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Ghaleb A. Husseini
ghaleb_husseini@hotmail.com

William G. Pitt
pitt@byu.edu

See next page for additional authors

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Authors
Ghaleb A. Husseini, William G. Pitt, and Ram Singh

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Modeling Phase Transitions of Nanoemulsion for Ultrasonic Gene Delivery

William G. Pitt1, Ram Singh1, and Ghaleb A. Husseini2

1Chemical Engineering Department, Brigham Young University, Provo, Utah, USA
2Chemical Engineering Department, American University of Sharjah, Sharjah, UAE
pitt@byu.edu

ABSTRACT SUMMARY:

A computer model was written that simulates the transformation of a nanoemulsion droplet to a gas bubble by the application of ultrasound. Experimentally such a technique could be used to enhance non-viral gene transfection beyond the boundary of the endothelial lining of the circulatory system, particularly in tumors exhibiting enhanced permeation and retention of nanoparticles.

INTRODUCTION:

Ultrasonic gene delivery is enhanced by the presence of microbubbles (MBs) that create cavitation events leading to the transient permeabilization of cell membranes, thus allowing the diffusion of DNA or other oligonucleotides into the cell. However, the size of microbubbles, usually 2-5 microns, restricts them to the circulatory system; thus gene transfection is limited to the endothelial cell layer, or perhaps 1 cell layer beyond if the cavitation disrupts the endothelium1. Microbubbles cannot pass this boundary to enhance gene transfection further into a tissue system.

However in some tumors, the capillary vasculature has much larger gaps and fenestrations which allow passage of much larger particles, sometimes up to 600 nm in diameter2; this is called “enhanced permeation and retention” (EPR). Thus sufficiently small nanoemulsion particles could escape from the capillaries. Our concept is to generate cavitation gas bubbles in “leaky” tumor tissues beyond the endothelium by creating submicron nanoemulsions containing perfluorohexane, a liquid (at body temperature) that boils at 55°C. But even at 37°, the liquid will transform to gas as the surrounding pressure is reduced, such as during the rarefaction phase of the ultrasonic cycle. Experiments in vitro have shown that 20 kHz and 500 kHz ultrasound (US) can produce cavitating gas bubbles from perfluorohexane emulsions (250 nm and 550 nm diameter) stabilized by perfluorooctanoic acid3.

In order to better understand the physics that govern the ultrasonic-induced phase transition of perfluorocarbon emulsion, and exploit them for gene delivery, we created a mathematical model and computer program of this phenomenon.

MATHEMATICAL AND COMPUTER MODEL:

We employ a model of a sphere of liquid with a surfactant layer producing a given surface tension. The ultrasonic sound wave is modeled as a sine wave oscillating around the local hydrostatic pressure. The pressure inside the emulsion liquid is the external pressure plus the Laplace pressure. As the US pressure decreases, the pressure inside the nanoemulsion eventually drops below the vapor pressure of the liquid at its local temperature. We assume the liquid boils to form a gas-phase-spherical-annulus between the emulsion liquid and the surrounding water. The expansion and movement of the gas/water interface is governed by a modified form of the Raleigh-Plesset equation4. As the emulsion liquid vaporizes, the surface temperature of the liquid core drops, thus reducing the vapor pressure in the gas phase and slowing the evaporation rate.

These highly coupled non-linear differential equations were solved using a Runga-Kutta solver within the Matlab (MathWorks Inc., Natick, MA) computer environment. Initial conditions were a resting perfluorohexane nanoemulsion at 25° or 37°, having a diameter of 250 or 550 nm. The bubble behavior was explored as a function of the ultrasonic amplitude and frequency, surface tension, bubble diameter, and initial temperature.

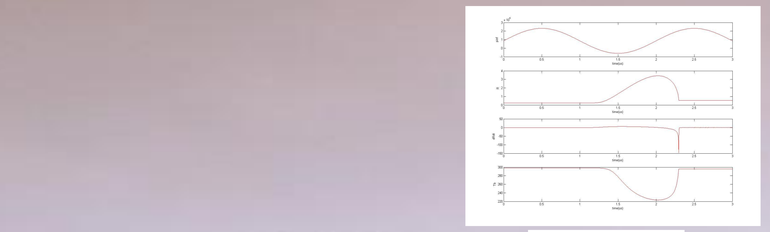

Fig 1

Fig 2

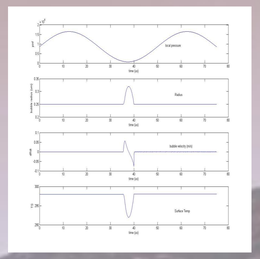

Fig 3

Fig 4

Fig 5

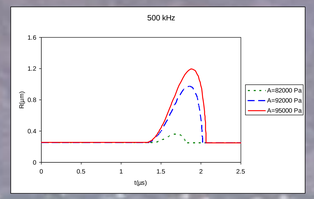

Fig 6

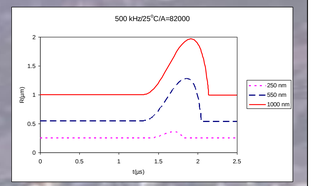

Fig 7

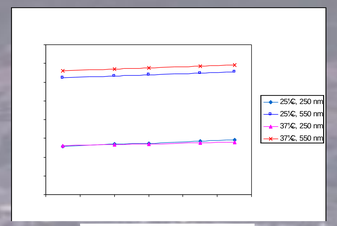

Fig 8

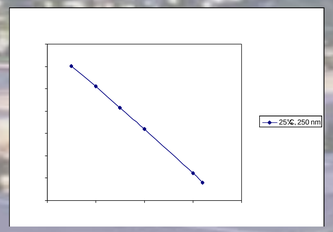

Fig 9

RESULTS AND DISCUSSION:

Figure 1 shows an example of the bubble radius, gas/water interface velocity, and gas temperature as a function of time at 500 kHz. The gas phase temperature is the same as that of the surface of the liquid interior drop that cools during evaporation. The computer code appeared to run properly in that the gas phase formed only after the external pressure plus the Laplace pressure dropped below the vapor pressure of the perfluorohexane. Larger emulsion nanodroplets formed larger bubbles than smaller ones because the decreased Laplace pressure allowed gas phase initiation to occur sooner and there was a longer time for bubble growth. At low acoustic pressures the gas phase condensed back into liquid phase as the acoustic pressure reversed. At some higher acoustic pressures, the gas phase grew large enough to persist through several cycles, and then finally collapsed. The velocity of the gas/water interface upon bubble collapse generally increased as the maximum diameter of the gas bubble increased. This is significant because higher collapse velocities produce higher shear stresses and more powerful shock waves.

The most significant parameter affecting maximum bubble diameter and collapse velocity is the ultrasonic frequency. At 20 kHz (compared to 500 kHz) there is much more time available for the expansion phase of bubble growth, and there is significant outward momentum that allows the bubble to keep expanding even after the pressure is no longer favorable for bubble growth. This leads to more energetic bubble behavior at low frequencies, as has been observed experimentally 4, 5.

The temperature of the interior gas (and liquid surface) dropped by as much as 40° during the bubble expansion phase, and then returned to the initial temperature as the evaporated perfluorocarbon condensed back onto the liquid nanodroplet.

DISCUSSION:

We were successful in generating a mathematical model and computer program that described the behavior of high-vapor-pressure nanoemulsions in the presence of an ultrasonic field. The results of the calculation qualitatively matched previous experimental data from our laboratory3 regarding the thresholds and intensity of collapse cavitation of perfluorohexane emulsions subjected to 20 and 500 kHz ultrasound.

This is a first-order model in that many of the more detailed physics were not included, such as the change in surface tension with expansion of the gas/water interface, and the evaporation (and condensation) of water at that (water) interface. The model also only considered an insolated emulsion nanodroplet, whereas in reality, the gas bubbles may interact with their neighbors and coalesce into larger bubbles. We have some confidence in the computer model because the thermal balance appears to be correct, within the numerical precision of the computer.

As for applications in drug and gene delivery, this model shows that nano-sized perfluorocarbon emulsions can be activated into oscillating gas bubbles that can create shear and stress on surrounding cells. DNA in the form of plasmids could be attached to the nanoemulsion surface or free (unattached) DNA could be mixed with the nanoemulsion before injection so the DNA is present when the liquid transforms to gas and stresses the local cell membranes. The model gives guidance as to which parameters most affect the generation of cavitating gas bubbles from nanoemulsions.

CONCLUSION:

We have successfully produced a mathematical model and computer program that simulates the generation of gas bubbles from perfluorocarbon nanoemulsion droplets. The results qualitatively match experimental data of the onset of collapse cavitation in this system. The advantage of using nanoemulsions in gene delivery is that the nanoemulsion droplets can pass through the leaky capillaries of tumors exhibiting EPR, and then by application of US, gas bubbles will be formed that will permeabilized the cells towards gene delivery.

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