



**University
of Victoria**

Graduate Studies

Notice of the Final Oral Examination
for the Degree of Master of Science

of

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BSc (University of Waterloo, 2013)
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“Properties of Vesicles Containing Natural and Synthetic Lipids Formed by
Microfluidic Mixing”

Department of Chemistry

November 18, 2015
3:00 P.M.
Engineering and Computer Science Building
Room 128

Supervisory Committee:

Dr. Tom Fyles, Department of Chemistry, University of Victoria (Supervisor)
Dr. Matthew Moffitt, Department of Chemistry, UVic (Member)

External Examiner:

Dr. Francis Choy, Department of Biology, UVic

Chair of Oral Examination:

Dr. Michael Nowlin, Department of English, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

A series of sulfonate anionic lipids esters derived from 4-sulfobenzoic acid (single chain) or 5-sulfoisophthalic acid (double chain) with chain length from C14 to C18 were synthesized and characterized. The sodium salts were uniformly insoluble in ethanol; the tetramethylammonium salts of the single chain derivative from oleyl alcohol and the double chain derivative from 2-octyldodecan-1-ol were sufficiently soluble for subsequent experiments.

Lipids in ethanol and aqueous buffers were mixed in a microfluidic system (NanoAssmblr ® microfluidic mixer) to prepare a lipid dispersion containing vesicles and/or nanoparticles.

Initial studies on prediction and controlling vesicle size based on lipid geometric parameters showed that particle size could be successfully affected and controlled by altering lipid compositions consistent with the formation of vesicles. A survey using high resolution cryo-Scanning Transmission Electron microscopy of the sample made by the microfluidic mixer demonstrated that vesicles were formed but a majority of the sample reformed to other aggregates, which complicated the interpretation of the initial product distribution. Further investigation on the efficiency of incorporation of phospholipids into vesicles indicated that 55% of the initial phospholipid appeared in the vesicle fractions. Sulfonate anionic lipids are incorporated into vesicles with lower efficiency and reach a threshold beyond which the sulfonate lipid is not incorporated. Entrapment efficiency was studied with three dyes. Different concentrations of the hydrophobic neural dye Nile red, the hydrophilic cationic dye neutral red and the hydrophilic anionic dye hydroxypyrene trisulfonate (HPTS) were prepared. The entrapment efficiency was quantitatively analyzed by HPLC, and electrospray mass spectrometry; up to 15% of the initial dye present could be entrapped. Vesicles permeability assays using the ion channel gramicidin and the ion carrier valinomycin with HPTS-loaded vesicle samples showed that vesicle samples made by the microfluidic mixer and made by a conventional extrusion method appeared to behave in the same manner. Addition of a sulfonate anionic lipid to the lipid mixture resulted in vesicle leakage. The unilamellar proportion of HPTS loaded vesicle samples was assessed using a mellitin assay. A vesicle sample made by the microfluidic mixer was 80% unilamellar; a vesicle sample made by the extrusion method on the same lipid mixture was 60% unilamellar.