10.1109/ULTSYM.2012.0103

Influence of microbubble size distribution on postexcitation thresholds for single ultrasound contrast agent using double passive cavitation detection

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***Abstract* — A recent study has shown that double passive cavitation detection (DPCD) is a valid method for determining cavitation characteristics including collapse thresholds of isolated microbubbles based on the detection of postexcitation signal (PES) occurring 1 to 5 µs after the principle excitation of the bubble. The hypothesis is that PES is associated with the collapse of a free gas bubble released from the ultrasound contrast agent (UCA) after the rupture of its shell. It has been shown that responses of UCAs depend on several parameters such as the shell and gas core as well as the size of the microbubble. However, a detailed study of the influence of size distribution only on single microbubble collapse thresholds is still not available. The aim of this study was to determine whether there was a significant correlation between microbubble size distribution and collapse thresholds. Experiments were performed using serum albumin and dextrose microbubbles. Four size distributions were obtained and we determined microbubble collapse thresholds for each distribution using the DPCD method (3 cycle tone bursts at the central frequency of 4.6 MHz). Statistical analysis was based on comparing the means of collapse threshold for the four size distributions using an analysis of variance. The microbubble collapse threshold was found to be significantly ($p<0.05$) correlated to the microbubble size distribution: the DPCD experiments demonstrated that, in the configuration of the experiments, PES thresholds were higher for microbubbles exhibiting smaller size distribution.**

***Keywords* — Ultrasound contrast agent, Passive cavitation detection, Collapse threshold**

I. Introduction

Ultrasound contrast agents (UCAs) are microbubbles exhibiting diameters in the range 0.1 – 10 µm, making them suitable for purely intra-vascular circulation. They consist of a gaseous core stabilized by a shell that can be composed of lipids, albumin, galactose or polymers [1-4]. Until now, the primary use for UCAs is to enhance diagnostic capabilities of ultrasonic imaging [5, 6]. Additional medical applications for them are being investigated focusing on their potential use in therapeutic ultrasound [7-9].

Among the existing techniques used to characterize the microbubbles is the passive cavitation detection (PCD) allowing the determination of the albumin and lipid based shell microbubble collapse threshold [10, 11]; it is based on determining the presence or the absence of a postexcitation signal (PES) occurring 1 to 5 µs after the principle excitation of the bubble. The hypothesis is that PES is due to the collapse of a free gas bubble released from the microbubble [12]. However, because the PCD technique only uses one receive transducer there is uncertainty of the spatial location of the bubble. To overcome that limitation, recent studies have been using the double passive cavitation detection (DPCD) [13, 14] involving two receive transducers limiting the confocal volume to a smaller region. Previous DPCD studies demonstrated that response of an UCA due to an ultrasonic pulse is dependent on material properties of the shell and the gas core as well as the size of the microbubbles [14]. However, until now, no studies have been performed to evaluate only the influence of size distribution on single microbubble collapse threshold.

Thus, the study aim was to evaluate the impact of serum albumin and dextrose microbubble size distribution on the 5% and 50% collapse thresholds evaluated using the DPCD method.

II. Materials and Methods

A. Microbubbles

Microbubbles were made based on the protocol proposed by Borrelli *et al* [15]. They were produced by mixing a solution of bovine serum albumin (BSA) (Sigma-Aldrich Co., St Louis, MO) and a solution of dextrose (Fisher Chemical, Fair Lawn, NJ). Solution was then saturated using perfluorocarbon gas (FluoroMed, L.P., Round Rock, TX) and sonicated with a 20 kHz Fisher 500 sonic dismembrator (ThermoFisher Scientific, Waltham, MA) using a 1.1 cm horn. Four different microbubble populations were made based on the different sonication settings.

B. Data collection

The DPCD experiments involved the confocal alignment of two receivers and one transmitter. The two receivers were placed at an angle of 90º with the transmitter in between them, at an angle of 45º (Fig. 1). The center frequencies of the two receivers were measured in a transmit mode to be 13.8 MHz and 14.6 MHz. Both receive transducers were f/2 with an element diameter of 0.5''. The transmitter exhibited a central frequency of 4.6 MHz, being f/2 with an element diameter of 0.75''. Data acquisition involved three cycle tone bursts with a pulse repetition frequency of 10 Hz at the central frequency of the transmitter. These were generated by a high-power pulser-receiver system (RITEC RAM500, Warwick, RI).

Each experiment involved placing the transducer holder in a Plexiglas water tank filled with degassed water at room temperature. Prior to each experiment, the transducers were aligned in pulse-echo mode using a 50-µm-diameter wire located in the confocal region. In addition, 50 signals were acquired without any UCA in the tank to determine the experimental system noise for each set of acquisitions. Then, the appropriate concentration of microbubbles was added to the water. The solution was then gently mixed using a magnetic stir bar to ensure the homogeneity of the microbubble distribution. Experiments involved the acquisition of several thousand signals for each incident peak rarefactional pressure amplitude (PRPA). The total experiment time was about 2 hours.

Once acquired by the receivers, the signals were amplified by 22 dB, digitized using a A/D converter (12-bit, 200 MS/s, Strategic Test digitizing board UF 3025, Cambridge, MA) and saved to a PC for offline processing using Matlab [14].

In addition, images of the tested microbubbles were acquired for each set of experiments using a microscope and camera system (Olympus BX51, Tokyo, Japan). Images were analyzed using a circular routine based on the Hough transform to determine the size distribution of the studied population.

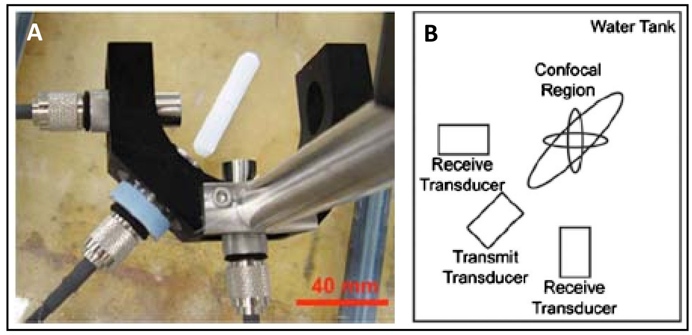

Fig. 1: (A) Photograph and (B) schematic of the DPCD experiments as described by King *et al [14]*

C. Signal analysis

In a previous study [14], a classification strategy of the detected signals has been described. Based on the characteristics of the signals received by each transducer, seven categories have been proposed: (1) no bubble within the confocal region, (2) multiple bubbles within the confocal region, (3) single bubble out of confocal region, (4) single bubble with PES in only one channel, (5) single bubble with PES in both channels, (6) single bubble with no PES, (7) unknown. In the context of the data analysis, only the signals including in categories 5 and 6 were used for subsequent statistical analysis as they were the only categories containing a single bubble in the confocal region and exhibiting symmetrical behavior.

D. Logistic curve fitting

A postexcitation threshold has been previously defined as the level at which a certain percentage of the total population of microbubbles transiently collapses with PES. Determination of such thresholds involved fitting the experimental data with a modified logistic regression curve:

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

where P(z) is the desired percentage of collapse, z is the log transform of the PRPA, Q is the maximum observed percentage of PES ($0 \leq Q \leq 1$) and α_0 and α_1 are the fitting coefficients. In the context of this study, thresholds at 5% and 50% and their confidence intervals were determined and compared [14].

E. Statistical analysis

The aim of the study was to determine if there were a significant relationship between the size distribution of the microbubbles and the collapse thresholds measured using the DPCD experiments. Four groups have been studied.

Size distribution: To determine the relationship between the size distributions of the four groups, we performed an analysis of variance (ANOVA). We compared the mean diameter evaluated among 300 random diameter measures performed using Matlab®. The hypotheses were:

$$H_0: m_1 = m_2 = m_3 = m_4$$
$$H_1: at\ least\ two\ of\ the\ group\ means\ are\ not\ equal$$

where m_k is the mean of the k^{th} group.

The hypotheses were tested for significance by comparing the corresponding likelihood ratio statistic with the reference *F* distribution with the appropriate degrees of freedom. Results were significant for *p*-values less than 0.05.

Collapse threshold: Statistical analysis was based on comparing the weighted means of the PRPA associated with the collapsing of the bubbles for the four tested groups. We performed an ANOVA between the four groups. Results were significant for *p*-values less than 0.05. Hypotheses were the same as for the size distribution study previously mentioned.

III. RESULTS

A. Size distribution

ANOVA revealed *p*-values less than 0.05 (p-value < 10^{-6}). We could reject H_0: there are at least two size distributions that are not equal. In addition to the ANOVA, we calculated the 95% confidence interval for each of the measured data. These are shown in Fig. 2.A. We could clearly divide the four groups into two sub-groups based on the confidence intervals; groups 1 and 2 exhibited similar size distribution as well as groups 3 and 4 (Fig. 2A) (overlapping of the confidence intervals).

B. Collapse threshold

ANOVA revealed *p*-value less than 0.05 (p-value < 10^{-6}). We could reject H_0: as for the size distribution, there are at least two weighted means that are not equal. Using (1), we determined the 5% and 50% collapse thresholds and their associated 95% intervals of confidence using Matlab®. As for the size distribution analysis, Fig. 2.B-C exhibited two distinct groups significantly different: groups 1 and 2 have similar collapse thresholds as does groups 3 and 4.

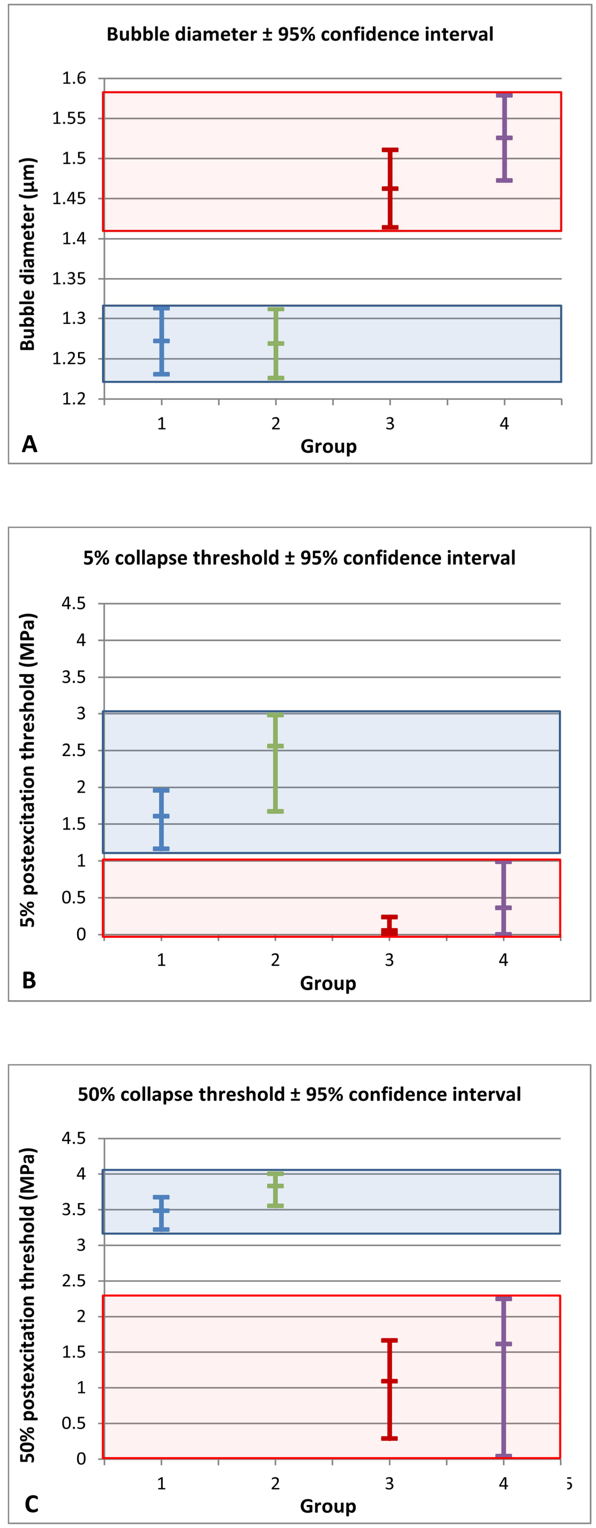

Fig. 2: Plot of 95% confidence intervals for the determined mean bubble diameters (A), 5% (B) and 50% (C) collapse thresholds for the 4 tested groups

IV. DISCUSSION

It has been previously demonstrated [14] that material properties of the shell and the gas core as well as the size of the bubbles influence the response of an UCA due to an ultrasonic pulse. However, until now no studies have been performed to evaluate only the impact of the size distribution on the PES-determined collapse threshold.

In this study, double passive cavitation detection was applied to determine the relationship between the bubble size distribution and the collapse threshold. The statistical analysis presented in that paper pointed out that the four groups could be divided in two distinct sub-groups based on their size distribution. The statistical analysis performed on the 5% and 50% collapse thresholds exhibited the same split between the four groups. These two sub-groups were then significantly different from the size distribution point of view as well as for the collapse thresholds one, thus leading to the conclusion that microbubble collapse threshold is correlated to the size distribution. In addition, we demonstrated that in the configuration of the experiments, PES threshold was higher for microbubbles exhibiting smaller size distribution.

Additional studies where other parameters are controlled independently of one another would be necessary to completely explain microbubbles behavior while using the DPCD experiments.

ACKNOWLEDGMENTS

The authors would like to acknowledge valuable discussions with Dr Douglas Simpson (UIUC Statistics Department) on logistic curve fitting. This research was supported by NIH under Grant No. R37EB002641.

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