M8006

Characterizing How Size Distribution and Concentration Affect Echogenicity of Ultrasound Contrast Agent Products: Scientific Considerations for an In Vitro Bioequivalence Approach

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2017 AAPS ANNUAL MEETING & EXPOSITION

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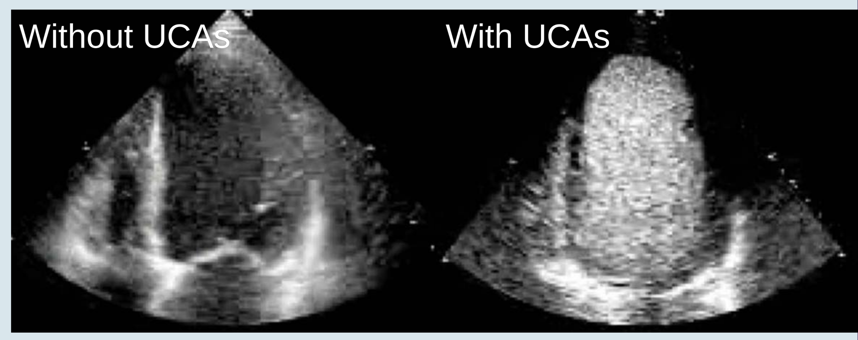
PURPOSE

Ultrasound contrast agents (UCAs), typically a suspension of gas-filled lipid microspheres (microbubbles), are used for improving contrast in suboptimal echocardiograms. There is a general correlation between the acoustic signal efficiency, microbubble size, concentration and ultrasound frequency used. Since the performance of these products is not directly correlated to the gas (the active ingredient) concentration in blood, it is challenging to develop and approve a generic UCA product based on traditional in vivo pharmacokinetic studies. Thus, alternative methods, based on an understanding of the critical quality attributes and appropriate in vitro tests, may be needed to support the equivalence of generic UCA products. Findings from this study will provide regulatory science support to develop product-specific guidance for generic UCA product development and approval.

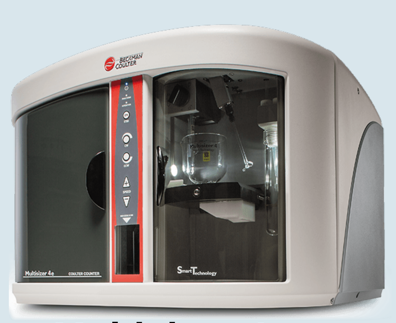


OBJECTIVES

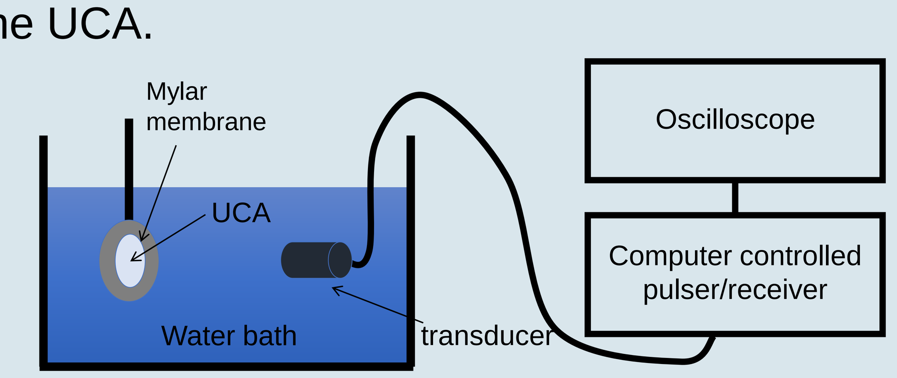
We investigated the correlation between size and concentration of a model UCA (approved sulfur hexafluoride lipid microbubble formulations) and their backscattering coefficient.

METHODS

1) Size distribution:
   - The size distribution was measured using a Multisizer 4 Coulter counter.
2) Bubble fractionation:
   - The agitated suspension was fractionated by differential centrifugation.
3) Backscattered acoustic signal:
   - Two transducers (2.25 MHz & 5 MHz) were separately used for different frequency range to cover 1 – 7 MHz (clinically relevant imaging range).
   - The transducers transmitted a broadband pulse and received a backscattered signal from the UCA.



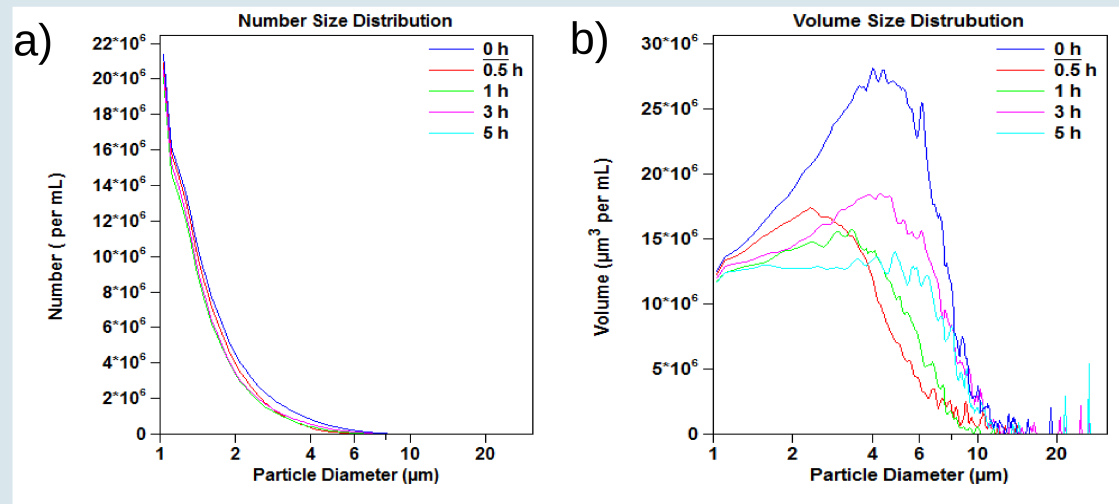
Multisizer 4



Experimental set-up for backscattering measurement

RESULTS

1. Stability of Microbubble in Sealed Vial



c)

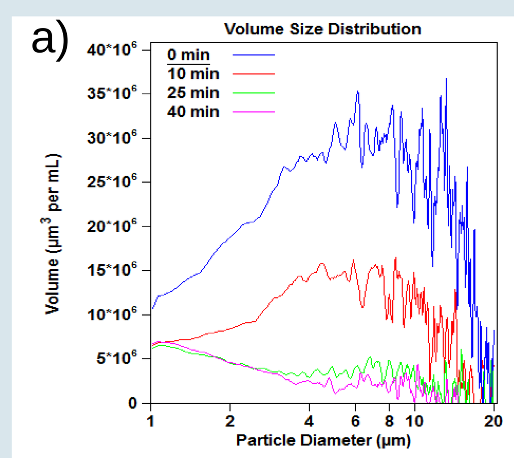
Diameter	Number (%)	Volume (%)
1 – 2 μm	64.2	4.7
2 – 6 μm	31.8	35.6
> 6 μm	3.9	59.7

d)

Time (h)	Number-weighted concentration (/mL)	Volume-weighted concentration (μm³/mL)
0	2.16×10^8	2.32×10^9
0.5	1.86×10^8	1.19×10^9
1	1.67×10^8	1.02×10^9
3	1.62×10^8	1.33×10^9
5	1.60×10^8	9.89×10^8

- Particle distribution shows that the majority of the bubbles in the formulation are small (< 2 μm) however, a large portion of particle volume fraction is from the large microbubbles (> 6 μm)
- Measure of bubble concentration and volume distribution with time (0 -5 h) reveals that larger microbubbles are less stable than smaller microbubbles, which corresponds to a large decrease in total bubble volume.
- Total microbubble concentration decreases in 30 min (14 % in number, 48.7 % in volume) and then remains relatively stable for 5 h (Table 1d).

2. Stability of Diluted Microbubble Suspension

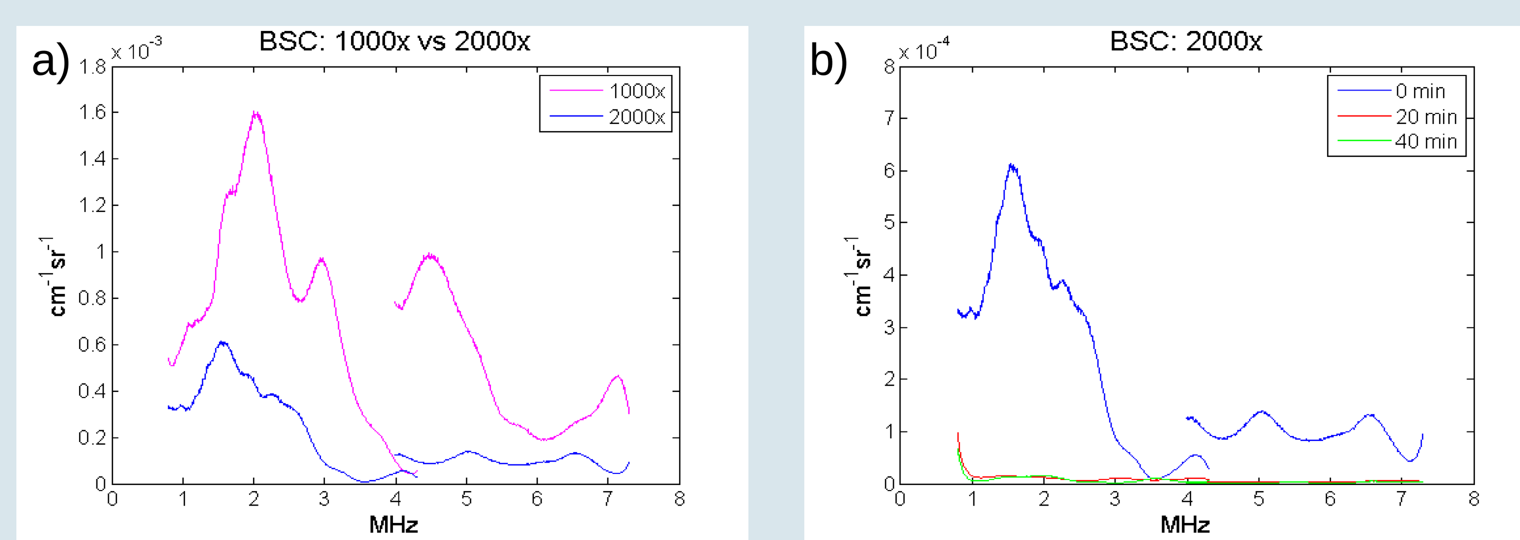


b)

Time (min)	Number-weighted concentration (/mL)	Volume-weighted concentration (μm³/mL)
0	3.22×10^8	8.58×10^9
10	1.64×10^8	3.39×10^9
25	1.09×10^8	1.02×10^9
40	1.12×10^8	0.62×10^9

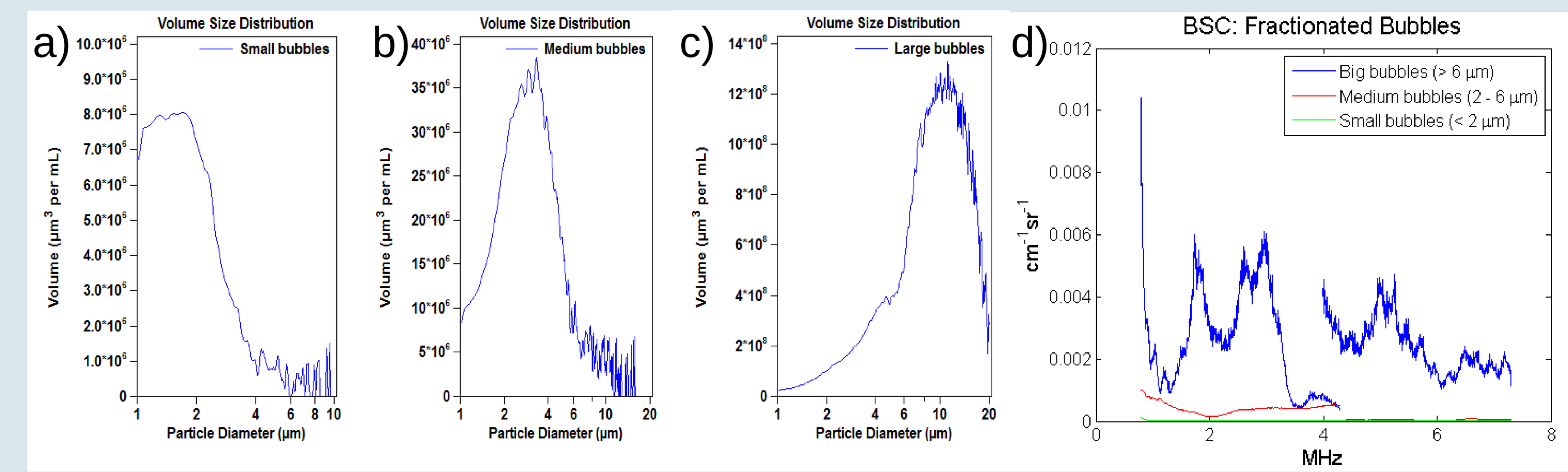
- UCA microbubbles degrade at a much faster rate when diluted (2000x) in ISOTON buffer compared to native formulation
- In 10 min, number-weighted and volume-weighted concentration drops 49 % and 60 %, respectively.

3. Effect of Microbubble Concentration on the Backscattering Coefficient (BSC)



- <u>Concentration (total volume of bubbles)</u> is a key factor in BSC of UCA microbubbles.
  - BSC is higher at higher bubble concentration, e.g. lower dilution (Fig 3a).
  - BSC significantly drops over time (Fig 3b), likely caused by the bubble instability and corresponding decrease in bubble volume over time (Fig 2)

4. BSC of Fractionated Microbubbles



- Three bubble populations of different size range (e.g. small, medium and large) were isolated from the same bubble stock using differential centrifugation.
- Fractionated large bubbles (> 6 μm) have 2 orders of magnitude larger volume compared to small (< 2 μm) and medium bubbles (2 – 6 μm) (Fig 4a – c).
- <u>Bubble size (volume)</u> is an important factor in BSC of UCAs (Fig 4d).
  - With the same number-weighted concentration, BSC results demonstrate that large bubbles generate much higher backscattering signal compared to others.
  - Small bubbles contribute negligible acoustic effect in 1 – 7 MHz.

CONCLUSIONS

- UCAs quickly degrade upon dilution, therefore, the stability of UCA should be carefully considered in a quantitative acoustic characterization, i.e., BSC.
- Large microbubbles generate higher backscattering signal than small microbubbles, indicating volume-weighted concentration is more meaningful than number-weighted concentration in terms of echogenicity.

ACKNOWLEDGEMENTS

- Dr. Bonhye Koo, and Dr. Bin Qin 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 (CDER) administered by the ORISE through an agreement between the U. S. Department of Energy and CDER.
- The authors would like to acknowledge the FDA Advanced Characterization Facility, Center for Drug Evaluation and Research/Office of Pharmaceutical Quality/Office of Biotechnology Products/Division of Biotechnology Review and Research II, Center for Devices and Radiological Health/Office of Science and Engineering Laboratories/Division of Applied Mechanics, and Center for Devices and Radiological Health/Office of Science and Engineering Laboratories/Division of Biology, Chemistry, and Material Science for instrument use.