Characterizing Drug Loading and Release in Liposomes

Ashley Velasquez and Lauren F. Sestito, Valparaiso University

Research Motivation

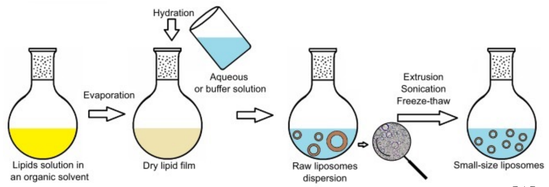
Lymphatic filariasis (LF) remains a significant global health burden, affecting millions of individuals worldwide. Current treatment methods have many limitations such as variable efficacy and adverse effects, underscoring the need for new and innovative approaches. The utilization of liposomes as drug delivery vehicles is a promising strategy for the treatment of LF, as they offer unique advantages including lymphatic targeting, biocompatibility, controlled drug release, and the ability to encapsulate hydrophilic and hydrophobic drugs simultaneously.

Objective

To characterize drug loading efficiency in and release rates from liposomes composed of phosphatidylcholine and cholesterol by using dye molecules as proxies for common LF therapeutics like doxycycline (DOX), albendazole (ALB), and diethylcarbamazine citrate.

Methods

The liposomes used for this experiment were generated using the thin-film hydration method, shown below. Phosphatidylcholine and cholesterol were dissolved at a 1:1 molar ratio in a chloroform solvent. After solvent evaporation, water was added to the residual lipid film and stirred to form a polydisperse liposome solution, which was downsized by sonication and filtration.



[1]

Oil Red-O (ORO) was selected because of its availability, bright red color, and some shared characteristics with LF drugs including albendazole (ALB) and doxycycline (DOX). ORO has a molar mass of 408.496 g/mol, an absorbance of 518 nm, and is hydrophobic [3]. DOX and ALB have molecular weights of 444.4 and 265 g/mol, respectively. While ALB is hydrophobic, DOX has limited solubility in water depending on its formulation [2]. ORO can thus serve as a proxy for such drugs to evaluate encapsulation efficiency and release rate from liposomes.

A 0.25M solution of ORO in isopropyl alcohol was created, and dilutions of this solution were used for all further experiments. To find the linear range of ORO absorbance, a standard curve was developed using a spectrometer at a wavelength of 518 nm. Dialysis was then used to evaluate the release rate of the dye from liposomes. ORO was encapsulated by mixing with a solution of liposomes in water, and naturally partitioned into the hydrophobic particles. The release of dye from liposomes was characterized over the course of hours by dialyzing a solution of ORO-loaded liposomes against deionized water, monitoring sample absorbance changes over time as dye was released.

References

[1] Minton, A. P. (2016). Recent applications of light scattering measurement in the biological and biopharmaceutical sciences. Anal Biochem, 501: 4–22. Academic Press Inc. https://doi.org/10.1016/J.AB.2016.02.007.
[2] PubChem, "Doxycycline," National Library of Medicine., 2019. https://pubchem.ncbi.nlm.nih.gov/compound/Doxycycline (accessed Apr. 19, 2024).
[3] PubChem, "1-((4-((2,5-Dimethylphenyl)diazenyl)-2,5-dimethylphenyl)diazenyl)naphthalen-2-ol," National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Solvent-Red-27 (accessed Apr. 19, 2024).

Results

Experiments 1 & 2: ORO Absorbance & Standard Curve

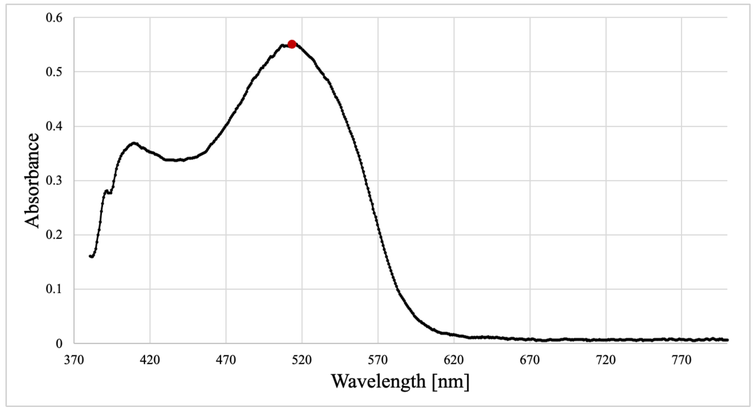


Figure 1. Using a 0.025 mM sample of ORO, the Absorbance vs. Wavelength plot using the spectrometer confirmed a peak wavelength of approximately 518 nm.

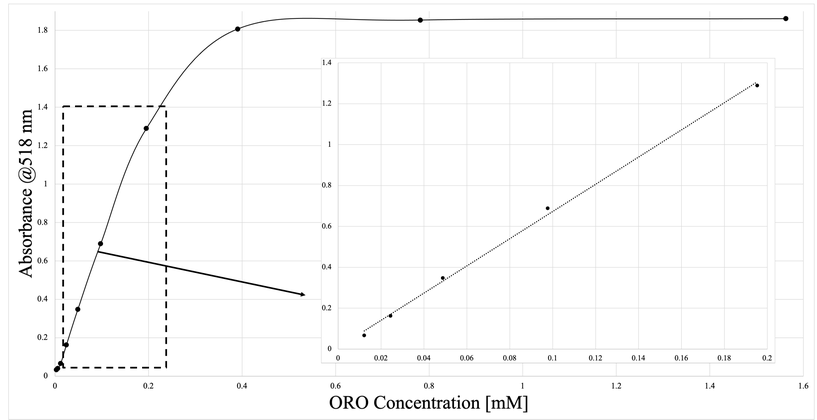


Figure 2. A standard curve was developed to show how the absorbance of ORO changes based on the concentration. The linear range was found to be between approximately 0.01-0.2 mM. Anything above or below this range will not generate accurate results when finding how the drug release relates to the concentration. Concentrations within the linear range were used for further data collection.

Experiments 3 & 4: ORO Release Using Dialysis Membrane

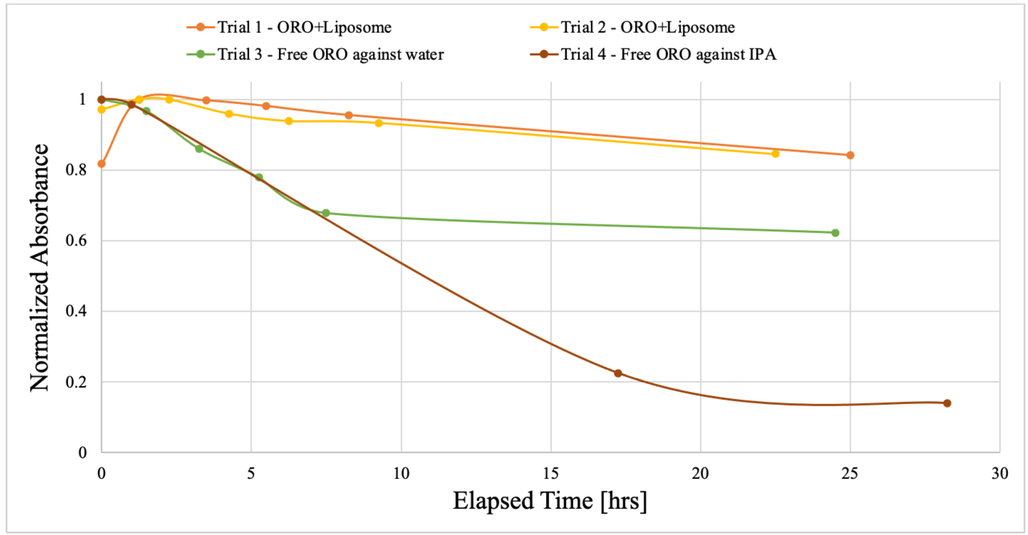


Figure 3. Absorbance at 518 nm was measured using the Vernier spectrometer of solutions with ORO over an elapsed period of time.
Trials 1 & 2 - 0.03 mL of 12.5 mM ORO solution was mixed with 2.47 mL of and 0.5 mL of pre-made liposomes. The liposomes used had a mean particle diameter of 0.469 µm after being heated and passed through a 0.22 µm filter. This mixture was added to a 12.4 kD cellulose membrane and placed in a large beaker of deionized water. At each time marking, the solution was removed from the membrane, absorbance and volume was measured, and then it was placed in a new membrane and put back in the water.
Trial 3 - 0.03 mL of 12.5 mM ORO solution was mixed with 1.47 mL of deionized water and 1 mL of isopropyl alcohol (so the ORO could dissolve). This mixture was added to a 12.4 kD cellulose membrane and placed in a large beaker of deionized water. At each time marking, the solution was removed from the membrane, absorbance and volume was measured, and then it was placed in a new membrane and put back in the water.
Trial 4 - The same procedure as trial three was repeated; however, a 30% isopropyl solution was used in order to provide an isotonic environment inside and out of the membrane.

Discussion

- There was no significant change in the absorbance of the liposome with ORO when undergoing the dialysis membrane experiment in Trials 1 & 2. This shows that almost all of the ORO has been encapsulated; however, over time, only a very small amount of it has been released from the liposome.
- Using ORO with no liposomes, the dye diffused out of the membrane over time at a much faster rate, decreasing the absorbance, as shown in Trials 3 & 4 of Figure 3. This is because the ORO dye is smaller than the pores of the dialysis tubing, so it diffused out when placed in deionized water, and especially in a solution of 30% isopropyl solution.
- The liposomes prove to encapsulate/hold on to the drug for a much longer period of time, making them more effective than if the drug was just released itself in the body.

Future Work

- Add the ORO to the liposome before the formation of the thin film and measure how it impacts encapsulation and release
- Characterize FITC or other dyes using similar procedures
- Characterize encapsulation efficiency using Size Exclusion Chromatography or similar methods

VALPARAISO UNIVERSITY
ENGINEERING