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Temperature and size-dependence of the vaporization threshold of phase-shift emulsions

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Acoustic cavitation has proven to be important for several therapeutic applications of ultrasound. However, acoustic cavitation is difficult to initiate and sustain in the absence of cavitation nuclei, particularly in tissue. Phase shift emulsions are ideal candidates for cavitation nuclei for *in vivo* applications. These emulsions, which consist of superheated liquid perfluorocarbon droplets enclosed by albumin shells, may be vaporized with acoustic pulses, a process known as acoustic droplet vaporization (ADV). In this study, we determined the ADV threshold at 2-MHz as a function of temperature and droplet size.

Studies were conducted with micro- and nano-sized emulsions in a closed-flow system immersed in a temperature controlled water bath. The emulsions were injected into a flow system and exposed to high intensity focused ultrasound (HIFU; acoustic parameters: 2-MHz, 10 cycles, 100Hz pulse repetition frequency). A portable diagnostic ultrasound scanner was used to monitor for vaporization. Upon vaporization, the peak rarefactional pressure of the acoustic pulse and the water bath temperature were recorded. It was determined that the vaporization threshold was independent of droplet size and inversely proportional to temperature. The utility of these emulsions in cancer therapy, particularly for bubble-enhanced heating during HIFU exposure, will be discussed.

1 Introduction

After serving as a reliable and successful imaging tool for decades, ultrasound has shown a promising future in therapeutics [1]. The inherent advantages of ultrasound, including few side effects, non-invasive application, and low-cost, makes it an effective tool for therapeutic applications. Several researchers have demonstrated the utility of High-Intensity Focused Ultrasound (HIFU) for therapy [2-4]. By focusing high intensity ultrasound to the treatment area, acoustic energy will be absorbed by diseased tissue, resulting in a rapid increase in temperature and ultimately cell death. This process, known as thermal necrosis, provides a non-invasive option to treating solid tumors without harming surrounding healthy tissues. Researchers have proposed using acoustic cavitation to accelerate HIFU-induced thermal necrosis. While the activity of bubbles generated by acoustic cavitation at the focus can increase the heating rate and, consequently, the efficiency of HIFU-induced heating [4-5], the onset of cavitation and the size and geometry of the bubble cloud is unpredictable. Additionally, the bubble cloud may act as an acoustic shield, causing pre-focal heating and migration of the lesion toward the transducer. Due to the difficulty with controlling the size, shape, and nature (stable vs inertial) of the cavitation field during HIFU exposure, it has been generally avoided in clinical trials despite its benefit in increasing HIFU efficiency.

The use of bubbles in HIFU thermal ablation may become more clinically acceptable if technology and strategies can be developed to control the location and extent of cavitation activity. Due to the low gas content in tissue, the pressure threshold for cavitation initiation can be as high as 10 MPa [6,7]. Unfortunately, at these pressures, one can expect violent inertial cavitation activity and primarily mechanical rather than thermal damage. This threshold can be reduced significantly with the introduction of exogenous cavitation nuclei. Due to leaky tumor vasculature and inefficient lymphatic drainage system, submicron cavitation nuclei injected systemically will accumulate in the tumor interstitial space [8]. These nuclei may include laser-activated gold nanoparticles [9] or ultrasound contrast agents [10]. However, the low penetration depth of lasers limits the utility of the gold nanoparticles, while ultrasound contrast agents are too large to extravasate through tumor vessels.

We propose the use of a phase shift emulsion (PSE), which consists of liquid perfluorocarbon droplets with an albumin shell, as a candidate for nucleating acoustic cavitation in solid tumors. In preliminary studies (unpublished), we have produced perfluorocarbon droplets with an average diameter of approximately 200nm, which is small enough to extravasate into the tumor interstitial space. The boiling point of perfluorocarbon is 29°C in bulk, but in droplet form, the perfluorocarbon will exist as a superheated liquid at temperatures in excess of 60°C. Droplet vaporization can be driven with an external stimulus, such as HIFU pulses, a process known as Acoustic Droplet Vaporization (ADV) [11]. In this paper we describe the formulation and synthesis of nanoemulsions and present the vaporization threshold for PSE as a function of temperature. With different methods, we prepared both micron-size and nano-size albumin-shell emulsions. By using diagnostic ultrasound and a temperature controlled closed-flow system, the ADV thresholds for both types of emulsions were investigated as a function of temperature. It was found that the ADV threshold is inversely proportional to temperature and independent of droplet size.

2 Phase shift emulsion preparation

To study the size-dependence, two sizes of emulsions were prepared with different methods:
The micron-size emulsion was prepared by following a protocol previously reported by Kripfgans *et al*. [9]: 0.9 ml of a 5 mg/ml albumin in phosphate buffered saline (PBS) solution (Bovine Serum Albumin, Sigma, CAS:9048-46-8) and 0.1 ml Dodecafluoropentane (C5F12, Synquestlabs, FL, CAS:138495-42-8) were mixed in a 1.5-ml vial and then shaken for 30 sec in an amalgamator (MSD Wig-L-Bug, Dentsply Rinn, Elgin, IL) at 4800 RPM. Images of the emulsion were captured with optical microscopy. The emulsion size distribution was determined using a Coulter Counter (Z2 Analyzer, Beckman Coulter, Inc, Fullerton, CA). The resulting emulsion contains droplets with a mean diameter of 4 μm (Figure 1).

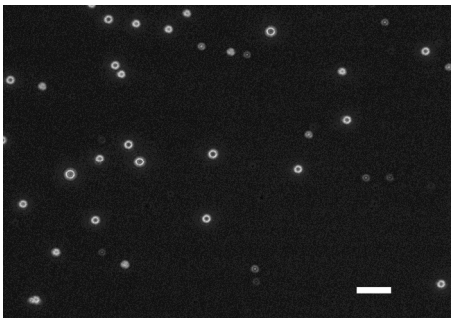


Figure 1 Micron-size droplets in phase-shift emulsion. Image was taken by phase contrast microscopy. The mean diameter of the droplets was 4 μm. The length of white bar in the bottom-right corner represents 30 um.

The nano-size droplets were prepared by the following procedure: 0.1 ml DDFP and 9.9 ml degassed distilled water were mixed and emulsified by an ultrasonic liquid processor (Model VC505, Sonic & Materials, Newton, CT) for 30 seconds. The resulting emulsion was slowing poured into 5 ml albumin-PBS solution ([albumin] = 20 mg/ml) to coat the droplet with an albumin shell and then the mixture was filtered through a disposable syringe filter (450nm pore size, PVDF membrane, Whatman). The mean diameter of droplets measured with a particle size analyzer (90Plus, Brookhaven Instruments, Inc., Worcestshire, UK) was 193.3 nm (Figure 2).

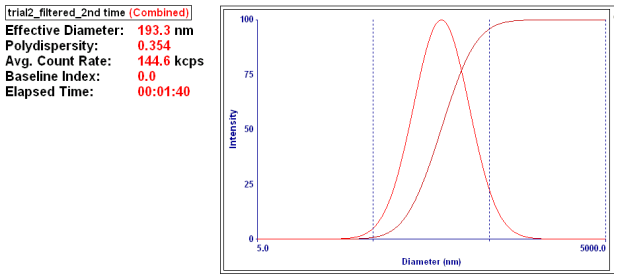


Figure 2 Size distribution of nano-size droplets. Measurements were made with a particle size analyzer.

Stored at atmospheric pressure and 2-8 °C, the micron-size emulsion was stable for more than a week and the nano-size emulsion was stable for at least two days. In our experiments, all emulsions were prepared in the morning and used in the same day. Because albumin is a surface-active molecule, air bubbles coated with an albumin shell would be entrained during preparation. To eliminate these unwanted bubbles, PSE suspensions were diluted by a factor of ten and sat for half an hour to allow large bubbles to rise to the surface of the suspension. The top layer was discarded and the remaining liquid was used in subsequent experiments. Additionally, because the density of DDFP (1.6g/cm^3) is greater than the albumin-saline solution, droplets would settle to the bottom. Before each measurement, emulsions were stirred with a pipette several times to resuspend the emulsion.

3 Transducer calibration

High amplitude pressure pulses used in the study were delivered by a 2-MHz HIFU transducer (Sonic Concepts, Woodinville, WA). The transducer was constructed from a single element piezoceramic crystal with 62.64 mm radius of curvature. The transducer was driven by a 150 W RF amplifier (A150, Electronic Navigation Industries) with sinusoidal waveforms provided by an arbitrary waveform generator (33250A, Agilent, Santa Clara, CA). The amplifier output impedance was matched to the transducer impedance via a matching network provided by the transducer manufacturer. The transducer input signal was monitored with an oscilloscope (Waverunner 6050A, Lecroy, Chestnut Ridge, NY), and the transducer output at the focus was calibrated as a function of voltage input with a fiber optic hydrophone (model 500, RP Acoustics, Stuttgart, Germany). A lateral scan of the spatial distribution of pressure at the focus is shown in Figure 3.

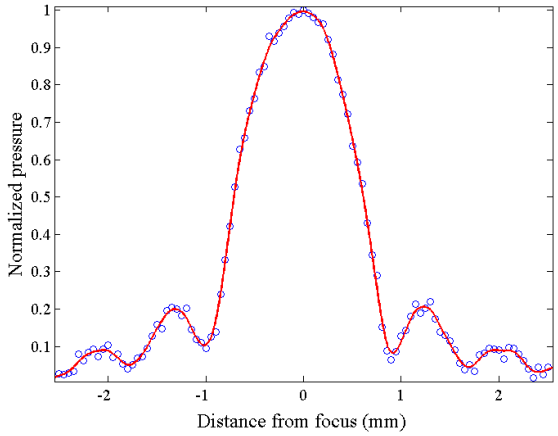
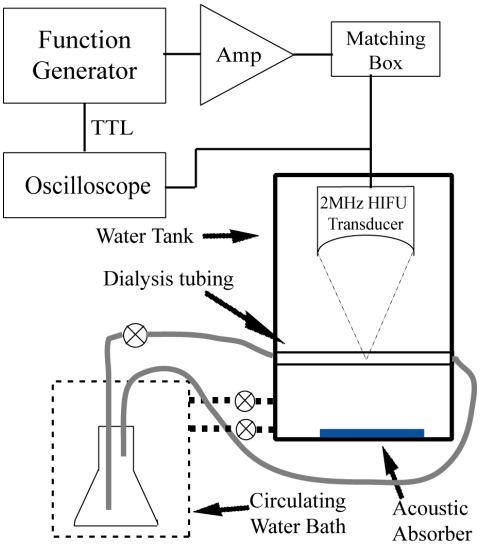


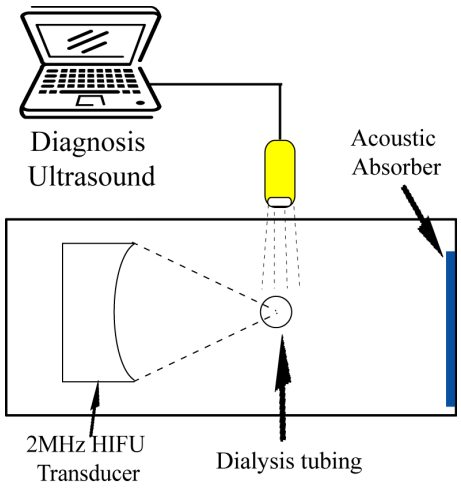
Figure 3 Lateral scan of HIFU pressure field at focus. Pressure measurements were normalized against the peak positive pressure at the focus.

4 Vaporization

Acoustic droplet vaporization (ADV) studies were conducted in a 40×17.5×16 cm water tank connected to a circulating heated water bath (Neslab EX-7, Thermo Fisher Scientific, Waltham, MA) for temperature control (Figure 4). PSE emulsions were circulated through dialysis tubing with a 6.5mm diameter (D2272, Sigma), which was positioned at the HIFU transducer focus. A thermocouple was attached at the outlet of the flow system to measure temperature. PSE suspensions were exposed to 2-MHz 10-cycle pulses with a 100Hz pulse repetition period (PRP). A portable laptop-based diagnostic ultrasound scanner (Terason 2000, Terason Ultrasound, Burlington, MA) was used to monitor vaporization of the emulsions. An imaging probe operating at 7.5-MHz was oriented normal to the propagation path of the HIFU pulses and positioned so that the HIFU focus was in the imaging plane. Pefluorocarbon vapor bubbles scatter ultrasound more effectively than perfluorocarbon liquid droplets. Thus, we anticipate a significant increase in echogenicity upon vaporization at the focus as well as downstream. The HIFU output was slowly increased (*i.e.* 0.1 MPa intervals) and the pressure at which a significant increase in echogenicity was detected was noted as the ADV threshold. The ambient water temperature in the tank was then changed and the process was repeated.



(a) Top view

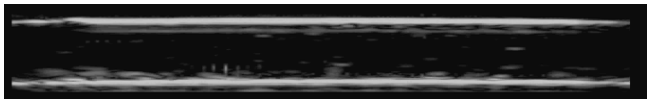


(b) Side view

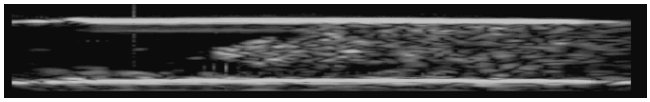
Figure 4 Sketch of experimental setup

5 Results

When the HIFU output exceeded a pressure threshold, the DDFP droplets transitioned from liquid to vapor. Due to the small size of the transducer focus to the dialysis tubing (*i.e.* 1 mm compared to 6.5 mm), vaporization was confined to a small region within the dialysis tubing. In the B-mode images, it was easy to identify where vaporization occurred. The resulting bubbles continued to flow downstream, creating a comet-like tail of highly-echogenic scatters (Figure 5).



(a) HIFU off



(b) HIFU on; applied pressure exceeds ADV threshold

Figure 5 B-mode images of dialysis tubing with and without acoustic exposure.

Figure 5 shows the experimental results of the relationship between ADV threshold and ambient temperature. To measure the threshold at a specific temperature, the transducer output was slowly increased at a set water temperature until increased scatter from perfluorocarbon bubbles was observed in the B-mode images. Upon vaporization, the peak negative pressure of the acoustic pulse and the water temperature were recorded. For each data point in figure 5, this procedure was repeated four times and the results were averaged. Due to the requirement of working temperature for transducer, the study was conducted from 8 - 45 °C.

As shown in Figure 5, the vaporization threshold was inversely proportional to the water temperature. It was also evident that vaporization of droplets that were not "superheated" was possible. Even when the temperature was reduced to 8°C, vaporization still occurred, most likely due to the applied negative pressure "tearing" the liquid perfluorocarbon. The resultant cavity could then be filled with solubilized gas from the surrounding aqueous medium or from perfluorocarbon gas that forms at the fracture point in the droplet. In our experiments, the emulsion size does not have an obvious affect on threshold. Additionally, we briefly investigated the role of the number of cycles and PRP on ADV. It was found that increasing the number of cycles or decreasing the PRP gave stronger echogenicity in B-mode imaging but did not significantly change the vaporization threshold.

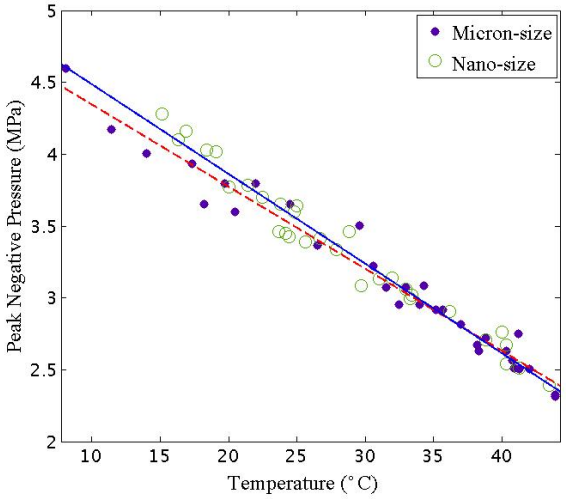


Figure 5 Relationship between ADV threshold and temperature

Although the mechanism of ADV phenomenon is still an open question, based on our study, we consider it is possible that simple boiling plays a key role in this acoustically driven phase-shift process. After DDFP liquid is emulsified, the small volume of each droplet limits the number of impurities present that may serve as nucleation sites for boiling. This results in a higher boiling temperature of DDFP in droplet form than bulk form at the same pressure. The acoustic pulse provides a high negative pressure on droplet that could significantly lower its boiling temperature. When the boiling temperature is reduced to the surrounding temperature, DDFP liquid would boil and vaporize. This is one possible explanation for ADV and why it is inversely proportional to temperature.

6 Conclusion

By varying ambient temperature and monitoring echogenicity in B-mode images, we identified the ADV threshold at different ambient temperatures ranging from 8 – 45 °C. It was found that the vaporization threshold is inversely proportional to temperature and independent of droplet size. This information can be used when developing a new strategy for HIFU cancer treatment based upon cavitation nucleation with PSE.

It has been reported that certain sizes of molecules, typically liposome or micelles, tend to accumulate in tumor tissue much more than they do in normal tissues, which is known as Enhanced Permeability and Retention effect [6]. The nano-size PSE presented in this report could potentially take advantage of this effect and be target-delivered into tumor tissue as cavitation nuclei. Knowing the vaporization threshold, these emulsions could be used to nucleate cavitation in a predictable manner and enhance the absorption of acoustic power in solid tumors. This would lead to more efficient treatment of cancer with HIFU, ultimately reducing treatment time and cost.

Acknowledgments

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