Ultrasound in Med. & Biol., Vol. 37, No. 2, pp. 338–342, 2011

0301-5629/$ - see front matter

doi:10.1016/j.ultrasmedbio.2010.11.014

● *Technical Note*

CHARACTERIZATION OF DEFINITY™ ULTRASOUND CONTRAST AGENT AT FREQUENCY RANGE OF 5–15 MHZ

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(*Received* 6 *July* 2010; *revised* 10 *November* 2010; *in final form* 23 *November* 2010)

Abstract—The status of vasa vasorum, which can be imaged using ultrasound contrast agents, is an indication for the progression of atherosclerosis. The preferred ultrasound frequency for this purpose is between 5 and 15 MHz. Therefore, it is essential to have knowledge about the acoustic properties of microbubbles such as elasticity and viscosity to be able to implement the current models for lipid encapsulated microbubbles developed for frequencies used in precordial imaging. In this study, the shell parameters, stiffness S_p and friction S_f, of Definity™ microbubbles have been calculated at frequency range of 5–15 MHz by comparing the theoretical modeling of acoustic bubble response and experimental measurements. Derived parameters are in good agreement with previous estimations on SonoVue™ and Sonazoid™ contrast agent. However, the value of S_f is higher than previously estimated for Definity™ between 12–28 MHz. (E-mail: t.faez@erasmusmc.nl) © **2011 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Contrast agents, Microbubbles, Carotid imaging, High frequency ultrasound.**

INTRODUCTION

Atherosclerosis is an inflammatory process that causes lesions in both large and medium-sized arteries. The disease progresses slowly over many decades causing clinical manifestations only at advanced stages of the atherosclerotic process.

The use of noninvasive imaging tools for *in vivo* assessment of atherosclerotic plaque composition holds considerable promise for clinical decision-making and patient treatment monitoring. Current knowledge regarding plaque composition and associated clinical endpoints has been largely derived from single-time-point histopathologic studies. However, serial monitoring of plaque progression using noninvasive imaging could provide insight into the process of plaque remodeling and disruption. Noninvasive ultrasound is the preferred methodology for the initial evaluation of carotid atherosclerosis. Carotid ultrasound is used to evaluate arterial stenosis or dissection in the cervical region. B-mode ultrasonography has been used in plaque progression/regression trials that involve either lipid-lowering drugs or calcium channel blockers (Crouse et al. 2004; O'Leary and Polak 2002). The preferred ultrasound frequency for carotid imaging is 5 to 15 MHz.

A new assessment of the progression of atherosclerosis is provided by the existence and volume of the vasa vasorum, which can be imaged using ultrasound contrast agents (Feinstein 2004). In healthy arteries, the vasa vasorum usually has a single vessel that runs parallel to each side of the epicardial artery being nourished with occasional interconnecting conduits from one side of the artery to the other (Hayden and Tyagi 2004).

The unstable, vulnerable plaques are associated with a malignant like invasion of the intima-media by adventitial derived fragile vasa vasorum vessels, which are prone to rupture resulting in intraplaque hemorrhage. These intraplaque hemorrhages accelerate plaque vulnerability and are associated with plaque rupture and acute vascular events (Hayden and Tyagi 2004).

This vascular volume is, therefore, an indication of the condition of the plaque and can be monitored by diagnostic ultrasound and ultrasound contrast agents.There is increasing interest in the use of microbubble contrast agents at higher frequencies. For frequencies between

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10 and 50 MHz, several studies have investigated their use in "linear" imaging mode using ultrasound biomicroscopy (Deng et al. 1998) and intravascular ultrasound (IVUS) systems (Cachard et al. 1997; Demos et al. 1999). More recent works have shown that it is also feasible to perform subharmonic and second harmonic imaging at high frequencies (Goertz et al. 2005, 2006). In these studies, both conventional commercial agents (Definity™, Lantheus Medical Imaging, North Billerica, MA, USA) and experimental liposomal preparations with micron to submicron mean diameters have been employed, since it is evident from linear theory that smaller bubbles are preferred for higher frequencies.

Current models for lipid encapsulated microbubbles, developed for frequencies used in precordial imaging (1–5 MHz) require input parameters like the viscosity and elasticity of the shell. These shell properties for Definity™ microbubbles have been estimated previously by Goertz et al. (2007) for the frequency range 12–29 MHz (*i.e.*, high frequency). This study focuses on examining the linear response of Definity™ microbubbles and estimating the shell properties for 5–15 MHz, which is a frequency range used clinically for carotid scanning. We use the same methodology as described by Goertz et al. (2007) except for the use of wide band transmission instead of pulse echo narrowband method in the attenuation experiments.

In the following, theoretical background for shell property estimation and the set-up used for attenuation measurements are described. At the end, the results of attenuation measurements and estimated values for shell parameters are deduced and discussed.

MATERIAL AND METHODS

Acoustic response modeling of encapsulated bubbles

Theoretical studies about the dynamics of encapsulated microbubbles in response to an ultrasound field started with modifying the so called Rayleigh-Plesset free gas bubble equations (Plesset and Prospretti 1977 and the references therein). de Jong et al. (1992) developed the first model by introducing ad hoc elasticity and friction shell parameters. Church (1995) enhanced the theory by considering the influence of the shell thickness and the surface tension in the nonlinear model. Hoff et al. (2000) formulated the so called Church's model for a thin shell, neglecting the surface tension term. The model proposed by Frinking and de Jong (1998) assumes that the shell behavior dominates the gas reaction inside the microbubbles.

The linearized approximations of all these models, which assume very small radial oscillation amplitude with respect to the resting radius, leads to very similar equations. Using the model of de Jong et al. (1992), which considers the bubbles covered with a viscoelastic solid shell, the undamped natural resonant frequency of encapsulated bubbles (f_0) for small amplitude oscillations is defined as below:

$$f_0(r) = \frac{1}{2\pi}\sqrt{\frac{3\gamma p_0}{\rho r^2} + \frac{2S_p}{\rho r^3}}, \tag{1}$$

where γ is the polytropic exponent, p_0 the static pressure, ρ the liquid density, r the bubble radius and S_p the shell material stiffness (N/m).

Neglecting thermal losses, the total damping (δ_{tot}) is the sum of three damping coefficients, viscosity (δ_{vis}), radiation (δ_{rad}) and internal shell friction (δ_{sh})

$$\begin{aligned}\delta_{tot} &= \delta_{rad} + \delta_{vis} + \delta_{sh}\\ \delta_{tot} &= \frac{\omega r}{c} + \frac{4\mu_l}{\omega\rho r^2} + \frac{S_f}{4\pi\omega\rho r^3},\end{aligned} \tag{2}$$

where ω is the angular frequency (2πf), c is the acoustic velocity, μ_l is the liquid viscosity and S_f is the shell friction parameter (Kg/s).

A preferable method to estimate the shell parameters is to measure the attenuation coefficient, α (dB/unit distance). An ultrasound wave experiences energy loss during propagation through a suspension of microbubbles. This attenuation is frequency dependent and is the product of number density (n) and scattering cross-section (σ_s) which are both function of the bubbles' radius r.

$$\sigma_s(r,f) = \frac{4\pi r^2}{\left\{\left(\frac{f_0(r)}{f}\right)^2 - 1\right\}^2 + \delta_{tot}^2(r,f)}, \tag{3}$$

$$\alpha(f) = \frac{10}{\ln(10)} \cdot \sum_r n(r) \cdot \sigma_s(r,f) \cdot \frac{\delta_{tot}(r,f)}{\delta_{rad}(r,f)}, \tag{4}$$

Hence, measuring number density of bubbles one can calculate the attenuation in a certain frequency range using eqn (4). Estimates for shell parameters are obtained by minimizing an error function, defined from the difference of the squares of the experimental and predicted coefficients, with the following form:

$$Err(S_p, S_f) = \sum_i \left(\alpha_{th}(f_i) - \alpha_{\exp}(f_i)\right)^2, \tag{5}$$

This method has been used previously by Gorce et al. (2000), Hoff et al. (2000) and Sarkar et al. (2005) at lower frequency ranges (0.8–8 MHz) to estimate the shell properties of lipid encapsulated contrast agents (SonoVue™, Bracco, Milan, Italy and Sonazoid™, GE Healthcare, Chalfont St. Giles, UK). Also, Goertz et al. (2007) reported the Definity™ shell parameters in the frequency range of 12–28 MHz for 15 min decanted and 2 μm filtered bubbles population.

Agent preparation

In this study, two types of Definity™ vials were used. One vial contained fresh microbubbles while the other contained microbubbles that were 2 years older than the printed expiration date; however, since their purchase they were kept in the fridge at 5°C. For convenience we will address them as "fresh" and "expired" Definity, respectively. The attenuation properties and size distribution of "expired" Definity™ were measured immediately after activation with the Vialmix™ agent activator (Bristol-Myers Squibb Medical Imaging, North Billerica, MA, USA). While for the "fresh" bottle of Definity™, the extraction was done after 5 min decantation.

Activation was performed at room temperature through a mechanical agitation process of 45 s duration according to company guidelines. After activation, two 19-gauge needles were inserted inside the inverted vial, one fully inserted for ventilation and the other up to the middle of the vial to gently extract the agent. Before extracting the agent, the vial was mildly mixed by hand again to avoid any decantation. For the "fresh" Definity™ vial, the sample was extracted from the vial bottom.

A measurement of 0.5 mL of the agent was withdrawn from both "fresh" and "expired" vials and diluted in Isotone II (Coulter Electronics, Luton, UK) in the sample chamber at a dilution ratio of 1:15000. All experiments were conducted at room temperature. To minimize the flotation effects and to ensure all different populations of bubbles interact with ultrasound beam, the agent was mixed with a magnetic stirrer within the chamber.

Measurement set-up

Figure 1 shows the scheme of the set-up for the "expired" bubbles. Attenuation measurements were done using two commercially available transducers (Panametrics Inc., Waltham, MA, USA) as transmitter and receiver aligned coaxially on opposite sides of a water tank. Both transducers had the center frequency of 10 MHz and covered the frequency range of 5 to 15 MHz. Microbubbles were injected in a sample chamber within the center of the water tank. On both sides of the chamber a Mylar window was mounted for optimal transmission of the ultrasound beam. An arbitrary waveform generator (AWG 520; Tektronix, Beaverton, OR, USA) was used to create broadband pulses. On receive, signals were attenuated (30 dB HAT-30; Mini-Circuits Inc.), amplified (AU-1519; Miteq Inc., Hauppauqe, NY, USA) and low pass filtered (BLP-30, Mini-Circuits Inc.). A digital oscilloscope (Tektronix TDS 3014B) and a computer were used for monitoring and recording the signals.

A wideband pulse was employed (25 MHz). The pulse repetition rate was 200 ms, which ensured independent sampling between the recordings. For each experiment, 128 signals were recorded. Recordings started 1 min after agent was diluted in the chamber and the FFT of the recorded signals were averaged. The pressure level used was 107 kPa at focus, measured with a 0.075 mm needle hydrophone (Precision Acoustic Ltd., Dorchester, UK) in a water tank. The experiment was repeated three times for three samples of the same population and the results were averaged.

For the "fresh" sample, protocol for attenuation measurements and data analysis was the same as what has been explained in Goertz et al. (2007). Two sets of measurements were done; each set was done for a different sample from the extraction and consisted of 10 frequency sweeps, spaced at 5-s intervals. The frequency sweeps were comprised of 20 cycles per pulse, with center frequencies ranging from 7–27.5 MHz at 0.5 MHz intervals (25 kPa pressures). The results were averaged for the analysis. These measurements were conducted with a transducer (Panametrics Inc., Waltham, MA, USA) with focal length of 38.1 mm and aperture diameter of 63.5 mm. The path length within chamber to reflector was 21 mm.

Size measurement

A Multisizer III (Beckman Coulter Inc., Fullerton, CA, USA) was used to measure the size distribution of the agent. A 20-μm aperture was used, which is able to measure in the size range of 0.46 to 12 μm with the

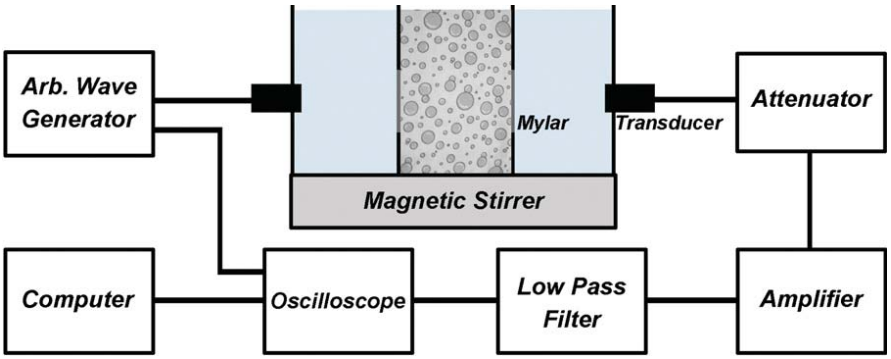

Fig. 1. Scheme of the measurement set-up.

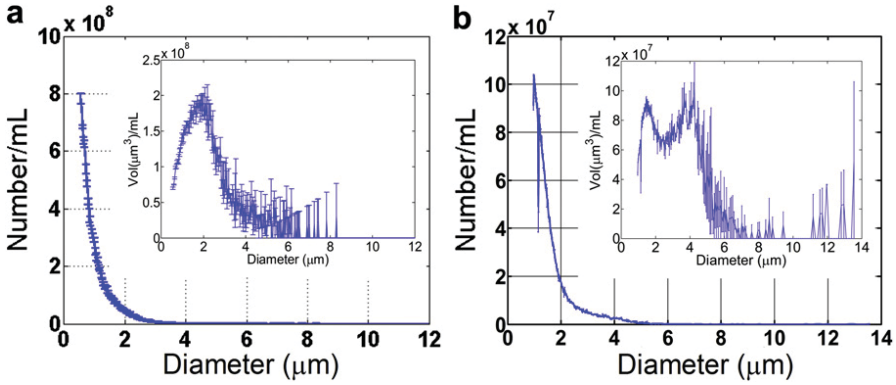

Fig. 2. Coulter counter size distribution measurement for Definity™ in number/mL (inset: vol/mL) for a) "expired" and b) "fresh" bubbles.

resolution (bin size) of 0.039 μm. For the "fresh" vial, a 30-μm aperture was used with the resolution of 0.01 μm. Microbubbles were diluted at the same dilution ratio of 1:15000 as for the acoustic measurements. Each measurement was repeated three times and averaged and background noise was subtracted. Figure 2 shows the output of the Coulter counter, number and volume per unit volume vs. the diameter of bubbles for both samples.

RESULTS

Figure 3 presents the attenuation as a function of frequency for both studied cases. The maximum attenuation for "expired" and "fresh" microbubbles occurs around 8 MHz (1.55 dB/cm) and 18 MHz (1.80 dB/cm), respectively. The two shell parameters S_p and S_f were estimated in the frequency range of 5–15 MHz in Figure 3a and 7–15 MHz in Figure 3b by minimizing the value of the error function in eqn (5). To find the limits for the confidence intervals of the shell stiffness and friction, the parameters S_p and S_f were varied separately until the error function indicator was doubled. The shell parameters are summarized in Table 1. For comparison, all previously published values for S_p and S_f of different contrast agents are also added including the applied frequency range.

DISCUSSION AND SUMMARY

Estimates on shell properties for both "expired" and "fresh" bubbles (Table 1) indicate a similar value for shell stiffness (S_p) compared with previous studies at lower and higher frequencies (Goertz et al. 2007; Gorce et al. 2000; Hoff 2000; Sarkar et al. 2005). The shell friction (S_f) estimates are very close to the reported values of other studied contrast agents at lower frequencies (*e.g.*, SonoVue™ and Sonazoid™, 1–8 MHz). However there is a difference between derived values for S_f in the two studied samples. The source of this difference in the range of 5–15 MHz can be due to the differences in the size distributions presented in Figure 2. The populations of larger bubbles, which were shown before by Goertz et al. (2007) and are still present after 5 min decantation, were not detected in the "expired" Definity™ sample. The size measurements by Coulter counter showed no evidence on the presence of bubbles larger than 3 μm in our samples (Fig. 2a). The absence of larger bubbles, which may be due to the aging of the vial, is the source of different attenuation measurements (Fig. 3). The shell stiffness parameter seems to be independent of the variations in the size distribution but it slightly affects the shell viscosity.

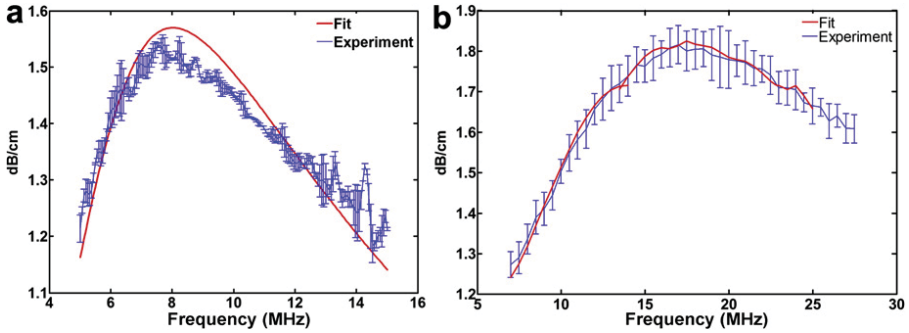

Fig. 3. Attenuation measurements as a function of frequency for Definity™ and the fit made for shell properties estimation on (a) "expired" and (b) "fresh" bubbles.

Table 1. Estimated shell parameters for Definity™ and previously published measurements

Agent	Study	Frequency range (MHz)	S_p (N/m)	$S_f\ 10^{-6}$ (kg/s)
SonoVue™	Gorce et al. (2000)	0.8–3	1.1	0.27
SonoVue™ (10' dec.)	Gorce et al. (2000)	3–7	1.1	0.56
Sonazoid™	Hoff (2000)	1.5–8	1.2 ± 0.07	0.48 ± 0.06
Sonazoid™	Sarkar et al. (2005)	2–8	1.25 ± 0.24	0.60 ± 0.18
Definity™ (15' dec.)	Goertz et al. (2007)	12–28	1.71 ± 0.24	0.015 ± 0.015
Definity™ (2 μm filt.)	Goertz et al. (2007)	13–29	1.51 ± 0.36	0.016 ± 0.016
Definity™ "Expired"	present	5–15	1.58 ± 0.16	0.44 ± 0.03
"Fresh"	present	7–15	1.64 ± 0.33	0.15 ± 0.02
	present	15–25	2.04 ± 0.67	0.01 ± 0.01

Moreover, the estimated values of S_f in the frequency range of 5–15 MHz are one order of magnitude higher than the estimations done by Goertz et al. (2007) on Definity™ at higher frequencies (12–28 MHz). This difference can originate from the frequency dependence of shell properties. To test this hypothesis, we estimated the values of S_p and S_f of the same "fresh" Definity™ sample in higher frequencies between 15–25 MHz. The values are presented in Table 1. The shell parameters in this frequency range are consistent with previously reported values in the similar frequency range (12–28 MHz, Goertz et al. 2007). These results are in line with the findings of Van der Meer et al. (2007) who observed the effect of shear thinning in phospholipid contrast agents. In Goertz et al. (2007), it was hypothesized that the lower shell viscosities estimated for smaller bubbles at higher frequencies could be due to two factors. First, shear thinning at higher excitation frequencies may lead to a reduction in the estimated shell viscosity. A second factor may be size-dependant shell properties, possibly due to issues related to shell microstructure, inhomogeneities and thickness. Regardless of the origin, it was proposed that the reduction in viscosity may be a key factor in enabling the generation of nonlinear oscillations in small bubbles at high frequencies.

In conclusion, the shell viscosities estimated in this study for 5–15 MHz were greater than previous reported values in higher frequencies (12–28 MHz). We propose that this difference originates from the frequency and size dependence of rheological properties of the lipid shell.

Acknowledgments—The authors would like to thank Emma Huo for performing measurements and analysis on "fresh" Definity™, Miranda Harteveld for her assistance with Coulter counter measurements and Marcia Emmer and Rik Vos for inspiring discussions.

REFERENCES

Cachard C, Finet G, Bouakaz A, Tabib A, Francon D, Gimenez G. Ultrasound contrast agent in intravascular echography: An *in vitro* study. Ultrasound Med Biol 1997;23:705–717.

Church CC. The effects of an elastic solid surface layer on the radial pulsations of gas bubbles. J Acoust Soc Am 1995;97:1510–1521.

Crouse JR III, Grobbee DE, O'Leary DH, Bots ML, Evans GW, Palmer MK, Riley WA, Raichlen JS. Measuring effects on intima media thickness: An evaluation of rosuvastatin in subclinical atherosclerosis–The rationale and methodology of the METEOR study. Cardiovasc Drugs Ther 2004;18:231–238.

De Jong N, Hoff L, Skotland T, Bom N. Absorption and scatter of encapsulated gas filled microspheres: Theoretical considerations and some measurements. Ultrasonics 1992;30:95–103.

Demos SM, Alkan-Onyuksel H, Kane BJ, Ramani K, Nagaraj A, Greene R, Klegerman M, McPherson DD. *In vivo* targeting of acoustically reflective liposomes for intravascular and transvascular ultrasonic enhancement. J Am Coll Cardiol 1999;33:867–875.

Deng CX, Lizzi FL, Silverman RH, Ursea R, Coleman DJ. Imaging and spectrum analysis of contrast agents in the *in vivo* rabbit eye using very-high-frequency ultrasound. Ultrasound Med Biol 1998;24:383–394.

Feinstein SB. The powerful microbubble: From bench to bedside, from intravascular indicator to therapeutic delivery system, and beyond. Am J Physiol Heart Circ Physiol 2004;287:H450–H457.

Frinking PJ, de Jong N. Acoustic modeling of shell-encapsulated gas bubbles. Ultrasound Med Biol 1998;24:523–533.

Goertz DE, Cherin E, Needles A, Karshafian R, Brown AS, Burns PN, Foster FS. High frequency nonlinear B-scan imaging of microbubble contrast agents. IEEE Trans Ultrason Ferroelectr Freq Control 2005;52:65–79.

Goertz DE, Frijlink ME, de Jong N, van der Steen AF. Nonlinear intravascular ultrasound contrast imaging. Ultrasound Med Biol 2006;32:491–502.

Goertz DE, de Jong N, van der Steen AFW. Attenuation and size distribution measurements of Definity™ and manipulated Definity™ populations. Ultrasound Med Biol 2007;33:1376–1388.

Gorce JM, Arditi M, Schneider M. Influence of bubble size distribution on the echogenicity of ultrasound contrast agents: A study of SonoVue. Invest Radiol 2000;35:661–671.

Hayden MR, Tyagi SC. Vasa vasorum in plaque angiogenesis, metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: A malignant transformation. Cardiovasc Diabetol 2004;3:1–16.

Hoff L. Acoustic characterization of contrast agents for medical ultrasound imaging. PhD. Thesis. Trondheim, Norway: Norwegian University of Science and Technology; 2000.

Hoff L, Sontum PC, Hovem JM. Oscillations of polymeric microbubbles: Effect of the encapsulating shell. J Acoust Soc Am 2000;107:2272–2280.

O'Leary DH, Polak JF. Intima-media thickness: A tool for atherosclerosis imaging and event prediction. Am J Cardiol 2002;90:18L–21L.

Plesset MS, Prosperetti A. Bubble dynamics and cavitation. Ann Rev Fluid Mech 1977;9:145–185.

Sarkar K, Shi WT, Chatterjee D, Forsberg F. Characterization of ultrasound contrast microbubbles using *in vitro* experiments and viscous and viscoelastic interface models for encapsulation. J Acoust Soc Am 2005;118:539–550.

Van der Meer SM, Dollet B, Voormolen MM, Chin CT, Bouakaz A, de Jong N, Versluis M, Lohse D. Microbubble spectroscopy of ultrasound contrast agents. J Acoust Soc Am 2007;121:648–656.