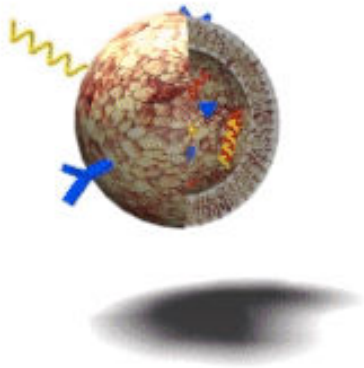


IN VITRO DISSOLUTION TESTING FOR NANO FORMULATIONS



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Deemed University, Elite status and Centre of Excellence (GOM),

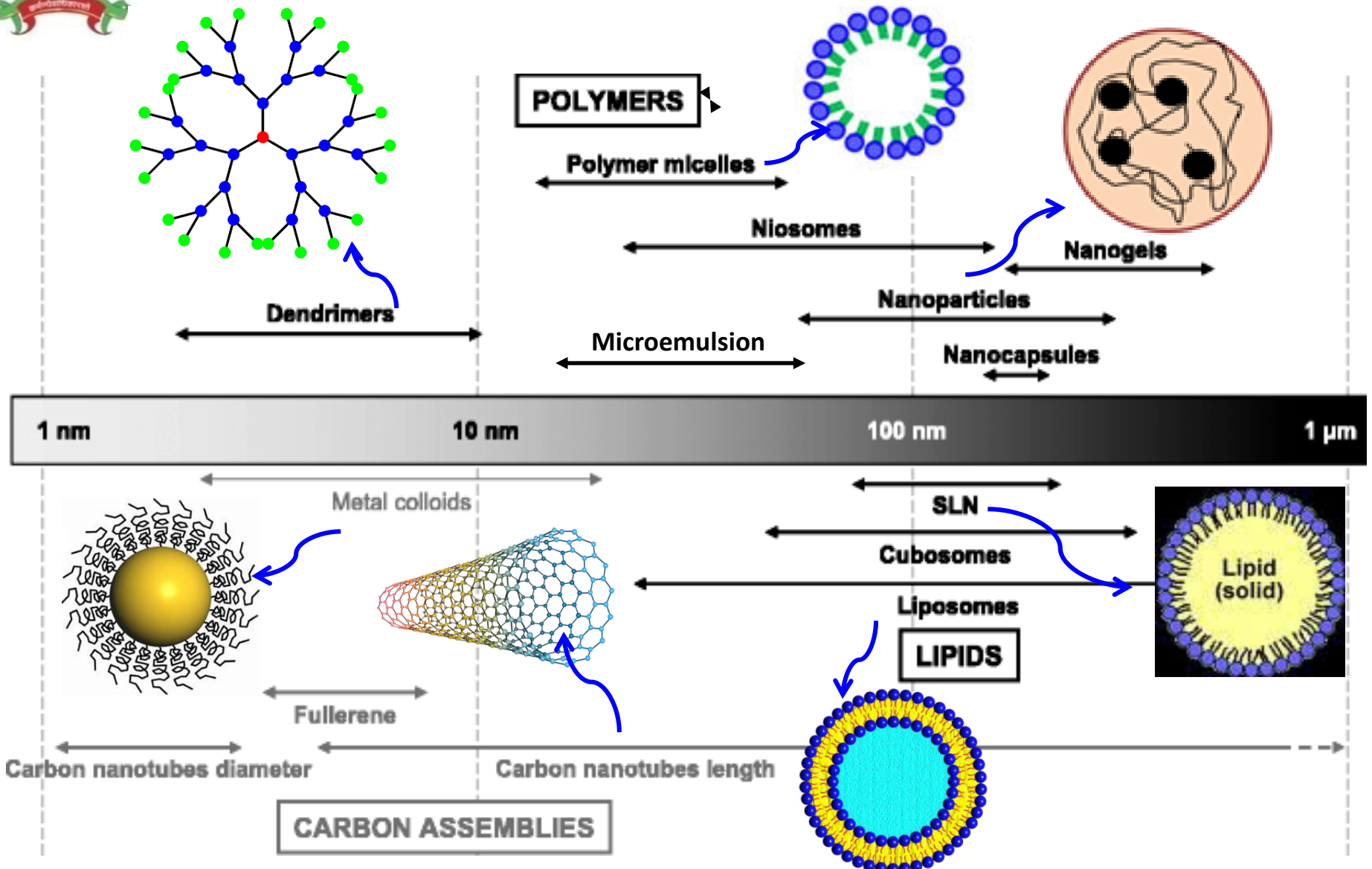
Mumbai 400 019, INDIA

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DISSO INDIA 2017- MUMBAI JUNE 8-9, 2017



NANOCARRIERS IN NANOMEDICINE





ADVANTAGE - NANOMEDICINE

- ✓ TARGETTED DELIVERY
- ✓ ENHANCED BIOAVAILABILITY & EFFICACY
- ✓ DECREASED SYSTEMIC TOXICITY

SUCCESS STORIES

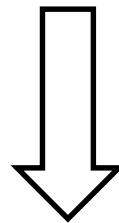
CANCER

INFECTIOUS DISEASES

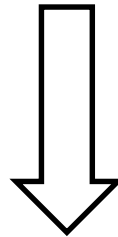


Nanosystems and Need for *in vitro* Dissolution testing

Nanosystems are promising



Unavailability of standardized *in vitro* dissolution method



Urgent Need to develop Standardized Testing Methods



CHALLENGES IN DISSOLUTION METHOD FOR NANOMEDICINES



Size &
Separation

- Difficulty in Separation of NP from medium

Complex
System

- Complexity of System type
- Target specific release
- Environment specific release (pH, temperature)
- Programmed Release
- Biorelevant media



DISSOLUTION METHODS FOR NANOMEDICINES

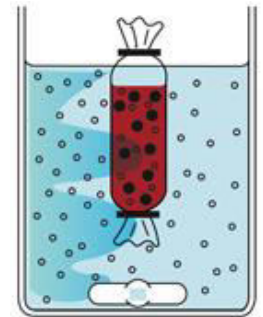


DISSOLUTION METHODS

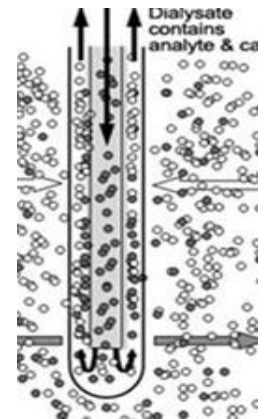
❑ Sample & Separation methods



❑ Membrane Diffusion (Dialysis Sac) methods



❑ Others (Micro dialysis, Dynamic dissolution & 2 stage reverse dialysis)

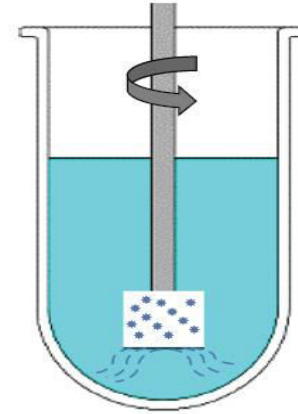




MODIFIED OFFICIAL APPARATUSES

□ Constant Volume

- USP I & II



□ Continuous Flow Methods

- USP IV



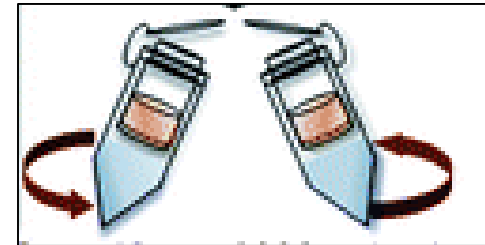


SAMPLE & SEPERATION METHODS

- NP directly added in medium & separation techniques applied
- Drug content in supernatant or filtrate is analyzed



Ultrafiltration



Ultracentrifugation



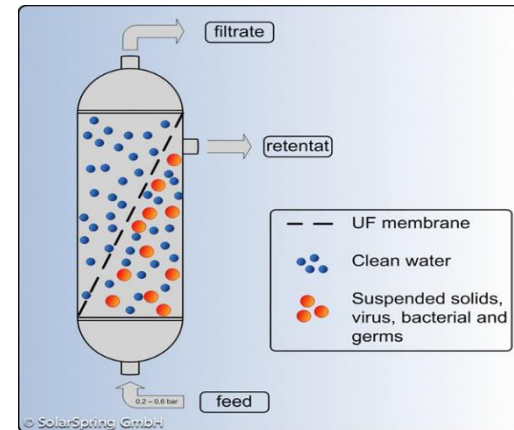
Key parameters : **Sample separation technique**
Agitation conditions



SAMPLE & SEPARATION METHODS

Pressure Ultrafiltration

- Completely separate Nanoparticles from release media within 5 min
- Prevent Clogging of filter pores



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Syringe Filtration

- Use of Syringe filters with smaller pore size (0.1 to 0.02 μm) has been used

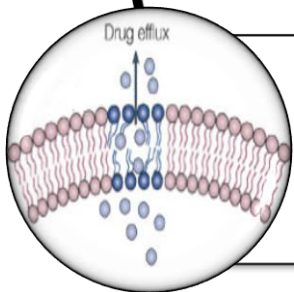




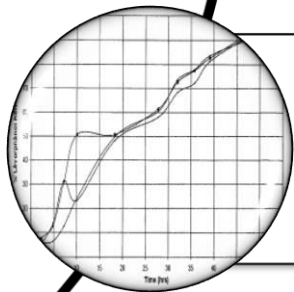
DISADVANTAGES OF SAMPLE & SEPARATION METHODS



Difficulty in separation of NP from media though high external energy applied



Long-time & High speed can result in destabilization of system (e. g. Nanoemulsion & Liposome)

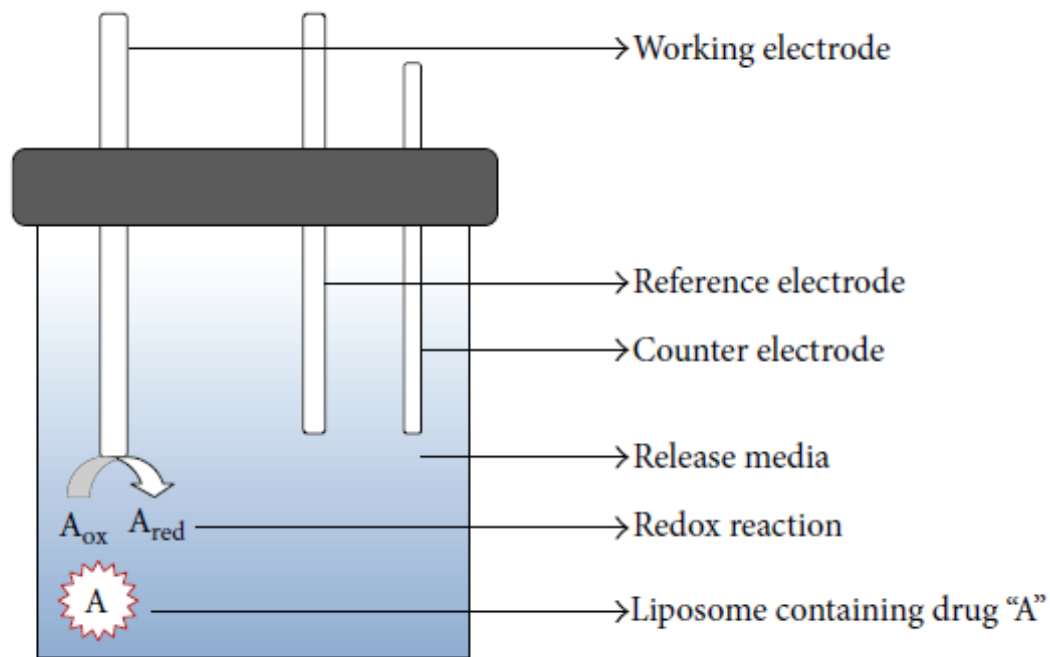


Drug release continues during separation process, which can lead to erroneous results



DYNAMIC DISSOLUTION

ADVANCED SAMPLE AND SEPARATE METHOD



Utilize ion- or drug-selective electrodes to monitor the dissolution/release profiles of electroactive drugs

Not suitable for non-electroactive drugs

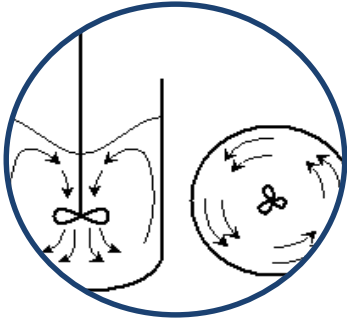


DIALYSIS METHODS

FIXED VOLUME



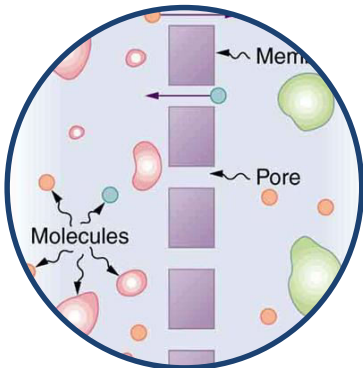
KEY PARAMETERS INFLUENCING DRUG RELEASE IN DIALYSIS METHODS



Agitation Conditions



Ratio between Donor & acceptor cell Volume
Inside Volume 6 to 10 fold less than medium volume



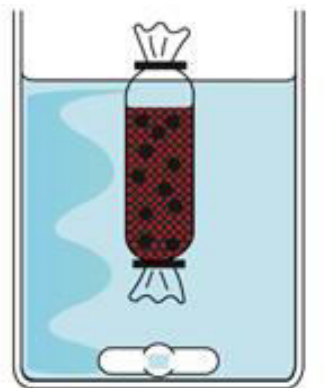
Molecular Weight Cut-Off (MWCO) of
membrane

MWCO 100 times more than drug MW

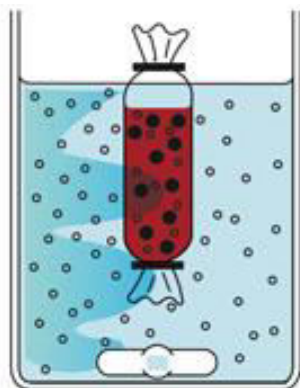


MEMBRANE DIFFUSION METHODS (DIALYSIS BAG)

Nanosystems separated from the release medium through dialysis membranes that are permeable to the free drug but impermeable to the nanosystems

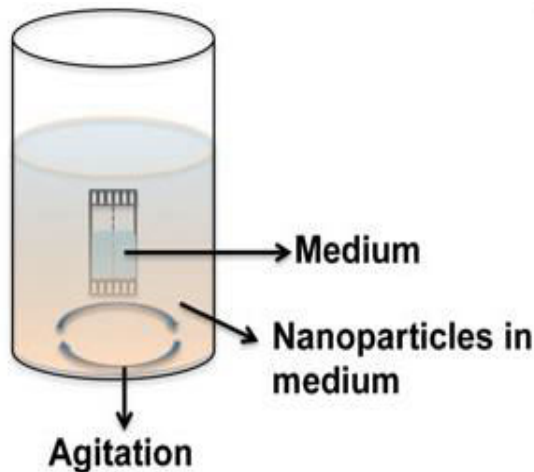


Start Dialysis
(high concentration gradient)

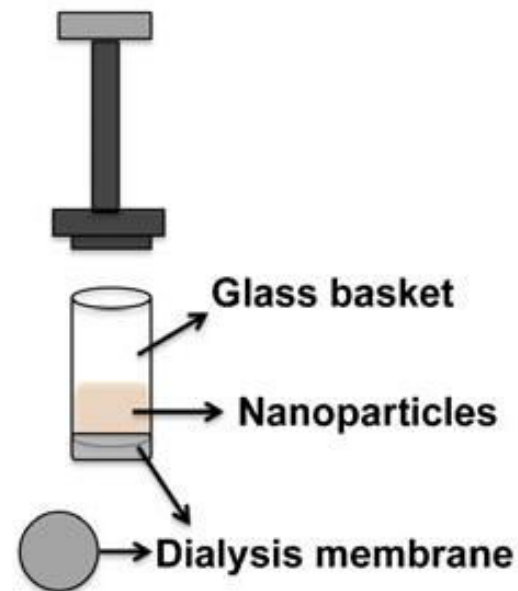


End Dialysis
(equilibrium)

Dialysis Sac Method



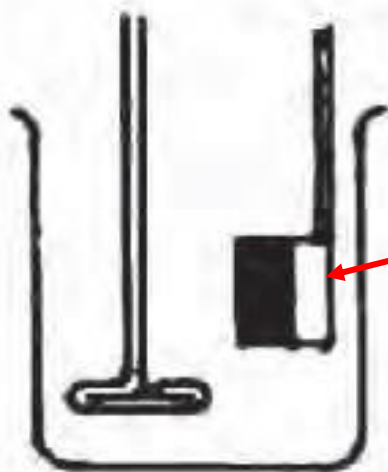
Reverse Dialysis Sac
Method



Side-by-Side-
Dialysis

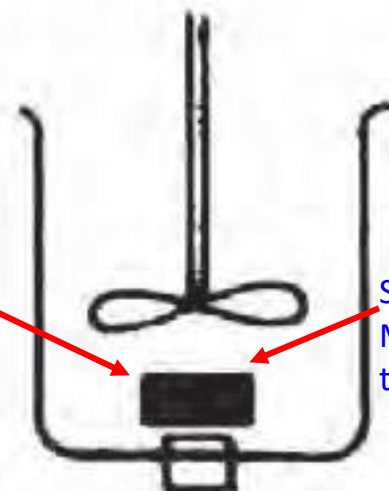


DEVELOPMENTS IN DISSOLUTION METHOD



Stationary basket
Cook (1968)

Dialysis Bag

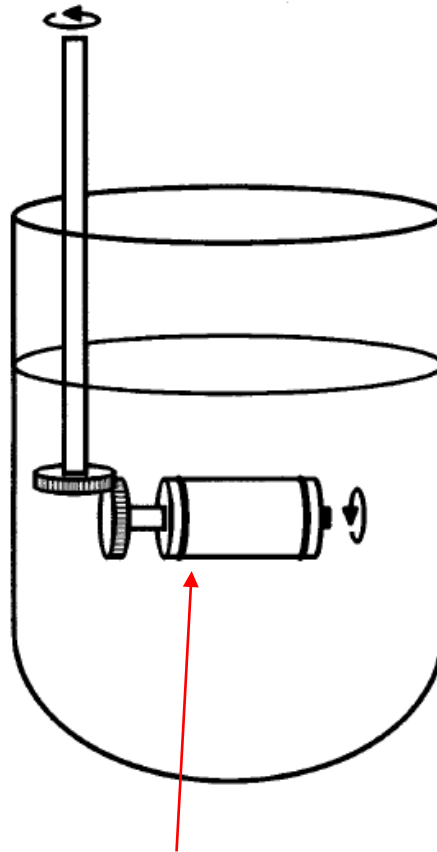


Stirring
Magnet inside
the bag

Magnetic basket
Shepherd, *et al* (1972)



ROTATING DIALYSIS CELL FOR PARENTERAL DEPOT FORMULATIONS

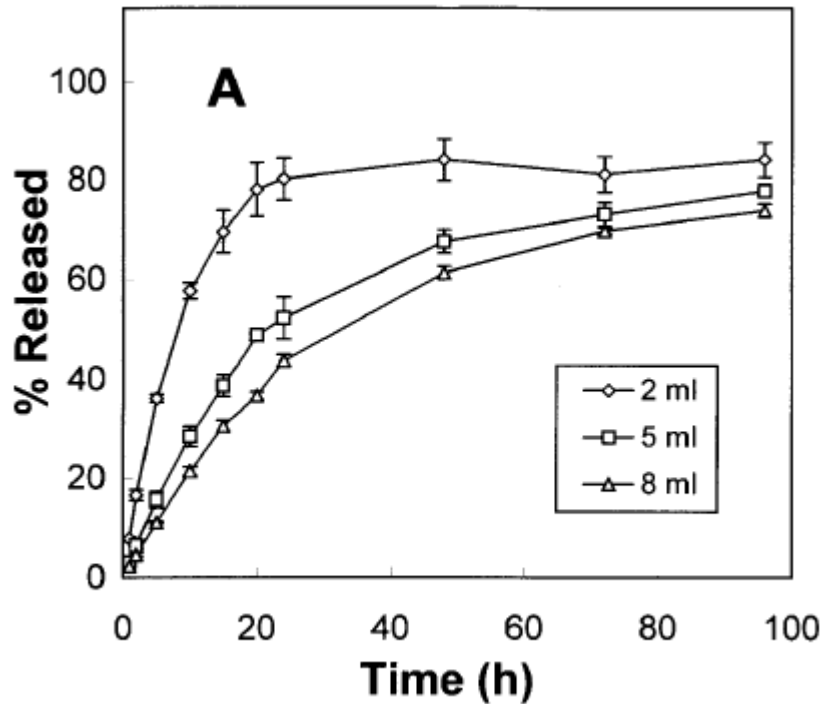


BASKET MODIFIED INTO A DIALYSIS CELL

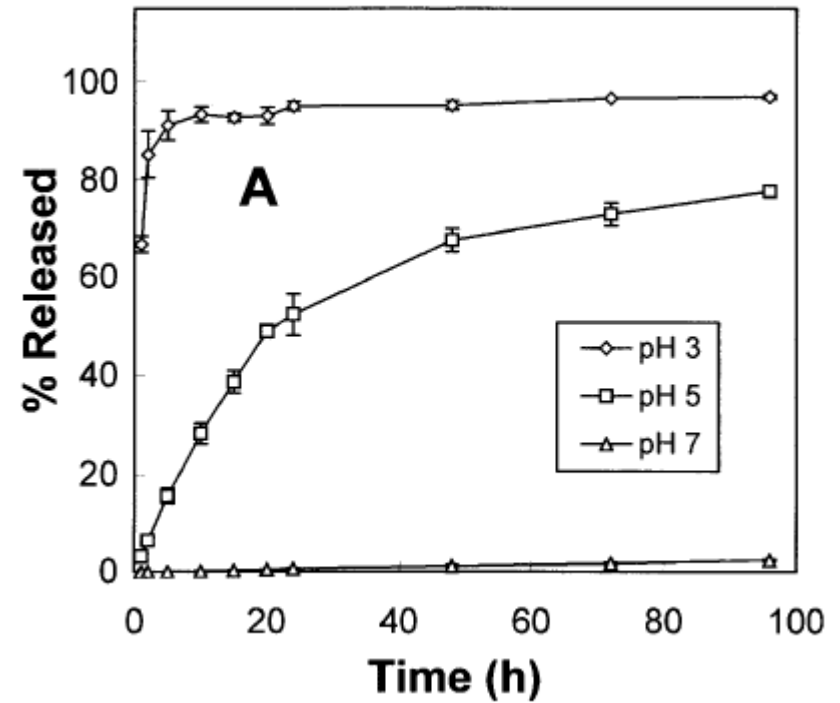
K. Schultz et al, Int. J. Phar., 1997



ROTATING DIALYSIS CELL FOR PARENTERAL DEPOT FORMULATIONS



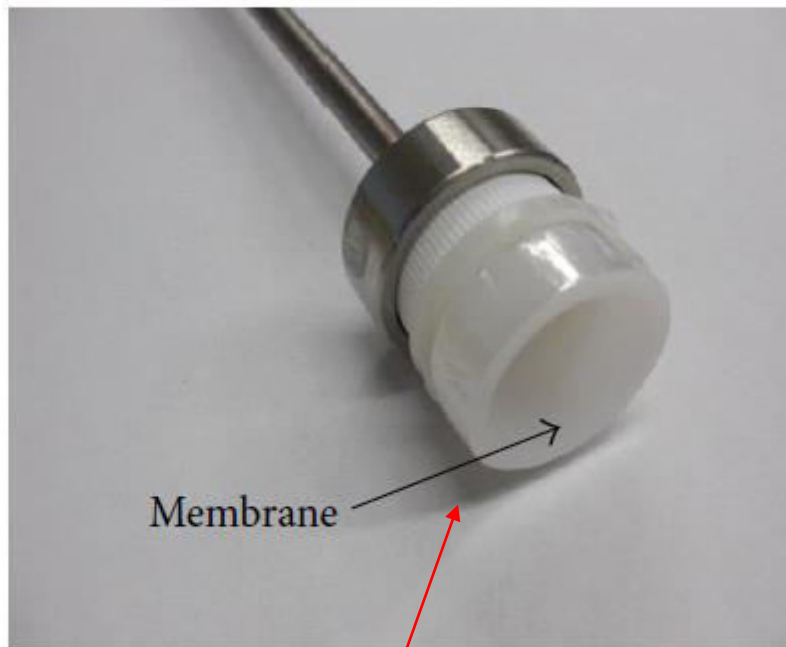
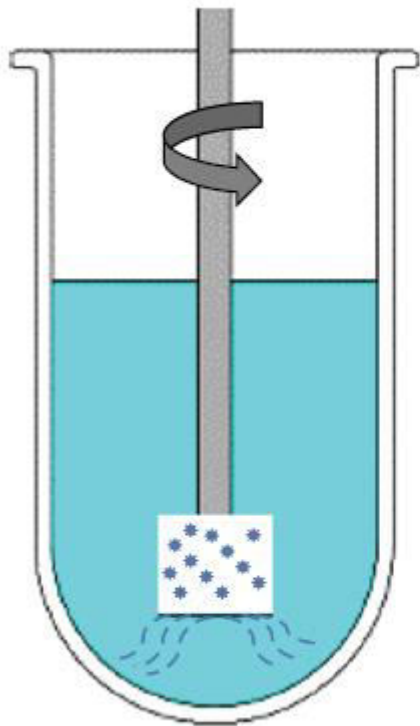
Release profiles at pH 5.00 for three different volumes of formulation. Flupentixol NP depot



Release profiles for 5 ml of formulation at three different pH values.



ADAPTATION OF DIALYSIS AND USP TYPE I

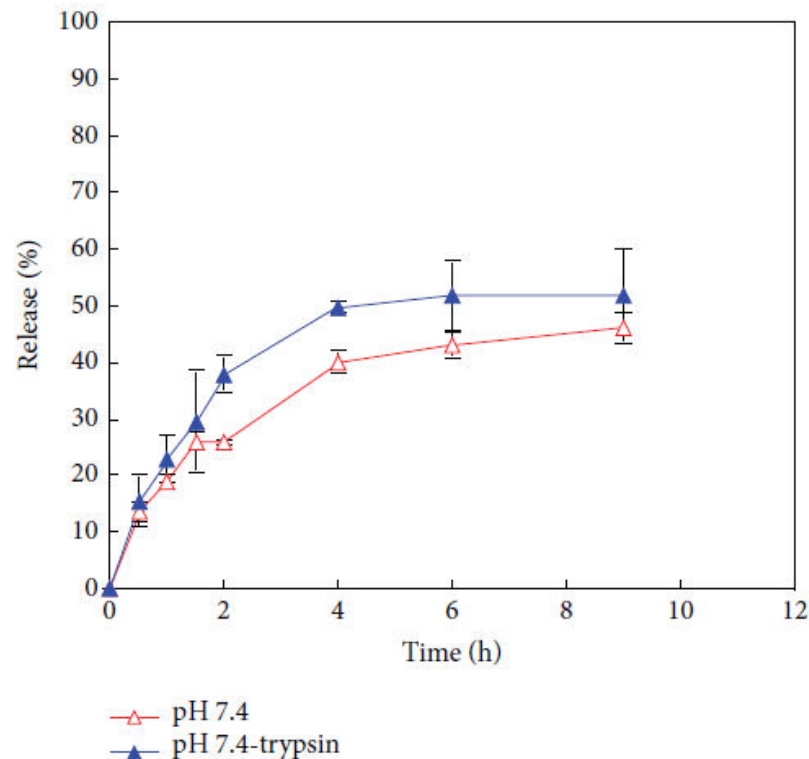
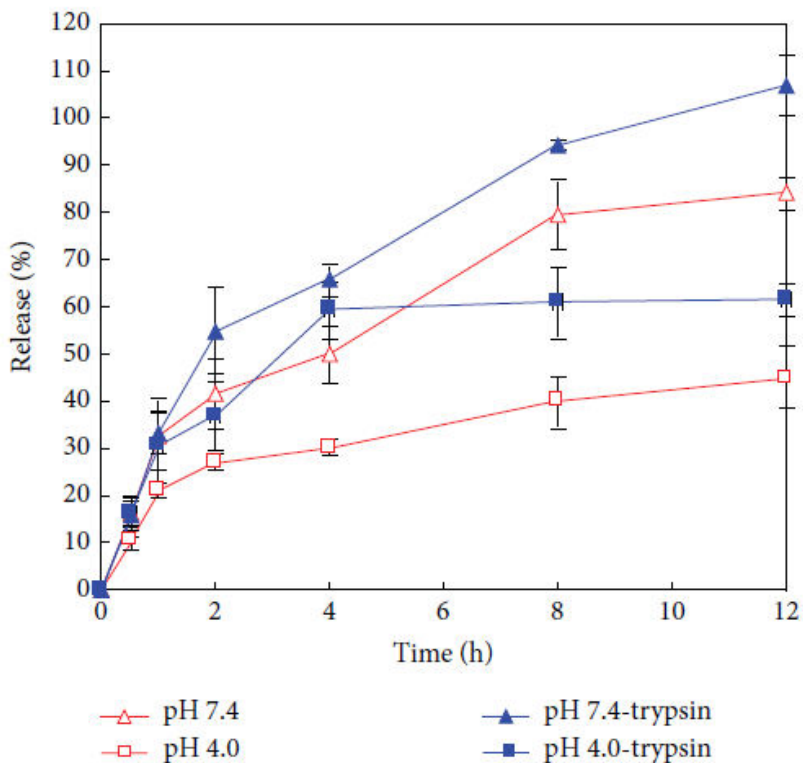


BASKET MODIFIED INTO A DIALYSIS CELL

Yuan Gao et al, BioMed Res. Inter., 2013



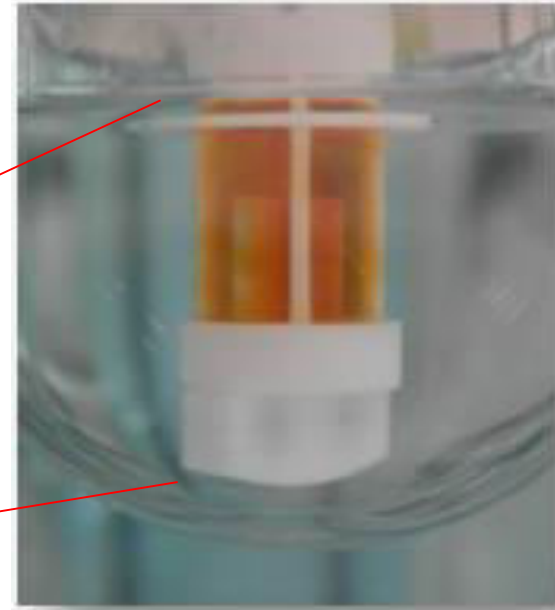
ADAPTATION OF DIALYSIS AND USP TYPE I



In vitro release curves of RIF-NPs using the modified cylinder method. (a) RIF-Gel-NPs with drug loading of 21.6% w/w in the presence and absence of trypsin; (b) RIF-Gel-NPs with drug loading of 56.7% w/w in the presence and absence of trypsin;



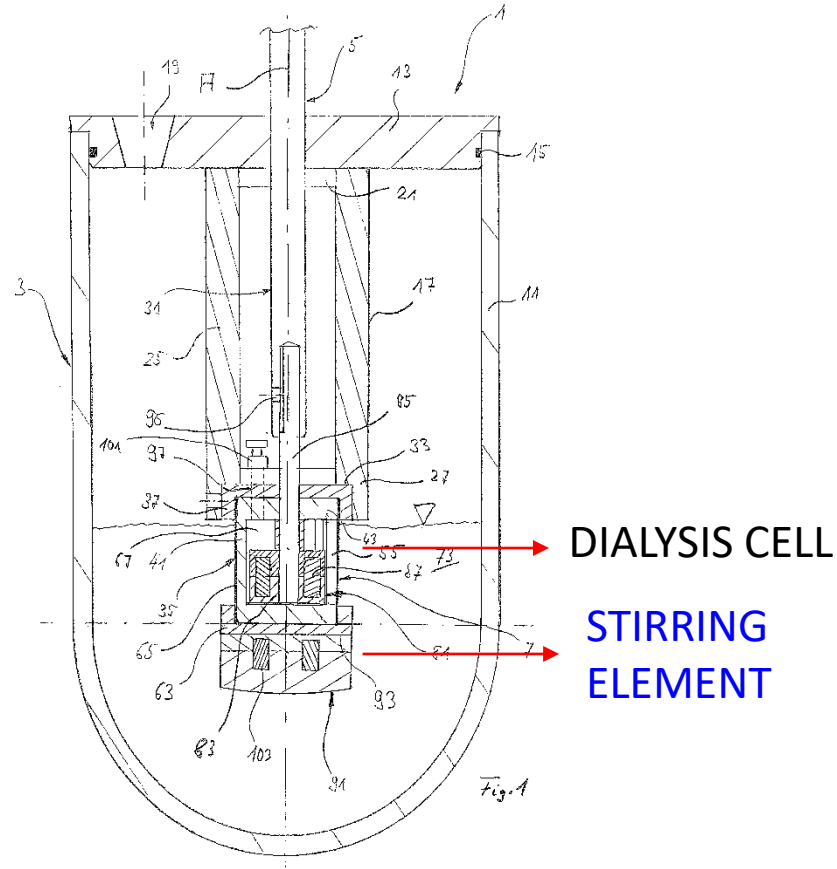
ADAPTATION OF DIALYSIS AND USP TYPE I & II (Phamatest)



- Pharma Test offers the “dispersion releaser”
- High sensitivity for fluctuations in release rate
- Works well for compounds **with poor, moderate and good solubility**



ADAPTATION OF DIALYSIS AND USP TYPE I & II (Phamatest)

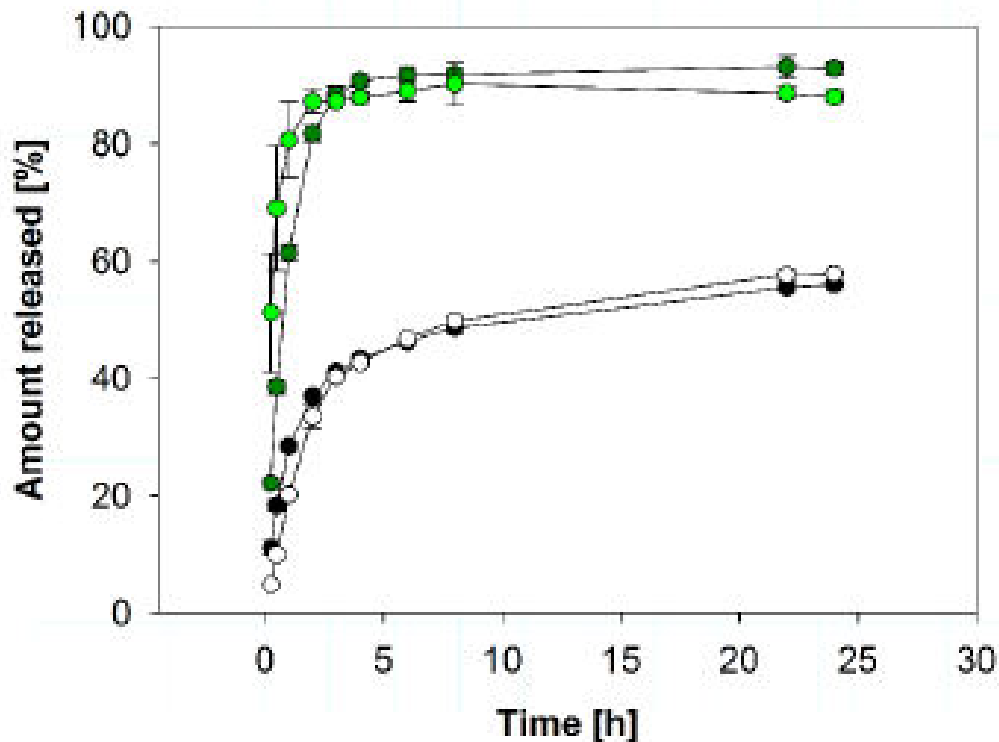


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Patent No. DE102013015522.3



ADAPTATION OF DIALYSIS AND USP TYPE I & II (Phamatest)



- Free drug, dispersion releaser
- Free drug, dialysis bag
- /○ SR formulation 1 /2

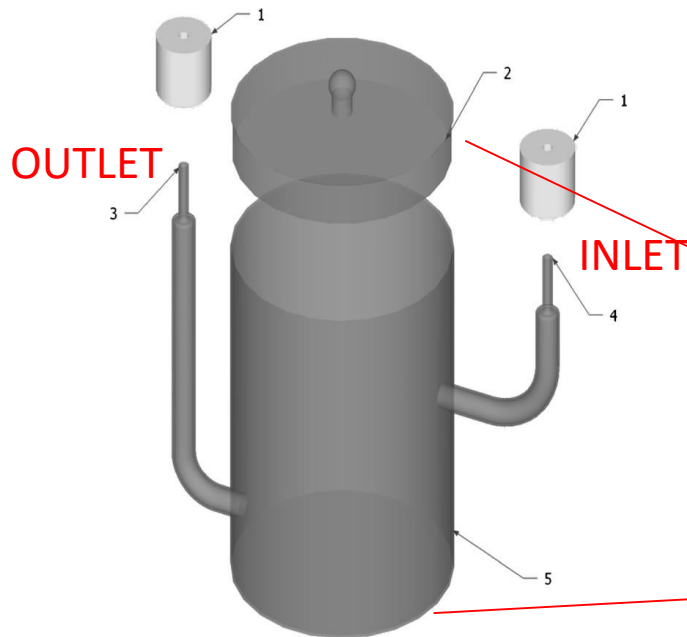
Setup USP2 / dialysis bag or dispersion releaser
pH 7.2
Medium phosphate buffer saline
 10% FCS

Batch-to-batch reproducibility

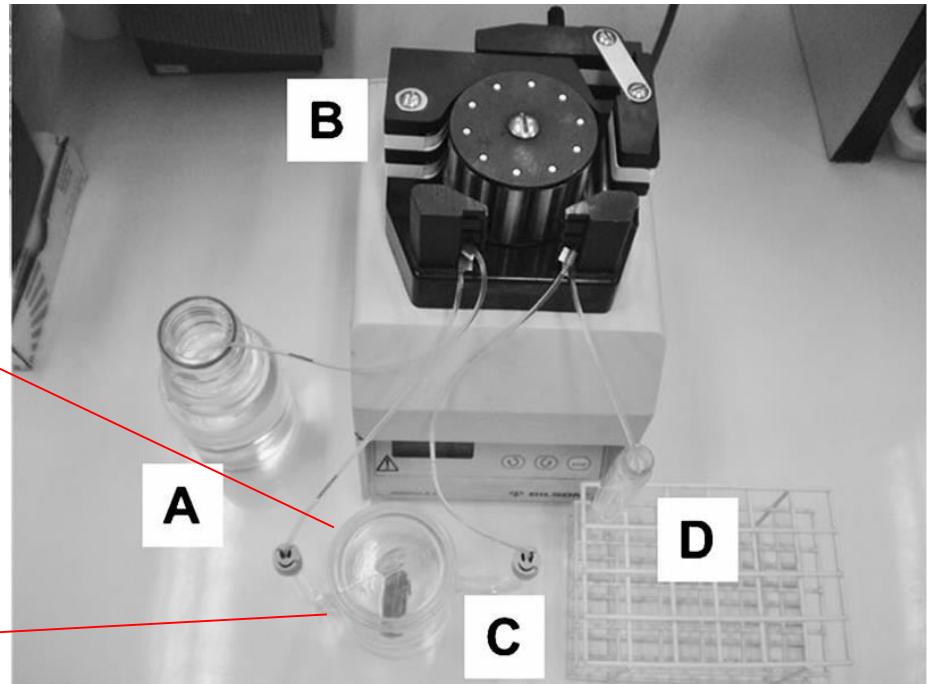
Useful for Low solubility API release study



ADAPTATION OF DIALYSIS AND CONTINUOUS FLOW CELL



- 1 rubber seals
- 2 glass lid
- 3 release medium outlet
- 4 release medium inlet
- 5 release device

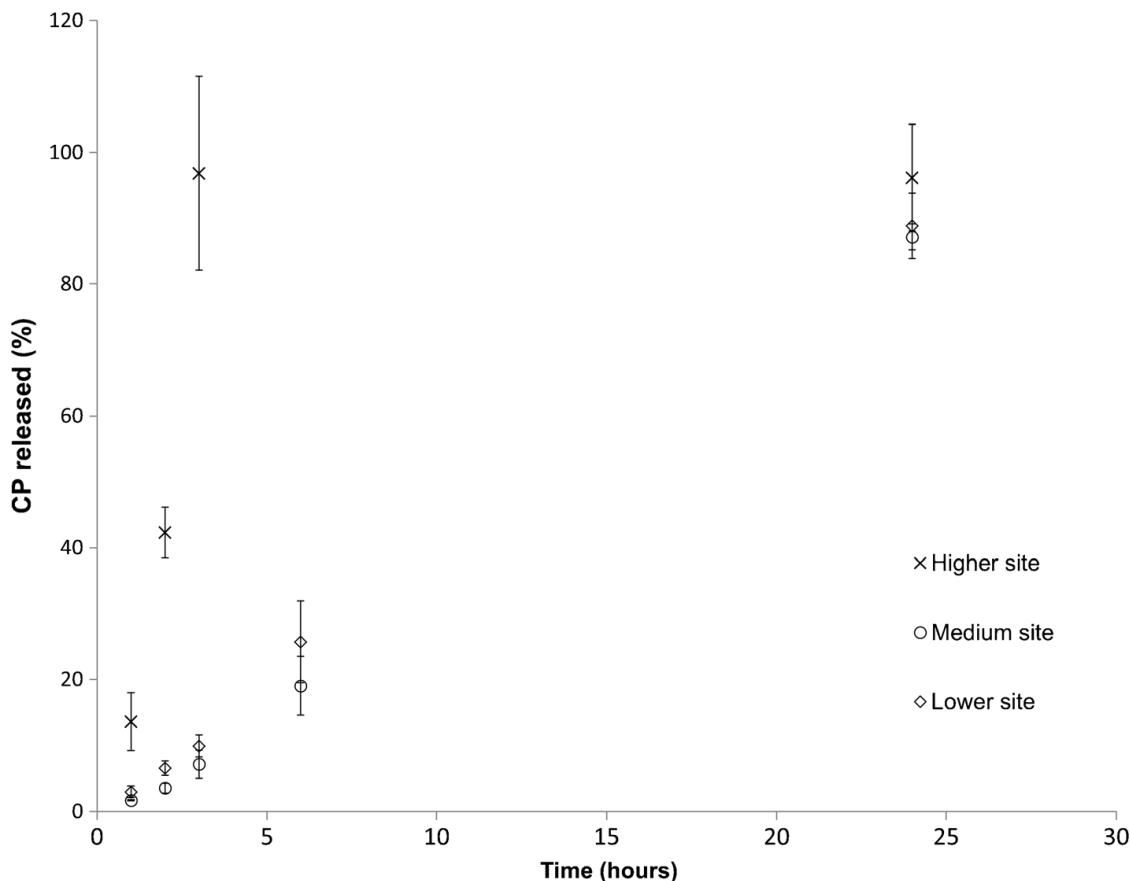


- A a flask containing fresh release medium
- B a peristaltic pump
- C the proposed release device
- D a sampling flask



ADAPTATION OF DIALYSIS AND CONTINUOUS FLOW CELL FOR NLC

Conventional Dialysis bag method with Proposed flow apparatus



In vitro CP (Clobetasol Propionate) release

**CONVENTIONAL DIALYSIS
SAC TECHNIQUE**

**SIGNIFICANT DIFFERENCE
BASED ON SITE OF
SAMPLING**



DISADVANTAGES OF DIALYSIS METHODS

Lack of adequate agitation inside membrane

Absence of sink condition
(fixed volume)

Hindrance to drug diffusion through membrane

Reverse system causes high dilution of Nano system thus medium loses its discriminatory ability

Disadvantages of Dialysis Methods



CONTINUOUS FLOW THROUGH CELL TYPE IV

This method has been widely used to investigate drug release from microspheres

But Nanoparticulate systems have very small particle size (<100nm), challenging to test their release in USP IV.

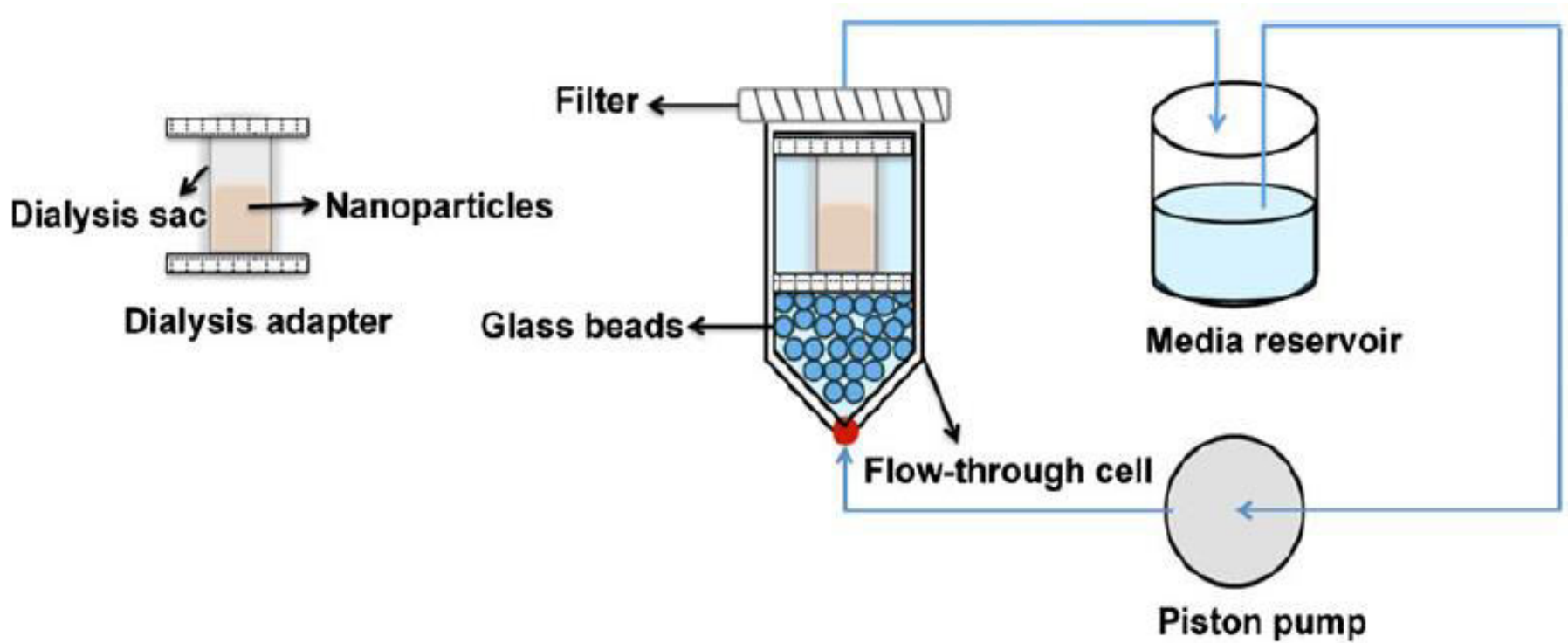
CHALLENGE:

- NP clog the filter leading to slow flow rates and high pressure build-up in the system
- Pass through filters, thus resulting in erroneous data.

SOLUTION: novel Dialysis Adaptor is introduced in USP type IV



CONTINUOUS FLOW THROUGH CELL TYPE IV





CONTINUOUS FLOW THROUGH CELL TYPE IV





CONTINUOUS FLOW THROUGH CELL TYPE IV - DIALYSIS CELL



- ✓ High Discriminative power
- ✓ Avoided Filter clogging
- ✓ Avoided violation of sink conditions
- ✓ Avoided lack of agitation

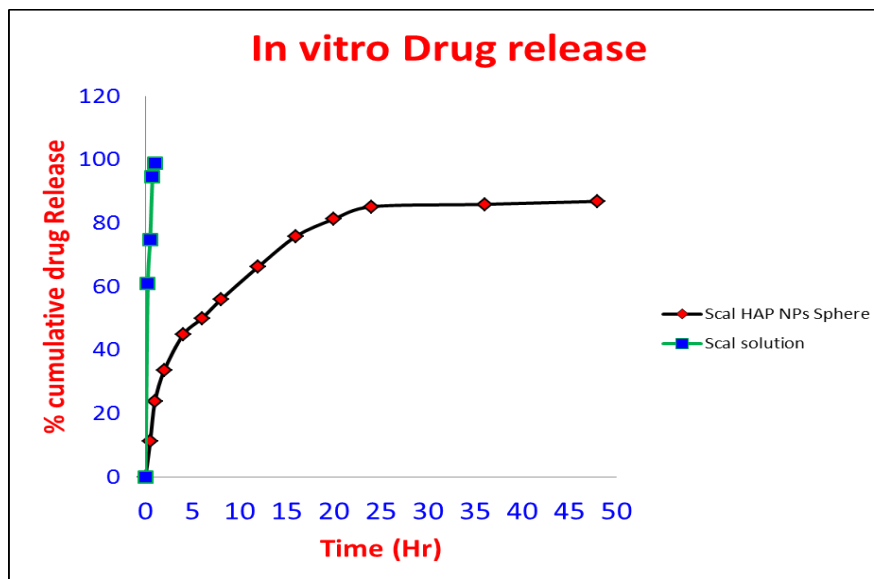


CASE STUDIES

USP APPARATUS IV WITH DIALYSIS CELL



INORGANIC NANOPARTICLES OF SALMON CALCITONIN USP IV



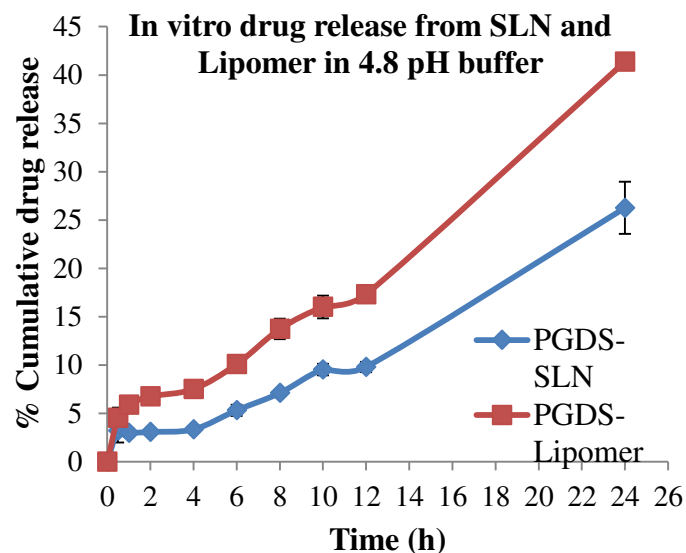
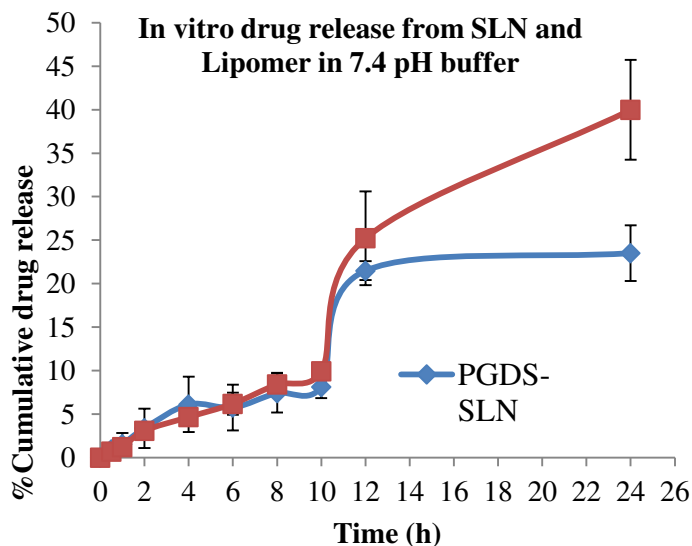
- 98 % of SCT high molecular weight drug (MW ~3000) in 1 hr indicates dialysis membrane not rate limiting
- Sustained release seen with SCT NPs



AMPHOTERICIN B NANOSYSTEM USP TYPE IV- LIPOMER vs SLN

Volume of Media-100mL
Flow rate-6mL/min

Sample volume-1mL
Aliquot volume-1mL



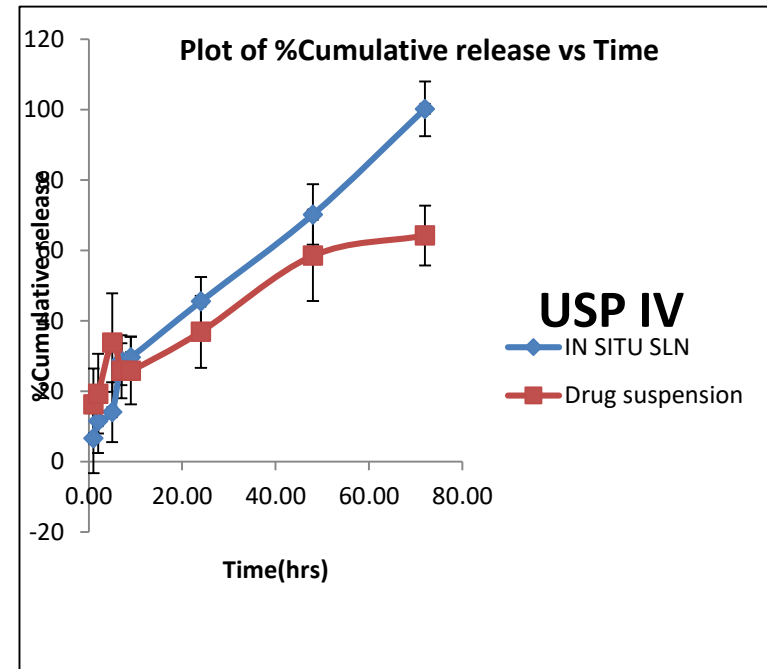
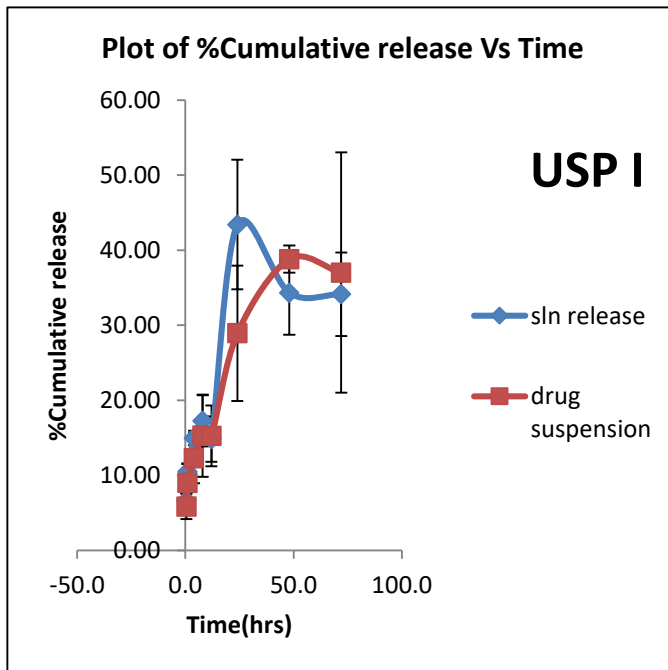
- SLN - $23.50 \pm 3.22\%$ AmB release after 24h
- Lipomer - $39.98 \pm 5.74\%$ AmB release after 24h

- SLN - $26.26 \pm 2.70\%$ AmB release after 24h
- Lipomer - $41.38 \pm 0.45\%$ AmB release after 24h

DISCRIMINATION BETWEEN TWO NANOSYSTEMS OBSERVED



BUPARVAQUONE SLN USP TYPE I vs IV



USP I – LOWER DRUG RELEASE DUE TO ABSENCE OF SINK CONDITION
USP IV – COMPLETE RELEASE AND LOWER STANDARD DEVIATIONS



SUMMARY

- **USP IV WITH DIALYSIS CELL APPEARS PROMISING**
 - DISPOSABLE DIALYSIS CELLS OVERCOME OPERATIONAL DIFFICULTIES OF THE DIALYSIS CELL
- **USP II MODIFIED APPARATUS PROMISING**
 - COULD LACK SINK CONDITION FOR POORLY SOLUBLE DRUGS



FUTURE PERSPECTIVES

- SYSTEMS THAT ADDRESS SPECIFIC REQUIREMENTS OF NANOSYSTEMS
- BIORELEVANT DISSOLUTION MEDIA
 - No release in circulation
 - Release prediction at site of delivery
- COST EFFECTIVE STRATEGIES MAY BE EXPLORED



PROF. DEVARAJAN'S RESEARCH GROUP





INSTITUTE OF CHEMICAL TECHNOLOGY



Deemed University , Elite status and Centre of Excellence (GOM)



ACKNOWLEDGEMENTS

- SOTAX INDIA PVT. LTD., FOR USP IV WITH DIALYSIS CELL
- Amit Lokhande



THANK YOU