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The Role of Ultrasound Cavitation in Liposome Size Reduction

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Abstract

Liposome size is a vital parameter of many quantitative biophysical studies. Sonication, or exposure to ultrasound, is used widely to manufacture artificial liposomes, yet little is known about the mechanism by which liposomes are affected by ultrasound. Cavitation, or the oscillation of small gas bubbles in a pressure-varying field, has been shown to be responsible for many biophysical effects of ultrasound on cells. Collapse cavitation is manifested in the acoustic spectrum by an f/2 subharmonic and an increase in broadband noise. In this study, we attempted to correlate the presence of cavitation with a decrease in liposome size. Lipid suspensions surrounding a hydrophone were exposed to various intensities of ultrasound and various hydrostatic pressures before measuring their size distribution with Dynamic Light Scattering. As expected, increasing ultrasound intensity with constant pressure decreased the average liposome diameter. Presence of collapse cavitation was manifested in the acoustic spectrum at high ultrasound intensities. Increasing hydrostatic pressure was shown to inhibit the presence of collapse cavitation. Interestingly, changes in liposome size still occurred when collapse cavitation was inhibited either by lowering the ultrasound intensity or by increasing the static pressure. Collapse cavitation did not correlate with decreases in liposome size. We propose a mechanism whereby stable cavitation, another type of cavitation present in sound fields, causes fluid shearing of liposomes and reduction of liposome size. A mathematical model was developed based on the Rayleigh-Plesset Equation of bubble dynamics and principles of acoustic microstreaming to estimate the shear field magnitude around an oscillating bubble. This model predicts the ultrasound intensities and pressures needed to create shear fields sufficient to cause liposome size change and correlates well with experimental data.

Introduction and Methods

Collapse cavitation is commonly detected by the presence of an f/2 subharmonic frequency and increased broadband emission[1]. It can be inhibited by increasing the static pressure[2]. An apparatus was built to expose lipid suspensions to various ultrasound intensities and various static pressures while listening to the acoustic spectra (figure 1). MATLAB code was developed to quantify the presence of the f/2 subharmonic and the broadband emission.

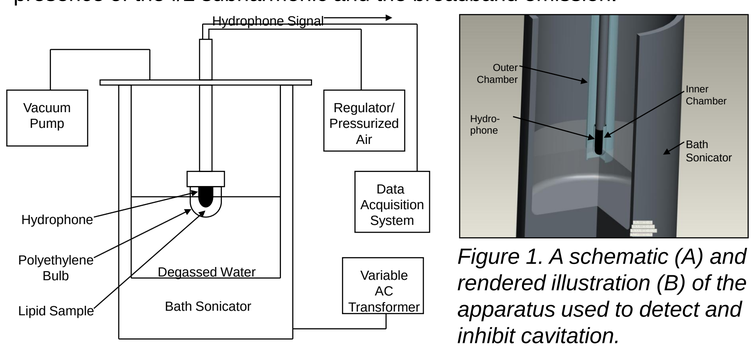


Figure 1. A schematic (A) and rendered illustration (B) of the apparatus used to detect and inhibit cavitation.

[1]Brennen, C.E. 1995. Cavitation and Bubble Dynamics. Oxford University Press, New York.
[2]Delius, M. 1997. Minimal Static Excess Pressure Minimises the Effect of Extracorporeal Shock Waves on Cells and Reduces it on Gallstones. *Ultrasound Med. Biol.* 23(4):611-617.
[3]Woodbury, D.J., E.S. Richardson, A.W. Grigg, R.D. Welling, and B.H. Knudson. 2006. Reducing liposome size with ultrasound: Bimodal size distributions. *Journal of Liposome Research* 16(1):57-80.
[4]Nyborg, W.L. 1968. Mechanisms for Nonthermal Effects of Sound. *J. Acoust. Soc. Amer.* 44(5):1302-1309.

Results

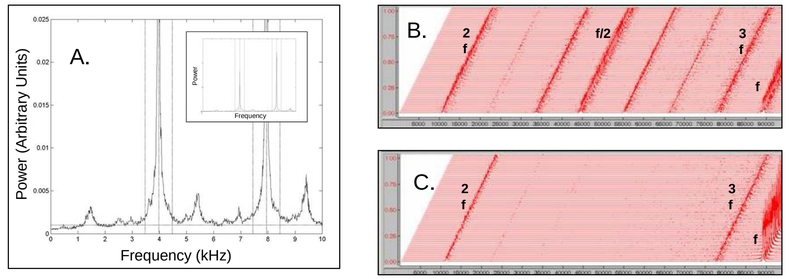
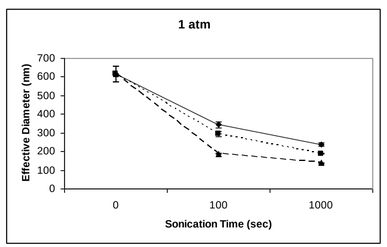
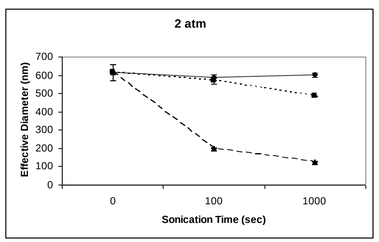


Figure 2. (A) is a representative FFT with lines indicating baseline noise and limits of integration as chosen by the MATLAB code. (B) shows the FFT of a sample receiving high intensity ultrasound at 1 atm static pressure, while (C) is the same sample at 4 atm static pressure. Note the inhibition of collapse cavitation as indicated by the loss of the f/2 subharmonic and lower baseline noise.

Inhibition of both broadband emission and subharmonic integration were achieved at higher intensities (figure 2B). There was size change without the presence of sonication, however (data not shown). As shown in figure 3, higher ultrasound intensities could produce a larger size change, but this could be inhibited at higher static pressures. We hypothesized previously[3] that stable cavitation, or the repeatable bubble oscillations always present in a sound field, could play a role in size change. We developed a mathematical model to determine if shear forces developed by an oscillating bubble could explain our results. Figure 4 shows that our mathematical model provides a feasible explanation for the observed effects.





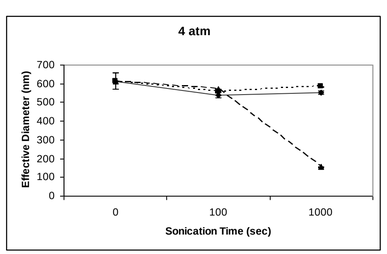


Figure 3. The effective diameters of liposomes formed at (A) 1 atm (B) 2 atm and (C) 4 atm static pressure under various ultrasound intensities (high (▲) medium (■) and low (♦)) and various time durations of exposure to ultrasound. Note that while increasing time exposure and intensity of ultrasound decreases the mean diameter, increasing static pressure can counteract this effect.

Mathematical Modeling

$$\frac{p_v(T_\infty)-p_\infty(t)}{\rho_L}+\frac{p_{G_O}}{\rho_L}\left(\frac{R_O}{R(t)}\right)^{3k}=R(t)\ddot{R}(t)+\frac{3}{2}(\dot{R}(t))^2+\frac{4\nu_L\dot{R}(t)}{R(t)}+\frac{2S}{\rho_L R(t)} \quad (1)$$

$$p_{G_O}=p_\infty(0)-p_v(T_\infty)+\frac{2S}{R_O} \quad (2)$$

$$Ca=\frac{Shear\ Forces}{Surface\ Tension\ Forces}=\frac{\mu GR^2}{SR}=\frac{\mu RG}{S} \quad (3)$$

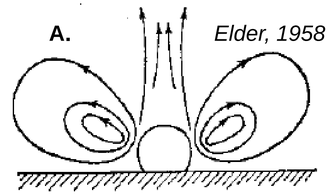
$$U_L=\frac{u_g^{\ 2}}{2\pi fR_0} \quad (4)$$

$$G\cong\frac{U_L}{\delta} \quad (5)$$

$$\delta=\left(\frac{\mu_L}{\pi\rho_L f}\right)^{\frac{1}{2}} \quad (6)$$

$p_v(T_\infty)$ = vapor pressure of liquid at T_∞ (the temperature away from the bubble),
$p_\infty(t)$ = time-dependant pressure of the liquid,
ρ_L = density of the liquid,
R_0 = initial bubble radius,
$R(t)$ = time-dependant bubble radius,
k = polytropic constant of enclosed gas,
ν_L = kinematic viscosity,
S = interfacial surface tension.
ug = velocity amplitude of bubble surface
UL = streaming velocity
δ = velocity boundary layer thickness
G = velocity gradient (shear rate)

A. Elder, 1958



B.

Crit. Diam. (nm)	1 atm	2 atm	4 atm
Low Intensity	Unaffected	Unaffected	Unaffected
Medium Intensity	350	Unaffected	Unaffected
High Intensity	200	450	610

Figure 4. We propose that shear created from oscillating bubbles, a phenomena called acoustic microstreaming that occurs with stable cavitation, is responsible for lowering mean liposome size. (A) shows an illustration of the flow fields around an oscillating bubble. Equations (1-6) constitute the equations of bubble dynamics[1], capillary number, and shear forces[4] used to calculate a critical liposome diameter above which liposomes are unstable and break into smaller vesicles. Critical diameters are shown in the table above (B) that agree with the data in figure 4. If the critical diameter was above the mean diameter at t=0, the liposomes were assumed to be unaffected.

Conclusions

It appears that pressurization during sonication inhibits the decrease in liposome size, but increased intensity can counteract the inhibition. Furthermore, increased static pressure also inhibits the subharmonic and broadband emissions, but size change is still present even without both of these elements, and neither type of acoustic emission is correlated with liposome size change. Finally, our mathematical models show that stable (non-collapse) cavitation, always present with or without subharmonic or broadband emissions, can generate sufficient shear through acoustic microstreaming to reduce liposome size. These mathematical models of acoustic microstreaming can qualitatively explain the effects of pressure and acoustic intensity on liposomes size reduction.
These observations and mathematical models support the hypothesis that it is microstreaming around oscillating bubbles, and not necessarily collapse cavitation events, that create shear sufficient to reduce the size of the liposomes during ultrasonic processing.