Ultrasound in Med. & Biol., Vol. 40, No. 10, pp. 2476–2487, 2014

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2014.05.018

● *Original Contribution*

ASSESSMENT OF THE VISCOELASTIC AND OSCILLATION PROPERTIES OF A NANO-ENGINEERED MULTIMODALITY CONTRAST AGENT

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(*Received* 25 *September* 2013; *revised* 20 *May* 2014; *in final form* 26 *May* 2014)

Abstract—Combinations of microbubbles (MBs) and superparamagnetic iron oxide nanoparticles (SPIONs) are used to fabricate dual contrast agents for ultrasound and MRI. This study examines the viscoelastic and oscillation characteristics of two MB types that are manufactured with SPIONs and either anchored chemically on the surface (MBs-chem) or physically embedded (MBs-phys) into a polymer shell. A linearized Church model was employed to simultaneously fit attenuation coefficients and phase velocity spectra that were acquired experimentally. The model predicted lower viscoelastic modulus values, undamped resonance frequencies and total damping ratios for MBs-chem. MBs-chem had a resonance frequency of approximately 13 MHz and a damping ratio of approximately 0.9; thus, MBs-chem can potentially be used as a conventional ultrasound contrast agent with the combined functionality of MRI detection. In contrast, MBs-phys had a resonance frequency and damping of 28 MHz and 1.2, respectively, and requires further modification of clinically available contrast pulse sequences to be visualized. (E-mail: dmitryg@kth.se) © 2014 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound contrast agent, Magnetic microbubbles, Fe_3O_4 nanoparticles, Harmonic oscillation, Viscoelastic properties.**

INTRODUCTION

Over the past two decades, highly echogenic microsized gas bubbles (MBs) confined by protein/lipid or polymer material have served as ultrasound contrast agents (UCAs) (de Jong et al. 1992). However, well-characterized thin protein/lipid-shelled MBs are affected by poor mechanical stability (Sirsi and Borden 2009). A biocompatible polymer shell made of polyvinyl alcohol (PVA) has been proposed (Cavalieri et al. 2005) to overcome this limitation. Because these MBs have thicker shells, they could, in principle, allow for the incorporation of larger payloads of drugs, ligands or nanoparticles (Cerroni et al. 2011).

Although ultrasound (US) imaging is free from ionizing radiation, inexpensive, bedside and non-invasive, the diagnostic accuracy of the detection of lesions is suboptimal compared with the magnetic resonance imaging (MRI) and computed tomography (CT) imaging modalities (Cosgrove 2006). A recent approach involves the integration of several diagnostic imaging modalities (*e.g.*, positron emission tomography [PET] combined with MRI [PET-MRI] or CT [PET-CT], or single-photon emission computed tomography [SPECT] combined with CT [SPECT-CT]) to allow for the acquisition of functional information without compromising image resolution, thereby increasing diagnostic specificity and sensitivity. Combinations of ultrasound with other techniques are not currently widely available. For example, MRI-US integrated technology has been proposed to improve prostate cancer diagnosis (Marks et al. 2013). The simultaneous use of combinations of different contrast agents further supports multimodality imaging.

Functionalized superparamagnetic iron oxide (Fe_3O_4) nanoparticles (SPIONs) are well-established MRI contrast agents (Salaklang et al. 2008). Marchal et al. (1989) reported that SPIONs are taken up by Kupffer cells in the liver through the macrophage system. This phenomenon has been used to enhance the detection of liver metastasis with ultrasound imaging and magneto-motive excitation (Oh et al. 2006). Recently,

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the combination of superparamagnetic nanoparticles and MBs has been proposed as a dual-modality contrast agent that would simultaneously support both US and MRI imaging modalities (Yang et al. 2008). Presently, this dual-modality contrast agent is manufactured by introducing the magnetic nanoparticles into MBs by physically embedding them into the solid encapsulated shell, chemically/electrostatically coupling them to the surface of shell or engrafting them in a special oil layer inside the shell (Cai et al. 2012). The backscattered signal is enhanced during ultrasound imaging, and the relaxation rate (T2) is increased in MRI by all three prototype dual-modality magnetic MBs compared with non-magnetic MBs (Brismar et al. 2012; Liu et al. 2011; Park et al. 2010; Yang et al. 2008). In addition to their applications for dual image enhancement, magnetic MBs may have potential applications in guided MR manometry. MR manometry is a MRI-based technique for the detection of intravascular pressure that estimates T2 changes relative to MB volume changes (Dharmakumar et al. 2005).

This article discusses the relationships between the viscoelastic, physical and structural properties of magnetically cross-linked poly PVA-shelled MBs by comparing acoustic experimental evidence and a linearized version of the non-linear equation of MBs. Most existing theoretical models that use non-linear equations of motion were developed and evaluated for thin-shelled UCAs with shell thickness-to-radius ratios less than 5% (Doinikov and Bouakaz 2011; Hoff 2001). The non-linear equation of motion derived by Church (1995) is free from these assumptions and accounts for finite shell thickness. As the Church model imposes no restriction on shell thickness, it is remarkably well suited for the characterization of thick-shelled MBs (Doinikov and Bouakaz 2011). Grishenkov et al. (2009) adapted the Church model and introduced a frequency-dependent complex dynamic modulus in the place of the frequency-independent shear modulus and shear viscosity parameters to characterize thick PVA-shelled MBs. In their model, the frequency-dependent complex viscoelastic parameters mainly account for the effects of the dynamic behavior of the relaxation mechanism of the cross-linked PVA network. Recently, magnetic thin phospholipid-shelled MBs were acoustically characterized with the modified Church model via the inclusion of an additional damping term that accounts for the interaction of the internal nanoparticle suspension layer with the outer phospholipid shell (Mulvana et al. 2012).

Notably, the viscoelastic parameters of the shell in the non-linear equation of motion are generally unknown. These parameters are obtained by fitting the attenuation coefficient, $\alpha(\omega)$, and phase velocity, $c(\omega)$, measurements to the linearized theoretical model. The identification of the viscoelastic characteristics of the shell becomes a tedious and ambiguous process because the number of unknown shell terms included in the non-linear equation of motion increases. In previous studies, the residual error/mean square error (MSE) has been used to assess the goodness of fit (Frinking and de Jong 1998; Hoff et al. 2000). The MSE does not account for the structural similarities between the curves (Wang and Bovik 2009), whereas the correlation coefficient (R) estimates the structural similarities between the curves and disregards the errors. In each fitting process, several combinations of R values and MSEs are obtained. R values vary between 0 and 1, and an R value of 1 indicates a strong linear relationship between data sets, whereas MSE values vary from 0 to ∞, and an MSE value of 0 represents the ideal fit between data sets. Therefore, the combination of maximum R and minimum MSE value is required to identify the best possible agreement between experiments and theory. However, it is challenging to identify the maximum R and minimum MSE values from direct comparison of a large range of R and MSE data sets. Incompatibility and incomparability scale comparison problems, especially in the fields of pattern reorganization, artificial intelligence and neural networks, have been solved with multiple classifier rank-based decision-combination methods (Saranli and Demirekler 2001). Hence, fitting processes have been automated and standardized with the assistance of rank-based decision algorithms (Saranli and Demirekler 2001) that identify the maximum R and minimum MSE combination.

Overall, we identified the frequency-dependent viscoelastic properties (*i.e.*, shear storage modulus, $G'(\omega)$, and shear loss modulus, $G''(\omega)$) of two structurally different magnetic PVA-shelled MBs by comparing the measured and theoretically calculated attenuation coefficients ($\alpha(\omega)$) and phase velocity ($c(\omega)$) spectra within −20-dB bandwidth frequencies. The simultaneous fits with best possible combinations of the statistical parameters R and MSE were identified with the help of a rank-based decision algorithm. The identification of the mechanical parameters allowed recalculation of the physical characteristics, that is, total damping ratio (ζ_{tot}) and undamped resonance frequency (f_0). These characteristics were further exploited to describe the oscillation characteristics of both types of MBs.

METHODS

MB preparation and properties

The manufacturing processes for MBs manufactured with SPIONS and anchored chemically on the surface (MBs-chem) and MBs manufactured with SPIONS and physically embedded into a polymer shell (MBs-phys) have been fully described in Brismar et al.