In Vitro Release Test for Complex Drug Product: What is Your Perspective?

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Outline

- The Problem: Drug Release from Ophthalmic Emulsions
- Inside vs. Outside the Box
- Examples

Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user – Drugs are no different.

Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is assuring *every* dose is safe and effective, free of contamination and defects.

It is what gives patients confidence in their ***next*** dose of medicine.

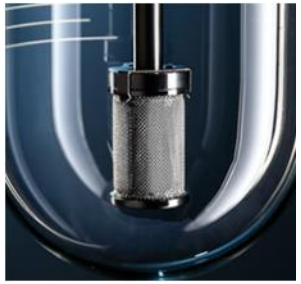
The Purpose of Studying Drug Release (IVRT)

- Estimate the bioavailability (rate and extent) of drug
- Product development (formulation screening, product understanding)
- Quality control (batch-to-batch consistency)
- Bioequivalence (sameness)
- In lieu of in vivo test (IVIVC, Post-approval changes)

IVRT is not the goal, but a means to an end.

How do we Normally study IVRT?

USP Apparatus 1

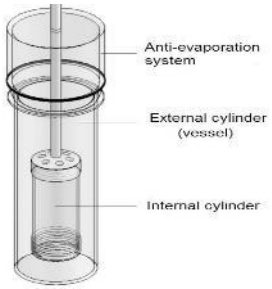


Basket

USP Apparatus 2

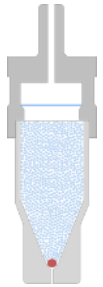
Paddle

USP Apparatus 3



Reciprocating Cylinder

USP Apparatus 4



Flow-through Cell

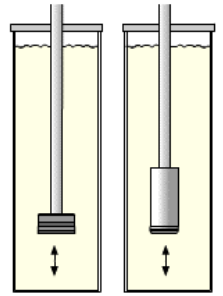
USP Apparatus 5

Paddle over Disk

USP Apparatus 6

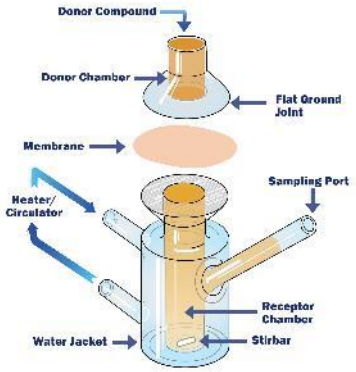
Rotating Cylinder

USP Apparatus 7

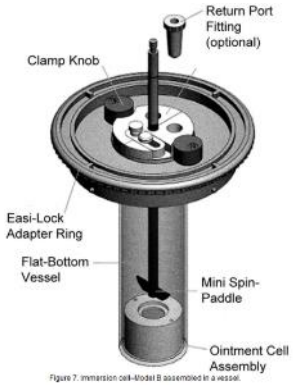


Reciprocating Holder

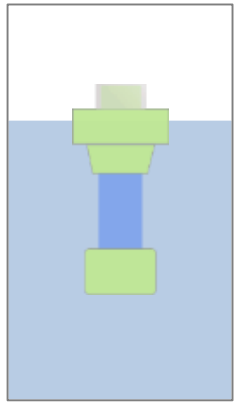
Vertical Diffusion Cell



USP 2 Immersion Cell



Dialysis



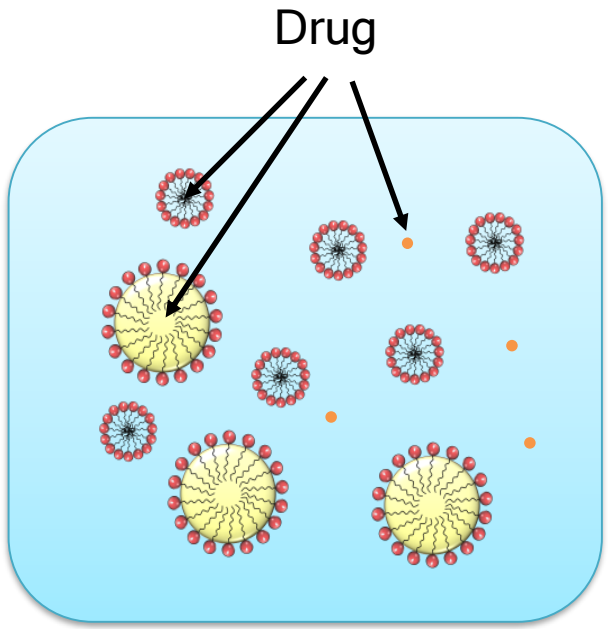
Bottle-shaking

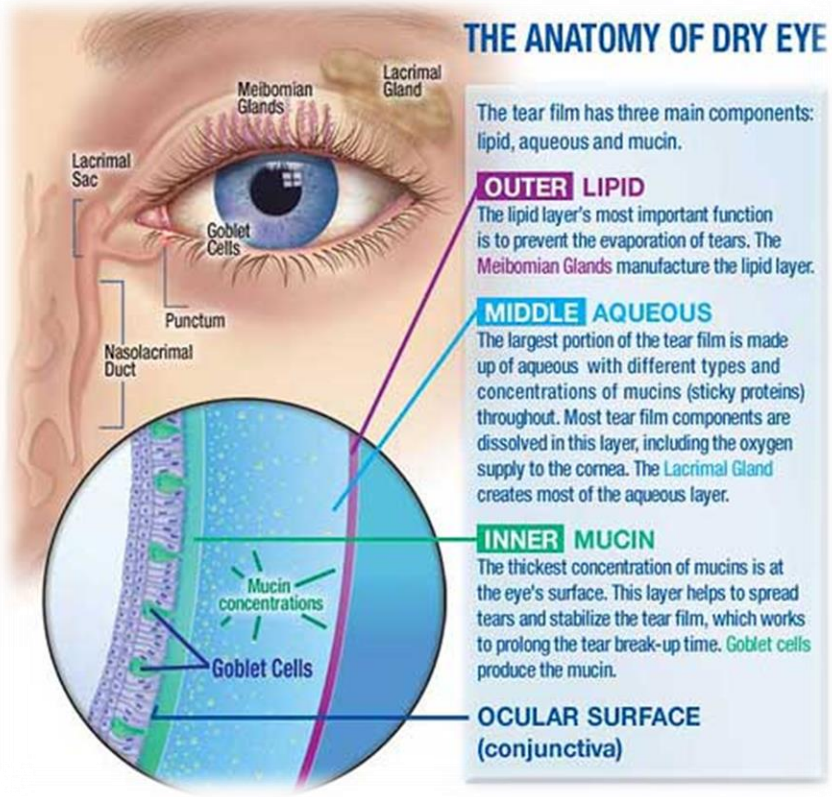
Sample-and-separate

Others

- Pulsatile Microdialysis (PMD)
- Miniatured flow-through cell
- MicroDiss™
- MicroFLUX™
- Scissor™ (sub-cutaneous)
- Adaptive Perfusion

The Problem: Drug Release from Ophthalmic Emulsions





http://www.swmeyecenter.com/tips-to-relieve-dry-eye-symptoms/

Two fundamental problems:

- Transfer kinetics
- Particle separation

Out

Thinking inside a box:

Solution

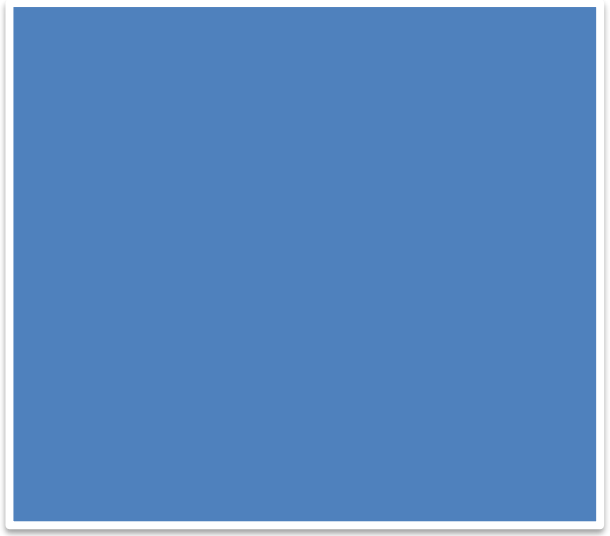
Problem

- How to measure distance?
- How to measure weight?
- How to measure volume?
- How to measure concentration?
- How to perform dissolution for IR tablet?

Most of the time, we can do well by just thinking inside the box (the purpose of training, education)

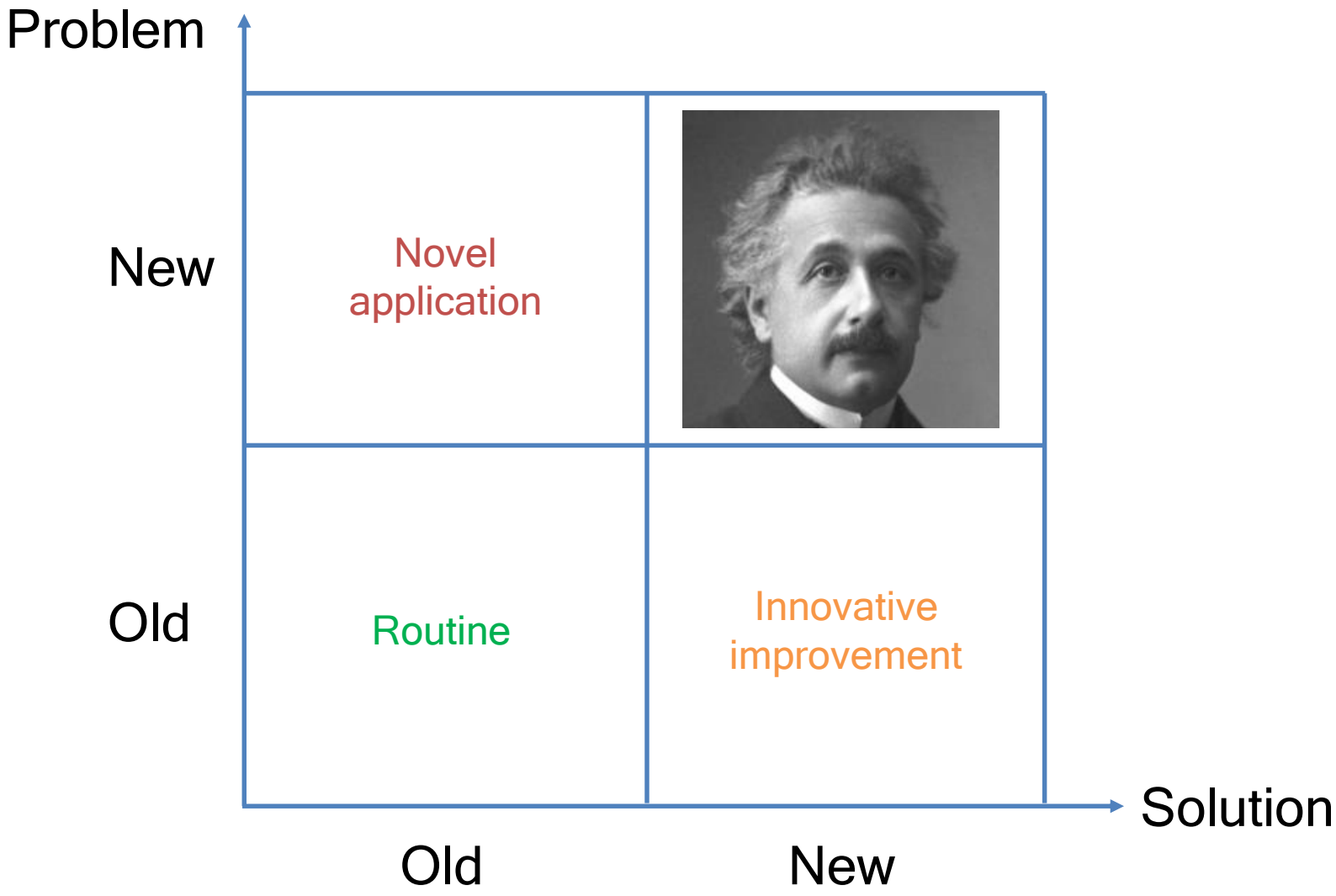
Solution

Problem

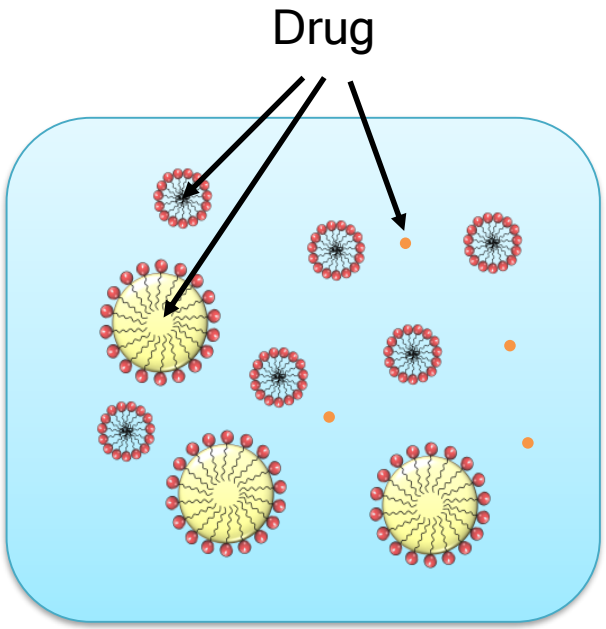


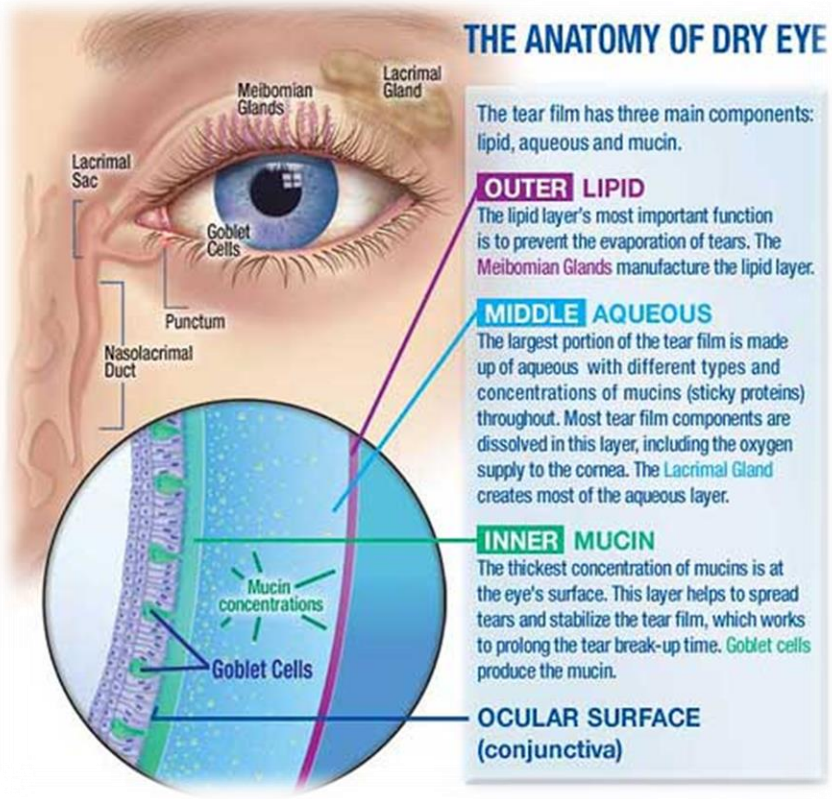
How to measure drug release from emulsions?

Thinking outside a box: New vs. Old



The Problems: Study drug release from nanoemulsions



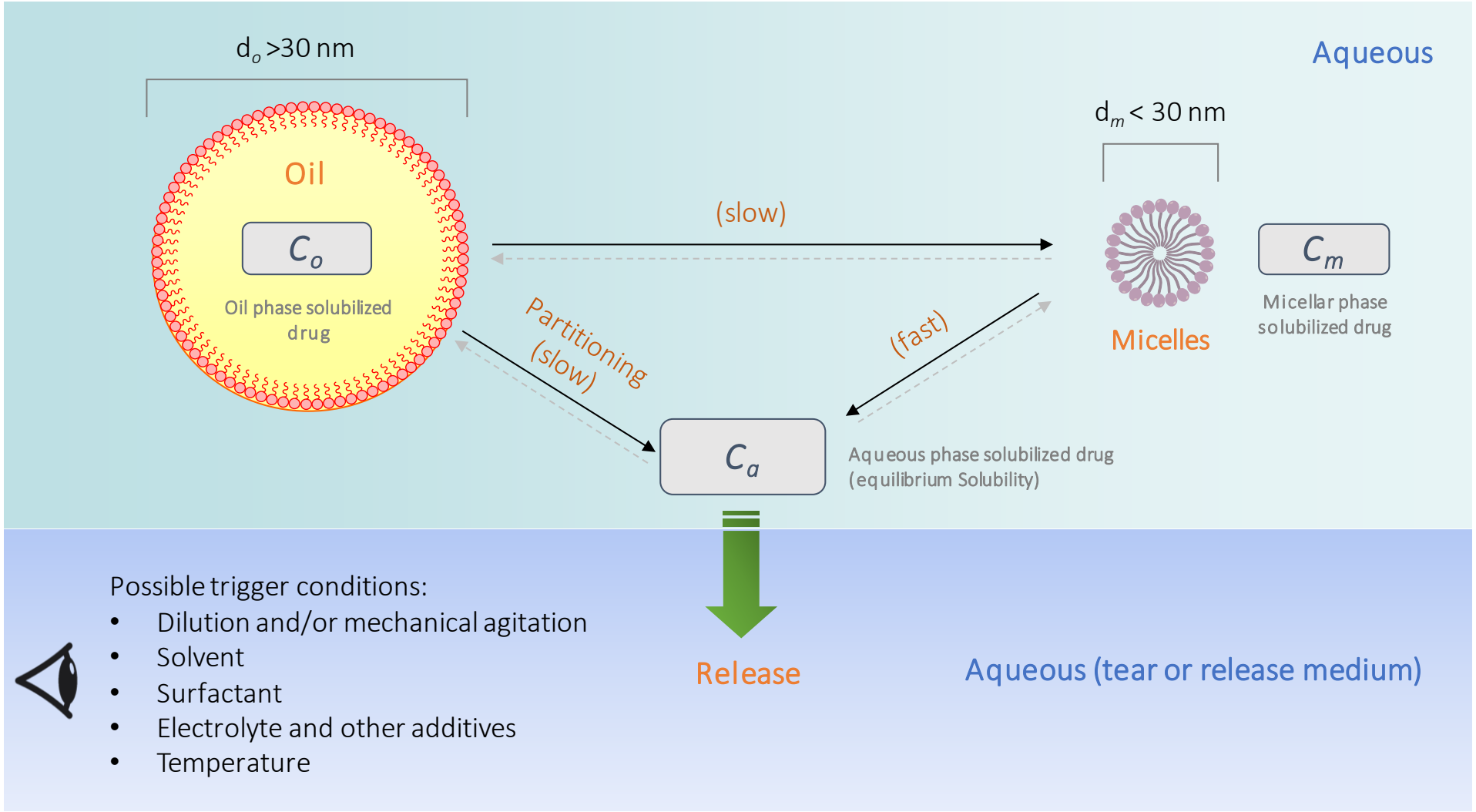


Two fundamental problems:

- Transfer kinetics (old problem, new solution)
- Particle separation (new problem, old solution)

First (old) problem: transfer kinetics

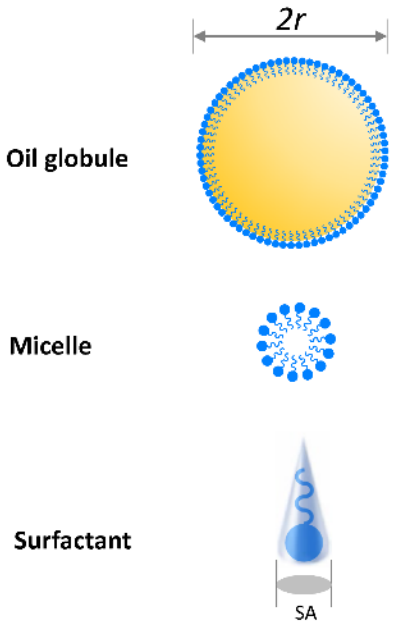
Problem: transfer kinetics in emulsions

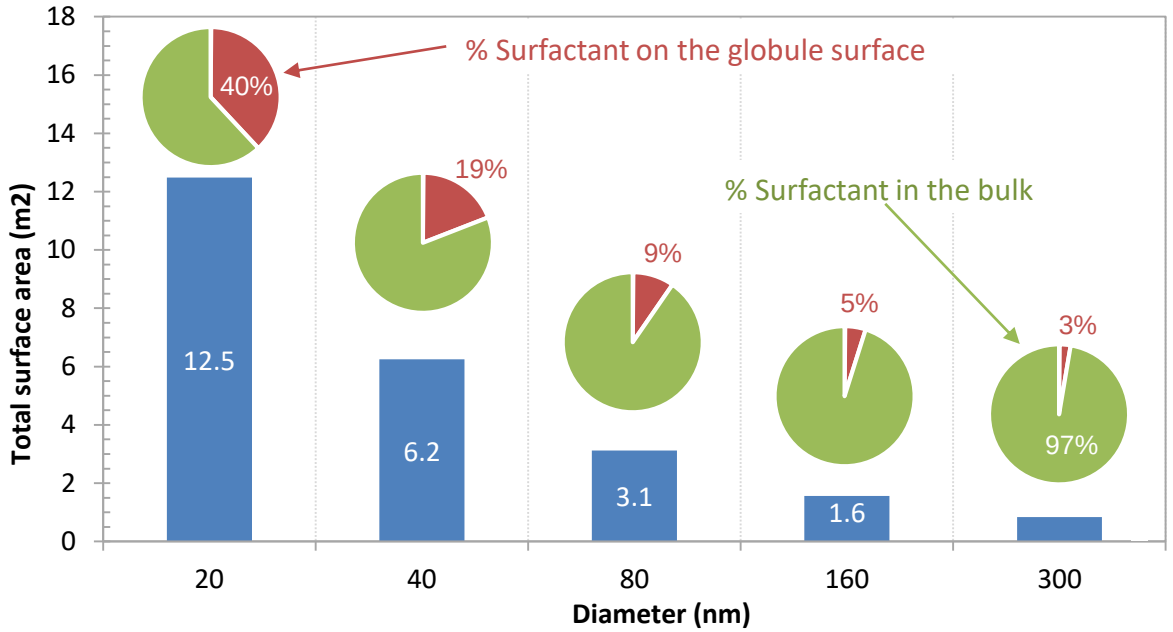


Y. Dong et al. Journal of Controlled Release (2020), 327, 360-370

Connecting the dots: relating GSD to drug distribution

Example below: 4% castor oil and 3% Tween 80 (we can easily switch the numbers with actual formulation composition)





Fraction covering the (globule) surface

$$F_s = \frac{\sum_i n_i \cdot k_i}{n_T} = \frac{\sum_i \frac{C_{oil}}{m_i} \cdot P_i \cdot \frac{S_i}{S_A}}{n_T} = \frac{3 \cdot C_{oil} \cdot MW_T}{S_A \cdot C_T \cdot \rho_{oil} \cdot N} \sum_i \frac{P_i}{r_i} \times 100\%$$

Fraction forming the micelles

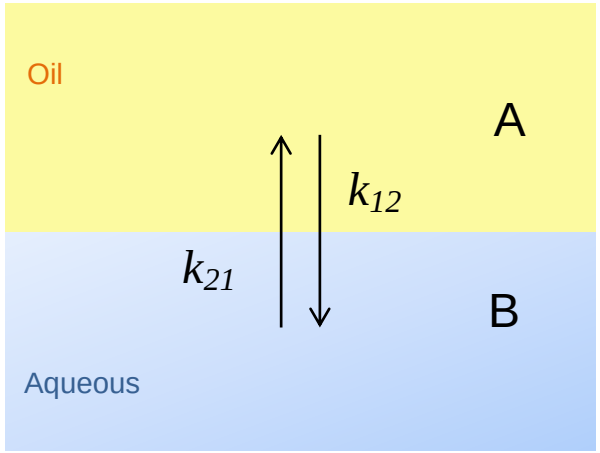
$$F_m = 1 - F_{cmc} - F_s$$

Smaller the globules, **larger surface area**, less micelles, and **less drug in micelle fraction**.

Y. Dong, et al. ***J of Controlled Release*** (2019), 313, pp 96-105

Q: Which one is more important for release (kinetics vs. equilibrium)?

A New Solution to An Old Problem: Determine Drug Diffusion (Distribution)



For a simplified scenario of drug diffusion between A (oil phase) and B (aq. phase)

$$A \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} B$$

$$\begin{cases} \frac{dA}{dt} = -k_{12} * A + k_{21} * B \\ \frac{dB}{dt} = -k_{21} * B + k_{12} * A \end{cases}$$

Assumption: one directional diffusion at time zero

B to A (aqueous to oil phase)

$$\frac{dB}{dt} = -k_{21} * B + k_{12} * A$$

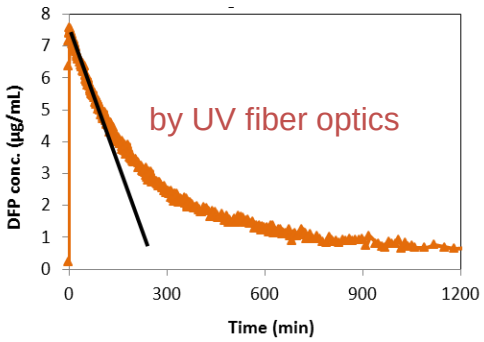
$$\frac{dB}{dt} = -k_{21} * B_0$$

$$\int_{B_0}^{B} B = -k_{21} \cdot B_0 \cdot \int_0^t t \cdot dt$$

$$B = B_0 - k_{21} \cdot B_0 \cdot t$$

$$k_{21} = -Initial\ slope/B_0$$

At t=0,
A_0=0



A to B (oil to aqueous phase)

$$\frac{dB}{dt} = -k_{21} * B + k_{12} * A$$

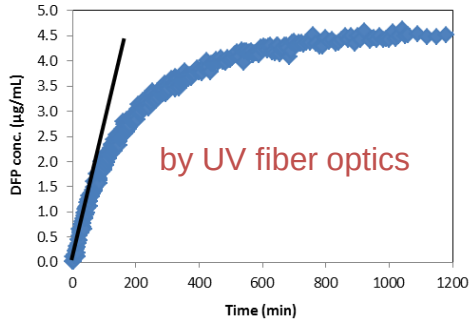
$$\frac{dB}{dt} = k_{12} * A_0$$

$$\int_{B_0}^{B} B = k_{12} \cdot A_0 \cdot \int_0^t t \cdot dt$$

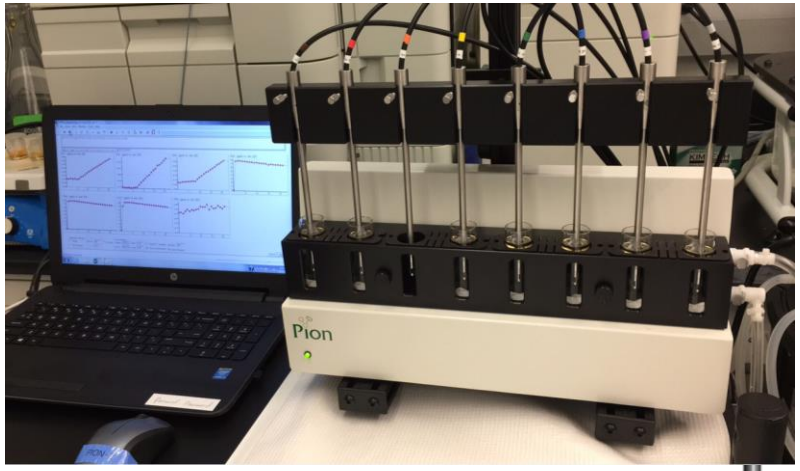
$$B = k_{12} \cdot A_0 \cdot t$$

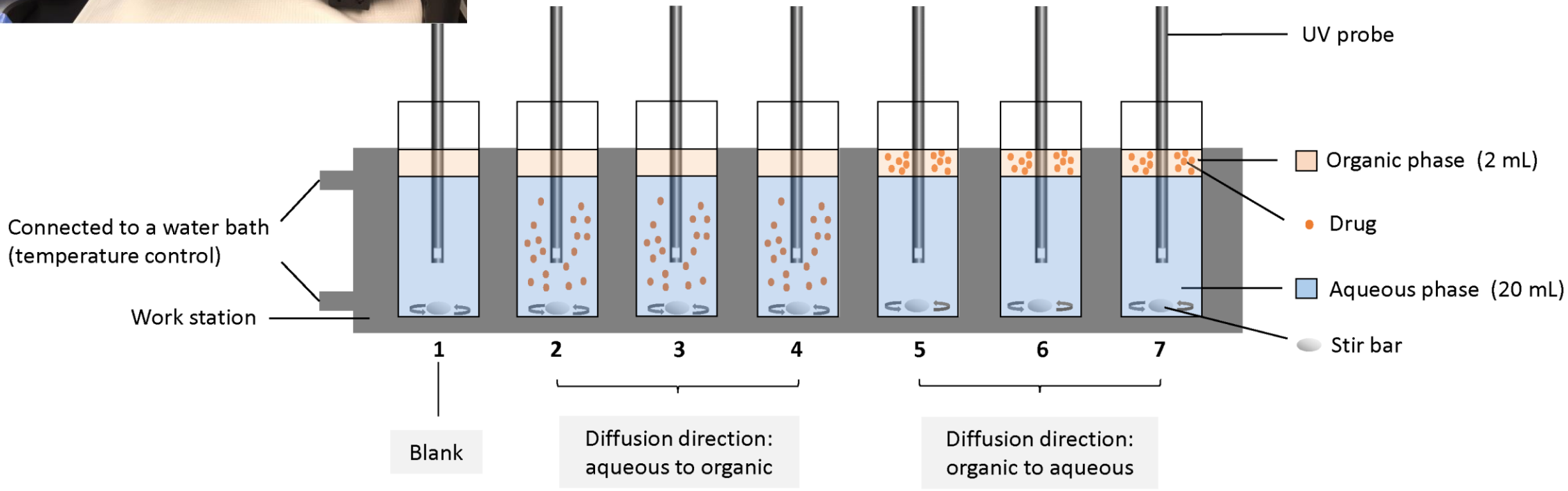
$$k_{12} = Initial\ slope/A_0$$

At t=0,
B_0=0



A New Method to Determine Drug Diffusion (Distribution)

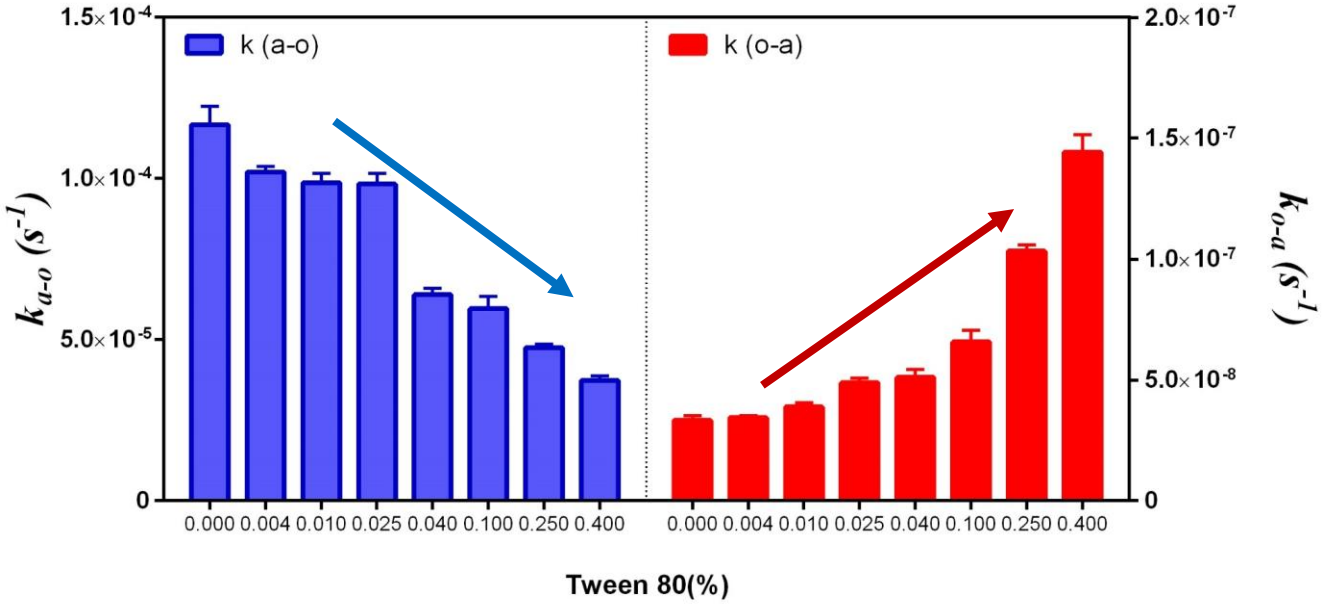


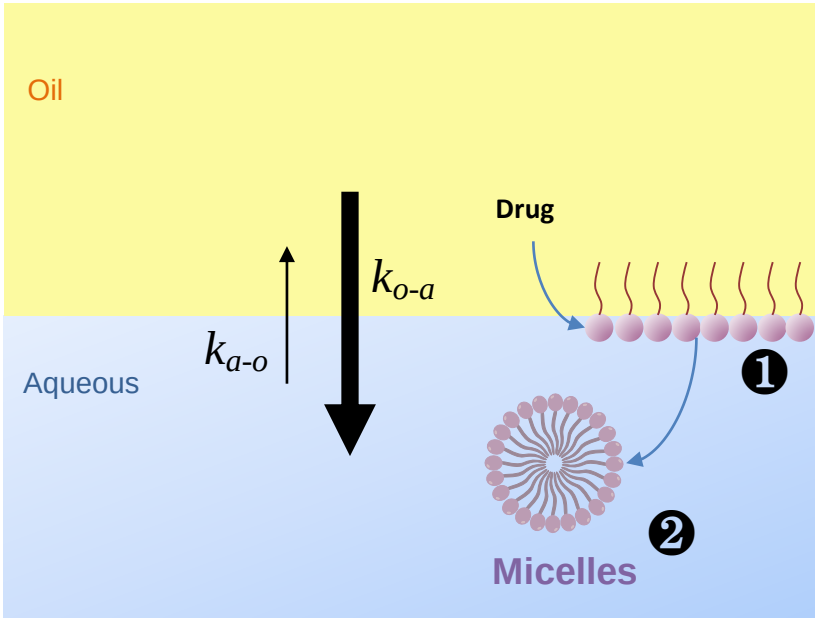


Y. Dong et al. Journal of Pharmaceutical Sciences (2019) 108, pp 2002-2011

Effect of Surfactant on Rate and Extent of Diffusion (cont.)

Difluprednate





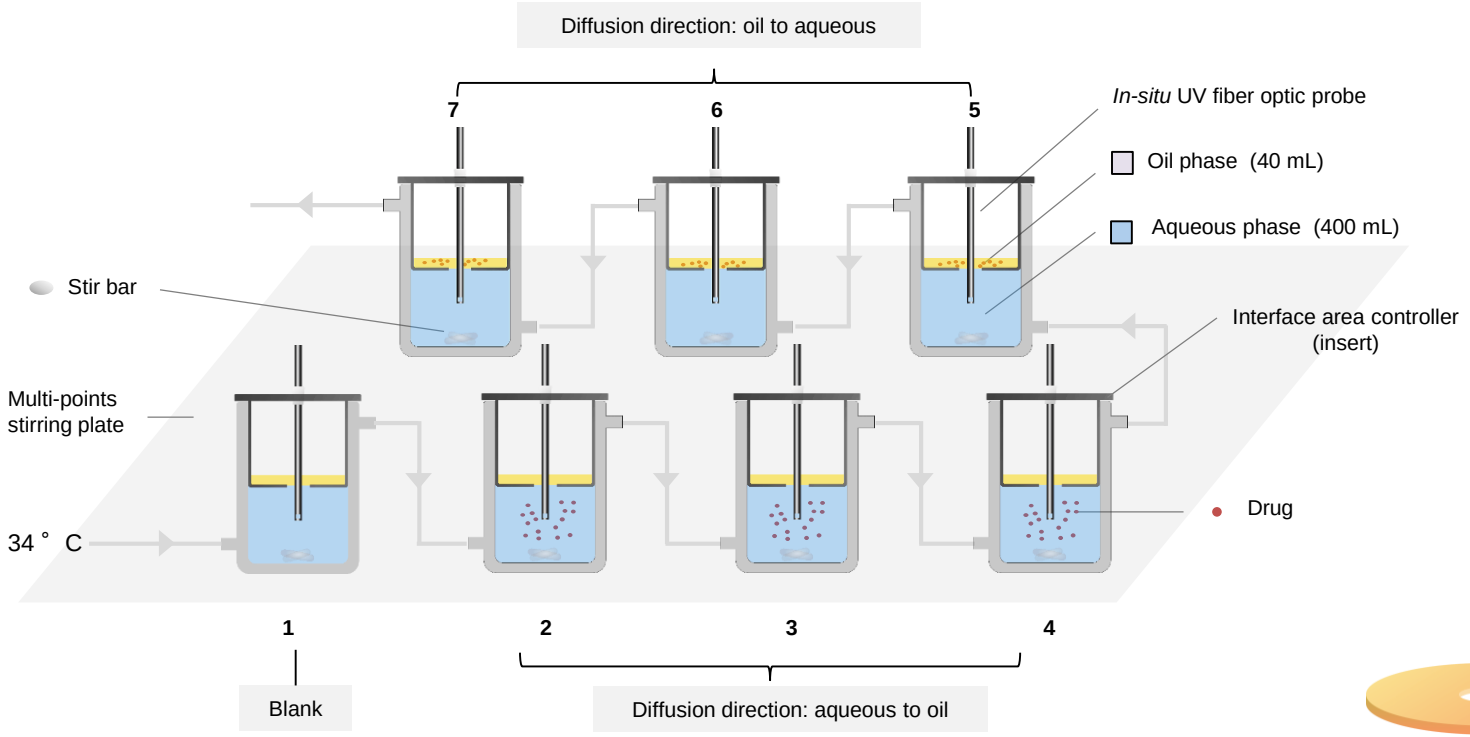
As Tween 80 conc. increased:

- Aqueous-to-oil phase transfer (k_{a-o}) decreased (*slower*)
- Oil-to-aqueous phase transfer (k_{o-a}) increased (*faster*)

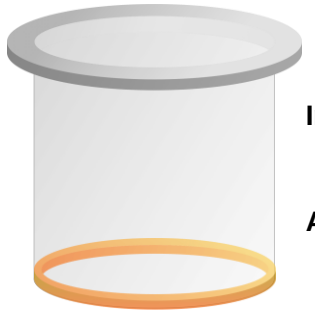
- Ratio of the rate constants, i.e. $\log_{10}\frac{k_{ao}}{k_{oa}}$, decreased from **3.61** to **2.38** (more in aqueous AND micelle phase)

Y. Dong, et al. Journal of Controlled Release (2019), 313, pp 96-105

Effect of Surface (Interfacial) Area



In-house fabricated interface area controller

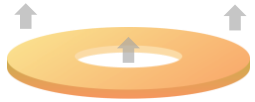


Insert Top

Area controller

83.00 cm^2

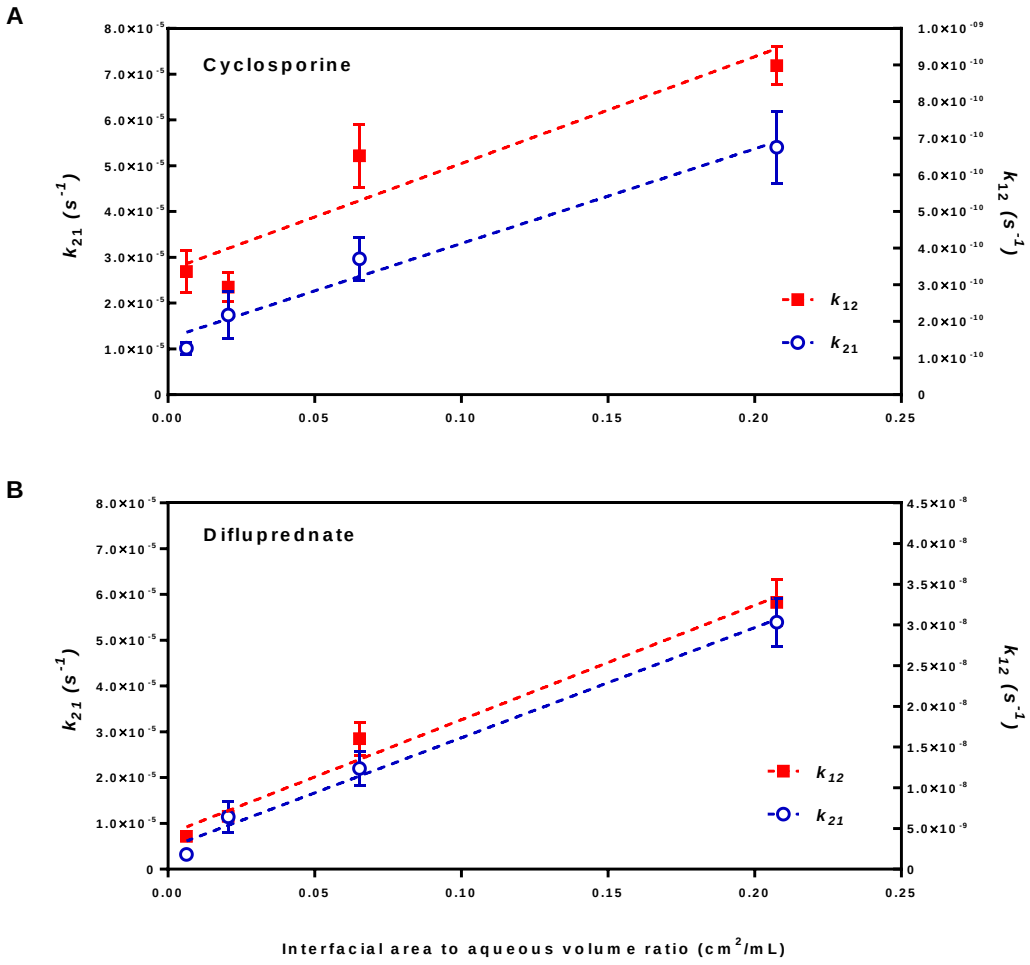
2.51 cm^2



8.23 cm^2

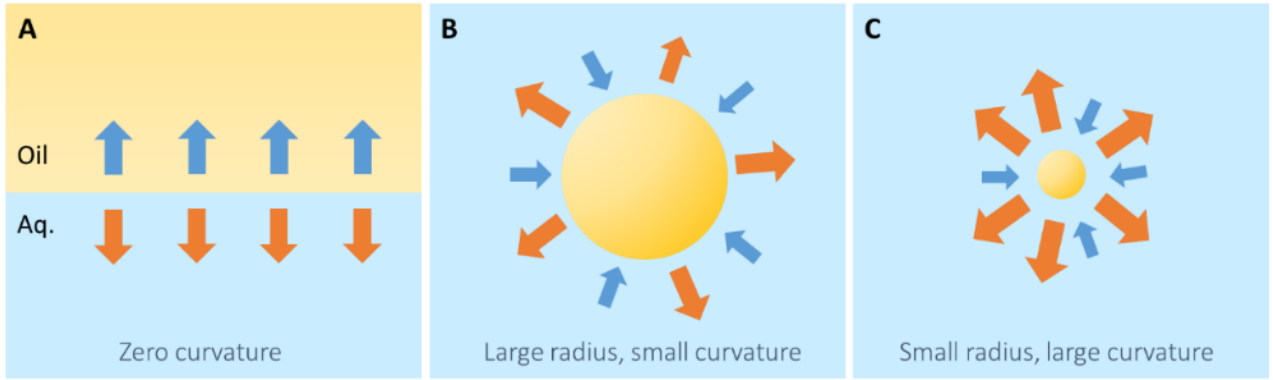
26.10 cm^2

Effect of Surface (Interfacial) Area



Increase in rate, but not in extent.

However,



$k_{12} \approx 10^{-9}\ s^{-1}$

If we scale based on the actual globule surface area (**1.5 m²**), the predicted transfer rate (from oil globule to aqueous) becomes:

$$K_{12} \approx 10^{-3}\ s^{-1}$$

and $$t_{1/2} = \frac{ln2}{K_{12}} \approx 12\ min$$

Release from oil globule is slow (relative to <2 min ocular retention time)

Y. Dong, et al. ***Journal of Controlled Release*** (2019), 313, pp 96-105

Value of $LogP_{app}$ (Predicting Drug Distribution and Drug Release)

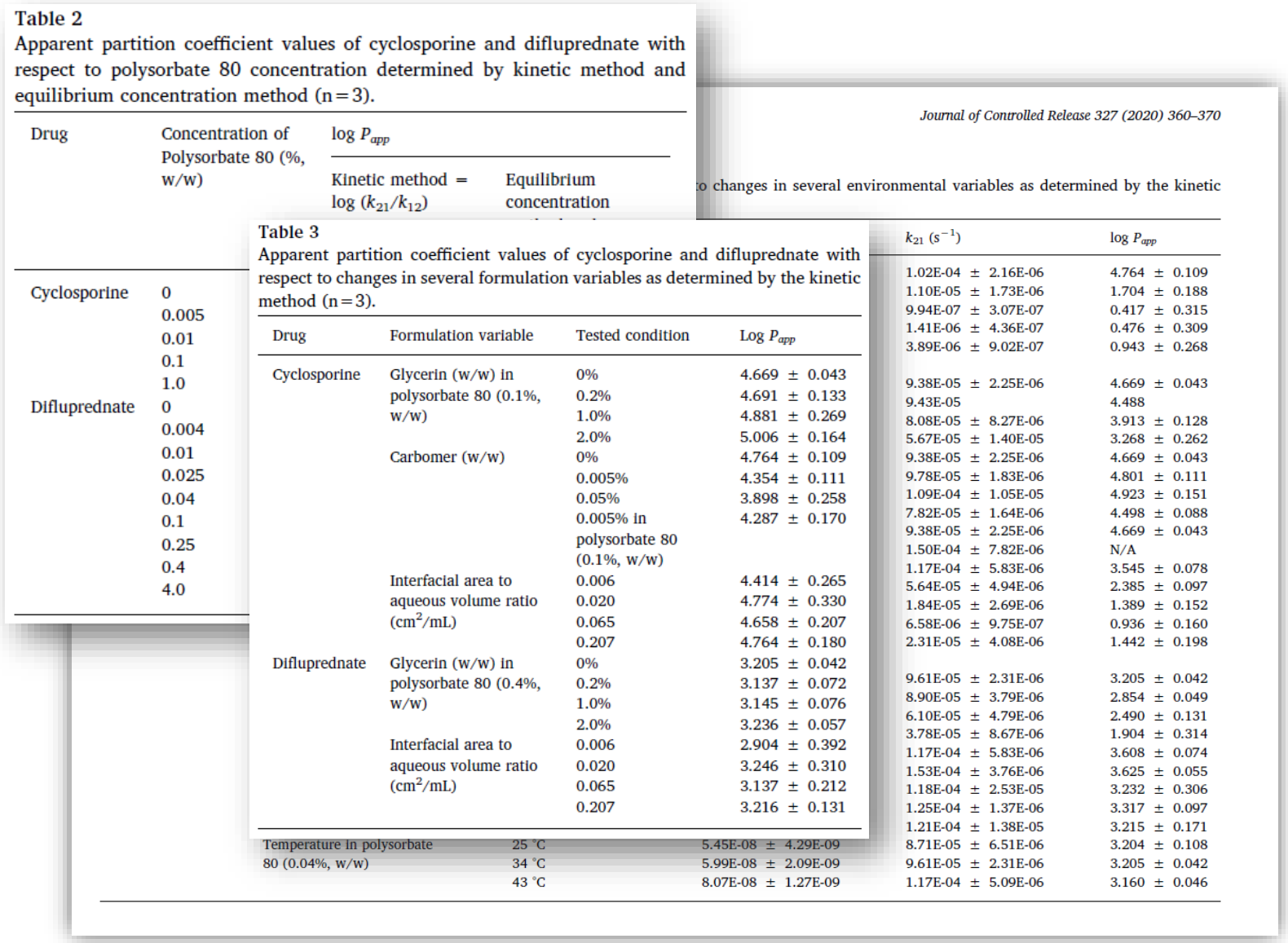


Table 2
Apparent partition coefficient values of cyclosporine and difluprednate with respect to polysorbate 80 concentration determined by kinetic method and equilibrium concentration method (n = 3).

Drug	Concentration of Polysorbate 80 (%, w/w)	log P_{app}	
		Kinetic method = log (k_{21}/k_{12})	Equilibrium concentration
Cyclosporine	0		
	0.005		
	0.01		
	0.1		
	1.0		
Difluprednate	0		
	0.004		
	0.01		
	0.025		
	0.04		
	0.1		
	0.25		
	0.4		
	4.0		

Table 3
Apparent partition coefficient values of cyclosporine and difluprednate with respect to changes in several formulation variables as determined by the kinetic method (n = 3).

Drug	Formulation variable	Tested condition	Log P_{app}
Cyclosporine	Glycerin (w/w) in polysorbate 80 (0.1%, w/w)	0%	4.669 ± 0.043
		0.2%	4.691 ± 0.133
		1.0%	4.881 ± 0.269
		2.0%	5.006 ± 0.164
	Carbomer (w/w)	0%	4.764 ± 0.109
		0.005%	4.354 ± 0.111
		0.05%	3.898 ± 0.258
		0.005% in polysorbate 80 (0.1%, w/w)	4.287 ± 0.170
	Interfacial area to aqueous volume ratio (cm^2/mL)	0.006	4.414 ± 0.265
		0.020	4.774 ± 0.330
		0.065	4.658 ± 0.207
		0.207	4.764 ± 0.180
Difluprednate	Glycerin (w/w) in polysorbate 80 (0.4%, w/w)	0%	3.205 ± 0.042
		0.2%	3.137 ± 0.072
		1.0%	3.145 ± 0.076
		2.0%	3.236 ± 0.057
	Interfacial area to aqueous volume ratio (cm^2/mL)	0.006	2.904 ± 0.392
		0.020	3.246 ± 0.310
		0.065	3.137 ± 0.212
		0.207	3.216 ± 0.131

...o changes in several environmental variables as determined by the kinetic

		k_{21} (s^{-1})	log P_{app}
		1.02E-04 ± 2.16E-06	4.764 ± 0.109
		1.10E-05 ± 1.73E-06	1.704 ± 0.188
		9.94E-07 ± 3.07E-07	0.417 ± 0.315
		1.41E-06 ± 4.36E-07	0.476 ± 0.309
		3.89E-06 ± 9.02E-07	0.943 ± 0.268
		9.38E-05 ± 2.25E-06	4.669 ± 0.043
		9.43E-05	4.488
		8.08E-05 ± 8.27E-06	3.913 ± 0.128
		5.67E-05 ± 1.40E-05	3.268 ± 0.262
		9.38E-05 ± 2.25E-06	4.669 ± 0.043
		9.78E-05 ± 1.83E-06	4.801 ± 0.111
		1.09E-04 ± 1.05E-05	4.923 ± 0.151
		7.82E-05 ± 1.64E-06	4.498 ± 0.088
		9.38E-05 ± 2.25E-06	4.669 ± 0.043
		1.50E-04 ± 7.82E-06	N/A
		1.17E-04 ± 5.83E-06	3.545 ± 0.078
		5.64E-05 ± 4.94E-06	2.385 ± 0.097
		1.84E-05 ± 2.69E-06	1.389 ± 0.152
		6.58E-06 ± 9.75E-07	0.936 ± 0.160
		2.31E-05 ± 4.08E-06	1.442 ± 0.198
		9.61E-05 ± 2.31E-06	3.205 ± 0.042
		8.90E-05 ± 3.79E-06	2.854 ± 0.049
		6.10E-05 ± 4.79E-06	2.490 ± 0.131
		3.78E-05 ± 8.67E-06	1.904 ± 0.314
		1.17E-04 ± 5.83E-06	3.608 ± 0.074
		1.53E-04 ± 3.76E-06	3.625 ± 0.055
		1.18E-04 ± 2.53E-05	3.232 ± 0.306
		1.25E-04 ± 1.37E-06	3.317 ± 0.097
		1.21E-04 ± 1.38E-05	3.215 ± 0.171
Temperature in polysorbate 80 (0.04%, w/w)	25 °C	8.71E-05 ± 6.51E-06	3.204 ± 0.108
	34 °C	9.61E-05 ± 2.31E-06	3.205 ± 0.042
	43 °C	1.17E-04 ± 5.09E-06	3.160 ± 0.046
		5.45E-08 ± 4.29E-09	
		5.99E-08 ± 2.09E-09	
		8.07E-08 ± 1.27E-09	

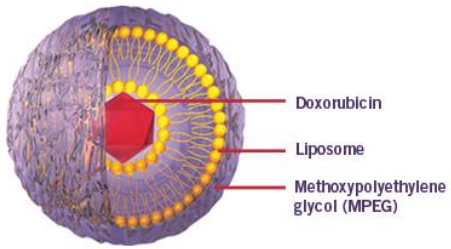
Over 50 $LogP_{app}$ values :

1. **Formulation (composition)**
2. **Release (medium, temperature)**

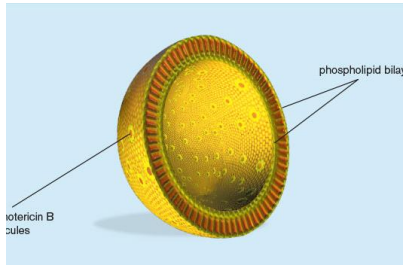
- Y. Dong et al. Journal of Controlled Release (2019), 313, 96-105
- Y. Dong et al. Journal of Controlled Release (2020), 327, 360-370

Now, the second problem…

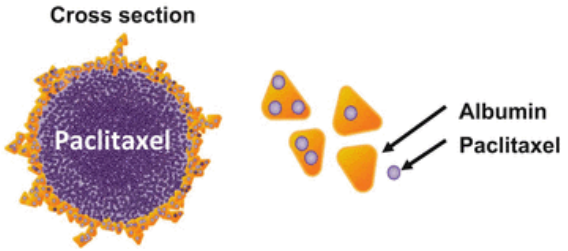
The problem: particle separation that triggers release



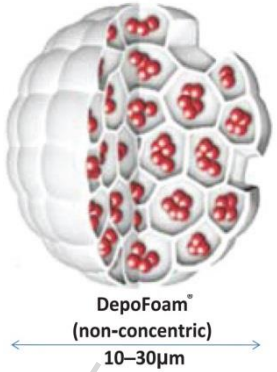
Doxil (80-90 nm)

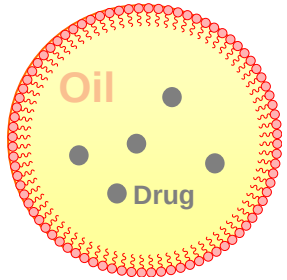


Ambisome (<80 nm)



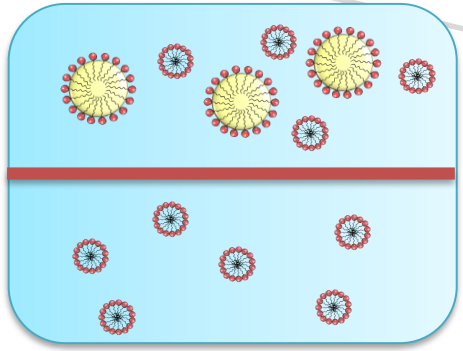
Abraxane (~130 nm)





Nanoemulsion (~100 nm)

Membrane

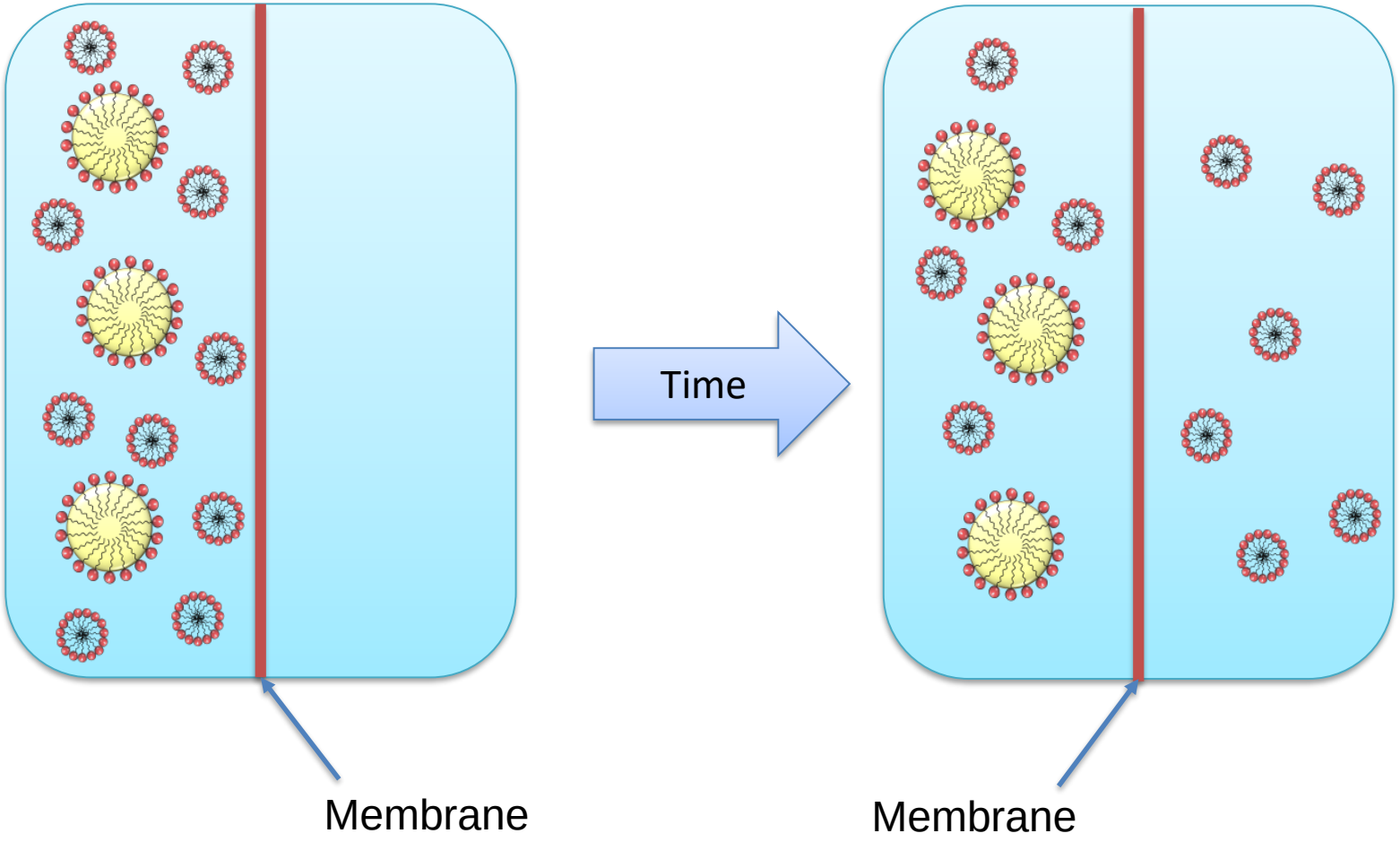


Free Drug

Separation!

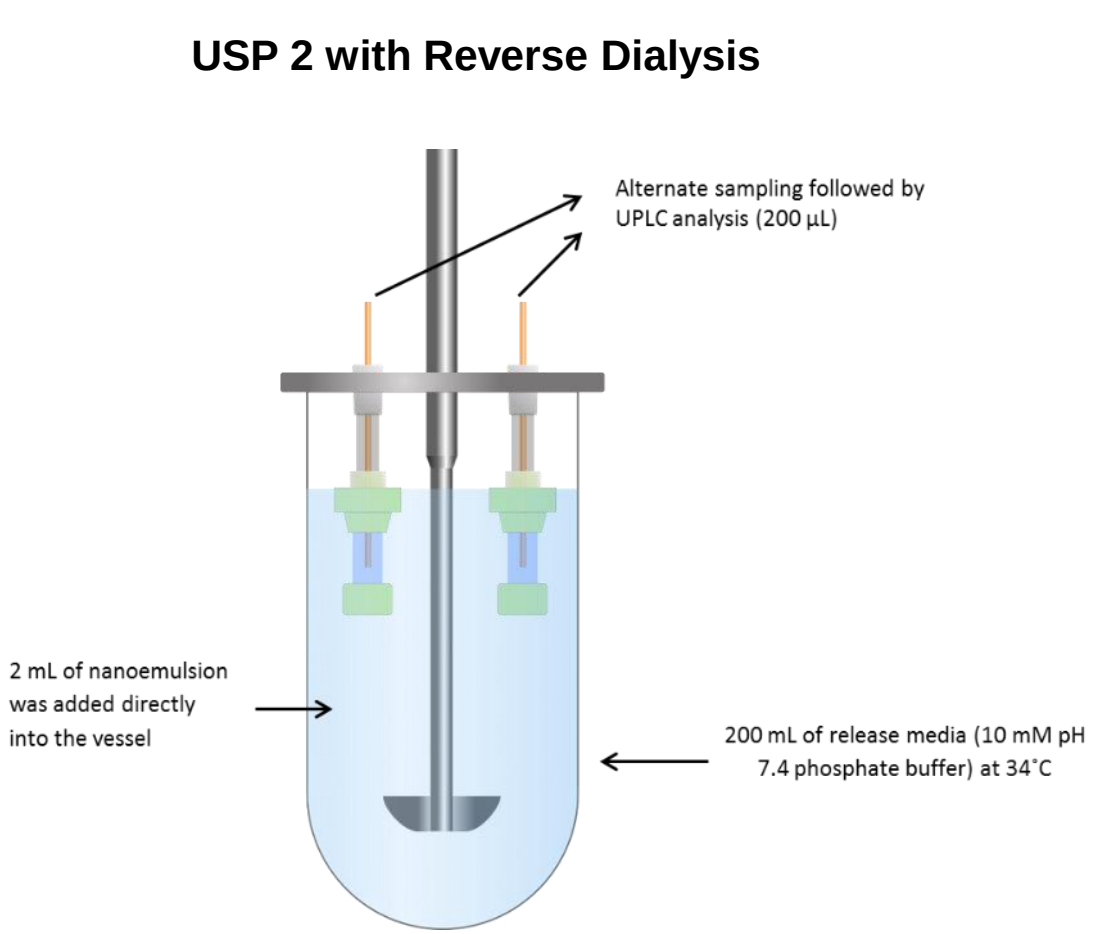
The first step towards analysis drug release from dispersed systems, such as liposomes, suspensions, micelles and emulsions, is the “separation of free drug”. Common approach uses dialysis membrane, which can become rate-limiting and severely impact IVRT method’s discriminatory power.

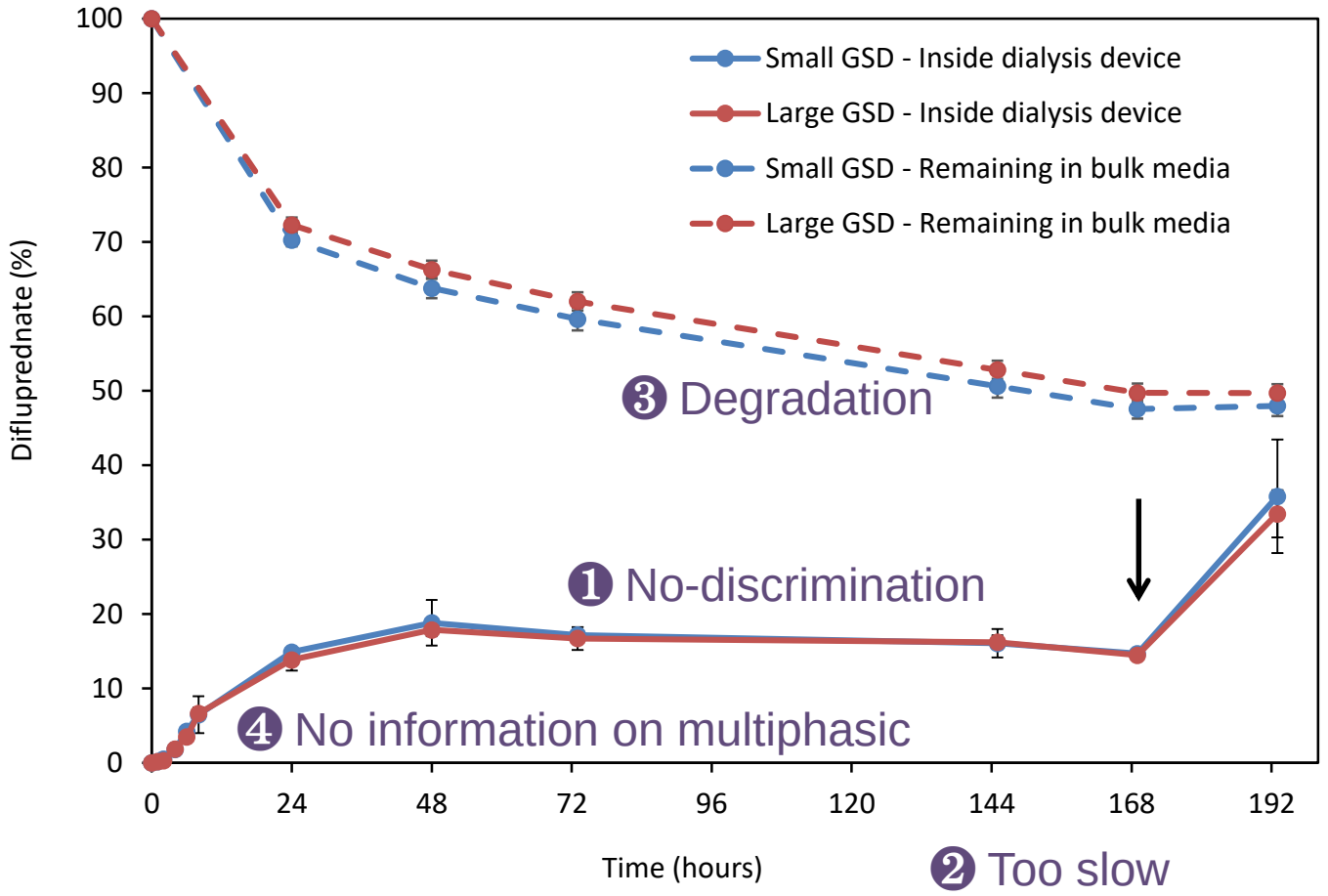
Drug release of nanoparticle: usual way



- Driven by concentration gradient: High to Low
- Membrane transfer may become a rate-limiting step

IVRT by (Reverse) Dialysis: A Typical Example





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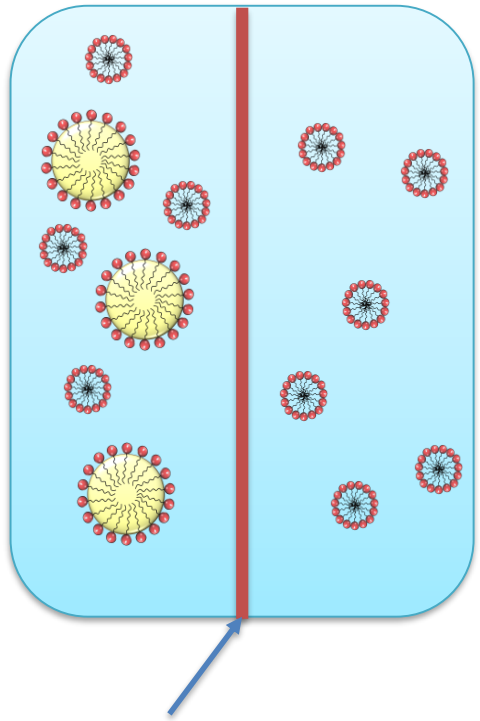
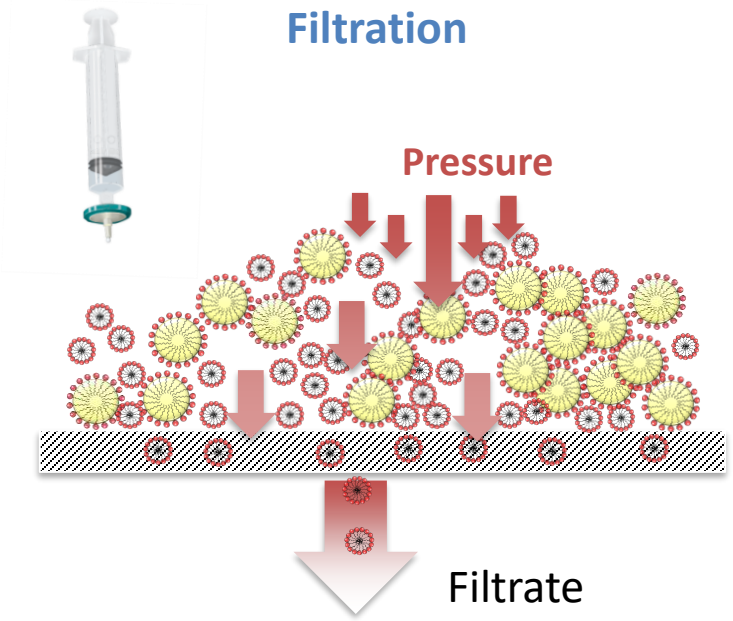
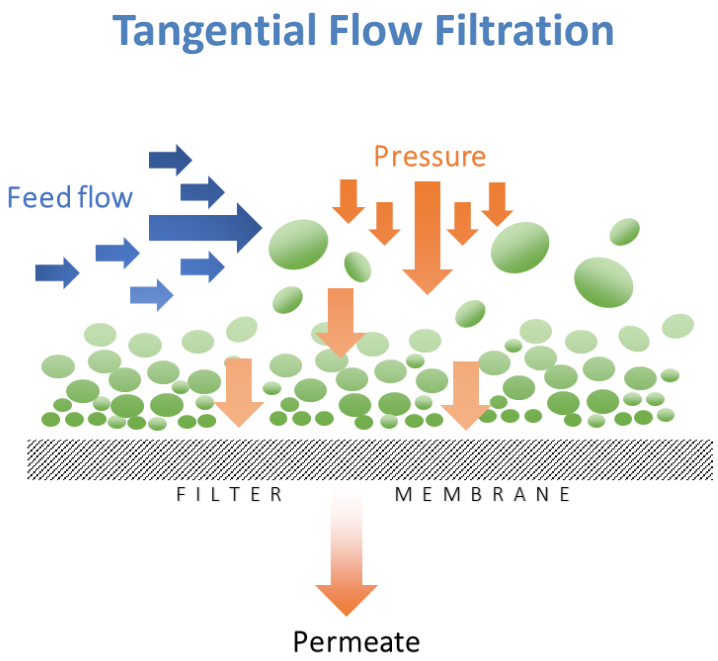
How can we solve it?

Filtration, Instead of Diffusion



Membrane



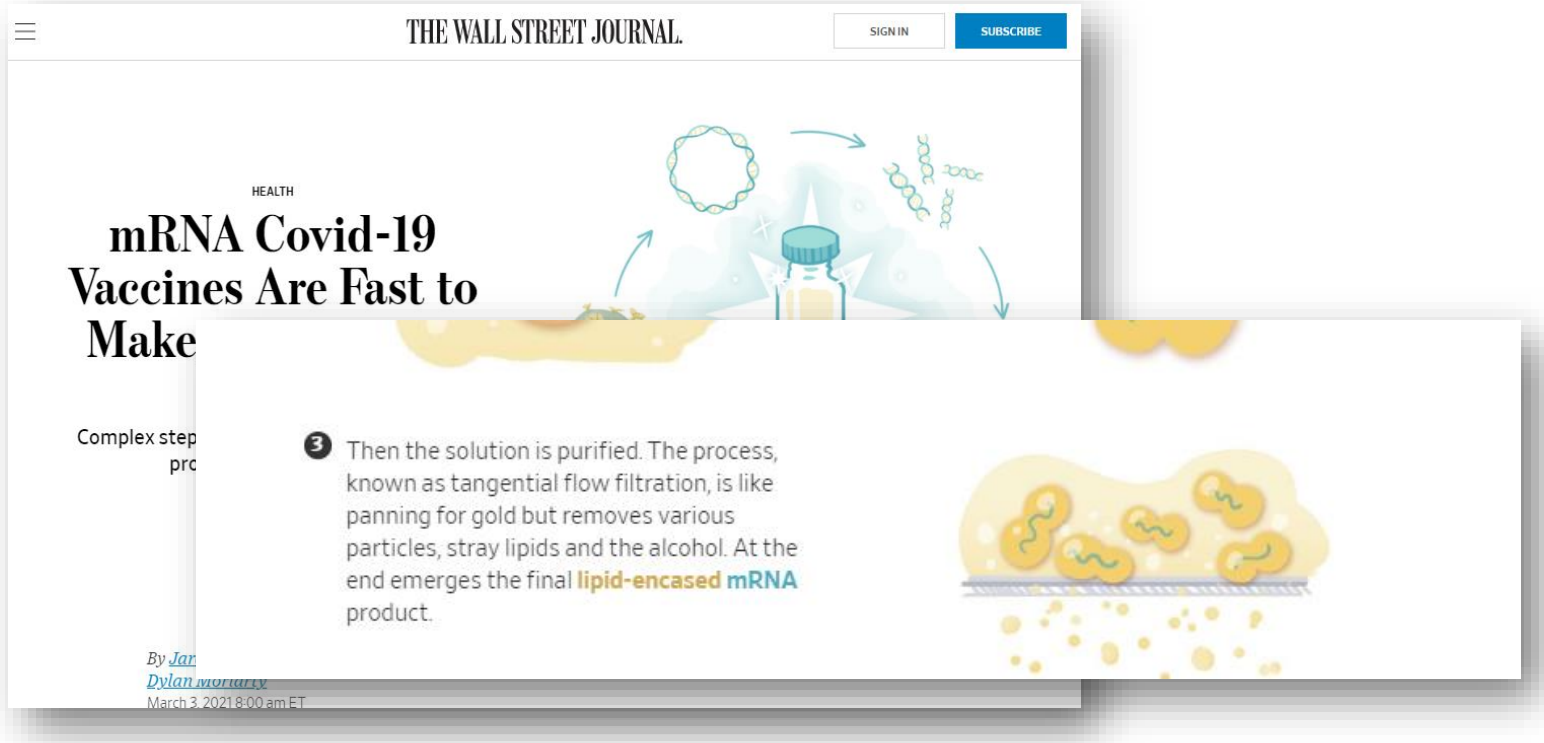


- Pressure driven
- Controllable flow by filtration
- Separation based on membrane size

- Tangential flow, thus avoiding build up at the membrane surface (swept away by flow)

TFF: old solution to old problem

- TFF is not a new technique. Widely used since 1960s in various industrial processes, e.g., de-salting, solvent-exchange, concentration.



Different Focus in Adaptive Perfusion

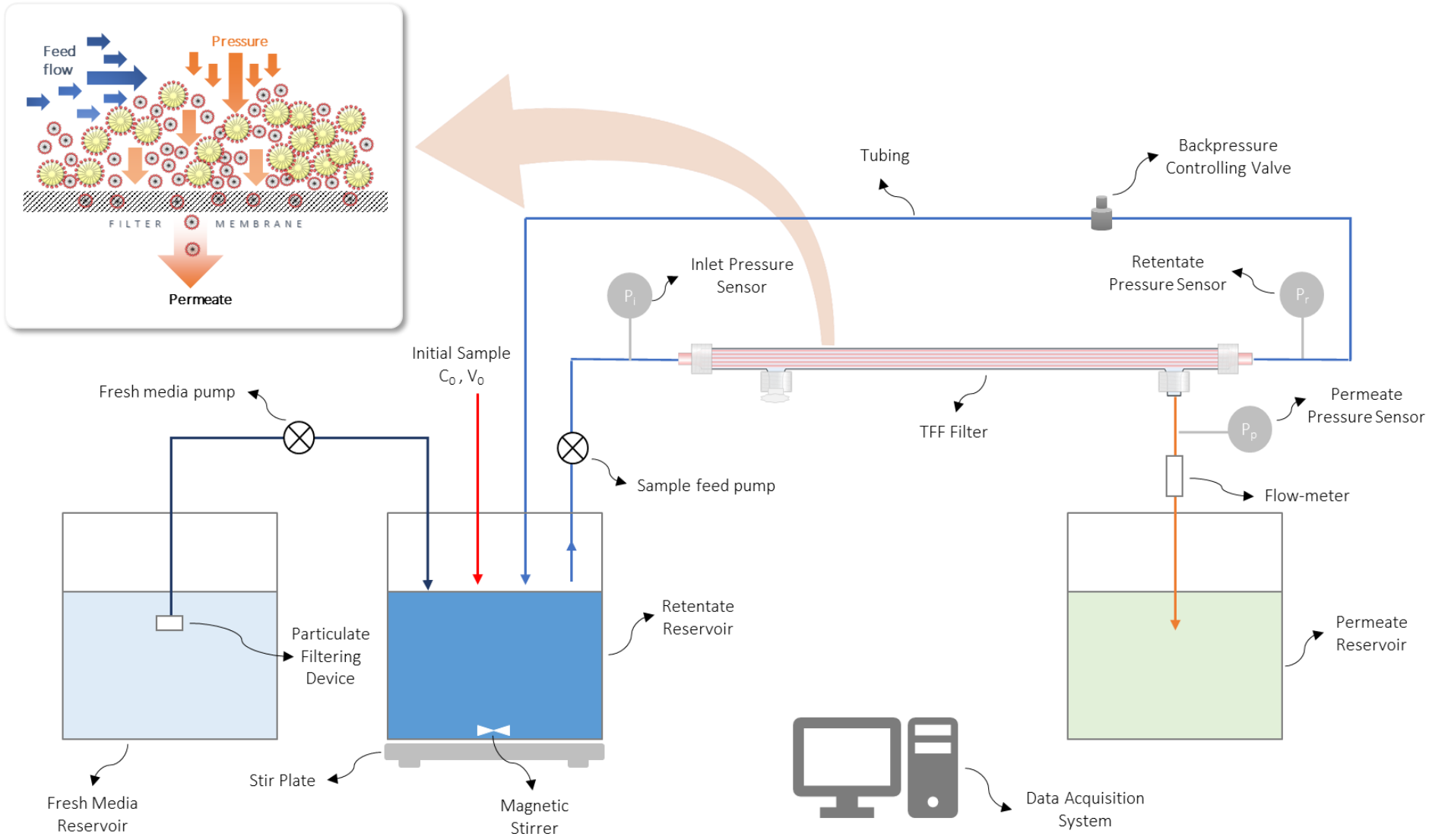
Common use:

- Only focus on retentate OR permeate
- Only focus on the extent (% recovery, purity)

In Adaptive Perfusion, goal is to obtain “Drug Release”:

- Retentate(drug remain) and permeate(drug remove)
- Rate AND Extent (how fast AND how much)

Schematic Diagram of Adaptive Perfusion



D. Patel et al. Adaptive Perfusion: An In Vitro Release Test (IVRT) for Complex Drug Products. Journal of Controlled Release (2021), 333, pp.65-75.

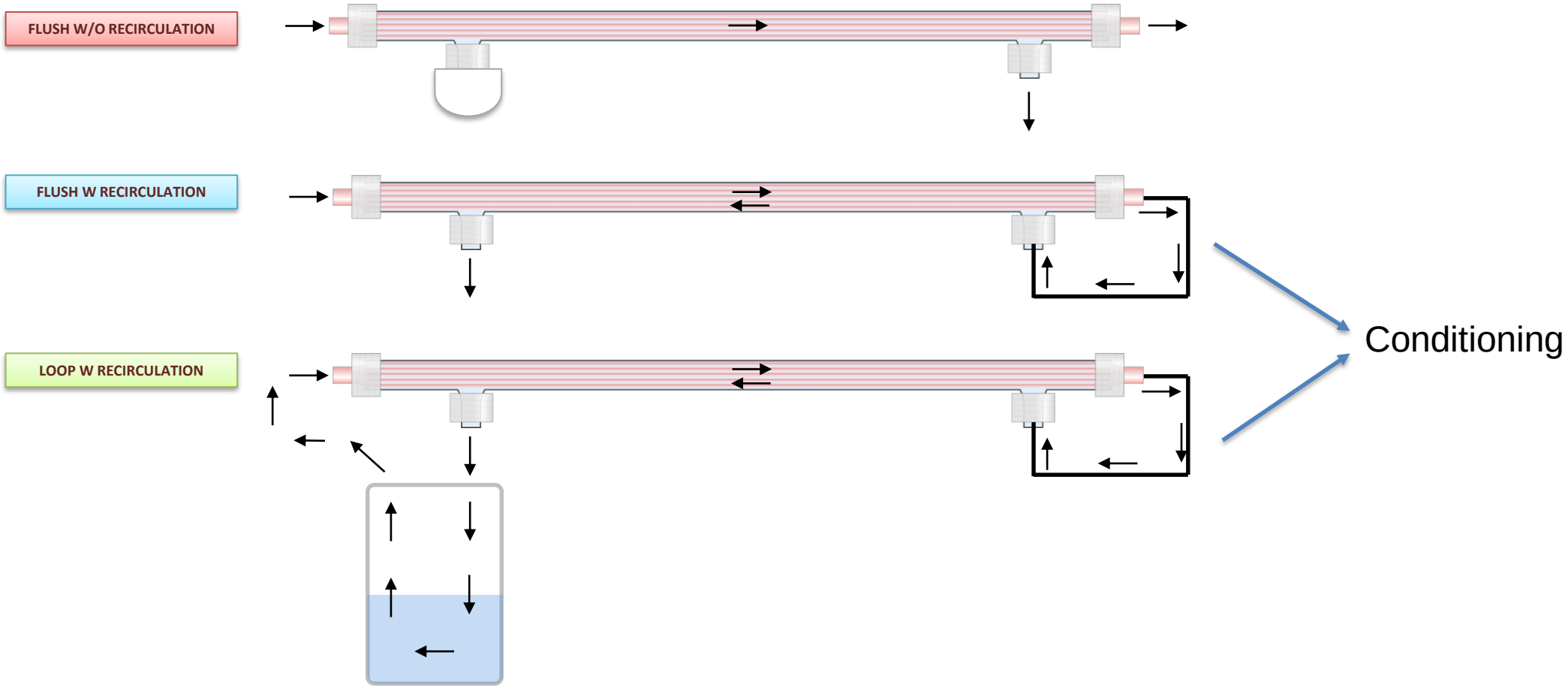
Where we started…

Key Challenges (Solved)

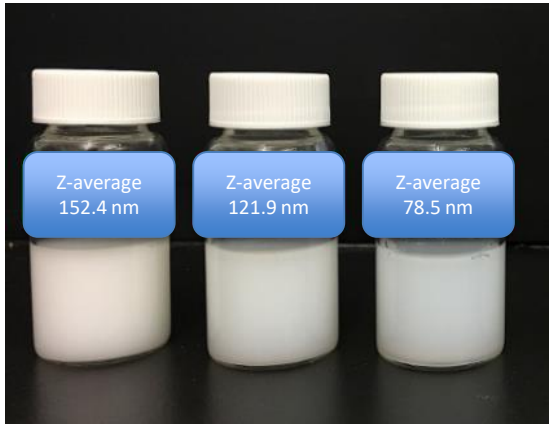
- Reproducibility:
  - Fiber to fiber (critical for switching fibers)
  - Run to run
- Fouling:
  - Performance degradation, lead to low flux -> can't see the difference between different GSD formulation
- Discriminatory capability

Solution: Membrane Conditioning

Both Medium and Configuration are important!!

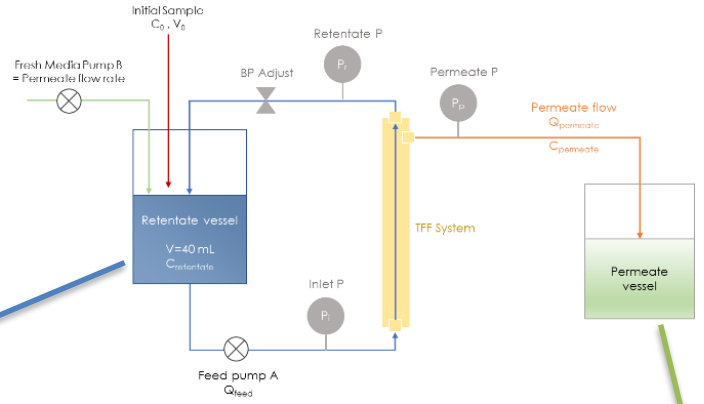


In-house Formulation with Intentionally Varied GSD

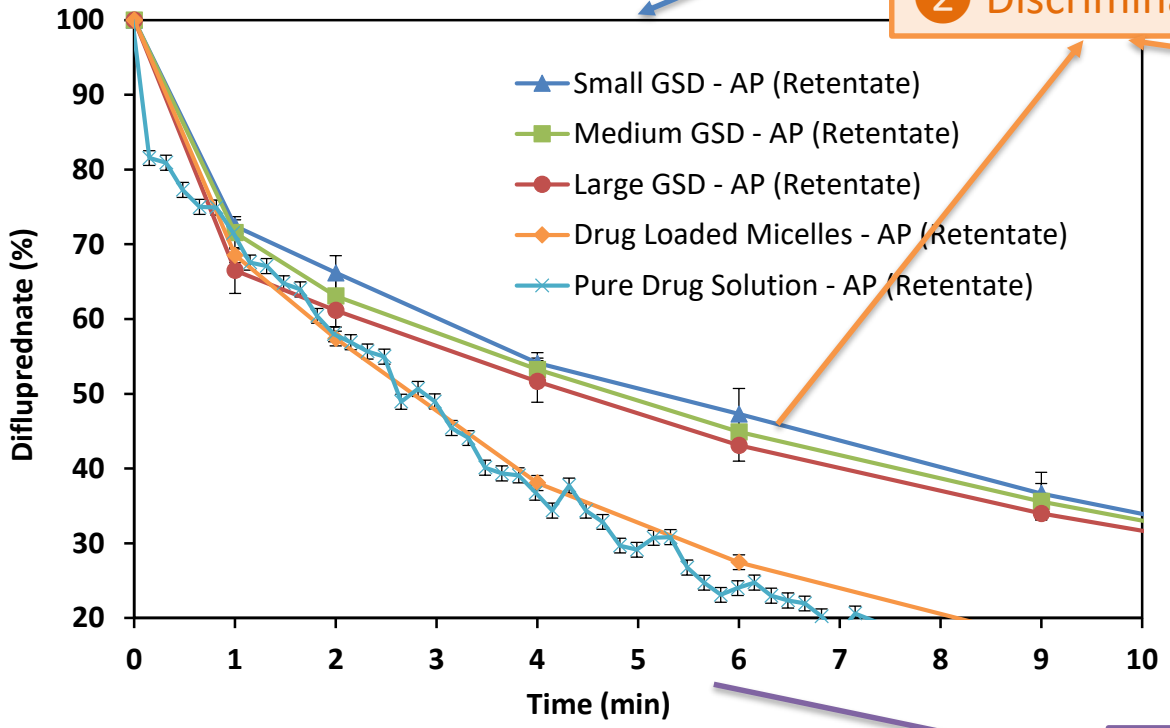


Sample	Z-Average (d.nm)	PdI
Large GSD	152.4 ± 1.3	0.181 ± 0.014
Medium GSD	121.9 ± 0.9	0.203 ± 0.010
Small GSD	78.5 ± 0.6	0.206 ± 0.008

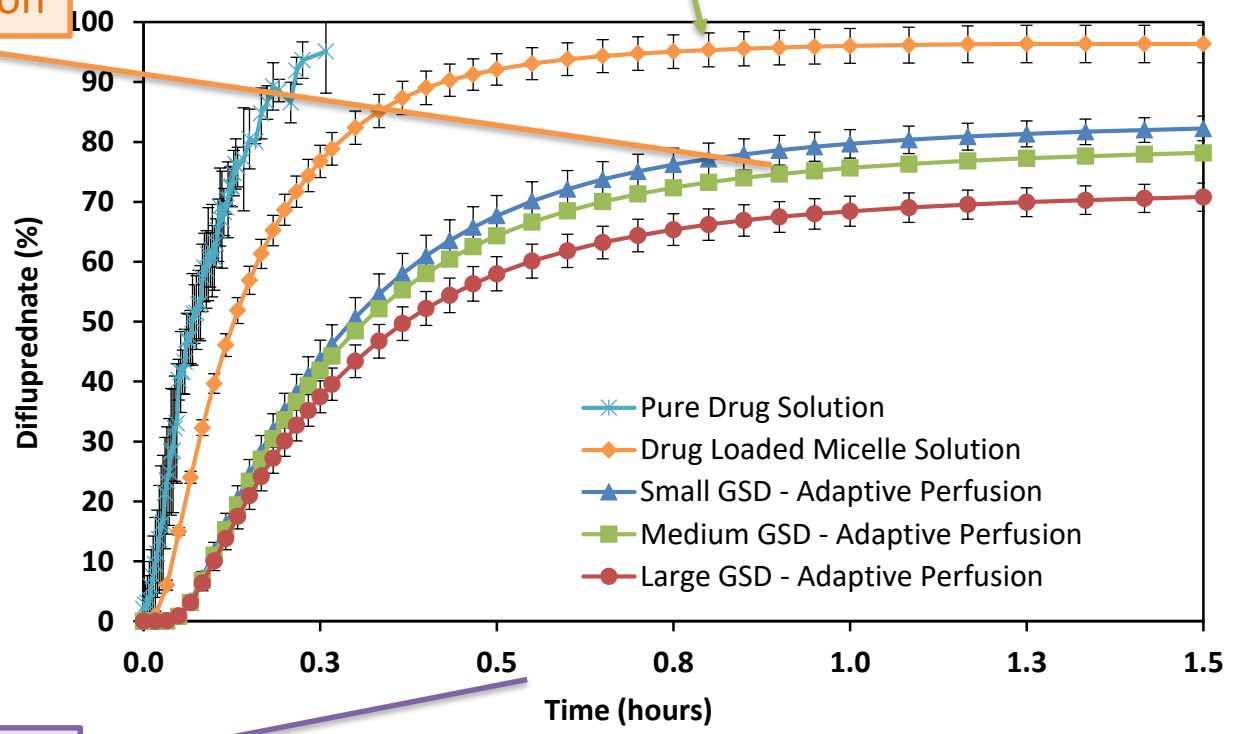
Results



Retentate Concentration Profile (n=3)



Permeate Concentration Profile (n=3)



2 Discrimination

1 Fast

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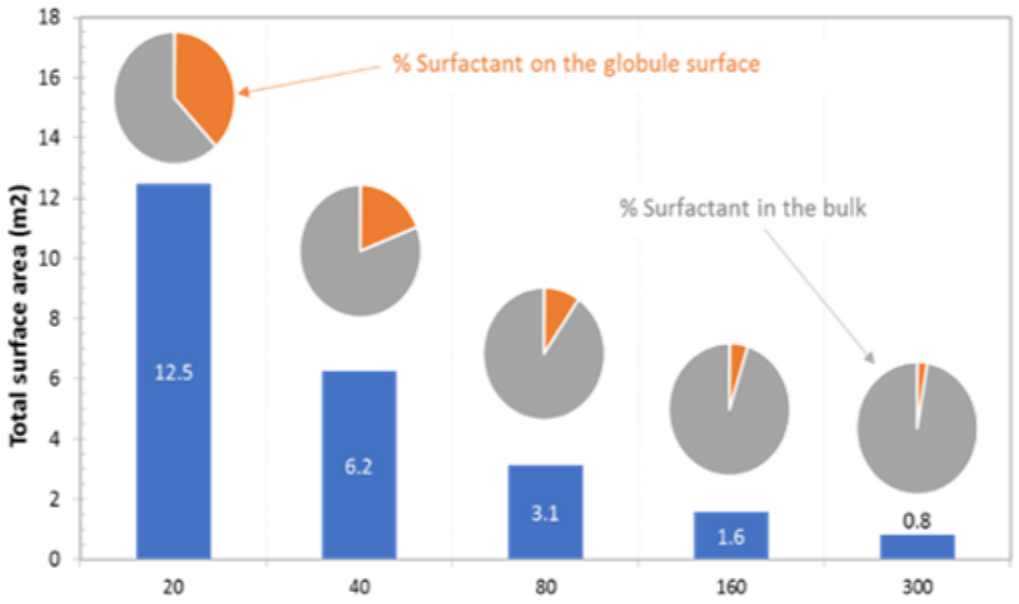
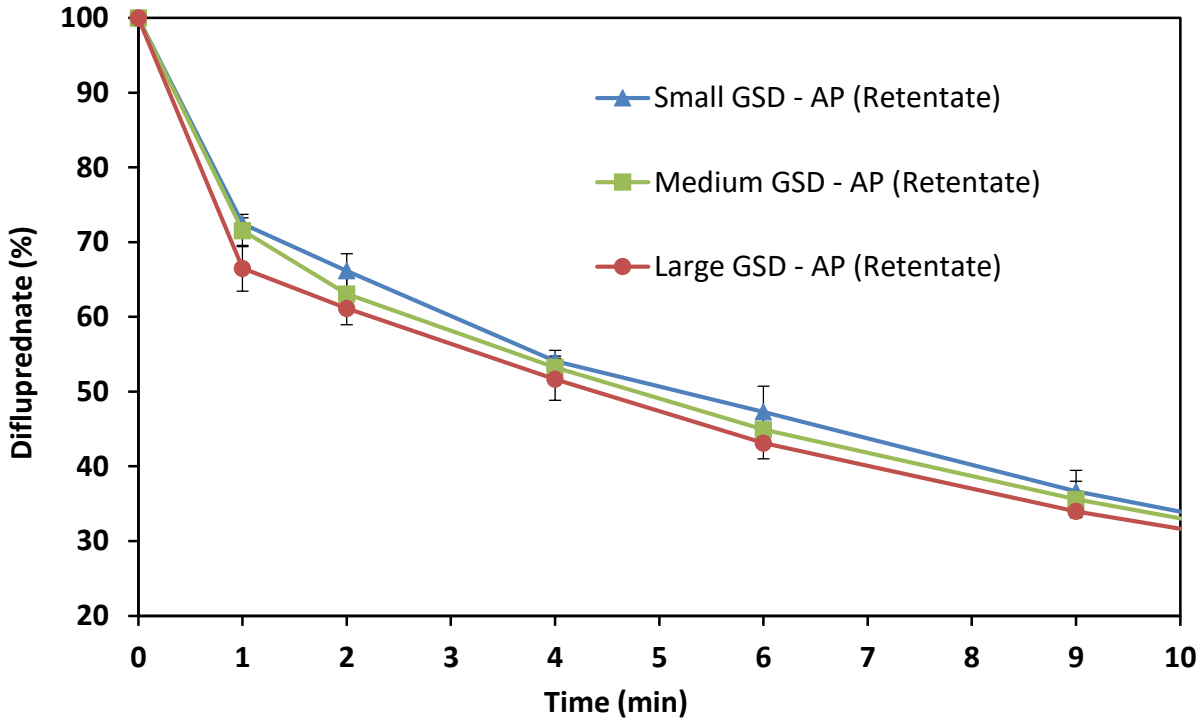
Surfactant Distribution Directly Influence the Rate of Drug Release

Particle Size Distribution



Retentate Concentration Profile (n=3)



Smaller GSD, less surfactant, slower first phase release
Larger GSD, more surfactant, faster first phase release

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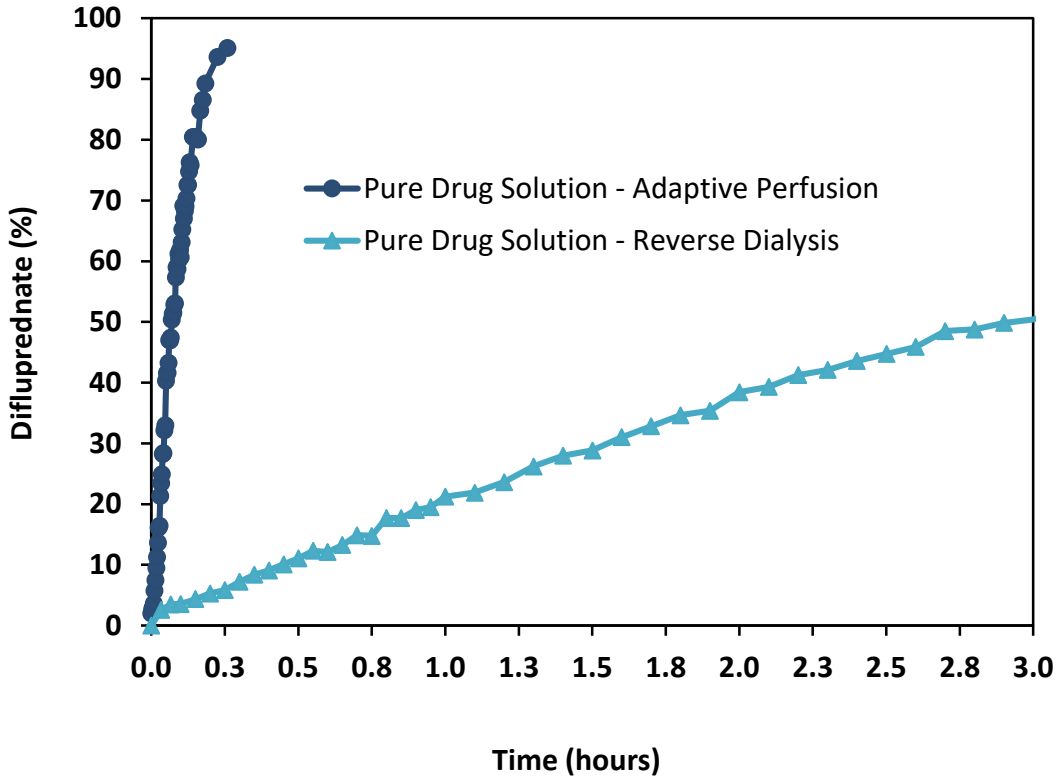
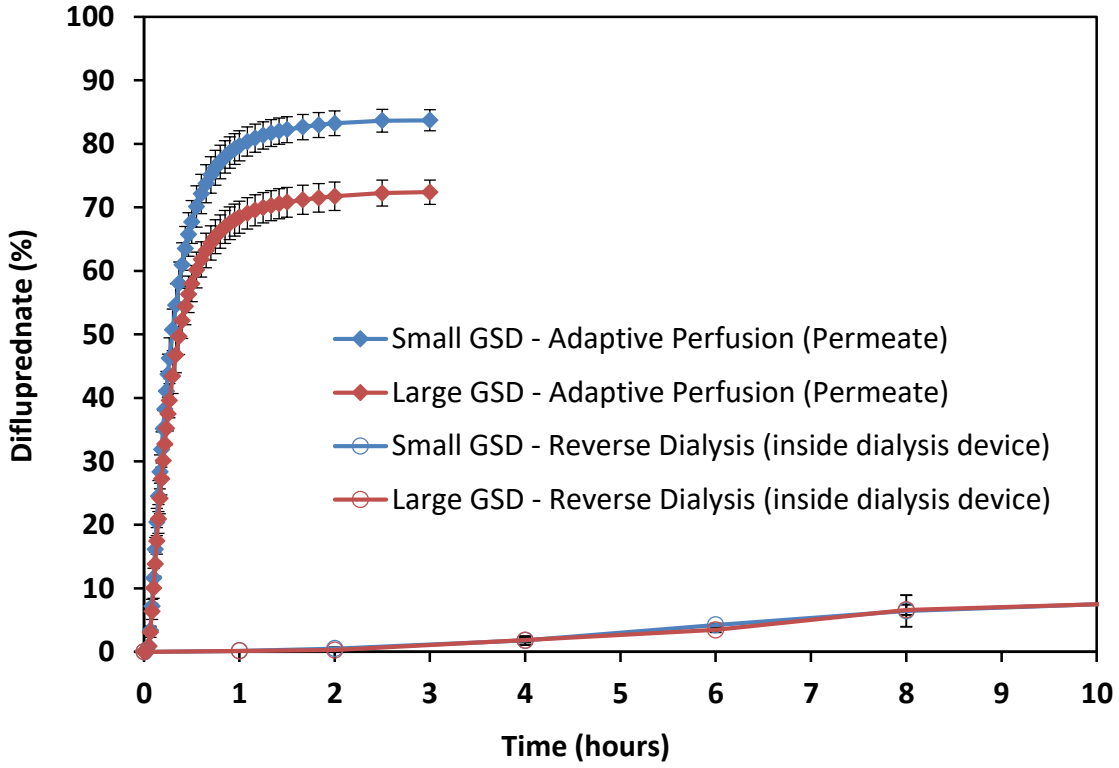
New vs. Old

Pure Drug Solution (n = 3)



Small and Large GSD nanoemulsions (n = 3)



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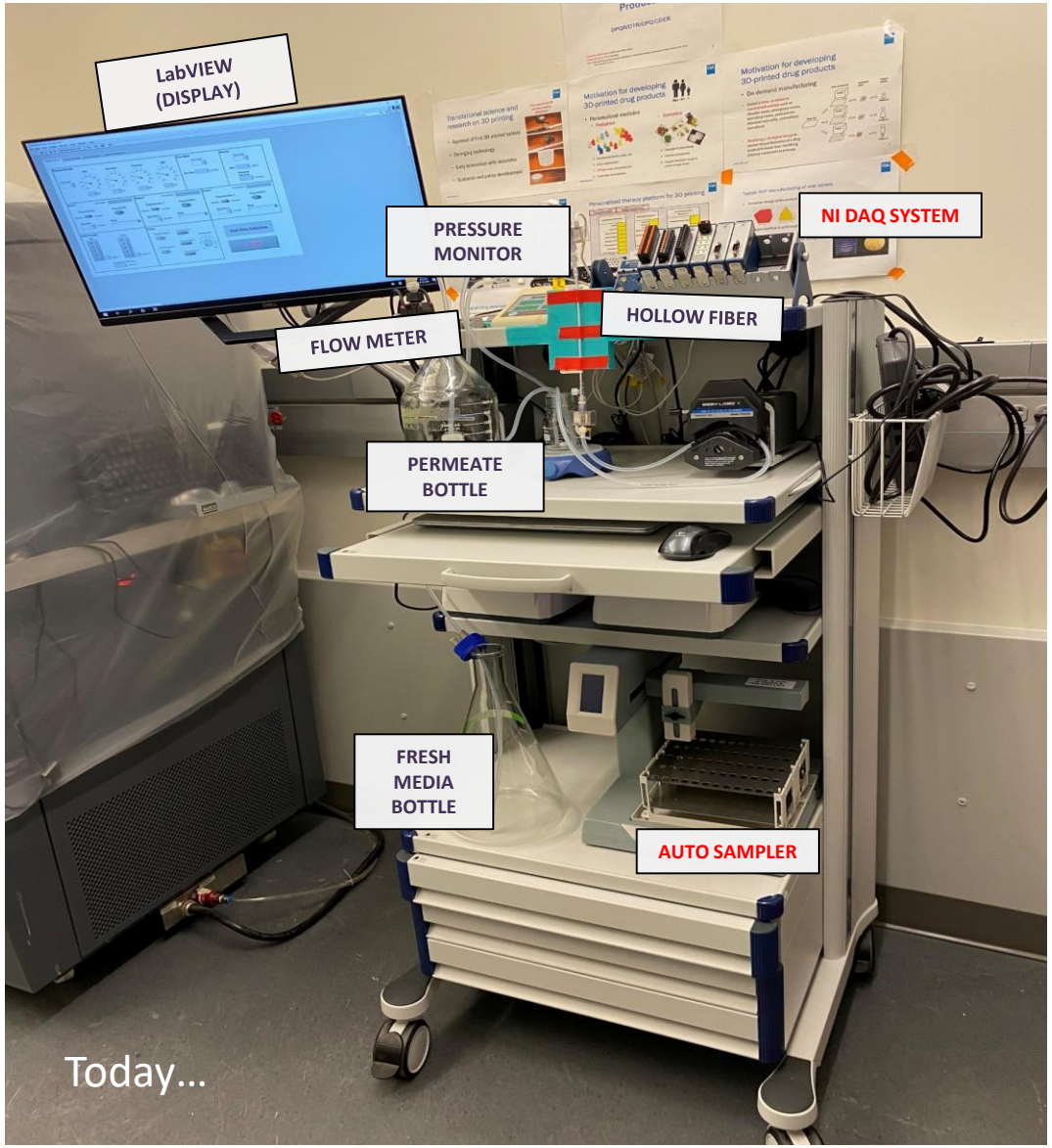
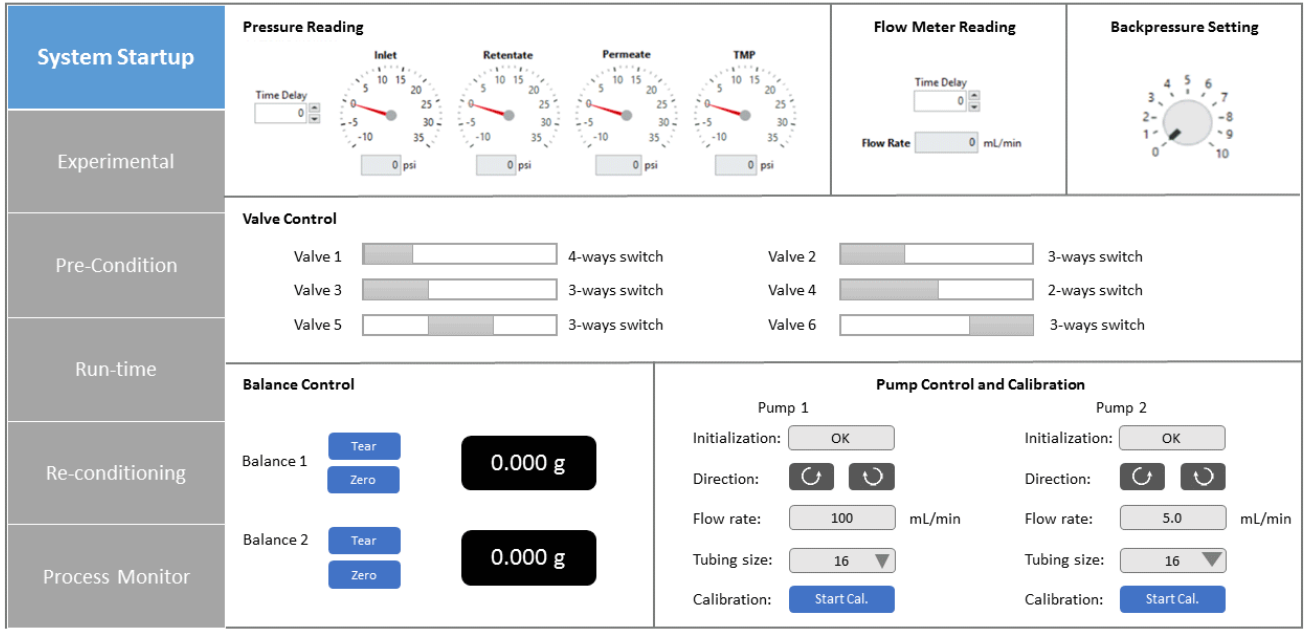
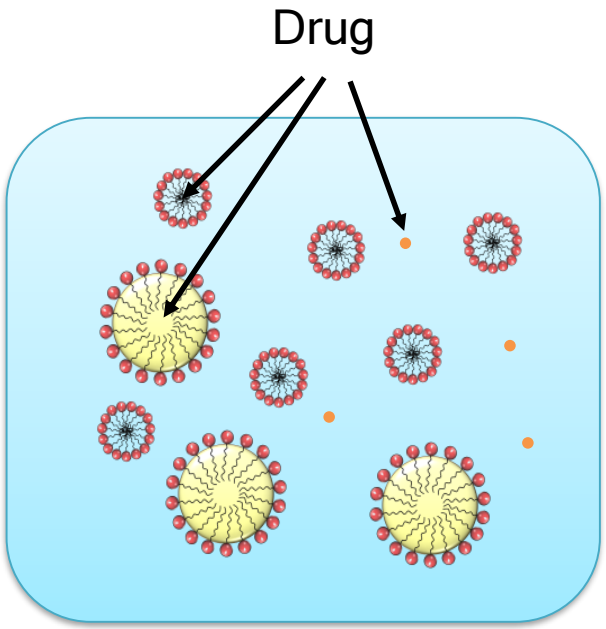
Future: A Turnkey Solution

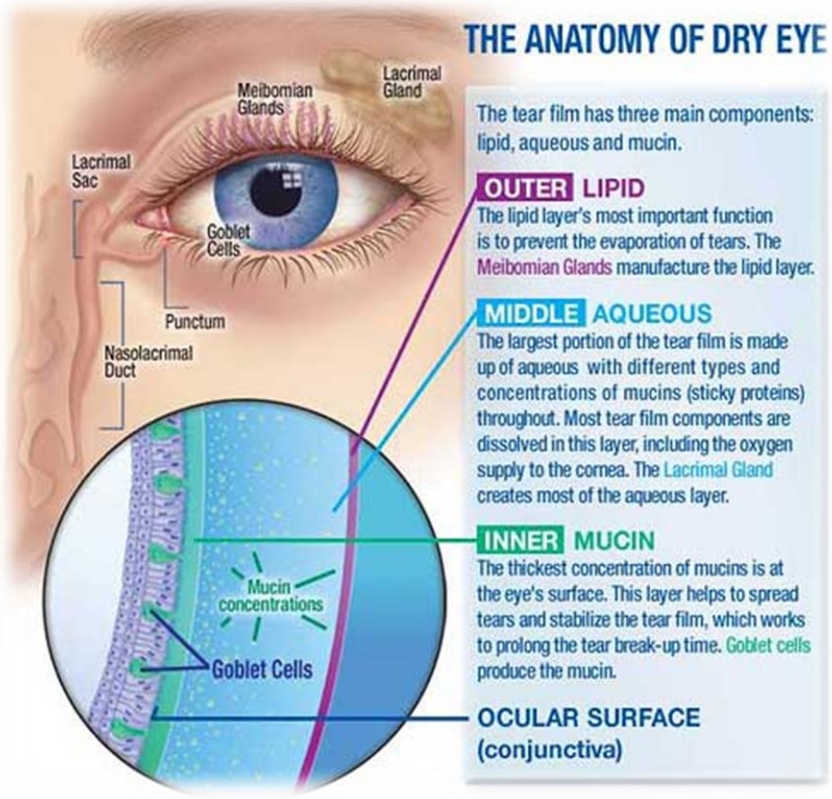


Custom Designed Control Interface



The Problems: Study drug release from nanoemulsions





Two fundamental problems:

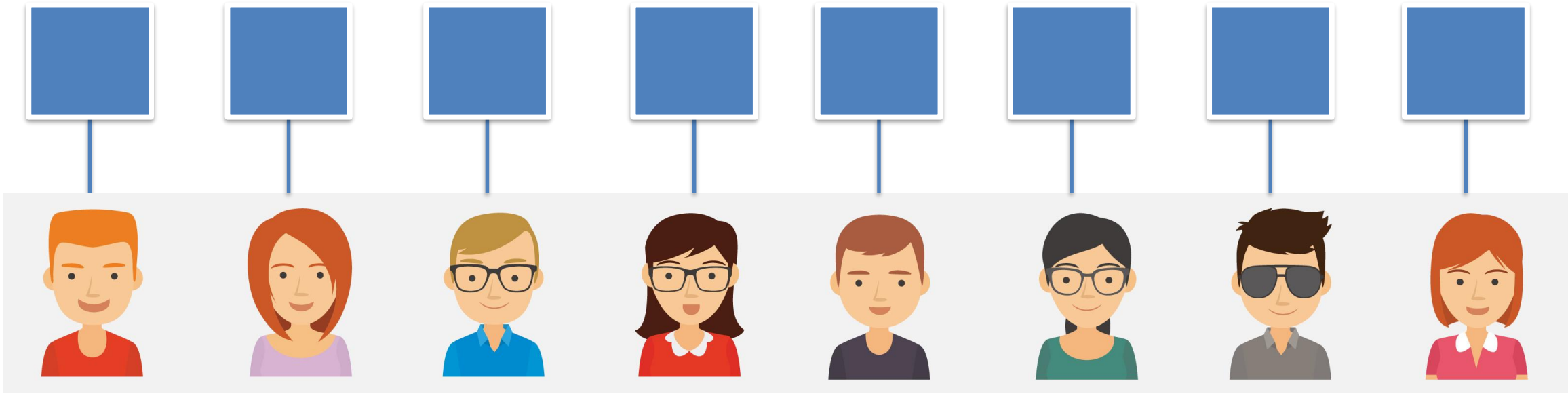
- Transfer kinetics (old problem, new solution)
- Particle separation (new problem, old solution)

Summary

Thinking critically

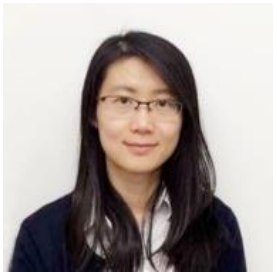
- Old problem, new solutions;
- New problem, old solutions;
- It’s all about perspectives (expand your boxes).

Thinking outside a box: Perspectives

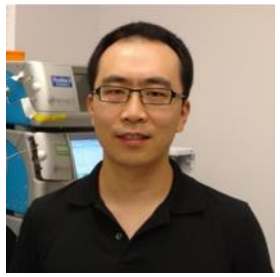


Acknowledgement

Deval Patel



Yixuan Dong



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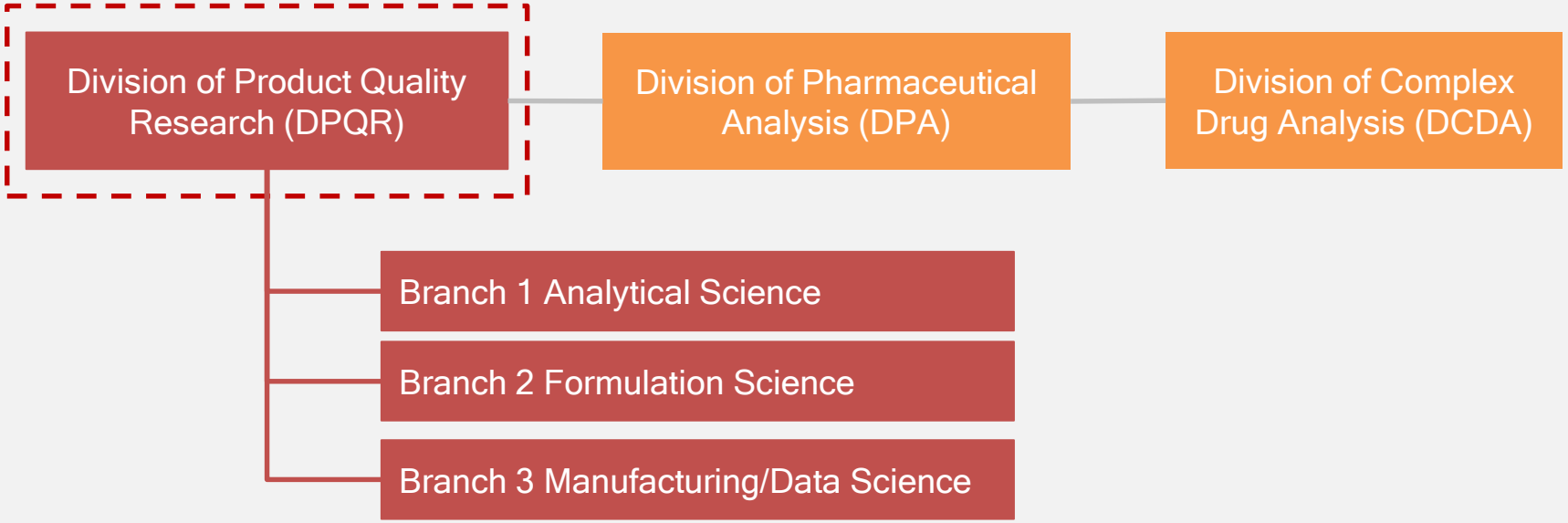
OTR Organization



Office Locations

White Oak Campus, MD

St. Louis, MO

Ammendale Facility, MD

New Facility


Building 64 First Floor

DPQR Research Areas

Analytical Science

- Chromatography (e.g., HPLC and UPLC core facility)
- Mass spectrometry (e.g., high throughput RapidFire)
- Nuclear magnetic resonance (NMR) spectroscopy
- Advanced separation (e.g., field flow fractionation)
- Product performance (e.g., dissolution, in vitro release test, IVRT)
- Bioanalytics
- Shelf-life Extension Program (SLEP)

Formulation Science

- Oral solids (e.g., tablets, capsules)
- Topicals and transdermal
- Ophthalmic
- Injectables (e.g., liposomes, lipid-nanoparticles, suspensions, emulsions, long-acting)
- Implantable (e.g., intravaginal, intrauterine, intramuscular)
- Biopharmaceutics (e.g., IVIVC, BCS, biowaivers, bioequivalence)
- Nanotechnology
- All other complex

Adv. Manufacturing

- Continuous manufacturing (drug substances, solid oral dosage forms, complex formulations)
- 3D printing
- Process analytical technology (PAT)
- Biomanufacturing (e.g., upstream/downstream processing, lyophilization)

Modeling & Simulation

- Digital twins
- In vitro in vivo correlation (IVIVC)
- Modeling, e.g., CFD, MD, DEM, RTD
- System/Process design (e.g., LabVIEW)
- Data science, e.g., AI/ML, chemometrics

Thank You!