Theoretical and Experimental Comparisons of Perfluoropropane (C_3F_8) Gas Volume in Bubble Contrast Agents for Ultrasound Applications

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Background, Motivation and Objective Gas volume of ultrasound contrast agents is critical to their acoustic activity and *in vivo* performance, and has been a parameter used to normalize bubbles of different sizes or shell contents. Typically, the total theoretical gas volume in a sample is calculated assuming a sphere based on a bubble radius and concentration measured experimentally via the Coulter Counter® and is used as ground truth (Song, K. et al. *Theranostics* 2017). However, the Coulter Counter cannot distinguish between buoyant and non-buoyant particles. This affects particle count accuracy and leads to inaccurate total gas volume calculations. Here, we address these limitations by: (1) using resonant mass measurement (RMM) to measure bubble size and concentration, and (2) utilizing headspace gas chromatography/mass spectrometry (GC/MS) to accurately measure the gas volume of the bubble sample.

Statement of Contribution/Methods Lipid shell-stabilized C_3F_8 microbubbles (MBs) and nanobubbles (NBs) were prepared and isolated via centrifugation and characterized with RMM. Concurrently, the same samples were added to headspace vials, sealed, and sonicated at 50 °C for 20 min to destroy all the bubbles and release the C_3F_8 gas to the vial headspace, which was analyzed using GC/MS. Gas volume was quantified based on a calibration curve of C_3F_8 gas in air (0-1% v/v). Theoretical gas volume was estimated using size and concentration from RMM while subtracting the bubble lipid shell thickness (~2.5 nm) and assuming a sphere with a gas density of 0.008 $g \cdot mL^{-1}$.

Results/Discussion Based on RMM, NBs and MBs had a diameter of 294±178 nm (6.5×10^{11} particles$\cdot mL^{-1}$) and 881±290 nm (1.1×10^{10} particles$\cdot mL^{-1}$), respectively. Experimental GC/MS gas volume (nL/mL) measurements agreed well with theoretical predictions and the difference was 1.4% for MBs and 9.9% for NBs. MB size measured using RMM and laser diffraction techniques, with a limit of detection (LOD) of 250 nm and 17 nm, respectively, showed comparable mean size. For the same samples the Coulter Counter measured a diameter of 1103±370 nm (1.7×10^{9} particles$\cdot mL^{-1}$) due to a higher LOD of 600 nm. This resulted in a predicted gas volume of 1150 nL/mL (70% lower than the experimental value). This study demonstrates that gas volume can be accurately measured using GC/MS. Accuracy of theoretical predictions is highly dependent on proper size and concentration measurements.

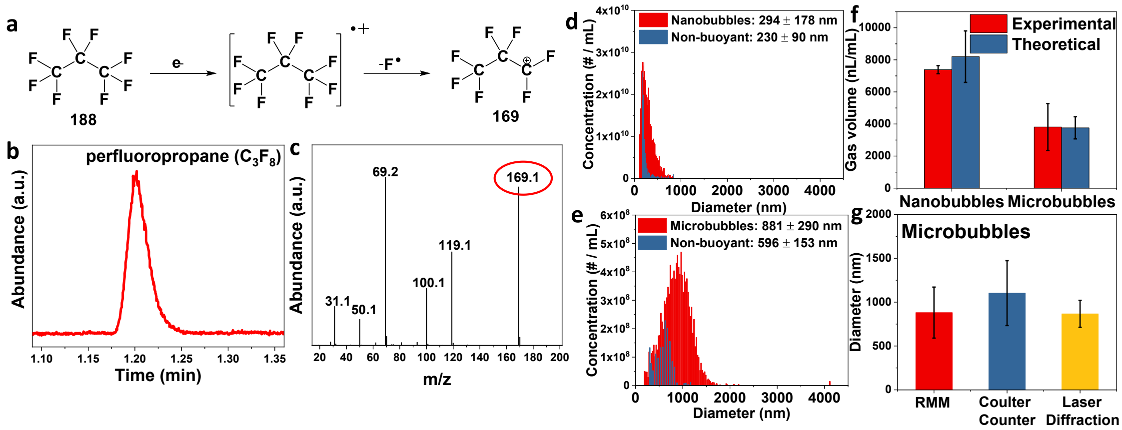

Fig. 1. (a) Mass spectrometry fragmentation pattern of C_3F_8. Headspace GC/MS analysis of the bubble sample shows the (b) gas chromatographic trace with a retention time of 1.2 min and (c) mass spectrum of C_3F_8 gas (m/z 169 used for analyses). Resonant mass measurement (RMM) shows the size and concentration of (d) nanobubble (NB) and (e) microbubble (MB) samples. (f) Experimental and theoretical comparison for NB and MB gas volume measurements via headspace GC/MS. (g) MB size characterization using three different techniques: RMM, Coulter Counter, and laser diffraction.