Detection of Microbubble Ultrasound Contrast Agent Destruction Applied to Definity®

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***Abstract*—For different applications such as imaging, drug delivery and tissue perfusion measurement, it is necessary to know the inertial cavitation (IC) threshold of ultrasonic contrast agent (UCA) microbubbles. The IC threshold for Definity® was determined using a passive cavitation detection (PCD) system. The IC threshold criterion was rebound. The incident ultrasonic field at a constant pulse repetition frequency of 10 Hz was varied with frequency (0.9, 2.8, 4.6 and 7.1 MHz), pulse duration (3, 5, and 7 cycles), and pressure level (ranging from 0.4 to 8.7 MPa). The transmit transducer excited the contrast agent while the 13-MHz receive transducer (mounted confocal to the transmitter) simultaneously received acoustic emissions from the microbubbles. The concentration of Definity® was chosen so that statistically only one microbubble was in this focal volume. Simulation results showed that a collapsing microbubble emits a characteristic broadband signal that was used to determine IC events. In order to classify the data, five different classes were created: Noise, Oscillation, Collapse, Multiple Bubbles and Not Classified. The peak rarefactional pressure thresholds of IC events were determined for three of the four frequencies (e.g., 1.6, 0.8, and 2.6 MPa for 0.9, 2.8, and 4.6 MHz, respectively). It was not possible to determine a threshold pressure at 7.1 MHz.**

I. Introduction

There are various applications for ultrasound contrast agents consist of small, stabilized microbubbles (diameter $<$ 10 μm). The contrast enhancement of blood vessels in ultrasonic images due to the good ultrasound scattering qualities of the microbubbles injected intravenously, was one of the earliest application [3]. Later, several image techniques were developed using linear and nonlinear microbubble responses to measure blood perfusion, blood volume or blood velocity rates in tissue [2]. A high-intensity ultrasound burst applied to a selected area in situ purposefully reduces the UCA concentration and the dynamically contrast enhancement can be used to evaluate blood volume and flow rates. These techniques require the UCA destruction threshold to be known in order to avoid false measurements due to unintentional modification of the UCA concentration. The knowledge of UCA destruction threshold is also fundamental for targeted drug and gene delivery.

Several techniques have been developed using noise emission from microbubble destruction measured by PCD to estimate UCA destruction thresholds. The use of high-speed cameras are currently the reference method to determine UCA destruction thresholds [1], but the expensive equipment limits its accessibility and is not usable for in vivo studies. However, we report on a technique using post-excitation broadband signals to identify microbubble destruction. These signals are linked to IC of bubbles released after UCA shell rupture. The minimum value of peak rarefactional pressure lead to the minimum rupture threshold. This technique has the advantage that the inertial collapse and rebound signals are not contaminated by nonlinear spectral contents from other sources [4].

II. Methods and Materials

A. Contrast Agent

For all experiments the FDA-approved contrast agent Definity® was used. The UCA is a lipid shell microbubble that contains Octafluoropropane (C_3F_8) gas. The manufacture's vials have a maximum concentration of 1.2 x 10^{10} microbubbles/mL. Before use, Definity® was activated using Vialmix™ [5]. The mean diameter of the microbubble distribution is between 1.1 and 3.3 μm, the maximum diameter is less than 20 μm and approximately 98% of the bubbles have a diameter less than 10 μm.

TABLE I
Peak rarefactional pressure (MPa) ranges for each transducer and pulse duration combination.

Cycles	0.9 MHz	2.8 MHz	4.6 MHz	7.1 MHz
Three	$0.56 - 8.69$	$0.38 - 2.91$	$1.07 - 6.04$	$0.23 - 1.93$
Five	$0.58 - 8.66$	$0.44 - 3.24$	$1.27 - 6.67$	$0.26 - 2.06$
Seven	$0.58 - 8.71$	$0.47 - 3.46$	$1.31 - 6.89$	$0.29 - 2.14$

B. Exposure Setup

The incident field was generated by four different confocal transducers with center frequencies at 0.9, 2.8, 4.6 and 7.1 MHz. The experiment was performed in a 50.5-cm long x 25.5-cm wide x 30-cm high tank, filled up with 25 $\pm$

0.6 L of degassed water. Sinusoidal tone bursts with pulse duration of 3, 5 or 7 cycles and frequency of 0.9, 2.8, 4.6 or 7.1 MHz were generated by a pulser-receiver (RITEC RAM5000, Warwick, RI). The pulse repetition frequency (PRF) was 10 Hz and the pulse phase was set to 180° (excitation pulse leads with negative amplitude) for all experiments. The pressure amplitude was varied using the output control of the pulser and an attenuation bar (Model 358, Arenberg Ultrasonic Laboratory, Boston, MA) was used to realize the low-pressure levels. The pressure amplitude of each frequency and pulse duration combination was calibrated with a PVDF hydrophone (Marconi 6999/1/00001/100; GEC Marconi Ltd., Great Baddow UK) in the center of the focus according to the well-established calibration procedures [7] [8]. The range of peak rarefactional pressure levels used in the experiment is shown in Table I.

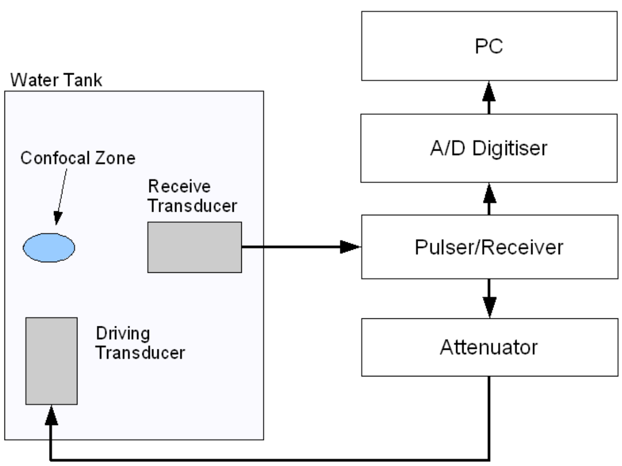

Fig. 1. Experimental setup of the passive cavitation detector.

C. Passive Cavitation Detector

The schematic lay out of the passive cavitation detector (PCD) is shown in Fig. 1. A focused 13-MHz receive transducer was positioned in a 90° and 45° angle to the 0.9, 7.1 and 2.8, 4.6 MHz transducers, respectively. The transducers were aligned so that the focal region of the receive transducer was completely within the confocal volume of the driving transducers. The -6 dB focal region of the PCD was characterized, using a wire technique [6], to be cigar shaped with a volume of 0.12 mm^3. The received signal scattered from the microbubbles was amplified (44 dB), digitized (12-bit, 200 MS/s, Strategic Test digitizing board UF 3025, Cambridge, MA) and saved to a PC using Matlab® (The Math Works, Inc., MA).

D. Data Acquisition

A syringe was used to inject a solution of 0.1-mL Definity® containing approximately 25 x 10^8 microbubbles in the tank to yield a concentration of 10 microbubbles/μL. This procedure resulted in an average of only one microbubble in the -6 dB focal volume of the PCD. The water was gently stirred using a magnetic stirring bar during data acquisition to maintain an even distribution of contrast agent in the focal volume of the PCD. For each PD (3, 5 or 7 cycle) one hundred PCD waveforms were acquired from the receive transducer. This procedure was repeated for each driving transducer (0.9, 2.8, 4.6 or 7.1 MHz) under the same condition. All waveforms were stored for off-line processing using Matlab®.

E. Data Processing and Classification

The offset of each waveform was removed by subtracting the the mean value and then the waveforms were filtered by a bandpass (FIR, 1 - 25 MHz passband) to reduce the amplitude of signal contents that were outside of the impulse response of the receive transducer. The waveforms were sorted from the highest to the lowest amplitude, and for every pressure level a spectrogram was generated from the ten highest amplitude waveforms. All spectrograms were generated by means of the implemented Matlab® function (spectrogram; 128 point Hanning window , 126 point overlap, 8192 point fft). Using the spectrograms, the acquired signals were visually sorted into five classes, Noise, Oscillation, Collapse, Multiple Bubbles and Not Classified.

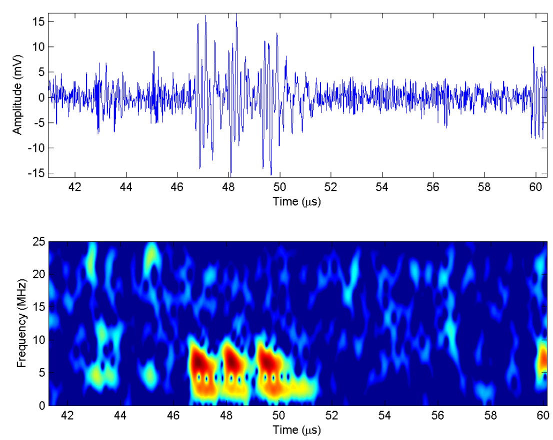

Fig. 2. An example of a waveform of the class Noise with signal artifacts.

Even though there should have been always just one microbubble in the focal volume of the PCD, there were cases when there were no bubbles in that volume, resulting in no bubble-generated echo but only noise. Data acquired with no UCA in the tank showed that there were also waveforms that could be interpreted incorrectly. Fig. 2 is a representative example for such a waveform. The signal content between 45 and 51 μs could possibly be interpreted as multiple, nonlinear oscillating microbubbles with fundamental and first harmonic modes.

Fig. 3 shows a representative PCD waveform for a single microbubble that was classified Oscillation. The content of the waveform between approximately 43 and 46 μs corresponds to the PCD response of the scattered microbubble echo. In the spectrogram, the fundamental mode, at approximately 3 MHz, and the harmonic modes, at 6, 9, 12 and 15 MHz, are visible; the harmonic modes may have been generated both by

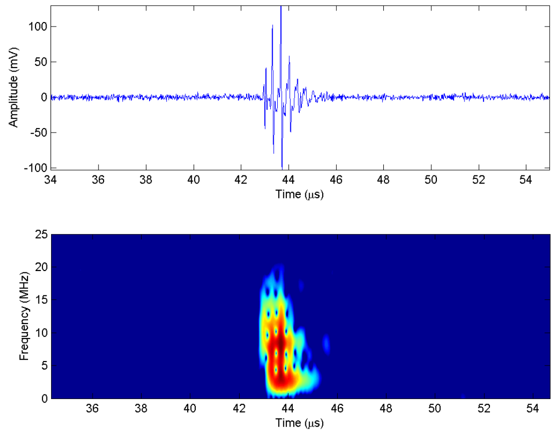

Fig. 3. An example of a waveform of the class Oscillation. A 2.8-MHz 3-cycle 2.9-MPa peak rarefactional pressure toneburst was used to exicte the bubble.

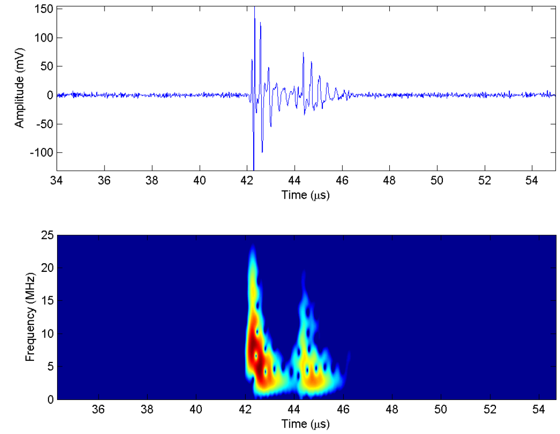

Fig. 5. An example of a waveform with two microbubbles of the class Oscillation. One of the responses is between 42 and 44 μs and the other between 44.8 and 45.8 μs. A 2.8-MHz 3-cycle 2.68-MPa peak rarefactional pressure sinusoidal toneburst was used to excite the bubbles.

nonlinear bubble dynamics and nonlinear propagation of the exciting pulse and scattered echo [4]. There was no acoustic emission after the end of the driving pulse.

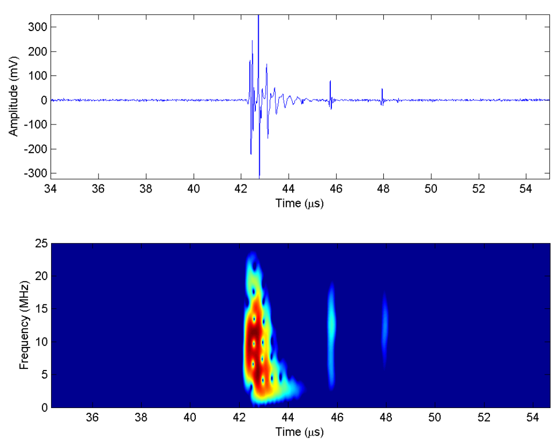

Fig. 4. A representative waveforms of the class Collapse. A 2.8-MHz 3-cycle 2.75-MPa peak rarefactional pressure sinusoidal tone burst was used to excite the bubble.

Fig. 4 shows a representative PCD waveform for a single microbubble that was classified Collapse. The principal response of the microbubble between 42 and 45 μs looks similar to the waveforms of the class Oscillation. In the spectrogram are frequency bands corresponding to the fundamental mode and harmonic modes. After the principal response are broadband signals (rebounds) with a frequency band between approximately 3 and 22 MHz, at 46 μs and 48 μs. This post-principal response feature indicates that inertial cavitation occurred [4].

Waveforms of the class Multiple Bubbles show characteristics of both classes Oscillation and Collapse but the PCD response contains signals of multiple microbubble responses. In Fig. 5 there are two microbubble responses, one between 42 and 44 μs and the other between 44.8 and 45.8 μs, each with features of the class Oscillation. In order to exclude false inertial cavitation thresholds because of the possible interaction among microbubbles, waveforms from this class were not used for threshold estimation.

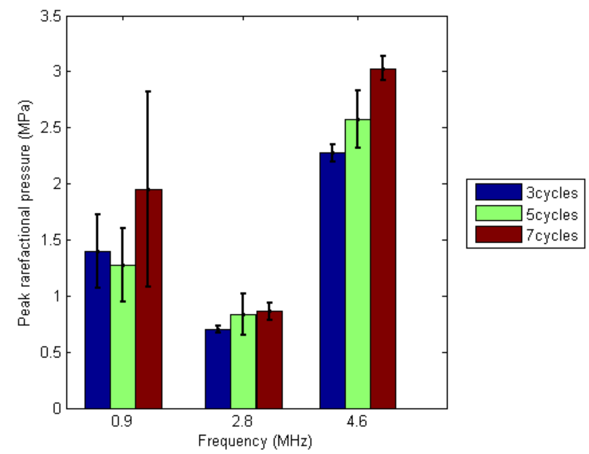

Fig. 6. Minimum rupture thresholds using IC criteria. The error bars represent the standard deviation.

All waveforms that could not be classified as Noise, Oscillation, Collapse or Multiple Bubble were marked as Not Classified.

F. Inertial Cavitation Threshold Estimation

The inertial cavitation threshold was estimated from the lowest peak rarefactional pressure level from the class Collapse. This procedure was repeated for all frequency and pulse duration combinations.

III. Experimental Results

For each frequency and PD combination, at least three data sets were acquired. After the data processing and visual spectrogram classification of the ten highest signal amplitudes of the microbbule responses, the inertial cavitation threshold was estimated from the lowest pressure level for which the waveforms occurred from the class Collapse. The IC threshold of Definity® could be found for 0.9, 2.8 and 4.6 MHz transducers but not for 7.1 MHz. For all PD and pressure levels at 7.1 MHz there were no waveforms that clearly belonged to the class Collapse. Fig. 6 shows the mean threshold values (out of at least three experiments).

IV. Discussion

There is a significant dependency of the IC threshold on frequency (Fig. 6). A significant dependency of the IC threshold on the PD was not observed. The lower IC threshold at 2.8 MHz may come from resonance effects of the microbubble distribution. In Fig. 7 the IC thresholds of Definity® and Optison™ are shown. The higher IC threshold of Definity® at 0.9 and 4.6MHz may come from the higher elasticity of the lipid shell compared to the albumin shell of Optison™.

V. Conclusion

The relatively simple technique of the PCD was applied to the lipid shelled UCA Definity®. The IC threshold for Definity®, using the post-excitation signal rebound as a criteria [4], was determined at 0.9, 2.8 and 4.6 MHz. The higher IC thresholds of Definity® at 0.9 and 4.6 MHz compared to Optison™ were found and may be explained due to the higher elasticity of the lipid shell of Definity®.

Acknowledgment

This work was supported in part by NIH Grant R37EB02641 and in part by Beckman Institute for Advanced Science and Technology's Research Initiative in Targeted Contrast and Therapeutic Agents for Molecular Biomedical Imaging.

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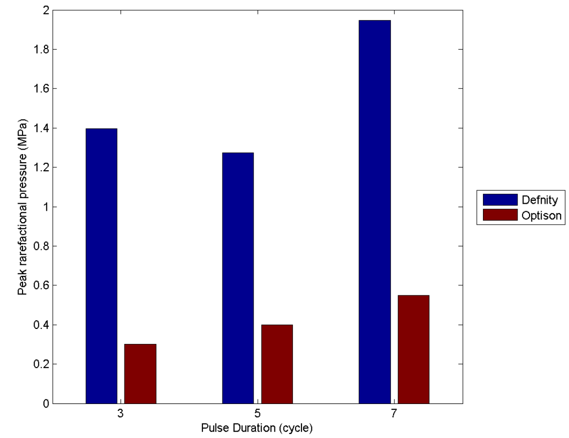
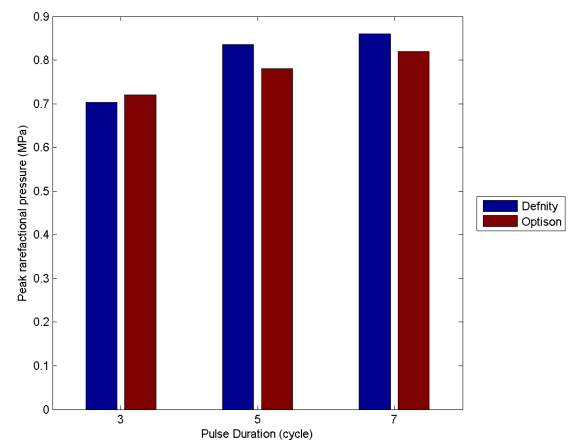
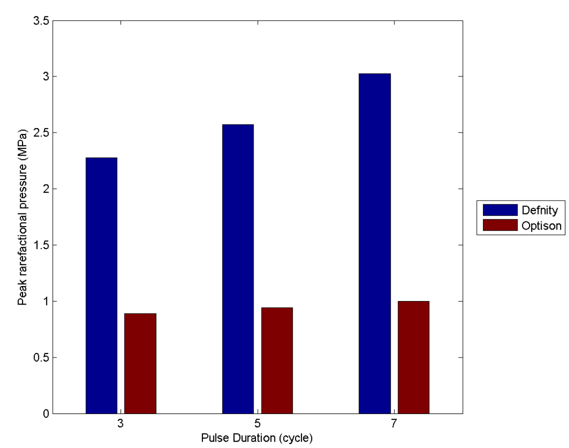


(a) 0.9 MHz



(b) 2.8 MHz



(c) 4.6 MHz

Fig. 7. Comparison of the inertial cavitation thresholds of Definity® and Optison™