Spring 2019

Manipulating Light: Creating a Biomimetic Photonic Crystal Material Inspired by the Chameleon

Love L. Richburg
Eastern Kentucky University, love_richburg@mymail.eku.edu

Eastern Kentucky University

Manipulating Light: Creating a Biomimetic Photonic Crystal Material Inspired by the Chameleon

Honors Thesis
Submitted
In Partial Fulfillment
Of The
Requirements of HON 420
Spring 2019

By
Love Richburg

Faculty Mentor
Dr. Thomas Jarvis
Department of Physics and Astronomy

Manipulating Light: Creating a Biomimetic Photonic Crystal Material Inspired by the Chameleon

Love Richburg
Dr. Thomas Jarvis of the Department of Physics and Astronomy

Abstract. The purpose of this project is to create a chameleon-inspired biomimetic material using guanine crystals and polydimethylsiloxane (PDMS) to form a tunable photonic crystal and then characterize the material. The method for developing this material is to first synthesize a photonic crystal made of a guanine microsphere lattice. Then embed the guanine lattice in PDMS. Since PDMS is an elastomer, it can be stretched to adjust the spacing between the guanine crystals. This should allow for the photonic crystal to be adjusted similarly to the way that the chameleon adjusts the photonic crystal in its skin by changing the spacing between the guanine particles. The material is characterized primarily through qualitative observations and scanning electron microscopy. It will be shown that the guanine microspheres are successfully synthesized. Two potential methods for embedding the guanine microspheres in PDMS are developed, experimented with, and explained. A third potential method is explored, but no experimentation was done with this method.

Keywords: chameleon, photonic crystal, pdms, material, color-change, physics, biology, chemistry.

Table of Contents

Introduction……..1

Literature Review…….3

Experimental Section……..11

- Pre-Experimental Background……..11
- Methods for PDMS……...12
- Data for PDMS……..17
- Commentary for PDMS…….18
- Methods for Guanine Crystal Synthesis……19
- Data for Guanine Crystal Synthesis……...21
- Commentary for Guanine Crystal Synthesis……..23
- Methods for Embedding Guanine in PDMS……..23
- Data for Embedding Guanine in PDMS…….28
- Commentary for Embedding Guanine in PDMS……28
- Future Experimentation……29

Conclusion……..29

Works Cited……31

Figures

Figure 1 – PDMS Preparation……...……..14

Figure 2 – Liquid PDMS in Desiccator……...…15

Figure 3 – Measuring Elasticity……..……15

Figure 4 – PDMS on Water…….....16

Figure 5 – PDMS Cured on Water…….……..……16

Figure 6 – Guanine in Weigh Boat……...…20

Figure 7 – Guanine on Surface……..…..21

Figure 8 – PDMS on Guanine Solution……..…….25

Figure 9 – Guanine Embedded in PDMS……...……..26

Figure 10 – Close-Up of Fig. 11……..26

Figure 11 – SEM Image 1……27

Figure 12 – SEM Image 2……27

Figure 13 – SEM Image 3……27

Tables

Table 1: Comparison of PDMS with Different Ratios of Base to Curing Agent…….16

Acknowledgements

Following are acknowledgements of individuals and entities that have been instrumental in supporting and motivating this project: Dr. Thomas Jarvis for continuous support throughout the formation of this honors thesis, for originating the idea for the project, for acquisitioning all the necessary materials, and for always being willing to sit down and talk things out; Eastern Kentucky University College of Science for the funding of this project; Vasantha Basavalingappa for key advice on the guanine synthesis process; the Eastern Kentucky University Chemistry department for the lending of a balance with the precision necessary for measuring out the guanine powder; the Eastern Kentucky University Forensics department for the use of their Scanning Electron Microscope; and the University of Kentucky for the use of the Microscopy Center.

Introduction

The chameleon has long been known for its color-changing abilities. Recent research revealed that the mechanisms behind this color change are not quite what one might expect after studying color change in species that move pigments around to affect their perceived color like the octopus. Despite what one might think, the chameleon does not actively manipulate the pigment concentration. The chameleon changes color by tuning a natural photonic crystal in its skin. This photonic crystal is made up of guanine crystals that form the upper layer of iridophores in the chameleon's skin. Photonic crystals are materials that selectively absorb electromagnetic waves depending on the energy of the incident wave. Guanine is often found in natural biological structures, but it may be better known for its role as a nucleic acid base. Iridophore is the name given to a structure that contributes to the color of an animal through reflection [1].

The chameleon tunes the photonic crystal by changing can change to match their surroundings. For example, if a chameleon were placed on a red and white checkered tablecloth, some might expect it to change to match both the color and pattern of the

tablecloth. However, the pattern of the chameleon is restricted to its natural pattern--some species display stripes or other defined markings--and the color change is typically in response to a stimulus. Potential stimuli for the chameleon include other chameleons and changes in temperature. When exposed to a stimulus that results in a shift to brighter colors, the chameleon is referred to as being in an excited state. Otherwise, it is in a relaxed state. While the chameleon does not change colors to match its background, its color in the relaxed state is close to the colors that exist in its natural habitat. This allows it to blend in quite thoroughly. Its nature to blend into its surroundings coupled with the ability for vibrant, rapid shifts in color is perhaps the origin of the misconception that has just been discussed. Another source of confusion may be the erroneous representation of chameleons in popular media. Moving forward it is important to know that chameleons change colors in response to certain stimuli by changing the spacing between the guanine crystals in their skin.

The color-changing process of the chameleon is of particular interest optically because of how the iridophores form an adjustable photonic crystal. The objective of this project is to create a material that mimics the color-changing process of the chameleon. This material will be formed by a guanine nanocrystal lattice embedded in polydimethylsiloxane. Polydimethylsiloxane will be referred to as PDMS after this. There are two processes that constitute this material synthesis. The first is a self-assembly process to form the guanine lattice. The second is capturing the guanine in the PDMS. The capture process involves applying liquid PDMS to the surface of the solution on which the guanine forms, then allowing it to cure, and later removing it from the container. These processes will be thoroughly discussed later. If this material successfully

mimics the tunable photonic crystal of the chameleon, then it could be used to better understand the process in nature. There are certain benefits to being able to work with a material instead of with a live animal. The material can be manipulated exactly instead of being dependent on the chameleon's mood. It can also be studied with methods that are not compatible with live chameleons. Furthermore, it is appealing to researchers who are not comfortable with taking skin biopsies from chameleons or otherwise collecting information from the specimen. This is a new material and whether or not it is able to fully encompass the properties of the structure in the chameleon it will exhibit distinct optical properties. Furthermore, depending on these optical properties, this material may be a candidate for augmentation to form new optical materials.

Literature Review

Due to the intersectionality of this project the literature review reaches into several different topics. This section will also serve as a background section. The literature will be presented in chronological order based on the publication date to provide the reader with a development leading up to this project.

"The Dermal Chromatophore Unit," by Bagnara, Taylor, and Hadley introduces and thoroughly describes the color-changing structure in amphibians. This structure is referred to as the dermal chromatophore unit. The dermal chromatophore unit is made up of three main structures: xanthophores, iridophores, and melanophores. These three structures work together to influence the displayed color of amphibians. In this study Bagnara, Taylor, and Hadley are focused on different types of color-changing tree frogs. They characterized the dermal chromatophore unit through close inspection of skin samples, electron microscopy, and light microscopy. The xanthophore is the uppermost

element of the unit. The xanthophores have a static contribution to the color of the frog. Below the xanthophores are iridophores. The iridophores are made up of reflective organelles. Below the iridophores are the melanophores. The melanophores have the ability to spread into finger-like structures that surround the iridophores and the space between the xanthophores. When the melanophores spread into these structures, it has a darkening effect on the color of the frog. They can recede just as rapidly resulting in a lightening of the color. The melanophores and iridophores work closely together to influence the color of the frog. Intermedin, a hormone, governs the changing of the melanophores and iridophores here [2]. The Dermal Chromatophore Unit was published in 1968. While this study relates to amphibians and not chameleons--chameleons are reptiles--it does serve to give an idea of the terminology surrounding color influencing structures in nature. It also provides a contrasting example of color change to the color change of the chameleon which is helpful in identifying what makes the chameleon a unique subject.

In "Biochemical Analysis of Crystals from the Dermal Iridophores of a Chameleon *Anolis Carolinensis,*" by Rohrlich and Rubin the dermal iridophore of the Anolis Carolinensis is identified as guanine crystals. Rohrlich and Rubin are quite thorough in their chemical characterization of the iridophores. They used chromatography, electron microscopy, and electron diffraction to characterize the iridophores both as crystalline guanine [3]. Published in 1975, this characterization is useful in future research of the chameleon's color-changing abilities as the chemical identity and structure of the iridophore can have a significant influence on how it behaves within the chameleon. It also provides support for this project because it gives evidence suggesting that the color

contribution of the iridophores relies on the arrangement of guanine crystals relative to each other rather than within each crystal.

In “Photonic Crystals: Semiconductors of Light,” Yablonovitch presents the photonic crystal in an accessible manner describing the characteristics necessary for a photonic crystal and the process that he and other researchers went through to make a man-made photonic crystal. A photonic crystal is a crystal that forbids a particular wavelength or range of wavelengths of an electromagnetic wave to pass through it. This range of wavelengths that are forbidden to pass through are defined as the photonic band gap for the given structure. Notice this is not restricted to just light. It can refer to any electromagnetic radiation. The first documented man-made photonic crystal was created in 1991 by Yablonovitch’s research group. This is interesting to think about because photonic crystals have existed in nature since long before 1991. Yablonovitch acknowledges towards the end of the article that there have now been discoveries of photonic band structures in nature in butterfly wings, opals, and in the hairs of a seamouse. However, in none of these is there a complete photonic band gap. Complete photonic band gap meaning that the specific wavelengths are forbidden despite their path of incidence. In the examples cited there are some angles at which the forbidden wavelength of light is allowed to pass through [4]. This article was published in 2001 perhaps since then a complete photonic band gap has been discovered in nature. This introduction to photonic crystals helps to understand what properties to expect and inspect in the material produced by this project.

In “Photonic Rubber Sheets with Tunable Color by Elastic Deformation,” by Fudouzi and Sawada a material composed of PDMS and carefully treated Polystyrene spheres is

shown to act as a tunable photonic rubber sheet. The polystyrene spheres form a colloidal, close-packed crystal. The spacing between the polystyrene spheres is increased by up to 10% after they are captured in the PDMS by swelling the material with silicon oil. The additional space is then filled with more PDMS. The figures throughout this article illustrate a material with a very clear color shift [5]. This article was published in 2006 this is nine years before the article detailing the color changing process of the chameleon surfaced. This material is nearly identical in function to the goal of this project--a material that contains a photonic crystal which can be tuned by deforming the PDMS that contains it.

"Photonic Crystals Cause Active Colour Change in Chameleons," by Teyssier et al. is the principle article on the color changing process of the chameleon. Teyssier et al. describes the structure and role of two separate layers of iridophores present in the panther chameleon through analysis of skin structure, Raman spectroscopy, nanocrystal measurements, photometry, optical modelling, single cell videography, and reflectivity measurements. The upper layer is referred to as superficial iridophores (s-iridophores). They contribute to color regulation while the deeper layer (d-iridophores) contributes primarily to thermoregulation. Teyssier et al. biopsied samples of chameleon skin and subjected these samples to osmotic pressure experiments that revealed that an excited skin sample could be forced to change from appearing white or yellow to blue by changing the spacing in the guanine crystal lattice. Through cell-tracking an individual cell was observed passing through all the colors in the range between the white/yellow and blue state. All of this led to the conclusion that chameleons change color by tuning the photonic crystal formed by the guanine crystal lattice of the s-iridophores. The s-

iridophores were found to measure 127.4±17.8 nm in diameter. The refractive index of the cytoplasm surrounding the guanine nanocrystals is $n_{cytoplasm}$= 1.33 and for the guanine it is $n_{guanine}$= 1.83 [1]. This article was published in 2015.

Chameleons change color by tuning a guanine crystal lattice in their skin. “Spectral Transition in Bio-Inspired Self-Assembled Peptide Nucleic Acid Photonic Crystals,” by Berger et al. presents a method for synthesizing a guanine crystal lattice using a double-protected PNA powder that when dissolved in water assembles itself into the organized structure that forms a photonic crystal. Here the spacing of the guanine crystals is adjusted by changing the salt concentration in the solution. Thus, it is shown that changing the spacing of the particles in the naturally assembled guanine structure results in an adjustment of the photonic crystal and the observable color of the structure. The guanine spheres have a diameter of 1.7 μm [6]. In this project, this same process for synthesizing a guanine photonic crystal can be used to form the photonic crystal portion of the biomimetic material. The difference will be that instead of keeping the guanine in the solution and adjusting salt content to change spacing the guanine structure will be captured in PDMS thus forming a material that can be stretched or contracted to influence the spacing of the guanine in the photonic crystal.

“Light Manipulation by Guanine Crystals in Organisms: Biogenic Scatterers, Mirrors, Multilayer Reflectors and Photonic Crystals,” by Gur et. al. provides an overview of different uses of guanine in organisms. According to Gur et. al. anhydrous crystallized guanine takes different forms in different organisms to manipulate light differently. In certain spiders, guanine is used to scatter light creating a bright white coloration to the spider. In certain fish guanine in a plate-like form creates a silvery, reflective effect. In

other fish it is used to create an iridescent effect. As discussed earlier on, and again in this article, guanine is also used in chameleons to form a three-dimensional, tunable photonic crystal that allows the chameleon to rapidly change colors. It is emphasized that the this is the only known example of a guanine-based three-dimensional photonic crystal is in the panther chameleon. The chemical formula for guanine is $C_5N_5H_5O$. It is part of the purine family. It is well-suited to light manipulation roles due to its relatively high refractive index. Pure guanine is rather insoluble in solutions with neutral pH (like water), so it is rather stable in biological systems. These factors are what makes it favorable for the role that it plays in nature. However, there are other crystallized purines that have similar properties. This leads to the question of why other purine crystals are not as prevalent as light manipulators in biological systems. This question requires further research to answer [7]. This article was published in 2016 which was soon after the article that explained how photonic crystals cause active color change in chameleons. It presents an interesting perspective on the use of guanine crystals in natural systems. Nature seems to favor guanine crystals for manipulating light. This leads to the concept of incorporating guanine crystals into synthetic optical components. In the particular case of this paper the focus is on implementing guanine crystals to mimic the tunable photonic crystal that exists in the chameleon.

"Progress and Opportunities in Soft Photonics and Biologically Inspired Optics," by Kolle and Lee is a review article. Kolle and Lee describe soft photonics as optical components that are flexible and sensitive to stimuli. They note that most optical components do not fall into the category of soft photonics. This is due in part to complications that arise in trying to design soft photonic elements. However, Kolle and

Lee turn to nature as an answer to this problem. They say nature has already designed numerous ways of controlling light that may be adapted to find applications in the field of optics in the form of soft photonics. They go into great depth on several examples of possible inspirations for soft photonic materials and explain the research that has already been done in these areas. For example, they discuss a cephalopod which has the ability to change colors and patterns making itself nearly visually imperceptible. While no known material has been created that can fully mimic these abilities, there have been advances is soft photonics that make use of the reflectin proteins in cephalopods. Interestingly, they also discuss the chameleon as a potential source for inspiration in creating photonic components [8]. This article was published in 2018, so they were able to refer to the article published by Nature Communications on the color-changing process of the chameleon. Furthermore, the attempt to create a material that captures the properties of the chameleon's color change is a relevant endeavor as a preliminary to possible advances in the bio-inspired soft photonic field.

These articles are all connected by their relationship to lending to the understanding of some part of the chameleon color-changing process. Hopefully they will also lend to the understanding of the project at hand. There are some important things to note before proceeding. When testing the samples to see if they will change colors when stretched, it is also important to consider that there may be better results with compressing the samples [9,10]. PDMS has a refractive index of n_{pdms}= 1.4 [11]. This is like that of cytoplasm (recall: $n_{cytoplasm}$= 1.33). The guanine spheres that are produced by the self-assembly process are 1.7 μm while the guanine in the chameleon has a diameter of approximately 127.4 nm. This means that the diameter of the guanine spheres formed by

the self-assembly process is roughly 10 times that of the guanine in the chameleon. In the chameleon the guanine is packed in a face-centered cubic structure while the guanine formed in the self-assembly process is in a hexagonal close-packed structure. These differences may become significant in the future.

Experimental Section

Pre-Experimental Background. A photonic crystal is a material that is typically made up of some arrangement of dielectric materials or a different material that changes the way an electromagnetic wave passes through it. The wavelength of the photon incident on the crystal determines whether or not it will propagate. Since different colors of light present with different wavelengths, a photonic crystal can act as a selector to reflect only particular colors at particular angles of incidence, creating an opalescent effect. Opalescent means that something appears to have a changing color as an opal does. It is possible to have photonic crystals of multiple dimensions: 1,2, and 3-dimensional are possible. The top layer of guanine crystals in a panther chameleon form a three-dimensional photonic crystal. The guanine is arranged in a face centered cubic geometry that creates the photonic crystal.

PDMS stands for polydimethylsiloxane. PDMS is a silicon-based organic polymer. For this project the type of PDMS that is employed is called Sylgard 184 Silicone Elastomer. Sylgard is hydrophobic, and comes in two parts, a base and a curing agent. These two parts are recommended to be mixed at a 10:1 ratio by mass or volume and then allowed to cure for 48 hours at room temperature. Variations on this are allowed and can be used to manipulate the nature of the final sample.

For the guanine crystal synthesis, a double protected guanine-based PNA monomer is used. This is referred to as Fmoc-G-(Bhoc)-aeg-OH. Fmoc stands for flourenylmethyloxycarbonyl and Bhoc stands for benzohydryloxycarbonyl. These two groups make the PNA double protected which means that the groups are bonded to the PNA to help keep the PNA from experiencing degradation. This material comes in a

powder form and can be dissolved in water to prompt it to self-assemble a photonic crystal. This powder will be referred to as guanine powder. This process is based off research reported in the paper, “Spectral Transition in Bio-Inspired Self-Assembled Peptide Nucleic Acid Photonic Crystals” by Berger et. al. [6].

Methods for PDMS. The recommended ratio of Sylgard base to curing agent is 10:1 by mass or volume. To learn whether ratios other than the standard 10:1 yield samples with useful mechanical properties, the ratio was altered for some of the samples that were fabricated. In general, the process to prepare a PDMS sample is as follows:

1. Measure out the base into a weigh boat on an electronic balance (use the amount that is necessary for whatever ratio is being prepared).
2. Add the curing agent to the weigh boat using a syringe to control the amount added so that the measurement is precise. Use a vent throughout this process to remove vapors coming from the mixture.
3. Mix the base and the curing agent in the weigh boat for ten minutes with a metal stirring rod.
4. Place the weigh boat in the desiccator. Make sure the weigh boat is positioned so that it will not spill while the sample is being desiccated.
5. Secure the lid of the desiccator.
6. Turn on the vacuum pump that is connected to it. The vacuum pump used for this experiment is capable of reaching pressures less than 2 mmHg. The recommended pressure for this process is 10-20 mmHg. For this process, with the strong capabilities of the vacuum pump, it was necessary to closely supervise the sample during the desiccation.

7. Frequently, open the release valve on the desiccator to raise the pressure in the chamber and pop bubbles in the sample. The goal is to pop all the air bubbles in the PDMS sample, so that the bubbles in the sample do not disrupt optical measurements later.
8. Once the bubbles are popped, place the sample in a dry place where it will not be disturbed and allow it to cure. Some of the samples were cured in the weigh boats and others were cured on glass microscope slides. Curing times can be varied for various levels of stickiness/flexibility. The recommended curing time is 48 hours.

Further experimentation was done with curing the PDMS on the surface of water. These are the steps that were taken:

1. Complete steps 1-3 from above. Use an 8:1 ratio of base to curing agent.
2. For the sake of visibility in the water add blue pen ink to the PDMS and mix thoroughly.
3. Complete steps 4-8 from above.
4. Prepare water by partially filling a beaker and adding one drop of pink food coloring. Pink is used to create a contrast in the color of the PDMS and the color of the water to further aid the visibility.
5. Slowly pour the PDMS onto the surface of the water.
6. Leave the beaker somewhere that it will not be disturbed for 48 hours for the PDMS to cure.

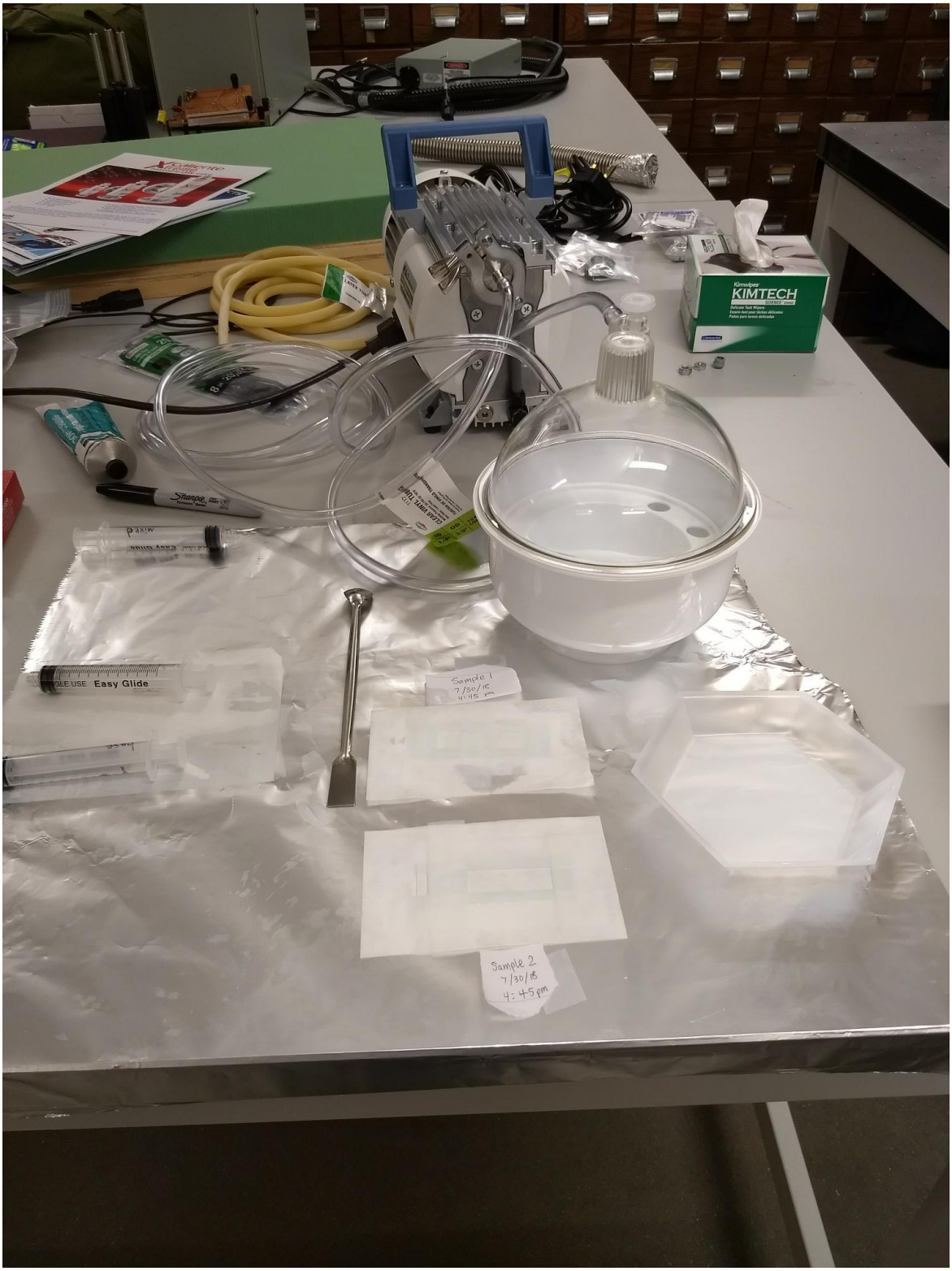

Figure 1 - PDMS Preparation: The set-up for preparing the PDMS samples. The vacuum pump is attached to the desiccator, and a weigh boat is in the front right.

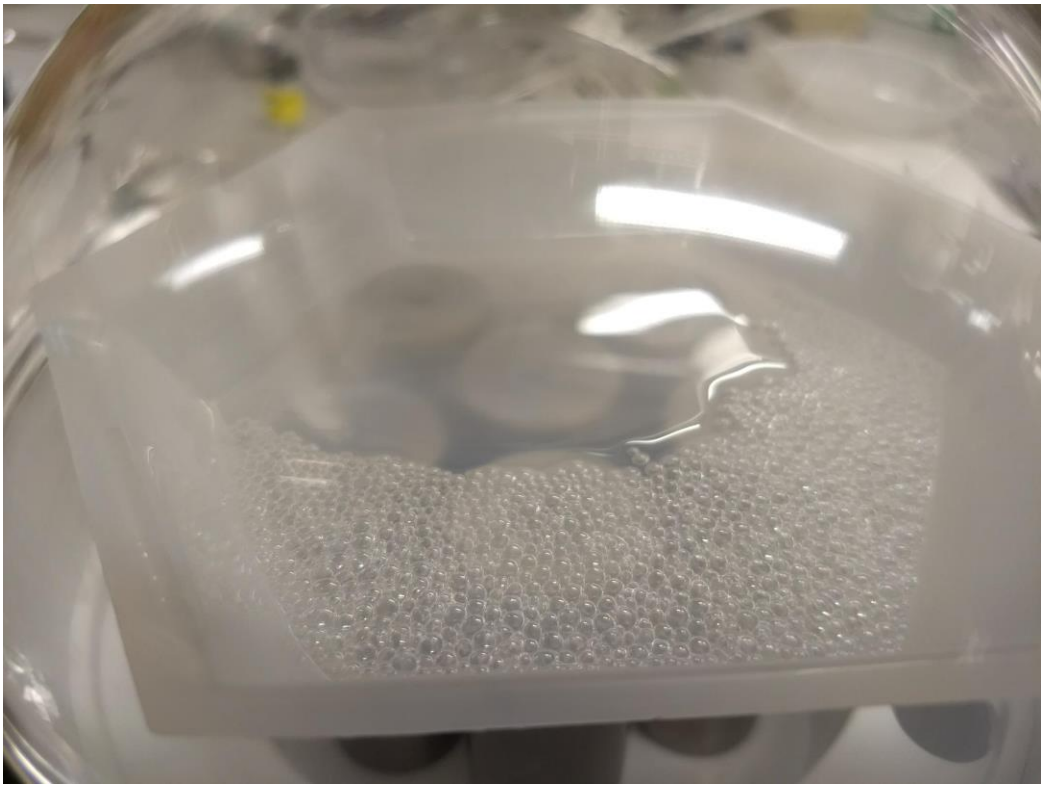

Figure 2 - **Liquid PDMS in Desiccator**: This is an image of the PDMS inside the desiccator. Once all the bubbles have popped, the sample will be ready to come out.

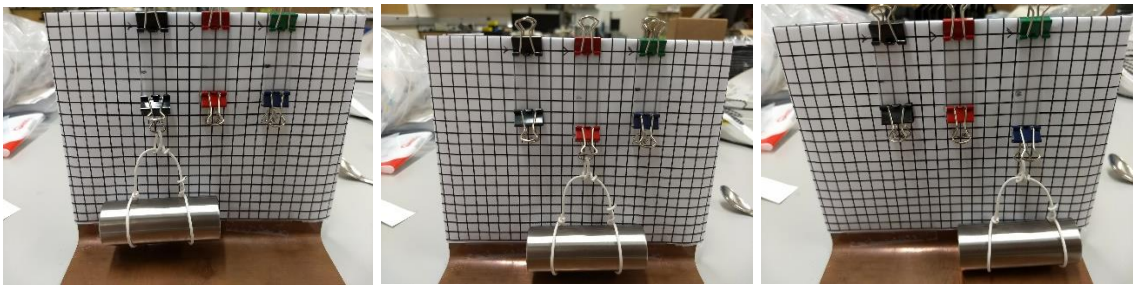

Figure 3 - Measuring Elasticity: This is the set-up for determining the relative elasticity of PDMS samples made with different ratios of base to curing agent. From left to right: 8:1, 10:1, 12:1.

Table 1: Comparison of PDMS with Different Ratios of Base to Curing Agent

Ratio of base to curing agent	Mass hung from sample (g)	Amount extended ("x" in inches)
8:1	260.1	$x < \frac{1}{8}$
10:1	260.1	$\frac{1}{8} < x < \frac{1}{4}$
12:1	260.1	$\frac{1}{4} < x < \frac{3}{8}$

Figure 4 - PDMS on Water

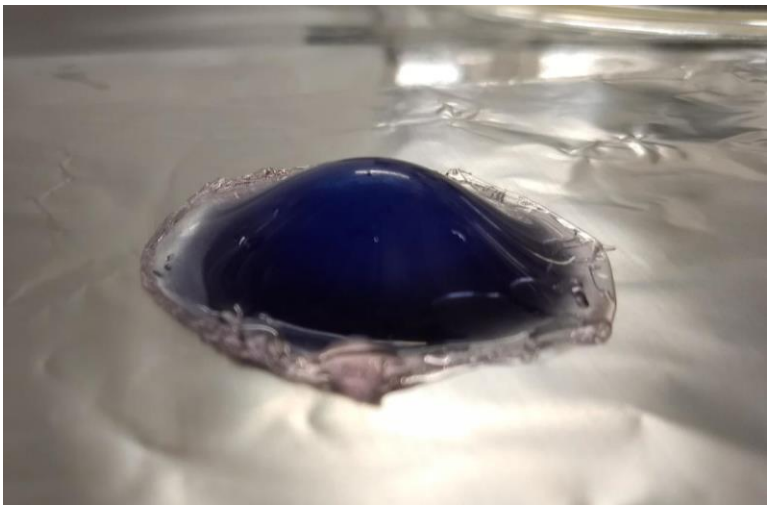

Figure 5 - PDMS Cured on Water

Data for PDMS. The PDMS samples that are prepared in weigh boats have varying thicknesses at different parts of the container. Generally, they are thicker towards the edge and thinner near the center of the weigh boat. After being allowed to cure for 24 hours, all samples, no matter what the ratio of base to curing agent, are very flexible. They peel off the plastic weigh boat material easily, but they are very sticky to gloves and to themselves. The samples that were cured on glass microscope slides would stick to the slides so much that an effort to remove a sample from the slide after only 24 hours would require scraping it off and damaging the sample in the process. After 48 hours or longer the samples become less sticky and therefore easier to work with. They could be easily peeled from the plastic weigh boats and from the glass microscope slides. They did not stick to gloves as much and did not stick to paper at all. The samples were overall less flexible than before but seemed to stretch more consistently. The samples cured on glass microscope slides seemed to have more consistent thicknesses.

Three samples of PDMS were prepared with different ratios of base to curing agent (8:1, 10:1, and 12:1) to determine the effect that varying the ratio has on the elasticity of the samples. These samples were prepared in weigh boats and allowed to cure for more than 72 hours. The samples undergoing measurements were cut out of the portion of the samples in the weigh boats that seemed to have consistent thickness and thicknesses that were close to one another. All samples that are used for measurements were ½ inch by 1½ inches. Inches are being used as the unit of measurement here because the graph paper used has ¼ by ¼ inch squares. Based on the data in Table 1 the higher the ratio of base to curing agent the more the sample will stretch under the same force. Observations after each stretching process indicate that all the samples, despite the ratio of base to

curing agent, returned to their original positions. The PDMS that was cured on the surface of water ended up forming a sort of bowl shape (See figures 4 and 5 for a visual representation). When the PDMS was first poured on the surface it spread out a little bit; as more was added, it started to dip down more significantly into the water. It seemed like the material was exhibiting hydrophobic properties even in its liquid form. This allowed it to stay relatively separate from the water and cure on the surface. See figure 4 for an image of the PDMS on the surface of the water. Due to its thickness after it cured it was difficult to obtain any measurements on its ability to stretch. It seemed to have absorbed some of the water. It was very smooth and not sticky.

Commentary for PDMS. The experimentation with different ratios of Sylgard base to curing agent revealed that all the ratios tested yielded well-behaved samples. For a sample that is able to stretch more with less effort use a higher ratio of base to curing agent and vice versa. Any ratio between 8:1 and 12:1 is suitable for the biomimetic material.

The three surfaces that the PDMS was cured on were: plastic, glass, and water. Each of them comes with advantages and disadvantages.

The PDMS peels off the plastic easily, but it cures with inconsistent thickness. Perhaps there is a process that could be performed to help the PDMS in the weigh boat cure with a more consistent thickness. For example, pushing the material while it is in liquid form to the center of the weigh boat and allowing it to slowly redistribute itself as it cures. Another disadvantage of using the weigh boat is that the sample size must be larger for it to cover the entire bottom of the container, and therefore each sample uses more material.

The PDMS does not peel off the glass as easily, though if left to cure long enough it becomes easier. It has a more consistent thickness than samples cured on plastic or water. The consistent thickness is favorable, and makes this option seem like it may be the best suited for the creating the biomimetic material. In addition, it requires less material, so it would be easy to prepare multiple samples at once using this method. It is also convenient for being able to place the microscope slide under a microscope to get a better look at what is happening on a microscopic level.

The PDMS cured on water is easily removed from the water, but the thickness is very inconsistent this makes it less ideal for the biomimetic material. Perhaps, using less material on the surface of the water would lead to a sample with more consistent thickness. This method of curing the material is attractive because the goal is to embed the photonic crystal formed by the guanine crystal array in the material, and the guanine crystals are being synthesized on the surface of water.

Methods for Guanine Crystal Synthesis. Multiple processes for the synthesis of guanine crystals were explored. For both processes a solution with a concentration of 4 mg/mL was prepared. For the first process the following steps were taken:

1. Measure 25 mL of ultra-pure water into a glass beaker.
2. Place the beaker of water on a hot plate on high heat. Heat until the water begins to boil.
3. Carefully measure out 0.1 grams of the guanine powder.
4. Add the powder to the boiling water and stir it with a metal stirring rod.
5. Once the powder is completely dissolved draw up the solution in a syringe.

6. Place a 0.22 μm PVDF syringe filter on the syringe and then filter the solution into the desired container. Potential containers include weigh boats, small vials, and microscope slides (microscope slides here have tape barriers on them to create contained areas on the slide).
7. Allow the filtered solution to cool.

There were two variations to this process. One variation was to add the guanine powder to the beaker before placing it on the hot plate. In this variation, heated water was added as necessary to keep the total volume of water at 25 mL. The other variation replaced the ultra-pure water with double distilled water.

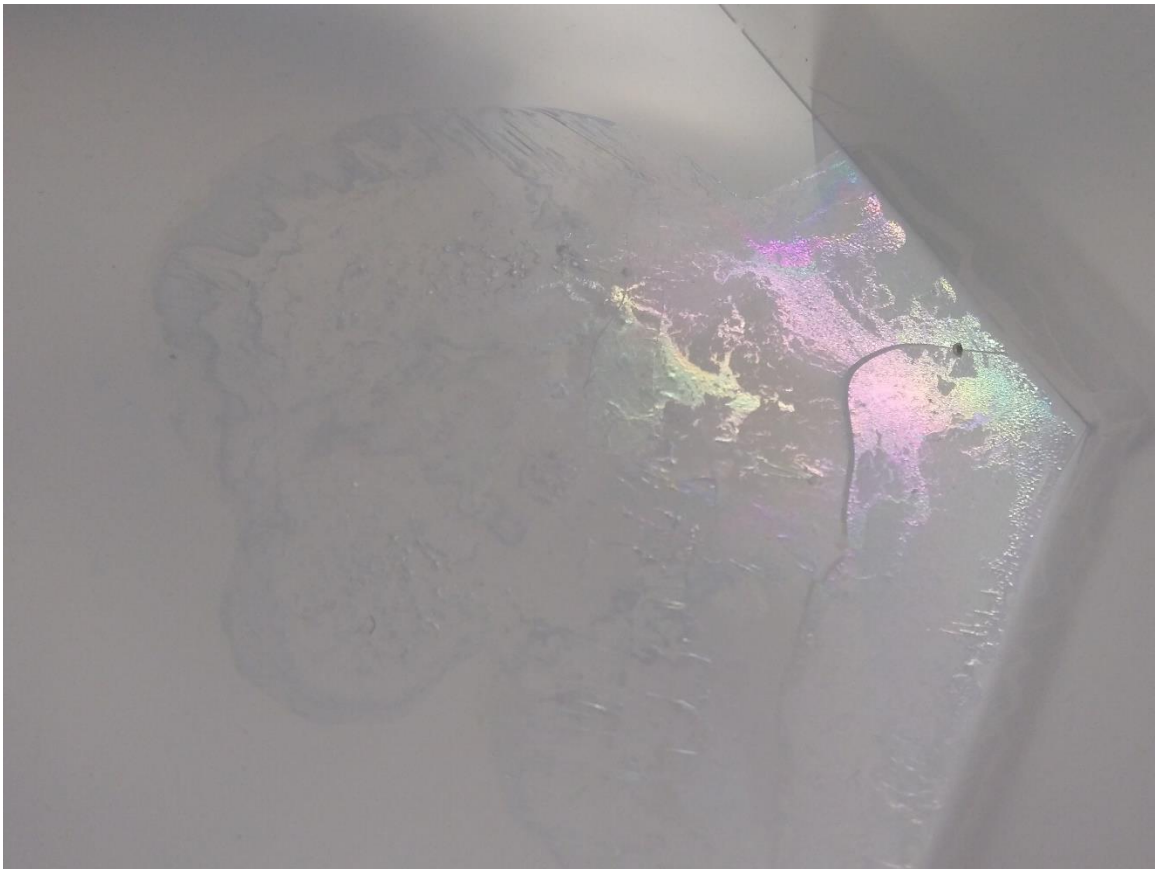

Figure 6 - Guanine in Weigh Boat

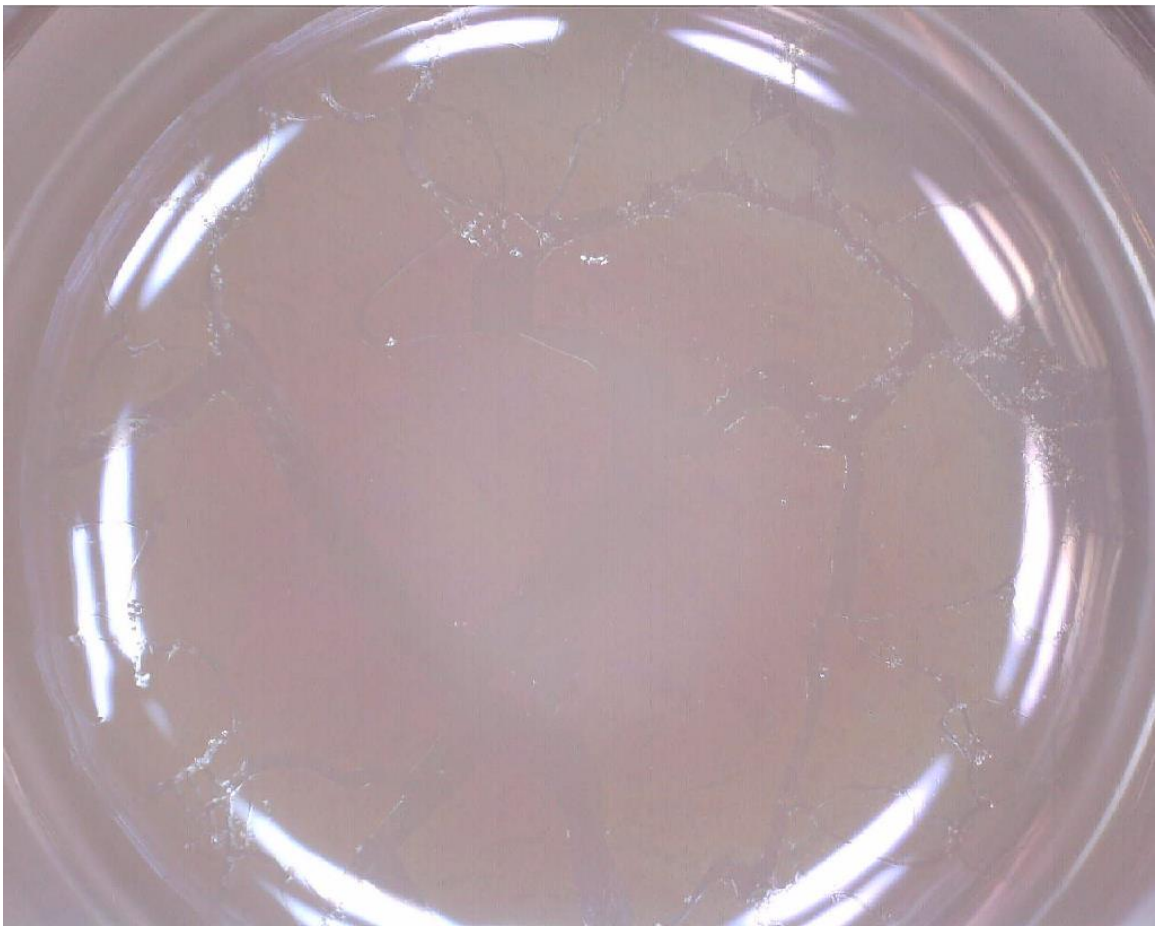

Figure 7 - Guanine on Surface: Look closely to observe color gradient.

Data for Guanine Crystal Synthesis. When the guanine powder was added after the water was boiling, a lot of the powder dissolved nearly on contact with the water. The PNA that remained undissolved started to clump up and then began to stick to the stirring rod. Efforts to get more of it to dissolve after it stuck to the stirring rod were unsuccessful. Once the solution was filtered and deposited into each of the containers described above it began to cool and a slight iridescence could be seen on the surface of the samples in the vials and on the surface of the sample in the weigh boat. The sample on the slide seemed like it was not well contained by the thick label tape. No iridescence was visible on the slide. After a day the samples in the vials were cloudy and there was

little to no iridescence visible on the surface. The sample in the weigh boat maintained some iridescence.

When the guanine powder was added while the water was at room temperature, it took more time for the powder to dissolve. However, it did seem like more of the powder ended up dissolving after the water was brought to a boil than in the previous method. There was still some of the powder that did not make it into solution and ended up stuck to the stirring rod. Once the solution was filtered and deposited into each of the containers described above it began to cool and a slight iridescence could be seen on the surface of all the samples. After a day the samples in the vials were cloudy and there was little to no iridescence visible on the surface. The sample in the weigh boat maintained some iridescence (Figure 6). The samples on the slides with parafilm and scotch tape barriers had solidified and maintained some iridescence while the one on the slide with thin label tape looked like it had seeped through and it did not have any iridescence. The samples that had dried completely did not have a consistent thickness.

When the samples were prepared by adding the guanine powder while the water was at room temperature and using double distilled water instead of ultrapure water nearly all the powder dissolved in these solutions, and the process for the powder to dissolve took less time. These samples were primarily deposited in scintillation jars, weigh boats, and 5mL beakers. All three samples displayed iridescence as they began to cool. After a day the iridescence was more difficult to observe in the scintillation jars and beakers. The sample in the weigh boat presented the most widespread iridescence and it was still visible after it was left to sit.

Commentary for Guanine Crystal Synthesis. The most promising results for the guanine crystal synthesis were yielded by adding the guanine powder to the water at room temperature and using double distilled water. The observed solubility of the guanine powder was significantly better in the double distilled water than in the ultra-pure water. Observations also indicate that adding the powder to the room temperature water yielded slightly better results for the guanine crystal synthesis because more of the powder dissolved resulting in more iridescent samples. The iridescence of the samples is attributed to the guanine crystals forming microspheres that form a well-ordered lattice. Scanning electron microscope images of the guanine embedded in PDMS indicates that the guanine synthesis process is successfully creating guanine microspheres. The samples in the vials that have iridescence near the surface at first and then appear cloudy after a day could be having the guanine going back into solution, so maybe drawing off the excess liquid before the iridescence goes away would better preserve the guanine crystal structure in these samples.

Methods for Embedding Guanine in PDMS. There are two processes for embedding the guanine crystal lattice in PDMS that have been attempted. The first process is as follows:

1. Prepare a guanine sample in a scintillation jar or small beaker using the steps defined above with both variations.
2. While the guanine sample is cooling, prepare a PDMS sample with a 12:1 ratio of base to curing agent. Leave the PDMS in the weigh boat that it is prepared in.

3. Once iridescence is observed in the guanine sample, carefully apply a small amount liquid PDMS to the surface of the guanine sample. This should be done slowly. Try to distribute the PDMS evenly on the surface of the guanine sample.
4. Allow 72 hours for the PDMS to cure. Then remove the sample from the container.

The second process is below:

1. Prepare a guanine sample in a weigh boat using the steps defined above with both variations.
2. Let the guanine sample sit until all the excess water has evaporated and only the guanine remains.
3. Prepare a PDMS sample with a 12:1 ratio of base to curing agent.
4. Evenly distribute the liquid PDMS over the guanine sample in the weigh boat. This can be achieved by pouring the PDMS from the weigh boat it was mixed in into the weigh boat with the guanine sample.
5. Allow 72 hours for the PDMS to cure. Then remove the sample from the container.

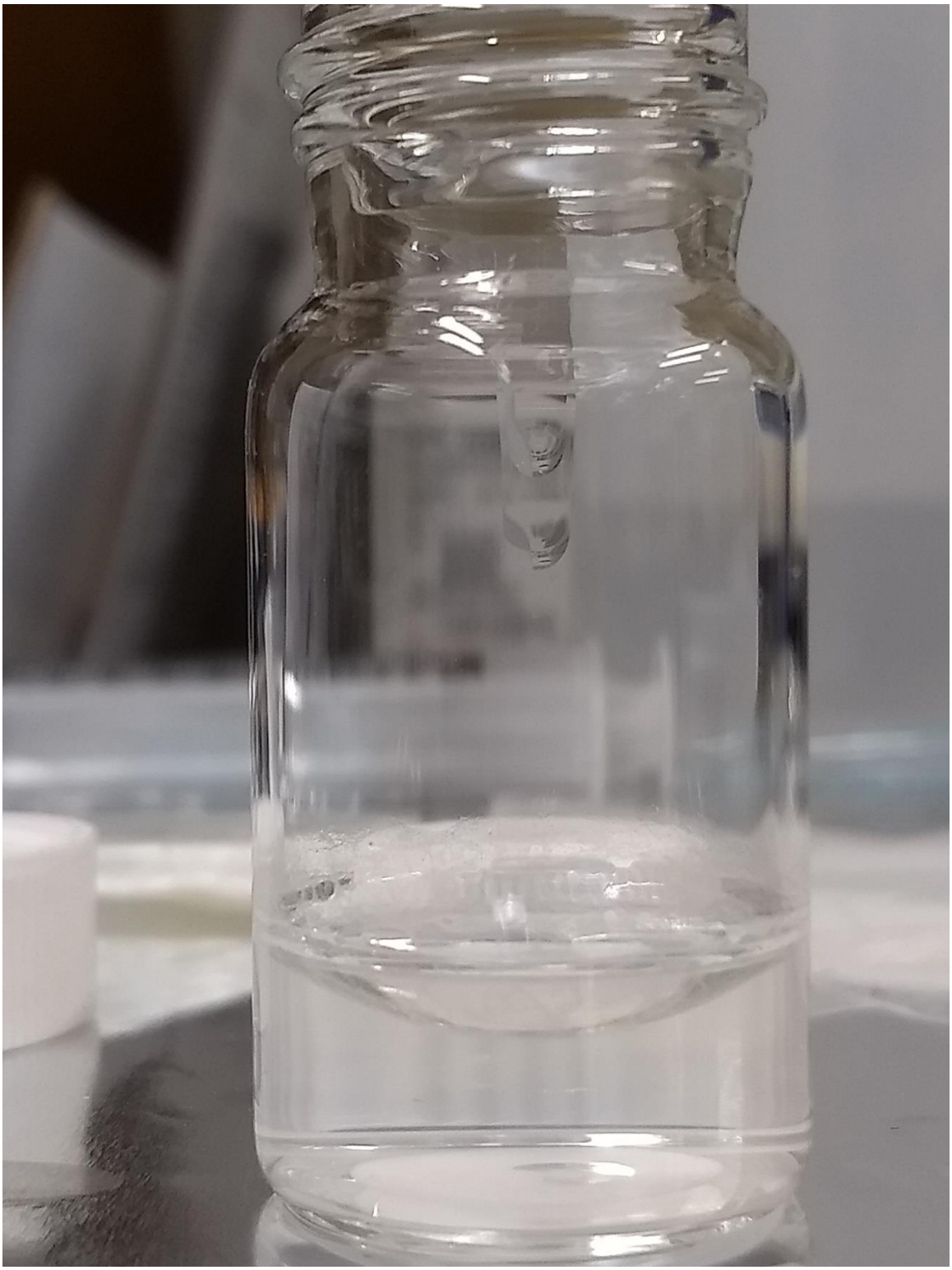

Figure 8 - PDMS on Guanine Solution

Figure 9 - Guanine Embedded in PDMS

Figure 10 - Close-Up of Fig. 11: Observe the iridescent patch.

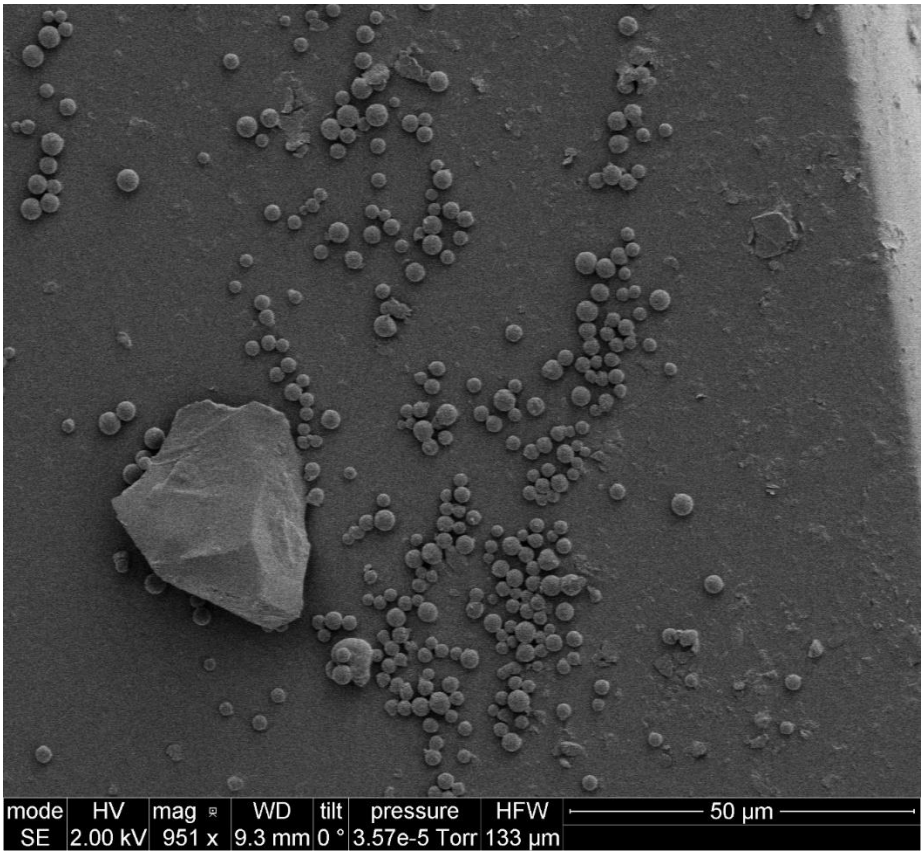

Figure 11 - SEM Image 1

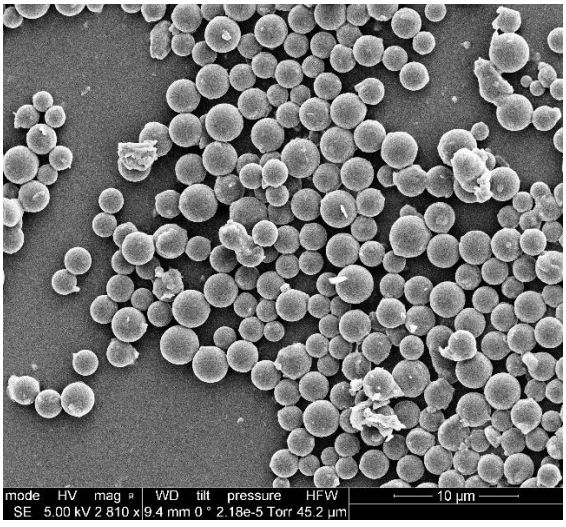

Figure 12 - SEM Image 2

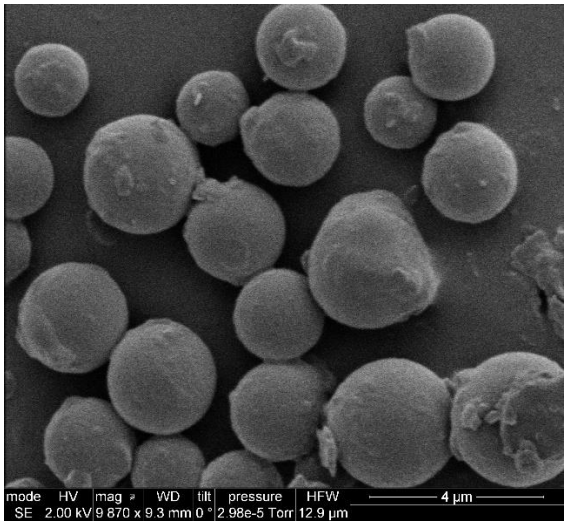

Figure 13 - SEM Image 3

Data for Embedding Guanine in PDMS. The samples prepared by applying liquid PDMS to the surface of the guanine solution were flat on one side and curved on the other. They had inconsistent thickness, but in places the iridescence was preserved (See figures 9 and 10). Scanning electron microscope (SEM) images were obtained for some of the samples (See figures 11-13). These images show that the ordering of the guanine microspheres is not consistent throughout the samples. In the images it also appears that the spheres have inconsistent diameters; however, this could be due to the spheres being at different depths in the material. The largest diameter of a guanine microsphere observed in these images is roughly 3 μm. This diameter differs from the 1.7 μm diameter of the guanine microspheres observed in [6].

The samples prepared by applying liquid PDMS to dry guanine samples in weigh boats were flatter. They still had inconsistent thickness. Attempts to remove the sample from the weigh boat led to the PDMS tearing in places. The iridescence of the guanine was not preserved with this method. Parts of the guanine remained stuck to the weigh boat.

Commentary for Embedding Guanine in PDMS. While guanine deposits in weigh boats maintained the most widespread iridescence, the method of pouring liquid PDMS over these samples was unsuccessful at capturing and preserving the guanine lattice. Applying liquid PDMS to the guanine while it was on the surface of the solution it formed in had results that preserved patches of iridescence in the samples. However, the SEM images reveal that the ordering of the guanine microspheres in these samples is not well-defined over a significant area of the samples. Therefore, this method for capturing

the guanine crystal lattice is also not succeeding as robustly as is necessary for the biomimetic material.

Further experimentation with methods of capturing the guanine crystal lattice in the PDMS would be advantageous.

Future Experimentation. There are a multitude of potential methods for capturing the guanine crystal lattice in PDMS. One such method is described here. This is the suggested next step to take in experimentation with embedding the guanine in PDMS. The potential method is as follows:

1. Prepare a sheet of PDMS allow it to cure in a mould of some kind (potentially a weigh boat). Consistent thickness is desirable in this sheet.
2. Prepare the guanine solution and filter it onto the sheet.
3. Allow the sample to sit until all the excess water has evaporated.
4. Apply liquid PDMS to the surface of the sample. Again, consistent thickness is desirable.
5. Allow the sample to cure.

This method is hypothesized to capture the guanine crystal lattice between the two layers of PDMS. The guanine would have the chance to form widespread iridescence as it does when it is deposited in a weigh boat, and since there is a layer of PDMS underneath it the problem of some of the guanine sticking to the weigh boat is eliminated. The samples prepared in this way could then be imaged with a scanning electron microscope to help determine how well the lattice is preserved.

Conclusion

The objective of this project was to create a material that mimics the color-changing process of the chameleon and characterize the material. The literature review revealed that there is a way to create a guanine microsphere lattice through a relatively simple self-assembly process. This process for creating guanine microspheres was successfully replicated here with a discrepancy in the diameter of the spheres. The largest observed spheres here had diameters of nearly 2 times that of the spheres in the study performed by Berger et. al. [6]. This is much larger than the guanine nanospheres found in chameleons. This implies that the color-changing effect would be created on a different scale in the material than in the chameleon. As seen in the section on embedding the guanine in PDMS, the guanine spheres in the material created here are too disordered to create the desired effect. This indicates that the method of applying the liquid PDMS to the surface of the guanine solution is not an effective way of preserving the ordering of the guanine microspheres. Further experimentation is recommended to find a method that preserves the ordering of the guanine. If the ordering is preserved over a significant area, it is hypothesized that the combination of a guanine crystal lattice and PDMS will result in a material that has characteristics like that of the tunable photonic crystal observed in chameleons. If this material is later successfully created, it would have applications as a reference when studying the color changing process of the chameleon, it could have potential in the field of soft photonics, and in creating other optically interesting materials. Despite the ability of the material to mimic the chameleon a well-ordered array of guanine in PDMS should have distinct optical properties with interesting applications.

Works Cited

[1] J. Teyssier, S.V. Saenko, D. van der Marel, and M.C. Milinkovitch, "Photonic crystals cause active colour change in chameleons," Nature Communications 6, 6368, (2015).

[2] J.T. Bagnara, J.D. Taylor, and M.E. Hadley, "The Dermal chromatophore unit," Journal of Cell Biology 38 (1), 67, (1968).

[3] S.T. Rohrlich, and R.W. Rubin, "Biochemical characterization of crystals from the dermal iridophores of a chameleon anolis carolinensis," Jour. of Cell Bio. 66 (3), 635, (1975).

[4] E. Yablanovitch, "Photonic crystals: semiconductors of light," Scientific American 285 (6), 46-55, (2001).

[5] H. Fudouzi, and T. Sawada, "Photonic rubber sheets with tunable color by elastic deformation," Langmuir 22 (3), 1365-1368, (2006).

[6] O. Berger, E. Yoskovitz, L. Adler-Abramovich, and E. Gazit, "Spectral transition in bio-inspired self-assembled peptide nucleic acid photonic crystals," Adv. Mater. 28, 2195-2200, (2016).

[7] D. Gur, B.A. Palmer, S. Weiner, and L. Addadi, "Light manipulation by guanine crystals in organisms: biogenic scatterers, mirrors, multilayer reflectors, and photonic crystals," Advanced Functional Materials 27 (6), 160351, (2016).

[8] M. Kolle, and S. Lee, "Progress and opportunity in soft photonics and biologically inspired optics," Advanced Materials 30, 1702669, (2018).

[9] S. H. Foulger, P. Jiang, Y. R. Ying, A. C. Lattam, D. W. Smith, J. Ballato Advanced Materials 13 (24), 1898-1901, (2001).

[10] S. H. Foulger, P. Jiang, A. Lattam, D. W. Smith, J. Ballato, D. E. Dausch, S. Grego, B. R. Stoner, Advanced Materials 15, 685-689, (2003).

[11] M. N. Polyanskiy, “Refractive index database,” refractiveindex.info. Accessed on 4-21-2019.