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SINGLE MICROBUBBLE MEASUREMENTS OF LIPID MONOLAYER VISCOELASTIC PROPERTIES FOR SMALL AMPLITUDE OSCILLATIONS

Jordan S. Lum¹, Jacob D. Dove¹, Todd W. Murray^{1,2}, and Mark A. Borden^{*,1,2}

¹Department of Mechanical Engineering, University of Colorado, Boulder, CO 80309, USA

²Materials Science and Engineering Program, University of Colorado, Boulder, CO 80309, USA

Abstract

Lipid monolayer rheology plays an important role in a variety of interfacial phenomena, the physics of biological membranes, and the dynamic response of acoustic bubbles and drops. We show here measurements of lipid monolayer elasticity and viscosity for very small strains at megahertz frequency. Individual plasmonic microbubbles of 2–6 μm radius were photothermally activated with a short laser pulse, and the subsequent nanometer-scale radial oscillations during ring-down were monitored by optical scatter. This method provided average dynamic response measurements of single microbubbles. Each microbubble was modeled as an underdamped, linear oscillator to determine the damping ratio and eigenfrequency, and thus the lipid monolayer viscosity and elasticity. Our non-isothermal measurement technique revealed viscoelastic trends for different lipid shell compositions. We observed a significant increase in surface elasticity with lipid acyl chain length for 16 to 20 carbons, and this effect was explained by an intermolecular forces model that accounts for lipid composition, packing and hydration. The surface viscosity was found to be equivalent for these lipid shells. We also observed an anomalous decrease in elasticity and increase in viscosity when increasing the acyl chain length from 20 to 22 carbons. These results illustrate the use of a novel nondestructive optical technique to investigate lipid monolayer rheology in new regimes of frequency and strain, possibly elucidating phase behavior, as well as how the dynamic response of a microbubble can be tuned by the lipid intermolecular forces.

Graphical abstract

*Corresponding Author: Mark A. Borden, PhD, Department of Mechanical Engineering, University of Colorado, 1111 Engineering Drive, Boulder, CO 80309-0427, Phone: 303.492.7750, Fax: 303.492.3498, mark.borden@colorado.edu.

Supporting Information.

Plots for total, viscous, radiation, thermal and shell damping, and the resulting shell viscosity as a function of resting radius for DPPC, DSPC, DAPC, and DBPC microbubble compositions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

surface elasticity; viscosity; resonance frequency; intermolecular forces

INTRODUCTION

Surfactant monolayer rheology plays an important role in a variety of interfacial phenomena, including surface-wave propagation, wetting, emulsification, emulsion stability, settling and multiphase flows.¹ A particular class of surfactant monolayers – lipid monolayers – has been the focus of research on the biophysics of lung surfactant² and cell membranes.³ In the ultrasonics community, there is interest in lipid-coated microbubbles and fluorocarbon nanodrops as imaging contrast agents, molecular probes, drug delivery vehicles and theranostics.⁴ The stability and dynamic behavior of such acoustic drops and bubbles depends on the lipid monolayer compression rheology.^{5,6} In these applications, and others employing microscale drops and bubbles, the relevant timescale for monolayer compression and expansion is given by the eigenfrequency for volumetric oscillations, which typically occurs in the megahertz range.^{5–7}

The most common method for measuring compression properties of lipid monolayers has been the Langmuir trough.⁸ Here, the compression elasticity (modulus) is calculated from the slope of the surface pressure-area isotherm, while the compression viscosity is obtained by the time variation of surface tension upon compression.¹ Models of microbubble acoustic behavior have used surface elasticity values determined in this manner.⁹ The Langmuir trough is limited, however, to frequencies of a few hertz and below. The Langmuir trough is also inaccurate for measuring surface tension in condensed monolayers that may accumulate anisotropic stresses.¹⁰ Sessile drop and captive bubble shape tensiometers, which rely on millimeter-scale particles to achieve a Bond number near unity, are likewise limited to frequencies below one hertz.¹¹ Capillary pressure tensiometer techniques can operate at frequencies up to 150 Hz,¹² and light-scattering methods can access capillary waves at the liquid surface with frequencies up to 100 kHz.¹ Unfortunately, these techniques remain limited to frequencies at least an order of magnitude below the typical eigenfrequencies of microscale (femtoliter) drops and bubbles.

Our group recently described a novel optical method for measuring the photoacoustic response of plasmonic microbubbles,^{13–15} which is developed here to explore monolayer rheology at megahertz frequencies, albeit in the linear regime under non-isothermal conditions. The method employs a 0.5-ns pulsed laser to stimulate a microbubble loaded

with 5-nm gold plasmonic nanoparticles. Heating of the gas core leads to rapid expansion and subsequent “ring down” of the microbubble, which is captured by light scatter from a second laser operating in continuous-wave mode. The lipid monolayer elasticity and viscosity are determined by the natural frequency and damping ratio, respectively. One advantage of this technique is that it measures the surfactant rheology at the characteristic response time of the particle to a thermal or mechanical impulse. This response time depends on the particle size, so it is important to measure over a range of megahertz frequencies. Additionally, the technique investigates perturbations from the resting state, where the particle is in mechanical equilibrium and has a very low surface tension. Such perturbations are relevant not only for surfactant-stabilized bubbles and drops, but also natural lung surfactant² and lipid rafts in the cell membrane.³

Another advantage of the optical technique is the high signal-to-noise ratio which manifests from averaging over thousands of nondestructive pulses per microbubble.¹⁴ This allows one to resolve sub-nanometer scale radial oscillations, or less than ~0.1% area strain, which is well within the linear regime.⁶ The intermolecular lipid displacements at such tiny strains are only ~0.1 – 1.0 pm. Thus, the method can approximately measure the intermolecular forces between lipids. In the present study, the method is expanded to explore the intermolecular forces for a homologous series of saturated diacyl phosphatidyl choline (PC) lipids commonly found in biological membranes and biomedical emulsions and foams.

MATERIALS AND METHODS

Fabrication of Plasmonic Lipid-Encapsulated Microbubbles

Plasmonic microbubbles were fabricated using 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, 16 carbons per acyl chain), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC, 18 carbons per acyl chain), 1,2-diarachidoyl-*sn*-glycero-3-phosphocholine (DAPC, 20 carbons per acyl chain), or 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine (DBPC, 22 carbons per acyl chain) purchased from NOF America (White Plains, NY, USA). The emulsifier, 1,2-distearoyl-*sn*-glycerol-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000, 18 carbons per acyl chain) and the biotinylated species, 1,2-distearoyl-*sn*-glycerol-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] (DSPE-PEG2000-B) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The gas core comprised perfluorobutane (PFB) at 99 wt% purity obtained from FluoroMed (Round Rock, TX, USA). The plasmonic nanoparticles, 5 nm avidin-tagged gold nanospheres, were purchased from Nanopartz (Loveland, CO, USA).

The microbubble shells comprised either DPPC, DSPC, DAPC or DBPC combined with the emulsifier and biotinylated species at a molar ratio of 90:9:1, respectively. All microbubbles were fabricated using probe sonication and size isolated to 2-6 μm radius using differential centrifugation. The procedure for synthesizing plasmonic nanoparticle-coated microbubbles was previously described by Dove et al.¹³ Briefly, the lipids were suspended in 0.01 M NaCl phosphate-buffered saline (PBS) pH 7.4 (4 mg/mL total lipid) and heated 10% above the phosphatidylcholine main phase transition temperature to promote lipid mixing. The lipid suspension was then cooled and further refined using a Branson 450 Sonifier (Branson, Dandurt, CT) by completely submerging the probe sonicator tip and sonicating on a low

setting. The headspace was then filled with PFB gas, and the sonicator tip was set to mechanically agitate the gas-liquid interface on the highest power setting to form microbubbles. Microbubbles were centrifuged at 300 RCF (relative centrifugal force) for 3 min (Eppendorf 5804, Hauppauge, NY, USA) and washed three times to remove free lipid. The resulting microbubbles were centrifuged at 100 RCF to allow removal of smaller bubbles ($<4\ \mu\text{m}$ diameter).

Microbubbles were mixed with plasmonic avidin-tagged gold nanoparticles, at a 1:1 ratio of biotin:avidin, where one nanoparticle contained one avidin. Microbubbles were sized and counted with an AccuSizer 780 Optical Particle Sizer (NICOMP Particle Sizing Systems, Santa Barbara, CA) to determine total microbubble surface area, and biotin was calculated by estimating $25,000\ \text{biotinylated-lipid}/\mu\text{m}^2$. The mixture of nanoparticles and microbubbles was incubated on an end-over-end rotator for 2 h. The gold nanoparticle-templated (plasmonic) microbubbles were then separated from the unconjugated nanoparticles through gravitational flotation. Figure 1a shows a diagram of the composition for a gold nanoparticle covered microbubble.

Single-Microbubble Photothermal Experiments

The procedure for detecting optically induced microbubble oscillations was described by Dove et al.¹⁴ Briefly, microbubble oscillations were forced by rapid thermal expansion using a pulsed microchip laser with a wavelength of 532 nm, a pulse width of 0.5 ns and a repetition rate of 200 Hz. The pulsed laser was passed through a microscope objective to illuminate a single microbubble. The gold nanospheres attached to the microbubble shell absorbed a portion of the pulsed laser energy and caused a rapid localized heating of the microbubble gas core, leading to an induced oscillation. The pulsed laser fluence was set to $10\ \text{mJ}/\text{cm}^2$ at the sample surface using a variable attenuator; this provided good signal-to-noise without overstimulating the microbubbles into dissolution. We estimate a maximum temperature rise of up to 9.1 K at the lipid shell and 6 K for the gas core.¹⁵ This temperature increase is not expected to have an effect on the lipid phase since the lowest lipid transition temperature is 41 °C for DPPC, and the microbubble starts at room temperature.¹⁶ The microbubble has sufficient time to return to thermal equilibrium (within microseconds) before the next excitation laser pulse (every 5 milliseconds). However, phospholipid monolayer viscoelastic properties are temperature-dependent, so our non-isothermal measurement technique will not provide the absolute viscoelastic properties.^{17,18}

To measure the oscillations, a continuous wave laser with 488 nm wavelength was sent to the sample through the same microscope objective. The width of both the pulsed and continuous beams were adjusted to achieve Gaussian spatial profiles with $1/e$ radii at the objective focal plane of $15\ \mu\text{m}$ and $10\ \mu\text{m}$, respectively. After passing through the sample, the scattered light from the continuous beam was collected, amplified and recorded using an oscilloscope. Figure 1b shows a schematic of the setup.

The relationship between the change in scattered light and microbubble radial displacement was found using a previously described calibration procedure.¹⁴ After measuring the scattered light of various sized microbubbles (sized by optical microscopy), a sensitivity curve was created to correlate the measured voltage to microbubble radius. Microbubble

radial displacements were calculated to be less than a few nanometers, resulting in an area strain of less than 0.01% during microbubble oscillations, well within the linear regime. The sub-nanometer resolution of radial extension was aided through signal averaging, where each signal was averaged ~100,000 times. Potential error sources for this optical procedure are associated with the calibration procedure, bubble size determination, and random noise in the measurements.

Microbubble Dynamic Response Analysis

This section describes the post-processing method used to determine the eigenfrequency and damping ratio for each microbubble. After measuring and recording a microbubble radius-time curve, the damped resonance frequency was determined using the fast Fourier transform (FFT). The radial oscillations of the microbubble were assumed to obey a linear equation of motion:⁶

$$\ddot{x} + 2\delta\omega_0\dot{x} + \omega_0^2x = 0 \quad (1)$$

where x is the microbubble radial displacement, $\dot{x}$ is the radial velocity, $\ddot{x}$ is the radial acceleration, δ is the damping ratio, and ω_0 is the angular eigenfrequency. It was also assumed that, after the initial thermal expansion from the pulsed laser heating of the gold nanoparticles, there is no forcing term present during the microbubble oscillations. To determine the damping ratio, equation (1) was solved for the underdamped case,¹⁹ and an empirical fitting parameter was added to account for a background exponential decay sometimes observed in the experimental data and likely associated with microbubble cooling. The experimentally measured data was then fit to the functional form:

$$x = \left[x_0 \cos(\omega_d t) + \frac{\delta\omega_0 x_0}{\omega_d} \sin(\omega_d t) \right] \cdot e^{-\delta\omega_0 t} + A e^{\alpha t} \quad (2)$$

where x_0 is the initial displacement owing to heating, ω_d is the damped natural frequency, t is the time and the term $A \exp(\alpha t)$ is an empirical fitting parameter. Equation 2 was fit to microbubble oscillation starting at the initial expansion peak, thus assuming an initial velocity equal to zero.

The damped natural frequency for each microbubble is determined by FFT, and the angular eigenfrequency is given by $\omega_0 = \omega_d / \sqrt{1 - \delta^2}$. There are four unknowns in the harmonic oscillator model: δ , x_0 , A and α . These unknowns were determined by a least-squares regression between equation (2) and the experimentally measured waveforms. The best-fit damping ratios ranged from 0.01 to 0.2 within the data set. We note that some of the measured waveforms with a root mean squared error greater than 0.12 (where the experimental waveforms were normalized prior to analysis) were excluded from analysis. In addition, waveforms that quickly decayed to zero without clear oscillatory behavior were not considered. In both cases, the behavior deviated from that of an ideal harmonic oscillator. The damping ratio found for each microbubble was then used to determine the eigenfrequency. Figure 1c shows an example of an experimental microbubble waveform fit

with the harmonic oscillator model, where the damping ratio was found to be $\delta=0.065$, giving an eigenfrequency of $f_0=1.73$ MHz.

RESULTS AND DISCUSSION

Lipid Shell Elasticity

Figure 2 shows the measured eigenfrequency for each microbubble as a function of resting radius. The power-law relationship seen between eigenfrequency and the resting radius has been established in literature.^{7,14,20} We show here for the first time, however, that the curves are shifted to higher frequencies for longer acyl chains, indicating stiffer shells. This result is consistent with prior results from Rooij et al.,²⁰ who showed that DSPC-coated microbubbles are stiffer than their DPPC counterparts.

The shell elasticity (χ) was calculated from the equation for the eigenfrequency of a shelled bubble undergoing linear (small amplitude) oscillations:^{7,9}

$$\chi = \pi^2 f_0^2 \rho_L R^3 - \frac{3}{4} \kappa P_0 R - \frac{(3\kappa - 1) \sigma_w}{2} \quad (3)$$

where ρ_L is the density of the surrounding water (10^3 kg/m³), κ is the polytropic exponent for the gas core, P_0 is the ambient pressure (10^5 Pa), R is the resting radius of the microbubble, and σ_w is the surface tension of the gas-liquid interface, which will be very low for a lipid coated microbubble in equilibrium and was assumed to be negligible owing to the presence of the lipid shell.²¹ The solid lines in Figure 2 show the eigenfrequencies calculated with equation (3) using the mean elasticity values determined below.

Selection of the value for the polytropic exponent requires further discussion. Although the microbubbles were originally fabricated with perfluorobutane gas cores for increased yield and stability during storage, it is likely that gas exchange with an air-saturated aqueous environment can occur within seconds to minutes, based on the modeling results of Sarkar and others.^{22–24} Therefore, it was assumed that the gas cores of the microbubbles were filled purely with air due to the washing and conjugation steps in air-saturated media. The polytropic exponent for the gas core was calculated by first considering the thermal diffusion length:⁶

$$l_D = \sqrt{\frac{K_g}{2\omega_d \rho_g C_p}} \quad (4)$$

where ω_d is the damped angular frequency, and K_g , ρ_g and C_p are the thermal conductivity, density and heat capacity at constant pressure of the gas, respectively. The polytropic exponent was then calculated by the following equation:⁶

$$\kappa = \text{Re} \left\{ \left[\frac{1}{\gamma} \left(1 + \frac{3(\gamma - 1)}{\Psi^2} (\Psi \coth \Psi - 1) \right) \right]^{-1} \right\} \quad (5)$$

where $\Psi = \frac{1}{2}(1+i)\frac{R}{l_D}$ and γ is the adiabatic constant (specific heat ratio) of the gas. Here, κ is equal to 1.0 for an isothermal process and 1.4 for an adiabatic process. The κ values calculated for our experimental conditions ranged from 1.04 to 1.10, indicating oscillation in the transition region where thermal damping is significant.⁶ The actual gas core may be a mixture of air with some perfluorobutane, resulting in our polytropic exponent for a purely air gas core being slightly overestimated.

The microbubbles in our study were resting against a glass coverslip. Theoretical studies employing an “image bubble” oscillating in phase to account for a rigid boundary have predicted that the apparent (measured) eigenfrequency decreases by 17% compared to that for an isotropic medium.^{7,25} We thus corrected our measured values to account for this effect.

Figure 3 shows corrected shell elasticity histograms for each lipid composition. Interestingly, the data followed a normal distribution. The average elasticity ranged from 2.4 to 4.0 N/m for DPPC to DAPC, which is consistent with prior studies of lipid-coated microbubbles driven to higher amplitude oscillations. For example, Overvelde et al.²⁶ obtained an elasticity of 2.5 N/m. It is again important to note that while our elasticity values are comparable to other studies, our experimental values were measured non-isothermally and may not be the absolute values for these lipid monolayers. As expected from the resonance curves above, the elasticity was found to increase with lipid acyl chain. A Student’s t-test showed that the increase between DPPC, DSPC and DAPC was statistically significant ($p < 0.05$). The elasticity of DBPC shells, however, decreased compared to DAPC ($p < 0.05$). This anomalous result for DBPC is discussed in greater detail below.

Intermolecular Forces

We derived a simple model of the intermolecular forces to identify the dominant interactions and examine the effect of lipid composition on monolayer elasticity. The model is based on elementary force fields between the lipid molecules taken from previously published data.²⁷ As derived here, the model provides an *a priori* prediction of the potential energy, force, surface pressure and elasticity as a function of lipid composition and surface density.

We start by assuming that the microbubble is at rest and the lipids are in mechanical equilibrium. The microbubble is neither dissolving nor growing, even as the gas core is in diffusive exchange with the surrounding medium. In this state, the lipids form a complete monolayer shell, and essentially zero gas/water interface is exposed. The microbubble is assumed to be perfectly spherical with purely dilatational deformation. The lipids are hexagonally-close-packed (HCP) (Figure 4a). Since each lipid has two acyl chains, we define two interaction regions: the head region and the tail region (Figure 4b). In the head region, we assume only lateral charge-charge interactions (Coulomb potential) and

dispersion interactions (Lennard-Jones potential) between the choline and phosphate groups, and dispersion interactions between the glycerol groups. In the tail region, we assume only dispersion interactions between the ester and methylene groups.²⁷

The intermolecular pair potential for diacyl phospholipids is split into the head and tail interactions with corresponding group separation distances (d):

$$W_H = \sum_{k=1}^2 \frac{Q_k^2}{4\pi\epsilon_0\epsilon_w d_H} + \sum_{i=1}^3 4\epsilon_i \left[\left(\frac{\sigma_i}{d_H} \right)^{12} - \left(\frac{\sigma_i}{d_H} \right)^6 \right] \quad (6)$$

$$W_T = 4\epsilon_E \left[\left(\frac{\sigma_E}{d_T} \right)^{12} - \left(\frac{\sigma_E}{d_T} \right)^6 \right] + \sum_{j=1}^N 4\epsilon_j \left[\left(\frac{\sigma_j}{d_T} \right)^{12} - \left(\frac{\sigma_j}{d_T} \right)^6 \right] \quad (7)$$

where the subscripts H and T refer to the head and tail groups, respectively. The subscripts k and i are 1 for choline and 2 for phosphate, and the subscript j is 3 for glycerol. Q is the charge of choline or phosphate, ϵ_0 is the vacuum permittivity, ϵ_w is the dielectric constant of water, ϵ is the depth of the potential energy well and σ is the van der Waals diameter. The subscript E refers to the ester group, and the subscript j refers to the methylene groups and ranges from 1 to N . N is the number of methylene groups and depends on the number, n , of carbons per acyl chain:

$$N = \frac{n-3}{3} \quad (8)$$

Each methylene group contains 3 carbons, and the 3-carbon ester group is counted separately.²⁷ For calculating the self-energy of a single lipid, we would sum the pair potentials for each group over all nearest neighbors. When deriving the elasticity, however, the relevant interaction is the pair potential, and the relevant length scales are the distance between the head-groups (d_H) and the distance between tail groups (d_T). These distances can be determined from the area per molecule, a , as follows for hexagonal packing:

$$d_H = \sqrt{\frac{2a}{\sqrt{3}}} \quad (9)$$

$$d_T = \sqrt{\frac{a}{\sqrt{3}}} \quad (10)$$

We neglect interactions between out-of-plane groups, as well as dipole interactions since the effect of water is taken by the dielectric constant ϵ_w . These assumptions are valid for

determination of the elastic modulus at small deformations, where van der Waals dispersion forces are dominant. This coarse graining methodology and the physical constants for each lipid group (Table 1) were found from a molecular dynamics study by Orsi et al.,²⁷ which provided realistic structural and physical properties for DSPC bilayers in the gel and fluid phases.

The intermolecular force between two lipids is given by summing the head group and chain contributions:

$$F = F_H + F_T = -\frac{\partial W_H}{\partial d_H} - \frac{\partial W_T}{\partial d_T} \quad (11)$$

where the contributions for the head and tail regions are given by:

$$F_H = \sum_{k=1}^2 \frac{Q_k^2}{4\pi\epsilon_0\epsilon_W d_H^2} + \sum_{i=1}^3 4\epsilon_i \left[\left(\frac{12\sigma_i^{12}}{d_H^{13}} \right) - \left(\frac{6\sigma_i^6}{d_H^7} \right) \right] \quad (12)$$

$$F_T = 4\epsilon_E \left[\left(\frac{12\sigma_E^{12}}{d_T^{13}} \right) - \left(\frac{6\sigma_E^6}{d_T^7} \right) \right] + \sum_{j=1}^N 4\epsilon_j \left[\left(\frac{12\sigma_j^{12}}{d_T^{13}} \right) - \left(\frac{6\sigma_j^6}{d_T^7} \right) \right] \quad (13)$$

The surface pressure is determined by dividing the intermolecular force over the intermolecular separation:

$$\Pi = \Pi_H + \Pi_T = \frac{F_H}{d_H} + \frac{F_T}{d_T} \quad (14)$$

The surface elasticity (equivalent to the surface compressibility modulus) is defined as:

$$\chi = -A \frac{\partial \Pi}{\partial A} = -a_H \frac{\partial \Pi}{\partial a_H} - a_T \frac{\partial \Pi}{\partial a_T} \quad (15)$$

Combining equation (15) with (9) and (10), the surface elasticity is given by:

$$\chi = \chi_H + \chi_T = -\frac{d_H}{2} \frac{\partial \Pi_H}{\partial d_H} - \frac{d_T}{2} \frac{\partial \Pi_T}{\partial d_T} \quad (16)$$

Thus,

$$\chi_H = \sum_{k=1}^2 \frac{3Q_k^2}{8\pi\epsilon_0\epsilon_w d_H^3} + \sum_{i=1}^3 2\epsilon_i \left[\left(\frac{168\sigma_i^{12}}{d_H^{14}} \right) - \left(\frac{48\sigma_i^6}{d_H^8} \right) \right] \quad (17)$$

$$\chi_T = 2\epsilon_E \left[\left(\frac{168\sigma_E^{12}}{d_T^{14}} \right) - \left(\frac{48\sigma_E^6}{d_T^8} \right) \right] + \sum_{j=1}^N 2\epsilon_j \left[\left(\frac{168\sigma_j^{12}}{d_T^{14}} \right) - \left(\frac{48\sigma_j^6}{d_T^8} \right) \right] \quad (18)$$

Figure 5 shows plots for energy, force, pressure and elasticity versus area per molecule for the different microbubble shell compositions in the fully hydrated state. Using equations (9) and (10), the equilibrium area per molecule when $F=0$ was found to be 0.434 nm^2 for all of these lipid compositions. Using this surface density, the resulting elasticity values for DPPC, DSPC, DAPC and DBPC were predicted to be 4.9, 5.5, 6.0 and 6.6 N/m , respectively. At the equilibrium area per molecule, strong cohesion in the tail group region was the main contribution to the shell elasticity. The head group region contributed negative elasticity owing to charge-charge repulsion, but this contribution was smaller than that for tail cohesion.

These equilibrium force fields do not account for thermal expansion at room temperature. Thermal energy kT , where k is Boltzmann's constant and T is absolute temperature, was added to the minimum potential energy for each lipid to find a new area per molecule, which was determined by calculating the arithmetic mean of the smallest and largest areas on each side of the potential well. The new equilibrium area per molecule after thermal expansion was predicted to be 0.44 nm^2 at 300 K (only a 1.3% increase).

Another important consideration is hydration, which would mainly affect charge-charge interactions between the phosphate and choline groups. Since we do not know the hydration level of these groups *a priori*, we compare the two extremes of dehydrated and fully hydrated states. The fully hydrated state was modeled by using the dielectric constant of water ($\epsilon_w=80$). The dielectric constant was set to 2 for the dehydrated state because lower values lead to unphysical charge-charge repulsion dominating the interaction at all separations. Figure 6 shows a comparison between the experimental elasticity values and the theoretical values, including limits for the fully hydrated and dehydrated states. The experimental data points lie between these two bounds. Good agreement between theory and experiment was obtained with a dielectric constant of 2.75, yielding an area per molecule of 0.465 nm^2 . This result suggests that even partial hydration is sufficient to screen the head group charge repulsion.

We also compared the model calculations with experimental elasticity values to estimate the "apparent" area per lipid in resting microbubbles of each composition (labeled on Figure 6). For DSPC-coated microbubbles, the apparent area per molecule was found to be 0.459 nm^2 , which is slightly less than the reported literature value for a DSPC bilayer of 0.472 nm^2 .^{27,28} Despite the simplifications in the model, it provides a reasonable estimate for the elasticity

as a function of lipid composition. To our knowledge, this is the first physical model to link lipid composition to microbubble dynamics.

For the sake of comparison between the intermolecular forces model and the experimental results, we assume that the microbubble shell is in mechanical equilibrium. Clearly, it is never in thermodynamic equilibrium, since Laplace pressure-driven dissolution forces the monolayer to condense beyond the equilibrium surface concentration. The equilibrium concentration for the lipids is lower than for a static microbubble and has a corresponding equilibrium surface tension measured by Lee et al.^{29,30} to be 25 mN/m for lipids at or above their main phase transition temperature, and >25 mN/m for lipids below their main phase transition temperature. Such a surface tension at this high curvature would induce a significant Laplace pressure and drive rapid dissolution, as modeled by Epstein and Plesset³¹ and experimentally validated by Duncan et al.²¹ The transition between mechanical equilibrium (the static microbubble) and thermodynamic equilibrium (complete microbubble dissolution) is hindered by the high activation energy barrier of lipid dissolution, as indicated by the very low critical micelle concentrations of these lipids.¹⁶ The gas molecules have a significantly higher aqueous solubility than the lipids,³² and hence lower activation energy barrier to solvation, and thus the gas core is assumed to be in thermodynamic equilibrium with the surrounding medium, even while the lipids are not.

Lipid Shell Viscosity

Shell damping is an important consideration in the quality of the microbubble echo and the rate of heat dissipation to the surrounding medium. The shell damping for a single microbubble was derived using known damping terms:⁷

$$\delta_{tot} = \delta_{vis} + \delta_{rad} + \delta_{therm} + \delta_{shell} \quad (19)$$

where δ_{vis} is due to viscous losses in the surrounding medium, δ_{rad} is due to radiation of sound, δ_{therm} is due to heat transfer between the gas and liquid, and δ_{shell} is due to the lipid shell. These damping terms are given by the following relations:⁶

$$\delta_{vis} = \frac{\mu}{\pi R^2 \rho_L f_0} \quad (20)$$

$$\delta_{rad} = \frac{\pi f_0 R}{c} \quad (21)$$

$$\delta_{therm} = \frac{3P_0}{8\pi^2 f_0^2 R^2 \rho_L} \text{Im} \left(\frac{1}{\Phi} \right) \quad (22)$$

$$\delta_{shell} = \frac{\kappa_s}{\pi R^3 \rho_L f_0} \quad (23)$$

where μ is the viscosity of water (8.9×10^{-4} Pa-s), c is the sound speed in water (1500 m/s) and κ_s is the shell viscosity. The shell damping term is taken by combining equations (19)-(22) and subtracting them from the experimentally determined damping value.

Figure 7 shows shell damping histograms and normal distribution curves for each microbubble shell composition. Note that there was significantly more bubble-to-bubble variation in the surface viscosity measurements than in the surface elasticity measurements, as demonstrated by the greater spread in viscosity data. The Supplementary Information section contains plots for total, viscous, radiation, thermal and shell damping, and the resulting shell viscosity from equation (23), as a function of resting radius for all four microbubble compositions. Interestingly, shell damping was found to be an important contributor to total damping, ranging from 44% to 58% for DPPC and DBPC microbubbles, respectively. This result agrees with van der Meer et al.,⁷ who showed that lipid shell viscosity contributes up to ~70% to total microbubble damping.

We did not observe a significant change in the shell damping with increasing acyl chain length from 16 to 20 carbons. This result is consistent with van Rooij et al.,²⁰ who also observed no difference in shell damping between DPPC and DSPC for much larger oscillations. However, the damping for DBPC microbubbles was statistically greater ($p < 0.05$) than the other compositions. This rise in DBPC surface viscosity coincided with the drop in surface elasticity compared to the trend for the other lipids, indicating softening of the lipid shell. This is contrary to prior work on microbubble stability, in which DBPC was shown to be more stable than the shorter chain lipids, such as DAPC.³³ It is an interesting paradox that the more stable DBPC shell appears to be softer under low-amplitude oscillation. One possible explanation for DBPC shell softening may be a different microstructure than the other shells, possibly arising from lateral phase separation and/or lipid folding. Alternatively, the DBPC shells might have been slightly stretched owing to a longer equilibration time during gas exchange.

CONCLUSIONS

In conclusion, we have demonstrated a novel optical method to explore lipid monolayer rheology at infinitesimal strain and megahertz frequency, which is relevant to the dynamic response of microscale bubbles and drops. While our optical method is a non-isothermal technique and will not provide the absolute viscoelastic property values of lipid monolayers, our method does reveal how lipid composition significantly affects the viscoelastic properties. The results revealed an increasing trend in microbubble shell elasticity for lipid acyl chain lengths of 16 to 20 carbons that could be accurately predicted by an intermolecular forces model accounting for the main chemical groups. The model may be useful for predicting effects of lipid composition on microbubble resonance. Additionally, shell damping was found to be a significant portion of overall damping, but was unaffected

by lipid acyl chain lengths between 16 and 20 carbons. This study also demonstrated a break from the trend for the viscoelastic properties of microbubbles with lipid shell chain length of 22 carbons, suggesting shell softening for this composition. Overall, these results illustrate how the dynamic response of a microbubble can be predicted by the lipid intermolecular forces, as well as the use of a novel nondestructive optical technique to investigate lipid monolayer rheology in new regimes of frequency and strain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PEG-lipid	poly(ethylene glycol)-lipid
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DAPC	1,2-diarachidoyl-sn-glycero-3-phosphocholine
DBPC	1,2-dibehenoyl-sn-glycero-3-phosphocholine
DSPE-PEG2000	1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]
DSPE-PEG2000-B	1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000]
PFB	perfluorobutane
PBS	phosphate-buffered saline
FFT	fast Fourier transform
HCP	hexagonally-close-packed

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Figure 1. Schematic showing (a) microbubble compositions, (b) experimental setup used to excite and detect microbubble oscillations and (c) an example of a microbubble dynamic response fitted to a model curve assuming a linear, underdamped harmonic oscillator. The damping ratio for this case was found to be $\delta=0.065$, giving an eigenfrequency of $f_0=1.73$ MHz.

Figure 2.

Experimentally measured eigenfrequency versus resting radius for each microbubble shell composition (blue data points). The calculated curves with average elasticity are shown by the solid red lines. Also shown is the number (n) of experimental data points for each shell composition.

Figure 3. Histograms of measured shell elasticity for microbubbles fabricated with (a) DPPC, (b) DSPC, (c) DAPC and (d) DBPC. The red curve represents a Gaussian distribution curve fitted to the data. Bin size is 0.14 N/m.

Figure 4. Cartoon schematic for (a) the in-plane ordering of the acyl chain tail groups in the monolayer lipid microbubble shell and (b) a side view of two lipid molecules that have been coarse grained with each main component of the head and tail regions shown.

Figure 5.

Model calculations of (a) intermolecular pair potential, (b) intermolecular force, (c) surface pressure and (d) elasticity for monolayers of DPPC, DSPC, DAPC and DBPC. The predicted equilibrium area per molecule (where $F=0$) for is 0.434 nm^2 for all compositions.

Figure 6.

Model results for shell elasticity for microbubbles comprising DPPC, DSPC, DAPC and DBPC lipid in the (bottom line) dehydrated ($\epsilon_w = 2$) or (top line) fully hydrated ($\epsilon_w = 80$) states. The experimental data values (mean $\pm$ standard deviation) fall between these two limits (shaded region), indicating partial hydration of the head groups. The line showing the model fit to the data is for a partially hydrated state ($\epsilon_w = 2.75$). Also shown for each lipid is the area per molecule from the model that gives the same elasticity as the average experimental value.

Figure 7. Histograms of measured shell damping for microbubbles fabricated with (a) DPPC, (b) DSPC, (c) DAPC and (d) DBPC. Also indicated is the percent contribution the shell has towards the total microbubble damping.

Table 1

Parameter values used in the model calculations. Subscripts C, P, G, E, M, and W are used to represent choline, phosphate, glycerol, ester, methylene and water, respectively.

Parameter	Values (Orsi and Essex ²⁷)
σ_C, σ_P	0.52 nm
σ_G, σ_E	0.46 nm
σ_M	0.45 nm
ϵ_C, ϵ_P	6.0 kJ/mol
ϵ_G, ϵ_E	4.0 kJ/mol
ϵ_M	3.5 kJ/mol
ϵ_W	80
Q_C	+0.7 e
Q_P	-0.7 e