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Harmonic responses and cavitation activity of encapsulated microbubbles coupled with magnetic nanoparticles

Yuyang Gu [a], Chuyi Chen [a], Juan Tu [a,*], Xiasheng Guo [a], Hongyi Wu [a], Dong Zhang [a,b,*]

[a] Key Laboratory of Modern Acoustics (MOE), Department of Physics, Collaborative Innovation Center of Advanced Microstructure, Nanjing University, Nanjing 210093, China
[b] The State Key Laboratory of Acoustics, Chinese Academy of Science, Beijing 10080, China

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ABSTRACT

Encapsulated microbubbles coupled with magnetic nanoparticles, one kind of hybrid agents that can integrate both ultrasound and magnetic resonance imaging/therapy functions, have attracted increasing interests in both research and clinic communities. However, there is a lack of comprehensive understanding of their dynamic behaviors generated in diagnostic and therapeutic applications. In the present work, a hybrid agent was synthesized by integrating superparamagnetic iron oxide nanoparticles (SPIOs) into albumin-shelled microbubbles (named as SPIO-albumin microbubbles). Then, both the stable and inertial cavitation thresholds of this hybrid agent were measured at varied SPIO concentrations and ultrasound parameters (e.g., frequency, pressure amplitude, and pulse length). The results show that, at a fixed acoustic driving frequency, both the stable and inertial cavitation thresholds of SPIO-albumin microbubble should decrease with the increasing SPIO concentration and acoustic driving pulse length. The inertial cavitation threshold of SPIO-albumin microbubbles also decreases with the raised driving frequency, while the minimum sub- and ultra-harmonic thresholds appear at twice and two thirds resonance frequency, respectively. It is also noticed that both the stable and inertial cavitation thresholds of SonoVue microbubbles are similar to those measured for hybrid microbubbles with a SPIO concentration of 114.7 μg/ml. The current work could provide better understanding on the impact of the integrated SPIOs on the dynamic responses (especially the cavitation activities) of hybrid microbubbles, and suggest the shell composition of hybrid agents should be appropriately designed to improve their clinical diagnostic and therapeutic performances of hybrid microbubble agents.

1. Introduction

Ultrasound contrast agent (UCA) microbubbles (microbubbles) are usually insoluble gas core (e.g., perfluorocarbon and sulfur hexafluoride) encapsulated by thin coatings (e.g., lipid, albumin and polymer) that can prevent bubbles from quick dissolution. Taking advantages from the compressible gas cores and resonant-induced intense scattering, UCA microbubbles injected into the bloodstream were initially utilized in diagnostic ultrasound (US) imaging to enhance the acoustic contrast of specific cardiographic or radiologic features. With more popular use of UCA microbubbles, their potential therapeutic applications relevant to US-induced bioeffects were also investigated both *in vitro* and *in vivo* [1–3]. More recently, due to the increasing demands of performance improvement of UCAs, hybrid contrast agent microbubbles, which can integrate more than one diagnostic/therapeutic modalities in one agent, have attracted broad interests in both research and clinic communities [4–6].

Superparamagnetic iron oxide nanoparticles (SPIOs) are one kind of powerful multifunctional agents that can both effectively enhance magnetic resonance imaging (MRI) contrast and potentially serve as magnetic nonviral vectors [7–9]. It has been reported in previous literatures that, by integrating SPIOs into the shells of UCA microbubbles, hybrid US/MRI contrast agents can be fabricated to achieve higher imaging resolution, deeper tissue penetration depth, better treatment efficacy and greater clinic safety [10–12]. However, the embedding of SPIOs to microbubble shell structures will also significantly affect the dynamic responses of the microbubbles, e.g., the generation of harmonics and US-induced cavitation activities [13–15].

It is well accepted that US-induced stable cavitation (SC) and inertial cavitation (IC) play an important role in the development of microbubble-assisted diagnostic and therapeutic applications, e.g., contrast harmonic imaging, low intensity pulsed US

* Corresponding authors at: Institute of Acoustics, Nanjing University, Nanjing 210093, China.
E-mail addresses: juantu@nju.edu.cn (J. Tu), dzhang@nju.edu.cn (D. Zhang).

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stimulated fracture healing, high intensity focused US cancer therapy, and US-induced gene/drug delivery [16–18]. It has been reported that the SC of UCAs is usually determined by sub-harmonic and ultra-harmonic responses, which vary with acoustic parameters such as the pulse length, amplitude, and frequency [19,20]. Other studies have also been performed to investigate the IC thresholds of UCAs [21–25]. However, up to our knowledge, currently it is lack of better understanding of how the embedding of SPIOs into the shells of UCA microbubbles affects the cavitation threshold and dose of the hybrid US/MRI contrast agents.

In the present work, hybrid US/MRI contrast agent microbubbles were synthesized by embedding SPIOs to albumin-shelled perfluorocarbon microbubbles (referred as SPIO-albumin microbubbles for short). Then, both the SC and IC doses generated by the SPIO-albumin microbubbles were quantitatively measured based on passive cavitation detection (PCD) method. As the results, the SC and IC thresholds of SPIO-albumin microbubbles could be determined at different SPIO concentrations and US parameters (e.g., frequency, pressure amplitude, and pulse length). For comparison, the same measurements were applied to commercially available SonoVue microbubbles as well. This work will be helpful for better understanding how the integration of SPIOs into the shells of UCAs plays an important impact on the microbubble dynamic response (especially their cavitation activities), which should be beneficial for improving the development of hybrid contrast agents and providing new insights for their diagnostic/therapeutic applications, while minimizing undesired side-effects (e.g., vascular endothelial damage, cell apoptosis and hemorrhage) [26–28].

2. Materials and methods

2.1. Synthesis of perfluorocarbon-filled SPIO-albumin microbubbles

The hybrid contrast agent used in the present work was synthesized by loading SPIOs into albumin-shelled perfluorocarbon microbubbles. The preparing of SPIO-albumin-shelled perfluorocarbon microbubbles involves two steps: synthesis of the SPIOs and assembly of the SPIO-albumin microbubbles. A brief description of the method is as follows. An aqueous solution of $FeCl_3{\cdot}6H_2O$ (1.0 mol/L, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and $FeSO_4{\cdot}7H_2O$ (0.5 mol/L, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was used as the iron source. Concentrated $NH_3{\cdot}H_2O$ (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was added to the iron-containing solution until the pH reached 11.0 under a nitrogen atmosphere to prevent oxidation. Oleic acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was then added to the alkaline solution to yield mono-disperse hydrophobic Fe_3O_4-oleic acid nanoparticles. Via a surface double-exchange reaction between oleic acid and meso-2,3-dimercaptosuccinic acid (DMSA; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), mono-disperse, water-soluble SPIOs (Fe_3O_4-DMSA) were obtained and collected through a magnetic separation procedure. After pH neutralization, the SPIO solution was dialyzed through dialysis membranes (Cellu-Sep T3, Membrane Filtration Products Inc., Seguin, Texas, USA) in pure water for 3 days to remove excess impurities. The final sample was filtrated through a 0.22-μm membrane and stored at 4 °C. In the second step, a sonication method, similar to that reported by Porter et al. [29], was used to synthesize the SPIO-albumin microbubbles. Briefly, 10% bovine serum albumin and 60% sucrose were mixed with a volume ratio of 1:1 in deionized water. A certain amount of SPIOs were then added to the mixed solution. The mixed solution was placed and kept overnight in a bell-like glass chamber (radius: 9 cm; height: 20 cm) saturated with perfluorocarbon (C_3F_8). The solution was then sonicated with a horn-type ultrasonic processor (VCX750, Sonics and Materials Inc., Newtown, CT, USA) for 2 min, while a syringe pump (LEGATO 270, KD Scientific Inc., Holliston, MA, USA) was used to continuously pump in C_3F_8 gas. The ultrasonic processor worked in pulsed mode with an on/off ratio of 3:1, a working frequency of 20 kHz, and an intensity of 300 W. The SPIO-albumin microbubbles were centrifuged and then washed to eliminate free SPIOs. Finally, the upper layer of the multibubble suspensions was collected for subsequent analysis.

For comparison, a commercially available UCA (SonoVue, Bracco Diagnostics Inc., Geneva, Switzerland) was studied also. SonoVue microbubbles consist of sulfur hexafluoride (SF_6) gas encapsulated by a thin lipid monolayer membrane. The microbubbles were reconstituted according to the manufacturer's instructions by venting the sample vial with a sterile 18-gauge needle, followed by a 5-mL injection of phosphate buffered saline into the vial. The vial contained 2–5 $\times$ 10^8 microbubbles/mL and through inversion agitation the microbubbles were evenly distributed. The reconstituted SonoVue suspension was diluted for analysis.

2.2. Experimental setup

The experimental apparatus are illustrated in Fig. 1. Tone-burst signals were generated by an arbitrary waveform generator (33250A, Agilent, Santa Clara, CA). While being amplified by a 53-dB power amplifier (2200L, E&I, Rochester, NY), signals with different parameters (e.g., frequencies, pulse lengths and pressures) were used to drive four unfocused transducers. The central frequencies of the transducers #1 to #4 were 1, 2.25, 3.5 and 5 MHz, respectively (V303-SU, V325-SU, V383-SU and V326-SU, Panametrics, Waltham, MA). The transmitted ultrasound waves were sonicated at a 1.5-mL hydrophilic polyester sample tube (1-cm diameter, 30-μm wall thickness; Vention Medical, Salem, NH) filled with diluted microbubble suspensions. The peak negative pressures generated by the source transducers were calibrated with a needle hydrophone (HNC-0100, Onda Corporation, Sunnyvale, CA) that was controlled by a three-dimensional positioning system (Newport, Irvine, CA) using the LABVIEW software (NI Corporation, TX), which allowed the peak negative acoustic driving pressure to be determined from the input electrical voltages.

To determine the SC and IC threshold of different microbubbles, the output voltage of the function generator was increased with a 5-mV step size every 2 s from the initial level (viz., 0 mV) over a 60-s period for each sample. During the experiment, in order to reduce the impact of the source signal on the detection of the broadband noise and sub- and ultra-harmonic signals, the needle hydrophone was positioned 90° to the source to detect the scattered and emitted signals from the microbubbles. The detected signals were digitalized by and displayed on a digital oscilloscope (54830B, Agilent, Santa Clara, CA) with a sampling frequency of 25 MHz; each signal contained 20,000 data points. For each experiment, five signals were measured with fresh microbubble suspensions. For microbubbles in individual series (e.g., SonoVue microbubbles and hybrid microbubbles with various SPIO concentrations), five replicate measurements were performed for sample suspensions withdrawn from different vials.

2.3. Determination of sub-harmonic and ultra-harmonic thresholds of SC microbubbles

Normally, when the microbubbles were excited by US pulses, SC will occur first as the acoustic driving pressure reaches a certain threshold. When the driving pressure increases further to exceed a higher threshold, IC takes place. The scattered and emitted signals can be detected by a passive cavitation detection (PCD) system and used to determine these two cavitation thresholds, which are