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Detection and characterization of higher order nonlinearities in the oscillations of Definity at higher frequencies and very low acoustic pressures

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***Abstract*—The oscillation dynamics of phospholipid shell microbubbles (MBs) (e.g. Definity) exhibiting subharmonic (SH) oscillations has been widely investigated for high frequency ultrasonic imaging of the vascular bed. However, many aspects of the behavior of these MBs have remained unexplored, partly due to an extra degree of complexity imposed by the nonlinear behavior of the shell, leading to "compression only" behavior. Full understanding of the dynamics of these MBs at higher frequencies will aid in the design of optimal ultrasonic exposure parameters and MB properties to improve the current SH imaging protocols. The dynamics of Definity MBs were investigated both numerically and experimentally. Polydisperse dilute solutions of Definity were sonicated separately at frequencies of 25 and 55 MHz using pulse trains of 30 cycles with a Vevo770 ultrasonic machine. For each sonication the driving pressure was varied between 100 kPa to 3.8 MPa. The backscatter signals from single MBs were isolated and analyzed. The Marmottant model for lipid shell MBs was numerically solved for a wide range of MB sizes and driving acoustic pressures at frequencies of 25 and 55 MHz. The results of the numerical simulations were visualized using bifurcations diagrams (MB expansion ratio versus MB size and acoustic pressure and buckling radius). Analysis of the signals extracted from the oscillations of single Definity MBs revealed, for the first time, that in addition to the conventional SH of order 1/2, SHs of the higher order of 1/3, 1/4 and 1/5 can be observed at very low acoustic pressures (~200 kPa) at both frequencies (25 and 55 MHz). These results contradict the predictions of the viscoelastic models (e.g. Hoff) which require very high acoustic pressures (~2.5 MPa) for the generation of higher order SHs at high frequencies (e.g. 55MHz). In order to investigate the origin of these oscillations, the bifurcation structure of the Marmottant model was generated. In very good agreement with experimental results, numerical results reveal the generation of higher order subharmonics in the dynamics of lipid shell MBs at pressure levels as low as 200kPa. Depending on the initial size of the MBs, simulations predict the generation of the oscillations including period 2 (P2), P3 and up to P5. Results of the simulations using the bifurcation diagrams showed that compression only behavior is the most probable reason for the occurrence of higher order SHs at very small pressure values. These results suggest that the value for the buckling radius is of great importance in the generation and order of higher order SHs. To our best knowledge, this is the first time that the numerical classification and experimental observation of the higher order SHs in the dynamics of MBs undergoing buckling and rupture is shown.**

***Keywords*—Lipid shell microbubbles, Buckling, Rupture, Subharmonic Imaging, Higher Order Subharmonics**

I. INTRODUCTION

The nonlinear dynamics of microbubbles (MBs) are not fully understood, especially for contrast enhanced ultrasound applications above 10 MHz [1]. The nonlinear shell properties of phospholipid MBs adds extra complexity to the intricate behaviour of the MBs. The Marmottant model [2] takes in to account the nonlinear shell properties by considering a radius dependent surface tension. According to the model, the shell remains elastic over a certain range of MB radii, above which the shell ruptures and below which the shell buckles. This model explains the generation of ½ order SHs at very low acoustic pressures [3] where the other models based on pure viscoelastic shell behaviour fail to predict these low pressure thresholds.

Despite the widespread recent interest on the use of high frequency contrast enhanced ultrasound, the occurrence of buckling and rupture at higher frequencies and their contributions to the nonlinear dynamics of the MBs at high frequencies is not well understood [1]. In order to optimize the imaging and therapeutic applications of MBs at higher frequencies, a detailed knowledge of the behaviour of the system is required.

In this study, the dynamics of the individual Definity MBs with different radii are experimentally studied at 25 and 55 MHz. According to experimental results, Definity MBs exhibit high order nonlinear behaviour (e.g. high order SHs) at very low acoustic pressures. This contradicts the predictions of the models considering the MB shell as a pure viscoelastic material (e.g. Hoff Model [4]). To investigate the cause of the enhanced nonlinear emissions at such low acoustic pressures and high frequencies, a numerical parametric study of the dynamics of the MBs undergoing buckling and rupture is performed and the results are visualized through bifurcation diagrams. It is seen that for appropriately sized MBs, the dynamic change in the surface tension from shell buckling and rupture can be used to explain the enhanced generation of higher order SHs at low acoustic pressures.

II. METHODS

A. Experimental procedure

Very dilute solutions of Definity MBs were sonicated with continuous pulse trains of 25 and 55 separately using Vevo 770 (VisualSonics Inc. Toronto, Ontario). The pulse length was held constant at 30 cycles while the applied acoustic pressure were varied over the range of 0.1-3.8 MPa. The backscattered signals from each UCA were extracted and different nonlinear modes of oscillations were identified.

B. Numerical simulation

The dynamics of the UCAs were simulated using the Marmattont model [2]:

$$\rho_L\left(\ddot{R}R+\frac{3}{2}\dot{R}^2\right)=[P_0+\frac{2\sigma(R_0)}{R_0}](\frac{R_0}{R})^{3\Gamma}(1-\frac{3\Gamma}{c}\dot{R})$$
$$-P_0-\frac{2\sigma(R)}{R}-4\mu_L\frac{\dot{R}}{R}-\frac{4\kappa_s}{R^2}\dot{R}-p_A(t) \qquad (1)$$

where R_0 is the initial radius, ρ_L is the density of the liquid, P_0 is the equilibrium pressure inside the bubble, Γ is the polytropic exponent, μ_L is the viscosity of the surrounding liquid, μ_L is the liquid viscosity, κ_s is the surface dilational viscosity of the shell, and $\sigma(R)$ is the initial surface tension which is given by equation 2:

$$\sigma(R)=\begin{cases}0 \quad if\ R\le R_b\ (buckled)\\ \chi(\frac{R}{R_0}-1)^2\ \text{if}\ R_b<R<R_r\ \ \text{(elastic)}\\ \sigma_{water}\ if\ R\ge R_r(ruptured) \qquad (2)\end{cases}$$

where χ is the shell elasticity, $R_b=\frac{R_0}{\sqrt{1+\frac{\sigma(R_0)}{\chi}}}$ and $R_r=R_b\sqrt{1+\frac{\sigma_{break-up}}{\chi}}$.

In eq. 1, $p_A(t)$ is the driving acoustic pressure and is given by equation 3:

$$p_A(t)=P_A\sin(2\pi ft)(3)$$

In order to replicate and understand the experimental results that we expect by sonicating a polydisperse dilute solution of MBs, the results of the numerical simulations were visualized using a very efficient method: bifurcation analysis. In this method the bifurcation structure of the normalized UCA oscillations (R/R0) are plotted versus a control parameter [4]. This gives insight into the nonlinear behaviour over a wide range of parameters, thus reducing the complexity of the analysis and interpretation of the results. In this paper the bifurcation diagram of the normalized radial oscillations of the Definity MBs were plotted versus the MB initial diameter for fixed frequencies of 25 MHz and 55 MHz at a fixed pressure value of 200 kPa. The bifurcation diagrams were generated for different values of σ_0. It has been shown that the surface tension σ_0 is very critical to the oscillation dynamics of a lipid shell MB. MBs with σ_0 close to 0 tend to exhibit compression only behavior while for σ_0 close toσ_{water}, MBs are close to the ruptured state and tend to exhibit mainly expansion dominated behavior [1].

III. RESULTS

A. Experimental observations

The backscattered signals from individual MBs were isolated and extracted. Figure 1 shows the sample isolated backscattered signals at ~200 kPa of driving pressure. As seen various MB sizes were able to exhibit different types of nonlinear behavior. Fig. 1a shows a case of period 2 (P2) oscillations. As seen the signal has two clear maxima and the

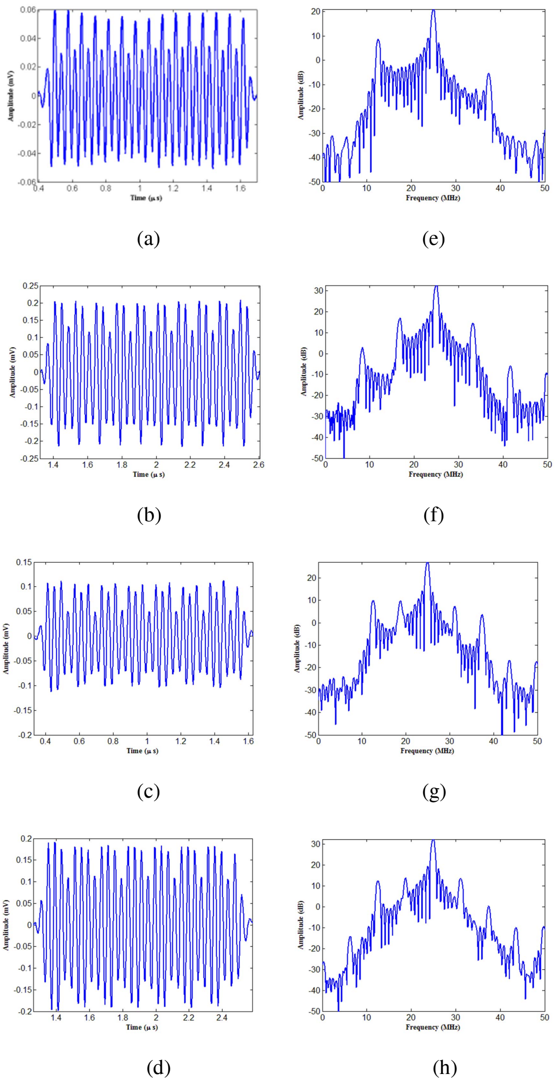

Figure 1: (right column) Experimentally detected signals of individual Definity MBs at 25 MHz and apressure of ~200 kPa. Oscillatory patterns of the MBs consistent with a) P2, 2b)P3, 2c)P4-2, and 2d)P4-1osicalltions.The corresponding frequency spectrum of the backscatter signals (left column):1e) P2, 1f)P3, 1g)P4-2 and 1h)P4-1.

corresponding frequency spectrum (fig. 1e) has a ½ order SH at 25 MHz. Fig. 1b shows the case of P3 oscillations. The signal has three distinct maxima while its corresponding frequency spectrum (fig. 1f) has two SHs at 8.33 MHz and 16.66MHz. Fig. 1c shows the P4-2 signal, with 4 distinct pressure maxima. The corresponding frequency spectrum (fig.

1f) has 3 SHs at 6.25, 12.5 and 18.75 MHz. Note the strength of the SHs at different frequencies (12.5MHz>18.75>>6.25). Fig. 1d shows the case of P4-1 oscillations. The signal has 4 distinct pressure maxima but with a different pattern compared to the P4-2 oscillations of fig. 1c. The corresponding frequency spectrum at fig. 1h has three distinct SHs at 6.25, 12.5 and 18.75 MHz. Note the strength of the SHs at different frequencies are different from P4-2 oscillations (18.75>12.5>6.25 MHz).

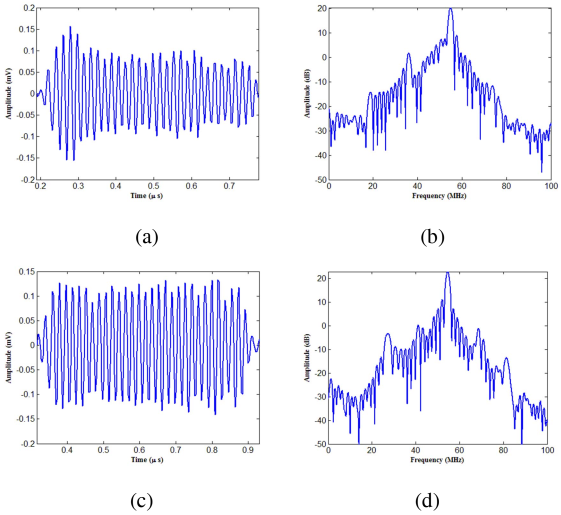

Figure 2: (right column) experimentally detected signals of individual Definity MBs at 55 MHz and pressure ~ YYY KPa. Oscillatory patterns of the MBs consistent witha)P3 and b)P4 oscillations, and their corresponding frequency spectrum (left column, c and d).

B. *Numerical simulations*

In Figure 3 the bifurcation structure of R/R0 versus the initial diameter of the MB for different values of σ_0 when driven by continuous pulses of 25 MHz frequency and 200 kPa pressure are shown. For MBs with σ_0=0 N/m (fig. 3a), the oscillations are dominated by compression only behavior (R/R0 <1) and one can witness P2, P3, P4 and P5 oscillations. MBs with σ_0=0.045 N/m are initially at an elastic state. As seen in fig. 3b, these MBs undergo resonant oscillations of high amplitude for MB sizes around 0.6 µm. A large size range of bigger MBs (X to Y µm) exhibit P2 oscillations. MBs with sizes of ~2.5 µm exhibit P3 oscillations.

Figure 2 illustrates the extracted signals at 55 MHz and ~950 kPa. Fig. 2a shows a case of P3 oscillations. After a short transient behaviour, the MB exhibit 3 pressure maxima in its dynamics. The corresponding frequency spectrum in fig. 2c shows 3 SHs at 18.33 MHz and 36.66 MHz. Fig. 2b illustrates the case of P4-1 oscillations. The corresponding frequency in fig. 2d has 3 SHs at 13.75, 27.5 and 41.25 MHz. It should be noted that the SH at 13.75 MHz is very weak and it is out of the effective bandwidth of the transducer. This may explain the absence of a pronounced peak at this frequency. However, the frequency spectrum displays pronounced ultraharmonics at 68.75 and 82.5 MHz.

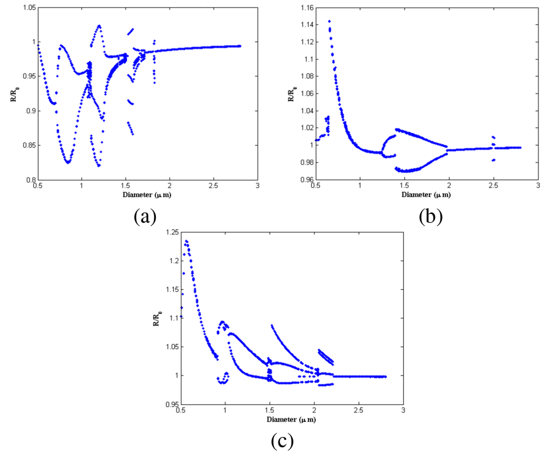

Figure 3: Bifurcation structure of the R/R0 of the MB versus the initial diameter of the MB excited by 25 MHz of frequency and 200 kPa of pressure. The MB shell properties are $\chi =$ 1.35 N/m, κ_s=1.99^{-10}kg/s and $\sigma_{\text{break-up}} = 0.072$ where: 3a)and$\sigma_0 = 0\ N/m$, 3b)$\sigma_0 = 0.045\ N/m$, 3c)$\sigma_0 = 0.068\ N/m$

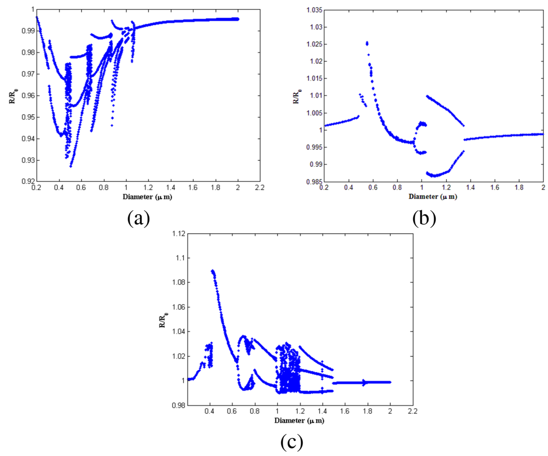

Figure 4: Bifurcation structure of the R/R0 oscillations of the MB versus the initial diameter of the MB excited by a 55 MHz pressure wave with200 kPa amplitude. The MB shell properties are $\chi =$ 2.35 N/m, κ_s=1.99^{-10}kg/sand $\sigma_{\text{break-up}} = 0.072$ .The bifurcation structures were generatedfora) $\sigma_0 = 0\ N/m$, b)$\sigma_0 = 0.045\ N/m$, c)$\sigma_0 = 0.06\ N/m$.

Dynamics of the MBs close to ruptured state with σ_0=0.068 N/m (fig. 3c) have a very different bifurcation structure. It consists of large amplitude linear oscillations for smaller MBs, while larger MBs exhibit P2, P4-2, P3 and P4-1oscillations.

Figure 4 shows the bifurcation structure of R/R0 of the MB versus its initial diameter for the same parameter as in figure 3 but for a 55MHz exposure. It can been seen in Figure 4athat MBs with $\sigma_0 = 0\frac{N}{m}$, exhibit compression only behavior (R/R0 <1) and nonlinear dynamics with P2, P3, P4 and P5 oscillations. MBs initially in elastic state with $\sigma_0 = 0.045\ N/m$ (fig. 4b) exhibit a different dynamical behavior. This includes resonant oscillations for MBs with ~0.55µm diameter and P2 oscillations for larger MBs. MBs initially close to ruptured state ($\sigma_0 = 0.06\frac{N}{m}$ fig. 4c), exhibit expansion dominated behaviour and depending on their initial size they undergo linear resonant, P2, P4-2,P3 and P4 oscillations.

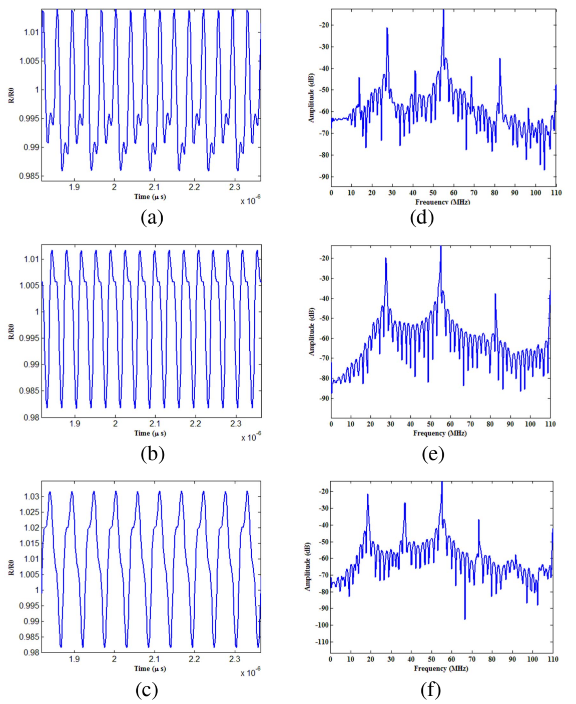

Figure 5: R/R0 oscillations of a 0.61μm MB versus time (right column) excited by a 55 MHz pulse with an amplitude of200 kPa. The MB shell properties are $\chi =$ 2.35 N/m, κ_s=1.99^{-10}kg/sand $\sigma_{\text{break-up}} = 0.072$. The bifurcation structures were generated for :a) $\sigma_0 = 0.02\,N/m$, b $\sigma_0 = 0.045\,N/m$, c$\sigma_0 = 0.06\,N/m$, (left column) the corresponding frequency spectrum of the backscatter pressure of: 5d) 5a, 5e) 5b and 5f) 5c.

Figure 5 shows the radial oscillations of the 0.61μm MB (right column) and the corresponding frequency spectrum of the backscattered pressure, for different initial shell conditions $\sigma_0$of 0.01, 0.045 and 0.068 N/m. As seen in fig. 5a, the MB initially in a buckling state exhibits compression only behavior and P4-2 oscillations which are clearly seen in the corresponding frequency spectrum (fig. 5d). The MB at elastic state $\sigma_0 = 0.045\,\frac{N}{m}$ (fig. 5b) exhibits symmetrical P2 oscillations. The corresponding frequency spectrum (fig. 5e) shows a clear SH at XYZ MHz. The MB initially close to ruptured state $\sigma_0 = 0.068\,\frac{N}{m}$exhibits expansion dominated P3 oscillations (Figure 5c). The corresponding frequency spectrum has 2 SHs at 8.33 and 16.66 MHz (Figure 5f) .

IV. Discussion

We have detected the higher order SHs in the oscillation of Definity MBs at high frequencies of 25 and 55 MHz and with pressures as low as 200 kPa. To our best knowledge this is the first experimental report of the generation of higher order nonlinearities at such low pressures. These results contradict the predictions of the free bubble and viscoelastic shell MB models (e.g Hoff [5]) which relates the higher order nonlinearities to very high acoustic pressures [4]. To investigate the origin of this phenomenon, a large parametric study was performed by numerically solving the Marmottant Model [2]. Our results are in agreement with the results of previous studies that suggest buckling of the shell and compression only behaviour can be used to explain the generation of ½ order SHs at low acoustic pressures. In addition, numerical results showed that buckling may also enhance the generation of higher order SHs.

We have also shown that in addition to compression only behavior, MBs undergoing symmetric and expansion dominated oscillations can also exhibit higher order nonlinearities at low pressures. Comparison between numerical simulations and experimental results suggest that our experimental observation of higher order SHs at low pressures may be due to MBs initially at elastic or ruptured condition which results in symmetric and expansion dominated oscillations. Helfield et al. [1] has recently shown that ½ SHs can be generated at 25 MHz and low acoustic pressures due to expansion dominated oscillations. Detailed numerical analysis showed that the dynamic change in $\sigma(R)$ during MB oscillations may explain our experimental observations. Furthermore, through analysing the bifurcation structure of the MBs, it was also shown that the value for the buckling radius is of great importance in the prediction of the generation and of the order of higher order SHs.

V. Conclusion

It was shown both experimentally and numerically that lipids coated MBs can exhibit higher order SHs at high frequencies and very low acoustic pressure thresholds. Comparison between numerical simulations and experimental observations suggest that dynamic changes of the effective surface tension incorporated in Marmottant model is the main reason in enhancing this phenomenon at low pressures.

VI. Acknowledgement

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