Probing The Mechanism Of Drug Release From Multivesicular Liposomes

Soumyarwit Manna[1,2], Yong Wu[2], Peter E Petrochenko[1], Bonhye Koo[1,2], Lynn Chen[1], Yixuan Dong[1], Xiaoming Xu[1], Stephanie Choi[1], Darby Kozak[1], Yan Wang[1], Jiwen Zheng[2]

1. Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD
2. Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, MD

BACKGROUND

- Bupivacaine (BPV), an amide type local anesthetic, has a relatively short half-life ($t_{1/2\text{-BPV}}$ ~ 2.7 hours)
- BPV, when encapsulated in a multivesicular liposome (MVL) formulation, exhibits *sustained release* characteristics ($t_{1/2\text{-BPV}}$ ~ 34 hours)
- Multivesicular liposomes (MVLs) consist of a non-lamellar honeycomb structure with *non-concentric* aqueous chambers
- *Limited* information on the mechanism of drug release from MVLs – required to advance MVL drug product development, quality assessment, and generic formulation equivalence
- Unique physicochemical properties and complex drug release characteristics *challenge* the design and development of appropriate in vitro release test (IVRT) methods
- It is *hypothesized* that altering IVRT conditions can affect the mechanism of drug release from the MVLs

➢ Speed and orientation of agitation ➢ Composition of release medium (presence of protein) ➢ pH ➢ Temperature	influences	➢ Change in liposomal vesicles (e.g. size and morphology of the outer lipid membrane and inner vesicles) ➢ Drug (e.g. solubility, ionization, and partitioning) ➢ Lipids (e.g. arrangement and phase transitions)

OBJECTIVES

- Obtain a mechanistic understanding of the physical phenomena involved in drug release from MVLs
- Characterize the vesicle size and morphology of the BPV-MVL complex formulation
- Conduct IVRTs to analyze the effect of different parameters such as a) agitation, b) composition of release medium, c) temperature, d) pH, and e) dissolution set-up on the release mechanism of the drug over a period of 7 days - expected complete drug release profile (5 times the $t_{1/2\text{-BPV}}$)

METHODS

- MVL vesicle size and morphology: Cryo-scanning electron microscopy (Cryo-SEM), laser diffraction, confocal microscopy
- IVRT set-up and drug release detection techniques:
  - ➢ Rotary shaker (Thermo Scientific™ Tube Revolver / Rotator)

Dissolution media	50 mM PBS (pH 7) with varied concentrations of HSA				
Temp.	37°C	*Rotation*	12 rpm	*Conc. BPV - MVLs*	0.782 mg / mL

HPLC Column	Agilent ZORBAX SB-CN 4.6 × 150 mm, 5µm		
Mobile phase	40% Acetonitrile + 0.01% TFA; pH 2.8		
Flow rate	1 mL/min	*Column Temp.*	30°C
λ_{BPV}	263 nm	*Ret. time*	~ 2.1 min

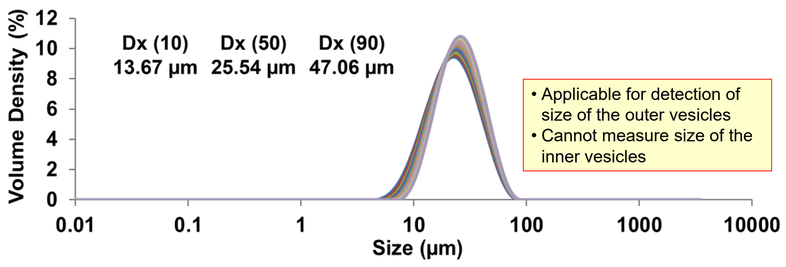
- ➢ USP II apparatus with Reverse dialysis (Teledyne Hanson Research)

Dissolution media	pH 5, pH 6 (citrate phosphate buffer) pH 7 (phosphate buffer) pH 10 (carbonate buffer)		
Temp.	25°C, 31°C, 37°C and 40°C	*Agitation*	120 rpm 240 rpm

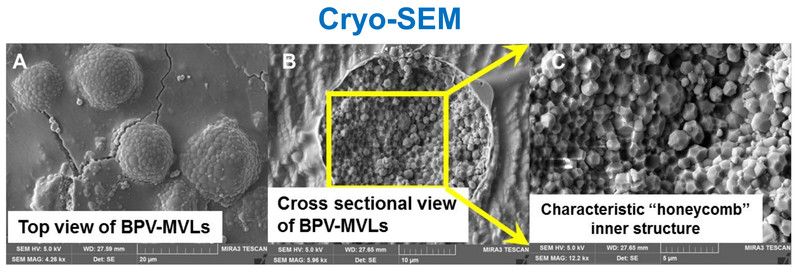
Dialysis cartridges	100 kDa (MWCO) cellulose ester membrane conditioned with SDS
Drug detection	In situ UV-Vis fiber optic probe
Conc. BPV - MVLs	0.58 mg / mL

RESULTS: MVL Vesicle Size and Morphology

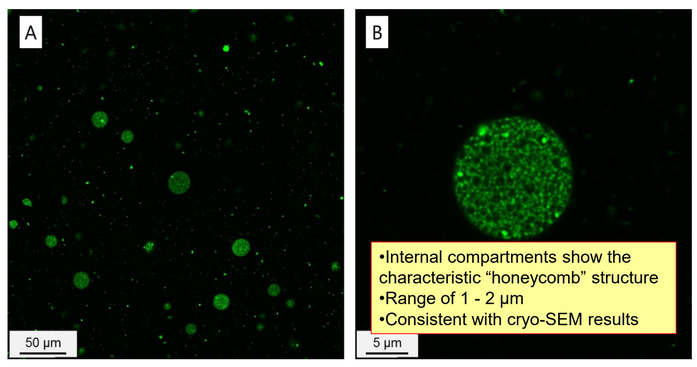
Laser Diffraction



Cryo-SEM

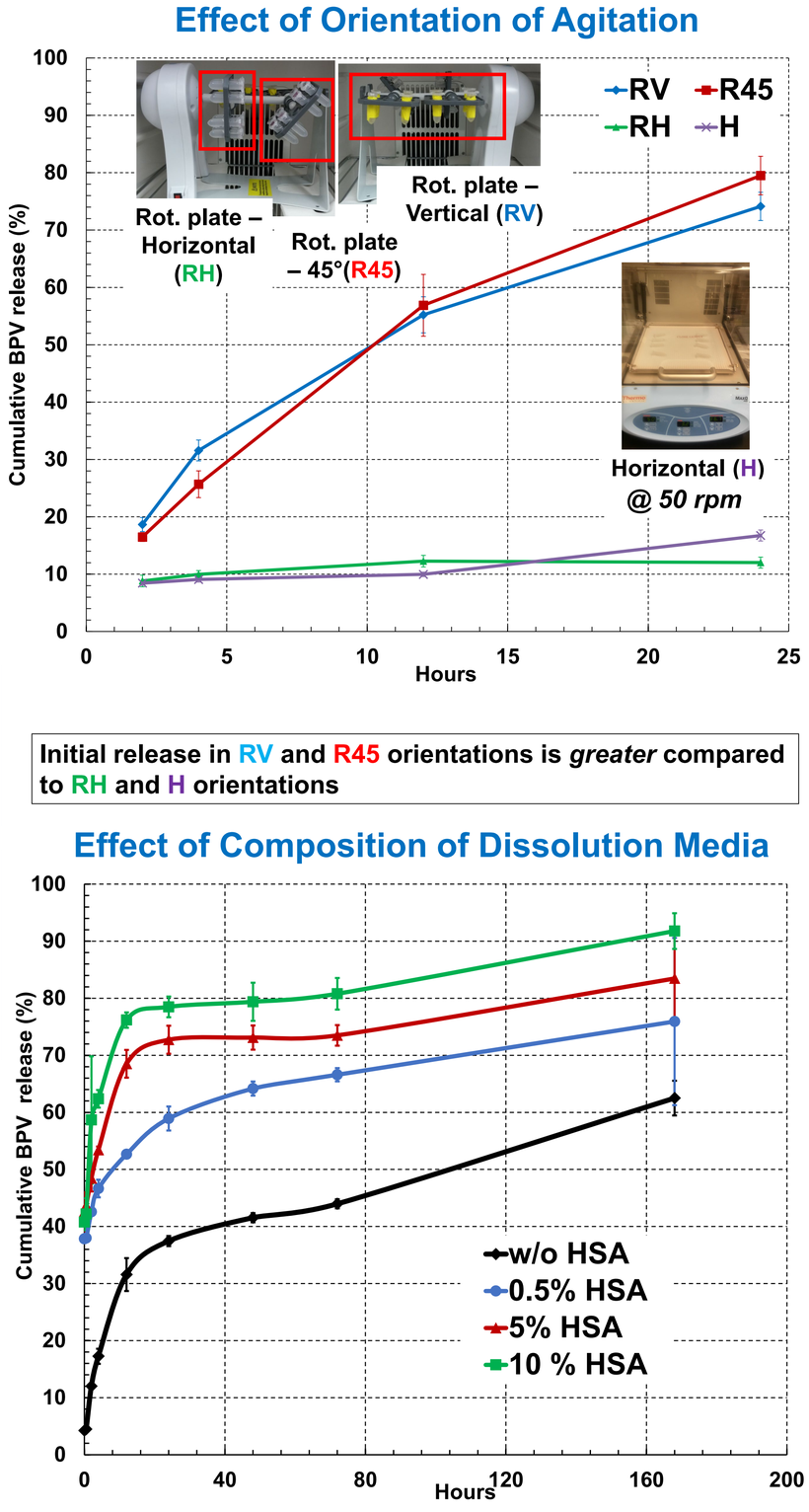


Confocal Microscopy



RESULTS: In vitro Drug Release – Rotary Shaker + HPLC

Effect of Orientation of Agitation



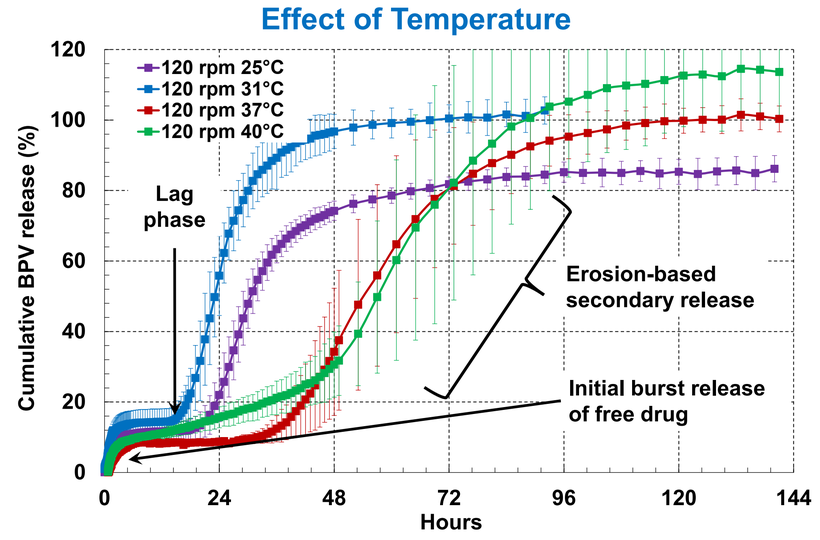
Initial release in RV and R45 orientations is *greater* compared to RH and H orientations

Effect of Composition of Dissolution Media

Presence of HSA in 50 mM PBS caused accelerated release of BPV from the MVLs under RV rotation

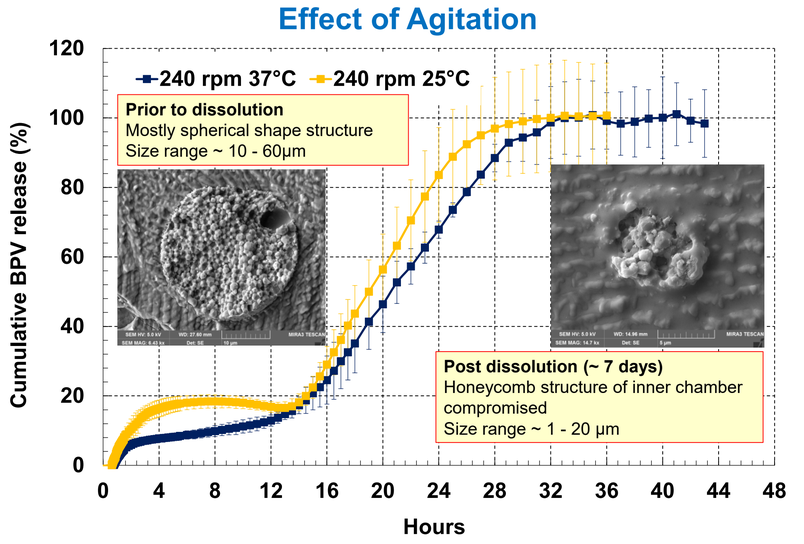
RESULTS: In vitro Drug Release – USP II with Reverse Dialysis + in situ UV

Effect of Temperature



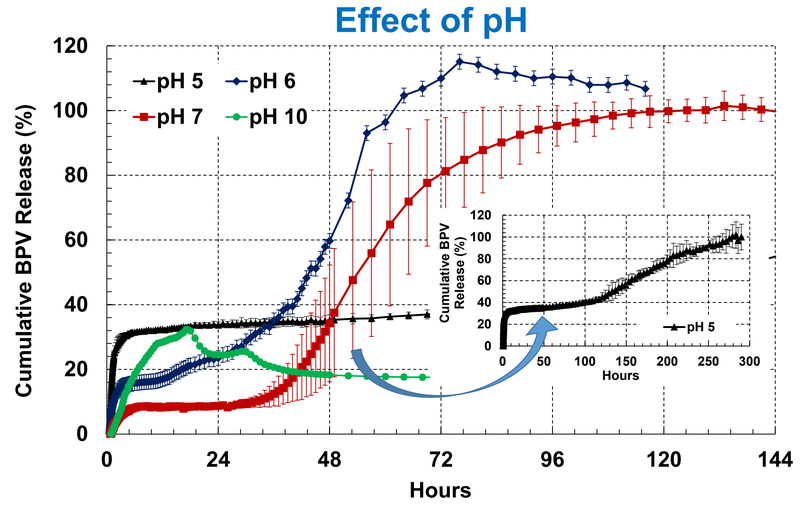
- Temp. influences the lag phase and the secondary release phase
- Temp. close to T_m of the lipids (~ 41°C) cause more variable release in the secondary release phase

Effect of Agitation



- Higher agitation releases the drug faster, irrespective of the temperature (in comparison to 120 rpm – please see above)

Effect of pH



- pH ⇩ higher water solubility of BPV (~ 40 mg/mL) ⇧
- pH ⇧ ionization of BPV (pKa = 8.4) ⇩, water solubility ⇩

CONCLUSIONS

- BPV MVLs are monodisperse spherical particles with complex internal compartment morphology
- Possible mechanism of release: combination of diffusion (initial burst), rearrangement of internal vesicles, physical degradation (secondary release)
- IVRT– Rotary shaker (captures a *biphasic* profile)
  - Orientation of agitation and presence of HSA influence BPV release
- IVRT– Reverse dialysis – USP II coupled with in-situ UV fiber optic (captures a *triphasic* profile)
  - Discern both the initial diffusion burst release (~ 2 – 4 hr) and the secondary release, likely triggered by physical degradation of MVLs
  - *Advantage* - continuous monitoring of BPV release
  - Initial burst release was primarily influenced by the pH, while the lag and secondary release were impacted by the temperature, agitation and pH

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