Improving drug loading in sub-micron bubbles using hydrophobic doxorubicin

Pinunta Nittayacharn[1], Al De Leon[2], Eric Abenojar[2], Agata A Exner[1,2]
[1] *Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio, USA.*
[2] *Department of Radiology, Case Western Reserve University, Cleveland, Ohio, USA.*

Background, Motivation and Objective

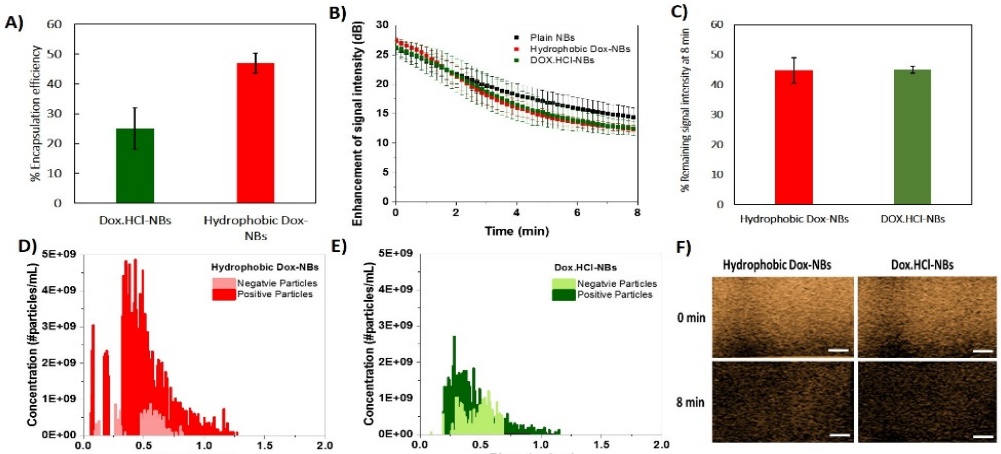
Drug-loaded submicron ultrasound contrast agents have the potential to augment the efficiency of drug delivery through improved drug extravasation and cell uptake, resulting in higher drug accumulation at the target site. The therapeutic efficiency of drug-loaded bubbles has been limited by the loading capacity of the shell which stabilizes the gas core. High drug loading can destabilize the bubbles, which can result in insufficient therapeutic effect. Here to improve drug loading directly into the bubble shell, deprotonated hydrophobic doxorubicin (hDox) was prepared and loaded into submicron C_3F_8 bubbles. Drug encapsulation efficiency, the size, concentration, and *in vitro* acoustic properties were characterized and compared to commercially available doxorubicin (Dox.HCl)-loaded bubbles.

Statement of Contribution/Methods

hDox was prepared by reacted Dox.HCl solution with trimethylamine overnight. hDox-NBs were prepared as previously reported (Nittayacharn P, et.al. 2018 *IEEE IUS,* Abenojar et al, 2019 *Langmuir*) by dissolving hDox or Dox.HCl and lipids (DBPC, DPPA, DPPE, DSPE-PEG2k) in propylene glycol and PBS. Following air exchange with C_3F_8, bubbles were formed by mechanical agitation and isolated by centrifugation. Encapsulation efficiency (%EE) was determined using fluorescent measurement after centrifuge filtration. Size and concentration of buoyant/non-buoyant particles were measured using resonant mass measurement (RMM) (Malvern). US signal enhancement and signal decay were measured by imaging using nonlinear contrast harmonic imaging (Toshiba) at 12 MHz, 1 fps, and 0.1 MI.

Results/Discussion

By using hDox, %EE was doubled from to 24.9±3.4 to 46.9±10.0% compared to standard Dox.HCl NBs. RMM showed NB size of 0.36±0.20 µm and an increased bubble concentration of $2.4x10^{11}$ elevated bubble to non-buoyant particle ratio for hDox NBs (Fig1 d). Acoustic properties of both formulations were comparable to NBs without Dox, indicating that hDox and Dox.HCl do not affect NB performance (Fig1b, c). These results provide evidence that incorporating deprotonated Dox into lipid shelled bubbles can increase the drug loading efficiency which could improve the therapeutic efficacy of Dox or other hydrophobic drug-loaded bubbles. Future experiments will study this effect on the isolated NBs/MBs with a narrow size distribution and will examine *in vitro* and *in vivo* efficacy.



Hydrophobic Dox-NBs	Buoyant particles	Non-buoyant particles
Mean diameter (µm)	0.36±0.20	0.59±0.1
Concentration (#/mL)	2.44E+11	1.39E+10

Dox.HCl-NBs	Buoyant particles	Non-buoyant particles
Mean diameter (µm)	0.30±0.09	0.49±0.13
Concentration (#/mL)	1.73E+11	1.57E+10

Figure 1. Characterization of hydrophobic Dox and Dox.HCl-loaded NBs. (A) % Encapsulation efficiency; (B) US signal decay; (C) Remaining US signal at the end of study (8 min). (D and E) Concentration and size distribution measured by RMM. The mean diameter of NBs was calculated based on the Gaussian distribution; (F) Representative US contrast images. The scale bar is 0.5 cm.