Effects of acoustic parameters and bulk fluid properties on acoustic droplet vaporization threshold of perfluoropentane droplets

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Abstract

Phase shift droplets vaporizable by acoustic stimulation are better alternatives to conventional ultrasound contrast agents in terms of higher stability and possibility of achieving smaller sizes. This study determines the threshold pressures required to induce acoustic droplet vaporization (ADV) and inertial cavitation (IC) of a suspension of droplets with a perfluoropentane (PFP) core (diameter 400-3000 nm) in a novel tubeless setup using acoustic methods. We further investigate the effects of excitation frequency, pulse lengths, temperature and dissolved gas concentration on both ADV and IC thresholds. We have found that ADV threshold decreases with temperature and increases with frequency. ADV thresholds at all the frequencies studied here occurred at lower rarefactional pressures than IC thresholds indicating that phase transition precedes inertial cavitation. The ADV and the IC thresholds did not change when the experiments were performed in degassed conditions. The scattered response from droplets above the ADV threshold was also found to qualitatively match with that of independently prepared lipid-coated microbubble suspensions in magnitude as well as trends.

Keywords: acoustic droplet vaporization; perfluoropentane droplets; scattered response; inertial cavitation; ultrasound contrast agents

Introduction

Gas-filled microbubbles encapsulated by lipids and other surfactants are highly responsive to ultrasound, which has led to their effective role as vascular agents for contrast enhanced ultrasound imaging[1]. More recently, emulsions of phase shift droplets of volatile perfluorocarbon liquids, that can be vaporized in situ into highly echogenic microbubbles by external application of ultrasound pulses, are being investigated[2, 3]. Note that phase shift droplets can be used for extravascular imaging and drug delivery mainly due to the fact that small size droplets can extravasate through leaky vasculature of cancerous tumors as a result of the enhanced permeability and retention (EPR) effect[4]. The diagnostic and therapeutic potentials of acoustic droplet vaporization (ADV) have been studied extensively [5, 6].

The phenomenon of acoustic droplet vaporization (ADV), specifically the threshold ultrasound excitation for vaporization, has been studied in the past using both acoustic and optical methods. Acoustic investigations determine ADV thresholds through the scattered signals. Optical investigations depend on direct observations of the vaporization using cameras which might suffer from limitation of resolution. Here, we have performed an acoustic investigation of the ADV threshold of lipid-coated perfluoropentane (PFP) droplets and its dependence on excitation frequency, pulse lengths, temperature and dissolved gas concentration in a tube-less setup that minimizes the effects of the containment apparatus. We also measure the acoustic signals from the lipid-coated perfluorobutane (PFB) microbubble suspension to offer a qualitative comparison with that from vaporized PFP droplets. This study also investigates the threshold pressures required to induce inertial cavitation of the vaporized droplets in the same setup.

Experimental methodology

Perfluorocarbons (PFP and PFB) and lipids (DPPC and DPPE-PEG-2000) were purchased from FluoroMed (Round Rock, Texas) and Avanti Polar lipids (Alabaster, AL), respectively. Lipid-coated PFP-droplets and PFB-microbubbles were prepared using sonication and mechanical agitation techniques, respectively. The details of the methods can be found in[7]. The size distribution and the concentration of droplets and microbubbles were determined using qNano (Izon Science™, Cambridge, MA). The droplet suspension had an average diameter of 890 nm and a total

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concentration of 2.1×10^9 droplets/ml whereas microbubbles had an average diameter of 1.6 μm and a total concentration of 2.05×10^8.

To measure the scattered responses, droplet and microbubble suspensions were passed through a metal tube mounted right above the focal region of the two confocally aligned transducers into a chamber filled with DI water, as shown in Fig.1. For the ADV experiments at room temperature, we employed two spherically focused immersion transducers (Panametrics Transducer, Olympus NDT Corporation, Waltham, MA), with central frequencies of 2.25, 5, 10 and 15 MHz. While transmitting transducers were excited at their central frequency, the receiving transducers for measuring fundamental, sub- and second- harmonic scattering at that excitation frequency could have different central frequencies depending on the transducer bandwidth. For all the experiments performed at 37C, a broadband transducer (Sonic concepts, Woodinville, WA, USA) was placed as the receiver. The same broadband transducer was used to monitor for broadband emissions from vaporized droplets undergoing inertial cavitation.

To determine the ADV threshold, we plotted fundamental, subharmonic and second harmonic components of the scattered signal, as the ultrasound excitation amplitude was progressively increased. Liquid core droplets scatter ultrasound weakly; however, upon vaporization and turning into a gaseous bubble, the droplet diameter increases by 5-6 times resulting in a sudden jump in their scattered response. To quantitatively determine the ADV value based on this observation, we followed a curve fitting and slope-based approach similar in [8].

Results and discussion

The typical raw RF data along with the corresponding frequency spectrum for the control and droplet suspensions at the excitation frequency of 2.25 MHz and excitation amplitudes of 1 and 1.5 MPa are displayed in Figures 2 (a) and (b), respectively. It is evident from this figure that the droplet signal is significantly higher upon vaporization. Figures 3(a), (b) and (c) plot the subharmonic (1.125 MHz) and second harmonic (4.5 MHz) and the fundamental responses respectively from the droplet emulsion at the excitation frequency of 2.25 MHz at room temperature. We determined the ADV threshold by the method described earlier to be 1.22 MPa (r^2=0.99), 1.22 MPa (r^2=0.84) and 0.72 MPa (r^2=0.96) for subharmonic, second harmonic and fundamental components, respectively. The average of the three values results in the threshold value of 1.05 MPa at 2.25 MHz.

The fundamental response from droplets and microbubbles also show a qualitative match above 1 MPa. Note that, we have also investigated the effects of nonlinear propagation by studying the second harmonic response from a non-vaporizing liquid such as propylene glycol. As can be seen in Figure 3 (b), the second harmonic response from the propylene glycol stream was found to be minimal indicating minimum contribution due to nonlinear propagation through the liquid.

Similarly, we determined the ADV thresholds for higher excitation frequencies and summarized in Table I. Note that, we did not find subharmonic response at the excitation frequency of 10 MHz. We see an increasing trend for ADV threshold with frequency, as was also seen in optical investigations[9, 10]. One would expect that increasing the frequency, i.e. decreasing the time-period when the liquid is continuously under negative pressure, would increase the threshold for vaporization. The focal zone also becomes smaller at higher frequency decreasing the probability of nucleation inside the PFP droplet core.

ADV measurements performed at 37°C resulted in a substantial decrease in the vaporization threshold. Figure 4(a) displays the average ADV threshold at two temperatures as a function of frequency. Figure 4(b) shows the effect of number of cycles on the ADV threshold at 37°C at the excitation frequency of 2.25 MHz in degassed DI water. As shown in this figure, ADV threshold was independent of the pulse lengths tested here. However, the amplitude of scattered responses (fundamental, second harmonic and subharmonic) substantially decreased in case of 1 cycle excitation which is expected (data not shown).

We also studied the effects of dissolved gas concentration on ADV and IC behaviors. For ADV, we plotted the subharmonic response of the droplet suspension excited at 2.25 MHz and 8 cycles. Experiments were conducted once in DI water and once in degassed DI water at 37 °C. Figure 5(a) shows that the dissolved gas concentration does not affect the ADV threshold, however vaporized droplets oscillate more strongly in DI water due to the influx of dissolved gas and the fact that bubble dissolution decreases as the dissolved gas concentration of the bulk fluid increases[11].

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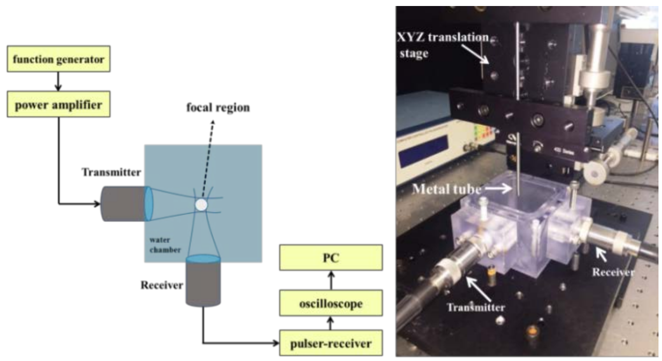
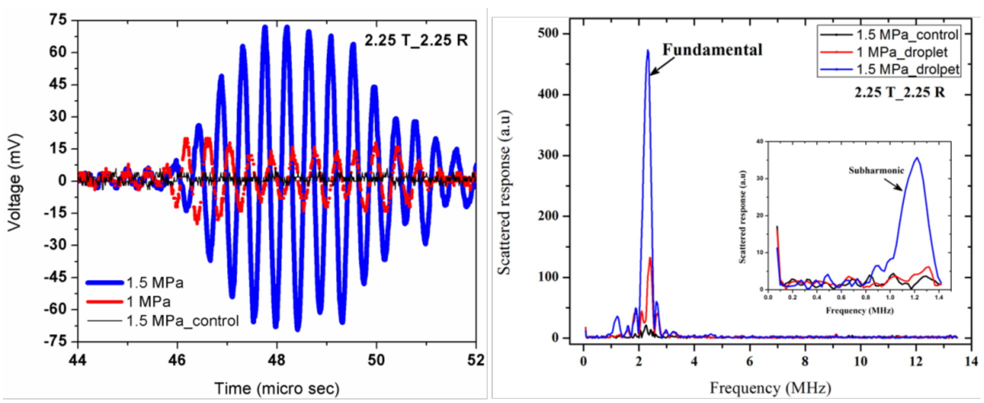
It is well-documented that perfluorocarbon microbubbles are effective cavitation nuclei[12]. Vaporized PFP droplets can undergo stable oscillation or inertial cavitation depending on the excitation parameters. Since inertial cavitation is associated with generation of broadband noise, we integrated the frequency power spectrum in the range of 0.5 to 1 MHz. The integrated power for this frequency band is plotted as a function of peak negative pressure in Figure 5 (b) for an excitation frequency of 2.25 MHz. The inertial cavitation threshold was defined as the pressure at which the integrated power at this frequency band increased for at least three experiments. We found the IC thresholds of 1.7 MPa peak negative pressure (peak positive pressure of 2.3 MPa) at 2.25 MHz and 3 MPa peak negative pressure (peak positive pressure of 8.3 MPa) at 5 MHz. We did not observe any IC activity at the excitation frequency of 10 MHz up to the highest peak negative pressure of 5 MPa tested in this study.



(a) (b)

Figure 1. Schematic representation (a) and a picture (b) of the experimental setup used for determining ADV threshold.



(a) (b)

Figure 2. (a) RF trace and (b) corresponding FFT from the control and droplet suspensions at 2.25 MHz.

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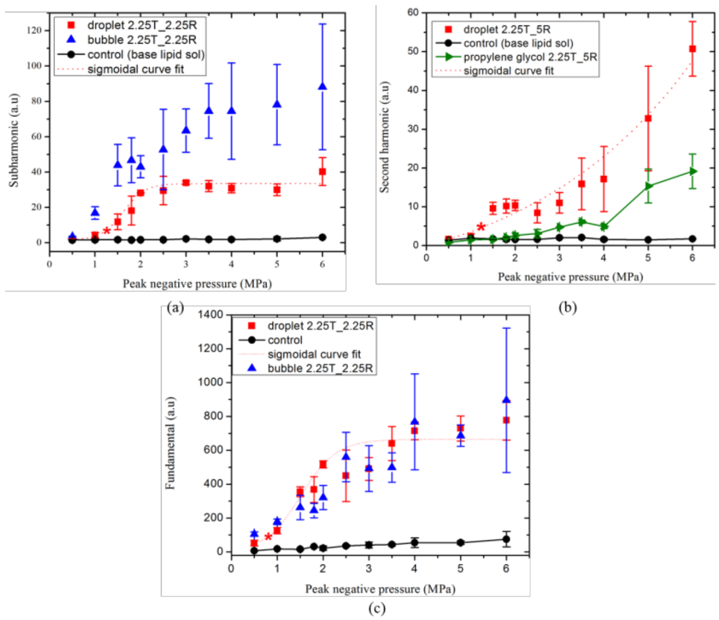

Figure 3. (a) Subharmonic, (b) second harmonic and (c) fundamental components of the scattered responses from droplets, microbubbles, propylene glycol stream and control at an excitation frequency of 2.25 MHz. Central frequencies of transmitting (T) and receiving (R) transducers are indicated in the legends. The data sets were scaled by 10^5 for easier display. The ADV threshold determined from each curve is denoted by (*).

Table I. Frequency dependence of ADV threshold.

Excitation frequency (MHz)	Threshold (fundamental) MPa	Threshold (subharmonic) MPa	Threshold (second harmonic) MPa	Threshold (average) MPa	Mechanical index
2.25	0.72	1.22	1.22	1.05	0.7
5	1.45	1.72	2.55	1.89	0.84
10	2.35	-	2.33	2.34	0.74

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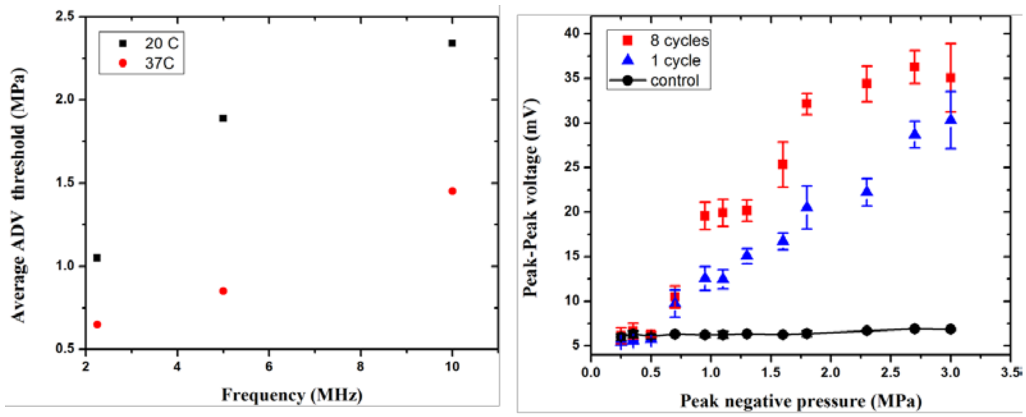
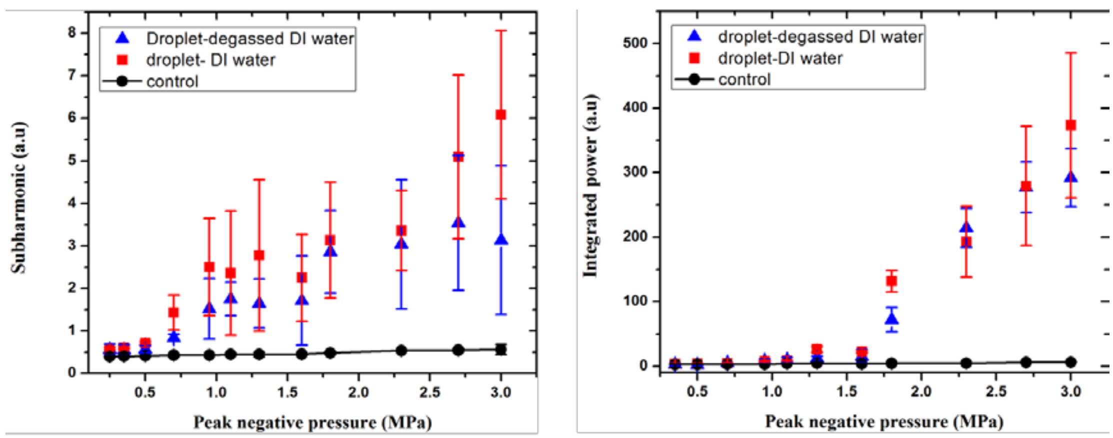


(a) (b)

Figure 4. (a) Effect of temperature on ADV threshold as a function of frequency, (b) effect of number of cycle on ADV threshold at 2.25 MHz and 37º C in degassed DI water.



(a) (b)

Figure 5. Effect of dissolved gas concentration on (a) subharmonic response of the scattered signal from droplet suspensions at 2.25 MHz, (b) inertial cavitation threshold of the vaporized droplets excited at 2.25 MHz. All experiments were done at 37°C.

Conclusion

Lipid coated PFP droplets were excited with ultrasound pulses at various excitation frequencies and pulse lengths at two different temperatures and dissolved gas concentrations to find the threshold excitation for acoustic vaporization. Fundamental, second harmonic and subharmonic components of the scattered response of the droplet suspensions were used to determine the threshold of vaporization. The scattered response from droplets was also compared with the scattered response from a PFB microbubble suspension at the corresponding excitation pressure and frequency. The ADV thresholds were found to increase with frequency and decrease with temperature. The pulse lengths and dissolved gas concentration affected the amplitude of the scattered response from the droplets post vaporization without changing the ADV threshold. IC was measured by calculating the integrated power frequency spectrum in the range of 0.5 to 1 MHz and resulted in the peak negative pressure of 1.7 MPa and 3 MPa for 2.25 and 5 MHz, respectively. For all the frequencies studied here, ADV threshold was much lower than the IC threshold.

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