In-vitro drug release studies for nanoparticulates: methodologies and challenges

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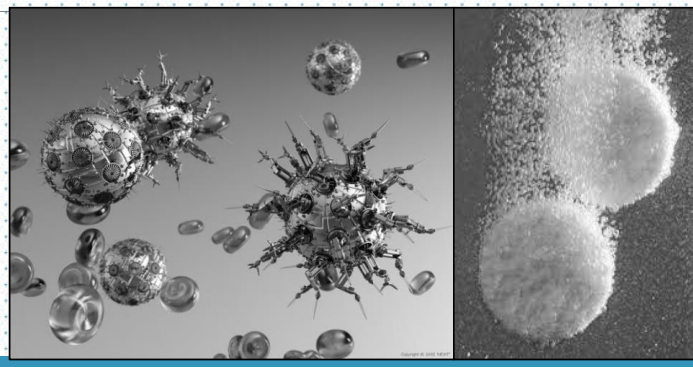

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- Conclusion

Nanoparticulates

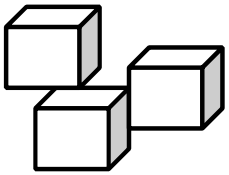
Nanotechnology - design, characterization, production, application of structures, devices, systems by controlling size in nanometer range (Royal society and the royal academy of engineering)

Nanomedicine is application of nanotechnology to health exploiting the properties of materials at the nanometric scale

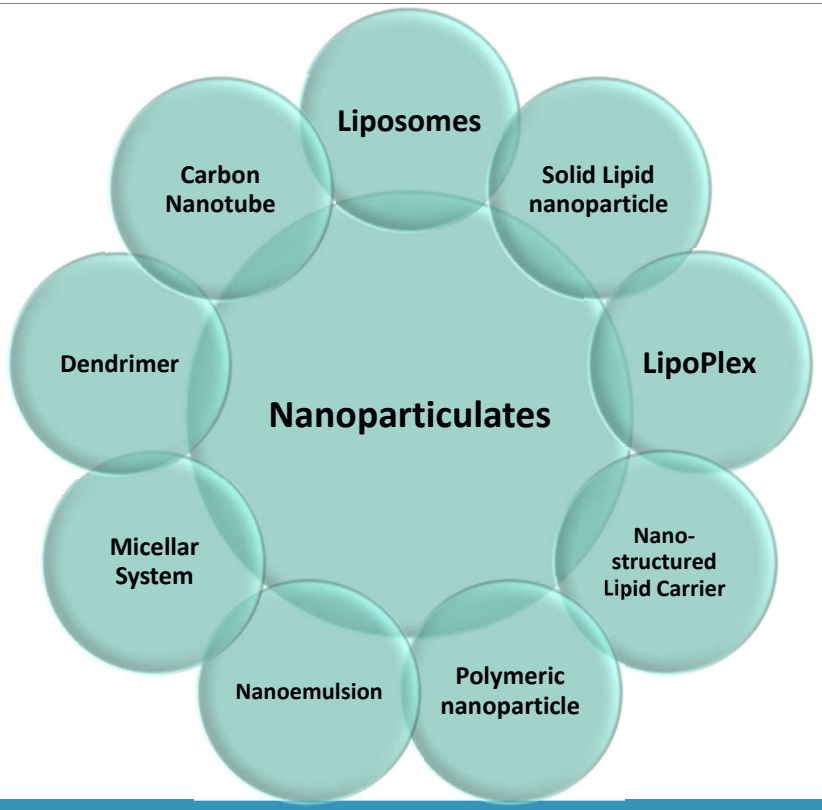
Dominant research field in nanomedicine is drug delivery systems

Need for nanoparticulates

- Addressing the drug-delivery problems
  - poor solubility,
  - Stability issues
  - poor bioavailability,
  - PK variability
  - Lack of specificity , off-target toxicities
- **Benefits**
  - **target specificity**
  - **faster dissolution, improved bioavailability**, diminished toxicity

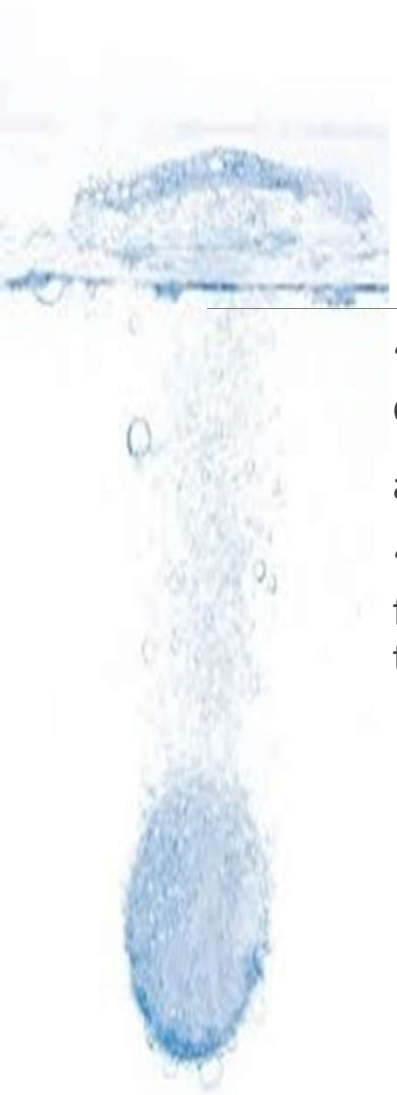


Types of nanoparticulates



Characterization Tests for nanoparticulates

- Morphological investigation of the system
- Particle size determination & Polydispersity index
- Zeta Potential/Surface charge
- Drug Loading
- ***In-vitro* drug release testing**
- Safety profile
- *In-vivo* pharmacokinetics and bio-distribution
- *In-vivo* efficacy testing

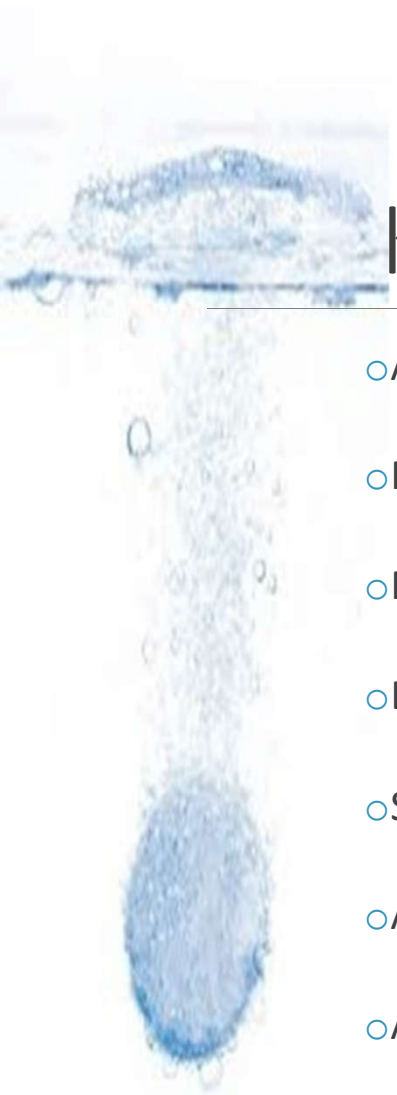


USP <1088>

"No product, including suspensions and chewable tablets, should be developed without dissolution or drug release **characterization** where a solid phase exists."

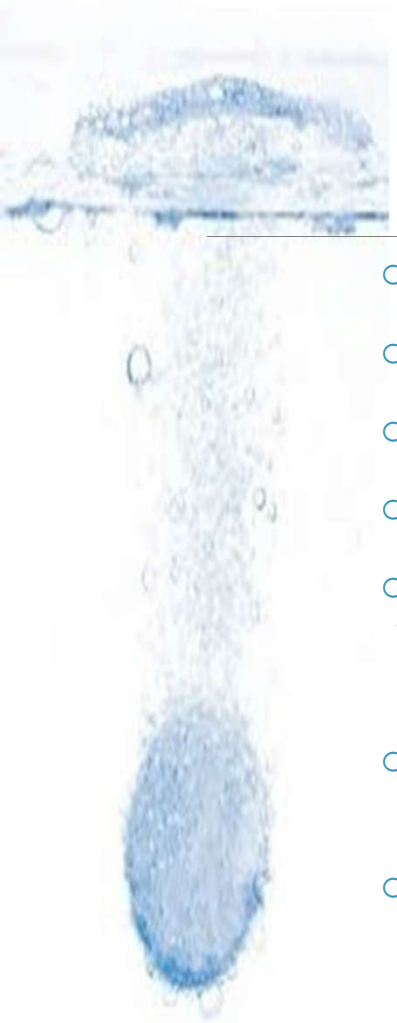
and

"Dissolution/ drug release **testing** is required for all solid oral Pharmacopoeial dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect"



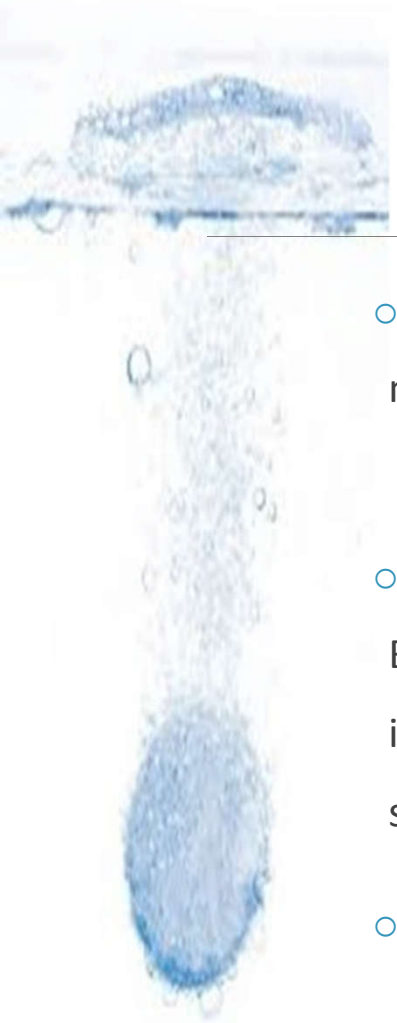
Key objectives

- Assess effect of formulation factors and method of preparation on product performance
- Routine quality control test to support batch release
- Provide information on the possible release mechanism of the system.
- Establishing in-vitro in-vivo correlation/relationship
- Substantiating label claims
- Assuring product sameness under SUPAC guidelines
- As a compendial requirement



USP Dissolution Apparatus

- Apparatus I - Basket (37 °C)
- Apparatus II - Paddle (37 °C)
- Apparatus III - Reciprocating Cylinder (37 °C)
- Apparatus IV – Flow-Through Cell (37 °C)
- Apparatus V – Paddle over Disk (32 °C), Transdermal Delivery System, use paddle and vessel from Apparatus 2 with a stainless steel disk assembly to hold the transdermal on the bottom of vessel.
- Apparatus VI, Cylinder (32 °C), Transdermal Delivery System, use Apparatus 1 except replace the basket shaft with a stainless steel cylinder element.
- Apparatus VII, Reciprocating Holder, for transdermal delivery systems and also a variety of dosage forms

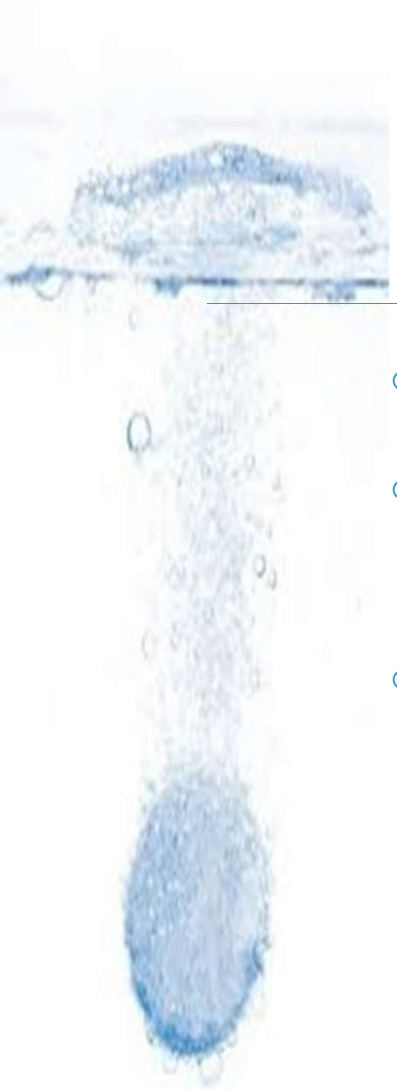


Nanoparticulates & in-vitro release

- Difficulty in separating nanoparticle from drug which is released and dissolved in the medium , effectively and rapidly.
  - Use of dialysis membrane ,filter or suitable process
- As in most of the cases, drugs that are loaded onto these delivery systems belong to BCS class II or IV category, a dissolution medium that can provide sink conditions is important. Use of surfactant, co-solvents like alcohol or liquid PEGs is useful in providing sink conditions.
- Analytical method which is Specific and selective for analyte.

Bombay COLLEGE OF PHARMACY

Methods and Case Studies for in-vitro dissolution testing



Sample and Separate Methods

- Nanoparticulates are directly added into the release medium
- Separation techniques like ultrafiltration, ultracentrifugation, and barrier membranes are used to separate nanoparticles from the continuous phase
- Drug content in the supernatant or filtrate is analyzed.

Sample and Separate Methods

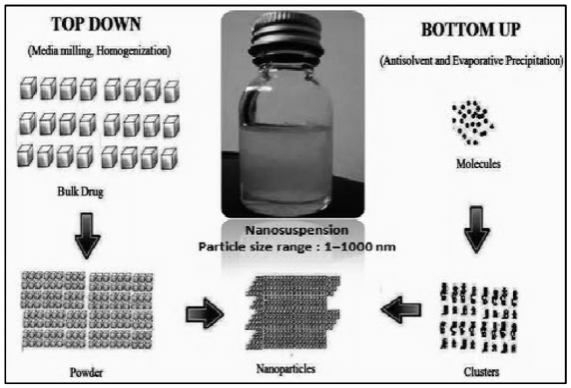
- No influence of barrier like dialysis membrane
- Easy sampling and agitation
- Separation from media is difficult
- filtered/centrifuged sample of nanoparticles is lost
- Clogging of and adsorption on filters can take place
- High energy and longer time for separation may destabilize nanoparticles
- Release continues during the separation process which may lead to erroneous results

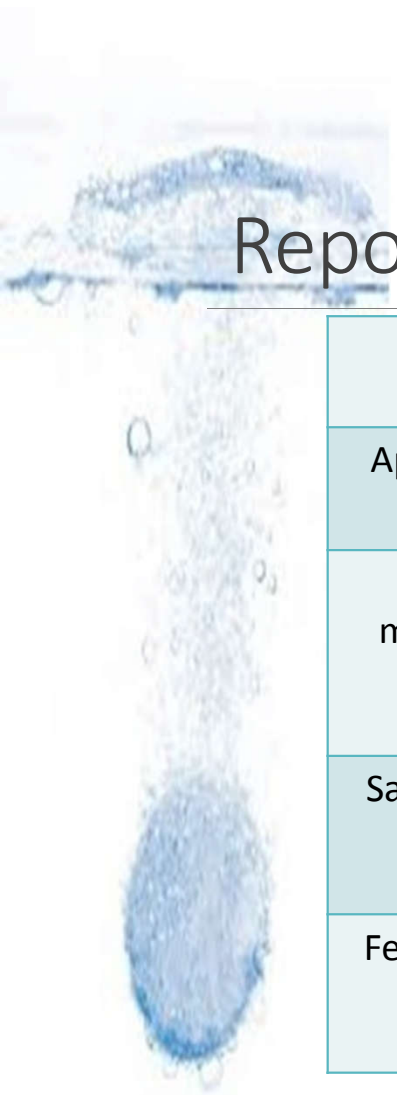
Nanosuspensions

Smaller particles take long time to settle, there may be more dissolution of these particles during this time leading to false concentrations.

Dissolution rate determination based on light scattering - relative dissolution profiles.

Dissolution rate is dependent on particle size and the nature of the polymorph as well as its crystallinity and amorphous nature.



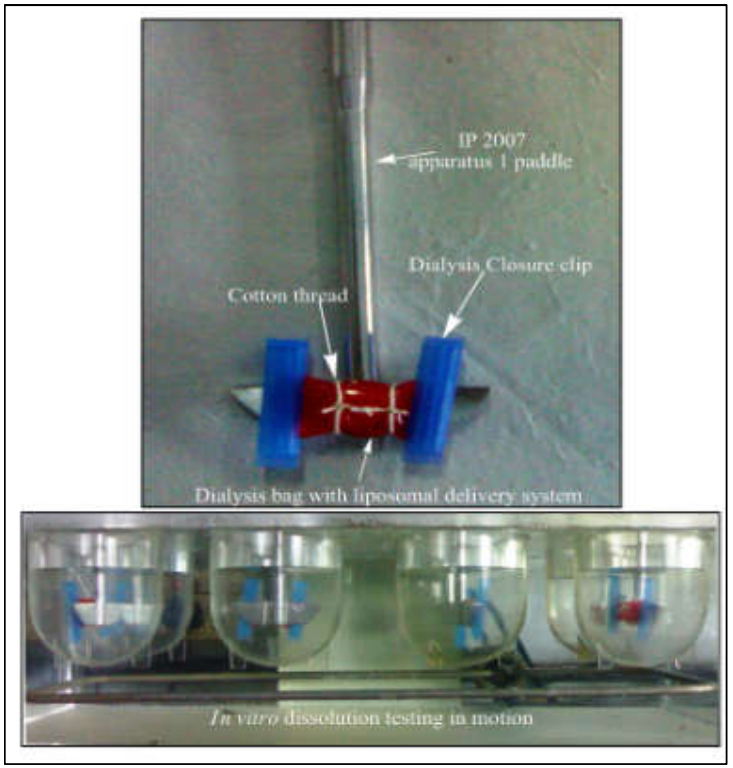


Reported literature: Sample-Separate method

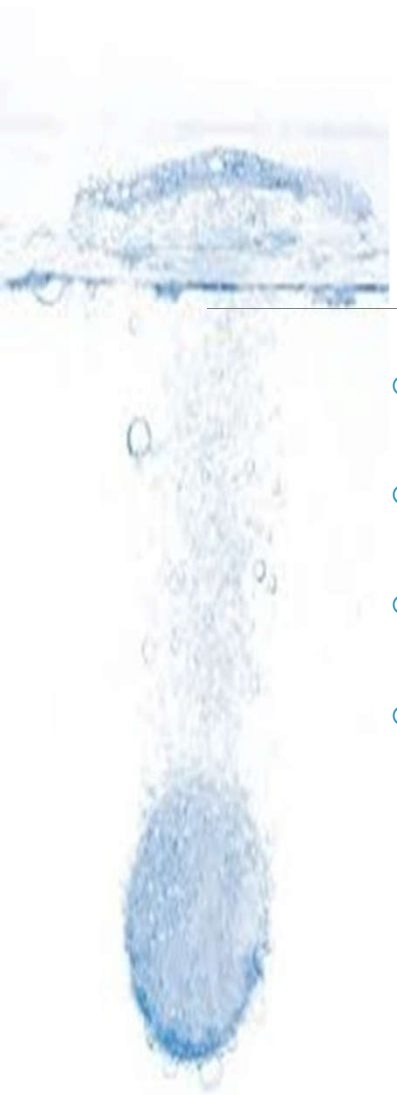
Drug	Formulation	Conditions	Considerations	References
Aprepitant	Micronized or Nanosize drug	Bio-relevant dissolution medium, filtration	in vitro- in vivo co-relation obtained	Shono Y et.al. 2010
Carba-mazepine	Nano-suspension	USP Type 2 apparatus, SGF without enzymes, filtration	Good correlation obtained between in vitro dissolution and in vivo performance.	Jain et.al. 2013
Savoxepine	PLGA Nanoparticles	Beaker, SLS added to maintain sink conditions, centrifugation	Sustained release obtained for more than a month.	E. Allemann 1993
Fenofibrate	Micronized or Nanosize drug	Bio-relevant dissolution medium, filtration	In-silico-in vitro- in vivo co-relation obtained	Juenemann D. et.al. 2011

Dialysis Sac Method#*

- Membrane diffusion method
- Formulation is sealed in bag which is placed in USP Type 1 or 2 apparatus
- Most feasible and economic
- Microspheres, liposomes, lipid nanoparticles, submicron emulsions, and self micro/nano emulsifying drug delivery system can be evaluated



SM SHAH AND MS NAGARSENKER ET AL, INTERNATIONAL JOURNAL OF PHARMACEUTICS 477 (1), 128-139
* AS JAIN AND MS NAGARSENKER ET AL, BIOMEDICINE & PHARMACOTHERAPY 68 (4), 429-438



Applications

- Liposomes
- SLN, NLCs
- Submicron emulsions
- Polymeric Nanoparticulates

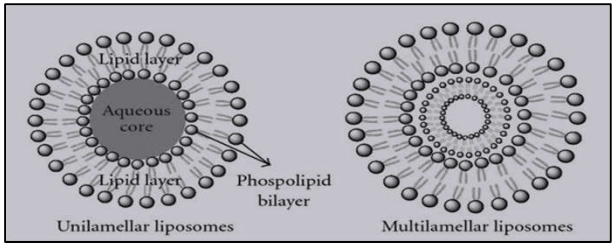
Liposomes :Dialysis Method

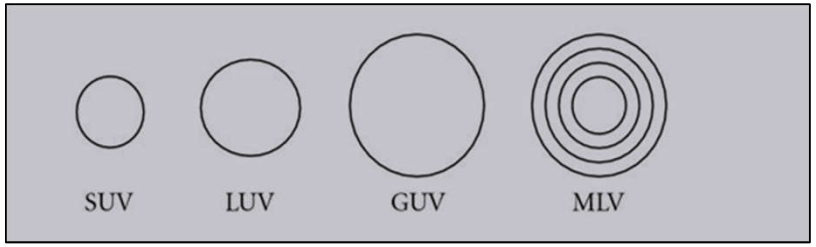
Efficacious therapy by liposomes loaded drug requires optimum spatial placement and temporal delivery.

FDA warrants in vitro release test as an important parameter for DOXIL

- Almost no release upon storage at 2 - 8ºC
- Minimum release during circulation time in vivo at 37ºC
- Sufficient doxorubicin release in under conditions that imitate tumor at 37 ºC

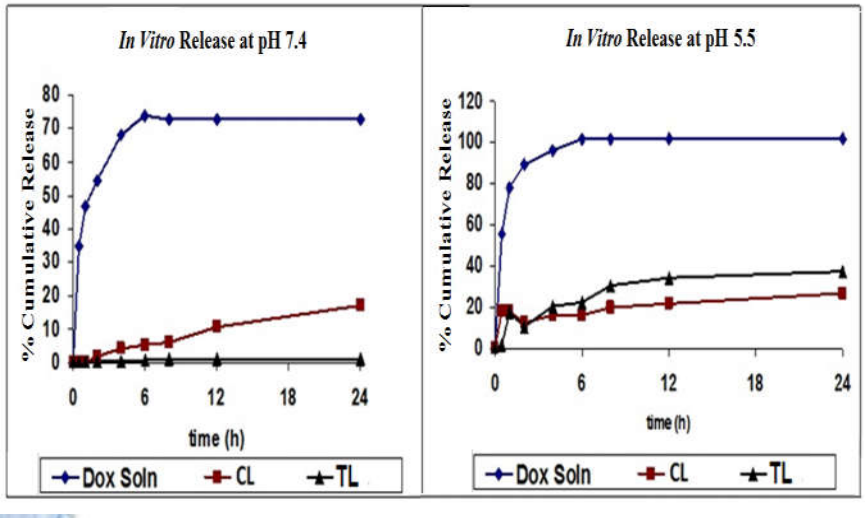
A physiological “Dissolution” test - a drug release assay is crucial for IVIVC and for the comparability studies*



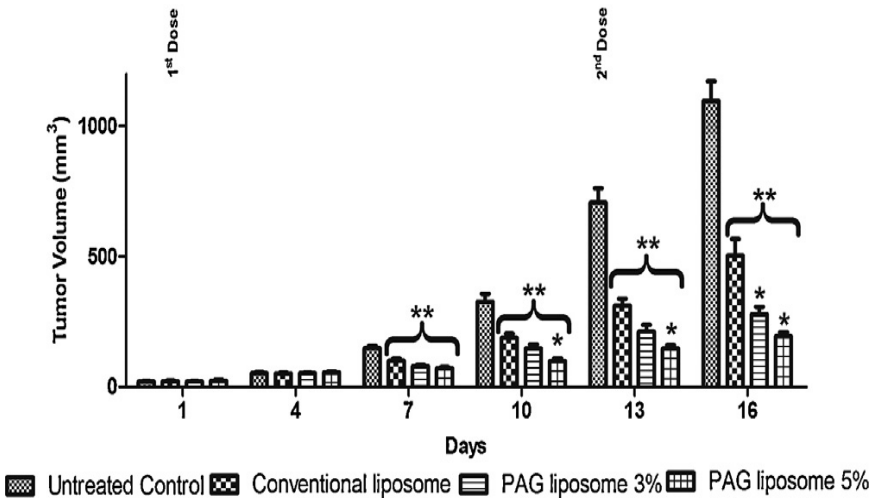


*BARENHOLZ C; PHOSPHOLIPID SYMPOSIUM 2015,HEIDELBERG

In-vitro release
Effect of formulation and pH of medium

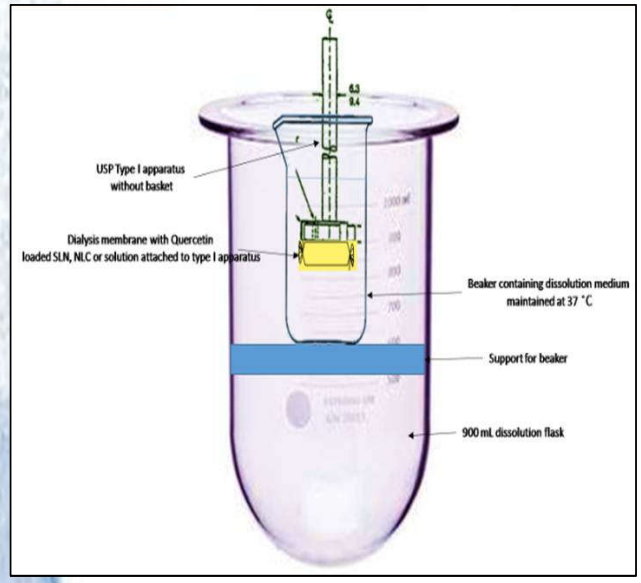


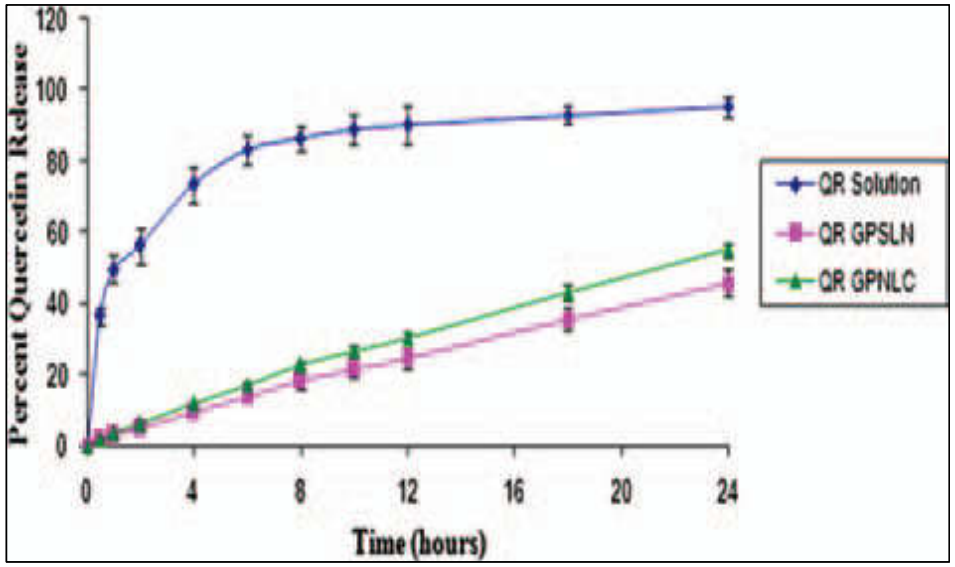
In-vitro release of various formulations



In-vivo efficacy in flank model

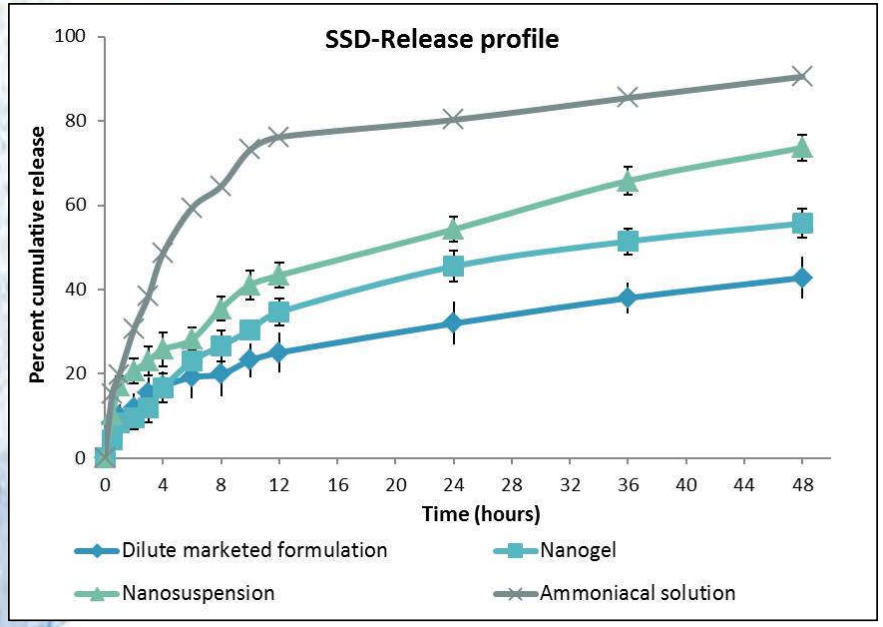
Lipid Nanoparticles : :Dialysis Method

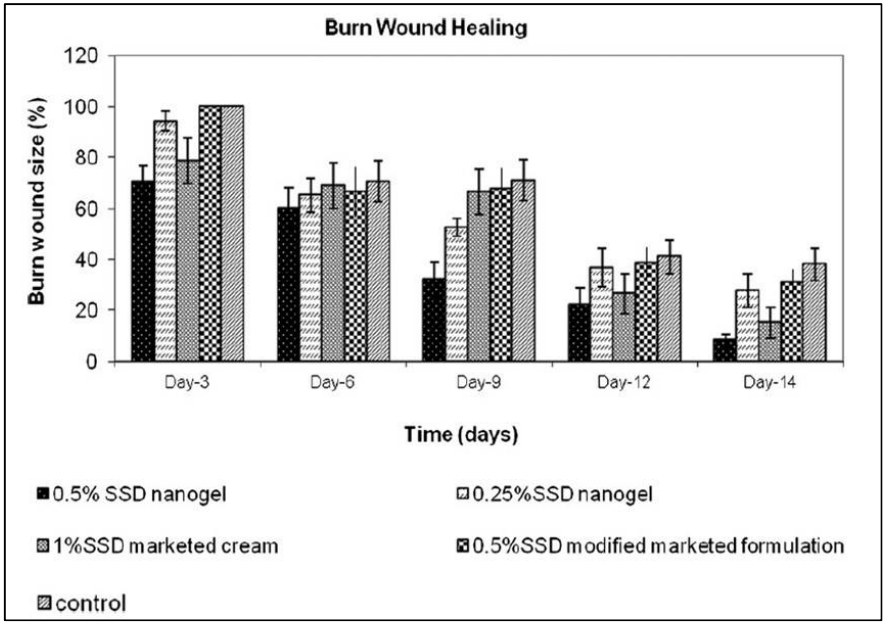




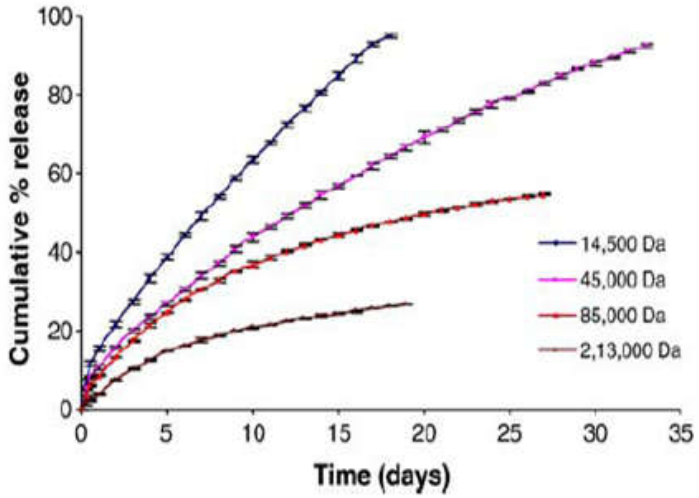
mechanism by which there is improvement in rate and extent of dissolution.

In-vitro release of silver sulfadiazine nanoparticles*:Dialysis Method





In-vitro release Estradiol loaded PLGA nanoparticles :Dialysis Method *



In vitro release profiles of estradiol loaded PLGA (50:50) nanoparticles of different molecular weights with DMAB as stabilizer in pH 7.4 phosphate buffer. Data points shown are mean±standard deviation ($n=3$).

- Low molecular weight PLGA showed greater release; zero order release indicating degradation mechanism of release.
- Diffusion prevailed for higher molecular weight PLGA .

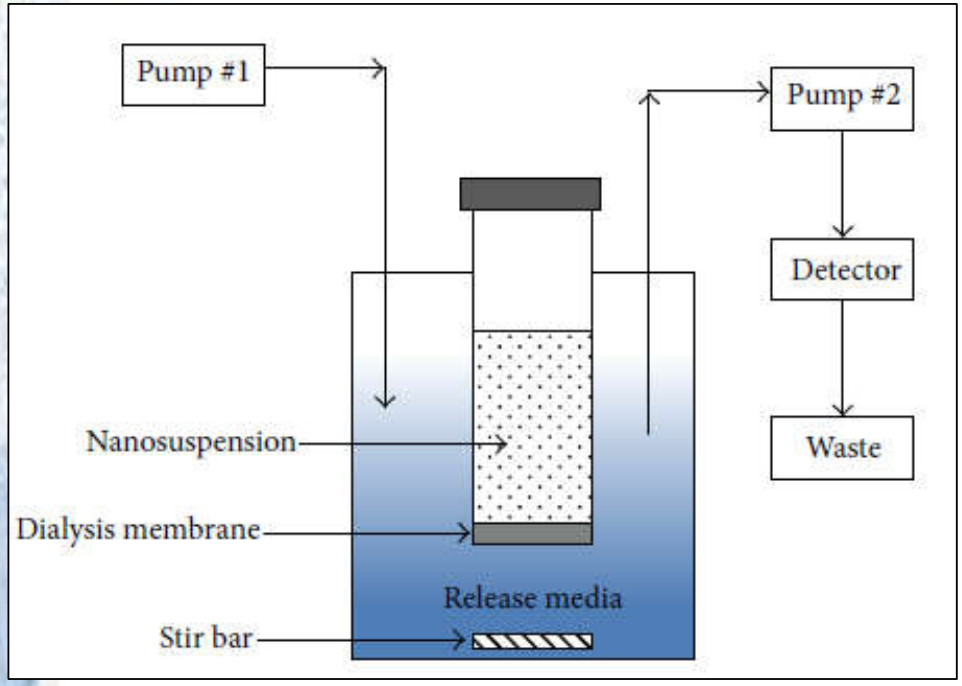
Modifications :Dialysis Method

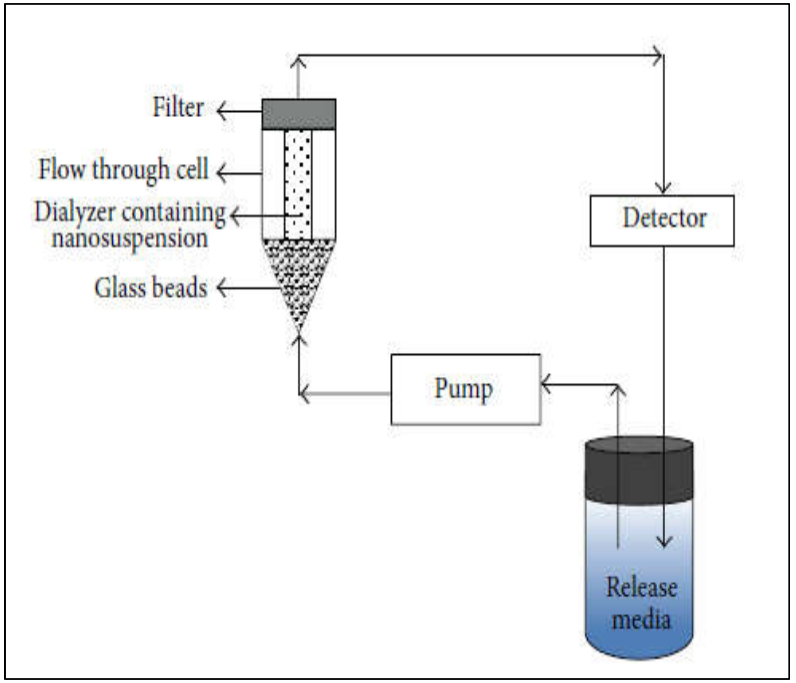
Reverse dialysis
Glass basket dialysis
Side by side dialysis

Dialysis Method

- MWCO, donor to acceptor volume, agitation conditions
- Determining release rate **requires k1 << k2(** diffusion through dialysis membrane**)**
- k2 is higher for compounds with **moderate or good solubility**

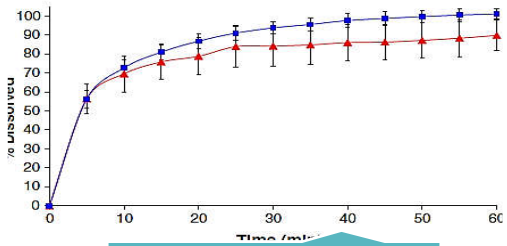
Continuous Flow Methods



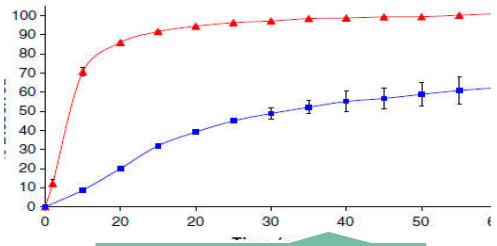


Case Study1: CF method

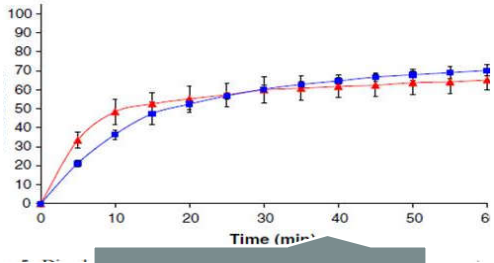
- Cefuroxime Axetil nanoparticles were evaluated using the CF method as well as other methods
- Complete drug release - achieved only with the USP II paddle and USP IV apparatus, release profiles with the USP IV method being well separated



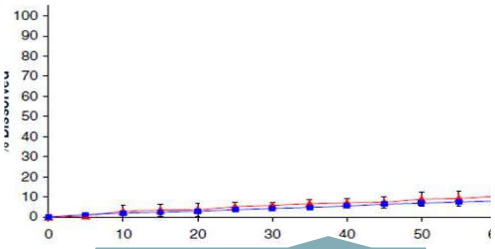
USP II



USP IV



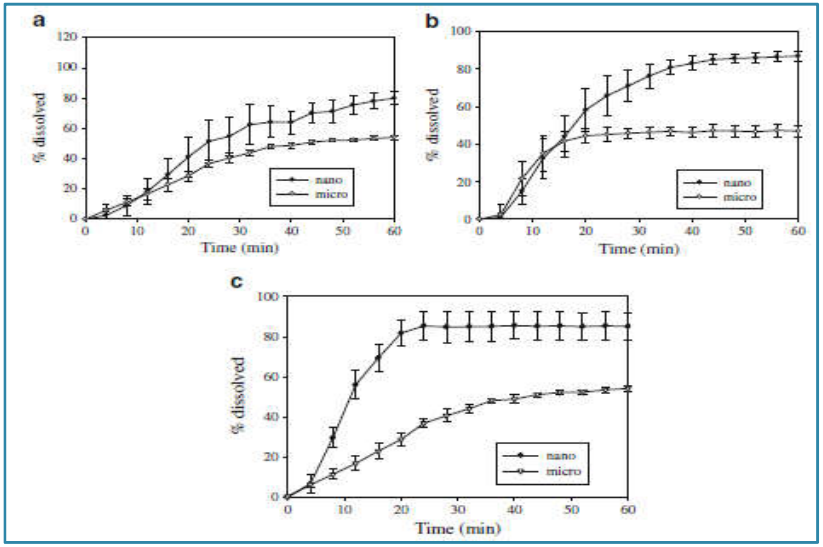
USP I



Dialysis

Case Study 2 : CF method

Dissolution carried out in USP Type 4 apparatus
with different flow rates



Nano- and micro-particle loaded strip films of BCS II drug, Griseofulvin

Combination of dialyis and continuous flow method

Novel dialysis adapter to be used with USP type 4

- Avoid problem of filter clogging
- Loss of nanoparticulate formulation.

Important factors that modulate the release on a flow through cell are

- flow rate - 4 mL/min, 8 mL/min, 16 mL/min used
- type of flow – laminar
- system configuration – open or closed loop
- media volume – composition and pH

Combination of dialysis and USP Type 2

- Dispersion releaser
- USP2 is a robust standard setup used for quality control of IR formulations
- Pharma Test offers the “dispersion releaser”
- High sensitivity for fluctuations in release rate
- Works well for compounds with poor, moderate and good solubility (high k2)
- Evaporation occurs in long-term experiments

Drug	Formulation	Apparatus and Method	In vivo studies	IVIVC correlation	References
Simvastatin	Nanostructured lipid carriers and solid lipid nanoparticles	Dialysis bag, 100 ml of phosphate buffer, pH 7.4	Balb/C mice	0.9404, 0.941	Tiwari R. et.al. 2011
Indomethacin	Gelatine nanoparticles	Dialysis bags, 40 mL PBS	Wistar Albino rats	0.981	Kumar R. et.al. 2011
Fenofibrate lipid matrix particles	Lipid matrix particles	In vitro lipolysis model , Bio-relevant Medium, 35 mL	Sprague Dawley rats	Rank Order	Borkar N. et.al. 2014
Silybin	72 hr SLB - Porous silica nanoparticles	Combination (USP I—dialysis bag), 0.08 M Na2CO3, 900 mL	Beagle dogs	0.9931	Cao X. et.al. 2013
Capsaicin	MPEG-PCL nanoparticles	dialysis bags, 100 mL (SGF; pH 1.2; 0.1 mol/L) or PBS (pH 7.4; 0.1 mol/L), contained Tween-80 (0.3%, w/v)	male Sprague-Dawley rats	0.998 ,0.996 in SGF, PBS	Peng W. et.al. 2015
Silybin meglumine	Hollow-sphere mesoporous silica nanoparticles	Combination (USP I—dialysis bag) 0.06 M Na2CO3, 900 mL	Beagle dogs	0.9741	Cao X. et.al. 2012

Dissolution discussion groups

- AAPS in-vitro dissolution/ release testing Focus group
- FDA Dissolution discussion group
- FIP Dissolution working group and joint workshops
- DIA joint workshops
- Indian chapters , SPDS

- **Groups work to highlight various aspects of dissolution as an important QC test**
- **Outcome of workshops published as papers and serve to formulate regulatory guidelines for novel as well as existing products.**

Conclusion

- No compendial/ standard procedure for in-vitro release test of complex systems of nanoparticulates.
- Greater significance to the test as indicator of quality , performance and guide to formulation
- combination with dialysis membrane with existing apparatus appear suitable for nanoparticulate and liposomal delivery systems
- More work on biorelevent method, mechanism and mathematical models will give future direction .

THANK YOU