

Release of Calcein from Liposomes using Low-Frequency Ultrasound

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Introduction

Several groups have reported the delivery of Doxorubicin and other drugs from liposomes. Liposomes possess a lipid bilayer capable of encapsulating and sequestering water soluble drugs until release¹. Liposomes that are masked with PEO remain in circulation longer than those without PEO². More recently, several groups have reported on the use of ultrasound to release drugs from liposomes by disrupting and spilling their contents³⁻⁵. In this paper, we examined how acoustic release of calcein from liposomes is affected by varying the power density at 20 kHz.

Materials and Methods

Liposomes are made by dissolving the appropriate amounts of phospholipids and cholesterol in methylene chloride and then drying these by N₂ flow and rotovap onto the sides of a glass test tube or round bottom flask. Then the liquid to be encapsulated inside the liposome is added to the container, such as PBS, calcein solution, or ammonium sulfate solution. The glass container is heated in a 60° C water bath for several minutes and then sonicated (20 kHz, 2 W/cm²) in a Sonicor 100 cleaning bath for 15 to 30 minutes until no lipid residue is left on the flask. This solution is then sheared several times in a "gas-tight-syringe" with a 0.8 μm filter to produce large micron-sized liposomes. The size distribution of the liposomes is measured on a *Brookhaven 90Plus* Particle Sizer (Brookhaven Instruments Co., Holtsville, New York). To measure the amount of calcein released from liposomes using ultrasound, a 20-kHz Ultrasonic probe (model VC130PB, Sonics & Materials Inc., Newtown, CT) was used. The probe of the sonicator was immersed in the quartz cuvette through an opening in the fluorimeter. The probe was immersed approximately 1.5 cm in to a 3 mL sample, initially containing only phosphate buffered saline (pH = 7.4). Sixty microliters of calcein encapsulated in a liposome stock solution (ca. 0.06 μmol calcein / μmol of lipid) were added through the opening in the fluorimeter. The fluorimeter was then completely covered using a black cloth to minimize the amount of light inside the fluorimeter chamber.

Excitation and emission wavelengths were set at 488 and 520 nm, respectively. After the liposome/calcein solution was added, the fluorescence signal was recorded for 100 s. Ultrasound was then applied for

100 seconds, during which time the fluorescence was continuously monitored. The ultrasound was then turned off for 100 s. This US ON/OFF cycle was repeated 4-6 times and then at the conclusion of each experiment, the detergent Triton X-100 (30 μL of 2wt%) was pipetted in the cuvette to destroy liposomes completely thus recording the fluorescence level of 100% release.

Results

Results showed that as the power density of the ultrasound increases, so did the amount of calcein release as evidenced by the increase in the level of fluorescence after each sonication period in Figure 1.

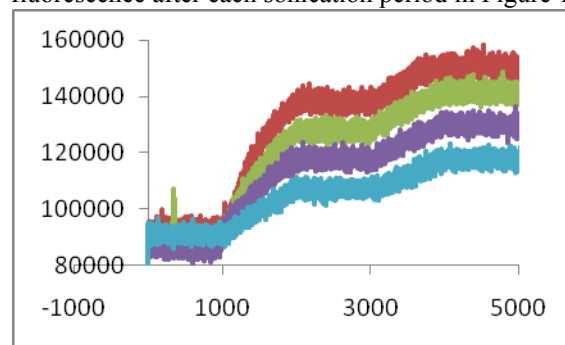


Figure 1: Fluorescence of calcein encapsulated in liposomes. Ultrasound was turned ON after 100 and 300 seconds. Sampling rate = 10 per second.

Conclusion

Ultrasound can be used to increase the rate of calcein and other molecules from liposomes. The percent and rate of release increases as the power density of ultrasound increases. More work need to be done at different ultrasonic frequencies and with different liposomal formulations in order to optimize release and to synthesize carriers that are more susceptible to ultrasound.

References

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