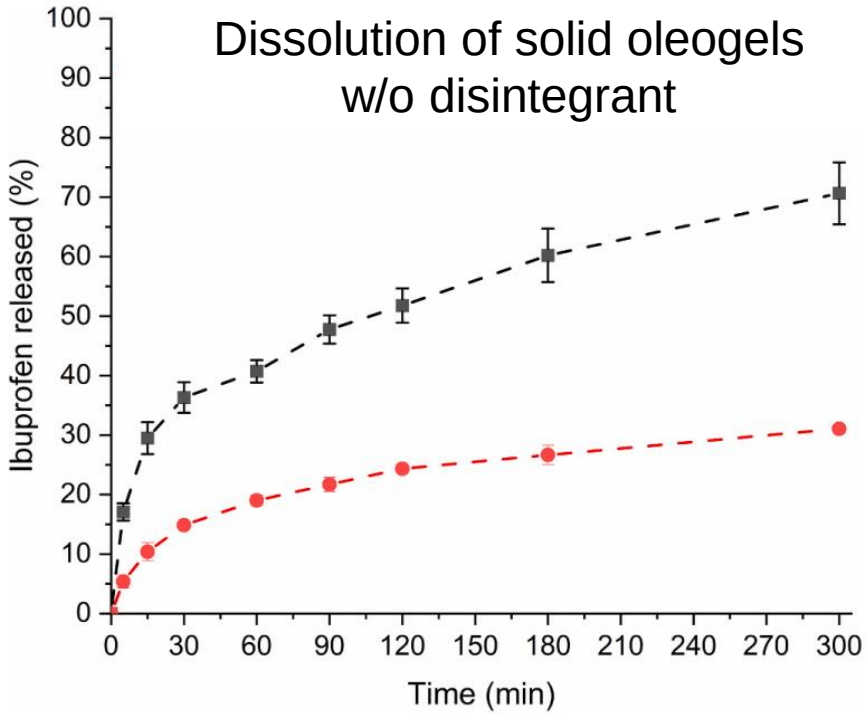
- Following the dissolution of a drug (from a dosage from) over time in aqueous medium
- Various instruments (e.g. USP paddle/basket)
- **Parameters to consider:**
  - Dissolution volume
  - Stirring speed / flow
  - Geometry
  - Sampling technique
  - Medium
- **Medium:**
  - pH
  - Buffer type, salt(s)
  - Enzymes, bile salts, **PLs**



Nernst-Brunner equation

$$\frac{dC}{dt} = \frac{D \times A}{V} \times \frac{c_s - c_t}{h}$$

Dissolution of solid oleogels w/o disintegrant

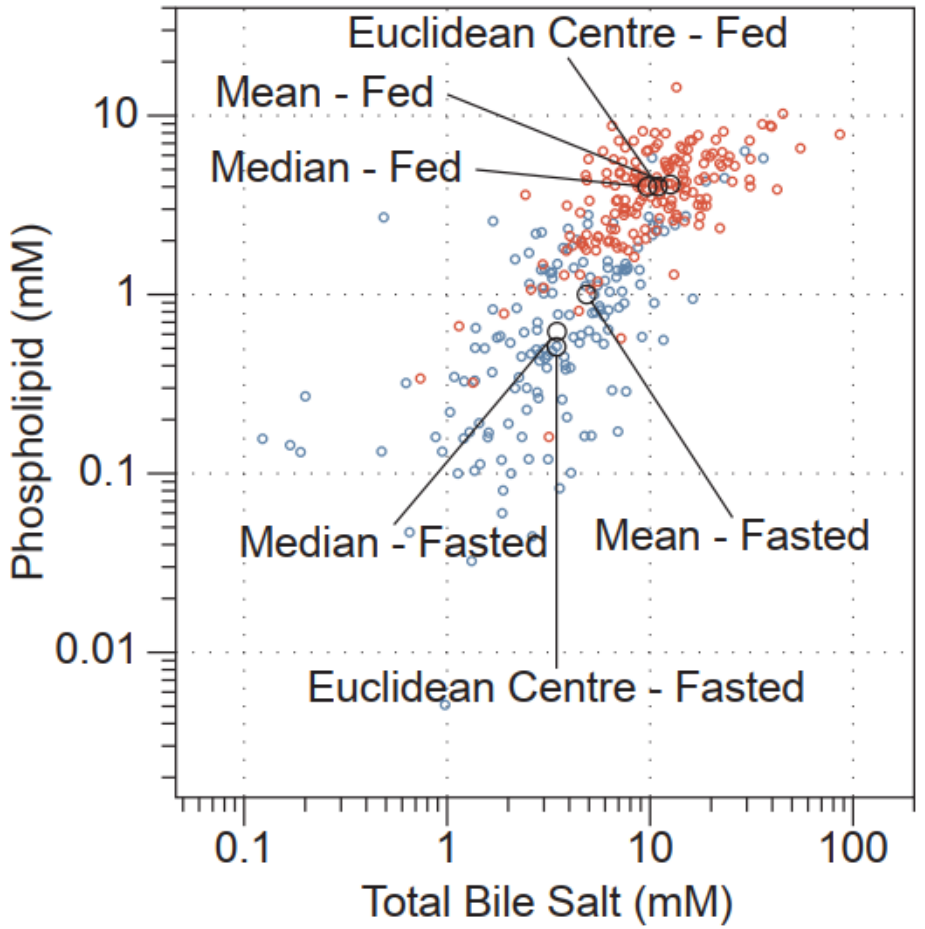


Kabels et al. 2025, *J. Control Release*, DOI:10.1016/j.jconrel.2025.114140

Phospholipids in intestinal fluids

- Phospholipids are present in (gastro)intestinal fluids
- Sources of PLs in GI fluids:
  - Bile
  - Food
- PLs in Bile:
  - >95 % PC
  - ~ 4:1 ratio between bile salts and PLs
- Assemblies:
  - Mixed bile salt-phospholipid micelles
  - Vesicles (fed)
- PL concentration in GI-fluids:
  - Intra- and interindividual variations
  - Variations with prandial state

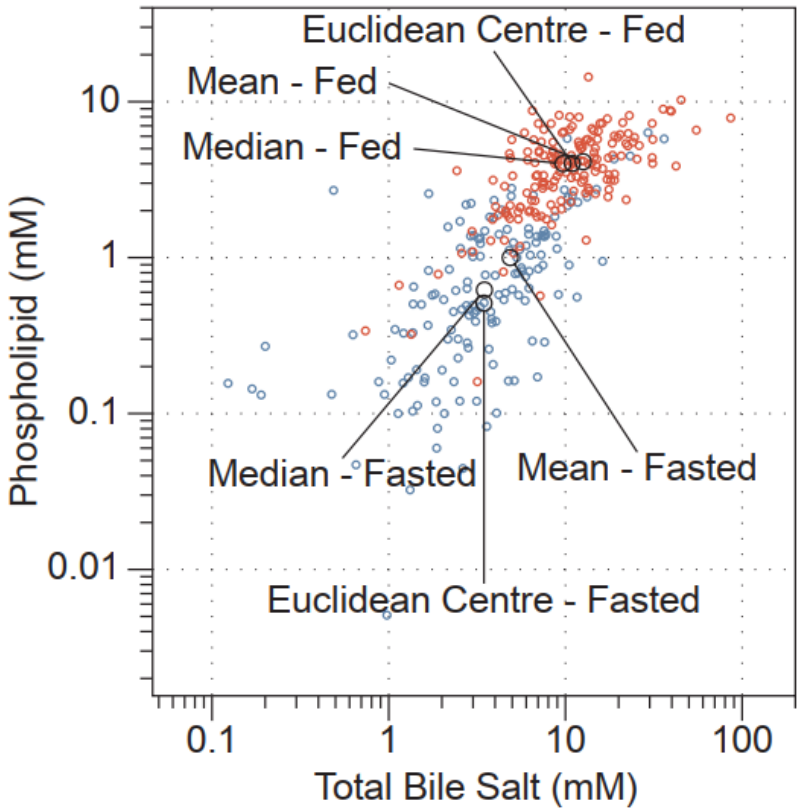
Solubilization of poorly soluble drugs!



Pyper et al. 2020, *Eur. J. Pharm. Biopharm.*, DOI: 10.1016/j.ejpb.2020.06.011.
Data from Riethorst et al. 2016, *J. Pharm. Sci.*, DOI: 10.1002/jps.24603

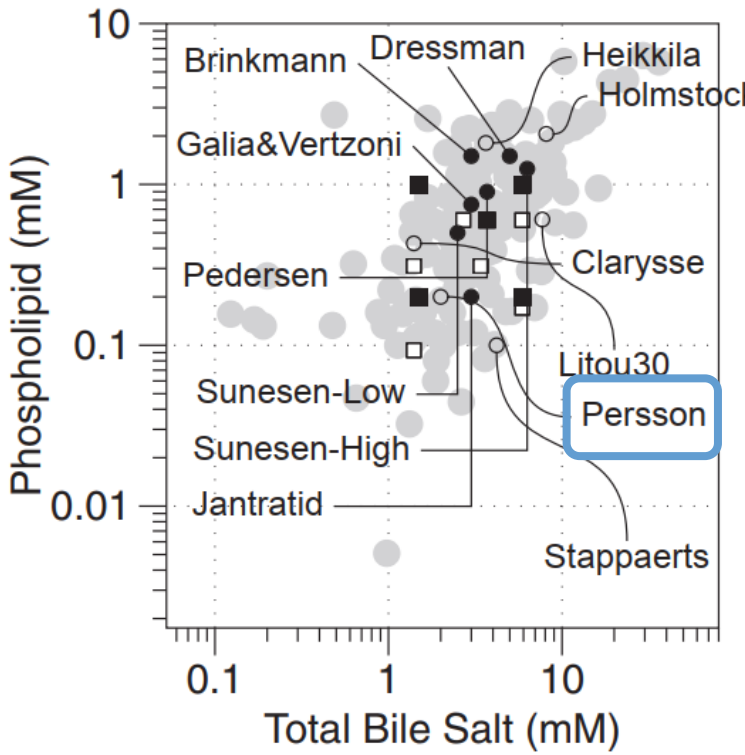
Phospholipids in intestinal fluids

Riethorst et al. 2016 data

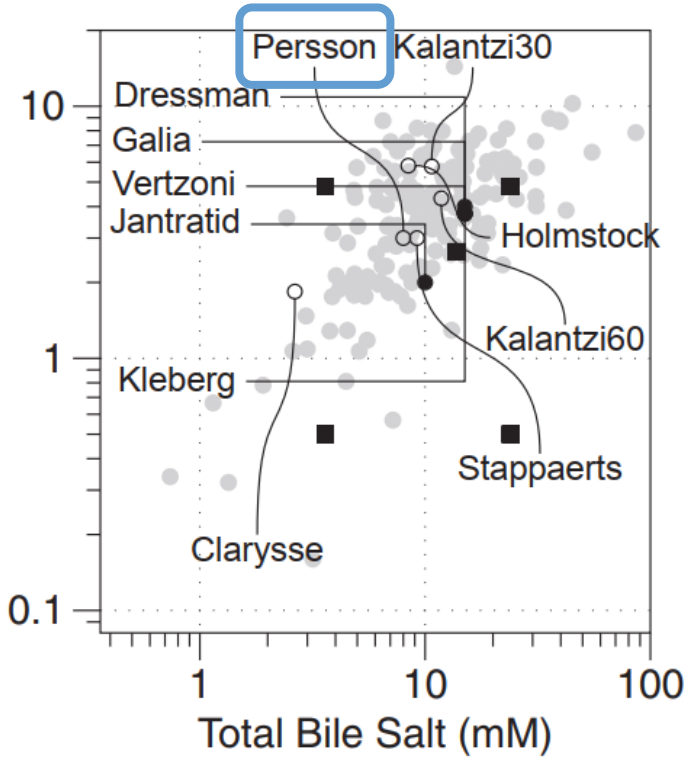


Compared to literature

Fasted-state HIF



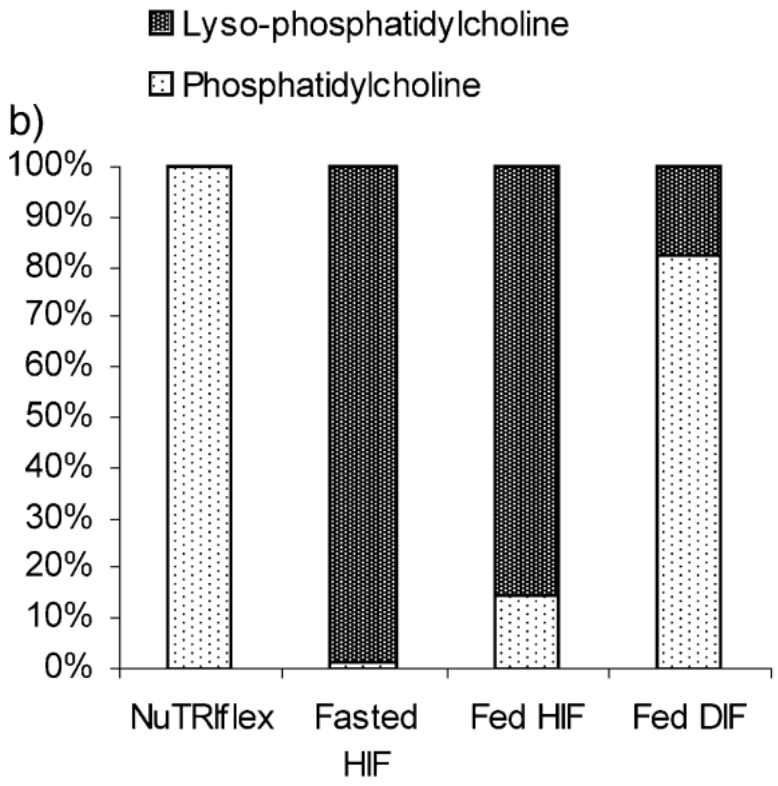
Fed-state HIF



Quantification of PLs (in most studies):
Enzymatic assay (choline)

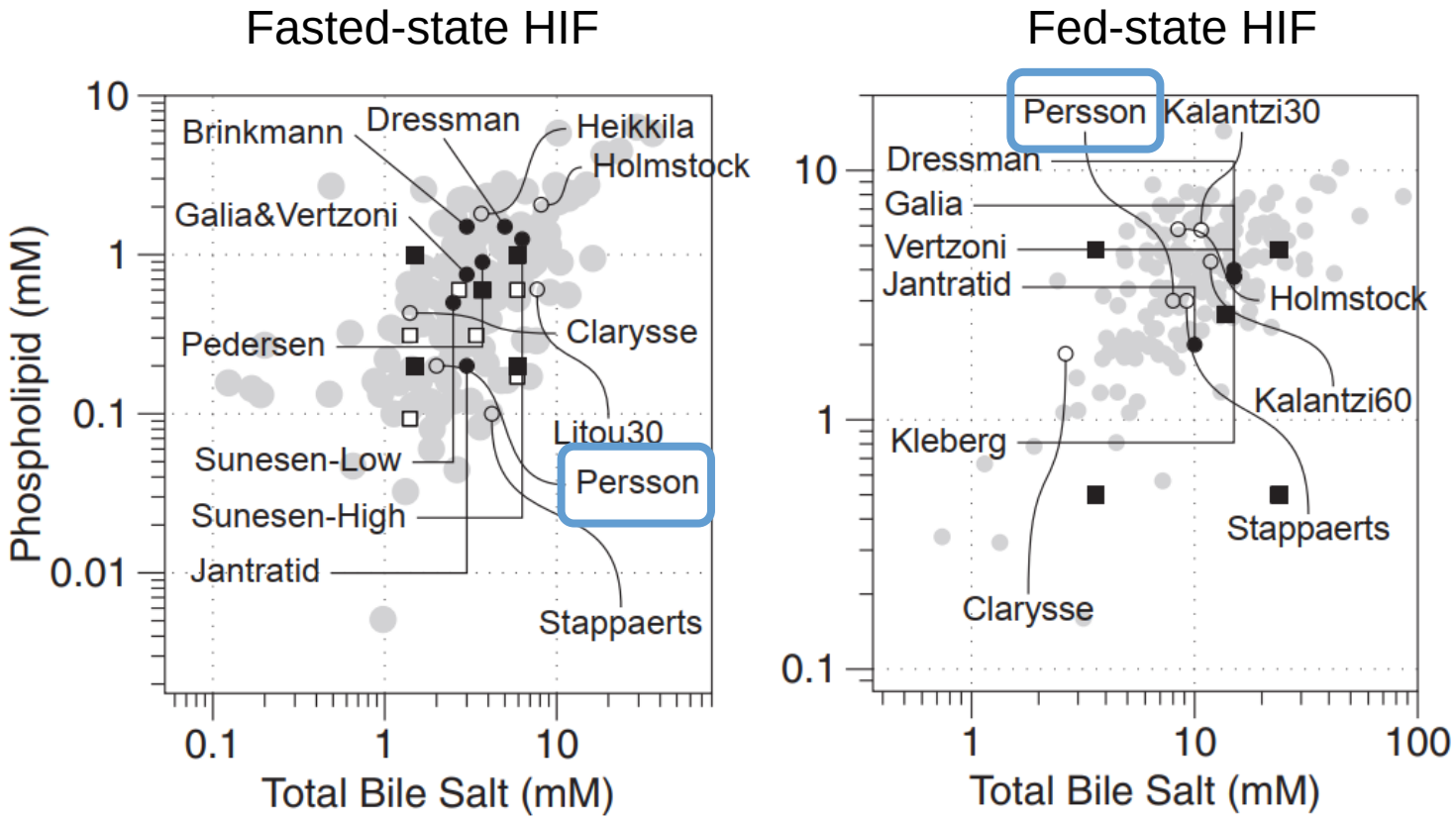
Pyper et al. 2020, *Eur. J. Pharm. Biopharm.*, DOI: 10.1016/j.ejpb.2020.06.011.

Phospholipids in intestinal fluids



Persson et al. 2005, *Pharm. Res.*, DOI:10.1007/s11095-005-8192-x

Compared to literature



Pyper et al. 2020, *Eur. J. Pharm. Biopharm.*, DOI: 10.1016/j.ejpb.2020.06.011.

Simulated intestinal fluids

- Mimicking (human) intestinal fluids
- Lecithin and bile salts (sodium taurocholate; NaTC)
- First described in 1998 by Galia et al. DOI: 10.1023/a:1011910801212
- Few variants

	FaSSIF V1*	FeSSIF V1*	FaSSIF V2*	FeSSIF V2*	FaSSIF V3**
Cholesterol (mM)	-	-	-	-	0.2
Glycerol monooleate	-	-	-	5	-
Lecithin (mM)	0.75	3.75	0.15	2	0.035
Lysolecithin (mM)	-	-	-	-	0.315
Sodium glycocholate (mM)	-	-	-	-	1.4
Sodium oleate (mM)	-	-	-	0.8	0.315
Sodium taurocholate (mM)	3	15	3	10	1.4
pH	6.5	5.0	6.5	5.8	6.7

* Commercially available from biorelevant.com, ** composition from Klumpp et al. 2020, DOI: 10.1016/j.ejps.2019.105138

Solubilization of poorly soluble drugs – simulated intestinal fluids

Table 2. Apparent Solubility and Intrinsic Dissolution Rate[a]

	apparent solubility (μg/mL)				IDR (μg/min/cm^2)			
	pH 6.5		pH 5.0		pH 6.5		pH 5.0	
compound	$FaSSIF_{blk}$	FaSSIF	$FeSSIF_{blk}$	FeSSIF	$FaSSIF_{blk}$	FaSSIF	$FeSSIF_{blk}$	FeSSIF
albendazole	0.85 ± 0.36	1.9 ± 0.0	1.1 ± 0.0	6.1 ± 0.1	0.09 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.30 ± 0.01
astemizole	19.2 ± 0.2	97.9 ± 2.1	248 ± 6	182 ± 1	1.80 ± 0.10	7.76 ± 0.17	22.7 ± 0.6	16.9 ± 0.1
carvedilol	46.0 ± 1.0	55.9 ± 1.0	243 ± 2	305 ± 2	4.40 ± 0.10	5.40 ± 0.09	23.2 ± 0.2	26.0 ± 0.2
cinnarizine	1.4 ± 2.5	13.4 ± 0.9	11.8 ± 0.2	112 ± 2	0.14 ± 0.25	0.33 ± 0.02	1.18 ± 0.17	4.44 ± 0.07
danazol	0.6 ± 0.0	8.4 ± 0.7	0.9 ± 0.1	28.8 ± 0.4	0.06 ± 0.01	0.16 ± 0.01	0.09 ± 0.01	0.95 ± 0.01
felodipine	1.2 ± 0.0	54.5 ± 3.7	1.1 ± 0.0	237 ± 1	0.12 ± 0.01	0.64 ± 0.04	0.11 ± 0.00	7.16 ± 0.01
glibenclamide	4.5 ± 1.1	4.7 ± 0.1	0.3 ± 0.0	2.5 ± 0.2	0.48 ± 0.11	0.41 ± 0.01	0.03 ± 0.00	0.10 ± 0.01
indomethacin	219 ± 78	443 ± 10	14.9 ± 0.2	109 ± 7	21.7 ± 7.8	33.5 ± 0.7	1.49 ± 0.02	4.63 ± 0.28
tamoxifen	5.9 ± 0.2	156 ± 2	22.6 ± 0.9	236 ± 13	0.58 ± 0.02	2.25 ± 0.03	2.21 ± 0.09	23.1 ± 1.3
tolfenamic acid	27.4 ± 0.5	63.0 ± 2.7	0.8 ± 0.0	41.0 ± 0.5	2.98 ± 0.06	4.01 ± 0.17	0.09 ± 0.01	1.31 ± 0.02
min	0.6	1.9	0.3	2.5	0.06	0.13	0.03	0.10
max	219	443	248	305	21.7	33.5	23.2	26.0

[a] Apparent solubility and intrinsic dissolution rate (IDR) in fasted state simulated intestinal fluid (FaSSIF), fed state simulated fluid (FeSSIF) and their corresponding blank buffers without taurocholic acid and lecithin ($FaSSIF_{blk}$ and $FeSSIF_{blk}$).

Fagerberg et al. 2010, *Mol. Pharm.,* DOI: 10.1021/mp100049m

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astemizole	19.2 ± 0.2	97.9 ± 2.1	5.10	248 ± 6	182 ± 1	0.73	1.80 ± 0.10	7.76 ± 0.17	22.7 ± 0.6	16.9 ± 0.1
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cinnarizine	1.4 ± 2.5	13.4 ± 0.9	9.57	11.8 ± 0.2	112 ± 2	9.49	0.14 ± 0.25	0.33 ± 0.02	1.18 ± 0.17	4.44 ± 0.07
danazol	0.6 ± 0.0	8.4 ± 0.7	14.00	0.9 ± 0.1	28.8 ± 0.4	32.00	0.06 ± 0.01	0.16 ± 0.01	0.09 ± 0.01	0.95 ± 0.01
felodipine	1.2 ± 0.0	54.5 ± 3.7	45.42	1.1 ± 0.0	237 ± 1	215.5	0.12 ± 0.01	0.64 ± 0.04	0.11 ± 0.00	7.16 ± 0.01
glibenclamide	4.5 ± 1.1	4.7 ± 0.1	1.04	0.3 ± 0.0	2.5 ± 0.2	8.33	0.48 ± 0.11	0.41 ± 0.01	0.03 ± 0.00	0.10 ± 0.01
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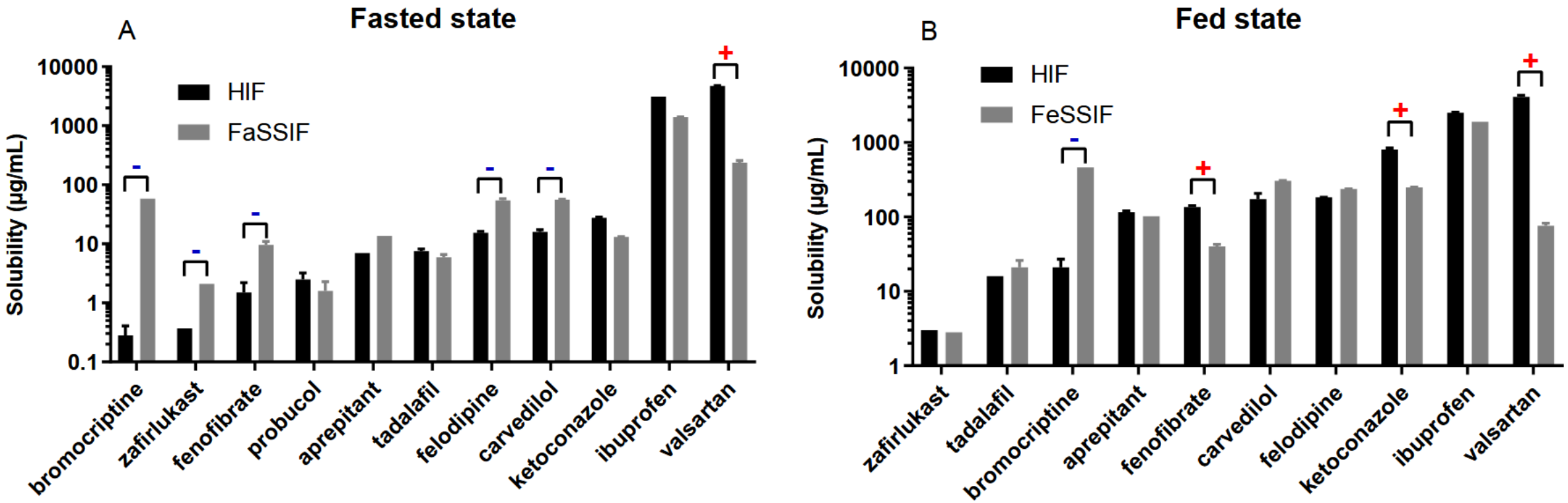
Modiefied from Fagerberg et al. 2010, *Mol. Pharm.,* DOI: 10.1021/mp100049m

Simulated vs human intestinal fluids

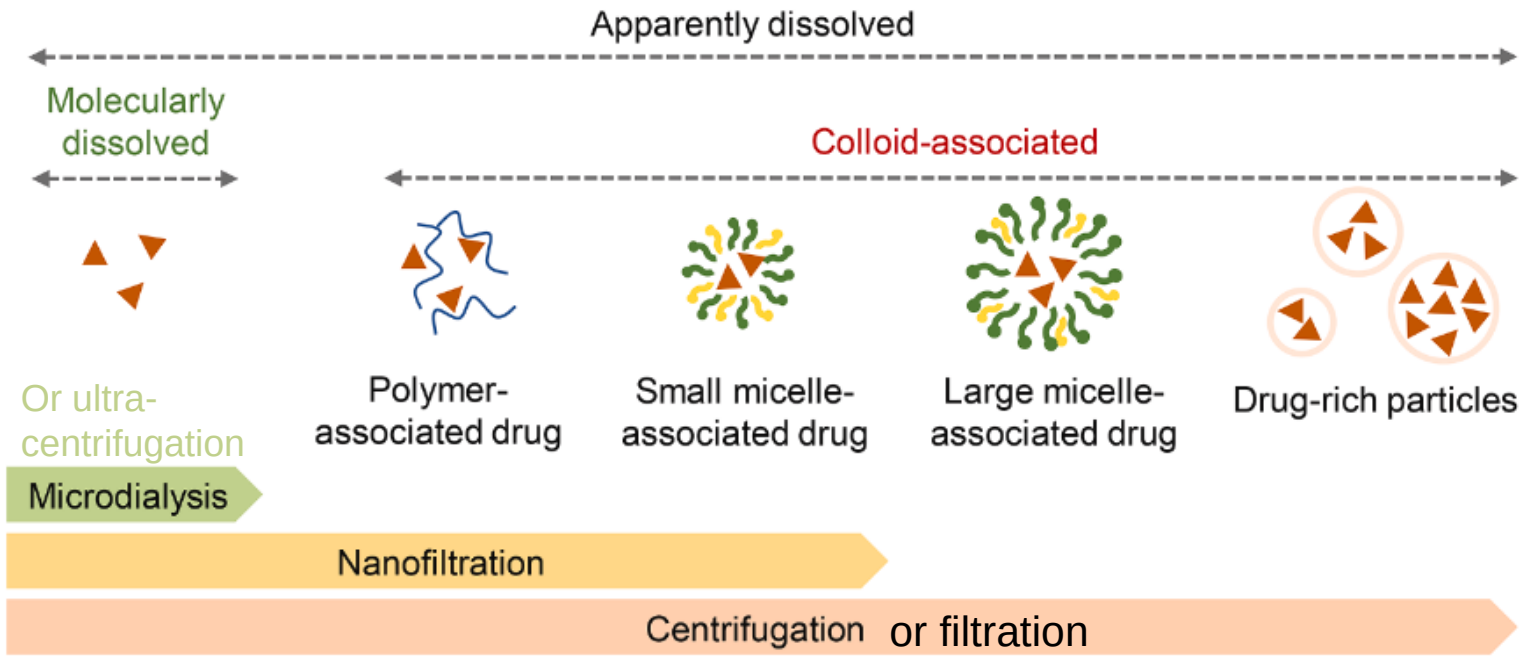


Dahlgren et al. 2021, *Eur. J. Pharm. Biopharm.*, DOI: 10.1016/j.ejpb.2021.04.005

Increased solubility in presence of mixed micelles: what are we measuring?

- Typical sample preparation during solubility or dissolution studies:
  - Filtration or bench-top centrifugation
  - Dilution
- Alternative sampling / sample preparation:
  - Microdialysis
  - Ultra-centrifugation

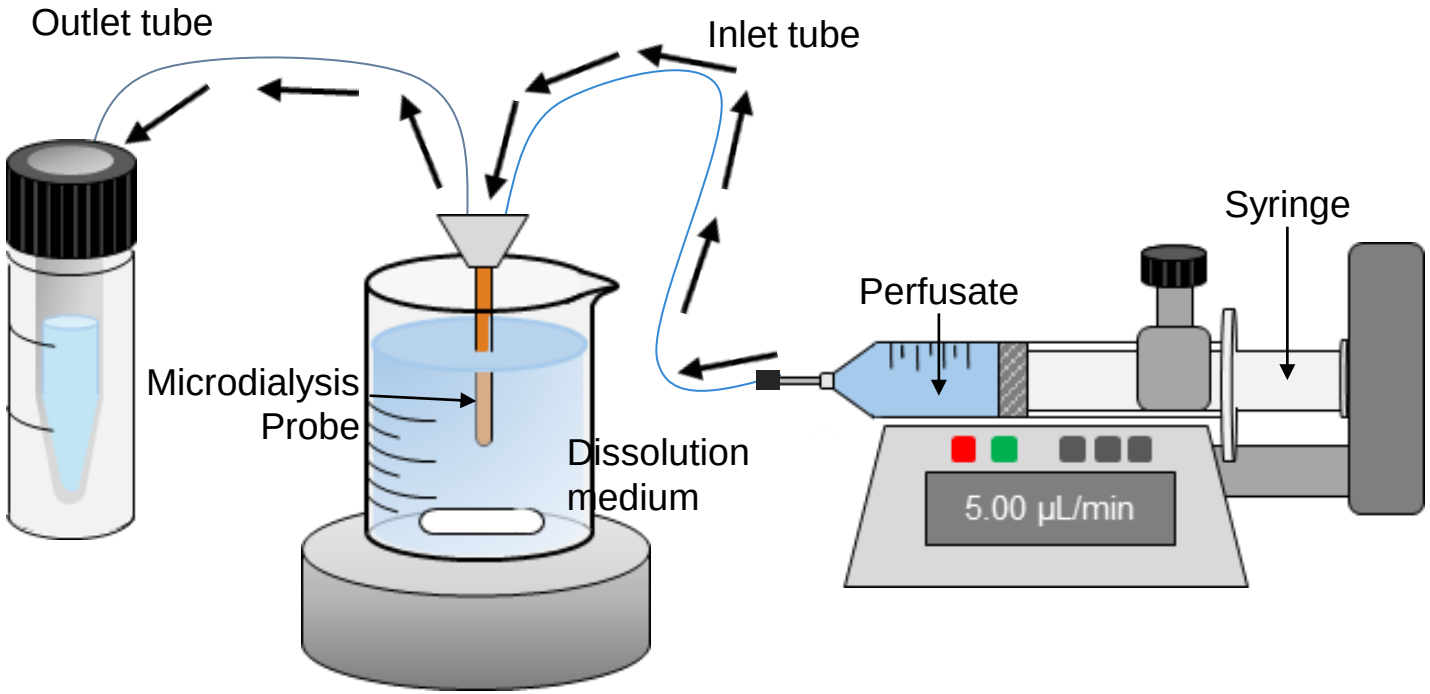
Species present in samples from dissolution studies on posaconazole ASDs

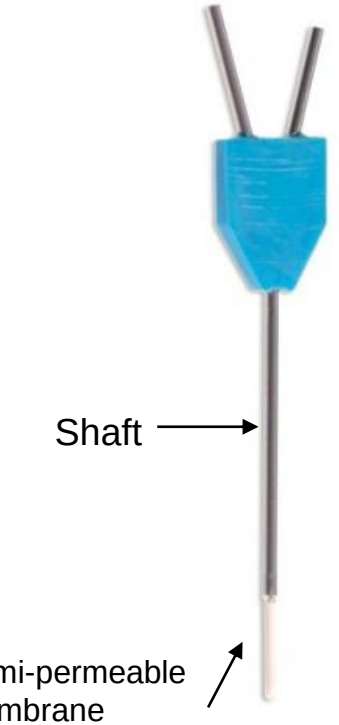


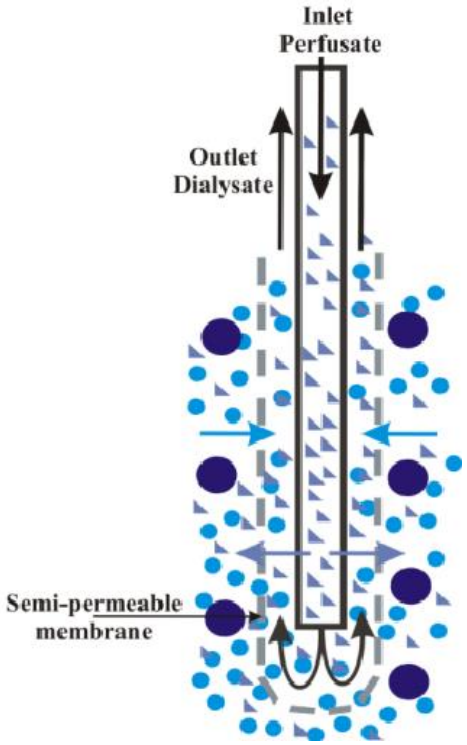
Holzem et al. 2022, *Eur. J. Pharm. Sci.*, DOI:10.1016/j.ejps.2022.106166

Microdialysis sampling

- Commonly used for minimal invasive tissue sampling (e.g., drug concentration in brain)
- Can separate molecules (or very small assemblies) from colloids (e.g. mixed micelles)





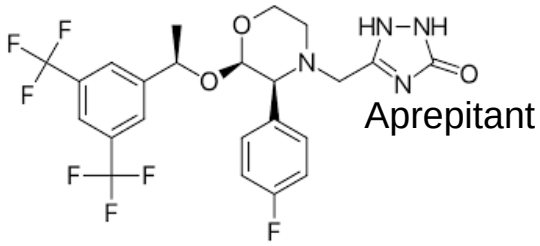


Tønning et al. 2025, Mol. Pharm., DOI: 10.1021/acs.molpharmaceut.5c00640

Small molecules Large molecules Salt from perfusate

Example: Apparent vs molecular solubility of aprepitant in simulated intestinal fluids

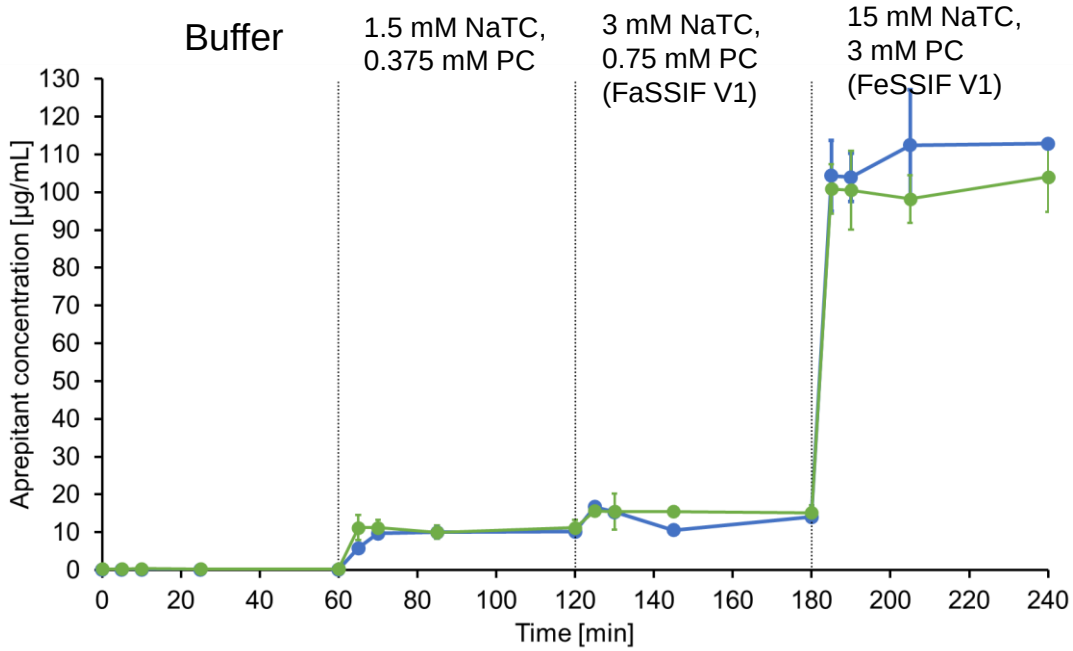
- Very poorly soluble in aqueous media (e.g., ~0.25 µg/ml in PBS pH 6.5)
- Simulated intestinal fluids substantially increase apparent solubility
- Microdialysis sampling:
  - Significant increase in molecular solubility in FeSSIF V1 (pH 6.5)



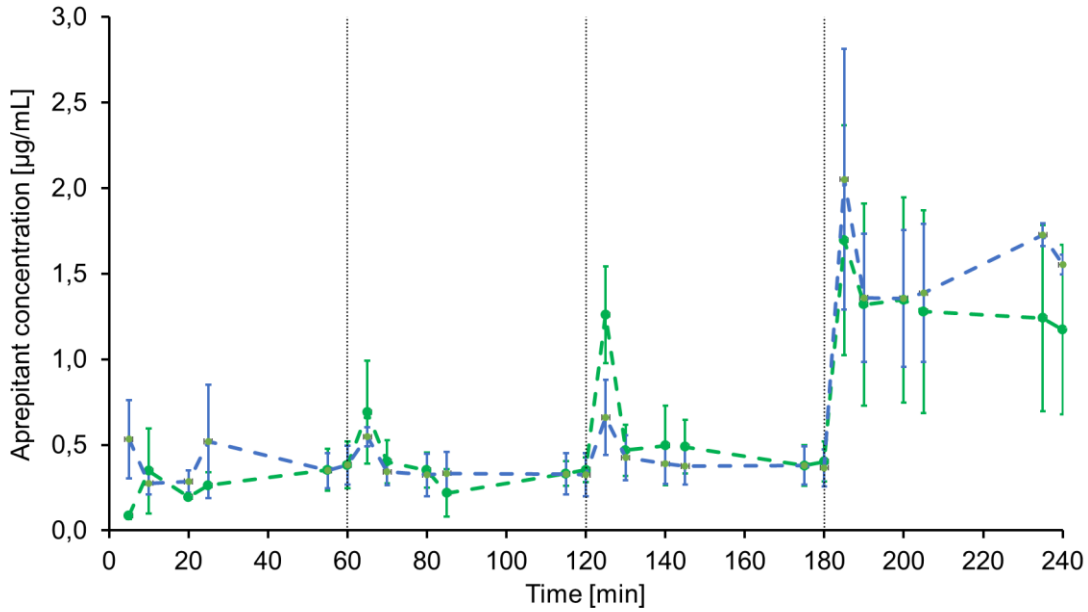
Aprepitant

20 kDa cut-off
100 kDa cut off

Bench-top centrifugation

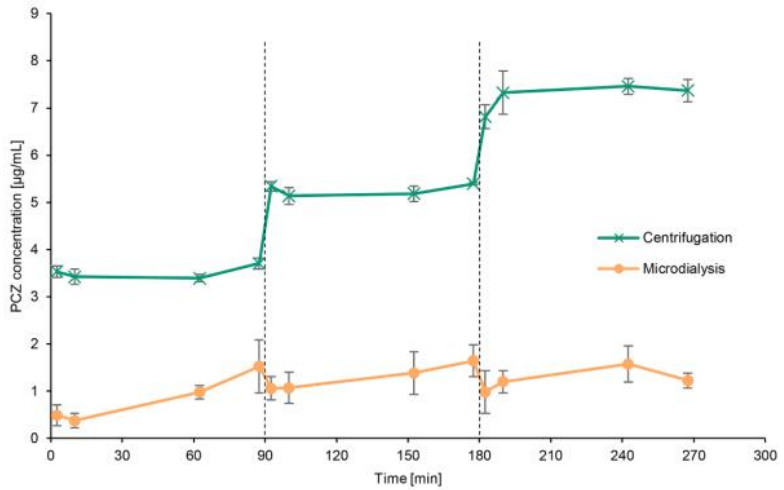


Microdialysis sampling



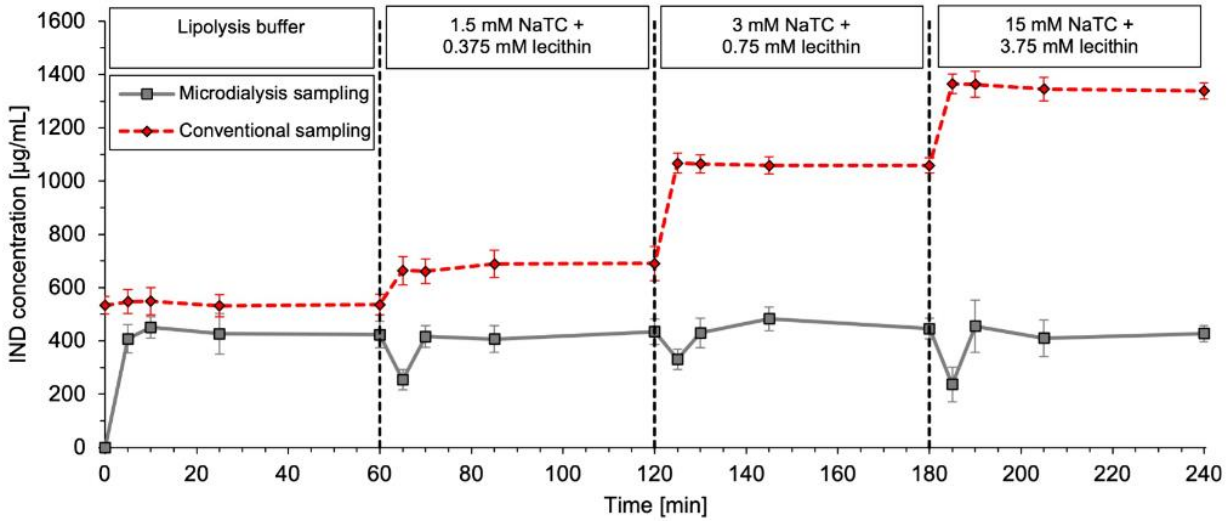
Comparison – Molecular solubility in presence of (mixed) micelles

Posaconazole

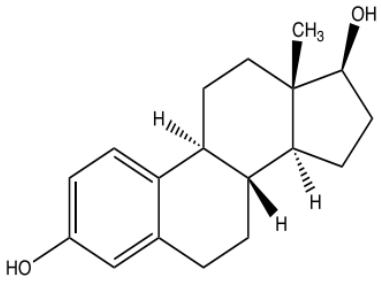


Holzem et al. 2022, *Eur. J. Pharm. Sci.,* DOI:10.1016/j.ejps.2022.106166

Indomethacin



Tønning et al. 2025, Mol. Pharm., DOI: 10.1021/acs.molpharmaceut.5c00640



(a) 17β-Estradiol (E2)

Ultra-centrifugation

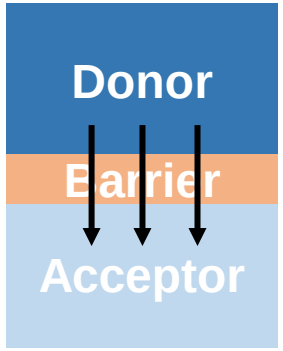
Without PS80: Crystalline and amorphous solubility of E2
2.2 and 13.9 µg/mL, respectively.
With 1% PS80: Crystalline and amorphous solubility of E2
4.6 µg/mL and 15.7 µg/mL

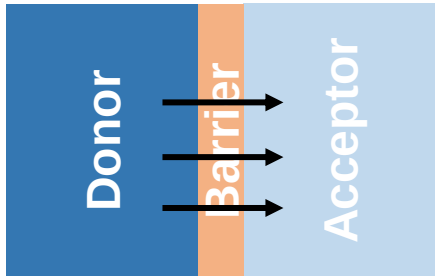
Arce et al. 2020, *Mol. Pharm.,* DOI:10.1021/acs.molpharmaceut.0c00599

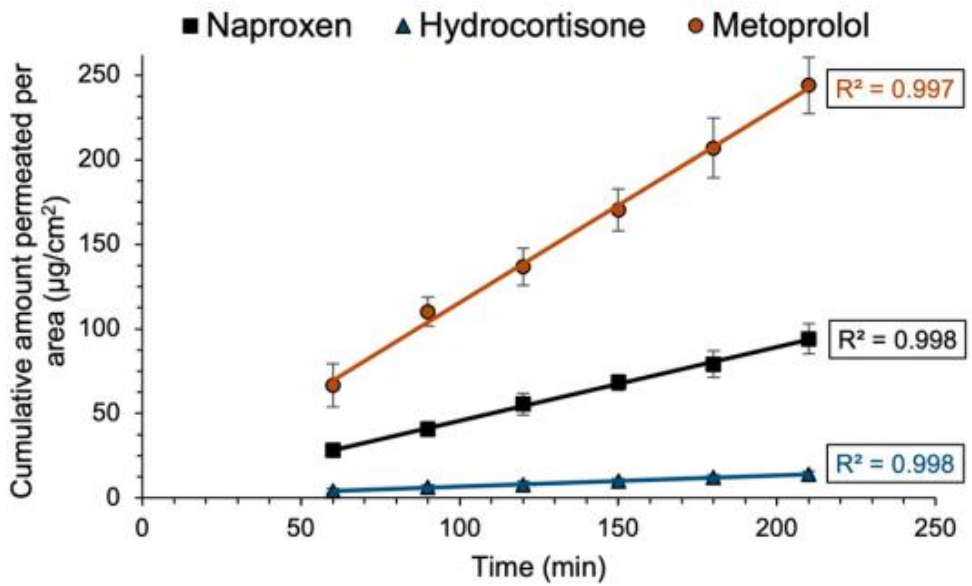
Phospholipids in permeation studies

Permeation studies

- Following the movement of drug molecules from one compartment (donor) across a barrier to another compartment (acceptor/receiver)
- **Barriers:**
  - tissues (e.g., intestinal tissue from rat),
  - cells (e.g **Caco-2** monolayers),
  - artificial barriers w/o PLs

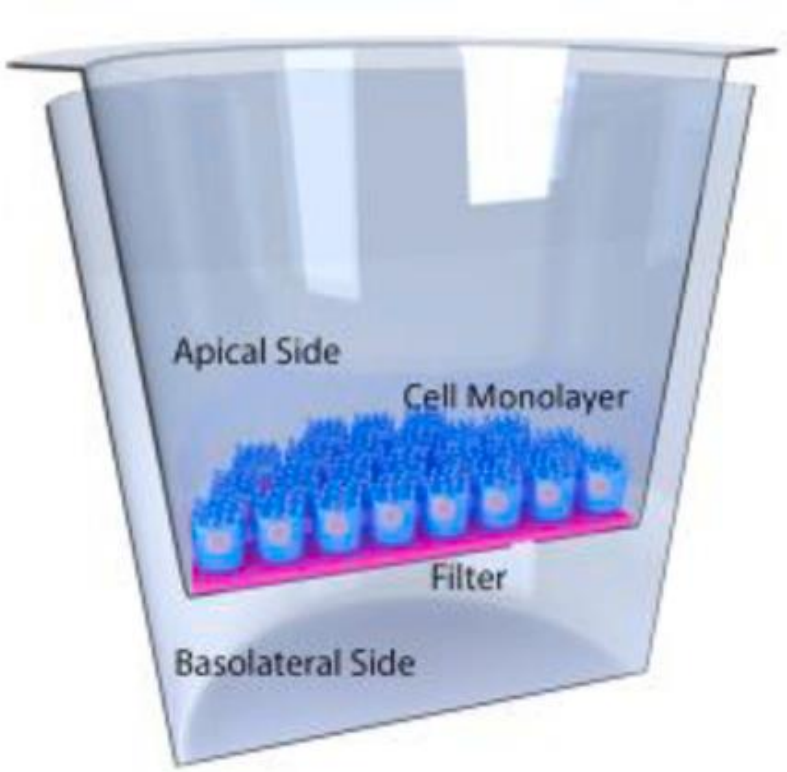






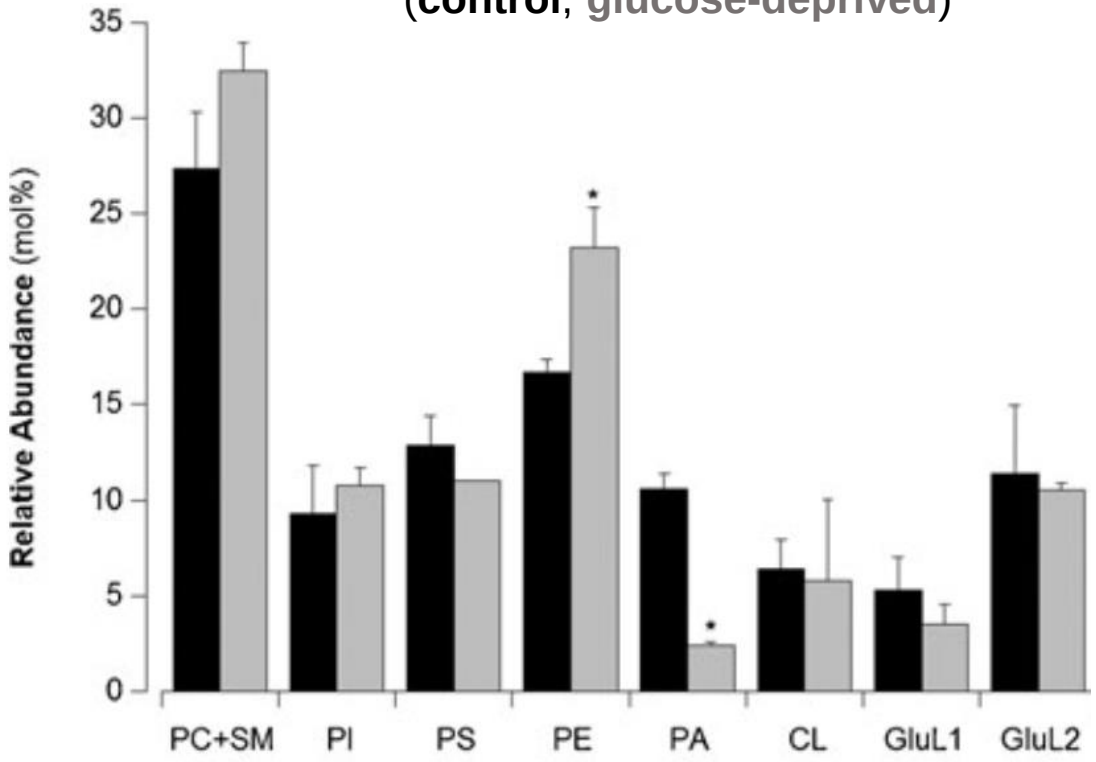
Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

Caco-2 permeability assay



Jarc et al. 2019, *J. Pharm. Pharmacol.*,
DOI: 10.1111/jphp.13111

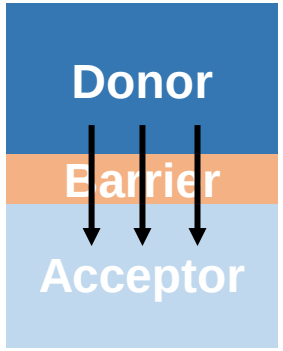
PL composition
(**control**, **glucose-deprived**)

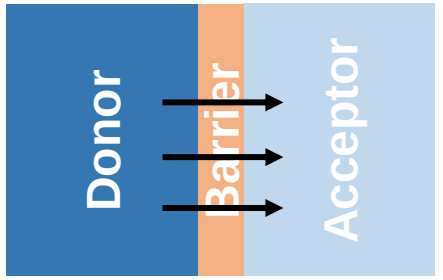


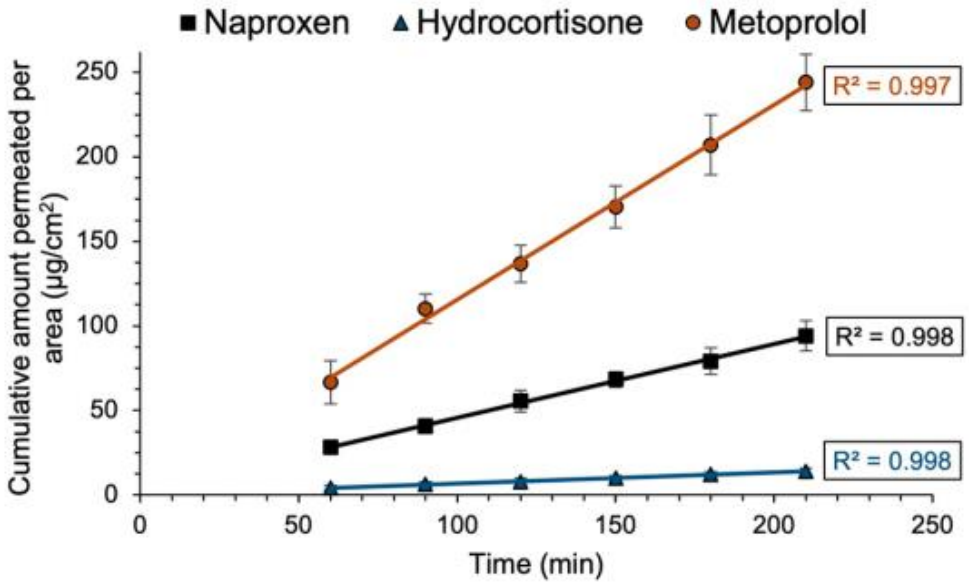
Monteiro-Cardoso et al. 2014, J. Bioenerg. Biomembr.,
DOI: 10.1007/s10863-013-9531-y

Permeation studies

- Following the movement of drug molecules from one compartment (donor) across a barrier to another compartment (acceptor/receiver)
- **Barriers:**
  - tissues (e.g., intestinal tissue from rat),
  - cells (e.g., Caco-2 monolayers),
  - artificial barriers w/o PLs
- **Artificial barriers with PLs:**
  - PAMPA
  - PVPA
  - Permeapad®







Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

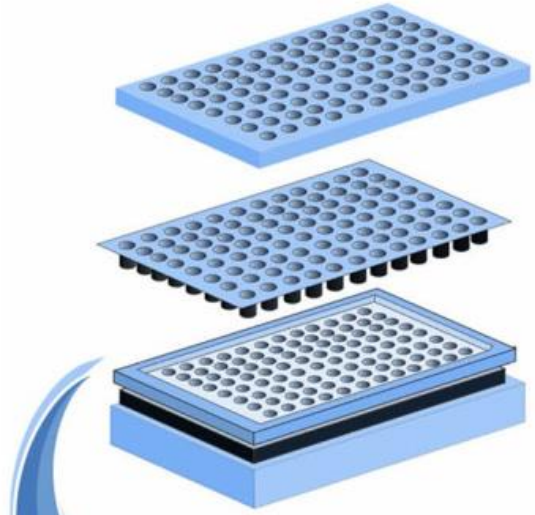
PAMPA

- Developed by Kansy and co-workers (F. Hoffmann-La Roche) in 1998
- Barrier composition: A filter impregnated with phospholipids dissolved in an organic solvent
- 96-well format most common
- Several variants

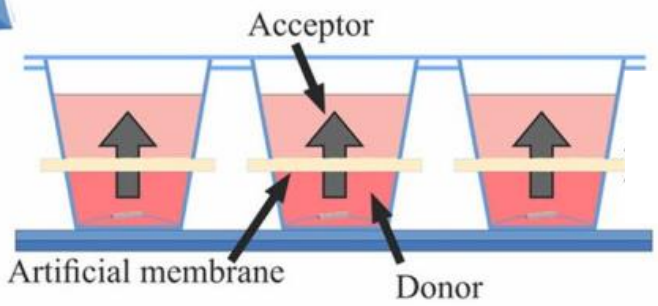
Table 1
Characteristics of PAMPA assays.

Assay short name	Barrier target	Support filter	Solvent	Membrane composition	Lit. ref
Egg-PAMPA	GI-tract	Hydrophobic PVDF (125 µm)	n-Dodecane	10% egg lecithin	(Kansy et al., 1998)
HDM-PAMPA	GI-tract	Polycarbonate 10 µm, non-tissue culture treated	n-Hexadecane	n-Hexadecane	(Wohnsland and Faller, 2001)
BM-PAMPA	GI-tract	Hydrophobic PVDF (125 µm)	1.7-Octadiene	3% of a phospholipid mixture	(Sugano et al., 2001)
	GI-tract	Hydrophilic PVDF (125 µm)	n-Dodecane	1% egg lecithin	(Zhu et al., 2002)
DOPC-PAMPA	GI-tract	Hydrophobic PVDF (125 µm)	n-Dodecane	2% DOPC	(Avdeef et al., 2001)
PAMPA-DS	GI-tract	Hydrophobic PVDF (125 µm)	n-Dodecane	20% (phospholipid mixture)	(Bermejo et al., 2004)
BBB-PAMPA	BBB	Hydrophobic PVDF (125 µm)	n-Dodecane	2% polar brain lipids	(Di et al., 2003)
PAMPA-skin	Skin	Hydrophobic PVDF (125 µm)	70% silicon oil, 30% iso-propyl myristate	70% silicon oil, 30% iso-propy myristate	(Ottaviani et al., 2006)
Precoated PAMPA	GI-tract	PVDF (0.45 µm)	Hexane	Lipid/oil/lipid trilayer	(Chen et al., 2008)

Modified from Berben et al. 2017, *Eur. J. Pharm. Sci.*, DOI: 10.1007/s11095-023-03499-9

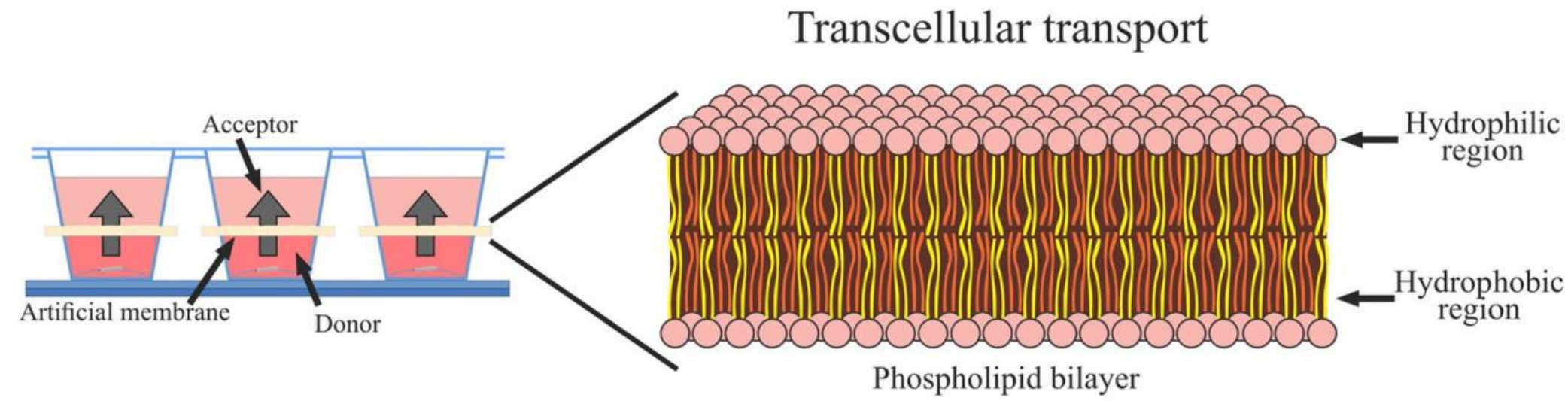


PAMPA ”sandwich” system



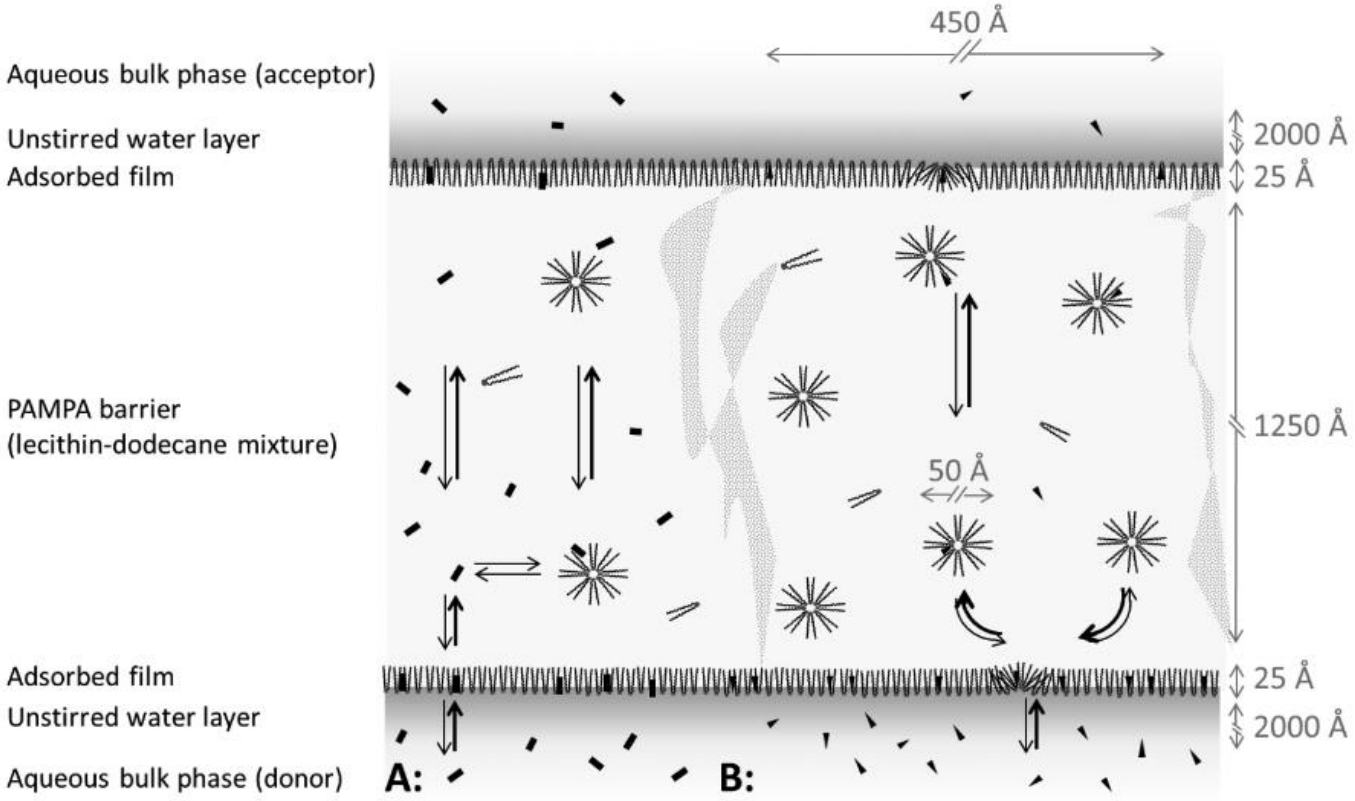
Modified from Biondo et al. 2023, *Pharm. Res.* DOI: 10.1007/s11095-023-03499-9

PAMPA barrier structure



Modified from Biondo et al. 2023, *Pharm. Res.* DOI: 10.1007/s11095-023-03499-9

PAMPA barrier structure



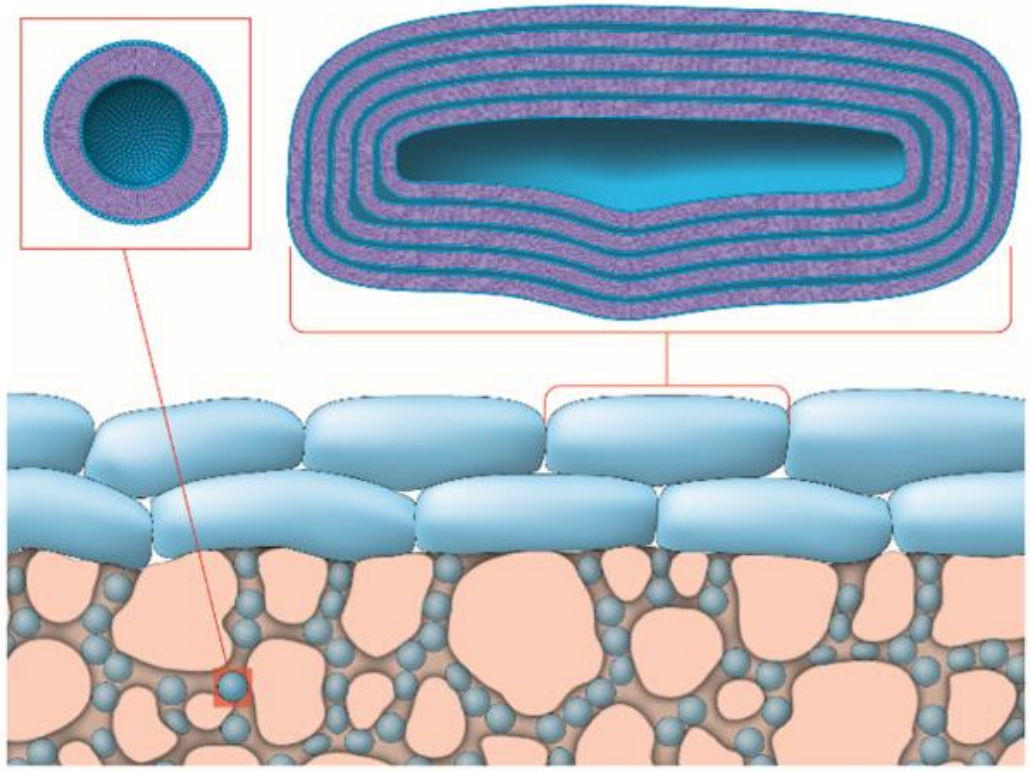
Reverse micelles: d=6.9 and 4.6 nm

- 31P, 1H NMR study
- Egg lecithin (>60% PC) in dodecane, PVDF filter
- Dodecane concentration:
  - low→ inversion from a bilayer to inverted hexagonal phase.
  - high(>10 mol %) → formation of an isotropic phase
- PVDF filter: unfavorable for providing aperture for BLM (rough and hydrophilic).
- Reverse micelles of PC and PE in dodecane
  - Carriers for amphiphilic molecules (e.g., propranolol)
- Monolayer at PAMPA−lipid solution/water interface is plausible but could not be verified due to excess bulk (3000:1).

Assmus et al. 2016, *Mol. Pharm.,* DOI:10.1021/acs.molpharmaceut.6b00889

PVPA

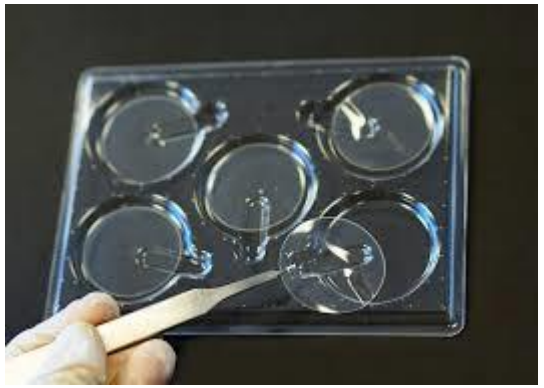
- Developed by Flaten, Brandl (University of Tromsø) and co-workers in 2006
- Barrier composition:
  Liposomes of different sizes/lamellarity deposited on a filter support in a multi-step process including centrifugation
- Format: Transwell (6-, 12-well)
- Several variants

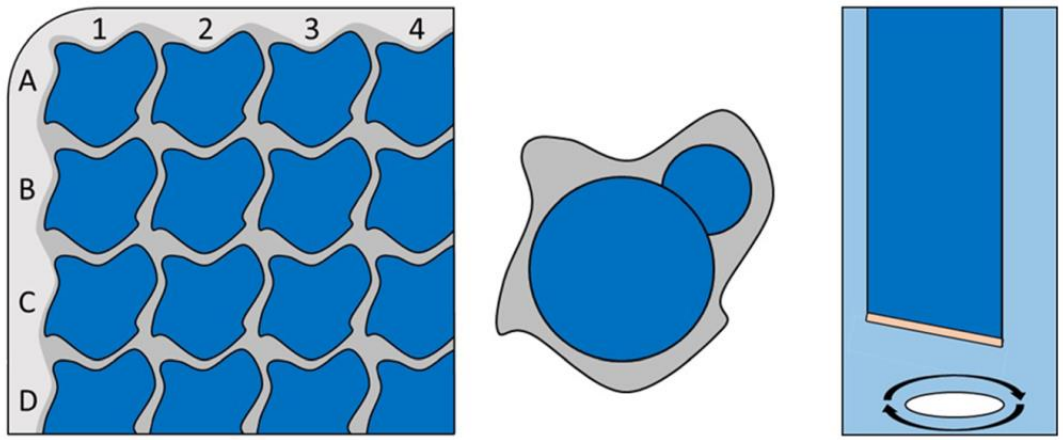


Berben et al. 2017, *Eur. J. Pharm. Sci.*,
DOI: 10.1007/s11095-023-03499-9

Permeapad®

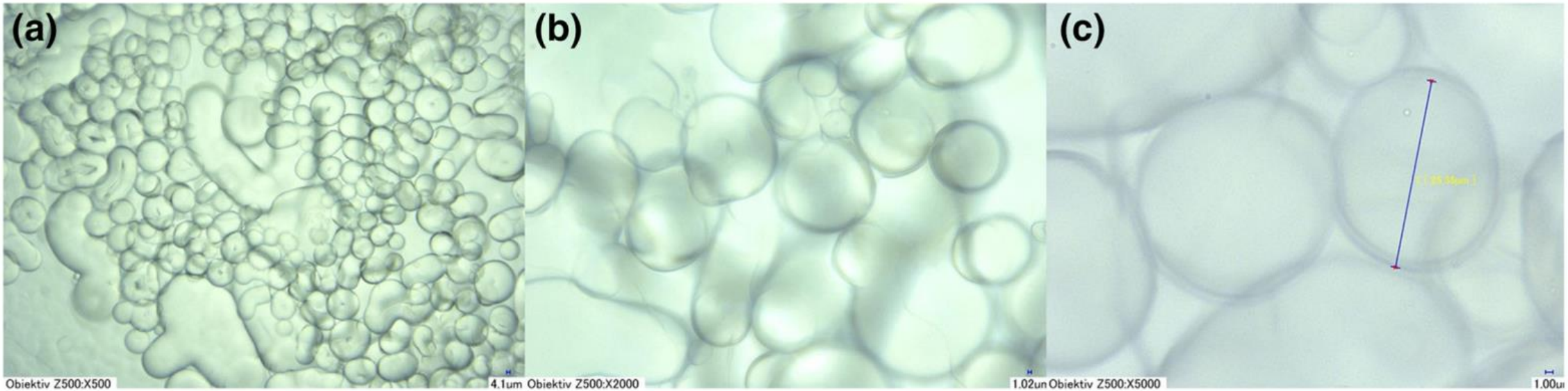
- Developed by di Cagno and Bauer-Brandl (University of Southern Denmark) in 2015
- Barrier composition:
  A sandwich of cellulose-hydrate membranes (support sheets) and dry phospholipid
- Formats:
  - Compatible with most permeation devices (side-by-side cells, Franz cells)
  - 96-well device





Jacobsen et al. 2020, *Pharm. Res.*, DOI: 0.1007/s11095-020-02807-x

Permeapad® barrier structure

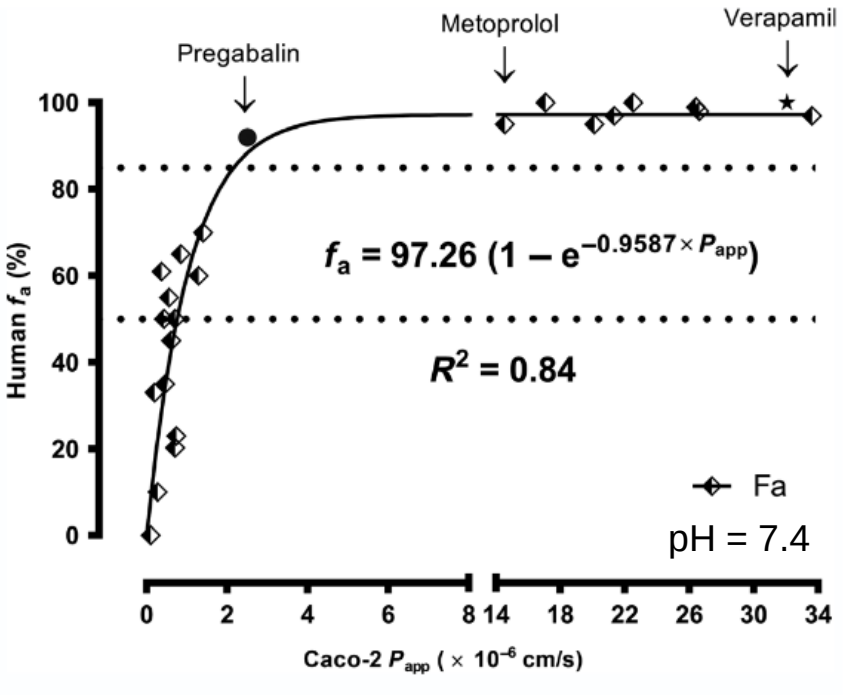


Jacobsen et al. 2020, *Pharm. Res*., DOI: 0.1007/s11095-020-02807-x

Oral absorption vs in vitro permeability

- Fraction absorbed in humans (f_a) *vs.* apparent permeability (P_{app} in $\times 10^{-6}$ cm/s)
- Steep slope region (low-moderate absorption), plateau region (high absorption)

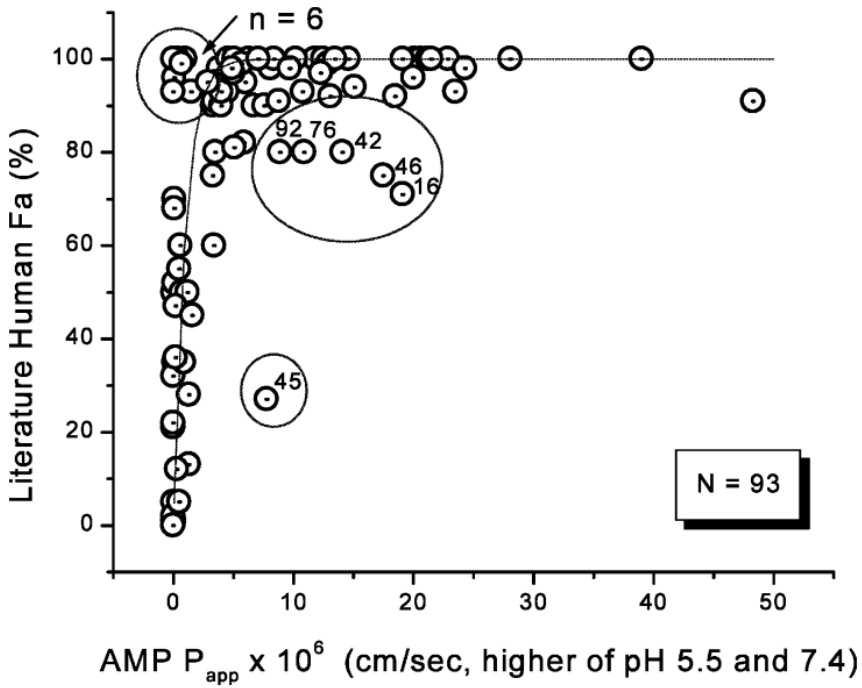
Caco-2



Jarc et al. 2019, *J. Pharm. Pharmacol.*,
DOI: 10.1111/jphp.13111

PAMPA

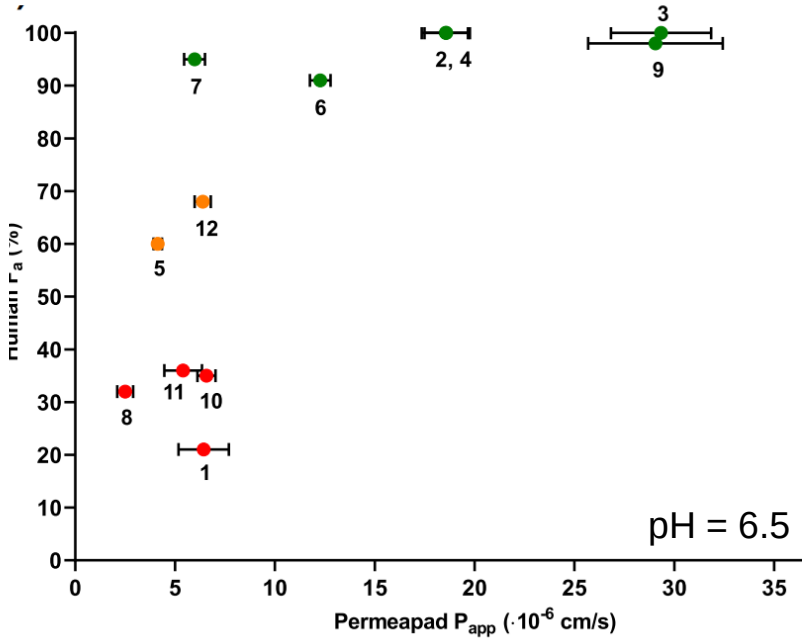
(1% egg lecithin in *n*-dodecane)



Zhu et al. 2002, *Eur. J. Med. Chem.*
DOI: 10.1016/s0223-5234(02)01360-0

Permeapad®

(96-well plate device)



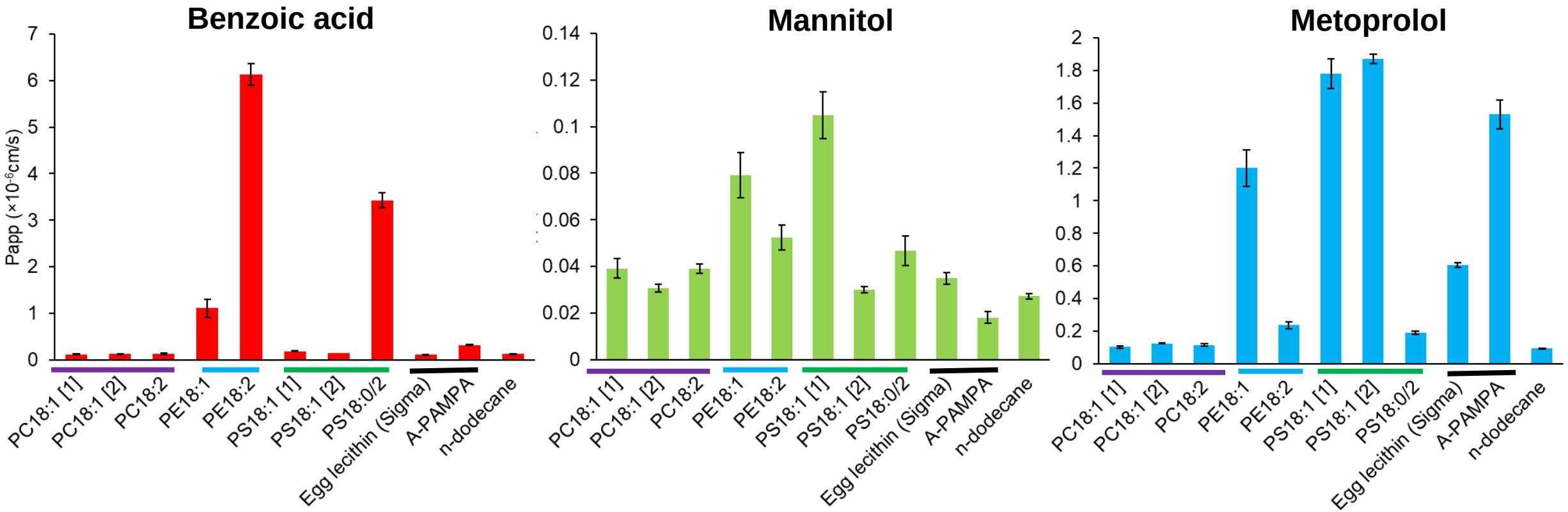
Jacobsen et al. 2020, *Pharm. Res.*,
DOI: 0.1007/s11095-020-02807-x

Influence of phospholipid composition on in vitro permeability determined with PAMPA, PVPA and Permeapad®

PAMPA: Influence of phospholipid composition

- 5 % phospholipid (w/v) in *n*-dodecane
- Either pure **PC**, **PE**, **PS** (varied unsaturation)
- Or mixture: **Egg lecithin**, **A-PAMPA** (52 % PC18:1, 18 % PS18:1, 30 % cholesterol)

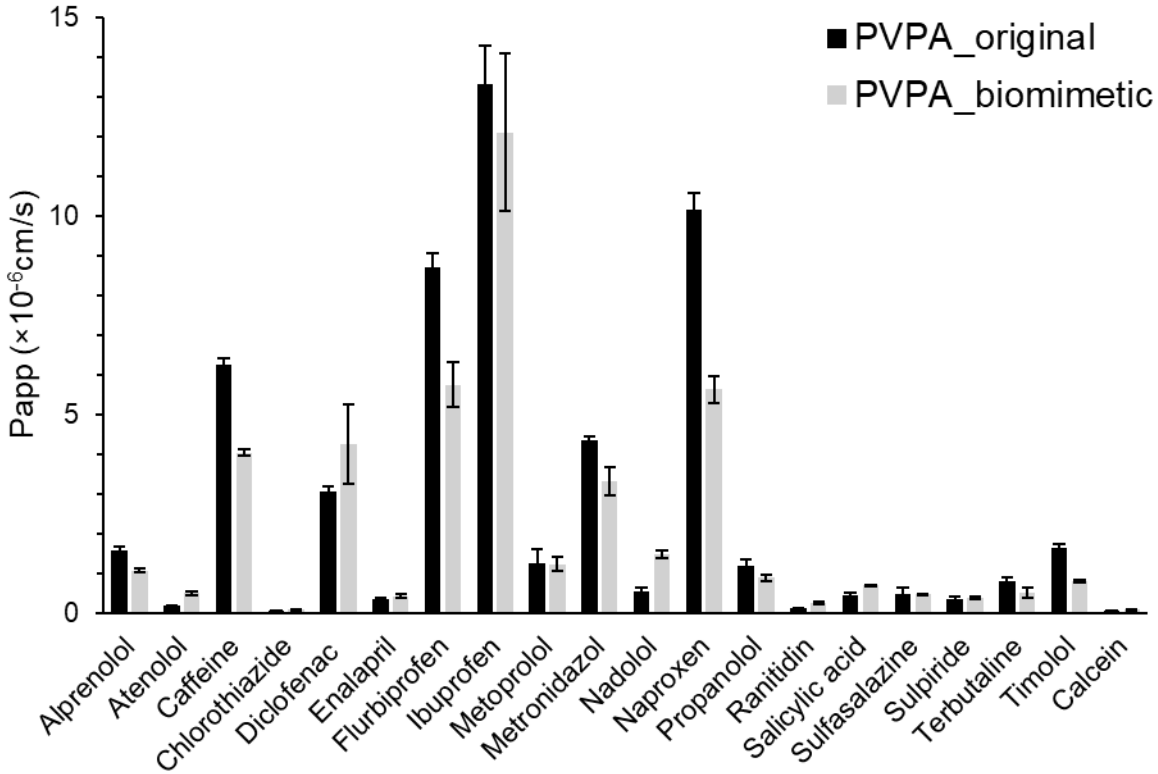
pH = 6.5

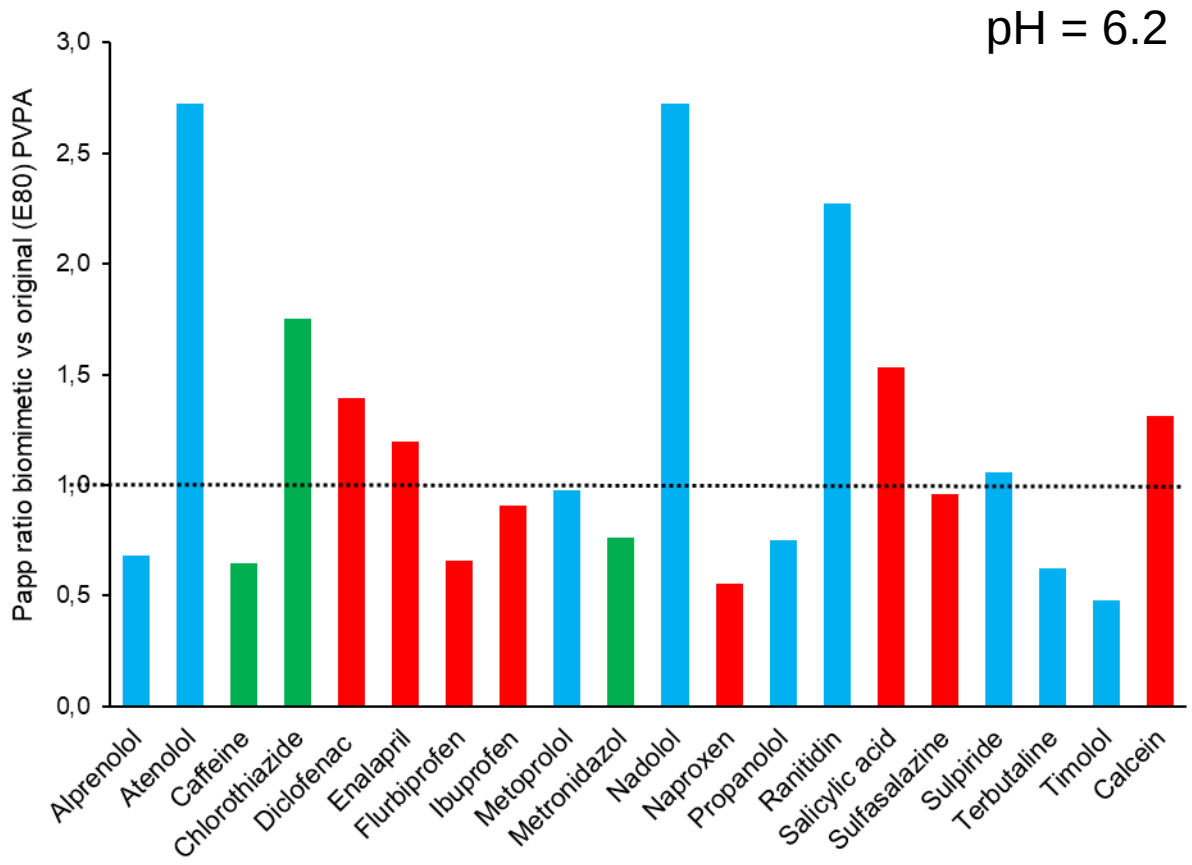


Figures prepared from Seo et al. 2006 [1], *Eur. J. Pharm. Sci.,* DOI: 10.1016/j.ejps.2006.04.012 and Teksin et al. 2006 [2], J. Control. Release, DOI: 10.1016/j.jconrel.2006.08.020

PVPA: Influence of phospholipid composition

- **Original PVPA:** 100% phosphatidylcholine from egg (Lipoid E80)
- **Biomimetic PVPA:** 26.5% phosphatidylcholine, 26.5% phosphatidylethanolamine, 7% phosphatidylserine, 7% phosphatidylinositol and 33% cholesterol





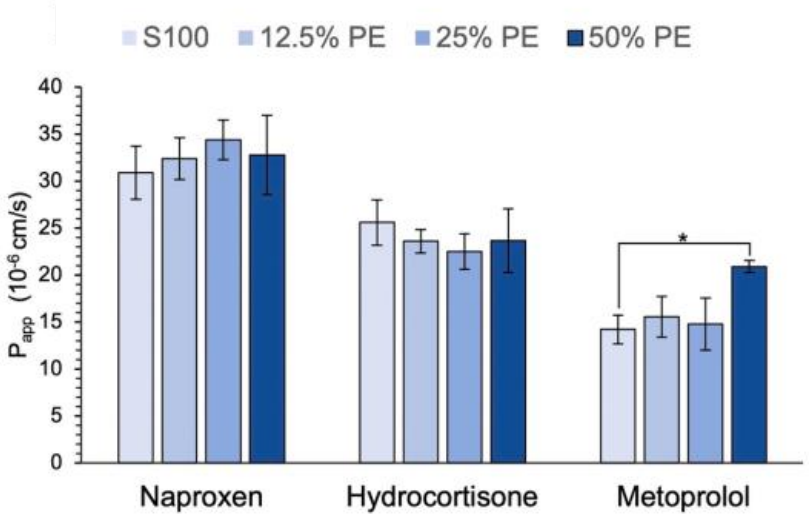
Figures prepared from Naderkhani et al. 2014, *J. Pharm. Sci.*, DOI: 10.1002/jps.23954

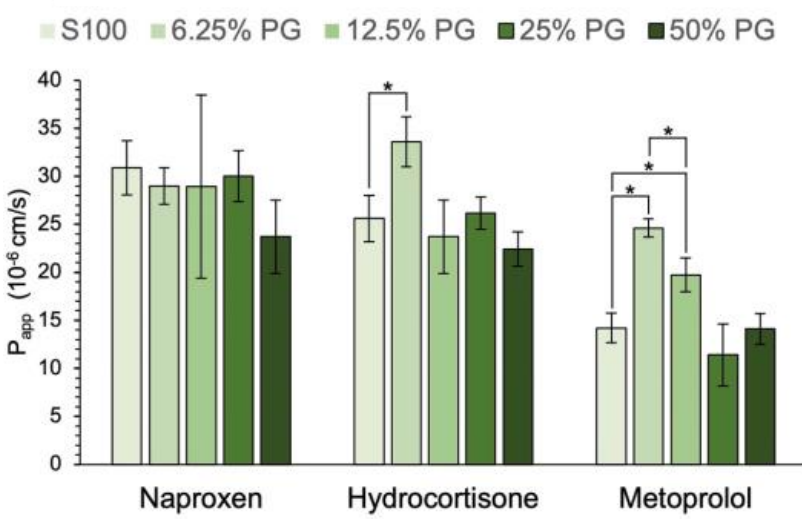
Permeapad®: Influence of phospholipid composition

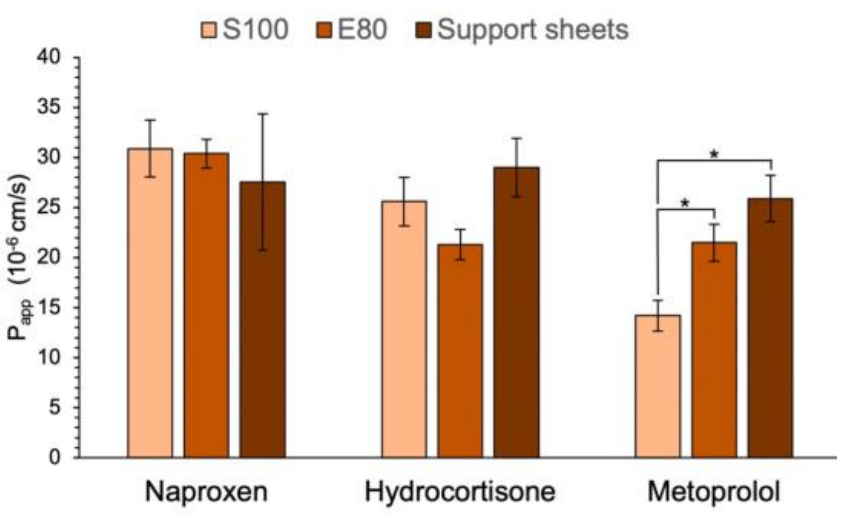
- Original and commercial Permeapad consist of 100% PC from soy (Lipoid S100)
- Series of Permeapad with 12.5-50 % phosphatidylethanolamine, 6.25-50 % phosphatidylglycerol, and 50-93.75 % phosphatidylcholine
- 100% Lipoid E80

Significant effects:

- 50 % PE, metoprolol
- 6.25 % PG, metoprolol and hydrocortisone
- 12.5 % PG, metoprolol

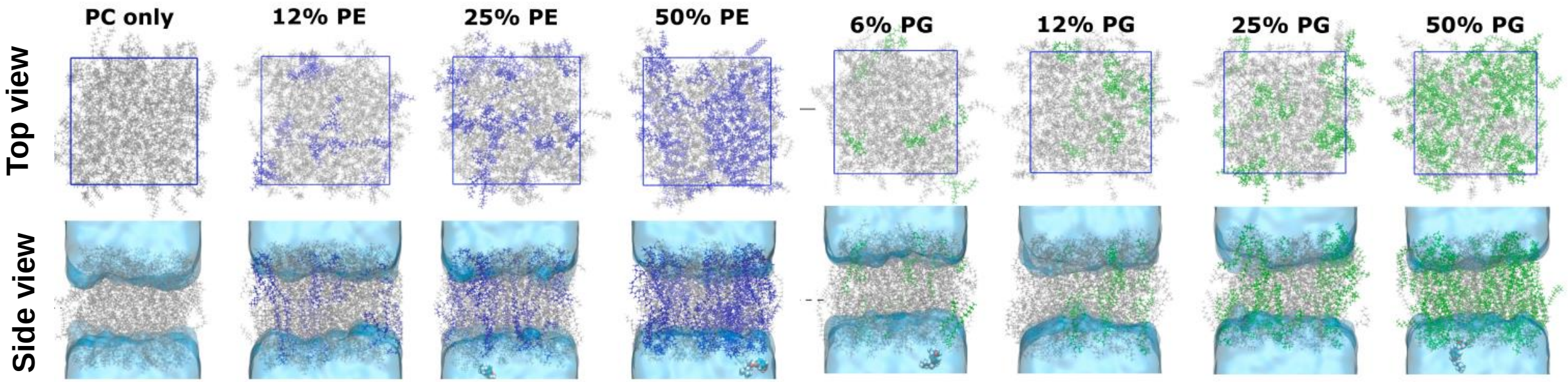






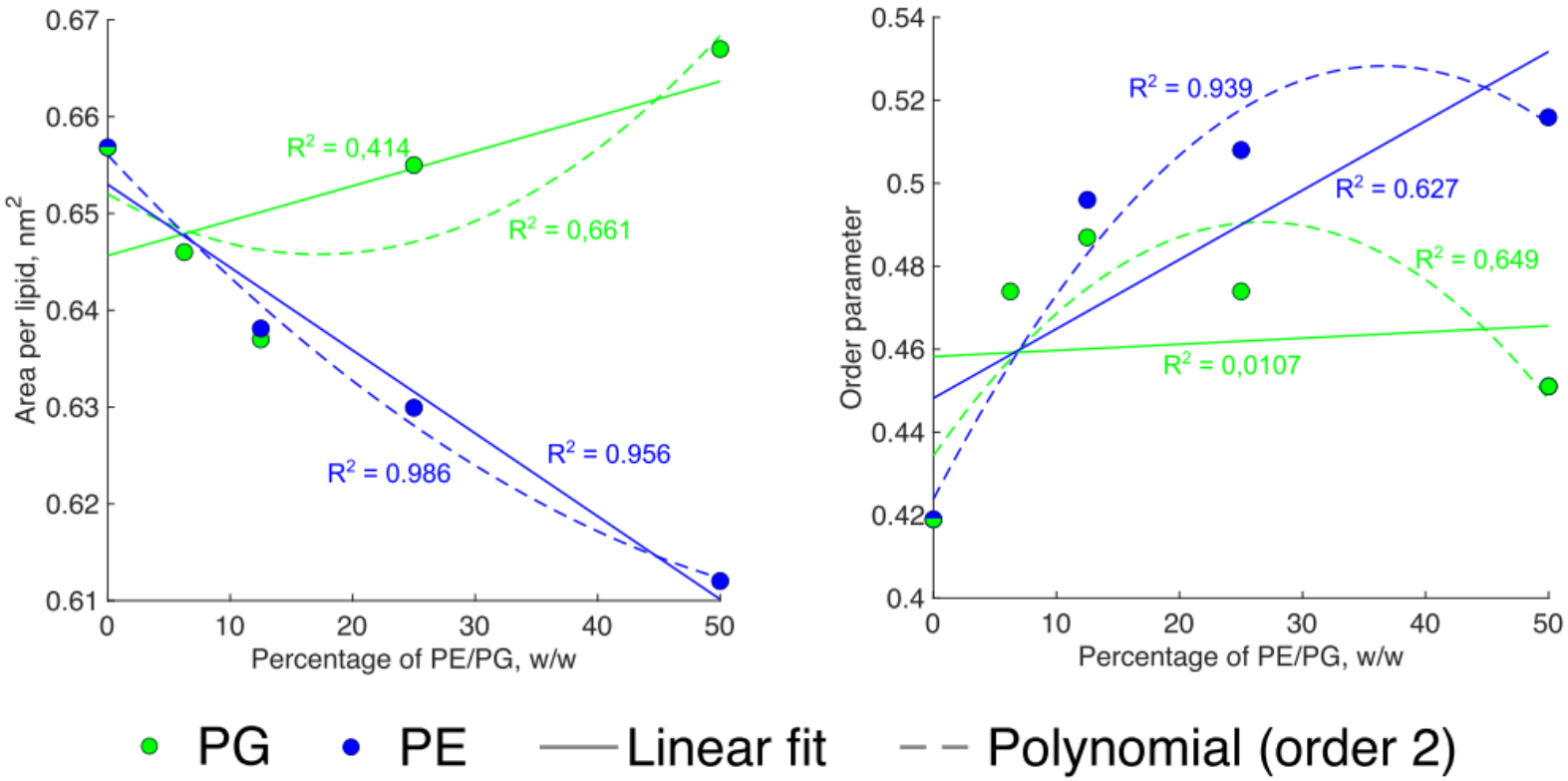
Modified from Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

Molecular dynamics simulations: PE, PG membranes



Modified from Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

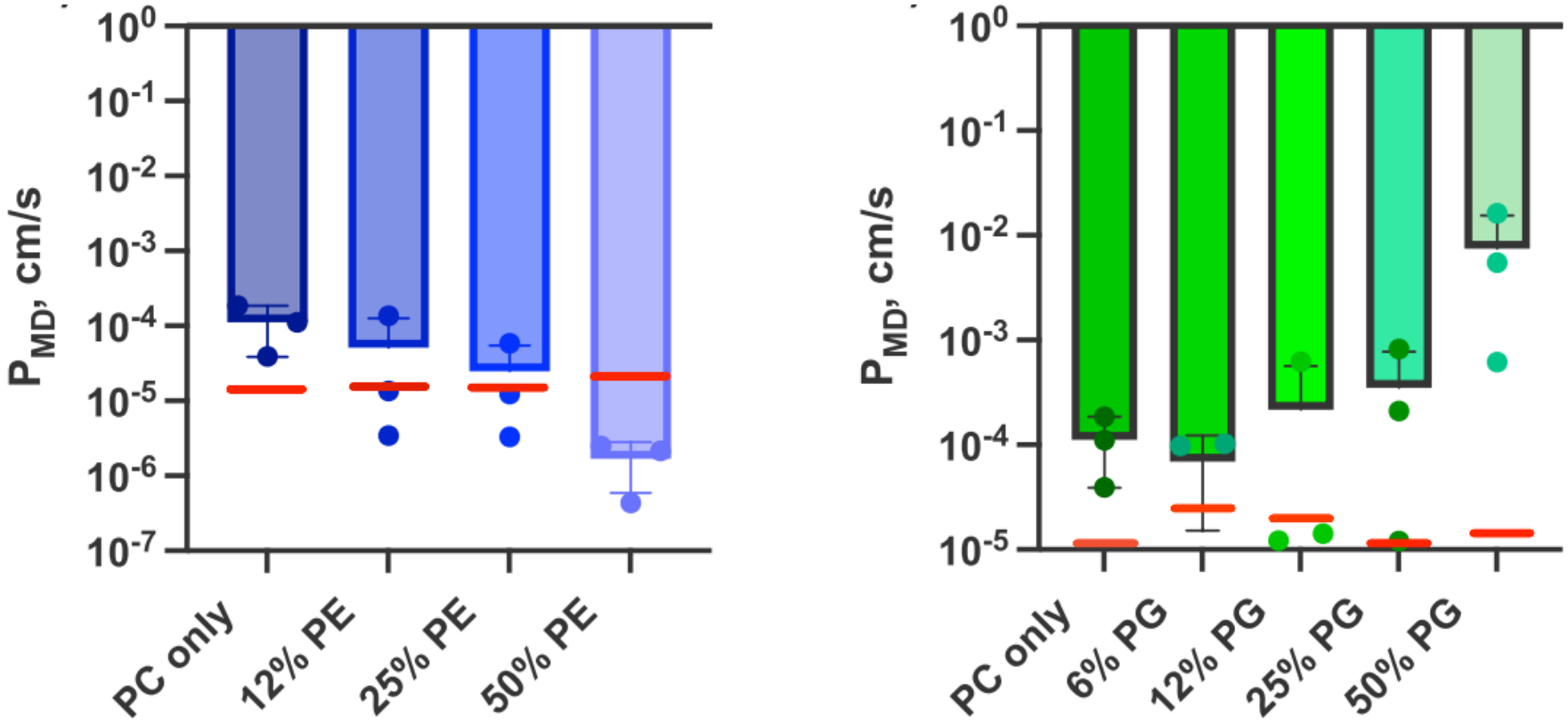
Molecular dynamics simulations: PE, PG membranes



Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

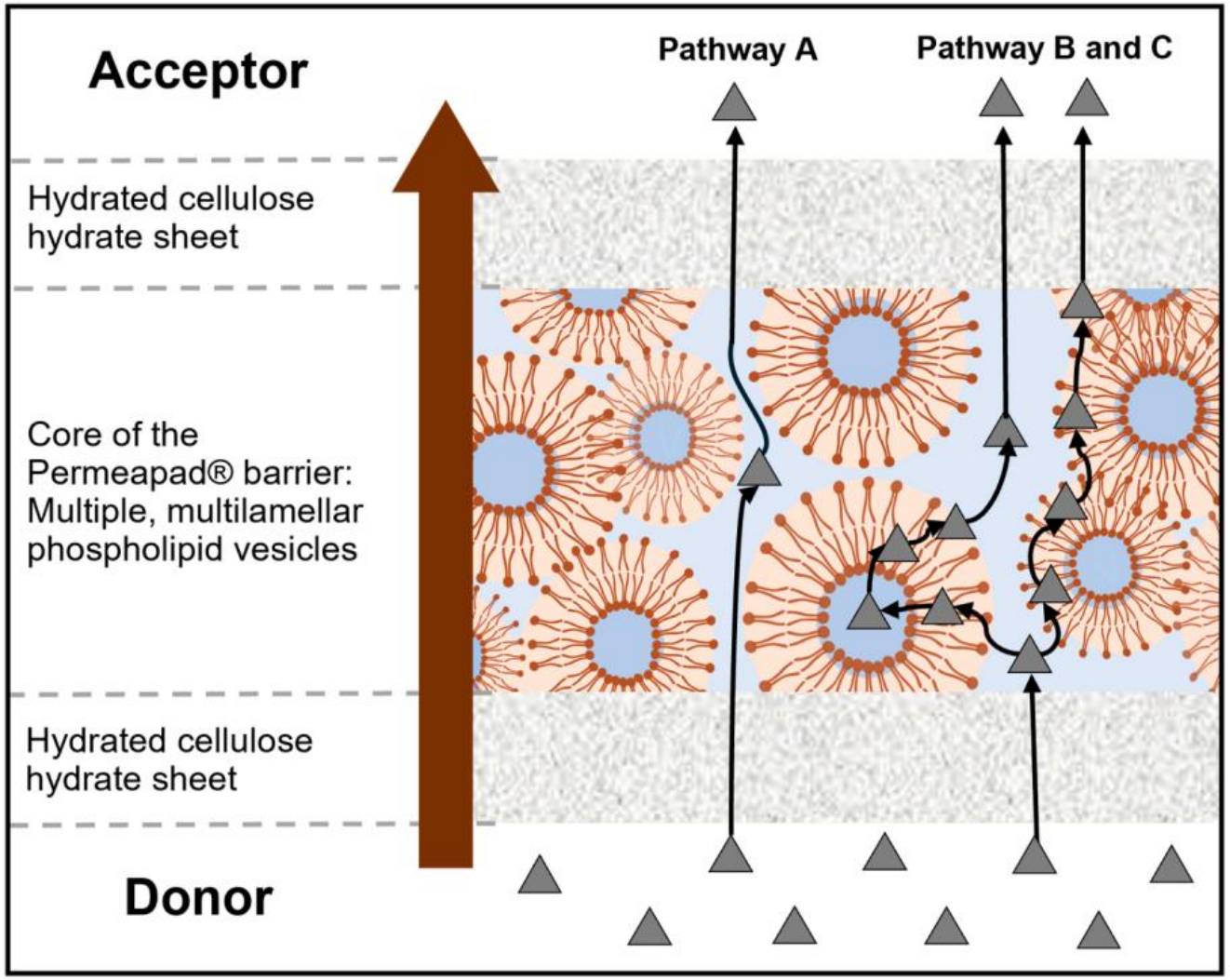
Molecular dynamics simulations: Metoprolol and PE, PG membranes

- In silico permeability values calculated from free energy profiles and diffusivity values at various distances from the membrane (umbrella sampling simulations)



Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

Permeation pathways in Permeapad



- Permeapad® is more complex than a single phospholipid bilayer
- Type of phospholipid in Permeapad® can affect:
  1. How drugs are attracted to the liposome surface,
  2. whether they will get internalized into the liposomes,
  3. whether they easily can redistribute.

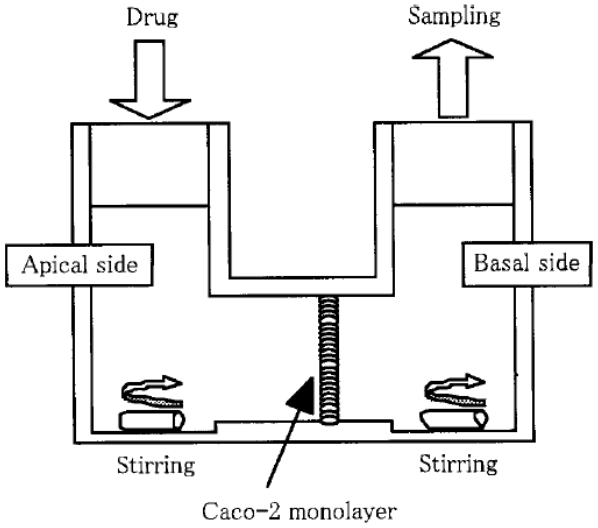
Kabedev et al. 2025, *Colloids Surf., B,* DOI: 10.1016/j.colsurfb.2025.114706

Phospholipids in combined dissolution/permeation studies

Combined dissolution/permeation studies

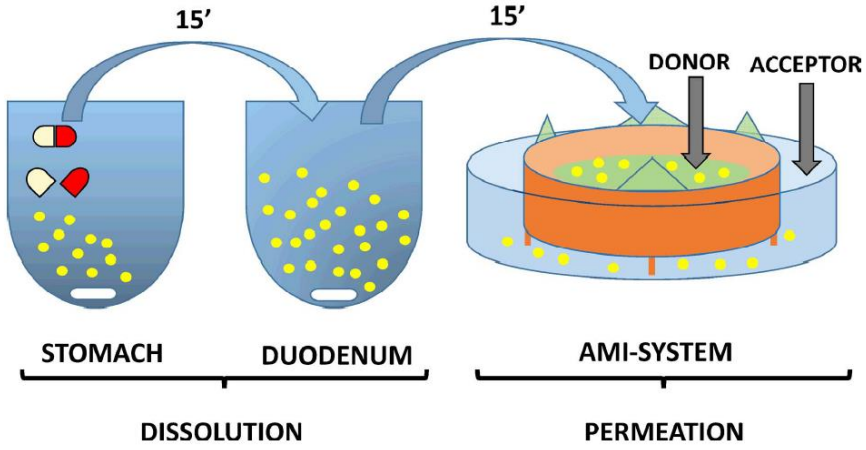
- Dissolution in an absorptive environment
- Performance evaluation of enabling formulations (e.g. amorphous solid dispersions; ASDs)
- **Basic set-up:**
  Two vessels, a donor and an acceptor, separated by a permeation barrier
- Simultaneous or sequential

D/P system



Kataoka, M., et al., 2003. Pharm. Res. 20, 1674–1680.

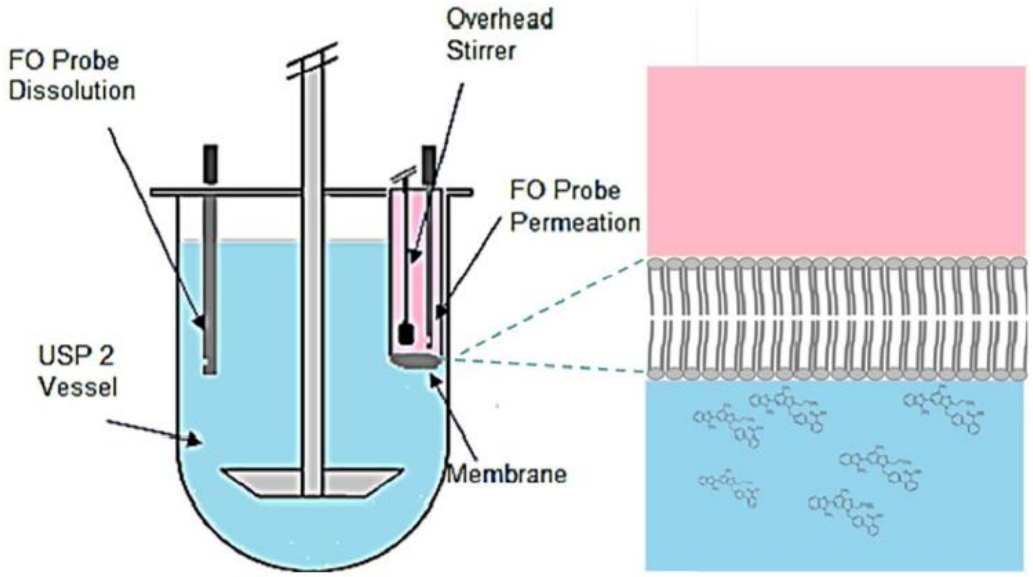
AMI system



Berben, P.,et al. 2018, Int. J. Pharm. 537, 22–29.

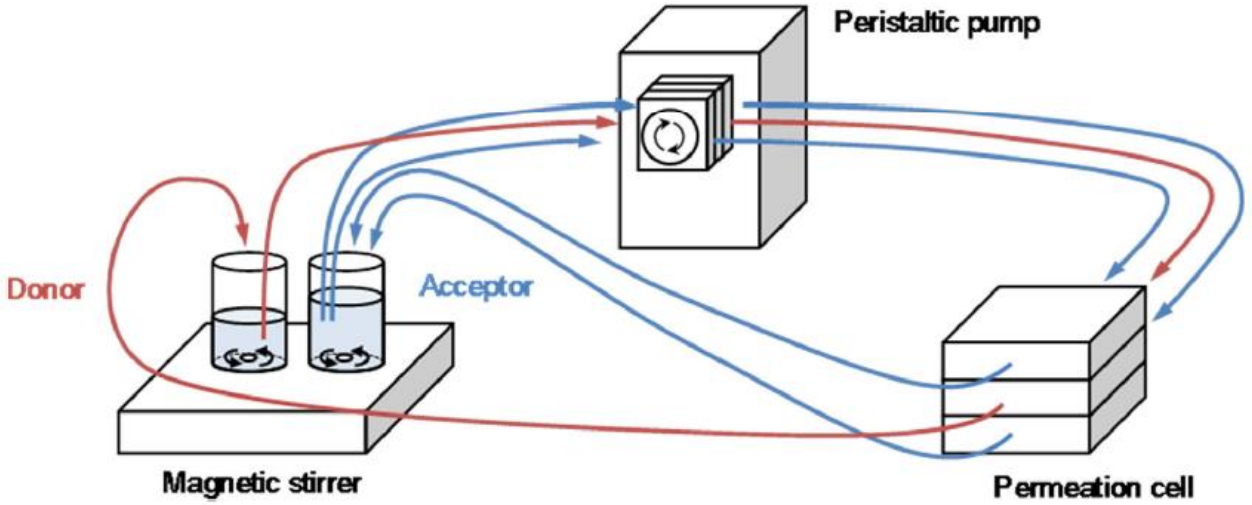
Combined dissolution/permeation studies

macroFLUX™



Borbás, E., et al., 2018. Eur. J. Pharm. Sci. 114, 310–317.

Permealoop™



Sironi, D., et al., 2018. J. Pharm. Biomed. Anal. 156, 247–251.

- Overview of set-ups in O'shea et al. 2022, DOI: 10.1016/j.ejps.2021.106098

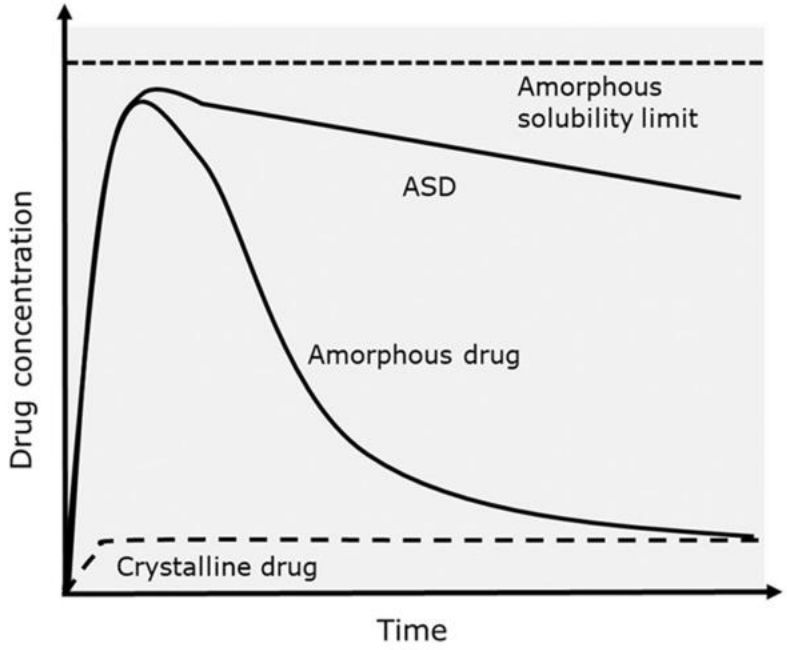
Example:
Influence of phospholipids on performance evaluation of ASDs

Dissolution of ASD → Transient supersaturation → Increased driving force for absorption

?

Effect of phospholipids on supersaturation?

- Nucleation induction time in presence/absence of phospholipids (a type of solvent shift assay)
- Combined dissolution/permeation studies in presence/absence of phospholipids

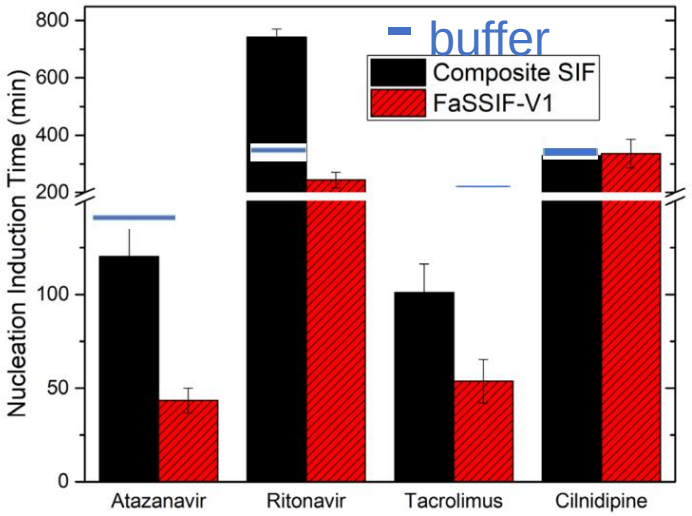


Hermans et al. 2023, *AAPS Journal*, DOI: 10.1208/s12248-022-00760-8

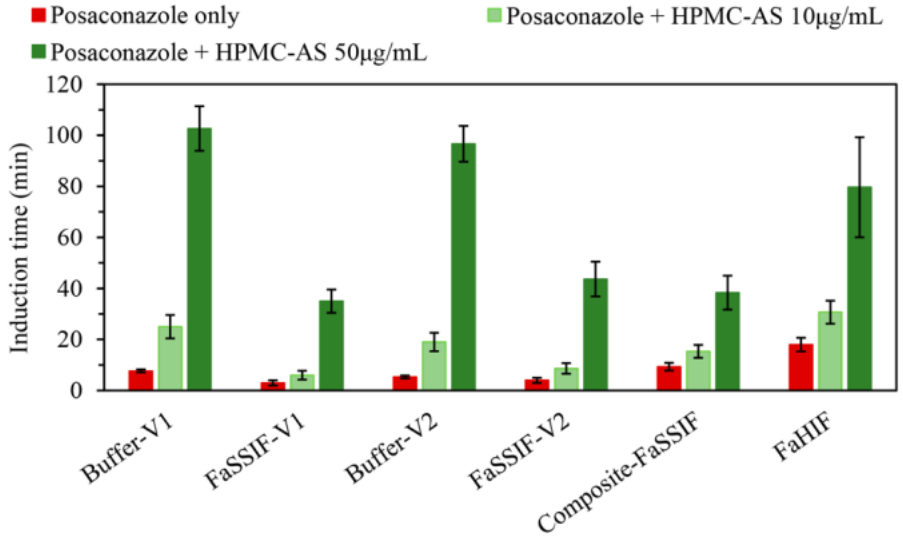
Effect of phospholipids on nucleation induction time (NIT)

Drug in methanol → Aliquot in test medium (e.g., FaSSIF V1) → UV/vis spectrophotometer with fiber optic dip probe: measure extinction at non-absorbing wavelength → Increase in scattering: **NIT**

- Presence of mixed micelles with lecithin (e.g. FaSSIF V1) decreases NIT of some drugs (but not all)
- Lyso-PC more potent than PC (atazanavir)
- Effect dependent on (atazanavir):
  - Concentration
  - Presence of bile salts

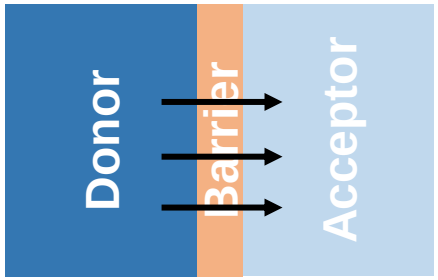


Indulkar et al. 2018, *Pharm. Res.*,
DOI: 10.1007/s11095-018-2441-2



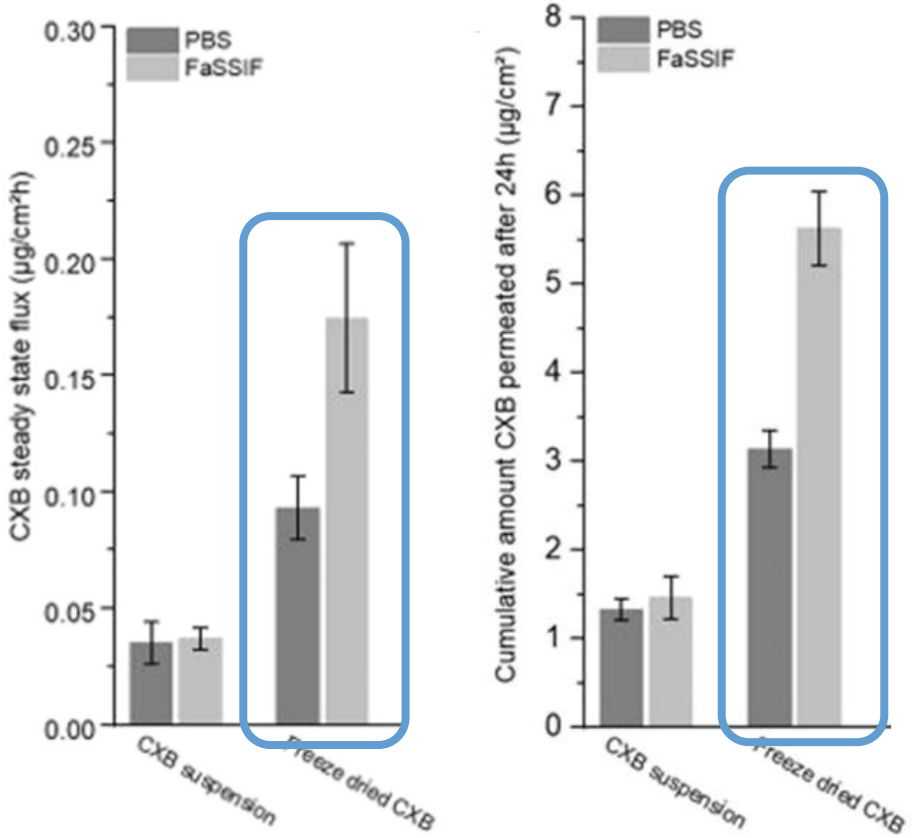
Elkhabaz et al. 2021, *Cryst. Growth Des.*,
DOI: 10.1021/acs.cgd.0c01730

Effect of phospholipids on permeation from amorphous solids



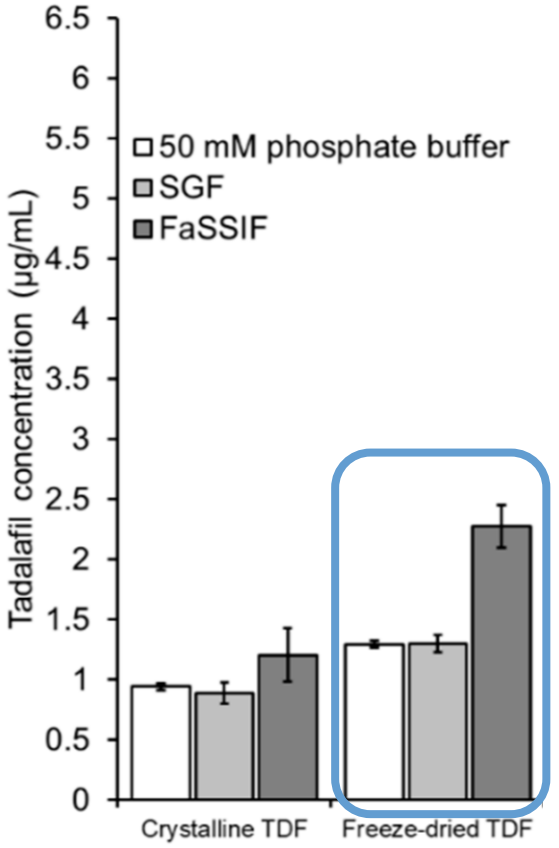
- Permeation as indirect measure for supersaturation
- Longer and/or more pronounced supersaturation
  → higher permeation
- Presence of FaSSIF V1 increased permeation from amorphous celecoxib and tadalafil

Celecoxib



Modified from Jacobsen et al. 2019,
Eur. J. Pharm. Sci., DOI: 10.1016/j.ejps.2018.11.003

Tadalafil



Modified from Jacobsen et al. 2019,
Pharmaceutics, DOI: 10.1016/j.ejps.2018.11.003

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- Prof. Alexandra Teleki
- Prof. Annette Bauer-Brandl
- Prof. Martin Brandl

Ann-Christin Jacobsen
Kiel University
Department of Pharmaceutics and Biopharmaceutics
Grasweg 9a
24118 Kiel, Germany
e-mail: ajacobsen@pharmazie.uni-kiel.de