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Nonamphiphilic Modification of the Self-Assembly Behavior of Surfactants and the Potential for Bolaamphiphile-based Vesicle Formation in the Presence of 5'Disodium Cromoglycate

A Capstone Project Submitted in Partial Fulfillment of the
Requirements of the Renée Crown University Honors Program at
Syracuse University

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and Renée Crown University Honors
May 2011

Honors Capstone Project in ________Chemistry________

Capstone Project Advisor: ___________________________
Yan-Yeung Luk

Honors Reader:___________________________________
Michael Sponsler

Honors Director:__________________________________
James Spencer, Interim Director

Date:____

Abstract

Emulsion systems stabilized by surfactants have historically been driven by the separation of hydrophobic oils from water, essentially creating surfactant micelles. As surfactant molecules aggregate around the oil, they self-assemble in such a way that the hydrophobic chains of the surfactants face inward toward the oil while the hydrophilic, or water-loving, “head” of the surfactant face outward toward the water medium. The emergence of water-in-water emulsions consisting of droplets of a water-solvated biocompatible liquid crystals, disodium cromoglycate (5’DSCG), with polymers has unlocked the potential for modifying the self-assembly behavior of surfactants. We report here on the capacity of 5’DSCG to drive vesicle formation in surfactants that would otherwise form micelles, as well in bolaamphiphiles that would otherwise form lamellar bilayers. This behavior is two-pronged with the ability of these surfactants to form shiny, colorful liquid crystal droplets (a phenomenon known as birefringence which results from materials that modify light in such a way that it can pass through double polarizers that would otherwise allow no light through) at concentrations of 5’DSCG much lower than systems containing just 5’DSCG alone and water, indicating that while 5’DSCG promotes vesicle formation, the surfactants promote the “compression” of solvated 5’DSCG molecules. We also report on the ability of samples prepared via stock solutions to exhibit an odd-even effect in regards to the length of the aliphatic chain in vesicle formation, such that surfactants that have an even number of carbons actually have more difficulty forming vesicles than surfactants with an odd number of carbons in the chain because they aggregate, or pack together, more efficiently.

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Preface

I do believe that my preface may very well need its own preface. After all, it is a fact that most scientific literature does not begin with a preface. In fact, most scientific literature simply jumps into an introduction of the material (after having wetted your appetite with a title and an abstract of course), and usually this introduction will be convoluted and incomprehensible to anyone but the very interested scientist. This is not simply because the scientist has succumbed to the hubris of his highly intellectual role in society; it is not because he carries with him some residual disdain for the common man that stems from his years of being a nerd (though this seems entirely plausible). The truth of the matter is: a writer, albeit a scientist writer, must write for his audience. He cannot dilly-dally upon the qualms of simplification and background information because more likely than not, the only people who will ever read his work are those who possess the knowledge and experience to comprehend it. And, of course, sometimes we pay by the word.

I regret that I cannot deliver a "readable" and dramatically simplified explanation of my work for those who have not had some experience in chemistry, though I have certainly endeavored in the Summary section at the very end of this capstone, which I have named "A (Moderately) Non-Technical Summary of this Capstone." It is here that I will drain my creative juices in an effort to make metaphors and visualizations of such clarity that even those most adverse to science can appreciate its capacity to grow, and respect its history of expansion.

The truth, the simple and unending truth, is best summarized in a quote from a letter sent to Robert Hooke from Sir Isaac Newton: “If I have seen further it is only by standing on the shoulders of giants.” If there is one thing that everyone should take away from the collection of scientific literature, it is the sheer volume of references. For every miniscule fragment of knowledge we acquire, it is only through the mosaic of science that we are able to continue reaching further, it is through the achievements and failures of the explorers that we have the maps that will guide us to the edges of our current reality.

And herein lies the challenge of the Honors capstone. These theses are a culmination of our college endeavors and experiences, a testament to our newfound knowledge these past years, and a lasting pillar that represents the beginnings of our future. No matter how very different we were before this accomplishment, and however far our paths diverge from this moment, we will always be united by this single accomplishment, and all the struggles and glory that comes with its completion. Thus, my work becomes more than mere scientific literature, it is my lasting legacy at Syracuse University. This will be the fragment of that greater mosaic that I leave behind. This will be shared, experienced, and perhaps even read by my friends and family, by scientists and photographers, by artists and engineers.

Acknowledgements

No professor in my four years of college has taught me more than Dr. Yan-Yeung Luk has. He has been a guiding mentor, a brilliant scientist, and a great human being. Without him, this capstone would not be what it is. Without him, I would not be who I am today. He has provided me with all the materials necessary to do research successfully, and I have had the opportunity to perform all types of research in this lab—from colloid and interfacial chemistry to organic synthesis in pursuit of anti-biofouling agents to bioorganic chemistry for water-driven reactions. And this is what I wanted most in college—to be able to experiment with different chemistries to ultimately widen my perspective and add depth to the multitude of courses I enrolled in.

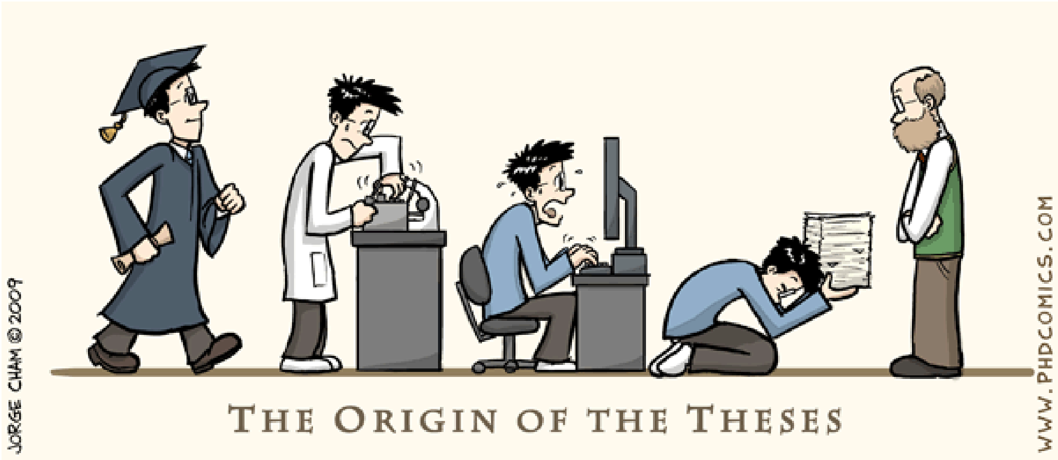
I would also like to thank all the graduate students in the Luk Group, who have taught me numerous things and given me so much advice. First I would like to thank Karen A. Simon, who—despite all the work she had—took time to teach me all about birefringence and phase diagrams and preparing water-in-water emulsions. I wish also to thank Debjyoti Bandyopadhyay who taught me everything I know about organic synthesis and purifying compounds—first as my Organic Chemistry Lab TA, then in the Luk Group as I worked on L-gulitol self-assembling monomers. I would also like to thank Nisha Varghese, who was crucial in helping me design and implement the experiments that essentially make this capstone thesis. Her expertise in colloidal and interfacial chemistry have helped me a great deal in writing this paper. We often joke about how I’ve been in the Luk Group longer than she has—but she is smart and gifted and as helped me so much. Furthermore, I would like to thank Sri Kamesh Narasimhan for his

advice and friendship—his humor and amiability made the laboratory environment one that I enjoyed to work in. And for all the little bits of advice and guidance over the years, I would also like to thank Deepali Prashar, DaWei Cui, Ricky Lei Wu, Preeti Sejwal, Erik Burton, Fei Cheng, Sijie Yang, Nischal Singh, and Gauri Shetye, as well as Akshay Shah, who first showed me around the laboratory. In addition, I wish to thank Troy Lam, Dakin Sharum, and Alex Augatis for their help in organic chemistry.

As faculty members that have opened numerous opportunities to me, I would also like to thank Dr. Michael Sponsler, Dr. Mark Braiman, Dr. James Kallmerten, Dr. Karin Ruhlandt-Senge, Dr. Matt Maye, Dr. Jim Spencer, and Dr. James Dabrowiak for all their guidance and help during my time in Syracuse. I would also like to thank Cathy Voorhees, Denice Buchanan, and Jodi Randall for everything they do. Again, I would like to especially thank Dr. Michael Sponsler for being my Honors Reader despite his very busy schedule.

Lastly, I would like to thank my friends and family for all their love and support. I would like to thank my Mom and Dad for their continued support, as well as my little sister Kavida who now heads off to college in pursuit of her own dreams. I would like to thank Jillian Ellis, my guiding spirit, for all her encouragement and assistance. And I would like to thank Christopher Walter and Clara Harris for their advice and guidance.

Advice to Honors Students



Adopted from PhD Comics, © Jorge Cham

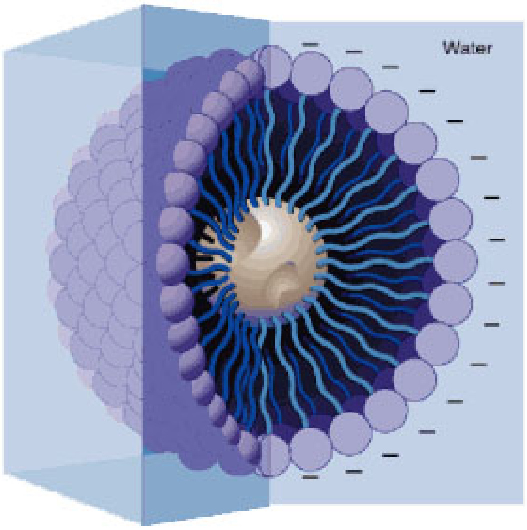
Let your entire body of work, everything you do, be the accumulation and the assimilation of thousands of small, well done, parts.

I. Introduction

When Scottish chemist Thomas Graham discovered that certain substances like gelatin or starch pass through membranes more slowly than inorganic salts, he distinguished them by calling the former colloids and the latter crystalloids.[1] It was here in 1854 that Graham discovered dialysis, and his work essentially began the field of interface and colloidal chemistry.[2] More specifically, this branch of science is concerned with particles, droplets, and bubbles dispersed in bulk phases, and the properties of the interfacial regions around them, as well as within them.[3] Colloids have become a common entity and aside from a gaseous phase dispersed in another gaseous bulk, they can exist as any combination of phase and medium.[4] For instance, a liquid phase dispersed in a bulk gas is an aerosol; a gas phase dispersed in a bulk liquid is a foam; and a liquid phase dispersed in a bulk liquid is an emulsion.[5] An emulsion is a colloid because the two liquids are immiscible—they will not mix.

One famous example of this is oil and water, two immiscible liquids that will often form two layers. Upon being emulsified (shaken vigorously, for instance), the oil exist as droplets in the bulk water, though this is often very short-lived.[6] This particular type of instability is referred to as coalescence, which is the process by which two or more droplets merge during contact to form a larger droplet.[7] As a result, the emulsion progressively separates, and the oil and water will eventually form two separate layers again. In an effort to stabilize these emulsions, surfactants have been used.[8]

This stabilization is why soap works. In fact, soap is the earliest known surfactant—it dates back to 2800 B.C.E in Ancient Babylon—as archaeologists have found soap-like substances in clay cylinders during excavations of the ancient civilization.[9] Soap is amphiphilic in nature, possessing both hydrophilic (water-loving) and lipophilic (fat-loving) properties. As a result, these soap surfactants form micelles around grease. Micelles (**Scheme 1**) are the aggregation of these surfactant subunits, which are oriented in such a way that the exterior of the micelle is hydrophilic while the interior (where the grease or oil is dissolved) is hydrophobic, or lipophilic.[10] Thus, surfactants stabilize the oil-in-water emulsion and have inconsequently created a dogma that emulsions require amphiphilic molecules.

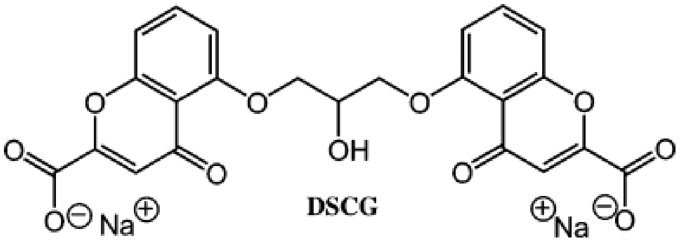


Scheme 1- Micelle Structure Around Grease
Image adopted from the University of Illinois-Chicago. http://www.uic.edu/classes/bios/bios100/lecturesf04am/micelle.jpg

Admittedly, assembling large quantities of molecules in water has historically involved amphiphilic molecules forming micelles, vesicles, or other assemblies based on the separations caused by hydrophobic and hydrophillic interactions, as aliphatic chains aggregate to avoid water molecules that surround them.[11] Generally, assembling large quantities of nonamphiphilic but water-soluble molecules requires overcoming incredible entropy that comes with organizing solute molecules in space.[12] Thus, without forming crystals or precipitates, such assemblies without amphiphilic molecules are considered exceptionally rare.

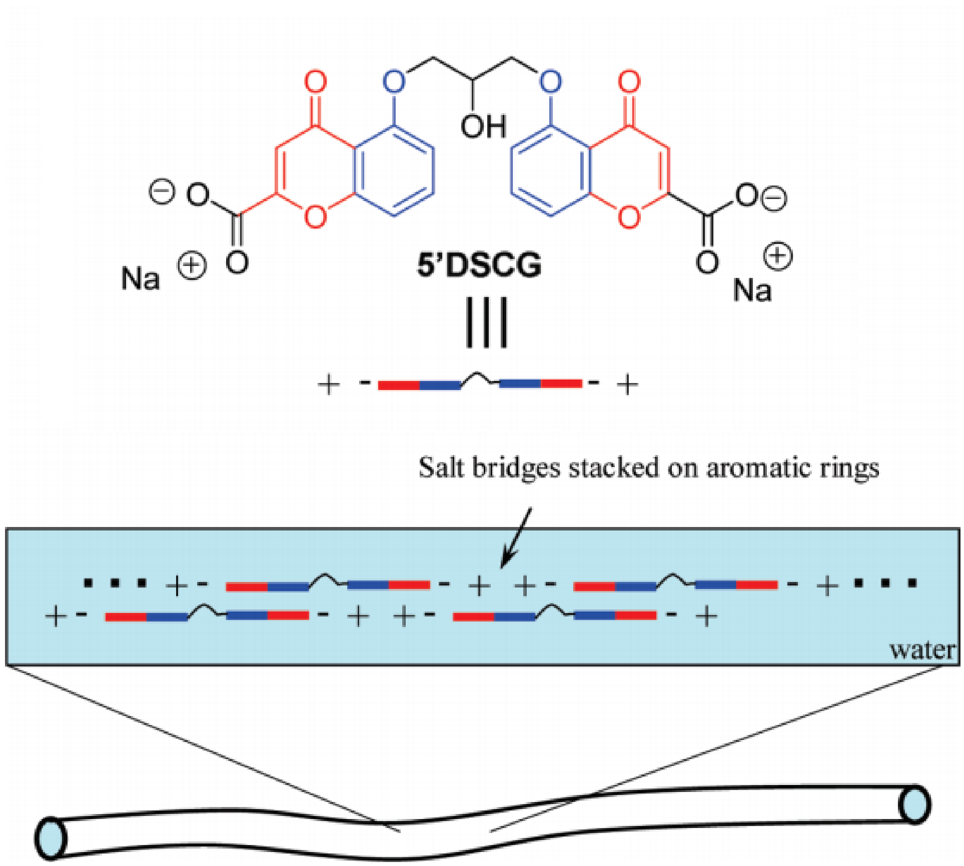
Nonamphiphilic assemblies are important because not all phenomena observed at the aqueous interface between a surface and a biological entity are as simple as mere hydrophobic/hydrophilic interactions. Proteins can adsorb to most surfaces, regardless of hydrophilicity and numerous mechanisms and chemistry have been proposed for controlling the adsorption of proteins[13] or the adhesion of cells to surfaces.[14,15]

Recently, our group has reported on the molecular interactions that are usually associated with biological molecules without aliphatic hydrophobic interactions to form water-in-water emulsions.[16] These emulsion systems involve dispersing water-solvated droplets of nonamphiphilic lyotropic liquid crystals in aqueous solutions containing different polymers. In this case, the lyotropic liquid crystal is composed of disodium cromoglycate (5’DSCG) dissolved in water (**Scheme 2**). A material is referred to as lyotropic if it forms liquid crystals because solvent is added; it is referred to as thermotropic if temperature determines the order of its components.



Scheme 2 – Disodium Cromoglycate forms lyotropic liquid crystals in water

5’DSCG, which is also known as cromolyn sodium salt was discovered in 1936,[17] but was first synthesized and developed into a potent anti-allergic drug in the 1960s.[18-20] At concentrations of 11 to 21 wt.% at room temperature in deionized water, this molecule forms highly birefringent liquid crystal phases.[21-23]



Scheme 3 - Thread Assembly Model for DSCG
Image adopted from Simon et. al J. Phys. Chem. B **2010**, *114*, 10358

Historically, lyotropic liquid crystals resulted from hydrophobic/hydrophilic interactions in solvents but this interaction cannot be applied to the 5’DSCG liquid crystal system, as 5’DSCG is a nonamphiphilic organic salt.[20,23,24]

Understanding the actual molecular organization of the liquid crystal phase formed by 5’DSCG has been a point of controversy over the years. After the liquid crystal phase of 5'DSCG in water was first observed in 1971,[25] the discoverers proposed a fairly simple model in 1973.[26] Seven years later in 1980, a harsh attack was made on the model without any experimental results and a new "chimney" model was proposed for the system.[27] In the following year, the original discoverers responded accordingly,[28] and the "chimney" model was quietly retracted by its author.[29] Nonetheless, this obsolete model is still cited in the literature today.

Over the years, different stacking models have emerged that have tried to explain the observed phenomena of 5'DSCG.[25,29,30-34] Our group recently proposed that instead of a stacking model,[35,36] the molecules of 5'DSCG actually self-assemble in water to form threads that are interconnected by salt bridges (Scheme 3).[37,38]

Taking into account the inconsistencies of the general stacking model, and the evidence for the thread assembly model, this new model is very compatible with existing knowledge of the system. When considering the stacking models, most describe smectic or discotic columnar liquid crystal phases very well; however, the liquid crystals formed by 5'DSCG are nematic.[39] Of the liquid crystal (LC) phases, nematic is one of the most common. Ironically, it comes from the Greek "nema" meaning "thread" but this term actual refers to the topological defects, which are called disclinations. These LC phases have long-range directional order, and are typically uniaxial—they have one preferred axis (cylinders or rod shaped).[40] On the other hand, smectic phases exist at lower temperatures to nematic, and form well-defined layers (stacked) that can slide over one another.[41] Similarly, discotic columnar phases are also packed into stacks, but are disk-shaped.[42]

The stacking model only considers the π-π interactions to drive assembly, and so ignore the importance of counter inorganic ions on the assembly. Our group discovered that by changing the sodium cation (Na^+) of 5'DSCG to lithium or potassium, the liquid crystal phase is almost entirely eliminated.[43] Given its name, the stacking model also emphasizes that stacking is the primary attribute for assembly, and slightly modified structures should not affect the success of stacking, but only ultimately change some properties (for instance, transition temperature) of the LC phase; however, nine out of eleven DSCG derivatives with small structural changes do not exhibit any LC phases.[37]

Upon analysis by small angle neutron scattering, 5'DSCG in water matches best with a thin rod model with neglible cross-sectional diameter. These dimensions are not at all consistent with the molecular stacking model, which has a fairly large cross-sectional area, but are consistent with a thread model. Finally, and perhaps most importantly, molecules similar to 5'DSCG can exhibit similar structures but different assembly structures under different conditions, a phenomenon referred to as polymorphism. The fact that different aggregates can formed by the same molecule is in direct violation of the stacking model.[37]

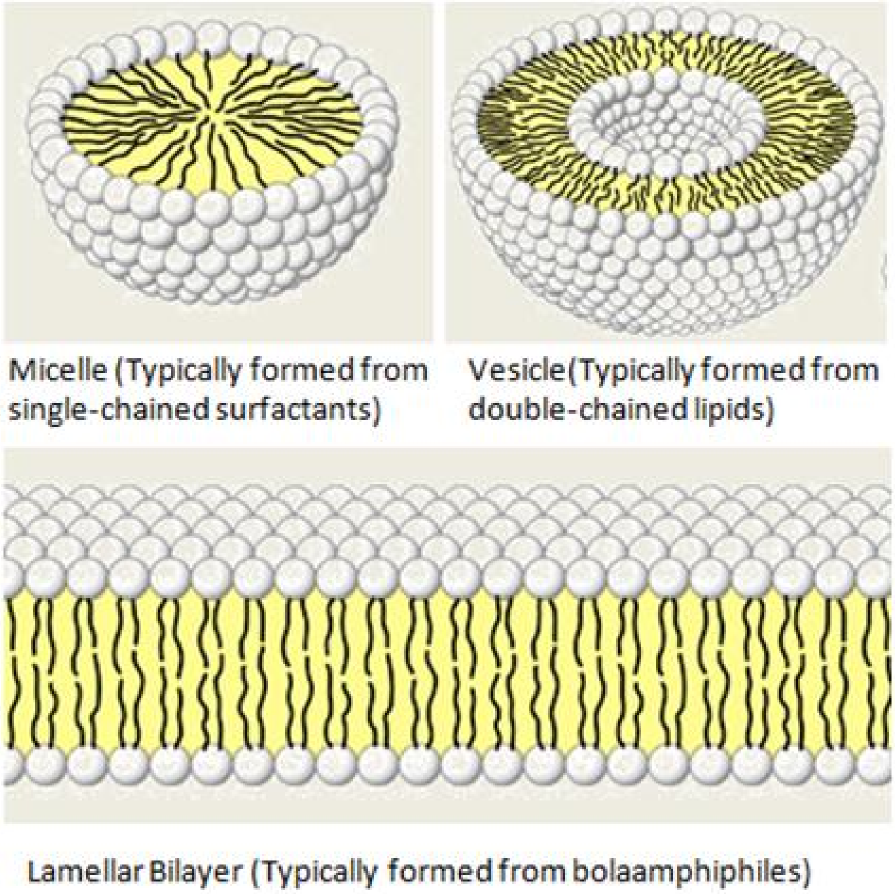
Additionally, support for the thread assembly was generated by synthesizing monocharged molecules of 5'DSCG, which eliminated the LC phase in water. Since the thread model requires two charges on the molecule to make the connections necessary to elongate threads, and the stacking model just requires stacking moieties, this further supports the thread assembly model.[37]

These thread assemblies form highly birefringent LC phases at roughly 11 wt.% 5'DSCG in water; at concentrations lower than this, no birefringence is visible—it is considered isotropic.[37] Birefringence is an important quality for analyzing liquid crystals, as these exhibit birefringence due to their anisotropic nature, that is, they demonstrate double refraction. As a result, when light enters a material like a nematic liquid crystal, the light is broken up into two components depending on its polarization. Two polarizers perpendicular to each other will block all (non-broken) light that attempts to pass through it; however, our birefringent material will be visible because it had been broken into two components, and one of those components can pass through—and so the region appears bright.[44] As a general rule, samples that appear dark between cross polarizers are classified as isotropic; those that transmitted light between crossed polarizers were classified as birefringent. Birefringent can be extended to splash, radial, tactoid, or amorphous—these will be expanded upon in the results section.

Interestingly, when nonionic polymers such as poly(vinyl alcohol) or poly(acrylamide) are added to an isotropic 5'DSCG solution (any solution less than 11 wt.%, for instance, 6wt.%) the 5'DSCG molecules are concentrated together to form water-based liquid crystal droplets.[37] The fact that 5'DSCG can form birefringent liquid crystals at much lower concentrations is an understudied and interesting field, especially in the case of surfactants. Surfactants, like soap described above, are typically amphiphilic organic compounds that can aggregate around oil to stabilize oil-in-water emulsions.

To make things more clear, there are three basic self-assemblies in aqueous solutions (Scheme 4): those that assemble into large, lamellar bilayers (e.g. the human body's lipid bilayer), those that assemble into spherical vesicles consiting of a bilayer (typically double-chained molecules with a hydrophilic head, referred to as a liposome), and simple micelles (formed from simple single-chained molecules with a hydrophilic head, like many surfactants).[45]

In this paper we will observe 5'DSCG's capacity to promote vesicle formation in surfactants and amphiphiles that typically exhibit only micelle or lemllar layer assembly, as in the cases of single-chained surfactants and bolaamphiphiles.



Scheme 4- Various Assemblies of Amphiphiles
Image adopted from The University of New South Wales, Cell Biology lecture.

Bolaamphiphiles (also known as bolaform surfactants) are amphiphilic molecules that have a hydrophilic group at both ends of a sufficiently long hydrophobic (or lipophilic) chain. Compared to single-headed amphiphiles, the introduction of a second head-group generally increases solubility in water, increases the critical micelle concentration (cmc), and decreases the aggregation number.[46] They are typically shown to produce lamellar layers, whereas single-chained surfactants typically form micelles; in nature, only double-chained lipids commonly form vesicles.

In the following sections, two endeavors will be carried out to study 5’DSCG’s capacity to change the self-assembly behavior of molecules. First, we synthesize a bolaamphiphile and observe its capacity to self-assemble into vesicles instead of lamellar bilayers, as well as induce birefringence in 5’DSCG at a concentration less than 11wt.%. For this bolaamphiphile, the hydrophilic heads will be L-gulitol, a biocompatible sugar that has been previously used in our group in anti-biofouling research. Second, we analyze a series of surfactants to observe any odd-even effects that result from favorable packing formations, as well as observe 5’DSCG capacity to force surfactants from micelles to vesicles, and the surfactant’s ability to induce birefringence at reduced 5’DSCG concentrations.

II. Materials and Methods

Materials and Equipment. Disodium cromoglycate (5’DSCG) was purchased from MP Biomedicals (Solon, OH). L(+)-Gulonic gamma-lactone is commercially available from Sigma-Aldrich and should be kept refrigerated.

Disodium cromoglycate was purchased from Sigma. 2,2-dimethoxypropane, p-toluenesulfonic acid, sodium borohydride, sodium hydride, potassium hydroxide pellets, reagent grades ethanol, methanol, and acetone, 37% hydrochloric acid, and triethylamine were all acquired from Sigma-Aldrich. Dimethyl sulfoxide (DMSO), 1,12-dibromo-dodecane, 1,9-dibromo-nonane, as well as 1-bromoheptane were purchased from TGI Chemicals. Sodium dodecyl sulfate (SDS, 96.8%), sodium N-lauroylsarcosinate hydrate (SLS, 98.0%), and sodium n-nonyl sulfate (SNS, 98.0%) were purchased from Sigma-Aldrich (St. Louis, MO). Fisherfinest glass microscope slides were purchased from Fisher-Scientific (Fairlawn, NJ). Deionized water with resistivity of 18.2 MΩ-cm was used for the preparation of all the liquid crystal samples. Birefringence was measured by plane polarized light in transmission mode on an Olympus BX51 polarizing microscope. Micrographs were taken using an Olympus C-5060 wide zoom digital camera.

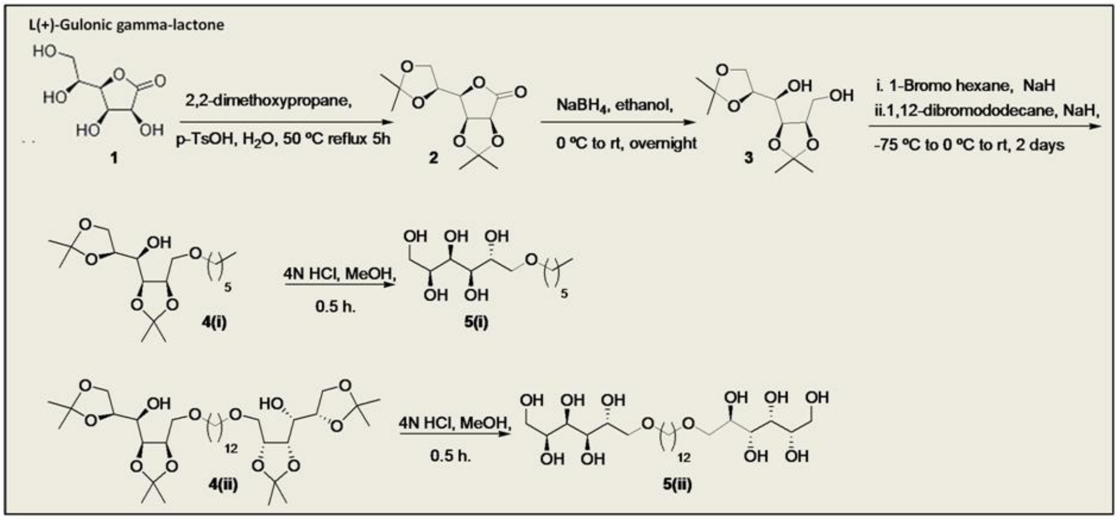
Sample Preparation. Two methods of sample preparation are utilized. For *Dilution Method*, stock solutions of the appropriate compounds were prepared with water and allowed to sit overnight before use. Targeted masses of 5’DSCG, surfactant stock solutions were added to a vial, and water was added for balance to acquire a targeted weight percent. For *Dissolution Method*, appropriate weights of solid compounds were measured out and water was added, the sample was then vortex mixed and allowed to sit overnight before being observed.

Optical Cell Assembly and Birefringence Analysis. Samples were placed between two glass microscope slides with a piece of Saran wrap folded twice as a spacer, with a small hole made in the middle. When the samples were ready, a specified amount was placed in the center of the Saran wrap by pipette and the other glass slide was placed on top, and the optical cell was secured with four binder clips to prevent evaporation. This assembled optical cell was immediately examined under a polarizing microscope. If the sample appeared generally dark, it was classified as *isotropic*; those that transmitted light that covered the entire slide were classified as *splash birefringence*; those that transmitted light with no shape, a tactoid shape, or a radial shape were classified as *amorphous, tactoid droplets,* or *radial droplets* respectively.

Synthesis Instrumentation. For the synthesis of the bolaamphiphile, several types of glassware are required, including several 250 mL round-bottom flasks with the appropriately-sized addition funnel, condenser, vacuum adapters, and seals. A column will be needed for purification (as well as silica gel and abundant quantities of hexanes and ethyl acetate for running them, sand, cotton, and an air source may also be needed). A scale balance, graduated cylinders, measuring syringes, and an argon air source are also required. Aluminum foil will be required for reactions that proceed in the dark. pH paper is sufficient for ensuring certain reactions are neutralized in the end.

For reactions, a hot plate with magnetic stirrer, stir bars, a mineral oil bath, septa, balloons or an air line, a vacuum line, and cork base are needed. Dry ice and acetone to achieve cold conditions, or liquid nitrogen, are sufficient. Some method of vaporizing the solvent is also needed, preferably a rota-vap.

All NMR analysis were performed on a 300 MHz ^{1}H NMR Brunker machine.



Scheme 5 - Synthesis Route for L-Gulitol-based Bolaamphiphile

Synthesis of Protected L(+)-Gulonic-gamma-lactone. All steps of the synthesis are outlined in Scheme 5. For the first two steps of the reaction, literature directions were followed. In step 1, the hydroxyl groups of the starting material are protected via 3.7eq. of 2,2-dimethoxypropane with catalytic p-toluenesulfonic acid in water at 50 C then reflux for 5h. The solution was neutralized with triethylamine to a pH of 7, then rotavapped and stored in the fridge. A column chromatography was carried out in roughly 30% ethyl acetate in hexane, and the fractions with matching TLC Rf values with that of the starting material were stored away. The remaining two spots were analyzed with ^{1}H NMR to confirm presence of the protected-starting material (**2**), and checked against a reference NMR of the starting material.

Synthesis of the Ring-Opened L-Gulonic-gamma-acetonine (3). For the second step, (**2**) underwent a ring-opening with excess sodium borohydride in ethanol, cooled with an ice bath initially and allowed to warm to room temperature overnight. The product, (**3**) was acquired by rota-vapping off the solvent and dissolving the residue in ethyl acetate and extracting with brine. The product was in the organic layer, and very pure, confirmed by NMR.

Synthesis of the Protected Bolaamphiphile (4ii). 1,12-dibromododecane was reacted with NaI in acetone first, in a classic Finklestein reaction, to obtain 1,12-diiodododecane, where there is a much better leaving group for the Sn2 reaction. The reaction was run for 1 day in complete darkness. The NaBr salts were then decanted off, and the 1,12-diiodododecane was isolated for the next step of the reaction by rota-vapping.

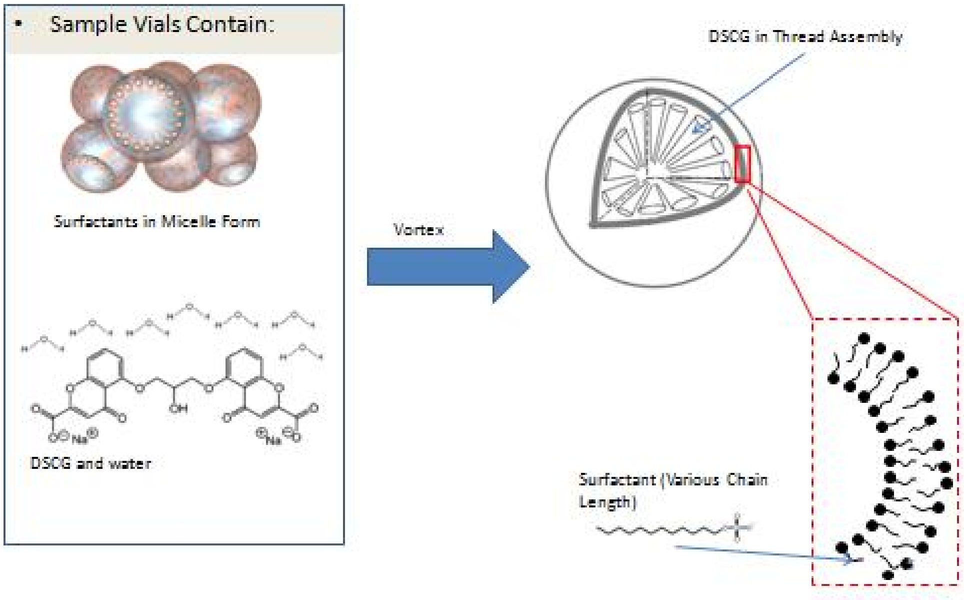
Then, (**3**) was dissolved in DMSO at 3.5 eq under argon atmosphere, then cooled to -75 C with dry ice and acetone for 30 min. To this, the 1,12-diiodododecane was added at roughly 1 eq to ensure a double-substitution was more favorable. This was stirred for several minutes before NaH was added, also at 3.5 eq. It is important that all glassware is as dry as possible, as NaH is highly reactive with water.

The solution was stirred at -75 C for an hour before behind warmed slowly to 0 C, then maintained at 0 C for 16 h then allowed to react at RT for 2 more days. Ultimately, single and double elimination and substitution occurred, resulting in 4 distinct products. A careful column was run with increasing polarity, initially at 2% ethyl acetate and finally at 30% ethyl acetate. All spots were analyzed by NMR and the protected bolaamphiphile (**5ii**) was isolated.

Deprotection of (5ii) to Acquire the L-Gulitol-based Bolaamphiphile. In the final step of the bolaamphiphile synthesis, (**5ii**) was refluxed with 2 mL of hydrochloric acid in methanol for 2h. The resulting solution was rotavapped and became a white powder, this compound was confirmed by NMR.

III. Results and Discussion

In this work, we have confirmed two properties of surfactants and 5’DSCG. Long-chain surfactants, which typically form micelles in water, produce vesicles in the presence of 5’DSCG (**Scheme 6**); additionally, the surfactants induce birefringence in 5’DSCG at concentrations much lower than that of 5’DSCG alone in water, essentially “compressing” 5’DSCG together as it simultaneously induces vesicle formation by means of its highly-hydrated thread assembly shells. Similarly, the presence of the bolaamphiphile induces birefringence at concentrations less than 11wt.% DSCG; however, the entire system is very unstable, as upon heating, the bolaamphiphile goes into solution but will crash out of solution rather quickly.



Scheme 6 - Schematic Representation of Micelle to Vesicle Formation
Micelle Image adopted from Sim Science (http://simscience.org/membranes/intermediate/page4.html)
DSCG Vesicle image adopted from Simon et. al J. Phys. Chem. B **2010**, *114*, 10358

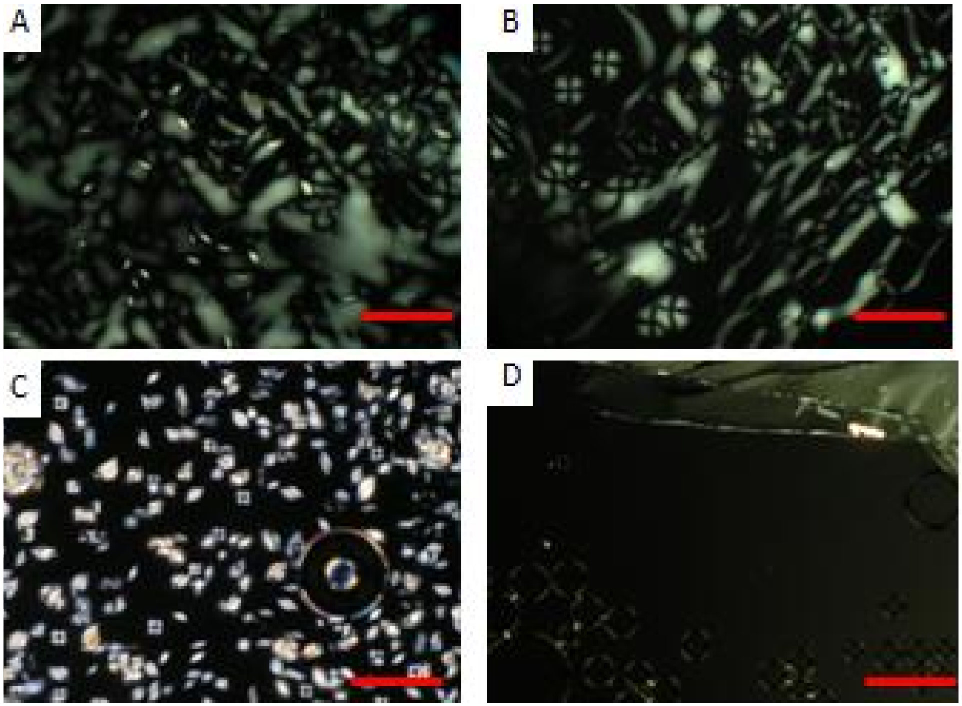


Figure 1. At sufficiently high wt.% DSCG (>7wt.%), there is no observable odd-even effect; however, it can be confirmed that the surfactant micelles become vesicles or at least encapsulate the 5'DSCG liquid crystal phase. (A) Sodium dodecyl sulfate (SDS; 12-chain surfactant)--there are distinct tactoid droplets visible which occur when 5'DSCG molecules orient in a bipolar droplet configuration; scale bar = 76µm. (B)Sodium undecyl sulfate (11-chain surfactant)--distinct radial droplets are visible, indicating vesicle formation; scale bar = 76µm. (C) Sodium decyl sulfate (10-chain surfactant)--there is a mixture of radial and tactoid droplets; scale bar = 76µm. (D) Sodium nonyl sulfate (SNS; 9-chain surfactant)--appears to be isotropic (there are only bubbles present), though this can be attributed to a low concentration of surfactant.

Various Chain-Length Surfactants Promote Liquid Crystal Droplet Formation by 5'DSCG in Water by Odd-Even Effect. The effect of an odd or even chain length on the packing factor, and subsequently the formation of micelles or vesicles has been an interesting subject of study.[43] At sufficiently high concentrations of 5'DSCG and surfactant, vesicles will form regardless of this effect (**Figure 1**); however, using minimalistic concentrations of 5'DSCG and surfactant, a distinct odd-even effect is clearly visible (**Figure 2**). In order to

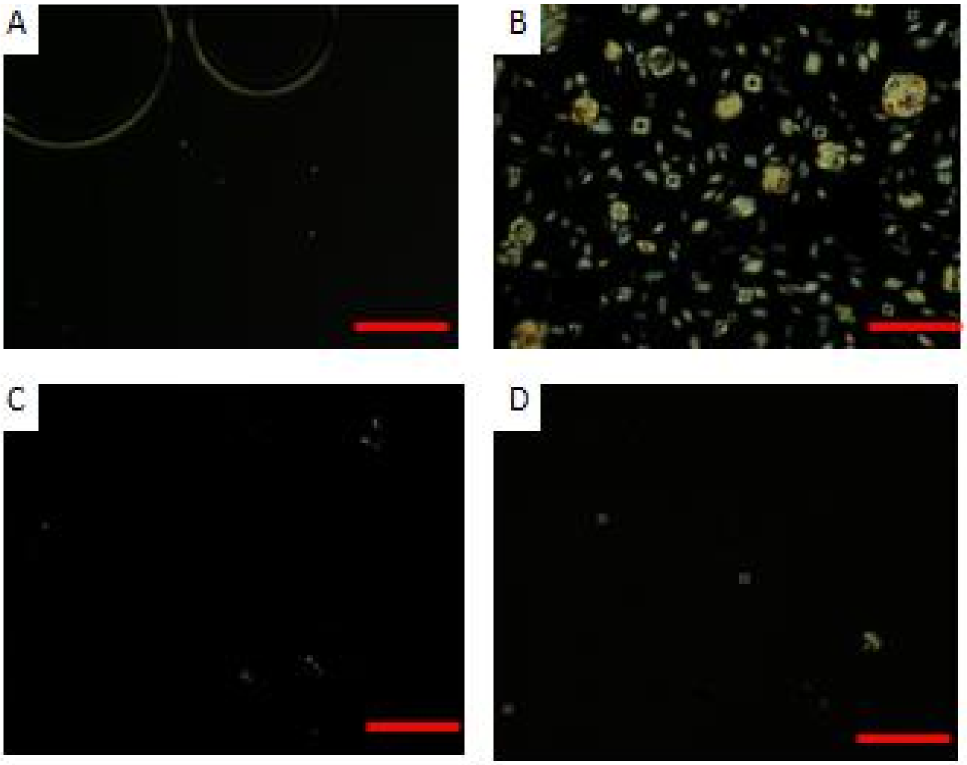
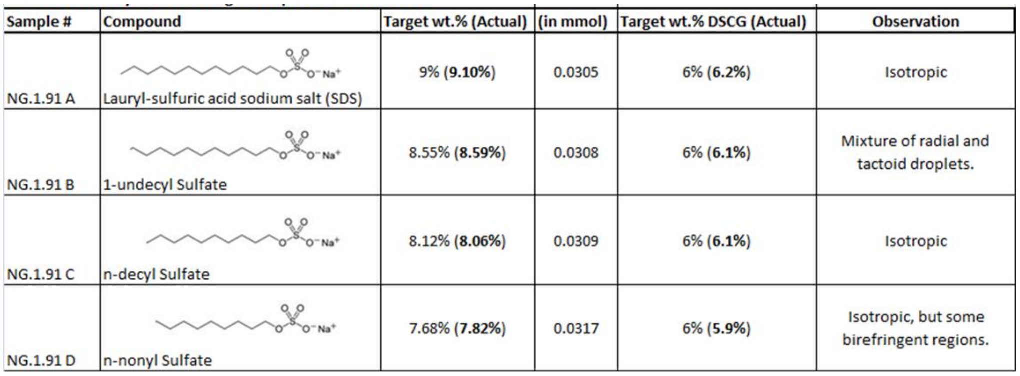


Figure 2. At sufficiently low wt.% DSCG (~6wt.%), there is an observable odd-even effect; however, it can be confirmed that the surfactant micelles become vesicles or at least encapsulate the 5'DSCG liquid crystal phase. (A) 9.1wt.% sodium dodecyl sulfate (SDS; 12-chain surfactant)--there is no observable birefringence; scale bar = 76µm. (B) 8.59wt.% sodium undecyl sulfate (11-chain surfactant)--distinct radial droplets are visible amongst some amorphous birefringent droplets and tactoid droplets, indicating vesicle formation; scale bar = 76µm. (C) 8.06wt% sodium decyl sulfate (10-chain surfactant)—the mixture is isotropic, any birefringence visible is a consequence of bubbles that formed; scale bar = 76µm. (D) 8.05 wt.% sodium nonyl sulfate (SNS; 9-chain surfactant)--appears to be mostly isotropic but there are distinct radial droplets visible, which indicate that vesicles did form, and occurred with the 9-chain and not the 10-chain.

ensure equal molecules of surfactant were present, constant moles of surfactant were used instead of a constant wt.%. Increasing the concentrations of the surfactant slightly, while keeping the 5'DSCG concentration at ~6wt.% offers the opportunity for sodium n-nonyl sulfate (SNS) to exhibit radial droplets while still ensuring the even chain compounds, sodium dodecyl sulfate and sodium decyl sulfate, still appear isotropic. At this slightly elevated concentration, the odd-even effect continues to be applicable and SNS exhibits a few distinct radial droplets not present in any of the even chain surfactants.



Sample #	Compound	Target wt.% (Actual)	(in mmol)	Target wt.% DSCG (Actual)	Observation
NG.1.91 A	Lauryl-sulfuric acid sodium salt (SDS)	9% (**9.10%**)	0.0305	6% (**6.2%**)	Isotropic
NG.1.91 B	1-undecyl Sulfate	8.55% (**8.59%**)	0.0308	6% (**6.1%**)	Mixture of radial and tactoid droplets.
NG.1.91 C	n-decyl Sulfate	8.12% (**8.06%**)	0.0309	6% (**6.1%**)	Isotropic
NG.1.91 D	n-nonyl Sulfate	7.68% (**7.82%**)	0.0317	6% (**5.9%**)	Isotropic, but some birefringent regions.

Table 1 - Summary of Odd-Even Effect Observations for Various Surfactants.

It is crucial to note that the odd-even effects in vesicle formation is observed for samples prepared by dilution but not by dissolution. In direct dissolution, the solid surfactant and 5’DSCG are added together, and water is added. In this case, the surfactant and 5’DSCG are competing for water for solvation. At the onset, 5’DSCG is gradually becoming hydrated, and so forms a mixture of solid 5’DSCG and LC phases. Solvation of surfactants immediately leads to micelle formation without individually solvated molecules, as long as the concentration exceeds that of the critical micelle concentration (cmc, the concentration at which micelles form for a surfactant). On the other hand, the dilution method is carried out by adding two stock solutions of both surfactant and 5’DSCG. In this case, the two components are still competing for water but the kinetics of solubilizing the 5’DSCG are already overcome, as the 5’DSCG is solvated individually and the surfactants continue to exist as micelles.

Thus, the key differences are in the behavior of the 5’DSCG. In both cases, the surfactant simply needs to be disassembled from micelles. For 5’DSCG, however, it needs to be solubilized in the dissolution method, but simple needs to be assembled in the dilution method.

In dilution, the presence of micelles will reduce the amount of water available for solubilizing 5’DSCG molecules, thus inducing LC droplets. In this case, the main energy barrier is the actual disassembly of the micelles to surround the LC droplets as vesicles. How well the aliphatic chains on the surfactants pack

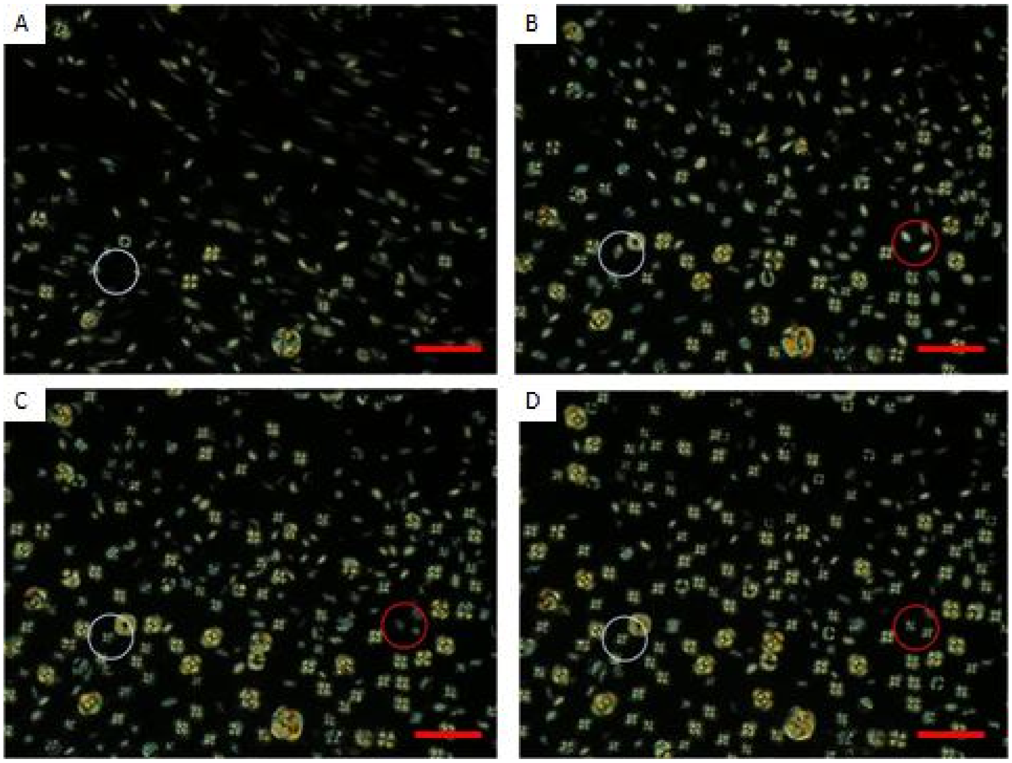


Figure 3. Overcoming the thermodynamic barrier with added heat, 5’DSCG appears to favor the radial droplet configuration over the bipolar or tactoid configuration. (A) At time = 0 minutes, a few radial droplets are visible amongst amorphous birefringent droplets. (B) At time = 5 minutes, more radial droplets begin to assemble as the solution cools. (C) At time = 10 minutes, more radial droplets are present. (D) At time = 15 minutes, radial droplets are almost exclusively present. For all images, Scale bar = 76μm

together is thus critical for vesicle formation. For dissolution, the sample must be mixed or heated to solubilize 5’DSCG. This heating or mixing provides the energy necessary to disassemble the micelles and form vesicles. As a result, the effect of the chain packing is essentially removed, and so the odd-even effect is also removed.

Stability of Radial Droplets Over Tactoid or Amorphous Droplets. To determine the most stable configuration of 5’DSCG (radial vs. tactoid), a sample containing some mixture of tactoid and radial droplets was warmed and allowed to cool. This removed any thermodynamic barrier present and allowed 5’DSCG to arrange in is most favorable form upon cooling. Interestingly, upon observing this gradual cooling over time, radial droplets were present almost exclusively under microscope after fifteen minutes (**Figure 3**). While this may not ultimately confirm the most stable configuration of the 5’DSCG thread assembly, it does indicate that there is some energy barrier to radial droplet formation.

Potential for More Stable Nonamphiphilic Modification of Bolaamphiphile Behavior. Upon acquiring and confirming the L-gulitol-based bolaamphiphile, several samples were prepared with 6wt.% DSCG and between 2 and 8wt.% bolaamphiphile, with water to balance. These were analyzed under the polarizing microscope (**Figure 4**). Though it has been confirmed that the bolaamphiphile induces liquid crystal phases at concentrations less than 11wt.% for DSCG, the solution itself is particularly unstable. Upon warming, the solution dissolves readily and appears birefringent but after a few minutes, the bolaamphiphile crashes out of solution. This may be a result of the L-gulitol hydrophilic head, with its numerous hydroxyl grounds being unable to adequately compete with 5'DSCG for water.

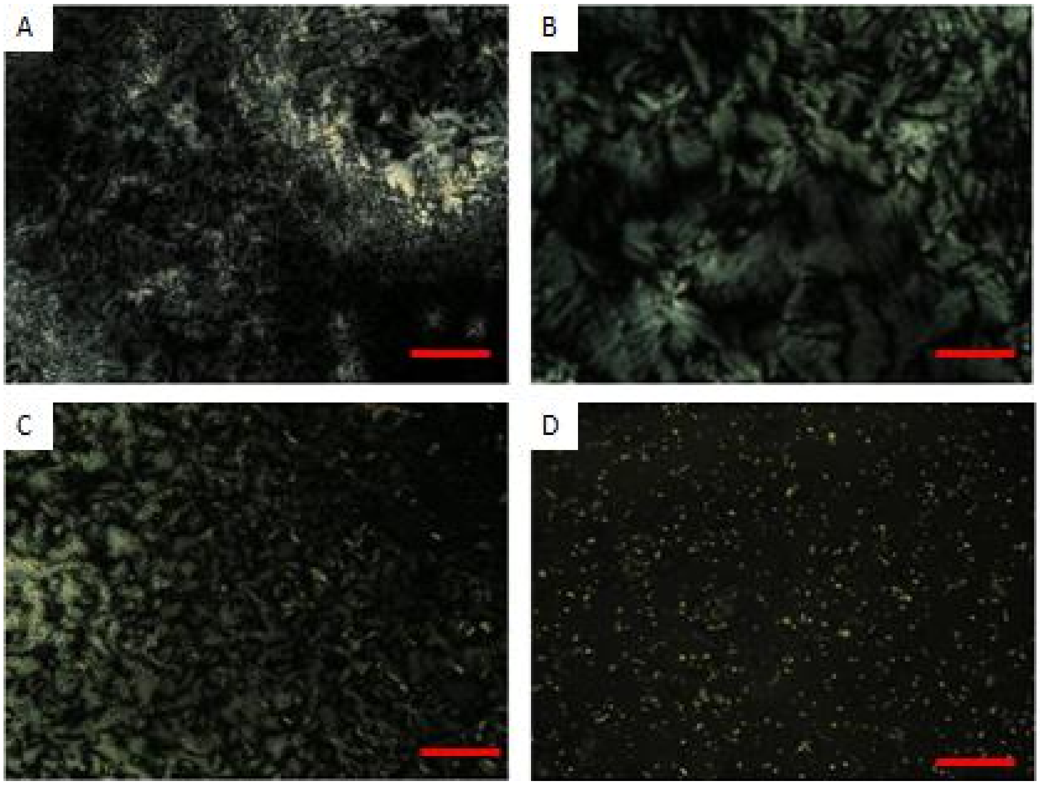


Figure 4. After slight warming, the microscopic analysis provided the above pictures. The solution was only stable for a few minutes, where these pictures were taken. In all cases, 6wt.% 5'DSCG was present, indicating that birefringence was exhibited at concentrations less than that of 5'DSCG alone. (A) At 8wt.% bolaamphiphile warmed, the solution exhibits some splash birefringence; scale bar = 76µm. (B) Reduced to 6wt.% bolaamphiphile warmed, the solution continues to exhibit some splash birefringence; scale bar = 76µm. (C) Reduced to 4wt.% bolaamphiphile, small amorphous birefringent spots appear amongst splash birefringence; scale bar = 76µm. (D) Reduced to 2wt.% bolaamphiphile, small amorphous pockets of birefringence are visible but radial droplets indicating vesicle formation continue to be absent; scale bar = 76µm.

IV. Conclusions

We have shown that surfactants, which usually aggregate to form simple micelles in emulsion systems can be disassembled from their micelle forms and realigned to create vesicles when promoted by 5'DSCG. At the same time, the creation of these vesicles essentially “compresses” 5’DSCG molecules together, causing them to exhibit birefringence at concentrations less than 11wt.% in water, below which 5’DSCG alone is isotropic when solvated in water. In the past, our group has shown that water-in-water emulsions can be established with polymers, as well as small, nonamphiphilic molecules with salt bridges stacked on aromatic rings.[47] Now, we have reported on surfactants, which have historically only been used to sequester oil , that can also take up water-based drops with 5’DSCG in water—forming one type of water-in-oil-in-water emulsion: a vesicle.

Although at first it may seem that the method of preparing these water-in-oil-in-water emulsions can be lenient, we have discovered that the method of preparation plays a critical role in vesicle formation. And odd-number aliphatic chain can show vesicle formation more readily than an even-number aliphatic chain when the sample is prepared by dilution method as opposed to dissolution method. By dissolution, the 5’DSCG becomes hydrated gradually—forming a mixture of solid compound and LC phase. By contrast, the dilution method solvates 5’DSCG individually, thus the kinetics of solubilizing 5’DSCG are already overcome. As a result, dilution requires only the assembly of 5’DSCG threads from solvated molecules, while dissolution requires the prerequisite of solubilization. With the main energy barrier being just the disassembly of the surfactant micelles, the packing of the chains plays a critical role in vesicle formation.

Although bolaamphiphiles show promise for vesicle formation at lower concentrations, creating structures with good solubility becomes a new challenge. For the bolaamphiphile, the spacer length has little influence on the melting points and the enthalpy of melting—this actually suggests that the packing of these compounds is predominantely determined by the amphiphilic head-group.[46]

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Appendix

A (Moderately) Non-Technical Summary of this Capstone

There is a well-known English saying that says, “Oil and water don’t mix.” Of course, today it is taken to mean that certain qualities or personalities are incompatible with each other, but the phrase itself derives from a certain truth: the two liquids are immiscible, they readily forms layers. They, without any special effort, will not mix. If you shake them vigorously, you’ll find the oil forms distinct droplets in the water, but after a few seconds, the smaller droplets combine with the larger droplets, and the larger droplets come together to inevitably form a separate layer again.

Science is about overcoming the seemingly impossible though. These mixtures of states of matter are called colloids, and they surround us thanks to men and women in laboratories. If you have a gas phase dispersed in a liquid medium, for instance, you have a foam. If you have a liquid dispersed in a gas medium, on the other hand, you have an aerosol. If you have a liquid dispersed in bulk solid, you have a gel. And if you have a liquid dispersed in a bulk liquid, you have something called an emulsion. So if anybody asks you what state of matter mayonnaise is, you simply say it’s a colloid—it’s an emulsion.

Emulsions only work, however, if the two immiscible liquids are mixed—otherwise you just have two liquids layered on each other, which brings us back to our oil and water example. Certainly, we know oil and water don’t mix—this is why it’s so hard to get grease off our dishware or hands—the oil simply doesn’t go with the water. We rectify this with detergents, soap—substances known as surfactants.

Surfactants are amphiphilic in nature—this means they have a water-loving part and a water-hating part. They are the bridge between these two worlds of opposites. They effectively change that old English saying, and bring about the mixing of oil and water.

The mechanism is quite simple. Surfactants in water are ambivalent—they like water and they hate water. These surfactant molecules notice the oil droplets we just introduced by vigorous shaking. The hydrophobic parts of the surfactants agree with the oil: water sucks. Meanwhile, the hydrophilic parts of the surfactant just love the water. These surfactants begin to aggregate around the oil droplet—they form a sphere around it. On the inside of this sphere, the hydrophobic parts are shielded from the water outside the system by the hydrophilic parts. This assembly is called a micelle, and it is one of many different assemblies that can form.

Similar to this example, our laboratory has done research on water-in-water emulsions—instead of oil, we utilize a biocompatible compound known as disodium cromoglycate, or 5'DSCG (Five-Prime-D-S-C-G). At 11 weight percent in water, 5'DSCG forms liquid crystals. It exists as threads of "super-" hydrated molecules. This 5'DSCG is essentially the water droplets, albeit liquid crystals, that are dispersed in water. These liquid crystals are visible under a microscope because they exhibit a property known as birefringence, which means they appear shiny and colorful under a double polarizer, which usually blocks all light but special compounds can break light up in such a way that it can still get through.

Normally, as described above, surfactants form micelles; however, in this paper we reveal that with the help of 5'DSCG, we can modify the behavior of these surfactants, and force them to disassemble from micelles and form vesicles around 5’DSCG. A vesicle consists of two layers of surfactants, the inner layer is essentially an inverse micelle—the water-loving parts face the 5’DSCG while the water-hating parts face outward. But since the bulk water is still everywhere around this system, a larger micelle forms around this inverse micelle. This creates a sort of double layer, so that water-loving parts face the outside and the inside—and the water-hating parts are safe inside the middle of this sphere.

The ability to modify the self-assembling behavior of these surfactants is very useful in designing drug carriers, techniques for protein recrystallization, and advancing the field of interfacial and colloidal chemistry. As such, we have discovered that surfactants can exhibit an interesting “odd-even” effect, such that depending on whether the chain-length on the surfactant is an odd or even number of carbons long, vesicles may or may not form. Specifically, we’ve discovered that even-numbered chains do not readily form vesicles unless a higher concentration of 5’DSCG is present. Odd-numbered chains do form vesicles, even at the very minimal concentrations. In our experiments, 12- and 10-chain surfactants were isotropic (exhibited no birefringence, or shininess associated with liquid crystals and vesicles), while 11- and 9-chain surfactants were birefringent, meaning they displayed these vesicles.

This is an invaluable observation for two reasons. First, this effect is observed only when the samples are prepared by a method referred to as the dilution method, in which stock solutions of the desired components are made before hand. This is opposed to the dissolution method, when solid compounds are added together and then water is added. As a result, more efficient methods of preparing vesicles can be taken from this work, in addition to the knowledge that some molecules can be encouraged to form vesicles.

Vesicles in human biology play an important role in storage, transport, digestion, and waste removal in a biological system like humans. They can fuse with the plasma membrane to release their contents outside of the cell. They also play a role in metabolism, buoyancy, and enzyme containment.

As such, these artificial vesicles have the capacity to be used in drug delivery. Since they are a bubble in a bubble, drugs that are dissolved in water cannot escape the interior until the vesicle reaches the target site. But at the same time, they can also carry drugs that are not readily dissolved in water because they have an interior region that is hydrophobic.

More than just a drug delivery method, vesicles can be used in the delivery of dyes to textiles, pesticides to plants, enzymes and supplements to foods, and cosmetics to skin. As of 2008, there are 11 drugs that have been approved for vesicle delivery systems. Truly, the potential applications of a compound that can induce vesicle formation are numerous and outstanding.

Furthermore, our chemical synthesis of a bolaamphiphile—which is a compound that has two water-loving parts on either end of a long water-fearing chain was successful but the compound did not dissolve well in water. When slightly heated, the compound exhibited some birefringence but quickly crashed out of solution. In the future, more soluble bolaamphiphiles could lead to very effective vesicles with a wide-range of applications. The advantage of bolaamphiphiles rests in the fact that they generally do have better solubility in water that their single-headed counterparts and require fewer molecules for a successful aggregation into a vesicle.