Numerical Study of the Dynamics of Microbubble Clusters in an Ultrasonic Field

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Abstract

In recent years, ultrasound and microbubble contrast agents have been shown to have potential to become very powerful diagnostic tools in medical applications, particularly in the areas of drug/gene delivery and site-targeted imaging. While a fairly substantial amount of experimental research has been conducted, their behaviour is yet to be fully understood. This is paramount in increasing the reliability and effectiveness of the microbubbles while ensuring patient safety.

The aim of this thesis is to investigate the effects of boundary, bubble size, bubble arrangement and cluster size on the dynamical behaviour of the cluster and to see if chaotic or bifurcation characteristics could be helpful in diagnostics.

Small encapsulated microbubbles subjected to appropriate variations in ultrasound pressure amplitude were considered to mimic practical applications. It was found that under these conditions, the system becomes numerically stiff. Therefore a code based on the numerical difference formula was written to solve the normalised Keller-Miksis-Parlitz model equation to trace the bubble dynamics. The resulting oscillations were analysed in terms of bifurcation diagrams, Poincaré plots, Floquet analysis and Fourier spectra.

It was found that as monodispersed microbubbles were clustered closer together, the oscillation amplitude for a given applied ultrasound power was reduced, and for inter-bubble spacing smaller than about ten bubble radii nonlinear subharmonics and ultraharmonics were eliminated. For clustered microbubbles, as for isolated microbubbles, an increase in the applied acoustic power caused bifurcations and transition to chaos. The bifurcations preceding chaotic behaviour were identified by Floquet analysis and confirmed

to be of the period-doubling type. As the number of microbubbles in a cluster increased, regularisation occurred at lower ultrasound power and more windows of order appeared.

The model was also modified to account for the presence of the wall. It was shown that for a monodispersed cluster of microbubbles near a wall, the route of chaos was altered. Microbubbles that were close to a boundary exhibited an intermittent route to chaos while microbubbles that were further away from the boundary exhibited period doubling route to chaos. In addition, strong boundary-microbubble coupling effects caused the natural frequency of the bubble system to decrease and produced larger shifts in the chaotic threshold.

By varying the arrangement, size of the microbubbles and the size of the bubble cluster, it was found that larger-sized microbubbles exhibited profound influence on the inter bubble interaction by either changing the resulting route to chaos in a cluster of mainly smaller microbubbles, or stabilising the cluster dynamics by suppressing chaotic oscillations in a cluster of larger microbubbles. It was also found that the cluster will exhibit the same type of attractor in spite of the different microbubble sizes within the cluster and the variation of the inter bubble distance.

It is anticipated that, when validated by experiment, the results obtained in this thesis have the potential to serve as a guide for the development of microbubbles in biomedical applications. This conceptual study, in conjunction with other approaches discussed in the literature, may serve the purpose of improving the diagnostic sensitivity of ultrasound targeting of medical agents and enabling more control and accuracy in medical therapeutic procedures.

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Chapter 1

Introduction

For many medical diagnoses, ultrasound represents a fast, cheap and safe method of scanning. However, when juxtaposed with other techniques, particularly magnetic resonance imaging, the image quality is often inferior. Thus, in order to enhance ultrasound backscatter, microbubble contrast agents have been employed. Upon application of acoustic excitation, microbubbles oscillate nonlinearly thus producing a unique sonogram with increased contrast which improves detection and characterization of tumour, image blood perfusion and other applications as well.

In addition to contrast enhancements, their potentials to be employed in diagnostic, therapeutic and theranostic applications are currently under investigation. By taking advantage of the sensitivity and specificity of ultrasound to detect microbubbles, molecular imaging can be realised by attaching antibodies or other targeting species to microbubble surfaces, which selectively binds to the targeted site. Besides diagnostic, microbubbles have been shown in preclinical studies to induce thrombolysis and to be promising agents for drug and gene delivery.

It is important to note that the operational transition of microbubbles from contrast agents to therapeutic, diagnostic and theranostic agents require departure from their normal behaviour that is similar to red blood cells in circulation. Consequently, the development of microbubbles in these applications demand understanding of the interaction between acoustic excitation and the bubble response due to different parameters such as bubble size and ultrasound pressure and frequency. Furthermore, it is important to be able to predict the bubble response in order to exploit specific properties to develop, improve and optimise the effectiveness and the efficacy of the techniques.

More importantly, is the ability to accurately characterise the bubble oscillation in order to further investigate and understand the possible risk and safety concerns that may arise. Despite considerable studies however, the behaviour of microbubbles are not fully understood. Many challenges remain to be addressed before microbubbles can be employed as therapy and therapeutic agents.

One of the challenges that has been considered in this thesis is the ability to identify the formation of clusters. This phenomenon may occur when it is grouped by ultrasound and upon microbubbles reaching the target lesions in diagnostic imaging and therapy. Consequently, it is important to clarify the effects of mutual interactions among bubbles. In many theoretical modelling of microbubbles, however, it has been assumed that the bubbles are sufficiently apart such that the effects they have on each other are negligible (see, for example,Carroll, Calvisi & Lauderbaugh (2013); Church (1995); Morgan et al. (2000)). While there have been several studies investigating the effects of coupling due to neighbouring bubbles, shell encapsulation however, was not considered (see, Chong et al. (2010); Ooi & Manasseh (2004). Experimental observations have shown to be a significant contributor to the stiffness of the bubble. Furthermore, it has been observed that the microbubble size may also affect the dynamical response of a group of microbubbles. This gap in the body of knowledge has led to the first research question, "What are the effects of inter bubble coupling and bubble sizes on the dynamical behaviour of the encapsulated microbubble?"

The second problem that has been considered in this thesis is the ability to distinguish between free-flowing microbubbles to a nearby boundary, for example the wall of a blood vessel or targeted tissue. It is important to investigate the complex interaction between oscillating microbubbles and the presence of a boundary, which may modify the incident and scattered acoustic field. This phenomenon is crucial for microbubbles as vehicle for drug/delivery to ensure that the therapeutic contents of the microbubble are not deposited during free-flow. It has been generally assumed that microbubbles oscillate in infinite medium. A single bubble oscillating near a boundary wall was recently studied by Suslov, Ooi & Manasseh (2012), however, there still remains a need to investigate the effects of the boundary to the dynamical response of a cluster of microbubbles. This gap in the body of knowledge has led to the second research question, "What are the effects of a boundary and inter bubble coupling on the dynamical behaviour of a cluster of encapsulated microbubbles?"

The third problem that has been considered in this thesis is in relation to the non-uniform sized microbubbles. Monodispersed microbubbles are highly desirable to improve consistency of acoustic response. However, the production of uniform sized microbubbles is still in the development stage. Consequently, most clinical studies utilise commercially available microbubbles, which are non uniform in size, to develop therapeutic, diagnostic and theranostic applications for microbubbles. In order to facilitate the investigation and development of medical applications for microbubbles, it is essential to better understand the microbubble-ultrasound interaction of a cluster of polydispersed microbubbles. Few studies have discussed the effects of an equidistant and uniform sized cluster to the oscillation response (see Chong et al. (2010); Ooi & Manasseh (2004)), but to date, the effects of different microbubble sizes in a cluster as well as their spatial arrangement have yet to be explored. This gap in the body of knowledge has lead to the third research question, "What are the effects of varying spatial arrangement of a non-uniform sized encapsulated microbubbles on the dynamical behaviour? and how does the effect of coupling influence the behaviour of a large cluster of non-uniform size?"

This thesis is divided into seven chapters and one Appendix. A preview, highlighting the novel contributions made in each chapter follows. A literature review tracing the development of microbubbles from a diagnostic tool to the possibility of a therapeutic and theranostic modality is presented in Chapter 2. In terms of microbubble behaviour, this development implies a departure from free circulation. Therefore, the demand to have a better understanding of the behaviour of microbubbles in ultrasonic fields and to characterise the oscillation of the microbubbles via modelling are emphasised. The remainder of Chapter 2 is dedicated to characterisation of the dynamical and chaotic phenomena of the nonlinear dynamical response of the microbubble system.

In order to analyse the microbubble oscillation upon acoustic excitation, Chapter 3 derives an existing model which is capable in solving for high amplitude oscillations for varying parameters. The model is extended to include a coupling term to allow the consideration for a larger microbubble cluster. The model is then non dimensionalised to recover the properties of the system. The numerical methods which form the basis of the written code is discussed prior to validating the code against other simulations and experimental observations with good agreement.

To answer the first research question, Chapter 4 applies the model developed in Chapter 3 to simulate the dynamical response of different sized microbubbles in varying cluster sizes. The effects of coupling is further investigated by studying different inter bubble spacing. Examinations of the resulting simulations are implemented in the form of bifurcation diagrams where bifurcations preceding chaotic oscillations were identified by the Floquet analysis. This work has been published in the Journal of the Acoustical Society of America, attached in Appendix A.

The second research question is investigated in Chapter 5. The Keller-Miksis-Parlitz equation used in Chapter 4 is modified to include the presence of a boundary. This modification has been published in a conference paper and attached in Appendix 2 and compared with experimental observations with good agreement. The effects of the boundary proximity on microbubble behaviour are investigated by comparing a bubble in infinite medium with a bubble at varying bubble-boundary distances. Examination is extended by adding bubbles into the cluster. These simulations are presented using bifurcation diagrams and oscillation stability diagrams that for identification of the route to chaos and visualization of the different oscillation modes. Furthermore, the effect of natural frequency the chaotic threshold are investigated.

The third research question is explored in Chapter 6. The model derived in Chapter 3 is used to simulate the effects of varying inter bubble distances in a cluster consisting different microbubble sizes. The study is then extended to include a large cluster of microbubbles consisting different sizes similar to commercially available microbubbles that are non-uniform in size.

The findings of the investigations concluded in this thesis are summarised in Chapter 7. Areas requiring further investigation and items in which the results of this thesis could be applied are suggested for future work. Appendix A contains published work from the results obtained in Chapter 4. Work from Chapter 5 has partially contributed to the published paper in Appendix B.

Chapter 2

Literature Review

2.1 Introduction

This chapter traces the development of microbubbles as a contrast agent for diagnostic ultrasound imaging, in Section 2.2 to the potential uses in therapeutic and theranostic applications, in Section 2.3. In terms of microbubble behaviour, this development implies a departure from free circulation which is discussed more thoroughly in Section 2.4. Consequently, to develop, improve and optimise the effectiveness and the efficacy of the techniques there is a demand to have better understanding of the behaviour of microbubbles in ultrasonic fields. In order to characterise the dynamical response of the microbubbles, theoretical modelling of the radial oscillation is discussed in Section 2.5. The remainder of this chapter, Section 2.6 is dedicated to nonlinear characterisation of the dynamical response by applying methods from theory of chaos.

2.2 Application of Microbubbles in Ultrasound Imaging

Microbubbles have been employed in medical diagnosis since 1970s (Hoff, 2001). Medical diagnostic imaging relies on echoes, scatter and reflection of sound. These echoes form compositions of the images of the body interior which is then interpreted in the ultrasound scanner to achieve diagnostic imaging. Ultrasound scatter of blood is weaker from other tissue. The objective of introducing ultrasound contrast agents (UCSA) or microbubbles, is to increase the scatter of sound from the blood, which has dramatically improved the image contrast in ultrasound radiography (Stride & Saffari, 2003). Incorporation of microbubbles in evaluations of cardiac conditions for example, has

facilitated visualisations of blood flow and accurate assessment of myocardial perfusion (Bloch, Dayton & Ferrara, 2004; Wu, Pepe & Dewitt, 2003). Detection of blood flow in abdominal and peripheral vascular structures as well as in breast, prostate and liver has been facilitated with ultrasound contrast agents (Bloch, Dayton & Ferrara, 2004).

Ultrasound contrast agents for imaging were first proposed in 1968 (Hoff, 2001) to enhance blood-pool image (hence its name *contrast agents*) and enable assessment of tissue blood flow at the micro vascular level (Lindner, 2004). Early solutions relied on hand agitating solution and sonicated solution (Gramiak & Shah, 1968) hence resulting in a wide bubble size range, some sufficiently large to cause entrapment in arterioles and capillaries (Lindner, 2004). A more systematic search for better ultrasound agents has been carried out since 1980 which include developments in stabilising the shell and gas content of bubbles. The research intensified ten years later resulting in the release of several shell encapsulated bubbles which are more stable in clinical trials on the market including Echovist® from Schering AG Co. in Germany in the year 1991 and two years later, Albunex® from Molecular Biosystems Inc. in San Diego (Hoff, 2001). Other shell encapsulated bubbles developed are shown in Table 2.1. To further increase intra vascular stability of the microbubbles, perfluorocarbon gas core are used (Lentacker, Smedt & Sanders, 2009). These developments prevent gas diffusion out of the microbubbles allowing for better stability and hence longer circulation time in the blood. In recent years, there has been a growing interest in using microbubbles as non invasive drug and gene delivery vehicles hence a need to understand the dynamics of microbubbles. Interestingly, despite the substantial experimental and theoretical research that has been conducted over the years, the characteristics and behaviour of microbubbles when subjected to ultrasound is not yet fully understood.

2.3 Potential of Microbubbles in Medical Applications

Ultrasound offers advantages over other non invasive imaging modalities, e.g. X-ray, computed tomography (CT) and magnetic resonance imaging (MRI), since it can be used for real-time imaging, it is relatively inexpensive and portable, hence allowing it to be utilised in the ambulance, battlefield, bedside or private practice office. The introduction of microbubbles have greatly increased the usefulness of ultrasound. For example, since microbubbles behave like red blood cells in the microcirculation and are

able to be destroyed with a change of acoustic input, the blood volume and velocity can be quantified hence allowing the unique opportunity to study various pathological conditions *in vivo* (Lindner, 2004). With further refinement, the use of microbubbles in conjunction with ultrasound has great potential in the development of targeted imaging to characterise diseases at a molecular level *in vivo*. In addition, the bioeffects produced by microbubbles provide a unique opportunity to extend the use of microbubbles beyond diagnostic imaging to include therapeutic applications using ultrasound mediated methods (Lindner, 2004).

Application of microbubbles and ultrasound in non invasive targeted imaging

Non invasive imaging has been successful in defining anatomical and physiological changes in a wide array of disease. Experimental formulation of targeted microbubbles which are designed to attach at disease-specific ligands have shown potential to expand its applications in non invasive imaging into the realm of non invasive targeted imaging which has been heralded as the solution to many problems in current medicine (Blasberg, 2003). A study by Lindner et al. (2001), among many others, have reported a successful use of microbubbles designed to target inflamed tissue in an animal model. The general underlying idea of employing microbubbles as targeting agents is to target molecular markers of a particular disease hence allowing non invasive assessment of inflammation, angiogenesis and early tumour formation (Kaufmann & Lindner, 2007; Lindner, 2004).

There are two key concepts in designing microbubbles as targeting agents. The first is to ensure retention of microbubbles at the location of the specific targeted pathological process. The second is to ensure non invasive detection of the attached microbubbles after the freely circulating microbubbles have cleared which are dependent on the stability of the microbubbles (Kaufmann & Lindner, 2007; Klibanov, 2005). In order to ensure microbubble retention, there are two general strategies that may be employed. The first strategy involves modification of the microbubble shell. Studies by Lindner et al. (2000a,b,c) has been shown that lipid and albumin microbubbles adhere to activated leukocytes hence are retained within the microcirculation of inflamed tissue. Subsequently, these regions can be detected when subjected to ultrasound (Lindner

et al., 2000b,c). The second strategy consists of attaching disease specific ligands, such as monoclonal antibodies, glycoproteins, carbohydrates, peptides or peptidomimetics to the microbubble shell.

Systematic development of microbubbles in the area of targeted imaging will facilitate non invasive detection of pathopysiological diseases, gene expression and delineation of molecular mechanism of diseases (Bloch, Dayton & Ferrara, 2004) which are important as means for early diagnosis for patients as well as research and development of novel therapies in patients and animal models. An example of the latter is the investigation of drug localisation. Current methods of observing the process involve long term monitoring of traditional critical end points (Christiansen & Lindner, 2005). In order to assess the response to local drug delivery it is imperative that instant information at molecular and cellular level are obtained which may be made possible with further refinement of application of microbubbles in targeted imaging.

Application of microbubbles and ultrasound as a therapeutic modality

Microbubbles and ultrasound are rapidly emerging as a promising tool for non invasive therapy and drug delivery. In the context of non invasive therapy, the operational transition of using microbubbles and ultrasound from an imaging modality to a therapeutic modality is an increase of average acoustic intensity (Coussios & Roy, 2008) for which the end result is a beam of high-intensity focused ultrasound (HIFU). The operation is dependent on inertial cavitation and aimed at producing bioeffects such as tissue destruction and vascular occlusion (Coussios & Roy, 2008). There are two mechanisms to produce these bioeffects. The first mechanism is therapy, purely via ultrasound i.e. in the absence of microbubbles. By exposing the tissue to high intensity ultrasound, microbubbles are formed *in vivo* by absorbing gas out of the solution in the surrounding tissue (Stride, 2009). The size of these bubbles grow via rectified diffusion (Yang, Roy & Holt, 2004) and subsequently undergo inertial cavitation producing shock waves and high velocity jets (Leighton, 1994), free radicals (Edmonds & Sancier, 1983) and high local temperatures (Apfel, 1982) which may cause unwanted tissue damage beyond the targeted region (Coussios & Roy, 2008; Kennedy, 2005). The second mechanism requires introduction of microbubbles which act as a focusing agent (Marmottant & Hilgenfeldt, 2003). Since microbubbles are contained in blood, the

oscillations are isolated to the blood vessel wall and provide efficient means for reflecting acoustic energy and/or localising mechanical effects for rapid and highly localised heat deposition (Unger et al., 2002) which significantly reduces the acoustic intensity threshold for inertial cavitation to occur (Hynynen et al., 2006b) making it a promising truly non invasive solution for deep-seated solid tumours.

In the context of drug and gene delivery, the efficacy of the drug action is significantly improved in the disease region and side effects and toxicity in the body are reduced (Coussios & Roy, 2008; Stride, 2009). Microbubbles and ultrasound are attractive for drug localisation since it is possible to design the microbubbles to selectively target delivery area. In addition, imaging of the delivery may be implemented in real-time *in vivo*. Unlike non invasive therapy, drug delivery depends on both inertial and stable cavitation of the bubble for which the latter allows shape oscillations that enhance mass transport over inaccessible interfaces (Coussios & Roy, 2008). There are four general strategies that have been suggested for drug/gene localisation with microbubbles. The first strategy is to enclose the exogenous material within the shell to ensure the protection of the drug and lower the chance of premature release prior to ultrasound application (Lentacker, Smedt & Sanders, 2009; Unger et al., 2001). The second and third strategy involves incorporating material on to the shell (Teupe et al., 2002) or attaching on to the shell surface (Hernot & Klibanov, 2008). The fourth strategy involves no chemical or physical binding between drug/gene material with the microbubble, in which the microbubbles and the drug/gene-filled particles are mixed in a suspension (Rapoport, Gao & Kennedy, 2007). Upon applying ultrasound, the microbubbles disrupt the micelles to release the material. There has also been considerable evidence that microbubble oscillation increases the permeability of cell membranes and endothelium (Ferrara, Pollard & M.Borden, 2007). Taking advantage of this property, the microbubbles will stimulate the uptake of the released material in nearby cells (Kheirolomoom et al., 2007). An exciting application includes the microbubble-based drug/gene delivery across the blood brain barrier (Ferrara, Pollard & M.Borden, 2007; Hynynen et al., 2001, 2006b; McDannold, Vykhodtseva & Hynynen, 2006). Hynynen et al. (2001) showed that it is possible to temporarily induce blood brain barrier destruction, for which only few alternative methods can change it's permeability (Hynynen et al., 2006a). This phenomenon allows microbubble payload to be delivered to the target area while the rest of the brain is still protected by the BBB.

2.4 Importance of Understanding Behaviour of Microbubbles Subjected to Ultrasound

It is important to appreciate that, unlike other medical imaging contrast agents, microbubbles have a complex nonlinear interaction with ultrasound. Therefore, despite the substantial amount of theoretical and experimental research that has been conducted, the dynamics of microbubbles subjected to ultrasound is yet to be fully understood. While use of materials with high atomic numbers are adopted for X-ray imaging applications, and likewise, paramagnetic materials are employed in MRI, compressibility and density of microbubbles are not the only factors that influence microbubble cavitation. Upon application of ultrasound, the microbubbles may oscillate, coalesce, jet or fragment, hence emitting pulses and variable spectral energy characteristics. Moreover the operational transition of microbubbles from contrast agents to diagnostic and therapeutic agents require departure of their normal behaviour similar to red blood cells in circulation (Jayaweera et al., 1994). Consequently, it is of paramount importance to gain understanding of the microbubble interaction with ultrasound in order to exploit specific properties to develop, improve and optimise the effectiveness and sensitivity of application.

The understanding of the complex nonlinear microbubble behaviour is important for the development in clinical applications. For example, a physical behaviour in acoustic cavitation (see Neppiras, 1984) is inertial cavitation for which microbubbles experience violent collapse. When the microbubbles are close to a wall, micro jets which are associated with puncturing the cell membrane (Prentice et al., 2005), may occur. While this is desirable for drug/gene delivery, it may also cause tissue damage if not properly controlled. Drug/gene delivery also undergo stable cavitation upon entering the blood stream until targeted site is reached. It is therefore important to identify the parameters that will result in such response and to avoid premature drug release due to inertial cavitation, for example (Dijkmans et al., 2004). It is thus important to understand and identify the ultrasound microbubble interaction such that the desirable effects are achieved and bubble-mediated applications are optimised and controlled. There has also been extensive research to improve microbubbles as contrast agents to develop better techniques for imaging (Shi & Forsberg, 2000) such as second harmonic (Morgan, Averkiou & Ferrara, 1998) and subharmonic (Shankar, Krishna & Newhouse,

1998) imaging which takes advantage of the frequencies emitted by the microbubbles compared to surrounding tissue. For example, non-destructible subharmonic imaging may be applied to estimate blood perfusion (Chomas et al., 2002). Microbubbles have the ability to redraw the boundaries of biomedical acoustics. The challenge however lies in the ability to predict bubble oscillation in an acoustic field which may be achieved with theoretical modelling.

2.5 Characterisation of Microbubble Behaviour: Modelling

The study of dynamical behaviour of microbubbles applied in the medical field is built on decades of theoretical and experimental models and research of underwater acoustics. The application of medical ultrasound contrast agents, however, introduces new phenomena not previously studied. For example, encapsulating the gas bubble with a shell, consequently alters acoustic properties such as scatter and transmission of sound (de Jong, Cornet & Lance, 1994) compared to free gas bubbles. In addition, microbubbles oscillate in blood, and unlike water, blood is a highly inhomogeneous liquid where particles constitute 40% of its volume (Hoff, 2001). There are also differences in terms of scale, where in the medical field, the parameters consist of a higher frequency, shorter distances and smaller sized bubbles and time scales thus introducing different absorption mechanisms that alter the sound energy transfer to heat.

Overview of Mathematical Models

The ability to model the oscillation of microbubbles driven by ultrasound is a determinant factor for diagnostic and therapeutic applications. The equations of motion that govern the bubble oscillation are generally based on air bubble models which are nonlinear in nature. The first theoretical model for the collapse of a spherical cavity was presented by Lord Rayleigh (Rayleigh, 1917) which formed the foundation of modern theoretical bubble dynamics. Oscillations of large bubbles were assumed to remain spherical throughout oscillation in surrounding liquid which were compressible and inviscid, and that the surface tension was negligible. For a period thereafter, theoretical dynamics centred around hydrodynamically generated cavities until Blake (1949) published a study of acoustically generated cavities.