Interfacial rheological properties of contrast microbubble Targestar P as a function of ambient pressure

Krishna N Kumar and Kausik Sarkar,
Department of Mechanical and Aerospace Engineering
The George Washington University
Washington DC 20052

Author to whom the correspondence should be addressed: Kausik Sarkar

Postal Address:

The George Washington University
800 22nd Street NW
Suite 3000
Washington DC 20052

Telephone: +1-(202)-994 2724

FAX: +1-(202) 994 0238

Email: sarkar@gwu.edu

Abstract

In this brief note, we determine the interfacial rheological parameters of the encapsulation of the contrast agent Targestar-P using ultrasound attenuation. The characteristic parameters are obtained according to two different interfacial rheological models. The properties—surface dilatational elasticity (0.09 ± 0.01 N/m) and surface dilatational viscosity ($8 \pm 0.1 \text{E-}9$ Ns/m)—are found to be of similar magnitude for both models. Contrast microbubbles experience different ambient pressure in different organs. We also measure these parameters as functions of ambient pressure using attenuation measured at different overpressures (0, 100 and 200 mm Hg). For each value of ambient hydrostatic pressure, we determine the rheological properties, accounting for changes in the size distribution due to the pressure change. We discuss different models of size distribution change under overpressure—pure adiabatic compression or gas exchange with surrounding medium. The dilatational surface elasticity and viscosity are found to increase with increasing ambient pressure.

Keywords: Contrast agents, interfacial rheology, encapsulation, shell, pressure estimation, bubble dynamics, attenuation, gas diffusion

Introduction

Use of lipid-, protein- or polymer-coated microbubbles—such as Optison (GE Healthcare, Oslo, Norway), Sonazoid (GE Healthcare, Oslo, Norway), Definity (Lantheus Imaging, N. Billerica, MA), Sonovue (Bracco), Targestar (Targeson Inc., San Diego, CA)—have led to significant enhancement in the quality of ultrasound imaging (Goldberg, Raichlen 2001, Nahire, Haldar 2014, Nahire, Haldar 2013, Paul, Nahire 2014). They all differ in size and coating and require careful characterization. Unlike Targestar, others mentioned above have been approved for clinical use in the US and Europe, and have been characterized using controlled *in vitro* acoustic experiments determining the mechanical properties of the coating, e.g. Definity (Goertz, de Jong 2007), Optison (Chatterjee and Sarkar 2003), Sonazoid (Hoff 2001, Paul, Katiyar 2010, Sarkar, Shi 2005) and Sonovue (Gorce, Arditi 2000). In contrast, Targestar has not been similarly characterized. Here, we measure the mechanical properties of Targestar contrast agent under different ambient hydrostatic pressures using *in vitro* broadband attenuation.

Targestar microbubbles have a perfluorobutane (PFB) gas core encapsulated by a layer of phospholipids (Shekhar, Awuor 2014). They been investigated for applications in high frequency subharmonic imaging (Shekhar and Doyley 2011, Shekhar and Doyley 2012, Shekhar and Doyley 2013, Shekhar, Rychak 2013) and molecular imaging (Knowles, Heath 2012, Saini, Warram 2011, Wang, Hagemeyer 2012, Warram, Sorace 2011). Targestar SA, Targestar P, Targestar HF, Visistar are few available variants of this microbubble each having different applications such as perfusion, labeling, targeting etc. Here, we investigate Targestar P agent, a non-targeted perfusion contrast agent designed to enhance and quantify blood flow.

Contrast agents experience widely different pressures in different organs—5-10 mmHg in portal vein (D'amico, Pagliaro 1995), 8-20 mmHg in pulmonary artery (Simonneau, Robbins

2009) and 100-140 mmHg in left ventricle (systolic) (Lawes, Vander Hoorn 2004). This motivates us to measure the properties of Targestar P under different ambient pressure in the range 0-200 mmHg. Previously, Hoff (Hoff 2001) investigated stability of encapsulated microbubbles under enhanced ambient pressure through attenuation measurement. Recently, we have performed a detailed characterization of Definity microbubbles determining pressure dependent shell properties using attenuation measured at different pressures (Kumar and Sarkar 2015). In this note, a similar procedure is followed for Targestar.

We will use ultrasound attenuation through a suspension of contrast agent Targestar under different ambient pressures to determine the interfacial rheological properties of the encapsulation using two different models due to strain-softening exponential elasticity model (EEM) (Paul, Katiyar 2010) and Marmottant model (Marmottant, van der Meer 2005).

Materials and Methods

A. Contrast agent

Targestar P agent was procured from Targeson Inc., (San Diego, CA), delivered in suspension from in glass vials with a perfluorobutane headspace. The agent was stored at 4° C, in accordance with the product specifications. Before use, the vial is shaken very gently 2-3 times to mix and then 10 μ l was extracted from the vial using a syringe and mixed in 100 ml of standard PBS solution. The resulting dispersion has 2.54×10^5 /mL bubbles (according to the information supplied by the vendor).

B. Estimating the encapsulation parameters using attenuation

In our earlier publications (Chatterjee and Sarkar 2003, Kumar and Sarkar 2015, Paul, Katiyar 2010, Paul, Russakow 2013, Sarkar, Shi 2005) we have described the procedure in detail for determining the encapsulation properties of an encapsulation based on different models using attenuation data. Here, attenuation is measured in an air-tight set-up that can control the ambient pressure with an accuracy of 0.07 kPa (Figure 1a and b) using an unfocused broadband transducer (Olympus NDT, Waltham, MA, USA) with a central frequency of 3.5 MHz (-6dB bandwidth 2.5 to 4.99 MHz). It is excited by a pulser/receiver (Model 5800; Panametrics-NDT, Waltham, MA, USA) to produce a broadband pulse of PRF of 100 Hz with peak amplitude of 35 kPa at 3.5 MHz, low enough so that the acoustic propagation through the microbubble suspension remains linear (Chatterjee, Jain 2005, Chatterjee, Sarkar 2005). The pulse traveled a total distance of 10 cm through the contrast agent suspension before being received and fed to the digital oscilloscope (Model TDS 2012; Tektronix, Beaverton, OR, USA) to observe the signal in real time. A pressure gauge (SSI Technologies, Janesville, WI, USA) was used to measure the static pressure of the chamber. Signals were acquired from the oscilloscope using LabView (National Instruments, Austin, TX, USA) software. Fifty voltage-time RF traces were acquired in an averaging mode (64 sequences are used for averaging) and saved. They are transformed to frequency domain and averaged for 50 acquisitions. The frequency dependent attenuation coefficient was calculated using the following expression

$$\alpha(\omega) = 20 \log_{10} \left(\frac{\bar{V}_{ref}(\omega)}{\bar{V}_{sig}(\omega)} \right) / d, \quad (1)$$

where $\bar{V}_{ref}(\omega)$ is the averaged response in the frequency domain without any contrast agent in the medium, $\bar{V}_{sig}(\omega)$ is the averaged response in the frequency domain microbubbles suspended in the medium, and $d=10$ cm is the total path traveled by the pulse before it is being received by

108 the transducer. Theoretically an expression for attenuation is obtained using a linearized version
 109 of the modified Rayleigh-Plesset equation with an effective surface tension $\gamma(R)$ and interfacial
 110 dilatational viscosity $\kappa^s(R)$ (Paul, Katiyar 2010) for bubble radius $R(t)$:

$$111 \quad \rho \left(R\ddot{R} + \frac{3}{2} \dot{R}^2 \right) = P_g \left(1 - 3\kappa \frac{\dot{R}}{c} \right) - 4\mu \frac{\dot{R}}{R} - \frac{4\kappa_s(R)\dot{R}}{R^2} - \frac{2\gamma(R)}{R} - P_0 + P_A \sin \omega t. \quad (2)$$

112 Here, ρ is the density of the liquid, P_g is the gas pressure inside the bubble which expands with
 113 a polytropic coefficient κ , c is the speed of sound in the liquid, μ is its viscosity, and P_0 is the
 114 ambient pressure. Since with oscillations at MHz frequency Peclet number $Pe = R_0^2 \omega / D_g \gg 1$ (
 115 D_g is the thermal diffusivity; for C_4F_{10} $2.57 \times 10^{-6} \text{m}^2/\text{s}$), we assume an adiabatic behavior for the
 116 gas inside ($\kappa = \kappa_{ad} = 1.07$ for C_4F_{10}). The bubble is responding to an ultrasound wave with
 117 amplitude P_A and circular frequency ω . EEM uses

$$118 \quad \gamma(R) = \gamma_0 + E^s \beta, \quad \beta = (R/R_E)^2 - 1, \quad \text{and } \kappa^s(R) = \kappa^s(\text{constant}), \quad (3)$$

$$E^s = E_0^s \exp(-\alpha^s \beta),$$

119 characterizing the encapsulation with a reference surface tension γ_0 , reference dilatational
 120 elasticity E_0^s and a coefficient α^s . R_E is the stressfree radius. Marmottant model uses

$$121 \quad \gamma(R) = \begin{cases} 0 & \text{for } R \leq R_{buckling} \\ \chi \left(\frac{R^2}{R_{buckling}^2} - 1 \right) & \text{for } R_{buckling} \leq R \leq R_{rupture} \text{ and } \kappa^s(R) = \kappa^s(\text{constant}), \\ \gamma_w & \text{for } R \geq R_{rupture} \end{cases} \quad (4)$$

122 where χ [same as E^s in (3)] is the elastic modulus of the shell, $R_{buckling} = R_0 \left[1 + \gamma(R_0) / \chi \right]^{-1/2}$
 123 and $R_{rupture} = R_{buckling} \left[1 + \gamma_w / \chi \right]^{1/2}$. Above $R_{rupture}$, the bubble is assumed to have a pure air-water

124 interface and below $R_{buckling}$, it is in a buckled state where the effective interfacial tension is zero.

125 Attenuation through a suspension of contrast agent $\theta(\omega)$ is computed as

$$126 \quad \theta(\omega) = 10 \log_{10} e \int_{R_{min}}^{R_{max}} \sigma_e(R; \omega) n(R) dR, \quad \sigma_e = 4\pi R_0^2 \frac{c\delta}{\omega_0 R_0} \frac{\Omega^2}{[(1-\Omega^2)^2 + \Omega^2 \delta^2]}, \quad \Omega = \frac{\omega_0}{\omega} \quad (5)$$

127 where $n(R)dR$ is the number of bubbles per unit volume with radius in the range $(R, R+dR)$, and

128 the range of bubble radii is given by (R_{min}, R_{max}) . The nondimensional damping term δ is given

129 by

$$\begin{aligned} \delta &= \delta_{liquid} + \delta_{encapsulation} + \delta_{radiation} + \delta_{thermal} \\ &= \frac{4\mu}{\rho\omega_0 R_0^2} + \frac{4\kappa^s}{\rho\omega_0 R_0^3} + \frac{3\kappa P_0}{\rho\omega_0 R_0 c} + \frac{1}{\omega\omega_0} \frac{3P_0}{\rho R_0^2} \text{Im} \left(\frac{1}{\Phi(R_0, \omega)} \right), \\ 130 \quad \Phi(R_0, \omega) &= \frac{1}{\kappa_{ad}} \left(1 + \frac{3(\kappa_{ad} - 1)}{X^2} (X \coth X - 1) \right), \\ X(R_0, \omega) &= R_0 \sqrt{\frac{i\omega}{D_g}}. \end{aligned} \quad (6)$$

131 An error function between the measured and the modeled attenuation is minimized to find the

132 interfacial rheological parameters, e.g. $\gamma, \kappa^s, E^s, \alpha^s$ etc (Sarkar, Shi 2005). The bubble count of

133 2.54×10^5 /mL with average bubble diameter of 2.2 μm indicates a bubble-bubble separation of

134 ~ 70 diameter. This validates the fundamental assumption underlying the attenuation analysis of

135 no interactions between individual bubbles. The linear dependence of attenuation with bubble

136 concentration has been previously investigated (Sarkar, Shi 2005).

137

138 ***C. The size distribution and changes with ambient pressure***

139 Figure 2 shows size distribution of Targestar microbubbles measured at atmospheric

140 pressure (0 mmHg of overpressure) provided by the manufacturer. The measurement could not

resolve bubble less than one micrometer in diameter. It contains 2.54×10^9 /mL bubbles with an
 average diameter of 2.2 μm . The size distribution is similar to the one obtained by (Shekhar and
 Doyley 2012). As noted above the dilution in PBS decreases the count to 2.54×10^5 /mL.
 Attenuation measured at 0 mm Hg after temporarily subjecting the suspension to 200 mm Hg for
 3 minutes showed result similar to that obtained without any pressure change indicating little
 irreversible destruction of microbubbles under pressure change (Kumar and Sarkar 2015). An
 identical conclusion was drawn by Hoff through similar attenuation measurement (Hoff 2001)).
 However microbubbles can reversibly change size with pressure change due to mechanical
 compression or gas diffusion. Sudden increase of overpressure leads to a transient disequilibrium
 which can give rise to gas diffusion process from/into the microbubble. While the air
 concentration far away from the bubble is determined by the saturation level at atmospheric
 pressure p_{atm} , concentration near the bubble interface is higher in equilibrium with the gas inside
 the bubble. We have previously developed a mathematical model for gas diffusion from
 encapsulated microbubbles (Borden and Longo 2002, Epstein and Plesset 1950, Katiyar, Sarkar
 2009, Sarkar, Katiyar 2009). Note that gas diffusion critically depends on the gas permeability
 through the encapsulation. The encapsulating shell is definitely more permeable to air than PFB
 due to the larger size of PFB molecules. We will assume that within the experimental time, due
 to its larger size and lower solubility, there is negligible diffusion of PFB, and the size change is
 effected only by air diffusion—below we refer this as model A1. However for comparison, we
 also consider two other models. In model A2, we completely ignore gas diffusion and assume
 adiabatic compression under pressure as was done by others (Frinking, Gaud 2010, Tremblay-
 Darveau, Williams 2014). The third case A3 considers a completely air-filled microbubble. Note
 that even with small amount of PFB diffusion, eventually all PFB will be replaced by air (Paul,

Katiyar 2010, Sarkar, Shi 2005). Although, within the time of the experiment, it is unlikely that such a state will be reached we consider it just for comparison.

With initial condition of $\dot{R} = \ddot{R} = 0$ equation (2) reduces to an equation for the initial gas pressure inside a bubble of initial radius R_0

$$P_{g0} = \frac{2\gamma(R_0)}{R_0} + P_0, \quad P_0 = p_{atm} + p_{over} . \quad (7)$$

A1) Bubble retains PFB and air diffuses out: Here we assume PFB content remains unchanged. At any instant the net gas pressure inside the microbubble described by (7), comprising of air and PFB at partial pressures p_A and p_F ,

$$P_{g0} = p_A + p_F = \frac{2\gamma(R)}{R_0} + p_{atm} + p_{over} . \quad (8)$$

At each overpressure level, the dissolved air concentration far away from bubble is $C_A(\infty)$ and in the liquid adjacent to the microbubble, containing air at pressure p_A is $C_A(R)$. They are given by Henry's law:

$$C_A(\infty) = L_A \frac{p_{atm}}{R_G T}, \quad C_A(R) = L_A \frac{p_A}{R_G T} . \quad (9)$$

L_A is the Ostwald coefficient relating dissolved concentration to adjacent gas concentration. R_G and T are the universal gas constant and the temperature respectively. After overpressure change, gas exchange and accompanying diffusion leads to a new equilibrium $C_A(\infty) = C_A(R)$ obtaining $p_A = p_{atm}$. At zero overpressure with initial radius is R_0^0 , Equation (8) therefore gives rise to

$$p_F = \frac{n_F R_G T}{\frac{4}{3} \pi (R_0^0)^3} = \frac{2\gamma(R_0^0)}{R_0^0} = \frac{2\gamma_0}{R_0^0} \quad (10)$$

The number of moles n_F of PFB remains constant. Unlike our previous publications, here we cannot assume $\gamma(R_0^0) = 0$. Instead, we assume $R_E = R_0^0$, i.e. a stress-free condition, which leads to the second equality in (10). At nonzero overpressure one obtains

$$p_F = \frac{n_F R_G T}{\frac{4}{3} \pi (R_0)^3} = \frac{2\gamma(R_0)}{R_0} + p_{over} \quad \text{or} \quad (11)$$

$$\frac{2\gamma_0}{R_0^0} \left(\frac{R_0^0}{R_0} \right)^3 = \frac{2}{R_0} \left(\gamma_0 + E^s \left(\left(\frac{R_0}{R_0^0} \right)^2 - 1 \right) \right) + p_{over}.$$

Solving it one obtains R_0 at new overpressure p_{over} . $\gamma(R_0)$ is expressed as in (3) using values of γ_0 and E^s obtained at zero overpressure as an approximation. This approximation is used in all three approaches. After interfacial rheological properties are estimated at nonzero overpressure, they are then used to iteratively improve this approximation to find little difference in result.

192

193

A2) *No gas exchange*: From equation (7), at $p_{over} = 0$, the initial gas pressure as $P_{g0}^0 = p_{atm}$, where $\gamma(R_0^0)$ is assumed to be zero at initial radius R_0^0 . Assuming an adiabatic process for the gas inside the bubble, the initial bubble radius would change from R_0^0 to R_0 when subjected to nonzero overpressure according to

$$\frac{2}{R_0} \left(\gamma_0 + E^s \left(\left(\frac{R_0}{R_E} \right)^2 - 1 \right) \right) + p_{atm} + p_{over} = p_{atm} \left(\frac{R_0^0}{R_0} \right)^{3\kappa} \quad (12)$$

Solving at nonzero p_{over} , one obtains the new radius.

A3. Air-filled bubble exchanging air with medium: Here we assume that all PFB had diffused out leaving air-filled microbubbles. At $p_{over} = 0$, assuming a steady bubble warrants $\gamma(R_0^0) = 0$. At other overpressures

$$\frac{2\gamma(R_0)}{R_0} + p_{over} = 0 \quad \text{or} \quad \frac{2}{R_0} \left(\gamma_0 + E^s \left(\left(\frac{R_0}{R_E} \right)^2 - 1 \right) \right) + p_{over} = 0 \quad (13)$$

which is solved for obtaining R_0 .

Figure 3 shows predicted change in radius when bubbles are subjected to 100 and 200 mm Hg overpressures using all three models. Model A2 that assumes no gas exchange results in least amount of decrease in radius. Model A3 that assumes purely air bubbles gives rise to the maximum radius change. They give rise to different modifications of the size distribution shown in Figure 2. They are all used to obtain the rheological properties.

Results and Discussion

Rheological parameters at different overpressures

Figure 4 plots the measured attenuation curves at three different overpressures. The attenuation decreases with the increase of ambient overpressure till frequency 3.75 MHz beyond which attenuation increases with increasing overpressure. We also show the fitted curves used to obtain the rheological parameters according to the EEM model. The fitting is similar for MM model. The properties are listed in Table 1 for EEM and Table 2 for MM. First note the

similarity between the MM and the EEM models for all pressures and different approaches of radius change, i.e. similar values of κ^s for both models and similar values of E^s (EEM) and χ (MM). The similarity in property values across different models demonstrates robustness of the approach. Also note that the linear theory underlying attenuation computation linearizes the bubble dynamics leading to very similar forms for both models.

We first note that the surface dilatational elasticity E^s value of Targestar-P microbubble is rather low 0.09N/m compared to other lipid coated microbubbles such as Sonazoid ($\sim 0.5\text{N/m}$) (Sarkar, Shi 2005), Sono Vue ($\sim 0.55\text{N/m}$) (van der Meer, Dollet 2007), Definity (0.5-2.5N/m) (Faez, Goertz 2011, Helfield and Goertz 2013, Kumar and Sarkar 2015, Raymond, Haworth 2014) or MicromarkerTM (3-5N/m) (Helfield and Goertz 2013). The surface dilatational viscosity value $\kappa^s \sim 8 \times 10^{-9}$ Ns/m is similar to that of Sono Vue (5×10^{-9} Ns/m) (van der Meer, Dollet 2007) and smaller than Sonazoid's (1.2×10^{-8} Ns/m) (Sarkar, Shi 2005) or Definity's (4×10^{-8} Ns/m) (Kumar and Sarkar 2015). Note that the attenuation (measured with a transducer with nominal central frequency 3.5 MHz) is limited to the frequency range lower than $\sim 6\text{MHz}$ (Figure 4). The shell properties listed here therefore are representative of this frequency range and may substantially differ outside, specifically above, this range. Also note that contrast agents show considerable variability from batch to batch. The properties estimated here are based on experiments performed on the particular vials of contrast microbubbles. The relatively low value of the surface elasticity leads to a low resonance frequency—from the attenuation curve the size averaged resonance at 0mm Hg is $\sim 2.2\text{MHz}$.

Figure 5 plots the variations of surface dilatational elasticity (Figure 5a) and surface dilatational viscosity (Figure 5b) with increasing pressure. We first look at the model of our choice A1, where we assume that PFB content remains constant and the bubble shrinks because

242 of air egress. We find that both E^s and κ^s increase with increasing ambient pressure. Note that
 243 the resonance frequency of a microbubble increases with increasing E^s . Therefore, the increasing
 244 trend of E^s with overpressure can be related to the upward shift of the peak of the attenuation in
 245 Figure 4. For a mono-dispersed suspension of microbubbles the peak of the attenuation curve
 246 occurs at the resonance frequency. For a poly-dispersed suspension, the peak represents a
 247 weighted average resonance frequency of the suspension. The weighted resonance frequency in
 248 Figure 4 increases from 2.2 MHz at 0 mm Hg to 2.4 MHz at 100 mm Hg and 2.6 MHz at 200
 249 mm Hg. In in vitro attenuation measurement, Hoff (2001) found that for lipid coated Sonazoid
 250 agent, the resonance frequency (peak in the attenuation) increases with overpressure
 251 increase—similar to the observation here for Targestar—but for polymeric microbubbles, it
 252 decreases with overpressure. The opposite trend for polymeric microbubble was surmised to be
 253 caused by curling/buckling of the stiff shell. Recently Tremblay-Darveau et al (2014) saw a
 254 similar increase in resonance frequency in backscattered signals from phospholipid coated agent
 255 under overpressure. They too ascribed the observation to pressure induced shell buckling. When
 256 one assumes no gas exchange but just mechanical compression under ambient pressure increase
 257 (curve for A2), the variation is similar but higher in magnitude. Note that this assumption, as
 258 noted before, has been used previously (Frinking, Gaud 2010, Tremblay-Darveau, Williams
 259 2014). The property values for this assumption are the highest arising from the minimum relative
 260 change in size distribution under A2 in Figure 4. The low value of surface elasticity is
 261 responsible for the relatively large decrease in radius under pressure increase. Finally, when one
 262 assumes the unlikely case of a fully air-filled microbubble (A3), one finds very little change in
 263 E^s and a decreasing trend in κ^s with increasing overpressure. We parenthetically note that this

wide variance in results underscore the importance of choosing the right model and limitation of the technique in absence of a right size distribution.

Conclusion

We use broadband attenuation of ultrasound measured at different hydrostatic pressures to estimate shell properties of Targestar contrast agent. Two interfacial rheological models—strain-softening exponential elasticity model (EEM) and Marmottant model (MM)—were applied to the attenuation data. The bubble size decrease with static pressure increase was accounted for using different gas diffusion models. Different models of encapsulation gave rise to similar shell property values but different assumption about gas diffusion results in different pressure dependence of interfacial properties. The dilatational elasticity increases from ~ 0.1 N/m at 0 mm Hg to ~ 0.2 - 0.3 N/m at 200 mm Hg, while the dilatational viscosity increases from $\sim 8 \times 10^{-9}$ Ns/m to $\sim 10 \times 10^{-9}$ Ns/m for the same pressure values. The property increase with increasing pressure arises due to bubble size decrease that causes contraction of the encapsulation and enhanced association of the lipid molecules.

Acknowledgement

This work is partially supported by NSF Grants No. CBET-1205322, DMR-1239105 and GWU Katzen Cancer Research Center Innovative Cancer Pilot Research Award. We also gratefully acknowledge Targeson and Dr. Joshua Rychack for the contrast microbubbles used here.

References

- Borden MA, Longo ML, Dissolution behavior of lipid monolayer-coated, air-filled microbubbles: Effect of lipid hydrophobic chain length. *Langmuir* 2002; 18:9225-33.
- Chatterjee D, Jain P, Sarkar K, Ultrasound-mediated destruction of contrast microbubbles used for medical imaging and drug delivery. *Physics of Fluids* 2005; 17:100603.
- Chatterjee D, Sarkar K, A Newtonian rheological model for the interface of microbubble contrast agents. *Ultrasound Med. Biol.* 2003; 29:1749-57.
- Chatterjee D, Sarkar K, Jain P, Schreppler NE, On the suitability of broadband attenuation measurement for characterizing contrast microbubbles. *Ultrasound Med. Biol.* 2005; 31:781-86.
- D'amico G, Pagliaro L, Bosch J, The treatment of portal hypertension: A meta-analytic review. *Hepatology* 1995; 22:332-54.
- Epstein PS, Plesset MS, On the Stability of Gas Bubbles in Liquid-Gas Solutions. *Journal of Chemical Physics* 1950; 18:1505-09.
- Faez T, Goertz D, De Jong N, Characterization of Definity (Tm) Ultrasound Contrast Agent at Frequency Range of 5-15 Mhz. *Ultrasound Med. Biol.* 2011; 37:338-42.
- Frinking PJA, Gaud E, Brochot J, Arditi M, Subharmonic scattering of phospholipid-shell microbubbles at low acoustic pressure amplitudes. *IEEE Transactions on Ultrasonics Ferroelectrics and Frequency Control* 2010; 57:1762.
- Goertz DE, de Jong N, van der Steen AFW, Attenuation and size distribution measurements of definity (TM) and manipulated definity (TM) populations. *Ultrasound Med. Biol.* 2007; 33:1376-88.
- Goldberg BB, Raichlen JS, Forsberg F. *Ultrasound Contrast Agents: Basic Principles and Clinical Applications*. London: Martin Dunitz 2001.
- Gorce JM, Arditi M, Schneider M, Influence of bubble size distribution on the echogenicity of ultrasound contrast agents - A study of SonoVue (TM). *Investigative Radiology* 2000; 35:661-71.
- Helfield BL, Goertz DE, Nonlinear resonance behavior and linear shell estimates for Definity (TM) and MicroMarker (TM) assessed with acoustic microbubble spectroscopy. *J. Acoust. Soc. Am.* 2013; 133:1158-68.
- Hoff L. *Acoustic Characterization of Contrast Agents for Medical Ultrasound Imaging*. Norwell: Kluwer Academic 2001.
- Katiyar A, Sarkar K, Jain P, Effects of Encapsulation Elasticity on the stability of an Encapsulated Microbubble. *Journal of Colloid and Interface Science* 2009; 336:519-25.
- Knowles JA, Heath CH, Saini R, Umphrey H, Warram J, Hoyt K, Rosenthal EL, Molecular targeting of ultrasonographic contrast agent for detection of head and neck squamous cell carcinoma. *Archives of Otolaryngology-Head & Neck Surgery* 2012; 138:662-68.
- Kumar KN, Sarkar K, Effects of ambient hydrostatic pressure on the material properties of the encapsulation of an ultrasound contrast microbubble. *J. Acoust. Soc. Am.* 2015; 138:624-34.
- Lawes CM, Vander Hoorn S, Law MR, Elliott P, MacMahon S, Rodgers A, High blood pressure. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization 2004:281-390.

Marmottant P, van der Meer S, Emmer M, Versluis M, de Jong N, Hilgenfeldt S, Lohse D, A model for large amplitude oscillations of coated bubbles accounting for buckling and rupture. *J. Acoust. Soc. Am.* 2005; 118:3499-505.

Nahire R, Haldar MK, Paul S, Ambre AH, Meghnani V, Layek B, Katti KS, Gange KN, Singh J, Sarkar K, Mallik S, Multifunctional polymersomes for cytosolic delivery of gemcitabine and doxorubicin to cancer cells. *Biomaterials* 2014; 35:6482-97.

Nahire R, Haldar MK, Paul S, Mergoum A, Ambre AH, Katti KS, Gange KN, Srivastava DK, Sarkar K, Mallik S, Polymer-Coated Echogenic Lipid Nanoparticles with Dual Release Triggers. *Biomacromolecules* 2013; 14:841-53.

Paul S, Katiyar A, Sarkar K, Chatterjee D, Shi WT, Forsberg F, Material characterization of the encapsulation of an ultrasound contrast microbubble and its subharmonic response: Strain-softening interfacial elasticity model. *J. Acoust. Soc. Am.* 2010; 127:3846-57.

Paul S, Nahire R, Mallik S, Sarkar K, Encapsulated microbubbles and echogenic liposomes for contrast ultrasound imaging and targeted drug delivery. *Comput. Mech.* 2014; 53:413-35.

Paul S, Russakow D, Rodgers T, Sarkar K, Cochran M, Wheatley MA, Determination of the interfacial rheological properties of a Poly(DL-lactic acid)-encapsulated contrast agent using in vitro attenuation and scattering. *Ultrasound Med. Biol.* 2013; 39:1277-91.

Raymond JL, Haworth KJ, Bader KB, Radhakrishnan K, Griffin JK, Huang SL, McPherson DD, Holland CK, BROADBAND ATTENUATION MEASUREMENTS OF PHOSPHOLIPID-SHELLED ULTRASOUND CONTRAST AGENTS. *Ultrasound Med. Biol.* 2014; 40:410-21.

Saini R, Warram JM, Sorace AG, Umphrey H, Zinn KR, Hoyt K, Model system using controlled receptor expression for evaluating targeted ultrasound contrast agents. *Ultrasound in medicine & biology* 2011; 37:1306-13.

Sarkar K, Katiyar A, Jain P, Growth and dissolution of an encapsulated contrast microbubble. *Ultrasound Med. Biol.* 2009; 35:1385-96.

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; 118:539-50.

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. *The Journal of the Acoustical Society of America* 2005; 118:539-50.

Shekhar H, Awuor I, Thomas K, Rychak JJ, Doyley MM, The delayed onset of subharmonic and ultraharmonic emissions from a phospholipid-shelled microbubble contrast agent. *Ultrasound in medicine & biology* 2014; 40:727-38.

Shekhar H, Doyley MM, A coded excitation technique for the functional imaging of coronary atherosclerosis using ultrasound contrast agents. *The Journal of the Acoustical Society of America* 2011; 129:2610-10.

Shekhar H, Doyley MM, Improving the sensitivity of high-frequency subharmonic imaging with coded excitation: A feasibility study. *Medical physics* 2012; 39:2049-60.

Shekhar H, Doyley MM, The response of phospholipid-encapsulated microbubbles to chirp-coded excitation: Implications for high-frequency nonlinear imaging. *The Journal of the Acoustical Society of America* 2013; 133:3145-58.

Shekhar H, Rychak JJ, Doyley MM, Modifying the size distribution of microbubble contrast agents for high-frequency subharmonic imaging. *Medical physics* 2013; 40:082903.

374 Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, Elliott CG,
 375 Gaine SP, Gladwin MT, Jing Z-C, Updated clinical classification of pulmonary
 376 hypertension. *Journal of the American College of Cardiology* 2009; 54:S43-S54.
 377 Tremblay-Darveau C, Williams R, Burns PN, MEASURING ABSOLUTE BLOOD PRESSURE
 378 USING MICROBUBBLES. *Ultrasound Med. Biol.* 2014; 40:775-87.
 379 van der Meer SM, Dollet B, Voormolen MM, Chin CT, Bouakaz A, de Jong N, Versluis M,
 380 Lohse D, Microbubble spectroscopy of ultrasound contrast agents. *J. Acoust. Soc. Am.*
 381 2007; 121:648-56.
 382 Wang X, Hagemeyer CE, Hohmann JD, Leitner E, Armstrong PC, Jia F, Olschewski M, Needles
 383 A, Peter K, Ahrens I, Novel Single-Chain Antibody-Targeted Microbubbles for
 384 Molecular Ultrasound Imaging of Thrombosis Validation of a Unique Noninvasive
 385 Method for Rapid and Sensitive Detection of Thrombi and Monitoring of Success or
 386 Failure of Thrombolysis in Mice. *Circulation* 2012; 125:3117-26.
 387 Warram JM, Sorace AG, Saini R, Umphrey HR, Zinn KR, Hoyt K, A triple-targeted ultrasound
 388 contrast agent provides improved localization to tumor vasculature. *Journal of Ultrasound*
 389 *in Medicine* 2011; 30:921-31.

390

391

392 **List of Figure Captions**

393 **Fig. 1** (a) Schematic of the experimental setup to measure acoustic attenuation.(b) Airtight
394 chamber (Reprinted with permission from Kumar & Sarkar, 2015, J. Acoust. Soc. Am. 138, 624-
395 634. Copyright 2015, Acoustical Society of America).

396 **Fig. 2** Size distribution of Targestar P microbubble.

397 **Fig. 3** Percentage change in radius over its value at the atmospheric pressure at two different
398 ambient overpressure according to three different models A1, A2 and A3 of bubble size change.

399 **Fig. 4** Measured frequency dependent attenuation curves at different ambient overpressures
400 along with EEM model predictions using A1 model for radius change.

401 **Fig. 5** Variation in surface dilatational elasticity (a) and surface dilatational viscosity (b) with
402 change in ambient pressure according to EEM model.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Approach of calculating initial radius at overpressure	Encapsulation parameters	0 mmHg	100 mmHg	200 mmHg
PFB content constant, air diffuses (A1)	$\kappa^s (\times 10^{-9} \text{ N.s/m})$	8.0 $\pm$ 0.1	8.17 $\pm$ 0.3	9.3 $\pm$ 0.3
	$E_0^s (\text{N/m})$	0.09 $\pm$ 0.01	0.085 $\pm$ 0.02	0.19 $\pm$ 0.01
	α	1.5 $\pm$ 0.5	2 $\pm$ 0.5	2 $\pm$ 0.5
	$\gamma_0 (\text{N/m})$	0.01 $\pm$ 0.005	0.001 $\pm$ 0.001	0.007 $\pm$ 0.002
No gas exchange, mechanical compression (A2)	$\kappa^s (\times 10^{-9} \text{ N.s/m})$	8.0 $\pm$ 0.1	8.68 $\pm$ 0.1	10.56 $\pm$ 0.1
	$E_0^s (\text{N/m})$	0.09 $\pm$ 0.01	0.11 $\pm$ 0.005	0.31 $\pm$ 0.005
	α	1.5 $\pm$ 0.5	1 $\pm$ 0.5	1 $\pm$ 0.5
	$\gamma_0 (\text{N/m})$	0.01 $\pm$ 0.005	0.01 $\pm$ 0.005	0.01 $\pm$ 0.005
Air-filled bubble exchanging air with medium (A3)	$\kappa^s (\times 10^{-9} \text{ N.s/m})$	8.22 $\pm$ 0.2	7.3 $\pm$ 0.2	6.55 $\pm$ 0.3
	$E_0^s (\text{N/m})$	0.035 $\pm$ 0.005	0.022 $\pm$ 0.005	0.032 $\pm$ 0.003
	α	1 $\pm$ 0.5	1 $\pm$ 0.5	1 $\pm$ 0.5
	$\gamma_0 (\text{N/m})$	0.01 $\pm$ 0.01	0.001 $\pm$ 0.001	0.001 $\pm$ 0.001

Table 1 Rheological parameters of Targestar P bubbles according to EEM model using three different approaches of radius change under overpressure.

Approach of calculating initial radius at overpressure	Encapsulation parameters	0 mmHg	100 mmHg	200 mmHg
PFB content constant, air diffuses (A1)	$\kappa^s (\times 10^{-9} \text{ N.s/m})$	8.32±0.2	8.37±0.3	9.14±0.3
	$\chi (\text{N/m})$	0.092±0.01	0.078±0.008	0.21±0.01
No gas exchange mechanical compression (A2)	$\kappa^s (\times 10^{-9} \text{ N.s/m})$	8.28±0.2	8.94±0.1	10.83±0.4
	$\chi (\text{N/m})$	0.089±0.008	0.10±0.005	0.316±0.005
Air-filled bubble exchanging air with medium (A3)	$\kappa^s (\times 10^{-9} \text{ N.s/m})$	8.65±0.2	7.46±0.4	6.79±0.2
	$\chi (\text{N/m})$	0.05±0.005	0.015±0.003	0.044±0.003

Table 2 Rheological parameters of Targestar P bubbles according to MM model using three different approaches of radius change under overpressure.