THÈSE DE DOCTORANT DE L’UNIVERSITÉ PARIS VI

Spécialité
Acoustique physique
(Imagerie médicale, produit de contraste ultrasonore)

Présentée par
Mr Azzdine Yahya AMMI

Pour l’obtention du grade de
DOCTEUR DE L’UNIVERSITÉ PARIS VI

Sujet de la thèse
DÉTECTION ET CARACTÉRISATION DE LA
DESTRUCTION DES MICROBULLES DE PRODUIT DE
CONTRASTE ULTRASONORE

Soutenue le 17 Mars 2006

Jury :
Mme S. Lori BRIDAL Directrice de thèse
M Olivier BOU MATAR Rapporteur
M Christian CACHARD Rapporteur
Mme Geneviève BERGER Examineur
M Ayache BOUAKAZ Examineur
M François TRANQUART Examineur
M Cyril LAFON Invité
M William D. O’BRIEN, Jr Invité
Acknowledgements

I would like to first thank my Advisor, Dr. S. Lori Bridal, for providing me the opportunity to become involved in this research as well as for her enthusiasm, guidance and support throughout my time in the lab.

I am more than grateful to Pr William D. O’Brien, Jr. I learned a great deal from him and he made me feel really at home in his lab. Bill, thanks for introducing me to the “American culture”, I had a lot of fun.

Many thanks to Dr Robin O. Cleveland from Boston University for his significant scientific contributions, ideas concerning theoretical development, help with experimental design and support. Robin, I will never thank you enough for believing in me.

I would like to thank the members of my committee Pr Olivier Boumatar, Pr Christian Cachard, Pr Geneviève Berger, Dr Ayache Bouakaz, Pr François Tranquart and Dr Cyril Lafon for the effort they have put into evaluating my work.

A special thank to Monica Spisar who worked in LIP on the project and pushed it forward during my time in BRL. She became one of my best friends during her stay in France and I would like to thank her also for her personal support.

Many thanks to Dr Amena Saied, she was always available and willing to give input on my research, especially during the last year.

Thanks to Cécile Baron, Dorothée Bossis, Fabienne Marescq, Najwa Elkadi and Matthieu Santin for their support and help during the final stage of my stay at LIP.

Zoulikha Kahlouche and Michèle Boudinet were always present when I needed them so thanks to both of you.

Thanks to Dr Jonathan Mamou a great colleague and friend. A French speaker in BRL definitely helped.
I would like to thanks the experimenters Grace Wang, Jeong-Ah Lee, Zachary Hafez for their hard work and overnight experiments.

Sue Clay one of the sweetest “lady” I have been given to meet during my stay in BRL. Thanks for making my life in Urbana-Champaign so much easier. Thank you for your kindness and your smiley face.

Thanks to Dr Michael Olze for the time he has given me talking about our existential matters: science and life!
A special thought from dude to dude to Dr Jim Blue great guy also from BRL, Shaquille O’neil’s challenger.

Thanks to Dr. Rita J. Miller without her the experiments would have been a nightmare to set and my stay in BRL less fun.

I would also like to thank all of the other members of both LIP and BRL for their assistance and collaboration towards this work.

Nahil Sobh, thank you for helping me with the forever lasting simulations by allowing me to use the NCSA computers. Thank you for your friendship, I appreciated our long discussions.

Thanks to Dr Susannah Bloch, a post doc from Pr Katherine W. Ferrara’s Lab, for your comments on this work.

Thanks to my friends: Djilali Ledhem, Matthieu Chambault and Mohamed Zerrouk for their faithful moral support.

I finally thank my Mom… otherwise I will get into real trouble!!! She was right the sight is wonderful from up here.
Table of Contents

ACKNOWLEDGEMENTS ..................................................................................................................i

NOTATION ....................................................................................................................................v

CHAPTER I – INTRODUCTION ....................................................................................................1
I. INTRODUCTION .......................................................................................................................3
   I. 1. BACKGROUND ..................................................................................................................3
      I. 1. a. UCA composition .....................................................................................................3
      I. 1. b. The regimes of the UCA acoustic response .........................................................7
      I. 1. c. Clinical applications .............................................................................................9
I. 2. MOTIVATION FOR THIS RESEARCH AND CURRENT STATE OF RESEARCH ON CONTRAST AGENT SHELL RUPTURE ..................................................11
I. 3. SCOPE OF THIS THESIS .................................................................................................13

CHAPTER II – MODELING THE UCA RESPONSE ..................................................................17
II. 1. AVAILABLE THEORETICAL MODELS DESCRIBING UCA OSCILLATION: .....................19
II. 2. DESCRIPTION OF THE MODIFIED HERRING EQUATION MODEL ............................20
II. 3. ESTIMATIONS OF PHYSICAL PARAMETERS USED TO DESCRIBE MICROBUBBLES IN THE MODEL ........................................................................................................23
II. 4. PREDICTING MICROBUBBLE ECHOES .........................................................................24
II. 5. EFFECT OF THE SHELL TERMS ON THE MODELED RADIUS-TIME CURVES .............25
II. 6. DISCUSSION ..................................................................................................................27

CHAPTER III - EXPERIMENTAL METHODS .........................................................................29
III. 1. INTRODUCTION ................................................................................................................31
III. 2. CHARACTERIZATION OF INCIDENT ULTRASONIC PULSE WAVEFORMS ......................31
      III. 2. a. Incident waveform characteristics for the PCD and DPCD ..................................33
            III. 2. b. Incident waveform characteristics for the measurement system allowing microbubble isolation with optical verification (optical/ acoustic measurement system) ...........................................37
III. 3. PASSIVE CAVITATION DETECTOR ...................................................................................38
III. 4. DOUBLE PASSIVE CAVITATION DETECTOR .................................................................41
III. 5. OPTICAL/ACOUSTIC MEASUREMENT SYSTEM ..........................................................41
III. 6. CONTRAST AGENT .......................................................................................................48
III. 7. SUMMARY .....................................................................................................................48

CHAPTER IV – ULTRASONIC CONTRAST AGENT SHELL RUPTURE DETECTED WITH A PASSIVE CAVITATION SYSTEM .................................................................................51
IV. 1. INTRODUCTION .............................................................................................................53
IV. 2. DATA ACQUISITION .......................................................................................................53
IV. 3. DATA ANALYSIS ...........................................................................................................54
IV. 4. RESULTS .......................................................................................................................54
IV. 5. DISCUSSION ..................................................................................................................60
IV. 6. CONCLUSION ................................................................................................................65

CHAPTER V – AUTOMATIC DETECTION OF IC EVENTS: EXPLORATION OF THE NOISE FLOOR AND DESTRUCTION OCCURRENCE THRESHOLDS ..............................................67
V. 1. INTRODUCTION ...............................................................................................................69
V. 2. AUTOMATIC IC DETECTION.................................................................................................................. 69
V. 3. SENSITIVITY OF IC DETECTION TO VOLTAGE LEVELS IN THE NOISE AS A FUNCTION OF INCIDENT PEAK
RAREFACTIONAL PRESSURE ...................................................................................................................... 76
V. 4. CALCULATION OF THE PERCENT OCCURRENCE OF DESTRUCTION EVENTS AT EACH INCIDENT PRESSURE... 78
V. 5. RESULTS................................................................................................................................................ 79
   V. 5. a. Peak voltage levels due to IC signals relative to that of the noise .................................................. 79
   V. 5. b. Percent occurrence of destruction events as a function of incident pressure ............................. 81
   V. 5. c. Exposure dose ............................................................................................................................... 84
   V. 5.d Comparison between theory and experiment ................................................................................. 85
V. 6. DISCUSSION AND CONCLUSIONS ................................................................................................. 86

CHAPTER VI – LARGE BANDWIDTH EXPLORATION OF THE SHELL
RUPTURE RESPONSE USING DOUBLE PASSIVE CAVITATION DETECTION..... 93

VI. 1 INTRODUCTION .................................................................................................................................... 95
VI. 2. DATA ACQUISITION ............................................................................................................................ 96
VI. 3. DATA ANALYSIS ............................................................................................................................... 96
   VI. 3. a. Evaluation of measurement variation ......................................................................................... 97
   VI. 3. b. Visual assessment of signals acquired simultaneously with the transmit transducer and the passive
   13 MHz receiver ........................................................................................................................................ 98
   VI. 3. c. Spectral evaluation of the destruction response .......................................................................... 98
VI. 4. RESULTS ............................................................................................................................................... 99
   VI. 4. a. Minimum pressure rupture threshold measurements: variations and significance of differences .. 99
   VI. 4. b. Visual assessment of signals acquired simultaneously with the transmit transducer and the passive
   13 MHz receiver .........................................................................................................................................101
   VI.4.c. Spectral evaluation of the destruction response ..........................................................................106
VI.5. DISCUSSION AND CONCLUSION .................................................................................................111

CHAPTER VII – OPTICAL AND ACOUSTICAL OBSERVATION OF ISOLATED
MICROBUBBLES..........................................................................................................................................115

VII. 1. INTRODUCTION .................................................................................................................................117
VII. 2. DATA ACQUISITION........................................................................................................................117
VII. 3. RESULTS ........................................................................................................................................119
VII. 4. DISCUSSION AND PERSPECTIVES .............................................................................................126

CHAPTER VIII – CONCLUSION..................................................................................................................131

REFERENCES................................................................................................................................................137
Notation

$\rho$  Density of the surrounding liquid
$\mu$  Viscosity of liquid
$c$  Speed of sound
$f_0$  Driving frequency
$P_0$  Hydrostatic pressure
$p_i$  Internal pressure
$P$  Time derivative of the pressure at the bubble wall
$P_{\text{drive}}$  Driving pressure
$t$  Time
$R_0$  Initial microbubble radius at equilibrium
$R_{\text{max}}$  Maximum microbubble radius
$R$  Microbubble radius
$\dot{R}$  Microbubble wall velocity
$\ddot{R}$  Microbubble wall acceleration
$y$  Non-dimensional microbubble radius
$\dot{y}$  Non-dimensional wall velocity
$\gamma$  Polytropic gas exponent
$\sigma$  Interfacial tension coefficient
$\chi$  Elasticity modulus of the shell
$\mu_{\text{sh}}$  Viscosity of the shell
$\varepsilon$  Shell thickness
$\omega_0$  Resonant frequency of the microbubble
$0^\circ$  Pulse leading with a phase of positive voltage
$180^\circ$  Pulse leading with a phase of negative voltage

DPCD  Double passive cavitation detector
FDA  Food and drug administration
HIFU  High intensity focused ultrasound
IC  Inertial cavitation
PCD  Passive cavitation detector
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>Pulse duration</td>
</tr>
<tr>
<td>PRF</td>
<td>Pulse repetition frequency</td>
</tr>
<tr>
<td>r-t</td>
<td>radius-time</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>TOF</td>
<td>Time-of-flight</td>
</tr>
<tr>
<td>UCA</td>
<td>Ultrasound contrast agent</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
</tbody>
</table>
Chapter I – Introduction
Chapter I

I. Introduction

In this chapter, the background and motivation for this research are introduced, and the thesis scope is presented. In the first section, the microbubble is introduced as an ultrasound contrast agent (UCA). The physical properties of the UCA (size, shell composition, and gas core) are described as well as the different regimes of the contrast microbubble acoustic response. Current and potential future applications of UCA are introduced. The second section of this chapter introduces the shell rupture of microbubbles. The practical importance of understanding the shell rupture of UCA motivating this study is explained and the current state of research concerning the characterization of UCA shell rupture is reviewed. In the third and final section of this chapter, the objectives of the research in this thesis are described and the scope of this dissertation is introduced with an outline of the chapter contents.

I. 1. Background

I. 1. a. UCA composition

Today’s ultrasonic contrast agents consist of encapsulated microbubbles on the order of 1 to 8 μm in diameter. They may be filled with air, or with a lower solubility gas. Gas-filled bodies are highly echogenic due to the difference in the compressibility between the microbubbles and the surrounding medium (water or blood). The compressibility of air is 7.65×10^{-6} \text{ m}^2/\text{N}, in comparison with 3.9×10^{-11} \text{ m}^2/\text{N} for blood. The compressibility for an encapsulated microbubble approaches that of air. For example, the compressibility of Albunex® microbubbles was measured by de Jong et al. to be 5×10^{-7} \text{ m}^2/\text{N} [1]. The shell, designed to reduce gas diffusion into the blood, can be rather stiff (e.g. human serum albumin) or more flexible (e.g. phospholipids), and the shell thickness can vary from approximately 1 to 200 nm. Ideally, a contrast agent should be easily administered by intravenous injection and must cross the microcirculation of the pulmonary bed. Its physical properties should remain stable during the ultrasonic examination and provide a strong acoustic response with optimized harmonic response and microbubble disruption thresholds.
It should be nontoxic and harmlessly eliminated from the system after the ultrasonic examination.

For an examination by contrast ultrasonography, a small amount of microbubble contrast agent (a few mL) is injected intravascularly. Following intravenous injection, the microbubbles remain in the blood pool for several minutes which enables examination of patients. The gas is gradually eliminated from the blood by exhalation via the lungs, and the shell material is metabolized. Preclinical and clinical studies have proven the efficacy and tolerance of the agent by patients [2-4]. Unlike contrast agents used for Computed Tomography or Magnetic Resonance Imaging, ultrasound contrast agents do not diffuse into the extra cellular compartment. They are strictly blood pool agents.

The ultrasonic response of a contrast agent depends on the size of the bubble, the composition of the shell and the gas core. The larger the microbubble, the larger is its scattering cross section. If microbubbles are too large however (diameter ≥ 8 μm, i.e larger than red blood size), they are unable to cross the pulmonary circulation [4, 5]. An ideal contrast agent would have dimensions at the resonant size (having a resonant frequency equal to that of the driving frequency) for optimal scattering of ultrasound. Table I.1 compares the mean diameter of Albunex® (the first UCA approved by the US Food and Drug Administration (FDA)) to the mean diameters of: newer contrast agents (Optison™, SonoVue, Levovist...etc), red blood cells and microbubbles formed in agitated saline solution.

<table>
<thead>
<tr>
<th>Type</th>
<th>Size (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell</td>
<td>6-8 (Approximate long axis)</td>
</tr>
<tr>
<td>Microbubbles in Agitated Saline</td>
<td>16 (Approximate diameter)</td>
</tr>
<tr>
<td>Albunex®</td>
<td>3-5 (Average diameter)</td>
</tr>
<tr>
<td>Newer UCAs</td>
<td>2.5 (Average diameter)</td>
</tr>
</tbody>
</table>

Table I.1 Characteristic sizes for contrast-producing media as compared to red blood cells [6, 7].
Chapter I

The radius of the microbubble and the variation of this radius during acoustically forced oscillation is an important parameter in the shell rupture process. It has been reported that larger microbubbles require less acoustic pressure to be applied than smaller microbubbles in order to collapse [8-10]. The relative expansion of a spherical microbubble, defined as the maximum radius, $R_{\text{max}}$, reached during microbubble oscillation divided by the initial equilibrium radius, $R_0$, has been used as an index for microbubble rupture thresholds and has been correlated with the occurrence of fragmentation [11, 12]. Empirical results in Albunex® indicated that relative expansion thresholds for cavitation ranged from 2.3 to 3.5 [11, 12]. Many studies have been based on a rupture threshold criterion at a maximum radius of $R_{\text{max}} \approx 2 \times R_0$ [13-15]. Another type of rupture threshold criterion has been suggested by Plesset and Mitchell who hypothesis that microbubbles become violently unstable and fragment when the minimum contraction decreases below one tenth of the maximum expansion achieved by the microbubble [16].

The shell has two major effects on the oscillation and scattering of sound from the microbubble. First, the shell makes the microbubble stiffer than a free gas bubble of equal size. This causes the resonant frequency of the shell-encapsulated microbubble to be at a higher incident ultrasonic frequency than for the free bubble, and it also dampens the oscillation amplitude. Stiffer-shelled contrast agents have been found to rupture with less acoustic pressure than agents with more flexible shells [17]. The more elastic the shell, the greater its range of contraction and expansion prior to rupture. Flexible-shelled agents demonstrate stronger nonlinear response at a given ultrasound field acoustic pressure than their stiffer counterparts. The second important effect introduced by the shell is viscosity. The additional viscosity of the shell causes more of the energy in the incident ultrasonic pulse to be converted to heat instead of being reradiated. This reduces the scattering-to-attenuation ratio of the microbubble defined by [18, 19] and estimated for Albunex® by [20]. The shell resistance to ultrasound exposure is proportional to its viscosity [21]. No threshold criterion described in the literature takes into account the shell’s intrinsic properties in spite of the fact that they clearly play an important role in the acoustic pressure required for shell rupture [17]. Research on this relationship is underway that should help to link shell properties to microbubble vibration and rupture [22]. Table I.2 summarizes characteristics of several ultrasound contrast agents with specific interest in the relative stiffness of the different shell materials.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Gas</th>
<th>Shell/Stiffness</th>
<th>Manufacturer</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI-700</td>
<td>Perfluorocarbon</td>
<td>Polymer/High</td>
<td>Acusphere pharm</td>
<td>Clinical trials in the USA</td>
</tr>
<tr>
<td>Albunex</td>
<td>Air</td>
<td>Human albumin/Medium</td>
<td>Mallinckrodt Medical</td>
<td>Withdrawn</td>
</tr>
<tr>
<td>Definity</td>
<td>Perfluorocarbon/Air</td>
<td>Lipids-surfactant/Low</td>
<td>Dupont Merck/ImaRx</td>
<td>Approved for echocardiography in North America</td>
</tr>
<tr>
<td>Bisphere</td>
<td>Air</td>
<td>Polymer/Albumin High</td>
<td>Point Biomedical</td>
<td>Clinical trials</td>
</tr>
<tr>
<td>BY963</td>
<td>Air</td>
<td>Lipid/Low</td>
<td>Byk-Gulden</td>
<td>Clinical trials completed, no approved application intended</td>
</tr>
<tr>
<td>EchoGen</td>
<td>Dodecafluoropentane</td>
<td>Surfactant/Low</td>
<td>Sonus</td>
<td>Approved in EU, not marketed</td>
</tr>
<tr>
<td>Echovist</td>
<td>Air</td>
<td>No shell/Very low</td>
<td>Schering</td>
<td>Approved for echocardiography in a few European countries</td>
</tr>
<tr>
<td>Imagent</td>
<td>Perfluorohexane/air</td>
<td>Surfactant/Low</td>
<td>Alliance</td>
<td>FDA approved in 2002</td>
</tr>
<tr>
<td>Levovist</td>
<td>Air</td>
<td>Palmitic acid/Low</td>
<td>Schering</td>
<td>Approved in 70 European and Asian countries, not in USA</td>
</tr>
<tr>
<td>Optison</td>
<td>Octofluoropropane (C₃F₈)</td>
<td>Albumin/Medium</td>
<td>MBI/Amersham Medical</td>
<td>Approved in the USA and Europe but not in France</td>
</tr>
<tr>
<td>Quantison</td>
<td>Air</td>
<td>Albumin/Medium</td>
<td>Upperton</td>
<td>Pre-clinical development</td>
</tr>
<tr>
<td>Sonovue</td>
<td>Sulfur hexafluoride (SF₆)</td>
<td>Lipids-surfactant/Low</td>
<td>Bracco/ALTANA</td>
<td>Approved in EU and several Asian countries, not in Japan and USA</td>
</tr>
<tr>
<td>Sonovist</td>
<td>Air</td>
<td>Cyanoacrylate/High</td>
<td>Schering</td>
<td>Clinical trials stopped</td>
</tr>
<tr>
<td>Sonozoid</td>
<td>Perfluorobutane</td>
<td>Lipids/Low</td>
<td>Amersham Medical</td>
<td>Clinical trials in Japan</td>
</tr>
</tbody>
</table>

Table 1.2 A summary of the characteristics of several ultrasound contrast agents [23, 24].
Table I.3 illustrates some of the principal differences arising due to the properties of
the encapsulated gas. The advantages of the air-filled microbubble are that it is a strong
scatterer of ultrasound and highly soluble; however, air-filled microbubbles have relatively
low persistence and lack stability because air diffuses rapidly. Gases of higher molecular
weight, on the other hand, are less soluble. Thus, they are more stable and have longer
persistence. Persistence is important in prolonging the contrast effect so that clinical
assessments can be made. Studies have shown that the rise in temperature of the gas during
compression may cause the microbubbles to rupture and temperature thresholds from 1550 K
to 5000 K have been proposed for collapse [25-27].

<table>
<thead>
<tr>
<th>Air</th>
<th>Heavier gases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly soluble</td>
<td>Low solubility</td>
</tr>
<tr>
<td>Low persistence and</td>
<td>High persistence and</td>
</tr>
<tr>
<td>stability</td>
<td>stability</td>
</tr>
<tr>
<td>Rapid diffusion</td>
<td>Reduced diffusion</td>
</tr>
</tbody>
</table>

Table I.3 Microbubbles typically contain either air or a gas of higher molecular weight. Principal
differences can be described in terms of acoustic stability and diffusion.

I. 1. b. The regimes of the UCA acoustic response

When exposed to ultrasound excitation, microbubbles respond by oscillating. The
oscillations of the microbubbles have a resonant frequency. A mathematical expression
linking the microbubble properties to its resonant frequency $\omega_r$, is given by [28].

$$\omega_r = \sqrt{\frac{1}{\rho R_0^2}} \left[ 3\gamma \left( \frac{P_0 + 2\sigma}{R_0} + \frac{2\chi}{R_0} - \frac{6\chi}{R_0} \right) - 2 \left( \frac{1}{\rho R_0^2} \left( 2\mu + \frac{6\mu_{ab}}{R_0} \right) \right) \right]$$  (I.1)

where $\rho$ is the density of the surrounding liquid, $R_0$ is the initial microbubble radius
at equilibrium, $\gamma$ is the polytropic gas exponent, $P_0$ is the hydrostatic pressure, $\sigma$ is the
interfacial tension coefficient, $\chi$ is the elasticity modulus of the shell, $\mu$ is the viscosity of
liquid, $\mu_{ab}$ is the viscosity of the shell and $\varepsilon$ is the shell thickness. Equation I.1 shows that all
the physical properties of the microbubble, as well as the properties of the surrounding
medium, affect the resonant frequency. The resonant frequencies of typical contrast agent microbubbles are within the frequency range used in medical ultrasound (2 to 8 MHz).

The acoustic response of a contrast microbubble will differ as a function of the pressure in the incident ultrasonic pulse. The measurement of transmitted ultrasonic pressure that is displayed by modern ultrasound imaging systems is the mechanical index (MI). It is defined as the derated (0.3 dB/cm-MHz) peak rarefational pressure (for a 1-cycle acoustic period) divided by the square root of the centre frequency of the transmitted pulse [27]. Both the frequency and the peak rarefational pressure are normalized by 1 MHz and 1 MPa respectively, the MI has no units. MI settings in clinical contrast-enhanced sonography are able to go up to 1.9.

At very low transmit ultrasonic pressure (MI ≤ 0.05), the oscillations of the microbubbles are symmetrical about its equilibrium radius. During the positive (compressional) phase of the ultrasound (US) pulse the microbubble is compressed and during the negative (rarefational) phase it is expanded, both to the same degree.

As the ultrasonic pressure amplitude is increased (0.05 ≤ MI ≤ 0.3), the microbubble becomes relatively more resistant to compression than to expansion which leads to asymmetrical or nonlinear oscillations. These nonlinear oscillations contain several “harmonic” frequencies. Harmonic signals occur at multiples (two, three and four times ... etc) of the insonating frequency and each successive harmonic is at a lower spectral amplitude than the previous one. The second harmonic (double the insonating frequency) has the highest amplitude among the harmonics and is therefore the most relevant for contrast-specific imaging. In order to provide a strong harmonic response of the microbubbles, the insonating frequency should be close to the resonant frequency of the microbubble. Other frequencies, in addition to the harmonics, can in fact be detected from microbubbles subject to a driving pressure of frequency $f_0$. These include subharmonics for which signals occurs at $f_0/2, f_0/3, f_0/4$... etc and ultraharmonics for which signals occurs between the harmonics e.g. at $3f_0/2, 5f_0/2$,... etc. Nappiras [29] summarized two theories for the generation of subharmonics that depend on the microbubble properties. He states that the low-frequency emissions might originate from surface waves on the microbubble wall, or might be due to excitation at a frequency above the resonant frequency. Wide-bandwidth transducers used in the clinic are
capable of transmitting at a fundamental frequency and receiving at the second harmonic. The resulting “harmonic imaging” improves the ratio of signal from contrast microbubbles relative to the ‘noise’ from surrounding tissues, [30-32]. Several signal analysis algorithms have been developed to separate the specific nonlinear response of contrast from tissue to optimize visualization of contrast microbubbles in the microcirculation [33, 34].

At even higher ultrasonic pressure amplitudes (MI ≈ 0.3), destruction of microbubbles occurs. The pressure threshold for destruction is variable and depends on a number of different factors such as intrinsic microbubble properties (the size, shell material and gas core) and also the ultrasound excitation settings (frequency, pulse duration, pressure and phase). For Albunex® (no longer commercially available), the threshold MI for microbubble destruction in vitro has been reported to be 0.3 [35].

The process of microbubble destruction is complex. Recent video-microscopic studies with extremely high temporal resolution have given some insight into the physical phenomena involved [8-10]. In [8], microbubble destruction has been observed to be preceded by a large expansion of the microbubble wall (up to eight times its initial radius). At this level of expansion, the subsequent compression is so important that the microbubbles are fragmented into several smaller bubbles. These fragmented microbubbles only last a few milliseconds after the onset of insonation. Microbubble disappearance has also been observed to be due to the deflation of the microbubbles by acoustically driven diffusion [36, 37] via which, the shell becomes more permeable during its extension and permits the evacuation of gas.

I. 1. c. Clinical applications

In recent years, ultrasound contrast agents have been shown to enhance the detection of blood flow in peripheral and vascular structures, providing a useful diagnostic tool in many medical specialties [38-40]. Enhanced sonographic contrast after UCA injection is used for early detection of a variety of diseases [41, 42]. Ultrasound contrast agent use is of particular interest in the field of oncology. Nonlinear imaging techniques allow sensitive detection of contrast microbubbles in tumor vessels of the liver, kidney, ovary, pancreas, prostate, and breast tumors [43-50]. The level of vascularization in such tumors provides an indicator of malignancy [51]. Harmonic imaging of contrast agents has also been shown to have
significant clinical importance in the field of cardiology with absence of vascularization associated with ischemic or infarcted myocardium [52, 53].

Microbubble destruction is the key to many of the techniques used or under evaluation in the clinic. By destroying contrast microbubbles with a high-amplitude acoustic pulse and then observing the refill of microbubbles at lower acoustic pressure levels, information on the kinetics of flow can be obtained. Contrast enhancement will return to regions of rapid flow faster than to regions of slow flow. Wei [54] introduced the concept of using the destruction of microbubbles and the rate of signal return to map perfusion quantitatively. In recent years, this technique has been applied in a number of organs [55, 56]. The acoustic rupture of contrast microbubble shells has also been proposed as a means to release more strongly-scattering, free bubbles that provide a short-lived but unique acoustic signature for imaging [57].

There is growing interest in using contrast agents for targeted imaging. The principle behind this is that contrast microbubbles would be targeted to bind to a specific site, where they would locally enhance image contrast in the zones containing the targeted tissue components (neovessels, tumor cells... etc). One method for targeting involves attaching binding molecules such as monoclonal antibodies, biotin, or other adhesion molecules to the microbubble shell [58]. This type of targeted microbubble has been shown experimentally to be effective in selectively binding to activated coronary artery endothelial cells and fibrin clots [59]. However, currently, there are no targeted microbubbles commercially available for clinical ultrasound use.

Several researchers are exploring microbubbles as drug delivery vehicles, which can be concentrated in a specific area for localized treatment [60]. Local, controlled delivery of drugs such as chemotherapeutic agents which can be systemically toxic should significantly reduce undesirable side effects. Researchers have shown that encapsulated microbubbles designed for transporting therapeutic agents can be ruptured with an acoustic pulse [61]. Such acoustic delivery could be applied to contrast microbubbles concentrated at a desired treatment site in vivo.
Chapter I

1.2. Motivation for this research and current state of research on contrast agent shell rupture

As described in the previous section, local acoustic destruction of contrast agent microbubbles in a selected region in situ can be used to purposefully modify contrast concentration. Dynamic evaluation of image contrast-enhancement can then be applied to quantify blood volume and flow rate [62, 63]. However, UCA-based blood perfusion quantification by such techniques is hampered by unintentional modification of UCA concentration during imaging and the unknown ultrasound backscattered echo contribution to the received signal by microbubble destruction. For these reasons, if the origin of the desired image enhancement is linked to the destruction of the microbubbles, then the transmit parameters should be set above the destruction threshold. On the contrary, if the imager’s goal is to follow intact UCAs, then the transmit settings need to minimize or eliminate UCA destruction. Knowledge of the UCA destruction thresholds is thus essential for robust application of these sensitive functional imaging techniques. An understanding of microbubble destruction is also fundamental for development of microbubble-aided delivery therapy techniques [64, 65]. With the increasing interest in attaching antibodies, DNA, and chemicals to microbubbles, understanding the shell rupture mechanism could be a powerful tool for design capability in drug delivery. Furthermore, increased knowledge concerning destruction thresholds should help to assess the risk of microbubble-related bioeffects [66-68].

A strong experimental basis is needed to guide theoretical model developments [17, 69, 70] so that modifications of microbubble dynamics described by these models can be applied to predict destruction thresholds based on UCA properties (e.g., composition of encapsulating shell, gas, size distribution). The need to respond to such practical concerns and the potential to develop new UCA applications have generated considerable interest in the elucidation and quantification of UCA destruction thresholds [7, 10, 37, 71]. Several techniques have been used. The passive cavitation detector (PCD) technique uses a receiver to listen passively for acoustic emissions from microbubbles excited by another source [72, 73]. Giesecke and Hynynen [71] used a PCD to determine the cavitation thresholds based on the peak rarefational pressure amplitude that caused an increase in the broadband noise emission. This increase was empirically chosen to be greater than one standard deviation above the baseline noise. As determined by this method, thresholds of Optison™ increased with frequency (approximately 0.1 MPa at 0.74 MHz, 0.2 MPa at 1.1 MHz, 0.4 MPa at 2.18 MHz and 1.6 MPa at 3.3 MHz). The study by Giesecke and Hynynen, which used long
incident pulse durations (from 2 to 100 ms), found that pressure thresholds did not vary strongly as a function of pulse duration (PD). Chen et al. [7] also used a PCD to estimate fragmentation pressure thresholds for four contrast agents (at 1.1 and 3.5 MHz). They selected the fragmentation threshold to correspond to the incident peak rarefractional pressure simultaneously satisfying two criteria. The first criterion was that 5 percent of “spikes” in the received signal exceeded a voltage threshold. The second criterion was that the signal’s spectral amplitude increased between harmonics (broadband noise). Using these criteria, the acoustic fragmentation thresholds of three UCAs (Optison™, Sonazoid and biSpheres) were estimated for various acoustic exposure conditions. Optison™ had mean fragmentation pressure thresholds of 0.13 MPa at 1.1 MHz and 0.48 MPa at 3.5 MHz. A PD of at least five cycles was necessary for Optison™ microbubbles to achieve steady-state fragmentation thresholds. Comparison of threshold levels reported for Optison™ under similar conditions [7, 71] reveals that the levels may vary depending on criteria applied to signals received during microbubble excitation.

Active cavitation detection (ACD) has also been used to estimate UCA collapse thresholds, where the receiver interrogates the region of interest with a low-pressure pulse amplitude in order to assess the potential cavitation effects from another ultrasound exposure of that same region [74-76]. Chen et al. [77] used an ACD to study the dependence of ultrasound-induced inertial cavitation (IC) on the PD. The dependence of IC activity and hemolysis generated by 1.15-MHz ultrasound as a function of PD (between 5 and 200 cycles) was conducted using a constant peak rarefractional pressure (3 MPa) and constant energy. Two UCAs were used (Optison™ and Albunex®). With Optison™, only 2 of 10 tests under the 50-cycle PD condition generated detectable microbubble signals. For the longer PDs (e.g., 100 and 200 cycles), 9 of 10 tests showed variable amounts of generated microbubbles. Hence, more microbubbles were generated under longer PD conditions. Using Optison™, up to 60% hemolysis was produced with the longer PDs (100 and 200 cycles), compared with <10% hemolysis with the shorter PDs (5 and 10 cycles). Albunex® generated considerably less IC activity and hemolysis than that produced using Optison™.

Acoustic attenuation modifications have also been used to evaluate UCA destruction. Chang et al. [74] defined the threshold as the peak rarefractional pressure that caused total destruction of UCAs in a suspension when exposed to High Intensity Focused Ultrasound (HIFU) at 1.1 MHz. The total destruction of UCAs was determined when the echo amplitude
from the rear wall of a tube filled with UCA returned to the amplitude observed prior to the introduction of UCA in the suspension. Their results showed that the total destruction pressure threshold increased with increasing Albunex® concentration and decreased with increasing pulse repetition frequency (PRF). This threshold decreased with increasing the PD in terms of the number of cycles N for N < 10 and was independent of the number of cycles for N > 10. However, because the return to baseline criterion detects total destruction of all UCA in a solution, onset of microbubble destruction cannot be assessed by this technique nor does it permit isolation of the response from a single microbubble.

Optical observation has also been used to investigate UCA response. Chomas et al. [10] used a high-speed camera to experimentally observe the destruction of isolated UCA microbubbles. Within the studied frequency range (1 to 5 MHz), the fragmentation pressure threshold of the experimental agent studied was shown to increase with frequency (resonant frequency not reported). This direct optical observation approach is currently the reference for measuring UCA fragmentation thresholds, but the expensive equipment necessary for its application limits the technique’s accessibility and is not usable for in vivo studies of optically opaque human organs.

I. 3. Scope of this thesis

The goal of this work is to combine experimental and theoretical tools to investigate the shell rupture of single UCA microbubbles. Three experimental configurations are used (passive cavitation detector, double passive cavitation detector and a system allowing microbubble isolation with optical verification). Acoustic signals acquired experimentally from collapsing microbubbles are used to identify an objective acoustic criterion for the detection of microbubble destruction [61]. Theoretical predictions of the microbubble dynamics are used to reconstruct radius-time (r-t) responses behaving in the same manner as experimentally acquired signals linked to destruction. This comparison between theory and experiment provides insight concerning the destruction process. The experimental investigations described in this work enabled the quantification of the minimum destruction threshold and the destruction occurrence threshold for Optison™. Temporal comparison of signals presenting rupture characteristics received from the same microbubbles with high and low frequency transducers is made. Spectral comparison between oscillating and collapsing
microbubbles is evaluated to assess spectral features that could be used to detect destruction events. Observations obtained with the system allowing microbubble isolation with optical verification are presented to validate the fact that the signal features associated with rupture in the PCD and DPCD experiments are associated with the rupture of single Optison™ microbubbles.

The next chapter, Chapter II, introduces the theoretical model used in this work to calculate the oscillatory response of a microbubble driven by the pressure of an incident acoustic pulse. This model is based on a modified version of the Herring equation. The microbubble radius, wall velocity and acceleration as a function of time determined from this model are then related to the acoustic pressure wave resulting from the microbubble response. The modified Herring equation incorporates two shell terms to describe the properties of an encapsulated microbubble. The driving pressures injected into the model to calculate the microbubble response are obtained from experimental calibrated hydrophone measurements.

No theoretical development currently exists that can predict the microbubble wall motion leading to shell rupture. To approach this problem, the first step is to establish a stronger experimental knowledge of contrast agent destruction. Thus, Chapter III will present the three in vitro experimental systems applied to study the destruction-response of individual contrast microbubbles. The first system consists of a widely accessible passive cavitation detection (PCD) configuration. It consists of a transmit transducer used for microbubble insonation and a receive transducer (of higher center frequency) used to passively collect signals from insonified microbubbles. The transducers are confocally placed in a tank containing a weak dilution of UCA. The dilution is calculated so that on average only one microbubble is within the confocal region of the transmit and receive transducers. The second system extends the PCD system to simultaneously capture signals received by the low-frequency transmit transducer (pulse-echo during the excitation and passively at post-excitation) and the higher frequency transducer (receiving passively at all times). This system is referred to as a double passive cavitation detector (DPCD). The third system, referred to as the optical/acoustic measurement system, allows microbubble isolation with optical verification and provides synchronized optical and acoustic observations of the isolated contrast microbubbles.
The experiments with the PCD and DPCD systems were carried out in the Bioacoustics Research Laboratory (BRL) at the University of Illinois at Urbana-Champaign (Illinois, USA)\(^1\). The synchronized optical and acoustic data were acquired in the Laboratoire d’Imagerie Paramétrique (LIP), UMR 7623 CNRS – Université Paris VI, Paris France. This system was entirely implemented at the Laboratoire d’Imagerie Paramétrique (LIP) during the course of my thesis research. The analysis of data acquired with the three systems was performed in both laboratories (in close collaborations with members of the BRL for the evaluation of PCD and DPCD data).

In Chapter IV the protocol used for PCD experiments is presented. The received acoustic signals are explained in terms of principal, inertial cavitation (IC) and rebound responses. IC signals (and rebounds, although not always present) are linked to shell rupture and are used as a criterion for assessing rupture occurrence. Using these signals (IC signals), the minimum pressure threshold for shell rupture is estimated and presented in this chapter. A comparison of the experimental time-pressure waveform measured with the PCD system at the minimum rupture threshold and the waveform predicted by the modified Herring equation is presented to explore the hypothesis relating shell rupture to the inertial cavitation signals.

Chapter V explains the implementation of an automatic signal analysis algorithm for detection of the post-excitation signal due to the IC response. The algorithm is applied to the full PCD data set to evaluate sensitivity of IC detection to system noise and to estimate the percentage of microbubble destruction as a function of the peak rarefactional pressure. The results of the automatic detection provide an evaluation of the percent destruction occurrence threshold for 0.9, 2.8 and 4.6 MHz center frequency pulses and 3-, 5- and 7- cycle pulse durations.

Chapter VI describes the experimental protocol used for data acquisition with the double passive cavitation detector (DPCD). Results include comparison of the signals acquired simultaneously with the passive receiver and the pulse-echo transmit transducer. Spectral analysis of these signals is made to examine the spectral response linked to destruction across a larger frequency bandwidth. The results provide additional insight as to

---

\(^1\) Exchange funded by the co-operative project for biomedical engineering between the University of Illinois at Urbana Champaign, USA, and the Centre National de Recherche Scientifique, France.
the characteristics of the UCA destruction response within a frequency range and transmission/reception configuration more typical of those used in diagnostic imaging.

Chapter VII presents results obtained with the optical/acoustic measurement system. This system allows the isolation of a single microbubble in a cellulose fiber at the shared focal point of the transmit and receive transducers and the focal zone of an optical microscope. Optical images acquired pre- and post-insonification are used to validate that the IC signals occur only when a microbubble is destroyed by an insonifying pulse. It is shown that the microbubble is no longer present in the post-insonification optical image when post-excitation acoustic signals are detected, but is still found optically when no post-excitation acoustic signals are observed. Optical data from groups of microbubble are displayed in a preliminary investigation of acoustically driven microbubble aggregation.

Lastly, Chapter VIII summarizes and discusses the results obtained in this thesis.
Chapter II – Modeling the UCA response
II. 1. Available theoretical models describing UCA oscillation:

The dynamics of microbubble oscillations have been an active field of research since the mid-1800s. A first theoretical description of the behavior of free bubbles exposed to an external pressure field was developed by Lord Rayleigh (1917) [78]. Herring [79, 80], Gilmore [81, 82], Keller and Kolodner [83], Flynn [84], and Tomita and Shima [85] have consecutively developed systems of equations that model the displacement, velocity and acceleration of the free bubble wall as well as the pressure and temperature fields in the liquid surrounding the bubble and in the gas within. For moderate oscillation amplitudes (up to 100% the initial radius) all these models behave essentially in the same way. However, for stronger oscillations, only a few give satisfactory results. Four free-bubble models have been compared by Vorkurka [86] (the Rayleigh-Plesset, Gilmore, Herring and the modified version of the Herring equation). He found that, at pressures which result in fluctuations in radius greater than 125% of the initial radius, of these four models, only the Herring model and its modified version accurately predict the response of the bubble.

To model the response of encapsulated gas microbubbles to a time-varying pressure, modifications in resonance and damping introduced by the shell must be considered. The first models to consider such factors added terms representing the elasticity and/or damping of the shell in an ad-hoc manner [87-90]. Roy et al. [87] treated the shell as a simple viscous liquid layer. de Jong et al. [88] assumed it to be a viscoelastic solid. These models, have been extensively compared with experimental results. However, the parameters describing the shell elasticity and damping were obtained by fitting the model results to the experimental measurements. Thus, although providing some initial insight, these models lack the ability to predict changes in the agent’s response as shell material parameter properties are modified. Work by Church [91] modeled the shell as a continuous layer of incompressible solid elastic material with a viscous dissipative component. Including these terms in the radial stresses at the solid-fluid interface in the constitutive equation, he derived a Rayleigh-Plesset-like equation for encapsulated microbubble dynamics. The results of this model were compared to experimental results for the encapsulated UCA Albunex and gave satisfactory results [91].

In this work, the modified Herring equation described by Morgan et al [28] was implemented. This model uses the visco-elastic terms derived by Church [91]. It applies to thin shells (between two and three thousandth of the dimensions of the initial radius) with a
fluid-like constitutive equation. The main difference between this equation and the Rayleigh-Plesset equation used by others is the treatment of the damping due to re-radiation. In the Rayleigh-Plesset formulation presented by Frinking and de Jong [92], the radiative losses are included as part of a total damping coefficient. In the modified Herring equation, the losses are each included as separate terms. Because of these terms, the modified Herring equation is more accurate than the Rayleigh-Plesset at driving pressures that result in radial fluctuations greater than 125% of the initial equilibrium radius [86]. Another difference is that the modified Herring equation takes surface tension into account which has been formerly neglected in other models [92]. The modified Herring equation has been validated by direct comparison of the radial expansion predicted by the model with optical microscope observations of radial displacement recorded with a high speed camera [28]. Since its introduction, this model has served as the foundation of most subsequent contrast agent dynamic models found in theoretical and experimental studies [10, 93-95]. The analytical development of the modified Herring equation which was used throughout this work is presented in the next subsection.

II. 2. Description of the Modified Herring Equation Model

In this subsection, the theoretical model used to calculate the wall dynamics of an encapsulated microbubble in response to an ultrasonic pulse is presented. The responses, obtained in terms of the microbubble radius as a function of time, are then used to predict the pressure variations produced by the oscillation. The notation used throughout this section is summarized at the beginning of this dissertation.

The model used in the formulation of the encapsulated microbubble echoes is based on the modified Herring equation for a free microbubble, Eq. II.1, described by Vorkurka et al. [86].

\[ \rho R \dddot{R} + \frac{3}{2} \rho R^2 \ddot{R} = p_i R^{3\gamma} + \frac{R}{c} \dot{P} - (P_0 + P_{\text{driv}}(t)) \]  

(II.1)
where \( \rho \) is the density of the surrounding liquid, \( R \) is the microbubble radius, \( \dot{R} \) is the wall velocity, \( \ddot{R} \) is the acceleration, \( p_i \) is the internal pressure, \( \gamma \) is the polytropic gas exponent, \( P_0 \) is the hydrostatic pressure, \( c \) is the speed of sound, \( \dot{P} \) is the time derivative of the pressure surrounding the bubble, \( P_{\text{drv}} \) is the driving pressure and \( t \) is time.

The final form of the modified Herring differential equation for radial fluctuations of an encapsulated microbubble, (II.2), (described by Morgan et al. [28]) is given by,

\[
\rho R \ddot{R} + \frac{3}{2} \rho \dot{R}^2 = \left( P_0 + \frac{2\sigma}{R_0} + \frac{2\chi}{R_0} \left( \frac{R_0}{R} \right)^\gamma \left( 1 - \frac{3\gamma}{c} R \right) - \frac{2\chi}{R} \left( \frac{R_0}{R} \right)^2 \left( 1 - \frac{3}{c} R \right) \right) - \frac{4\mu R}{R} \left( 1 - \frac{1}{c} R \right) - 12\mu_{sh} \varepsilon \frac{R}{R(R - \varepsilon)} - (P_0 + P_{\text{drv}}(t))
\]  
(II.2)

where \( R_0 \) is the initial microbubble radius at equilibrium, \( \sigma \) is the interfacial tension coefficient, \( \chi \) is the elasticity modulus of the shell, \( \mu \) is the viscosity of liquid, \( \mu_{sh} \) is the viscosity of the shell and \( \varepsilon \) is the shell thickness. The values used for propagation in room temperature water for \( c, P_0, \mu \) and \( \rho \) are:

\( c = 1489 \text{ m/s}, P_0 = 101 \text{ kPa}, \mu = 0.001 \text{ Pa.s} \) and \( \rho = 998 \text{ kg/m}^3 \).

In equation (2) the viscosity of the surrounding liquid is included in the model as \( 4\mu \frac{R}{R} \). The first shell term incorporates the elasticity of the shell and is given by \( \frac{2\chi}{R} \left( \frac{R_0}{R} \right)^2 \).

The second shell term is a damping term due to the viscosity of the shell, which has the form \( 12\mu_{sh} \varepsilon \frac{R}{R(R - \varepsilon)} \). The derivation of these two shell terms can be found in Church [91]. The term \( \sigma \) represents the total surface tension and equals the sum of the gas-shell and the shell-water surface tensions.

The differential equation, (II.2), is put into a non-dimensional form using the variables \( \frac{R(t)}{R_0} = y \) and \( \frac{\dot{R}(t)}{R_0} = \dot{y} \).
The equation (3), below, represents the non-dimensional form of (II.2):

\[
\begin{align*}
\frac{\dot{y}_1}{y_1} &= y_2 \\
\frac{\dot{y}_2}{y_1^2} &= \frac{1}{2} \left( -\frac{3}{2} y_2^2 + \frac{1}{\rho R_0^2} \left( P_0 + \frac{2\sigma}{R_0} + \frac{2\chi}{R_0} \right) y_1^{-3/2} \left( 1 - \frac{3\pi R_0}{c} y_2 \right) - 4\mu \frac{y_2}{y_1^2} \right) \\
&\quad - 2\sigma \left( \frac{1}{R_0} - \frac{1}{c} \right) - \frac{2\chi}{R_0} y_1^{-3/2} \left( 1 - \frac{3\pi R_0}{c} y_2 \right) - 12\mu \epsilon \frac{y_2}{y_1^2} \left( \frac{1}{y_1 R_0 - \epsilon} - \epsilon \right) - (P_0 + P_{\text{driv}}(t)) \right) \end{align*}
\] (II.3)

This equation has been implemented in Matlab (The Mathworks, Natick, MA). An ordinary differential equation solver in Matlab (ode45 based on an explicit Runge-Kutta) is then used to solve for the non-dimensional radius and wall velocity for a microbubble subject to a given driving pressure, \( P_{\text{driv}}(t) \). The driving waveform can be specified by an equation or loaded into Matlab from a text file containing the acoustic pressure vs. time at the focal position of a transducer as measured experimentally with a hydrophone. The initial conditions used are \( R = R_0 \) and \( \dot{R} = 0 \) at time \( t = 0 \).

This model relies on the following assumptions:

- The microbubble is a spherical, gas-filled cavity that remains spherical throughout its oscillation.
- The medium surrounding the microbubble is a viscous liquid of infinite extent.
- The shell is insoluble and viscoelastic with a non-variable thickness.
- The gas obeys a polytropic pressure-volume relationship.
- The gas content inside the shell is constant. \textit{i.e.} There is no mass exchange as, for example, via diffusion across the shell.
- Thermal damping is not included. (This is negligible compared to the viscoelastic damping terms for the range of bubble sizes considered.)
II. 3. Estimations of physical parameters used to describe microbubbles in the model

To apply equation II.3 to predict microbubble wall motion, it is necessary to have estimated values of the microbubble’s physical parameters. The shell parameters remain the most difficult to characterize. Typically, shell terms are estimated in one of the three ways described below. Shell terms can be estimated based on measured bulk properties of the shell material. Estimations are also achieved by matching the scattering and attenuation responses predicted by the model with experimental measurements from a population of microbubbles with a known distribution of resting radii [21, 87-90]. Alternately, the values for the shell terms have been determined from fitting high-speed optical microscope radius-time (r-t) curves from a microbubble with a known equilibrium radius to the r-t curve from the models [28]. In general, the shell parameters can be frequency dependent, and the estimate is made in the frequency range used in medical ultrasound imaging, i.e. between approximately 2 and 8 MHz.

Other parameters can be evaluated independently with reference techniques. The microbubble diameter range in a suspension of UCA can be estimated using a Coulter counter [96]. For surface tension, a standard measurement method uses a thin plate (perimeter about 40 mm) that is lowered to the surface of a shell and the downward force applied to the plate when the plate is just touching the shell is measured. Surface tension is directly equal to this force divided by the perimeter of the plate in contact with the shell. Specific heat can be measured for a given gas composition using a thermal analyzer. Shell thickness can be evaluated with an electronic microscope. The density of the gas is measured using conventional methods widely described in the literature [97].

The Table II.1, below, shows the different values describing microbubble physical properties reported in the literature for three contrast agents.
<table>
<thead>
<tr>
<th>Contrast Agent</th>
<th>Optison</th>
<th>SonoVue</th>
<th>Albunex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell</td>
<td>Albumin</td>
<td>Phospholipid</td>
<td>Albumin</td>
</tr>
<tr>
<td>Mean diameter (µm)</td>
<td>2-4.5</td>
<td>1.5-3.5</td>
<td>2.5-3.5</td>
</tr>
<tr>
<td>Surface tension σ (N/m)</td>
<td>0.073</td>
<td>0.051</td>
<td>0.073</td>
</tr>
<tr>
<td>Ratio of specific heat γ</td>
<td>1.07</td>
<td>1.10</td>
<td>1.40</td>
</tr>
<tr>
<td>Shell thickness ε (nm)</td>
<td>15</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Shell Viscosity μ (Pa.s)</td>
<td>0.2-0.4</td>
<td>0.2-0.45</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>Shell elasticity χ (N/m)</td>
<td>0-4</td>
<td>0-4</td>
<td>0-4</td>
</tr>
</tbody>
</table>

Table II.1 Estimations of the physical properties for three contrast agents [28].

II. 4. Predicting microbubble echoes

Once the non-dimensionalized form of (II.2) has been solved for the time-dependent radius, wall velocity, and acceleration (\(R(t), \dot{R}(t)\) and \(\ddot{R}(t)\), respectively), these are used to determine pressure \(P\) received from the oscillating microbubble as described by Leighton [98] and used by [99]:

\[
P = \rho \cdot r^{-1} \left( R^2 \dot{R} + R \ddot{R} \right) \quad \text{(II.4)}
\]

where \(r\) is the distance between the microbubble and the receiving transducer (0.0381 m for our passive cavitation detection and double passive cavitation detection experiments and 0.0508 m for the optical/acoustic experiments).

The predicted r-t curves produced by Matlab are not sampled on a uniform grid, and therefore each modeled r-t trace is resampled before applying Equation II.4. In the simulations compared with experimental results in this thesis, modeled radius time curves were calculated and recorded for Optison™ microbubbles submitted to a range of ultrasonic driving pressures (0.5 – 5 MPa), pulse durations (three, five and seven-cycle transducer excitations) and frequencies (0.9, 2.8 and 4.6 MHz). Radius-time curves could then be used to calculate the pressure –time curves according to Equation (4).
II. 5. Effect of the shell terms on the modeled radius-time curves

All simulations presented in this section are performed with the same excitation: a
5-cycle pulse duration with a center frequency of 2.8 MHz and a peak rarefractional pressure
of 2.38 MPa. The initial diameter was also kept constant at 3.2 μm.

Fig. II.2(a) shows three curves corresponding to simulated radius-times (r-t) traces for
microbubbles with surface tension of 0.030, 0.050 and 0.073 N/m plotted in blue, green and
red, respectively. Little difference is observed between these curves. Once several cycles of
the excitation have elapsed (between times of approximately 2 and 3 μs in the Figure, the
microbubble does not always contract below its initial radius during the compression phase of
the excitation pulse. The maximum amplitude of radial oscillations varied by only
approximately 1% over the full range of surface tensions 0.030 – 0.073 N/m. This indicates
that the choice of the value of the surface tension used in the model within this range should
have a small effect on the predicted oscillatory behavior.

Figure II.1. Simulations representing the microbubble wall motion using an identical excitation
for three different values of surface tension for μ<sub>sh</sub> = 0.3 Pa.s and χ = 3 N/m.
Figure II.2. Simulations representing the microbubble wall motion using an identical excitation for three different values of the shell viscosity at (a) 0.1 Pa·s, (b) 0.2 Pa·s and (c) 0.3 Pa·s. Each figure display simulations for three value of elasticity (1, 2 and 4 N/m).
Fig. II.2 shows three curves corresponding to simulated r-t traces for microbubbles with shell elasticity of 0, 2 and 4 N/m. The difference between panel (a), (b) and (c) in Figure II.2 is the shell viscosity 0.2, 0.3 and 0.4 Pa.s, respectively. Initially the modeled r-t traces are very similar with differences increasing towards later times in the oscillation. As mentioned in the previous chapter, the more elastic the shell the greater its range of expansion. Thus, it is anticipated that the maximum radial expansion, in the simulated r-t curves, will be observed for the most elastic microbubble (4 N/m, plotted in red), although for the shell viscosity of 0.2 Pa.s in Figure II.2(a), this is not the case. The difference in the maximum radial expansion is more significant from one value of viscosity to another than when the shell elasticity parameter is varied for a constant shell viscosity. Overall differences in the radii maximum expansion predicted for the ranges of 1 and 4 N/m shell elasticity and from 0.2 to 0.4 Pa.s shell viscosity are modest (7% on average). The shell elasticity and shell viscosity for the contrast agent used in this work is estimated to remain in these range of values.

II. 6. Discussion

Various models that describe the dynamic behavior of free bubbles under acoustic insonification are available (Rayleigh, Herring, Gilmore...etc). These models have been extended to include shell terms to better describe the dynamic oscillation of encapsulated microbubbles. The Herring equation, modified to include radiation damping, was chosen for this work. The shell is described using Church’s visco-elastic terms, with an exponential stress-strain relationship. This model also includes more terms describing dissipation than do other models, and it has been show that it described microbubble dynamics more accurately at important radial expansions. This model applies to microbubbles that have small radii and very thin shells (compared to the microbubble equilibrium radius). These conditions and the other hypothesis described in the preceding sections of this chapter are met by the ultrasound contrast agent studied in this thesis.

The values describing medium and contrast agent properties used in the implemented code of the modified Herring equation were taken from the literature. The values for Optison™ surface tension (0.07 N/m), the shell thickness (15 nm) and ratio of specific heat (1.07) were kept constant for all simulations. The driving pressures were measured experimentally using a PVDF calibrated hydrophone at the focal position of each transmitting
transducer for a wide range of peak negative pressures (0.5 – 5 MPa). The radius-time trace calculated from equation (II.3) is related to the backscattered pressure from equation (II.4).

Varying the shell parameters showed that the shell viscosity value plays a more important role in the microbubble response than the shell elasticity value in terms of maximum expansion. However, in the range of viscosity values estimated for Optison™ (0.2-0.4 Pa.s), the model does not show a very important variation in the estimated radius-time curves. The surface tension is expected to have a negligible effect on the simulation.
Chapter III - Experimental Methods
III. 1. Introduction

In this work, experiments were made using three different systems that were designed and implemented to study the interaction between the acoustic response and destruction of ultrasound contrast agent (UCA) microbubbles. The first of the following sections describes measurements performed to characterize the incident pulses produced with the transmit transducers used in each of the three experimental systems. The specific characteristics of each of the three experimental configurations (a passive cavitation detector, a double passive cavitation detector and the optical/acoustic setup) and the UCA studied (Optison™) are then described. The original passive cavitation detector (PCD) system was developed by colleagues at the Bioacoustics Research Laboratory (BRL) at the University of Illinois at Urbana-Champaign (UIUC). The PCD system, and its modified dual passive cavitation detector (DPCD) system, were used for extensive data acquisition in the course of this thesis. The optical/acoustic measurement system was progressively implemented at the LIP during this thesis entailing the testing of the individual system components, interfacing and the development of apparatus for specimen alignment.

Different sets of ultrasonic transducers and transmit electronics were available at the BRL and the LIP. The first section below describes the steps taken to characterize the transmit waveforms in preparation for experimental measurements.

III. 2. Characterization of incident ultrasonic pulse waveforms

An initial series of measurements was made to quantify the center frequency, effective pulse duration and peak rarefactional pressure produced by each transmit transducer over the full range of excitation conditions applied in subsequent experiments. Measurements were performed according to a well-established calibration technique [100, 101]. Each transmit transducer was carefully aligned with a calibrated hydrophone in a Plexiglas tank containing degassed water (boiled, sealed and then cooled) between 20 and 22°C. The hydrophone was positioned in the field to obtain maximum amplitude in the hydrophone response (focal zone). Using the transmit electronics and each combination of transmit amplitude, frequency and pulse duration (PD) that was to be used in subsequent experiments, the waveform received by
the hydrophone was digitized (8-bit 500-Ms/s LeCroy Model 9354TM digital oscilloscope, Chestnut Ridge, NY) and saved for processing off-line using Matlab®.

A PVDF bilaminar shielded membrane hydrophone (diameter of the active element: 0.5 mm, Marconi 699/1/00001/100; GEC Marconi Ltd., Great Baddow UK) was used for evaluation of the pulses produced for PCD and DPCD experiments conducted at the UIUC. A PVDF needle hydrophone, recalibrated with respect to the PVDF bilaminar shielded membrane hydrophone of the BRL, was used for evaluation of the pulses produced for the optical/acoustic experiments conducted at the LIP. Estimated precisions in pressure measurements obtained with these hydrophones were 7 and 15 %, respectively.

![Diagram](image.png)

**Fig. III.1.** Diagram presenting a typical waveform measured with a hydrophone and its envelope. The peak rarefactual pressure and the –6dB pulse duration are indicated on the waveform and its envelope, respectively.

Figure III.1 shows a schematic diagram of a waveform typical of those measured with the hydrophone at the transducer’s focus. The peak rarefactual pressure was measured as shown in the diagram. The pulse duration was determined from the time between the positions on the waveform envelope at which the amplitude decreased to a level 6 dB below the absolute value of the peak pressure (corresponding to half the peak pressure). The center frequencies were estimated for the receive transducer and each transmit transducer by
measuring the scattered signal from a 50-μm-diameter wire reflector translated throughout the focal region in a pulse-echo configuration (using either a Panametrics 5800PR or 5900PR pulser-receiver, Waltham, MA) [102]. The pulse-echo signal presenting maximum amplitude (wire at the focal position) was gated with a rectangular window, from which a fast Fourier transform (FFT) was performed to determine frequency content. The frequency with maximum spectral amplitude provided an estimate of transducer center frequency.

III. 2. a. Incident waveform characteristics for the PCD and DPCD

Four 19.1-mm-diameter single-element focused (f number = 2) transducers (measured center frequencies of 0.9, 2.8, 4.6 or 7.1 MHz) were characterized for use in the passive cavitation and double passive cavitation measurement set-ups. Sinusoidal tone bursts were generated by a pulser-receiver (Ritec Advanced Measurement System RAM5000, Warwick, RI) for a selected combination of frequency (0.9, 2.8, 4.6 or 7.1 MHz) and PD (3, 5 or 7 cycles). The pulse repetition frequency (PRF) was 10 Hz and pulse phase was 0° for all experiments. A 0° polarity waveform is defined as one having the first of the two largest half cycles as a compression, while the 180° polarity waveform has the first of the two largest half cycles as a rarefaction. The transmit pressure amplitude was varied using the pulser-receiver’s output control settings. To fine tune (obtain smaller changes in) the transmit pressure amplitude, a step-variable attenuation bar (Model 358, Arenberg Ultrasonic Laboratory, Boston, MA) was used. Transmit electronics for the PCD and DPCD systems are diagrammed in (Fig III.2).

![Fig. III.2. Transmit electronics for PCD and DPCD.](image-url)
Figure III.3(a) shows the hydrophone-measured waveform (at the focus) of the 4.6-MHz center frequency transducer for a 7-cycle PD at the lowest excitation level. The waveform was symmetric with peak rarefactive pressure and peak compressional pressure of 0.35 MPa.

![Waveform](image)

![Spectral Amplitude](image)

**Fig. III.3.** Lowest amplitude excitation for the 4.6 MHz, 7-cycle pulse (a) Pressure waveform measured at the transducer focus (b) Corresponding spectral amplitude.
The temporal waveform was gated with a 10-μs Blackman window, zero padded to 8192 points from which a fast Fourier transform (FFT) was performed to determine frequency content (Fig III.3(b)). The principal frequency component was observed at 4.6 MHz, corresponding to the measured transducer center frequency. A smaller harmonic component was observed at 9.2 MHz. Its amplitude was half that of the fundamental frequency.

Fig. III.4. Highest amplitude excitation for the 4.6 MHz, 7-cycle pulse (a) Pressure waveform measured at the transducer focus (b) Corresponding amplitude spectrum.
At the highest output setting applied for the 4.6-MHz measured center-frequency transducer, the 7-cycle PD hydrophone-measured waveform (at the focus, 50.8 mm) is displayed in Fig III.4(a). The waveform was highly asymmetric with a peak rarefational pressure of 5.39 MPa and peak compressional pressure of 11.9 MPa. This sort of asymmetry is characteristic of a diffracted beam that has undergone nonlinear propagation distortion [103].

Figure III.4(b) shows the corresponding spectrum in which the nonlinear propagation distortion manifests itself as the transfer of the energy from the fundamental (4.6 MHz) to the higher harmonics. This behavior demonstrates that harmonic signals scattered by a microbubble may not be solely due to nonlinear microbubble dynamics but likely include harmonic components present in the insonating pulse.

By repeating hydrophone measurements for all combinations of transducer frequency, PD and output setting, the incident waveforms used in the PCD and DPCD set-ups were calibrated over the peak rarefational pressure range between 0.07 and 5.39 MPa (Table III.1). The measured centre frequencies were 0.9, 2.8, 4.6 and 7.1 MHz. At 0.9 MHz, the measured PDs were 5.8, 8.0 and 11.1 µs; at 2.8 MHz, were 1.5, 2.6, and 3.3 µs; at 4.6 MHz were 0.9, 1.4 and 2.0 µs; and at 7.1 MHz, were 0.6, 0.9 and 1.2 µs for PDs of 3, 5 and 7 cycles, respectively.

The maximum pressure difference measured between consecutive amplifier output settings was 0.5 MPa, and the minimum pressure change obtained using the external attenuation bar was 0.05 kPa.

<table>
<thead>
<tr>
<th></th>
<th>0.9 MHz</th>
<th>2.8 MHz</th>
<th>4.6 MHz</th>
<th>7.1 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 cycles</td>
<td>0.07 – 1.30</td>
<td>0.39 – 3.83</td>
<td>0.28 – 4.85</td>
<td>0.30 – 5.23</td>
</tr>
<tr>
<td>5 cycles</td>
<td>0.09 – 1.52</td>
<td>0.48 – 4.13</td>
<td>0.32 – 5.40</td>
<td>0.35 – 5.02</td>
</tr>
<tr>
<td>7 cycles</td>
<td>0.10 – 1.60</td>
<td>0.51 – 4.31</td>
<td>0.35 – 5.39</td>
<td>0.42 – 4.98</td>
</tr>
</tbody>
</table>

Table III.1. Peak rarefational pressures (MPa) of incident pressure waveforms for lowest and highest pulser/receiver settings.
A 19.1-mm-diameter, single-element 5-cm focal length transducer (nominal center frequency of 1 MHz) was used for insonification in the optical/acoustic isolated microbubble measurement set-up. Sinusoidal tone bursts were generated with an arbitrary waveform generator (Agilent 33250A, Thousand Oaks, CA) for one nominal transducer frequency (1 MHz) and one PD (7 cycles). The output of the function generator was amplified by 55 dB with an RF amplifier (ENI A150, Rochester, NY) and used to excite the transducer. The transmit pressure amplitude was varied by adjusting the function generator’s output voltage control settings (range: ± 10 Volts and resolution: 10 mV). The transmission system is described schematically in Figure III.5. Pulse phase was 0° and PRF was 25 Hz for all experiments.

Fig. III.5. Transmit electronics of the optical/acoustic isolated microbubble measurement system.
The incident waveforms used in the optical/acoustical measurement system were calibrated for two peak rarefational pressures: 0.2 and 0.6 MPa (obtained at output voltage levels of the Agilent function generator of 0.1 to 0.3 Volts, respectively). The measured PD for these pressures at 7 cycles was 6.9 μs. The measured center frequency was at 0.99 MHz.

III. 3. Passive cavitation detector

Within a Plexiglas tank (50.5-cm long x 25.5-cm wide x 30.0-cm high) containing 9.6 ± 0.3 L of degassed water between 20 and 22°C, a 13-MHz measured-center-frequency, focused transducer (12.7-mm active element diameter and 15.4-mm focal length) was mounted confocal and at a 60° angle to the transmit beam axis. This transducer was used to passively collect emissions from the microbubbles (Fig. III.6).

![Diagram](image)

Fig. III.6. Passive cavitation detection experimental configuration.

The -6-dB field limits were determined for the receive transducer and each transmit transducer by measuring the scattered signal from a 50-μm-diameter wire reflector translated throughout the focal region in a pulse-echo configuration [102]. The characteristics of the transducer fields are summarized in Table III.2.
<table>
<thead>
<tr>
<th>Center frequency (MHz)</th>
<th>-6-dB bandwidth (MHz)</th>
<th>-6-dB beamwidth at focus (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>0.8 - 1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>2.8</td>
<td>2.6 - 3.0</td>
<td>2.3</td>
</tr>
<tr>
<td>4.6</td>
<td>4.3 - 4.8</td>
<td>1.9</td>
</tr>
<tr>
<td>7.1</td>
<td>6.7 - 7.5</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>9.3 - 17.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table III.2. Measured transducer field characteristics for transducers used in the PCD and DPCD systems.

The receiver’s -6-dB field limits were more limited than those of the transmit transducers. Thus, the effective confocal volume corresponded to the 13-MHz transducer’s -6-dB field limits: a cigar-shaped volume 3.38-mm long and 0.25 mm in diameter (approximate volume of 0.12 mm³). The -6-dB field limits are presented graphically in the Figure III.7 and the overlapping beam pattern is demonstrated in Figure III.8.

![Graphical representation of field limits](image)

Fig. III.7. -6-dB field limits of the 13 MHz receiver transducer. The principal beam dimensions were estimated from data obtained with the characterization technique and the elliptical shape was completed based on these dimensions using Matlab. X-axis represents the beam axis.
The PCD passively detected signals from this volume and was primarily sensitive to frequencies greater than the transmit frequency range. The mean concentration of contrast microbubbles injected into the tank was calculated so that, on average, only one microbubble should be within the effective confocal volume at any given time. The output from the receive transducer was amplified (44 dB), digitized (12-bit, 200 MHz, Strategic Test digitizing board UF 3025, Cambridge, MA) and saved to a personal computer.

Fig. III.8. Overlapping beam pattern of the 2.6 and the 13 MHz transducers. This image is a combination of two images obtained from the characterization of the transducers. The characterization was performed by measuring the amplitude of pulse echo reflections from a 50-μm-diameter wire. The wire was moved with a motorized micromanipulator in a 4mm×4mm box centered at the transducer focus. The color of each point in the image represents the amplitude of the signal reflected in dB with white being the highest amplitude and black being a zero amplitude.
III. 4. Double passive cavitation detector

This experimental setup is based on the PCD system. The experiments were carried out in the same Plexiglas tank (50.5-cm long x 25.5-cm wide x 30.0-cm high), however, it contained 19.2 ± 0.3 L of degassed water between 20 and 22°C. In addition to the acquisition of signals passively received by the 13-MHz transducer, the receive electronics were modified so that the signals incident upon the transmit transducer, coinciding-with (pulse-echo) and following (passive) the excitation, could be acquired. The resulting system, allowing simultaneous acquisition of the signals received with the two transducers for a single excitation pulse, formed the DPCD.

The receiver’s -6-dB field limits were again fully inside those of the transmit transducers. However, the effective -6-dB focal volume for signals acquired with the transmit transducer corresponded to the transmit transducer’s -6-dB field. Because the transmit transducer also acted as a receiver in the DPCD system and the aim of the study is to observe individual microbubbles only, it was necessary to reduce the mean concentration of UCA microbubbles injected into the tank relative to the UCA concentrations used in the PCD system. This ensured that, on average, only one microbubble was within the ‘pulse-echo’ volume at any given time. The output from the 13-MHz transducer was amplified by 44 dB and the output from the transmit transducer was amplified by 22 dB. The data were digitized (12-bit, 100 MHz, Strategic Test digitizing board UF 3025, Cambridge, MA) and saved to a personal computer.

III. 5. Optical/acoustic measurement system

It has been noted that the interpretation of data acquired with the PCD and DPCD systems relies upon the hypothesis that, on average, only a single bubble should lie within the interrogated -6-dB beam volume at any given time. Using an optical microscope, the isolated UCA microbubble measurement setup provides an independent means: to verify that only a single microbubble is insonified, to estimate the size of that microbubble and to confirm microbubble destruction (or persistence) following the application of an acoustic pulse.
The global system configuration is schematically shown in Figure III.9 with front and top views of the measurement cell presented in Figures III.10 and III.11, respectively.

Fig. III.9 The global optical/acoustic system configuration.
Fig. III.10 Front view of the optical/acoustic system configuration.

Fig. III.11 Top view of the optical/acoustic system configuration.
An optically transparent, 200-µm inner-diameter hollow cellulose fiber (MWCO, Spectrum Labs Inc., Rancho Dominguez, CA, USA) is positioned in a Plexiglas tank (11.5-cm long 11.5-cm wide 6-cm high) containing degassed water between 20 and 23°C. Each end of the fiber is inserted within a polyethylene tube having an inner diameter of 305 µm (20300300631, Degania Silicone, Israel), and the joints are sealed with glue. Each end of the polyethylene tubing is connected to a needle (G27). The needle has been bent manually to an angle of approximately 80° and each syringe is fixed to a post on an L-shaped plate. Adjustment of syringe fixation at the posts is used to vary the level of inclination of the fiber. The L-shaped plate (Figures III.9 and III.11) is mounted on a micromanipulation system (MDT616, ThoroLab, UK) that allows micrometric positioning of the fiber along x, y and z axes. The syringe at the inflow end of the tube consists of the HI-7 furnished with a system for manual microinjection (IM-5B, Narishige Inc., Japan). The syringe at the outflow end consists of a 1-mL syringe without a piston that is subsequently connected to a larger outflow tube.

A solution of contrast agent was diluted in saline to approximately 1 microbubble/µL before use. This dilution provided for approximately one microbubble per field of view. The dilute contrast agent solution was pumped through the tube. To ensure the presence of only one microbubble, the objective of the microscope was moved throughout the entire depth of the cellulose fiber to visually scan the entire tube depth. Then the focus of the objective was adjusted to optimize visualization of the isolated microbubble.

The ultrasonic transducers were fitted in two orthogonal sides of the tank such that the cellulose fiber was at the focal distance of each and the long axis of the cellulose fiber was at an angle of 45° with respect to the insonification axis of each transducer. The transducers were mounted at an angle of approximately ten degrees above horizontal to avoid acoustic reflection from the rear walls. The 0.99 MHz measured center frequency transducer (V302, Parametrics, MA) was used to transmit and a 9.8 MHz measured center frequency transducer (ILD-1006-GP, Sofratest, France) was used in reception. The -6-dB transducer beamwidths (manufacturer’s values) are summarized in Table III.3.
<table>
<thead>
<tr>
<th>Centre frequency (MHz)</th>
<th>-6-dB bandwidth (MHz)</th>
<th>-6-dB beamwidth at focus (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.99</td>
<td>0.67 – 1.30</td>
<td>3.1</td>
</tr>
<tr>
<td>9.8</td>
<td>6.64 – 13.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table III.3 Transducer field characteristics for transducers used in the system allowing microbubble isolation with optical verification as measured by the manufacturer’s using impulsive sources. The 1-MHz transducer was characterized using a Panametrics 5030A, the source used for the 10-MHz transducer was not reported.

The transmitting electronics (described in the previous section) of this system consist of an arbitrary waveform generator (Agilent 33250A, Thousand Oaks, CA), which is used to create the pulse train, and an RF power amplifier (ENI A150, Rochester, NY), which amplifies the signal at 55 dB. The received signals were amplified (BR 640A, Ritec Inc, Warwick, RI) and recorded by a digitizing oscilloscope (6051A, Lecroy Inc. Chestnut Ridge, NY) controlled by LabView (National Instruments, Austin, TX). Each time trace containing 20000 points at 100 Msamples per second sampled at 8 bits was stored for data analysis using Matlab® (MathWorks, Natick, MA).

To align the cellulose fiber at the mutual foci of both ultrasonic transducers, the fiber was filled with air and the amplitude of the acoustic signal reflected by the fiber was optimized in pulse-echo mode for each of the two transducers. For this alignment, a short pulse produced and received by a Panametrics 5052PRX was used.

To demonstrate the relative acoustic transparency of the cellulose fiber two acquisitions were performed using a 7-cycle, 0.82 MPa and 0.99 MHz center frequency insonification pulse. Figures III.12 (a) and (b) present the signal received with the 9.8 MHz transducer from a fiber containing non diluted contrast agent solution and from a fiber filled with degassed fluid, respectively.
A Leica Z16 APO macroscope (Z16 APO, Leica, Bannockburn, IL, USA) interfaced to a camera was used for optical observation of the fiber and its contents. A 9.2x zoom level with a 20X objective (Leica Achromat 100X, NA = 0.42) provided sufficient magnification for the visualization of UCA microbubbles to approximately 0.8-μm diameter while providing...
a significant working distance of 20 mm. The large working distance of this objective offers the advantage that no acoustic echoes are reflected by the microscope objective (the objective tip remains above the water’s surface). A high-intensity, continuous halogen source (250 Watts, Techni-Quip Inc., Eastlake, OH) was used to illuminate the fiber in transmission. The light was transmitted through a 2-m fiber-optic cable positioned approximately 20 mm below the Plexiglas tank and 37 mm below the microscope objective. A 20-mm focused optical lens was fixed into the bottom of the tank for light transmission and focusing on the fiber. Once the transducers have been aligned with the fiber, the optical focus is adjusted to optimize the image of a microbubble within the fiber. Figure III.13 shows a single microbubble in the cellulose tube. The tank and the microscope are mounted on an active antivibration table (07 OTI 031, Melles Griot Ltd, UK).

![Image](image.png)

**Fig. III.13.** Example of an image taken with the camera showing a single microbubble in the field of view at 20×9.2 total magnification.

A short exposure-time camera was mounted on the macroscope (LH 509 ULL, France). The black and white CCD camera can capture images for exposure times as short as 100 ns and benefits from maximum light intensity because the light beam is not split in the core of the macroscope tube. Maximum image acquisition frequency is 25 Hz. Images received by the camera were recorded via a digital card (Matrox Meteor-II, Matrox imaging, Canada) and saved to PC using commercial software produced by the camera and card
retailers. The spatial resolution is 0.12 μm/pixel and the number of pixels is 768X576 in an image.

III. 6. Contrast agent

Experiments were conducted with the three measurement systems using Optison™ (Mallinckrodt and Molecular Biosystems, San Diego, CA), an FDA-approved UCA. Optison™ microbubbles have an albumin shell, approximately 15-nm thick, encapsulating perfluor C₃F₈ gas. The solution in the manufacturer’s vial has a concentration between 5 and 8 × 10⁸ microbubbles/ml. Approximately 93% of the microbubble diameters are less than 10 μm, with a maximum diameter of 32 μm and a mean diameter in the range of 2-4.5 μm [104]. The vials of Optison™ are stored in a refrigerator at –6°C. Before use, the vials need to be gently rolled between the hands to eliminate bubble aggregates.

III. 7. Summary

Initially, all the transducers used in experiments were carefully calibrated and characterized in order to have a precise knowledge of the pulse interacting with the microbubbles and the active volume of the measurement region. The pressure waveforms recorded at the focal distance of each transducer with a hydrophone provide precise data describing the incident acoustic pulse for numerical modeling of microbubble oscillations. The waveforms demonstrate that the nonlinear components in the incident pulse originating from nonlinear wave propagation in water can be very significant. This phenomenon in the distortion of the incident pulse becomes more and more important as its pressure amplitude increases.

Three experimental systems are presented. Each was used to study the acoustic response and destruction of single UCA microbubbles. First, the PCD system provided a straightforward and easily implemented means to detect the broadband microbubble destruction response using a passive receiver with a frequency range above the transmit frequency. Second, this system was adapted to what we refer to as a DPCD. The DPCD completes passive measurements at high frequency with the simultaneous acquisition of
signals received in the transmit bandwidth. The optical observation provided by the system allowing microbubble isolation with optical verification added two important capabilities not available with the PCD and DPCD systems: the possibility to verify the presence of a single microbubble in the insonified zone optically and the possibility to confirm its acoustic destruction or persistence. However, these capabilities are obtained at the cost of a much more complex measurement system with a lower data acquisition throughput. Results obtained with each of the experimental systems are presented in the following chapters.
Chapter IV – Ultrasonic contrast agent shell rupture detected with a passive cavitation system\textsuperscript{2}

IV. 1. Introduction

Using a passive cavitation detector (PCD), this work evaluates rupture based on acoustic emissions from single encapsulated gas-filled microbubbles. Sinusoidal ultrasound pulses were transmitted into weak solutions of Optison™ at different center frequencies (0.9, 2.8 and 4.6 MHz), pulse durations (3, 5 and 7 cycles of the center frequencies), and peak rarefractional pressures (0.07 to 5.39 MPa). Pulse repetition frequency was 10 Hz. Signals detected with a 13-MHz center-frequency transducer revealed post-excitation acoustic emissions (between 1 and 5 μs after excitation) with broadband spectral content. The observed acoustic emissions were consistent with the acoustic signature that would be anticipated from inertial collapse followed in some cases by “rebounds” when a microbubble ruptures and thus generates daughter/free bubbles that grow and collapse. The peak rarefractional pressure threshold for detection of these emissions increased with frequency (e.g., 0.53, 0.87 and 0.99 MPa for 0.9, 2.8 and 4.6 MHz, respectively; 5-cycle pulse duration) and decreased with pulse duration. The emissions identified in this work were separated from the excitation in time and spectral content, and provide a novel determination of microbubble shell rupture.

IV. 2. Data acquisition

The measurement protocol, using the passive cavitation detector (PCD) described in chapter III, was as follows. The tank was filled with degassed water. The selected transmit transducer was aligned with the PCD receiver. A baseline data acquisition was made in the absence of contrast agent by acquiring 128 received waveforms at each transmit pressure amplitude. Using a graduated syringe, 0.2 ml of Optison™ was injected into the tank corresponding to approximately 10^8 microbubbles, and resulting in a mean concentration in the tank of about 10 microbubbles/μl. Thus, on average, only one microbubble should be within the -6-dB receiver volume. The water was gently stirred with a pump before and during data acquisition to maintain an even distribution of the UCA in the water and to ensure its replenishment in the active volume. A 3-cycle PD at the transducer center frequency was generated. For each transmit pressure amplitude (varied from highest to lowest), 128 consecutive received waveforms were acquired. This acquisition procedure was repeated for 5-cycle PDs and then 7-cycle PDs. Total acquisition time after introduction of Optison™ into
solution did not exceed 2 hours. Samples of a degassed water and Optison™ solution under the same conditions and at the same concentration as used for the experiments were observed under a microscope. Microbubbles were observed to remain intact throughout the experimental duration.

IV. 3. Data analysis

The DC component of each received waveform was set to zero by subtracting its mean value. The noise level was evaluated from each baseline acquisition made in the absence of Optison™. Each set of waveforms acquired with Optison™ was then sorted from highest to lowest echo amplitudes. Then, each waveform was inspected and segments with signals above the noise level (indicating UCA response) were selected for further analysis. A sliding Blackman window (120 points, 0.6 μs) was moved along the selected portion of each zero-mean received waveform in steps of 0.025 μs. Windowed signals were zero padded to 8192 points and their FFT was calculated (frequency resolution of 24 kHz). The frequency content of signals received from a microbubble in the interrogation volume during and after insonification was evaluated graphically by plotting these spectra as a function of time (spectrogram).

IV. 4. Results

Figure IV.1 shows a representative echo waveform and its time-frequency spectrogram for a single microbubble. The 4.6-MHz, 7-cycle PD transmit pressure waveform had a peak rarefactive pressure of 0.95 MPa. The echo waveform between approximately 2 and 3 μs corresponds to the PCD response of the microbubble due to the excitation. We refer to this as the principal response. In the spectrogram, the band centered near 9.3 MHz contains harmonics that may have been generated both by nonlinear bubble dynamics and nonlinear propagation distortion of the pulse incident on the microbubble. The thin vertical lines (at discrete times across a large range of frequencies) demonstrate that broadband content appears periodically during the compression phases. After the end of the excitation (end of the principal response), no acoustic emissions are detected in the time trace.
Figure IV.2 shows an echo waveform and its time-frequency spectrogram for a single microbubble excited by 4.6-MHz, 7-cycle PD transmit pressure waveform with a peak rarefractional pressure of 2.82 MPa. The echo waveform between approximately 2 and 3 μs corresponds to the PCD response of the microbubble due to the excitation (principal response). In the spectrogram the strong bands near 10 and 15 MHz are harmonics generated both by nonlinear propagation distortion of the incident pulse and nonlinear bubble dynamics. The spectral amplitude near 9.3 MHz is approximately 9 times higher in Figure IV.2 than in Figure IV.1. Again, thin vertical lines with broadband content appear periodically during the compression phases. After the end of the excitation, at around 4.5 μs (Fig IV.2(a)), a short-duration response is seen in the time trace, and a corresponding broadband signature (from 3 to 19 MHz) is observed in the spectrogram (Fig IV.2(b)). Such post-excitation short-duration broadband response corresponds well to the anticipated acoustic signature due to the inertial collapse followed in some cases by “rebounds” of daughter bubbles [105]. There is also evidence of a rebound at 5.5 μs. Such a repeated rebound event is also consistent with the existence of a free bubble undergoing inertial oscillations. The release of daughter bubbles following UCA shell rupture has previously been observed optically [37, 106]. After the shell has ruptured, unconstrained daughter bubbles are formed [105] that are able to grow and collapse essentially as free IC bubbles [106]. It is highly unlikely that the rebound signature could be generated by a microbubble that is still encapsulated because encapsulated microbubble oscillations are damped by the shell properties after the excitation. In this work inertial cavitation is defined as a pulse occurring after the end of the excitation, this signal can be followed by broadband pulses of less intensity called rebounds. Thus we hypothesize that absence of post-excitation inertial collapse signals (Fig IV.1) indicates that the shell has not ruptured. Therefore we used the presence of the inertial collapse signal (followed in some cases by additional signals corresponding to rebounds) to define a minimum rupture threshold for the UCA: the lowest excitation peak rarefractional pressure for which an IC is detected from any of the acquired signals.
Fig. IV.1. PCD measurements (13-MHz receiver) for a 4.6-MHz, 7-cycle, 0.95-MPa peak rarefactual pressure excitation (a) Time waveform. (b) Corresponding time-frequency spectrogram.
Fig. IV.2. PCD measurements (13-MHz receiver) for a 4.6-MHz, 7-cycle, 2.82-MPa excitation
(a) Time waveform. (b) Corresponding time-frequency spectrogram.
Fig. IV.3. Peak positive and negative voltages measured by the 13-MHz PCD receiver as a function of incident peak rarefractional pressure for Optison™ microbubbles excited with a 4.6-MHz pressure waveform. (a) 3-cycle PD, (b) 5-cycle PD and (c) 7-cycle PD. Error bars show standard deviations. The arrows indicate the incident peak rarefractional pressure thresholds at which the first IC pulse was detected (minimum rupture threshold).
Figure IV.3 shows the peak positive and peak negative voltages from the 13-MHz PCD receiver of the principal microbubble response as a function of the incident peak rarefational pressure. Data are shown for 3-, 5- and 7-cycle PDs at 4.6-MHz transmit signal. Each point is the average value from the subset of the 128 acquired time traces where a response was detected. Error bars represent the standard deviation of this average.

For incident peak rarefational pressures up to about 1.5 MPa (Fig IV.3), there is a smooth and gradual increase in the peak voltage signals received during insonification. For incident peak rarefational pressures greater than 1.5 MPa there is a marked increase in the standard deviation of the 13-MHz PCD receive signals. This increase can be attributed to large variations in the pulse-to-pulse response of the microbubbles, presumably due to rupture of the contrast agents. The arrows in Fig IV.3 indicate the incident peak rarefational pressure thresholds at which the first (lowest incident peak rarefational pressure) IC pulse was detected for each PD at 4.6 MHz. Inertial collapse and rebound signals were detected for peak rarefational pressures around 0.8 MPa (7-cycle data). This is well below the onset of unsteady behavior of the peak PCD receive voltages. Thus, occurrence of IC and rebound signals appears to identify rupture at lower transmit pressures than would be identified based on modification of the voltage amplitude of the principal bubble response (percent of spikes in the principal microbubble response [7]).

The lowest incident peak rarefational pressures for which IC pulses were observed in the detected data are shown in Fig IV.4 as a function of the center frequency and PD. The data show that the peak rarefational pressure required at rupture threshold increases with increasing frequency and decreases with increasing PD. This peak rarefational pressure response is consistent with frequency and PD dependencies reported in the literature [7, 10, 71, 74, 107].
Fig. IV.4. Minimum rupture thresholds obtained using inertial collapse criterion (error bars represent uncertainties in the hydrophone measurement of the incident peak rarefactional pressure)

IV. 5. Discussion

We contend that the post-excitation acoustic signal we have identified is a unique signature of IC collapse and a robust indicator of UCA rupture. Although we do not have the capability of high-speed camera imaging, there is substantial circumstantial evidence to support our contention. The most telling feature is that the signal is only present above a certain pressure threshold and that the timing of the signal is such that it occurs after the passage of the acoustic excitation. It has been shown in the shock wave lithotripsy literature (albeit on longer time scales) that this post-excitation acoustic signal is a unique signature of IC collapse (see e.g., [105, 108, 109]). It has also been shown with optical observations that microbubble rupture is followed by the creation of unshelled daughter bubbles [10] that could then be nuclei for IC. In another study of the rupture of Optison™ contrast agent, similar post-excitation signals were visible in time-traces from the PCD [110-112] but no interpretation of these signals was provided by the authors.
We have found observations of signals identified as IC and rebound responses are consistent with predictions of existing microbubble dynamics models. We modeled the microbubble dynamics using the shelled microbubble model described by Morgan et al. [28]. This model, described in chapter II, employs the modified Herring equation for microbubble dynamics with two terms augmented to account for the elasticity and viscosity of the shell of the microbubble. The initial microbubble radius used in simulations was 2 μm and the shell thickness was 15 nm, which correspond to the mean values reported for Optison™ [104]. The shell elasticity was 4 N/m and the shell viscosity was 0.073 Pa.s. The values of these two parameters were taken from the values given by Morgan et al. [28]. The measured acoustic waveforms were used as the input acoustic driving pressures to the microbubble dynamics model.

Figure IV.5(a) shows the 7-cycle incident pressure waveform measured at the experimentally determined shell rupture threshold of 0.89-MPa peak rarefactual pressure. The modeled microbubble radius as a function of time (r-t curve) is shown in Fig IV.5(b) assuming an intact shell. It can be seen that the microbubble only responds when the driving pressure is present, and the oscillations die out as soon as the drive signal stops. The maximum radius attained with this simulation was 3.3 μm and we assumed that shell rupture occurred near this radius because at this pressure IC signal was observed experimentally. In a second simulation, the shell elasticity and viscosity were initially 4 N/m and 0.533 Pa.s, but once the microbubble radius exceeded 3 μm, the values for both of these parameters were set to zero so that the effect of the shell was no longer included in the simulation. The resulting r-t curve is shown in Fig IV.5(c). In this case, once the shell was removed the bubble underwent large oscillations that continued well after the driving pressure had ceased. In particular, there was an inertial growth and collapse phase that started at 3 μs and ended at 4 μs (Fig IV.5 (c)). The inertial collapse was followed by many rebounds.
Fig. IV.5. Bubble dynamics simulations. (a) Waveform measured at the focal distance with a hydrophone for a 4.6 MHz, 7-cycle excitation used as driving pressure (b) Radius-time curve for a microbubble with an intact shell modeled with the modified Herring equation. (c) Radius-time curve for a microbubble with a shell that ruptures when the radius first exceeds 3 micrometers. (d) Radiated pressure from intact bubble (b). (e) Radiated pressure from ruptured bubble (c) with the inertial collapse and rebounds identified.
Figures IV.5 (d) and (e) show the radiated pressure from the simulated microbubbles as a function of time calculated using the Equation II.4 in chapter II. For the intact shell (Fig. IV.5 (d)) the radiation was only emitted when the driving pulse was present and appeared to be primarily scattering of the driving pulse. The ruptured microbubble (Fig. IV.5 (e)) generated scattering during the excitation but there were also distinct spikes that could be attributed to strong collapses. These collapses are possible because once the shell has ruptured it can no longer act to dampen the response when the driving pressure is present. After the passage of the excitation pulse, the bubble continued to emit a number of distinct spikes due to both the inertial collapse (at 3 μs) and the subsequent rebounds (e.g., 4 μs). The simulated pressure waveform of a ruptured bubble (Fig IV.5(e)) indicates that the first post-excitation signals identified in PCD traces (e.g., Fig IV.2) should correspond to the IC of a ruptured microbubble. The regular temporal separation between post-excitation emissions in the PCD traces is consistent with regularity of IC and free-bubble rebounds in the simulation.

We note that the measured IC time (t_i) could be used to estimate the maximum bubble radius of the ruptured contrast agent through application of the Rayleigh collapse model (see, e.g., [113, 114]). We define the inertial time t_i as the time from when the microbubble is ruptured during excitation by the negative phase of the ultrasound pulse to its collapse, for example, in Fig IV.5(c) t_i ≈ 1 μs. The Rayleigh collapse model relates the initial radius of a vapor cavity at rest to the time required by the fluid to collapse the radius to zero. From the predicted r-t curve in Fig IV.5(c) we observe that the collapse time, that is, the time from its maximum radius to its collapse, is approximately t_i/2. The corresponding maximum bubble radius, predicted using Rayleigh’s expression, is

\[
R_{\text{Max}} = \frac{t_i}{2} \times \sqrt{\frac{P_0}{\rho}} \times 0.915
\]

(1)

where P_0 and ρ are the ambient pressure and density of the liquid, respectively. For water, R_{Max} ≈ 5.5 t_i, where R_{Max} is measured in micrometers and t_i in microseconds. For t_i ≈ 1 μs in Fig IV.5 (c), the Rayleigh model predicts a maximum bubble radius of 5.5 μm. This predicted value corresponds well to the value obtained in the simulation (Fig. IV.5 (c)) (even though the
r-t curve is still partially affected by the incident pressure when the maximum radius is reached). By measuring $t_1$ it is therefore possible to estimate the maximum size of the unconfined bubble.

Based on identification of the rebound signal, we have experimentally determined minimum shell rupture thresholds (based on peak rarefactive pressure) of Optison™ in terms of frequency (0.9, 2.8 or 4.6 MHz), and PD (3, 5 or 7 cycles). The minimum thresholds varied from 0.29 to 0.99 MPa. For a 0.9-MHz, 7-cycle incident pulse (PRF 10 Hz), we found the collapse threshold of Optison™ to be 0.29 MPa. This compares favorably with the value of 0.13 MPa for a longer pulse (10 cycles at 1.1 MHz) reported by Chen et al. [7], who used broadband noise to identify destruction thresholds. For much longer PDs (in the continuous wave regime), Geisecke and Hynynen [71] estimated peak rarefactive pressure thresholds for Optison™ fragmentation to be 0.4 MPa at 2.18 MHz and 1.6 MPa at 3.3 MHz. Although we only applied a 7-cycle PD as our longest PD, the range of their values encompasses our estimation of 0.71 MPa obtained with an intermediate frequency of 2.8 MHz. Miller and Thomas [107] assessed the IC occurrence based on hydrogen peroxide levels produced during continuous wave ultrasonic exposure of Albunex® and Levovist® solutions. Thresholds were thus determined to be 0.41 MPa at 2.17 MHz and 0.58 MPa at 2.95 MHz (spatial peak pressure amplitude; temporal peaks not reported), compares with our thresholds for Optison™. Chomas et al. [10] used high-speed photography to measure fragmentation of MP1950 UCA. Although they do not report thresholds they show that fragmentation occurs at a peak rarefactive pressure of 1.2 MPa for a 2.4-MHz 2-cycle pulse indicating that the threshold must be less than 1.2 MPa which is consistent with our results although true comparison can’t be made considering the important differences in the shell properties of Optison™ and MP1960. Holland et al. [76] exposed Albunex solutions to 1-cycle M-mode and 4-cycle Doppler mode clinical ultrasound (at 2.5 MHz and 5.0 MHz). Using a 30-MHz active cavitation detection system, they did not detect cavitation for peak rarefactive pressures up to 1.2 MPa. With the exception of the results reported by Holland et al. [76], pressure thresholds reported for IC in UCA solutions are in the same general range as pressures estimated for contrast rupture in our study.

We found that the minimum incident peak rarefactive pressure necessary to induce UCA rupture increased with the excitation frequency. For this case where the studied frequency range was centered above the resonant frequency of the UCA (resonant
frequency \approx 1 \text{ MHz for Optison}^{\text{TM}} [115]), this result was expected. At lower frequencies the duration of the rarefactual pressure (which drives the microbubble growth) is longer. Longer duration rarefaction should make microbubbles grow to a larger size and thus be more likely to provoke shell failure. Also we found that shorter PDs required larger peak rarefactual pressures to induce rupture. This was also expected. Rupture in most materials is a stochastic process, and therefore, by applying more cycles per pulse, shell rupture would be more likely. Similar variations of shell rupture thresholds have been demonstrated as a function of frequency and insonification PD in an experimental contrast agent using a high-speed camera [10] and as a function of PD based on attenuation measurements in Albunex [74].

IV. 6. Conclusion

We report on a technique that utilizes passive cavitation detection. However, instead of analyzing signals received during acoustic excitation, broadband emissions occurring between 1 and 5 \mu s after excitation were identified. We have linked these signals to the inertial cavitation of bubbles released after UCA shell rupture, and refer to the minimum incident peak rarefactual pressure leading to such an event as the minimum rupture threshold. Because these emissions were separated in time and spectral content from the main echo, they were easily distinguished from the principal microbubble response (e.g., oscillations and shell rupture). Thus, the detection of shell rupture thresholds using IC emissions as proposed herein is different from previously reported approaches using passive cavitation detection and has several potential advantages over other techniques described in the literature. Detecting UCA rupture from signals during the acoustic excitation is confounded by the presence of spectral components from many sources, for example, nonlinear propagation of the incident pressure pulse and nonlinear microbubble dynamics. The inertial collapse and rebound signals are not contaminated by nonlinear spectral content from other sources. Thus, detection of these post-excitation signals provides a more robust detector of UCA rupture than can be obtained through analysis of the principal response. Second, and this is true for all PCD-based techniques, the equipment necessary to make the measurements is less complex and expensive than that required for a high-speed camera, and the PCD has the potential to detect the rupture thresholds \textit{in vivo} as has been done in lithotripsy [116]. Finally, the literature related to UCA destruction applies a variety of terms to describe destruction thresholds such as fragmentation, cavitation, rupture and collapse. In
effect, each term refers to specific aspects of the destruction process [105, 117]. By comparing our experimental results with numerical simulations of the dynamics of shelled microbubbles, we find that our observations are consistent with the inertial cavitation response of a free bubble that is released after the rupture of the shell of a UCA.

This work links post-excitation acoustic emissions, detected with a passive cavitation system, to inertial collapse and rebounds following rupture of single UCA microbubbles. Minimum incident rarefactional excitation pressure thresholds detected using this signal (IC signal) are consistent with anticipated frequency-dependent and pulse-duration-dependent behavior. Experimental results were consistent with numerical simulations employing existing models for microbubble dynamics that considered the process of shell rupture, inertial cavitation and acoustic emissions. The separation of emissions from the excitation in time and spectral content, the simplicity of measurement equipment and potential for in vivo application represent significant advantages of this technique compared to techniques previously applied to detect UCA destruction.
Chapter V – Automatic detection of IC events:
Exploration of the noise floor and destruction occurrence
thresholds
V. 1. Introduction

Detection of IC events in the initial data set acquired with the PCD system, as presented in the Chapter IV, relied heavily upon time-consuming visual inspection of the data. To permit more efficient and automated analysis of the data, a detection algorithm for IC and rebound signals was developed. This automated detection algorithm was then applied to the data obtained with the PCD system to evaluate the sensitivity of IC detection relative to voltage levels in the noise as a function of incident peak rarefractional pressure. Furthermore, results in Chapter IV characterize only the lowest incident peak rarefractional pressures for which IC pulses were observed. Thus, once an automated IC detection technique was developed, it was used to evaluate the percent occurrence of IC events sampled as a function of incident peak rarefractional pressure. Finally, for incident peak rarefractional pressures at 50% destruction occurrence, the modified Herring equation was used to model the maximum expansion and the velocity of the microbubble wall associated with this destruction occurrence.

V. 2. Automatic IC detection

During data acquisition, 128 transmit pulses were initiated at a PRF of 10 Hz (every 100 ms) and for each transmitted pulse a data segment was acquired with the 13-MHz PCD receiver. Each of the 128 received signals has a t = 0 reference at the time of initiation of the transmit pulse. Each signal is truncated to keep a 4000-sample segment (20-μs duration) centered at the position corresponding to the time-of-flight (TOF) to the center of the confocal zone of the PCD system (at t = 42 μs). The TOF to a microbubble detected in the confocal zone is estimated to be 42 ±0.3 μs based on estimations of the times-of-flight to a microbubble located at either extremity of the −6 dB confocal volume.

Figure V.1 shows an example of a 4000 sample-point truncated segment. The PCD response shown represents the microbubble response during excitation, defined as “the principal response”. The principal response does not include IC or rebound responses which occur post-excitation. The principal response is centered at about 42 μs. Because the maximum duration of a principal response observed with the lowest frequency and longest pulse duration (0.9 MHz, 7-cycle) is approximately 10 μs, the principal response is entirely
captured in the 4000 sample truncated segment. Hence, as the segment is 20 μs in duration, there is a security margin of approximately 10 μs in duration so that data can be acquired prior to and after the principal response. Prior to the principal response, the first 100 samples (0.5 μs) in this security margin are assumed to correspond to noise (no microbubble or related responses) and are used for evaluation of signal noise amplitude. After the principal response, the security margin is assumed long enough to include any post-excitation signals related to IC. In the course of data analysis performed previously by visual observation of signals (reported in Chapter IV), post-excitation (IC and rebound) signals were never observed at times greater than 5 μs after the end of principal response.

![Graph](image.png)

**Fig. V.1.** A 20-μs duration segment of the signal centered on the TOF to the confocal zone of the PCD system (13-MHz receiver) for a 0.9-MHz, 7-cycle, 0.6-MPa peak rarefractional pressure excitation. The principal response arising from the microbubble response during acoustic excitation has a total duration of less than 10-μs. The additional 10 μs of data kept for analysis is divided between an approximately 5 μs data record prior to and a 5 μs data record following the principal response.

For signal processing, the truncated PCD response signals are presented in units of linear voltage as a function of time. The DC component of each truncated signal is set equal to zero by subtracting the signal’s mean value. The central sub-segment (10 μs in duration) of one such signal, centered on the TOF to the confocal zone, is presented in Fig. V.2, Step 1 to illustrate the first steps of the automatic detection algorithm. For each of the 128 segments,
the maximum absolute voltage amplitude in the first 100 samples of the 4000 sample-point truncated segment was calculated to evaluate the maximum signal amplitude in the noise prior to microbubble excitation, $V_{\text{max, noise}}$.

Once the maximum voltage in the noise has been characterized, for each 4000-point segment, the peak maximum and minimum voltage amplitudes are detected. The first two such detected peaks are illustrated by the positions of the two asterisks in Fig. V.2, Step 2. Of these two peaks (not necessarily adjacent), the one occurring at the greater time is defined as the principal peak (regardless of its amplitude or sign). In the example in Fig. V.2, Step 2 the principal peak is the positive peak because it occurs at a time greater than that of the negative peak. The principal peak will be denoted peak #1 and quantitatively described by its amplitude and the time when the peak occurs. The time of the nearest zero-crossing immediately adjacent to and at a time greater than that of the principal peak is also recorded. Because the principal peak is systematically observed towards the middle of the principal response (during microbubble excitation), the algorithm searching for an IC event need not consider any point prior to this recorded zero-crossing (the time of the zero crossing immediately adjacent to and at a time greater than that of the principal peak). Points exempted from analysis are illustrated in Fig. V.2 by a black color. In practice, this exemption is performed by setting all excluded points in the signal segment to zero. Removing these points from further analysis prevents the detection of irrelevant peaks occurring prior to the principal peak.

Then, iteratively in order of decreasing absolute magnitude (steps 2-6), subsequent half-cycle peak minima and maxima are detected. As each half-cycle peak is detected (and the time and amplitude of the peak are recorded), the points in the signal between the two zero-crossings on either side of the half cycle are exempted from the subsequent search for the IC peak. This exemption of half-cycles avoids multiple detection of the same peak as well as false detection of intermediate local peaks in subsequent iterations. The automatic peak detection algorithm continues detecting peaks sequentially in the order of decreasing absolute magnitude, until one of the following two criteria is satisfied:

- The absolute magnitude of a detected peak is less than $V_{\text{max, noise}}$.
- The temporal width of a detected peak has only three points (zero-crossing/peak/zero-crossing). Three points are not sufficient to describe a half-cycle of the pulse; sampling frequency is 200 MHz.
These are the two criteria (in terms of amplitude and time) for ending the peak detection algorithm. Once all peaks (maxima and minima) have been detected, they are numbered according to their magnitudes as illustrated in Fig. V.2, Step 6.

Visual observation of the microbubble response in Fig. V.2, Step 1 shows that the principal response of the microbubble is between approximately 1 and 3 μs. Within the principal response, and at times greater than that of the principal peak, the peaks (both positive and negative) of this signal continuously decrease in voltage amplitude with increasing TOF. In other words, the principal response amplitude decreases as a function of time beyond the time of the principal peak. After the end of the principal response, at about 4.2 μs, a broadband pulse (composed of detected peaks #3 and #8) appears. The voltage amplitude of this pulse is higher than that of the last pulse (detected peaks #9 and #10) in the principal response. This pulse (peaks #3 and #8) is the inertial cavitation signal. At a greater time (~5.7 μs; peak #7) a rebound signal is detected. All broadband pulses present after the IC signal are defined as rebounds. In this example only one rebound signal is present. Sometimes multiple rebounds are observed. The maximum number of rebounds observed in this PCD data set was three. Sometimes, however, no rebounds are detected after an IC signal. Because post-excitation signals are present in the example presented in Figure V.2, the microbubble is assumed to have ruptured. The aim of the IC detection algorithm is to detect such behaviors automatically instead of by visual inspection. The steps that the algorithm applies to the detected peaks to determine automatically whether or not an IC signal is present are presented in the next paragraph and illustrated based on the example presented in Figure V.2.
Fig. V.2. The six first steps of microbubble peak detection applied to the PCD response received with a 13 MHz receiver from a microbubble excited by a 3-cycle incident pulse at 2.8 MHz with a peak rarefactive pressure of 1.3 MPa. Step 1 presents the central 10 μs of the truncated signal selected for automatic signal analysis. Visual inspection reveals that the time-trace has the signature typical of a ruptured microbubble: the principal response is followed by an IC (and rebound signals). The asterisks in Step 2 show the locations of the maximum positive and negative peaks. Of these two peaks, the one occurring latest in time is defined as the principal peak (in this case it is a positive peak). All points prior to the last zero-crossing of the principal peak are exempted from further signal analysis. At each step the sections of the curve in red represents the segments of the signal that will be searched for peaks in the next iteration of the algorithm and the black sections of the curve represents the segments of the signal that have been eliminated from the peak search. Steps 3 to 6 indicate subsequent detections of the next most important positive and negative peaks. These half-cycle peak minima and maxima are detected in order of decreasing absolute magnitude. Numbers on the peaks in step 6 represent the order in which the peaks were detected.
Table V.1 summarizes the relative TOF and amplitude of each peak, the time delay between each pair of consecutive peaks and their amplitude differences for the signal in Figure V.2. Once all the peaks have been detected, the peaks, numbered in the chronological order of their detection from maximum to minimum amplitudes, are ranked in order of increasing TOFs. For the example in Figure V.2, the peaks occur in the following orders: 1, 5, 9, 3 and 7 for the positive peaks and 2, 4, 6, 10 and 8 for the negative peaks (it is coincidental that positive peaks are odd numbers and negative peaks are even numbers in this example). For peaks of the same sign, amplitudes of consecutive peaks are then compared. The aim is to determine whether the amplitudes of the detected peaks decrease steadily with increasing TOF or if an increase in peak amplitude is observed with increasing TOF. For example, for the positive peaks, as a function of increasing TOF, the peak amplitudes are 34.2, 12.7, 6.8, 12.9 and 8.0 mV. Note that the amplitude decreases for the first three peaks (34.2 to 12.7 to 6.8 mV) and then increases (to 12.9 mV). A similar trend also occurs for the negative peaks. In general, this trend is detected for both the positive and negative peaks when IC is present. The amplitude comparison is made automatically based on the differences between amplitudes as illustrated by column 6 of Table V.1 (the difference changes sign from negative to positive). This decreasing, then increasing signal amplitude is an indicator that an IC response is present in the signal.

Next, the temporal separation between consecutive peaks of the same sign is inspected by examining the differences in the TOF as illustrated in column 5 of Table V.1. For the example in Figure V.2, the positive peaks 1, 5, 9, 3 and 7 have time separations of 0.24, 0.35, 1.84 and 1.51 µs peaks and the negative peaks 2, 4, 6, 10 and 8 for have time separations of 0.09, 0.26, 0.35 and 1.50 µs. Results show that peak number 3 is 1.84 µs after the preceding peak 9 and that peak number 8 is 1.80 µs after the preceding peak 10. These temporal separations are greater than the spacing that should occur between two peaks of the same sign due to the period of the excitation pulse (The temporal separation between consecutive peaks during excitation at 2.8 MHz should be on the order of 0.36 µs). A temporal separation of consecutive peaks greater than the period of the excitation pulse by 30% is a second indicator that IC response is present in the signal.
<table>
<thead>
<tr>
<th>Peak # (ranked in order of increasing TOF)</th>
<th>TOF (µs)</th>
<th>Amplitude (V)</th>
<th>Pair compared</th>
<th>TOF Differences (µs)</th>
<th>Amplitude differences (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.79</td>
<td>0.0342</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2.03</td>
<td>0.0127</td>
<td>1-5</td>
<td>0.24</td>
<td>-0.0215</td>
</tr>
<tr>
<td>9</td>
<td>2.35</td>
<td>0.0068</td>
<td>5-9</td>
<td>0.35</td>
<td>-0.0059</td>
</tr>
<tr>
<td>3</td>
<td>4.19</td>
<td>0.0129</td>
<td>9-3</td>
<td>1.84*</td>
<td>0.0061**</td>
</tr>
<tr>
<td>7</td>
<td>5.70</td>
<td>0.0080</td>
<td>3-7</td>
<td>1.51*</td>
<td>-0.0049</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak # (ranked in order of increasing TOF)</th>
<th>TOF (µs)</th>
<th>Absolute Amplitude (V)</th>
<th>Pair compared</th>
<th>TOF Differences (µs)</th>
<th>Amplitude differences (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.73</td>
<td>0.0232</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.82</td>
<td>0.0173</td>
<td>2-4</td>
<td>0.09</td>
<td>-0.0059</td>
</tr>
<tr>
<td>6</td>
<td>2.08</td>
<td>0.0144</td>
<td>4-6</td>
<td>0.26</td>
<td>-0.0029</td>
</tr>
<tr>
<td>10</td>
<td>2.43</td>
<td>0.0046</td>
<td>6-10</td>
<td>0.35</td>
<td>-0.0101</td>
</tr>
<tr>
<td>8</td>
<td>4.23</td>
<td>0.0048</td>
<td>10-8</td>
<td>1.80*</td>
<td>0.0002**</td>
</tr>
</tbody>
</table>

Table V.1. TOFs and amplitudes of the positive and the negative peaks detected automatically with comparison between consecutive peaks in terms of temporal spacing and relative amplitude. * Spacing greater than that related to the period of the excitation pulse (0.35 µs, in this case). ** Absolute amplitude of consecutive peaks increases with increasing TOF.

Based on the results of the peak comparison described above, the automatic detection algorithm classifies each signal segment into one of the four classes described below:

- Microbubbles detected with no post-excitation response were classified “0”. The criteria for this classification are, first, that three peaks or more were detected. Second that these detected peaks steadily decreased in amplitude with time after the principal peak and the temporal separation between peaks was consistent with the spacing expected based on the period of the excitation pulse.
Microbubbles detected with post-excitation response were classified “1”. For this case the peak amplitude had to decrease and then increase for a greater TOF or the temporal spacing between consecutive peaks of the same sign was greater than the period of an excitation cycle.

Cases with no microbubble detected were classified “2”. This implies that no peak was detected above the level of \( V_{\text{max, noise}} \) or that less than three peaks were detected.

All cases that did not fit into those described above were classified as undefined “4”. These signals were then evaluated visually.

V. 3. Sensitivity of IC detection to voltage levels in the noise as a function of incident peak rarefactional pressure

The IC signals were found to be present at high insonation pressure and absent at low insonation pressure. A minimum rupture threshold was defined is the previous chapter (IV) to be the peak rarefactional pressure for which the first IC event is observed. In this subsection a critical look at the data is taken to evaluate how reliably the first IC event can be estimated. For this purpose, we study the sensitivity of the IC detection to voltage levels in the noise as a function of the incident rarefractional pressure. In other words, the amplitude of the post-excitation segment of signals for all insonation pressures is evaluated to estimate its increase when the first IC event occurs. The sensitivity of the minimum destruction threshold is related to the relative increase of voltage amplitude observed at the first IC event.

The automatic detection routine described in section V.2 was applied to each of the 128 waveforms at a given incident pressure, frequency and pulse duration of the PCD data to determine the nature of the each signal (oscillation, collapse, no microbubble or undefined corresponding to the classifications “0”, “1”, “2” and “3”, respectively). If no IC signals were detected for any of the 128 waveforms, then the incident rarefractional pressure was defined to be below the minimum pressure threshold for rupture. For each waveform classified as “0” (oscillating microbubble) in such a group of waveforms, a 2-\( \mu \)s segment of the signal, beginning 0.6 \( \mu \)s after the principal response (at 6 \( \mu \)s in the example of a microbubble classified as “0” in Figure V.3), was extracted. The time to the end of the principal response could not be determined automatically, thus for this reduced number of signals (only those signals with detected microbubbles need be considered), the end of the principal response was
determined visually to position the 2-μs post-excitation, analysis time-window. In the 2-μs segment, the maximum absolute voltage amplitude was determined. For all microbubbles classified “0” submitted to the same insonification pressure and pulse characteristics, the average maximum voltage amplitude in the post-excitation segment and its standard deviation were then calculated. This characterized the maximum voltage amplitude due to noise in the absence of post-excitation emissions.

![Diagram](image)

**Fig. V.3.** PCD waveform (13-MHz receiver) for a 2.8-MHz, 7-cycle, 0.8-MPa peak rarefactual pressure excitation. This waveform was classified as an oscillating microbubble with no detectible IC. The signal due to the principal response of the oscillating microbubble during excitation and the post-excitation segment of 2 μs used for analysis of the voltage level in the noise are illustrated by brackets.

For groups of signals acquired at incident rarefactual pressures equal to or above the incident peak rarefactual pressure threshold leading to automatic detection of at least one IC event among the 128 waveforms, the maximum voltage amplitude of each post-excitation signal identified as IC (Figure V.4) was determined. Then for all signals received with the same insonification characteristics, these maximum voltage amplitudes were averaged. This provides an estimate of the average of the maximum voltage amplitude of the IC signals as well as its standard deviation.
The algorithm for automatic IC detection identified a post-excitation, IC signal with a maximum absolute voltage amplitude of 0.04 Volts.

The results for the averaged maximum voltage amplitude of post-excitation signals were then plotted as a function of incident peak rarefactional pressure. This permitted to evaluate the change in the peak voltage levels obtained from IC signals detected from microbubbles classified “1” (as collapsed) with respect to the peak voltage levels due to noise. The resulting plots are presented and discussed in section V.5.

V. 4. Calculation of the percent occurrence of destruction events at each incident pressure

The automatic IC detection routine was then applied to the PCD data to evaluate the number of microbubbles destroyed at each incident peak rarefactional pressure for a fixed set of excitation parameters (frequency and pulse duration). The percent occurrence of destruction was estimated by dividing the number of microbubbles with IC detection by the total number of detected microbubbles (the sum of the number of detected oscillating
microbubbles and the number of detected ruptured microbubbles). This simple calculation is described in equation (V.1)

$$ POD = \frac{N_f}{N_f + N_0} $$ (V.1)

where $POD$ is the percent occurrence of destruction, $N_f$ is the number of signals classified as destroyed, “1”, and $N_0$ is the number of signals classified as detected but not destroyed, “0”.

Percent occurrence of destruction data as a function of incident peak rarefractional pressure were fit to a cumulative probability curve for a logistic distribution which is widely used in to model binary responses [118-120]. Logistic regression uses binomial probability theory to predict the state of a dichotomous dependent variable. The mathematical formula of the logistic regression is given by [121]:

$$ P = \frac{1}{1-\exp(-a-bx)} $$ (V.2)

where $P$ is the probability of microbubble rupture, $a$ and $b$ are the coefficients of the linear regression of the percent occurrence of destruction ($POD$) and $x$ is the independent variable (incident peak rarefractional pressure). Logistic regressions best fitting the experimental data were calculated using Matlab’s routine glmfit (generalized linear model) with a binomial distribution to determine the coefficients $a$ and $b$. This Matlab routine returns also the p-values and the standard errors of these coefficients. Then another Matlab routine glmval is used to derive the values of the logistic regression $P$ as in Eq. 2 using the coefficients derived with glmfit. Matlab routine glmval returns the 95% confidence bounds for the predicted values of $P$.

V. 5. Results:

V. 5. a. Peak voltage levels due to IC signals relative to that of the noise

Figure V.5 presents the evolution of the post-excitation maximum voltage amplitude as a function of the incident peak rarefractional pressure at each of the three frequencies (0.9,
2.8 and 4.6 MHz) and three pulse durations (3 cycles, 5 cycles and 7 cycles). The incident pressure level at which the first inertial cavitation event was detected is indicated in the each graph by an arrow. For incident pressures lower than this level, the voltage levels indicate average peak voltages in the noise, and for incident pressures greater than or equal to this level, the peak voltages correspond to those from detected IC signals. In all graphs, the amplitude of the average peak voltage due to the noise is relatively stable as a function of the incident peak rarefational pressure.

Fig. V.5. Evolution of the post-excitation amplitude as a function of the incident peak rarefational pressure for each frequency (0.9, 2.8 and 4.6 MHz) and pulse duration (3, 5 and 7 cycles). Arrows in the figures indicate the pressure level of the first detection of an inertial cavitation event. For incident pressures lower than this level, the voltage levels indicate average peak voltages in the noise, and for incident pressures greater than or equal to this level, the peak voltages correspond to those from detected IC signals. The error bars represent the standard deviation of the mean.
The average peak voltage level in the noise is 0.003 +/- 0.001 volts for all transmit frequencies and pulse durations. For all excitation settings, the peak voltage amplitude of the first inertial cavitation was 0.009 +/- 0.001 Volts. This represents an increase of between 6 and 14 dB above the peak voltage in the noise (minimum increase of 6 dB for insonification with a 0.9 MHz, 3-cycle pulse). As the incident peak rarefactive pressure increased above the pressure for first inertial cavitation event detection, the average maximum amplitude of post-excitation IC voltage level increased progressively as well as its standard deviation. The larger range of incident pressures used at 2.8 and 4.6 MHz permitted to observe this increase in IC peak voltage amplitude better than at 0.9 MHz.

V. 5. b. Percent occurrence of destruction events as a function of incident pressure

For each frequency and pulse duration, microbubble rupture occurrence (Eq. V.1) is plotted as a function of the incident rarefactive pressure in Figure V.6 (blue squares). The s-shaped logistic regression curves, fit to the experimental data as described in section V. 4, Eq V.2, are plotted with solid curves in this figure. For all the curves, the 95% confidence bounds for the predicted values of P were on average equal to 0.01. The error bar bounds (upper and lower) were not displayed because they were not distinct from the logistic regression curve on the scale of the graphs’ display.

In all the cases, microbubble rupture occurrence increases progressively from the minimum incident peak rarefactive pressure at which IC is first detected. The limited range of incident pressures over which data were acquired at 0.9 MHz did not include the pressures leading to 100% microbubble destruction. The greater range of incident pressures considered at incident frequencies of 2.8 and 4.6 MHz provided measurements of bubble destruction from 0 to nearly 100% in all cases except for the case of a 4.6 MHz, 3-cycle incident pulse. The microbubble rupture occurrence curves are generally well described by the logistic regression curve-fits. Table V.2 summarizes, for each set of incident pulse characteristics, the values for the coefficients a and b used by the logistic regressions (red curves), their standard errors and p-values.
<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>Number of cycles (cycles)</th>
<th>Coefficient $a$</th>
<th>Coefficient $b$</th>
<th>Standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>3</td>
<td>-6.71</td>
<td>5.28</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-7.19</td>
<td>6.41</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-4.70</td>
<td>4.13</td>
<td>0.15</td>
</tr>
<tr>
<td>2.8</td>
<td>3</td>
<td>-6.41</td>
<td>3.68</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-6.14</td>
<td>3.83</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-6.13</td>
<td>3.84</td>
<td>0.10</td>
</tr>
<tr>
<td>4.6</td>
<td>3</td>
<td>-5.39</td>
<td>1.69</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-5.08</td>
<td>2.00</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-4.88</td>
<td>2.12</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table V.2 Coefficient used for the logistic regression fit, their standard errors and p-values. The p-value were < 0.0001 in every case, indicating that the coefficients $a$ and $b$ fit the experimental data very well.
Fig. V.6. At each frequency (0.9, 2.8 and 4.6) and pulse duration (3 cycles, 5 cycles and 7 cycles), the square symbols represent the percent number of collapsed microbubbles detected at each incident pulse pressure. The S-shaped curves represent the logistic regression fit to the experimental data as described in Section V.4.
V. 5. c. Exposure dose

Based on the logistic regression statistics fit to the data, the 5% and 50% microbubble rupture occurrence levels were calculated. Table V.3 summarizes the results for peak rarefractional pressure thresholds yielding 5% and 50% destruction in Optison™. For all peak rarefractional pressure exposure levels at a given frequency, increasing the pulse duration decreases the incident peak rarefractional pressure required to reach a fixed microbubble rupture occurrence level. Also, for a fixed pulse duration, increasing the frequency increases the incident peak rarefractional pressure required to reach a fixed microbubble ruptured occurrence level. For 5% microbubble destruction, the incident peak rarefractional pressure is below 1 MPa for all frequencies and pulse durations except at the highest frequency (4.6 MHz) and shortest pulse duration (3 cycles) (The 5% microbubble ruptured occurrence is reached at 1.45 MPa in this case). The 50% destruction thresholds are lowest at 0.9 MHz. Figure V.7 displays the pressures reported in Table V.3 in a graphical format for visual comparison.

<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>Number of cycles (cycles)</th>
<th>First IC event (MPa)</th>
<th>5 % destruction (MPa)</th>
<th>50 % destruction (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>3</td>
<td>0.53</td>
<td>0.71</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.40</td>
<td>0.66</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.29</td>
<td>0.42</td>
<td>1.13</td>
</tr>
<tr>
<td>2.8</td>
<td>3</td>
<td>0.87</td>
<td>0.93</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.77</td>
<td>0.83</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.71</td>
<td>0.82</td>
<td>1.59</td>
</tr>
<tr>
<td>4.6</td>
<td>3</td>
<td>0.99</td>
<td>1.45</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.93</td>
<td>1.07</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.89</td>
<td>0.91</td>
<td>2.29</td>
</tr>
</tbody>
</table>

Table V.3. Summary of the peak rarefractional pressure thresholds for first event, 5 and 50 % destruction as a function of incident frequency and pulse duration.
Fig. V.7. Peak rarefactive pressure for the first IC event, 5% destruction and 50% destruction at the difference excitation settings used for the PCD experiments.

V. 5.d Comparison between theory and experiment

The maximum relative radial expansion and peak velocity were calculated with the modified Herring model for each pulse duration and frequency at the rarefactive pressure yielding 50% destruction. The microbubble diameter was chosen at the upper limit of the size distribution for Optison™ (4.6 μm). The relative radial expansion is defined as the radial expansion divided by the initial radius and was calculated from the radius-time curve obtained with Equation II.2 given in chapter II of this thesis. The peak velocity was obtained from the time derivative of the radius-time curve and occurs during the compression phase of the oscillation. For all the pulse durations considered, the microbubble experiences a greater relative expansion at the lowest frequency studied (0.9 MHz), see Fig. V.8 (a). At 0.9 MHz the microbubble experiences expansions about twice as great as those reached at other frequencies. Variations of the maximum relative expansion with respect to the pulse duration are much less sensitive to change. Pooling data from all pulse durations, the average value of the maximum relative expansion modeled for 50 % destruction at 0.9 MHz is 6.4 +/- 0.5. At 2.8 and 4.6 MHz, the maximum expansion varies between 2.6 and 3.1 with an average value of 2.8 +/- 0.2, respectively.
Fig V. 8. Maximum normalized radial expansion (a) and peak velocity (b) of the microbubble wall modeled using the incident pulses with peak rarefactive pressures yielding 50% destruction.

The maximum wall velocity follows the same trend as that of the wall expansion Fig. V.8 (b). It does not exceed 170 m/s for 2.8 and 4.6 MHz incident pulses for any pulse duration (average = 150.7 +/- 25.8 m/s). However it exceeds 330 m/s for a 0.9-MHz pulse reaching 450 m/s for a 0.9-MHz, 7-cycle incident pulse (average = 381.4 +/- 62.3 m/s). In others words at 0.9 MHz the Mach number in air was always exceeded.

V. 6. Discussion and Conclusions

A technique was developed for the automatic detection of IC signals. The principal of the algorithm can be summarized as follows. During the excitation pulse, the peaks in the detected signal from the principal response are repeated at the frequency of excitation (0.9, 2.8 or 4.6 MHz). Following the position of the maximum peak within this principal response, peak amplitudes decrease until excitation ceases. Thus, temporal spacing between peaks which does not correspond to the period of the excitation frequency, or increased pulse amplitude following the end of the principal response are criteria which indicate post-excitation signals related to IC. To enable the detection of peaks indicating IC, an algorithm was developed to detect all peaks in the signal and then to compare their spacing and amplitudes. This automatic process was applied to classify signals as unruptured oscillating microbubbles or ruptured microbubbles. However, near the pressure where the first IC event
was observed for each set of insonification parameters, a few signals (from 5 to 8 out of 128) were marked “4” by the automatic algorithm (not defined). When signals were classified by the algorithm as 4, they were classified based on visual analysis of the signal. Although some signals still required visual assessment, the automatic detection algorithm succeeding in classifying all but a very small percentage of the signals. This made it possible to rapidly evaluate all the signals \((128\text{(waveforms)}\times3\text{(frequencies)}\times3\text{(pulse durations)}\times62\text{(pressures)} = 71424\text{ signals})\) obtained with the passive cavitation detector. The time taken for the automatic analysis of a set of 128 signals was on the order of 7 minutes using a Xeon™ CPU 2.66 GHz. This automatic IC-detection ability is central to making the passive cavitation detection approach a practical tool for examining the stochastic process of microbubble destruction.

Using the automatic algorithm, the amplitudes of peak voltages in post-excitation segments of signals with and without IC events were evaluated as a function of incident peak rarefational pressure. The maximum voltage amplitude of the first detected IC events was always at least 6 dB above the maximum voltage amplitude of post-excitation signal segments without an IC event. This step-like increase in voltage amplitude due to the presence of IC signals should allow sensitive detection of microbubble rupture. Increasing the incident peak rarefational pressure beyond the threshold or the first IC event increased the maximum IC voltage amplitude as well as its standard deviation.

The current work extends previous estimations of the minimum peak rarefational pressure leading to a single microbubble rupture event to estimate the percent occurrence of microbubble rupture as a function of pressure. This original approach permitted the study of the evolution of the percentage of ruptured microbubble as a function of the incident peak rarefational pressure. The 50% thresholds were on the order of 2 to 3 times higher than the pressures producing a single rupture event at all frequencies except for the case of 7 cycles at 0.9 MHz. The more detailed threshold information obtained in this study should be useful in selecting the pulse characteristics to apply for imaging (minimized microbubble destruction) and therapeutic (maximized microbubble destruction) applications.

The normalized radial expansion estimated with the modified Herring model for the incident pulse characteristics leading to 50% destruction was between approximately 2.6 and 3.1 for the 2.8- and 4.6-MHz excitations (microbubble diameter of 4.6 μm at equilibrium).
At 0.9 MHz, the normalized radial expansion modeled for the 50% destruction level was 6.4 +/- 0.5. The maximum wall velocity follows the same trend as that of the wall expansion with an average value of 150.7 +/- 25.8 m/s for 2.8 and 4.6 MHz pulses and 381.4 +/- 62.3 m/s for the 0.9-MHz pulses (average = 381.4 +/- 62.3 m/s). The greater expansions and wall velocities found at 0.9 MHz might be due to the fact that the resonant frequency of Optison\textsuperscript{TM} is closer to this frequency than to the other frequencies considered. Modification of the pulse duration from 3 to 7 cycles had a relatively limited effect on the modeled expansion and velocity. Relative expansion thresholds for cavitation have been predicted theoretically to be on the order of 2 to 3 [11, 12], but little precise experimental data describing the wall motion associated with microbubble rupture is available. For an experimental ultrasound contrast agent (MP190, Mallinckrodt, Inc., St Louis, MO), Chomas et al. [10] have reported optical microscopy evaluations of images of microbubble wall movement recorded with a high speed camera. Relative expansion observed for fragmented microbubbles varied from approximately 3.3 to 5.5, from 3 to 4.6 and from 2.5 to 3.5 for 2-cycle incident pulses at 1, 1.5 and 2 MHz, respectively. Varying the pulse length from 3 to 7 cycles did not seem to change the range of relative expansion values significantly in the work presented by Chomas et al. Thus, the relative expansions modeled for the 2.8 and 4.6 MHz 50% destruction conditions identified with the PCD system appear to be consistent with relative expansions for destruction cited in the literature.

Percent destruction occurrence thresholds have been reported for a few other ultrasound contrast agents using several different techniques for the estimation. Chomas et al. [37] presented results from both acoustical and optical evaluations of the destruction occurrence for an experimental lipid-shelled contrast agent, MP2211. Their acoustical PCD setup consisted of a calibrated transmit transducer (5.6 MHz) mutually focused with a receive transducer (10.5 MHz) at an angle of 15°. The microbubble solution was pumped at low flow rate through a hollow 200-μm cellulose fiber placed in the confocal volume of the transducers. Each microbubble was insonified by a 5-MHz, 1.5-cycle acoustic pulse with a calibrated transmission peak rarefactive pressure (0.8, 1.4, 2.0 or 2.6 MPa). The intermittent pulsing scheme involved a train of 10 pulses with a pulse repetition frequency of 1 kHz. The 10-pulse train was followed by a delay on the order of 1 s before transmission of the next 10 pulses. Thus a single microbubble is interrogated by each 10-pulse train and a new bubble arrives in the confocal volume for the next 10-pulse train. The percentage destruction occurrence was calculated from groups of 500 microbubbles interrogated at 0.8, 1.4, 2.0 and
2.6 MPa. A microbubble was classified as being destroyed if the signal received with the 10.5 MHz transducer did not persist for each pulse in the entire 10-pulse train transmitted with the 5 MHz transducer. Otherwise, the microbubble is considered to be intact. About 5% of the insonified microbubbles were destroyed at a transmission pressure of 800 kPa. Over 90% of microbubbles were destroyed when insonified with transmission pressures of 1.4 MPa and above.

A separate series of optical microscopy measurements was made by Chomas et al. using the same contrast agent and the same acoustical insonifying pulse characteristics (5 MHz center frequency, 1.5 cycles). Images of the microscopic field of view (100X objective with a 2X zoom lens) of the 200-µm fiber placed at the transducer focal zone were acquired with the camera at a frame rate of 240 frames/s with a shutter duration of 1/500 s. For cases in which the microbubble diameter decreased by more than 25% relative to the original diameter or in which multiple bubble fragments were observed, the microbubble was considered to have been destroyed. For a transmission pressure of 800 kPa, 10% of the microbubbles studied were classified as destroyed. For transmission pressures greater than 800 kPa, more than 70% of the microbubble were classified as destroyed (more than 55% of the bubbles studied at 2.6 MPa were observed to fragment).

We obtained percent destruction occurrence thresholds of only 1% at 800 kPa and 27% at 2.6 MPa with Optison™ for the set of excitation settings (3 cycles, at 4.6 MHz) that most closely approximates the settings used by Chomas et al. in the acoustic and optical evaluations described above. The lower percent destruction occurrence observed for Optison™ relative to MP2211 may indicate that the lipid-shelled MP2211 is a more fragile agent. The fragility of MP2211 could potentially be overestimated by the optical technique, however, if some of the microbubbles observed optically to decrease in diameter by more than 25 % (limitation of optical resolution of the microscope system) were not truly destroyed. One important difference in the acoustical approach used by Chomas et al. with respect to our PCD approach is that Chomas et al. exposed each contrast microbubble to a series of ten pulses whereas we only applied a single (multiple-cycle) pulse to each microbubble. It is reasonable to expect that it is more likely to rupture a microbubble by repeated applying multiple pulses. Furthermore, the percent destruction occurrence thresholds summarized for the acoustical experiments reported by Chomas et al. include both very rapid destruction events they attribute to fragmentation and slower destruction that they attribute to acoustically
Driven diffusion. They report that 53% of the microbubbles interrogated with a pulse at 2.0 MPa were destroyed within the first two pulses, but do not give similar values for the other insonification pressures. Only such rapid fragmentation type events should be expected to give rise to IC.

Using a high-speed optical observation microscopic technique, Postma et al. [122] reported the average percent of cracked microbubbles for two contrast agent. The first was an albumin-encapsulated air bubble Quantison™ (Upperton, Limited, Nottingham, UK) and the second was a bilayered shell (outer layer is albumin and inner layer is composed of a biodegradable polymer) encapsulated gas bubble, PB127 (POINT Biomedical corporation, San Carlos, CA). For each set of acquired data, approximately 10 to 15 contrast agent microbubbles present in their field-of-view were insonified with eight cycles of ultrasound. Evidence of gas escaping from the bubble on the optical images was used to determine that a microbubble had been cracked. The two contrast agents, for MI > 0.8, had on average 8% and 22% of cracked microbubbles at 0.5 MHz (peak negative pressure >0.56 MPa) and 32% and 38% of cracked microbubbles at 1.7 MHz (peak negative pressure >1.04 MPa), for Quantison™ and PB127, respectively. The pulse applied at 0.9 MHz with 7 cycles in our PCD experiments most nearly approaches the frequencies and pulse-length of the insonification pulses used by Postema et al. With this pulse at peak rarefational pressures of 0.56 and 1.04 MPa, the percentage of ruptured microbubbles measured in Optison™ were 8.4% and 40%. It is interesting to note that the values estimated for percent destruction occurrence of Optison™ with the PCD IC detection is similar to the percent of cracked microbubbles estimated by this optical technique for the similarly-shelled agent, Quantison™. Frinking et al. have previously also estimated that pressure thresholds for shell destruction are similar for the agents Quantison™ and Optison™ [123].

In conclusion, experimental techniques and data responding to questions concerning the percent occurrence of microbubble contrast agent destruction are beginning to emerge. The nature of the information offered by these different techniques and their relative advantages are somewhat different. The acoustical technique applied by Chomas et al. considers a destruction response to multiple pulses such as may be experienced by microbubbles during a rapid ultrasound scanning sequence. Furthermore, it seems that microbubble destruction from a variety of destruction mechanisms including acoustically driven diffusion is included in the estimations of the percent destruction. Optical techniques
potentially provide the most precise evaluation of the mechanism that leads to microbubble destruction but can be limited by optical resolution. Furthermore, optical techniques rely on visual assessment of the images of individual microbubbles strongly limiting the number of microbubbles that can be evaluated in a given time. The PCD technique described in this chapter specifically detects destruction events leading to IC. The step-like rise in the post-excitation signal associated with the occurrence of the IC response and the automatic algorithm demonstrated in this work should make this technique a particularly robust and practical approach for the large-scale characterization of microbubble rupture thresholds.
Chapter VI – Large bandwidth exploration of the shell rupture response using double passive cavitation detection
VI. 1 Introduction

The relatively strongly focused, 13-MHz receiver used in the PCD system experiments described in chapter IV was chosen to favor detection of the broadband response from a cavitation event and to limit the size of the active measurement volume. However, it is also of interest to examine the ruptured microbubble response in the bandwidth of the transmitted excitation pulse. Thus, to further explore the shell rupture dynamics and response, a double passive cavitation detector (DPCD) was implemented as described in Chapter III. The data acquired with this system were used to respond to three general objectives.

Firstly, a total of seven independent series of data were acquired with the DPCD system by four operators (Azzdine Y. Ammi, Grace I. Wang, Zac Hafez and Jeong-Ah Lee). The data acquired with the 13 MHz passive receiver by each experimenter were analyzed in the same way that data from the PCD system were analyzed previously. This analysis consisted of the measurement of the minimum peak rarefactional pressure thresholds based on IC detection in the signal received with the 13 MHz receiver. Analysis of these results provides an evaluation of the measurement variability and of the statistical significance of differences in thresholds observed at different frequencies and pulse duration.

Secondly, the signals obtained simultaneously with the 13-MHz receiver and the transmit transducer were compared in the time domain to evaluate if the IC signal could be detected in the signals received with the transmit transducer in pulse-echo mode. Furthermore, pairs of signals acquired simultaneously with the transmit transducer and the 13 MHz receiver were examined visually for any features that only occurred when post-excitation IC signals were detected. Such time-domain response features could potentially be linked to a shell rupture event.

Thirdly, the average spectral content within both the 13-MHz receiver’s bandwidth and the frequency range of the transmit transducer was compared for groups of signals with and without IC events to evaluate how this spectral content was modified by the destruction event and whether such a modification could be detectable.
VI. 2. Data acquisition

The measurement protocol was essentially the same as that used with the PCD. The double passive cavitation detector (DPCD) tank, described in Chapter III, was filled with degassed water. The selected transmit transducer was aligned with the receiver. At each transmit pressure amplitude, a baseline data acquisition was made in the absence of contrast agent by acquiring 128 received waveforms. Using a graduated syringe, 0.1 mL of Optison™ was injected into the tank. This volume of contrast agent contains approximately $0.5 \times 10^8$ microbubbles, and resulted in a mean concentration in the tank of about 2 microbubbles/µL. The volume injected for DPCD measurements was half of the volume used in previous PCD measurements. This reduction in injected volume of UCA was necessary to ensure that, on average, only one microbubble should be within the -6-dB focal volume of the transmit transducer. In this work, the active volume is considered to be the −6-dB volume of the transmit transducer because all the transducers used in transmission (0.9, 2.8, 4.6 or 7.1 MHz, center frequencies) have beam dimensions at -6-dB that are larger than those of the receiver (13 MHz center frequency). The water was gently stirred with a pump before and during data acquisition to maintain an even distribution of the UCA in the water and to ensure the replenishment of contrast microbubbles in the active volume. Initially, a 3-cycle pulse duration at the transmit transducer’s center frequency was generated. For each transmit pressure amplitude (varied from highest to lowest), 128 consecutive received waveforms were acquired simultaneously from the 13-MHz center frequency passive receiver and from the transmit transducer. The simultaneously acquired waveforms were sampled by the data acquisition card at a sampling rate of 100 MHz (half that obtained in the PDC configuration). This acquisition procedure was then repeated for 5-cycle and then 7-cycle pulse durations. Total acquisition time after introduction of Optison™ into solution did not exceed 2 hours. A total of seven data runs were repeated with the DPCD system by four operators (Azzdine Y. Ammi, Grace I. Wang, Zac Hafez and Jeong-Ah Lee).

VI. 3. Data analysis

In the work described in this chapter, the post-excitation signals were again used to detect microbubble rupture. For both sets of received signals, each of the 128 waveforms was truncated to keep a 4000 sample segment (20 µs) centered at the position corresponding to the
time-of-flight to the active volume. The maximum duration of a microbubble response observed with the longest pulse duration (1-MHz, 7-cycle) is approximately 10 μs. Thus, the entire useful signal is included in the 20 μs segment. The time-of-flight with respect to the active volume was 42 μs for the signal received with the 13 MHz transducer and 50 μs for the signals received with the transmitting transducer. The four transmit transducers had the same focal distances, thus the same time of flight. The DC component of each signal, considered in units of linear voltage as a function of time, was eliminated by subtracting the signal mean. The detection of the post-excitation signals was then performed visually for all received signals by three of the experimenters (Grace I. Wang, Zac Hafez and Jeong-Ah Lee).

VI. 3. a. Evaluation of measurement variation

For each transmitted frequency and pulse duration, the minimum peak rarefractional pressure threshold for Optison™ microbubble shell rupture was estimated from this new data set based on the lowest pressure for which at least one post-excitation signal was detected in a set of 128 waveforms acquired at 13 MHz.

The minimum peak rarefactional pressure thresholds thus estimated by the seven independent data runs with the DPCD system and the single data run performed previously with the PCD system were pooled for statistical analysis. The Matlab® function ‘anova’ was applied to this data to perform a balanced one-way analysis of variances (ANOVA) to study the statistical significance of threshold variations with pulse duration and with frequency. The ‘anova’ function compares the means of two or more samples containing mutually independent observations. The most common p-value thresholds used to decide if a difference is significant or not are 0.01 and 0.05. A threshold of 0.05 means that one can be 95% confident that the result is significant. In our analysis, a difference was judged statistically significant if the p-value is less 0.001. The Wilcoxon rank sum test is run to ensure that pairs of mean threshold values measured at different frequencies but the same pulse duration are significantly different (Matlab® function ‘ranksum’).
VI. 3. b. Visual assessment of signals acquired simultaneously with the transmit transducer and the passive 13 MHz receiver

The time-of-flight with respect to the active volume was 42 μs for the signal received with the 13 MHz transducer and 50 μs for the signals received with the transmit transducer (the four transmit transducers had the same focal distances). Signals were initially aligned based on these respective times-of-flight. However, further fine alignment was necessary to compensate for variations linked to the precise position of the microbubble in the active volume (0.25×0.27×3.4 mm³). For pairs of signals presenting post-excitation IC responses, the fine alignment was based on the alignment of the maximum positive peak amplitude of the inertial cavitation response. The time of occurrence of these peaks is marked T in the figures in the case where the inertial cavitation signature is detected. For pairs of signals without post-excitation IC responses (or with detection of an IC response in only one of the two signals), fine alignment was based on the position of the maximum voltage amplitude of the first pulse in the principal response in both signals.

VI. 3. c. Spectral evaluation of the destruction response

For 3-, 5- and 7-cycle, 0.9, 2.8- and 4.6-MHz incident pulses at acoustic pressures identified as the pressures yielding 50% microbubble destruction (see the previous chapter), each pulse-echo and 13 MHz signal in each group of 128 signals was analyzed with the automatic IC detection algorithm described in Section V.2. Each waveform was classified as: (1) indicating an oscillation (without post-excitation signal), (2) indicating collapse (inertial cavitation signals detected) or (3) presenting no evidence of a microbubble. For each of the approximately 50 % of the waveforms classified as an oscillating, non destroyed microbubble, the 20 μs segment of the waveform, centered at the position corresponding to the time-of-flight to the active measurement zone, was windowed and the Fast Fourier Transform of the windowed segment was calculated to estimate the spectrum. The average spectrum was then calculated. Similarly, the average spectra were calculated for the 20 μs segments of the approximately 50 % of the waveforms that were classified as ruptured microbubbles. Spectral data were considered to be above the noise within the – 20 dB bandwidth of each transducer (as estimated from the spectrum of a planer reflector placed perpendicular to the transducer’s beam at the focal distance). Signals with no evidence of a microbubble were excluded from
analysis. The 7.1 MHz data were also excluded from this analysis because the estimation of the pressure threshold for 50 % destruction occurrence required the higher number of microbubbles interrogated previously in the PCD configuration. (No data were acquired with the 7.1 MHz transmit transducer at the microbubble concentrations used in the original PCD experiments.)

VI. 4. Results

VI. 4. a. Minimum pressure rupture threshold measurements: variations and significance of differences

Figure VI.1 shows the average minimum peak rarefractional pressure rupture threshold for each transmit frequency and pulse duration estimated from the waveforms acquired in seven series of data acquisition with the 13-MHz centre frequency receiver of the DPCD system. Results show that the lowest peak rarefractional pressure rupture thresholds are found for a 0.9 MHz incident pulse and the highest are found for 7.1 MHz.

![Graph showing peak rarefractional pressure (MPa) vs. frequency (MHz) for 3 cycles, 5 cycles, and 7 cycles.]

Fig. 1. (a) Minimum rupture thresholds estimated from the DPCD data at 13 MHz using the inertial collapse criterion (error bars represent the standard error). Averages and standard deviations from seven independent series of data acquisition.
The ANOVA tests revealed the existence of at least one statistically significant difference in the minimum rupture threshold at fixed PD as a function of the transmit frequency (for all pulse durations). Increasing the center frequency of the transmit transducer leads to an increase in the rupture threshold. However, there was no statistically significant variation in the threshold at fixed frequency as a function of pulse duration. Table VI.1 summarizes the p-value estimated for each group of data.

<table>
<thead>
<tr>
<th>Number of cycles</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>0.00006</td>
<td>0.00070</td>
<td>0.00018</td>
</tr>
</tbody>
</table>

(a)

<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>0.9</th>
<th>2.8</th>
<th>4.6</th>
<th>7.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>0.7979</td>
<td>0.7684</td>
<td>0.4536</td>
<td>0.7133</td>
</tr>
</tbody>
</table>

(b)

Table VI.1. Results of the ANOVA tests for significance of differences between the minimum peak rarefactional pressure leading to a single rupture event for (a) values from data sets with the same number of cycles in the pulse duration as a function of frequency (b) values from data sets with the same incident frequency as a function of pulse duration.

The ANOVA test being significant only insures that at least one of the mean values is significantly different from the others. The Wilcoxon rank sum test was then run to ensure that pairs of thresholds estimated at the same PD but at two different frequencies are significantly different. The results of this test are summarized in Table VI.2

<table>
<thead>
<tr>
<th>Pair of frequencies (MHz)</th>
<th>0.9 - 2.8</th>
<th>2.8 - 4.6</th>
<th>4.6 - 7.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability for 3 cycles (%)</td>
<td>3</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>Probability for 5 cycles (%)</td>
<td>4</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Probability for 7 cycles (%)</td>
<td>8</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

Table VI.2. The probability that the difference between thresholds estimated at two different frequencies are not significantly different as determined using the Wilcoxon test.

Results in Table VI.2 show that the probability that thresholds estimated at 0.9 and 2.8 MHz are not significantly different is less than 4% at all pulse durations. Those compared between 4.6 and 7.1 MHz is less than 8% at all pulse durations. However, the threshold values are not strongly different for measurements made at 2.8 and 4.6 MHz at all pulse durations.
VI. 4. b. Visual assessment of signals acquired simultaneously with the transmit transducer and the passive 13 MHz receiver

To illustrate the features that were identified in the time-domain only when post-excitation IC signals were detected, example pairs of signals acquired simultaneously are presented for each frequency combination in the following paragraphs.

Fig VI.2 shows an example of simultaneous waveforms acquired with the DPCD system for a 0.9-MHz 7-cycle, 1.3 MPa peak rarefactive pressure excitation pulse. From top to bottom the four panels represent: (1) the passively detected signal (2) its corresponding spectrum (3) the pulse-echo signal (4) the excitation pulse. The top panel in Figure VI.2 presents two broadband peaks during the microbubble excitation detected with the 13-MHz passive receiver. Between these peaks, weak, lower amplitude oscillations are apparent. These oscillations arise from the microbubble oscillatory response to the 7-cycle incident excitation pulse, but the bandwidth of this response, which has a fundamental frequency of 0.9 MHz (as observed in the spectrogram), is highly filtered by the receive bandwidth of the 13 MHz receiver. The signal acquired with the 0.9-MHz transducer consists of the pulse-echo response during microbubble excitation followed by the post-excitation, inertial cavitation response. The 0.9 MHz pulse-echo response follows the cyclic variations in the pressure of the incident ultrasonic pulse (third panel in Figure VI.2). Indentations at the peak amplitudes received by the 0.9 MHz transducer occur during the 2\textsuperscript{nd} and 6\textsuperscript{th} cycles of the microbubble oscillation. The indentations coincide with the broadband peaks detected in the 13-MHz signal (times marked A and B on Figure VI.2). When post-excitation signals were present, many of the waveform pairs detected with the 0.9 MHz and 13 MHz transducers demonstrated peak amplitude indentation in the 0.9 MHz signal occurring simultaneously with broadband peaks in the signal detected with the 13 MHz transducer. This observation held true for all the studied pulse durations.
Fig. VI.2. DPCD signals received for a 0.9-MHz center frequency, 7-cycle, 1.3 MPa peak rarefactional pressure excitation pulse. From top to bottom the panels represent: Signal received with the 13-MHz transducer. Corresponding spectrogram. Signal simultaneously received with the 0.9-MHz transducer. Excitation pulse as measured with a hydrophone at the focal position of the transmitting transducer. The post-excitation peaks at the time marked, T, indicates inertial cavitation of a collapsing microbubble.

When no post-excitation signals were present (example DPCD waveforms for such a case are presented in Figure VI.3), no broadband peaks were detected with the 13-MHz receiver during the 0.9-MHz excitation. Furthermore, no indentations were observed about the peak amplitudes of the 0.9 MHz signal received during microbubble excitation.
Fig. VI.3. DPCD signals received for a 0.9-MHz center frequency, 3-cycle, 0.9 MPa peak rarefactive pressure excitation pulse. From top to bottom the panels represent: Signal received with the 13-MHz transducer. Corresponding spectrogram. Signal simultaneously received with the 0.9-MHz transducer. Excitation pulse as measured with a hydrophone at the focal position of the transmitting transducer. No post-excitation peaks are present to indicate inertial cavitation of a collapsing microbubble. The microbubble is oscillating.

For transmit frequencies of 2.8 and 4.6 MHz, waveforms received with the transmit transducer and the 13 MHz receiver are compared in Figures VI.4 and VI.5, respectively. Post-excitation signals in the 13 MHz waveforms (top panels in Figure VI.4 and VI.5) indicate that microbubbles were ruptured in both cases. On the contrary, however, post excitation signals are not detectable in the pulse-echo signals recorded with the transmitting transducers (third panels in Figure VI.4 and VI.5). The amplitude of the forced oscillatory response during acoustic excitation is still well above the noise in the 2.8 and 4.6 MHz pulse-echo waveforms at the times when the inertial cavitation events are detected in the 13 MHz received waveforms. In both 2.8 and 4.6 MHz cases, no indentations in the amplitudes of the peaks in the low frequency signals were observed as had been observed in the 0.9 MHz data (Figure VI.2). Similar observations were made for all studied pulse durations.
Fig. VI.4. DPCD signals received for a 2.8-MHz center frequency, 3-cycle, 2 MPa peak rarefactual pressure excitation pulse. From top to bottom the panels represent: Signal received with the 13-MHz transducer. Corresponding spectrogram. Signal simultaneously received with the 2.8-MHz transducer. Excitation pulse as measured with a hydrophone at the focal position of the transmitting transducer. The post-excitation peak at the time marked, T, indicates inertial cavitation of a collapsing microbubble.
Fig. VI.5. DPCD signals received for a 4.6-MHz center frequency, 3-cycle, 2.1 MPa peak rarefactive pressure excitation pulse. From top to bottom the panels represent: Signal received with the 13-MHz transducer. Corresponding spectrum. Signal simultaneously received with the 4.6-MHz transducer. Excitation pulse as measured with a hydrophone at the focal position of the transmitting transducer. The post-excitation peak at the time marked, T, indicates inertial cavitation of a collapsing microbubble.

At the highest transmit frequency studied (7.1 MHz), Figure VI.6 shows the presence of post-excitation signals in both the 7.1- and 13-MHz signals. The signal acquired with the 7.1-MHz centre frequency transducer contains a pulse-echo response during excitation and evidence of passively detected post excitation response. The 13 MHz waveform also shows the post-excitation pulse. No indentations in the amplitudes of the peaks in the low frequency signals were observed as had been observed in the 0.9 MHz data (Figure VI.2). Observations were similar for all studied pulse durations.
Fig. VI.6. DPCD signals received for a 7.1-MHz, 7-cycle, 1.3 MPa peak rarefractional pressure excitation pulse. Top panel: Signal received with the 13-MHz transducer of a collapsing microbubble (presence of post-excitation signal). Second panel from the top: corresponding spectrogram. Third panel from the top: Signal simultaneously received with the 7.1-MHz transducer. Bottom panel: Incident pulse. The post-excitation peaks at the time marked, T, indicate inertial cavitation of a collapsing microbubble.

Finally, a general observation is that as the transmit frequency approaches the bandwidth of the 13-MHz passive receiver, the oscillatory response of the microbubble during excitation is more fully described in the 13-MHz signal.

VI.4.c. Spectral evaluation of the destruction response

The average spectra measured at the 50 % destruction occurrence threshold from a group of ruptured and nonruptured microbubbles are compared for both the transmit and 13-MHz receive bandwidths in the Figures VI.7, VI.8 and VI.9. The average spectra from signal groups acquired at a 0.9 MHz transmit frequency are compared for cases with and without IC detection in Fig VI.7. For all pulse durations, the spectral amplitude in the
bandwidth of the 13 MHz passive receiver (black and red solid curves) is detectably higher for collapsing microbubbles. Spectra of the pulse echo data acquired with the 0.9 MHz transducer demonstrate no detectable difference for the 5 and 7 cycle PDs. For the 3 cycle pulse duration, the spectral amplitude is higher for the collapsing microbubbles in the transmit bandwidth.

Figure VI.8 presents the averaged spectra of the signals with and without post-excitation IC signals for the 2.8 MHz transmit frequency. For all pulse durations and for both high and low frequency bandwidths, the spectral amplitude is consistently higher when microbubble collapse is detected. Spectral components at harmonics of the transmit frequency are clearly visible for 5 and 7 pulse duration excitations. The harmonic response appears slightly stronger for microbubbles with an IC response than for those without an IC response.

Average spectra for data acquired at a transmit frequency of 4.6 MHz are summarized in Figure VI.9. Additional broadband noise is again observed over much of the bandwidth when microbubble collapse is detected. The peak amplitudes at the fundamental frequency are not different for the shortest PD. Harmonics of the transmit frequency were present in all the spectra for all pulse durations with and without bubble collapse.
Fig. VI.7. Data for an incident peak negative pressure of leading to a 50 % destruction occurrence. The black solid curves present the mean FFT from 13-MHz waveforms with IC. The red solid curves present the mean FFT from 13-MHz waveforms without IC but with microbubble oscillations during excitation. The dash-dotted and dotted curves present the mean FFT from waveforms acquired with the excitation transducer at 0.9 MHz with and without evidence of IC, respectively. For excitation settings of (a) 3 cycle, 1.26 MPa (b) 5 cycle, 1.14 MPa and (c) 7 cycle, 1.13 MPa.
Fig. VI.8. Data for an incident peak negative pressure leading to a 50 % destruction occurrence. The black solid curves present the mean FFT from 13-MHz waveforms with IC. The red solid curves present the mean FFT from 13-MHz waveforms without IC but with microbubble oscillations during excitation. The dash-dotted and dotted curves present the mean FFT from waveforms acquired with the excitation transducer at 2.8 MHz with and without evidence of IC, respectively. For excitation settings of (a) 3 cycles, 1.74 MPa (b) 5 cycles, 1.60 MPa and (c) 7 cycles, 1.59 MPa.
Fig. VI.9. Data for an incident peak negative pressure leading to a 50% destruction occurrence. The black solid curves present the mean FFT from 13-MHz waveforms with IC. The red solid curves present the mean FFT from 13-MHz waveforms without IC but with microbubble oscillations during excitation. The dash-dotted and dotted curves present the mean FFT from waveforms acquired with the excitation transducer at 4.6 MHz with and without evidence of IC, respectively. For excitation settings of (a) 3 cycles, 3.19 MPa (b) 5 cycles, 2.54 MPa and (c) 7 cycles, 2.29 MPa.
Table VI.3 summarizes the average differences in the spectral amplitudes observed between spectra from oscillating and ruptured microbubbles. Averages were calculated across the -6-dB bandwidth of the transducer in each case.

<table>
<thead>
<tr>
<th>Transmit Frequency (MHz)</th>
<th>0.9</th>
<th>2.8</th>
<th>4.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>–6-dB bandwidth (MHz)</td>
<td>0.8-1.0</td>
<td>9.3-17.6</td>
<td>2.6-3.0</td>
</tr>
<tr>
<td>3 cycles</td>
<td>8.94</td>
<td>6.52</td>
<td>6.93</td>
</tr>
<tr>
<td>5 cycles</td>
<td>0.66</td>
<td>9.20</td>
<td>5.47</td>
</tr>
<tr>
<td>7 cycles</td>
<td>2.71</td>
<td>13.08</td>
<td>7.32</td>
</tr>
</tbody>
</table>

Table VI.3 Average differences between spectra from collapsing and oscillating microbubbles in dBs.

VI.5. Discussion and conclusion

Using the signals acquired with the DPCD system by several independent experimenters, the minimum peak rarefational pressure thresholds for microbubble rupture were estimated for each series of acquired data. These results showed the same general trends as the ones found using the PCD in Chapter IV. However the pressure thresholds estimated with the initial set of PCD data are generally lower than the average estimates obtained with the DPCD system. Potential sources of experimental variation include the level of operator experience, slight potential differences in the quality of the transducer alignment and the difference in the contrast concentration in solution. Another source of variation between these two series of experiments may include the differences in the visual assessment criteria applied by each operator to determine the presence of IC.

Results showed a statistical significance in the variation of the minimum destruction threshold as a function of frequency but no statistical significance due to changes in the pulse duration for 3, 5 and 7- cycle pulses. Giesecke et al. [71] also reported very small variations in destruction thresholds of Optison™ at different, but very long, pulse durations (several milliseconds). Chen et al. [7] showed that more than five cycles were necessary to reach a steady-state amplitude for which the fragmentation threshold only depended on the pressure amplitude. On average, the peak amplitude of the 4-cycle signal was 94.5% of the steady
state, and was 83.9% for a 2-cycle pulse and thus observed a small difference at low number of cycles. However, in another study Chen et al. [77] found that with Optison™, up to 60% hemolysis was produced with long pulses (100 and 200 cycles), compared with less than 10% with short pulses (5 and 10 cycles).

Previous data acquired with a single PCD (13 MHz) had been difficult to interpret especially when a 0.9-MHz transmit frequency was applied. In effect, at this transmit frequency, only isolated, large bandwidth peaks were recorded at 13 MHz allowing questions to be raised concerning as to whether these signals were truly from the same microbubble or from several microbubbles in the effective volume. The DPCD data allowed the comparison of simultaneously acquired low and high frequency signals received from the measurement volume. Post-excitation broadband signals attributed to IC were observed in both the high and low frequency signals (0.9 and 7.1 MHz). This IC event was probably detectable in both receive pass bands due to its very large bandwidth frequency content. During the time over which the principal response of a single microbubble was detected with the 0.9 MHz transducer, isolated, broadband peaks were observed with the 13 MHz transducer. The presence of these broadband peaks during microbubble excitation observed with the 13 MHz transducer might be linked to collapses during excitation. The 13 MHz passive receiver filters out the low frequency component of the microbubble principal response and detects mainly the events with broadband content. This interpretation helps to explain the impulsive shape of the response acquired with the 13 MHz transducer at the lowest transmit frequency. Indentations at the peak amplitudes received by the 0.9 MHz transducer were observed to coincide with the broadband peaks detected in the 13-MHz signal. Further exploration and modeling is necessary to understand this very interesting finding.

When IC signals were detected on the 13 MHz signals, they were also detected in the corresponding signals received with the 0.9 and the 7.1 MHz transducers. For signals received with the transmitting transducers for transmit frequencies of 2.8 and 4.6 MHz, the signal did not show evidence of post-excitation signals even when such signals were detected on the corresponding signal received with the 13 MHz transducer. A potential explanation of this may be related to the fact that 2.8 and 4.6 MHz transducers had a higher $Q$ factor than the 0.9 and 7.1 MHz transducers. A "high $Q$" transducer will respond to a short voltage pulse with a relatively long lasting vibration. A "low $Q$" transducer, on the other hand, will vibrate for a shorter time, emitting a shorter pulse. In the case of the 2.8 and 4.6 MHz transducers used in
these experiments, the ring down time was perhaps too long and hid the inertial cavitation
signature that followed the microbubble’s principal response.

Comparison of the spectral response for groups of ruptured and non-ruptured bubbles
insonified with the same incident pulse demonstrated an increase in broadband noise added to
spectra with otherwise similar spectral content in the signals received with the 13 MHz
transducer. Chen et al. [7] used such an increase in the broadband noise of spectra as a
criterion for microbubble collapse detection. This criterion does not appear very robust,
however, as the amplitude of the increase appears to vary considerably from one data set to
another.

This chapter explored the IC response of ruptured microbubbles in terms of
measurement variation and significance of the differences between rupture thresholds
measured by different experimenters. Measurements obtained with the DPCD were used to
examine the IC response across a large bandwidth, including the transmit transducer’s
bandwidth. Based on our results, it appears that detection of IC events using the transmitting
transducer in pulse-echo mode is possible but that the transducer characteristics (bandwidth
and Q) should be carefully chosen. Other than a variable increase in the broadband noise of
spectra, no regularly occurring, spectral features linked to IC events were identified. Thus, the
time-domain detection technique described in chapter V still appears to be the most promising
approach for IC detection. The next chapter looks at the IC event from yet another perspective
by combining PCD acoustical detection of the event with optical microscope observations.
Chapter VII – Optical and acoustical observation of isolated microbubbles
VII. 1. Introduction

In the precedent chapters the identification of the microbubbles of ultrasound contrast agent shell rupture was based on analysis of the radio frequency (RF) signals. In this chapter additional information was gained by coupling a passive cavitation detector to an optical microscope. For the measurements reported in the previous chapters, the microbubbles were freely circulating in a tank. In this work, static microbubbles were isolated in a cellulose tube. This setup allows to verify optically that only a signal microbubble is present at the measurement site and that the microbubble disappearance is well correlated with the detection of post-excitation signals in the collected RF data. The images obtained with the microscope were also used to provide an estimate of the equilibrium microbubble size for theoretical models so that the modeled microbubble response can more precisely be compared to experimental results. Optical observations of the movement of groups of microbubbles during insonification showed the complexity of the microbubble dynamics at very high concentrations in a confined medium.

VII. 2. Data acquisition

A 200-μm cellulose fiber (Spectrum Labs, Inc.) was placed in the centre of the microscope optical field of view. The transducers were then aligned on the fiber as described in Chapter III, section 5. A baseline data acquisition was made in the absence of contrast agent. A solution of contrast agent was diluted in saline to approximately 1 microsphere/μL providing approximately one microbubble per optical field of view inside the cellulose fiber. The dilute contrast agent solution was pumped through the aligned cellulose fiber with a manual microinjector (IM-5B, Narishige, Inc.). Once a flowing microbubble was optically observed the injection process was stopped and the microbubble was approximately centered in the optical field of view. An excitation at the transducer centre frequency (0.99 MHz) was generated. For each transmit excitation, received signals were acquired with the 9.8 MHz receiving transducer and images of the microscope field were digitized with the camera.

Figure VII.1 shows a chronogram of the acquisition sequence. The camera provided the driving clock which limited the data acquisition to one image every 40 ms, as shown by the first trace in Figure VII.1. The function generator sent a 7-cycle, 0.99-MHz excitation
pulse at the desired pressure to the transducer when the first rising edge of the driving clock occurred (at $t=0$). Also at the rising edge of the master clock (time $t=0$), an impulsion generator (HP 8114A, Germany) is triggered to send a very narrow pulse of 100 ns after a selected delay to trigger the camera. A 30 μs delay was selected so that the camera was triggered prior to the acoustic excitation of the microbubble (The TOF from the transmit transducer to the microbubble is about 37.5 μs). Once the camera is triggered, the shutter is opened for the duration of this open-gate pulse (100 ns) and an image is recorded. (The delay between the ultrasonic pulse and the camera image acquisition can be increased by a fixed time at each rising edge of the master clock if desired to investigate microbubble oscillation at different times during its excitation but this function was not used in the current study). A signal was acquired with the 9.8 MHz receiving transducers beginning at $t=0$ and continuing until $t=200$ μs with respect to the master clock (microbubble response occurs between 71 and 77 μs). The image, transmit and receive sequence is repeated at the next rising edge of the master clock. The total number of consecutive acoustic excitations to apply to an isolated microbubble is specified by the user. It can be as high as 500 excitations, but was generally on the order of 100 for the experiments reported here.

![Diagram](image)

**Fig. VII.1.** Chronogram of the acquisition sequence. At a rising edge of the driving clock the function generator, the impulsion generator and the oscilloscope are triggered. The function generator sends an acoustic waveform through a power amplifier to excite the transducer. The impulsion generator After a delay of 30 μs, a pulse generator transmits a 100 ns-long square wave to the camera to open the time window for optical microscope image acquisition. The oscilloscope records all the signal received by the 9.8 MHz transducer from $t = 0$ until well after the microbubble response is finished.
VII. 3. Results

Figure VII.2 shows an image of an Optison™ microbubble prior to excitation. The radius was estimated to be 10.3 μm. This microbubble was excited by a 0.99-MHz, 7-cycle PD transmit pressure waveform with a peak rarefactional pressure of 0.27-MPa.

Fig. VII.2. Images of a single microbubble before excitation with a 0.27-MPa, 7-cycle 0.99 MHz acoustic pulse. A total of fifty acoustic waves were sent to the microbubble but the microbubble did not collapse. The numbers on the bottom left corner represent the image frame number. Frames rate was 25 Hz. The arrow in the first image represents the direction of propagation of the incident US pulse.
To study this microbubble, 50 consecutive image/transmit/receive sequences were acquired. Figure VII.2 shows the microbubble at every 10 transmit pulses. The direction of propagation of the ultrasound (US) pulse was horizontal with respect to the image (from the right to the left) as indicated by the arrow in the first image. The microbubble did not appear to have a detectably different diameter after repeated ultrasonic excitation, however it clearly moved from its initial position. This movement is consistent with the movement that would result from radiation forces as described by Beissner [124, 125] and Leighton [126] and observed for UCA by Dayton et al. [94].

Figure VII.3 (a) shows the corresponding acoustic response received at 9.8 MHz of the microbubble in Fig. VII.2. The responses were identical for all of the 60 excitations. The response of the microbubble is detected between 71 and 77 μs. No post-excitation signal was detected. Thus, the acoustical evaluation indicating that the microbubble did not collapse, is confirmed by the optical observations. The corresponding spectrogram Fig. VII.3 (b) shows a strong spectral component at 0.99 MHz with thin vertical lines demonstrating the presence of signals at higher frequencies during the collapse phases of the principal response.
Fig. VII.3 (a) Signal received with a 9.8-MHz receiver for a 0.99-MHz, 7-cycle, 0.27-MPa peak rarefractional pressure excitation. (b) Corresponding spectrogram. The microbubble is oscillating and has not ruptured (no post excitation signals are present).
Figure VII.4 (a) shows an image of an Optison™ microbubble in the confocal area prior to excitation (with a low zoom magnitude). The radius was estimated to be 4.9 µm at higher magnification. This microbubble was excited with a 0.99-MHz, 7-cycle PD transmit pressure waveform with a peak rarefractional pressure of 0.82-MPa. To study this microbubble, 10 consecutive image/transmit/receive sequences were acquired. Figure VII.4 (b) shows the same field of view after the first pulse. The microbubble disappeared from the field of view indicating that the microbubble had collapsed.

![Image](image-url)  
**Fig. VII.4.** Images of a single microbubble before (a) and after (b) insonification with a 0.82-MPa, 7-cycle 0.99-MHz acoustic pulse. The arrow in the first image represents the direction of propagation of US.

Figure VII.5 (a) shows the corresponding acoustic response received with the 9.8 MHz transducer of the microbubble for the image/transmit/receive sequence corresponding to the image in Fig. VII.4 (a). The response of the microbubble is again approximately between 71 and 77 µs. In this case, a post-excitation signal was detected in the segment (near 77 µs). Thus the acoustical data indicates the microbubble is collapsed by this acoustic pulse and this is confirmed by the optical image acquired prior to the next transmit/receive sequence in Figure VII.4 (b). The corresponding spectrogram in Fig. VII.5 (b) shows a strong spectral component at 0.99 MHz with brighter vertical line compared to the ones in Fig VII.3 (b) demonstrating a stronger spectral activity at high frequencies in the collapsing case. As in the PCD case the post-excitation signal show a very large bandwidth content.
Fig. VII.5 (a) First received waveform with a 9.8-MHz receiver for a 0.99-MHz, 7-cycle, 0.82-MPa peak rarefractional pressure excitation. (b) Corresponding spectrogram. The microbubble response shows evidence of post-excitation signal indicating its rupture.
Figure VII.6 (a) shows a simulated microbubble response of a 4.9 µm microbubble to the excitation applied to the bubble in Figure VII.4. When the microbubble first expanded to a radius equal to twice its initial radius, the shell parameters were set to zero to simulate shell rupture.

![Graph](image1)

(a)

![Graph](image2)

(b)

Fig. VII.6 (a) Simulated received echo from a microbubble excited with a 0.99-MHz, 7-cycle, 0.82-MPa peak rarefactive pressure pulse (b) Corresponding spectrogram.
The modeled radiated pressure in Figure VII.6 demonstrates sharp peaks during excitation at 7.7 and 9.5 μs. Post-excitation peaks appear in Figure VII.6 (a). The corresponding spectrogram in Figure VII. (b) shows very large bandwidth spectral content at the times of the sharp peaks.

A total of 11 microbubbles were studied with synchronized optical and acoustical data. Of these, the 5 exposed to pulses with a peak rarefational pressure of 0.27 MPa did not present IC signal and remained visible in the microscopic images throughout the experiment. The other 6 microbubbles exposed to a peak rarefational pressure of 0.82 MPa demonstrated post-excitation IC signals. All of these microbubbles were confirmed to be absent in the microscopic image acquired following the excitation-IC response sequence.

Table VII.1 summarizes the size of microbubbles with IC response as well as the characteristics of their respective echoes. The diameter of the studied microbubbles ranged from 2.6 to 8.5 μm. Increasing the microbubble diameter led to an increase in the absolute peak voltage in the principal response. Although the distribution of microbubble diameters interrogated was relatively broad, neither the time-delay relative to the end of the principal response (25±0.10μs) nor the amplitude (1.7±0.5 mV peak to peak ) of the IC signal appear to vary as a function of the initial microbubble size 1.

<table>
<thead>
<tr>
<th>Initial microbubble diameter (μm)</th>
<th>Principal response peak maximum voltage (V)</th>
<th>Principal response peak minimum voltage (V)</th>
<th>IC signal time-delay after the end of principal response (μs)</th>
<th>IC Peak maximum voltage (V)</th>
<th>IC Peak minimum voltage (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.64</td>
<td>0.56</td>
<td>-0.74</td>
<td>1.28</td>
<td>0.21</td>
<td>-0.23</td>
</tr>
<tr>
<td>2.76</td>
<td>0.60</td>
<td>-0.68</td>
<td>1.34</td>
<td>0.15</td>
<td>-0.26</td>
</tr>
<tr>
<td>4.08</td>
<td>0.79</td>
<td>-0.68</td>
<td>1.27</td>
<td>0.23</td>
<td>-0.42</td>
</tr>
<tr>
<td>4.92</td>
<td>0.60</td>
<td>-0.68</td>
<td>1.30</td>
<td>0.16</td>
<td>-0.26</td>
</tr>
<tr>
<td>6.72</td>
<td>1.07</td>
<td>-1.16</td>
<td>1.07</td>
<td>0.26</td>
<td>-0.35</td>
</tr>
<tr>
<td>8.52</td>
<td>1.32</td>
<td>-1.29</td>
<td>1.18</td>
<td>0.33</td>
<td>-0.56</td>
</tr>
</tbody>
</table>

Table VII.1. Characteristics of the echo from microbubbles identified as having collapsed. The excitation was 7-cycle pulse centered at 0.99 MHz with a peak rarefactual pressure of 0.82 MPa.
VII. 4. Discussion and perspectives

The acoustical/optic experimental system was demonstrated to allow microscopic observation of an isolated microbubble synchronized with the insonification of the microbubble and the passive detection of the microbubble acoustic response.

Applying a 0.9-MHz, 7-cycle pulse duration with a peak rarefactual pressure of 0.27 MPa, 5 microbubbles were studied. All of these microbubbles were observed to remain in the optical field of view throughout the entire series of applied acoustic pulses (a hundred excitations with a PRF of 25 Hz). The signals received passively from each of these microbubbles with a 9.8 MHz transducer presented the same voltage-time and frequency-time content from one excitation to another with no evidence of a post-excitation response. Thus, the post-excitation signals were not detected when the microbubble was confirmed to persist in the microscopic image data.

Using the same transmit frequency and pulse duration but with a higher peak rarefactual pressure (0.82 MPa), 6 microbubbles of different initial diameters were studied. The acoustic responses received with the 9.8 MHz transducer clearly showed the presence of a small, broadband peak occurring shortly after the end of the principal response. Optically, for all these cases, the microbubbles were visible in the image acquired prior to the application of the pulse leading to an IC response and no longer visible in the image acquired after this response. Thus the post excitation IC signals were confirmed optically to correspond to microbubble disappearance.

However, some aspects of the passive receiver signals acquired with this system were notably different from those acquired previously with the PCD system at the BRL (chapter IV). As noted in Chapter VI, section 4.b, the data acquired with the 13 MHz transducer during microbubble excitation with a 0.9 MHz pulse filtered out most of the principal response of the microbubble and only a few isolated broadband peaks were detected during microbubble oscillation associated with IC events. The data acquired with the 9.8 MHz transducer during microbubble excitation with a 0.99 MHz pulse presents a smooth cyclic voltage variation during the principal response. This is probably due to a less strong filtering effect with this slightly lower frequency receiving transducer. A second and more puzzling difference is that the time-delay between the end of the principal response and the IC signals detected with the
PCD system at the BRL were considerably longer and more variable than delays observed with the acoustical/optic measurement system. This could possibly be due to the fact that microbubbles flowed freely in a tank in the BRL PCD system and were confined to a cellulose fiber in the acoustical/optic system. Work reported by [127] showed that in shock wave lithotripsy the inertial cavitation time dramatically decreased as compared to that of free bubbles when bubbles flowed inside a 200 μm-diameter tube. For this study they used Albunex® as cavitation nuclei. The IC time was up to 20 times shorter when produced in a fiber, depending on the incident pressure. Such a reduction of the IC time would reduce the time between the detected IC response and the end of the principal response, but further investigation is required to probe this hypothesis.

Groups of microbubbles were also observed using the acoustical/optic measurement system. Fig. VII.7 presents a sequence of images recorded for a group of Optison™ microbubbles. The frame rate was 1 frame every 40 ms, however, for display convenience, only 1 of every 40 frames are shown in the Figure. The transmitted pulse had a center frequency of 0.99-MHz, 7-cycle pulse duration and a peak rarefactional pressure of 0.27-MPa. Initially the microbubbles are distributed throughout the fiber although the larger bubbles are already concentrated along the central axis of the fiber. Forty frames (1.6 seconds) later, most of the microbubbles are gathered in a column along the middle of the fiber. The column grows thinner and thinner and begins to compress along its length as well (frame 120). Finally, the microbubbles are tightly clustered in an ellipsoidal aggregate that begins to move out of the optical field of view. Observation at a lower magnification (Figure VII.8) demonstrates that several microbubble clusters are formed along the length of the fiber exposed to insonification.
Fig. VII.7 Optical Images of a group of Optison™ microbubbles. Every 40th frame is displayed (original frame rate 25 Hz). The formation of a bubble cluster is apparent.
Fig. VII.8 Optical Images of a group of Optison™ microbubbles. Every 40\textsuperscript{th} frame is displayed (original frame rate 25 Hz).
Chapter VIII – Conclusion
Hypotheses relating signal intensities to microbubble concentration assume that microbubbles remain intact during imaging. However experimental evidence suggests that the microbubbles are significantly destroyed under many clinical scanning conditions. Under other conditions, destruction of ultrasound contrast agent microbubbles may be desirable. For example, the destruction of microbubbles may contribute to new techniques under development such as targeted contrast imaging as well as acoustically aided drug and gene delivery. Thus, the destruction of contrast agent microbubbles is a key to quantitative contrast imaging and to opening new perspectives for contrast agent application. The exact mechanisms of destruction have been the subject of many studies. Recent experimental evidence indicates that a variety of processes are involved, depending upon both the type of ultrasound contrast agent microbubbles and the acoustic insonification parameters. A better understanding of these mechanisms should help to optimize the ultrasound parameters, scanning strategies and UCA properties for specific applications.

This work is focused on the detection and the characterization of the destruction of UCA microbubbles, and an objective acoustic signature permitting the detection of microbubble destruction has been identified.

Experimental results obtained in this work were completed with predictions of the microbubble dynamics performed using the modified version of Herring equation. This equation was chosen because it has been widely used in the literature and radius time curves predicted with this equation have been previously validated under a variety of insonification conditions by following the movement of the microbubble wall with a high speed camera mounted on an optical microscope [28]. The originality of our application of this model lies in the fact that we relaxed the terms describing the constraint due to the microbubble shell when the radius-time curve obtained with the model reached a particular radial expansion limit. The resulting radius-time response (linked to the pressure-time response) was shown to well mimic the experimentally observed pressure-time response of ruptured microbubbles.

Three experimental systems were used: a passive cavitation detector, a double passive cavitation detector and a system allowing microbubble isolation in the field of view of a microscope. Each system had its own specificity. The passive cavitation detector allowed the sensitive detection of broad bandwidth signals related to microbubble destruction and allowed
the acoustic interrogation of a large number of microbubbles in a short time using a passive detector with a center frequency well above that of the transmitting transducer. The double passive cavitation detector added the capacity to simultaneously capture signals due to the microbubble response with the transmit transducer. The acoustical/optic experimental system had a much lower throughput but insured that only a single microbubble was in the acoustically active zone, provided an estimation of the microbubble diameter and allowed optical verification that the microbubble remained (or disappeared) following each insonification.

The signals recorded with the passive cavitation detection were evaluated in the temporal and spectral domains. Broadband emissions were observed between 1 and 5 µs after the principal response of the microbubble. These signals were linked to the inertial cavitation of bubbles released after UCA shell rupture. The minimum incident peak rarefractional pressure at which IC signals were detected was characterized for Optison™ at three frequencies and pulse durations. Because these IC signals were separated in time and spectral content from the principal response, they were easily detected.

An algorithm was implemented to automatically detect IC signals. By this mean, the amplitudes of peak voltages in post-excitation segments of signals with and without IC events were evaluated as a function of incident peak rarefractional pressure. The robustness of using the first IC event to quantify the minimum threshold as a function of frequency and pulse duration was demonstrated by comparing the post-excitation voltage level in signals with and without IC response. The IC responses detected at the lowest pressure threshold for destruction were above the noise by 10 +/- 1.9 dB. In this work, using the automatic detection algorithm, the occurrence of microbubble destruction has been evaluated over a large range of incident peak rarefractional pressures from 0.5 to 4.5 MPa with a very fine resolution in the pressure step-size.

Detecting UCA rupture from signals during the acoustic excitation is confounded by the presence of spectral components from many sources, for example, nonlinear propagation of the incident pressure pulse and nonlinear microbubble dynamics. Because IC signals were separated in time and spectral content from the main echo, they were easily distinguished from the principal microbubble response (e.g., oscillations and shell rupture). Thus, detection of these post-excitation signals provides a more robust detector of UCA rupture than can be
obtained through analysis of the principal response. The equipment necessary to make the measurements is less complex and expensive than that required for a high-speed camera, and the PCD (which can, in principal, be applied in optically opaque organs) has the potential to detect the rupture thresholds \textit{in vivo} as has been done in lithotripsy [116]. Additionally, the PCD technique may sensitively detect microbubble destruction events yielding fragments that are below the resolution for detection with optical microscopy systems.

The reproducibility of the threshold measurements and the statistical significance of threshold differences were evaluated. Results showed a statistical significance in the variation of the minimum destruction threshold as a function of frequency but not with pulse duration. This is consistent with the findings reported by several other researchers. However, only a rather limited range of pulse durations were considered in our experimental studies. The simultaneous reception of signals with a 13 MHz passive receiver and the transmit transducer with the DPCD system demonstrated that IC signals could be detected with the transmit transducer for the 0.9 and 7.1 MHz center-frequency transducers. The success of IC detection with the transmit transducer seemed to be related to the ring-down time of the microbubble principal response and thus was detected better for low Q transducers. Multiple broadband peaks detected with the 0.9 MHz transducer during the microbubble’s principal response only occurred when IC signals were also present and were hypothesized to be due to collapses during excitation after microbubble shell rupture. The spectral response for groups of ruptured and non-ruptured bubbles insonified at the pressures leading to 50% rupture was also compared for the DPCD data. Although an increase in broadband noise was observed, the amplitude of this increase was variable and it was not always present in the low frequency data acquired with the transmit transducers.

The system allowing microbubble isolation with optical verification allowed the observation of the microbubble in the confocal area of the transducers. Observations validate the fact that the IC signal, associated with rupture in the PCD and DPCD experiments, is correlated to the disappearance of Optison\textsuperscript{TM} microbubble from the optical field of view. Future work should apply this experimental system to determine pressure thresholds for destruction as a function of the initial microbubble radius based on both optical observation (as performed by [8-10]) and post-excitation confirmations. The combination of optical and acoustical techniques is a powerful tool for UCA characterization. This combination is made
possible, without interferences with the US field, because of the large working distance that the microscope objective offers.

To date, theoretical analysis of the destruction process has been based on approximate instability criteria developed for free bubbles. A rigorous analysis of the mechanical behaviour of the shelled ultrasound contrast microbubbles during destruction has not been reported. More extensive experimental data should help develop theoretical models for ultrasound contrast agent microbubble destruction. The data describing pressure thresholds for shell rupture described in this work can be used to test and adjust such models. Another original contribution of this thesis research is the automatic algorithm developed to detect IC signals associated with microbubble rupture. This algorithm enables rapid throughput for the assessment of microbubble rupture thresholds, and its use should help assessing rupture for large variety of acoustic parameters and different ultrasound contrast agents.

Estimates of UCA destruction thresholds reported in this thesis or obtained with the methods described herein should contribute to ultrasound contrast imaging in many ways. Derated values for ultrasound contrast destruction should help select machine settings for minimal destruction of ultrasound contrast agent during perfusion assessment sequences in vivo. Information on destruction thresholds can be applied to select the excitation settings to release free bubbles in a vessel for targeted imaging or to release encapsulated medicines for targeted therapy. Occurrence of IC events and the amplitude of these events can be explored in terms of safety levels for ultrasonic exposure when contrast microbubbles have been injected. Increasing knowledge concerning UCA destruction mechanisms, acoustic signature and microbubble dynamics should be a leading factor contributing to the exciting developments emerging for new ultrasound contrast agents and imaging technologies.
References
References

References

References


References

78. Rayleigh, J.W., On the pressure developed in a liquid during the collapse of a spherical cavity. Philos. Mag. 1917. 34.
References

PUBLICATIONS

Publication in peer-review journals


Proceedings


Oral presentations


S Lori Bridal, J-M Correas, O Lucidarme, A Ammi, E Jouannot, P Laugier “Emerging ultrasound contrast functional imaging techniques”. IMVIE 2 Imaging for life and medical sciences, Strasbourg 1-3 mars 2005


DETECTION AND CHARACTERIZATION OF ULTRASOUND CONTRAST AGENT MICROBUBBLE DESTRUCTION

Abstract: Ultrasound contrast agents present a significant potential for imaging and therapy. This potential is linked to a better understanding of the destruction phenomena of the microbubbles present in these agents.

The aim of this work was to detect, characterize and quantify destruction of isolated ultrasound contrast agent microbubbles. The tools applied toward this objective were a model for nonlinear shell-bubble-wall dynamics (modified Herring equation) and three in vitro experimental systems. Post-excitation acoustic emissions were detected using the first experimental system (passive cavitation detector). These emissions are shown to be linked to inertial cavitation events following microbubble shell rupture. This link is illustrated with the microbubble dynamics model. Thereupon, an automatic algorithm is developed to detect the post-excitation signals. Destruction thresholds and percent occurrence of destruction for Optison™ microbubbles are estimated using these post-excitation signals for three frequencies (0.9, 2.8 and 4.6 MHz) and three durations (3, 5 and 7) of the incident acoustic pulse across a range of incident peak rarefractional pressures from 0.07 to 5.39 MPa. The second system consisting of a double passive cavitation detector was used to compare the temporal and spectral response of oscillating and ruptured microbubbles across a large bandwidth, including the transmit bandwidth. The third system permitted verification by optical microscopy that post-excitation signals were present only when the microbubble was destroyed.

The post-excitation signals provide an acoustic signature of the microbubble destruction and enable the characterization and the quantification of destruction.

Key words: Ultrasound contrast agent, threshold, Optison™, post-excitation, inertial cavitation, shell rupture, microbubble.

Résumé: Les produits de contraste ultrasonore présent un grand potentiel pour l’imagerie et la thérapie. Ce potentiel est lié notamment à une meilleure connaissance du phénomène de destruction des microbulles constituant ces produits.

Le but de ce travail de thèse était de détecter, caractériser et quantifier la destruction d’une microbulle isolée. Pour ce faire, la dynamique de la microbulle fut modélisée en utilisant une version modifiée de l’équation d’Herring en parallèle à trois systèmes expérimentaux in vitro. Le premier système expérimental (détecteur passif de cavitation) a permis la détection d’émissions acoustiques se produisant après le passage de l’excitation ultrasonore sur la microbulle. Ces émissions ont été reliées aux cavitations inertiels du gaz interne de la microbulle libéré suite à la rupture de la coque. Le lien entre ces émissions post-excitation, la cavitation inertielle et la rupture de la coque d’une microbulle a été illustré par la modélisation de la dynamique de la microbulle. A partir de ces observations, un algorithme a été développé afin de détecter automatiquement les signaux post-excitation. Cet algorithme a permis de mesurer le pourcentage de microbulles d’Optison détruites en fonction du pic de dépression de l’onde incidente pour trois fréquences (0.9, 2.8 et 4.6 MHz) et trois longueurs d’impulsion (3, 5 et 7 cycles) sur une gamme de pics de dépression allant de 0.07 à 5.39 MPa. Le second système expérimental est un double détecteur passif de cavitation utilisé pour, comparer dans le domaine temporel et spectral, les réponses de microbulles oscillantes et rompues sur une large bande passante. Le troisième système a permis de vérifier par microscopie optique que les signaux post excitation sont présents uniquement lorsque la microbulle était détruite en faisant une critère robuste et fiable.

Les signaux post excitation sont une signature acoustique de destruction de la microbulle et peuvent permettre la caractérisation et la quantification de la destruction.

Mots clés: Agent de contraste ultrasonore, seuil, Optison™, post-excitation, cavitation inertielle, rupture de la coque, microbulle.

Laboratoire d’Imagerie Paramétrique CNRS UMR 7623 – Université Pierre et Marie Curie Paris VI 15, rue de l’école de médecine, 75006 Paris