

Using Passive Cavitation Detection to Observe Postexcitation Response of Ultrasound Contrast Agents

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Abstract— Passive cavitation detection was used to improve the experimental characterization of single ultrasound contrast agent microbubble responses to short, large amplitude pulses. Two situations were examined: isolated microbubbles in an unconstrained environment, and isolated microbubbles flowing through a tube. The microbubbles were categorized according to a classification scheme based on the presence or absence of postexcitation signals, which are secondary broadband spikes that may follow the principle oscillation of the ultrasound contrast agent in response to an insonifying pulse. Experiments were conducted for different frequencies, peak rarefactional pressures, flow rates, and types of microbubble. Postexcitation activity was found to increase as frequency decreased, acoustic pressure increased, and flow rate increased. Additionally, lipid-shelled microbubbles were found to exhibit greater postexcitation at lower acoustic pressure thresholds than albumin-shelled microbubbles.

Keywords - microbubbles; postexcitation; passive cavitation detection; inertial cavitation; flow rate

I. INTRODUCTION

Ultrasound contrast agents (UCAs) are thin-shelled microbubbles with a gas core typically ranging in diameter from 1-10 μm . Current clinical usage of UCAs consists primarily of diagnostic imaging, but much of the focus of ongoing research involves the functional potential of microbubbles for therapeutic ultrasound. Among other procedures, recent experimental studies have shown that use of UCAs in conjunction with ultrasound enhances thrombolysis [1], sonoporation across cellular membranes [2], and molecular transport across the blood brain barrier [3].

While UCAs have been shown to be successful in increasing the effectiveness of such therapies, the precise physical mechanisms leading to these bioeffects have been insufficiently explained so far. In response to an ultrasonic pressure field, UCAs may undergo a wide range of dynamic responses ranging from linear oscillation to transient inertial cavitation and fragmentation. The bubble response leads in turn to the generation of a variety of fluid behaviors such as streaming and jetting, which are highly significant in producing

biological responses. However, experimental cavitation responses for varying types of shelled microbubbles in reaction to large amplitude pulses remain poorly documented, limiting understanding of the necessary conditions needed to maximize bioeffects.

Therefore, the goal of this research is to improve experimental acoustic characterization of microbubble behavior in the large amplitude regime, including the determination of accurate collapse thresholds. Two distinct experiments are presented: (1) a more theoretically useful situation of isolated microbubbles in an unconstrained environment, and (2) a more physiologically relevant situation of microbubbles flowing through a tube with varying rates of speed.

II. METHODS AND MATERIALS

A. Experimental

The setup for the double passive cavitation detection (DPCD) system involved the confocal alignment of three single element transducers (Valpey Fisher, Hopkinton, MA). Two passive receive transducers were placed at a 90-degree angle with one active transmit transducer positioned halfway between them in a custom holder as shown in Fig. 1. The fields of all transducers were characterized using a 50 μm diameter wire [4]; alignment of the transducers was also performed using this wire technique. The center frequencies of the two receive transducers were nominally 15 MHz, but measured to be 14.6 and 13.8 MHz in pulse-echo mode; both were $f/2$, with an element diameter of 0.5". The center frequencies of the four transducers used to generate the transmitted pulse were 0.95, 2.8, 4.6 and 7.1 MHz; all were $f/2$, with an element diameter of 0.75".

The holder was placed in a Plexiglas tank filled with 15 to 25 L of degassed water at temperatures ranging from 20 to 22° C. The appropriate concentration of UCAs was added to the tank so that only a single microbubble should be present within the confocal region at any instant, and the mixture was gently stirred with a magnetic stir bar to ensure uniformity of the UCA distribution.

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Three cycle tone bursts with a pulse repetition frequency of 10 Hz at the center frequency of each transmit transducer were generated using a pulser-receiver system (RITEC RAM5000, Warwick, RI). To achieve the lowest pressure settings, an attenuation bar (Model 358, Arenberg Ultrasonic Laboratory, Boston, MA) was used. To determine the pressure amplitude of the generated waveform, all settings were calibrated using a PVDF hydrophone (0.5 mm diameter, Marconi 6999/1/00001/100; GEC Marconi Ltd., Great Baddow UK) at the center of the confocal region of the receive transducers according to established procedures [5, 6].

The experimental setup for the flow rate experiments was similar, but not identical. As illustrated in Fig. 1, a 2.25 MHz transmit and 13 MHz receive transducer (Technisonic Research Inc., Fairfield, CT) were confocally aligned with a microscope (Z16 APO microscope, Leica, Bannockburn, IL, USA) with 20X magnifying objective lens (Leica Achromplan 100X, NA = 0.42) on a cellulose fiber with an inner diameter of 200 μm (MWCO, Spectrum Labs Inc., Rancho Dominguez, CA, USA). Both transducers had an element diameter of 0.75" and a focal length of 2". Five cycle tone bursts with a pulse repetition frequency of 4 Hz were used in these experiments.

Degassed water at 20 to 24° C. was mixed with an appropriate concentration of UCAs so that only a single microbubble was present within the confocal region. This mixture was injected at varying flow rates through the fiber using an electronic syringe pump (11 Plus Syringe Pump, Harvard Apparatus, Holliston, MA, USA). Visual confirmation that only a single microbubble was present during each insonifying pulse was achieved with the microscope when flow was stopped.

The two commercially available contrast agents used in the DPCD experiments were lipid-shelled Definity® (Lantheus Medical Imaging, N. Billerica, MA) and albumin-shelled Optison™ (GE Healthcare Inc., Princeton, NJ). Both of these microbubbles contain octafluoropropane as the gas core. For the flow experiments, the two UCAs used were Definity and BR14 (Bracco Research Inc., Geneva, Switzerland). Both are lipid-shelled agents; BR14 contains perfluorobutane in the gas core.

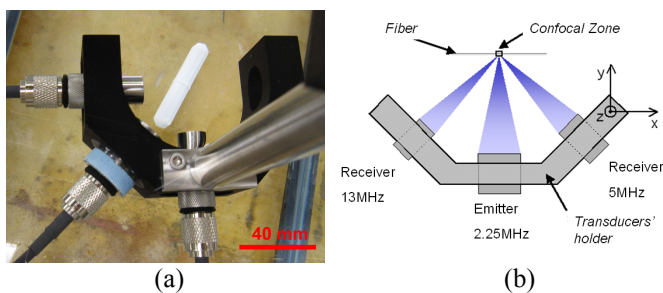


Figure 1. (a) Holder and transducer alignment used for the double passive cavitation detection experiments. (b) Illustration of confocal alignment of transducers on fiber used in flow experiments.

B. Signal Analysis

In both double passive cavitation detection and flow rate studies, signals were acquired and then manually classified according to two characteristic features of the temporal response of isolated microbubbles.

Primary response: The initial harmonic response of the microbubble lasting in duration up to the length of the transmitted pulse.

Postexcitation signal (PES): A secondary broadband response separated in time from the principle response, typically 1 to 5 μs later.

Signals were categorized according to the presence or absence of PES, which is a specific type of bubble rebound that may occur after the driving pulse is no longer present as illustrated in Fig. 2. It has been hypothesized that this type of rebound signal only occurs for free (i.e. unshelled) gas bubbles and therefore identification of PES indicates rupture of the UCA shell and subsequent collapse or destruction of the microbubble [7].

III. RESULTS AND DISCUSSION

A. Double Passive Cavitation Detection

The signals acquired in the DPCD experiment were processed to remove the DC component from the signal and then low pass filtered with a cutoff frequency of 20 MHz to remove undesirable system noise. Experimental noise thresholds were determined by collecting 50 signals with no UCAs present prior to adding the microbubbles.

While there should be on average only one UCA per confocal volume at any time, this does not preclude the possibility that there may be greater or fewer than one microbubble present in the region. Therefore, the received signals must be classified to eliminate those which contain

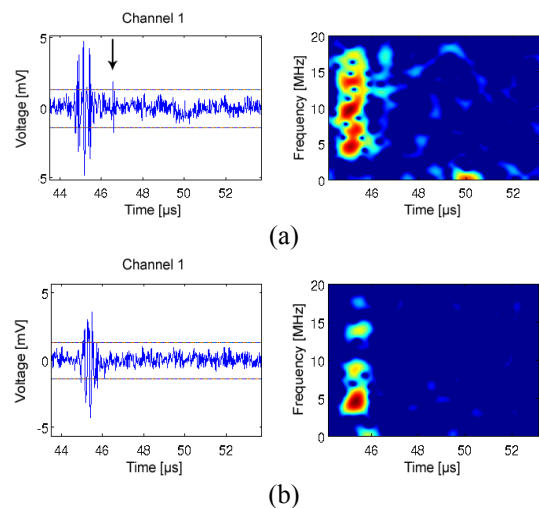


Figure 2. Examples of voltage-time and corresponding frequency-time signals. (a) Single bubble with postexcitation marked by an arrow. (b) Single bubble with no PES, only the principle harmonic response.

multiple bubbles, no bubbles, or a single bubble outside of the confocal zone. Only those signals categorized as arising from a single bubble within the confocal region were used for subsequent analysis.

In a typical experiment, several thousand signals at each pressure level were acquired. Approximately 80% to 90% of acquired signals were automatically eliminated from classification using empirical rules based on the noise thresholds and cross-correlation of the two receive channels. The remaining signals were classified through visual analysis of both the voltage-time signal and frequency-time signal calculated with a sliding Hanning window (1.28 μ s, in steps of 0.02 μ s). Of these manually classified signals, approximately 10% to 40% were classified as containing a single microbubble within the confocal region.

To determine percentage collapse thresholds, the percent of signals exhibiting PES relative to the total number of single bubble signals was fit with a curve using peak rarefactional pressure (PRP) as the independent variable. While a postexcitation signal may be indicative of shell rupture and transient collapse of the bubble, the converse is not necessarily true. A bubble may transiently fragment such that the gas content is not of a critical size and diffuses into the liquid without a violent rebound, a response which has been shown in both experiment [8] and modeling [9]. Therefore, 100% collapse is not a direct equivalent with 100% PES, which may never be reached for given acoustic parameters as PRP increases.

To accommodate this constraint, a modified logistic regression curve was used, beginning at zero for zero acoustic pressure and increasing to the maximum observed percentage of PES.

$$P(z) = \frac{Qe^{\alpha_0 + \alpha_1 z}}{1 + e^{\alpha_0 + \alpha_1 z}} \quad (1)$$

Here, $P(z)$ is the percentage of collapse, z is the log transform of the PRP, and Q is the maximum observed percentage of PES ($0 \leq Q \leq 1$).

For every given acoustic frequency, pressure, and contrast agent, at least ten signals were classified as a single bubble within the confocal region according to the scheme described above. The classification results, presented in Fig. 3, indicate that the percentage of occurrence of a postexcitation signal increases from zero to some maximum as the peak rarefactional pressure is increased while holding other insonifying parameters constant for signals identified as a single bubble within the confocal region. Such a result is consistent with other experimental results of ultrasound contrast agent collapse [10, 11].

Specific thresholds relative to the maximum observed PES can be chosen from the logistic curves, and the results for 5% and 50% are listed in Table 1. The pressure values for these thresholds are consistently lower for Definity than for Optison at a given frequency.

It has been suggested that different shell compositions may lead to different mechanisms for collapse; for example, stiffer

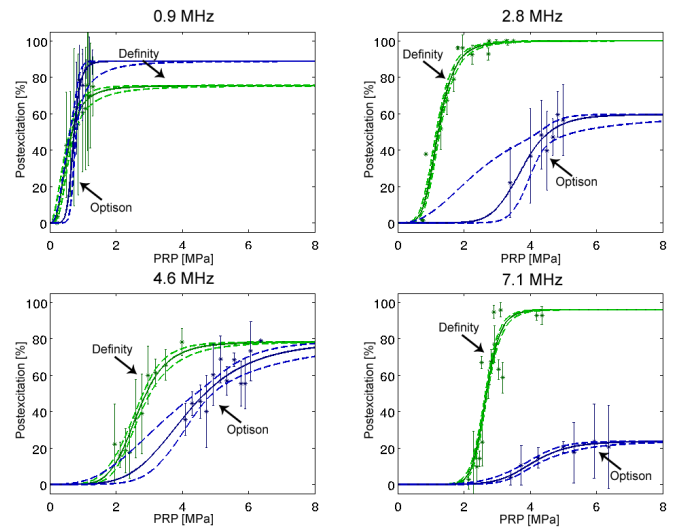


Figure 3. Logistic regression curves of the percentage of signals with postexcitation plotted against peak rarefactional pressure. The asterisks represent the experimental averages of three independent classifiers. The solid curve (—) is the best fit and the dotted curves (---) represent the 95% confidence intervals.

albumin-shelled agents may be more prone to acquiring shell defects, while more flexible lipid-shelled agents may be more likely to fragment [12]. In this research, it is shown that both types of UCAs collapse with PES signals. However, the maximum percentage of postexcitation was closer to 100% for Definity microbubbles than for Optison microbubbles in several cases, suggesting that collapse of this UCA type may be more likely to produce postexcitation signals.

B. Flow Speed

In a first set of flow rate experiments, BR14 microbubbles were isolated visually and positioned within the acoustic field. No acoustic responses of microbubbles insonified with 0.4 to 1.8 MPa peak rarefactional pressure contained postexcitation signals even when destruction was confirmed by microscopic imaging (N = 5).

Next, signals from single microbubbles in highly diluted solutions of Definity and BR14 were acquired with insonification at 0.6 MPa PRP as flow rate was varied from 5 to 200 mm/s. At least ten microbubble responses at each flow rate were examined visually for detection of PES. The percentage of signals observed with postexcitation emissions increased as a function of flow rate, as shown in Fig. 4.

When the flow was stopped or slowly flowing, microbubbles rose toward the surface and rested against the

TABLE I. PERCENTAGE POSTEXCITATION THRESHOLDS [MPa]

Frequency [MHz]	Definity		Optison	
	5%	50%	5%	50%
0.9	0.19	0.54	0.47	0.72
2.8	0.68	1.22	2.62	3.75
4.6	1.63	2.65	2.20	4.17
7.1	2.10	2.67	2.73	4.07

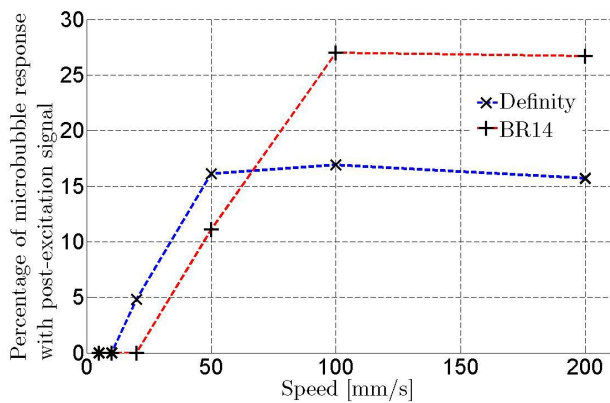


Figure 4. Percentage of microbubbles with postexcitation as a function of flow speed.

wall of the cellulose tube. In contrast, the microbubbles were carried through the whole tube and were less likely to come into contact with the cellulose tube at more rapid flow speeds. It is noted that at higher flow rates, the percentage of Definity UCAs with postexcitation at 2.25 MHz, 0.6 MPa PRP insonification is slightly greater than 15%, a value that compares favourably with results obtained in the unconstrained DPCD experiments.

IV. CONCLUSIONS

The relative percentage of signals exhibiting postexcitation was generally found to increase as peak rarefactional pressure increased for a specific frequency. Higher frequencies required greater PRP to reach the same percentage postexcitation thresholds as lower frequencies, in most cases. For matched frequencies, Definity microbubbles were also found to have lower percentage postexcitation thresholds than Optison microbubbles, a result which may be explained by different size distributions, different shell properties, or a combination of these factors. Under low-flow or no-flow conditions in the capillary tube, a smaller percentage of events contained postexcitation signals. The fact that postexcitation signals were observed more often when microbubbles were freely flowing but less under low or no-flow conditions may indicate that inertial cavitation is reduced in slowly flowing capillary networks.

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REFERENCES

- [1] S. Datta, C. C. Coussios, A. Y. Ammi, T. D. Mast, G. M. de Courten-Myers, and C. K. Holland. "Ultrasound enhanced thrombolysis using Definity as a cavitation nucleation agent." *Ultrasound in Medicine and Biology*, vol. 34, pp. 1421-1433, 2008.
- [2] M. M. Forbes, R. L. Steinberg, and W. D. O'Brien Jr. "Examination of inertial cavitation of Optison in producing sonoporation of chinese hamster ovary cells." *Ultrasound in Medicine and Biology*, vol. 34, pp. 2009-2018, 2008.
- [3] N. McDannold, N. Vykhodtseva, and K. Hynynen. "Blood brain barrier disruption induced by focused ultrasound and circulating preformed microbubbles appears to be characterized by the mechanical index." *Ultrasound in Medicine and Biology*, vol. 34, pp. 834-840, 2008.
- [4] K. Raum, and W. D. O'Brien Jr. "Pulse-echo field distribution measurements technique for high-frequency ultrasound sources. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, vol 44, pp. 810-815, 1997.
- [5] R. C. Preston, D. R. Bacon, A. J. Livett, K. and Rajendran. "PVDF membrane hydrophone performance properties and their relevance to the measurement of the acoustic output of medical ultrasound equipment." *Journal of Physics E: Scientific Instruments*, vol. 16, pp. 786-796, 1983.
- [6] J. F. Zachary, J. M. Sepsrott, L. A. Frizzell, D. G. Simpson, and W. D. O'Brien Jr. "Superthreshold behavior and threshold estimation of ultrasound-induced lung hemorrhage in adult mice and rats." *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, vol. 34, pp. 581-592, 2001.
- [7] A. Y. Ammi, R. O. Cleveland, J. Mamou, G. I. Wang, S. L. Bridal, and W. D. O'Brien, Jr. "Ultrasonic contrast agent shell rupture detected by inertial cavitation and rebound signals," *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, vol. 53, pp. 126-136, 2006.
- [8] S. L. Cessio, and C. E. Brennen. "Observations of the dynamics and acoustics of traveling bubble cavitation." *Journal of Fluid Mechanics*, vol. 233, pp. 633-660, 1991.
- [9] V. A. Bogoyavlenskiy. "Differential criterion of a bubble collapse in viscous liquids." *Physical Review E*, vol. 60, pp. 504-508, 1999.
- [10] J. E. Chomas, P. Dayton, D. May, and K. Ferrara. "Threshold of fragmentation for ultrasonic contrast agents." *Journal of Biomedical Optics* vol. 6, pp. 141-150, 2001.
- [11] T. Giesecke, and K. Hynynen. "Ultrasound mediated cavitation thresholds of liquid perfluorocarbon droplets in vitro." *Ultrasound in Medicine and Biology*, vol. 29, pp. 1359-1365, 2003.
- [12] P. A. Dayton, K. E. Morgan, A. L. Klibanov, G. H. Brandenburger, and K. W. Ferrara. "Optical and acoustical observations of effects of ultrasound on contrast agents." *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, vol. 46, pp. 220-232, 1999.