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Ambient pressure dependence of the ultra-harmonic response from contrast microbubbles

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Sub-harmonic response from ultrasound contrast agent microbubbles has been demonstrated to be an effective modality for noninvasive pressure measurement. In the present study, the dependence of ultra-harmonic response on the ambient overpressure was investigated by both experimental measurements and simulations. In the measurements, the microbubbles were exposed to Gaussian pulses with varied driving frequencies and pulse lengths, at an acoustic pressure of 0.3 MPa. The amplitudes of sub- and ultra-harmonic components were measured when the ambient overpressures varied from 0–25 kPa. At the driving frequency of 1.33 MHz, the ultra-harmonic energy decreased but the sub-harmonic energy increased with the increasing overpressure; while at the driving frequency of 4 MHz, both the sub- and ultra-harmonic components showed the same tendency that the corresponding energy decreased as the overpressure was increased. A 4-MHz Gaussian pulse with 64 cycles could provide an ultra-harmonic response with both good ambient pressure sensitivity and high linearity. Furthermore, the effects of shell parameters of a microbubble on the generation of ultra- and sub-harmonic responses were discussed based on simulations using Marmottant's model. This study suggests that the ultra-harmonic response from contrast microbubbles might be applicable for noninvasive pressure measurement. © *2012 Acoustical Society of America*. [http://dx.doi.org/10.1121/1.4707512]

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I. INTRODUCTION

Pulmonary hypertension (PH) refers to high blood pressure in the vessel that carries blood from the heart to the lungs.[1] Since the symptoms of PH are not specific, accurate diagnosis of PH is still a great obstacle facing the researchers and clinicians, although many tests might give clues to the possibility of PH. For instance, the right heart catheterization (RHC) is currently considered as the best diagnostic modality of PH.[1] During the test of RHC, a catheter would be inserted through the groin into the femoral vein, then advanced to the right side of the heart to measure the local blood pressure in the right side of the heart and pulmonary arteries. However, this technique is invasive and subjected to the high risk of patients' infection.[2] Therefore, exploration of more safe and accurate diagnostic methods is always in high demand for improving PH treatment.

Ultrasound contrast agents (UCAs) are usually comprised by suspensions of gas-filled microbubbles with micrometer diameters encapsulated by a thin stabilization coating (e.g., lipid, albumin, or polymer).[3–5] UCA was first introduced clinically to improve echocardiographic image contrast since microbubbles in fluids could create an acoustic impedance mismatch between the host fluids (e.g., blood) and the surrounding tissues. Besides enhancing the reflection of ultrasound, the UCA microbubbles also exhibit nonlinear vibrations under insonation, which will produce harmonic responses (response at $2f$, $3f$,… when excited at f), sub-harmonic response (response at $f/2$ when excited at f), and ultra-harmonic response (response at $3f/2$ when excited at f).[6,7] In the past three decades, much attention has been paid on noninvasive blood pressure measurements using UCAs microbubbles combined with diagnostic ultrasound. Fairbank and Scully first reported the cardiac pressure measurements by the use of UCAs.[8] They showed that the resonance frequency of an air bubble would vary with the changing of the ambient pressure. However, the accuracy of the measurement is significantly affected by the large size distribution of the first-generation UCA. Bouakaz *et al*. proposed an approach based on the measurement of the disappearance time of free bubbles which were generated by destroying UCA microbubbles at a certain region of interest.[9] However, the sensitivity of this approach was also limited due to the quick vanishment of free bubbles.[10] Recently, sub-harmonic response was proposed as a possible means for local blood pressure measurements on account of an almost linear reduction in sub-harmonic response as the ambient overpressure increases.[11–13] Shi *et al*. experimentally found that the sub-harmonic response is robustly dependent on the ambient pressure.[14] They reported a 9.6 dB linear decrease in the peak

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amplitude of the sub-harmonics for a 24.8 kPa (186 mm Hg) rise in the ambient pressure. Furthermore, several UCAs (Optison®, Definity®, and Sonazoid®) also demonstrated a decrease in the sub-harmonic response with a 188 mm Hg increase of ambient pressure.[12] However, at different excitation levels, the sub-harmonic responses of UCAs might exhibit different motions with the increasing ambient pressure. Frinking *et al*. revealed that the amplitudes of sub-harmonic echoes may increase by 28.9 dB for 180 mm Hg increase of ambient pressure at a very low excitation level of 50 kPa, while a 9.6 dB decrease in sub-harmonic response was observed at a higher excitation pressure of 400 kPa.[15] Andersen and Jensen theoretically revealed that the variation of the sub-harmonic component is related to the driving parameters, such as shape, length, and amplitude.[16] Katiyar *et al*. claimed that the ratio of the excitation frequency to the natural frequency of the bubble is the dominant parameter of the change in sub-harmonics.[2] Meanwhile, similar to sub-harmonics, ultra-harmonics might also be generated as UCA microbubbles are insonified with ultrasound. It has been claimed that the developments of both sub- and ultra-harmonics have the same trend (i.e., occurrence, growth, and saturation) with the increase of exciting acoustic pressure, but the generation of ultra-harmonic has a lower threshold than that of sub-harmonic.[17] However, so far there is no attempt for ambient pressure measurements via ultra-harmonics.

In the present study, the possibility of utilizing ultra-harmonic response for the ambient pressure measurements was examined both theoretically and experimentally, and comparisons were made between the results of the ultra- and sub-harmonic responses. Furthermore, dependences of the ultra-harmonic response on the driving frequency and pulse length were discussed. Finally, the effects of microbubble parameter on the ultra-harmonic generation were also investigated in this paper.

II. THEORY AND METHOD

A. Encapsulated microbubble dynamics

Microbubble dynamics in an incompressible liquid has been studied widely using various types of modified Rayleigh–Plesset (RP) equations.[6,18–21] In the present study, the Marmottant model is used for describing the dynamic behavior of microbubbles encapsulated with a thin lipid shell.[22] Compared to other coated microbubble dynamic models, the Marmottant model specifically considers the nonlinear surface tension modifications arising from the buckling and rupture of a bubble shell. The dynamic equation of the model is written as[22]

$$\rho R\ddot{R}+\frac{3}{2}\rho\dot{R}^2=\left[P_0+P_{ov}+\frac{2\sigma(R_0)}{R_0}\right]\left(\frac{R_0}{R}\right)^{3\kappa}\left(1-\frac{3\kappa}{c}\dot{R}\right)-(P_0+P_{ov})-\frac{2\sigma(R)}{R}-\frac{4\mu\dot{R}}{R}-\frac{4\kappa_s\dot{R}}{R^2}-p_{ac}(t), \quad (1)$$

where R is the instantaneous radius of the microbubble; $\dot{R}$, $\ddot{R}$ are the first and second time derivatives of R; R_0 is the equilibrium radius; ρ is the density of the surrounding medium; κ is the polytropic exponent; μ is the surrounding liquid viscosity; κ_s is the surface dilatational viscosity; p_0 is the hydrostatic pressure; $p_{\rm ov}$ is the overpressure (to evaluate the variation of ambient pressure); $p_{\rm ac}(t)$ is the exciting acoustic pressure; c is the sound velocity. The Marmottant model underlines the effective surface tension of a microbubble during its oscillation and the movement of the bubble is defined as three successive stages as the surface tension rises. The effective surface tension $\sigma(R)$ is expressed as[22]

$$\sigma(R)=\begin{cases}0 & \text{if } R\le R_{buckling}\\ \chi\left(\frac{R^2}{R^2_{buckling}}-1\right) & \text{if } R_{buckling}\le R\le R_{break\text{-}up}\\ \sigma_{water} & \text{if ruptured and } R\ge R_{ruptured}.\end{cases} \quad (2)$$

As the bubble radius is below $R_{buckling}$, the surface buckles and $\sigma=0$; The elastic modulus χ gives the slope of the elastic regime where the bubble radius is between $R_{buckling}$ and $R_{break\text{-}up}$; $R_{ruptured}$ describes the moment of rupture and the maximum surface tension saturates at σ_{water}.

The scattered acoustic response is calculated from the radial response at a distance of d from the microbubble:[15]

$$P_s(d,t)=\rho\frac{R}{d}(2\dot{R}^2+R\ddot{R}). \quad (3)$$

Given that UCA microbubbles have a wide size distribution, we adapt a weighting scheme[23] on the basis of the size distribution of microbubbles to determine the cumulative echoes. $S(f)$ stands for the total scattered pressure from microbubbles with sizes ranging from i to j at a frequency f (Ref. 23)

$$S(f)=\sum_{k=i}^{j}P_{SC}(r_k,f)w_k\%, \quad (4)$$

where $P_{SC}(r_k,f)$ is the scattered pressure from microbubbles with size k at the frequency f; w_k is the number percentage weight of bubbles with size k.

B. Simulations

Equations (1) and (2) were solved by using an ordinary differential equation solver ODE45 implemented in MATLAB (Mathworks Inc., Natick, MA) with initial conditions $R=R_0$ and $\dot{R}=0$. In the simulation, some physical constants were set as follows:[22,24,25] shell surface dilatational viscosity, $\kappa_s=15\times10^{-9}$ N; shell elastic compression modulus, $\chi=1.1$N/m; surface tension in the equilibrium, $\sigma_0=0.035$ N/m; critical tension where shell breaks, $\sigma_{break\text{-}up}=0.13$ N/m; water surface tension, $\sigma_{water}=0.072$ N/m; hydrostatic pressure, $p_0=1.01\times10^5$ N/m^2; polytropic exponent of the gas, $\kappa=1.4$; density of surrounding liquid, $\rho=998$ Kg/m^3; sound speed in liquid, $c=1500$ m/s; viscous in liquid, $\mu=1.0\times10^{-3}$N s/m^2. The equilibrium radius R_0 was 2 μm, and the shell buckling radius was set as 1.8 μm. The size distribution of SonoVue® was adapted from Gorce *et al*.[24] and bubbles smaller than 1 μm or bigger than 10 μm in diameter were discarded in this study.

C. Experiments

The UCA used here is SonoVue® (Bracco Diagnostics Inc., Geneva, Switzerland) with a mean radius of 2 μm. According to the instructions of the manufacturer, the SonoVue® vial was vented with a sterile 18-gauge needle, followed by 5-ml phosphate-buffered saline (PBS) injection. Then UCA microbubbles were evenly distributed by inversion agitation of the vial. Finally, the microbubbles were diluted in saline to a concentration of about 2×10^6 microbubbles/ml. Figure 1 shows the diagram of the experimental setup for measuring the sub- and ultra-harmonics scattered from UCA microbubbles. An arbitrary waveform generator (Agilent 33250, CA) was used to produce tone-burst signals with varied pulse lengths (20, 64, or 128 cycles) at a pulse repetitive frequency (PRF) of 100 Hz. Then, the signals were used to drive a transmitter, after being amplified by a radio frequency (RF) power amplifier (A150, ENI, Rochester, NY). Two different transducers were used as the transmitters for the experiments. Transducer #1 was a 3.5-MHz focused transducer (V382-SU, Panametrics, MA, focal length = 5.0 cm, diameter = 1 cm), and transducer #2 was a 1.0-MHz focused transducer (V303-SU, Panametrics, MA, focal length = 5.0 cm, diameter = 1 cm). The scattered wave was received by a needle hydrophone (TNU001A, NTR Systems, Inc. Seattle, WA). After passing through a pre-amplifier (fixed gain of 30 dB, HPA30, NTR Systems, Inc. Seattle, WA), the received signal was captured by a digital oscilloscope (Agilent 54810, Santa Clara, CA). As shown in Fig. 1, in order to reduce the impact of the insonating signal on the detection of the sub- and ultra-harmonic signals, the transmitter and receiver were orthogonally positioned away from the center of a sample container by 5.0 cm (focal area of the transmitter) and 4.0 cm, respectively. The sample container used to hold the microbubble suspension was a plastic test tube (13.3 mm diameter and 70 mm length). The ambient pressure was controlled by using the valve on the top of the tube and the pressure gage (Y-150, Tianchuan Instrument, Shanghai, China) on the tube's side wall. A linear 3-axial scanning system (ESP7000, Newport, Irvine, CA) controlled by a PC via LABVIEW software (NI Corp., TX) was used for aligning the transmitter and receiver. The *in situ* acoustic pressure of the transmitting transducer was calibrated by using a needle hydrophone (TNU001A, NTR Systems, Inc. Seattle, WA) with a 30-dB preamplifier (HPA30, NTR Systems, Inc. Seattle, WA). All measurements were performed at room temperature (approximately 23 °C). Five replicated measurements were performed at each setting for the sub- and ultra-harmonic emissions with refreshed microbubbles suspensions. For each measurement, the response of the microbubbles was averaged over 25 traces and then analyzed by use of a fast-Fourier-transform (FFT) program.

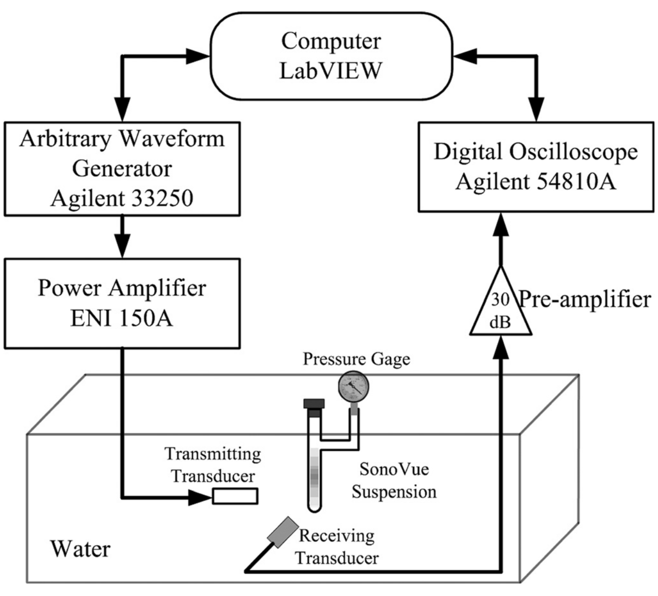

FIG. 1. Diagram of the experimental system for measuring sub- and ultra-harmonics scattered from microbubbles.

III. RESULTS

It has been reported that sub- and ultra-harmonic components are only generated from encapsulated microbubbles when the exciting acoustic pressure exceeds certain thresholds, and the threshold pressures of the sub- and the ultra-harmonics would reach minimum levels at $f = 2f_0$ and $f = 2/3f_0$, respectively.[17] As the resonant frequency of SonoVue® microbubbles is around 2 MHz, two different driving frequencies of 4 MHz ($f = 2f_0$) and 1.33 MHz ($f = 2/3f_0$) were used in the simulation and the measurements. Figures 2(a) and 2(b) plot the spectra of the scattered signals from SonoVue® microbubbles when driven by ultrasound pulses with 64 cycles at driving frequencies of 4 and 1.33 MHz. The acoustic pressure was kept at 0.3 MPa. Significant discrete sub- and ultra-harmonic components were observed for both 4 and 1.33 MHz driving pulses. This indicates that both frequencies could be utilized to generate sufficient sub- and ultra-harmonic signals.

Figure 3 illustrates the changes in the FFT amplitudes of ultra- [Fig. 3(a)] and sub-harmonics [Fig. 3(b)] generated from microbubbles as a function of ambient pressure when driven by 1.33 MHz Gaussian pulses with varied pulse lengths. In Fig. 3, the calculated results are plotted as solid (20 cycles), dashed (64 cycles), and dotted (128 cycles) lines; and the measured ones are represented as rectangles (20 cycles), up-triangles (64 cycles), and down-triangles (128 cycles). During the experiments, the acoustic pressure amplitude was kept at 0.3 MPa. It is shown in Fig. 3 that the ultra-harmonic energy decreases as the overpressure is increased. Over the ambient pressure range between 0 to 25 kPa (corresponding to 0–188 mmHg), the measured ultra-harmonic components reduced by about 5.5, 8.5, and 16.8 dB corresponding to 20, 64, and 128 cycles. However, the measured sub-harmonic component showed the adverse tendency over the same ambient pressure range; the sub-harmonic components increased by about 3.5, 10.3, and 14.1 dB, corresponding to 20, 64, and 128 cycles.

While keeping other parameters fixed, the same measurements were performed for the microbubbles exposed to ultrasound pulses driven at 4-MHz central frequency. The FFT amplitude changes of ultra- [Fig. 4(a)] and sub-harmonics [Fig. 4(b)] are plotted as a function of ambient pressure. Both

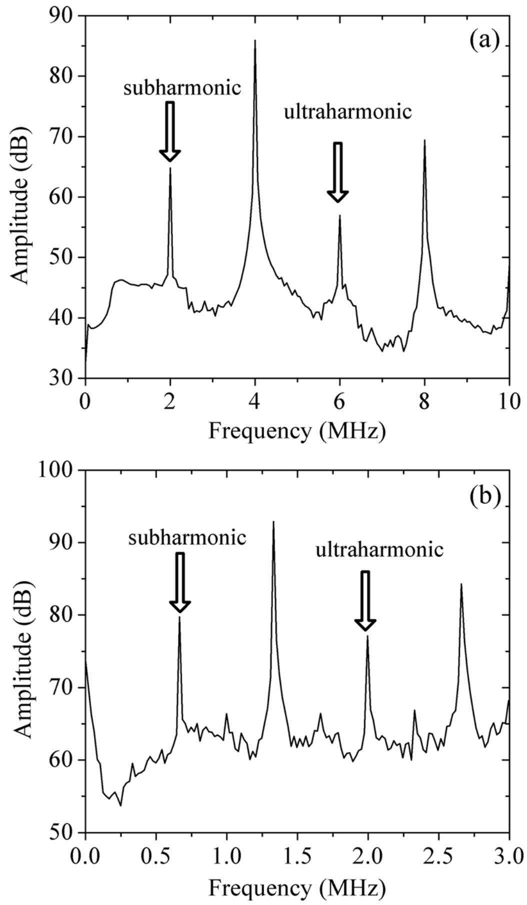

FIG. 2. Measured spectra of scattered signals from SonoVue microbubbles exposed to 0.35-MPa ultrasound pulses with 64 cycles. The driving frequencies are (a) 4 MHz and (b) 1.33 MHz.

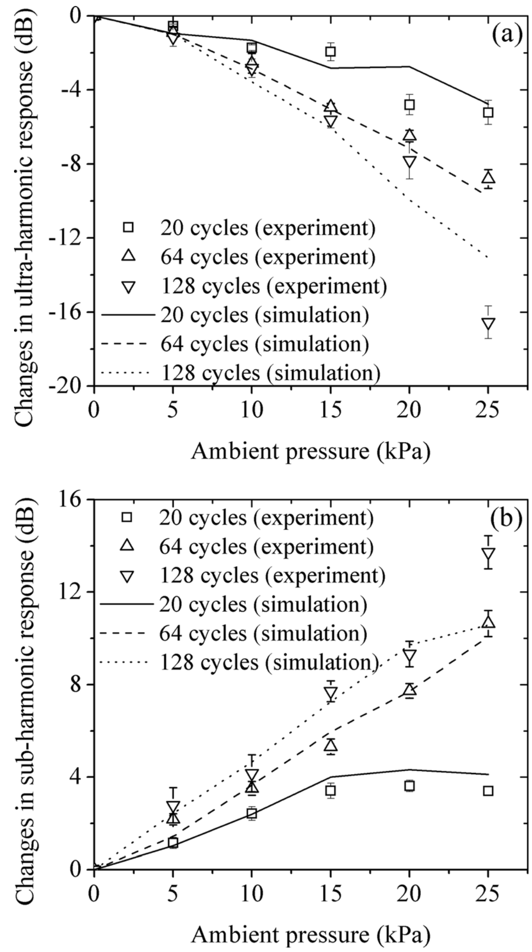

FIG. 3. Calculated and measured changes in sub- and ultra-harmonic pressure amplitude as a function of ambient pressure at 1.33 MHz excitation, (a) ultra- and (b) sub-harmonics.

the sub- and ultra-harmonic components showed the same tendency that the corresponding energy decreases as the overpressure is increased. Over the ambient pressure range of 0–25 kPa, the measured ultra-harmonic components reduced by 6.2, 9.8, and 19.9 dB corresponding to 20, 64, and 128 cycles, while the measured sub-harmonic components reduced by about 0.2, 9.0, and 13.5 dB, respectively.

As shown in Table I, over the ambient pressure range of 0–25 kPa, the variation of the ultra-harmonic amplitudes is greater than that of the sub-harmonic responses. For example, for a 4-MHz driving pulse with 128 cycles at 0.3 MPa, the sensitivities of ultra- and sub-harmonic responses corresponding to change of the ambient pressure are 0.79 and 0.54 dB/kPa, respectively. It is suggested that the ultra-harmonic component might be utilized as a more sensitive parameter to evaluate the variation of the ambient pressure. Shi *et al*. reported that the sensitivity of the sub-harmonic response from Levovist® is 0.4 dB/kPa when driven by 2-MHz ultrasound with 64 cycles at 0.39 MPa;[14] Andersen and Jensen observed the SonoVue® sub-harmonic pressure sensitivity of 0.42 dB/kPa;[13] Frinking *et al*. reported a sensitivity of sub-harmonic response 0.39 dB/kPa from a phospholipid-coated contrast microbubble (similar to BR14 or SonoVue®) at a excitation pressure of 0.4 MPa.[15] Our measured results for the sub-harmonic response are in good agreement with those reported experimental results. Moreover, Table I also shows the influence of pulse length (represented by the number of pulse cycles) on the variations of ultra- and sub-harmonic responses. It is observed that a longer pulse length would ensure higher sensitivities for the ultra- or sub-harmonic responses. The result indicates that more ultra- or sub-harmonic energy should be deposited by longer driving pulses, which is inconsistent with theoretical results obtained by Andersen and Jensen.[16] However, longer pulse length might lead to quicker microbubble destruction. In addition, the pulse length is related to the linearity of the curve. Both sub- and ultra-harmonic responses show the best linear decrease for the pulse length of 64.

The simulated results in Figs. 3 and 4 are in good agreement with the measured ones, except for the pulse with 128 cycles at the ambient pressure of 25 kPa. Microbubble destruction could explain this discrepancy.

IV. DISCUSSION

A. Effects of ambient pressure on the variation trend of ultra-harmonics

It has been demonstrated in Sec. III that the sub-harmonic either increases or decreases with increasing ambient pressure, while the ultra-harmonic tends to keep the

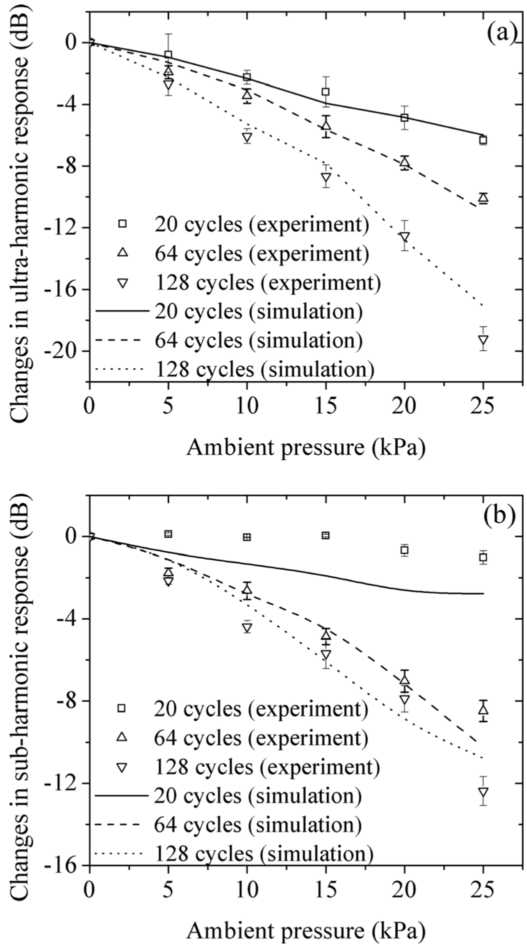

FIG. 4. The FFT amplitudes of (a) ultra- and (b) sub-harmonics plotted as a function of ambient pressure at 4 MHz excitation.

descendant trend. Katiyar *et al*. has suggested that the ratio of the excitation frequency (f) to the natural frequency (f_0) of the bubble might be considered as the dominant factor for the variations of ultra- and sub-harmonic motions.[2] It has been reported that the amplitude of the sub- or ultra-harmonics would be highly correlated to the value of f/f_0.[15] The nature frequency f_0 might be raised by the increasing ambient pressure, which would result in the decrease of f/f_0. If the increase in the ambient pressure leads the ratio of f/f_0 to move to the peak value of sub- or ultra-harmonic amplitude, the sub- or ultra-harmonic response shows an increasing tendency. In contrast, if the increase in the ambient pressure leads the ratio to move away from the peak, the sub- or ultra-harmonic response shows a decreasing tendency. It is suggested that the driving frequency $f = 2f_0$ or $f = 2/3f_0$ cannot ensure the most robust change in sub- or

TABLE I. Comparison of sensitivities for sub- and ultra-harmonic responses with different duty cycles excited by 4-MHz ultrasound at 0.3 MPa.

Pulse cycles	Sub-harmonic (dB/kPa)	Ultra-harmonic (dB/kPa)
20	0.008	0.25
64	0.36	0.39
128	0.54	0.79

ultra-harmonic generation. Since the ratio f/f_0 might be affected by many factors, such as the exciting frequency, the exciting acoustic pressure, the ambient pressure, and the bubble parameters, it is difficult to propose a certain factor to determine this behavior. Further investigation is required in a future study.

B. Effects of bubble shell parameters on the ultra-harmonic generation

The simulations in this paper were based on the Marmottant model, which does not allow the shell parameters changing with the ambient pressure varying in the simulation. First, the initial surface tension $\sigma(R_0)$ is a crucial parameter for determining the initial state (buckling or elastic states) of a microbubble.[6,12,18] Microbubbles with the same size might have different values of the initial surface tension, showing different dynamic behaviors.[26] Figure 5 shows the spectra of the simulated echo responses generated from a microbubble ($R_0 = 2.0$ μm) excited with a 4.0-MHz Gaussian pulse with 64 cycles at 0.3 MPa, where the elastic state is plotted as solid line calculated with an initial surface tension $\sigma(R_0) = 0.035$ N/m, while the buckling state is plotted as dashed lines calculated with $\sigma(R_0) = 0$ N/m. As shown in Fig. 5, the microbubbles with the elastic initial state would emit stronger sub- and ultra-harmonic signals, which is different from the observation by Frinking *et al*.[15] Note that Frinking *et al*. indicated that sub-harmonic oscillations are highly related to compression-only behavior[26] at the relatively low excitation levels (<50 kPa). However, the results of our simulation are just on the contrary at the relatively large pressures. The variation of the ratio $\Delta R^+/\Delta R^-$ [defined by $\Delta R^+ = \max(R) - R_0$, $\Delta R^- = R_0 - \min(R)$][22] might give clues to explain this finding. Asymmetry in radius excursion is a key to the nonlinearity of bubble vibration. At the low-amplitude excitation, the compression-only asymmetry occurs when $\Delta R^+/\Delta R^- < 1$ (Ref. 22) and bubbles are ready to be buckled under compression rather than expansion. However, under large-amplitude excitation, the ratio $\Delta R^+/\Delta R^-$ turns out to be >1, suggesting a longer excursion in the elastic state.

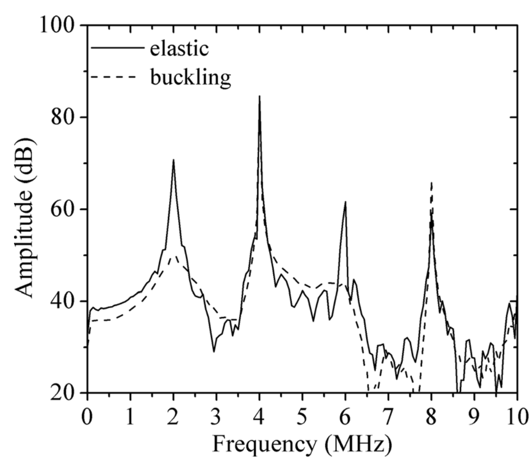

FIG. 5. Calculated scattered spectra for a single 2.0-μm bubble which is excited at 0.3 MPa in the elastic state (solid line) and in the buckling state (dashed line).

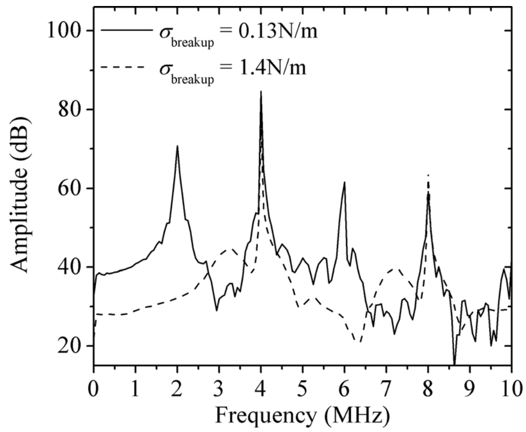

FIG. 6. Simulated scattered spectra for a single 2.0-μm bubble which is excited at 0.3 MPa for $\sigma_{breakup} = 0.13\,\mathrm{N/m}$ (solid line) and $\sigma_{breakup} = 1.4\,\mathrm{N/m}$ (dashed line).

Second, the critical surface tension $\sigma_{breakup}$, which determines the upper limit of the elastic region,[22] might influence the generations of the sub- and ultra-harmonics. Figure 6 illustrates the spectra obtained from simulated echo responses of a microbubble ($R_0 = 2.0$ μm, $\sigma(R_0) = 0.035$ N/m) excited with a 4.0 MHz Gaussian pulse with 64 cycles at 0.3 MPa, where the solid lines are calculated with $\sigma_{breakup} = 0.13\,\mathrm{N/m}$ and the dashed lines are obtained with $\sigma_{breakup} = 1.4\,\mathrm{N/m}$. Results indicate that the shorter elastic region induces stronger sub- and ultra-harmonic scatterings.

Besides, the buckling radius $R_{buckling}$ is another vital parameter for describing the dynamics of a microbubble. Figure 7 plots the variation of the calculated ultra-harmonic responses vs the ambient overpressure for a single 2.0-μm microbubble with varying buckling radius, where the microbubble is exposed to 4-MHz pulses with a driving amplitude of 0.3 MPa. It is observed that the value of the buckling radius has a significant impact on the ultra-harmonic response. A buckling radius $R_{buckling}$ of 2.3 μm generated the largest dynamic variation (-8 dB) of the ultra-harmonic response, while with a small buckling radius of 1.2 μm, much smaller dynamic variation (-4.5 dB) of the ultra-harmonic response were reached. The buckling radius is the separate point between the buckling and elastic state. With the rising of $R_{buckling}$, the buckled region becomes larger while the elastic one is shorter. As discussed above, the larger buckling region induces stronger sub- and ultra-harmonic scatterings at a high level of excitation. As a result, the bubbles with a larger buckling region may be more readily affected by the ambient pressure.

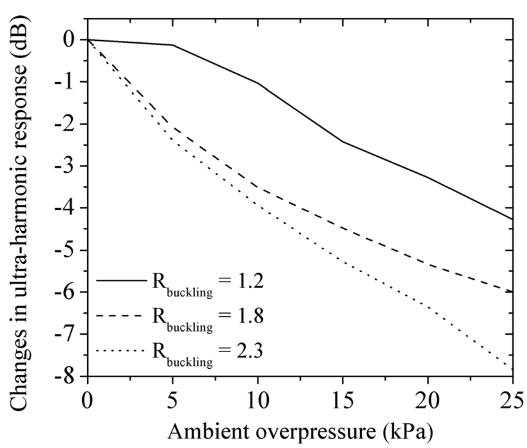

FIG. 7. Simulated change in ultra-harmonic response for a single 2.0-μm bubble as the ambient overpressure increases, varying the buckling radius at 1.2, 1.8, and 2.3 μm.

In summary, under relative large amplitude excitations, the elastic initial state, larger buckling region, and shorter elastic region of contrast microbubble are crucial to the generation of ultra-harmonic response. Furthermore, bubbles with a larger buckling region might be affected by ambient pressure more easily. This observation may be exploited for improving sensitivity of pressure measurement by utilizing specifically designed contrast agents with optimized buckling state and initial surface tension.

C. Discrepancies between the experiment and simulation

It is noted that there are still discrepancies between the theoretical and the experimental curves of sub- and ultra-harmonic responses vs the ambient pressure (as shown in Figs. 3 and 4). One possible reason is the incomplete consideration of the effective surface tension. The uncontrollable initial surface tension might lead microbubbles with the same size to undergo different behaviors, buckling state or elastic state, even a combination of these two states.[12] Another possible reason is the bubble size distribution used in the simulation might not be exactly consistent with the situation applied in practice. Bubble size distribution may play an important role in determining the spectrum of the scattered signal. The actual size distribution could be affected by many experimental conditions. Furthermore, other factors, such as the un-consideration of the interaction among microbubbles, disparate shell parameters for microbubbles with different sizes, and microbubble rupture, might also have contributions to the discrepancies between the simulation and measurements.

V. CONCLUSION

The dependence of the ultra- and sub-harmonic responses of contrast agent microbubbles on the ambient pressure has been investigated under different acoustic driving conditions. The behaviors of the sub- and ultra-harmonic responses show either increasing or decreasing tendency with the increasing ambient pressure, depending on the driving frequency. The results also demonstrate that the energy variations of the sub- and ultra-harmonic components are highly correlated to the pulse lengths of the acoustic driving signals. Choosing an appropriate driving frequency and pulse length could optimize the sensitivity and linearity of the microbubble-based ultra-harmonic responses.

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[1]J. I. Benisty, "Pulmonary hypertension," Circulation **106**, 192–194 (2002).

[2]A. Katiyar, K. Sarkar, and F. Forsberg, "Modeling subharmonic response from contrast microbubbles as a function of ambient static pressure," J. Acoust. Soc. Am. **129**, 2325–2335 (2011).

[3]P. Frinking, A. Bouakaz, J. Kirkhorn, C. F. Ten, and N. de Jong, "Ultrasound contrast imaging: Current and new potential methods," Ultrasound Med. Biol. **26**, 965–975 (2000).

[4]F. Goldberg, J. B. Liu, and F. Forsberg, "Ultrasound contrast agents: A review," Ultrasound Med. Biol. **20**, 319–333 (1994).

[5]F. A. Duck, "Nonlinear acoustics in diagnostic ultrasound," Ultrasound Med. Biol. **28**, 1–18 (2002).

[6]N. de Jong, R. Corner, and C. T. Lancee, "Higher harmonics of vibrating gas-filled microspheres. Part one: Simulations," Ultrasonics **32**, 447–453 (1994).

[7]W. T. Shi and F. Forsberg, "Ultrasonic characterization of the nonlinear properties of contrast microbubbles," Ultrasound Med. Biol. **26**, 93–104 (2000).

[8]W. M. Fairbank and M. O. Scully, "New noninvasive technique for cardiac pressure measurement-resonant scattering of ultrasound from bubbles," IEEE Trans. Biomed. Eng. **24**, 107–110 (1977).

[9]A. Bouakaz, P. J. A. Frinking, N. de Jong, and N. Bom, "Noninvasive measurement of the hydrostatic pressure in a fluid-filled cavity based on the disappearance time of micrometer-sized free gas bubbles," Ultrasound Med. Biol. **25**, 1407–1415 (1999).

[10]A. Brayman, M. Azadniv, M. W. Miller, and R. S. Meltzer, "Effect of static pressure on acoustic transmittance of Albunex® microbubble suspensions," J. Acoust. Soc. Am. **99**, 2403–2408 (1996).

[11]F. Forsberg, J. B. Liu, W. T. Shi, J. Furuse, M. Shimizu, and B. B. Goldberg, "In vivo pressure estimation using subharmonic contrast microbubble signals: Proof of concept," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **52**, 581–583 (2005).

[12]L. M. Leodore, F. Forsberg, and W. T. Shi, "In vitro pressure estimation obtained from subharmonic contrast microbubble signals," *2007 IEEE International Ultrasonics Symposium Proceedings*, P5B-6 (2007).

[13]K. S. Andersen and J. A. Jensen, "Impact of acoustic pressure on ambient pressure estimation using ultrasound contrast agent," Ultrasonics **50**, 294–299 (2010).

[14]W. T. Shi, F. Forsberg, J. S. Raichlen, L. Needleman, and B. B. Goldberg, "Pressure dependence of subharmonic signals from contrast microbubbles," Ultrasound Med. Biol. **25**, 275–283 (1999).

[15]P. J. A. Frinking, E. Gaud, J. Brochot, and M. Arditi, "Subharmonic scattering of phospholipid-shell microbubbles at low acoustic pressure amplitudes," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **57**, 1762–1771 (2010).

[16]K. S. Andersen and J. A. Jensen, "Ambient pressure sensitivity of microbubbles investigated through a parameter study," J. Acoust. Soc. Am. **126**, 3350–3358 (2009).

[17]Y. J. Gong, D. Zhang, and X. F. Gong, "Subharmonic and ultraharmonic emissions based on the nonlinear oscillation of encapsulated microbubbles in ultrasound contrast agents," Chin. Sci. Bull. **50**, 1975–1978 (2005).

[18]C. C. Church, "The effects of an elastic solid interface layer on the radial pulsation of gas bubbles," J. Acoust. Soc. Am **97**, 1510–1521 (1995).

[19]X. Yang and C. C. Church, "A model for the dynamics of gas bubbles in soft tissue," J. Acoust. Soc. Am. **118**, 3595–3606 (2005).

[20]P. A. Dayton, J. S. Allen, and K. W. Ferrara, "The magnitude of radiation force on ultrasound contrast agents," J. Acoust. Soc. Am. **112**, 2183–2192 (2002).

[21]B. B. Khismatullin and A. Nadim, "Radial oscillations of encapsulated microbubbles in viscoelastic liquids," Phys. Fluids **14**, 3534–3557 (2002).

[22]P. Marmottant, S. van der Meer, M. Emmer, M. Versluis, N. de Jong, S. Hilgenfeldt, and D. Lohse, "A model for large amplitude oscillations of coated bubbles accounting for buckling and rupture," J. Acoust. Soc. Am. **118**, 3499–3505 (2005).

[23]H. Zheng, A. Barker, and R. Shandas, "Predicting backscatter characteristics from micron- and submicron-scale ultrasound contrast agents using a size-Integration technique" IEEE Trans. Ultrason. Ferroelectr. Freq. Control **53**, 639–644 (2006).

[24]J. M. Gorce, M. Arditi, and M. Schneider, "Influence of bubble size distribution on the echogenicity of ultrasound contrast agents—A Study of SonoVue™," Invest. Radiol. **35**, 661–671 (2000).

[25] M. Tang, J. Loughran, E. Stride, D. Zhang, and R. J. Eckersley, "Effect of bubble shell nonlinearity on ultrasound nonlinear propagation through microbubble populations," J. Acoust. Soc. Am. **129**, EL76–EL82 (2011).

[26]N. de Jong, M. Emmer, C. T. Chin, A. Bouskaz, F. Mastik, D. Lohse, and M. Versluis, "Compression-only behavior of phospholipid-coated contrast bubbles," Ultrasound Med. Biol. **37**, 653–656 (2007).