COLLAPSE DYNAMICS OF ULTRASOUND
CONTRAST AGENT MICROBUBBLES

BY

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DISSENTATION

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ABSTRACT

Ultrasound contrast agents (UCAs) are micron-sized gas bubbles encapsulated with thin shells on the order of nanometers thick. The damping effects of these viscoelastic coatings are widely known to significantly alter the bubble dynamics for linear and low-amplitude behavior; however, their effects on strongly nonlinear and destruction responses are much less studied.

This dissertation examines the behaviors of single collapsing shelled microbubbles using experimental and theoretical methods. The study of their dynamics is particularly relevant for emerging experimental uses of UCAs which seek to leverage localized mechanical forces to create or avoid specialized biomedical effects. The central component in this work is the study of postexcitation rebound and collapse, observed acoustically to identify shell rupture and transient inertial cavitation of single UCA microbubbles. This time-domain analysis of the acoustic response provides a unique method for characterization of UCA destruction dynamics.

The research contains a systematic documentation of single bubble postexcitation collapse through experimental measurement with the double passive cavitation detection (PCD) system at frequencies ranging from 0.9 to 7.1 MHz and peak rarefactional pressure amplitudes (PRPA) ranging from 230 kPa to 6.37 MPa. The double PCD setup is shown to improve the quality of collected data over previous setups by allowing symmetric responses from a localized confocal region to be identified. Postexcitation signal percentages are shown to generally follow trends consistent with other similar cavitation metrics such as inertial cavitation, with greater destruction observed at both increased PRPA and lower frequency over the tested ranges. Two different types of commercially available UCAs are characterized and found to have very different collapse
thresholds; lipid-shelled Definity exhibits greater postexcitation at lower PRPAs than albumin-shelled Optison. Furthermore, by altering the size distributions of these UCAs, it is shown that the shell material has a large influence on the occurrence of postexcitation rebound at all tested frequencies while moderate alteration of the size distribution may only play a significant role within certain frequency ranges.

Finally, the conditions which generate the experimental postexcitation signal are examined theoretically using several forms of single bubble models. Evidence is provided for the usefulness of modeling this large amplitude UCA behavior with a size-varying surface tension as described in the Marmottant model; better agreement for lipid-shelled Definity UCAs is obtained by considering the dynamic response with a rupturing shell rather than either a non-rupturing or nonexistent shell. Moreover, the modeling indicates that maximum radial expansion from the initial UCA size is a suitable metric to predict postexcitation collapse, and that both shell rupture and inertial cavitation are necessary conditions to generate this behavior.

Postexcitation analysis is found to be a beneficial characterization metric for studying the destruction behaviors of single UCAs when measured with the double PCD setup. This work provides quantitative documentation of UCA collapse, exploration into UCA material properties which affect this collapse, and comparison of existing single bubble models with experimentally measured postexcitation signals.
Dedicated to my family.
ACKNOWLEDGEMENTS

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>A/D</td>
<td>Analog-to-digital</td>
</tr>
<tr>
<td>ACD</td>
<td>Active cavitation detection</td>
</tr>
<tr>
<td>CEUS</td>
<td>Contrast enhanced ultrasound</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>IC</td>
<td>Inertial cavitation</td>
</tr>
<tr>
<td>KZK</td>
<td>Khokhlov-Zabolotskaya-Kuznetsov (equation)</td>
</tr>
<tr>
<td>MI</td>
<td>Mechanical index</td>
</tr>
<tr>
<td>PCD</td>
<td>Passive cavitation detection</td>
</tr>
<tr>
<td>PES</td>
<td>Postexcitation signal</td>
</tr>
<tr>
<td>PI</td>
<td>Pulse inversion</td>
</tr>
<tr>
<td>PM</td>
<td>Power modulation</td>
</tr>
<tr>
<td>PRPA</td>
<td>Peak rarefactional pressure amplitude</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>RPNNP</td>
<td>Rayleigh, Plesset, Noltingk, Neppiras, Poritsky (equation)</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>UCA</td>
<td>Ultrasound contrast agent</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
</tbody>
</table>
LIST OF SYMBOLS

\( \alpha, \beta \) van der Waals’ constants
\( \alpha(f) \) Threshold value for indicator function
\( \beta \) Damping constant
\( c \) Speed of sound
\( f \) Frequency
\( g \) Gravitational acceleration
\( h \) Enthalpy
\( k \) Spring constant
\( m \) Mass constant
\( n \) Number of moles
\( P \) Pressure
\( P_a(t) \) Acoustic pressure
\( P(x), P(z) \) Outcomes of logistic regression fitting
\( p_\infty \) Pressure far from bubble
\( p_g \) Gas pressure
\( p_i \) Internal bubble pressure
\( p_l \) Pressure at fluid boundary
\( p_0 \) Initial bubble pressure
\( p_v \) Vapor pressure
\( p_\sigma \) Laplace pressure
\( Q \) Maximum observed PES percentage
\( R^* \) Universal gas constant
\( R(t) \) Bubble radius
\( R_c \) van der Waals hard-core radius
\( R_0 \) Initial bubble radius
\( R_{\text{breakup}} \) Bubble breakup radius
\( R_{\text{buckling}} \) Bubble buckling radius
\( R_{\text{max}} \) Maximum radial expansion of bubble
\( R_{\text{rupture}} \) Bubble rupture radius
\( r \) Distance from center of bubble
\( S_f \) Shell friction
\( S_p \) Shell elasticity
\( T \) Acoustic period
\( T_k \) Absolute temperature
\( u \) Velocity
\( V \) Volume
\( V_{\text{Stokes}} \) Stokes terminal velocity
\( z \) Logarithmic transform of PRPA
\( \alpha_{0.1} \quad \text{Logistic regression fitting coefficients} \\
\delta_{\text{radiation}} \quad \text{Acoustic damping} \\
\delta_{\text{shell}} \quad \text{Shell damping} \\
\delta_{\text{total}} \quad \text{Total damping} \\
\delta_{\text{viscous}} \quad \text{Viscous damping} \\
\varepsilon \quad \text{Small perturbation} \\
\kappa \quad \text{Polytropic index of gas} \\
\kappa_s \quad \text{Monolayer surface dilatational viscosity} \\
\lambda \quad \text{Arbitrary bubble model parameter} \\
\mu \quad \text{Dynamic viscosity} \\
\xi \quad \text{Two times experimental noise threshold} \\
\rho_0 \quad \text{Constant liquid density} \\
\rho_f \quad \text{Constant fluid density} \\
\rho_p \quad \text{Constant particle density} \\
\sigma \quad \text{Surface tension} \\
\sigma(R) \quad \text{Size-varying surface tension} \\
\sigma_{\text{water}} \quad \text{Surface tension of water} \\
\phi \quad \text{Velocity potential} \\
\chi \quad \text{Shell elastic compression modulus} \\
\omega \quad \text{Angular frequency} \\
\omega_d \quad \text{Damped resonant frequency} \\
\omega_0 \quad \text{Undamped natural resonant frequency}
CHAPTER 1

INTRODUCTION

1.1 Motivation

The development of ultrasound contrast agents (UCAs) over the past 30 years has been revolutionary within the context of medical ultrasound. UCAs are micron-sized, encapsulated gas bubbles with a thin shell coating typically consisting of albumin, lipids, or other surfactants providing stability from rapid dissolution. Currently, the primary clinical applications for these microbubbles are for improved imaging of the heart and liver. In looking toward the future, it has been recognized that the dynamic behavior of UCAs in response to an ultrasound pressure wave opens up numerous new possibilities for emerging diagnostic and therapeutic uses of these microbubbles, including targeted drug delivery and gene therapy (sonoporation) [1], blood-brain barrier disruption [2], enhanced angiogenic response [3], and enhanced thrombolysis [4].
However, there are still significant barriers to widespread acceptance of these groundbreaking procedures. Fundamentally, many of the challenges can be attributed to the fact that the UCA response itself is insufficiently understood and incompletely characterized during large amplitude, destruction dynamics. Even the seemingly simple experimental measurement of the collapse threshold of UCAs is a tricky proposition, due to the many influencing factors and the wide variation of behavior that can occur. Numerous developing clinical uses of UCAs utilize mechanical forces generated by ultrasound-microbubble interactions; in many cases, the underlying mechanisms for these applications are poorly understood due in part to the bubble response being unclear. Therefore, it is important to study the basic behavior exhibited by shelled microbubbles under similar acoustic settings to those that are being explored therapeutically; otherwise this gap in knowledge will limit the efficacy and slow the adoption of these techniques.

A greater comprehension of the fundamental dynamics of UCAs is of significant importance for understanding and improving a wide range of ultrasound techniques. This provides a strong motivation for this specific research, which is to investigate a certain form of UCA rebound and collapse behavior; the anticipation is that the measurements will not only be useful as characterization information, but that they will also prove to be beneficial for interpreting and designing other studies within the broad context of ultrasound contrast agent mediated biomechanical effects (bioeffects).

### 1.2 General Aims of Dissertation Research

The dynamic behavior of collapsing shelled microbubbles was the central physical issue under investigation. More precisely, the scope of this dissertation
research was focused on the acoustic response due to ultrasound interaction near resonant frequencies and under large pressures when destruction occurs frequently. To date, most experimental and theoretical studies of UCAs have concentrated on small or moderate amplitude oscillatory regimes while relatively few have explored large amplitude behaviors such as shell rupture, inertial cavitation, and bubble destruction. In contrast, the multifaceted approach of comparing experiments and theories in this research explored less understood issues of rupture thresholds, rebound thresholds, and the influence of shell properties on UCA fragmentation.

Three general aims were identified for the current research. The first aim was to acoustically study the collapse responses of single UCAs, with the goal of identifying and quantifying useful experimental metrics for UCA characterization. The second aim was to study manipulation of the destruction thresholds, which were dependent not only on acoustic parameters, but also on material makeup of the microbubbles. Finally, the third aim was to provide a link between experiment and theory by comparing the collapse response with modeling, in order to understand the UCA collapse process and to be able to predict future results. These three broad goals all served to augment the existing knowledge of UCA dynamics by closely examining postexcitation rebound and collapse, a distinctive response of collapsing UCAs.

1.3 Specific Objectives of Dissertation Research

Four specific objectives were developed to accomplish the more broadly identified goals of characterizing, altering, and modeling UCA collapse behavior. The first objective was to measure thresholds of destruction of single ultrasound contrast agents using an acoustic detection setup. The experimental technique
developed was double passive cavitation detection (PCD), a refinement of previous PCD techniques which involved careful characterization and confocal alignment of two receive transducers. This system was used to study two of the primary acoustic parameters affecting UCA collapse, frequency and peak rarefational pressure amplitude (PRPA), while determining collapse thresholds for different populations of microbubbles.

The second objective was to develop an automated methodology for signal analysis that was user-independent. While previous work had identified the utility of the postexcitation signal analysis, this analysis was primarily qualitative and subject to variance even among well-trained observers. Therefore, a quantitative definition of the postexcitation signal was desired for the acoustic time domain characterization of large amplitude, single UCA behavior. Additionally, the development of this analysis method allowed for rapid analysis of large amounts of data, improving the ability to test for statistical significance.

The third specific objective was to determine the importance of UCA material properties for varying postexcitation thresholds. Basic bubble equations identified two properties which were likely to have a central impact on the UCA response: shell composition and size distribution. Multiple groups of UCAs were therefore studied varying shell properties and size distribution to determine their importance, if any, on the collapse behavior of UCAs. The effect of various thin shell coatings was of particular interest, to see if they had a significant impact on large amplitude destruction behavior as they do with small amplitude oscillation.

The fourth objective was to use theoretical modeling to develop an explanation for the reason of the observed postexcitation destruction. The experimental data was selected for unconstrained, symmetric UCA responses,
meaning that widely developed spherically symmetric bubble models were prime candidates for comparison. Adaptation of the standard Rayleigh-Plesset type models to incorporate viscoelastic effects of a thin shell had been shown to yield good agreement with small amplitude UCA oscillations, but this work would be among the first reported instances attempting to experimentally corroborate these types of models in the regime of large amplitude behavior around the upper limits of their validity. A principal goal was to use this modeling to explain the basis of postexcitation collapse in terms of non-dimensional threshold parameters, without frequency dependence.

1.4 Contributions from Research

Among the questions analyzed in the course of these studies were: What physical conditions lead to rebound and postexcitation collapse? Under what conditions is the postexcitation signal measureable experimentally? How does the shell influence the fragmentation of the destroyed UCA? The answers to these questions points to the most direct impacts from this research, which are briefly summarized here.

The major contributions from the series of studies presented in this dissertation to the body of ultrasound contrast agent literature are threefold. First is the refinement of a uniquely defined method for experimentally characterizing single bubble UCA collapse with the postexcitation signal. A central claim for this work is that the measurable postexcitation signal (PES), a broadband emission separated in time from the harmonic principal response that is observed in these experiments, is indicative of a specific category of collapsing UCAs, and is useful in characterizing both the properties and behavior of the UCAs. The work presented herein solidly links PES with bubble destruction, and
quantifies the level of destruction for a range of frequencies, pressures, and UCA populations. Since the determination of accurate collapse thresholds for UCAs holds the potential not only for increased understanding related to biosafety concerns, but also for improved theoretical modeling and elucidation of physical mechanisms for bioeffects resulting from functional usage of UCAs, these are important outcomes.

Second, the double PCD method was used to study responses from several UCA populations with varying shell composition and size distribution. These studies increased understanding as to what causes different characteristic curves. Such experiments are distinctive for using the same metric to compare the collapse of different types of contrast agents, and this research demonstrates that while altering the size distribution may play a minor role in collapse thresholds, particularly for certain frequencies, shell composition clearly has a significant impact across a much broader range. Such results may have particular importance for optimizing UCAs to obtain a desired response.

Third, this work also presents results comparing experimentally measured UCA collapse with predictions from existing shelled bubble models, which previously had been validated with small amplitude responses. Unshelled or free bubbles have been more widely studied than shelled bubbles; however, it is well known that the responses of UCAs are altered from unshelled bubbles by their coated interface when they are insonified by megahertz ultrasound pulses. This work augments the previous understanding by demonstrating the shell also affects large amplitude destruction behavior, not just small amplitude oscillations. When modeling collapsing contrast agents, it is necessary to take the shell properties into account. While the outcome of this comparison was not direct confirmation that such spherical models exactly describe the destruction
behavior, it was demonstrated they can be used for prediction of the thresholds for postexcitation collapse, particularly for lipid-shelled UCAs.

1.5 Outline of Dissertation

In Chapter 2, relevant background information is presented on experimental studies and theoretical descriptions of ultrasound, bubbles, and UCAs. Previous experimental studies on UCA response are reviewed, with particular emphasis on those studies which considered collapsing or destroyed UCAs. Additionally, basic theoretical bubble dynamics are described, along with various cavitation definitions previously identified in the literature.

In Chapter 3, the implementation of the double passive cavitation detection experiment is thoroughly described. The processes for data acquisition and analysis are introduced, including description of various observed signals. The method of statistical analysis used to define postexcitation thresholds and determine statistical significance, modified logistic regression curve fitting, is also presented in this chapter. Additionally, this section includes the description of the automatic classification procedure and its comparison with manual classification. Finally, initial results are presented to contrast the responses from two different types of commercially available UCAs, Definity and Optison.

Chapter 4 further explores the material changes that lead to different collapse thresholds by considering altered size distributions of the UCAs studied previously. The size measurement technique and two methods for altering size distributions are presented. Experimentally, two very similar size distributions of Definity and Optison are compared to consider the composition of the shell on the measured postexcitation collapse, removing the dependence of size.
In Chapter 5, the theoretical framework based on the Marmottant spherical shelled bubble model for analyzing the experimental results is introduced. Bubble models incorporating size-varying shell properties are compared with two simpler, alternate cases in which there is either no shell considered or the shell has constant properties. Additionally, the simulations are used to test the initial hypothesis that experimental measurements of postexcitation are for those bubbles undergoing shell rupture and inertial cavitation. The simulations are further used to explore a number of different material alterations to UCAs to predict their effect on postexcitation collapse.

An alteration of the experimental setup to use receive transducers of a different frequency is considered in Chapter 6. Definity UCAs are analyzed using lower frequency receive transducers to test if and how the experimental setup affects the data collection and observance of the postexcitation signal. Moreover, the results from this chapter are used to re-examine potential frequency independent metrics to describe postexcitation collapse.

Finally, in Chapter 7 the outcomes of the research are again summarized, along with limitations from the studies and suggestions for future work.
CHAPTER 2

BACKGROUND

2.1 History and Uses of Ultrasound

Since the first recognition of its potential in the early twentieth century, ultrasound has been studied and developed for both nonmedical and medical applications. The dynamic response of bubbles has also been widely researched over a similar time frame, particularly from a theoretical perspective, and particularly for single, isolated bubbles. The existence of ultrasound contrast agents as a medical device is newer than the study of either ultrasound or bubbles, but even they have been increasingly researched over the past twenty years, especially for certain regimes of behavior. Therefore, the purpose of this chapter is to introduce relevant background information for the dissertation on these three topics as found in the literature.
2.1.1 Ultrasound

Ultrasound (US) refers to pressure waves with a frequency greater than 20 kHz, the upper limit of the audible range for most humans. The existence of sound outside the audible frequency range has been recognized since at least the late 18th century, when Spallanzani and Jurine discovered bats could navigate while blinded, but not while their ear canals were plugged [5]. By 1914, ultrasound echolocation detection of icebergs had been suggested by Richardson and successfully demonstrated by Fessenden, a feat that built upon both the modern theory of acoustics described by Lord Rayleigh in 1877 and the discovery of the piezoelectric effect by the Currie brothers in the mid-1880s [6]. It was also recognized very early on that ultrasound could affect biology, as Langevin observed in 1917 that fish were killed when placed close to a powerful 150 kHz quartz transducer and that observers felt pain when putting their hands in the beam [7].

Alongside the nonmedical beginnings of echolocation, ultrasonic techniques related to medical applications arose. Medical ultrasound has been in development since the early twentieth century for a wide variety of purposes, from physiotherapeutic tissue heating beginning in the 1930s to diagnostic imaging beginning in the 1940s [8]. In practice, clinically relevant ultrasound today is typically is around 1 MHz for therapeutic uses and from approximately 1 to 15 MHz range for diagnostic imaging [9]. For therapeutic applications, ultrasound uses higher intensities to take advantage of some combination of thermal generation, mechanical stimulation, or cavitation to produce biological responses, or bioeffects. As an imaging modality, US techniques process the reflected waves from density and compressibility variations into an image and are valuable for being high speed, safe, portable, and cost-effective [10].
2.1.2 Ultrasound Contrast Agents

Ultrasound contrast agents (UCAs) are coated microbubbles typically ranging in diameter from 1-10 µm. They consist of a gaseous core surrounded by a thin, biocompatible shell which acts as a stabilizing interfacial boundary from the fluid outside. The first documentation of using air microbubbles for increased contrast in ultrasonic imaging of the vasculature was over 40 years ago [11], but the lack of persistence of bubbles at the necessarily small size limited the effectiveness of this approach. By the early 1980s, it was shown that thin coatings of materials such as gelatin could greatly increase the lifespan of small gaseous bubbles [12] and UCAs were approved for clinical use, first in Germany in the early 1980s and later in the United States and other regions of the world in the 1990s.

The earliest UCAs were filled with air and typically coated by galactose or albumin-based shells. More recently, it was recognized that using less soluble and less diffusive gases increased the stability of the contrast agents. Later generations of microbubbles have been introduced with phospholipid-based shells which are thinner and exhibit less stiffness than earlier shell materials. The two FDA-approved contrast agents studied in this dissertation are Optison™ (GE Healthcare Inc., Little Chalfont, UK) and Definity® (Lantheus Medical Imaging, North Billerica, MA), but a wide variety of contrast agents are currently available and others are under development. A selected list of UCAs most commonly cited in the literature is presented in Table 2.1.
<table>
<thead>
<tr>
<th>Name</th>
<th>Gas</th>
<th>Coating</th>
<th>Availability</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echovist</strong></td>
<td>Air</td>
<td>Galactose</td>
<td>EU, Canada, Japan, 1982</td>
<td>Bayer Schering Pharma AG (Berlin, Germany)</td>
</tr>
<tr>
<td><strong>Levovist</strong></td>
<td>Air</td>
<td>Galactose/Palmitin</td>
<td>EU, Canada, Japan, 1985</td>
<td>Bayer Schering Pharma AG (Berlin, Germany)</td>
</tr>
<tr>
<td><strong>Albunex</strong></td>
<td>Air</td>
<td>Human Albumin</td>
<td>EU, USA, Canada, 1994</td>
<td>Molecular Biosystems (San Diego, CA)</td>
</tr>
<tr>
<td><strong>Optison</strong></td>
<td>C₃F₈</td>
<td>Human Albumin</td>
<td>EU, USA, 1997</td>
<td>GE Healthcare (Little Chalfont, UK)</td>
</tr>
<tr>
<td><strong>Definity/Luminity</strong></td>
<td>C₃F₈</td>
<td>Phospholipid</td>
<td>EU, USA, Canada, 2001</td>
<td>Lantheus Medical Imaging (North Billerica, MA)</td>
</tr>
<tr>
<td><strong>SonoVue</strong></td>
<td>SF₆</td>
<td>Phospholipid</td>
<td>EU, China, South America, 2001</td>
<td>Bracco Imaging (Milano, Italy)</td>
</tr>
<tr>
<td><strong>Imagent</strong></td>
<td>C₆F₁₄</td>
<td>Phospholipid</td>
<td>USA, 2002</td>
<td>Alliance Pharm. Group (San Diego, CA)</td>
</tr>
<tr>
<td><strong>Sonozoid</strong></td>
<td>C₄F₁₀</td>
<td>Lipid</td>
<td>Japan, 2006</td>
<td>Daiichi Pharmaceutical (Tokyo, Japan)</td>
</tr>
<tr>
<td><strong>BiSphere</strong></td>
<td>N₂</td>
<td>Polyactide/Albumin</td>
<td>Development</td>
<td>POINT Biomedical (San Carlos, CA)</td>
</tr>
<tr>
<td><strong>BR14</strong></td>
<td>C₄F₁₀</td>
<td>Phospholipid</td>
<td>Development</td>
<td>Bracco Imaging (Milano, Italy)</td>
</tr>
</tbody>
</table>

Table 2.1. Selected list of UCAs.

2.1.3 Contrast Agents in Imaging

Ultrasound contrast agents improve ultrasound imaging of the vasculature because of their large scattering in comparison to tissue and liquid; this elevated scattering is due to the high compressibility of the gas relative to its surroundings. The current clinical usage of UCAs in the United States is
primarily their enhancement of imaging in diagnostic ultrasound, specifically for contrast echocardiography [13, 14]. Contrast enhanced ultrasound (CEUS) is widely used in Europe and Asia for radiological imaging of various organs including the liver and kidneys, but no UCAs are currently approved for these uses in the United States [15].

Many imaging techniques have been developed to take advantage of specific properties of UCA microbubbles. Because UCAs can display strongly nonlinear oscillation and the harmonic character of their scattered signal can be greater than tissue, harmonic imaging techniques such as Pulse Inversion (PI) or Power Modulation (PM) have been developed to enhance the UCA signal [16]. Doppler imaging techniques of blood flow are also improved by the enhanced scattered signal from UCAs, such as harmonic power Doppler for detection of small vessels in organs [17]. Additional methods for imaging perfusion, such as release burst imaging, whereby the UCAs are allowed to infuse into a region of interest before being eliminated with a strong destruction pulse, are reliant upon the possibility of transient UCA responses [18]. The shell surrounding of UCAs may also be functionalized for targeting biochemical receptors; this allows targeted imaging techniques to be developed [19].

2.1.4 Contrast Agents in Therapy

While the principal clinical usage of ultrasound contrast agents today remains their enhancement of imaging in diagnostic ultrasound, much of the focus of recent research has shifted to the potential use of UCAs in therapeutic ultrasound. Among other procedures, recent experimental studies have shown that use of UCAs in conjunction with ultrasound enhances thrombolysis [20, 4],
sonoporation across cellular membranes [21, 22, 1], and molecular transport across the blood brain barrier [2, 23, 24].

UCAs have been shown to be successful in increasing the effectiveness of such therapies, but the precise physical mechanisms leading to these bioeffects remain inadequately explained. In response to an ultrasonic pressure field, UCAs may undergo a wide range of dynamic responses ranging from linear oscillation to transient inertial collapse and fragmentation [25, 26]. The bubble response leads in turn to the generation of a variety of fluid behaviors including streaming, jetting, and shock waves. However, cavitation responses for different types of shelled microbubbles in reaction to large amplitude pulses are insufficiently documented. Greater understanding of microbubbles undergoing large amplitude oscillatory behavior, including the experimental determination of accurate collapse thresholds, is needed both to improve modeling of shelled bubble dynamics and also to elucidate the physical mechanisms for bioeffects resulting from functional usage of UCAs.

2.2 Bubble Dynamics

All micron sized bubbles, including ultrasound contrast agents, respond dynamically to the presence of a megahertz ultrasonic pressure wave by expanding and contracting in conjunction with the rarefational and compressional phases, respectively. As the size of the UCA microbubble is well below the wavelength of typical ultrasonic frequencies used, the time-varying pressure field is usually considered to be spatially uniform and the primary mode of response in the absence of nearby boundaries or other bubbles is the spherically symmetric ‘breathing’ mode.
The forced behavior of a bubble in general can be considered most straightforwardly as a damped nonlinear oscillator [27]. The Rayleigh-Plesset equation is the most widely used model specifically derived for bubble dynamics; this model considers a spherically symmetric unshelled gas bubble in an incompressible Newtonian liquid. The Rayleigh-Plesset model and other variations of it have been shown to be successful at elucidating many characteristics of free bubble behavior even in the extreme cases where the assumptions of the model are no longer strictly valid, such as during single bubble sonoluminescence [28].

At small amplitudes, the bubble oscillation in growth and compression is linear. At larger amplitudes, this oscillation becomes nonlinear, as the expansion phase may be much larger than the contraction phase. Finally, when the amplitude becomes great enough, the microbubble may strongly collapse upon itself, either once (transiently) or multiple times (stably). During the collapse, temperatures and pressures become extremely high in the interior of the bubble and shock waves may be emitted [29].

The presence of the shell in UCAs complicates theoretical treatments by acting as an additional damping force on the expansion and contraction of the free gas bubble [30]. The debate over how to best characterize the material properties of the shell has led to numerous models of varying complexity for UCA dynamics, as will be discussed further.

2.2.1 Basic Rayleigh-Plesset Derivation
There are several methods to derive the most commonly used nonlinear bubble equation, widely known as the Rayleigh-Plesset equation for its two principal contributors, following either energy balance or from the Navier-Stokes fluid
equations [31]. To outline the latter method, consider the Euler equations for inviscid flow,

\[ \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} = - \nabla P \frac{\rho_0}{\rho_0}, \quad (2.1) \]

where \( \mathbf{u} \) is the velocity, \( P \) is the liquid pressure, and \( \rho_0 \) is the constant fluid density. Using the velocity potential and integrating this equation yields the unsteady Bernoulli equation relating pressure and velocity of the fluid,

\[ \frac{p_L}{\rho_0} = \frac{p_\infty}{\rho_0} - \frac{\partial \phi}{\partial t} - \frac{1}{2} |\mathbf{u}|^2, \quad (2.2) \]

where \( p_L \) is the pressure within the fluid at a boundary, \( p_\infty \) is the pressure far from the bubble, and \( \phi \) is the velocity potential,

\[ \mathbf{u} = \nabla \phi. \quad (2.3) \]

The use of this form implies incompressible, irrotational, inviscid flow. By inserting the spherically symmetric velocity potential under these circumstances,

\[ \phi = -\frac{R^2 \dot{R}}{r}, \quad (2.4) \]

where \( R(t) \) is the time varying bubble radius and \( r \) is the distance from the center of measurement, the equation at the boundary becomes

\[ R\dot{\ddot{R}} + \frac{3}{2} \dot{R}^2 = \frac{1}{\rho_0} \left( p_L - p_\infty \right). \quad (2.5) \]

This is the basic form of the Rayleigh-Plesset equation, which has error in terms of the bubble wall motion of order, \( O(\dot{R}/c) \). The pressure far from the bubble is typically written as the sum of the static pressure, \( p_0 \), and the time-varying dynamic pressure, \( p_\infty(t) \),

\[ p_\infty = p_0 + P_\infty(t). \quad (2.6) \]

To describe the liquid pressure, various forms are used; commonly, the internal gas pressure is modified by the Laplace pressure to account for surface tension,
\[ p_L = p_i - p_\sigma = p_g + p_v - \frac{2\sigma}{R}, \]  
\( (2.7) \)

where \( p_g \) is the gas pressure, \( p_v \) is the vapor pressure, and \( \sigma \) is the surface tension. Equation 2.7 is also often written to introduce an approximation for sources of dissipation, which takes into account viscous losses but ignores thermal and radiation losses. This correction is obtained by dynamically matching normal stresses at the bubble wall and is written as

\[ p_L = p_g + p_v - \frac{2\sigma}{R} - \frac{4\mu \dot{R}}{R}, \]  
\( (2.8) \)

where \( \mu \) is the dynamic viscosity of the liquid.

Usually, a polytropic form for gas law is introduced for the gas pressure,

\[ p_g = p_{g0} \left( \frac{R_0}{R} \right)^{3\kappa} = \left( p_0 + \frac{2\sigma}{R_0} - p_v \right) \left( \frac{R_0}{R} \right)^{3\kappa}, \]  
\( (2.9) \)

where \( R_0 \) is the initial radius and \( \kappa \) is the polytropic index of the gas, most commonly assumed to be adiabatic.

Taking all these factors into account by combining equations 2.5 – 2.10 gives the so-called RPNNP model,

\[ R\ddot{R} + \frac{3}{2} \dot{R}^2 = \frac{1}{\rho_0} \left[ \left( p_0 + \frac{2\sigma}{R_0} - p_v \right) \left( \frac{R_0}{R} \right)^{3\kappa} + p_v - \frac{2\sigma}{R} - \frac{4\mu \dot{R}}{R} - p_0 - P_{ac}(t) \right], \]  
\( (2.10) \)

which is named after its five primary contributors: Rayleigh, Plesset, Noltingk, Neppiras, and Poritsky [32].

### 2.2.2 Other Unshelled Bubble Models

Numerous other models of unshelled single bubble dynamics [32, 33, 34, 35] have been developed to varying orders of accuracy based on the bubble wall Mach velocity [36]. Many of these have been shown to be capable of describing the majority of the bubble oscillatory cycle even in cases of extreme bubble
expansion such as occurs in single bubble sonoluminescence [28], where the bubble expansion may be ten times the initial radius [37].

The most widely used bubble equations other than Rayleigh-Plesset are the Keller-Herring family of equations which use the Kirkwood-Bethe approximation to include effects of liquid compressibility. Compressibility acts to decrease the velocity of collapse, and has a more significant impact on the formation of shock waves during bubble rebound than on the bubble dynamics themselves [29]. The Keller-Herring equation as written by Prosperetti and Lezzi [36] is

\[
\left[ 1 - \frac{(\lambda + 1)}{c} \dot{R} \right] R \dot{R} + \frac{3}{2} \left[ 1 - \frac{1}{2} \left( \frac{3\lambda + 1}{c} \right) \dot{R} \right] \dot{R}^2
\]

\[
= \left[ 1 - \frac{(\lambda + 1)}{c} \dot{R} \right] \left( h - \frac{p_v}{\rho_\infty} \right) + \frac{R}{c} \frac{d}{dt} \left( h - \frac{p_v}{\rho_\infty} \right),
\]

where \( c \) is the speed of sound in the liquid, \( h \) is the enthalpy, and \( \lambda \) is an arbitrary parameter of small order. The original Keller form of this equation is obtained when \( \lambda = 0 \), while the original Herring form of the equation is obtained when \( \lambda = 1 \). For an incompressible liquid, enthalpy can be written in terms of pressure and density in the more familiar form,

\[
h = \frac{1}{\rho_0} \left( p - p_\infty \right).
\]

Other bubble models, such as the Flynn equation [34] or the Gilmore equation [33], are only accurate mathematically speaking to the same first order Mach velocity of the wall, \( O \left( \frac{\dot{R}}{c} \right) \), as this family of equations. In general, these more complex, higher-order formulations for single bubble dynamics are not usually considered for models of shelled bubble dynamics, where the influence of the interface dominates the response.
2.2.3 Shelled Bubble Models

The effect of the interfacial shell alters the response of UCAs from non-encapsulated bubbles; for example, an observed effect of the damping from an albumin shell is an increase in resonance frequency \[25\]. Moreover, newer generation lipid-shelled microbubbles such as Definity, SonoVue, and Sonozoid have also been shown to exhibit unusual dynamic behaviors including thresholding to onset of oscillation \[26\] and ‘compression-only’ response \[38, 39\].

These and other experimental UCA results have indicated the inadequacy of directly applying free bubble models to shelled UCAs undergoing small oscillations. Therefore, existing free bubble models have been modified to incorporate terms corresponding to the damping and elasticity of the shell material. Albumin-coated UCAs were first modeled by adding additional damped linear oscillator terms in ad hoc fashion to the right hand side of Equation 2.10 to describe a viscoelastic solid effect of the shell \[30\],

\[
R \dddot{R} + \frac{3}{2} \ddot{R}^2 = \frac{1}{\rho_0} \left[ p_{g0} \left( \frac{R_0}{R} \right)^3 + p_v - \frac{2\sigma}{R} - 2S_f \left( \frac{1}{R_0} - \frac{1}{R} \right) - \delta_{total} \omega \rho_0 R \dddot{R} - p_0 - P_{ac}(t) \right], \quad (2.13)
\]

where \( \omega \) is the angular frequency, \( S_f \) is the shell elasticity, and \( \delta \) is the total damping excluding negligible thermal damping. This damping term includes both liquid viscosity and an additional shell friction term, \( S_f \), and is given by

\[
\delta_{total} = \delta_{radiation} + \delta_{viscous} + \delta_{shell} = \frac{\omega R_0}{c} + \frac{4\mu}{\omega \rho R_0^2} + \frac{S_f}{4\pi \omega \rho R_0^3}. \quad (2.14)
\]

Later models more rigorously derived the effect of the shell assuming shell behavior as an elastic solid \[40, 41\], or with an infinitesimally thin Newtonian rheology \[42\]. Increasingly complicated behaviors observed with UCAs containing more flexible lipid shells have prompted the introduction of a
rich variety of UCA models, incorporating descriptions such as Maxwell rheology [43], shear thinning [44], and strain-softening [45, 46] among others.

The first model to capture a wide range of nonlinear lipid-based UCA responses was the Marmottant model which postulated that the UCA shell behaves as a two-dimensional monolayer with size-varying surface tension [47]. This model is given by the equations,

\[
\rho \left( R \frac{d}{dt} + \frac{3}{2} \frac{R^2}{R} \right) = p_{\text{gas}} + \frac{R}{c} \frac{d}{dt} p_{\text{gas}} - \frac{2\sigma(R)}{R} - 4\mu\frac{\dot{R}}{R} - 4\kappa \frac{\dot{R}}{R^2} - p_0 - p_{ac}(t), \tag{2.15}
\]

\[
p_{\text{gas}} + \frac{R}{c} \frac{d}{dt} p_{\text{gas}} = \left( p_0 + \frac{2\sigma(R_0)}{R_0} \right) \left( \frac{R}{R_0} \right)^{-3\kappa} \left( 1 - \frac{3\kappa}{c} \frac{\dot{R}}{R} \right), \tag{2.16}
\]

\[
\sigma(R) = \begin{cases} 
0 & \text{if } R \leq R_{\text{buckling}} \\
\chi \left( \frac{R^2}{R_{\text{buckling}}^2} - 1 \right) & \text{if } R_{\text{buckling}} \leq R \leq R_{\text{breakup}} \\
\sigma_{\text{water}} & \text{if ruptured, } R_{\text{rupture}} \leq R
\end{cases} \tag{2.17}
\]

In these equations, \(\kappa\) is the monolayer surface dilatational viscosity and the size dependent surface tension, \(\sigma(R)\), includes an elastic compression modulus, \(\chi\), in addition to buckling, breakup, and rupture radii to define different bubble states. The relationship between shell friction and surface dilatational viscosity is

\[
S_f = 12\pi\kappa_s, \tag{2.18}
\]

while the relationship between shell elasticity and the elastic compression modulus is

\[
S_p = 2\chi. \tag{2.19}
\]

In contrast to most other shelled bubble models which assume a continuous shell state throughout the entire oscillation cycle, a unique feature of the Marmottant model is the explicitly incorporated rupture or breakup tension.
based on radial growth. After reaching a specified rupture radius, the bubble is assumed to continue to behave as a single entity but with new characteristics—as an unshelled bubble. However, the validity of this claim for large amplitude UCA oscillation and collapse was unsupported by the examples provided in the original paper and therefore requires further experimental validation.

### 2.2.4 Resonance

An important consideration for a dynamical system is the consideration of resonant frequencies; this is particularly crucial when analyzing ultrasound contrast agents, since their micron size is roughly resonant with typical megahertz insonifying frequencies. Nonlinear behavior due to the large response around resonance is certainly relevant to the collapse responses considered in this dissertation.

By considering small perturbations, $\varepsilon$, in the Rayleigh-Plesset equation and reordering the terms as mass-damper-spring equations,

$$m\ddot{\varepsilon} + b\dot{\varepsilon} + k\varepsilon = f(t),$$

(2.20)

the natural resonant frequency of the system is

$$\omega_0 = \left(k \frac{1}{m}\right)^{\frac{1}{2}} = \left(\frac{3kP_0}{\rho R_0^3}\right)^{\frac{1}{2}},$$

(2.21)

neglecting the effects of vapor pressure, viscosity, and surface tension. When shelled UCAs are considered, the elasticity from the shell adds an additional term to the equation,

$$\omega_0 = \left(\frac{3kP_0}{\rho R_0^3} + \frac{2S_p}{\rho R_0^3}\right)^{\frac{1}{2}},$$

(2.22)
Figure 2.1. Natural frequency as a function of initial bubble radius for unshelled, Definity, and Optison microbubbles.

The theoretical natural resonant frequencies for unshelled, Definity, and Optison UCAs across a range of sizes are plotted in Figure 2.1, where it is seen that the effect of the shell is to increase the natural frequency for a given radius.

The damped resonant frequency for these systems can be written as

$$\omega_d = \omega_0 \sqrt{1 - \frac{2\delta_{\text{total}}^2}{2}}.$$  \hspace{1cm} (2.23)

Since the damping terms in the UCA model are frequency dependent, a subset of small UCAs with large damping coefficients will not have a damped resonant frequency. One additional challenge when considering resonance effects with bubbles is that these equations are only strictly true for small, linear perturbations. The largest, 'resonant', response for a given frequency is also pressure dependent [32]. This is demonstrated in Chapter 5, where numerical solutions of the full UCA equations show that as pressure increases for a pulse with fixed frequency, the strongest response skews toward smaller bubbles.
Table 2.2. Natural and damped resonant sizes for unshelled, Definity, and Optison microbubbles.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Undamped</th>
<th>Damped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unshelled</td>
<td>Definity</td>
</tr>
<tr>
<td>0.9 MHz</td>
<td>3.17 µm</td>
<td>4.53 µm</td>
</tr>
<tr>
<td>2.8 MHz</td>
<td>1.02 µm</td>
<td>1.90 µm</td>
</tr>
<tr>
<td>4.6 MHz</td>
<td>0.62 µm</td>
<td>1.33 µm</td>
</tr>
<tr>
<td>7.1 MHz</td>
<td>0.40 µm</td>
<td>0.97 µm</td>
</tr>
</tbody>
</table>

Nevertheless, using the properties of Definity and Optison UCAs along with the Marmottant model considered in this paper, we can calculate the resonant size of the bubble for each of the four center frequencies studied. The damped resonant sizes, when they exist, are predicted to be very similar to the natural resonant sizes for the estimated properties of these UCAs (Table 2.2).

### 2.3 Bubble Collapse Dynamics

A plethora of theoretical and experimental methods to characterize collapsing bubble responses have been developed. Theoretical definitions are primarily based upon free bubble spherical models, and hypothesize varied threshold criteria to specify transient cavitation dynamics since the models themselves do not directly indicate when this occurs. Similarly, experimental studies of contrast agents have utilized multiple definitions to designate collapse. The reason for variation in the experimental studies has to do with the strengths and weaknesses inherent in each experimental setup, which generally fit into either the optic or acoustic category. The following sections review some of the variety of approaches which have been used to study bubble collapse. Due to the varied terminology to describe the possible large amplitude behaviors of both free and
### Term | Definition
--- | ---
**Inertial Cavitation (IC)** | Refers to bubble whose contraction phase is dominated by inertial forces; typically defined in terms of radial expansion

**Stable IC** | Refers to bubble undergoing IC which remains intact over multiple cycles

**Transient IC** | Refers to bubble undergoing violent IC which fragments and disappears

**Fragmentation** | Refers to the breaking apart of gas bubbles or shell of UCAs

**Shell Rupture** | Refers to the compromising of shell of UCAs such that gas is ejected

**Collapse** | Refers (1) to transient IC for free bubbles and (2) to shell rupture for UCAs

**Principal response** | Refers to the initial reaction of UCAs due to ultrasound

**Postexcitation (PES)** | Refers specifically to a broadband spike in acoustic signal from UCAs following principal response; associated with shell rupture and UCA destruction

| **Table 2.3. Definitions of terms describing large amplitude behavior of free bubbles and UCAs.**

shelled bubbles, and some inconsistency in its usage throughout the literature, a list of terms as they are defined in this dissertation is presented in Table 2.3.

### 2.3.1 Theoretical Definitions

The inertial cavitation response of a bubble is perhaps the most widely studied large amplitude bubble dynamic behavior. In a series of papers, Flynn [34, 48] examined dissipative and inertial effects for his model of a collapsing free bubble. First, by comparing the ratio of energy dissipated by a cycle of the bubble oscillation to the mechanical work done on the bubble cavity during the contraction phase (an energy dissipation modulus), he determined that this ratio always reached a maximum value when $R_{\text{max}}$, was in the range of 2 to 3, with maximum radial expansion given by

24
Thus, above this transition value, decreasing amounts of energy were dissipated by the bubble despite increasing amounts of energy being supplied it.

Furthermore, Flynn noted that after decomposing the equation of motion into inertial terms and pressure terms, the inertial terms dominated the collapse phase for an expansion greater than a critical $R_{\text{max}}$, found to be in the range of 1.9 to 2.3 for the initial radii he studied. Flynn then showed that for bubbles with an initial radius below 5 µm, the collapse phase was dominated by dissipative effects, while for bubbles larger than 5 µm, the collapse was dominated by inertial forces. He therefore hypothesized that transient cavitation occurred when $R_{\text{max}}$ exceeded both the dissipative and inertial thresholds, whereas stable cavitation existed below them.

A different criterion for collapse was proposed by Apfel [49], who suggested that the wall of the bubble must reach supersonic collapse speed in order to undergo transient cavitation. This threshold was reached upon radial expansion of $R_{\text{max}} \approx 2.3$. Still another condition was proposed by Holland and Apfel [50], in which they hypothesized that the maximum temperature of the gas inside the collapsing cavity must reach 5000 K, a temperature that was found to lead to the formation of free radicals under certain experimental conditions.

Due in part to the complexity of applying these different criteria for transient cavitation, another definition of transient cavitation for the free bubble was that that the ratio $R_{\text{max}}$ must exceed 2 [51, 27]. The simplicity of this criterion has led to its widespread adoption. In the course of relating maximum temperature to maximum radial expansion in order to compare results with other models, it was found that a temperature of 960 K corresponded to $R_{\text{max}} = 2$. 

$$R_{\text{max}} = \frac{\text{Max}(R(t))}{R_0}.$$  \hspace{1cm} (2.24)
and matched the results of the comparison study closely, though not identically [50].

In 1991, Apfel and Holland [52] applied their temperature-based definition of cavitation to acoustic conditions encountered in diagnostic ultrasound, employing short pulses (fewer than 10 cycles) and low duty cycles (less than 1:100). From this, they were able to derive an index of intensity proportional to the mechanical work done on a bubble,

\[ I = \frac{PRPA^2 \text{ [MPa]}}{f \text{ [MHz]}} \]

where the derated peak rarefractional pressure amplitude (PRPA) was given in MPa and the frequency, \( f \), was given in MHz. The widely recognized mechanical index (MI) was introduced as a result of that work. The MI is used as a guide for judging the likelihood for non-thermal cavitation activity \textit{in vivo} from ultrasound and is defined as

\[ MI = \frac{PRPA \text{ [MPa]}}{\sqrt{f} \text{ [MHz]}}. \]

While MI may be useful for roughly representing basic trends of bubble behavior there is little clear theoretical or experimental evidence for this particular scaling metric with contrast agents, despite it being extensively reported in UCA literature [9].

\subsection*{2.3.2 Optical Studies of Contrast Agent Destruction}

Optical observations are usually considered the standard by which microbubble responses are judged due to their ability to distinguish initial conditions of the microbubble as well as radial expansion and compression. Visual experiments normally leave little doubt as to the presence and disappearance of a microbubble, though the typical viewing field and time duration during which
signals are recorded may be sources of uncertainty. While the majority of optical experiments focus on small amplitude UCA responses to observe radial motion and determine bubble shell properties, a few studies have examined the behaviors which are associated with the destruction of UCAs due to large amplitude pulses, including fragmentation, gas release, and rebound.

Chomas et al. [53], using a streak imaging based imaging approach, found fragmentation for a phospholipid-based UCA when the relative expansion reached three times the initial radius. For a single sinusoid pulse at 2.25 MHz, 800 kPa peak negative pressure, this corresponded to microbubbles with an initial radius smaller than 2.5 µm. They also found increased likelihood of fragmentation for decreased frequency, increased pressure amplitude, and increased pulse duration.

Bouakaz et al. [54], using a high speed camera, observed the release of gas from double-walled, albumin based UCAs, which escapes as a transient free bubble. They reported destruction of microbubbles greater than 5 µm at a mechanical index as low as 0.3, while destruction of smaller microbubbles occurred above MI = 0.6 for 10 cycle pulses at 1.7 MHz. In related experiments, Postema et al. detected similar shell cracking behavior with thick single-walled albumin UCAs [55] and also observed fragmentation of lipid shelled contrast agents at low pressures below MI = 1 at 0.5 MHz while predicting that radial excursions much less than 2R₀ led to this destruction [56]. Optical studies provide valuable insight into microbubble behavior, but there are also drawbacks to this approach including limited temporal and spatial resolution, limited size of the data set, and expense involved in the necessary equipment [57]. The difficulty in overcoming these challenges is significant, and therefore the majority of UCA collapse studies have been acoustically based rather than optically based.
2.3.3 Acoustic Studies of Contrast Agent Destruction

The primary challenge involved with acoustic studies of ultrasound contrast agent collapse is determining a consistent and fundamental indicator of transient destruction. This difficulty in signal interpretation has led to a variety of approaches. It is generally agreed that a large amplitude UCA response as is involved with inertial cavitation (IC) or microbubble fragmentation is also associated with an increase in broadband spectral content; however, the definitions found in literature are often qualitative and arbitrary.

While there is greater difficulty in interpretation of signals for acoustic methods, they also have appealing qualities. One of strengths is the widespread dissemination of the technology to make the measurements and thus, the potential to be practical in a wider variety of situations than optical methods. Another advantage is greater temporal resolution and a longer measurement period. One of the fastest high speed cameras currently in use, the Brandaris 128, has an imaging frequency of up to 25 MHz but only over a time span of 128 frames [58], while an acoustic experiment can easily acquire data at 100 MHz or higher for relatively long periods of time. For these reasons, as well as for potential use in vivo [59], acoustic approaches for monitoring microbubble activity are appealing.

Acoustic studies can be divided into two subcategories: active cavitation detection (ACD) and passive cavitation detection (PCD). ACD uses a secondary low pressure pulse to investigate changes caused by the initial pulse [60, 61]. However, while the measurement pulse in ACD has significantly less energy than the primary pulse, it still may have the potential to affect the cavitation process [62]. In contrast, passive cavitation detection only involves receiving the response.
Shi et al. [60] used active cavitation detection to investigate the destruction of single lipid shelled UCAs. They found damage to UCAs using 2.5 MHz, 2 to 16 cycle pulses at a MI ranging from 0.4 to 1.0. By loosely defining IC as a qualitatively different signal that disappears after a single tone burst, they determined that IC occurs above a MI of 1.0. Church and Carstensen [63] commented that this data indicated stable IC was occurring even at the lower MI values since the microbubbles were expanding to more than twice their initial size, and that the experiment was consistent with an observation of both stable and transient inertial cavitation.

Chen et al. [64], using a passive cavitation detection approach, defined the fragmentation threshold as the pressure at which at least 5% of spikes in the time trace exceeded a certain voltage threshold and simultaneously defined the inertial cavitation threshold as a sudden increase in broadband noise in the frequency spectrum between the harmonics. They reported that fragmentation produced an increase in the inertial cavitation ‘dose’ of the UCA population.

Giesecke and Hynynen [65] also used the increase in broadband noise as a method to define their inertial cavitation threshold when analyzing the response of albumin shelled Optison. They proposed an increase of one standard deviation greater than the background noise as the threshold, and reported that increasing the frequency increased the threshold for long (20-100 ms) tone bursts. In a later study, the sudden spectral power increase of at least 20 dB above the background noise [66] was used to identify inertial cavitation.

Chatterjee et al. [67] observed changes in attenuation over time which were attributed to destruction of lipid shelled Definity. They found moderate decreases in attenuation at 5 MHz insonification for pressures greater than 1.2 MPa, and increasingly rapid destruction as pressures increased further.
Similarly, Moran et al. [68] detected decreases in integrated backscatter coefficient over time for both Definity and Optison using a clinical array at 4 MHz. As peak rarefractional pressures were increased from 0.23 to 0.72 MPa, a larger drop in this quantity was observed, suggesting qualitatively that there was increased destruction of the microbubbles.

Yeh and Su [61], using an active cavitation detection system for UCAs flowing in a tube, proposed using the ratio of backscattered power with and without insonation to determine the destruction percentages for a group of Definity microbubbles. They found an increase in destruction for increased pressure, increased pulse length, and decreased frequency, through compared to other UCA studies they also reported unusual results of 50% destruction at an MI of about 0.1 and 95% destruction at an MI around 0.5 for short (1 and 3 cycle) pulses at 1 to 7.5 MHz.

### 2.3.4 Postexcitation Signals in Passive Cavitation Detection

Much of the research in this dissertation deals with a thorough exploration of what is termed postexcitation rebound and collapse, as observed using passive cavitation detection. Previous work related to the current study has proposed using passive cavitation detection to monitor microbubble destruction based on the relationship of two characteristic features of the acquired temporal signals: the principal response, defined as the initial harmonic response of the microbubble lasting in duration up to the length of the transmitted pulse, and the presence or absence of a postexcitation signal (PES), defined as a secondary broadband response separated in time from the principal response—typically 1 to 5 μs later [69]. In this work, it was hypothesized that this type of rebound signal only occurs for free (unshelled) gas bubbles emitted during rebound of the
UCA and consequently was linked to shell rupture and transient collapse of the UCA. This categorization approach for characterizing UCA responses is attractive for being, in principal, a non-arbitrary, quantitative definition of transient microbubble collapse activity.

The physical origin of the postexcitation emissions from single UCAs is believed to be related to similar examples of free bubble rebound and re-collapse which occur with larger clouds of bubbles. For example, such behavior has been observed in simultaneous optic and PCD lithotripsy experiments [70] and during sonoluminescence [71]. In the proposed scenario, the encapsulated UCA undergoing inertial cavitation emits one or more free gas bubbles due to shell rupture, bubbles which then serve as the source for the postexcitation signal.

Previous simulation work using the Marmottant model has suggested that the presence of postexcitation rebound is associated with an increase in broadband content, demonstrating a relationship with the strength of the inertial collapse; additionally, both shell rupture and inertial cavitation were found to be necessary conditions for the occurrence of PES in UCAs [72]. In practice, this establishes a minimum bound on UCA postexcitation at the inertial cavitation threshold $2R_0$, since the shell rupture threshold for the Marmottant model is typically set to $1.5R_0$ or less. While the survival of microbubbles without postexcitation is unclear, these theoretical and previous experimental observations solidly link postexcitation emissions with the transient collapse of the UCA microbubble.

Using a single focused receive PCD transducer on isolated UCAs has been found to be adequate to determine minimum destruction thresholds of isolated, unconstrained microbubbles [73]. However, due to variability in the spatial location of the microbubble relative to the focus of the incident pulse, previous studies were generally unable to establish any obvious trend between the
amount of microbubble collapse and the peak rarefactional pressure amplitude, a relationship which might be expected for a robust measure of cavitation activity. To address the challenge of determining spatial location, the current work utilized two matched receive transducers to limit the confocal region from which acceptable responses were obtained.

Double passive cavitation detection of UCAs using two receive transducers with different center frequencies has been previously reported [74]. However, that particular experimental setup only allowed for imprecise comparisons between the two received signals due to the differences in the transducers. The current double PCD study avoids these limitations by using matched high frequency receive transducers, thereby reducing both spatial uncertainty of the microbubble and incidence of asymmetrical behavior potentially occurring from interactions with surrounding microbubbles.
CHAPTER 3
DOUBLE PASSIVE CAVITATION DETECTION

3.1 Introduction
The experimentally measured response of single, unconstrained ultrasound contrast agents (UCAs) was used to facilitate both direct understanding of microbubble behavior and to provide a benchmark for comparison with mathematical modeling. The experimental setup developed to assess UCA response was called double passive cavitation detection (PCD) and consisted of three confocally aligned transducers, one for transmission and two for reception. Interpretation of the received signals from bubble responses led to observation of a characteristic response of shelled microbubbles termed postexcitation collapse. This chapter provides details on the experimental methodology, data analysis procedures, and initial results comparing two distinct UCA types.
Figure 3.1. (a) Photograph and (b) schematic of the double PCD experimental setup.

3.2 Double Passive Cavitation Detection

The double PCD technique remained fundamentally the same for all data acquisition trials over the course of this research with a few minor variations as noted. Two higher frequency passive receive transducers were placed at a 90-degree angle with one lower frequency active transmit transducer placed equally between them at a 45-degree angle (Figure 3.1). A custom holder gave alignment stability to this arrangement and allowed simple exchange of the transmit transducer once the initial positioning was complete. A small concentration of UCAs was introduced into the tank containing degassed water at room temperature, and short, large amplitude pulses were used to insonify the gently stirred UCAs. Subsequently, a variety of signal processing techniques were implemented to categorize the acquired UCA responses and to provide insight into the acoustic collapse behavior of UCAs.
<table>
<thead>
<tr>
<th>Transducer Model</th>
<th>Center Frequency [MHz]</th>
<th>-3 dB Fractional Bandwidth [%]</th>
<th>-6 dB Beamwidth at Focus [mm]</th>
<th>Focal Length [mm]</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>IS1504GP</td>
<td>14.6</td>
<td>25.56</td>
<td>0.27</td>
<td>27.18</td>
</tr>
<tr>
<td>IS1504GP</td>
<td>13.8</td>
<td>20.90</td>
<td>0.27</td>
<td>27.30</td>
</tr>
</tbody>
</table>

Table 3.1. Measured characteristics of the transducers.

3.2.1 Transducer Characterization

Four different single element focused transducers (Valpey Fisher, Hopkinton, MA) were used to generate the transmitted pulses. The center frequencies of these transducers were nominally 1, 3, 5, and 8 MHz, but were measured in pulse-echo mode to be 0.9, 2.8, 4.6 and 7.1 MHz, respectively; all were f/2, with an element diameter of 0.75 in. Two single element focused transducers were used concurrently to passively receive the signals. The center frequencies of the two receive transducers were nominally 15 MHz, but measured to be 14.6 and 13.8 MHz; both were f/2, with an element diameter of 0.5 in. Measured transducer characteristics were obtained using established wire scattering characterization procedures with a 30 – 100 μm diameter wire as applicable [75]; a summary of the most important characteristics is presented in Table 3.1.

3.2.2 Transducer Calibration

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). An attenuation bar (Model 358,
Arenberg Ultrasonic Laboratory, Boston, MA) was inserted in the transmit chain to achieve the lowest pressure settings. All settings were calibrated to determine the pressure amplitudes of the generated waveforms using a PVDF hydrophone (0.5 mm diameter, Marconi 6999/1/00001/100; GEC Marconi Ltd., Great Baddow UK) located at the center of the confocal region of the receive transducers according to established procedures [76, 77]. The pulses were nonlinear across most of the pressure ranges, but as is typical in bubble literature, insonifying pressures will be primarily reported using peak rarefactual pressure amplitude (PRPA).

The mechanical index (MI) is commonly used to gauge the likelihood of biomechanical effects in vivo due to cavitation activity from ultrasound. While use of the MI with UCAs is not well supported by experiments, it is commonly reported in the literature and the short pulse length and low duty cycle parameters used in this double PCD experiment were within the requirements given in its development. According to this theory, acoustic insonations related by Equation 3.1 may result in similar cavitation activity [52, 78].

\[
MI = \frac{PRPA \ [MPa]}{\sqrt{f} \ [MHz]} \tag{3.1}
\]

The ranges of peak rarefactual pressure amplitudes (PRPA) and equivalent mechanical indices (MI) used in the initial experiments for each transducer frequency are listed in Table 3.2.

### 3.2.3 Transducer Alignment

The transducers were aligned in pulse-echo mode using a 50 μm diameter wire located at the center of the confocal region. The two receive transducers were well-aligned (Figure 3.2). However, the confocal zone of the receive transducers
Table 3.2. Peak rarefactional pressure amplitudes used in double PCD experiment.

<table>
<thead>
<tr>
<th>Transmit Transducer [MHz]</th>
<th>PRPA Range [MPa]</th>
<th>MI Range [MPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>0.23 – 1.30</td>
<td>0.24 – 1.37</td>
</tr>
<tr>
<td>2.8</td>
<td>0.72 – 5.00</td>
<td>0.43 – 2.99</td>
</tr>
<tr>
<td>4.6</td>
<td>1.96 – 6.37</td>
<td>0.91 – 2.97</td>
</tr>
<tr>
<td>7.1</td>
<td>2.15 – 6.37</td>
<td>0.81 – 2.39</td>
</tr>
</tbody>
</table>

was less well-aligned with the foci of the transmit transducers (Figure 3.3), being separated by 0.28 mm in all cases. To gauge the importance of this misalignment, the calibration of the transmit transducers was performed both at the transmit transducer focus and at the center of receive transducer confocal zone. The average decrease in PRPA from the former to the latter location was small but non-negligible, particularly for higher frequencies which have a smaller beamwidth (Table 3.3). Therefore, results are presented using the calibrated data from the center of the receiver confocal region, which was the location of greatest sensitivity to the obtained response.

Table 3.3. Measured variation between focus of the transmit transducer and confocal region of receive transducers.

<table>
<thead>
<tr>
<th>Transmit Transducer [MHz]</th>
<th>Lateral Distance to Confocal Region [mm]</th>
<th>PRPA Percent Difference Between Confocal Region and Transmit Transducer Focus, Mean (St. Dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>0.28</td>
<td>-1% (2%)</td>
</tr>
<tr>
<td>2.8</td>
<td>0.28</td>
<td>-5% (1%)</td>
</tr>
<tr>
<td>4.6</td>
<td>0.28</td>
<td>-10% (1%)</td>
</tr>
<tr>
<td>7.1</td>
<td>0.28</td>
<td>-35% (6%)</td>
</tr>
</tbody>
</table>
Figure 3.2. Overlapping confocal region of the receive transducers. This image was created by overlapping the pulse intensity integral obtained using the wire characterization technique [75]. The scale is in dB.

Figure 3.3. Overlapping confocal region of 4.6 MHz transmit transducer with receive transducers. The scale is in dB.
3.3 Data Acquisition Procedures

3.3.1 Materials

The transducer holder was placed in a Plexiglas tank with dimensions 50.5 cm x 25.5 cm x 30.0 cm which was filled with 15 to 25 L of deionized, degassed water at room temperature (21.0° ± 1.0° C). A smaller tank with dimensions 22.5 cm x 23.5 cm x 12.5 cm was filled with 5 ± 0.5 L of water in later studies. In both cases, the transducers were located 3.0 ± 1.0 cm from the open surface of the water.

Signals acquired by the receive transducers were bandpass filtered from 1 to 20 MHz, amplified by 22 dB, digitized using an A/D converter (12-bit, 100 MS/s, Strategic Test digitizing board UF 3025, Cambridge, MA or 12-bit, 100 MS/s, Gage CS8247, Lockport, IL), and saved to a PC for offline processing using Matlab® (The Math Works, Inc., Natick, MA). A block diagram of the complete data acquisition system is presented in Figure 3.4.

![Figure 3.4. Double PCD block diagram.](image)
3.3.2 Experimental Determination of Noise Thresholds

Prior to the addition of microbubbles in the water tank, 50 signals were collected to establish the experimental system noise for each trial. Noise levels were determined by binning the amplitudes of all samples from these signals, assuming Gaussian noise, and setting the required signal threshold greater than 3.29 standard deviations from the mean. This was approximately equivalent to a noise limit set at 99.9% of the absolute value maximum obtained from these control signals.

3.3.3 Experimental Determination of Focal Region

For the later studies which relied on automatic classification, a 50 \( \mu \text{m} \) wire target was inserted into the water tank prior to the addition of microbubbles to establish the confocal region. The confocal location was determined by manually adjusting the wire to find the position of greatest scattering amplitude for both receive transducers simultaneously. Fifty signals were collected and averaged together, and the time to the focus for each channel was determined by the maximum reflection from the wire target (Figure 3.5).

3.3.4 Commercial Ultrasound Contrast Agents

The two commercial contrast agents used in these experiments were lipid-shelled Definity® (Lantheus Medical Imaging, N. Billerica, MA) and albumin-shelled Optison™ (GE Healthcare Inc., Little Chalfont, UK). The reported mean diameter range of Definity was 1.1 to 3.3 \( \mu \text{m} \), with 98% having a diameter less than 10 \( \mu \text{m} \); the maximum initial concentration was 1.2 x \( 10^{10} \) microspheres/mL [79]. The reported mean diameter range of Optison was 3.0 to 4.5 \( \mu \text{m} \), with 95% having a diameter less than 10 \( \mu \text{m} \); the initial concentration was 5.0 to 8.0 x \( 10^{8} \)
Figure 3.5. Reflections from 50 μm wire target for 7.1 MHz transducer. In this example, there is a 0.29 μs difference in time of arrival between each channel. The vertical lines represent ±3 insonifying periods from the maximum, and delineate the confocal region.

Both types of microbubbles contained octafluoropropane (C₃F₈, also known as perfluoropropane) as the gas core. For the standard experiments, the contrast agents were re-activated according to package instructions. Since each experimental trial used only a small amount of contrast agents, vials were reused for several trials, up to approximately 10 times.

The appropriate concentration of UCAs was added to the water tank to ensure dilution to less than 1 bubble per confocal volume (approximately 5000 bubbles/mL), and the mixture was gently stirred with a magnetic stir bar to ensure uniformity of the UCA distribution. Loss of acoustically active microbubbles in each trial occurred due to buoyancy and also due to gas diffusion across the shelled surface [81]. Therefore, microbubbles were
replenished when the rate of observable events decreased noticeably, up to every 5-10 minutes for Definity and every 10-20 minutes for Optison. In a typical experiment, several thousand signals at each pressure level were acquired continuously; the total experimental time was typically less than 1 hour following reactivation of the UCAs.

3.4 Data Analysis

Following the data collection, the acquired signals were processed to remove the DC component from the signal and then low pass filtered with cutoff frequency 20 MHz to remove excessive system noise frequencies. The data was then classified into one of seven categories based on the acoustic response of the UCA.

While the concentration of UCAs was chosen such that there should have been approximately one microbubble per the confocal volume on average, this did not preclude the possibility that there were either no microbubbles or greater than one microbubble present in the receiving region at any given time. Therefore, the received signals needed to be classified to eliminate those which did not contain a single bubble in the confocal region. Seven categories were used for classification: (1) no UCAs within the receiving region, (2) multiple UCAs within the receiving region, (3) a single UCA out of the confocal region, (4) a single UCA with postexcitation signals (PES) in only one channel, (5) a single UCA with PES in both channels, (6) a single UCA with no PES in either channel, or (7) a single broadband peak. These characteristics of categories are described in the following sections.
3.4.1 Automatically Excluded Signals

A majority of the total acquired signals were not used in the final analysis, which was expected since the larger total receiving region was more likely to contain microbubbles than the overlapping confocal region. Undesirable signals – those obviously containing no bubbles, multiple bubbles, or bubbles out of the confocal zone – were filtered from the data set. Signals with no samples in either channel greater than the predetermined noise limits were classified as category 1, no bubbles within the receiving region. Signals where the duration of the envelope exceeding the signal threshold was 3 times as long as the transmitted pulse length were classified as category 2, multiple bubbles within the confocal region. Category 3, a single bubble out of the confocal region, was defined using two criteria that were indicative of a signal source location significantly closer to one receive transducer than the other and thus outside the confocal region. If the difference in time of arrival determined through maximizing the cross-correlation between the two channels exceeded 1 µs, or if the maximum amplitude of the response in one channel exceeded 5 times that in the second channel, the signal was classified into this category. Approximately 80% to 90% of a data set was automatically classified in one of these first three categories and was immediately rejected from further analysis.

After excluding these unambiguous signals that did not contain the response of a single bubble from the confocal region, it was then necessary to further classify the remaining signals. This was accomplished using two methods. In the first study, final analysis was performed through manual classification by multiple observers following a prescribed set of guidelines. In subsequent studies, the secondary categorization was achieved using an automatic classification routine developed to mimic manual classification.
3.4.2 Manual Classification

Manual classification from visual inspection formed the basis for the initial studies comparing Definity and Optison UCAs as well as the baseline standard for evaluating the automatic classification routine. Two important features were defined to identify single UCA response. The principal response was specified as the initial harmonic response of the microbubble lasting in duration up to the length of the transmitted pulse. The postexcitation signal (PES) was specified as a secondary broadband response separated in time from the principal response, typically 1 to 5 µs later.

Visual analysis of both the voltage-time signals and frequency-time spectrograms calculated with a sliding Hanning window (1.28 µs, in steps of 0.02 µs) were used to classify the signals remaining after automatic filtering. Many additional signals did not satisfy the automatic exclusion criteria but were nonetheless identified as belonging to one of the first three categories, which left approximately 10% to 40% of the manually classified signals categorized as containing a single microbubble within the confocal region.

Since this classification was performed manually, there was some variability in what was determined to be a postexcitation or non-postexcitation signal for each classifier; nevertheless, the overall trend of increasing postexcitation with increasing PRPA was clear and consistent. Three individuals with varying levels of familiarity to the project were trained to classify the experimental data; the average number of single bubble signals with or without PES used for analysis per unique pressure and frequency settings for Definity and Optison were 30 (± 9) and 20 (± 8), respectively. The characteristics used to manually classify each signal are described with examples in the following sections.
Figure 3.6. Noise. No bubbles are present in channel 1. All signals presented here as examples of manual classification were acquired at 4.6 MHz with 4.47 MPa PRPA.

**Category 1: No bubbles**

A signal was classified as containing no bubbles when the only portion of the signal greater than the noise threshold was determined to be random noise (Figure 3.6). If no portion of the signal was greater than the noise threshold, the signal was automatically classified in this category. These signals were always excluded from further analysis.

**Category 2: Multiple bubbles**

A signal was classified as multiple bubbles within the confocal region when there were two or more responses separated in time (Figure 3.7), or when the principal response was significantly longer in duration than the measured transmit pulse at the focus (Figure 3.8). For example, at 2.8 MHz, the duration of a 3 cycle pulse is approximately 1 µs; if the principal response was 3 µs long, it was considered to have originated from several bubbles in close proximity to one another. If the
duration of signal greater than the noise threshold exceeded 3 times the duration of the measured transmit pulse, the signal was automatically classified in this category. These signals were always excluded from further analysis.

Figure 3.7. Multiple bubbles. Several bubbles are present in channel 1.

Figure 3.8. Multiple bubbles. Several bubbles are present in both channels.
Category 3: Single bubble out of confocal region

When each channel displayed a single principal response, but that response was widely separated in either time (Figure 3.9) or magnitude (Figure 3.10), the UCA was considered to be outside the confocal region of the two receive transducers. If the lag time for maximum correlation between the two channels exceeded 1 µs, or if the ratio of maximum values between the two channels exceeded 5, the signal was automatically classified in this category. These signals were always excluded from further analysis.

Category 4: Single bubble with PES in only one channel

When each channel displayed a single principal response, but one channel contained both a principal response and PES while the other channel had only a principal response (Figure 3.11), the signal was classified in this category. Possible scenarios which could explain this type of signal include multiple bubbles picked up separately by each receiver, a single bubble out of the confocal region such that the PES was only received by one channel, or a single bubble which collapsed with strong asymmetry, potentially due to proximity to other UCAs. Due to the non-spherically symmetric response and the large degree of uncertainty as to the proper classification, these signals were usually excluded from further analysis.
Figure 3.9. Single bubble out of confocal region. The lag time for the correlation maximum is 2.28 µs.

Figure 3.10. Single bubble out of confocal region. The signal in channel 2 is much stronger than in channel 1.
Figure 3.11. Single bubble with PES only present in channel 1.

Figure 3.12. Single bubble with PES in both channels.
**Category 5: Single bubble with PES in both channels**

When both channels displayed a single principal response followed by one or more PES responses, while satisfying the requirements to be within the confocal region, the bubble was considered to have collapsed transiently (Figure 3.12). These signals were always included in the analysis as collapsed microbubbles exhibiting postexcitation.

**Category 6: Single bubble with no PES**

When both channels displayed a single principal response without any accompanying PES signal, while satisfying the requirements to be within the confocal region, the bubble was considered to have responded without postexcitation collapse (Figure 3.13). These signals were always included in the analysis as microbubbles not exhibiting postexcitation rebound.

![Figure 3.13. Single bubble with no PES.](image)
Figure 3.14. Unknown. The single broadband spike is similar to the PES, but without the preceding principal response.

Category 7: A single broadband peak

The final class of signals contained a single broadband spike, without any secondary rebound (Figure 3.14). According to the classification scheme, it was unknown whether such a peak represents the transient collapse of a UCA, a cycle of the oscillatory behavior of a non-transient UCA, the collapse of a free gaseous bubble, or something else. These signals were usually excluded from further analysis due to their indeterminate origin.

3.4.3 Automatic Classification

While manual classification from multiple individuals was used to successfully obtain initial results, there were several weaknesses to this method. Visual inspection was both inherently susceptible to varying qualitative interpretation and also time consuming, limiting the amount of data that could be reasonably analyzed in a study. To address those issues, an automatic classification routine
based on peak detection parameters was developed to standardize postexcitation
data analysis and provide a quantitative details describing postexcitation
collapse.

This method of analysis was developed to imitate manual classification
guidelines and results. Fixed definitions for signal characteristics such as
detected peaks and grouped peaks were used across all frequencies and PRPAs
in order to reduce unintentional or intentional bias arising from manual
classification. However, since these new definitions were determined empirically
rather than arising out of a theoretical framework, the chosen values were still
influenced by the prior analysis.

The automatic classification routine was comprised of a multistep process.
First, the dataset characteristics – the noise thresholds and the confocal region –
were identified from the pre-experimental data. These features were used to
automatically eliminate the majority of signals from further classification in a
similar manner to that described previously. By including information about the
confocal region location and small time offset between each channel in every
data set, the automatic classifier improved this exclusion procedure; for example,
trial to trial speed of sound differences arising from water temperature
differences were now taken into account.

The period, $T$, associated with the insonifying frequency was used to
provide the frequency independent time metric. Due to the large amplitude
pulses used, the forced UCA oscillations were primarily at the insonifying
frequency rather than the natural frequency of each UCA. The confocal region
was therefore defined as the time $\pm 3T$ from the focus of the wire (Figure 3.5), and
its physical size varied depending on the insonifying frequency, ranging from
approximately 1 to 10 mm.
A peak detection algorithm was used to detect local maxima and minima in remaining signals. A peak was explicitly defined as a sample differing from its surrounding samples by some multiple of the noise threshold; typically, this was on the order of a few mV in this experimental setup. This method of definition proved to be more robust with the noisy PCD signals than low-pass filter and derivative-based approaches.

Next, the relationships among the detected peaks were determined, using multiples of $T$ for defining grouped or non-grouped peaks. Isolated peaks with no other peak detected within $8T$ were considered to be random noise fluctuations and were ignored. Closely spaced peaks separated by no more than a specified, empirically determined multiple of $T$ were considered to be harmonic and to have originated from the same source (i.e. a bubble). The longest unbroken sequence of peaks was considered to be the principal response; any peaks detected following the end of the principal response were considered to be postexcitation peaks.

Finally, with the determination of the principal response and any other peaks, the signal was classified in one of the seven previously identified categories. If no closely spaced peaks were identified within the confocal region, the signal was considered to contain no UCAs (Figure 3.15). If the principal response was longer than the specified multiple of $T$ or if several peaks were detected prior to the principal response, it was considered to have arisen from multiple bubbles (Figure 3.16). If the principal response from either channel occurred outside the confocal region or if different responses were observed in each channel, the signal was considered out of focus (Figure 3.17). If the principal response was within the confocal region while satisfying minimum and maximum length requirements, it was considered to have arisen from a single UCA and was classified depending on if postexcitation peaks were detected and
in which channels (Figures 3.18, 3.19, 3.20). Finally, if the principal response was shorter than 0.5T, the signal was considered to have only one broadband peak (Figure 3.21).

![Figure 3.15](image)

Figure 3.15. No UCA signal detected. Peaks are detected in channel 2, but are widely spaced and therefore are considered unassociated with UCA activity. All signals presented here as examples were acquired at 2.8 MHz with 1.11 to 2.91 MPa PRPA. The color range of the spectrograms is 20 dB from the signal maximum.
Figure 3.16. Multiple UCAs detected. In channel 1, the principal response is longer than the maximum allowed, while in channel 2, numerous peaks are observed prior to the principal response.

Figure 3.17. Out of focus UCA detected. While the correlation time is close, a principal response region is only observed in channel 1 which receives a much stronger response.
Figure 3.18. Single UCA with postexcitation detected in channel 1 only.

Figure 3.19. Single UCA with postexcitation detected in both channels.
Figure 3.20. Single UCA with no postexcitation detected, only the principal response.

Figure 3.21. Unknown signal detected. The single spike is shorter than 0.5T, so there is no harmonic principal response.
3.4.4 Logistic Regression Curve Fitting

Only those categories containing a single bubble that exhibited symmetric behavior within the confocal region were used for subsequent regression analysis. The two categories of signals used were those containing a principal response followed by a secondary broadband postexcitation signal (Figures 3.12, 3.19), and those with a principal harmonic response only (Figures 3.13, 3.20). By comparing the number of signals in the former category to the total number of signals in both categories, the percent of transiently collapsing bubbles for a particular transmit frequency and pressure amplitude was defined as

\[ \text{Postexcitation [%]} = \frac{\text{PES in both channels}}{\text{PES in both} + \text{PES in neither}} \times 100\% . \]  

(3.2)

A common approach which is useful for fitting data with a discrete, binary outcome variable is logistic regression [82]. The basic form of the logistic model is

\[ P(x) = \frac{e^{\alpha_0 + \alpha_1 x}}{1 + e^{\alpha_0 + \alpha_1 x}} , \]  

(3.3)

where \( P(x) \) is the outcome, \( x \) is the independent variable, and the coefficients \( \alpha_0 \) and \( \alpha_1 \) are estimated using a maximum likelihood method. In the double PCD experiments, \( x \) was chosen to be the PRPA and \( P(x) \times 100\% \) corresponded to the percent collapse at a given PRPA.

It is expected that at zero PRPA (i.e. no transmitted pulse), the number of UCA collapsing transiently must go to zero. However, no PCD signals are able to be collected at especially low pressures, and the lowest observed PRPA may contain nonzero PES. To account for this constraint on the fit, a transformation of the regression variable was used,

\[ z = \log(x - m) \]  

(3.4)
Using this transform, the probability of collapse will go to zero as the peak rarefractional pressure amplitude, \( x \), approaches the minimum threshold \( m = 0 \). In experiments where very few signals were acquired at the smallest pressures, an additional low PRPA at which no signals of any category were observed was incorporated with a value of zero PES and a weight equal to the mean number of signals at the other PRPAs to further reduce the uncertainty at the lower end of the logistic curve fit.

Furthermore, it was observed in most cases that the percentage of postexcitation increased from a minimum to a maximum value which was usually less than 100%. While PES is 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 [83]. Therefore, 100% destruction is not directly equivalent with 100% PES, the latter of which may never be reached for given acoustic parameters as PRPA increases. This physical motivation led to use of a modified logistic model,

\[
P(z) = \frac{e^{\alpha_0 + \alpha_1 z}}{1 + e^{\alpha_0 + \alpha_1 z}}.
\]

(3.5)

where \( Q \) was the maximum observed percentage of PES (0 \( \leq \) \( Q \leq \) 1).

A percentage postexcitation threshold was defined as the level at which a certain percentage of the total population of microbubbles transiently collapsed with PES. For example, a 5% threshold occurred near the inception of PES, while a 50% threshold occurred at a higher PRPA. To determine these thresholds from the experimental data, a generalized linear regression fit was performed using Equation 3.6. This curve established the amount of postexcitation present at a
certain PRPA proportional to the maximum amount observed for a specific frequency, and the thresholds were used as a metric for comparing similar relative amounts of cavitation activity across different insonifying conditions. In most cases, the 50% threshold was less prone to variation from trial to trial than the 5% or 95% thresholds.

The standard error for these collapse thresholds was approximated using a first order Taylor approximation,

$$SE(x_{5\%}) \approx \left( \frac{\partial x_{5\%}}{\partial \alpha_0} \frac{\partial x_{5\%}}{\partial \alpha_1} \right)^{1/2} \text{cov}(x_{5\%}) \left( \frac{\partial x_{5\%}}{\partial \alpha_0} \frac{\partial x_{5\%}}{\partial \alpha_1} \right)^{T} ,$$

where \( \text{cov}(x_{5\%}) \) was the covariance matrix returned from the maximum likelihood fit.

The regression analysis using the model presented in Equation 3.6 was implemented using the Matlab function ‘glmfit’ to determine the percentage collapse thresholds. When only one classification of data was available – for example, when using the automatic classifier – the standard deviation for the experimental data points was estimated from the fitted logistic curves using the binomial standard deviation formula,

$$SD(x) = 100 \times \sqrt{\frac{P(x)(1 - P(x))}{N(x)}} ,$$

where \( P(x) \times 100\% \) was the estimated percentage of collapse and \( N(x) \) was the total number of signals at a given pressure.

3.5 Results

3.5.1 Standard Definity and Optison

The three individual classifications for Definity UCAs at 2.8 MHz are presented in Figure 3.22. This example demonstrates the major features of the classification
Figure 3.22. Percentage postexcitation determined by three individual classifiers plotted against peak rarefactual pressure for Definity UCAs at 2.8 MHz. The average number of signals per data point per classifier (mean ± standard deviation) is 34 ± 11.

analysis as described above; at the lowest peak rarefractional pressures where single UCA signals were able to be identified, little to no postexcitation was observed. As PRPA was increased, the percentage of PES relative to the total number of individual microbubble signals also increased—in this instance, to a maximum at 100%.

The classification results for all four frequencies and both microbubble types are presented as mean ± standard deviation from the three individual classifiers in Figure 3.23. Additionally, the logistic curve fits to these averages and 95% confidence interval regions are shown. The maximum observed percentage of postexcitation for Definity UCAs ranged from approximately 70% to 100%, while this maximum for Optison was lower in most cases, ranging from approximately 20% to 90%.
Specific percentage postexcitation thresholds proportional to the maximum at each frequency were also determined from the logistic curves. Results for the 5% and 50% thresholds are listed in Table 3.4, along with their corresponding 95% confidence intervals. As frequency was increased for each type of UCA, the PRPA required to reach a specified threshold increased in most instances. The PRPA values for these thresholds were also consistently lower for Definity than for Optison, indicating that the Definity bubble population undergoes greater postexcitation collapse activity for an insonation with a high enough PRPA at a specified frequency.

Figure 3.23. Percentage postexcitation curves for Definity and Optison UCAs, plotted against PRPA and grouped by frequency. The asterisks (*) represent averages plotted with standard deviations from three persons who classified the experimental data. The solid (−) curve is the logistic fit, and the dotted (−−) curves represent the 95% confidence intervals. The average number of signals per data point (mean ± standard deviation) is 30 ± 9 for Definity and 20 ± 8 for Optison.
<table>
<thead>
<tr>
<th></th>
<th>Definity</th>
<th>Optison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>50%</td>
</tr>
<tr>
<td>0.9 MHz</td>
<td>0.19 MPa</td>
<td>0.54 MPa</td>
</tr>
<tr>
<td></td>
<td>(0.12 – 0.26)</td>
<td>(0.46 – 0.60)</td>
</tr>
<tr>
<td>2.8 MHz</td>
<td>0.68 MPa</td>
<td>1.22 MPa</td>
</tr>
<tr>
<td></td>
<td>(0.62 – 0.74)</td>
<td>(1.17 – 1.28)</td>
</tr>
<tr>
<td>4.6 MHz</td>
<td>1.63 MPa</td>
<td>2.65 MPa</td>
</tr>
<tr>
<td></td>
<td>(1.45 – 1.77)</td>
<td>(2.57 – 2.74)</td>
</tr>
<tr>
<td>7.1 MHz</td>
<td>2.10 MPa</td>
<td>2.67 MPa</td>
</tr>
<tr>
<td></td>
<td>(2.03 – 2.16)</td>
<td>(2.64 – 2.70)</td>
</tr>
</tbody>
</table>

Table 3.4. Percentage postexcitation thresholds with 95% confidence intervals in MPa PRPA, proportional to the maximum postexcitation observed at each frequency.

### 3.5.2 Automatic and Manual Classification

To validate the automatic classification, datasets were acquired and manually classified for Definity UCAs at 2.8, 4.6, and 7.1 MHz. A least-squares optimization was performed on the three parameters deemed most important for classification: maximum length of the principal response, maximum peak separation for harmonic response, and peak detection sensitivity. The values which minimized the sums of the squared differences compared to manual classification across the full range of pressures and frequencies were selected (Figure 3.24).

For the maximum principal response length, 4\( T \) yielded the most similar fit. This value was slightly longer than the 3\( T \) which might be expected from a 3 cycle insonification, but is appropriate when accounting for transducer ring down time. For the maximum peak spacing within the principal response, 1.02\( T \) was found to be most similar. This value is slightly larger than the 1\( T \) which might be expected of a purely forced harmonic response, but can be understood as helping to account for UCAs which are being insonified at a higher frequency than their natural frequency (i.e. larger UCAs). This value also establishes the
minimum time of detectability for a postexcitation peak, which must be greater than 102% of the insonifying period. Because this minimum detectability time varies with frequency, the average accompanying bubble growth and collapse associated with the postexcitation signal may also vary with frequency.

It is also apparent from Figure 3.24 that the best matching values vary with frequency, especially for 2.8 MHz compared to the two higher frequencies. This indicates that peaks and groupings of peaks were observed slightly differently at each frequency; in other words, there was some inconsistency in manual classification when applying the desired guidelines across frequencies. The automatic classifier only used a single value for these parameters regardless of frequency, which was more similar to manual classification at 4.6 and 7.1 MHz than at 2.8 MHz.

Figure 3.24. Sums of squared differences for 3 important automatic classifier optimization parameters, showing the local minima. (a) Maximum principal response length: 4T, (b) maximum principal response spacing: 1.02T, and (c) peak sensitivity 0.9ξ, where ξ equals twice the noise threshold.
Figure 3.25 shows the comparison between the automatic and manually classified signals for the 3 datasets. While the agreement was not exact, it captured similar trends to what was previously observed – namely, that PES percentage increased as PRPA increased, and increased as frequency decreased. With this result, the automatic classification routine was considered acceptable for use in subsequent double PCD research.

3.6 Discussion

In this study, double passive cavitation detection was successfully developed to use for observation of single ultrasound contrast agents and identify the presence
or absence of symmetric postexcitation signals. By reducing spatial variability in the location of the analyzed microbubbles with two matched receive transducers, a clear relationship between postexcitation occurrence and peak rarefactive pressure amplitude of the incident pulse was observed. Generally, this postexcitation percentage increased as PRPA increased for a given frequency, and this percentage decreased as frequency increased for a given PRPA. The observed trends for both types of microbubbles in this experiment are consistent with other experimental results of ultrasound contrast agent collapse [53, 54, 61, 64].

Bubble rebound will likely involve fragmentation of the gas content during transient collapse of the UCA. Inertial cavitation of spherical bubbles is often unstable and prone to broken symmetry, as demonstrated using perturbation analysis [84, 27]. Additionally, it has been observed both experimentally [85] and theoretically [83] that if the fragmentation of the bubble is such that the gas content is not of a critical size, it diffuses into the liquid without a violent rebound. Thus, it may be expected that while the PES is indicative of shell rupture and transient collapse of the bubble, the converse may not always be true when the experimental conditions are designed to capture a spherically symmetric response. While postexcitation is indicative of transient collapse, the potential for destruction of UCAs not involving PES suggests these reported thresholds should be considered lower limits on the percentage of UCAs being irreversibly altered. Equivalently, the experiment provides an upper bound for the PRPA threshold of inertially-cavitating, unstable UCAs.

The PES threshold behavior was significantly different between Definity and Optison at the 3 highest frequencies studied. This result indicates that the double PCD setup can be used to characterize UCAs by observation of PES, a property of collapse behavior that is dependent on the material makeup of the
The primary physical distinctions between these two types of UCAs are size distribution and shell composition, both of which may contribute to measured differences in thresholds and in maximum observed levels of PES. These differences are explored further in the following chapter.

Finally, these cavitation results were tested with the mechanical index. To compare the postexcitation cavitation results with the MI, the PES thresholds and 95% confidence intervals listed in Table 3.4 are plotted on the MI scale (Figure 3.26). A mechanical index around 0.1 is considered low MI, 0.2-0.7 is considered moderate MI, and above 0.8 is considered high MI, with an FDA regulatory limit of 1.9 [9]. The double PCD results show that postexcitation activity of these UCAs is particularly divergent from the MI scaling at the two lowest frequencies tested. Large percentages (50% and greater) of the populations of both types of microbubbles exhibit postexcitation at moderate MI levels for the lowest frequency tested, 0.9 MHz; at higher frequencies, Definity also undergoes PES activity within moderate MI, while Optison requires higher MI to achieve similar levels of PES activity at these higher frequencies. The lack of agreement with MI scaling adds to evidence that MI is an inadequate predictor of UCA cavitation activity [9].
Figure 3.26. Postexcitation thresholds and 95% confidence intervals at 5% (circles) and 50% (squares), plotted versus frequency on the mechanical index scale. Definity is plotted with open symbols, and Optison is plotted with closed symbols. Low, moderate, high, and above regulatory limit regimes are indicated with the horizontal dashed-dotted (−.) lines.
CHAPTER 4
POSTEXCITATION WITH ALTERED SIZE DISTRIBUTIONS

4.1 Introduction
In the initial PCD experiments, the two commercially available UCAs had noticeably different curves for percentage postexcitation collapse at the measured frequencies. While both of these contrast agents contained the same gas, they had two primary differences between them: shell composition and size distribution. Definity UCAs had a lipid-based shell and a reported mean diameter of 1.1 – 3.3 microns [79], while Optison had an albumin-based shell and a reported mean diameter of 3.0 – 4.5 microns [80]. Therefore, modified populations of commercial agents were created to change the size distributions and eliminate this difference. These altered UCAs were compared to each other and to standard population distributions at three insonification frequencies to study the influence of the shell and size distribution on postexcitation collapse percentages.
4.2 Bubble Sizing

There are several methods which can be used to size tiny particles like UCAs. Devices such as a coulter counter, which measure particle volume from electrical resistance changes, are frequently used [86, 87] to obtain distributions from large populations. Scattered laser light may be used to infer the bubble radius from a single bubble using Mie theory [88]. It is also common to measure size directly from microscopic images, particularly for single microbubbles [89, 39]. The latter method was used for this work, with a custom routine developed to address certain challenges of properly detecting and sizing many bubbles from microscope bright field images.

4.2.1 Methodology

After contrast agent activation and immediately prior to PCD experiment, a small drop of the bubbly mixture was placed between two microscope cover slips to size the UCAs. Images were acquired using a microscope and digital camera system (Axiovert 200M/Zeiss AxioCam MRm, Carl Zeiss Inc., Thornwood, NY or BX51TF, Olympus Corp, Tokyo) which yielded varying resolutions depending on the objective lens used. The images were calibrated using a stage micrometer; for 20x, 63x, and 100x objectives, the calibrated resolutions were 0.34, 0.10, and 0.06 µm/pixel, respectively. In typical grayscale images, UCAs appeared with a white center surrounded by a dark ring caused by light diffraction.

Due to relatively high concentrations, it was often necessary to distinguish among partially overlapping bubbles as well as those which were in and out of focus. A multi-step, semi-automatic process was used to extract the radii of detected circles from the images; this was implemented as a graphical user
interface in Matlab to provide immediate feedback for the results. First, the image was opened and the background was detected using a disk dilatation morphological operation. Next, preliminary binary segmentation was performed, and then the image was returned to grayscale. Following that, a Hough transform was performed on the image to detect circles from the background image. Finally, the identified circles were overlaid on top of the original image, and the user was given the ability to adjust any incorrectly or poorly detected bubbles (Figure 4.1). The estimated error in sizing was up to 2 pixels after performing these steps.

4.2.2 Validation of sizing code

The sizing process was tested by measuring known glass particles (Duke Standards 9000 Series Glass Particles, Thermo Scientific, Waltham, MA). Two distributions of spheres with different diameters were tested using a 20x objective with a resolution of 0.34 μm/pixel; the first was quoted as 42.3 ± 1.1 μm.
4.3 Measuring UCA Size Distributions

4.3.1 Standard Definity and Optison Distributions

The size distributions for the native Definity and Optison populations were measured after following normal activation procedures. The mean diameter for Definity was measured to be 1.99 μm with less than 0.1% of UCAs larger than 10 μm, consistent with the reported mean diameter range of 1.1 to 3.3 μm, with 98% having a diameter less than 10 μm. The mean diameter for Optison was
measured to be 4.24 µm with less than 4% of UCAs larger than 10 µm, also consistent with the reported mean diameter range of 3.0 to 4.5 µm, with 95% having a diameter less than 10 µm. Histograms of these two measured distributions are shown in Figure 4.3, while the mean, median, standard deviation, and skewness of the distributions are listed in Table 4.1.

![Figure 4.3. Measured size distribution of UCAs with standard preparation, with a count of approximately 6500 Definity microbubbles and approximately 3200 Optison microbubbles.](image)

<table>
<thead>
<tr>
<th>UCA Type</th>
<th>Mean Diameter</th>
<th>Median Diameter</th>
<th>Standard Deviation</th>
<th>Skewness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Definity</td>
<td>1.99 µm</td>
<td>1.80 µm</td>
<td>0.54</td>
<td>2.90</td>
</tr>
<tr>
<td>Standard Optison</td>
<td>4.24 µm</td>
<td>3.60 µm</td>
<td>2.44</td>
<td>2.23</td>
</tr>
<tr>
<td>Altered Definity</td>
<td>2.50 µm</td>
<td>1.80 µm</td>
<td>2.32</td>
<td>5.31</td>
</tr>
<tr>
<td>Altered Optison</td>
<td>2.61 µm</td>
<td>2.04 µm</td>
<td>1.68</td>
<td>3.08</td>
</tr>
</tbody>
</table>

Table 4.1. Statistics of the four tested UCA population distributions.
4.3.2 Altered UCA Size Distributions

Since the populations of Definity and Optison were substantially different (Figure 4.3), altering the size distributions of both of these UCAs was necessary to match their sizes. These changes could be accomplished in multiple ways; ultimately, one method was chosen to decrease the size of Optison and a different method was used to increase the size of Definity.

Optison was normally activated by gently rocking and inverting the vial to resuspend the microbubbles. A decantation technique was used to decrease the size of Optison in order to take advantage of the inherent buoyancy of the bubbles. Since large bubbles rise faster than small microbubbles, the decantation method provides natural filtering for the UCA population [41, 86]. This alternate activation procedure involved resuspending the microbubbles in the vial as before but letting it stand upright for 30 minutes rather than immediately extracting the UCAs. Optison was then withdrawn from a location approximately 1 cm from bottom of the vial. This change in the activation process decreased the mean size of Optison by nearly 50% to 2.61 µm. One downside was a significant decrease in concentration as decantation time increased; waiting longer than 30 minutes reduced the concentration of UCAs so much as to make subsequent analysis difficult.

A simplistic prediction of the change in size distribution from the bubble buoyancy may be obtained by assuming UCAs rise at Stokes terminal velocity for small particles. This velocity is given by

\[
\nu_{\text{stokes}} \approx \frac{2(\rho_p - \rho_f)g}{9\mu} R^2 ,
\]

where \( \rho_p \) is the particle density, \( \rho_f \) is the fluid density, \( g \) is gravitational acceleration, \( \mu \) is the dynamic viscosity, and \( R \) is the bubble radius. A model
based on this expression relies on assumptions of no edge effects, no bubble interaction, and no variation of background density of the fluid medium; these approximations are violated to varying extents as time progresses and bubbles rise and cluster.

Assuming that the initial size distribution of UCAs is randomly distributed in depth, the distribution of bubbles which will be at a specified depth can be predicted at any subsequent time using this formula. Using a withdrawal height of 1 cm after 30 minutes, this predicts a mean diameter of 2.58 µm which is very close to the measured mean diameter. However, the histogram shows that the predicted distribution is not very similar to the measured altered distribution in actuality (Figure 4.4). While such a model has been suggested in the literature [86] and may be accurate enough for quick calculations with relatively small changes in distribution, it was not found to be particularly useful for the current UCA study.

In contrast to the gentle activation of Optison, the standard method for activating Definity microbubbles was to insert the vial into a rapidly shaking agitator (VialMix, Lantheus Medical Imaging, N. Billerica, MA) for 45 seconds. To broaden the size distribution of these lipid shelled contrast agents, it was necessary to decrease the agitation time so that fewer large Definity microbubbles were broken up. It was determined that reducing the activation time to 10 seconds increased the mean size of Definity by approximately 25% to 2.50 µm, due primarily to the increased amount of larger UCAs. The altered Definity distribution closely matched the altered Optison distribution, as shown in Figure 4.5; additional statistical measures of these distributions are listed in Table 4.1.
Figure 4.4. Measured size distributions of standard and altered Optison, shown with the prediction from the Stokes velocity model.

Figure 4.5. Measured size distribution of UCAs with alternate preparation, approximately 3800 Definity microbubbles and approximately 2500 Optison microbubbles.
4.4 Results

As presented in Figure 4.5, the two altered populations of Definity and Optison had nearly the same size distribution. These altered microbubble populations were tested for postexcitation rebound using same double PCD experiment described previously. The percentage postexcitation comparison between these two altered populations is shown in Figure 4.6. This figure demonstrates that significant differences were still detected between lipid-shelled and albumin-shelled UCAs at these frequencies, as was observed previously with standard Definity and Optison populations (Figure 3.23). Results for the 5% and 50% curve fit thresholds at 2.8, 4.6, and 7.1 MHz are listed in Table 4.2, along with their corresponding 95% confidence intervals. As frequency increased for each type of UCA, the PRPA required for reaching a specified threshold typically increased as before. The PRPA values for the 50% thresholds again remained consistently lower for altered Definity than for altered Optison, though the shallower slope for the latter UCAs meant this was not always the case for the 5% thresholds.

It is also instructive to consider the comparison between the standard distribution and the altered distribution for each type of UCA. The percentage postexcitation curves for automatically classified altered and standard Definity distributions are shown in Figure 4.7, while those for the both Optison distributions are shown in Figure 4.8. From these figures, it is clear that there was substantial overlap between standard and altered populations for both types of UCAs. The largest exception occurred for Definity at 4.6 MHz, though Optison at 4.6 MHz also showed differences. Nevertheless, in nearly all of the other cases, differences between the standard and altered population curves were within the 95% confidence intervals and were less different than similarly sized UCA populations with different surface coatings.
Figure 4.6. Percentage postexcitation curves for altered Definity and Optison UCA populations at a) 2.8 MHz, b) 4.6 MHz, and c) 7.1 MHz. The asterisks (*) represent autoclassifier results plotted with estimated standard deviations from the experimental data. The solid (–) curves are the logistic fits, and the dotted (---) curves represent the 95% confidence intervals.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Altered Definity</th>
<th>Altered Optison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>50%</td>
</tr>
<tr>
<td>2.8 MHz</td>
<td>0.58 MPa</td>
<td>0.80 MPa</td>
</tr>
<tr>
<td></td>
<td>(0.51 – 0.64)</td>
<td>(0.75 – 0.85)</td>
</tr>
<tr>
<td>4.6 MHz</td>
<td>1.16 MPa</td>
<td>1.65 MPa</td>
</tr>
<tr>
<td></td>
<td>(1.01 – 1.26)</td>
<td>(1.56 – 1.72)</td>
</tr>
<tr>
<td>7.1 MHz</td>
<td>1.64 MPa</td>
<td>3.61 MPa</td>
</tr>
<tr>
<td></td>
<td>(1.08 – 1.98)</td>
<td>(3.30 – 4.19)</td>
</tr>
</tbody>
</table>

Table 4.2. Percentage postexcitation thresholds with 95% confidence intervals in MPa PRPA, proportional to the maximum postexcitation observed at each frequency.
Figure 4.7. Percentage postexcitation curves for standard and altered Definity UCA populations at a) 2.8 MHz, b) 4.6 MHz, and c) 7.1 MHz.

4.5 Discussion

First and foremost, the results from this study demonstrate experimentally that shell composition has a significant and noticeable impact on UCA collapse dynamics, as seen in Figure 4.6. While initial studies of Definity and Optison showed behavioral variations between contrast agents, identifying the primary cause of these disparities was obscured by differences in several material parameters. In contrast, the altered distributions of two types of UCAs in these experiments had very similar size distributions but still yielded distinct postexcitation curves.
Figure 4.8. Percentage postexcitation curves for standard and altered Optison UCA populations at a) 2.8 MHz, b) 4.6 MHz, and c) 7.1 MHz.

While the surface coating clearly affects postexcitation collapse, it does not immediately follow that it is the only factor and that size has no impact. In particular, the results for both UCAs at 4.6 MHz suggest that within certain frequency ranges it will matter significantly. But it still holds that, in comparing the postexcitation curves generated under the same conditions for standard and altered distributions, much smaller differences were generally observed across these frequencies than when comparing shell types. This was even the case for Optison where the mean diameter decreased by nearly 50%, and suggests that within the range of sizes tested, the initial bubble radius usually plays a lesser role in postexcitation collapse.
Furthermore, while the altered distribution curves overlap to a significant degree with the native distribution curves, a certain trend for the slopes may be observed. The altered populations for lipid-shelled Definity UCAs each had a slightly steeper slope than the standard populations. If bubble size were the dominant factor in explaining differences in postexcitation curves, a faster transition between no postexcitation and maximum postexcitation would be indicative of a narrower UCA population; however, for Definity, this is contradicted by the size distribution measurements which indicated the altered populations had a broader size distribution than the standard distribution.

Likewise, though to a lesser extent, differences in slope trends may be observed for albumin-shelled Optison. For the two lowest of three frequencies tested, the altered populations have a slightly shallower slope, which would be expected to be linked to a broader population were size the dominant factor. Again, this is in direct contrast to what was measured for the altered Optison size distribution which had a smaller standard deviation. This adds to the evidence that the bubble diameter probably only plays a minor role relative to shell composition in altering postexcitation percentage curves for UCAs over a typical range of sizes and insonifying frequencies.
CHAPTER 5
SPHERICAL MODELING OF SHELLED BUBBLES

5.1 Introduction
The percentage postexcitation results obtained from the double PCD experiment described large amplitude microbubble responses that were similar at multiple receiving angles. Because these postexcitation responses considered were largely symmetric, several variations of single bubble UCA models were fitted to the experimental results. This procedure evaluated the impact of shell model parameters on theoretical responses and also provided insight into the strength of UCA collapse needed to generate postexcitation rebound signals. The goal of this modeling was not to accurately replicate the full dynamics of a collapsing, fragmenting, and rebounding bubble, but rather to propose that experimental thresholds obtained from the rebound signal associated with shell rupture could be linked to a simplified model as in the case of the transient inertial collapse of a free bubble. This theoretical process was found to yield a good agreement with experimental data in many cases, and it was further extended to evaluate several alterations to the material makeup of the UCAs.
5.2 Theoretical Modeling

By assuming spherical symmetry, existing single bubble models describe bubble dynamics in terms of a bubble’s time dependent radius, $R(t)$ and its associated derivatives. The primary theoretical model used was a modified version of the Marmottant equation, which was originally written as \[\rho \left( R\ddot{R} + \frac{3}{2} \dot{R}^2 \right) = p_{\text{gas}} + \frac{R}{c} \frac{d}{dt} p_{\text{gas}} - \frac{2\sigma(R)}{R} \frac{\dot{R}}{R} - \frac{4\mu R}{R^2} - \frac{4\kappa R}{R^2} - P_0 - P_{ac}(t), \] (5.1)

where

\[p_{\text{gas}} + \frac{R}{c} \frac{d}{dt} p_{\text{gas}} = \left( P_0 + \frac{2\sigma(R_0)}{R_0} \right) \left( \frac{R}{R_0} \right)^{-3\kappa} \left( 1 - \frac{3\kappa}{c} \dot{R} \right). \] (5.2)

In these equations, $\rho$ and $c$ were the density and speed of sound of the surrounding medium, $P_0$ and $P_{ac}(t)$ were the ambient pressure and acoustic pressure, $\mu$ was the surrounding liquid viscosity, $\kappa$ was the polytropic gas exponent (assumed to be adiabatic), and $\kappa_s$ was the monolayer surface dilatational viscosity. The size dependent surface tension, $\sigma(R)$, was given by

\[\sigma(R) = \begin{cases} 0 & \text{if } R \leq R_{\text{buckling}} \\ \chi \left( \frac{R^2}{R_{\text{buckling}}^2} - 1 \right) & \text{if } R_{\text{buckling}} \leq R \leq R_{\text{breakup}} \\ \sigma_{\text{water}} & \text{if ruptured, } R_{\text{rupture}} \leq R \end{cases}, \] (5.3)

where $\chi$ was the elastic compression modulus.

5.2.1 Van der Waals

For especially strong single bubble collapses such as those studied in sonoluminescence literature, it is not uncommon to model the gas pressure inside the bubble as a van der Waals gas rather than an ideal gas [37, 28]. Using this form restricts the minimum size to which a bubble may collapse and
subsequently affects the rebound profile. Because the modeled UCAs in the postexcitation simulations were undergoing large amplitude responses including inertial cavitation and rebound, the Marmottant model was modified to incorporate a van der Waals gas rather than an ideal gas, a change given by [91]

\[ p_{\text{gas}} + \frac{R_c}{c} \frac{d}{dt} p_{\text{gas}} = \left( P_0 + \frac{2\sigma(R_0)}{R_0} \right) \left( \frac{R^3 - R_c^3}{R_0^3 - R_c^3} \right)^{-\kappa} \left( 1 - \frac{3\kappa}{c} \frac{R^3}{R_0^3 - R_c^3} \right) \dot{R}. \]  

(5.4)

Using this description of the interior gas pressure required the determination of the van der Waals’ hard-core radius, \( R_c \). The van der Waals equation of state is

\[ (P + \frac{n^2 a}{V^2})(V - nb) = nR^*T_k, \]

(5.5)

where \( V \) is the volume, \( n \) is the number of moles, \( R^* \) is the universal gas constant, \( T_k \) is the absolute temperature, and \( a \) and \( b \) are the van der Waals’ constants [92]. From this equation, the excluded volume of the system is \( V = nb \). Therefore the hard core radius can be approximated using the equation

\[ R_c \approx \left( \frac{Pb}{RT} \right)^{1/3} R_0. \]

(5.6)

For perfluoropropane, the constant \( b = 0.0001338 \text{ [m}^3/\text{mol]} \) [93], and therefore the hard core radius is approximately \( R_c \approx R_0/5.6 \). By way of comparison, the hard core radius for air is \( R_c \approx R_0/8.54 \) [37].

While there were only slight differences in the UCA responses across the majority of the tested range of pressures and radii (Figure 5.1), the largest percentage difference in terms of maximum radial expansion between the original and modified models was approximately 130%. This suggested that there was some value in using the more complex equation for the tested parameter space.
5.2.2 Non-Ruptured Shell Model

The full UCA model (Equations 5.1, 5.3, 5.4) was also simplified to other existing bubble equations to help evaluate the role of the shell parameters in modeling. By considering a constant surface tension, $\sigma$, rather than a size dependent surface tension (Equation 5.3), the model was used to evaluate a shell that remained in the purely elastic regime rather than buckled or ruptured during large amplitude behavior. This form of the model used was closely related to the earliest UCA models, as initially proposed by de Jong [30] and later rewritten by van der Meer as [94]

$$
\rho \left( R\ddot{R} + \frac{3}{2} \dot{R}^2 \right)
$$

$$
= p_{\text{gas}} + \frac{R}{c} \frac{d}{dt} p_{\text{gas}} - \frac{2\sigma}{R} - 4\kappa \left( \frac{1}{R_0} - \frac{1}{R} \right) - 4\mu \dot{R} - \frac{4\kappa \dot{R}}{R^2} - P_0 - P_a(t). 
$$

(5.7)
5.2.3 Unshelled Model

A comparison with unshelled bubble models was also considered by simplifying the primary model to remove shell terms. The modified Rayleigh-Plesset equation previously used in large amplitude free bubble studies [36, 28] is recovered when the shell term $\kappa$ is set to zero and the surface tension $\sigma(R)$ from Equation 5.3 is always set to a constant (in this case, water),

$$
\rho \left( R \ddot{R} + \frac{3}{2} \dot{R}^2 \right) = p_{\text{gas}} + \frac{R}{c} \frac{d}{dt} p_{\text{gas}} - \frac{2 \sigma}{R} - \frac{4 \mu \dot{R}}{R} - P_0 - P_{ac}(t). 
\tag{5.8}
$$

While all three shell models were idealizations of the actual collapse dynamics, two of the models were more obviously used outside their range of applicability. Certainly, the non-rupturing shell model would not apply to the case of a collapsing, fragmenting bubble. Likewise, the free bubble model would not describe the dynamics of unruptured UCAs, as it lacks the necessary damping terms on the motion. These cases were considered primarily to contrast their results with that of the Marmottant model.

A comparison of the effects of these three modeling forms for the shell impact is shown in Figure 5.2. With no shell present, the UCA responded more strongly and had less viscosity to quickly damp out oscillations, while a non-rupturing elastic shell significantly restricted the maximum growth of the UCA.

5.3 Simulation Procedures

Numerous variables affect the modeled response of an ultrasound contrast agent, including the forcing acoustic pressure waveform, the bubble size, and the material properties of the bubble. These parameters were determined either by direct measurement or from values reported in the literature. After evaluating
Figure 5.2. Simulated radial expansion of 1 µm radius Definity UCA for a 1.53 MPa PRPA pulse at 2.8 MHz, comparing three different forms of shell effect models.

the response of the bubble, thresholding criteria were used to compare the simulated UCA responses to the experimental responses.

5.3.1 UCA Properties
The fluid medium properties were chosen for water at room temperature and pressure. The interior gas properties of the UCAs were chosen for either octafluoropropane, used in both Definity or Optison, or air. Viscoelastic shell properties were determined by converting values previously obtained in other experiments, usually attenuation measurements of the linear UCA response, to the current model parameters. The complete list of parameter values used in these simulations is recorded in Table 5.1.

The two size dependent shell state parameters, $R_{\text{buckling}}$ and $R_{\text{breakup}}$, deserve special mention. In the original paper, several fitted examples are provided for the buckling radius, using values very close to the initial radius from $0.99R_0$ to $R_0$. 

87
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluid Medium Properties</strong></td>
<td></td>
</tr>
<tr>
<td>$\rho$</td>
<td>1000 [kg/m$^3$]</td>
</tr>
<tr>
<td>$P_0$</td>
<td>101.325 [kPa]</td>
</tr>
<tr>
<td>$c$</td>
<td>1480 [m/s]</td>
</tr>
<tr>
<td>$\mu$</td>
<td>0.001 [Pa s]</td>
</tr>
<tr>
<td>$\sigma_{\text{water}}$</td>
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</tr>
<tr>
<td><strong>Gas Properties</strong></td>
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</tr>
<tr>
<td>$\kappa$ (C$_3$F$_8$)</td>
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</tr>
<tr>
<td>$R_c$ (C$_3$F$_8$)</td>
<td>$R_0/5.6$ [93]</td>
</tr>
<tr>
<td>$\kappa$ (Air)</td>
<td>1.4 [37]</td>
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<tr>
<td>$R_c$ (Air)</td>
<td>$R_0/8.54$ [37]</td>
</tr>
<tr>
<td><strong>Original Definity Shell Properties</strong></td>
<td></td>
</tr>
<tr>
<td>$\kappa_s$</td>
<td>$2.4 \times 10^{-9}$ [kg/s] [72, 97]</td>
</tr>
<tr>
<td>$\chi$</td>
<td>0.38 [N/m] [72, 97]</td>
</tr>
<tr>
<td>$R_{\text{buckling}}$</td>
<td>0.99$R_0$ [47]</td>
</tr>
<tr>
<td>$R_{\text{breakup}}$</td>
<td>$R_{\text{rupture}}$ (=1.08$R_0$), 1.5$R_0$ [47, 46]</td>
</tr>
<tr>
<td><strong>Optison Shell Properties</strong></td>
<td></td>
</tr>
<tr>
<td>$\kappa_s$</td>
<td>$76.5 \times 10^{-9}$ [kg/s] [42]</td>
</tr>
<tr>
<td>$\chi$</td>
<td>0.932 [N/m] [42]</td>
</tr>
<tr>
<td>$R_{\text{buckling}}$</td>
<td>0.99$R_0$</td>
</tr>
<tr>
<td>$R_{\text{breakup}}$</td>
<td>$R_{\text{rupture}}$ (=1.32$R_0$)</td>
</tr>
<tr>
<td><strong>Alternate Definity Shell Properties</strong></td>
<td></td>
</tr>
<tr>
<td>‘Fresh’ $\kappa_s$</td>
<td>$3.98 \times 10^{-9}$ [kg/s] [98]</td>
</tr>
<tr>
<td>‘Fresh’ $\chi$</td>
<td>0.82 [N/m] [98]</td>
</tr>
<tr>
<td>‘Expired’ $\kappa_s$</td>
<td>$11.67 \times 10^{-9}$ [kg/s] [98]</td>
</tr>
<tr>
<td>‘Expired’ $\chi$</td>
<td>0.79 [N/m] [98]</td>
</tr>
</tbody>
</table>

Table 5.1. Values used for simulations.

It was recently demonstrated that changes in the initial surfactant concentration, which is related to both the initial surface tension and the buckling radius, influenced several common behaviors closely associated with lipid UCAs [95]. However, variation of this parameter was not considered for simplicity.
The $R_{\text{breakup}}$ parameter choice also deserves further discussion. As given, the rupture radius is determined by

$$R_{\text{rupture}} = R_{\text{buckling}} \left(1 + \frac{\sigma_{\text{water}}}{\chi}\right)^{\frac{1}{2}}. \quad (5.9)$$

However, the breakup radius was left as an undetermined variable in the original paper since the breakup surface tension was unknown (and potentially variable from bubble to bubble). From the two fitted examples in the Marmottant paper, it is observed that one example used $R_{\text{breakup}} = 1.06R_0$ while the other used $R_{\text{breakup}} > 1.4R_0$. In a separate study involving slow, quasi-static lipid microbubble growth and dissolution [96], it has been reported that the best fit surface tension for their model is a 7-fold increase over that of water; assuming an elastic compression modulus of 0.5 to 1.0 N/m as is typical for a lipid bubble shell gives $1.22R_0 \leq R_{\text{breakup}} \leq 1.41R_0$. In other studies which use the Marmottant model, $R_{\text{breakup}}$ has been chosen to be $1.2R_0$ [72] and $1.5R_0$ [46]. Therefore, several breakup radii were tested in the range from $R_{\text{rupture}}$ to $1.5R_0$; in nearly all cases, only minor differences (less than 10% in terms of predicted pressure thresholds) were observed among this range of breakup radii. Results are therefore reported using two values for the breakup radius, $R_{\text{rupture}}$ and $1.5R_0$, which give the greatest differences.

### 5.3.2 Simulated Pulses

Given that water is a highly nonlinear medium, the large acoustic pressures used experimentally led to an asymmetrical waveform at the transducer focus. In order to match more closely the conditions at the confocal region while also allowing for more pressure levels to be tested than were used experimentally, simulated waveforms were generated as 3 cycle Gaussian-weighted pulses.
modified using a time-domain form of the KZK equation to account for the effects of diffraction, absorption, and nonlinearity during propagation in water [99, 100, 101, 102]. The resulting simulated waveforms yielded better agreement with calibration measurements in terms of peak compression and peak rarefaction at the focal region than did symmetric waveforms (Figure 5.3).

5.3.3 Code Evaluation

The simulations were evaluated using a stiff solver routine (ode23s) in Matlab (The MathWorks, Natick, MA). The initial radii ranged from 0.2 µm to 10.0 µm in increments of 0.1 µm and the bubble wall was assumed to be initially at rest. Three-thousand UCAs were randomly sampled from the population distribution at each pressure; this number was two orders of magnitude greater than the quantity of signals collected in a typical experiment but of the same order of magnitude as the number of UCAs sized for the population distributions. The
Figure 5.4. Simulated radial expansion of 1 µm radius Definity UCA for a 1.28 MPa PRPA pulse at 2.8 MHz.

PRPAs covered the range for each of the four frequencies used in the double PCD experiment: 0.9 MHz (0.1 to 1.6 MPa), 2.8 MHz (0.1 to 5.8 MPa), 4.6 MHz (0.2 to 6.7 MPa), and 7.1 MHz (0.1 to 6.4 MPa), in increments of approximately 0.1 MPa. A sample solution plotted with the insonifying pulse is shown in Figure 5.4.

5.3.4 Thresholding for Postexcitation

Rather than assuming any postexcitation peak from these spherically modeled bubbles will directly correspond with experimental PES measurements of a collapsing UCA, the resulting radius-time curves were instead interrogated for several other thresholding criteria: maximum radius, minimum radius, and maximum velocity, each in non-dimensional and dimensional forms. These
values were used to set up indicator functions for whether or not a bubble would be considered to have undergone postexcitation as a function of insonifying frequency, PRPA, and initial microbubble radius. For example, the indicator function for maximum radial expansion, $R_{\text{max}}$, relative to the initial radius was written as

$$I(f, \text{PRPA}, R_0) = \begin{cases} 1 & \text{if } R_{\text{max}} \geq a(f)R_0 \\ 0 & \text{otherwise} \end{cases}$$

(5.10)

The threshold value, $a(f)$, was similar in form to that which is often used to define the inertial cavitation threshold of a bubble, and this threshold was individually determined for each frequency. The test function was weighted by the measured bubble distribution to determine the predicted percentage of postexcitation signals at each simulated pressure level; the threshold values were
then chosen by minimizing the sum of the squared difference to the experimental data points (Figure 5.5). As indicated in Figure 5.6, the non-dimensional maximum radial expansion criterion had the greatest similarity to the experimental data, and was therefore the selected criterion.

5.4 Results

5.4.1 Definity

The predicted $R_{\text{max}}$ values for each frequency as a function of initial radius and peak rarefactional pressure amplitude from the Marmottant equation for the case where $R_{\text{breakup}} = R_{\text{capture}}$ are plotted in Figure 5.5. These figures demonstrate trends in the response of microbubbles. As pressure is increased for a given frequency, the microbubble size which responds most strongly shifts toward smaller bubbles – a downward shift in ‘resonance’ frequency which has been observed at
Figure 5.7. Maximum radial expansion $R_{\text{max}}$ of Definity bubbles calculated using the Marmottant equation with $R_{\text{breakup}} = R_{\text{rupture}}$. The black lines indicate the threshold $R_{\text{max}}$ above which postexcitation is assumed to occur for optimal fit to the experimental results. (a) 0.9 MHz, $12.1R_0$ (b) 2.8 MHz, $5.0R_0$ (c) 4.6 MHz, $5.3R_0$ and (d) 7.1 MHz, $3.3R_0$.

increasing pressures for both free and shelled bubbles \([32, 103]\). For a specified PRPA, an increase in frequency is seen to narrow the range of bubble sizes expanding to a specific $R_{\text{max}}$.

The black curves on Figure 5.7 indicate the lowest pressure at which a specified microbubble size reaches the threshold $R_{\text{max}}$ value yielding the best comparison with the experimental postexcitation results. These threshold $R_{\text{max}}$ values are $12.1R_0$ at 0.9 MHz, $5.0R_0$ at 2.8 MHz, $5.3R_0$ at 4.6 MHz, and $3.3R_0$ at 7.1 MHz. For PRPAs higher than the threshold curves, the indicated microbubble size was assumed to undergo collapse with observable postexcitation emissions.
By weighting the threshold results (Figure 5.7) with the standard Definity population distribution (Figure 4.3), the percentage of PES as a function of PRPA was obtained. The curve fitting results using the logistic equation (Equation 3.6) for each frequency are presented in Figure 5.8, comparing the Definity experimental data and four simulated results: the Marmottant model with $R_{\text{breakup}} = R_{\text{rupture}}$, the Marmottant model with $R_{\text{breakup}} = 1.5R_0$, the constant shell model, and the modified Rayleigh-Plesset (free) model. Table 5.2 lists the 5% and 50% postexcitation thresholds obtained from these curves, as well as the threshold $R_{\text{max}}$ values for each model giving an optimal fit for the simulated data. The threshold $R_{\text{max}}$ was larger for those bubbles modeled as free rather than shelled, and was smallest for those bubbles with non-rupturing shells.

At 0.9 MHz and 2.8 MHz, all models fit the experimental results similarly. At 4.6 MHz, the Marmottant and unshelled models still fit the data with reasonably good accuracy, but the constant shell model fit less well, with a slope that was overly steep. Both Marmottant curves also fit the data well at 7.1 MHz; however, the slope of the fit using the Rayleigh-Plesset model was insufficiently steep compared to the experimental data. The large standard deviations for the 0.9 MHz experimental data compared to other frequencies suggests there may be only limited value in trying to interpret fitted simulation results to these data.
Figure 5.8. Simulated percentage postexcitation curves for Definity using a free bubble model, constant shell model, and Marmottant model at (a) 0.9 MHz, (b) 2.8 MHz, (c) 4.6 MHz, and (d) 7.1 MHz. Experimental data points (mean ± standard error) are shown as asterisks (*).
<table>
<thead>
<tr>
<th>Frequency</th>
<th>Experiment Definity</th>
<th>Free</th>
<th>Simulations ( R_{\text{rupture}} = \frac{R_{\text{breakup}}}{1.5R_0} )</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( R_{\text{breakup}} = 1.5R_0 )</td>
<td>( R_{\text{breakup}} = R_{\text{rupture}} )</td>
<td></td>
</tr>
<tr>
<td>0.9 MHz</td>
<td>5%</td>
<td>0.59</td>
<td>0.62</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>(0.12 – 0.26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.85</td>
<td>0.86</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>(0.46 – 0.60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Threshold ( R_{\text{max}} )</td>
<td>13.0</td>
<td>12.1</td>
<td>7.2</td>
</tr>
<tr>
<td>2.8 MHz</td>
<td>5%</td>
<td>0.75</td>
<td>0.79</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>(0.62 – 0.74)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>1.22</td>
<td>1.20</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>(1.17 – 1.28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Threshold ( R_{\text{max}} )</td>
<td>5.9</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>4.6 MHz</td>
<td>5%</td>
<td>1.53</td>
<td>1.93</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>(1.45 – 1.77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>2.65</td>
<td>2.70</td>
<td>2.85</td>
</tr>
<tr>
<td></td>
<td>(2.57 – 2.74)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Threshold ( R_{\text{max}} )</td>
<td>5.7</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>7.1 MHz</td>
<td>5%</td>
<td>1.24</td>
<td>1.83</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>(2.03 – 2.16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>2.52</td>
<td>2.52</td>
<td>2.55</td>
</tr>
<tr>
<td></td>
<td>(2.64 – 2.70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Threshold ( R_{\text{max}} )</td>
<td>3.5</td>
<td>3.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table 5.2. Experimental percentage postexcitation thresholds (with 95% confidence intervals) in MPa PRPA, proportional to the maximum observed in the pressure range at each frequency, compared with simulated thresholds given by the specified \( R_{\text{max}} \).

### 5.4.2 Optison

In contrast to the case of Definity, the validity of using the Marmottant model with the Optison shell surface is perhaps less likely, since it is unclear if these types of thin albumin layers exhibit similar buckling and rupturing behaviors as thin lipid layers. Nevertheless, the three types of models were tested with the Optison experimental results using the same procedures: the Marmottant model...
Figure 5.9. Maximum radial expansion $R_{\text{max}}$ of Optison bubbles calculated using the Marmottant equation with $R_{\text{breakup}} = R_{\text{rupture}}$. Note the difference in axes and scaling from Figure 5.7. The black lines indicate the threshold $R_{\text{max}}$ above which postexcitation is assumed to occur for optimal fit to the experimental results. (a) 0.9 MHz, 3.0$R_0$ (b) 2.8 MHz, 3.8$R_0$ (c) 4.6 MHz, 2.3$R_0$ and (d) 7.1 MHz, 2.0$R_0$.

with $R_{\text{breakup}} = R_{\text{rupture}}$, the constant shell model, and the modified Rayleigh-Plesset (free) model.

As demonstrated in Figure 5.9, the large increase in shell dilatational viscosity greatly restricts the growth of these UCAs compared to Definity, and therefore the $R_{\text{max}}$ threshold was correspondingly smaller. In general, the fitting results presented in Table 5.3 and Figure 5.10 were not as good as for Definity UCAs. The unshelled model failed much more dramatically than in the previous case, and while the shelled models matched the experiment reasonably well at the lower frequencies, they differed from the 7.1 MHz results considerably. The
Marmottant model does appear to be the best option again at the majority of the frequencies, but the fit was not as good as for the lipid shelled Definity.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Experiment Optison</th>
<th>Simulations</th>
<th>Simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Free</td>
<td>$R_{breakup} = R_{rupture}$</td>
</tr>
<tr>
<td>0.9 MHz</td>
<td>5%</td>
<td>0.47 MPa (0.19 – 0.59)</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.72 MPa (0.56 – 0.78)</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td><strong>Threshold $R_{max}$</strong></td>
<td>6.9</td>
<td>3.0</td>
</tr>
<tr>
<td>2.8 MHz</td>
<td>5%</td>
<td>2.62 MPa (0.76 – 3.27)</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>3.75 MPa (2.53 – 4.09)</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td><strong>Threshold $R_{max}$</strong></td>
<td>5.9</td>
<td>3.8</td>
</tr>
<tr>
<td>4.6 MHz</td>
<td>5%</td>
<td>2.20 MPa (1.32 – 2.75)</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>4.17 MPa (3.67 – 4.40)</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td><strong>Threshold $R_{max}$</strong></td>
<td>3.8</td>
<td>2.3</td>
</tr>
<tr>
<td>7.1 MHz</td>
<td>5%</td>
<td>2.73 MPa (2.27 – 3.02)</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>4.07 MPa (3.87 – 4.28)</td>
<td>3.82</td>
</tr>
<tr>
<td></td>
<td><strong>Threshold $R_{max}$</strong></td>
<td>3.9</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table 5.3. Experimental percentage postexcitation thresholds (with 95% confidence intervals) in MPa PRPA, proportional to the maximum observed in the pressure range at each frequency, compared with simulated thresholds given by the specified $R_{max}$. 
5.4.3 Altered Size Distributions

The altered size distributions measured in Chapter 4 for Definity and Optison UCAs were used to model this change using the Marmottant model. In these simulations, the threshold $R_{\text{max}}$ value from the original distribution was used; the only modification was changing the size distribution to the altered ones. Therefore, larger Definity UCAs received a greater sampling of the weighting, as did smaller Optison UCAs.

The effects are plotted in Figures 5.11 and 5.12. Only minor changes are observed, most notably for Definity UCAs at higher frequencies. In these cases,
there is a slight downward shift toward more postexcitation at lower PRPAs but also a shallower slope and smaller maximum postexcitation percentage; this trend is most evident at 4.6 MHz and 7.1 MHz, the two highest frequencies tested. Almost no changes are observed for Optison. Since both altered distributions are roughly the same size and the results of these simulations continue to show large differences between altered Definity and altered Optison UCAs, the theoretical results also suggest that the shell composition does play a very significant role in the postexcitation collapse response.

Figure 5.11. Predicted effects of altering the size distribution for Definity UCAs at (a) 0.9 MHz, (b) 2.8 MHz, (c) 4.6 MHz, and (d) 7.1 MHz.
5.4.4 Interior gas

The effect of replacing the inner gas from octafluoropropane to air was another material variable that was simulated using the Marmottant model with Definity UCAs. One strong motivation for investigating this effect was that UCA vials were reused for multiple trials. Once the vial seal was broken and the contents were exposed to air, gas diffusion is present to some degree, altering the concentration of C₃F₈ and air mixture within the UCAs upon reactivation.

The inner gas material does influence on the growth of the bubble, particularly later in the oscillation cycle after strong rebounds (Figure 5.13). Air, being less dense of a gas, may compress more tightly and subsequently rebound
to a larger size as the forcing pulse ends. However, the impact is minor under the tested conditions; computation of the full postexcitation curves reveals that altering the gas content has little effect on their appearance (Figure 5.14). The $R_{\text{max}}$ fitting threshold is also largely unchanged, being $12.1R_0$ at 0.9 MHz, $5.5R_0$ at 2.8 MHz, $5.5R_0$ at 4.6 MHz, and $3.5R_0$ at 7.1 MHz. These simulations suggest that, absent any other changes occurring to the UCA makeup, altering the gas content has only a minor effect on postexcitation collapse.

Figure 5.13. Simulated radial expansion of 1 µm radius Definity UCA for a 1.53 MPa PRPA pulse at 2.8 MHz, showing minor differences for the effect of using air rather than octafluoropropane.
5.4.5 Lipid shell aging

Recently, additional estimates for Definity shell parameters were made demonstrating that an aging effect alters the viscoelastic shell properties between ‘fresh’ and ‘expired’ UCAs, where the expired UCAs were greater than two years old [98]. In these experiments, the elastic compression modulus, $\chi$, was largely unchanged from fresh to expired, going from 0.82 to 0.79, but the surface dilatational viscosity, $\kappa_s$, increased by nearly an order of magnitude from $3.98 \times 10^{-9}$ to $11.67 \times 10^{-9}$. These measurements provide new values for shell properties filling the large gap between standard Definity and standard Optison values, and
therefore allow additional consideration of the effects that intermediate changes to shell dilatational viscosity might have on observed postexcitation.

The changes in the $R_{\text{max}}$ threshold are shown in Figures 5.15 and 5.16. While not as great as the shift from Definity to Optison, it is seen that an increase in surface dilatational viscosity again acts to restrict the growth of these UCAs, and the $R_{\text{max}}$ threshold is therefore smaller. Additionally, the computed postexcitation curves fitted to the experimental results are shown in Figure 5.17 and are very similar, with the expired Definity having a slightly steeper slope at 4.6 and 7.1 MHz than the fresh Definity.

Figure 5.15. Maximum radial expansion $R_{\text{max}}$ of Definity bubbles calculated with alternate, ‘fresh’ shell parameters using the Marmottant equation with $R_{\text{breakup}} = R_{\text{rupture}}$. The black lines indicate the threshold $R_{\text{max}}$ above which postexcitation is assumed to occur for optimal fit to the experimental results. (a) 0.9 MHz, 11.7$R_0$ (b) 2.8 MHz, 5.2$R_0$ (c) 4.6 MHz, 5.2$R_0$ and (d) 7.1 MHz, 3.2$R_0$. 
Figure 5.16. Maximum radial expansion $R_{\text{max}}$ of Definity bubbles calculated with alternate, ‘expired’ shell parameters using the Marmottant equation with $R_{\text{breakup}} = R_{\text{rupture}}$. The black lines indicate the threshold $R_{\text{max}}$ above which postexcitation is assumed to occur for optimal fit to the experimental results. (a) 0.9 MHz, 9.9 $R_0$ (b) 2.8 MHz, 4.0 $R_0$ (c) 4.6 MHz, 4.2 $R_0$ and (d) 7.1 MHz, 2.4 $R_0$. 
5.5 Discussion

The Marmottant framework for modeling lipid UCAs as having distinct regimes of shell effects had previously been shown to be useful for describing several nonlinear UCA behaviors, including ‘compression-only’ [47], ‘threshold’ to onset of oscillation [95], and enhanced subharmonic content [104]. However, it had not been validated against any type of large amplitude growth and collapse prior to this comparison with postexcitation collapse.

In this simulation work, the primary factors which affect the modeled response of a single ultrasound contrast agent are considered known \textit{a priori} from experiments: the peak rarefactual pressure amplitude measured by a calibrated hydrophone measurements at the PCD transducers’ confocal region,
the bubble size distribution measured from microscope images, and the bubble material properties obtained from other experiments documented in the literature. Therefore, the only available fitting parameters come from the simulated bubble responses themselves; using a threshold maximum expansion ratio from the initial radius in this work was chosen based on its usefulness in previous studies on free bubble cavitation [27] and its better fit to experimental data than other tested metrics. It is noted that such a choice of threshold considers measured PES to be equally sensitive to all responses regardless of size; despite the fact that larger UCAs may collapse with greater energy, such effects are not considered.

Both the free bubble model and the Marmottant model fit the Definity experimental data quite well at the lowest frequencies, suggesting that lipid shelled UCAs do indeed respond as free bubbles for large oscillations. Furthermore, where the threshold $R_{\text{max}}$ was smaller at 7.1 MHz, the Marmottant model significantly outperformed the free bubble model. This result may indicate that it is necessary to consider the impact of a lipid shell even beyond the inertial cavitation threshold, despite the fact that it only impacts a small portion of the overall oscillatory cycle.

None of the tested models performed equally as well for matching the Optison experimental data, though the shelled models fit reasonably well at the lower frequencies. The fact that the unshelled model fit the Optison results quite poorly overall, unlike in the case of Definity, suggests a very distinct difference between the large amplitude collapse processes of these two types of UCAs.

The simulations further indicate that the microbubble size distribution can have an effect on postexcitation curves, and the effect is more significant at 4.6 MHz than at the other tested frequencies – as was seen previously in the experimental data. A shift in the mean microbubble radius will affect the slope of
the PES curve as can be ascertained from maximum radial expansion images (Figure 5.7). However, for most frequencies, a large change in distribution is needed; shifting the mean size of both of these UCAs by 1-2 microns was found to have only minor effects. Definity UCAs do appear to be more sensitive to size changes than Optison UCAs, as shown in Figures 5.11 and 5.12. The presence of many UCAs far from the resonant size may limit the maximum observable percentage of postexcitation, particularly at higher insonifying frequencies since the largest microbubbles may not grow to sufficient size to collapse with postexcitation.

Good agreement was found between the model’s predicted shift of the 50% PES threshold and the measured shift from standard to altered populations for Definity UCAs at all tested frequencies (Table 5.4). The model also predicted larger changes at 4.6 MHz than at other frequencies, a trend observed in the experiments. At 2.8 and 7.1 MHz, the predicted shifts for Optison were also within the 95% confidence range; only at 4.6 MHz did the model fail to predict the observed shift.

<table>
<thead>
<tr>
<th>UCA Population</th>
<th>50% Threshold at 2.8 MHz (MPa)</th>
<th>50% Threshold at 4.6 MHz (MPa)</th>
<th>50% Threshold at 7.1 MHz (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted Definity Change</td>
<td>-0.01 MPa</td>
<td>-0.48 MPa</td>
<td>-0.35 MPa</td>
</tr>
<tr>
<td>Measured Definity Change</td>
<td>-0.01 MPa</td>
<td>-0.61 MPa</td>
<td>-0.29 MPa</td>
</tr>
<tr>
<td>Predicted Optison Change</td>
<td>-0.07 MPa</td>
<td>-0.16 MPa</td>
<td>-0.06 MPa</td>
</tr>
<tr>
<td>Measured Optison Change</td>
<td>-0.03 MPa</td>
<td>+0.86 MPa</td>
<td>-0.20 MPa</td>
</tr>
</tbody>
</table>

Table 5.4. Predicted and measured changes of the 50% threshold due to altering the UCA sizes from the standard distribution. Measured changes also include the 95% confidence interval.
A variety of material properties were also explored by basing the simulations on previously measured values. Shell rheology, particularly for such thin and small surfaces, is challenging to measure; a variety of estimates were tested spanning a broad range, particularly for dilatational viscosity. The primary method for determining UCA shell viscoelastic properties was from low pressure attenuation experiments which assume linear behavior, so an inherent assumption in using these models was that shell property values can be extended to large amplitude behavior. These shell property parameters did exhibit a much stronger influence on predicted postexcitation than did internal gas composition. Not only did the manner in which they were implemented matter (breaking versus non-breaking shells), but so did the order of magnitude of the surface dilatational viscosity.

The quality of the obtained fits between simulation and experiment in most tested cases gives confidence that a simplified model can predict the occurrence of UCA postexcitation activity. Nevertheless, the variation of this threshold with frequency deserves further investigation. All fitted postexcitation curves found a threshold $R_{\text{max}}$ above the inertial cavitation threshold, noted previously to be a prerequisite for postexcitation emissions. However, the threshold for Definity at 7.1 MHz was $3.3R_0$, while the threshold at 0.9 MHz was $12.1R_0$, a significant discrepancy.

Two explanations seem plausible. The first is that the observed frequency variability for a threshold growth leading to collapse with postexcitation emission is a real dependency based on the underlying dynamics of shelled bubble collapse. In other words, the bubble may grow to a larger size at lower frequencies than at higher frequencies before its inertial collapse with postexcitation activity.
However, another possible explanation may be that the ratio of receiving center frequency to insonifying frequency plays an appreciable role in the experimental results. As mentioned earlier, the definition of the PES criterion involves two components: a fundamental harmonic response, followed by a secondary broadband response. If the receiving center frequency is considerably higher than the insonifying frequency, as was the case for the lowest frequency in the described double PCD experiment, the sensitivity to the initial response will be low unless significant higher harmonic content is present. In the experiment, a microbubble insonified at a lower frequency such as 0.9 MHz is significantly further from the receive transducers’ center frequencies at 15 MHz than one insonified at 7.1 MHz. The microbubble insonified at the lower frequency must therefore grow to greater initial amplitude of oscillation before being identified as collapsing with PES, because it will not be classified until both the initial harmonic and secondary broadband response are observed. The significance of the dependence of postexcitation curves on the separation between receiving and insonifying frequencies is further investigated in the following chapter.

The Marmottant model with a maximum radial expansion threshold was shown to provide effective predictions for large amplitude oscillations of Definity UCAs due to its assumption that an ultrasound contrast agent with a lipid shell ruptures and behaves as a free bubble under large expansion. Additionally, while the free bubble model was limited in only being able to closely match experimental data assuming expansions greater than five times the initial radius, the Marmottant model showed no such restrictions down to 3.3 times the initial radius, suggesting shell fragments have a lingering effect on the evolution of collapsing UCAs.
CHAPTER 6

DOUBLE PCD WITH ALTERNATE RECEIVE TRANSDUCERS

6.1 Introduction
The initial double PCD experiments were developed with receive transducers at 15 MHz nominal frequency in order to tightly limit the confocal volume while maintaining good sensitivity to the broadband postexcitation signal. However, neither a mechanical index scaling nor a theoretical maximum radial expansion threshold criterion as described in the previous chapter initially appeared to yield a flat scaling with respect to frequency for fitting experimental results. The unanswered question then was whether or not the receiver sensitivity to the response at the very lowest frequencies was different than the sensitivity at the highest frequencies; in other words, whether this variation was a physical effect or a measurement effect.
Therefore, the postexcitation measurements at 2.8, 4.6, and 7.1 MHz were repeated for standard Definity UCAs using lower frequency receive transducers at a nominal frequency of 7.5 MHz to test the transferability of PES analysis to an alternate experimental setup. The obtained results demonstrated a slight increase in sensitivity to the PES at lower pressures, and furthermore suggest that PES collapse for Definity occurs for a maximum radial expansion of nearly $4R_0$.

6.2 Transducer Characterization and Alignment

Two single element receive transducers with similar characteristics to the previous receivers were used; the primary differences were that they had a lower frequency than before and a correspondingly larger focal volume. The center frequencies of these new, alternate transducers were nominally 7.5 MHz but were measured to be 8.2 and 8.3 MHz; as before, both were f/2 with an element diameter of 0.5". The measured transducer characteristics as obtained with a 100 μm diameter wire are presented in Table 6.1.

The transducers were aligned as described previously. Since the focal lengths of these two transducers were slightly shorter than the original

<table>
<thead>
<tr>
<th>Transducer Model</th>
<th>Center Frequency [MHz]</th>
<th>-3 dB Fractional Bandwidth [%]</th>
<th>-6 dB Beamwidth at Focus [mm]</th>
<th>Focal Length [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New Receivers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS0704</td>
<td>8.2</td>
<td>29.2</td>
<td>0.40</td>
<td>25.8</td>
</tr>
<tr>
<td>IS0704</td>
<td>8.3</td>
<td>29.4</td>
<td>0.40</td>
<td>26.1</td>
</tr>
<tr>
<td><strong>Original Receivers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS1504GP</td>
<td>14.6</td>
<td>25.56</td>
<td>0.27</td>
<td>27.18</td>
</tr>
<tr>
<td>IS1504GP</td>
<td>13.8</td>
<td>20.90</td>
<td>0.27</td>
<td>27.30</td>
</tr>
</tbody>
</table>

Table 6.1. Measured characteristics of the new lower frequency receive transducers, compared with the original receive transducers.
transducers, the alignment did not overlap as centrally as was the case with the original higher frequency receive transducers; nevertheless, the new receive transducers did intersect within their -6 dB volumes (Figure 6.1). The insonifying transducers also intersected within this volume, demonstrating reasonably good confocal alignment (Figure 6.2).

Figure 6.1. Overlapping confocal region of the lower frequency receive transducers. The color scale is in dB.
6.3 Data Acquisition and Analysis

Experiments were conducted in the smaller tank experimental setup as described previously. Noise thresholds and the confocal region were determined prior to the addition of UCAs into the tank. Definity UCAs were activated normally with the standard 45 second VialMix agitation and were added to the gently stirred, degassed water. Several thousand signals were acquired as before within an approximately 1 hour time frame for each trial.

The only necessary alteration to the procedure was a decrease in the amount of UCAs added to the tank. Due to the increased overall focal volume for the receive transducers, the concentration of UCAs added to the tank needed further reduction beyond what was previously found to provide a good number of single bubble signals. Using the new transducers, the concentration of UCAs used was about 20% of the original amount (approximately 1000 bubbles/mL),
meaning that the dilution contained well under 1 microbubble per confocal volume.

After acquiring the data, signal analysis was performed using the same autoclassification peak detection parameters used previously. The primary difference in signal appearance was a reduced sensitivity to higher frequencies, as was expected. Examples of single UCAs with and without postexcitation signals are shown in Figures 6.3 and 6.4, respectively.

Figure 6.3. Single UCA with postexcitation detected in both receive channels. The example signals presented here were acquired at 2.8 MHz with a peak rarefational pressure of 2.28 MPa.
6.4 Results

The percentage postexcitation comparison between the lower and higher frequency receive transducers for native Definity is presented in Figure 6.5. Additionally, results for the 5% and 50% curve fit thresholds are listed in Table 6.2. Both sets of transducers captured the basic trends of PES UCA collapse behavior, but slight differences between the two transducers were noticeable, particularly at 4.6 MHz. The differences were greatest at low pressures, as evidenced by the consistently lower values obtained for the 5% and 50% thresholds with the alternate transducers. At 7.1 MHz, the results were nearly indistinguishable.
Figure 6.5. Percentage postexcitation curves for Definity with original and alternate receive transducers at a) 2.8 MHz, b) 4.6 MHz, and c) 7.1 MHz. The asterisks (*) represent autoclassifier results plotted with estimated standard deviations from the experimental data. The solid (--) curves are the logistic fits, and the dotted (---) curves represent the 95% confidence intervals.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Definity – Alternate Transducers</th>
<th>Definity – Original Transducers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>50%</td>
</tr>
<tr>
<td>2.8 MHz</td>
<td>0.12 MPa (0.06 – 0.17)</td>
<td>0.73 MPa (0.63 – 0.81)</td>
</tr>
<tr>
<td>4.6 MHz</td>
<td>0.17 MPa (0.05 – 0.30)</td>
<td>0.85 MPa (0.59 – 1.04)</td>
</tr>
<tr>
<td>7.1 MHz</td>
<td>0.92 MPa (0.22 – 1.46)</td>
<td>3.79 MPa (3.16 – 5.08)</td>
</tr>
</tbody>
</table>

Table 6.2. Percentage postexcitation thresholds with 95% confidence intervals in MPa PRPA, proportional to the maximum postexcitation observed at each frequency.
6.5 Discussion

The initial postexcitation collapse results were inconclusive as to whether the observation frequency relative to insonification frequency influenced the resulting trends. Therefore, in order to test the effect of measurement setup on the postexcitation signal data, two lower frequency transducers were substituted for the original receivers.

The only difference in the data acquisition between the original and altered setups was a reduction in the amount of UCAs added to the water tank. While it may seem counterintuitive that fewer UCAs should be added with a larger overlapping confocal volume, the overall receiving volume was also greater and therefore would increase the instances of multiple bubbles within the receiving region. For this reason, fewer usable signals were able to be acquired using this setup in an equivalent amount of time.

The results with the alternate transducers found slightly increased sensitivity to postexcitation, a trend that was most pronounced with lower pressures at lower frequencies. As hypothesized earlier, an explanation for this may be that weaker, less broadband responses are more easily observed when the receiving frequency is close to the insonification frequency. On the other hand, if the observation frequency is quite a bit higher than the excitation frequency, the receive transducers may be too insensitive to the principal response.

Returning to the question of using the mechanical index with UCAs, it is observed that the alternate transducers had a nearly flat response with this scaling for the two lower tested frequencies; however, the highest frequency did not fit this trend (Figure 6.6). Again, the conclusion is that the mechanical index is not a good predictor of PES cavitation activity. There was continued evidence
Figure 6.6. Postexcitation thresholds and 95% confidence intervals at 5% (circles) and 50% (squares), plotted versus frequency on the mechanical index scale for Definity UCAs. The original transducers are plotted with open symbols, and the alternate transducers are plotted with closed symbols. Low, moderate, high, and above regulatory limit regimes are indicated with the horizontal dotted (– –) lines.

of UCA postexcitation collapse at low mechanical index at 2.8 and 4.6 MHz.

Finally, the maximum radial expansion criterion was again tested with the Marmottant equation by fitting the simulated results for standard Definity parameters to the experimental results. From the previous manually classified results, it appeared that there was a strong dependence on this ratio and that increasing the similarity between the receiving and insonifying transducers would noticeably decrease the predicted expansion (Figure 6.7) – in other words, that experimental postexcitation collapse was very dependent on its measurement. However, this observation was also highly influenced by one unusual data point: the 15 MHz receive transducers with the 0.9 MHz insonifying transducer.
Figure 6.7. Prediction maximum radial expansion from Marmottant equation fit to Definity experimental data, plotted against the ratio of receiving to insonifying transducers.

The data from the alternate transducers help establish that PES measurement dependence is not a strong effect within a reasonable range. By only including the automatically classified results (therefore omitting the outlier data point), a nearly flat trend for maximum radial expansion is seen across several receiving to insonifying frequency ratios ranging from approximately 1 to 5 (Figure 6.8). Thus, it appears that postexcitation collapse for Definity is clearly related to radial growth of the UCA. Moreover, this maximum radial expansion threshold of approximately $3.9R_0$ is predicted to be nearly frequency-independent within this tested range of 2.8 to 7.1 MHz using the Marmottant model. Such growth is consistent with the previous conclusion that the postexcitation signal arises from strong collapses, requiring that bubble growth be greater than shell rupture and inertial cavitation thresholds.
Figure 6.8. Prediction maximum radial expansion from Marmottant equation fit to automatically classified Definity experimental data, plotted against the ratio of receiving to insonifying transducers.
CHAPTER 7

CONCLUSIONS

7.1 Review of Outcomes

Fundamental studies of ultrasound contrast agent dynamics are important for their ultimate applicability in biomedical ultrasound applications. The research presented in this dissertation has focused on the acoustic response of single ultrasound contrast agent microbubble collapse including both experimental and theoretical work. The direct results from this study include postexcitation signal characterization of multiple types of ultrasound contrast agents across a range of diagnostically relevant acoustic conditions and development of the relationship between this experimental response and large amplitude behavior of single bubble UCA models.
Experimentally, double passive cavitation detection was implemented and demonstrated to offer improvement over previous PCD setups at isolating single, symmetrically responding UCAs. This experimental technique was used to measure destruction thresholds of single UCAs, characterization information which is useful to compare with theory and potentially other experimental circumstances. Postexcitation signal analysis was refined through the development of automated classifying routines which quantified principal response and PES parameters as well as allowing for a significant increase in the amount of data analyzed. Multiple types of commercial UCAs were tested, and the parameter variation in those studies showed that shell composition has a significant impact on postexcitation collapse while size variation has a smaller impact for certain frequencies.

It was also demonstrated theoretically that the Marmottant UCA model could be used to predict postexcitation collapse through the use of maximum radial expansion threshold criteria. This procedure worked particularly well for lipid shelled bubbles, predicting both the shape of the resulting curves as well as 50% PES threshold variations that occurred due to altering the size distribution. Moreover, multiple trials of Definity UCAs were insonified using several frequencies and received at several other frequencies, and the experimental data taken as a whole suggests that postexcitation collapse may be predicted by a simple criterion from this model, approximately $3.9R_0$ maximum radial expansion. This $R_{\text{max}}$ bubble growth is significantly larger than the theoretical inertial cavitation threshold, and indicates that postexcitation rebound is indeed preceded by a strong cavitation collapse.
7.2 Limitations

It should be noted again that the postexcitation rebound observed as an indicator of UCA destruction is a subset of the larger group of UCA collapse behaviors and therefore the PES results are not necessarily indicative of the entire domain of possibilities. Indeed, it is quite likely that other modes of single bubble UCA destruction exist, given that postexcitation is predicted by nearly $4R_0$ maximum radial expansion, while shell rupture and inertial cavitation thresholds are much lower. Direct visual confirmation for postexcitation rebound and other types of collapse may be necessary to fully explore and classify these possibilities – without this link, it will remain unclear how representative postexcitation rebound is of all UCA destruction. Nevertheless, it is contended that the experimental results obtained in this dissertation still form a unique and useful characterization of UCA destruction, despite the limited scope.

A second constraint on the results of this work is the possibility of limited applicability to the clinically relevant situations of many UCAs and UCAs in confined geometries, neither of which is addressed in this research. The outcomes of this dissertation might not be directly equatable with, for example, *in vivo* animal models to ascertain at what pressure bubbles are assuredly collapsing, jetting with a certain velocity, or only oscillating, because the complexities of those specific situations are not considered. However, the results do form a benchmark for comparison and will likely aid in understanding of these other results even if the situations are not completely analogous.

7.3 Connection with other studies

As a follow up to the second limitation of scope, one question to consider is whether single bubble characterization of UCA destruction is at all useful in the
broader context of medical ultrasound; does the information gained have any utility when compared with biological studies which are performed under very different circumstances? To date, two separate studies have directly used postexcitation collapse to try to explain bioeffects resulting from UCAs, and are worth highlighting here.

A particular success in the application of postexcitation collapse thresholds to another study dealt with *in vitro* sonoporation across cellular membranes [105]. In this study, cells were grown as monolayers on well plates, and UCAs were mixed into solutions containing normally impermeable dextran marker molecules. It was shown that increased membrane permeability required the presence of oscillating UCAs during insonification; the PRPA onset of measured postexcitation collapse indicating UCA destruction correlated very well with the PRPA above which there was steep drop in amount of sonoporation, despite the fact that both of these results were measured in separate experimental setups. This suggests the single bubble postexcitation threshold correlates well with the onset of rapid destruction in bubble clouds.

The second study considered the *in vivo* angiogenic response resulting from ultrasound contrast agents insonified by high PRPAs [3]. In this work, it was found that this response was pressure dependent, as PRPAs associated with increasing percentages of postexcitation destruction of UCAs led to greater vascular permeability. In contrast, the lower PRPAs below a significant destruction threshold showed insignificant changes in permeability. Therefore, unlike the previously described sonoporation experiment, it was found that strong destruction of UCAs was needed to provide the necessary mechanical stimulation for the resulting permeability bioeffects in this case.
7.4 Future Studies

There are several directions for future work related to these studies. More experimental characterizations of how UCA properties affect postexcitation collapse remain unexplored, such as confirmation of theoretical predictions that inner gas has little effect and further exploration into the frequency dependence of altered size distributions. Testing other types of bubbles might also increase understanding about shell effects if, for example, rigid or thick-shelled particles are studied.

As suggested above, one of the next major steps related to a fuller understanding of shelled bubble destruction involves understanding the mode or modes of collapse that lead to postexcitation rebound signals. Do the stronger acoustic pulses lead to more symmetry during inertial cavitation, do they generate a larger free bubble, or do they create multiple collapsing daughter bubbles? Multiple methods could be utilized to explore this, including joint acoustic-visual experiments using double passive cavitation detection. A second approach would involve more complicated modeling of bubble behavior and shell rupture, such as an axisymmetric or fully three-dimensional boundary element model to test how shell fragments may affect the evolution of collapse.

Another important direction for future research is greater understanding of how the generation of free bubbles during UCA destruction may affect the dynamics of collapsing UCA clouds. Experimental measurements of UCA cloud destruction thresholds and parameters are needed for such a study to provide a benchmark for comparison. Theoretical development examining the differences between destruction thresholds as well as mechanical forces generated by single collapsing bubbles versus clouds of collapsing bubbles would be of particular relevance to other biomedical work. How these forces vary spatially and in
magnitude when there is no longer simple symmetry would be an important future step to improve understanding of UCA-mediated bioeffects.

7.5 Final Statement

In summary, the experimental and theoretical evidence from this thesis has been used to gain greater understanding about the nature of single bubble ultrasound contrast agent collapse. It is anticipated that the knowledge obtained in these studies will be valuable for providing quantitative documentation of UCA collapse, for providing guidance in appropriate features and thresholds of shelled bubble models when used to predict collapse, and, in the broader picture, for providing assistance in the interpretation of UCA biomechanical effects in a wide variety of experiments.
REFERENCES


