# Microfluidics Guided Self-Assembly of Magnetoliposomes

**Tiffany Moeller**

**Chemical Engineering, University of Nebraska**

***NNIN REU Site: Cornell NanoScale Science and Technology Facility, Cornell University, Ithaca, NY***
*NNIN REU Principal Investigator(s): Carl Batt, Department of Food Science, Cornell University*
*NNIN REU Mentor(s): Matthew Kennedy, Dickson Kirui, Department of Food Science, Cornell University*
*Contact: tiffanymoeller@msn.com, cab10@cornell.edu, mjk67@cornell.edu, dk322@cornell.edu*

## Abstract:

**Liposomes in the range of 100 to 500 nm were created using a microfluidic flow-focusing device by varying composition and flow rates of the lipid and sheath fluids. Due to their amphiphilic structure, liposomes can be engineered to encapsulate magnetic nanoparticles, forming magnetoliposomes. The project aims were to (1) optimize reagent parameters to modify liposome size and (2) encapsulate nanoparticles. A three-dimensional microfluidic focusing manifold was employed to enhance the liposomes' monodispersity to improve *in vivo* pharmacokinetics. At flow rate ratios (FRRs)—sheath to lipid solution—from 10 to 25, increasing phospholipid concentration from 0.10 to 10.0 mM decreased liposome size from 215 to 120 nm. At the same FRRs, increasing the concentration of potassium chloride from 0.10 to 10.0 mM in the sheath fluid increased the liposomes' size from 120 to 470 nm; liposomes aggregated at FRRs less than 10.0. Magnetoliposomes were formed via an *in situ* precipitation of magnetite in the interior compartment of the liposomes.**

## Background:

Monodisperse liposomes are more desirable for *in vivo* applications because they display more uniform pharmacokinetics [1,2]. Additionally, liposomes with diameters of 150 to 200 nm demonstrate prolonged circulation half-lives and more efficient extravasation of tumor microvasculature compared to liposomes of other sizes [3,4]. Therefore, microfluidic-directed formation of liposomes is very useful as it allows for controlled size and more monodisperse liposomes over other synthesis methods [4].

Magnetoliposomes have been engineered to encapsulate magnetic nanoparticles to concentrate therapeutics at the delivery site [4]. An alternating magnetic field can induce hyperthermia, allowing a burst-release of the encapsulated therapeutic [2]. Magnetoliposomes have been synthesized using other methods [5], but we employed the microfluidic platform to produce more monodisperse product of controlled size.

## Experimental Procedure:

**Microfluidic Device.** The microfluidic device was fabricated using general photolithography techniques. Polydimethylsiloxane (PDMS) was spun onto SU-8 patterned wafers, and the two pieces of PDMS were aligned. The cross-sectional dimensions of the square microchannel were 125 $\mu$m; the channel was 2.0 cm in length.

**Liposomes for Size Experiments.** The phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG) were dissolved with cholesterol (Chol) in ethanol in a 10.1:1.0:9.9 (DPPC/DSPE-PEG/Chol) molar ratio. The sheath fluid was deionized water or a potassium chloride solution. A syringe pump flowed the lipid solution through the center input of the device and the sheath fluid in the remaining four inputs. Product was collected at varying flow rate ratios (FRRs)—sheath to lipid solution—with a constant bulk flow rate of 20.39 $\mu$L/min and a Reynolds number of 2.72.

***In situ* Synthesis of Magnetoliposomes (MLs).** The sheath fluid was 5.0 mM $FeCl_3$ and $FeCl_2 \cdot 4H_2O$ (2:1 molar ratio). The lipid solution (10.1:1.0:8.1; DPPC/DSPE-PEG/Chol) was pumped at 1.3 $\mu$L/min and the sheath at 4.8 $\mu$L/min (FRR = 14.7). $NH_4OH$ solution was added to the liposomes to precipitate magnetite ($Fe_3O_4$) according to the following chemical reaction:

$$2FeCl_3 + FeCl_2 + 8NH_3 + 4H_2O \rightarrow Fe_3O_4 + 8NH_4Cl$$

**Size Determination**. Hydrodynamic radii were determined with dynamic light scattering (DLS) at 0.010 mM phospholipid concentrations. Measurements were taken at 90° with a Melles Griot HeNe 632.8 nm laser.

**Characterization by Transmission Electron Microscopy (TEM).** A 5.0 $\mu$L aliquot of sample was pipetted onto a carbon-coated 200 nm mesh copper grid and stained with 5.0 $\mu$L of sodium phosphotungstate negative stain. Images were taken with a Philips Tecnai T-12 scope at 120kV.

## Results and Discussion:

**Monodispersity of Liposomes.** The microfluidic device was based upon Kennedy et al. [6]. The three-dimensional design focused the lipid solution to the microchannel's

center. Diffusion of the lipids into the surrounding aqueous sheath fluid directed liposome self-assembly. Doing so narrowed the velocity distribution of the molecules, potentially narrowing the size distribution of the liposomes as well.

**Effect of FRR on Liposomes.** At FRRs below 10.0, there was no trend in liposome size (Figure 1). An insufficient volume of sheath fluid in the microchannel prevented complete diffusion of the lipids, resulting in incomplete liposome assembly. At FRRs of 10.0 and above, increasing the phospholipid concentration from 0.10 to 10.0 mM decreased liposome diameter from 215 to 130 nm. At the higher concentration, lipids had a shorter diffusion time before reaching the critical aqueous concentration at which liposomes self-assemble, resulting in smaller liposomes.

Increasing the sheath's salt concentration from 0.10 to 10.0 mM increased the liposomes from 150 to 470 nm. At the lower salt concentration, the solvating action of the water molecules stabilized the liposomes thereby minimizing their size. With increasing ionic strength, water molecules increasingly solvated salt ions. The instability created by decreased solvation resulted in larger liposomes which are prone to aggregation (Figure 2). Characterization by TEM showed all liposomes to be generally spherical; however, lipid aggregates predominated most samples (Figure 3).

**Characterization of Magnetoliposomes (MLs).** TEM images before and after addition of $NH_4OH$ verified encapsulation of magnetite. Before $NH_4OH$ addition, the liposomes were spherical with an average mean diameter of 160 nm. Precipitated magnetite appeared as black spots in the liposomes' interior (Figure 4). TEM verified DLS size measurements.


## Acknowledgments:

The author thanks the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program and the National Science Foundation (NSF), the Cornell NanoScale Science and Technology Facility (CNF) and program coordinators Melanie-Claire Mallison and Rob Ilic, as well as the Batt Group—especially Matthew Kennedy, Dickson Kirui, and Carl Batt—for their assistance and support.


## References:


[1] Xu, Q., et al.; Small Journal, 5.13, 1575-1581 (2009).

[2] Arruebo, M., et al.; Nanotoday, 2.3, 22-32 (2007).

[3] Litzinger, D., et al.; Biochimica et Biophysica Acta, 1190, 99-107 (1994).

[4] Gu, F., et al.; Nanotoday, 2.3, 14-21 (2007).

[5] Sabaté, R., et al.; International Journal of Pharmaceutics, 347, 156-162 (2008).

[6] Kennedy, M., et al.; "A Microfluidic Hydrodynamic Diffusion Mixer with Reduced Taylor Dispersion"; (2009).


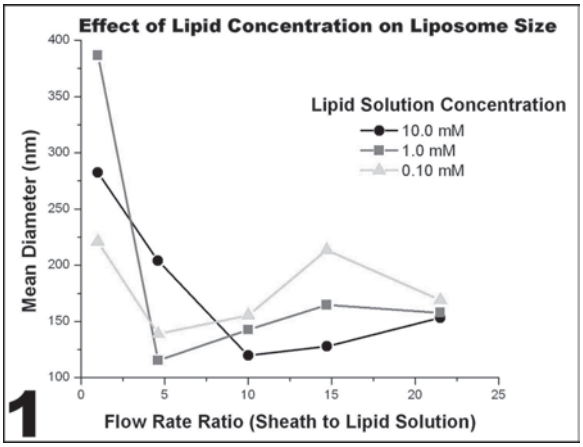


*Figure 1: Increasing phospholipid concentration resulted in smaller liposomes.*

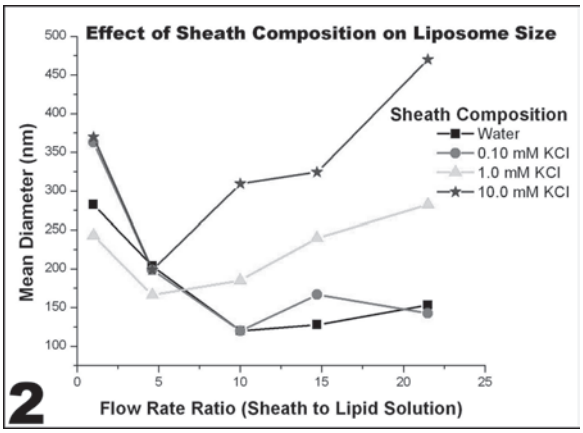


*Figure 2: Increasing ionic strength of the sheath fluid resulted in larger liposomes.*

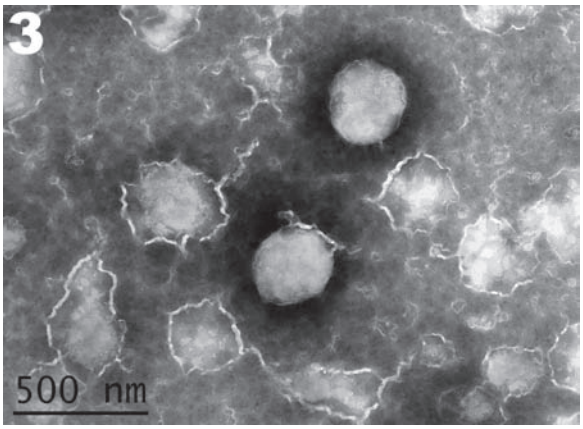


*Figure 3: TEM image of liposomes with a mean diameter of 240 nm.*

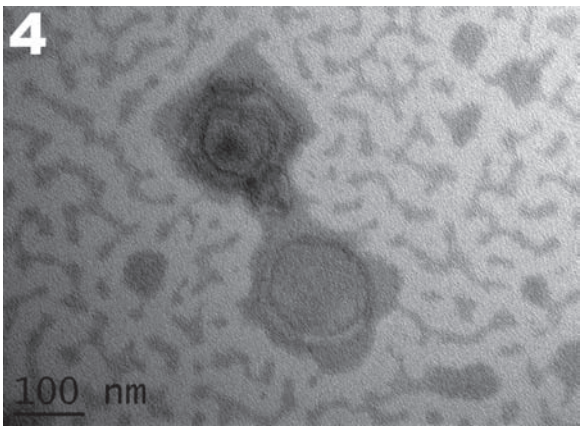


*Figure 4: TEM image of a magnetoliposome. The top liposome is encapsulating magnetite while the bottom liposome is not.*