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High frequency attenuation measurements of lipid encapsulated contrast agents

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Abstract

A number of recent studies have indicated the potential of ultrasound contrast agent imaging at high ultrasound frequencies. However, the acoustic properties of microbubbles at frequencies above 10 MHz remain poorly understood at present. In this study we characterize the high frequency attenuation properties of (1) BR14, (2) BR14 that has been mechanically filtered (1 and 2 μm pore sizes) to exclude larger bubbles, and (3) the micron to submicron agent BG2423. A narrowband pulse-echo substitution method is employed with a series of four transducers covering the frequency range from 2 to 50 MHz. For BR14, attenuation decreases rapidly from 2 to 10 MHz and then more gradually from 10 to 50 MHz. For 2 μm filtration, the attenuation peaks between 10 and 15 MHz. For 1 μm filtration, attenuation continues to rise until 50 MHz. The agent BG2423 exhibits a diffuse attenuation peak in the range of 15–25 MHz and remains high until 50 MHz. These results demonstrate a strong influence of bubble size on high frequency attenuation curves, with bubble diameters of 1–2 μm and below having more pronounced acoustic activity at frequencies above 10 MHz.

Keywords: Contrast agents; Harmonic; Subharmonic; Microbubbles; High frequencies; Intravascular ultrasound

1. Introduction

There is increasing interest in the use of microbubble contrast agents at frequencies in the 10–50 MHz range, where applications are possible in ophthalmology, small animal imaging, and cardiology. Several studies have investigated their use in 'linear' imaging mode using ultrasound biomicroscopy [1] and intravascular ultrasound (IVUS) systems [2,3]. More recent work has shown that it is also feasible to perform subharmonic and second harmonic imaging at high frequencies [4,5]. In these studies, both conventional commercial agents and experimental liposomal preparations with micron to submicron mean diameters have been employed.

The acoustic properties of microbubbles at high frequencies are not well understood at present, particularly with regards to knowledge of resonant bubble sizes and nonlinear scattering mechanisms. A reasonable hypothesis is that smaller bubbles are more active at high frequencies: for example, the resonant diameter of a free bubble at 20 MHz is 0.65 μm. The application of current models for lipid encapsulated microbubbles also predicts increased acoustic activity in bubbles of diameters 1–2 μm and below for frequencies above 10 MHz (e.g. [6]). However, these models are not validated at high frequencies and shell properties required as input parameters are unknown. From an experimental perspective, it has been shown that mechanical filtration of BR14 (Bracco Research, Geneva)

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using 1 and 2 μm pore sizes improves nonlinear scattering at 20 and 30 MHz transmit frequencies [6]. Further, the experimental micron to submicron lipid encapsulated agent BG2423 (Bracco Research, Geneva) has been shown to have substantial nonlinear activity (subharmonics and second harmonics) for transmit frequencies of 20–40 MHz [5,7].

While attenuation measurements have been widely employed at lower frequencies (<10 MHz) to gain insight into bubble resonant frequencies and shell properties [8–10], very little work has been done at high ultrasound frequencies (>10 MHz) [11]. In this study we investigate the attenuation properties of lipid encapsulated contrast agents in the frequency range from 2 to 50 MHz.

2. Methods

2.1. Measurement system overview

Attenuation measurements were made using the pulse-echo substitution method, an approach described in detail elsewhere [8]. A series of four commercially available transducers (Panametrics Inc., Waltham, MA) was used to cover the range of 2–50 MHz (Table 1). Agent was situated in a sample chamber located within the transducer beam, which entered the chamber through a mylar window and was then reflected off a steel plate located at focus. Two sample chambers were used, each one having two transducers (Fig. 1).

An overview of the hardware configuration is shown in Fig. 2. An arbitrary waveform generator (AWG 520 Tektronix, Beaverton, OR) was used to create low amplitude pulses, which were amplified with an RF power amplifier (2100 L, ENI, Rochester, NY for transducers 1 and 2; LPI-10, ENI, Rochester, NY for transducers 3 and 4) and sent to one of four transducers. On receive, signals were amplified (AU-1189 or AU-1263, Miteq Inc., Hauppage, NY), bandpass filtered, and then digitized (DP310, Acqiris, Geneva). A sequence of narrowband pulses was employed (20 cycles up to 10 MHz; 40 cycles for 10–50 MHz) to sweep the bandwidth of each transducer individually. The inter-pulse spacing was 200 ms, with sequences repeated at a rate of 8 min^{-1}. The use of narrowband pulses permitted the use of low transmit pressure levels that were controlled across a wide frequency range, in order to avoid potential pressure dependent attenuation effects [12] and thereby to enable comparison with linearized bubble models. This approach also extended the effective frequency range of each individual transducer, in terms

Table 1
Transducer models (Panametrics), characteristics, and frequency range

Transducer and model	Focal length (mm)	Aperture (mm)	Path length in chamber	Nom. center frequency (MHz)	Frequency range (MHz)
1. V304	75	25	42	2.25	2–3.5
2. V311	75	13	42	10	3–14
3. V317	50	6	29	20	8–30
4. V377	51	6	29	30	25–50

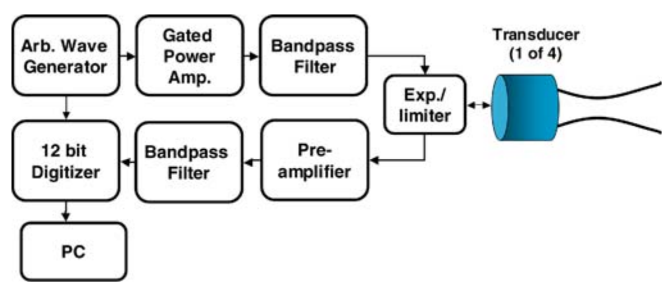

Fig. 2. Schematic overview of the hardware employed.

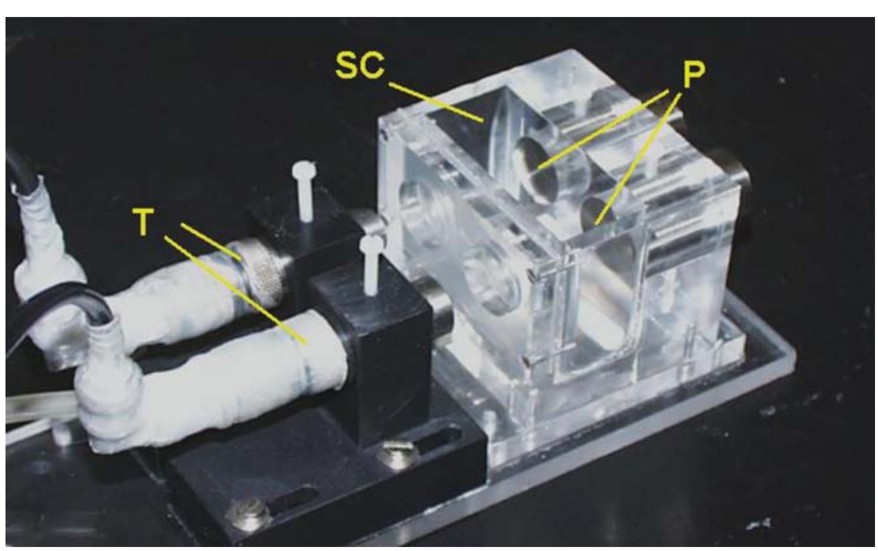

Fig. 1. Photograph of one of the sample chambers (SC) indicating the transducers (T), and reflective steel plates (P). These components were immersed in a water tank.

of signal to noise ratio, relative to that which could be achieved with a wideband pulse. The pressure level used was 25 kPa, as measured with a 0.075 mm needle hydrophone (Precision Acoustics Ltd., Dorchester, UK) at focus in a water tank.

2.2. Agent handling

The agents examined were (1) BR14; (2) BR14 with its population modified by mechanical filtering (1 and 2 μm pores sizes) to isolate small bubbles; and (3) BG2423, all experimental lipid encapsulated agents from Bracco Research (Geneva). According to size information provided by the manufacturer, BR14 has a mean volume weighted diameter of 6.8 μm and BG2423 is comprised substantially of micron to submicron bubbles. Agent was diluted in Isotone^{TM} to the ratios shown in Table 2. Filtration was performed by gravity feed of diluted agent through a porous polycarbonate membrane (Poretics 1 or 2 μm, GE Osmonics Inc), as also described in [6,8]. Measurements began 1 min after the agent was placed within the sample chamber, with agent undergoing gentle mixing with a magnetic stirrer. For a given sample, a total of eight measurements at each frequency were then performed during 1 min. A total of four independent sample measurements were made for native BR14 and BG2423 (i.e. averaging 32 values per frequency in total), and two samples for filtered cases (i.e. averaging 16 values per frequency in total). All experiments were performed at room temperature.

Table 2
Agent dilution ratios

Agent	Dilution ratio
B14	1:2000
BR14 (2 μm filter)	1:300
BR14 (1 μm filter)	1:150
BG2423	1:700

3. Results and discussion

The attenuation results are shown in Fig. 3(a)–(d). For native BR14 (Fig. 3(a)), attenuation decreased steeply from 2 to 10 MHz and more gradually from 10 to 50 MHz. These results indicate that its acoustic activity is most pronounced at lower diagnostic frequencies (<10 MHz), as may be expected with a mean bubble size of 6.8 μm.

When BR14 is passed through a 2 μm filter, the attenuation peak is shifted towards 10–15 MHz before leveling off until 50 MHz (Fig. 3(b)). This result suggests that there are bubbles below 2 μm in diameter present within BR14 that have significant acoustic activity above 10 MHz. When BR14 is passed through a 1 μm filter the attenuation at 10 MHz is low, but continues to rise until 50 MHz (Fig. 3(c)). This suggests that there are bubbles below 1 μm in diameter present within BR14 that have significant acoustic activity up to 50 MHz. These data are consistent with the results of [6], which showed that high frequency (20 and 30 MHz transmit) nonlinear scattering (subharmonic and second harmonic) from BR14 is improved with 1 and 2 μm filtering. It should be noted however that while

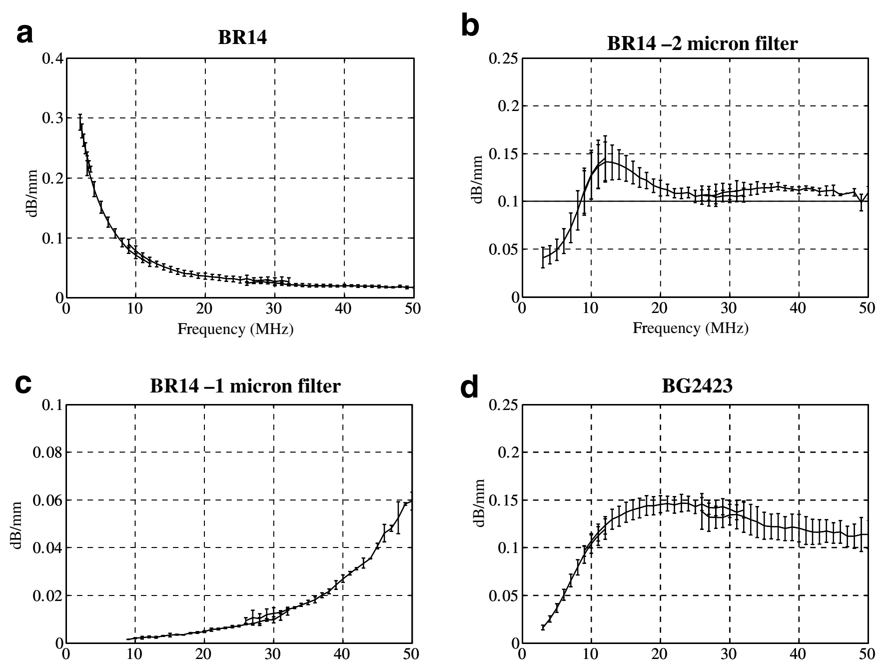

Fig. 3. Frequency dependent attenuation data (mean and standard deviations shown) for (a) BR14, (b) BR14 with 2 μm filtering, (c) BR14 with 1 μm filtering, and (d) BG2423.

this particular approach to mechanical filtering was done under 'gentle' gravity fed conditions, it is not known if the filtration process may have modified the shell properties of the agent.

Attenuation for BG2423 exhibits a diffuse peak in the range of 15–25 MHz, and remains high until 50 MHz (Fig. 3(d)). This agent has been found to have significant nonlinear activity at transmit frequencies from 20 to 40 MHz [5,7]. It is also notable that the form of the attenuation curve for this agent (comprised of micron to submicron sized bubbles) is intermediate between 1 and 2 μm filtration results.

4. Conclusions

Frequency dependent attenuation has been measured in the 2–50 MHz range for several lipid encapsulated bubble populations. The results of this study demonstrate a strong influence of bubble size on high frequency attenuation curves, with bubbles below 1–2 μm in diameter exhibiting more pronounced acoustic activity at frequencies above 10 MHz. This is consistent with the results of previous studies examining nonlinear activity of small bubble populations at high frequencies. These results also support the conclusion that agents with improved activity at high frequencies can be obtained through either the manufacture of small bubble agents or the modification of existing agents designed for use at lower frequencies. Future work with attenuation measurements will entail analyzing the data in combination with size distribution information to gain insight into shell properties and the relationship between bubble size and resonant frequencies above 10 MHz.

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