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● *Original Contribution*

ON THE SUITABILITY OF BROADBAND ATTENUATION MEASUREMENT FOR CHARACTERIZING CONTRAST MICROBUBBLES

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Abstract—Broadband attenuation measurement has been widely used for characterizing ultrasound contrast agents. Chen et al. (2002) recently suggested that broadband attenuation data depend on the center frequency of the broadband excitation pulse and, therefore, that they are not a reliable measure of the bubble behavior. We investigated the suitability of measurement of broadband attenuation as a characterizing tool using the contrast agent Definity® as a test case. Analyzing the attenuation data obtained with three broadband unfocused transducers with different center frequencies (2.25, 3.5 and 5 MHz), we found that attenuation is independent of the transducer used and matches in the overlap regions of any two transducers. Attenuation does not depend on excitation pressure amplitude as long as the excitation amplitude remains below a critical value (≈ 0.26 MPa), indicating that the measurement of broadband attenuation below critical excitation can, indeed, be used for characterization. Furthermore, the linear relationship of attenuation with concentrations of Definity® is also investigated. (E-mail: sarkar@me.udel.edu)

Key Words: **Broadband attenuation, Ultrasound, Contrast agent, Definity®, Concentration, Pressure amplitude.**

INTRODUCTION

Ultrasound (US) contrast agents are micrometer-sized encapsulated gas bubbles injected IV in patients to improve the quality of US blood flow images. The agents scatter sound and improve the signal-to-tissue contrast. Researchers have performed *in vitro* attenuation and scattering experiments (see, for example, de Jong and Hoff 1993; Frinking and de Jong 1998; Shi and Forsberg 2000; Hoff et al. 2000; Morgan et al. 2000) to characterize the behaviors of these microbubbles. Several theoretical models have been developed (de Jong and Hoff 1993; de Jong et al. 1992; Frinking and de Jong 1998; Church 1995; Morgan et al. 2000; Chin and Burns 2000; Chatterjee and Sarkar 2003) to describe them. The stabilizing encapsulation is modeled as a layer or an interface with material properties such as viscosity, elasticity and surface tension. The single-bubble model is fitted to measured attenuation data, along with a population size distribution to estimate these material properties (de Jong and Hoff 1993; Hoff et al. 2000; Chatterjee and Sarkar 2003; Sarkar et al. 2005). Chatterjee and Sarkar (2003) proposed a two-step process for model development. Small-amplitude broadband attenuation data were fitted with a linearized model for an agent to determine the parameters. The model was then validated by comparing its prediction with scattering measurement in a regime distinctly different from that where the model was fitted. Chatterjee and Sarkar (2003) and Sarkar et al. (2005) successfully predicted the subharmonic response for Optison® (GE Healthcare, Princeton, NJ, USA) and Sonazoid® (GE Healthcare, Oslo, Norway).

In the attenuation experiments reported in the literature (see, for example, de Jong and Hoff 1993; Hoff et al. 2000; Shi and Forsberg 2000), it is implicitly assumed that the driven bubble experiences small-amplitude oscillation and its response is linearly dependent on the acoustic pressure amplitude. However, the bubble dynamic is a nonlinear phenomenon and driving acoustic pressure plays a crucial role. Recently, Chen et al. (2002) have studied the effect of the driving pressure on attenuation through contrast agents Optison® and Definity® (Bristol-Myers Squibb Medical Imaging, North Billerica, MA, USA). They found that the broadband attenuation curves depend on the center frequency of the excitation pulse. They concluded that ". . .results showed great inconsistency when the center frequency of the incident

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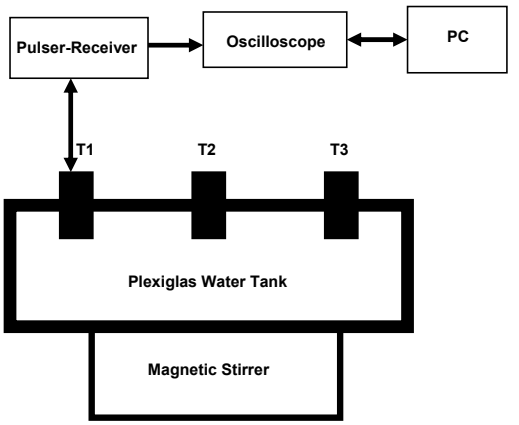
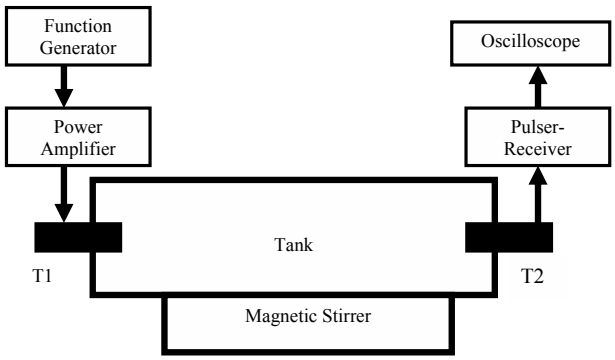
broadband pulse was changed, indicating that broadband techniques may not be suitable for contrast-agent attenuation measurements." They attributed the behavior to the nonlinear response of the bubbles to a broadband signal. For a strong excitation, nonlinearity can effectively transfer energy across the frequency range. The attenuation, therefore, attains its maximum value for the center frequency of the exciting pulse, where the excitation is at its maximum. In this study, we addressed this issue by performing a detailed investigation of attenuation of broadband US through the contrast agent Definity®, using three different transducers with different center frequencies and varying excitation amplitude and agent concentration.

EXPERIMENTAL SETUP AND METHOD

A schematic of the attenuation setup is shown in Fig. 1a. A pulser-receiver (model 5800, Panametrics, Waltham, MA, USA) was used to excite an unfocused broadband transducer at a PRF of 100 Hz. It transmitted a single-cycle pulse of length 1 μs into the bath. Three different broadband transducers were used with center frequencies of 2.25, 3.5 and 5 MHz. The −6-dB band widths for these transducers are 1.58 to 2.95 MHz, 2.5 to 4.99 MHz and 3.13 to 6.19 MHz, respectively. Ultrasound travels through the tank containing the contrast agent solution and the signal reflected from the back wall is received by the same transducer. The total travel path was 8.2 cm. The received signal was amplified by the pulser-receiver and fed into an oscilloscope (TDS2012, Tektronix, Beaverton, OR, USA) and a computer. Matlab® (Mathworks Inc, Natick, MA, USA) was used for postprocessing of the data. A 0.4-mm needle hydrophone (PZT-Z44-0400, Onda Corporation, Sunnyvale, CA, USA) was used to measure the acoustic pressure. The lowest value of the peak negative excitation amplitude produced by this setup using the pulser-receiver was 0.67 MPa. To obtain lower excitation amplitudes, we used an in-line tunable dB attenuator (0 to 60 dB, model 432D, Kay Elemetrics Corporation, Lincoln Park, NJ, USA) between the pulse-receiver and the transducer. Part of the data were also obtained using a second setup, where a function generator (33250A, Agilent, Palo Alto, CA, USA) and a power amplifier (ENI A150, Rochester, NY, USA), as shown in Fig. 1b, were used to produce one-cycle bursts. In these experiments, we used an unfocused broadband 5-MHz transducer as transmitter. Another unfocused broadband transducer with 3.5 MHz center frequency was used as receiver. Both were mounted on the wall of a bath and were separated by a distance of 10.5 cm. Two setups have been found to produce the same result within the variability accepted between different realizations of the same experiments.



(a)



(b)

Fig. 1. Schematics of two *in vitro* attenuation measurement setups and necessary instrumentation. (a) First set-up used same transducer both as a source and a receiver. Connection between transducer T1 and pulser-receiver is shown. During experiments, all transducers were connected to pulser receiver, one after another. (b) Second setup used two transducers for sending and receiving the signal (this setup was used to obtain results presented in Fig. 4).

The contrast agent used in these experiments was Definity® (Bristol-Myers Squibb Medical Imaging). Definity® microbubbles were prepared by shaking the suspension in a Vial-Mix™ (Bristol-Myers Squibb) for 45 s, according to the manufacturer's recommendations. The bubble suspension was then injected into Isoton-II (Beckman Coulter, Miami, FL, USA) 2 min after preparation. Excitation was started 1 min after injection. A magnetic stirrer (Fisher Scientific) was used to maintain a homogeneous solution containing microbubbles.

In a typical experiment, the received signal was recorded before Definity® was introduced into the solution to obtain a reference signal V_{ref}. Then, the measurement was repeated after injection of Definity® into the solution to find the signal V_{sig} in the presence of contrast agent. For each measurement, 64 sequences of signals were averaged in the oscilloscope and then transferred to a PC *via* an IEEE-488 (GPIB) interface for further analysis. The data transfer was controlled using Labview® (National Instruments, Austin, TX, USA).

With a plane linear wave assumption, different frequency components of a broadband pulse travel independently of each other:

$$P(x,t) = \mathrm{Re} P_0 e^{i\omega(t-x/c)} e^{-\frac{\alpha(\omega)}{2}x} \tag{1a}$$

$$I(x) = \left|P(x,t)\right|^2 = I_0 e^{-\alpha(\omega)x}, \tag{1b}$$

where Re indicates the real part, P_0 and I_0 are the incident pressure amplitude and intensity, c is the sound speed in the liquid, ω is the circular frequency and $\alpha(\omega)$ is the attenuation per unit distance. Figure 2a shows typical original and attenuated (because of bubbles) signals in the time domain. Each bubble contributes to the attenuation of a particular frequency component according to its extinction cross-section $\sigma_e(a;\omega)$:

$$\alpha(\omega) = 10(\log_{10} e) N \int_{a_{min}}^{a_{max}} \sigma_e(a;\omega) f(a) da\,, \tag{2}$$

where $f(a)$ is the normalized number of bubbles per unit radius a and N is the number of bubbles per unit volume. Equation (2) indicates that attenuation in this regime varies linearly with bubble density or concentration. Note that, here, attenuation is independent of the excitation amplitude (Sarkar and Prosperetti 1994; Foldy 1945). Equations (1) and (2) were obtained in the limit of small bubble concentration for a bubbly medium.

From the measured data, attenuation is computed:

$$\alpha(\omega) = 10 \log_{10}\left(\frac{V_{ref}^2(\omega)}{V_{sig}^2(\omega)}\right) \Big/ d\,, \tag{3}$$

where $\alpha(\omega)$ is in dB/cm, d is the distance between the receiver and the transmitter and $V_{ref}(\omega)$ and $V_{sig}(\omega)$ are the received sound in the absence and presence of contrast agents, respectively. Fast Fourier transforms of the time domain signals shown in Fig. 2a are shown in Fig. 2b.

RESULTS AND DISCUSSION

We investigated the dependence of attenuation on transducers with different center frequencies. Figure 3a shows the frequency spectra in the absence of bubbles for three transducers. Corresponding attenuation data obtained with Definity® are plotted in Fig. 3b. The experiments were performed at a concentration of 40 μL of Definity® in 1 L of Isoton-II and repeated four times. The peak negative excitation pressure was 0.1 MPa. The attenuations measured with different transducers match with each other in the overlap region, indicating that the computed attenuation is independent of the transducer as well as the center frequency of the excitation pulse. Using three transducers, we obtained the attenuation because of contrast microbubbles in the frequency range of 2 to 6.5 MHz. The spectrum shows a continuous increase with frequency with a slight peak at $\approx$ 6 MHz and is a characteristic of the contrast agent, rather than of the excitation or the transducer center frequencies (2.25, 3.5 and 5 MHz). Note that the continuous increase with frequency contrasts with the observation by Chen et al. (2002); they found that the attenuation as a function of frequency (figure 3 in Chen et al. 2002), measured with narrow-band incident pulses decreased steadily with in-

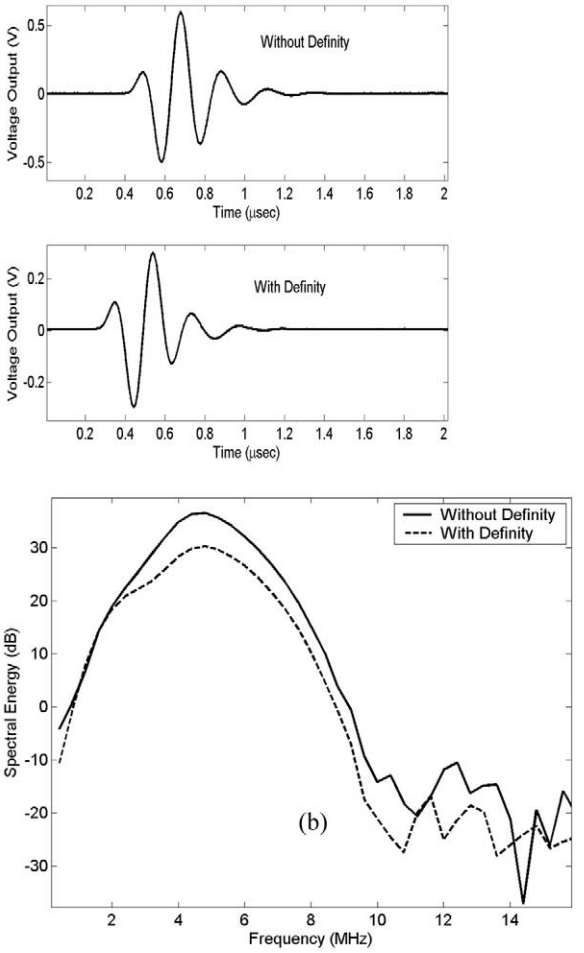


Fig. 2. Signal received by transducer (center frequency 5 MHz) with and without Definity® at a concentration of 40 μL/L (a) In time domain and (b) Their frequency content. Excitation pressure amplitude used was 0.67 MPa.

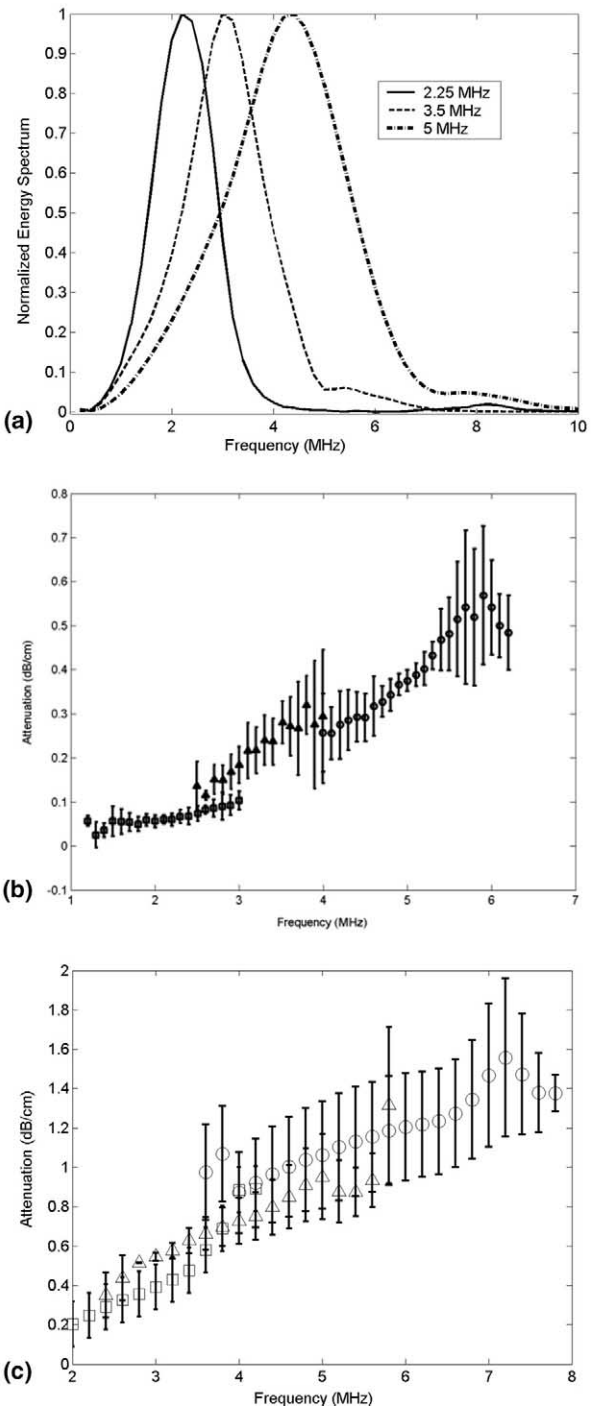


Fig. 3. (a) Normalized broadband signal sent by the pulser-receiver to transducers. Attenuation measured by three transducers with central frequencies of 2.25 (□), 3.5 (△) and 5 (○) MHz at pressure amplitudes (b) 0.1 MPa and (c) 0.67 MPa. PRF was 100 Hz and Definity® concentration was 40 μL/L.

creasing frequency in the range 1 to 5 MHz. Definity® microbubbles are small (≈ 1 μm radius). For free bubbles, the resonance frequency and radius are related by an empirical relation $f_0 = 3.25/r$, where r is the bubble radius in μm and f_0 is the resonance frequency in MHz (Leighton 1994). For the size of Definity®, the corresponding free-bubble resonance frequency is ≈ 3.25 MHz. The lipid encapsulation-induced elasticity further increases the resonance frequency. Therefore, one would expect the attenuation to increase with frequency for the range of 1 to 3.25 MHz and beyond, as is, indeed, observed here (Fig. 3b). Similar observations were obtained even for a higher pressure amplitude of 0.67 MPa (Fig. 3c). No bubble destruction took place under these conditions, as became evident from the constancy of attenuation data over a time interval of 10 min. Note that Chen et al. (2002) observed that using different center frequencies for excitation pulse (at a much lower amplitude of 0.15 MPa) resulted in very different attenuation, with peaks at those frequencies (figure 7 in Chen et al. 2002). They argued that the nonlinear dynamics of suspended microbubbles are responsible for this behavior. We proceeded to investigate this issue in further detail by explicitly computing attenuation at different excitation amplitudes. Effects of nonlinearity only become significant with increasing excitation amplitude and one would expect that attenuation would be independent of the excitation amplitude below a critical value.

We investigated the effect of excitation pressure amplitude by measuring attenuation at varying excitation amplitudes, using the setup of Fig. 1b. A transducer with center frequency of 5 MHz was used as a transmitter, and one with 3.5 MHz as a receiver. In Fig. 4, we computed attenuation (dB/cm) curves for increasing excitation amplitudes at concentrations of 40 μL/L and 120 μL/L. The experiments were repeated four times. We observed that the attenuation did not change with amplitude for excitation of less than 0.26 MPa, but those above (top four curves) increased with amplitude. We conclude that nonlinearity sets in above the critical value of ≈ 0.26 MPa. The linear propagation theory eqns (1) and (2) are not valid above this critical value of pressure amplitude. Wu and Tong (1997) and Zhang et al. (2000) observed increased nonlinearity in the medium with increased concentration, in that concentration may accentuate nonlinear wave propagation. However, Fig. 4b, for a concentration of 120 μL/L, a threefold increase, resulted in the same critical excitation amplitude (≈ 0.26 MPa). Note that Chen et al. (2002) found the broadband attenuation measurement to depend on the excitation amplitude for a far lower value of 30 kPa.

Next, we investigated the effects of concentration at varying pressure amplitudes. At lower concentrations, bubble-bubble interactions can be neglected, as though the

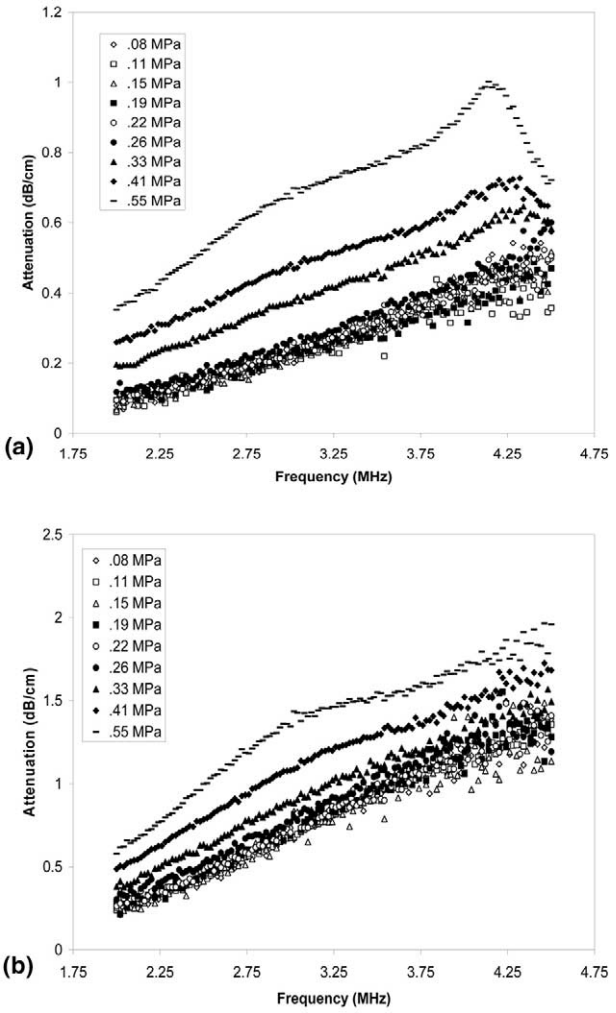


Fig. 4. Variation of attenuation with incident pressure amplitude. Concentrations used were (a) 40 μL/L and (b) 120 μL/L.

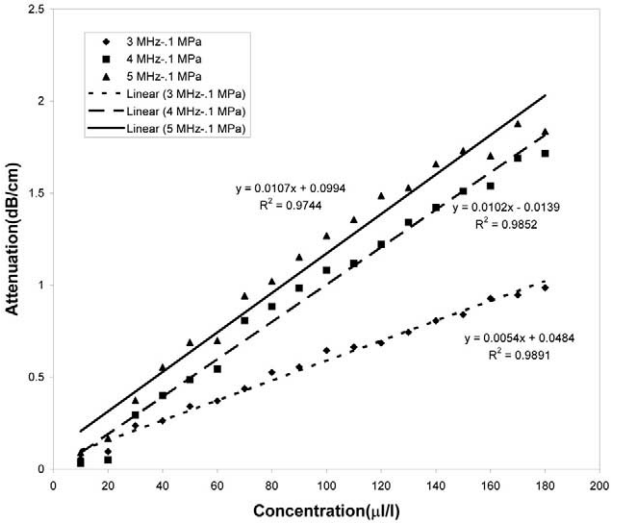


Fig. 5. Variation of attenuation with concentration at excitation amplitude of 0.1 MPa.

bubbles are acting in isolation, being excited only by the incident wave. Equations (1) and (2) describe such noninteracting dynamics of the bubbly medium, with attenuation varying linearly with concentration. At higher concentrations, interactions between bubbles and multiple scattering might play important roles. We measured attenuation with varying concentrations using a broadband transducer with a center frequency of 5 MHz and excitation amplitude of 0.1 MPa. In Fig. 5, attenuation corresponding to three different frequencies of 3, 4 and 5 MHz are plotted as a function of concentration. The figure shows that attenuation varies linearly with concentration in the entire range of concentration studied.

SUMMARY

In summary, we have found that attenuation of US through Definity® computed with different transducers matches in the overlap region for 40 μL/L. Chen et al. (2002) found it to depend on excitation pulse with amplitudes even in the low range of 0.03 to 0.15 MPa and concentration 114 μL/L. However, more careful consideration does indicate that the linear model of attenuation, as described by eqns (1) and (2), becomes invalid above a critical value of excitation amplitude, in that the computed attenuation becomes dependent on excitation amplitude. For Definity®, the critical excitation amplitude at the two concentrations of 40 μL/L and 120 μL/L considered is $\approx$ 0.26 MPa, a value higher than indicated by Chen et al. (2002). Chen and colleagues also found that the attenuation measured with narrow-band pulses decreased with frequency in the range of 1 to 3 MHz. Using the empirical relation between radius and resonance frequency, one would expect the attenuation to increase in the same range, as is observed in the current work. Their setup did differ from the one adopted here. However, the underlying theory for analyzing the data are the same. The reason behind the discrepancy is not apparent at this point and further investigation is warranted. We conclude that measurement of broadband attenuation can be used to characterize contrast microbubbles.

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