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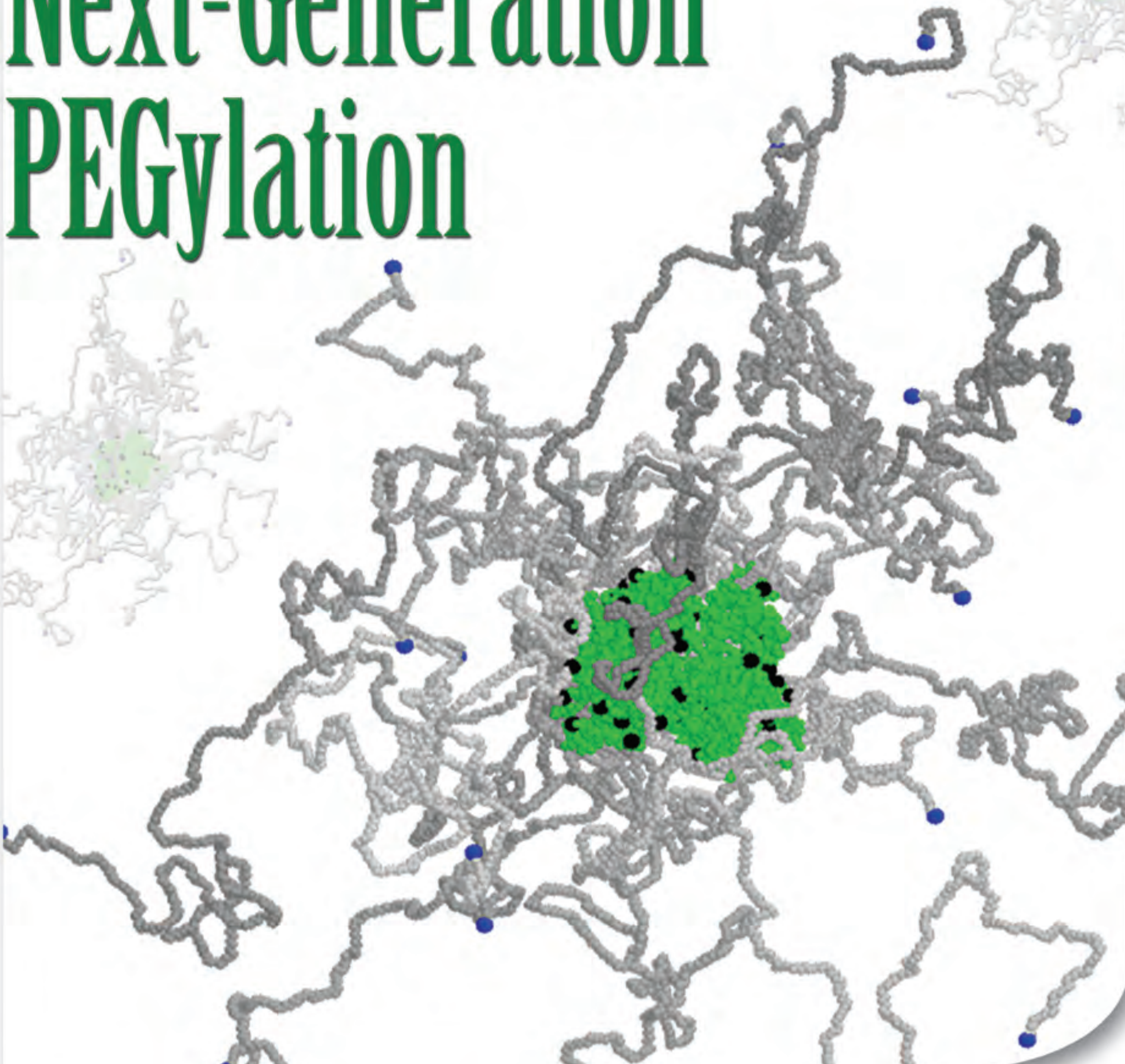
Next-Generation PEGylation



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Focused Ultrasound - A Novel Tool for Liposome Formulation

By: Srikanth Kakumanu, PhD, and Avi Schroeder, PhD

INTRODUCTION

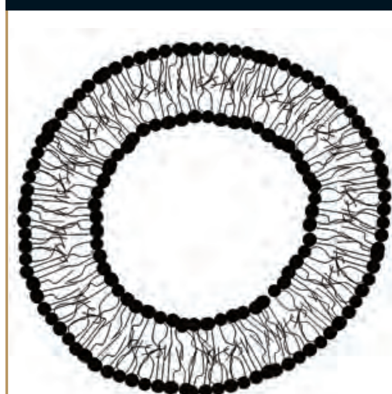
Liposomes are excellent carriers of active pharmaceutical ingredients and cosmetic agents. Their vesicular structure, housed by lipid bilayers, resembles that of natural cells and is shown in Figure 1. The building blocks of liposomes, ie, lipids, can be tuned to enhance the bioavailability of an active compound in specific tissues, improve the therapeutic index, and decrease side effects, such as toxicity. Clinically, liposomes are already FDA approved for delivering a wide scale of small molecule drugs, and their development for the delivery of more sophisticated macromolecules, such as DNA, siRNA, proteins, and peptides, is being sought by industry and academia. Producing liposomes at the nanoscale is of great interest; current technologies, such as extrusion, high-pressure homogenization/ultrasonication, and microfluidic chambers, are either non-suitable for delicate compounds or difficult to scale up. Herein, we describe a novel technology - Adaptive Focused Acoustics™ (AFA) - capable of efficiently producing nanoliposome formulations at the bench or in a pilot plant. The technology eliminates the need to heat the lipids or to dissolve them in a co-solvent during the formulation process. The computer-guided process ensures batch-to-batch repeatability, and the disposable closed flow-system prevents inter-batch contamination and alleviates the need for exhaustive wash cycles.

To date, among the approved liposomal drugs, liposomal doxorubicin (known as Doxil in the US and Caelyx in the EU), is the leading drug on the market, with annual sales that exceed $650 million. Doxil liposomes are composed of three major lipids (HSPC, PEG-DSPE, and cholesterol). In order to achieve proper liposome construction, traditional preparation methods call for high-temperature extrusion or other mechanical down-sizing processes. We tested the ability of AFA to formulate Doxil-like liposomes at 4°C. In addition, we used AFA to co-formulate nanoliposomes with the highly hydrophobic drug paclitaxel (Taxol) at 4°C and without the need for any co-solvent.

CURRENT PROCESSES & LIMITATIONS

Traditional liposome preparation methods include detergent depletion, ethanol injection, reverse-phase evaporation, and emulsion methods. Processing methods include high-pressure homogenization, extrusion, and ultrasound. One disadvantage of the preparation methods is the usage of large amounts of volatile organic solvents, multiple lengthy steps, and heat/degradation of the sample. These issues become even more problematic when scaling from small lab scales to those needed for volume manufacturing. The use of organic solvents can affect the chemical integrity of the active ingredient intended to be encapsulated and requires purification and separation steps, not to mention the environmental impact and associated

FIGURE 1



Representation of liposome structure showing a lipid bilayer with an aqueous core.

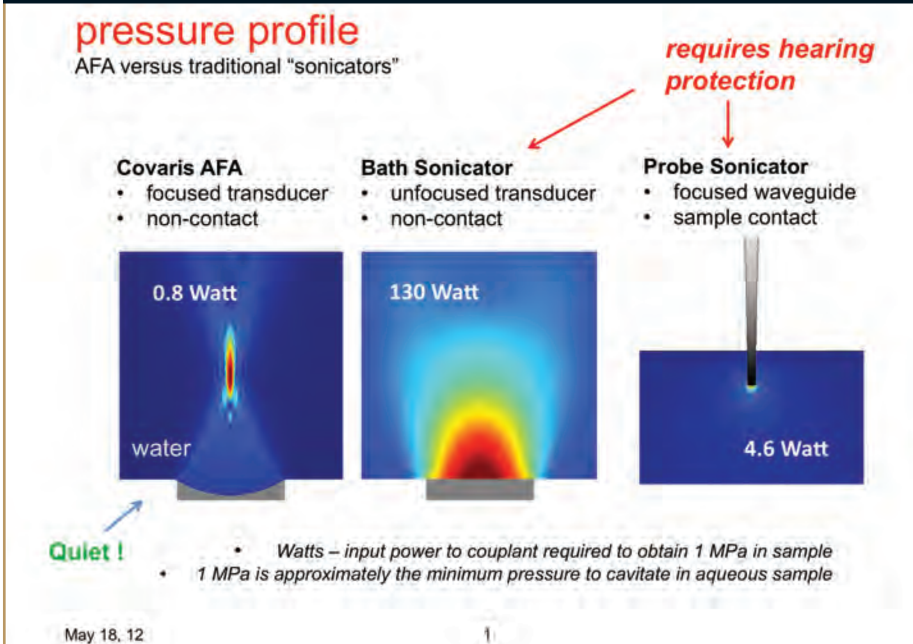
costs. New techniques, such as dense gas liposome production, are not widely employed because of the high operating pressures required for these processes.

High-pressure homogenization, in which the lipid emulsion is passed multiple times through a confined nozzle at speeds of 400 m/s and high shear rates, can generate heat and sample degradation, even when active cooling is implemented in the system.

Probe sonicators can be used to form liposomes; however, because the probe is in contact with the lipid/water during processing, concerns over contamination and scalability are inherent, and with a relatively low efficiency, the probe tip and adjacent sample material can experience extremely high temperatures. A model of pressure/temperature distribution of a probe sonicator is presented in Figures 2 and 3. In the case of a bath sonicator, the energy diverges away from the source, which reduces the intensity thus lowering the efficiency. With a broad divergent energy field, the acoustic waves can reflect and converge or diverge on a given area, thus creating "hot" or "cold" spots of uneven energy distribution.

Figure 4 represents how a sample is processed to form liposomes using AFA technology. A concave transducer directs the energy to a focal point, where the sample is placed inside of a closed vessel. Temperature is controlled by a surrounding water bath, allowing isothermal processing during liposome formation. The non-contact nature of this process ensures no contamination, and enables a sterile and disposable processing chamber.

FIGURE 2



Comparison between Covaris Adaptive Focused Acoustics (AFA), bath sonicater, and probe sonicater.

MATERIALS

Phospholipon 90G (Lipoid, Ludwigshafen, Germany), Egg Lecithin (Lipoid), HSPC: L-alpha-phosphatidylcholine, hydrogenated (Soy) (Avanti Polar Lipids, Alabastar, AL), PEG-DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (Avanti Polar Lipids), Cholesterol (Sigma), Paclitaxel (LC Laboratories, MA, US).

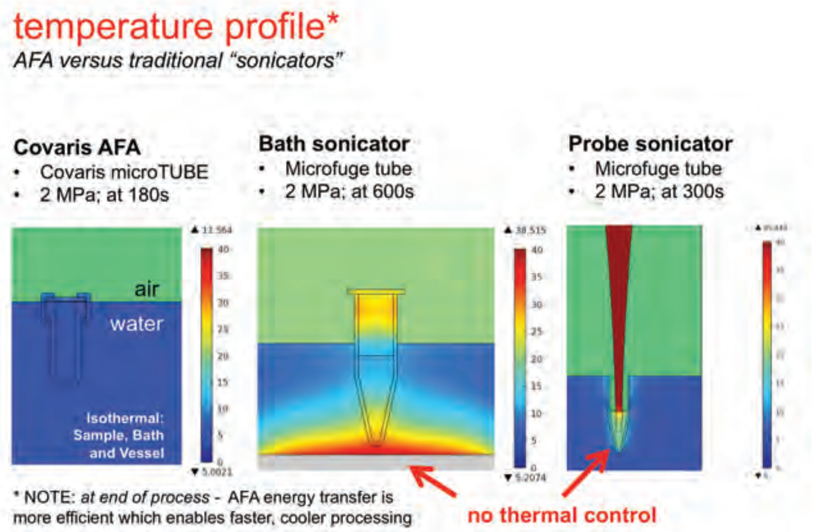
AFA Technologies (Covaris, MA, USA): S220x, Vessel: 12x24 sample vessel P/N 520056, Holder: 12x24 sample holder P/N 500199, Flow Cell, Flow Cell Holder, Flow System SF220X, Particle Sizing: Malvern Zetasizer S90/ZS90.

METHODS

To prepare 2 mL of Phospholipon 90G-based blank liposomes, 20 mg of dry Phospholipon was added to 2 mL DI water. The vessel was loaded into the instrument and processed at AFA conditions of 300 PIP, 50% Duty Factor, and 200 Cycles per Burst for 30 seconds. Particle size was measured using a Malvern Zetasizer using volume distribution analysis. Similarly, other natural lipids, such as Egg lecithin, can be processed to produce liposomes. Egg lecithin at 10 mg/ml with the same protocol produced 105-nm particles of liposomes. The concentration of lipids can be varied so it can be increased or decreased according to the needs.

Adding 20 mg of the hydrophobic anti-cancer drug Paclitaxel to 40 mg of Phsopholipon (both in their dry form, into 2-mL phosphate buffered saline) increased the particle size to 400 nm, even after 20 minutes of AFA processing. This is explained by stabilization of the particles by the drug and by the extremely high drug-to-lipid ratio. Increasing the lipid content will enable further reduction of particle size, while maintaining stability over time.

FIGURE 3



Temperature profile comparison between Covaris Adaptive Focused Acoustics (AFA), bath sonicator, and probe sonicator.

Pilot-Scale Production

The ability to scale up the lab system was tested using an AFA flow system. Here, the processed solution can be pumped through the AFA apparatus one or more times, depending on the target size of the particles. The smaller the particle size, the longer the needed exposure to AFA. A 250-mL batch was processed with 2.5 g of dry Phospholipon 90G in PBS. The dispersion was allowed to mix for 20 minutes before recirculation started. The flow rate through the acoustic field was adjusted to 65 mL/min. Figure 5 presents the particle size as a function of AFA processing time. As expected, as the processing time increases, particle size converges and the polydispersity index (PDI) decreases. It should be noted here that "over processing" can occur, in which samples reach their target size and then start agglomerating due to the continuation of the acoustic process. This highlights the need for the integrated control unit that indicates the real-time particle size by measuring absorbance in the system. In the current process, particles reached a uniform size of approximately 200 nm after 30 minutes of processing.

Low-Temperature Doxil Liposome Formulation + Taxol Formulation

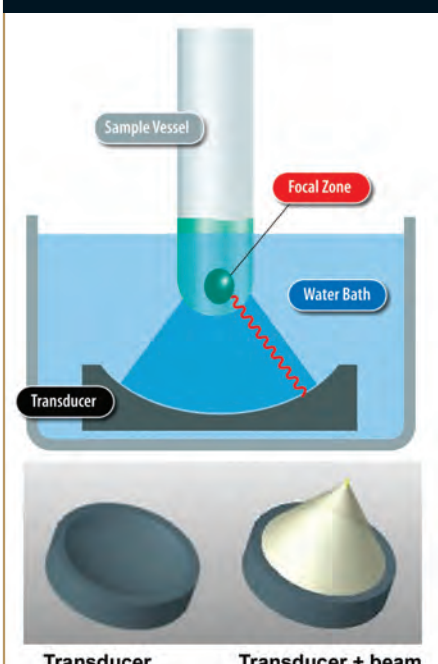
To prepare a 2-mL sample of Doxil liposomes, HSPC (11.4 mg), PEG-DSPE (3.8 mg), and cholesterol (4.2 mg) were placed dry in the sonication vessel, and PBS was added to fill the vessel to the lid (~2 mL). The vessel was processed at 200 PIP, 50% Duty Factor, 200 Cycles per Burst for 25 minutes, and particle size was measured. The particles reached a homogeneous size of < 100 nm. Doxil preparation is by remote loading, ie, the drug is loaded via an osmotic pumping mechanism post liposome formulation; thereby, these liposomes resemble those currently used in industry.

SUMMARY & CONCLUSION

Adaptive Focused Acoustic (AFA) technology was demonstrated to be a promising and novel tool for the formulation of lipid-based drug delivery systems. With the ability to formulate at low temperatures , non-contact, and without the use of solvents, AFA allows liposome formation across a variety of lipid compositions. The ability to form a Doxil liposome blank is demonstrated in small 2-ml batches while recovering 100% of the material. A Phospholipon 90G-based blank processed for 30 seconds produces a high- quality liposome distribution.

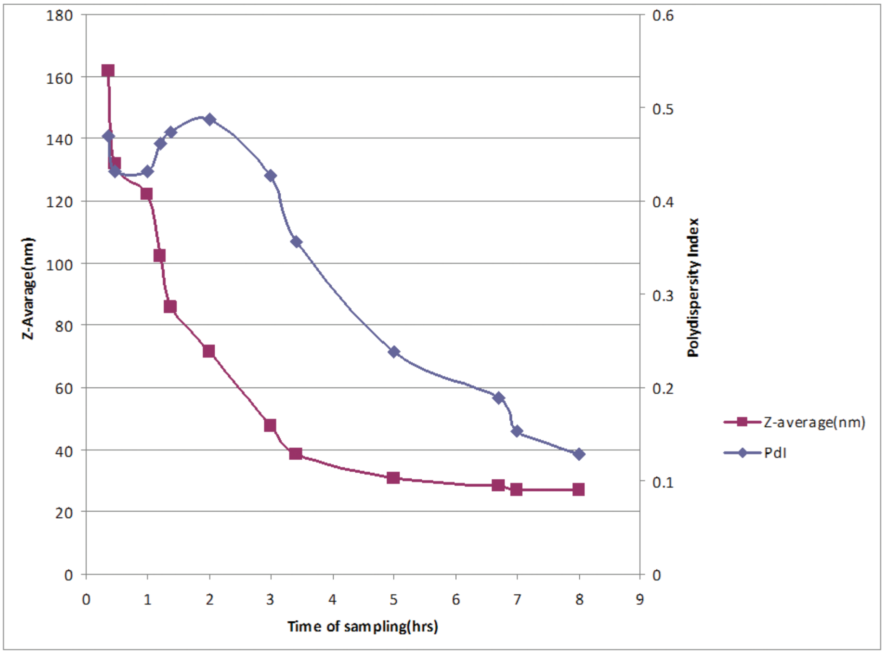
Scaling of AFA is accomplished using the same equipment utilizing a flow cell configuration. This was demonstrated to effectively produce a 250-ml batch of Phospholipon 90G-based liposome, again without contact or the use of solvents. The particle size distribution is controllable by adjusting AFA process settings and time,

FIGURE 4



Covaris Adaptive Focused Acoustics (AFA) Technology

FIGURE 5



Lipsome Upscaling 250 ml (10 mg/ml of Phospholipon 90G)

with a distribution of approximately 30-nm size particles achievable. We thus conclude that the AFA technology is a promising and novel tool for the formulation of lipid-based drug delivery systems at the bench and pilot scale. ◆

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BIOGRAPHIES

Dr. Srikanth Kakumanu earned his PhD from the Department of Biomedical Engineering and Biotechnology at University of Massachusetts in 2010. Since June, 2010 he has been working as a Research Scientist at Covaris Incorporated, where he heads the research in the application of Adaptive Focused Acoustics in formulations (dissolution, micronization, nano-suspension, and liposome production) and cell lysis. His major focus of research is scaling the AFA process to pilot scale and continuous flow sample volumes.

Dr. Avi Schroeder is a Post-doctoral Fellow in Robert Langer's Lab at the Department of Chemical Engineering and Koch Institute for Integrative Cancer Research, the Massachusetts Institute of Technology. Previously, he earned all three degrees in Chemical Engineering from the Ben Gurion and Hebrew Universities, Israel. Dr. Schroeder is an author of 19 peer-reviewed papers and inventor on 9 patents. He is a recipient of the Intel Nanotechnology, TEVA Pharmaceuticals, Hebrew University Nanoscience and Nanotechnology, and the Wolf PhD Awards.