



Modification of USP Apparatus 4 for Application to Liposomes and Other Nanoparticulates.

Diane J. Burgess, Ph.D.

Board of Trustees Distinguished Professor
School of Pharmacy, University of Connecticut

Marketed Liposome Products

Product	Company	Drug	Indication
Ambisome	Gilead	Amphotericin B	Fungal Infection, leishmaniasis
DaunoXome	Gilead	Daunorubicin	Kaposi's sarcoma
Doxil	Ortho Biotech	Doxorubicin	Ovarian cancer
Myocet	Elan	Doxorubicin	Ovarian cancer, AIDS related Kaposi's sarcoma
Depocyt	SkyePharma/Enzon	Cytarabine	Lymphomatous meningitis
Abelcet	Enzon	Amphotericin B	Fungal Infection
Caelyx	Schering-Plough	Doxorubicin	Ovarian cancer
Amphotec	InterMune	Amphotericin B	Fungal Infection
DepoDur	Endo/SkyePharma	Morphine sulphate	Epidural management of pain following major surgery
Visudyne	Novartis	Verteprofin	Age-related macular degeneration
Epaxal	Berna Biotech	Hemagglutinin	Hepatitis A
Inflexal	Berna Biotech	Influenza virus antigen	Influenza
Pevaryl	Janssen-Cilag	Econazole	Dermatocytosis
LMX4	Ferndale	Lidocaine	Topical Anesthetic

Marketed Nanosuspension Products

Drug	Indication	Route	Marketed	Company
Rapamune (sirolimus)	Immuno- suppresant	Oral	2001	Wyeth/Elan
Emend (aprepitant)	Anti-emetic	Oral	2003	Merck/Elan
Tricor (fenofibrate)	Lipid regulation	Oral	2004	Abbott/Elan
Megace ES (megesterol acetate)	Eating disorders	Oral	2005	Par/Elan
Trigilde (fenofibrate)	Lipid regulation	Oral	2005	Sciele Pharma /Skyepharma
Abraxane (paclitaxel)	Anti-cancer	I.V	2005	Abraxis Bioscience/ AstraZeneca

Key Issues Controlled Release Parenteral Products

- ▶ *In vitro* release testing (quality assessment, *in vivo* relevance),
 - ▶ *In vitro-in vivo* correlation
 - ▶ Drug stability during manufacture
 - ▶ Shelf-life stability testing (physical and chemical)
 - ▶ *In vivo* stability
 - ▶ Sterility assurance, sterility testing, particulate matter
 - ▶ Particle size analysis
 - ▶ Bioavailability, bioequivalence assessment
 - ▶ Qualification of new biopolymers
 - ▶ Residual solvent levels
 - ▶ Reconstitution
 - ▶ ⁴Nomenclature
-

Lack of *In Vitro* Release Testing Methods

- There is no standard method to test *in vitro* release for the purposes of:

- Routine assessment of process quality control;
- Formulation optimization in product development;
- As well as for the development of *in vitro-in vivo* relationships (IVIVR).



***In Vitro* Drug Release For MR Parenteral Dosage Forms**

- **Can we use one apparatus?**
 - ▶ Different methods for different dosage forms
 - ▶ Different APIs

FDA and USP now in process of adopting Apparatus 4 as method of choice for microspheres and possibly other CR parenterals

- **Media?**
- **Sampling method others?**
- **Total percent release?**

In Vivo Factors Affecting Drug Release

- **Delivery System Independent (Type I)**
 - **Barriers to drug diffusion: fluid viscosity,**
 - **tissue barriers (e.g. connective tissue)**
 - **Drug partitioning at the site**
 - **Available volume at the site**
 - **Motion at Site**
- **Delivery System Dependent (Type II)**
 - **Enzymatic degradation of delivery system**
 - **Protein adsorption**
 - **Phagocytosis**
 - **Inflammatory response**

In Vitro Release Test for Liposomes?

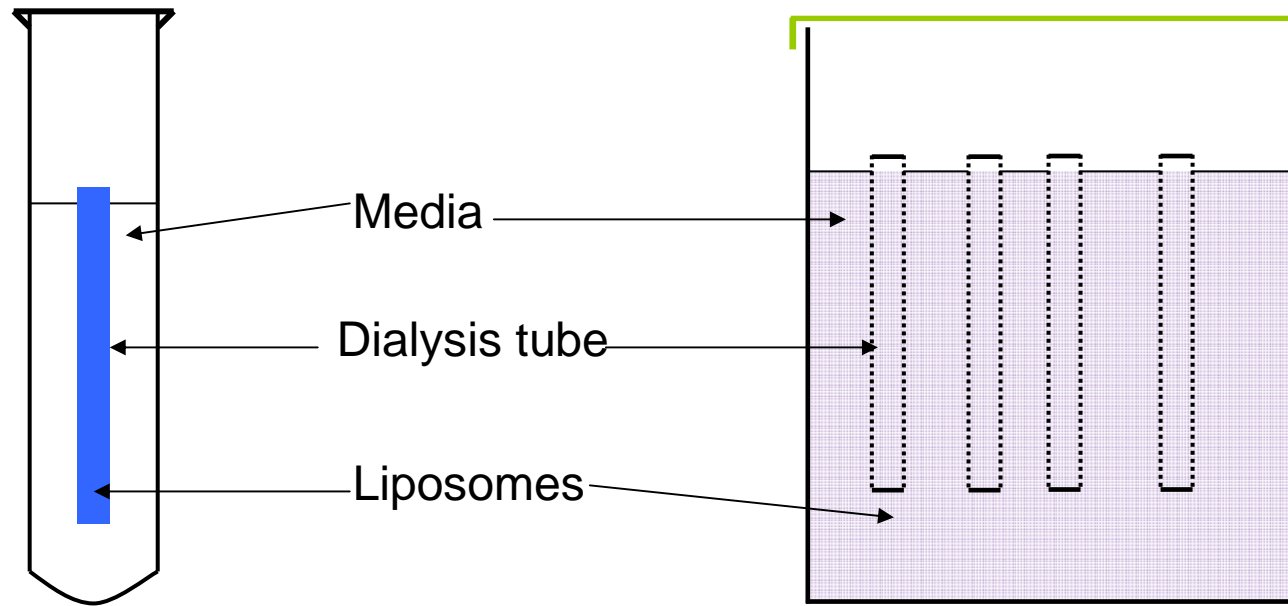
Different Routes of Administration

Liposomes

Considered three methods:

- **Dialysis sac** – may correlate with less perfused route of administration.
- **Reverse dialysis sac** – may correlate with more perfused route of administration
- **USP apparatus 4** – may correlate with less perfused route of administration

In Vitro release



Dialysis sac

Media: 50 ml HEPES buffer, 10 mM, pH 7.4

Membrane: Spectrapor Dispodialyzer 50 kDa
MWCO

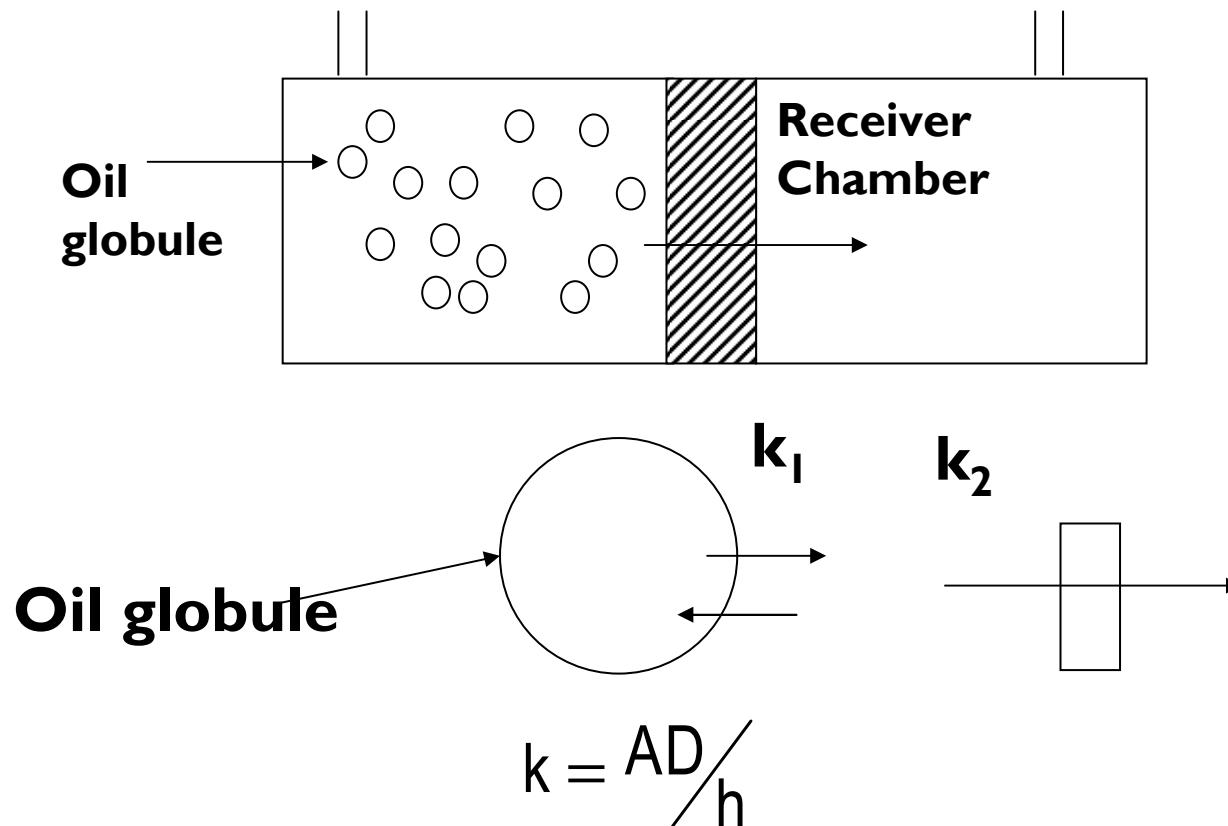
Reverse dialysis sac

Media: 125 ml HEPES buffer, 10 mM, pH 7.4

Membrane: Spectrapor Dispodialyzer 50 kDa
MWCO

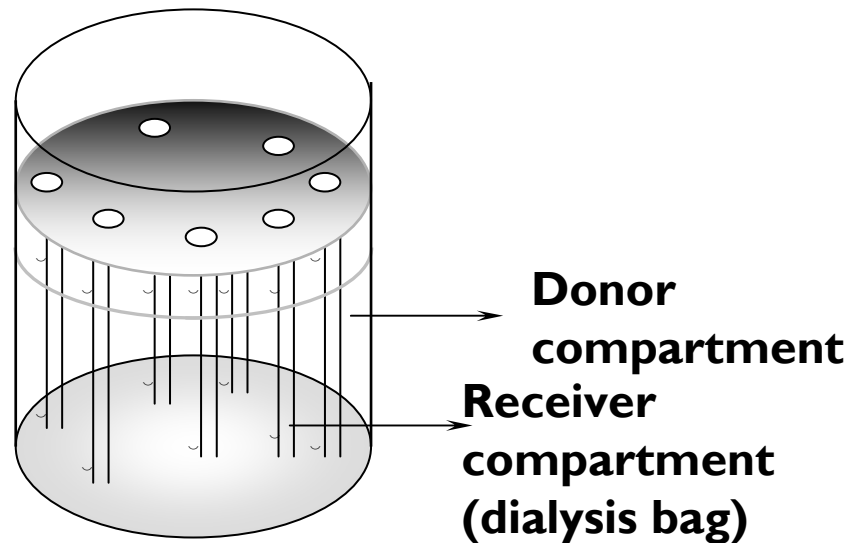
Conventional *In Vitro* Technique

- Side-by-side diffusion cell technique



Equilibrium Reverse Dialysis Bag Technique

Overcome limitations of side-by-side diffusion cell technique,

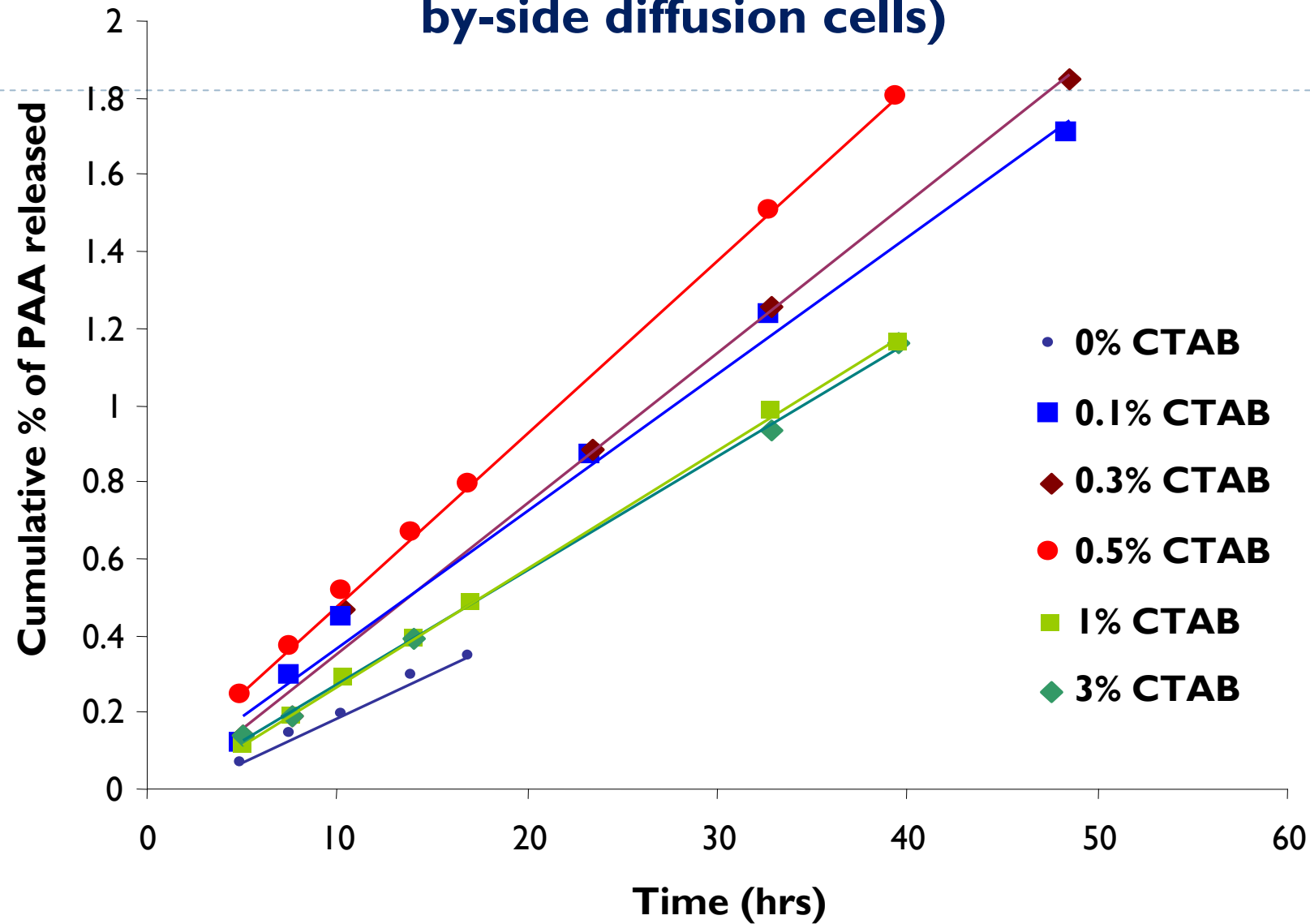


Continuous phase of the donor chamber is diluted infinitely (~100 times)

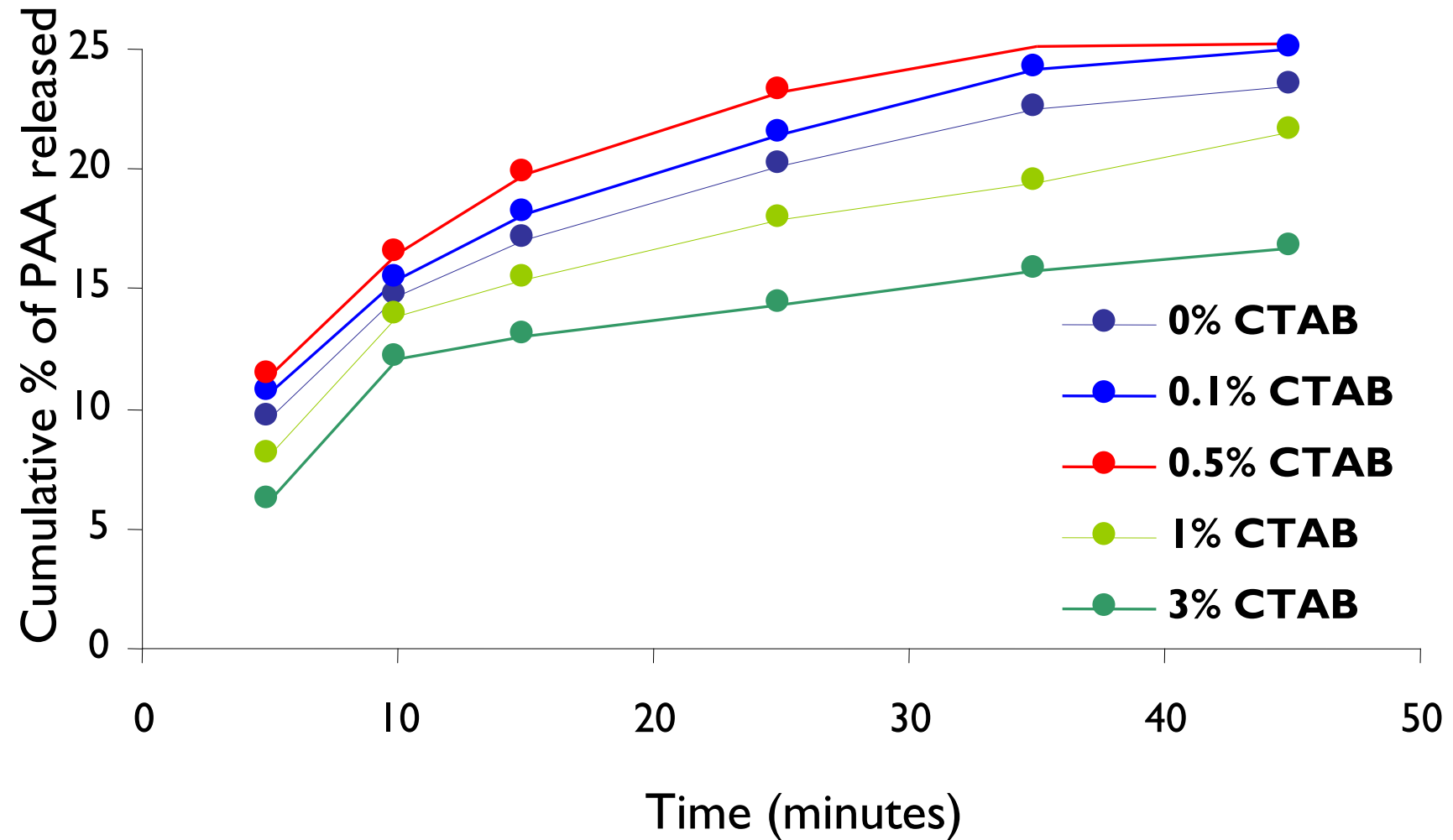
Membrane surface area is increased

N. Chidambaram and D.J. Burgess: A novel method to characterize *in vitro* release from submicron emulsions. *AAPS PharmSci.*, (1999), August 31, 1999; 1(3): Approx. size: 33k + 350k in images. Available at www.aapspharmaceutica.org

Release Rate of PAA Through 1 KD Membrane (side-by-side diffusion cells)



Release Rate of PAA through IKD



In Vitro Release of 5-FU: Microdialysis

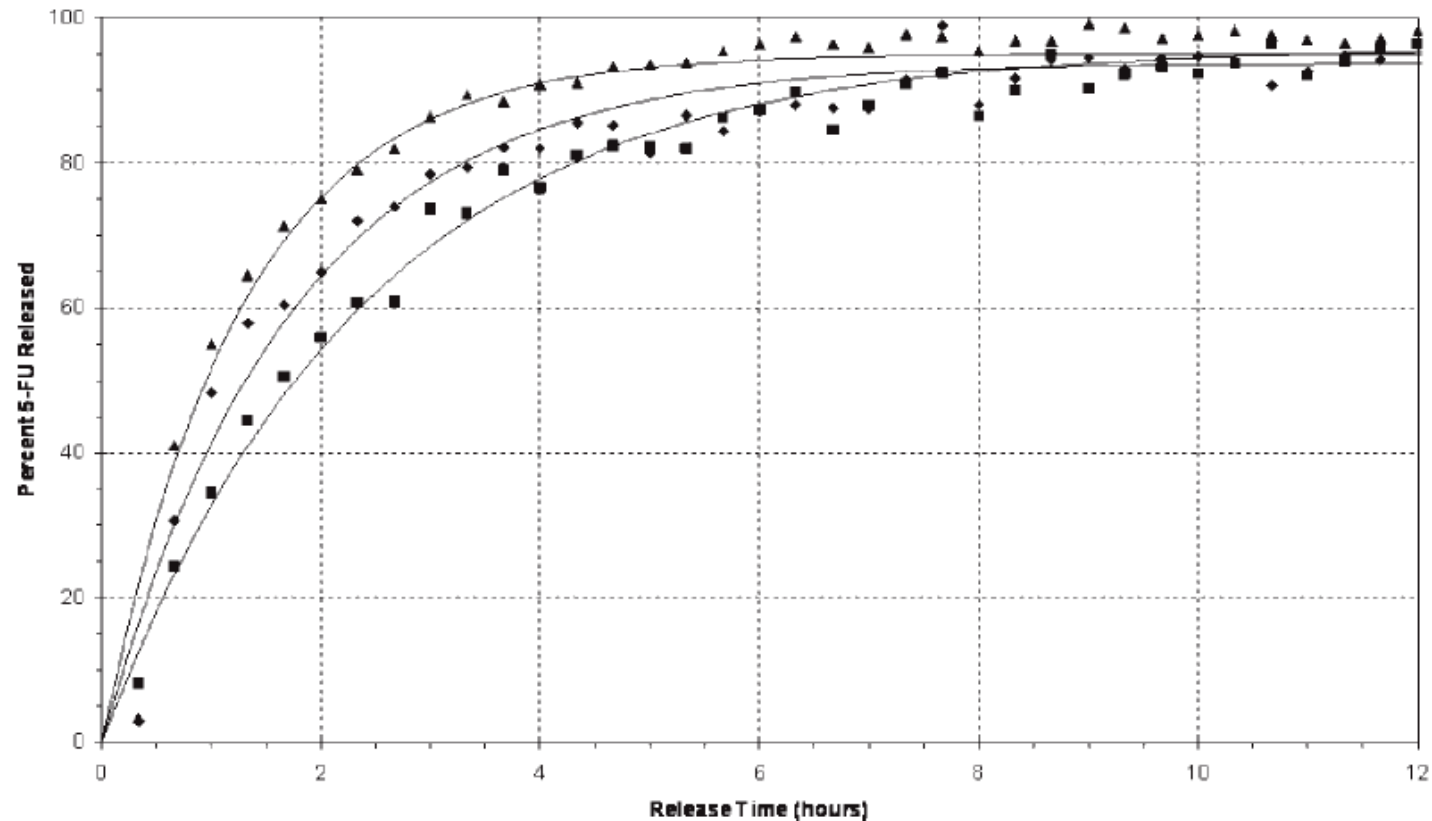
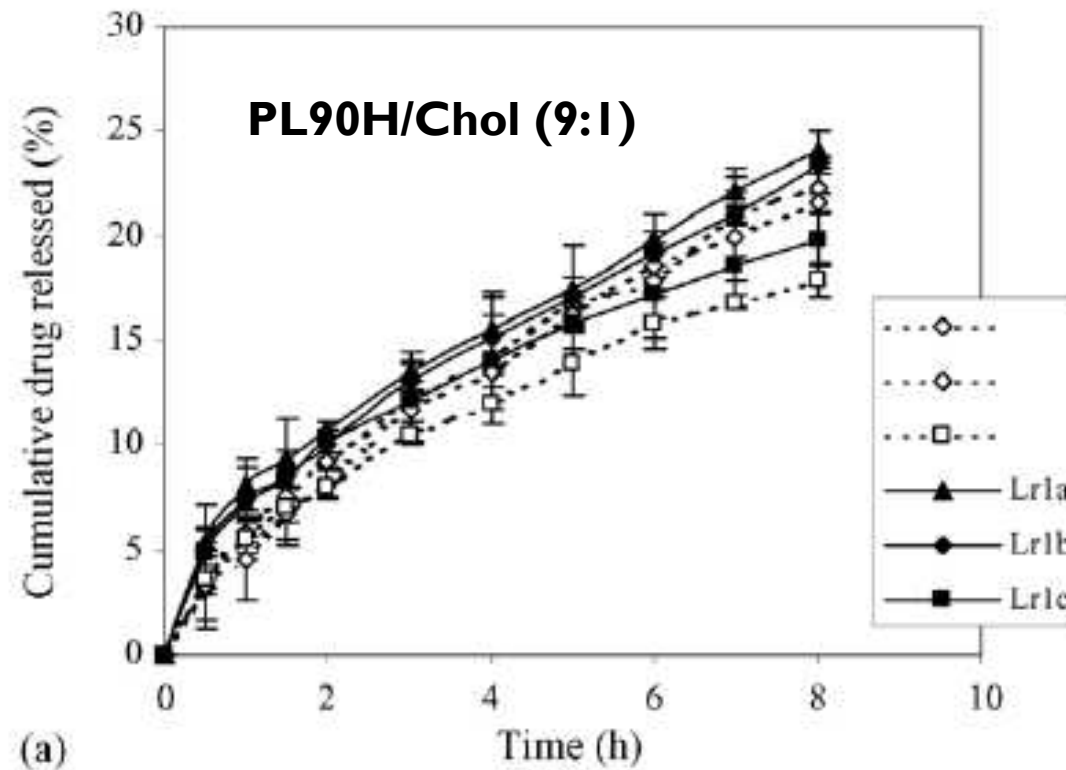


Figure Liposome release profiles: (\blacktriangle): HSPC:Chol:DPPA liposomes (65:25:10 mol%, extruded); (\blacklozenge): DPPC:Chol:DPPA liposomes (65:25:10 mol%); (\blacksquare): HSPC:Chol:DPPG liposomes (65:25:10 mol%).

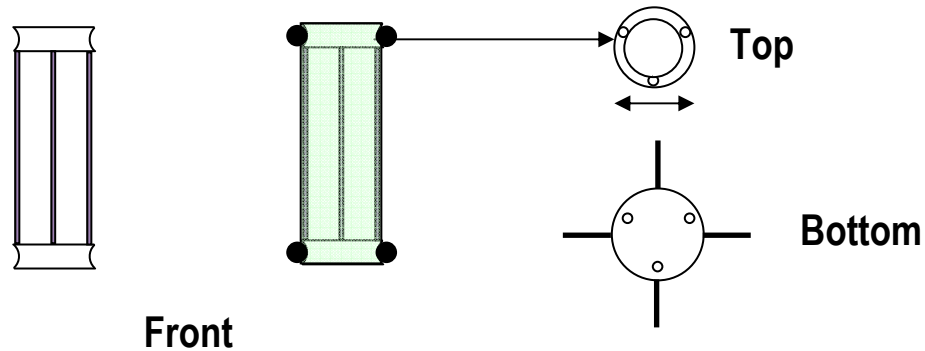
In Vitro Release of 5-FU: Dialysis



- Dialysis sac, 100 rpm, MWCO not mentioned
- Reconstituted lyophilized MLVs

No study comparing different methods for CR liposome formulation

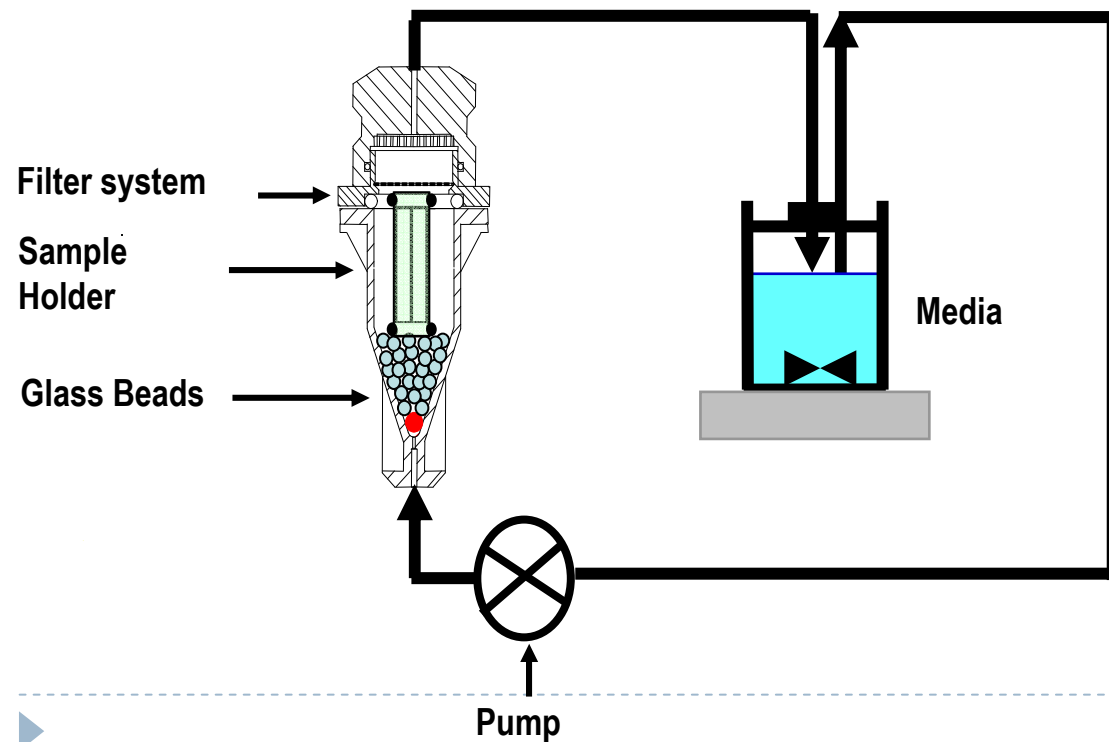
USP Dissolution Apparatus 4 Dialysis Adapter



Media: 100 ml HEPES buffer, 10 mM,
pH 7.4

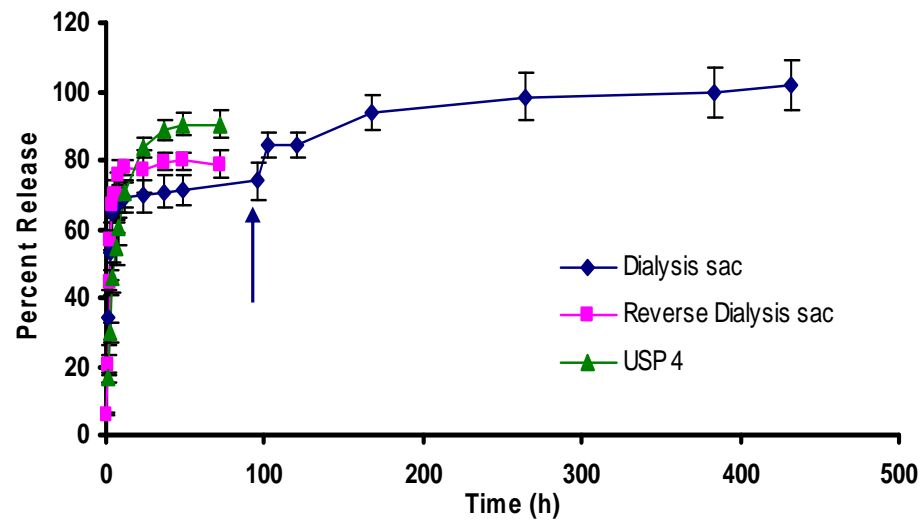
Membrane: Spectrapor 50 kDa MWCO

Flow rate: 16 ml/min

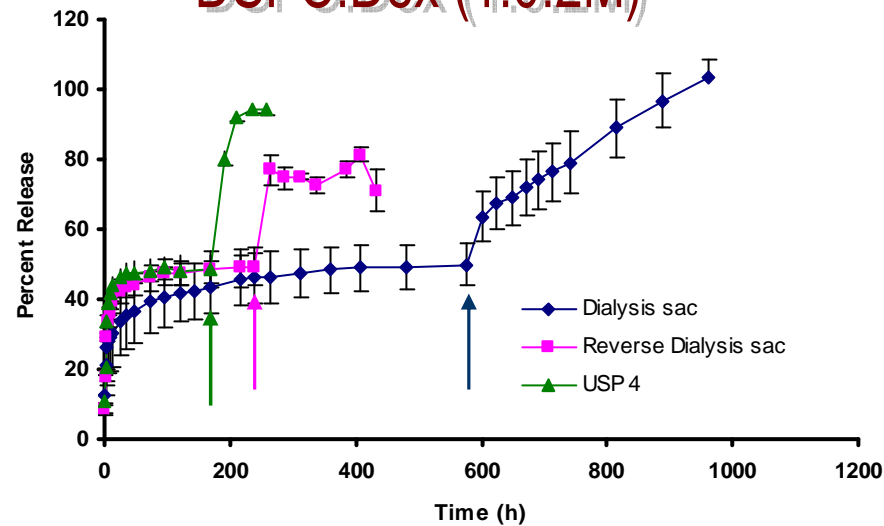


In Vitro Release : Method Comparison

DMPC:Dex (1:0.2M)

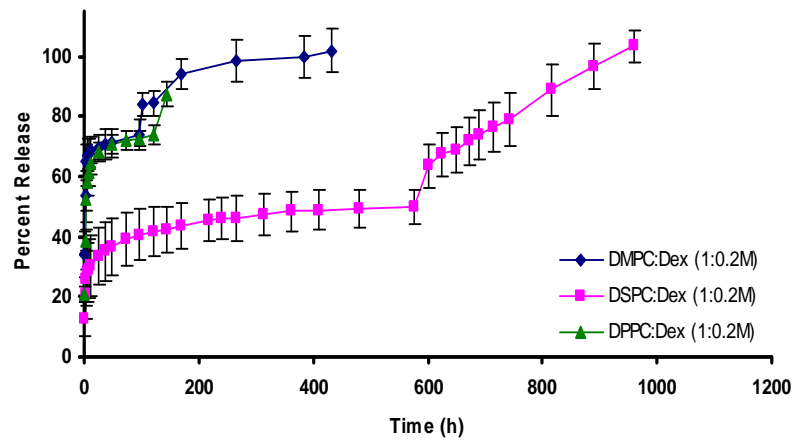


DSPC:Dex (1:0.2M)

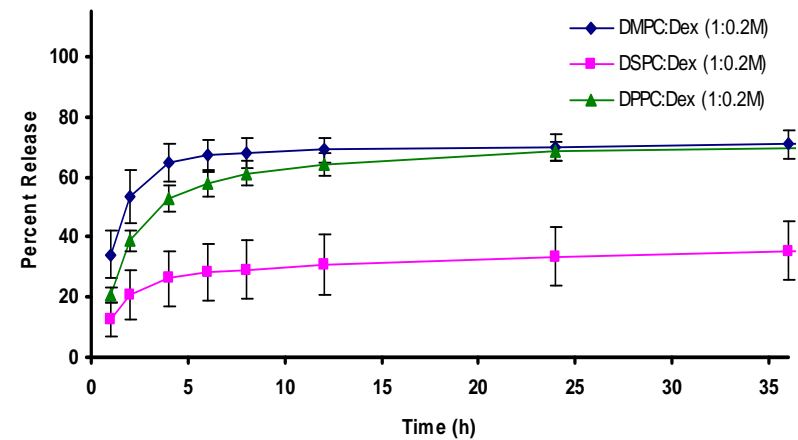


In Vitro Release : Method Comparison

Discrimination with dialysis method

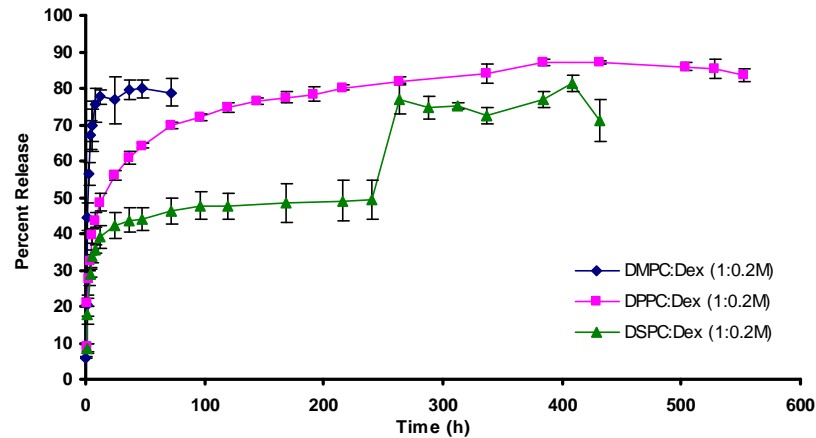


Initial release phase

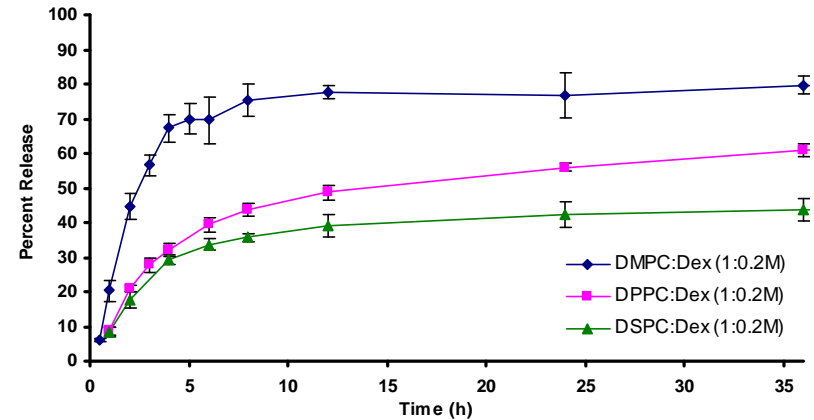


In Vitro Release : Method Comparison

Discrimination with Reverse dialysis method

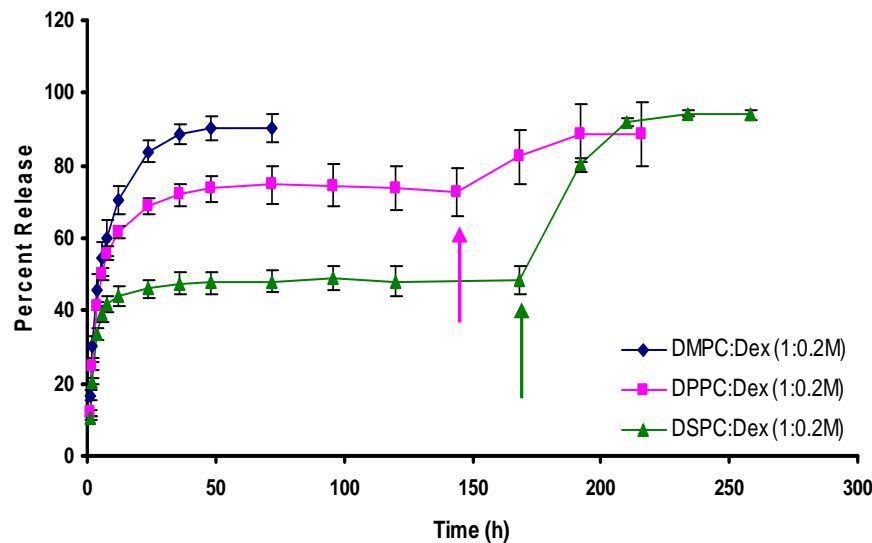


Initial release phase

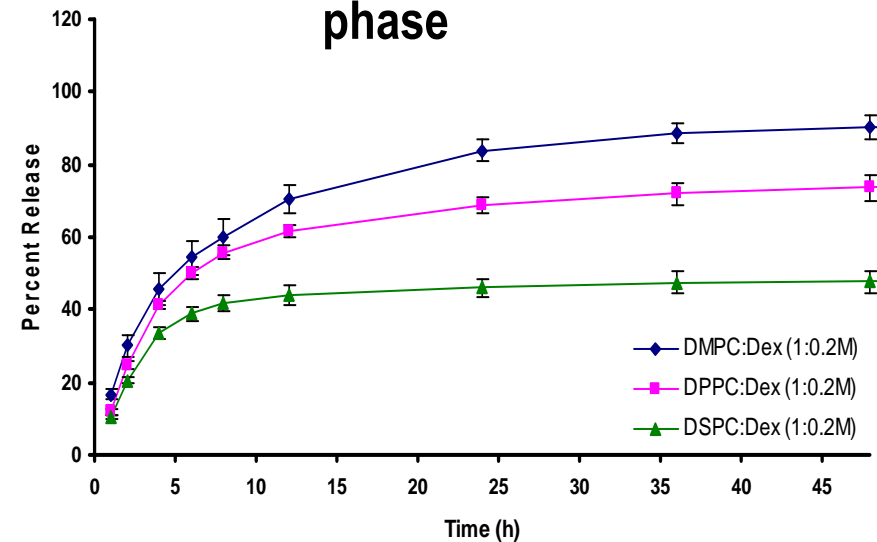


In Vitro Release: Method Comparison

Discrimination with USP Apparatus 4 adapter



Initial release phase

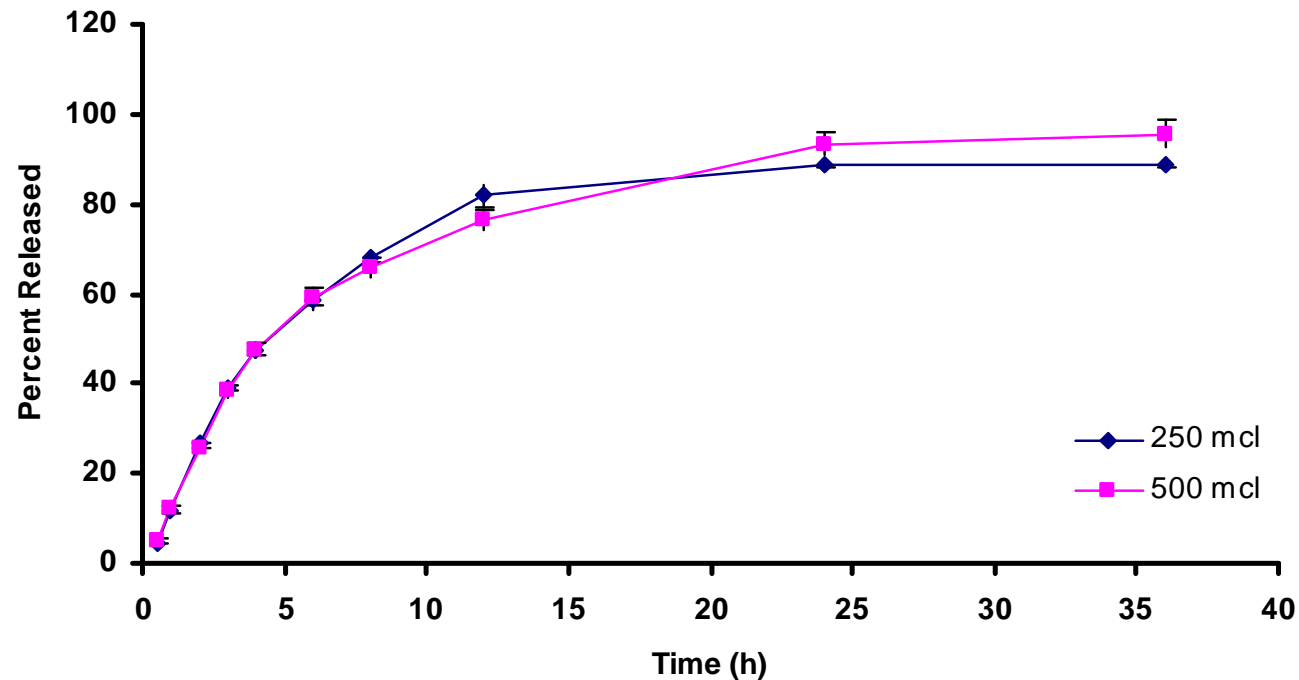


- USP 4 can show discrimination between different liposome formulations of dexamethasone.
- USP 4 is compendial and can be standardized for Dispersed/liquid dosage forms.



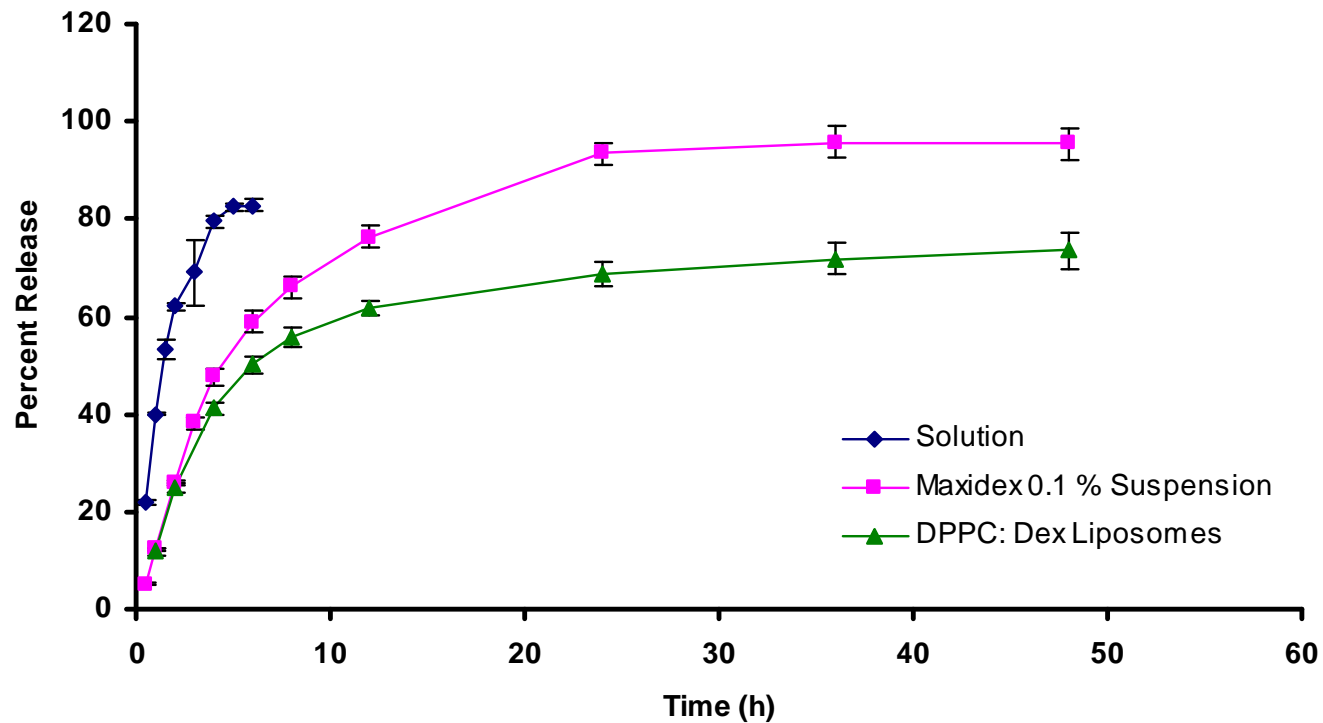
USP 4: *Suspension Using App. 4 Adapter*

Maxidex® 0.1 % ophthalmic Dexamethasone suspension (Alcon labs)



USP 4: *Release from Different Formulations*

Comparison of solution, suspension and DMPC liposomes release using USP 4

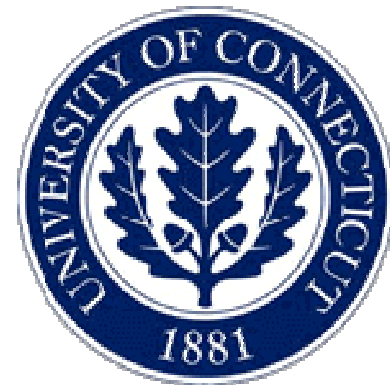


Acknowledgements

- ▶ Dr. Tae-Kyoung Kim
- ▶ Dr. Young-Ho Song
- ▶ Dr. Nachiappan Chidambaram
- ▶ Dr. Jie Shen

- ▶ Dr. Siddhesh Patil
- ▶ Dr. Jim Jiao
- ▶ Dr. Banu Zolnik
- ▶ Dr. Charudharshini Shrinivasan
- ▶ Dr. Jacqueline Morais
- ▶ Dr. Upkar Bhardwaj
- ▶ Dr.. Sarah Eccleston
- ▶ Dr. Sudhir Verma
- ▶ Dr. Archana Rawat
- ▶ Dr. Jennifer Voisine
- ▶ Ms. Tammy Hickey
- ▶ Ms. Misiya Norman
- ▶ Ms. Tiriza Saziru
- ▶ Mr. Don Shin
- ▶ Mr. Hyungchul Kim
- ▶ Mr. Xiaoming Xu
- ▶ Ms. Mamta Kapoor
- ▶ Ms. Yan Wang
- ▶ Mr. Sumit Kumar
- ▶ Mr. Mikail Kastellorizios

Professor Fotios Papadimitrakopoulos, Polymer Program,
UCONN
Dr. Izabela Galeska, Dr . Santhisagar Vaddiraju



- ▶ 28 Ms. Emma Vaughan
- ▶ Ms. Emma Hardy

Acknowledgements

- ▶ **NIH (1R01RR14171)**
- ▶ **US Army Medical Research Grants (#DAHD17-02-1 0713, #W81XWH-04-1-0779, # W81XWH-05-1-0539, and # W81XWH-07-1-0688).**
- ▶ **Juvenile Diabetes Foundation (JRFI-5-2001-687)**
- ▶ **Patterson Trust Foundation**
- ▶ **Parenteral Drug Association Foundation**
- ▶ **Boehringer Ingelheim Pharmaceuticals Inc.**
- ▶ **NSF Processing Center**
- ▶ **USP (Vinod Shah, Erika Stippler, Joseph Eaton)**
- ▶ **FDA**
- ▶ **GloboMax LLC**
- ▶ **Sotax Corp**
- ▶ **pion Inc (Delphian)**
- ▶ **Pfizer**
- ▶ **AFPE**

