

# Release from Nanoscale Water-in-Oil-in-Water Double Emulsions Stabilized by Block Copolypeptide Surfactants

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## Abstract

Typical microscale double emulsions are restricted from use in a variety of sustained release applications for a number of reasons. First, they are difficult to manufacture: they often require multiple carefully chosen surfactants and several formulation steps. Second, they are not always stable for long periods of time, and finally, they are too large for most intravenous drug delivery applications. The goal of this project is to expand upon previous research which showed that block copolypeptide surfactants are able to stabilize nanoscale double emulsions in a one step process. This method of preparation largely negates the restrictions typically hindering the application of double emulsions. Emulsions prepared with poly(L-lysine hydrochloride)-block-poly(*racemic*-leucine) ( $K_{55}(\text{rac-L})_{20}$ ) were studied to determine the release rates of mock cargos and stability with various hydrophobic phases. The aqueous phase containing surfactant and rhodamine-b was emulsified using tip sonication along with polydimethyl siloxane (PDMS) or vegetable oil. The emulsions were loaded into dialysis bags and dialyzed against ultrapure water (UPW). Samples taken from the dialysate were analyzed using a fluorimeter. Cetyl trimethylammonium bromide (CTAB) and polyethylene oxide – block polypropylene oxide (PEO-PPO) were used as control surfactants to generate single emulsions. Results suggest that  $K_{55}(\text{rac-L})_{20}$  is able to successfully encapsulate more than 95% of the loaded dye, and demonstrate stable release profiles over several days. Temperature and osmotic pressure studies suggest that the emulsions remain stable even in conditions that mimic *in vivo* application. The facile production of nanoscale water-in-oil-in-water emulsions with high encapsulation efficiencies could lead to new methods for controlled release applications in agriculture, cosmetics, food preservation, and intravenous drug delivery.

## Surfactant and Emulsion Background

### Double Emulsions:

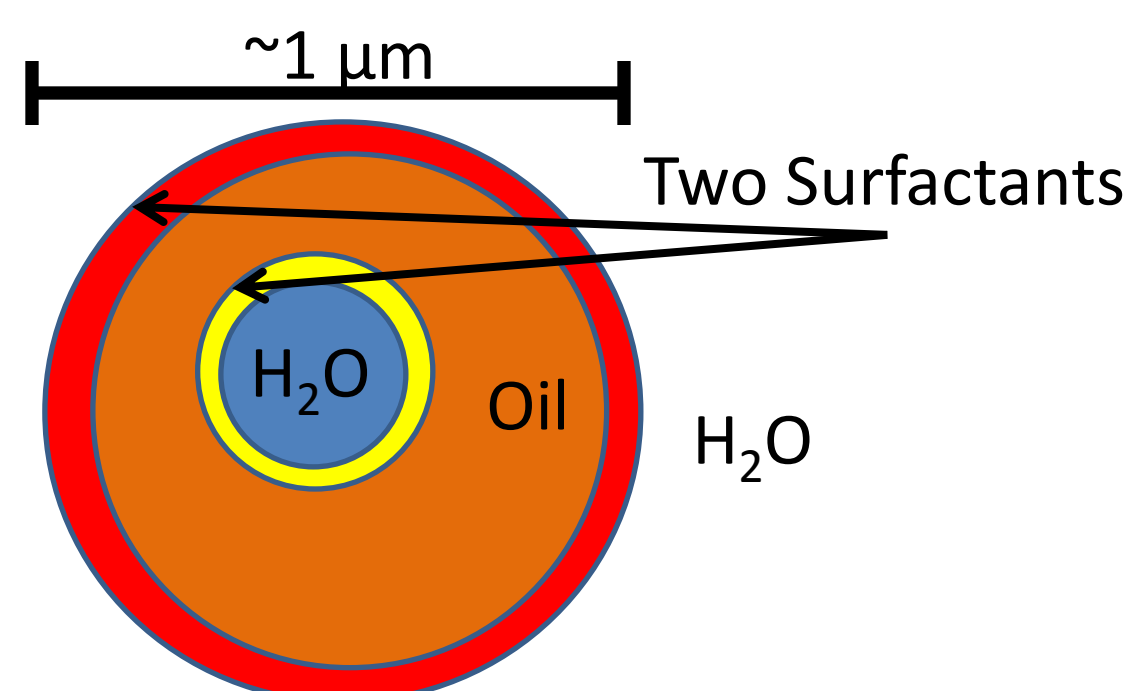
**Emulsion:** a suspension of droplets of one liquid in another liquid

(Examples: Milk, Salad Dressing, Creams)

**W/O/W Double Emulsions:** water dispersed in an oil phase which is itself dispersed in a larger aqueous phase. (eg. oil 'bubbles')

- Typically produced using a two step emulsification procedure
- Require complicated surfactant balance
- Large! Typically on the order of  $\sim 1 \mu\text{m}$

### Microscale Double Emulsion:

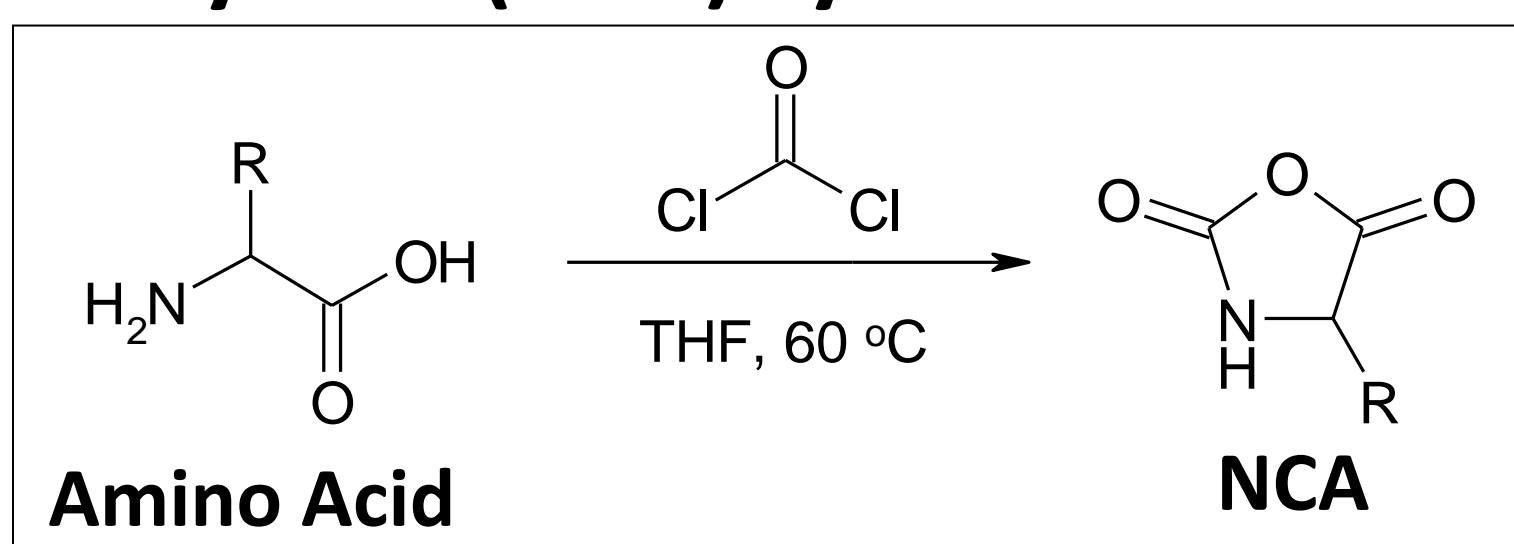


### Requirements for Drug Delivery:

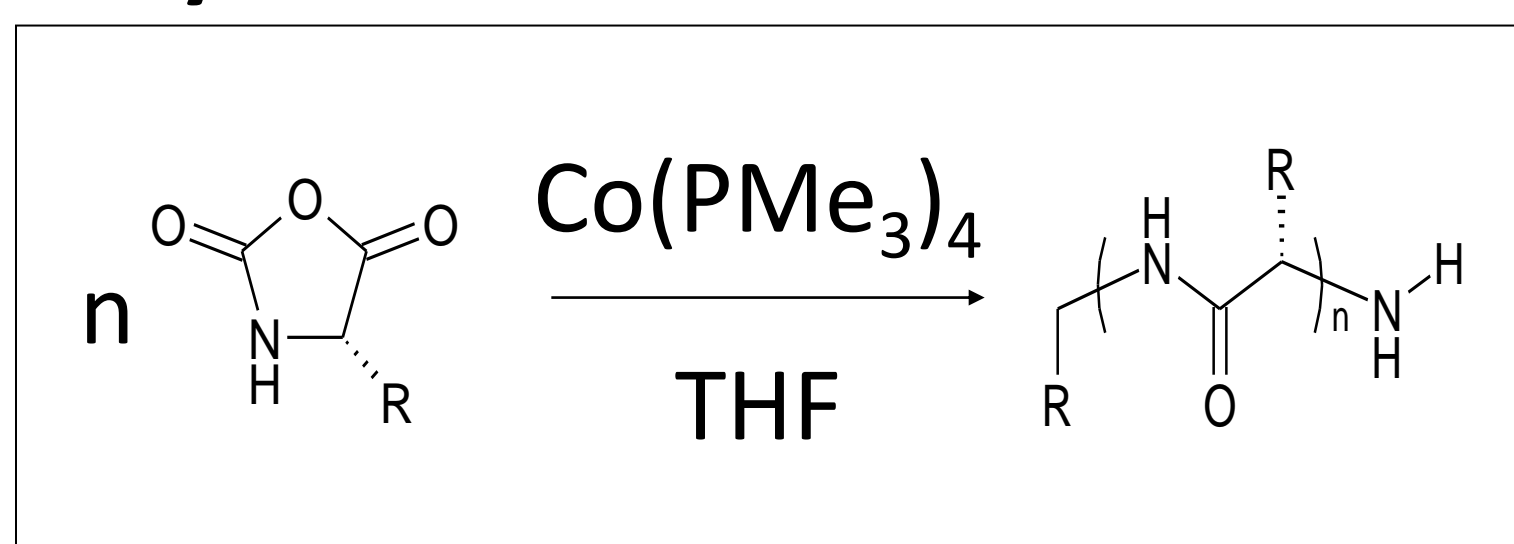
- Between 20 – 100 nm in diameter
- Remain stable in *in vivo* conditions
- Encapsulate both hydrophilic and hydrophobic cargos
- Easily fabricated
- Safe, biocompatible and biodegradable

## Synthesis of Block Copolypeptide Surfactant:

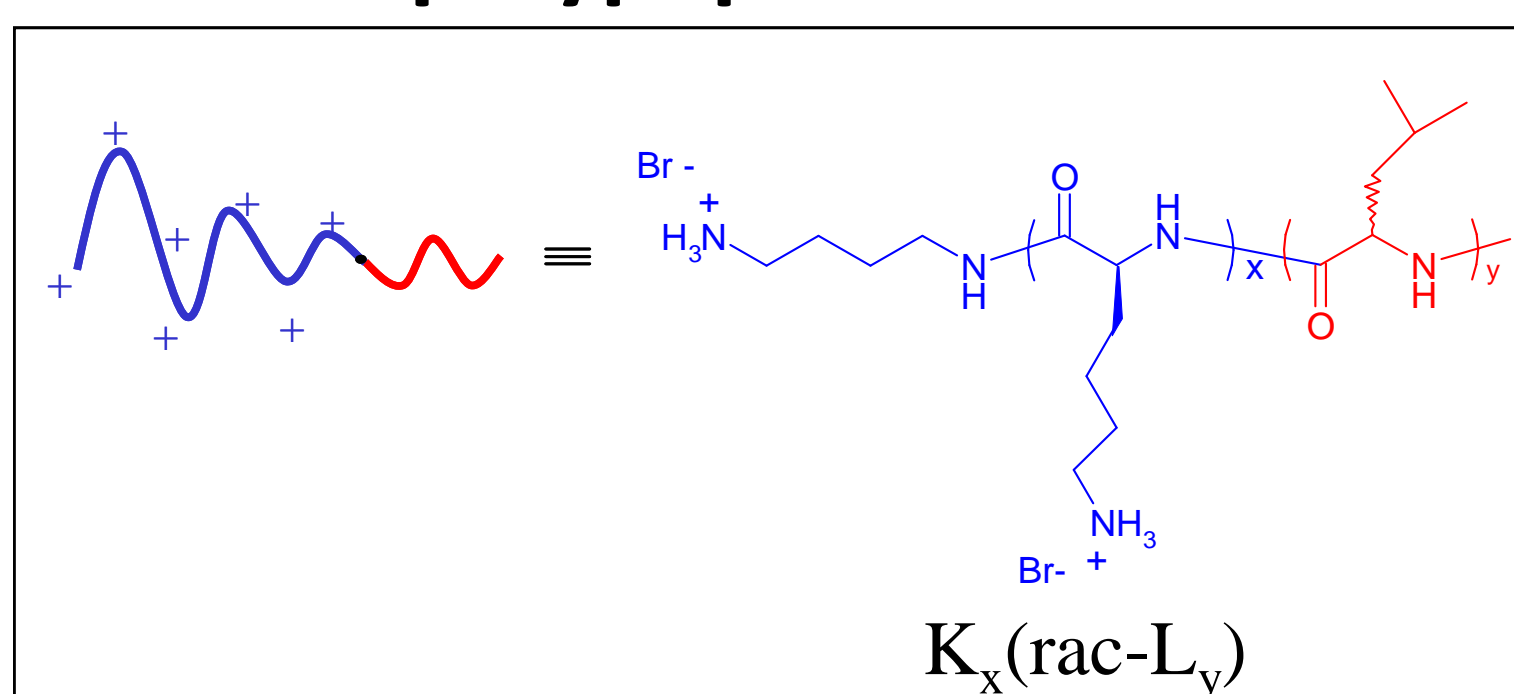
### $\alpha$ -amino acid-N-carboxy anhydride(NCA) synthesis:



### Polymerization reaction:



### Block Copolypeptide:



$K_{55}(\text{rac-L})_{20}$  surfactants have been shown to stabilize nanoscale double emulsions.

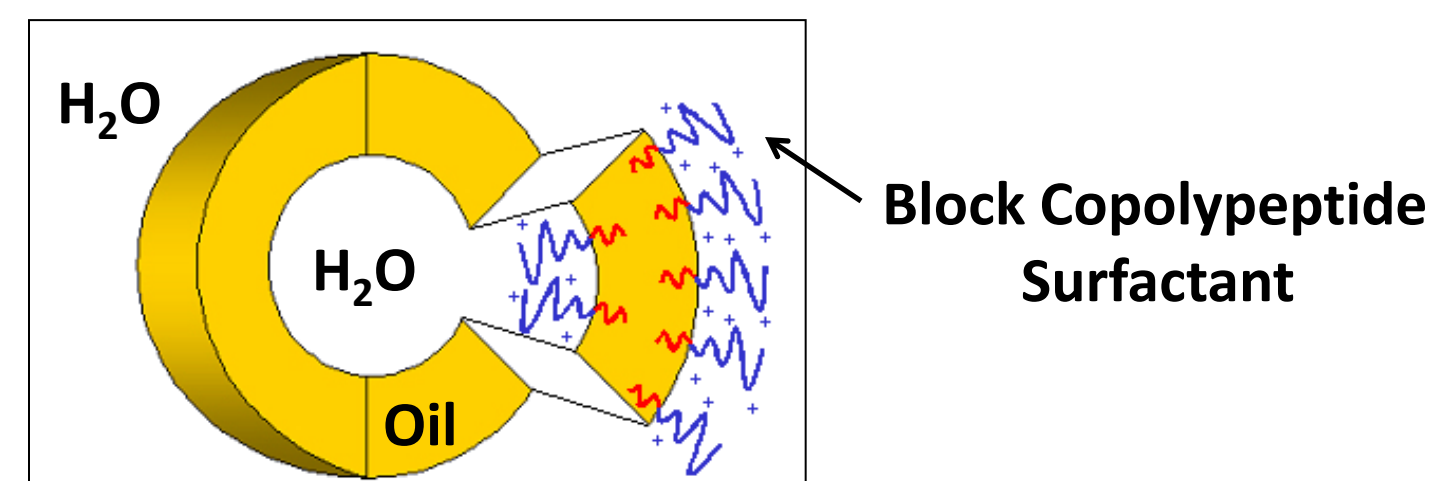
Hydrophilic poly(L-lysine) head group, hydrophobic oliyoleucine tail  $K_{55}(\text{rac-L})_{20}$  was selected for further study

## Nanoscale $K_{55}(\text{rac-L})_{20}$ Double Emulsions

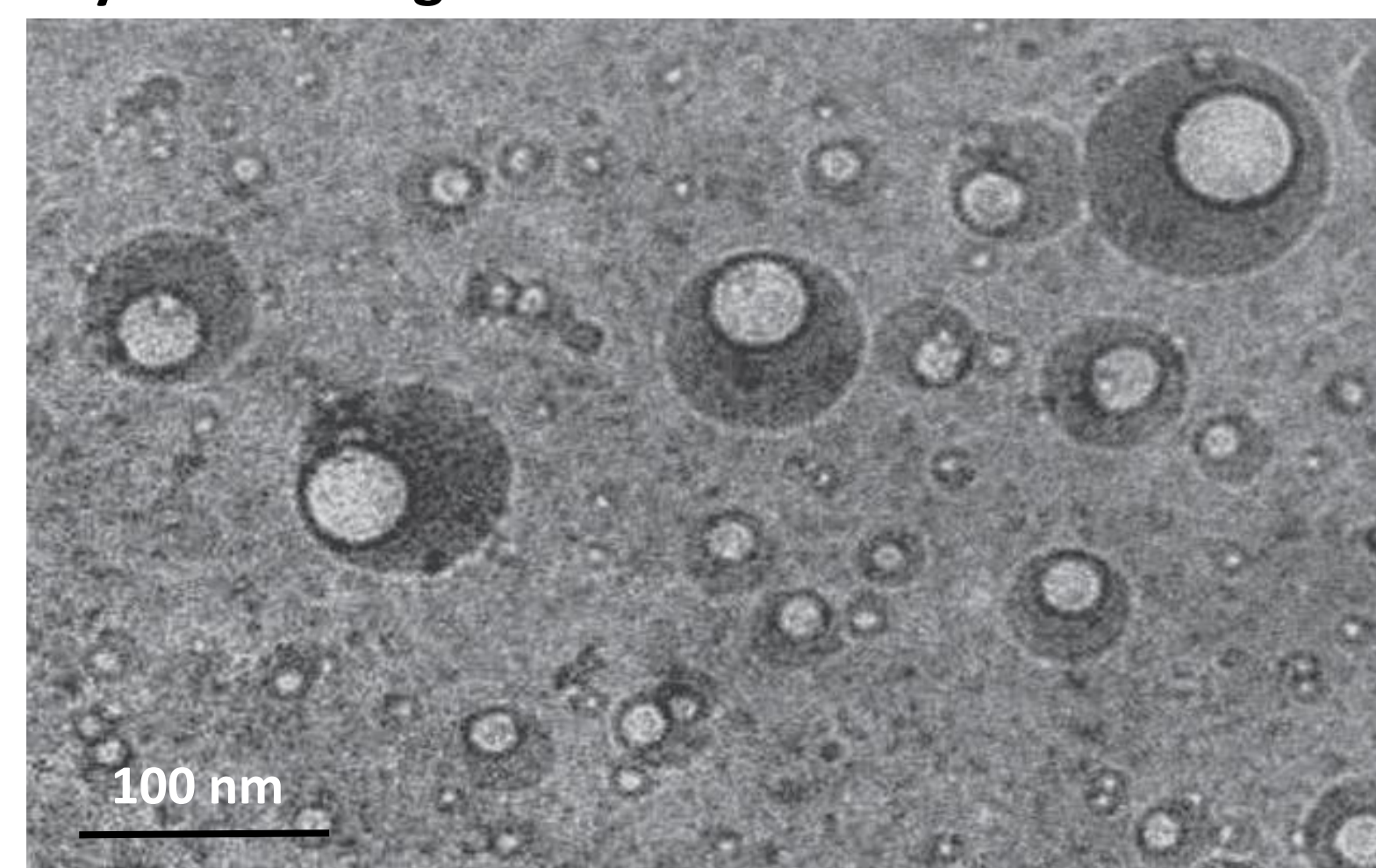
### Advantages:

- Single step, single surfactant
- Stable for long time periods
- Nanoscale (can reach  $\sim 100 \text{ nm}$  in diameter)

### Double Emulsion Structure:



### Cryo-TEM image:

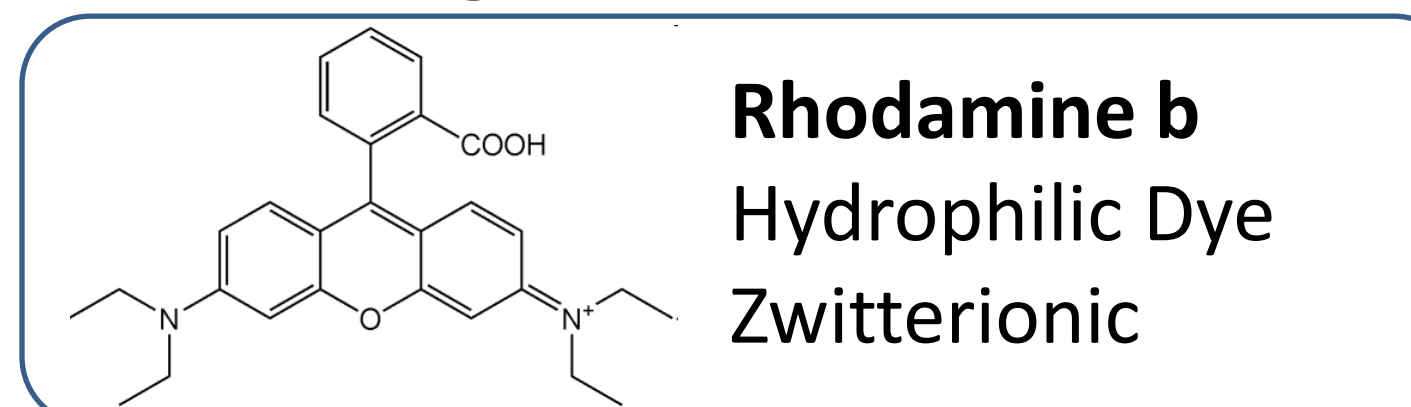


(Cryogenic Transmission Electron Microscopy)

## Encapsulation of Model Cargos

### Surfactant and Model Cargo Chemical Structures:

#### Model Cargos:

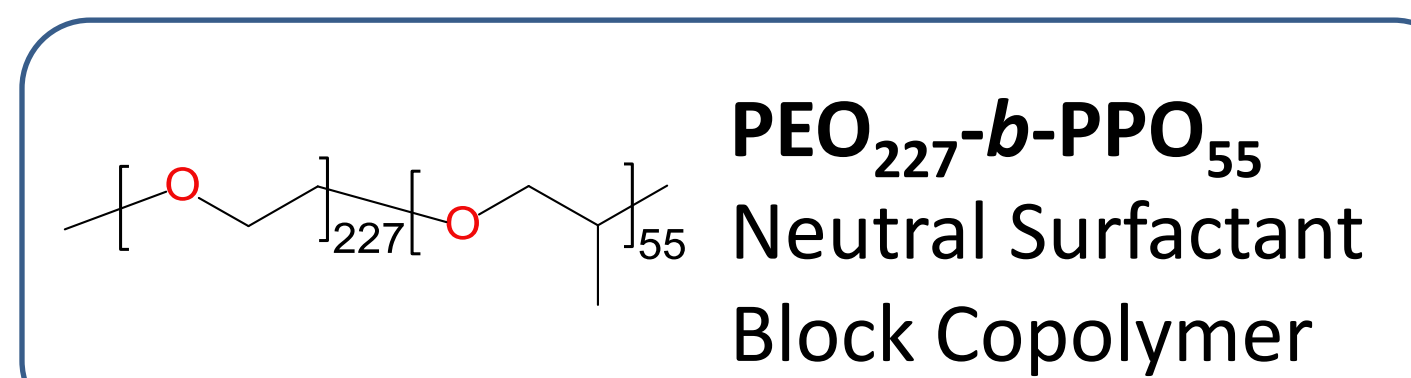


**Rhodamine b**  
Hydrophilic Dye  
Zwitterionic



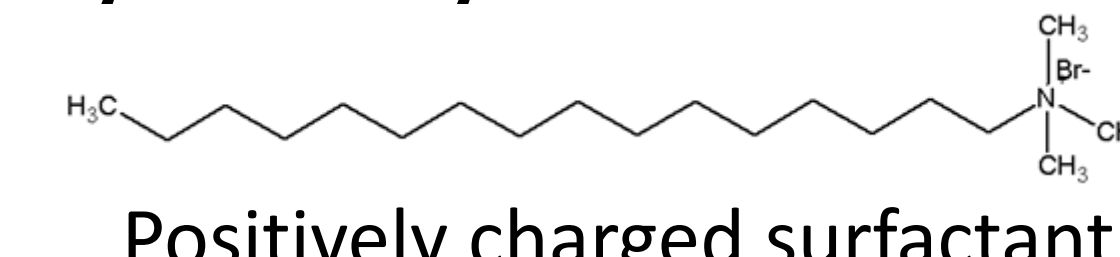
**Pyrene**  
Hydrophobic Dye  
Neutral  
Water Solubility: 0.135 mg/L

#### Control Surfactants:



**PEO<sub>227</sub>-b-PPO<sub>55</sub>**  
Neutral Surfactant  
Block Copolymer

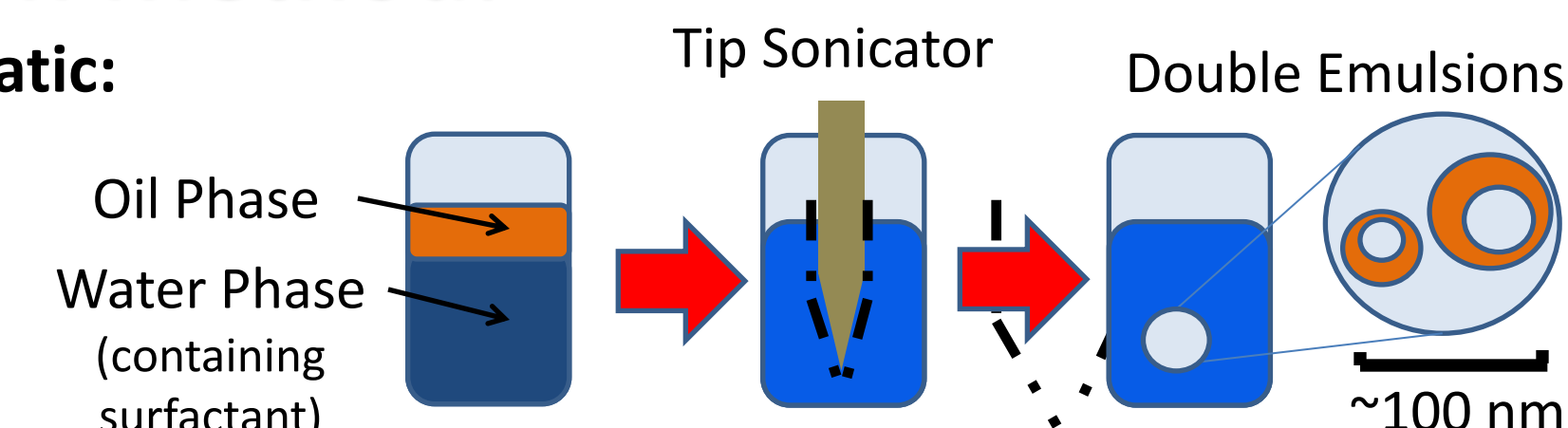
#### Cetyl Trimethyl Ammonium Bromide



Positively charged surfactant

### Emulsification Method:

#### Preparation Schematic:



#### Dialysis Setup:

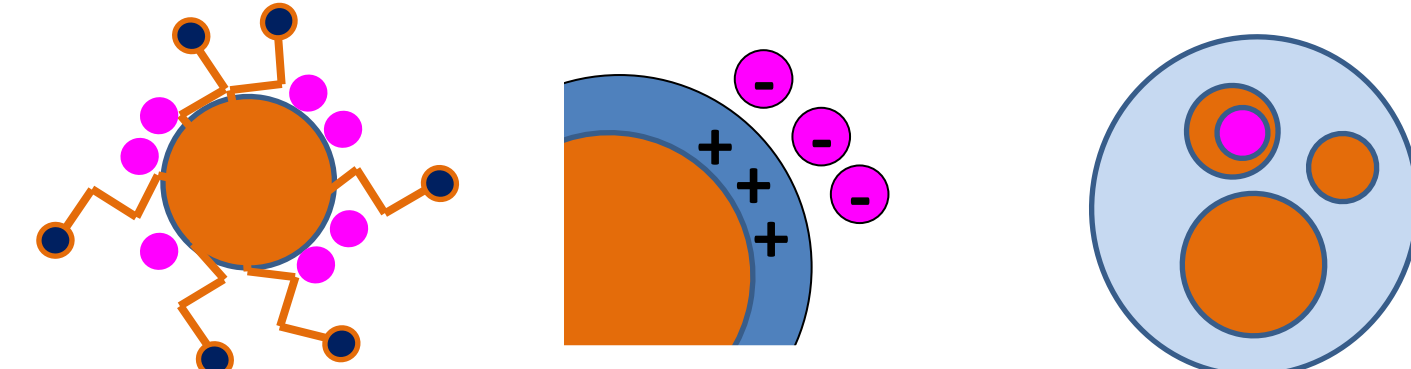
- Dialysis membrane loaded with emulsion containing dye loaded into known volume of dialysate
- Concentration of dye in dialysate can be determined by fluorescence

#### Experimental Setup



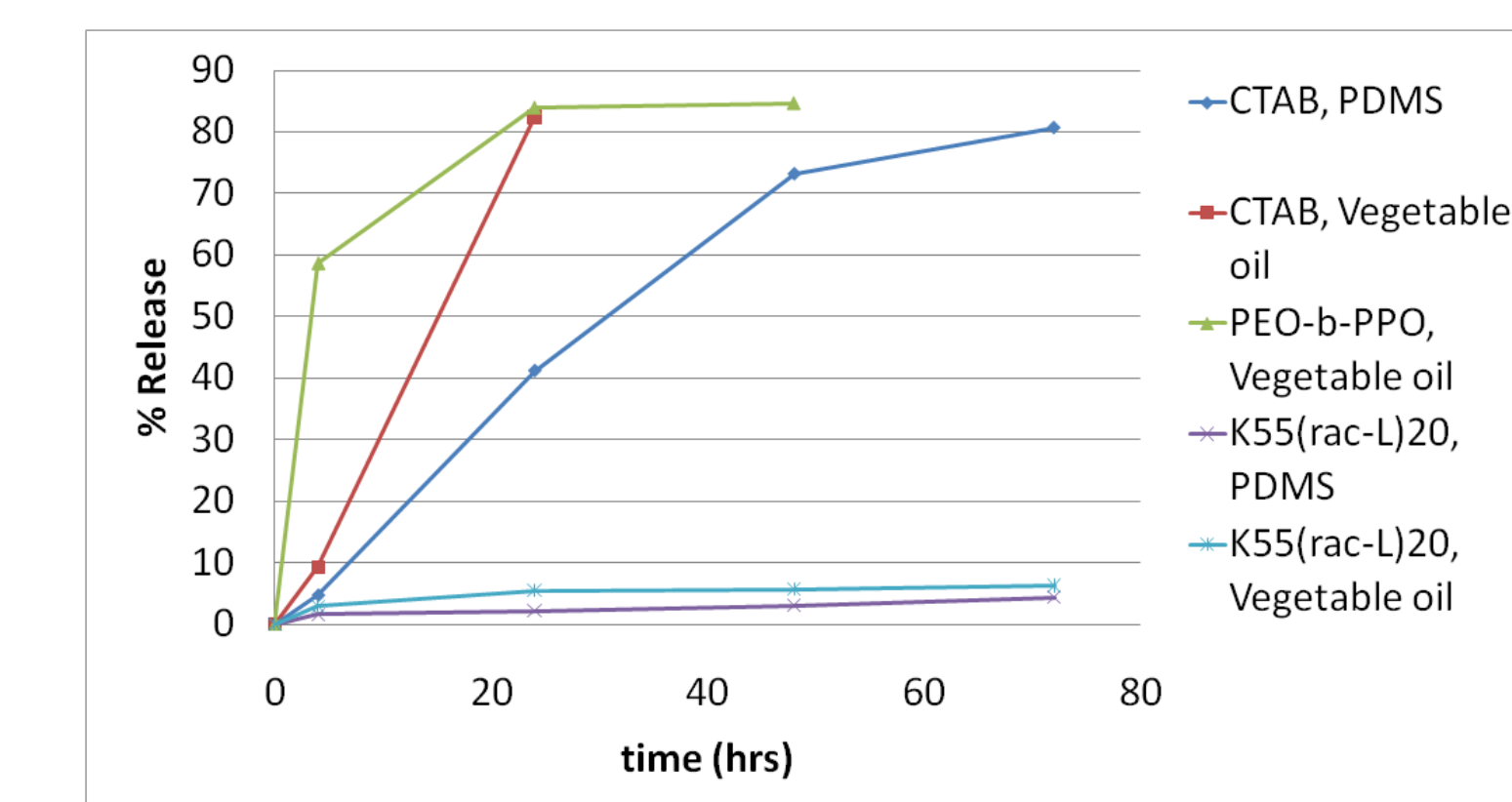
### Dye Retention by Emulsions:

Surface Adsorption Charge Interaction Encapsulation



- Dye delayed from release by three main methods
- Control single emulsion system needed to verify sustained release is due to encapsulation

### Release Profiles of Rhodamine B from Various Oil Phases:

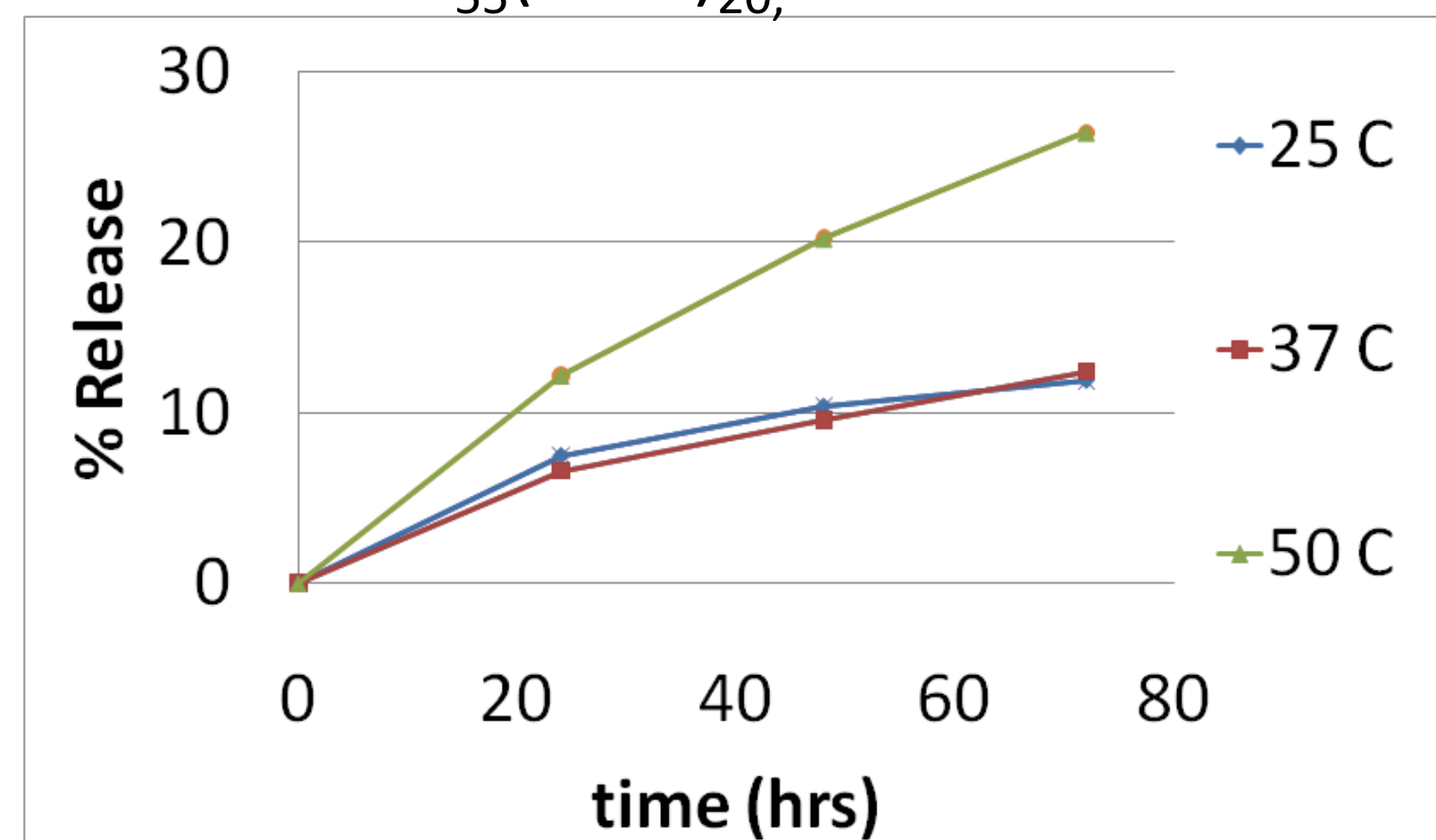


- Near complete release from single emulsion samples
- Very delayed, slow release from  $K_{55}(\text{rac-L})_{20}$  samples
- Good indication of applicability for drug release

### Release Profiles of Rhodamine B Under External Stresses:

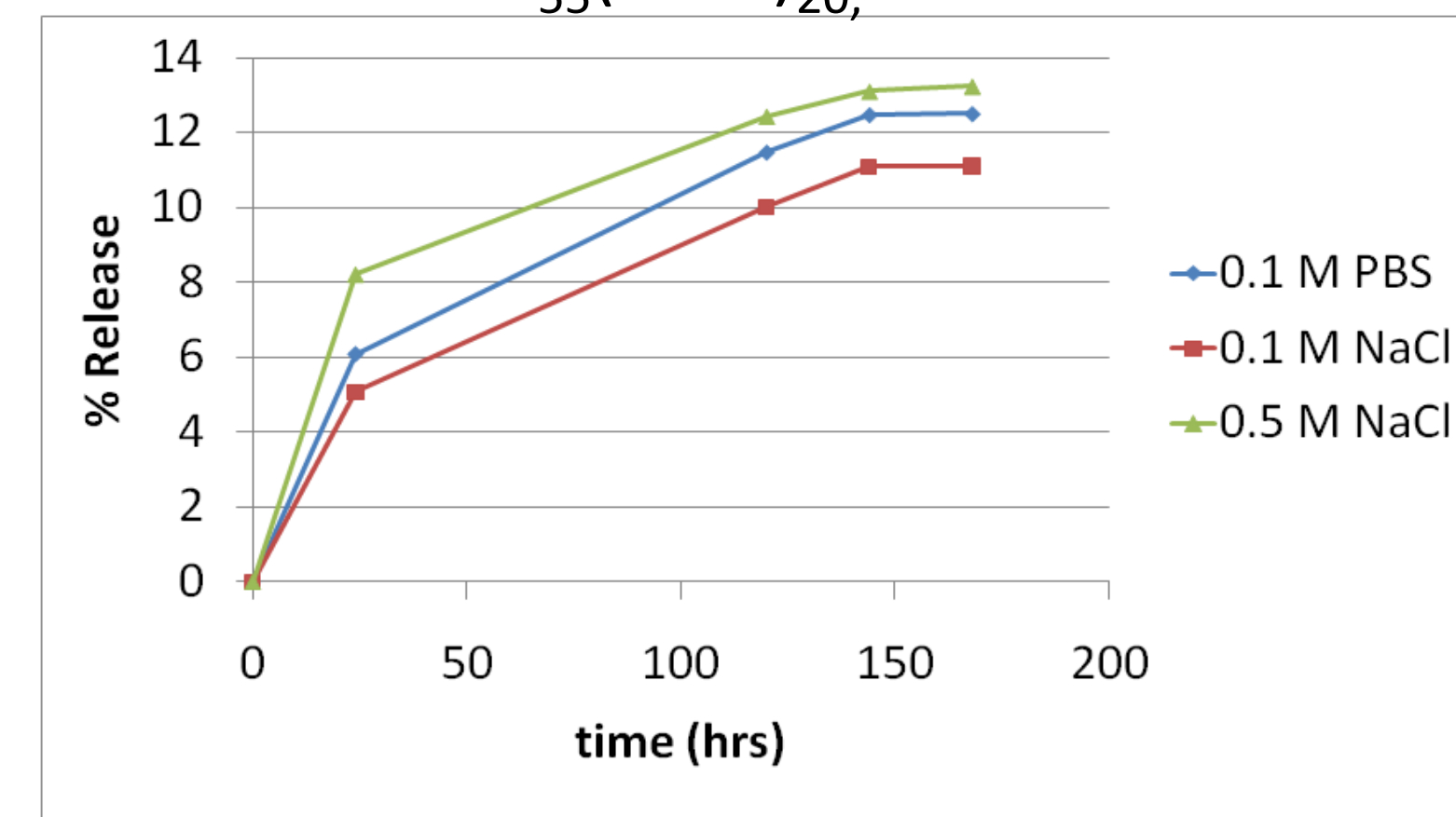
#### Effects of Increased Temperature:

$K_{55}(\text{rac-L})_{20}$ , 0.1 M PBS



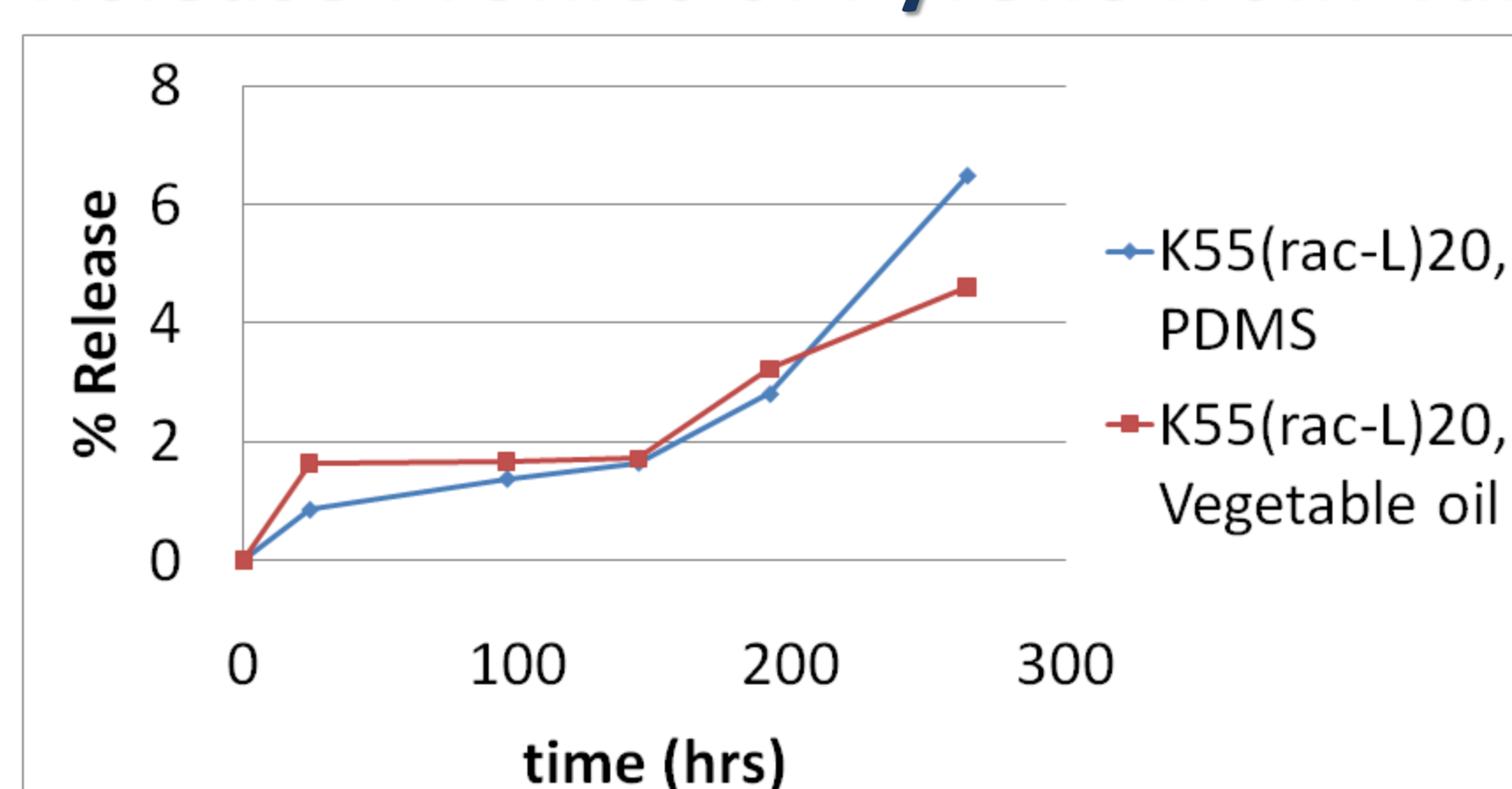
#### Effects of Dialysate Salt Content:

$K_{55}(\text{rac-L})_{20}$ , 25°C



- At simulated *in vivo* conditions,  $K_{55}(\text{rac-L})_{20}$  demonstrated stability and gradual release
- Increased osmotic pressure differences between internal and external aqueous phases increased release

### Release Profiles of Pyrene from Various Oil Phases:



- Release profile of mock hydrophobic cargo is nearly identical in PDMS and Vegetable oil
- Demonstrates very low, stable release

## Future Work

- Additional quantitative studies using more accurate dialysis techniques and longer time periods
- Encapsulation of additional mock cargos, including potential drugs
- Simultaneous encapsulation of both hydrophilic and hydrophobic compounds

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C (N) S I

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