U.S. FOOD & DRUG ADMINISTRATION

Significance of cryo-scanning electron microscopy (cryo-SEM) in evaluating the morphology of multivesicular liposomes

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Disclaimer

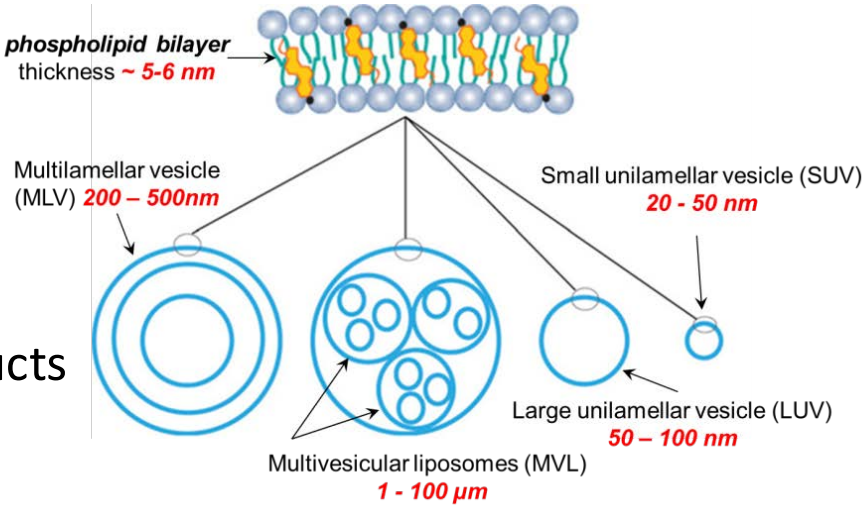
The views and opinions expressed in this presentation are those of the author and do not necessarily represent official policy or position of the U.S. Food and Drug Administration.

Outline

- Complex drug formulations – an introduction
- Current product specific guidance on bupivacaine-multivesicular liposome (BPV-MVL)
- FDA internal research
  - Physicochemical characterization of BPV-MVL – ***significance of evaluation of drug particle morphology***
  - In vitro drug release study on BPV-MVL
- Take home messages

Complex Drug Formulations – an introduction

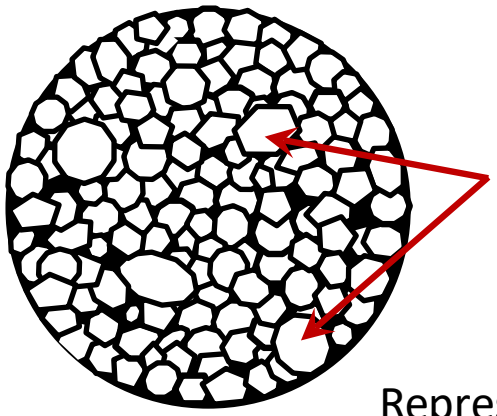
- Complex injectable drug formulations/dosage forms
  - Injectable drug products involving nanotechnology
    - Liposomes
    - Iron complex
    - Nano-suspensions
    - Protein/drug complex
  - Long-acting (LAI) parenteral drug products
    - Microparticles
    - Implants/inserts
    - Liposomes – unilamellar , multilamellar, multivesicular
    - Suspensions



Limited research conducted on Multivesicular Liposomes – lipid based micro-particles

Case Study- Exparel®

- Model Complex Formulation - Exparel®
  - Bupivacaine-Liposome Injectable Injection - 13.3 mg/mL, in 10 mL and 20 mL (single use vial) approved on 10/28/2011
  - An amide local anesthetic - postsurgical analgesia
  - Sterile, non-pyrogenic white to off-white preservative-free aqueous suspension of ***multivesicular liposomes (MVL)*** - based on DepoFoam® drug delivery system

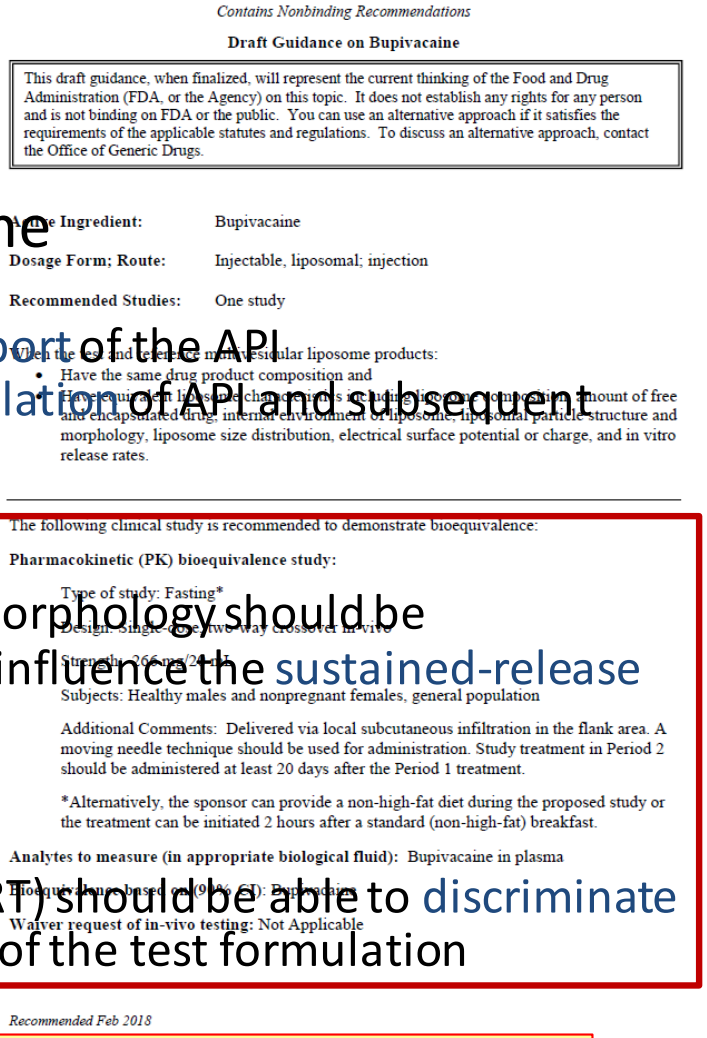


Characteristic polyhedral (honeycomb) inner vesicles

Representative MVL

Current Draft Product-Specific Guidance for BPV-MVL

- Composition
  - Lipid and non-lipid components (Q1, Q2 sameness)
  - Free and encapsulated drug
- Internal aqueous environment of liposome
  - pH – influence the ionization of the API
  - Osmolality – influence the transmembrane transport of the API
  - Volume and composition – influence the encapsulation of API and subsequent drug release mechanism
- Particle structure and morphology
  - Unique non-lamellar honeycomb structure and morphology should be comparable between the RLD and test product – influence the sustained-release of API
- In vitro release kinetics
  - Methodology used for in vitro release testing (IVRT) should be able to discriminate the effect of process variability in the production of the test formulation



Contains Nonbinding Recommendations

Draft Guidance on Bupivacaine

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

Active Ingredient: Bupivacaine

Dosage Form; Route: Injectable, liposomal; injection

Recommended Studies: One study

When the test and reference multivesicular liposome products:

- Have the same drug product composition and
- [illegible] physicochemical characteristics including liposome composition, amount of free and encapsulated drug, internal environment of liposome, liposomal particle structure and morphology, liposome size distribution, electrical surface potential or charge, and in vitro release rates.

The following clinical study is recommended to demonstrate bioequivalence:

Pharmacokinetic (PK) bioequivalence study:

Type of study: Fasting*

Design: Single-dose, two-way crossover in vivo

Strength: 266 mg/20 mL

Subjects: Healthy males and nonpregnant females, general population

Additional Comments: Delivered via local subcutaneous infiltration in the flank area. A moving needle technique should be used for administration. Study treatment in Period 2 should be administered at least 20 days after the Period 1 treatment.

*Alternatively, the sponsor can provide a non-high-fat diet during the proposed study or the treatment can be initiated 2 hours after a standard (non-high-fat) breakfast.

Analytes to measure (in appropriate biological fluid): Bupivacaine in plasma

Bioequivalence based on (90% CI): Bupivacaine

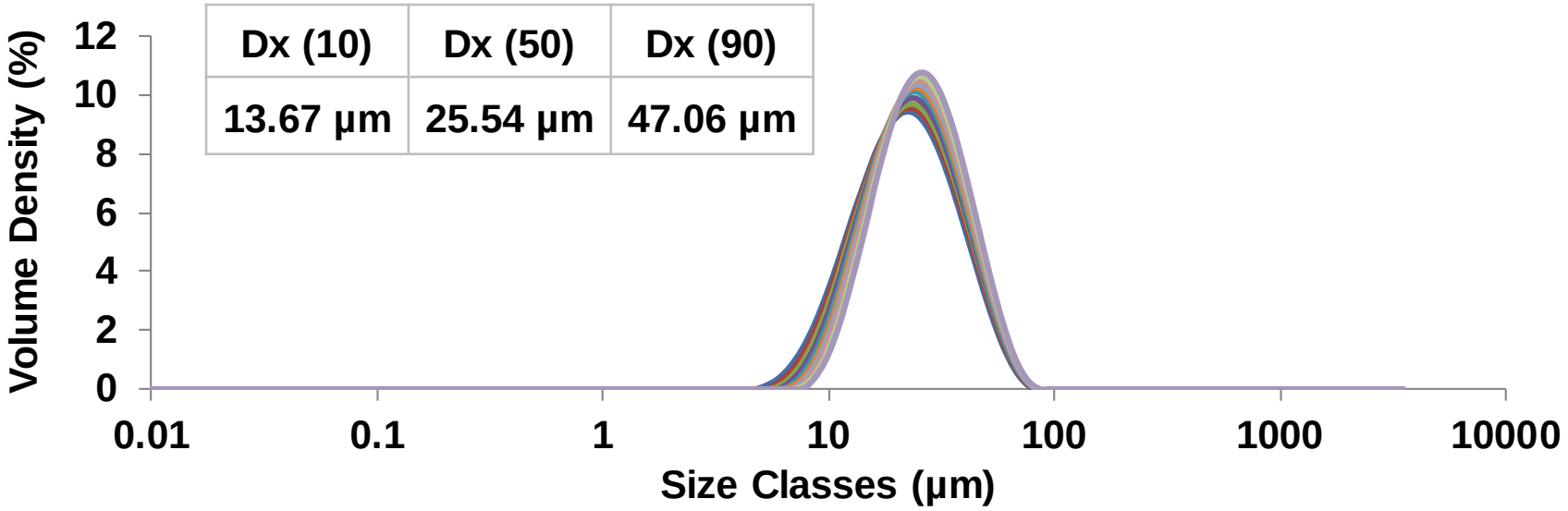
Waiver request of in-vivo testing: Not Applicable

Recommended Feb 2018

Scientific gaps in understanding the effect of change in morphology on the mechanism of release

Particle Size Characterization

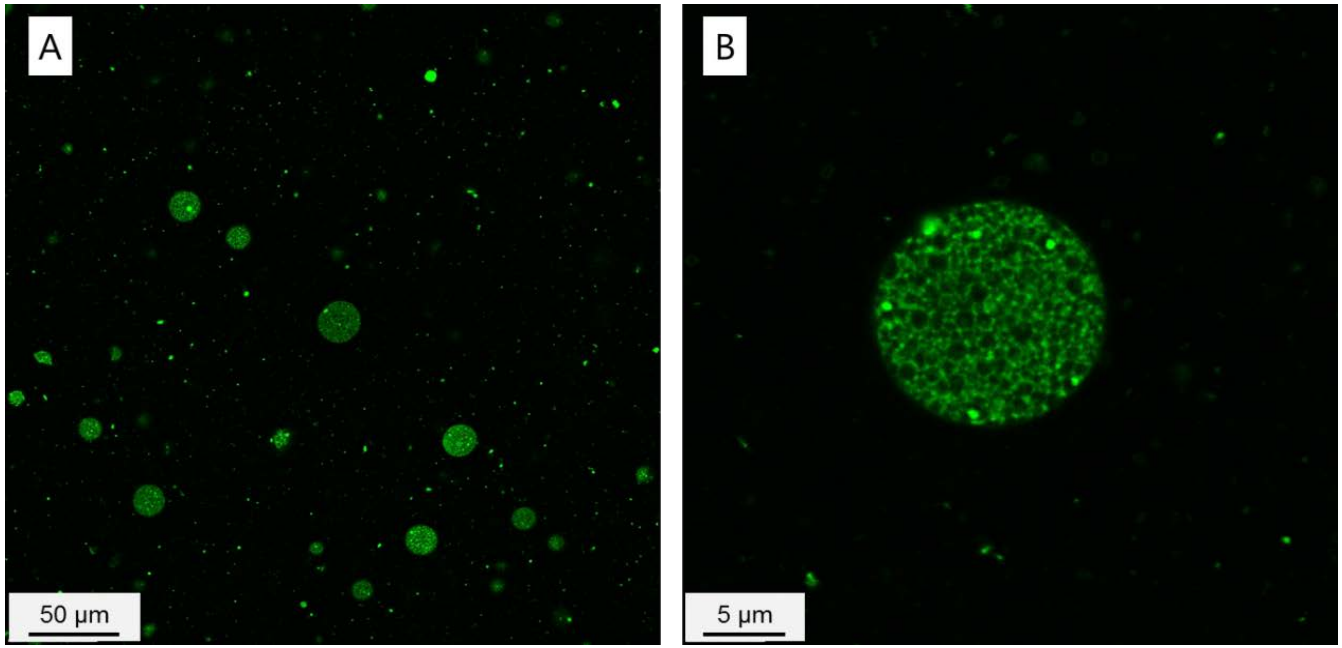
Method: Laser Diffraction



- Applicable for detection of size of the outer vesicles
- Cannot measure size of the inner vesicles
- Cannot be used for detection of size degradation of the vesicles during drug release
- Potential application restricted to assessment of size of the MVLs prior to any drug release study

Particle Structure and Morphology Characterization (Cont.)

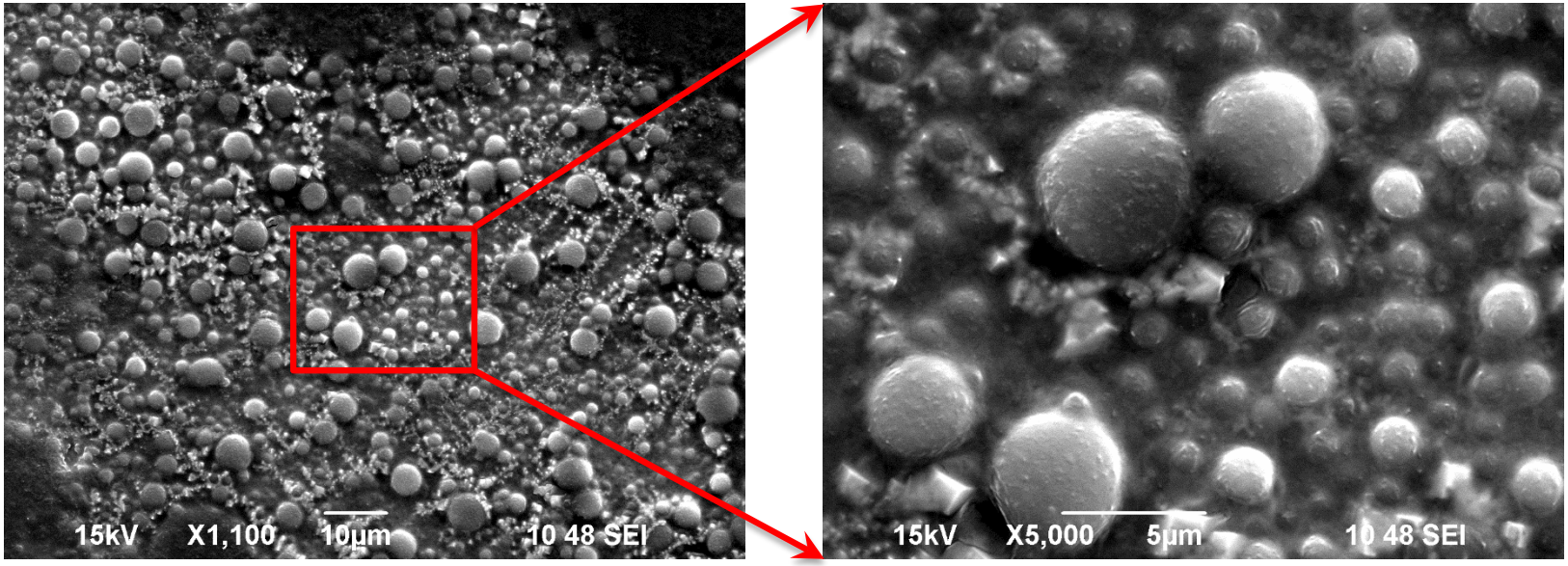
Method: Confocal Microscopy



- Internal compartments show the characteristic “honeycomb” structure
- Range of 1 - 2 µm - consistent with cryo-SEM results
- Can be used as a complimentary method to cryo-SEM

Particle Structure and Morphology Characterization (Cont.)

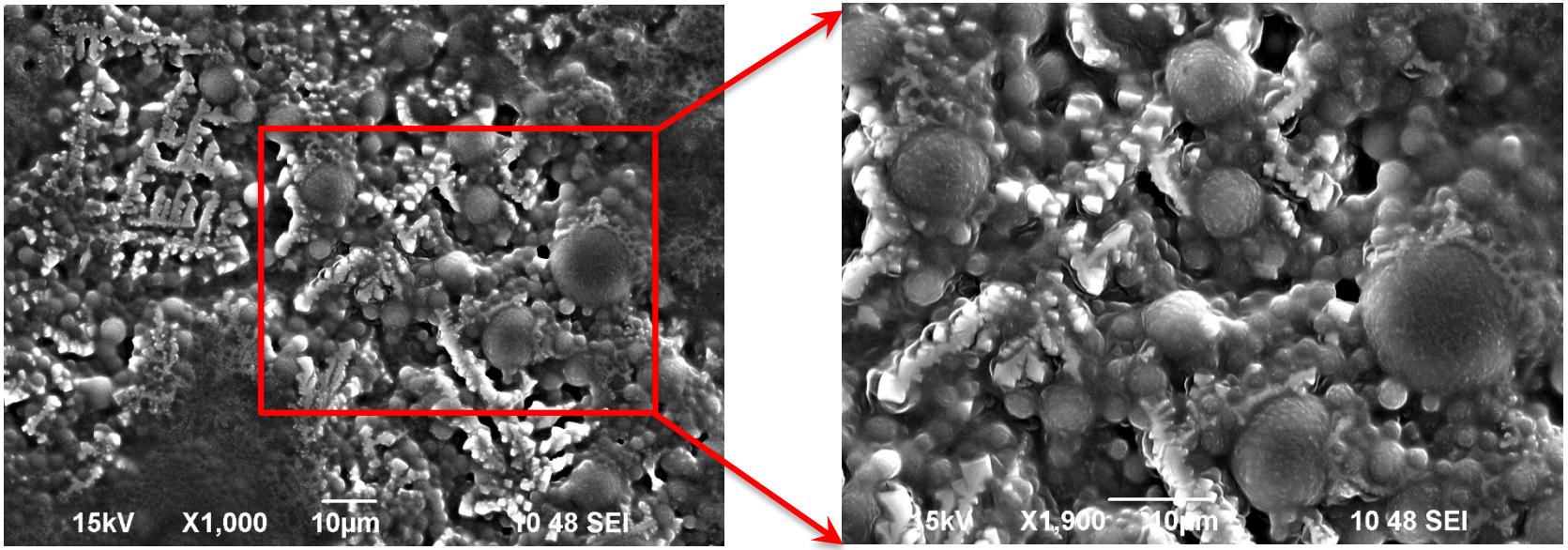
Method: Conventional High Vacuum SEM – samples dried at **25 C** and Au sputter coated



- Characteristic "honeycomb" structure not adequately observed
- Particle size ~ 3 µm – inconsistent with Confocal Microscopy and Laser diffraction results

Particle Structure and Morphology Characterization (Cont.)

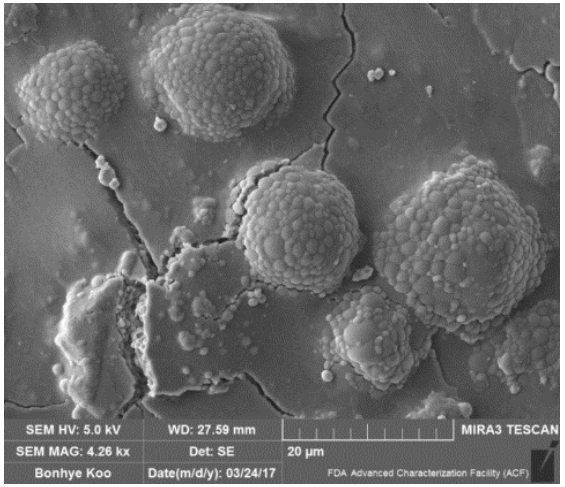
Method: Conventional High Vacuum SEM – samples dried at **37 C** and Au sputter coated

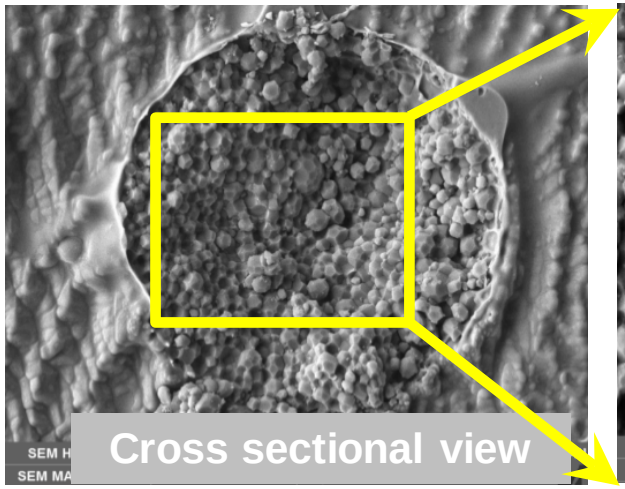


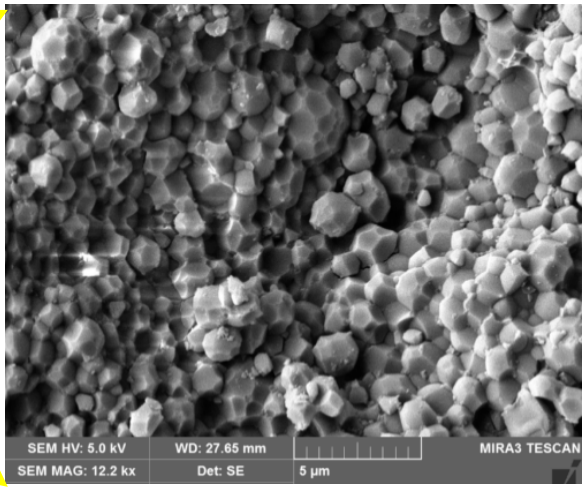
- Characteristic "honeycomb" structure not adequately observed
- Particle size ~ 3 µm – inconsistent with Confocal Microscopy and Laser diffraction results

Particle Structure and Morphology Characterization – **Cryo SEM**

Method: Cryo-Scanning Electron Microscopy



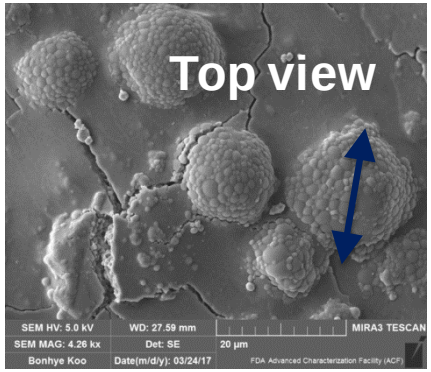


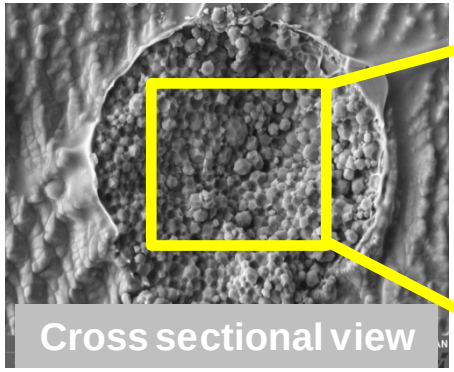


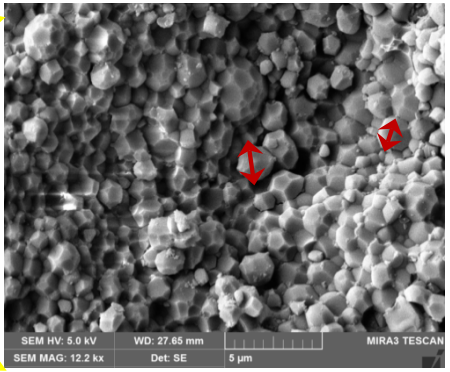
- Characteristic "honeycomb" observed
- Particle size ~ 20 µm –consistent with Confocal Microscopy and Laser diffraction results

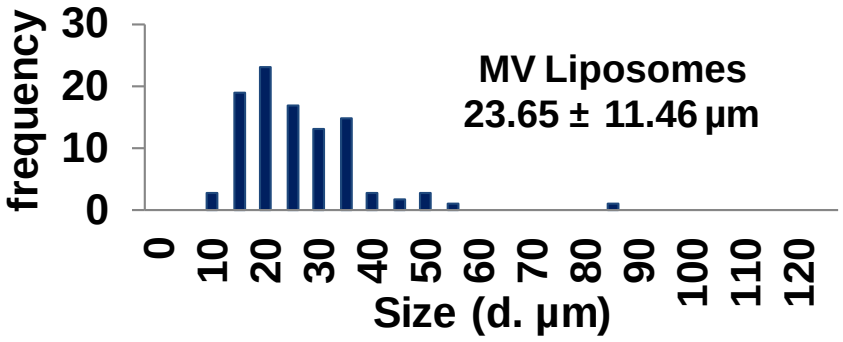
Particle Structure and Morphology Characterization – **Cryo-SEM**

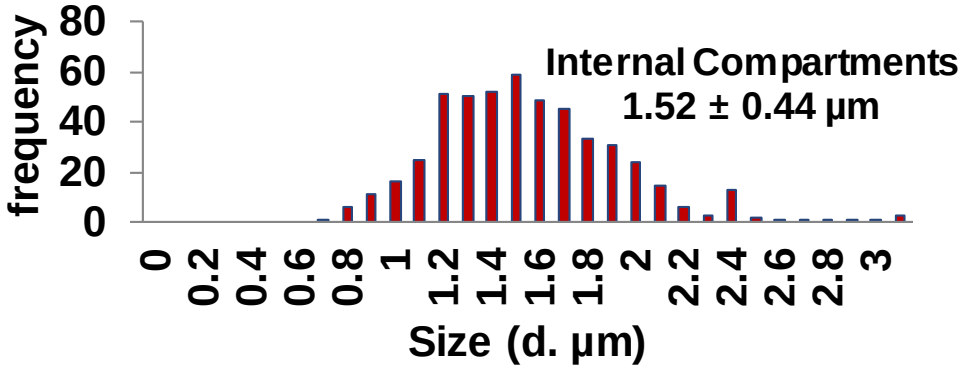
Method: Cryo-Scanning Electron Microscopy – Particle Size Analysis









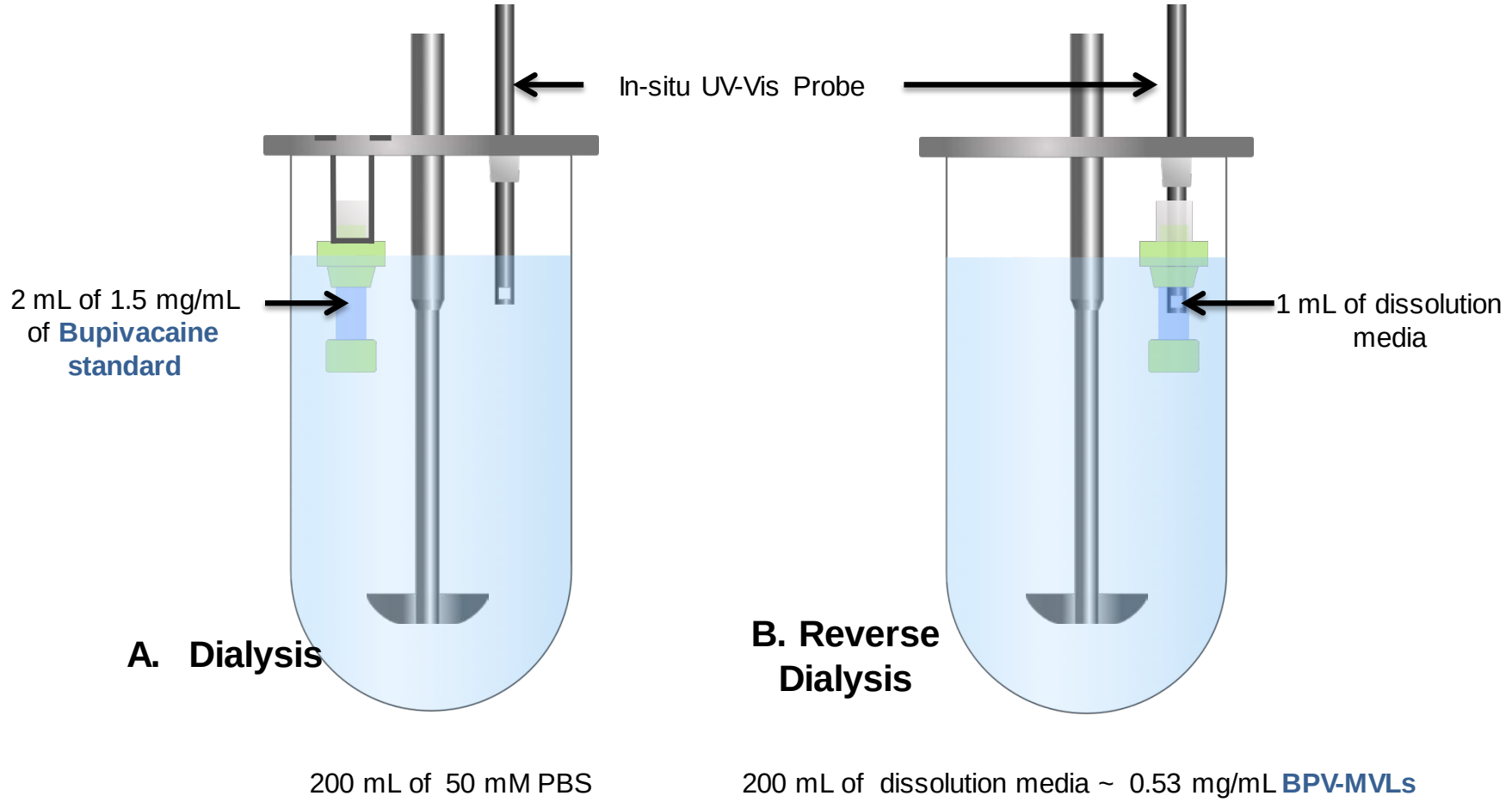


Mostly spherical shape structure; Size range of 10 - 60µm

In Vitro Release Test (IVRT) – Release Mechanism

- Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus
  - MWCO of dialysis membrane – 10 kDa, 20 kDa, 50 kDa and 100 kDa
  - Temperature - 25°C, 31°C, 37°C, 40°C at pH 7, 120 rpm
  - pH of dissolution media – pH 5, pH 6, pH 7 at 37°C, 120 rpm
  - Agitation speed – 120 rpm and 240 rpm at pH 7, 37°C

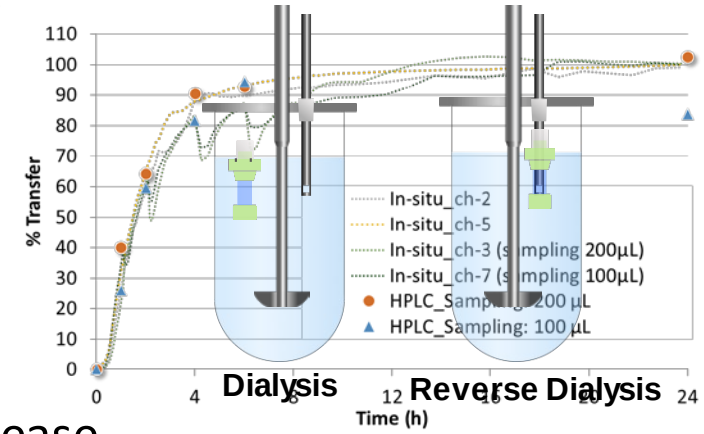
Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus



Figures provided by Xiaoming Xu and Yixuan Dong

Advantages - Dialysis Set-up in USP II Apparatus with in-situ UV-Vis Probe

- USP II apparatus
  - Compendial apparatus (more controllable)
- Reverse dialysis v/s Dialysis
  - Better agitation of the BPV MVLs obtained in the outer media compared to that in the dialysis cartridge (dialysis set-up)
    - Prevents BPV-MVLs from settling down
  - Proper sink conditions
  - No UV-interference from drug aggregates

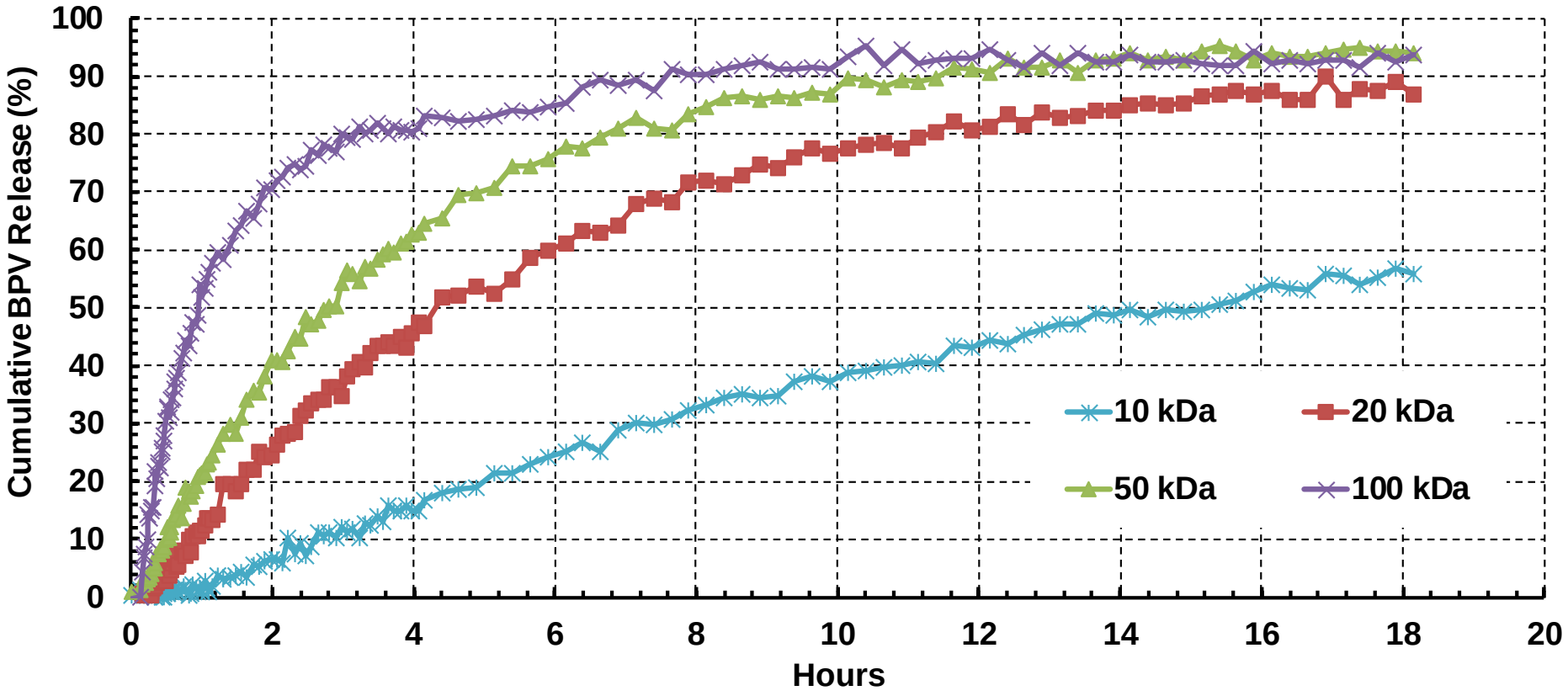


- In-situ UV-Vis probe
  - Continuous monitoring of real-time drug release
  - Use of 2nd derivative of the concentration data minimizes possible interferences from turbidity at the characteristic UV-wavelength
  - Provide more details regarding release kinetics

Figures provided by Xiaoming Xu and Yixuan Dong

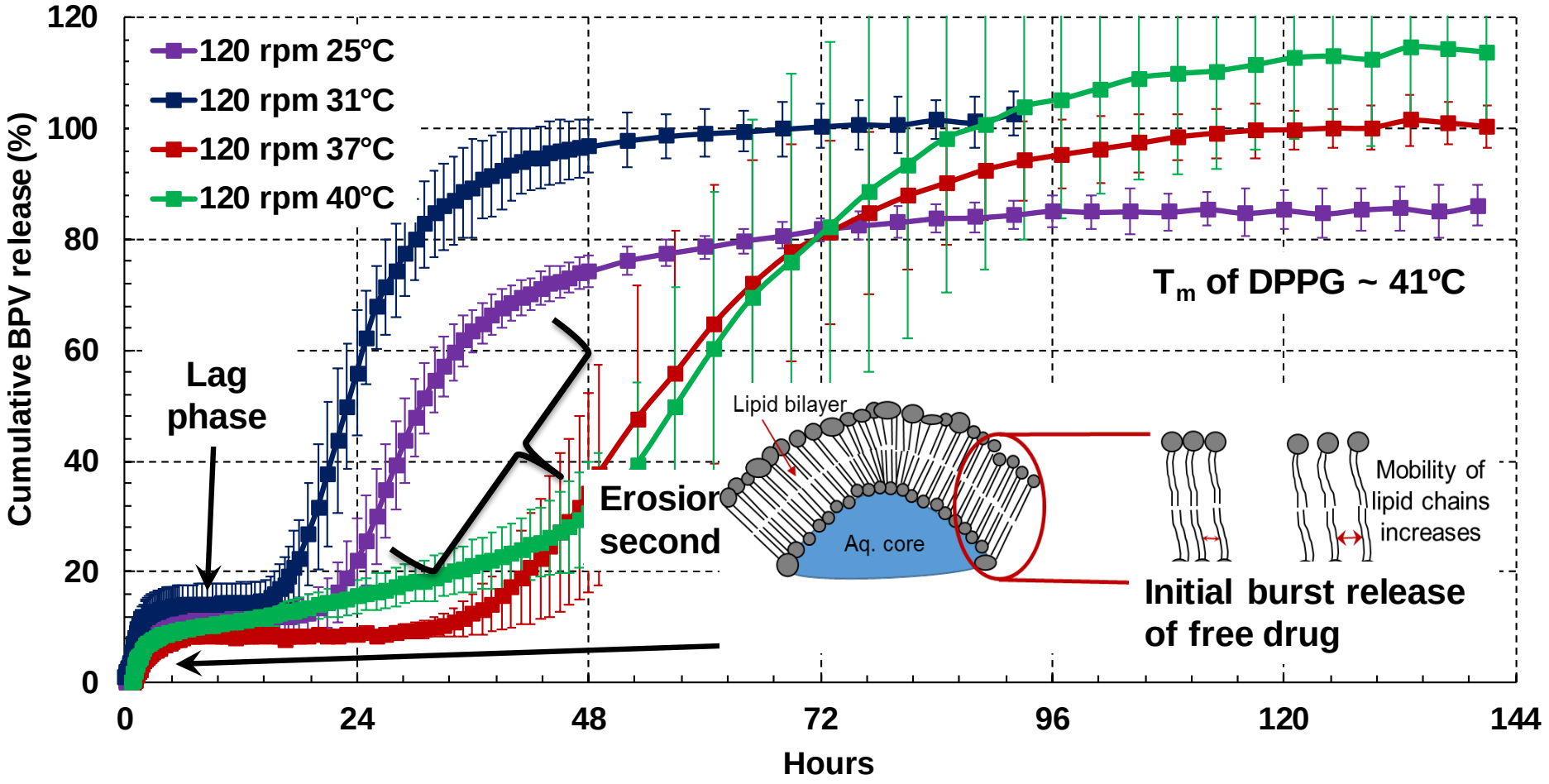
Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus

- Effect of MWCO of Dialysis Membrane on the Diffusivity of BPV



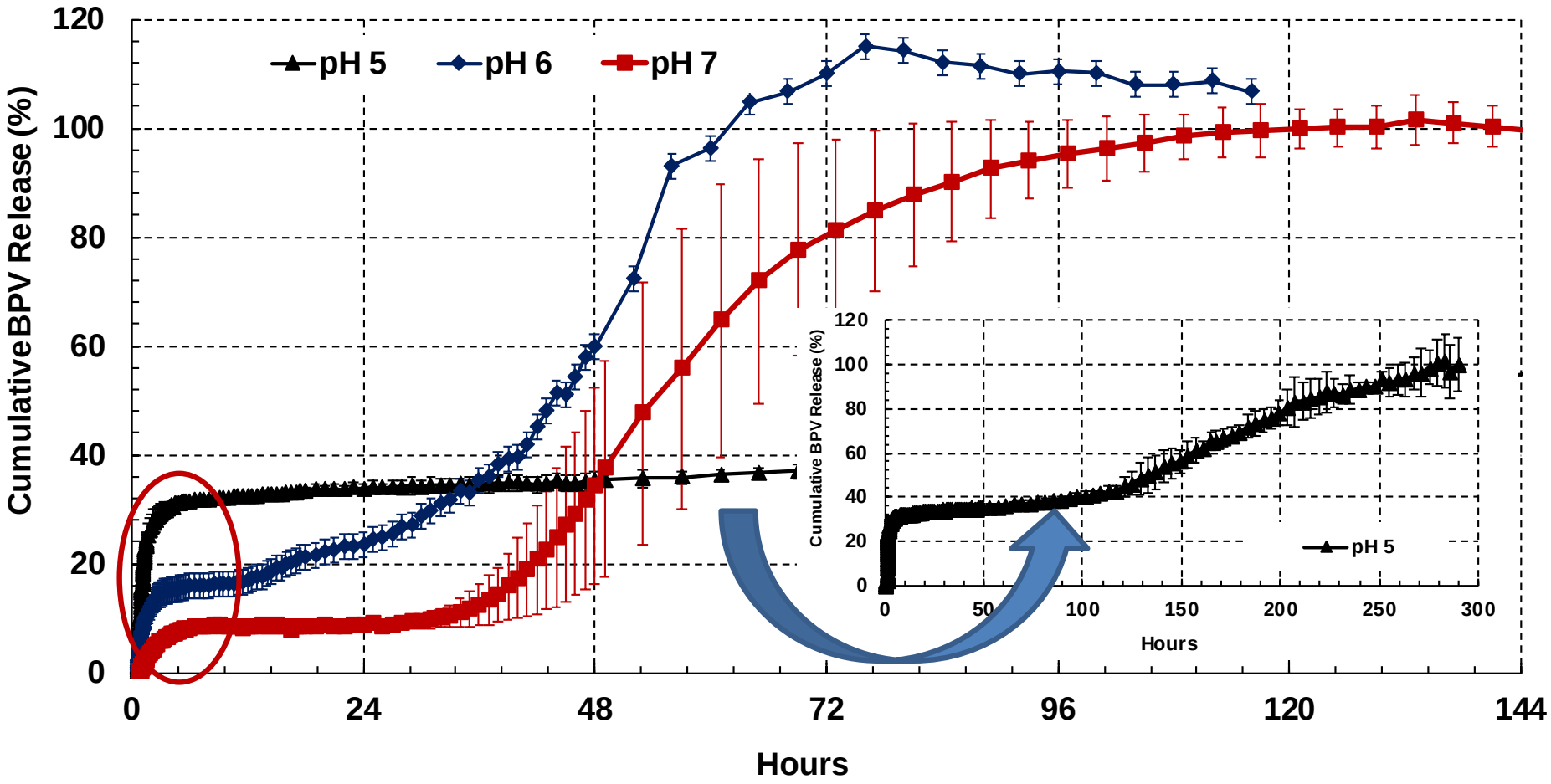
- Initial diffusion rate of BPV was proportional to the MWCO of the dialysis membrane
- 100 kDa membranes exhibited the fastest rate of diffusion among the tested membranes
- 100 kDa membranes also showed the typical diffusion profile ~ 2 h for most drugs

Reverse Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus
- Effect of Temperature



- Change in temperature influences the lag phase and the secondary release phase
- Temperatures close to T_m of the lipids cause more variable release in the secondary release phase

Reverse Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus
- Effect of pH

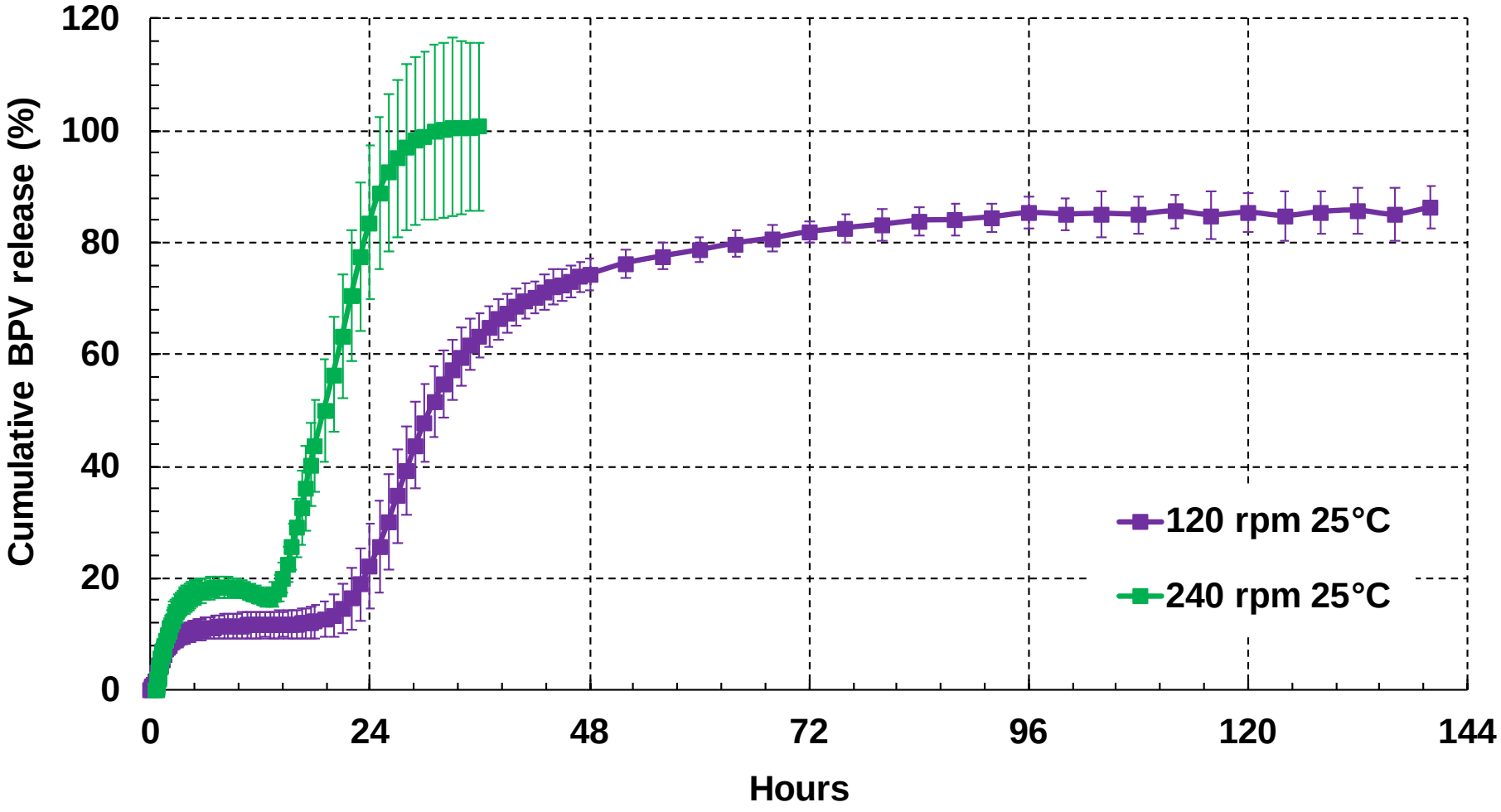


- pH ↓ higher solubility of BPV (~ 40 mg/mL)[1] ↑
- pH ↑ ionization of BPV (pKa = 8.4) ↓

[1] *Shah and Maniar, J Con Rel; 1993*

Reverse Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus

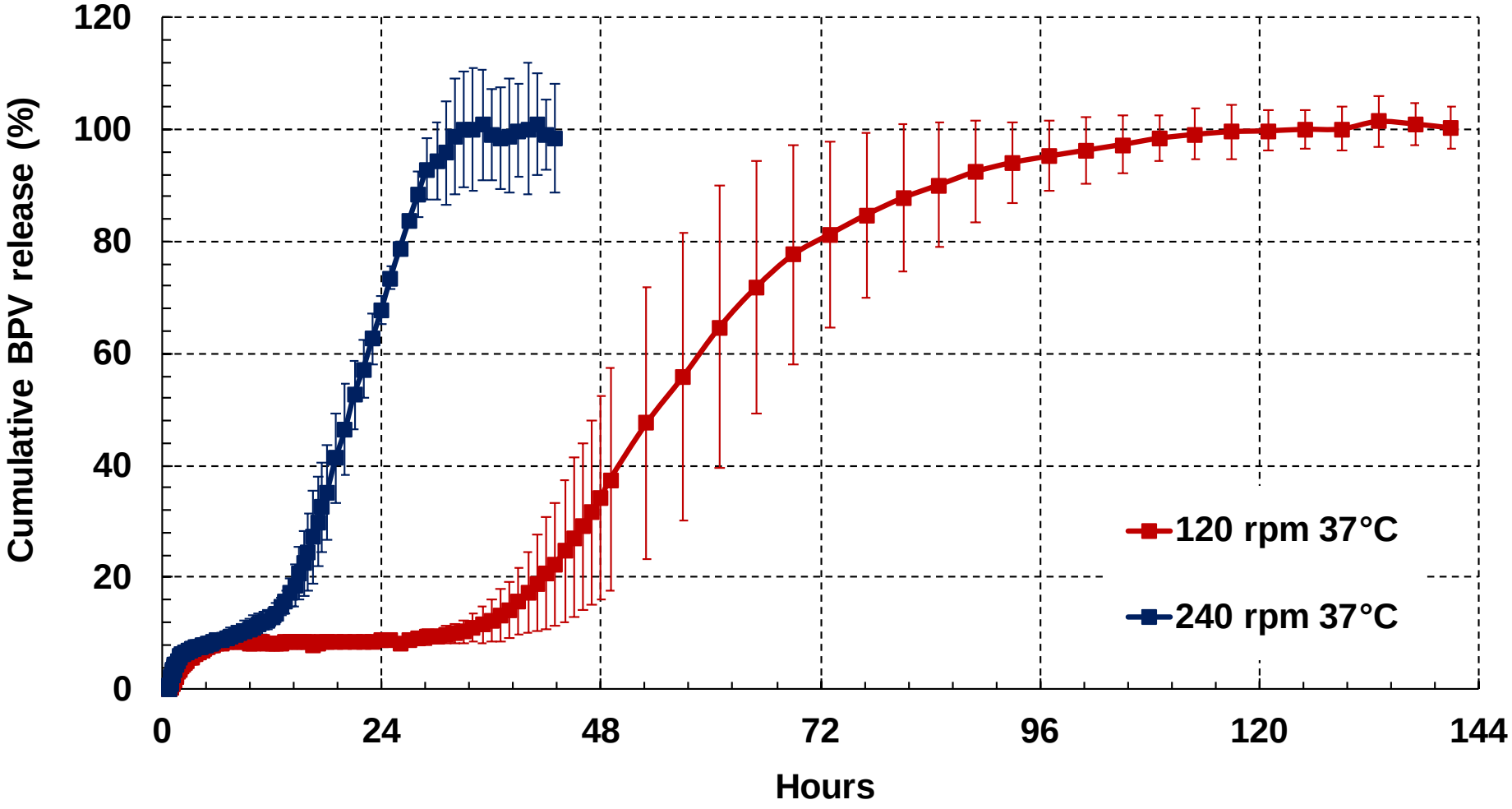
- Effect of Agitation - 25°C



Higher agitation releases the drug faster

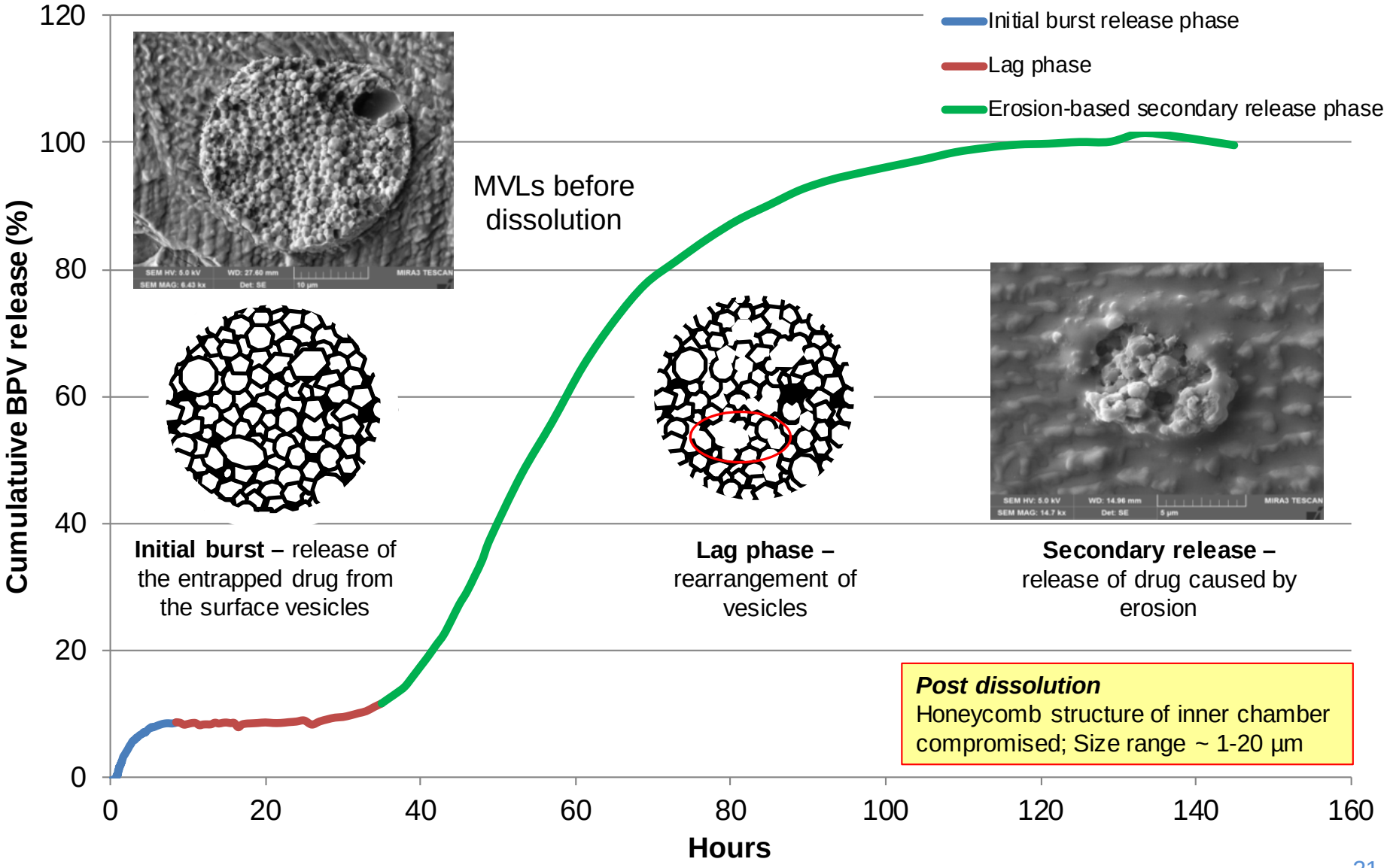
Reverse Dialysis coupled with in-situ UV-Vis probe in a USP II apparatus

- Effect of Agitation - 37°C



Higher agitation releases the drug faster, irrespective of the temperature

Potential Release Mechanism



Take Home Messages

- Cryo-SEM provides not only particle size, but also the internal structure of liposome

- Cryo-SEM provides the necessary correlation of the change in the morphology of the MVL structures with the change in the release mechanism dynamics

- The release mechanism of BPV from the MVLs are sensitive to the IVRT conditions
  - Temperature – changes the mobility of the lipid chains
  - pH – change the ionization of the drug
  - Agitation – causes disruption of liposomes
  - Composition of the release media – causes disruption of liposomes
  - MWCO of the dialysis membrane if applicable – influences diffusion of drug

Cover Story – J. of Controlled Release, January 2019

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journal of controlled release

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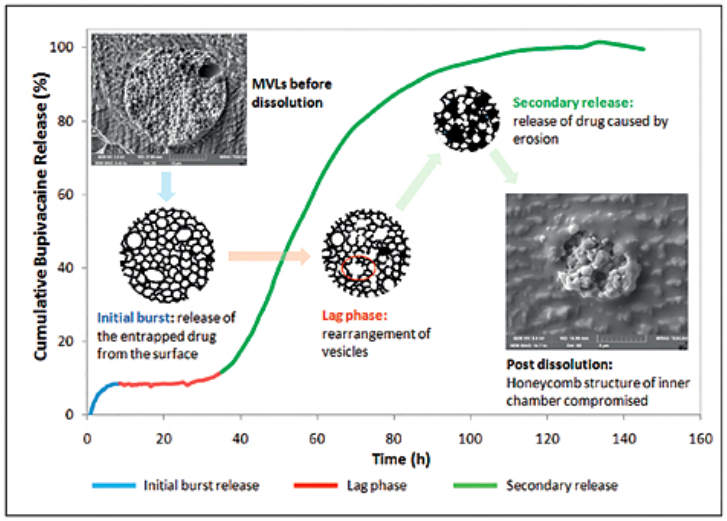

COVER STORY

Probing the mechanism of drug release from liposomes

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