Ultrasound Contrast Agents Loaded with Magnetic Nanoparticles: Acoustic and Mechanical Characterization

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Licentiate Thesis

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Bubble is the symbol of joy, now it is the part of my life

Dedicated to Acharya (Teacher)
The current methodologies in body scanning diagnostic uses different simultaneous imaging modalities like Ultrasound (US), magnetic resonance imaging (MRI), single photon emission tomography (SPECT) and positron emission tomography (PET). The field requires combination of different modalities for effective use in clinical diagnostics. Such incorporation of different modalities has already been achieved. For example, PET-CT hybrid scanner is designed to acquire align functional and anatomical images and recently US-MRI scanner has successfully shown to improve diagnosis of prostate cancer. The non ionizing radiation hybrid US-MRI is of great interest in health care industry. Further these US and MRI modalities uses different contrast agents like micro-sized gas bubbles (MBs) encapsulated by surfactant for US and superparamagnetic nanoparticles for MRI imaging modalities to further enables new diagnostic opportunities and therapeutic applications. Recently in our 3MiCRON project, we have developed the multimodal contrast agent that could be supported for both US and MRI. This was achieved by coating the magnetic nanoparticles to the poly vinyl alcohol (PVA) surfactant shelled MBs. The nanoparticles in the shell effect the structure can alter the MBs performance as an ultrasound contrast agent. The present thesis is conducted to examine the acoustic and mechanical properties of such multimodal contrast agents.

These multimodal contrast agents were prepared by coating the surface of PVA-shelled MBs by two following strategies: (1) The superparamagnetic iron oxide ($\text{Fe}_3\text{O}_4$) nano-particles (SPIONs) were chemically anchored to the surface of poly vinyl alcohol (PVA) shelled MBs namely MBs-chem and (2) in the second strategy the SPIONs were physical entrapped into the PVA shell while formation of PVA surface on the gas bubble were named as MBs-phys. To understand the scattering efficiency and viscoelastic properties of these modified agents, we investigated the backscattering power, attenuation coefficient and phase velocity measurements. Our acoustic experimental results indicate that both the modified MBs and non-modified plain PVA-shelled ultrasound contrast agents have the same echogenic response. The investigation of mechanical properties of modified MBs revealed that the attached SPIONs on the PVA shell has reduced the stiffness of MBs-chem shell, while, the SPIONs inside the shell has increased MBs-phys stiffness. As a result, MBs-chem exhibits soft shell behavior under ultrasound exposure than both MBs-phys. Finally, the images were obtained through the MRI investigations at the department of Radiology, Karolinka Institute, has demonstrated that both MB types have enough magnetic susceptibility that further provides good detectability in vitro and in vivo. As an outlook, the modified magnetic gas bubbles, i.e. both MBs-chem and MBs-phys can be proposed as a potential contrast agent for both US and MR imaging and can be further utilized in potential therapeutic applications.

**Keywords:** Ultrasound contrast agents, SPION nanoparticles, harmonic oscillation, backscattering power, attenuation coefficient, phase velocity, nonlinear equation of motion.
Acknowledgement

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Stockholm, October 2013
Satya Kothapalli.
List of Included Papers

Paper I

Paper II

Paper III

Other Scientific Contributions

Division of Work between Authors

**Paper I.** This paper includes contribution from 5 research groups, department of Radiology, Karolinska Institute (KI), department of Medical Engineering, KTH, department of Molecular Medicine and Surgery, KI, department of Structural Biology, KTH, department of Chemical Science and Technology, University of University of Rome Tor Vergata (UNITV), within the 3MiCRON project. The aim of the paper is to assess and characterize structural, acoustic, magnetic properties as well as demonstrate possibility to visualize two types of novel dual modal contrast agent using MRI both in vitro and in vivo using animal model. In this paper, Kothapalli and Grishenkov were responsible for assessing the acoustic properties of dual modal contrasts. Kothapalli performed the backscattering power and attenuation coefficient experiments and the scientific writing part was done by Grishenkov.

**Paper II.** This paper is the contribution of three research groups, University of Bayreuth (UBT), Physical Chemistry II, department of Medical Engineering, KTH, department of Structural Biology, KTH, within the 3MiCRON project. The objective of this paper is to correlate the structural and mechanical properties of three novel types of air encapsulated PVA shell loaded with and without magnetic nanoparticles. The structural properties were obtained by atomic force microscope (AFM) and Transmission electron microscope, whereas, the mechanical properties were obtained by AFM at low (kHz) frequencies and acoustic measurements at high (MHz) frequencies. In this paper, Kothapalli and Grishenkov were responsible for assessment of shell mechanical properties though acoustic measurements. Kothapalli performed both acoustical experiments and simulations and the scientific writing was done by Grishenkov.

**Paper III.** In this paper, Kothapalli had implemented the algorithm for simulations to fit theoretical attenuation coefficient and phase velocity spectra with measurements. From best fits we were identified the viscoelastic and physical properties of magnetic PVA-shelled MBs. The scientific writing part was done by Kothapalli under the supervision of Grishenkov.
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1. Introduction

Currently in healthcare industry integrating different diagnostic imaging modalities is one of the leading researches as it enables new diagnostic opportunities and therapeutic applications. Each imaging model has its own limitation. For example, ultrasound has low spatial resolution, MRI has less temporal resolution and X-ray based techniques has radiation dosage effects. The limitations of single modalities can be overcome by the synergic effect of a combination of two or more diagnostic imaging methods. For example, PET-CT hybrid scanners that are able to acquire and align functional and anatomical images are available in current clinical practice.

The complementary and non ionizing radiation modalities US and MR combination is of great interest. In particular, MRI contributes with an unlimited sensitivity and high spatial resolution in depth and an excellent soft tissue contrast to the benefits of US, which are high temporal resolution in the range of seconds, real time scanning and thus fast diagnosis, portability and bed-side applicability. In combination, MRI is able to overcome the limitations of US imaging whose acquisition is only possible through acoustic windows, like between ribs or a water-filled stomach. US will be reflected by bone and adsorbed by air-filled organs. US imaging on the other hand, is able to resolve the perfusion. Recently, MRI-US integrated technology has been proposed to improve the prostate cancer diagnosis.

Highly echogenic micro-sized gas bubbles (MBs) are encapsulated by protein or lipid or polymer materials have been served as contrast agents (UCAs) for US imaging. Commercially available thin protein- and lipid-shelled MBs are regularly utilized for echocardiography, detection of focal liver lesions and for monitoring abdominal aorta aneurysm. On the other hand, functionalized superparamagnetic iron oxide (Fe₃O₄) nanoparticles (SPIONs) are well established MRI contrast agents. These are intravenously injected and used for liver, lymph node or bone marrow imaging. Further, Marchal et al. showed that SPIONs were taken up by Kupffer cells in the liver through the macrophage system, which is further utilized to enhance the detection of liver metastasis by using ultrasound imaging under the magneto-motive excitation.

Although MRI-US integrated technology has a synergic effect to overcome the limitations of each individual modality, in some areas like vascular molecular imaging and therapeutic applications, an additional assistance of contrast agents are required. Thus for MRI-US hybrid scanner, it could be the combination of magnetic nanoparticles and MBs in one device would be of great potential for new emerging diagnostic and therapeutic fields. Here the challenge is to design such a hybrid contrast agent for US and MRI:
1. The contrast agent has to find the right compromise between different requirements.
2. The stability in the circulation within the cardiovascular system$^{12}$.
3. The size of the contrast agent should be smaller than 10 $\mu$m$^{13}$ and bigger than 0.1 $\mu$m to avoid any leakage through the endothelium as well as to avoid an immune reaction$^{14}$.
4. The production of power of an echo signal should be above 20 dB$^{15}$ and the ultrasound energy absorption should be close to soft tissue to avoid shadowing artifacts during organ distal part visualization with US imaging$^{16}$.
5. At the same time, the particle should give enough magnetization for MR imaging.

Thin, mechanically unstable and well-characterized protein-/lipid-shelled MBs are poor choice to attach or embed the nanoparticles into shell$^{17}$. In order to overcome this limitation a biocompatible, robust, thick polymer shell has been proposed$^{18}$. Certainly a thick shell reduces the MBs echogenicity of the MB, because it dampens the oscillation of MBs under ultrasound excitation$^{19}$. Until now the dual-modality contrast agent was manufactured by introducing the magnetic nanoparticles to MBs by physically embedding them in the solid encapsulated shell with chemical or electrostatic coupling on the surface of shell and engrafted in a special oil layer inside the shell$^{20}$. The physical and chemical modifications or different synthesis strategies can affect the structural or mechanical properties of the MB’s shell, which can alter their acoustic response$^{21}$. Hence, the structural design and structure property relations of hybrid probes are extremely important to optimize contrast agents for their particular application.

In this thesis work we have investigated the acoustic, viscoelastic and structural properties of two types of US/MRI contrast agents. These are manufactured by air-filled MBs and stabilized by a shell made of poly vinyl alcohol, PVA. SPIONs are either covalently attached to the microbubble surface (MBs-chem) or physically embedded inside the polymer shell (MBs-phys). The measurements are compared with the previous studies on plain PVA-shelled MBs.

**1.1 Outline of the Thesis**

This thesis is based on the work from three papers and is organized into 9 chapters. After the introduction the objective of thesis is stated in Chapter 2. Thereafter, the background of the gas bubbles, their evolution, and the nonlinear equation of motion are described in Chapter 3. Chapter 4 presents the methodologies and the research contributions within the thesis work. The following three chapters summarize the results, discussion and conclusions. The future perspectives and contributions are presented in Chapter 8 and the summary of the thesis is listed in Chapter 9, followed by References. The full versions of the articles are attached in the appendix.
2. **Objective**

The general aim of this thesis is to characterize the acoustic and mechanical properties of micro-sized multi modal contrast agents. These multimodal contrast agents are proposed to be a unique contrast agent for both US and MR imaging. Further, the acoustic properties are useful to provide the feedback for production, quality control, up-scaling and US imaging applications. The specific objectives for each paper are listed below

- To identify whether these novel multimodal contrast agents works as an ultrasound contrast agents. The investigation method includes ultrasound scattering efficiency and attenuation coefficient at the different dilution rates of these multimodal contrast agents.

- To investigate the viscoelastic (mechanical) properties of both modified PVA-shelled multi modal contrast agents and plain PVA-shelled MBs. Further the estimated viscoelastic properties corroborate with the estimated mechanical properties obtained by the atomic force microscope (AFM) technique at low (kHz) frequencies.

- To evaluate the viscoelastic and oscillatory behavior of multi modal contrast agents by using both attenuation coefficient and phase velocity data, which are further compare with the oscillatory behavior of plain PVA-shelled MBs and thin lipid-shelled MBs.
3. Background

This chapter focuses on the brief revision of micro-sized gas bubbles (MBs), especially explored their evolution from an echocardiography contrast agent to a blood pool therapeutic agent and as well as a multimodal contrast agent. This chapter begins with the retrospective study of gas bubbles in the human body and continues with their physical principles under the ultrasound excitation. Finally, the mathematical models of the nonlinear equations of motion of MBs are discussed briefly, which are still subject of ongoing research.22

3.1 Bubbles in the Body

In 1670, Sir Robert Boyle had reported the possibilities of the formation of nitrogen gas bubbles in the body at high atmospheric pressures (e.g. in deep water). The nitrogen gas bubbles, i.e. more than 100 μm sized gas bubbles, can be trapped anywhere in the body. Especially, if they are trapped in joints or muscles, they can cause pain, paralysis, and sometimes deaths.23 On the other hand, in 1968, Gramiak and Shah24 had reported that the small gas bubbles produced by agitated saline are useful in the detection of the aortic root during echocardiography examination. Later, stabilized gas bubbles were proposed as ultrasound contrast agents (UCAs).5,6 Therefore the gas bubble play two extreme roles in the human body, both as a harmful agent and as a handy tool to improve the visualization sensitivity in ultrasound imaging. These two major innovations were opened the door to an extensive scope of research on gas bubbles.

3.2 Ultrasound Contrast Agents

Ultrasound contrast agents, UCAs, are composed of micro-sized, gas-filled bubbles confined by a biocompatible stabilizing shell. The encapsulating shell on the gas bubbles is typically made of protein, lipid, or more recently, a polymeric materials.6 Initially a highly echogenic UCA were utilized as an intravascular contrast agent to enhance the blood pool signal for a transit period during US imaging examination13. The size of the ideal UCA should be less than 10 μm to avoid the blood flow from blocking in pulmonary capillaries.6 At the same time it should be larger than 0.1 μm to avoid the leakage of UCAs across the vessel wall.14 Currently available UCAs are well below the size of 10 μm.25

3.3 Physical Principle of UCA

In order to understand the physical principle of gas bubbles or UCAs under ultrasound excitation, it is important to start with a brief review of the fundamentals of the
ultrasound imaging technique. Ultrasound is a pressure wave that contains frequencies above the human audible range, which is typically above 20 kHz. In routine clinical diagnostic ultrasound have been utilizing the 1 MHz to 15 MHz frequency range scanners. Ultrasound imaging is based on the echoes or reflections from the object. As the ultrasound travels from one medium to another (acoustic boundary), part of the ultrasound wave is reflected (echo), and part is further transmitted. The magnitude of the echoes depends on the acoustic impedance mismatch between two media on either side of the acoustic boundary. Thus the larger the mismatch the brighter the image.

For example, the tissue consists of different fibrous, highly non-homogeneous and dense materials. Thus, it has more scatters per unit volume. As a reason, it appears as brighter (hyper-echogenic), while blood, which consists of blood cells and blood plasma (contains ~99% of water) is approximately a homogenous material. Thus, it has less scatters per unit volume and appears dark (hypo-echoic) in ultrasound B-mode image. This can be observed in Fig. 1a, where the wall of a heart chamber is observed as hyper-echoic and the cavity with the blood flow is visualized as hypo-echoic. On the other hand, the heart cavity is become brighter with the arrival of gas bubbles compared to the tissue signal as shown in Fig. 1b, thereby the contrast to tissue ratio (CTR) is increased. Worth to mention is that the contrast of the image depends on the difference in scatters available in the insonified volume. The pig echocardiograms were obtained by a Philips machine (iE33, Philips Healthcare, Amsterdam, the Netherlands) at 1.3/3.2 MHz contrast mode.

![Figure 1](image1.png)

**Figure 1.** Two-dimensional ultrasound images of the heart. The images were taken (a) before and (b) after the injection of MBs. The figures were taken with the permission from Malin Larsson at the Department of Medical Engineering, School of Technology, at KTH Royal Institute of Technology.

### 3.4 Evolution of UCA

The air bubbles generated in an agitated saline are named as zero generation UCAs. These are utilized to determine cardiac insufficiency and cardiac shunts. Due to high surface tension (which is equal to pressure difference, $\Delta P$, multiplied with the radius, $R_0$, of bubble), the air bubble disappears quickly. For example, a 3 μm sized air bubble will disappear in 0.02s. That means, the air bubbles will disappear or dissolve in surrounding blood before they reach to lungs if they administrated intravenously by
Thus, zero generation UCAs are not useful in monitoring the left cardiac chambers. In addition to the lack of stability, it is impossible to control their sizes.

Thus size controlled air bubbles encapsulated by surfactant shell definitely benefit the circulation period. Such stabilized UCAs produced in 1984, where the air gas bubbles were managed to encapsulated with the human albumin protein. Consequently, the protected shell decreased the surface tension and prolonged the circulation lifetime, which given an opportunity to monitor the heart left ventricles. This air encapsulated protein material is considered to be the first generation of UCAs.

The gas diffusion back to liquid is inversely proportional to the square root of its molecular weight. Thus, the use of high molecular weighted gases instead of air further reduces solubility rate. Researchers found such biodegradable high molecular weighted gases (e.g., perfluorocarbons and sulphur hexafluoride, SF$_6$) and managed to encapsulate them with either by protein or lipid materials. As a result, the gas persistence length has been increased. These are considered to be the second generation of UCAs. Currently, these UCAs are approved by FDA and EMA for left vertical opacification (LVO), endocardial border delineation (EBD) and Doppler applications. Commercially available UCAs in clinical practice are given in Table II. Note that the size of the UCAs is usually below 10 μm and the shell thickness of both first and second generation UCAs is less than 15 nm. The resonance peak where the maximum of oscillation occurs from these UCAs is found between 2 MHz to 5 MHz, which is typically in the routine clinically operated frequency range.

However, the second generation of MBs with high molecular weighted gases is solved the problem of rapid gas dissolution, but thin shelled UCAs are mechanically not stiff enough to have a stable circulation in the body. In addition, biological effects such as premature ventricular contraction, micro vascular rupture and petechial hemorrhage were observed at high pressures, typically, 300 kPa at 1 MHz frequency.

To address these problems, the gas bubbles are encapsulated with a thicker and more robust polymer material which has been developed. These polymer-shelled UCAs are called as third generation of MBs. Their shell thickness ranges from 100 nm to 1000 nm. Thick and stiff polymer-shelled UCAs prevent gas diffusion which

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacture</th>
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<tbody>
<tr>
<td>Albunex</td>
<td>Molecular biosystems</td>
<td>EU, USA, Canada</td>
</tr>
<tr>
<td>Levovist®</td>
<td>Bayer Schering Pharma AG</td>
<td>Worldwide</td>
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<tr>
<td>Optison™</td>
<td>GE Healthcare</td>
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<td>Definity®</td>
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<td>SonoVue®</td>
<td>Bracco SpA</td>
<td>Europe, China, South Korea, India, Hong Kong, Singapore</td>
</tr>
<tr>
<td>Imagent®</td>
<td>Alliance Pharmaceutical Corporation</td>
<td>USA</td>
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<tr>
<td>Sonazoid™</td>
<td>Amersham Health</td>
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Table 1. Commercially Available Ultrasound Contrast Agents, Data was adopted from Telli et al.
improves the circulation period from minutes to several hours. On the other hand, the echogenicity of MBs is significantly reduced as compared to first and second generation UCAs, due to larger damping and stiffness from thick shell. Further the resonance frequencies are also increased. The resonance frequencies of polymer-shelled MBs were reported between 5 MHz to 15 MHz. Moreover, polymer-shelled MBs are inactive upon insonation when the driving pressure was below 200 kPa. And at high pressures (approximately 1 MPa) gas releases through shell defects instead of rupturing. The release of gas through shell defects makes them suitable for intermittent harmonic power Doppler imaging, intermittent perfusion imaging, and therapeutic applications. More advanced imaging techniques, for example, multi-pulse coding excitation, chirp coding excitation are applied to enhance the acoustic response of these UCAs. To the best of our knowledge, none of the polymer-shelled UCAs are available in the market.

Current researchers focused on theuraptics applications with UCAs. The UCA shell is modified with nano-engineered techniques to attach drug, gene moieties or nanoparticles. These modified UCAs are referred as fifth generation of UCAs. The drug or gene biotin ligands are attached using non-covalent streptavidin interaction. This technique is widely utilized in vitro and in vivo studies. The streptavidin is a foreign protein for humans. Thus, drug/gene loaded through streptavidin cannot be used in human clinical trials. In this case, the drug/gene loaded UCAs through chemically conjugation technique is much safer for human trails. This technique is still in the research stage. The soft and thin lipid-shelled second generations of UCAs are not preferable for loading the drug or nanoparticles due to their mechanical instability. Moreover, the recommended polymer-shelled UCAs are not ideal UCAs. The additional coating on the polymer-shelled MBs with ligands or nanoparticles even further decreases the echogenicity of UCAs. Until recent, the multimodal contrast agents was obtained by either oil-layered or by the chemical conjugation or by the physical embedding of nanoparticles into coated MBs for supporting the other imaging modalities. The evolution of UCAs is summarized and its graphical representation is given in below.

**Figure 2.** The evolution of ultrasound contrast agents.
3.5 Radial Oscillation of UCA

In general, the radial oscillation of UCAs is classified into three regimes based on the incidental peak negative pressure, $P_{\text{neg}}$. As shown in Fig. 3, the radial oscillation of the UCAs consists of a fundamental frequency tone with overtones, i.e. the integer multiple of fundamental frequency at a certain threshold of acoustic pressures.\(^\text{10-13}\) Note that the pressure threshold for the three regimes depend on the encapsulated material, and the size and shell thickness of the UCA, the number of cycles and the frequency of the driving pulse. At low acoustic pressure (typically below 100 kPa), UCAs oscillate in a linear fashion, with the gas having the same expansion and compression amplitude and the frequency being exactly equal to the driving frequency.\(^\text{10}\) As increases the $P_{\text{neg}}$ (above 100kPa), it is easier to expand the shell than to compress it since repulsion forces between the shell molecules start to appear. Consequently, the nonlinear oscillation of behavior leads to higher frequency harmonic peaks,\(^\text{10}\) i.e. an integer multiple of driving frequencies appears in the Fourier domain spectra. At very high $P_{\text{neg}}$ (between 300kPa-1MPa), UCAs become ruptures and gas escapes to the surrounding media, thereby the broadband frequency spectrum can be observed as illustrated in Fig. 3.

![Figure 3. Schematic diagram showing the interaction of an ultrasound contrast agent with an insonation. The left panel is the incidental pulse in the time domain, the middle panel is the scattered signals from UCA at different pressures in the time domain, and the right panel is the frequency domain of the scattered signal from UCAs.](image)

Usually, the clinical diagnostic ultrasound uses the frequency between 1 MHz to 10 MHz. Thus the radial oscillation of the UCAs occurs in 1 MHz to 10 MHz frequency range. In order to visualize them we should have a high speed camera with at least a 2 to 20 million frame rate. Currently only one such high speed camera
equipped with ultrasound probes is available (Brandaries camera with a 25 million frames/second). On the other hand, the mathematical models of the nonlinear equation of motion (NME) of the UCAs are constantly developing, improving the understanding of the interaction between pressure waves and MB oscillation. In the next section we have discussed the present available mathematical models of UCAs, which are still subject of ongoing research.

3.6 Nonlinear Equation of Motion of UCA

3.6.1 Gas Bubble

The fundamental mathematical expression of the dynamics of gas bubble was derived by Lord Rayleigh and Plesset and extended by Noltingk-Neppiras-Poritsky. The nonlinear equation of motion (NEM) of MBs that is suspended in an unbounded liquid was formulated based on Newton’s third law. Briefly, the forces acting on the MB surface from the inside (i.e. gas pressure) are equals to the forces the acting from outside (i.e. both liquid pressure and driving pressure) as shown in Fig. 4. The resultant NEM is expressed in Eq. (1),

$$\rho_L \left( R \ddot{R} + \frac{3}{2} \dot{R}^2 \right) = p_G \left( \frac{R}{R} \right)^{\frac{3}{2}} - 4 \mu_L \frac{\dot{R}}{R} - \frac{2\sigma}{R} - p_0 - p_\infty,$$

and backscattered signals from a MB which is located at a distance of $r = 1$ cm is given in Eq. 2.

$$p_s(r,t) = \frac{R}{r} \left( \frac{1}{2} \rho R^2 + p_L - p_\infty \right)$$

where the left side terms in the Eq. (1) represents the inertia of the surrounding liquid due to the oscillating bubble; on the right side of Eq. (2) the term $4 \mu_L \frac{\dot{R}}{R}$ represents the damping in liquid. The rest of the terms represents the restoring forces: $\rho_L$ is the density of the surrounding liquid; $R$ is the instantaneous gas bubble radius that varies with the time, $t$, during the oscillation; the dots on top of the $R$ represent the differentiation with respect to time; $p_\infty$ denotes the sum of ambient, $p_0$, and incidental, $p_{inc}(t)$, pressures respectively; $\mu_L$ is the shear viscosity of the surrounding liquid;

$p_G$ is the gas pressure at equilibrium; $\sigma$ is the surface tension; $k$ represents the polytrophic gas constant that varies between isothermal state (when ratio of specific heats, $\gamma = 1$) and adiabatic state (when $\gamma = 1.4$); the value of $k$ is estimated by using Peclet number, $P_t = R_0^2 \omega / D$, where $\omega$ is the angular frequency and $D$ is the thermal
diffusivity of the gas. Most of the UCAs models were derived based on the extended version of the Rayleigh-Plesset (RP) equation. In Eq. (1), the surrounding liquid was assumed to be incompressible, thus the radiation damping caused by the surrounding liquid was neglected. This assumption is not valid when the velocity of the bubble wall is comparable with the speed of sound in liquid. Keller-Miksis, Herring and Gilmore derived NEM with the inclusion of radiation damping, \( \ddot{R}/c \), which are given in Eq. (3). By setting the parameter \( \lambda \) equals to 0, Eq. (3) becomes the Keller equation, whereas \( \lambda \) equals to 1 gives the Herring equation.

\[
\rho \dot{c} \left[ \frac{1}{2}(\dot{R}^2) \right] \ddot{R} + \frac{3}{2} \dot{R}^2 \left[ 1 - \left( \frac{\lambda + 1}{3} \right) \frac{\dot{R}}{c} \right] = \left[ 1 + \left( \frac{1 - \lambda}{c} \right) \frac{\dot{R}}{c} \right] p_b(t) - p_0 - \rho \dot{c} \left( t + \frac{R}{c} \right) + \frac{R}{c} \dot{p}_b(t)
\]

Where, \( p_b(t) = p_s(t) - \frac{4\eta \dot{R}}{R} - \frac{2\sigma}{R} \)

3.6.2 Encapsulated UCAs

In the case of encapsulated bubbles (or UCAs), the model should consider the contribution of both damping and elasticity’s due to an additional encapsulated layer which can be manufactured by protein (or) lipid (or) polymer material. So far, the thickness of shell is reported between 1 and 1000 nm. Therefore, the radial oscillation of UCAs depends on the properties of the shell material as shown in Fig. 5.

Initially, Roy et al. derived the NEM for gas encapsulated by shell, where the shell assumed to be a simple viscous liquid. They found good agreements between in vitro cavitation threshold measurements and theoretical predictions. In 1992, de Jong et al. modeled the Albunex® UCA (with an air encapsulated albumin protein shell) by considering the shell as an elastic solid. They derived the NEM by ad-hoc inclusion of the shell surface elastic modulus (\( S_p \)) and the shell surface viscosity (\( S_f \)) parameters to Eq. (1). The resultant NEM is given in Eq. (5),

\[
\left( R \dddot{R} + \frac{3}{2} \dot{R}^2 \right) = \frac{1}{\rho_s} \left[ \dot{p}_b \left( \frac{R_0}{R} \right)^3 + p_r - p_s(t) \right] - \frac{2\sigma}{R} - \delta \omega \rho \dot{L} R \ddot{R} - 2S_f \left[ \frac{1}{R_0} - \frac{1}{R} \right] - \frac{S_f \dot{R}}{4\pi R^2}
\]
where, $\delta_{tot}$ is the total dimensional damping coefficient characterized by the sum of four damping factors: thermal damping $\delta_{th}$, radiation damping $\delta_C$, liquid viscosity $\delta_L$, and shell damping $\delta_S$.

$$\delta_{tot} = \delta_L + \delta_C + \delta_R + \delta_S.$$  \hspace{1cm} (6)

The detailed description of these damping factors associated with bubble oscillation is given in Lighton$^{46}$ and Medwin$^{47}$. Mathematical expressions for $\delta_{tot}$ will be given in Chapter 4. Although the de Jong et al.$^{45}$ were found the good agreement between experiments and theoretical estimation, the use of the frequency dependent nonlinear damping term $\propto op_{L,R} \dot{R}$ in the linearized model is questionable. In this model the authors assumed the shell thickness was very small when compared to the total diameter of the UCA. In 1994, Church$^{48}$ derived the similar to the RP equation, by assuming the shell material as an incompressible and a viscoelastic solid. This assumption was allowed to adapt the Kelvin-Voigt constitutive equation for shell mechanics and the final NEM is given in Eq. (7),

$$R_i \dot{R}_i \left[ 1 + \frac{\rho_L - \rho_s}{\rho_s} \frac{R_{2i}}{R_{1i}} \right] + \left( \dot{R}_1 \right)^3 \left( 1 \right) \left[ \frac{3}{2} + \left( \frac{\rho_L - \rho_s}{\rho_s} \right) \left( 4 \frac{R_2 - R_1}{2R_1^3} \frac{R_1}{R_2} \right) \right] =$$

$$\frac{1}{\rho_s} \left[ \int P_{G_s} \left( \frac{R_0^1}{R_1^0} \right)^{3 \kappa} - \frac{P_s (t)}{R_1^0} \right] - 4 \mu_s \left( \frac{R_2^2 \frac{\dot{R}_1}{R_1^2} - 2 \left( \frac{\sigma_1 + \sigma_2}{R} \right) \dot{R}_1 V_s \mu_s - 4 \frac{\dot{R}_1 V_s G_S \left( 1 - \frac{R_{2i}}{R_1^0} \right)}{R_2^3} \right) \right) \right] \right],$$

where $R_{10}$ and $R_{20}$ are the inner outer radius of the shell at rest, and $\sigma_1$ and $\sigma_2$ are the surface tension coefficients for the gas-shell and the shell-liquid interfaces. The term $\tau_{rr}^S$ is the radial component of the stress deviator in the shell,

$$\tau_{rr}^S = 2G_s \frac{\partial u}{\partial r} + 2\mu_s \frac{\partial v}{\partial r},$$

by letting the Eq. (7) in Eq. (8), the resultant equation is given in Eq. (9),

$$R_i \dot{R}_i \left[ 1 + \frac{\rho_L - \rho_s}{\rho_s} \frac{R_{2i}}{R_{1i}} \right] + \left( \dot{R}_1 \right)^3 \left( 1 \right) \left[ \frac{3}{2} + \left( \frac{\rho_L - \rho_s}{\rho_s} \right) \left( 4 \frac{R_2 - R_1}{2R_1^3} \frac{R_1}{R_2} \right) \right] =$$

$$\frac{1}{\rho_s} \left[ \int P_{G_s} \left( \frac{R_0^1}{R_1^0} \right)^{3 \kappa} - \frac{P_s (t)}{R_1^0} \right] - 4 \mu_s \left( \frac{R_2^2 \frac{\dot{R}_1}{R_1^2} - 2 \left( \frac{\sigma_1 + \sigma_2}{R} \right) \dot{R}_1 V_s \mu_s - 4 \frac{\dot{R}_1 V_s G_S \left( 1 - \frac{R_{2i}}{R_1^0} \right)}{R_2^3} \right) \right],$$

where, $V_s = R_0^3 - R_{1i}^3 = R_2^3 - R_1^3$, describes an incompressibility of the shell, gas pressure, $P_{G_s}$ is the gas pressure equal to $p_\theta$ at equilibrium, $G_s$, is the shear modulus, and $\eta_s$ is the shear viscosity. Current existing UCAs models today are mostly derived based on the Church model$^{48}$. For example, in 2000, Hoff et al.$^{32}$ simplified the Church model$^{48}$ by letting the $R_{20} = R_{10} + \epsilon_0$, where $\epsilon_0$ is the finite shell thickness. The resulting NEM is given in Eq. (10),
\[ R\dddot{R} + \frac{3}{2} \dddot{R}^2 = \]

\[ \frac{1}{\rho_L} \left[ p_G \left( \frac{R_0}{R} \right)^{3\kappa} - p_{ac}(t) - \frac{p_0}{R} - \frac{2\sigma}{R} - 4\mu_L \frac{\dddot{R}}{R^3} \frac{12\epsilon G_s R_0^2}{R^3} \left( 1 - \frac{R_0}{R} \right) - \frac{12\kappa_s \mu_s R_0^2 \dddot{R}}{R^3} \right] \]  

(10)

Using this model Hoff characterized the acoustic behavior of polymer, protein- (Albumin®), and phospholipid- (Sonozoid™) shelled UCAs\(^{19}\).

In the same year, Morgan et al.\(^{49}\) derived the NEM for lipid-coated UCAs by including the shell elasticity (\(\chi\)) and the viscosity (\(\mu_s\)) terms to the Herring equation\(^{45}\) and the final form of the NEM is given in Eq. (11),

\[ R\dddot{R} + \frac{3}{2} \dddot{R}^2 = \]

\[ \frac{1}{\rho_L} \left[ \left( \frac{p_0}{R_0} + \frac{2\sigma}{R_0} \right) \left( \frac{R_0}{R} \right)^{3\kappa} - p_{ac}(t) - \frac{2\sigma}{R} \left( 1 - \frac{\dddot{R}}{c} \right) - 2\chi \left( \frac{R_0}{R} \right)^2 \left( 1 - \frac{\dddot{R}}{c} \right) - \frac{12\kappa_s \mu_s R_0^2 \dddot{R}}{R(R - c)} \right] \]  

(11)

In this model, the shell viscosity, \(\mu_s\), was adapted from the Church model\(^{48}\) and the elasticity, \(\chi\), was taken from Glazman\(^{50}\). Here the shell shear viscosity varies with the radial oscillation of UCAs, while the shell elasticity kept almost constant.

Chatterjee and Sarkar\(^{51}\) and Sarkar et al.\(^{52}\) proposed the encapsulated shell is an interface of infinitesimal thickness and Newtonian viscous fluid interface (where shear stress is linearly proportional to shear strain). The resultant mathematical derivation is simply the additional viscous term to the RP NEM as in Eq. (12),

\[ R\dddot{R} + \frac{3}{2} \dddot{R}^2 = \frac{1}{\rho_L} \left[ p_G \left( \frac{R_0}{R} \right)^{3\kappa} - p_{ac}(t) - \frac{p_0}{R} - \frac{2\sigma(R)}{R} - 4\mu_L \frac{\dddot{R}}{R^3} - 4\kappa_s \frac{\dddot{R}}{R^3} \right] \]  

(12)

Where, the shell surface viscosity, \(\kappa_s\), was adapted from the de Jong et al. model\(^{45}\) and the shell was presumed to be an adsorption film on the surface. The surface tension of such films depends on the surface area. Therefore surface tension of the UCA, \(\sigma(R)\), is,

\[ \sigma(R) = \sigma_0 + E^S \left( R^2 - R_E^2 \right) / R_E^2 \]  

(13)

where \(R_E\) is an unstrained equilibrium radius, \(\sigma_0\) is the initial surface tension at zero area change and \(E^S\) is the dilatational elasticity. Note that this model was validated only against the linear oscillation of UCAs and failed to predict the nonlinear oscillation of UCAs. Moreover, the all above mentioned models were not taken an account of multiple reflection effect at high UCA concentration.

At high concentrations typically above \(10^7\) MBs/ml, Hughes et al.\(^{54}\) found the significant effect of multiple scattering in acoustic attenuation spectra at very low acoustic pressures. To account the multiple reflections at high concentrations,
Stride et al.\textsuperscript{53} included the acoustic radiation damping term, $b_{rad}$, to the Church model.\textsuperscript{48}

$$R_t \ddot{R}_t \left(1 + \frac{\rho_t - \rho_s}{\rho_s} \frac{R_1}{R_2} \right) + \left( \ddot{R}_t \right) \left\{ \frac{3}{2} + \frac{\left( \rho_t - \rho_s \right)}{\rho_s} \right\} \times \left( \frac{4 \rho_t^3 - \rho_s^3}{2 \rho_t^3} \right) \frac{R_1}{R_2} = 1$$

$$\left[ \frac{1}{\rho_s} \left( \frac{R_{01}}{R_1} \right)^{3/2} - P_n(t) - \frac{4 \mu_L}{R_2} \frac{R_t^2 \ddot{R}_t}{R_2} - \frac{2}{R} \left( \sigma_1 + \sigma_2 \right) + \frac{4}{R_1} \frac{V_s \mu_s}{R_2} - 4 \frac{V_s G_s}{R_2^3} \left( 1 - \frac{R_{el}}{R_1} \right) - b_{rad} \right]$$

where the term $b_{rad}$ is equal to the radiation damping factor, mentioned in Eq. (6) and further described in Chapter 4. Using this model, the authors were successfully predicted the multiple scattering effect at higher concentrations.

In general, the additional encapsulated material should increase the damping and resonance frequency compared to gas bubbles.\textsuperscript{19} The contrary effect was observed in lipid-shelled UCAs, Definity\textsuperscript{®}, where the resonance peak is close to that of the gas bubble.\textsuperscript{55} This result is simply the negative hypothesis from the previous assumption. In order to predict this kind of behavior, Doinikov and Dayton\textsuperscript{56} treated the lipid-encapsulated shell as a “viscoelastic fluid” instead of “viscoelastic solid”, which was allowed them to use the Maxwell constitutive equation for shear forces on the shell,

$$\tau^{s}_{rr} = 2\mu_s \frac{\partial v}{\partial r} - \lambda_s \frac{\partial \tau^{(s)}}{\partial r},$$

where, $\mu_s$ is the shear viscosity as in the Church model\textsuperscript{48} and $\lambda_s$ is the stress relaxation time. The final version of the NEM is given in Eq. (16), is similar to the Church model\textsuperscript{48} except the shell terms included the $D(t)$ function which is expressed in Eq. (17),

$$R_t \ddot{R}_t \left(1 + \frac{\rho_t - \rho_s}{\rho_s} \frac{R_1}{R_2} \right) + \left( \ddot{R}_t \right) \left\{ \frac{3}{2} + \frac{\left( \rho_t - \rho_s \right)}{\rho_s} \right\} \times \left( \frac{4 \rho_t^3 - \rho_s^3}{2 \rho_t^3} \right) \frac{R_1}{R_2} = 1$$

$$\left[ \frac{1}{\rho_s} \left( \frac{R_{01}}{R_1} \right)^{3/2} - P_n(t) - \frac{4 \mu_L}{R_2} \frac{R_t^2 \ddot{R}_t}{R_2} - \frac{2}{R} \left( \sigma_1 + \sigma_2 \right) + \frac{4}{R_1} \frac{V_s \mu_s}{R_2} - 4 \frac{V_s G_s D(t)}{R_2^3} \right]$$

$$D(t) = R_t^3 \ddot{R}_t - \lambda_s \dot{D}(t).$$

Previous models were utilized the acoustic measurements in the linear regime of oscillation to predict the constant shell viscosity and elasticity. These parameters were further utilized in the NEM simulation. As described in Chapter 1, the compression-only behavior in the linear regime of the lipid-shelled MBs cannot be predicted with the previously explained models. To predict the lipid-shelled MB behavior, more sophisticated theories are required. Emmer et al.\textsuperscript{57} observed the single lipid-shelled MB oscillation using optical high-speed recordings, which exhibited both thresholding and compression-only behavior. Marmottant et al.\textsuperscript{58} proposed a theory to predict such thresholding and compression-only behavior by adding the additional variable surface
tension to the de Jong model as similar to the Eq. (13) proposed by the Sarkar model. The variable surface tension, \( \sigma(R) \), is given in Eq. (18).

\[
\sigma(R) = \begin{cases} 
0 & \text{if } R \leq R_{\text{buckling}} \\
\sigma_{\text{water}} & \text{if } R_{\text{buckling}} \leq R \leq R_{\text{break-up}} \\
\text{if ruptured and } R \geq R_{\text{rupture}} 
\end{cases}
\]

If the instantaneous radius \( R \) is less than or equal to the buckling radius \( R_{\text{buckling}} \), then MB exhibits compression-only behavior. If \( R \) is in between \( R_{\text{buckling}} \) and ruptured radius, \( R_{\text{ruptured}} \), then the \( \sigma(R) \) is converted to constant shell elastic modulus, \( \chi \) term is equal to in the elasticity term in the de Jong model. If \( R \) is above the buckling state, then shells rupture and the \( \sigma(R) \) become the surface tension of water, \( \sigma_{\text{water}} \), and the elastic term becomes zero.

In 2005, Cavalieri et al. produced air encapsulated the poly vinyl alcohol, PVA, shelled MBs. The shell thickness of these MBs are in the ranges of 700-1000nm. The PVA shell is formed by the cross-linked networks during polymerization. Grishenkov et al. introduced the complex shear modulus \( G^s(\omega) \) instead constant shear modulus, \( G_s \), in the Church model. This means the modulus of rigidity of the encapsulated shell varies with the driving frequency. Such phenomenon was observed in polymer entangled or cross-linked networks. The resultant NEM is given in Eq. (19),

\[
R \ddot{R} + \frac{1}{\rho_s} \left[ \frac{R_{eq}^3}{R_1} \right]^{\lambda - 1} - 4 \mu_c \frac{R_1^2 R}{R_2^3} \frac{4V_s}{R_2^3 R_1^2} \int_{-\infty}^{\infty} G(t-\tau) \left[ \frac{\partial}{\partial \tau} \left[ R_1^2 (R_1(t) - R_{\text{eq}}) \right] \right] d\tau,
\]

where \( \tau \) is the relaxation time of the cross-linked networks. This model was validated against the experimental data in the linear regime and it is not validated in nonlinear regime. Currently in this thesis we are considering this vacuum space for future study. We will validate this model against the plain PVA-shelled MBs which are further utilized to extend the conference paper presented at ICSV19.

Tsighifis and Pelekasis included the shell viscosity term from the Hoff model and an elastic term \( S_{el} \) to the Keller-Miksis equation. The elastic term, \( S_{el} \), was derived for three constitutive equations: the Kelvin-Voigt, the Mooney-Rivlin, and the Skalak laws.
\[
\left(1 - \frac{\dot{R}}{c}\right)\ddot{R} + \frac{3}{2}\dot{R}^2\left(1 - \frac{\dot{R}}{3c}\right) = \left(1 + \frac{\dot{R}}{c} + \frac{R}{c} \frac{d}{dt}\right)T
\]

\[
T = \frac{1}{p_L} \left[p_G\left(\frac{R_0}{R}\right)^3 - p_\infty(t) - p_0 - \frac{2\sigma}{R} - 4\eta_L \frac{\dot{R}}{R} - 4\chi \frac{R_0}{R} \left(1 - \frac{1}{R}\right) - 12\chi \frac{R}{R^2} - S_{el}\right]
\]

(20)

For small radial oscillations of MBs, the Kelvin-Voigt equation becomes the Hoff equation; Mooney-Rivlin law is used in the strain-softening materials, i.e. the elastic modulus decreases as the deformation increases; whereas, the Skalak law is used in strain-hardening materials, i.e. the elastic modulus increases with the deformation. The strain-softening method exhibit the decrease in resonance frequency as the incidental acoustic pressure increases. This kind of behavior was observed in phospholipid MBs. Thus the nonlinear models of soft-shelled MBs could be efficient if they consider the strain-softening material behavior into their models. Till date, there is no evidence of strain-softening or strain-hardening behavior in polymer shelled microbubbles. Therefore, the examination of the stress-strain relation in on polymer-shelled MBs at different pressure could be of great interest for nonlinear modeling.

Doinikov et al. derived the NEM which is similar to the Sarkar model by introducing the nonlinear viscosity term \(\kappa_S(\dot{R}/R)\) instead of the constant \(\kappa_S\). The shell elasticity term, \(\chi\) was adapted from the de Jong model. The final expression of the NEM is given in Eq. (21), and the expression of the nonlinear viscosity, \(\kappa_S\), is given in Eq. (22).

\[
R\ddot{R} + \frac{3}{2}(\dot{R})^2 = \frac{1}{p_L} \left[p_G\left(\frac{R_0}{R}\right)^3 - p_\infty(t) - p_0 - \frac{2\sigma}{R} - 4\eta_L \frac{\dot{R}}{R} - 4\chi \frac{R_0}{R} \left(1 - \frac{1}{R}\right) - 12\chi \frac{R}{R^2} - S_{el}\right]
\]

(21)

\[
\kappa_S(\dot{R}/R) = \frac{\kappa_0}{1 + \alpha(\dot{R}/R)} + \kappa_f \frac{\dot{R}}{R}
\]

(22)

The constants \(\kappa_0\) and \(\kappa_f\) can be estimated by fitting the linear version of Eq. (22) with the attenuation coefficient measurements. The first term follows the cross law which describes the shear thinning or pseudo-plastic fluid behavior (where shear strain is exponentially increasing with shear stress) and the second term describes the compression only behavior.

The Qin and Ferrara proposed the NEM which is similar to the Church model. However, they were included terms from van der Waals gas law instead of the polytropic gas law and the compressibility of the surrounding liquid from the Keller-Miksis model. Also, the surrounding medium was treated as a viscoelastic solid. The resultant equation is given in Eq. (23).
\[
R_2 \dot{R}_2 + \rho_L \left( \frac{1 - \dot{R}_2}{c} \right) + \rho_s \left( 1 + \frac{\dot{R}_2}{c} \left( \frac{R_2}{R_1} - 1 \right) \right) \\
+ \dot{R}_2 \left[ \frac{3}{2} \rho_L \left( 1 - \frac{\dot{R}_2}{3c} \right) + \rho_s \left( 1 + \frac{\dot{R}_2}{c} \left( \frac{2R_2}{R_1} - \frac{3}{2} - \frac{2R_2^4}{2R_1^3} \right) \right) \right] = \\
\left( 1 + \frac{\dot{R}_2}{c} \right) \left[ P_G(t) - P_\infty(t) - 4 \mu_L \frac{\dot{R}_2}{R_2} - \frac{4G_L}{3} \left( 1 - \frac{R_{20}^3}{R_2^3} \right) - \frac{2\sigma_1}{R_1} - \frac{2\sigma_2}{R_2} \right] \\
- 3\gamma \frac{\dot{R}_2}{c} \frac{R_2^3}{R_{10}^3} \left( \frac{R_1^3}{R_{10}^3} - \frac{a}{V_m} \right)^{-1} P_G(t),
\]

(23)

where, \( P_G(t) = \left( p_0 + \frac{2\sigma_1}{R_{10}} + \frac{2\sigma_2}{R_{20}} \right) \left( \frac{R_1^3}{R_{10}^3} - \frac{a}{V_m} \right)^{-\gamma} \).”

(24)

Where, \( G_L \) represents the shear modulus of the surrounding medium, ‘\( a \)’ is van der Waals constant, and \( V_m \) is the universal molar volume.

Paul et al.\(^{63}\) extended the Sarkar model\(^{52}\) by including the variable dilatational elasticity, \( E^\delta(\beta) \), which is a function of the surface area change instead of the constant elasticity \( E^\delta \). The authors have named it as the nonlinear elasticity model. In this model, the variable dilatational elasticity follows two laws as proposed in the Qin and Ferrera model\(^{62}\): (1) the quadratic elasticity model (QEM) and (2) the exponential elastic model (EEM). The QEM model encounters the effect of strain softening, whereas EEM accounts both strain softening and compression-only behavior. However, both models work fine in predictions of sub-harmonic response of lipid-shelled MBs at low acoustic pressure. To the best of our knowledge no comparison study made between RT curves and modeling were reported.

Recently, Mulvana et al.\(^{64}\) characterized experimentally and theoretically magnetized phospholipid-shelled MB. These magnetic MBs were produced by perfluorocarbon (C\textsubscript{3}F\textsubscript{8}) encapsulated by superparamagnetic nanoparticles in oil layer which was again covered by phospholipid layer. The authors included the additional damping term in the Church model\(^{48}\) to encounter the shell viscosity from nanoparticles. Later the phospholipid layer modeled as an adsorbed film which was derived by Stride et al.\(^{53}\) The resultant NEM follows the Church equation\(^{48}\) with an additional functional term \( f(\Gamma) \), which is the function of surface molecular concentration \( \Gamma \). This term encounters the effect of the outer surface coating and the mathematical representation of this function is given in Eq. (25),

\[
f(\Gamma) = \frac{4h_{30} \dot{R}_2}{R_2^2} + \frac{2K}{R_2} \left( 1 - \left( \frac{R_{02}}{R_2} \right)^2 \right),
\]

(25)
where, $h_{30}$ and $K$ are given constants for the surfactant shell. Theoretical model validated against both attenuation coefficient measurements and radius-time curves from optical high speed recordings and found good agreements.

The summary of NEM models of UCAs to date is given in Table 2. The gas and shell thickness restrictions are given in the end of the table. The author’s name is mentioned in bold letters, followed by the shell assumption, the gas law, the shell restrictions and the shell assumption.

<table>
<thead>
<tr>
<th>Author: Roy et al. (1990)</th>
<th>Validated Against: Hydrophobic polystyrene spheres cavitation.</th>
<th>Gas law and shell status: (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shell Assumption:</strong> Assumed to be simple viscous liquid and the shell term was included to the Rayleigh Plesset nonlinear equation of motion.</td>
<td><strong>de Jong et al. (1992)</strong> Albumin® UCAs oscillation at the linear regime.</td>
<td>(a)</td>
</tr>
<tr>
<td><strong>Church (1994)</strong> Albumin® UCAs oscillations at the linear regimes.</td>
<td><strong>Hoff et al. (2000)</strong> Polymer, Albumin®, Sonozoid™ UCAs in both linear and nonlinear regime oscillation.</td>
<td>(a)</td>
</tr>
<tr>
<td>Shell was assumed to be a layer of incompressible viscoelastic solid. The viscoelastic solid material follows the Kelvin-Voigt law and final derivation follows the RP equation with an additional shell shear modulus and shear viscosity terms.</td>
<td>An un bounded shell thickness in the Church model was simplified to finite shell thickness.</td>
<td></td>
</tr>
<tr>
<td><strong>Morgan et al. (2000)</strong> MP1950 (Mallinckrodt, Inc., St. Louis, MO), phospholipid-shelled UCAs oscillation in the nonlinear regime.</td>
<td><strong>Allen (2004)</strong> Radius-time curves of 500 nm thick shelled triacetin-shelled UCAs.</td>
<td>(b)</td>
</tr>
<tr>
<td>Shell was assumed to be an elastic solid. The shell terms, both viscosity and elasticity terms were included to the Herring equation.</td>
<td>Shell was assumed to be a visco-fluidic material and derived an equation similar to the Church model.</td>
<td></td>
</tr>
<tr>
<td><strong>Chatterjee and Sarkar (2003)</strong> Albu nex®, Optison®, and Quantison® UCAs oscillation in the linear regime.</td>
<td><strong>Sarkar et al. (2005)</strong> Sonozoid™ UCA oscillation in the linear regime and sub-harmonic responses at nonlinear regime.</td>
<td>(a)</td>
</tr>
<tr>
<td>Shell was assumed to be a Newtonian viscous fluid. As a result, viscous term is included to the RP equation.</td>
<td>Authors were introduced the variable surface tension instead of constant surface tension in the Chatterjee and Sarkar model.</td>
<td></td>
</tr>
<tr>
<td><strong>Marmottant et al. (2005)</strong> Radius-time curves of SonoVue® and BR14 UCAs (Bracco Research SA, Geneva, Switzerland).</td>
<td><strong>Stride et al. (2005)</strong> Albumin coated octofluoropropane and polymer coated isobutane UCA oscillation in the linear regime.</td>
<td>(a)</td>
</tr>
<tr>
<td>The authors were considered the effective surface tension term to the RP equation to accounts the thresholding behavior of MBs at different pressure levels.</td>
<td><strong>Doinkov and Dayton (2005)</strong> Phosolipid-shelled and Definity® UCA oscillation in the linear regime.</td>
<td>(b)</td>
</tr>
<tr>
<td>Authors were included an additional radiation damping term to the Church model for account of multiple-reflection effect.</td>
<td>Shell was assumed to be a viscoelastic fluid behavior which follows the Maxwell constitutes equation that include to the RP equation.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Tsigailis-Pelekasis Model (2008)</strong></td>
<td>Behavior of lipid-shelled MBs at linear regime oscillation and at nonlinear sub-harmonic events.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The shell material was assumed to be a viscoelastic solid as in the Church model. But instead of Hooke’s law, authors had proposed two laws to identify the strain-softening and strain-hardening behaviors.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The shell was assumed to be a viscoelastic solid as in the Church model. But the surrounding media is treated as a visco-elastic solid material instead of Newtonian liquid.</td>
<td></td>
</tr>
<tr>
<td>Grishenkov et al. (2009)</td>
<td>Thick polymer-shelled MB oscillation in the linear regime.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The shell was assumed to be frequency dependant complex viscoelastic moduli rather frequency independent modules in the Church model.</td>
<td></td>
</tr>
<tr>
<td>Paul et al. (2011)</td>
<td>Lipid-shelled UCAs oscillation at linear and nonlinear regimes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Authors utilized the Sarakar model to include the nonlinear elasticity term which encounters the nonlinear scattering.</td>
<td></td>
</tr>
<tr>
<td>Mulvana et al. (2012)</td>
<td>Lipid-shelled UCAs loaded with magnetic nanoparticles.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extended version of the Church model, where an additional viscosity term is included. The elasticity term that is effective surface tension from Sarkar model was considered.</td>
<td></td>
</tr>
</tbody>
</table>

a) **Gas law**: Polytropic gas law; **Shell**: Very thin
b) **Gas law**: Polytropic gas law; **Shell**: No restriction on shell thickness
c) **Gas law**: Vander-Wall hard core gas law; **Shell**: No restriction on shell thickness
4. METHODS AND MATERIALS

The following section describes the methods employed for assessing the acoustic and mechanical properties of multimodal contrast agents. The methods include backscattering, absorption and nonlinear equation of motion, which are adapted from Paper-I, Paper-II, and Paper-III.

4.1. Dual Modal Contrast Agent

The two types of multimodal contrast agents were prepared by combining the superparamagnetic iron oxide (Fe₃O₄) nanoparticles (SPIONs) and PVA-shelled micro-sized gas bubbles. The combination of SPIONS and PVA was performed in two different strategies and the micrographs of modified and non-modified PVA-shelled MBs are shown in Fig. 6

(i) SPIONs were anchored on the air encapsulated poly vinyl alcohol (PVA) shelled MBs by means of chemical reaction (MBs-chem)

(ii) In the second strategy the SPIONs were entrapped in PVA shell during the bubble formation (MBs-phys).

Figure 6. Micrographs of (a) plain poly vinyl alcohol, PVA-shelled MBs (MB-pH5-RT), (b) SPIONs are chemically attached on the PVA-shelled MBs (MBs-chem), and (c) SPIONs are physically entrapped inside the PVA-shelled MBs (MBs-phys). The images were taken with permission from Johan Härmak at the department of Structural Biotechnology, KTH.

4.2 Structural Parameters of MBs

The structural parameters such as shell thickness, size, and iron composition of the modified and non-modified PVA-shelled MBs were analyzed by using parameters as mentioned in Table 3. The MBs were mainly characterized by three different imaging techniques namely confocal laser scanning microscopy (Nikon Eclipse Ti-E, Japan), atomic force microscopy AFM (JPK instruments AG, Germany), and transmission electron microscopy TEM (CEM 902, Carl Zeiss AG, and Germany). In addition the iron content was measured by TGA and DTA techniques.
Table 3. Average diameter, shell thickness, and iron content of the particles.

<table>
<thead>
<tr>
<th>MB name</th>
<th>Concentration</th>
<th>Diameter</th>
<th>Shell thickness</th>
<th>Iron Content % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-chem</td>
<td>1.78×10⁸</td>
<td>3.8±0.6</td>
<td>315±44</td>
<td>29±6</td>
</tr>
<tr>
<td>MB-phys</td>
<td>4.0×10⁴</td>
<td>3.8±0.6</td>
<td>415±54</td>
<td>15±1</td>
</tr>
</tbody>
</table>

In Paper III, both diameter and shell thickness values from the AFM measurements were utilized.

<table>
<thead>
<tr>
<th>MB-pH5-RT</th>
<th>Concentration</th>
<th>Diameter</th>
<th>Shell thickness</th>
<th>Iron Content % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-chem</td>
<td>1.78×10⁸</td>
<td>3.6±1.3</td>
<td>215±133</td>
<td>0</td>
</tr>
<tr>
<td>MB-phys</td>
<td>4.0×10⁴</td>
<td>3.6±1.3</td>
<td>215±133</td>
<td>29±6</td>
</tr>
</tbody>
</table>

4.3 Acoustic measurements

To assess the acoustic characterization of MBs-chem and MBs-phys, the backscattering power (BSP), absorption measurements were performed. All these measurements were carried on different dilution rates of MBs at low incidental acoustic pressure (below 100 kPa) and at room temperature (at 24ºC).

4.3.1 Backscattering Power

The method for BSP estimation was described in Grishenkov et al.31 In brief, a 5 MHz focused transducer (Panametrics, V311, Waltham, MA, USA) with −6 dB bandwidth ranges between 2.5 MHz and 6.8 MHz was utilized as both transmitter and receiver and it was electrically excited by a Panametric pulser/receiver (Panametrics PR 5072, Waltham, MA, USA) as illustrated in Fig. 7a. The mathematical expression for the BSP spectra of MB suspension27 is given in Eq. (26),

\[
BSP_{MB} = 10\log_{10}\left(\frac{P_{MB}}{P_{ref}}\right),
\]

where, \(P_{MB}\) and \(P_{ref}\) are the scattered power from MBs suspension and water respectively.

4.3.2 Absorption

In the second experimental set-up, we investigated the energy of wave is absorbed by the MBs, which is both in magnitude reduction (called as attenuation coefficient) and the wave distortion (phase velocity). To perform these experiments, we utilized a flat transducer (Panametrics V311, Waltham, MA, USA) with a central frequency of 10 MHz. It was placed 10 cm above and perpendicular to aluminum plate in a water tank as shown in Fig. 7b. A custom made sample cell was placed 1 cm above the aluminum plate. The acquired scattered signals in time-domain were obtained from the metal reflector when the sample cell was filled with water and MB suspension respectively and further processed using Matlab®.
Figure 7. Experimental set-up for measuring (a) the backscattering power of MBs and (b) attenuation coefficient and phase velocity of the scattered acoustic wave.

The mathematical representation of scattered time-domain signal from the metal reflector can be expressed by considering the plane wave travelling in positive $z$-direction and is assumed to be unbounded in $x$ and $y$ direction as follows,

$$ p(z,t) = p_0 e^{i(\omega z - k_z z)}, $$

(27)

where the complex wave number $k_c$ is equal to $k_r + ik_i$ and the Eq. (27) becomes,

$$ p(z,t) = p_0 e^{i(\omega z - k_z z)} = p_0 e^{k_z z} |e^{i(\omega z - k_z z)}|, $$

(28)

and the Fourier transform of Eq. (28) is given in Eq. (29),

$$ F(\omega) = |F_0(\omega)| e^{i(\omega - k_z z)}. $$

(29)

The imaginary terms of complex wave number $k_i$ gives the attenuation coefficient, $\alpha(\omega)$, and real parts $k_r$ gives the phase velocity, $c(\omega)$, and acoustical path of the wave to be equal twice the length of the sample chamber, $L$, is

$$ F(\omega) = |F_0(\omega)| \exp(-\alpha(\omega)2L) \exp\left(-i \frac{2\omega L}{c(\omega)} \right). $$

(30)

Likewise, the Fourier transform of the signal which is acquired from the MB suspension, $F_{MB}$, and water, $F_{ref}$, are given in Eq. (31) and (32),

$$ F_{MB}(\omega) = F(\omega) \exp(-2\alpha_{MB} L) \exp\left(-i \frac{2\omega L}{c_{MB}(\omega)} \right). $$

(31)

$$ F_{ref}(\omega) = F(\omega) \exp(-2\alpha_{ref}(\omega) L) \exp\left(-i \frac{2\omega L}{c_{ref}(\omega)} \right). $$

(32)

Subtraction of real parts in Eqs. (31) and (32) give the attenuation coefficient, $\alpha(\omega)$, in Np and later it is converted to dB by multiplying with 20 (loge) which is given in Eq. (33). The subtraction of imaginary parts give the phase shift that further used to retrieve the phase velocity, $c(\omega)$, which is given in the Eq. (34).
\[
\alpha(\omega) = -\frac{1}{2L} \left[ \frac{F_{MB}(\omega, L)}{F_{ref}(\omega, L)} \right] Np/cm \quad \text{20 loge} \quad \alpha(\omega) = -\frac{20}{2L} \log \left( \frac{F_{MB}(\omega, L)}{F_{ref}(\omega, L)} \right) \text{dB/cm}, \tag{33}
\]

\[
\frac{1}{c_{MB}(\omega)} = \frac{1}{c_{ref}} \left[ \frac{\phi(F_{MB}(\omega, L)) - \phi(F_{ref}(\omega, L))}{2L\omega} \right] \text{ (m/s).} \tag{34}
\]

The terms \( |F_{MB}(\omega)|, |F_{water}(\omega)|, \phi_{MB}, \) and \( \phi_{water} \) are magnitudes and phase of retrieved signals from MB suspensions and water respectively. The speed of sound in pure water \( c_{ref} \) at room temperature 24°C is approximately 1494 m/s.

### 4.4 Mechanical Properties of Shell

The mechanical properties of the shell are usually retrieved by comparing the attenuation coefficient and phase velocity measurements with the linearized theoretical model. The attenuation coefficient can be calculated by two ways, i.e., (1) using extension scattering cross section, \( \sigma_e \), and (2) mathematical expression of complex speed of sound in bubbly liquid, \( c_m \).

In Paper-II the \( \sigma_e \) is used to calculate the attenuation coefficient. The calculated attenuation coefficient spectra were compared with the attenuation coefficient measurements of MB-pH5-RT, MBs-chem and MBs-phys to retrieve their viscoelastic properties. The mathematical expression for \( \sigma_e \) is given in Eq. (35),

\[
\sigma_e = \sigma_S \frac{\delta_{tot}}{\delta_c} = 4\pi R_{01}^2 \frac{\Omega^4}{(1 - \Omega^2)^2 + (\Omega \delta_{tot})^2} \frac{\delta_{tot}}{\delta_c}, \tag{35}
\]

where, \( \sigma_S \) is the scattering cross section, \( \delta_{tot} \) is the total damping ratio, \( \delta_c \) is the radiation damping, \( \Omega(=\omega/\omega_o) \) is the normalized frequency and \( R_{01} \) is the radius of MB at rest. The mathematical expression for attenuation coefficient, \( \alpha(\omega) \), is given in Eq. (36),

\[
\alpha(\omega) = 10 \log e \int_0^\infty \sigma_e(R_{01}, \omega) n(R_{01}) dR_{01}. \tag{36}
\]

In paper II, the viscoelastic properties are obtained by fitting only attenuation coefficient spectra. For more corrobororation in Paper III the phase velocity data is included to identify the viscoelastic parameters. The simultaneous spectra of attenuation coefficient and phase velocity were achieved by using the \( c_e \) expression, which is given in Eq. (37).

\[
\left( \frac{c_{ref}}{c_e} \right)^2 = 1 + \frac{4\pi c_{ref}}{\alpha \rho_s} R L \int_{\omega_o^2}^{R} \frac{R}{\omega_o^2 - \omega^2 + 2j \zeta_{tot} \omega_o \omega} n(R) dR \tag{37}
\]

Where, \( n(R)dR \) is the number of bubbles per unit volume within the radius of \( (R, R + dR) \), \( \sqrt{-1} \) is equal to \( -1 \), and \( \zeta_{tot} \) is the total damping ratio which is equal to \( \delta_{tot}/2 \). The complex wave number can be written as,
\[ k_v = k_{ref} (u + iv) = \frac{\omega}{c_{ref}} (u + iv) = \frac{\omega u}{c_{ref}} + \frac{\omega iv}{c_{ref}}. \]

Where, \( k_v = \omega/c_v \) and the real term in right side of the Eq. (38), gives the phase velocity \( c(\omega) = \omega/|k_v| \) and the imaginary term gives \( \alpha(\omega) = -20(\log(e) \times \text{Im}(k_v)) \). When the changes in phase velocity due to bubbles are small, the expressions can be simplified further by setting \( u \approx 1 \) in the calculation of \( v \). This gives

\[ u = 1 - 2\pi c_{ref}^2 \int_0^{\infty} \frac{R_{ij}}{\omega_0} \frac{\Omega^2 - 1}{(1 - \Omega^2)^2 + (\Omega \delta_{tot})^2} n(R_{0i}) dR_{0i} \]  

\[ \alpha(\omega) = 20 \log(e) \times 2\pi \int_0^{\infty} \frac{R_{ij}}{\omega_0} \frac{\Omega \delta}{(1 - \Omega^2)^2 + (\Omega \delta_{tot})^2} n(R_{0i}) dR_{0i} \]  

\[ \frac{1}{c(\omega)} = 1 - 2\pi c_{ref}^2 \int_0^{\infty} \frac{R_{ij}}{\omega_0} \frac{\Omega^2 - 1}{(1 - \Omega^2)^2 + (\Omega \delta_{tot})^2} n(R_{0i}) dR_{0i} \]  

By inserting \( u \) and \( \nu \) values in Eq. (38) give the both \( \alpha(\omega) \) and \( c(\omega) \) which are given in Eq. (41) and (42),

\[ \delta_{tot} = \delta_L + \delta_{th} + \delta_s \]  

The total damping ratio, \( \delta_{tot} \), is characterized by sum of four damping factors: thermal damping \( \delta_{th} \), radiation damping \( \delta_s \), liquid viscosity \( \delta_L \), and shell viscosity \( \delta_S \). The radiation damping, \( \delta_{th} \), and thermal damping, \( \delta_{th} \), are given in Eq. (43) and (44).

\[ \delta_s = \frac{\omega^2 R_{02}}{c} \left[ 1 + \left( \frac{\omega R_{02}}{c} \right)^2 \right]^{-1}, \]  

\[ \delta_{th} = \frac{3\rho_0}{\omega \rho_s R_{01}^2 \alpha} \text{Im} \left( \frac{1}{\phi(R_{01}, \omega)} \right). \]  

### 4.5 Nonlinear Equation of Motion

Both \( \delta_L \) and \( \delta_S \) are obtained from linearized version of the nonlinear equation of motion, NEM. In this thesis we utilized the linearized version of NEM is proposed by Grishenkov et al. which was stated in Eq. (19) and the Fourier decomposition of linearized version of this NEM is given in Eq. (45).

\[ -\omega^2 + \frac{4}{\alpha \rho_s R_{01}^2 R_{02}^3} \left( R_{01}^2 \mu L \omega + V_3 G^*(\omega) \right) + \frac{1}{\alpha \rho_s R_{01}^2} \left( 3\kappa P_{eq} + \frac{4V_3}{R_{02}} \right) G'(\omega) \right] X(\omega) \]

\[ = \frac{\Delta P}{\alpha \rho_s R_{01}^2}. \]

where, \( \alpha = \left[ 1 + \left( \frac{\rho_L - \rho_S}{\rho_S} \right) \frac{R_{01}}{R_{02}} \right] \]
Damped harmonic oscillator consists of a dashpot (or damper), a spring, and a mass system as illustrated in Fig. 8. The mathematical representation of linear harmonic oscillator is given in the Eq. (46),

\[ (-\omega^2 + 2j\zeta\omega_0 + \omega_0^2)X(\omega) = \frac{\Delta P}{\alpha\rho_s R_{01}^2} \quad (46) \]

By comparing the Eq. (45) and (46), then we can identify the \( \delta_s \) and \( \delta_L \) which are given Eq. (47) and (48), and natural frequency, \( \omega_0 \) is given in Eq. (49),

\[
\begin{align*}
\delta_s &= \frac{4V_s G'(\omega)}{\alpha\rho_s R_{01}^2 R_{02}^3}, \\
\delta_L &= \frac{4R_{01}^3 \mu_L}{\alpha\rho_s R_{01}^2 R_{02}^3}, \\
\omega_0 &= \sqrt{\frac{1}{\alpha\rho_s R_{01}^2} \left( 3\kappa P_{eq} + \frac{4V_s}{R_{02}^3} G'(\omega) \right)}.
\end{align*}
\] (47) \( \quad \) (48) \( \quad \) (49)

The frequency dependant storage modulus, \( G'(\omega) \), and loose modulus, \( G''(\omega) \), are expressed as,

\[
\begin{align*}
G'(\omega) &= G_{eq} + G_f \omega^{3/4}, \\
G''(\omega) &= \omega (\mu_e - \mu_0).
\end{align*}
\] (50) \( \quad \) (51)

In Eq. (50) and (51), four unknown variables, i.e. equilibrium storage modulus, \( G_{eq} \), frequency dependant storage modulus, \( G_f \), equilibrium loss modulus, \( \mu_{eq} \), and frequency dependant loss modulus, \( \mu_f \), are recalculated by fitting the experimental data with the mathematical model. In the simulation, the following parameters are utilized: Liquid density, \( \rho_L = 1000 \text{ kg/m}^3 \); Shell density, \( \rho_S = 1774 \text{ kg/m}^3 \) for MB-chem; 1393 \( \text{ kg/m}^3 \) for MB-phys; Shear viscosity in liquid; \( \mu_L = 1 \times 10^{-3} \text{ Pa.s} \); Driving pressure \( p_{ac}(t) \approx 25 \text{ kPa} \); Ambient pressure, \( p_0 = 0.1 \text{ MPa} \).

4.6. Rank-Based Algorithm

In the Paper-III, during the curve fitting process both mean square error (MSE) and correlation coefficient (R) were utilized to assess the goodness of fit. In each fitting process, several combinations of Rs and MSEs are obtained. R values vary between 0 to 1 and an R value of 1 indicates a strong linear relationship between data sets, while MSE values vary from 0 to \( \infty \), and an MSE value of 0 represents the ideal fit between data sets.67

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Therefore, the combination of maximum R and minimum MSE values is required to identify the best possible agreement between experiments and theory. The combination of maximum R and minimum MSE is identified with the assistance of rank-based decision-combination method\textsuperscript{68}. The architecture of rank-based decision algorithm is shown in Fig.9. In each simulation, R and MSE non-metric data was classified into two data sets and then data sets are weighted with integer values (from 0 to N) which are named as rank vectors $r_{Ri}$ and $r_{MSEi}$ (where $i = 1, 2, ... N$). The rank vectors $r_{Ri}$ and $r_{MSEi}$ were further combined at second layer and the minimum value of $r_{Ri} + r_{MSEi}$ was corresponded to best combination maximum R and minimum MSE. The index, i, at minimum of $r_{Ri} + r_{MSEi}$ vector was stored to retrieve the unknown variables $G_{eq}$, $G_1$, $\mu_0$, and $\mu_1$. 

Figure 9. Modular view of rank-based decision algorithm: in first layer data sets are converted to rank vectors and ranking data collected in second layer and find the optimal output.
5. Results

This chapter presents an overview of the results obtained from Paper I to III. The detailed result can be found in the full version of the papers in the appendix.

5.1. Acoustic Characterization

The average maximum BSP values with respect to concentration are presented in Fig. 10a. The $a(\omega)$ and $c(\omega)$ spectra of MBs-chem and MBs-phys at various concentrations are presented in Fig. 10b and their mean and standard deviation values at driving frequency, 10 MHz, is illustrated in Fig. 10c.

As listed in Table III, the radii of both MBs-chem and MBs-phys are far below the wavelength of the incidental pressure. Therefore, at low incidental acoustic pressures the scattering from MBs follows the Rayleigh scattering,

$$ I/I_0 = \left( \frac{1}{9} \right) NV_\sigma, $$

where $N$ is the number of scatters in the scattering volume $V$, and $\sigma_s$ has given in Eq. (35). According to Eq. (52), the backscattering intensity of MB suspension is directly proportional to $N$ and $\sigma_s$. As shown in Fig. 10a, the BSP of MBs-chem is linearly increases with the concentration until it reaches $8.8 \times 10^5$ MBs/ml (where the maximum 20 dB BSP is achieved) and decreases above this concentration. On the other hand, $2.0 \times 10^6$ MBs/ml concentration is required to reach 20 dB BSP for MBs-phys and above this concentration BSP is saturated. As shown in Figs. 10b and 10c, the attenuation coefficient values increased, while, the phase velocity values decreased linearly as the number of MBs increased in the insonified volume.
5.2. Mechanical Parameters

In Paper-II, theoretically calculated attenuation spectra fitted with the measured attenuation coefficient spectra at high, moderate and low concentration of MB-pH5-RT, MBs-chem, and MBs-phys. The resultant fittings are given in Fig.11.

![Figure 11](image1.png)

**Figure 11.** Attenuation coefficient vs. frequency for three types of MBs: (a) MB-pH5-RT, (b) MBs-chem and (c) MBs-phys; at three different concentrations. Solid lines in each plot indicate the experimental data, while dotted lines shows the theoretical predictions.

In Paper-III, the simultaneous fittings of both attenuation coefficient and phase velocity spectra are accompanied by the 95% confidential interval for both the MBs-chem and MBs-phys, which are illustrated in Fig. 12. In curve fitting analysis, both statistical parameters R values above 99% and MSE values below 99% are obtained.

![Figure 12](image2.png)

**Figure 12.** Comparison among experimental attenuation coefficient, phase velocity (solid black colour) and theory (solid green colour) with 95% CI (dashed red colour): attenuation coefficient and phase velocity spectra of (a, b) MBs-chem (c, d) MBs-phys.

The predicted unknown variables $G_{eq}$, $G_{1}$, $\mu_{eq}$, $\mu_{1}$, and resonance frequency, $f_{0}$, in both Paper-II and Paper-III studies are reported in Table 4. In both studies, storage (elastic) moduli, $G'(\omega)$, loss (viscous) moduli of MBs-phys is higher than MBs-chem.
According Eq. (47) and (49), the resonance frequency, \( f_0 \), is directly proportional to \( G'(\omega) \) and the total damping ratio, \( \zeta_{tot} \), is directly proportional to \( G''(\omega) \). Thus the larger \( G'(\omega) \) and \( G''(\omega) \) values of MBs-phys resulted in larger \( f_0 \) and \( \zeta_{tot} \) values.

The resonance frequency, \( f_0 \), of MBs-chem is predicted approximately 13 ± 2.2 MHz. It is also evident from the experimental data that the heavily damped resonance peak is located between 12 MHz and 14 MHz as shown in Figs. 12a and 12b. As illustrated in Figs.12c and 12d, the \( a(\omega) \) increased linearly with the driving frequency \( f_0 \), and \( c(\omega) \) was almost constant below \( c_{ref} \) for the MBs-phys. These findings indicate that the resonance peak is far from the upper limit of the investigated bandwidth. The model prediction of \( f_0 \) for the MBs-phys is approximately 27.65 ± 5.99 MHz, which is too far from the available bandwidth of the transducer.

Table 4. The four unknown viscoelastic coefficients \( G_{eq}, G_1, \mu_{eq} \) and \( \mu_1 \) of MBs-chem and MBs-phys were estimated from ultrasound attenuation coefficient measurements fitting with theory.

<table>
<thead>
<tr>
<th>Type of MB</th>
<th>Shell thickness [nm]</th>
<th>Concentration [MBs/ml]</th>
<th>( G_{eq} ) [MPa]</th>
<th>( G_1 ) [(Pa/Hz)^{3/4}]</th>
<th>( \mu_{eq} ) [Pa.sec]</th>
<th>( \mu_1 ) [(Pa.sec)/Hz]</th>
<th>( f_0 ) [MHz] at 10 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-pH5-RT</td>
<td>215±133</td>
<td>1.75×10^9</td>
<td>8.75×10^7</td>
<td>1.75×10^5</td>
<td>10.5</td>
<td>5.5</td>
<td>0.6</td>
</tr>
<tr>
<td>MB-chem</td>
<td>245±92</td>
<td>1.75×10^9</td>
<td>8.75×10^7</td>
<td>4.37×10^5</td>
<td>4.0</td>
<td>10.7</td>
<td>0.3</td>
</tr>
<tr>
<td>MB-phys</td>
<td>205±80</td>
<td>2×10^6</td>
<td>1×10^6</td>
<td>5×10^7</td>
<td>28.0</td>
<td>10.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Mechanical properties of MBs-chem, MBs-phys and Plain MBs adopted from Paper III.

<table>
<thead>
<tr>
<th>Type of MB</th>
<th>Shell thickness [nm]</th>
<th>Concentration [MBs/ml]</th>
<th>( G_{eq} ) [MPa]</th>
<th>( G_1 ) [(Pa/Hz)^{3/4}]</th>
<th>( \mu_{eq} ) [Pa.sec]</th>
<th>( \mu_1 ) [(Pa.sec)/Hz]</th>
<th>( f_0 ) [MHz] at 10 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-chem</td>
<td>315±44</td>
<td>8.75×10^9</td>
<td>9.5±1.0</td>
<td>1.8571±1.25</td>
<td>0.2886±0.02</td>
<td>0.4286±0.2</td>
<td>13.4±2.2</td>
</tr>
<tr>
<td>MBs-phys</td>
<td>415±54</td>
<td>2×10^6</td>
<td>22.0±5.0</td>
<td>17.6±2.5</td>
<td>0.75±0.01</td>
<td>4.4±0.7</td>
<td>27.6±5.9</td>
</tr>
</tbody>
</table>

For all three types of PVA-shelled MBs, i.e. MBs-chem, MBs-phys and MB-pH5-RT the damping caused by the encapsulating shells \( \zeta_{shell}(=\delta_{shell}/2) \) is completely dominating with the contribution of about 90% of its total damping as shown in Figs. 13a-c. Due to the term \( G''/\omega \) in Eq. (47) the \( \zeta_{shell} \) decreases as increases the driving frequency, \( \omega \). As a result the total damping ratio \( \zeta_{tot} \) of PVA-shelled MBs is decreased as increased the driving frequency. On the other hand, the \( \zeta_{tot} \) of lipid-shelled UCA increases with the driving frequency. It is due to the frequency dependant radiation damping, \( \zeta_c \), increases significantly and it starts to be comparable with the shell damping at a driving frequency equal approximately 10 MHz. As a result total damping ratio increases with increase of frequency for Sonazoid^TM UCA as shown in Fig. 13d.
Figure 13. Total damping ratio, (in pink colour), which is sum of thermal damping (in blue colour), shell damping (in green colour), liquid damping (in red colour) and radiation damping (in aqua colour) of MBs-chem and MB-phys, MB-pH5-RT and Sonazoid™ MBs.
6. Discussion

This chapter contains the general discussion of the results presented in this thesis and the relevant clinical applications and future work.

6.1. Acoustic Characterization

The acoustic experiments in the linear regime were performed to identify the optimal concentration of MBs for utilized in ultrasound imaging applications. The attenuation coefficient, $\alpha(\omega)$, and phase velocity, $c(\omega)$, values at this optimal concentration should be close to soft tissue, i.e. between 0.3 dB/(cm×MHz) and 1 dB/(cm×MHz) and 1490 m/s at 24°C to avoid the shadowing artifacts during organ distal part visualization.\textsuperscript{16} And also the BSP values should be above or at least equal to 20 dB, in order to discriminate the blood signal contrast from surrounding tissue.\textsuperscript{31} The maximum BSP is approximately 20 dB and the attenuation coefficient values are close to or below the 10 dB/cm at 10 MHz, and the phase velocity values are close to the speed of sound in surrounding media were identified at $8.8 \times 10^5$ MBs/ml and $2.0 \times 10^6$ MBs/ml concentrations for MBs-chem MBs-phys respectively.

According Rayleigh scattering which was mentioned in Eq. (52), the BSP should increase as increases the number particles in measured volume. But the BSP of MBs-chem decreases when the concentration is above $8.8 \times 10^5$ MBs/ml. Similar findings were reported\textsuperscript{53,54} on Albunex® (protein-shelled MBs) when the concentration is more than $10^6$ MBs/ml. This effect was assumed to be multiple reflection effect which generated from the secondary radial oscillation of MBs. And in MBs-phys the BSP increases as increases the number scatters which follows the Rayleigh scattering. This means that MBs-phys behaves like rigid particles with the density substantially different to surrounding media. Therefore, the MBs-chem are echogenically more active than MBs-phys.

Further, the clinically recommended concentration of MBs in the human body is between $2 \times 10^4$ MBs/ml and $2 \times 10^6$ MBs/ml.\textsuperscript{68} Thus, the MB concentration is a crucial parameter for ultrasound imaging and as well as for pharmacological and toxicological point of view. Accordingly, $8.75 \times 10^5$ MBs/ml and $2 \times 10^6$ MBs/ml concentrations corresponding to MBs-chem and MBs-phys were recommended for ultrasound imaging applications.

6.2. Ambiguities in the estimation of Shell Mechanical Properties

As mentioned in Table. 3, the techniques were used to quantify the geometrical parameters (such as diameter and shell thickness) of MBs possess their own limitations and complications. Unfortunately, there is no an efficient method or a gold standard to quantify these parameters. For example, when the characterizing MB sizes in optical microscope, the floating bubble moving away from the focusing plane appears as a smaller bubble, and when moving towards focal plane it’s assumed to be a bigger
bubble. The random slicing process in TEM measurements considers only a limited amount of MBs. Furthermore, TEM measurements are time consuming. Chemically extensive sample preparation in TEM might cause deviations in the initial bubble shape, and further, if the slice cutting is not precise enough during the slicing process, then it leads to over or under estimation of shell thickness. As in TEM, AFM measurements on estimating bubble diameter and shell thickness were performed on a limited amount bubbles typically less than 250 bubbles. Moreover during the experiments, the bubble dried by removing gas through a vacuum drying process where the bubble diameter and the shell thickness was estimated from flat folded bubble. Later, the dried stage measurements were converted to wet stage, but during the dried state the bubble lost its “hairy” structure and the gas or water pockets in the shell were squeezed out.

The estimation of shell mechanical parameters, i.e. shear modulus, \( G' \), and loss modulus, \( G'' \), are radius and shell thickness dependants. As mentioned in Eq. (53) and (54), the higher the shell thickness, \( V_S = R_{02}^3 - R_{01}^3 \), the lower the \( G' \) and \( G'' \) values; and the higher the \( R_{02} \) the larger the \( G' \) and \( G'' \) values will be obtained.

\[
G' = \frac{R_{02}^3}{4V_S} \left[ \alpha \rho S R_{01}^2 \omega_b^2 - 3\kappa R_{02}^3 \rho \right] \\
G'' = \frac{\alpha \rho S R_{01}^3 R_{02}^3 \delta S}{4V_S}
\]

In Paper-II we were utilized the total diameter of both MBs is equal to 3.6±1.6 and their shell thickness is equal to 215±133, whereas, in Paper-III we were used the diameter values is equal to 3.8±0.6 for both MB types and their shell thickness equals to 315±44 and 415±54 (for MBs-chem and MBs-phys) respectively. Thus, two \( G' \) and \( G'' \) values were identified from two studies for the same type of MBs, which were reported in Table. 4.

According to the Eq. (53), relatively large shell thickness of MBs-chem and MBs-phys gave smaller \( G' \) values in Paper-III study and vice versa in Paper-II study. As given in Eq. (48), the resonance frequency, \( f_0 \), is directly proportional to the \( G' \) and inversely proportional to square root of inner radius of MB. Thus the ratio of shell thickness and radius cancel out in the estimation \( f_0 \), as a result minor deviation between the Paper-II and Paper-III studies in the estimation of \( f_0 \) was observed. Moreover, the deviation is within the 95% confidential interval. On the other hand, \( G'' \) is directly proportional to radius and inversely proportional to shell thickness terms. Although we have utilized higher shell thickness values in the Paper-III, but the 100 nm difference in radius differences between Paper-II and Paper-II studies certainly counteracts the uncertainty in the estimation \( G'' \) as reported in Table. 4. The \( G'' \) values were further utilized to calculate the damping coefficients that describe the type of oscillation of MBs upon insonation.
6.3. Oscillatory behavior of MBs

The post-excitation (i.e., after removal of the incidental pressure) oscillations of the MBs are defined into three types of behavior depending on magnitude of the total damping ratio $\zeta_{\text{tot}}$:

1. Underdamped harmonic oscillator for $\zeta_{\text{tot}} < 1$, i.e., after post-excitation the MB oscillates radially in sinusoidal fashion around its equilibrium with the gradual reduction of amplitude,
2. Critically damped harmonic oscillator for $\zeta_{\text{tot}} = 1$, i.e., MBs radial oscillation returns quickly to equilibrium without oscillations,
3. Overdamped harmonic oscillator for $\zeta_{\text{tot}} > 1$, i.e., where the radial oscillation of MBs returns to equilibrium in an exponential manner when the incidental excitation is removed.

As shown in Fig.13d, $\zeta_{\text{tot}}$ of lipid-shelled UCA is well below unity, thus the radial oscillation of lipid-shelled MBs are classified as an underdamped harmonic oscillator. For this reason, they behaved most echogenically and exhibited an active oscillatory behavior under ultrasound exposures. Instead, $\zeta_{\text{tot}}$ value is just above the unity for both plain PVA-shelled MBs and MBs-phys, which identified them as overdamped harmonic oscillator systems. The $\zeta_{\text{tot}}$ value for MBs-chem is just below the unity, thus the radial oscillation of MB to post-excitation is expected as in phospholipid-shelled MBs, but high damping certainly reduces their magnitudes.

6.4. Cross-linked Network vs. Stiffness

The results from both Paper-II and III indicate that the nanoparticles integrated into MBs can alter MBs acoustic response and depend on the method of nanoparticles integration. The PVA cross-linked network reaction with SPIONs introduction can explain the change of acoustic response from MBs-chem to MBs-phys. The cross-linked polymer network stiffness is directly proportional to filaments length and to the cross-linker concentration. The attached SPIONs in MBs-chem might act as an additional preload to cross-link network upon insonation as in Fig. 14a, as a reason the stiffness of the shell could be decreased. This picture matches our previous observations wherein the loss of the cross-linked structure’s crystalline is observed by differential scanning calorimetry, DSC, measurements.
The inclusion of SPIONs inside the PVA shell in MB-phys acts as an additional cross-linker to the cross-linked PVA network as shown in Fig. 15b. The isotropically cross-linked network acts as a composite or bundle cross-linked network that increases the modulus of rigidity and exhibit stiffness behavior under ultrasound excitation.

6.5. Correlation with Other Techniques

6.5.1. Thermo gravimetric Analysis (TGA)/ Differential Thermal Analysis (DTA)

PVA network around the gas is formed based on the cross-linking and crystallization technique. The degree of crystallinity is estimated by using the TGA/DTA combined analysis. In Paper-I we have reported the degree of crystallinity of MBs-chem and MBs-phys with reference to plain PVA-shelled MBs using TGA/DTA analysis. The outcome of TGA/DTA study revealed that the anchored SPIONs on the PVA shell surface (MBs-chem) destroyed the PVA shell crystalline structure, whereas, PVA crystalline structure was identified even after introduction of SPIONs in PVA shell (MBs-phys). This finding is in good agreement with both acoustic and mechanical properties of MBs-chem and MBs-phys.

6.5.2. MR imaging

The MBs-chem and MBs-phys were investigated both in vitro and vivo in magnetic resonance imaging at the recommended concentrations form ultrasound measurements. The investigation on MBs-chem and MBs-phys in MRI has exhibited that, the hard-shelled MBs-phys have higher net magnetization approximately 43% higher than MBs-chem. Surprisingly the number of SPOIN particles were attached in the shell (~ 15 %) is approximately two folds below the SPOINs attached on the MBs-chem (~ 29 %). We theorized that the clusters in MBs-phys (as shown in Fig.6c) have synergic effect to generate stronger SPIN-SPIN relaxation time than sum of individual anchored SPIO nanoparticles in MBs-chem. The magnetization results, i.e., M-H loops of MBs-chem and MBs-phys are briefly discussed in Paper-I. To overall, the echogenicity and the magnetic enhancement can be appreciated in one micro particle by integrating the superparamagnetic nanoparticles and polymer-shelled micro gas bubbles.
In this thesis, we characterized both acoustic and mechanical properties of micro-sized gas bubbles encapsulated by PVA shell loaded with SPIO magnetic nanoparticles.

The introduction of the SPIONs to the PVA-shelled MBs was done in two ways: (1) SPIONs were attached to the chemically modified surface of the PVA-shell (MBs-chem) (2) SPIONs were introduced during the PVA shell formation around the gas bubble where the SPIONs were entrapped in the PVA shell due to physical interaction (MBs-phys). The outcome of the acoustic measurements at different concentrations of MBs-chem and MBs-phys shows that at least $8.8 \times 10^5$ MBs/ml of MBs-chem and $2 \times 10^6$ MBs/ml of MBs-phys (i.e. almost twice fold higher than the MBs-chem concentration) should be injected for ultrasound imaging applications in order to have a better visualization with less artifacts. The study was further continued to investigate their shell mechanical properties, i.e. viscous (loose) moduli and elastic (storage) moduli parameters which were further used to estimate the physical properties of the bubbles, i.e., the resonance frequency and the damping factors.

The viscoelastic parameters were estimated by fitting the acoustic experimental results with the modified Church model\(^{48}\). The model predicted lower viscoelastic moduli values, and thereby lower resonance frequency and total damping values were obtained for the MBs-chem. The storage moduli from bubble suspensions at high frequency (MHz) showed a good agreement with the elastic modulus values were estimated by atomic force microscope (AFM) technique on single bubble at low frequency (kHz). This result concludes that, attached SPIONs on PVA shell soften the MBs-chem under ultrasound exposures, while, embedded SPIONs in PVA shell hardening the MBs-phys under insonation. The MBs-chem had a resonance frequency of within the diagnostic ultrasound frequency range (1MHz-15MHz) and relatively lower damping compared to MBs-phys. Thus, the MBs-chem can potentially be exploited as a conventional ultrasound contrast agent with the combined functionality of MRI detection. In contrast, MBs-phys had a resonance frequency far away from the diagnostic ultrasound range and the damping ratio value is identified as overdamped harmonic oscillator, thus, further modification of clinically available contrast pulse sequences are required to visualize them. Moreover, the surface of MBs-phys is free for any modification, where, the drug (or) gene moieties can be attached to become a drug carrier and can be modified with chelating agents for radioisotopes to even support SPECT imaging in addition to US and MR imaging.
8. Future Work

From our experimental results and the feedback from the imaging group, both modified and non-modified PVA-shelled MBs are the best echo enhancers in the linear regime. To explore the nonlinear signature oscillations of these MBs, obviously, novel signal processing techniques or special pulse sequences are required. As we mentioned in Chapter 3, first the nonlinear oscillations of plain PVA-shelled MBs will be implemented by using the modified Church model which was mentioned in Eq. (9). This will give the freedom to investigate their oscillations behavior to the different nonlinear pulse sequences and different windowing techniques. Currently we are developing the Matlab™ code for modified Church’s model to examine the roles of different driving pulse shapes in the detection of nonlinear harmonic oscillation of PVA-shelled MBs. This work is partially extension to the ICSV19 conference paper, which was mentioned in “List of Publications” section. Once the code is implemented the work will be utilized to test the different pulse sequences for example, amplitude modulation, frequency modulations, Chirp, and heterodyne techniques.

Recently, the strain soft behavior was identified in phospholipid-shelled MBs. Strain-softening behavior means material becomes softer as increases the stress. Qin and Ferrera had included additional terms to the nonlinear equation of motion to simulate the strain-softening behavior of lipid-shelled MBs. Till now, such experiments were not performed and no evidences found in polymer-shelled MBs. Thus we will study the pressure dependant attenuation profile in PVA-shelled MBs.

In this thesis we have assessed the acoustic properties of magnetized microbubbles without any external magnetic field presence. In future acoustic characterization will be performed in the presence of an external magnetic field. The estimation of viscoelastic properties of magnetic MBs will be correlated with magnetic resin study on train damping materials.
9. Summaries of Papers

In the Paper-I, both preparation and quantification of two types of novel magnetic microbubbles (MBs) were discussed. These magnetic MBs were proposed to be multimodal contrast agents for supporting the US and MR diagnostic imaging. These were prepared by combining the iron oxide, Fe$_3$O$_4$, nanoparticles (SPIONs) on the surface of PVA shell (MBs-chem) and inside the PVA shell (MBs-phys). The outcome of this study described that modified MBs does not change their acoustic properties compared to non-modified PVA-shelled MBs and they have enough magnetic susceptibility that can useful as MR contrast agents.

In the Paper-II, mechanical properties of plain PVA-shelled MBs, MBs-chem, and MBs-phys were investigated at high and low frequency regimes. The quasi-static force measurements at low frequency (kHz) were performed using atomic force microscopy (AFM), whereas, modulus of rigidity was investigated through acoustic characterization at high frequency. The experimental results at both low and high frequencies conclude that the encapsulated MBs-chem shell has a soft behavior than both MB-phys and MB-pH5-RT.

In Paper-III, theoretically calculated attenuation coefficient and phase velocity spectra were compared with measurements of MBs-chem and MBs-phys. The viscoelastic properties of both MB types were identified in the comparison study, which was further utilized to estimate their resonance frequency, damping ratio. The model predicted a resonance frequency of approximately 13 MHz and a damping of approximately 0.9 for MBs-chem, whereas 28 MHz and 1.2 for MBs-phys. The damping profile of MBs-chem was identified as underdamped harmonic oscillators, whereas MBs-phys identified as an overdamped harmonic oscillators. Having the resonance frequency within clinical medical US limit of 15 MHz and lower damping MBs-chem can be utilized as a conventional UCA. On the contrary, MB-phys can preferably be applied only in relatively stationary internal organs after modification of clinically available contrast pulse sequences for example by using secondary radiation forces.


