Mechanistic Understanding of In Vitro Drug Release of Bupivacaine from Multivesicular Liposomes

M1130-07-055

Soumyarwit Manna[1,2], Peter Petrochenko[1], Yong Wu[2], Bonhye Koo[1,2], Lynn Chen[1,2], Yixuan Dong[3], Xiaoming Xu[3], Stephanie Choi[1], Darby Kozak[1], Yan Wang[1], Jiwen Zheng[2]

[1.] Office of Research and Standards, Center for Drug Evaluation and Research, FDA, Silver Spring, MD

[2.] Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, FDA, Silver Spring, MD

[3.] Office of Testing and Research, Center for Drug Evaluation and Research, FDA, Silver Spring, MD

Email: Soumyarwit.Manna@fda.hhs.gov

aaps PharmSci 360 ADVANCING PHARMACEUTICAL SCIENCES, CAREERS, AND COMMUNITY

PURPOSE

- Bupivacaine (BPV), an amino-amide local anesthetic, has a short half-life ($t_{1/2\text{-BPV}}$ ~ 2.7 h)
- BPV, when encapsulated in a multivesicular liposome (MVL) formulation, exhibits *sustained release* characteristics ($t_{1/2\text{-BPV}}$ ~ 34 h)
- MVLs consist of a non-lamellar honeycomb structure with *non-concentric* aqueous chambers
- Information is limited 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 evaluate 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), n = 3

Dissolution media	50 mM PBS (pH 7) with varied conc. of human serum albumin (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 Temperature*	30°C
λ_{BPV}	263 nm	*Retention time*	~ 2.1 min

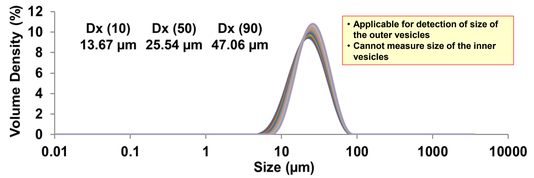
➢ USP II apparatus with Reverse dialysis (Teledyne Hanson Research), n = 3

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

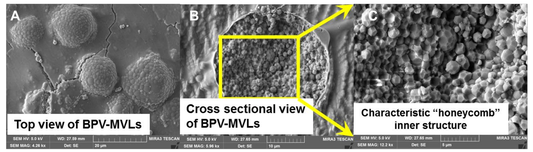
Dialysis cartridges	100 kDa (MWCO) cellulose ester membrane conditioned with SDS
Drug detection	In situ UV-Vis fiber optic probe
Conc. BPV - MVLs	0.58 mg / mL

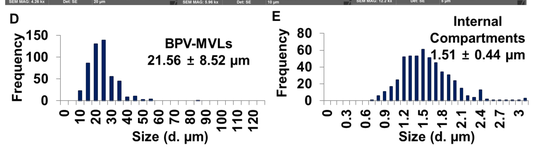
RESULTS: MVL Vesicle Size and Morphology

Laser Diffraction

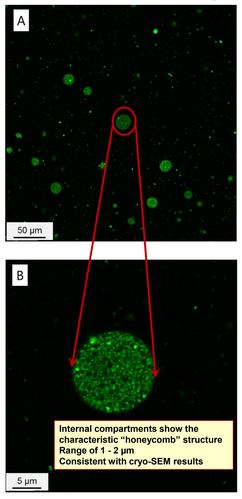


Cryo-SEM



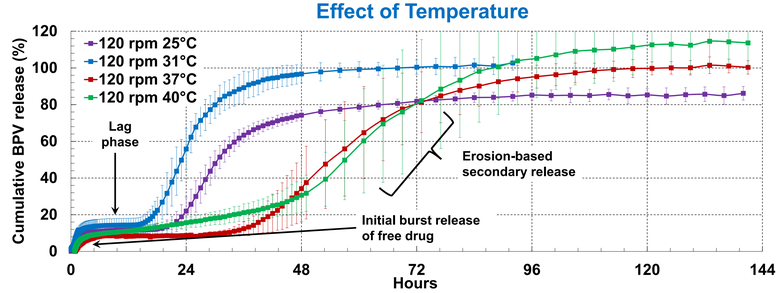


Confocal Microscopy



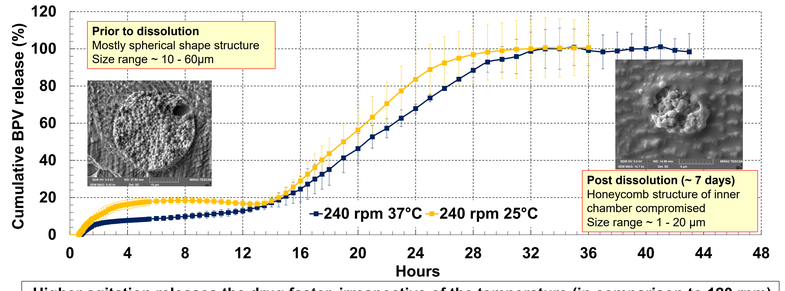
RESULTS: IVRT– USP II with Reverse Dialysis + in situ UV

Effect of Temperature



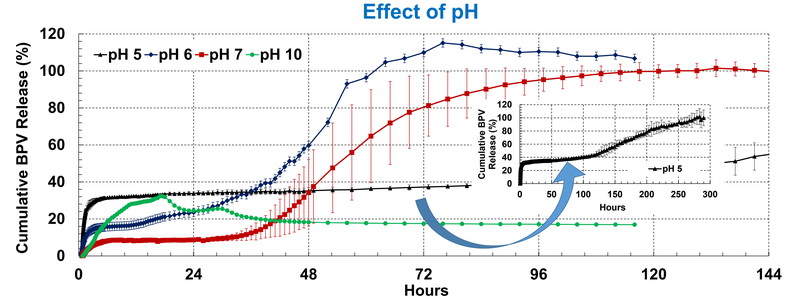
•Temp. influences the lag phase and the secondary release phase
•Temp. close to T_m of the lipids (~ 41°C) cause more variable release in the secondary release phase

Effect of Agitation



•Higher agitation releases the drug faster, irrespective of the temperature (in comparison to 120 rpm)

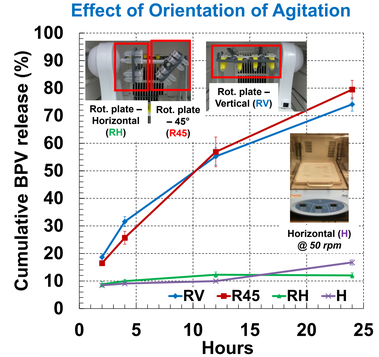
Effect of pH



• pH ↓ higher water solubility of BPV (~ 40 mg/mL) ↑ ionization of BPV (pKa = 8.4) ↑

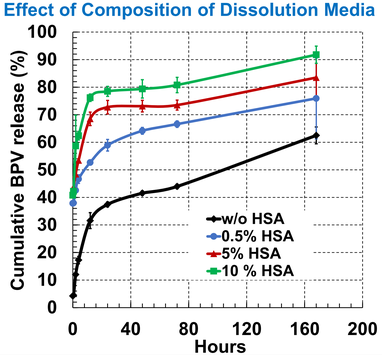
RESULTS: IVRT – Rotary Shaker + HPLC

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

CONCLUSIONS

- BPV MVLs - spherical particles with complex internal compartment morphology
- IVRT– Rotary shaker (*biphasic* profile)
  - Orientation of agitation and presence of HSA influence BPV release
- IVRT– Reverse dialysis – USP II coupled with in-situ UV fiber optic (*triphasic* profile)
  - *Continuous monitoring* of BPV release, discerning both the initial diffusion burst release (~ 2 – 4 h) and the secondary release, likely triggered by physical degradation of MVLs
  - Initial burst - primarily influenced by the pH
  - Lag and secondary release - impacted by the temperature, agitation and pH

ACKNOWLEDGEMENTS

The authors would like to thank the FDA Advanced Characterization Facility (ACF) and CDRH/Office of Science and Engineering laboratories/Division of Biology, Chemistry and Material Science for instrument use. Dr. Manna was supported in part by an appointment to the Oak Ridge Institute for Science and Education (ORISE) Research Participation Program at the Center for Drug Evaluation and Research administered by the ORISE through an agreement between the U. S. Department of Energy and FDA/CDER.

DISCLAIMER

FDA U.S. FOOD & DRUG ADMINISTRATION