Abstract:
The kinetics of Zn$^{2+}$ metal incorporation into porphyrin encapsulated phosphatidylcholine (PC) vesicles (or liposomes) was studied using spectrophotometry. (Figure 1) Liposomes, made by controlled detergent dialysis, were characterized by multiangle static light scattering to prove that they were unilamellar and monodisperse in size. The average radius of the vesicles was engineered to be 40 to 127 nm and was varied by changing the lipid-detergent molar ratio.

Both the positively charged Tetra (N-Methyl-4-Pyridyl) Porphyrin (TMPyP[4]) and the negatively charged Tetra (4-Sulfonatophenyl) Porphyrin (TPPS) were used for zinc incorporation into the vesicles. (Figure 2) This method of making porphyrin encapsulated vesicles that are homogenous in size, may prove useful in the removal of metal ions from wastewater.

Introduction:
Liposomes, or vesicles, consist of one or more lipid bilayers enclosing an aqueous compartment. They are capable of encapsulating either hydrophilic components in their aqueous interior or hydrophobic compounds in their bilayer membrane [1, 2]. Unilamellar vesicles have been widely used as models for biological cell membranes [2-5]. They have also been employed in the delivery of drugs, and other substances, to cells [1-5].

Porphyrins were encapsulated by liposomes because they bind to metal ions in a non-reversible reaction. Consequently, these types of vesicles may have applications in wastewater cleanup. The removal of metal ions from industrial effluent is an extensive environmental problem [6-8]. Metal ions are extremely toxic and pose a considerable threat to human and aquatic life when present in the environment. Porphyrin encapsulated vesicles may have an advantage over other cleanup processes because after metals have been incorporated into the vesicles, they can easily be filtered out. Additionally, liposomes are biodegradable and can be separated from the metalated porphyrins and recycled.

Methods:
The lipid film was prepared by evaporating the chloroform solvent using a rotary evaporator. Variable amounts of sodium cholate detergent was added to the dried lipid film obtaining a lipid-detergent ratio in the range of 0.29 - 1.24. The mixture was then dissolved in 1mL of 0.9-wt. % NaCl buffer, pH 6.8. Solid porphyrins were then dissolved in this solution. The final lipid concentration was 50 mg/ml and the final porphyrin concentration was varied from 5-50 mg/ml. Detergent removal was carried out using a Mini LipoPrep instrument (AmiKa Corp., Columbia MD). The dialysis procedure took place at room temperature. Vesicle samples were kept at room temperature in the dark until use.

Results and Discussion:
Light scattering studies were performed on several sets of TMPyP[4] encapsulated vesicles. Changing the lipid-detergent ratio varied the vesicle radius. The data indicates that the vesicle size increased as the lipid-detergent ratio was increased. This trend was experimentally verified by other investigators [3, 4].
At a constant lipid-detergent ratio, both negative (TPPS) and positive (TMPyP[4]) porphyrins were encapsulated by vesicles. These vesicles were characterized and compared to the experimental control consisting of pure vesicles with no encapsulated material.

We found that both the negative and positive porphyrin encapsulated vesicles had larger radii than the experimental control (Figure 3). Furthermore, the TPPS encapsulated vesicles were found to be slightly larger than the TMPyP[4] vesicles. While it is not entirely clear why this phenomenon occurs, we believe that vesicle size increases after porphyrins are encapsulated simply because the large number of porphyrin molecules enclosed within the vesicles causes them to enlarge. The minor change in size between the TPPS vesicles and the TMPyP[4] vesicles can probably be attributed to the difference in structures of the two porphyrins.

In aqueous solution, ionic strength 1M, TMPyP[4] presents absorption maxima at 422.0, 518.4, 553.8, 584.0, and 640.4 nm [10]. After zinc addition the resulting spectrum displays maxima at 436, 560, and 602 nm [10]. The absorption spectrum of TMPyP[4] encapsulated vesicles before and after zinc addition (Figure 4) reflects this data indicating that zinc ions successfully crossed the lipid bilayer and were incorporated into the porphyrin molecules. In our experiments we calculated the equilibrium constant for the reaction to be 4.02 X 10⁻³ M⁻¹ at ionic strength of approximately zero.

Conclusion:

Both TPPS and TMPyP[4] encapsulated vesicles were prepared by controlled detergent dialysis and characterized by light scattering to determine size and homogeneity. Zinc was successfully incorporated by the vesicles and into TMPyP[4] porphyrins. These results were confirmed by analyzing the absorption spectrum of the TMPyP[4] vesicles prior to and following zinc addition. The size of porphyrin encapsulated vesicles makes it possible for them to be filtered out of solution. Therefore, these types of vesicle may have application in wastewater cleanup.

References:
2. JH Van Zanten, “Characterization of Vesicles and Vesicular Dispersions via Scattering Techniques.”

Acknowledgements:

Thanks to Dr. Andre Palmer, Dr. Peter Hambright, the Materials Science, Chemistry, and Engineering Departments of Howard University, the National Nanofabrication Users Network, and the National Science Foundation.