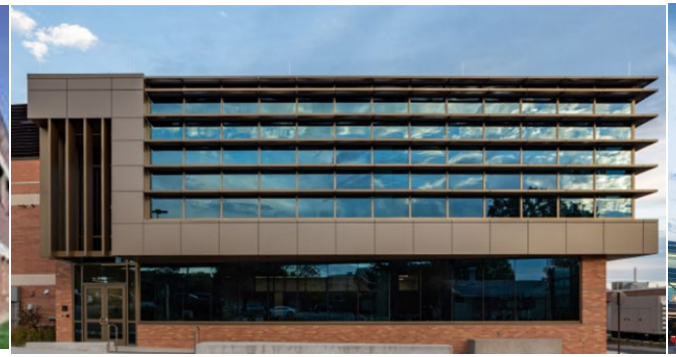
KU Biopharmaceutical Innovation & Optimization Center (BIOC)

~~ AND ~~

Physical Pharmacy & Drug Delivery (P_2D_2) Research Labs

McCollum Laboratories (Office 1188)

Simons Laboratories (Office 274; Labs 275)



KU Medical Center Labs

KU BIOPHARMACEUTICAL INNOVATION & OPTIMIZATION CENTER
The University of Kansas

P_2D_2 Labs
School of Pharmacy

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

Presentation to 2024 Drug Delivery Summit

Cyclodextrin-Facilitated Drug Delivery: Modulating Gelation of Peptides and Improving Dissolution of ASDs

Michael J. Hageman

Valentino J. Stella Distinguished Professor

Department of Pharmaceutical Chemistry

Director of the Biopharmaceutical Innovation & Optimization Center

Kansas University Center for Research

16 September 2024 at 3:40 PM in Room 1

KU | KU School of Pharmacy Pharmaceutical Chemistry

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Rapidly Changing Landscape and Challenges In Drug Delivery with New Molecular Modalities

- **The Modalities**
  - Evolving Chemical Space in Discovery – ***"Toto, I've a feeling we're not in Kansas anymore.***
  - Evolving Solubilization Technologies
- **Integrating Pharmaceutics into Decisions for Molecule Progression**
  - Pharmaceutics Enabling the Discovery Process with Line-of-Sight Strategies
  - Expanding solubilization strategy solutions through coupling with cyclodextrins
- **Expanding Cyclodextrin Technologies to De-risk Progression**
  - Modulating cyclic peptide aggregation for SC delivery
  - Expanding the limits of amorphous solid dispersions (ASD)
    - Physical form stabilization (in solid phase, during dissolution)
    - Facilitating supersaturation & preventing colloidal phase separation
  - Enabling supersaturation upon dilution at site of drug delivery
- **Acknowledgements**
  - KU – Negar Jafari, Indeewara Munasinghe, Kyle Gross, Jack Rider, Josephine Banks, Hao Lou
  - Ligand Pharmaceuticals – Lian Rajewski, Jo Krise, JD Pipkin
  - Funding by Val Stella Endowment Fund & by Ligand Pharmaceuticals LIGAND

Definitions of "Drug-Like" are Evolving with Advent of New Drug Modalities

Lipinski rule of 5

- Poor absorption and permeation are likely when
  - H-bond donors > 5 … MW > 500
  - logP > 5 … d Acceptors > 10

Veber Rules

- Good o… ailability in rat
  - Rot… onds ≤ 10
  - Polar Surface Area(PSA) ≤ 14… 12 H-bonds (acceptors+donors)

Pardridge Rules

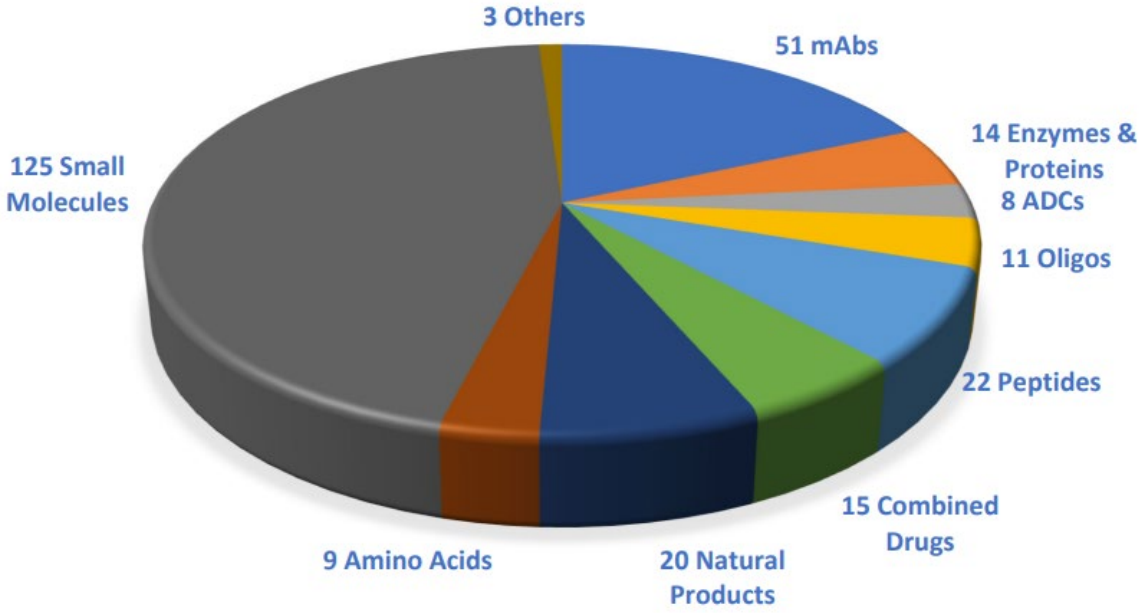
- Good probability of p… g the blood-brain barrier(BBB) when
  - H-bonds (accept… rs) ≤8-10
  - MW < 400-500 ar… idic

Spraklin

- Further states that … l H-bonds for BBB permeation
  - H-bond donors < … bond acceptors < 6

Clark & Lobell

- For good BBB barrier permeability
  - MW < 450 logD = 1-3
  - #N + #O < 6 clogP – (#N-#O) > 0
  - Polar Surface Area (PSA) < 60-70 Å^2

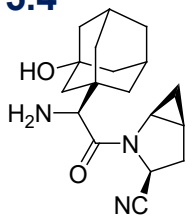


278 new drugs approved by FDA from 2016-2021

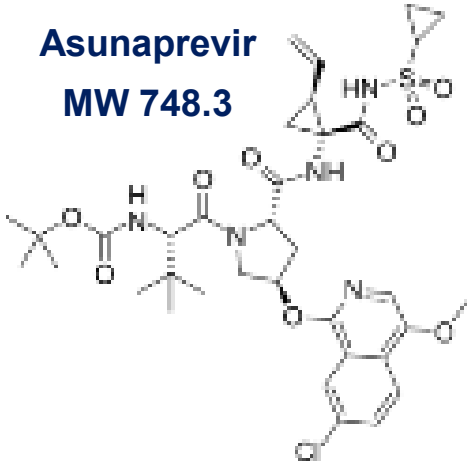
Shaer, Musaimi, Albericio, de la Torre, *Pharmaceuticals* 2022, *15*(2), 222; https://doi.org/10.3390/ph15020222

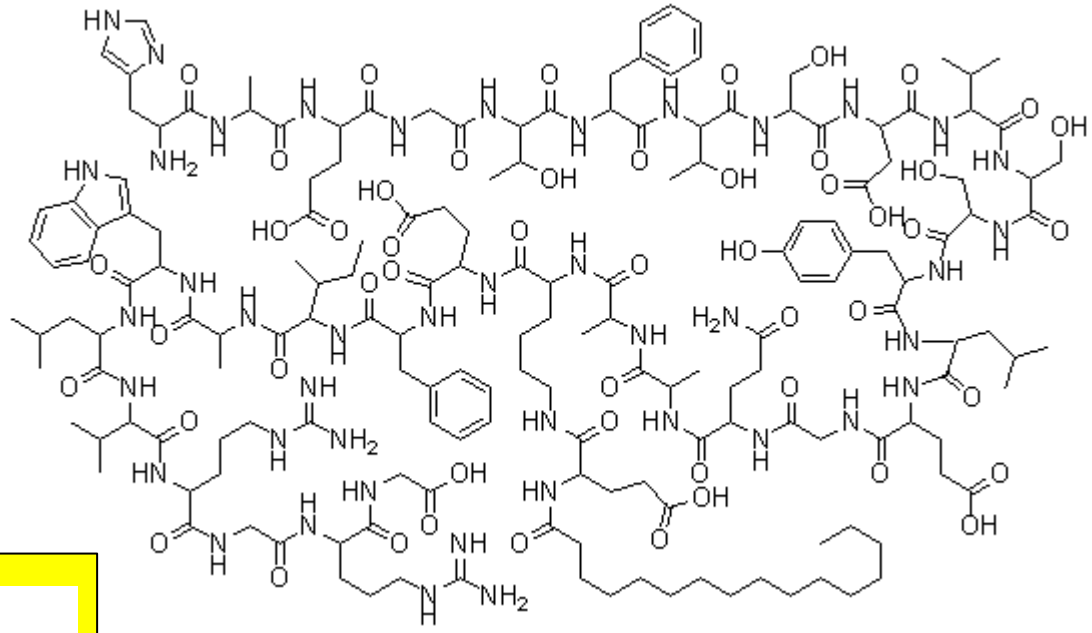
Evolving Chemical Space -- the Present

Saxagliptin
315.4



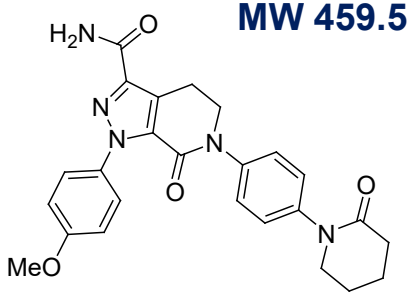
Asunaprevir
MW 748.3



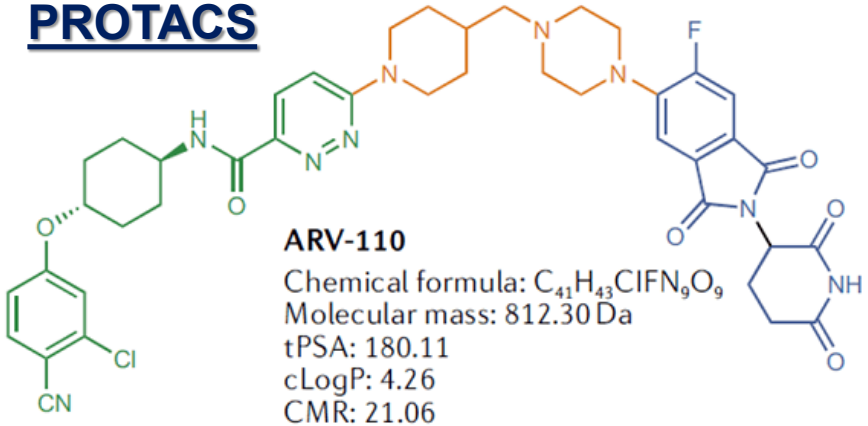


GLP1 Analogs
Liraglutide
MW 3751

Apixaban
MW 459.5

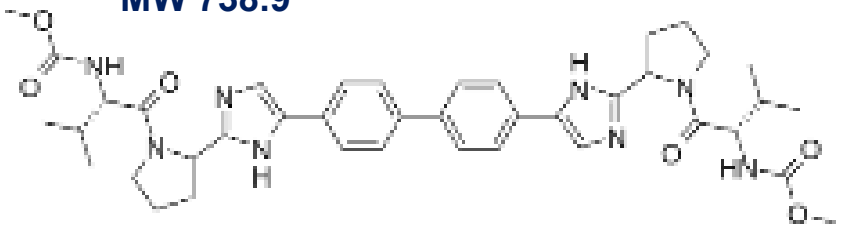


PROTACS



ARV-110
Chemical formula: $C_{41}H_{43}ClFN_9O_9$
Molecular mass: 812.30 Da
tPSA: 180.11
cLogP: 4.26
CMR: 21.06
LogS: −9.434
pK_a: 7.85

Daclatasvir
MW 738.9



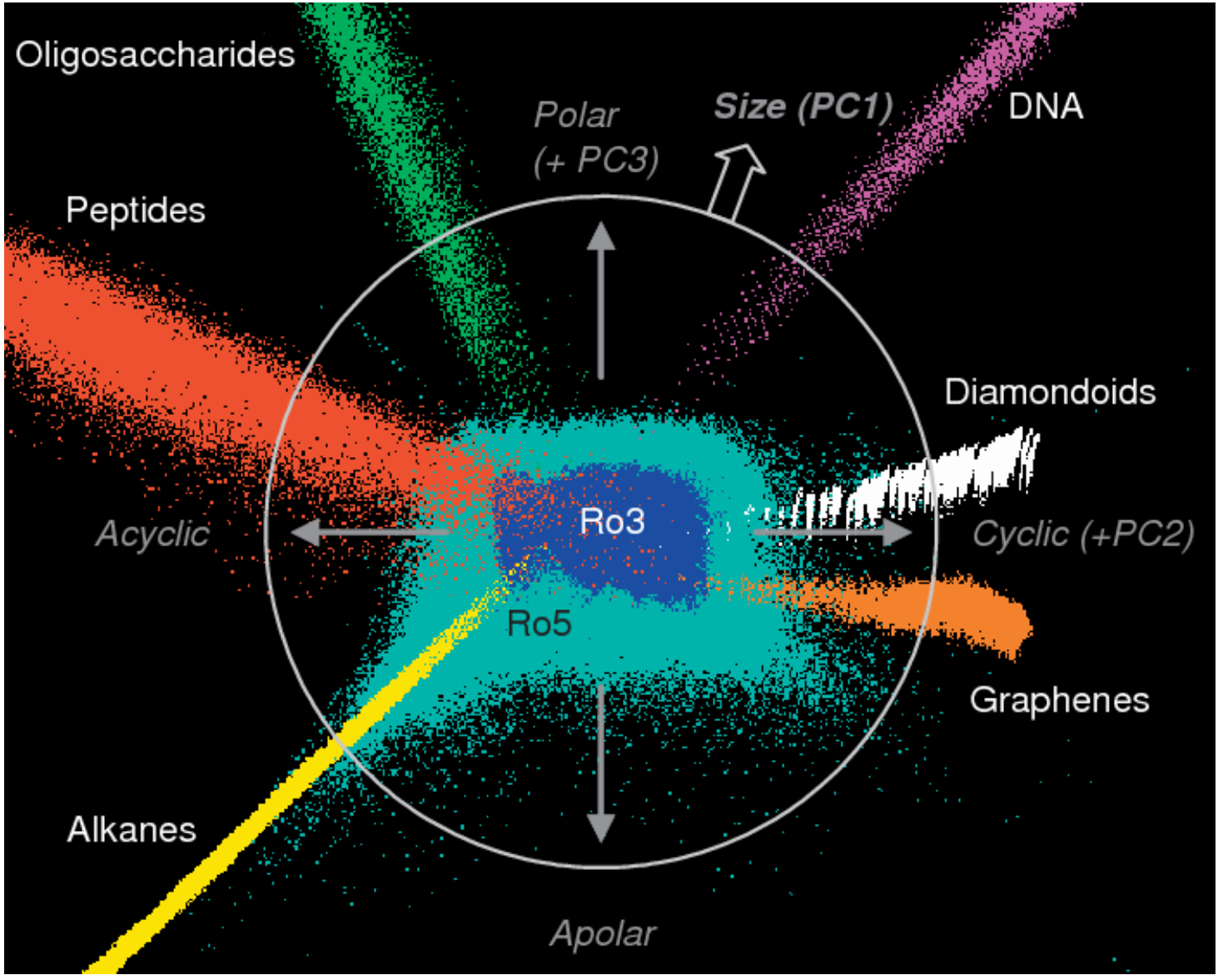
Preparing for The Future
- A Role for Cyclodextrins? -

DOI:10.2533/chimia.2011.863

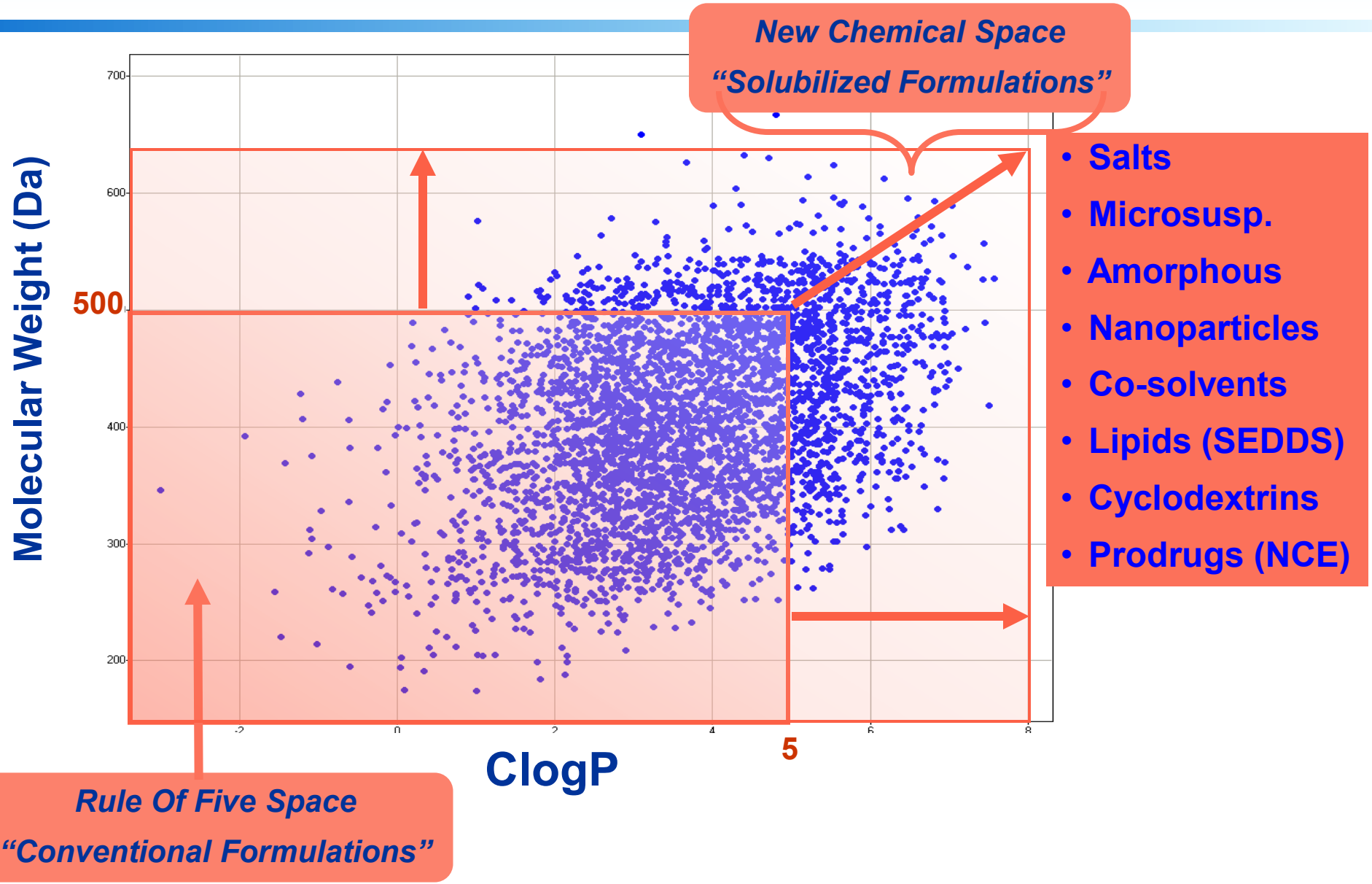
Exploring the chemical space of known and unknown organic small molecules at www.gdb.unibe.ch.

J. Reymond, Lorenz C. Blum, Ruud Van Deursen

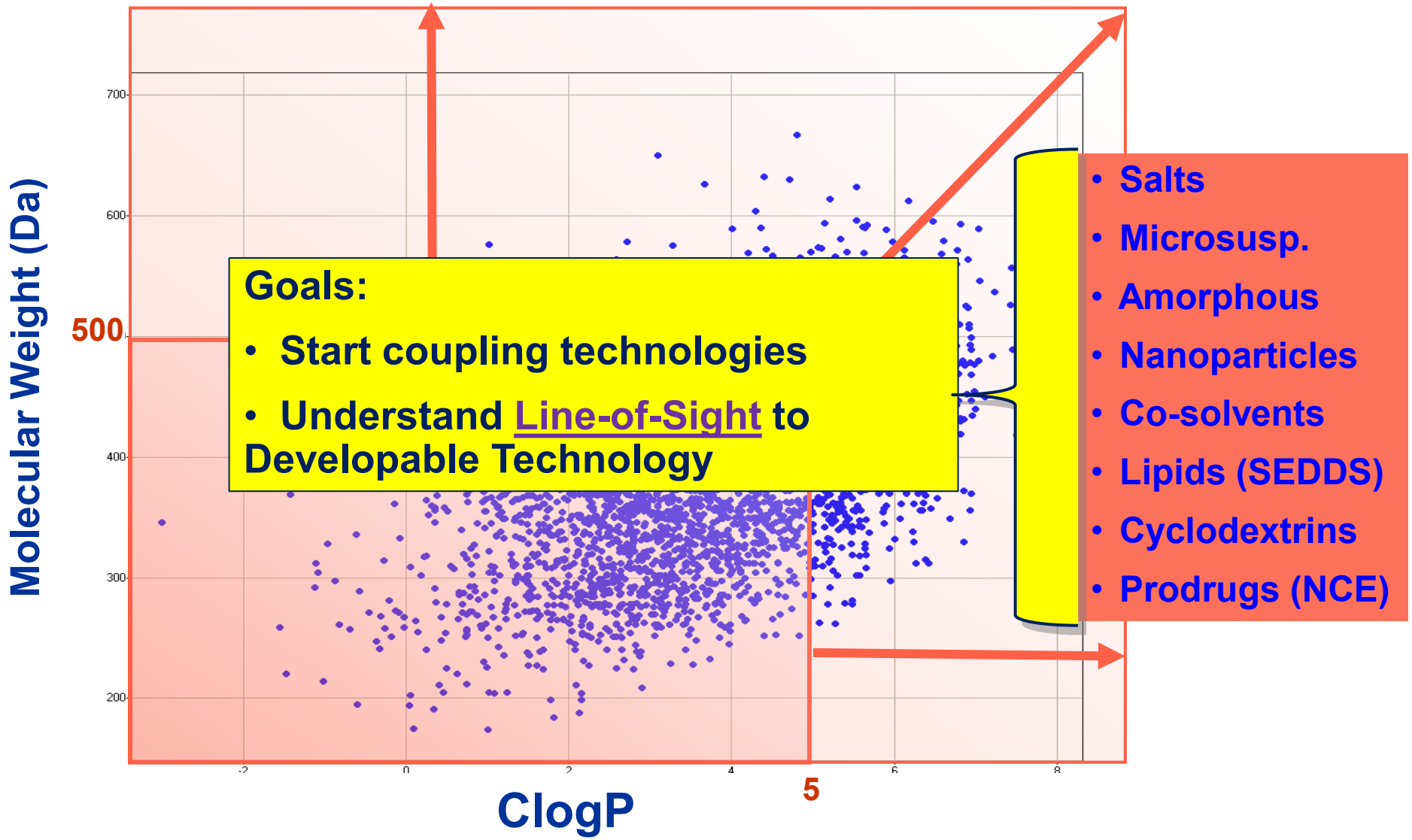
•Published in Chimia (Basel) 2011



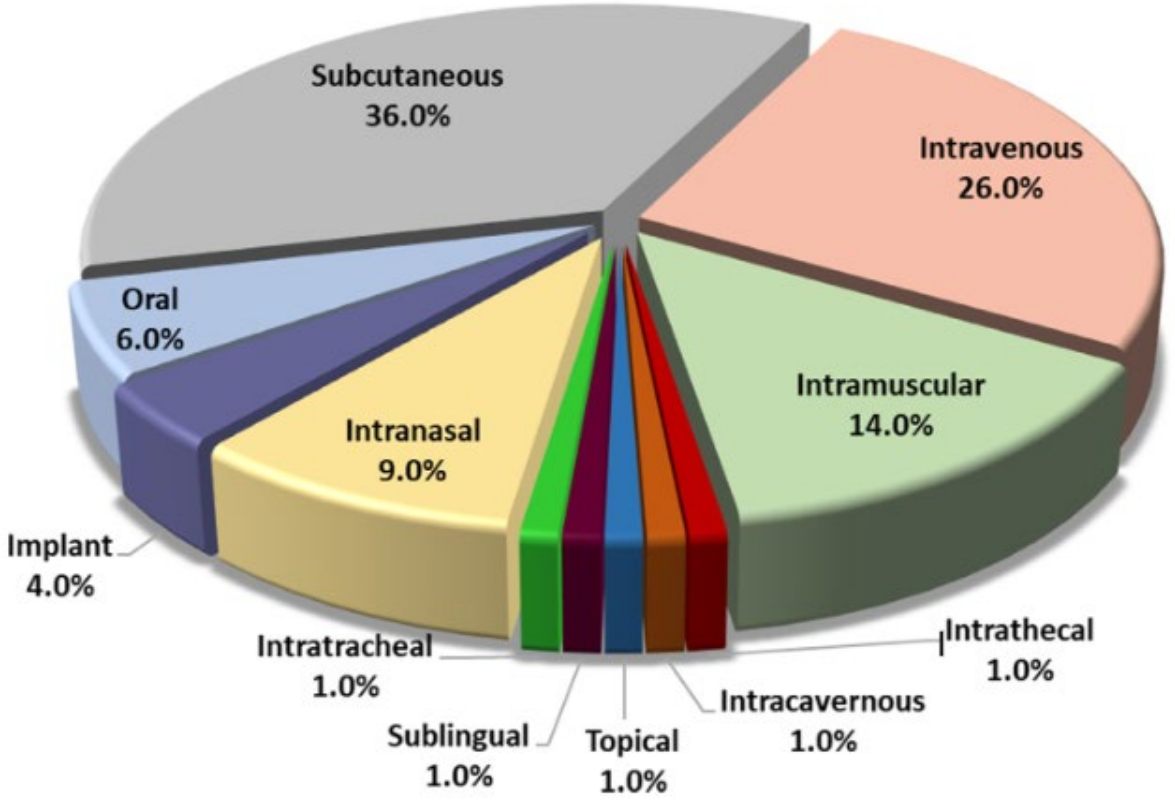
Typical Use of Formulation Technology to Expand Accessible Small Molecule Chemistry Space For Oral Delivery



Expanding Accessible Small Molecule Chemistry Space with Cyclodextrin-Enhanced Technologies



Parenteral Routes are Favored for Peptide Administration



- ✓ Do not pass through the GI tract
- ✓ Protect peptide from degradation by pH changes and enzymatic digestion process
- ✓ Maintain improved bioavailability and efficacy
- ✓ Avoid limitation of biological membrane permeations

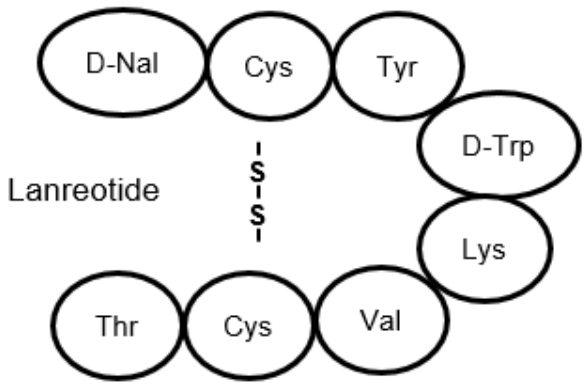
Zhang Y, Zhang H, Ghosh, D, Williams R 3rd, Int J Pharm. 2020 Sep 25:587:119491

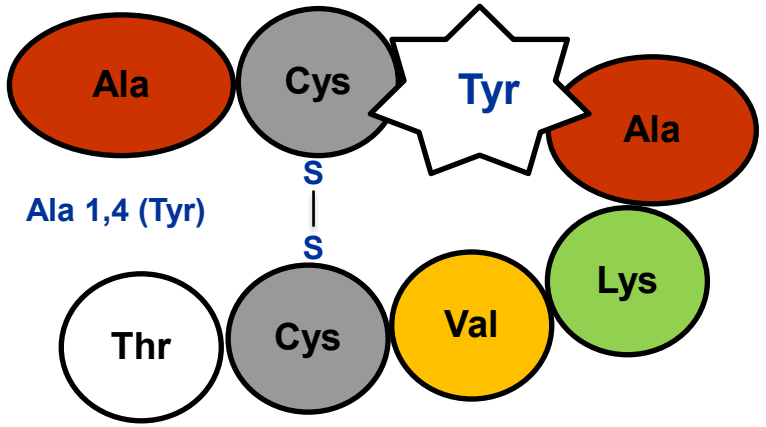
Why Cyclodextrins Might Help Formulation & Delivery of Peptides

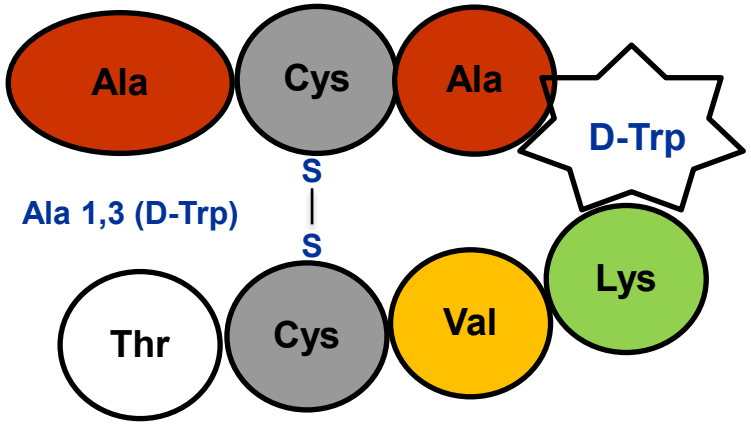
Cyclodextrin complexation with amino acid functional groups to modulate physicochemical properties.

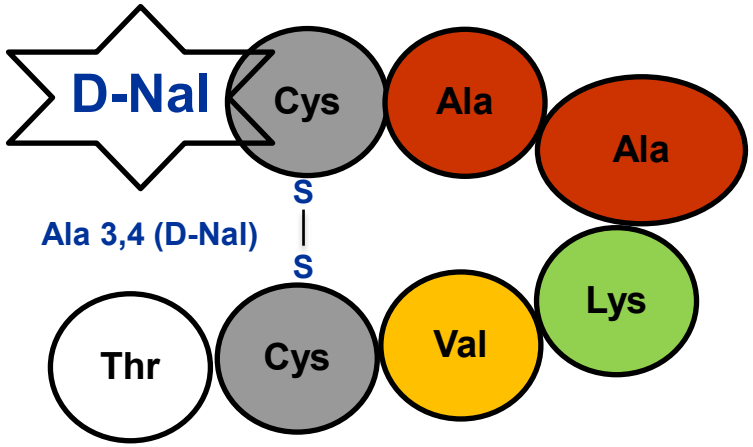
- Enhancing solubility of peptides, minimizing the impact of counter-ions and ionic strength?
- Providing an alternative equilibrium to prevent irreversible aggregation and to modulate peptide self-assembly or gelation?
- Minimize electrostatic peptide interactions with hyaluronic acid at subcutaneous site or bile acid mixed micelles orally? Facilitate lymphatic uptake of complex, especially higher order complexes?
- Modulate enzymatic liability of peptides either subcutaneously or orally?

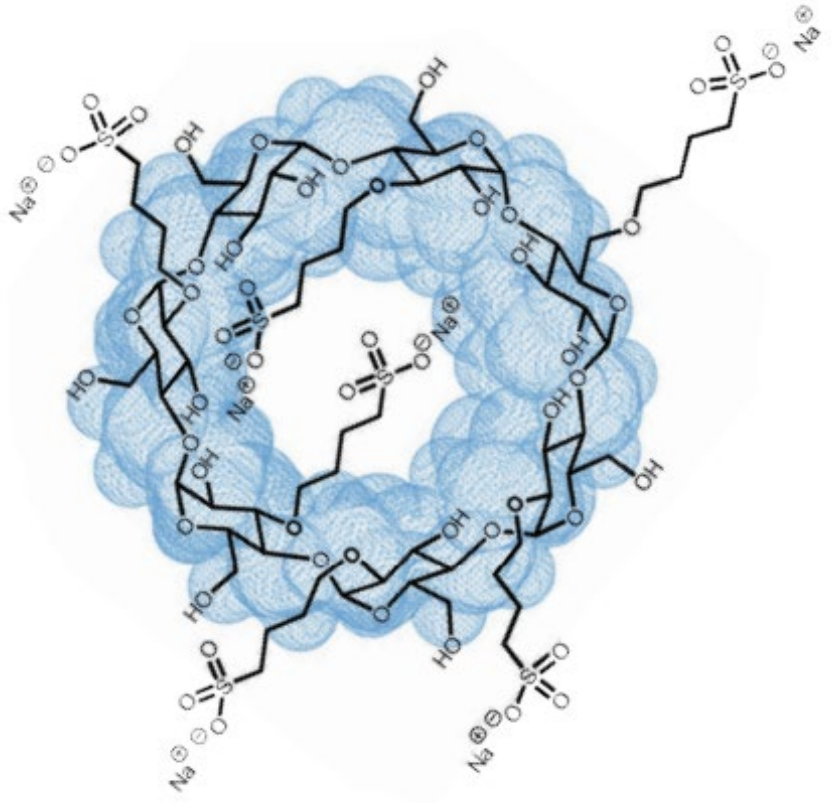
Alanine Analogs of Lanreotide used to Study the Side Chain Interactions with SBEβCD









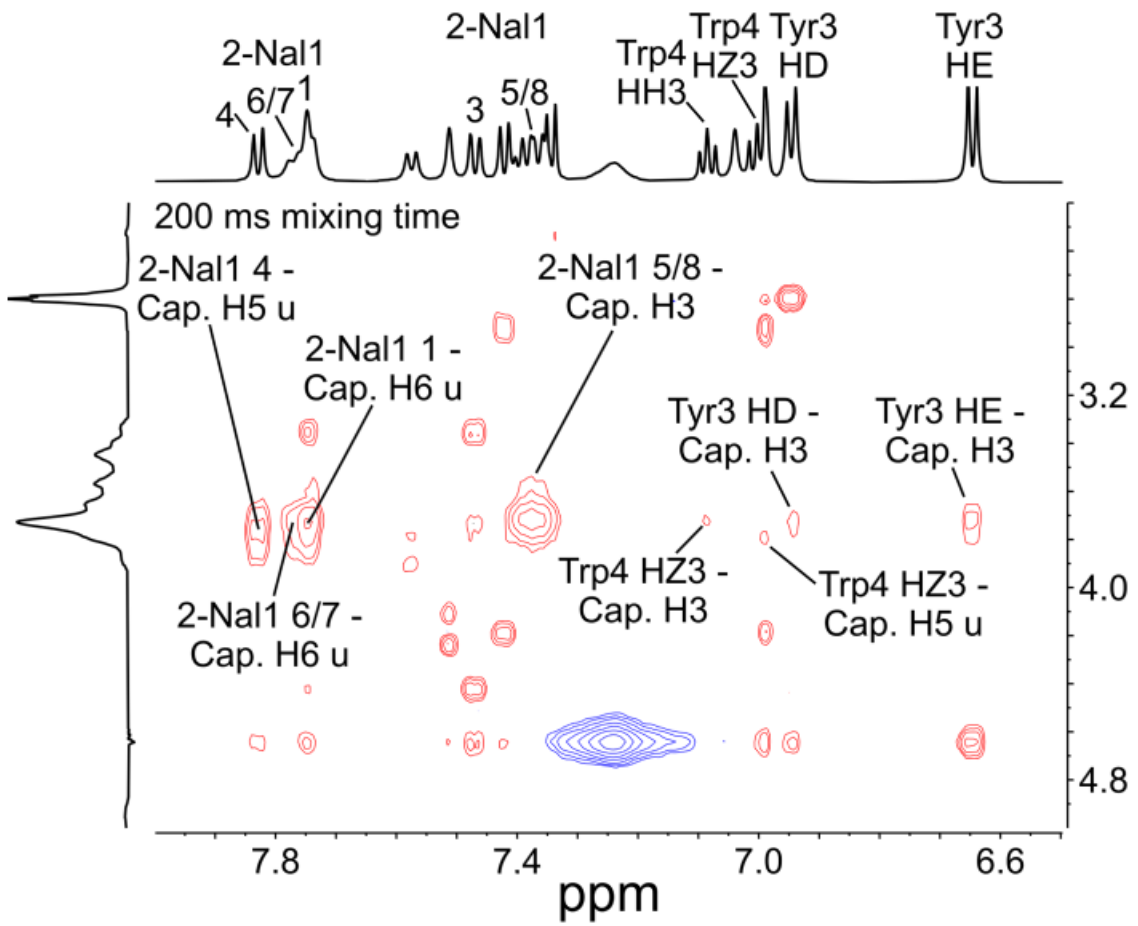


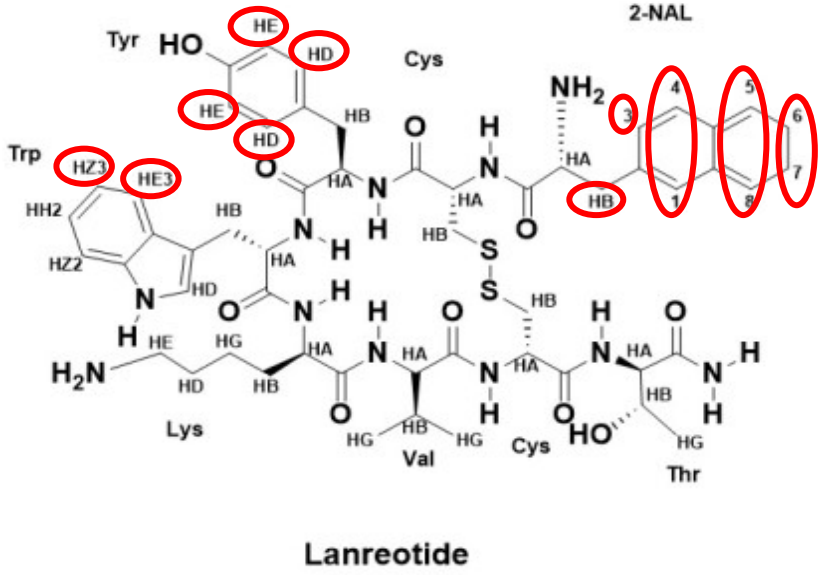
SBEβCD

CAPTISOL®

A LIGAND TECHNOLOGY

ROESY NMR of Lanreotide : SBEβCD

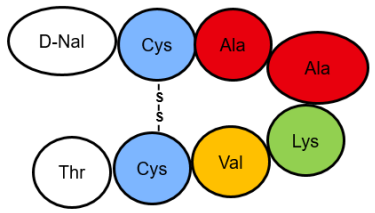




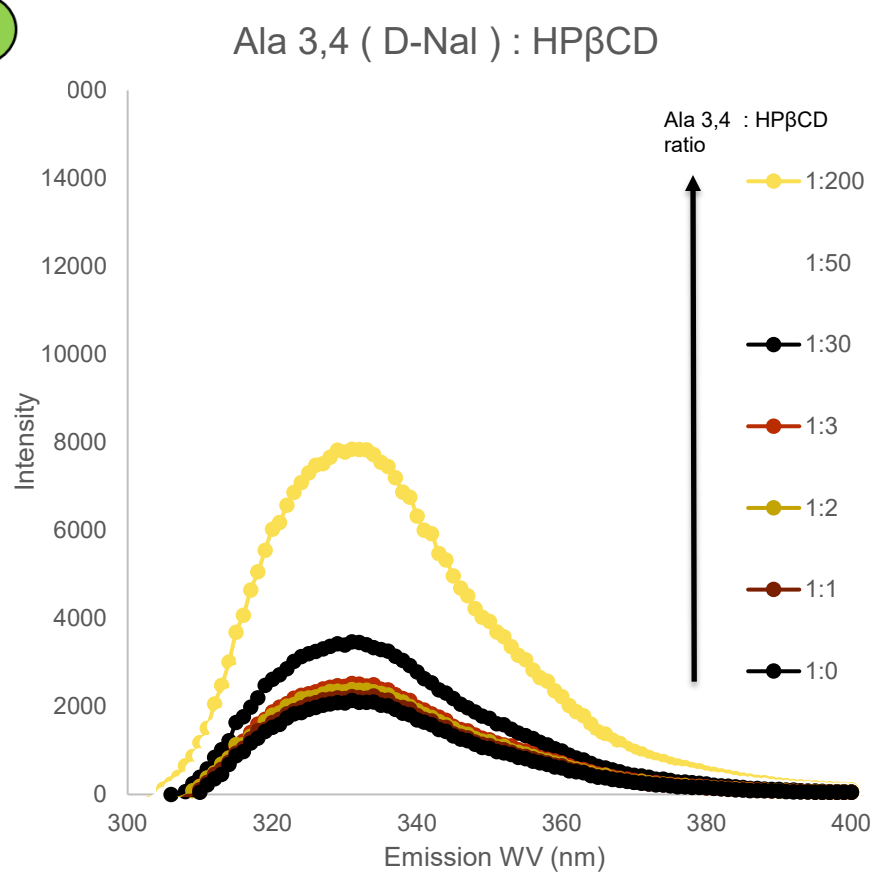
Lanreotide

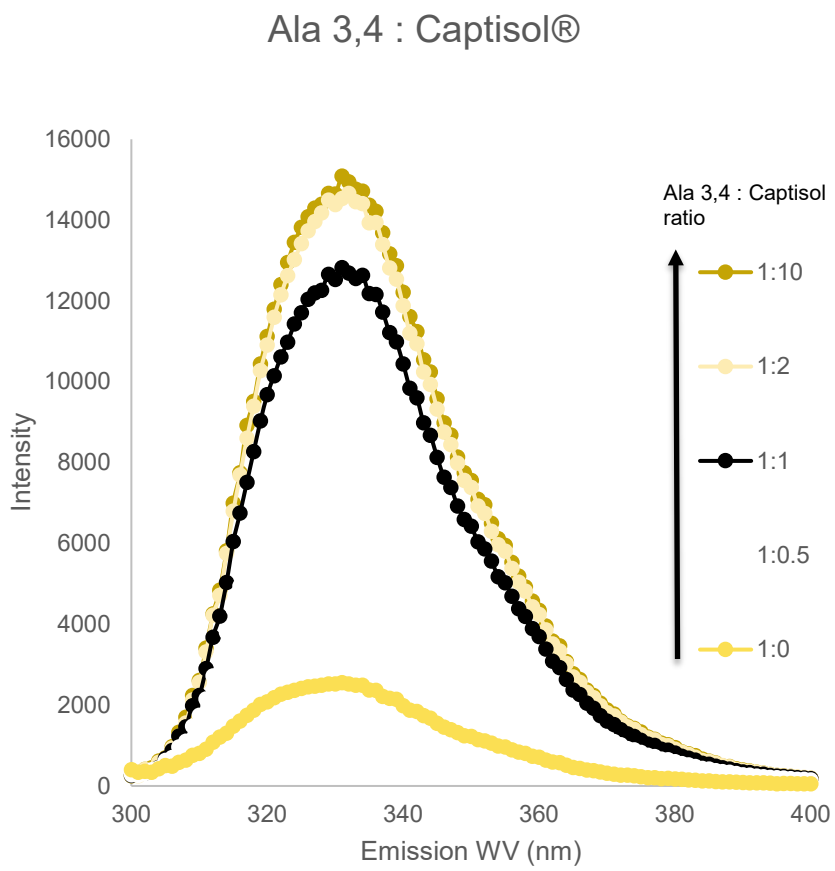
- The protons of aromatic side chains of lanreotide shows cross peaks with protons of SBEβCD reveals the complexation of Tyr, D-Trp and D-2NAL with SBEβCD .

Example of Fluorscence Changes with β-Cyclodextrin Complexation with Lanreotide Analog



Ala 3,4 (D-Nal)

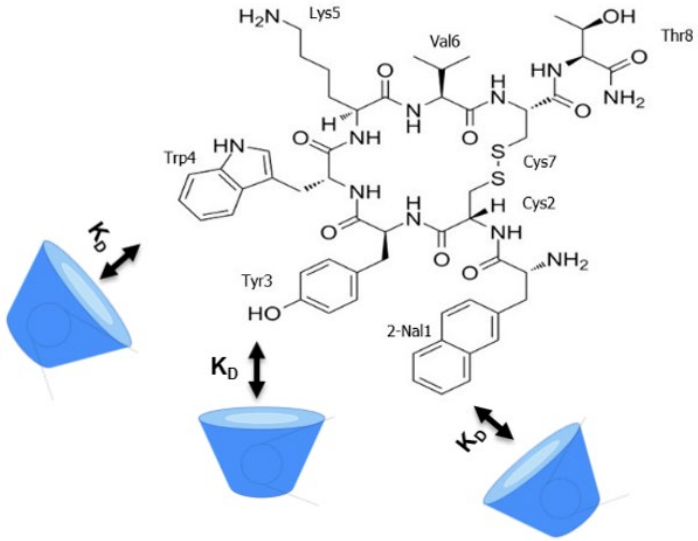




Summary of β-Cyclodextrin Complexation with Lanreotide

- DOSY NMR showed decrease in diffusion coefficient & increase in hydrodynamic radius, consistent with the complexation. ROESY NMR showed cross peaks of the cyclodextrin hydrogens with all three aromatic side chains indicating the potential complexation on more than one side chain, but not necessarily simultaneously.
- Fluorescence showed an increase in intensity and blue shift upon complexation. The binding constant of Captisol® to alanine analogs are 30-300 times greater than HPβCD. The D-Nal has the highest binding constant with HPβCD of the other 2 aromatic side chains. Whereas Captisol® binds slightly more tightly to Tyrosine. The binding constant of Captisol® to Lanreotide was 30-300 times greater than HPβCD.

Binding constant of three analogs with Cyclodextrins n=5		
Alanine substituent	**HPβCD (M^{-1})**	**Captisol®(SBEβCD) (M^{-1})**
Ala 3,4 (**2- Nal**)	**61.7 ± 20.7**	1833.3 ± 830
Ala 1,3 (**Trp**)	12.6 ± 4.2	2180 ± 800
Ala 1,4 (**Tyr**)	10.4 ± 3.7	**3583 ± 510**



Jafari N, Douglas J, Neuenswander A, Kelich P, Hageman M., β -Cyclodextrins Bind Aromatic Side Chains of Cyclic Peptides: A Fluorescence, Molecular Docking and NMR Study, submitted to J Pharm Sci Sept 2024

Ways to Study the SC Route

Open the Blackbox?
No / Yes

Not Study Mechanism

<u>Approach 1:</u>
Machine Learning: mAb SC Bioavailability Prediction

- Lou H, Hageman MJ. Pharm Res. 2021;38(3).

Study Mechanism

Diffusion

<u>Approach 2:</u>
Protein Diffusion in SC Extracellular Matrix (ECM)

- Lou H, Hageman MJ. Anal Methods. 2022;14.

Multiple Mechanisms in an Integrated System

In-Vitro Models to Simulate SC Site

<u>Approach 3:</u>
Evaluate/Apply a Commercial Model:
SCISSOR® (SubCutaneous Injection Site Simulator)

- Lou H, Berkland C, Hageman MJ. Int J Pharm. 2021;605.

<u>Approach 4: Develop a New Model:</u>
ESCAR (Emulator of SubCutaneous Absorption and Release)

1. Design & Fabrication
2. Applications
3. Intellectual Property (IP) & Commercialization

- Lou H, Hageman MJ. Mol Pharm 2022; 19(11)
- Lou H, Hageman MJ. AAPS J. 2023; 25(23)

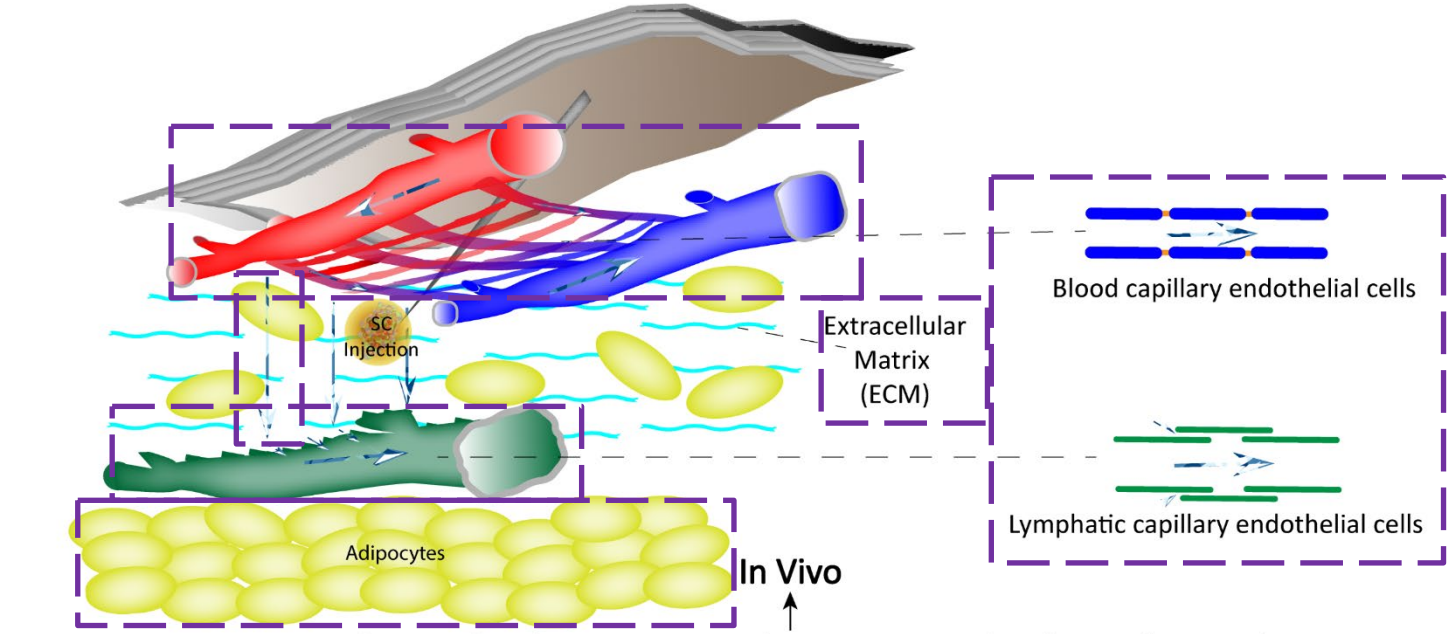
ESCAR Applications

- Molecule Screening

Small molecule: Griseofulvin & APAP
Peptide: Lanreotide Protein: BSA

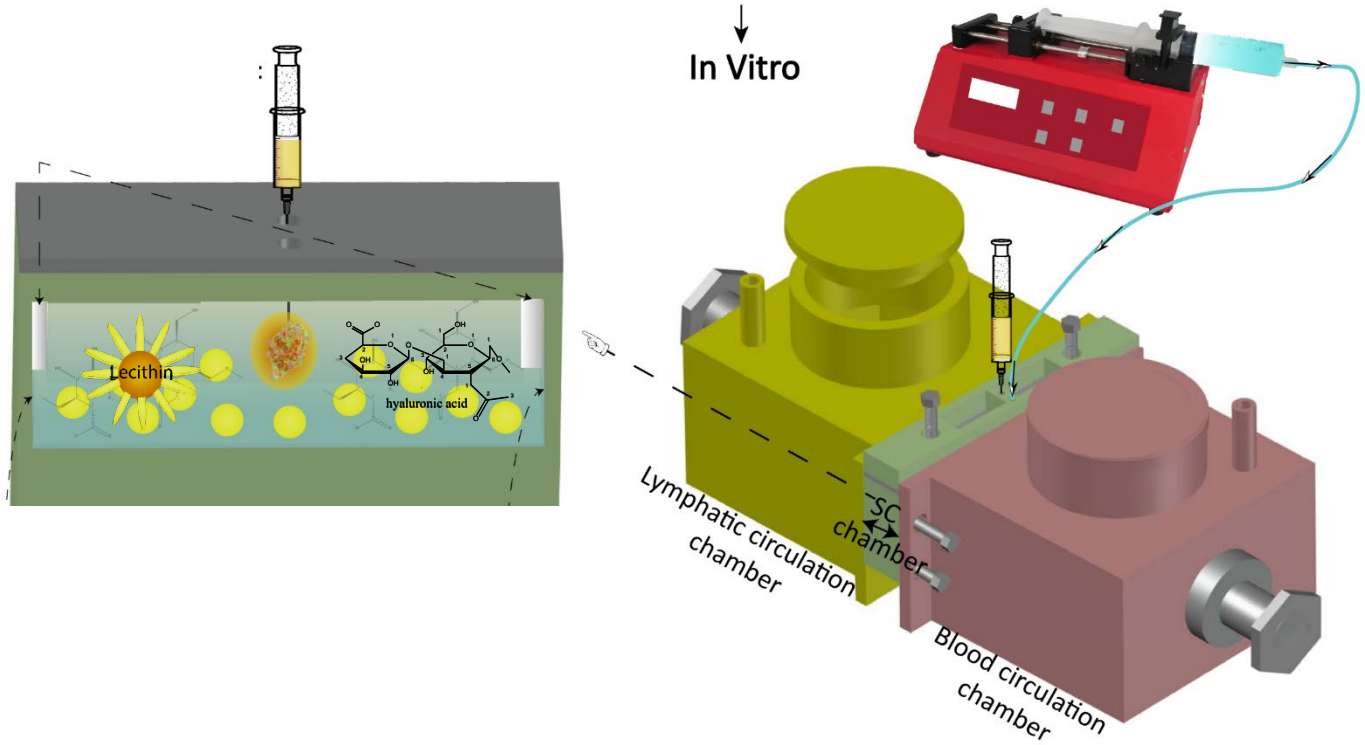
- Formulation Screening

Solution, Suspension, Complex, Emulsion, Gel, Nanoparticle, Microparticle, Depot, etc.



Emulator of SubCutaneous Absorption and Release (ESCAR)

In Vitro



ESCAR Design

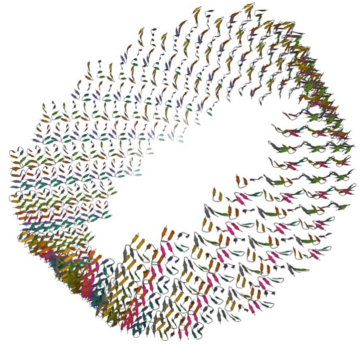
- Extracellular Matrix (hyaluronic acid)
- Fat Tissue/Lipids
- Drug Uptake
- Tight Junctions
- Convection/Liquid Flow
- pH & Temperature & Ionic Strength

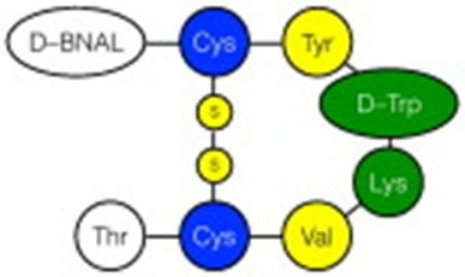
Development of an In Vitro System To Emulate an In Vivo Subcutaneous Environment: Small Molecule Drug Assessment. Lou H, Hageman MJ. Mol Pharm. 2022 Nov 7;19(11):4017-4025.

ESCAR Applications to Peptides & Proteins

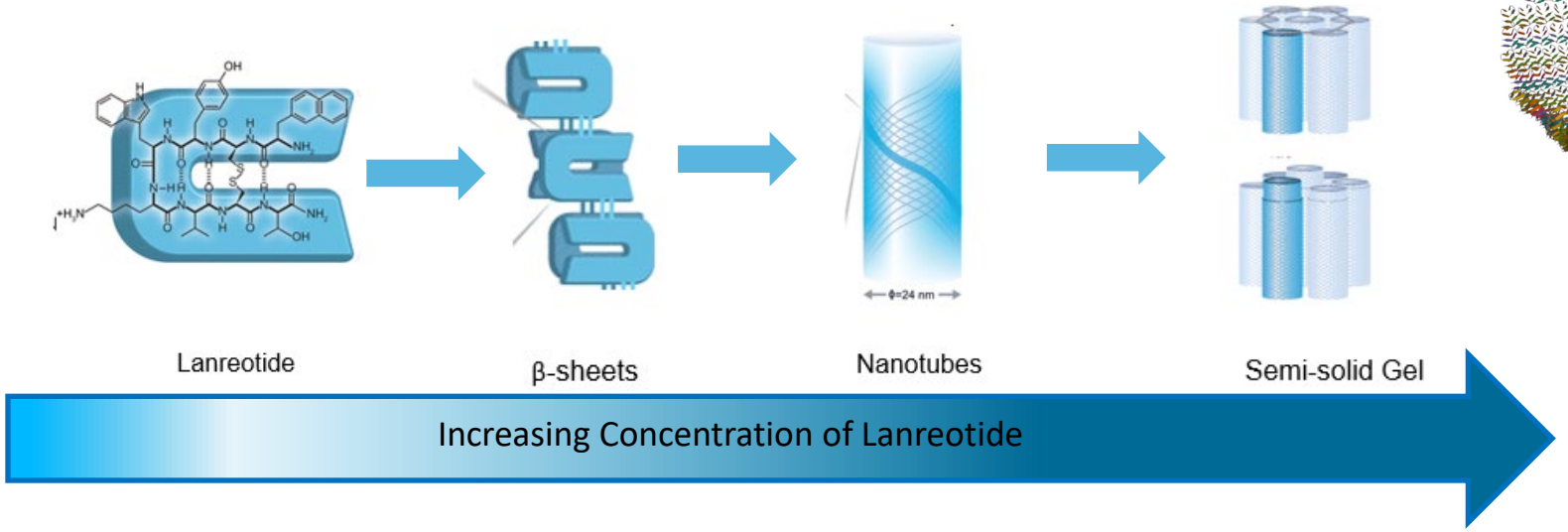
Can ESCAR be used to study peptide aggregation in SC Space?

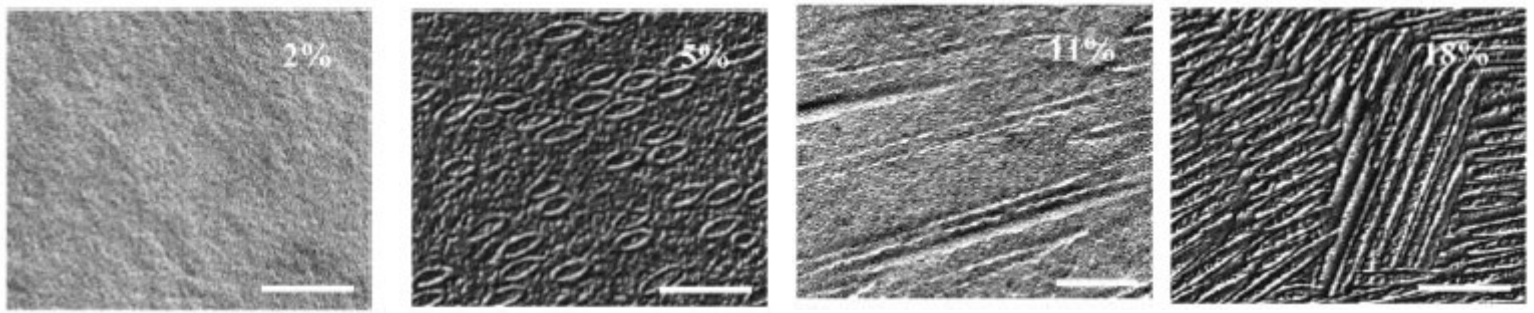
Lanreotide (3% w/v) aggregates in water.





Lanreotide

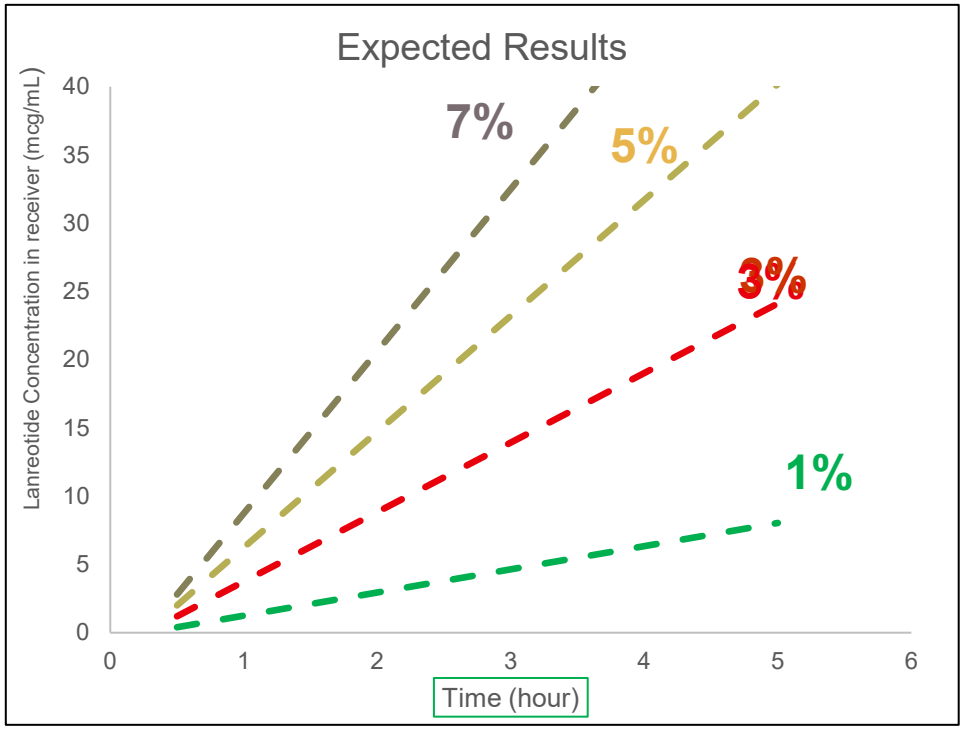


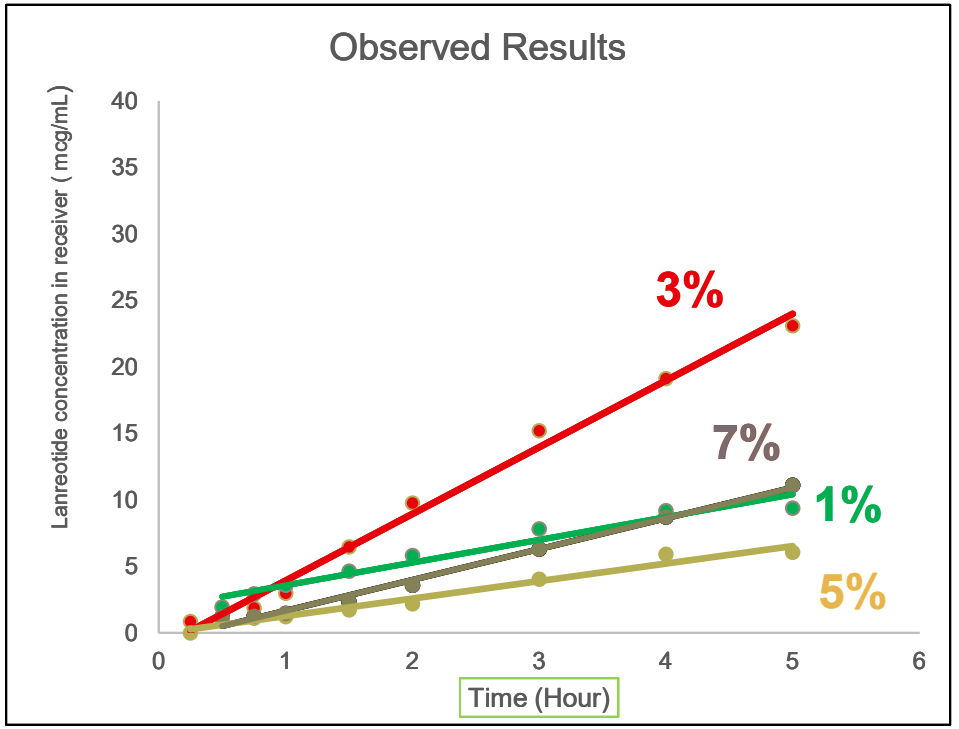


Wolin, E. M., A. Menon, (2016). Journal of Gastrointestinal Cancer **47(4): 366-374.**

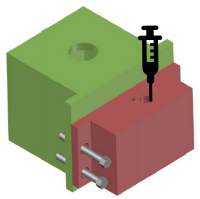
Valéry, C., F (2004). Biophysical Journal **86(4): 2484-2501.**

ESCAR Flux as a Function of Lanreotide Concentration

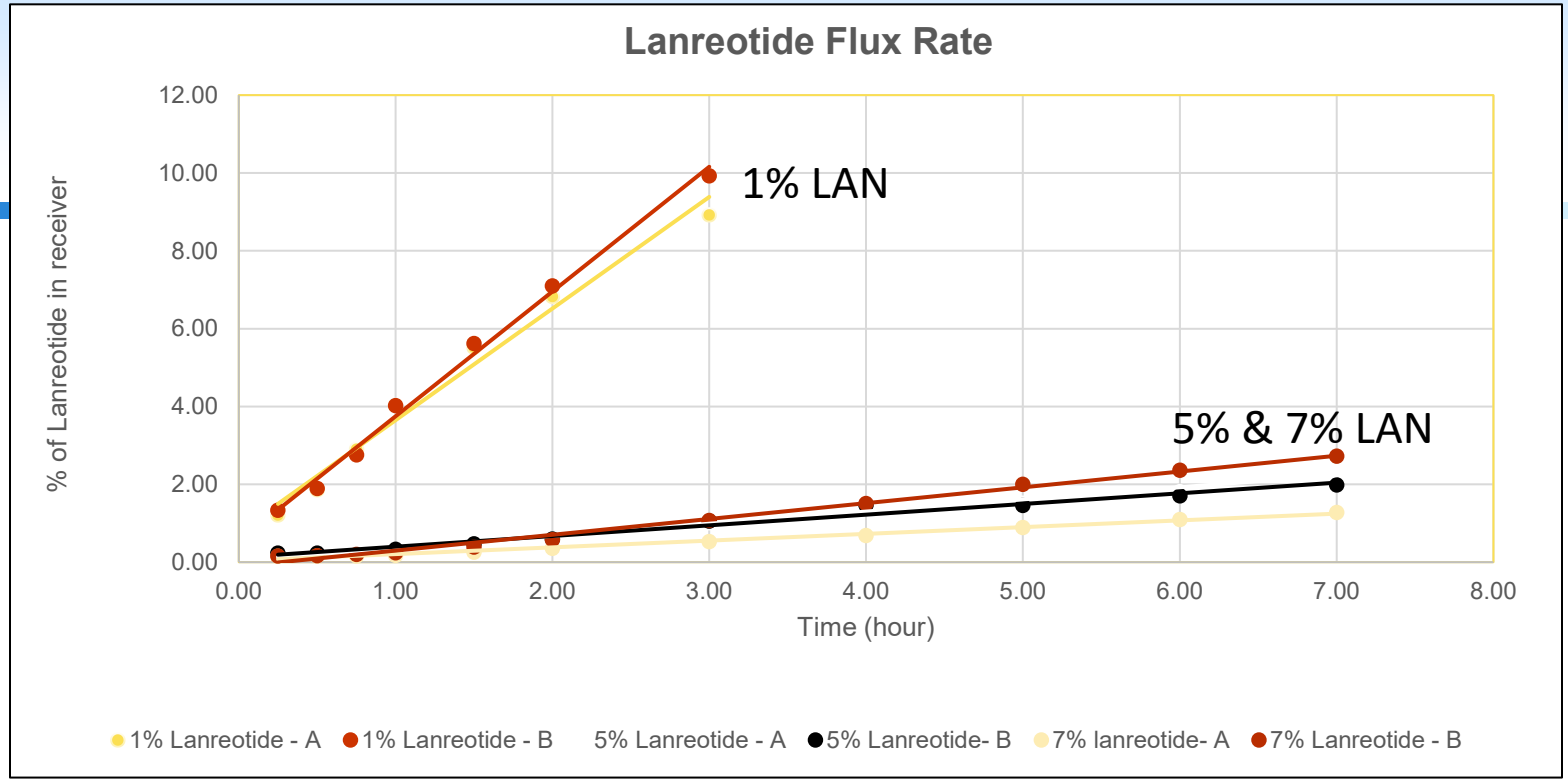




Temperature 37 ℃ , 50 kDa MWCO , Hyaluronic acid 5% , PBS pH 7.4



Obvious reduction in absolute amount (flux) when concentration exceeds 3% and reversible gelation plays a role

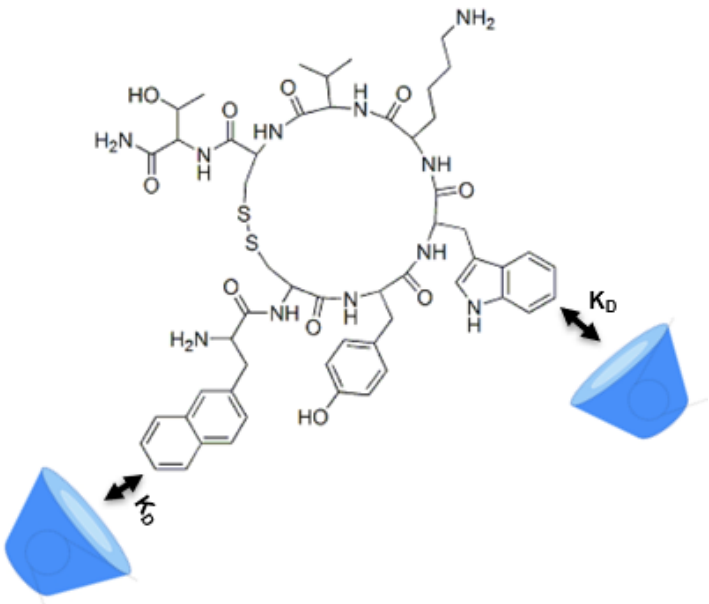


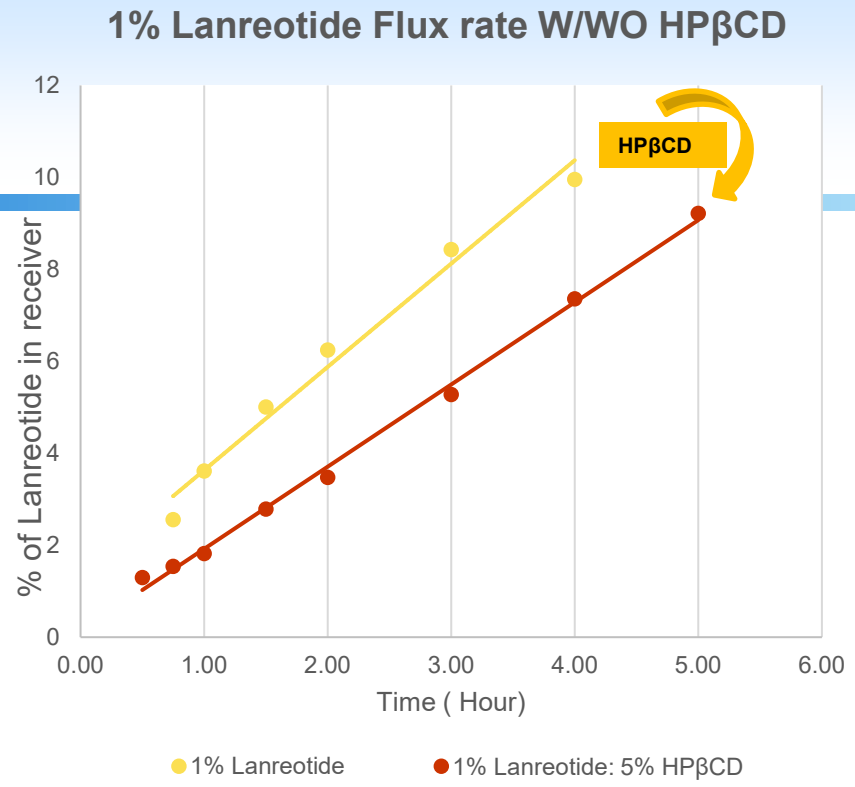
Same Injection Volume / Different concentration / Different Dose		
Flux Rate (µg /hr.cm^2)		
1 % Lanreotide (80 µg dose)	5% Lanreotide (400 µg dose)	7% Lanreotide (560 µg dose)
75.90	7.32	7.24

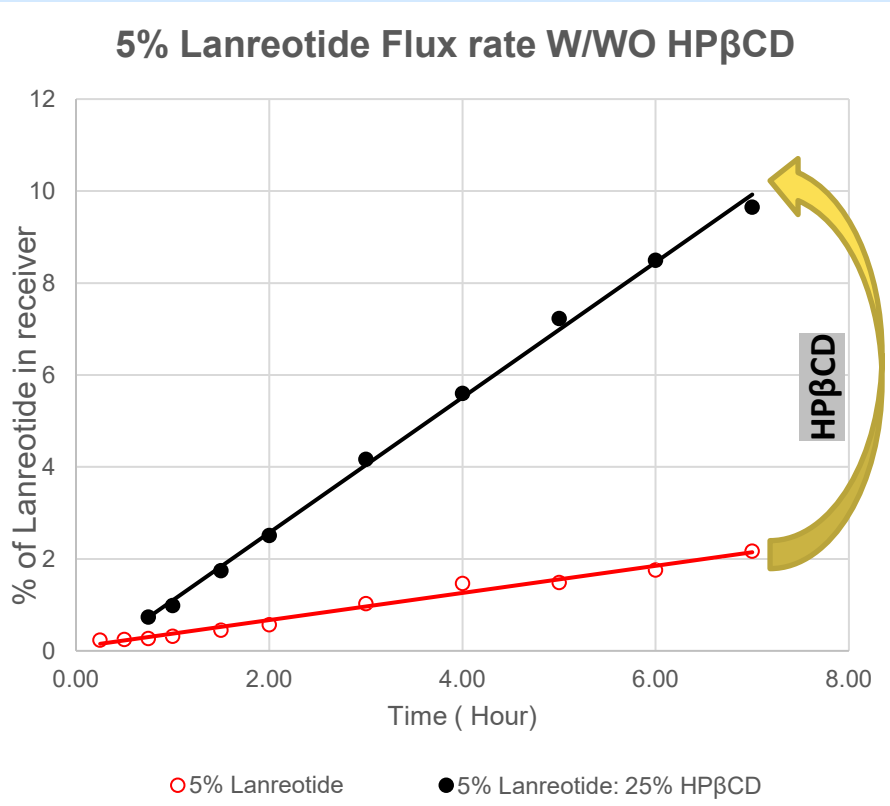
Temperature 34˚C , 50 kDa MWCO , HLA 5% , PBS pH 7.4

Jafari N, Addison C, Lou H, Hageman M. **Hydroxypropyl β Cyclodextrins Effects on Self-Assembly of Cyclic Peptide, Lanreotide Acetate, in Water and Subsequent Release rate from an in vitro Emulator of Subcutaneous Delivery,** submitted to J Pharm Sci Sept 2024

- LAN binding with HPβCD to inhibit aggregation



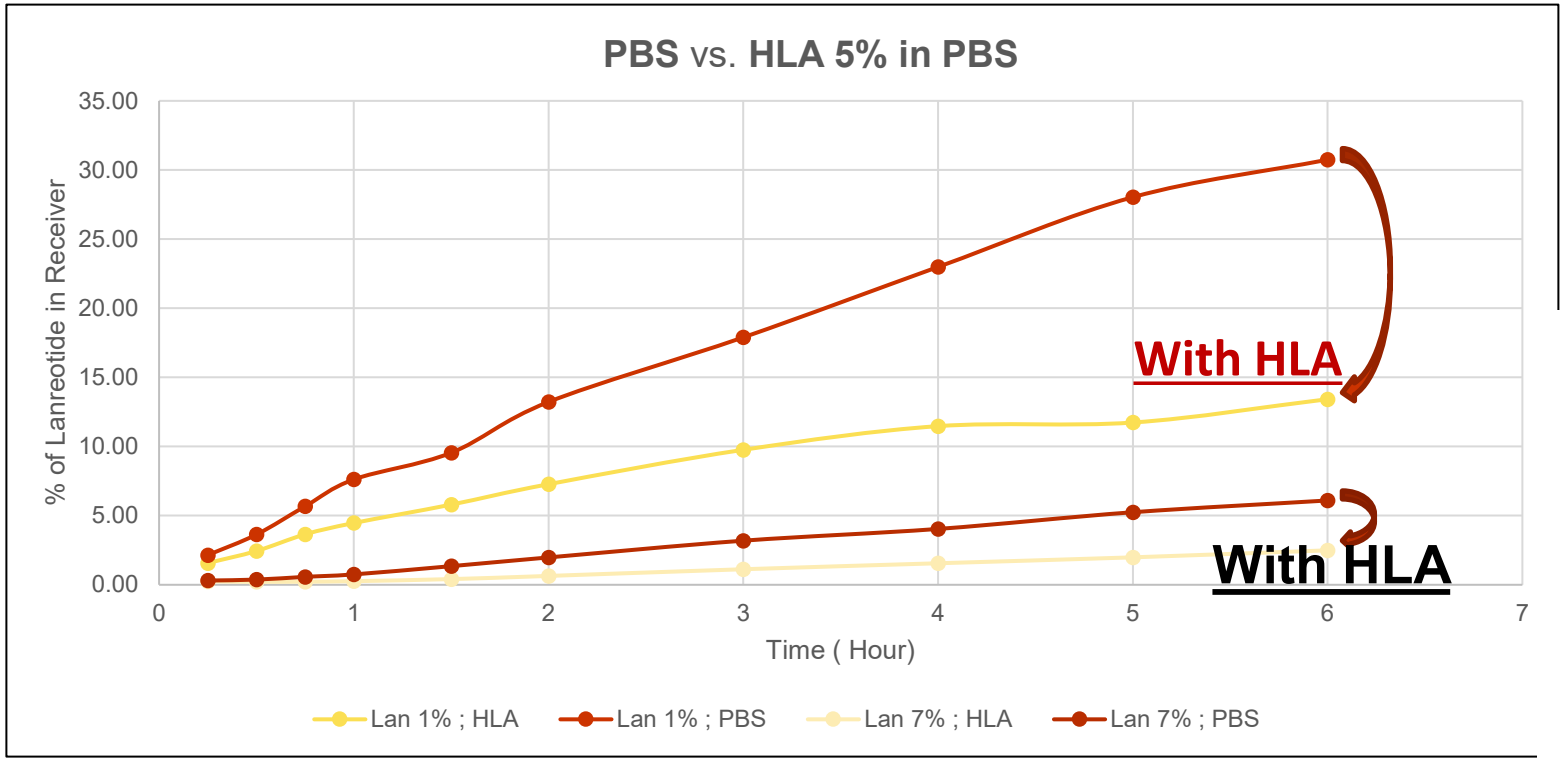




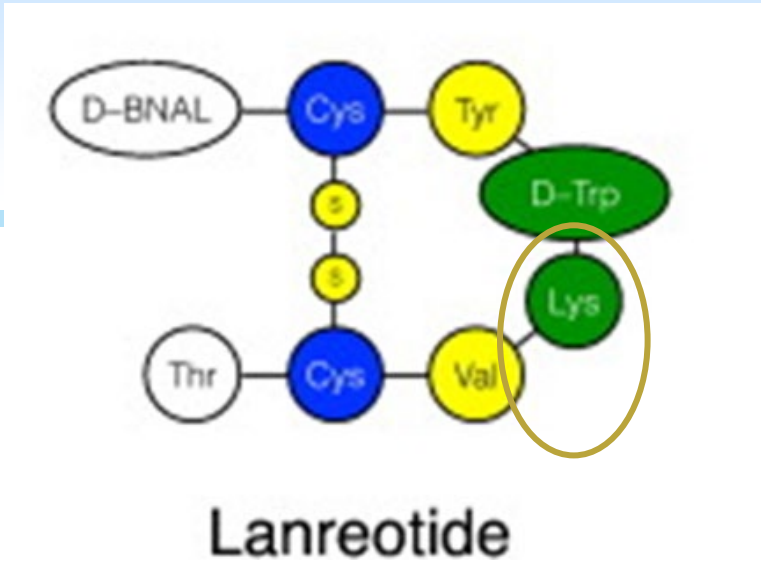
Flux Rate (µg /hr.cm^2) , Ratio of 1:5 (LAN:HPβCD)			
1% Lanreotide	1% Lanreotide:5% HPβCD	5% Lanreotide	5% Lanreotide:25%HPβCD
56.17	44.7	7.375	36.775

Temperature 34 ̊C , 50 kDa MWCO , HLA 5% , PBS pH 7.4

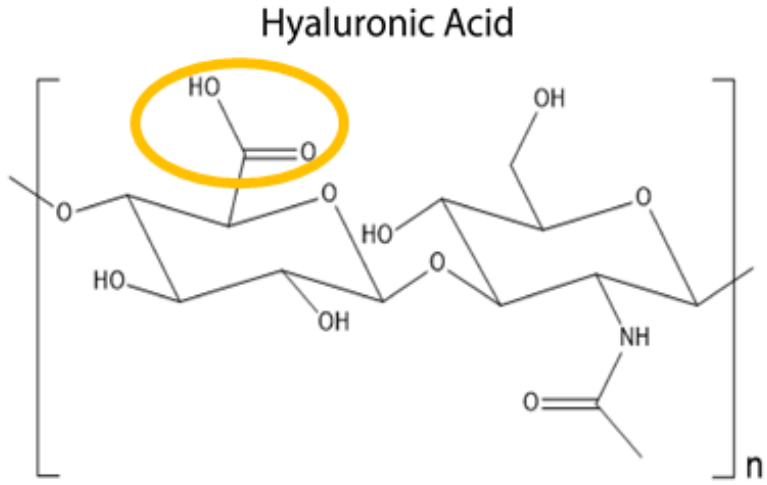
Hyaluronic Acid Electrostatic Interactions



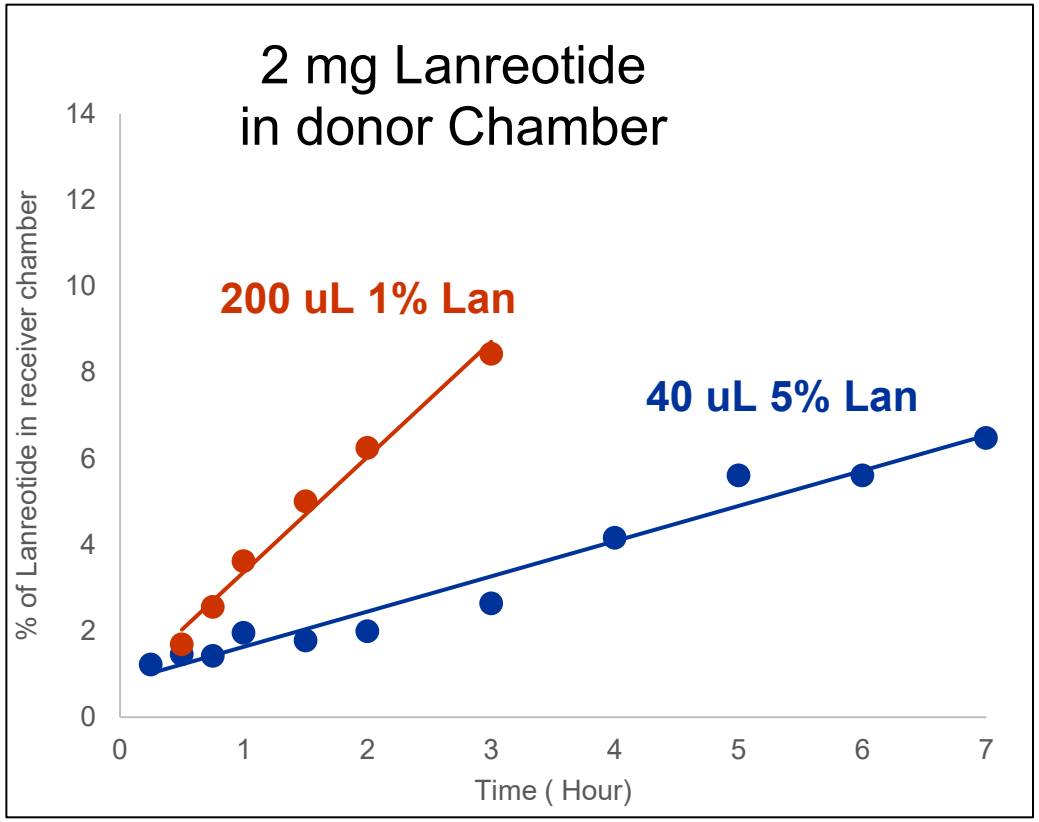
Temperature 34 ̊C , 50 kDa MWCO , HLA 5% , PBS pH 7.4

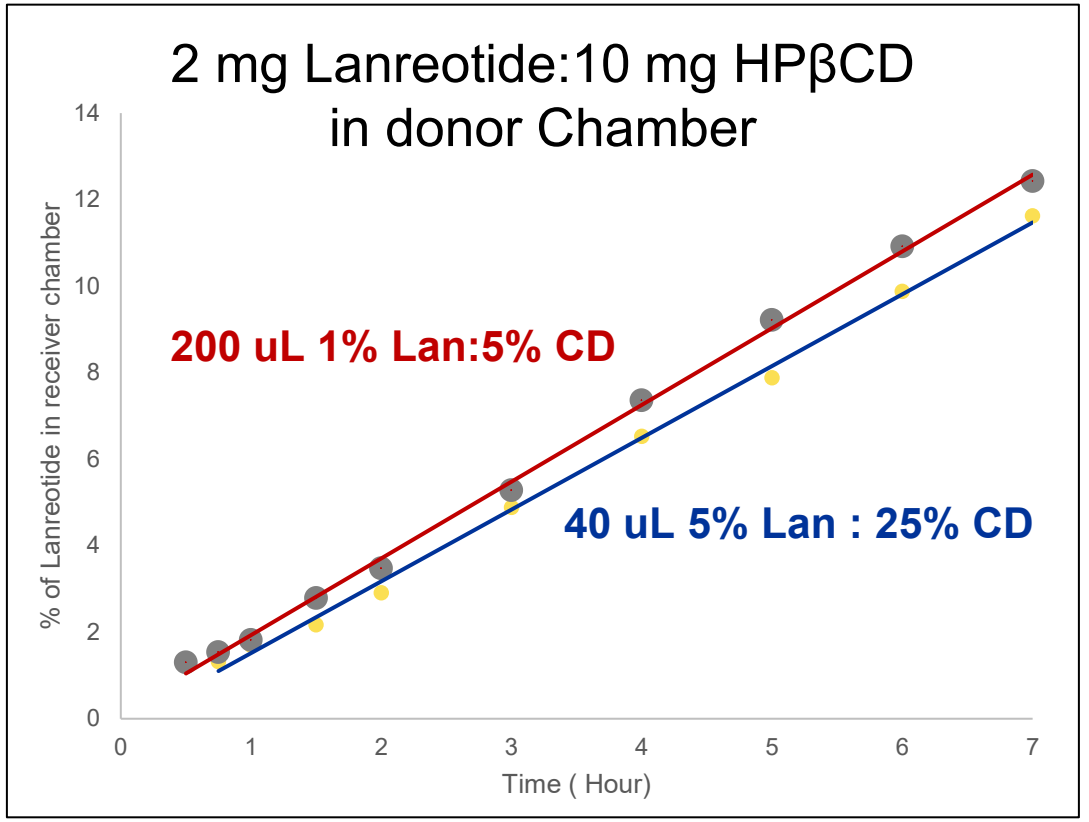


Lanreotide



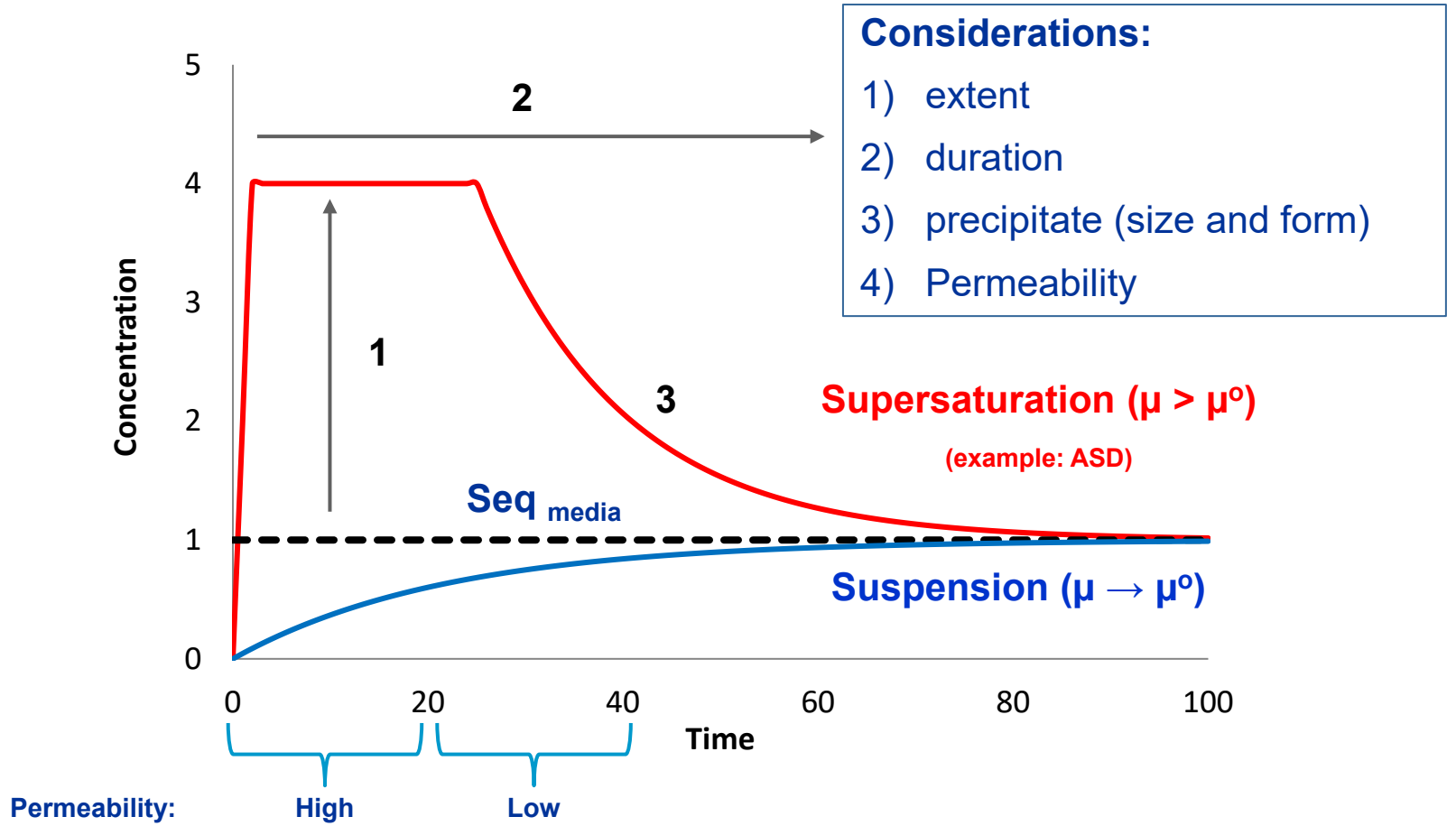
The HPβCD Formulation Mitigates the Gelation and Flux Rate Differences





Injection Volume	% Lanreotide	% HPβCD	n Lanreotide : m HPβCD	Flux Rate mcg/hr.cm^2
200 uL	1%	5%	1:5	44.35
40 uL	5%	25%	1:5	41.47

Overcoming Poor Solubility / Permeability
(The Advantages of Supersaturation in Oral Bioperformance)



μ - chemical potential

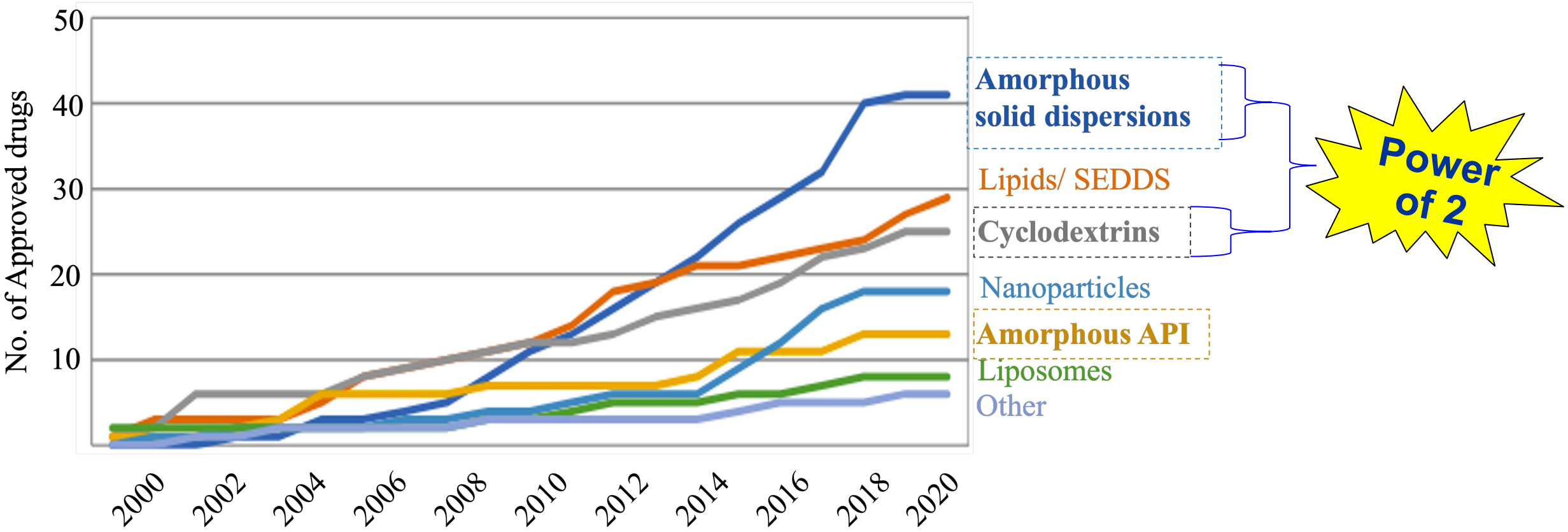
μ° - chemical potential @ Seq

Seq – equilibrium solubility

ASD – amorphous solid dispersion

Supersaturation stages: generation and maintenance

Increasingly Broad Applicability of Solid Dispersions

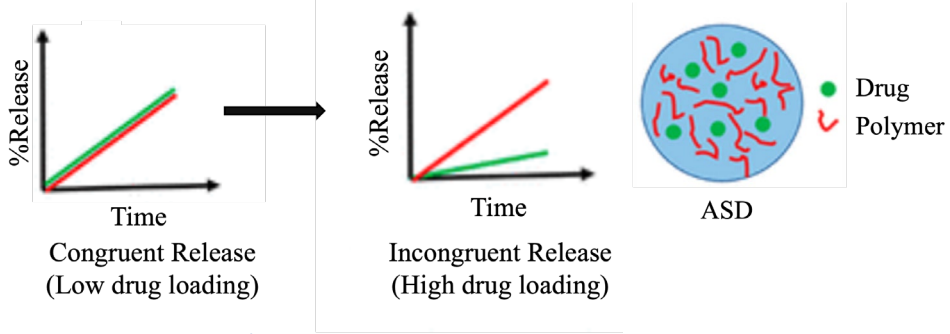


Broad applicability & technological advances in preparation

Combined 70+ products in the last two decades

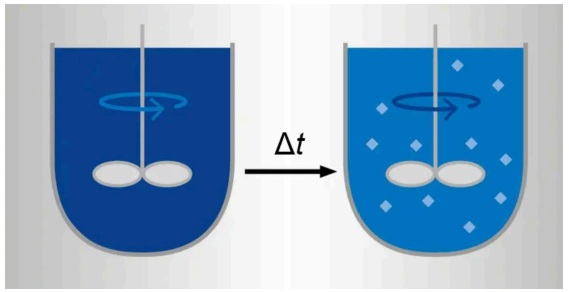
Iyer et al., *Pharmaceutics* **2021,** *13* (10).

Limitations of ASDs



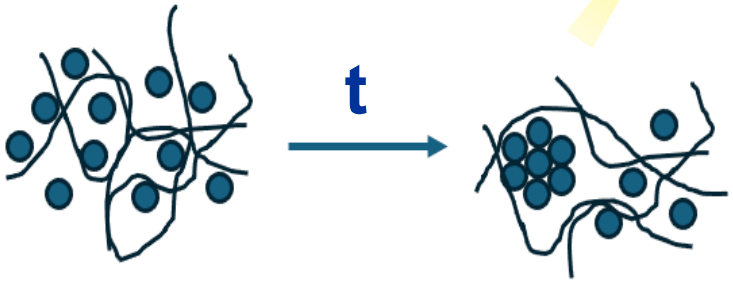
Drug loading threshold

(<20-30%)

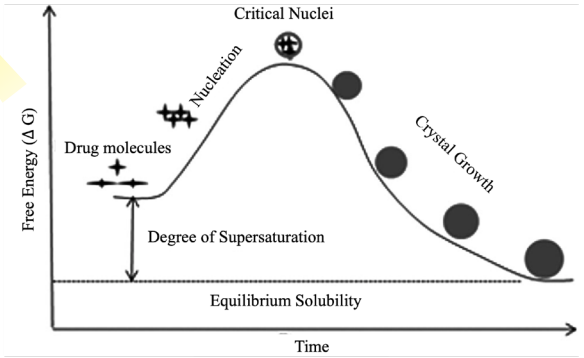


Crystallization during dissolution

Limitations of ASDs



Recrystallization or/and drug degradation during storage



Supersaturation leads to drug precipitation

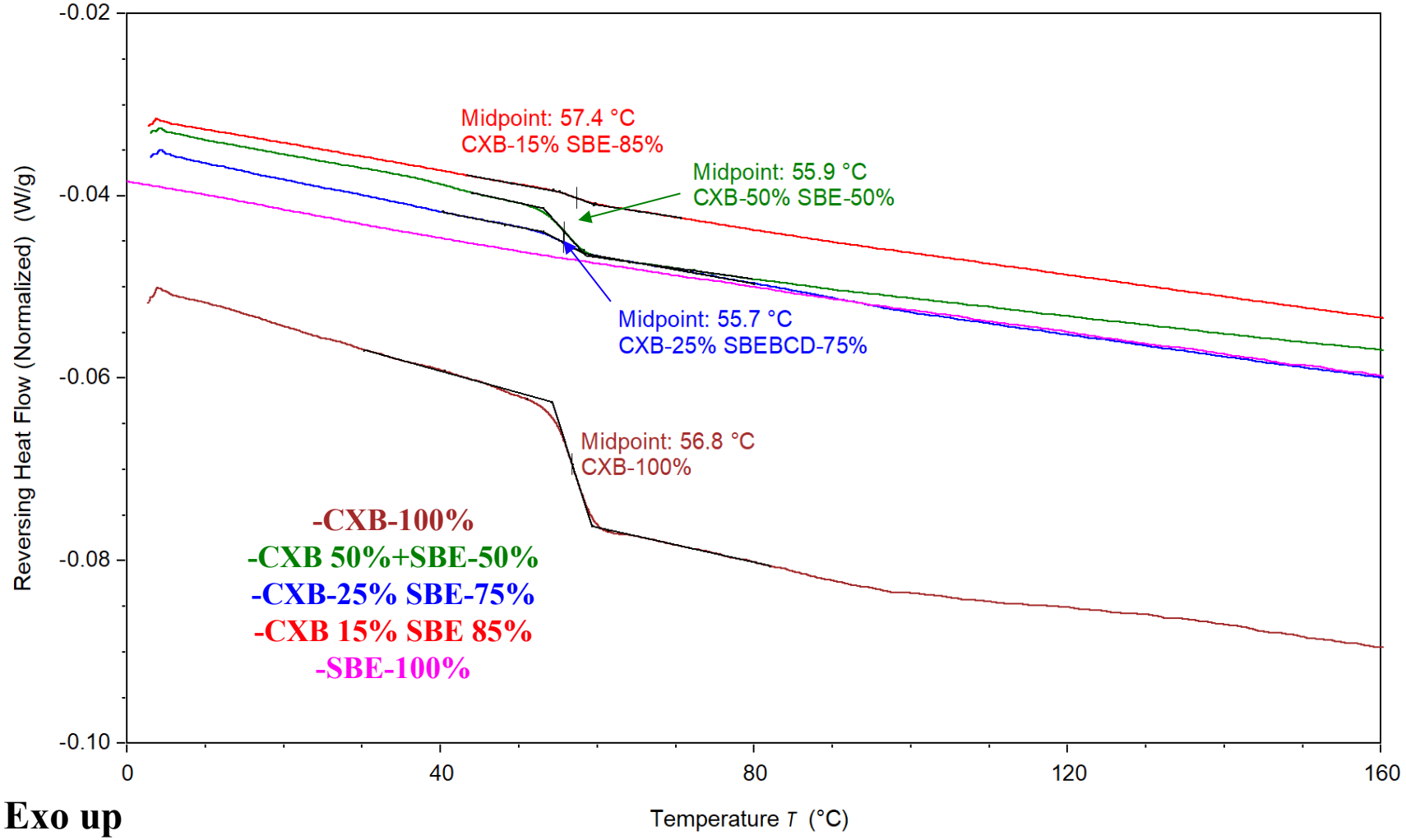
Saboo et al., *Molecular Pharmaceutics* 2020, *17* (4), 1261-1275

https://microporetech.com/making-products-better/active-pharmaceutical-ingredient-api-crystallization; accessed on 05/01/2024

Why Cyclodextrins Might Help ASD Performance

- The accessible free drug in the solid state to recrystallize is decreased because some fraction of the drug is bound to CD? As solvent evaporates complexation increases?
- Increase the apparent solubility at the dissolving surface and the gradient in the aqueous solubility diffusion layer (ADL) to enhance dissolution and limit precipitation or crystallization during dissolution?
- Provide alternative equilibria for drugs in the ADL and in bulk solution to limit phase separation and precipitation, yet provide a rapidly (relative to API dissolution) accessible source for free drug?
- Allows the ASD to perform while tolerating the presence of small amounts of crystallization which occur on manufacturing or storage.

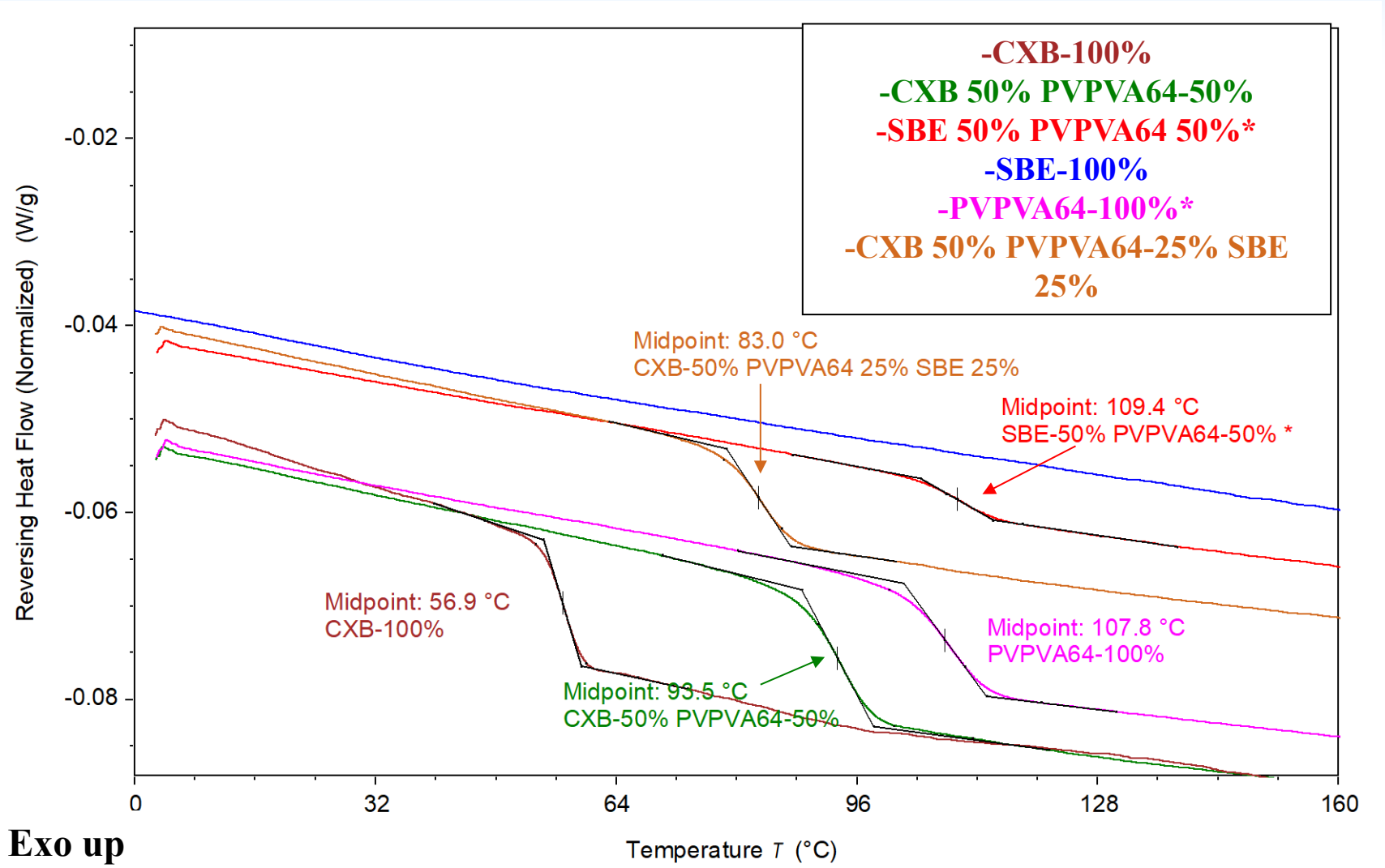
CXB-SBE Binary Solid Dispersion: Effect on T_g



% CXB	T_g (°C)
15	57.4
25	55.7
50	55.9
100	56.8
SBE	–

Immiscible Phases?

CXB-SBE-PVPVA64 Ternary SD: Effects on T_g



PVPVA64 and Celecoxib Appear Miscible

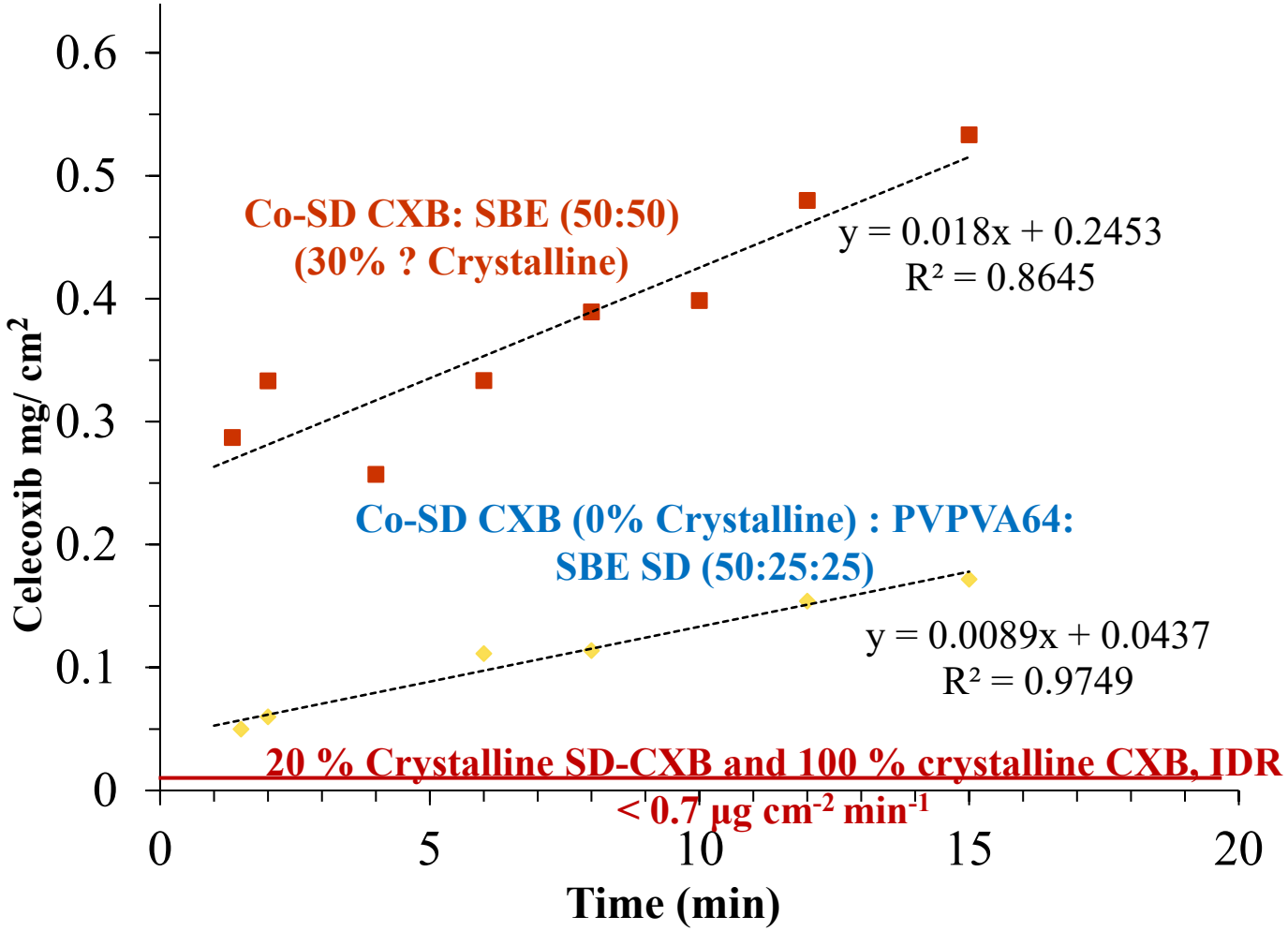
SBEßCD doesn't appear to be miscible with either celecoxib or PVPVA64?

Gordon Taylor equation predictions

CXB 50% PVPVA64-50% 78 °C (Exp 93.5 °C)

CXB 50% PVPVA64-25% SBE 25% (SBE excluded) 69.5 °C (Exp 83.0 °C)

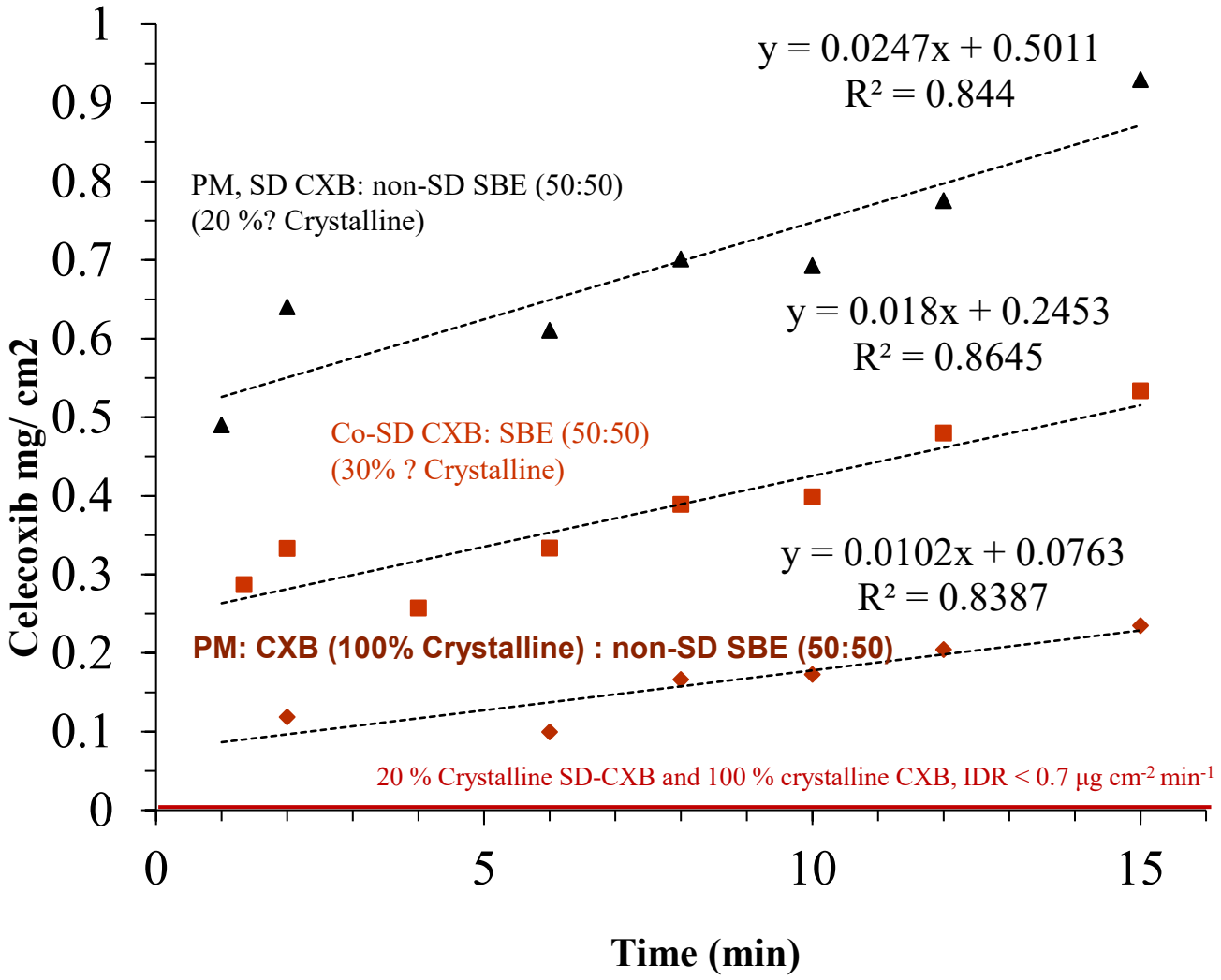
Intrinsic Dissolution Rate (IDR)
Binary & Ternary "Amorphous" ASD



Ternary ASD (drug:Captisol®:polymer) is amorphous and provides enhanced dissolution over both amorphous and crystalline drug alone.

Binary ASD (drug:Captisol®) containing residual crystalline material dissolves as well as or better than the ternary amorphous system.

IDR of Physical Mix vs Co-Spraydried Celecoxib and SBE



Both co-spray dried and Physical Mixture of SBE with celecoxib aids in enhanced dissolution similarly, helping to tolerate the presence of crystalline material.

Summary Of IDR for Celecoxib and Captisol®

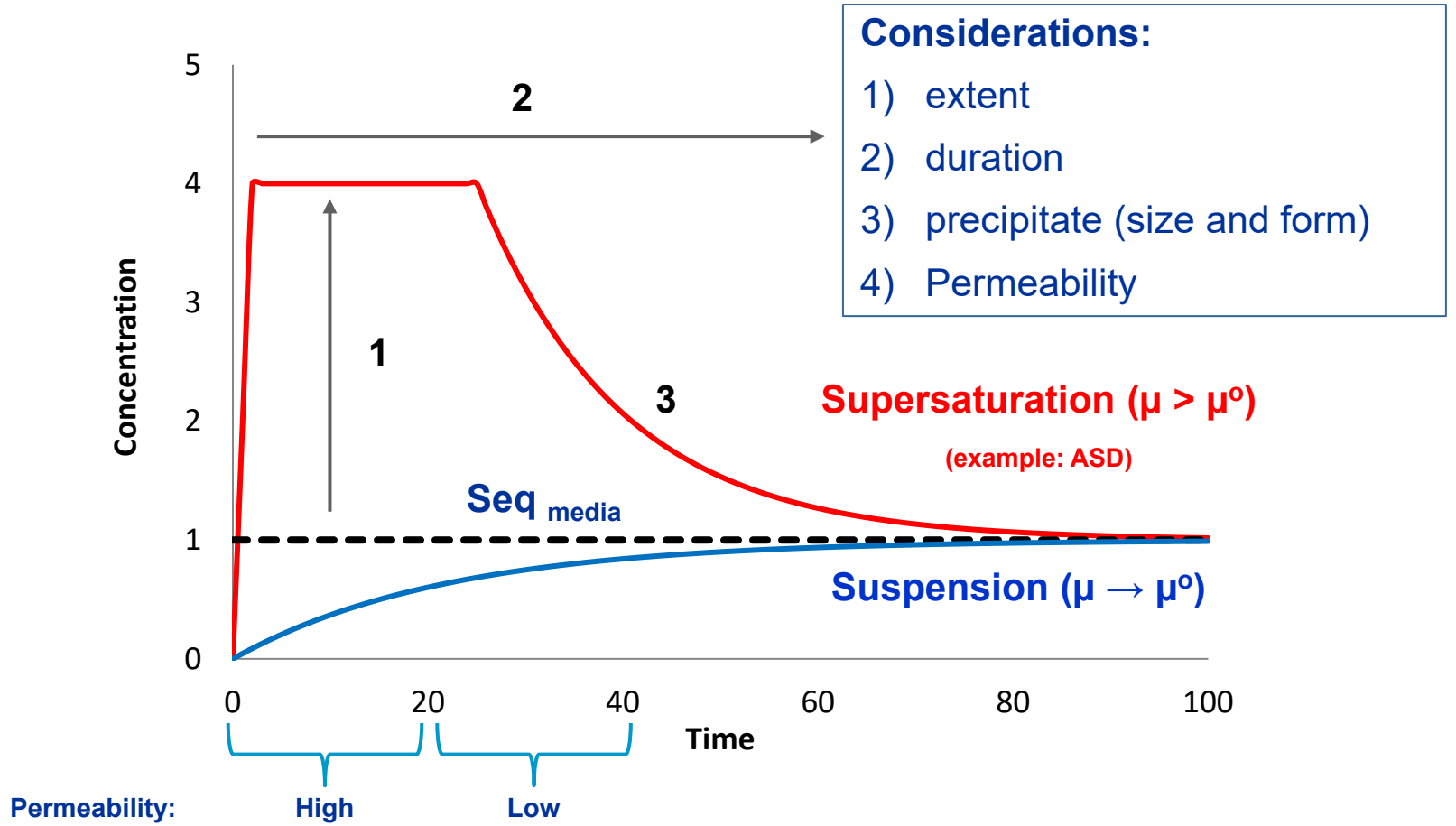
Solid Dispersion	Normalized by CXB% IDR=(dm/dt)/A µg/cm²/min
50% **CXB (31% Crystalline)** : 50% **SBE** mixture spray-dried (SD) from MeOH	36.0
50% **SD CXB (20% Crystalline)** : 50% **non-SD SBE** (physically mixed)	49.4
50% **Non-SD CXB (100% Crystalline)**: 50% **Non-SD SBE** (physically mixed)	20.4
50% **CXB (0% Crystalline)**: 25% **PVP**: 25% **SBE** mixture SD	17.9
Pure CXB (SD 20% Crystalline and non-SD 100% Crystalline)	< 0.7 (Calculated)

Background: Intraperitoneal Administration (IP)

- Rarely used in humans
- Commonly used in laboratory animals for preclinical “proof-of concept” studies where drug formulation and PK profile isn’t translated to later clinical studies?
- Valuable in the preclinical space for a variety of reasons:
  -Large volumes of solution (10 ml/kg) can be safely administered
  -Allows for repetitive chronic administration which is challenging for the IV route?
  -Avoids potential degradation or modification in the in IP cavity or in GI tract common in the oral route?
  -Drug Absorption is very quick (slightly slower but similar to IV)?
  -Facilitates absorption of both small and large molecules well?

Al Shoyaib A, Archie SR, Karamyan VT. Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies? Pharm Res. 2019 Dec 23;37(1):12. doi: 10.1007/s11095-019-2745-x. PMID: 31873819; PMCID: PMC7412579.

Overcoming Poor Solubility / Permeability
(The Advantages of Supersaturation in Intraperitoneal Bioperformance)



μ - chemical potential

μ° - chemical potential @ Seq

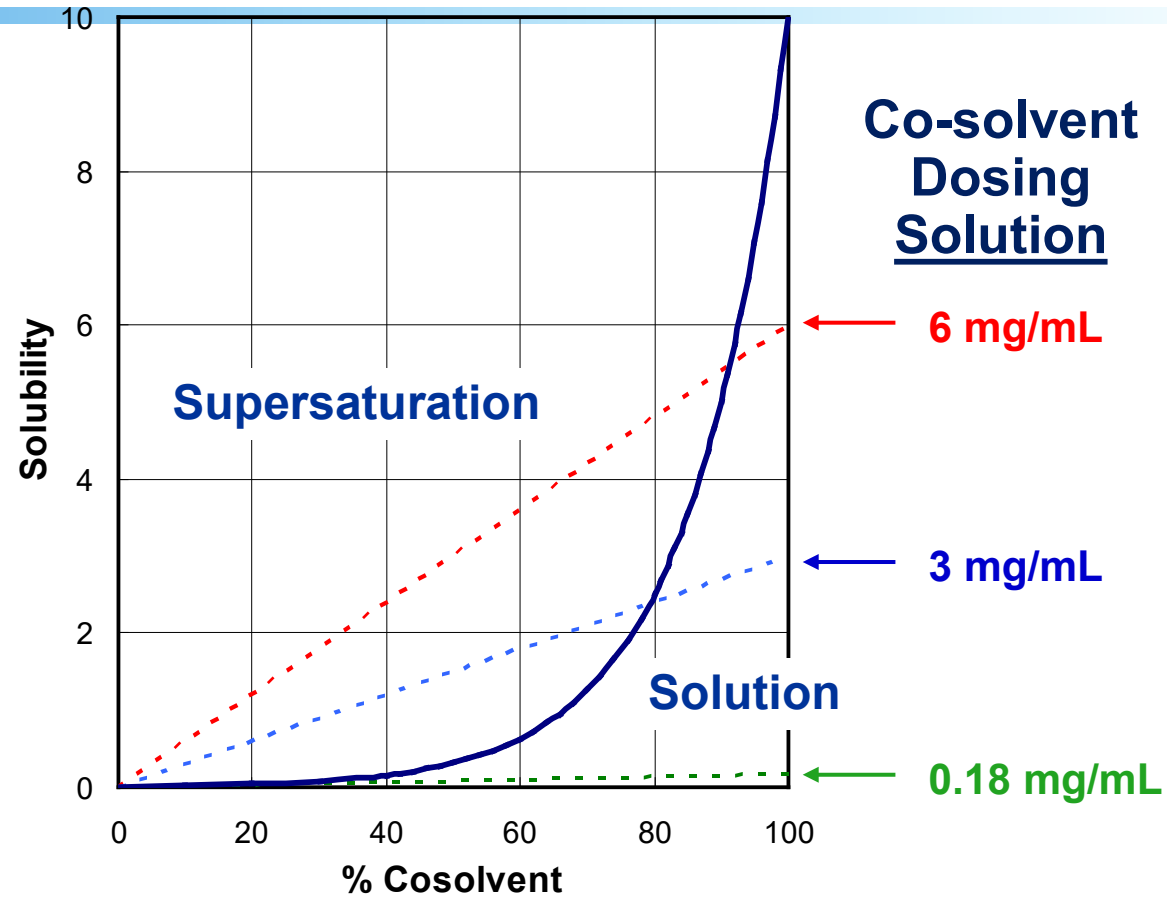
Seq – equilibrium solubility

ASD – amorphous solid dispersion

Supersaturation stages: generation and maintenance

Co-solvent Formulations Tend to Select For Propensity to Supersaturate as Well as Solubilization in Co-solvent

%Cosovent	Sol/(mg/mL)
0	0.01
20	0.04
40	0.16
50	0.32
60	0.63
70	1.26
80	2.51
90	5.01
100	10.00

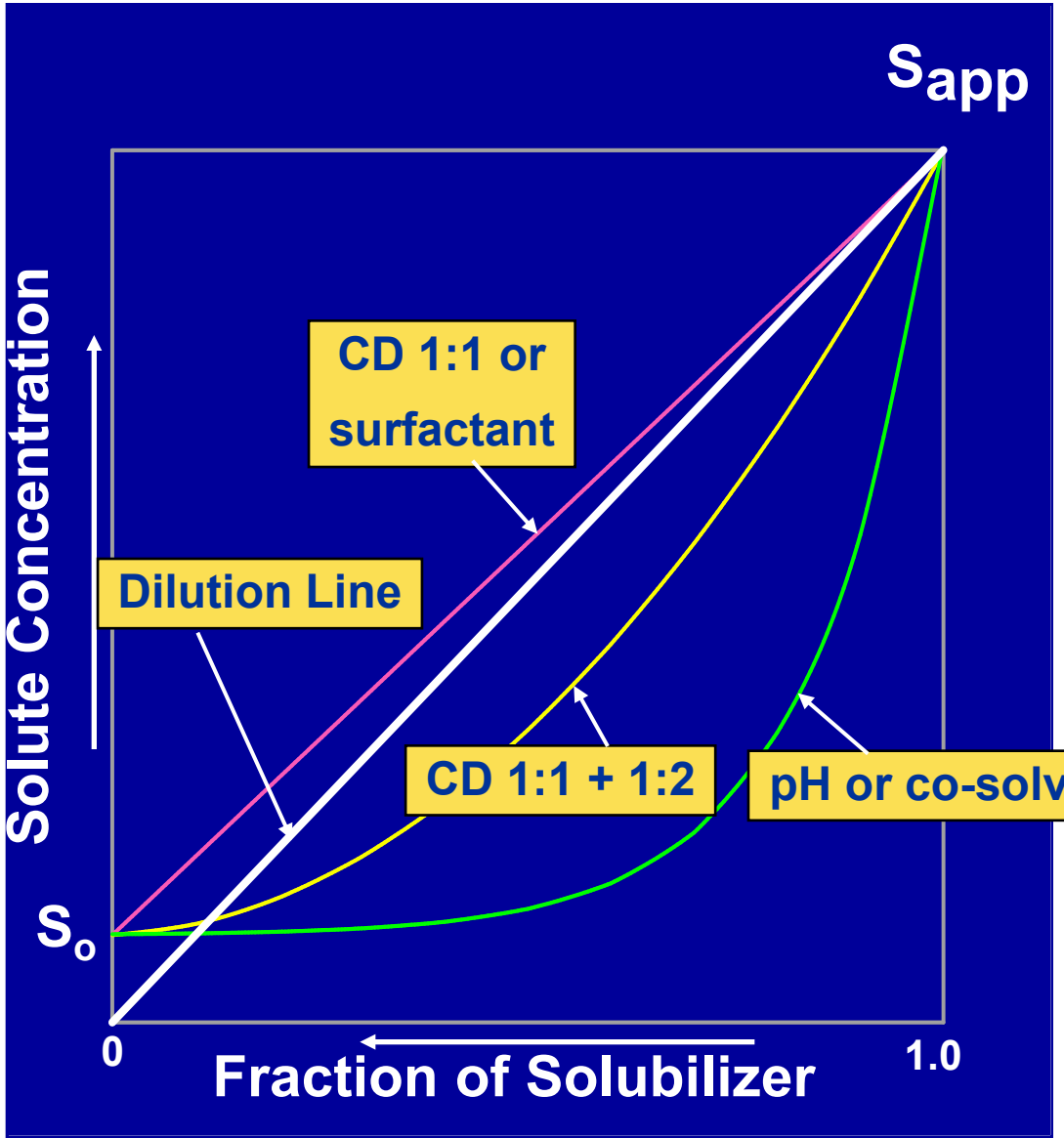


Precipitation from Supersaturated Solution depends on:

- Time spent in supersaturated state
- Extent of supersaturation (vertical distance above solubility curve)
- Ability of solute to nucleate/grow in the medium (use of precipitation inhibitors)

Adapted from Victor Guarino

Behavior of Solubilized Formulations on Dilution



Dilution of solubilized solution from apparent concentration of S_{app} can result in supersaturation and/or precipitation.

Note: Competitive displacement from complexing agents can also lead to supersaturation and/or precipitation .

Solubility enhancement from increasing cosolvent ratio

Increasing the amount of PEG 400 in our formulation should improve solubility in a Log-Linear fashion

Solubility is theoretically defined by the equation below

$$\log S_T = \log S_0 + \sigma * f$$

Solubilization power of cosolvent is defin

$$\sigma = M * \log k_{ow} + N$$

M=0.74 and N=1.26 for PEG 400

LogP of Celecoxib is 3.53

M and N are cosolvent constants

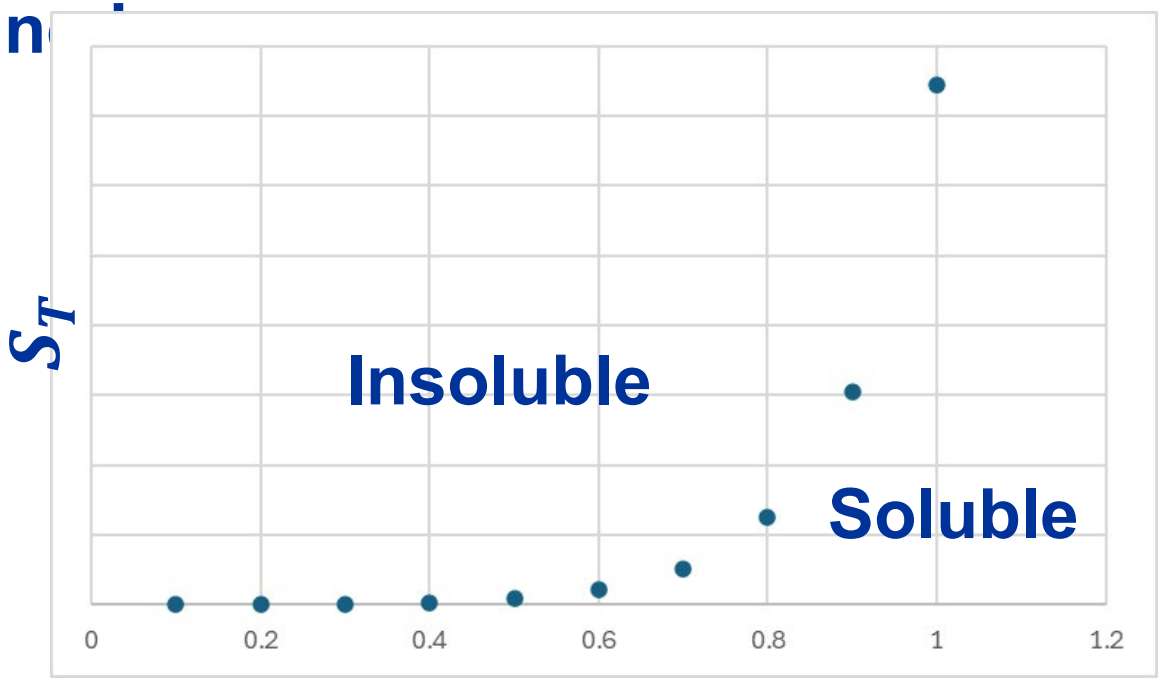
$\log k_{ow}$=Partition coefficient

S_T=Total solubility

S_0=Intrinsic solubility

σ=Solubilization power

f=Fraction cosolvent



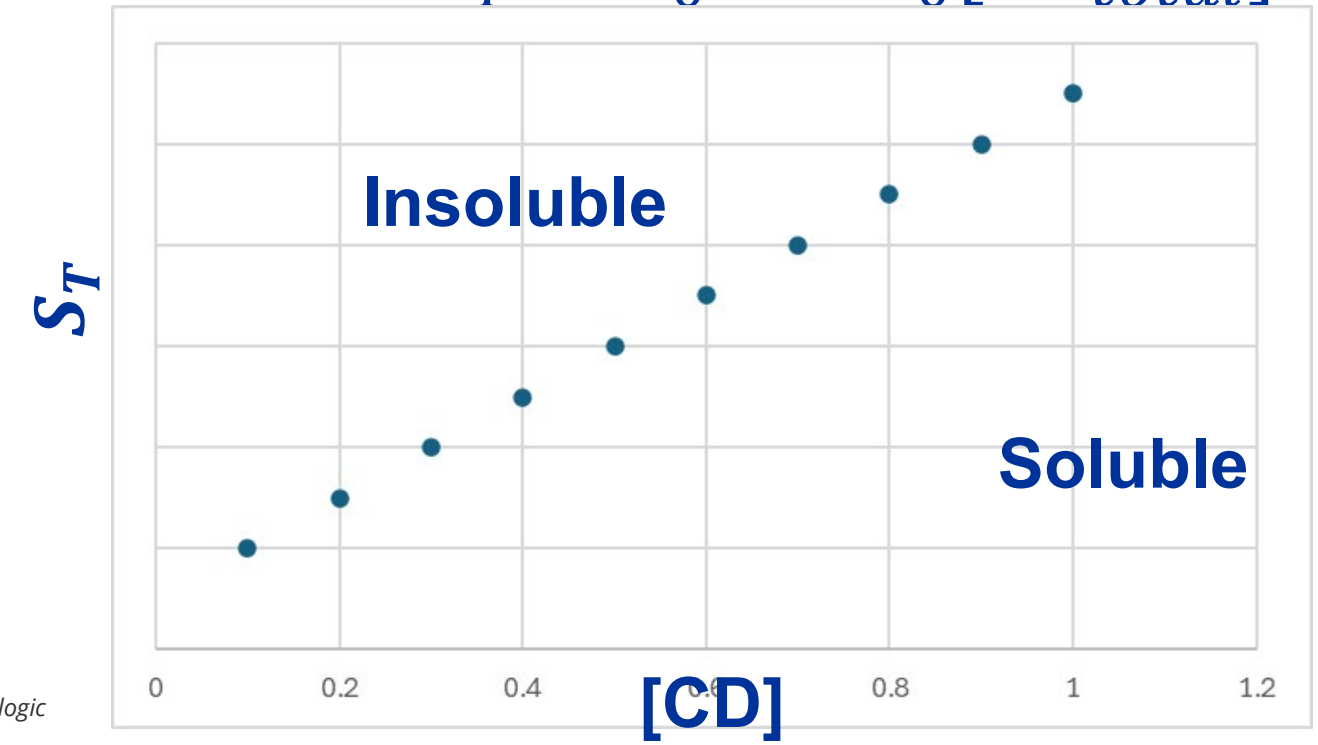
Jouyban, A. (2008). Review of the cosolvency models for predicting solubility of drugs in water-cosolvent mixtures. *Journal of Pharmacy & Pharmaceutical Sciences*, *11*(1), 32–58.

Solubility Enhancement from Increasing Captisol® Concentration

Increasing concentration of SBEßCD in our formulation should increase solubility in a linear fashion

Solubility is theoretically defined by the equation below

$$S_T = S_0 + KS_0[CD_{total}]$$



S_T=Total solubility

S_0=Intrinsic solubility

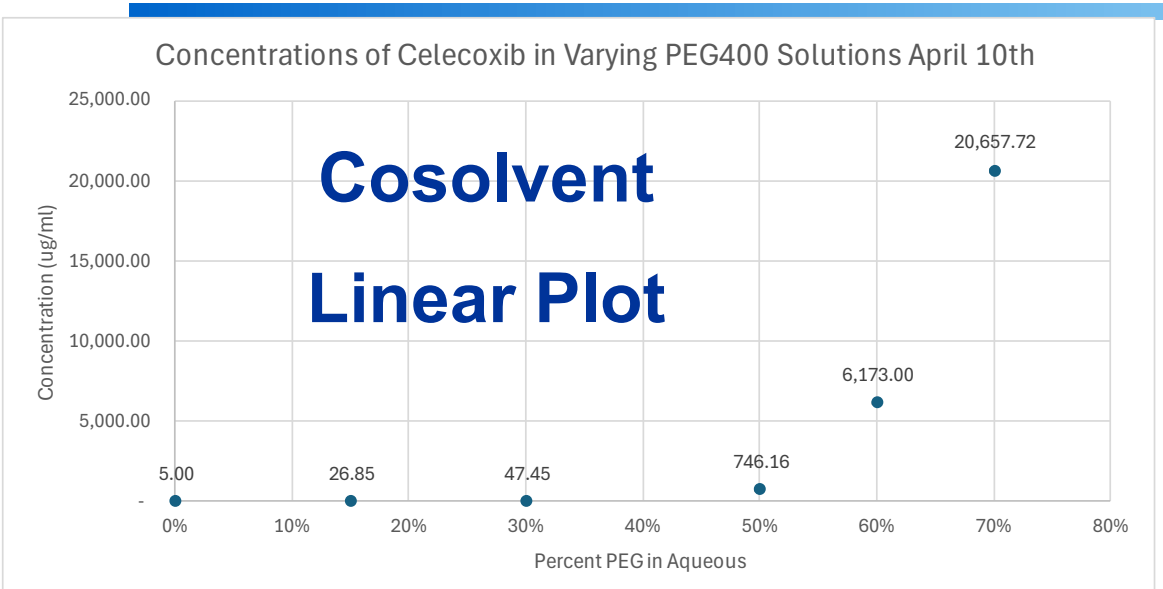
K=1:1 binding constant

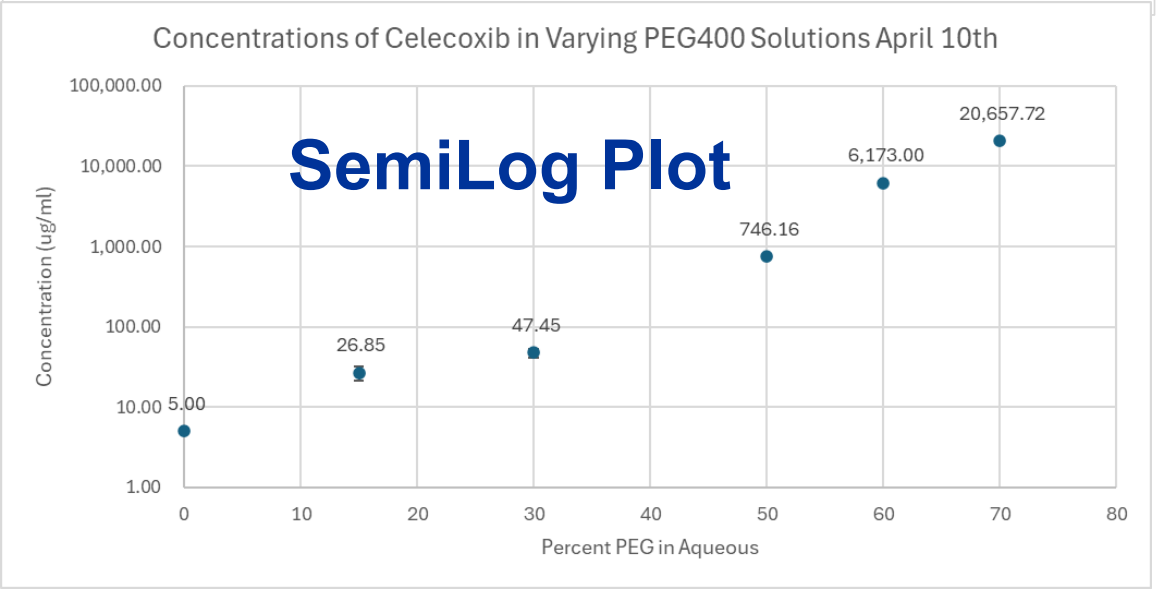
Stella VJ, He Q. Cyclodextrins. *Toxicologic Pathology*. 2008;36(1):30-42. doi:10.1177/0192623307310945

Why Cyclodextrins Might Help Intraperitoneal Delivery for Longterm Efficacy or Toxicology Studies

- Cyclodextrin-induced solubilization tends to be more linear upon dilution and can help mitigate the exponential drops incurred by pH or cosolvents?
- As solubilizing effect of pH or cosolvent is either diluted or moved toward physiological conditions the complexation constant will increase?
- The accessible free drug available to phase separate, precipitate and crystallize is decreased while still providing a rapidly accessible source of drug for absorption?
- Increase in apparent solubility gradient in the aqueous solubility diffusion layer (ADL) to facilitate the dissolution of any drug that has precipitated?
- Putatively, an excipient with reduced clearance from intraperitoneal cavity, to maintain it's solubilization effect longer? Relative to cosolvent?

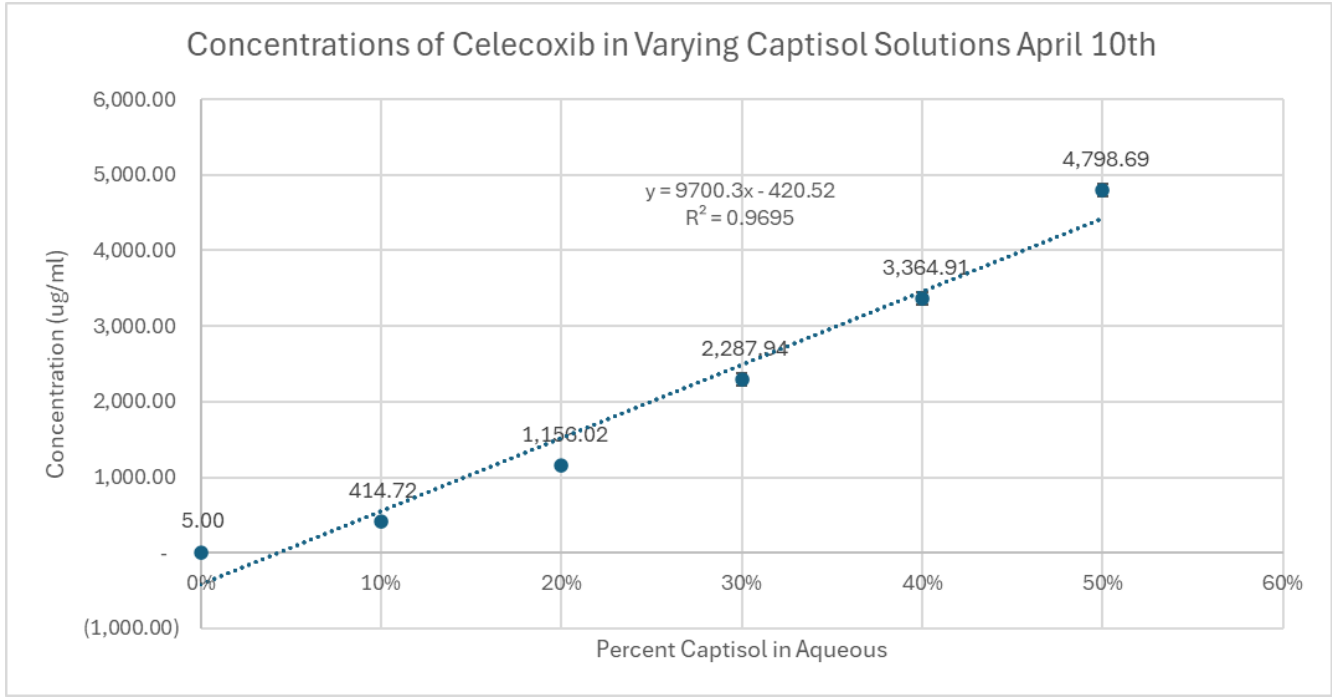
Celecoxib Solubility in Single Component Systems

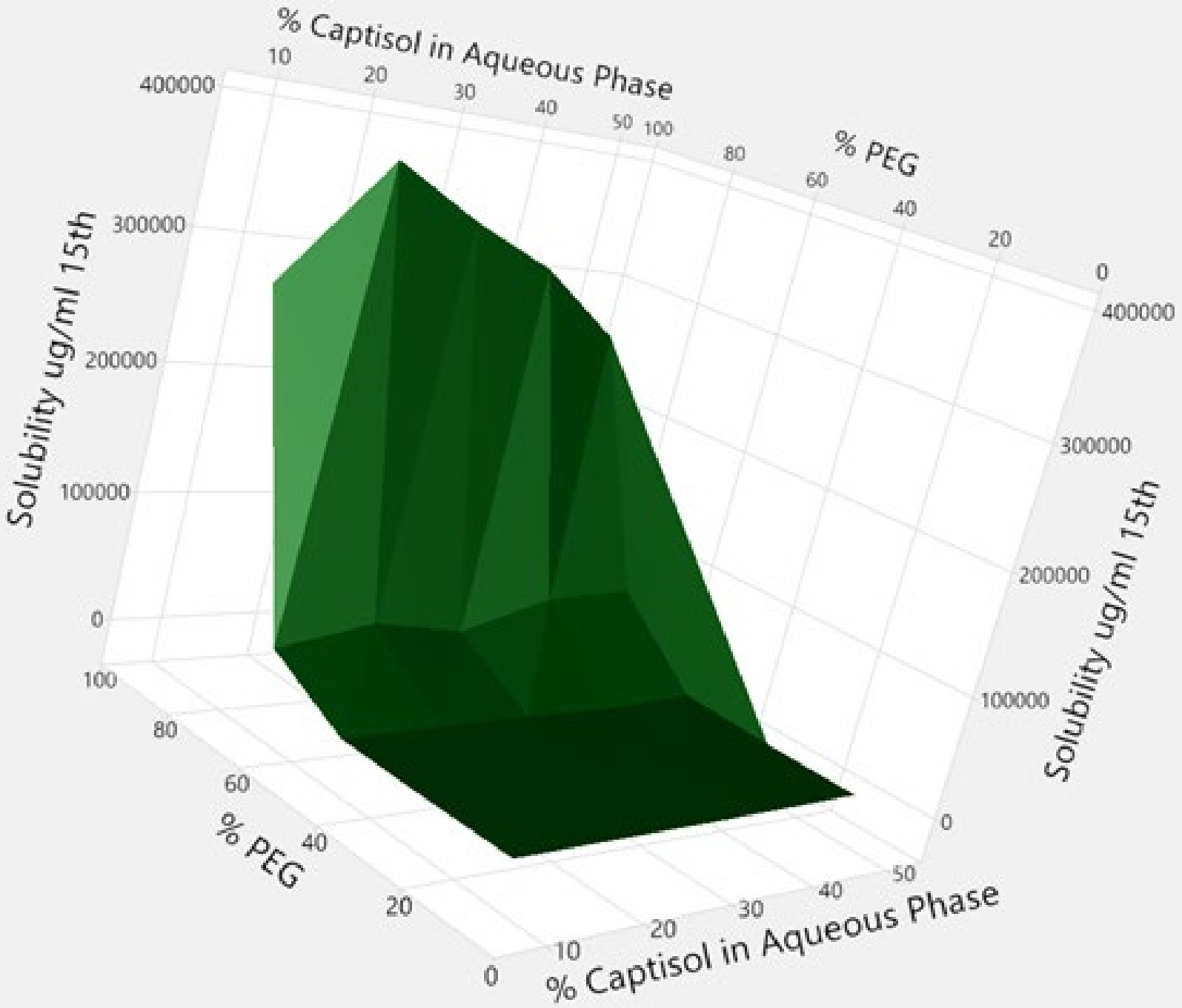




Captisol®

Linear Plot





Celecoxib Solubility in Two Component Systems

For Precipitation Studies

Drug Dissolved in PEG

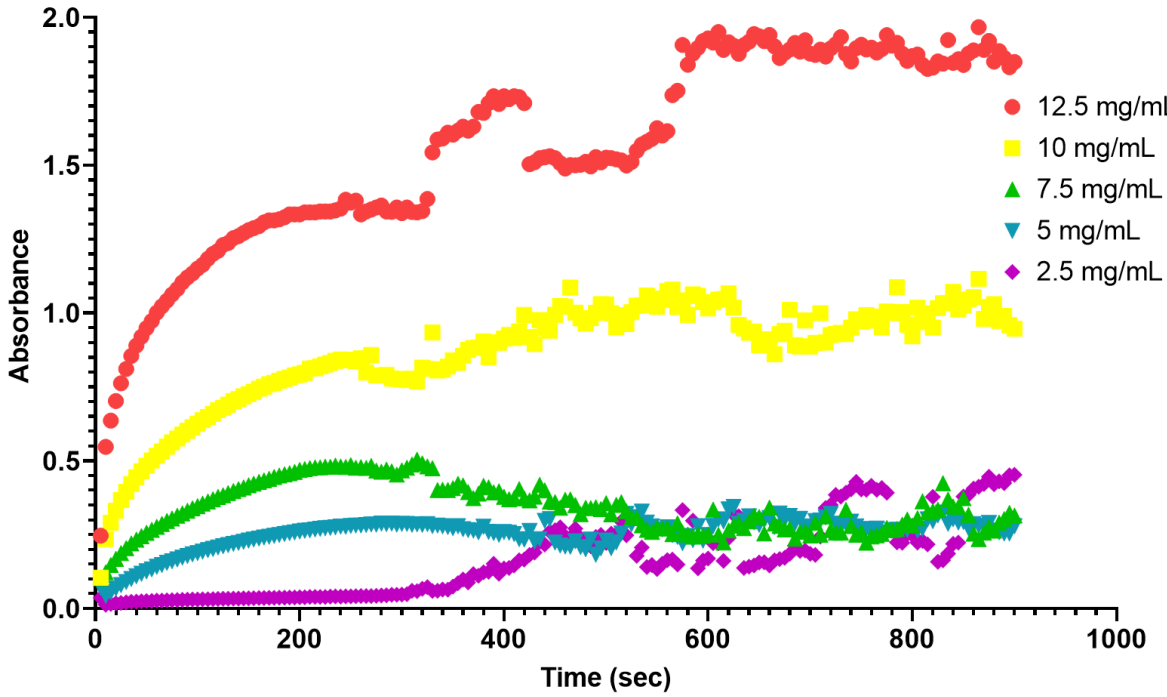
SBEßCD in water at various concentrations mixed with PEG drug containing solution

Resulting solutions diluted 1:100 with water

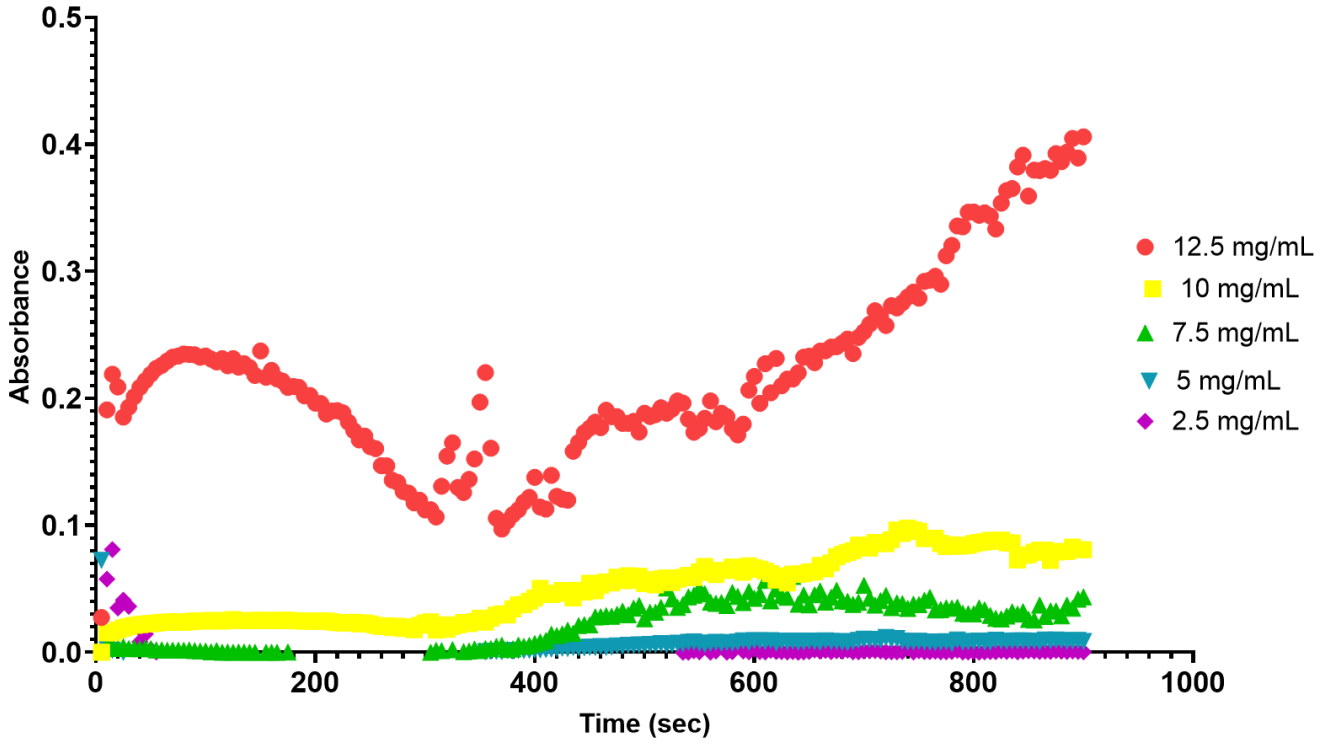
Precipitation (450 nm) Upon 100 fold Dilution of Designated Solution Concentrations in Water

(note: Solubility of celecoxib is about 5 mcg/ml in dilution media; i.e. dilution of 2.5 mg/ml would give 25 mcg/ml; dilution of 12.5 mg/ml would give 125 mcg/ml; i.e ~ 5-25 times saturation solubility)

70% PEG only



30% Captisol only



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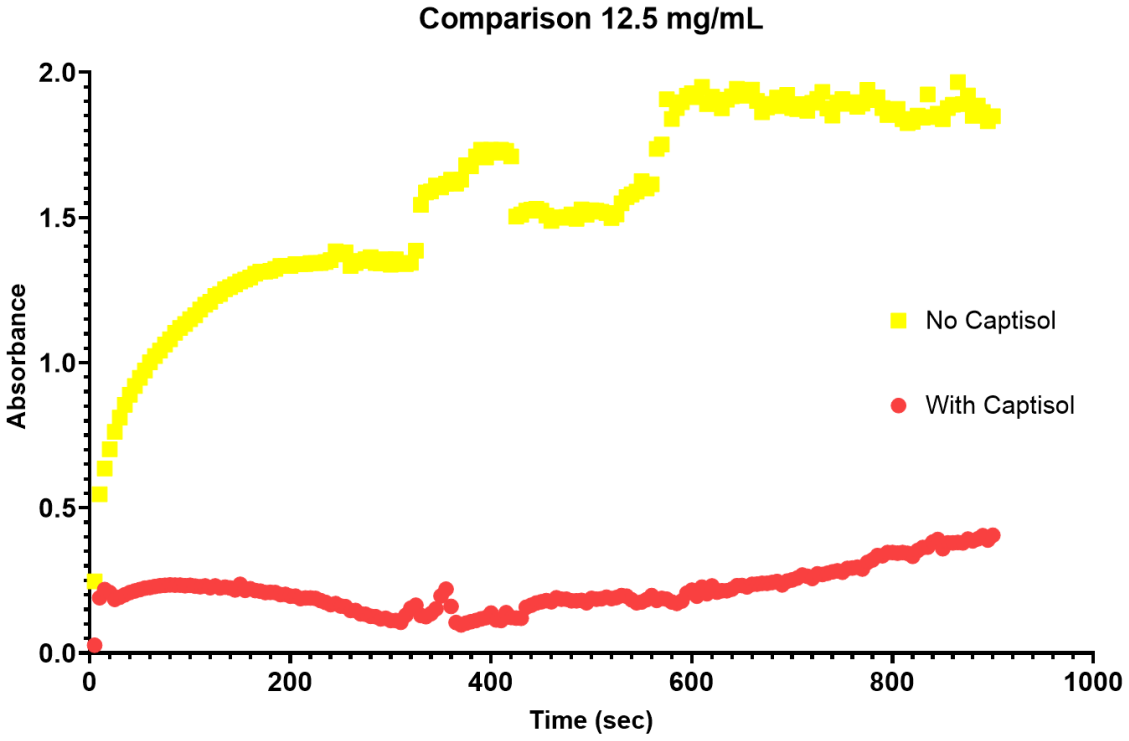
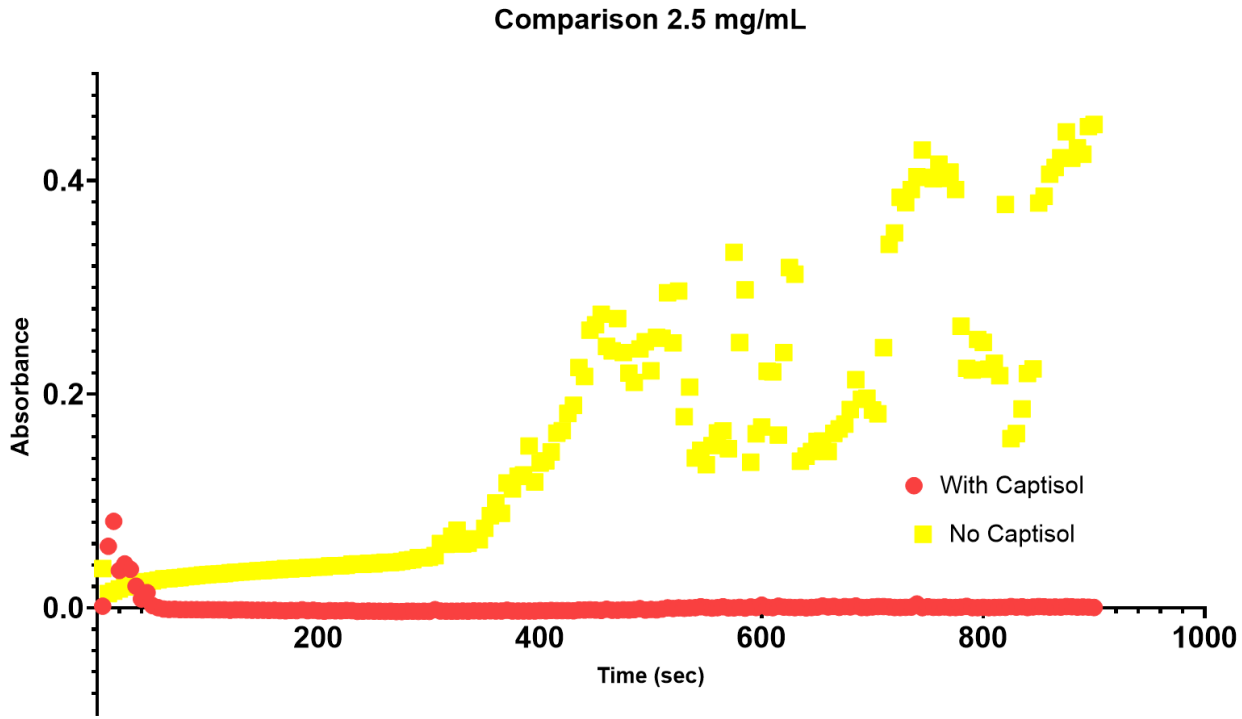
(70% PEG:30% Captisol)





Cyclodextrin-Facilitated Drug Delivery: Modulating Gelation of Peptides and Improving Dissolution of ASDs

- **New Challenging Modalities requiring Advanced Delivery Technology – Advantages of Coupling Technologies**
- **Integrating Pharmaceutics and Delivery into Decisions for Molecule Progression**
- **Expanding Cyclodextrin Technologies to De-risk and Enable Molecule Progression**
  - Modulating cyclic peptide aggregation for SC delivery
  - Expanding the limits of amorphous solid dispersions (ASD)
    - Physical form stabilization (in solid phase, during dissolution)
    - Facilitating supersaturation & preventing colloidal phase separation
  - Enabling supersaturation upon dilution at site of drug delivery

Posters with more detail presented at DDF Summit

Impact of Sulfobutylether-β-Cyclodextrin in Preparation and Dissolution of Celecoxib ASDs

Indeewara Munasinghe[1,3], Kyle Gross[1], Michael Hageman[1,2]
[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS
[2]Biopharmaceutical Innovation and Optimization Center, Lawrence, Kansas
[3]Synthetic Chemical Biology Core Facility, University of Kansas

Self-assembly of Cyclic Peptide, Lanreotide Acetate: Impact of βCyclodextrins on the Subsequent Diffusional Release from an *in vitro* Emulator of Subcutaneous Absorption

Negar Jafari, Camille Addison, Hao Luo, Michael J. Hageman
Pharmaceutical Chemistry, University of Kansas, USA

Builiding a High Speed Trains is Fine But ….

The Infrastructure and Technology Supporting the Chemical Entities Will Dictate the Pathways and the Probabilities of Success