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Oxidative Protection Using Pluronic Micelles

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Oxidative Protection Using Pluronic Micelles

by

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Abstract
Methods for drug delivery are currently being researched in many different applications. One significant obstacle to drug delivery is a drug's inherent hydrophobic character. Drugs that are hydrophobic are difficult to successfully administer since the body consists mostly of water. Therefore, a greater variety of drugs could potentially be used for the treatment of disease if they could successfully be administered. Recent research in oncology and virology has attempted to overcome the obstacles posed by hydrophobic drugs by encapsulating them in micelles. Micelles form spontaneously from compounds that contain both hydrophilic and hydrophobic regions and can be used to increase the solubility of hydrophobic drugs in water. Commonly used compounds that form micelles are Pluronic copolymers, which have a hydrophilic block that consists of polyethylene oxide (PEO) subunits and two hydrophobic blocks that consist of polypropylene oxide (PPO) subunits. Different types of Pluronic such as Pluronic F68 and F127 each have a triblock structure made of the same PEO and PPO subunits but differ in the number of these subunit repeats. This difference affects both size and hydrophilic lipophilic ratio, which is described as hydrophilic lipophilic balance (HLB). It was hypothesized that micelles offer encapsulated drugs protection from oxidation and that polymer properties such as HLB influence the degree of protection. Results from this experiment indicate that Pluronic can be used to form micelles that offer a degree of oxidative protection to encapsulated quercetin. Results also showed that the Pluronic polymers F68 and F127 may offer different levels of oxidative protection as well. The data suggests that Pluronic F127 may offer greater protection than Pluronic F68 which is thought to be due to differences in the inherent properties of each copolymer. Among these properties, HLB is thought to be a significant influencing factor.

Acknowledgments
A special thanks is given to Dr. David Britt who acted as a mentor, overseeing and giving input throughout this project. Funding for this research was provided by Utah State University Department of Biological Engineering, Office of Undergraduate Research, and Honors Program. special thanks to Andrew Kjar, who acted as a research partner during preliminary studies and gave counsel that helped develop this project. Acknowledgement is also to be given to Ian Wadsworth, who gave input with regards to experimental procedure. Last but not least, special thanks are given to Dr. Elizabeth Vargis who provided significant review and revision of this report.

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Background

Researchers have given significant attention to drug carrier systems as a means of addressing some of the current challenges of drug delivery. Research has shown that the properties of these drug delivery systems can be manipulated to apply to a wide range of biological applications [1]. One commonality of many of these drug delivery systems is the use of an amphiphilic copolymer that encapsulates a drug of interest in a nanostructure called a micelle. At specific concentrations of copolymer, hydrophilic and hydrophobic interactions drive the copolymer to self-aggregate into an arrangement that minimizes entropic penalty Hydrophobic portions of polymer congregate toward the center of the micelle along with hydrophobic drugs. A diagram of the resulting micelle is shown in **Figure 1**. Micelles increase the aqueous solubility of an otherwise insoluble drug [2], provide passive targeting by increasing permeability and retention time of anticancer drugs in tumor cells [3], and in some cases, influence encapsulated drugs to exert an alternative mechanism of action [4]. Much of the recent studies have been aimed at cancer treatment but a significant amount of research has also been focused on antiviral therapies [5].

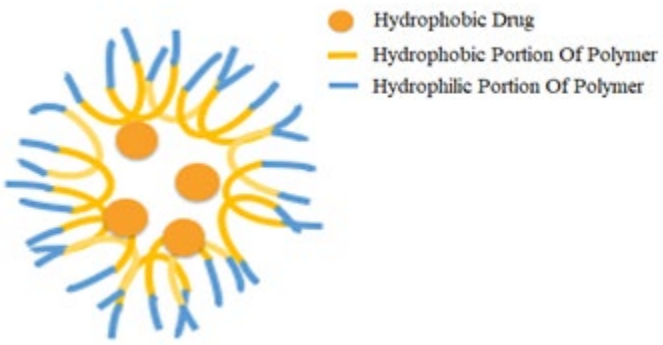

Figure 1: Schematic of drug loaded micelle in aqueous solvent. Yellow circles represent hydrophobic drug while curved yellow lines represent hydrophobic portions of copolymer. Blue represents hydrophilic portion of copolymer.

The copolymers used in these drug delivery systems vary based on application. One promising class of copolymers being researched are Pluronics. These copolymers are composed of polyethylene oxide (PEO) and polypropylene oxide (PPO) subunits that repeat [6]. Rather than repeating in a random fashion, PEO and PPO subunits form an X-Y-X tri-block structure, where X represents a continuous section of recurring PEO units and Y represents a continuous section of repeating PPO units, see **Figure 2**. Because of its higher carbon content, blocks of PPO are relatively hydrophobic while blocks of PEO are relatively hydrophilic.

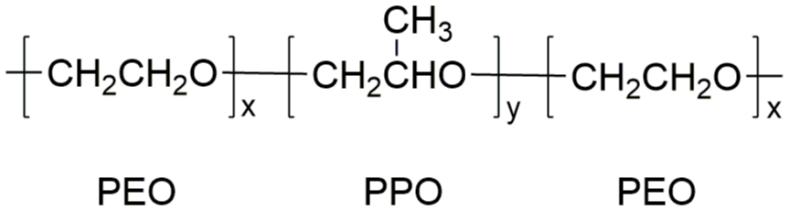

Figure 2: Generic structure of Pluronic copolymers, which are each composed of polyethylene oxide (PEO) and polypropylene oxide subunits (PPO). Symbols X and Y represent variable numbers of repeating subunits.

Pluronic copolymers have this general structure, but many variations are commercially available. These variations have different numbers of PEO or PPO subunit repeats. In **Figure 2**, the number of PEO and PPO repeats correspond to X and Y respectively. Variations in the number of subunit repeats give the copolymers unique characteristics that effect how they form micelles and interact with the drugs inside [7]. The concentration at which micelles self-form, drug loading efficiency, and hydrophilic lipophilic balance (HLB) are a few prominent characteristics that are affected by differing numbers of PEO and PPO subunits. The HLB parameter is generated by dividing the number of hydrophilic subunits by the number of hydrophobic subunits to obtain a ratio that can be used to compare copolymers' relative amphiphilic character. Despite the study of these properties and the differences in structure that drive them, literature lacks answers to whether Pluronic copolymers can protect encapsulated drugs from oxidation; it also lacks indications of how polymer structure influences this protection.

Quercetin is a flavonoid that is found in a variety of fruits and vegetables and is inexpensive to obtain in an isolated form. It has also been implicated as both an antiviral and anticancer compound when in its unoxidized form [8], [9]. One of the draw backs of using quercetin is that it is easily subjected to oxidation and UV degradation [10]. While UV degradation is documented to occur under direct exposure to UV lights, the degradation is thought to occur slowly in conditions where UV exposure is not as strong, such as under normal fluorescent lighting in labs. Oxidation readily occurs but fortunately, oxidation of quercetin has been well studied using spectrophotometry. Spectrophotometry has been used to measure quercetin's absorbance properties. Quercetin possesses a unique conjugated double bound structure that absorbs certain wavelengths of light. When a spectrophotometer shines a laser through a solution containing quercetin, it produces a graph of absorbance that can be used to confirm the presence of non-degraded quercetin or its degradation products. It is reported in literature that non-degraded quercetin, when analyzed via spectrophotometry, produces peak absorbance at 250 and 375 nm while products of oxidized quercetin produces peak absorbance at 225, 293, and 335 nm [11]. **Figure 3** below shows quercetin in its non-oxidized state and indicates the conjugated double bounds that researchers believe correspond to each of the aforementioned wavelengths. **Figure 3** also shows the oxidation products of quercetin and each corresponding wavelength of their unique conjugated double bond structure.

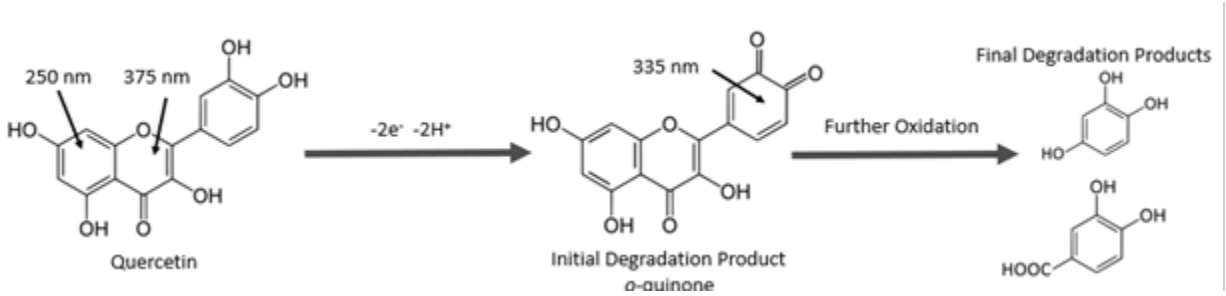

Figure 3: Chemical structure of quercetin, o-quinone, and further degradation products caused by oxidation. Arrows indicate which conjugated bond system produces absorbance at wavelengths specified by each of the labels.

Preliminary Observations and Studies

While working with Pluronic micelles that had been loaded with quercetin, it was observed over time that the color of some of these solutions began to change from a light yellow to a darker yellow brown color. It was theorized that this observation indicated quercetin was oxidizing and producing a color change similar to an apple turning brown after the flesh has been exposed to oxygen. Investigation into literature indicated that atmospheric oxidation was possible in the aqueous samples, so a testing method was devised to further investigate the occurrence of oxidation. New samples of quercetin loaded micelles were prepared and periodically tested using spectrophotometry. The samples were protected from UV degradation and only exposed to atmospheric oxygen. Absorbance spectra were gathered over a period of 6 weeks and a change in peak absorbance was observed in solutions containing quercetin that was encapsulated in micelles made of Pluronic F68. However significant changes were not seen in solutions that contained quercetin encapsulated in Pluronic F127 (**Figure 4**). In order to gain better perspective, this data was compared to a solution of unencapsulated quercetin, which is also shown in **Figure 4**.

Absorbance spectra for unencapsulated quercetin showed a dramatic shift in peak absorbance to wavelengths that are characterized by degradation products of quercetin. Lack of absorbance at 375 nm during week 2, suggests that unencapsulated quercetin degraded completely in less than 2 weeks, see **Figure 4**. Furthermore, quercetin encapsulated F127 showed consistent peak wavelength over the period of 6 weeks, indicating that the encapsulated quercetin did not degrade at a significant level over that time. However, quercetin encapsulated in F68, when compared to the control, showed a delayed shift in peak absorbance, indicating that micelles composed of F68 slowed degradation compared to the control.

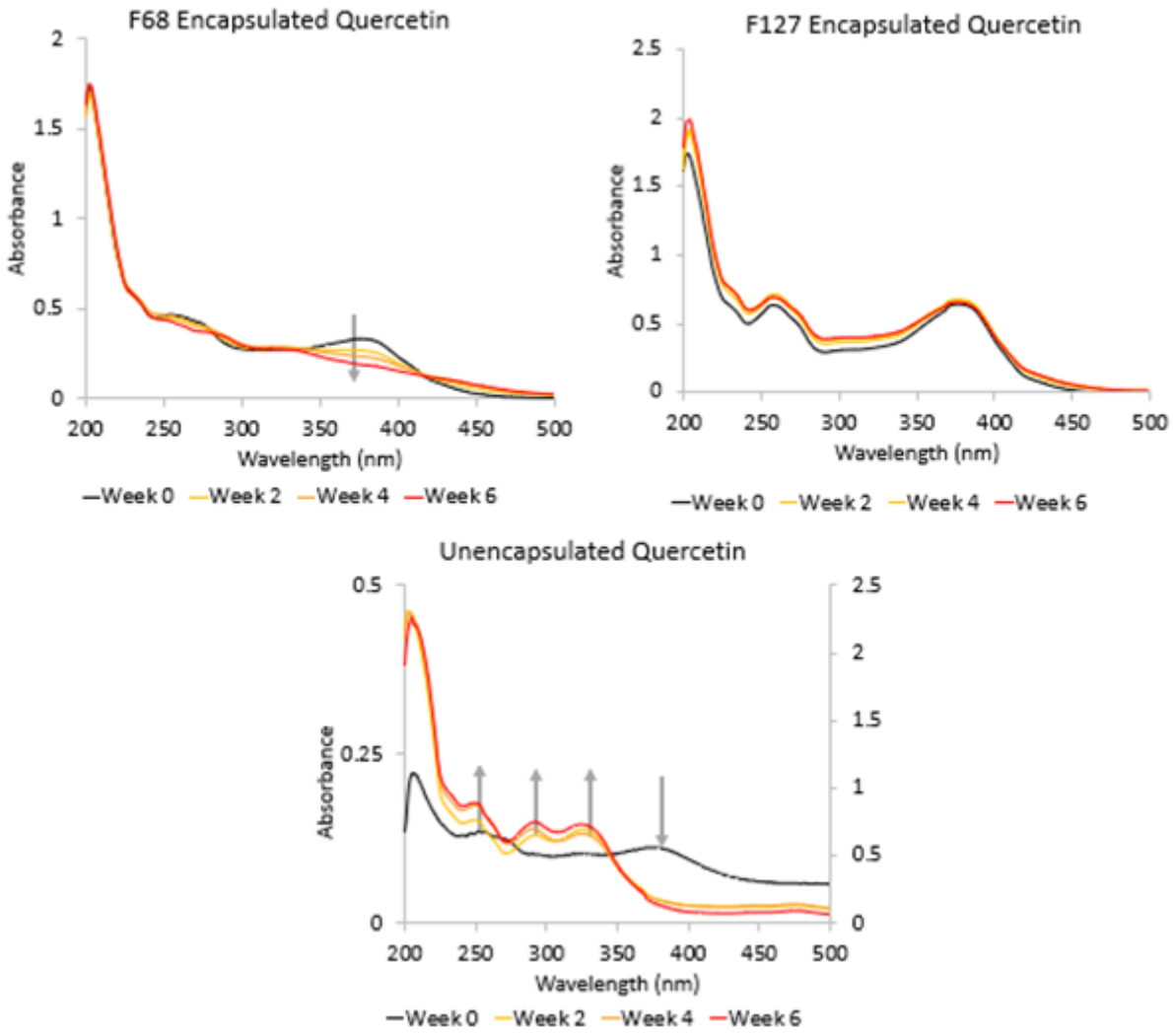

Figure 4: Absorbance spectra for encapsulated and unencapsulated quercetin over time. Arrows indicate increase or decrease in absorbance over time for corresponding wavelengths. Peaks at 250 and 375 nm indicate native quercetin, while peaks at 225, 293, and 335 nm indicate oxidation products of quercetin.

The exact reason for differences in degradation between F127 and F68 encapsulated quercetin is unknown. The copolymers F127 and F68 are made of identical subunits of polyethylene oxide (PEO) and polypropylene oxide (PPO), see **Figure 2**. However, they differ in the number of repeating PEO and PPO subunits. **Figure 5** depicts the scaled ratio of PEO and PPO subunits of each copolymer, note that the specific numbers of repeating subunits are also indicated.

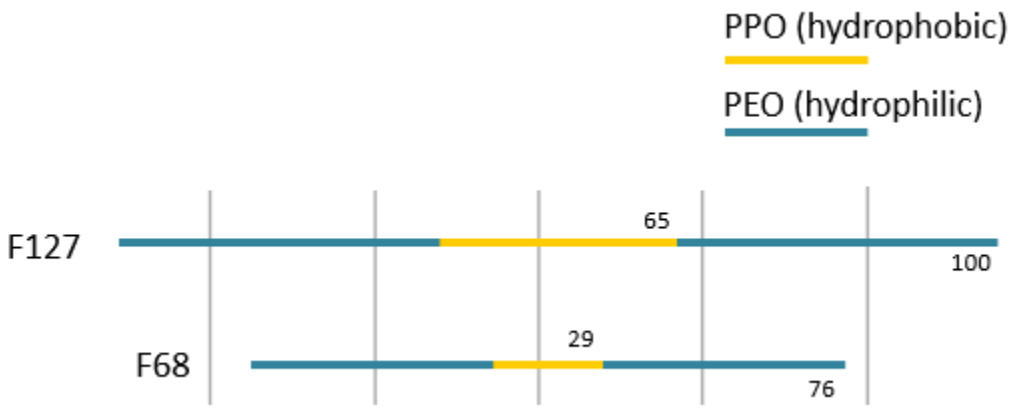

Figure 5: Pluronic copolymers F127 and F68 compared by number of PPO and PEO subunits. Polypropylene oxide (PPO) subunits represented in yellow and polyethylene oxide (PEO) subunits represented in blue. Image is to scale proportionally, and the numbers represent the number of repeating subunits in each corresponding block.

Differences in polymer structure and hydrophobicity are thought to be characteristics that effect how well a micelle protects quercetin from oxidation. The ratio of hydrophobic to hydrophilic portions predicts many of the behaviors of Pluronic micelles and was of particular interest moving forward. From these data, however, it was difficult to determine a cause. The data gave indications of oxidation, but greater data collection was needed to form statistics that would provide better certainty. The experiment took 6 weeks, which was thought to be problematic because solutes began to settle; yellow accumulation of quercetin began to be observed at the bottom of the storage flask. To remedy these problems, a different experiment was planned. The new experiment utilized a stronger oxidizing environment that would oxidize the micelles in more manageable time periods. After a few iterations and refining, more reliable data was produced with the new method. Below is a full description of that method and the resulting data.

Materials and Methods

Experimentation was carried out in two steps: the first was micelle formation and the second was oxidation testing. For the first step, 5 mixtures of Pluronic and quercetin were dissolved in 12.5 mL reagent grade acetone (Pharmco-AAPER). The 5 mixtures each contained 1 mg of quercetin dihydrate (Alfa Aesar) and 0.1 mmol of Pluronic F68 and F127 (BASF). While the total moles of Pluronic in each mixture remained constant at 0.1 mmol, the molar ratio of Pluronic F68 to F127 differed. **Table 1** below shows specific formulations for each sample.

Table 1: Table listing the mass of F68 and F127 added to each micelle formulation. Molar ratios of F68 to F127 varied, but total moles of polymer were kept constant across mixtures.

Mixture	Ratio of F68:F127 (mol:mol)	Mass of F68 (mg)	Mass of F127
1	1:0	840	0
2	5:1	700	208
3	1:1	420	625
4	1:5	140	1041
5	0:1	0	1250

A 10 mM final concentration of Pluronic was chosen to ensure that concentrations of Pluronic remained well above reported critical micelle concentration values throughout formulation and testing.

The solution was mixed without heat until both quercetin and the Pluronic had visibly solubilized. Mixtures were then transferred to 50 mL round bottom flasks. While larger flask sizes may be used, a 50 mL flask is recommended for easier resuspension later. Neck size may vary as well but must be selected to fit the rotary evaporator used. For this experiment, 24/40 taper neck flasks were used. Next, the bulk of acetone was evaporated off using a rotary evaporator (Heidolph). Rotation was set at 200 rpm and bath temperature was set at 40 C°. The bulk of acetone was evaporated until a viscous mass remained. Each flask was then covered loosely with aluminum foil to allow further evaporation and left overnight at room temperature to dry fully.

The thin film remaining in the flask was then resuspended in 10 mL of 1X phosphate buffer solution (PBS, MP Biomedicals LLC). Resuspension was done over the course of a day in a refrigerator with occasional repositioning and stirring. These steps are collectively referred to as a thin film hydration approach, which is abundantly found in literature for loading micelles [12]. In order to remove any non-encapsulated quercetin, each resuspension was then filtered using luer-lock syringes (Henke-Sass) and .22 µm hydrophilic polyvinyl difluoride filters (Thermo Fisher Scientific).

Next, each of the 5 samples were put in an oxidative environment and absorbance data was collected over time with a spectrophotometer (BioTek Synergy HTX). Pluronic F68 and F127 possess different loading efficiencies that are sensitive to various factors, so each sample was diluted with PBS until starting absorbance intensity at 375 nm was found to be approximately equal. By doing so, the mass of quercetin was made constant across all samples during testing. Then, 100 µL of each diluted sample was transferred to a clear ultraviolet sensitive 96 well plate (Corning). Next, 100 µL of 5% of hydrogen peroxide (Fisher Chemical) was added to each sample. Testing began immediately after the addition of hydrogen peroxide. A testing protocol was set to shake the samples for 1 minute, collect the spectral scans of absorbance of each sample immediately following, and wait 24 minutes before repeating. In total, absorbance data ranging from 200 nm to 550 nm were collected every 30 minutes for approximately 8 hrs.

To compare encapsulated quercetin to non-encapsulated quercetin, 1 mg of free quercetin was solubilized in 10 mL of 1.5 % ethanol and PBS. 100 µL of this mixture were combined with 100 µL of 5 % hydrogen peroxide and absorbance spectra were collected every 30 minutes for 8 hours following the same procedure used on the other samples.

Analysis

After these data were collected, they were analyzed in Excel. Each of the 6 samples generated 350 data points at each time point and each of the samples were read 17 times over the period of 8 hours. In all, there were 35,700 data points to sift through. One advantage this experiment had over previous studies was sterile procedure. The final data produced was done under sterile conditions, using sterile materials, and the testing occurred so soon after preparation that microbial influence can be discounted. During preliminary tests, sterile protocol was not strictly followed and time periods were long enough that microbial interference was an uncontrolled possibility. By decreasing testing times, quercetin falling out of solution was also ruled out definitively as an interfering factor.

Data analysis began with plotting the data to visually observe what was occurring. After working with the data in this way it became apparent that data for every half hour was only needed for the unencapsulated control. To the eye, it appeared that the peak signifying the presence of non-oxidized quercetin (375 nm) disappeared within about an hour. Absorbance spectra shown in **Figure 6** show a steady decrease in absorbance at this wavelength. It should be noted that the addition of hydrogen peroxide created interference below 300 nm, forcing the naturally occurring peak at 375 nm to be the only indicator of non-oxidized quercetin.

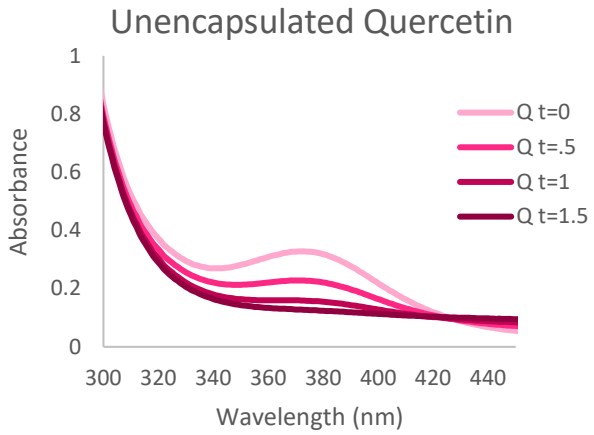

Figure 6: Absorbance spectrum for unencapsulated quercetin control. Darker shades of violet signify increasing time. Time steps were measuered every half hour.

Once an absorbance spectrum was determined for each of the samples, it also become apperent that comparing rates of oxidation would be aided by a less objective aproach than visually examining each spectra with the eye. Thus, a quantifiable approach was used to compare the data. When examining a decrease in peak intensity visually, the eye is comparing the intesity of a peak to the points around it. A flat horizontal spectra would be considered to have no peak intensity. These aspects can be described in a quantifiable manner by the slope. A simplified visual example shown in **Figure 7** illustrates this point. As illustrated, absorbance at two points

may be decreasing at different rates, but relative to one another the slope can be used to quantify how flat the spectra is becoming.

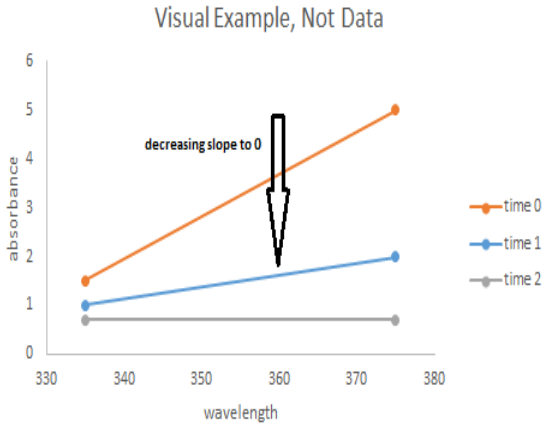

Figure 7: A simplified visual example of how slope can describe peak intensity.

It was desired that the slope of each spectra would be calculated between the most commonly occurring peak and valley of each initial spectra. A numerical derivative of the data points was then used to find these peaks and valleys. The most commonly occurring peak was found to occur at 375 nm, matching literature values for unoxidized quercetin. The most commonly occurring valley was found to occur at 335 nm, matching reported values for the first oxidation product of quercetin, o-quinone [11]. This correlation made sense because the derivatives were calculated with data gathered at the initial time point which was before oxidation could occur. This finding further supported assumptions that peroxide would not create interfering absorbance at higher wavelengths. These two wavelengths were then chosen to be the points at which a slope would be calculated for each spectrum at each time. **Figure 8** shows a plot of slope against time for the unencapsulated quercetin control.

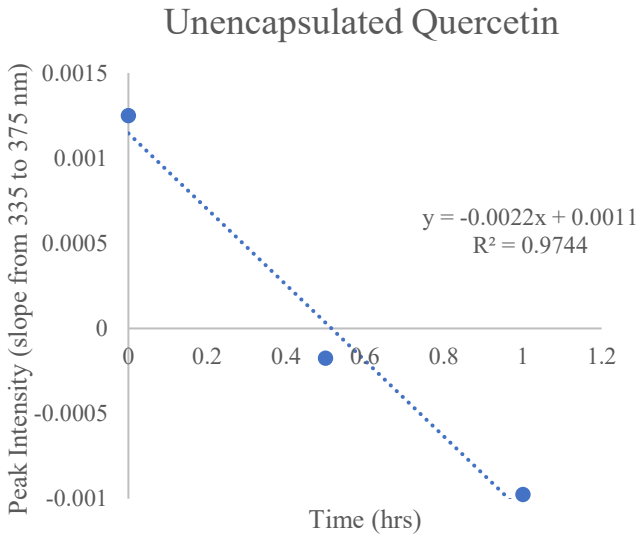

Figure 8: Plot of peak intensity measured as slope between 335nm and 375 nm.

The same procedure was then performed on the data for each of the samples. **Figure 9** shows the plotted data for quercetin loaded micelles made of pure F68. On the left is the graph showing each absorbance spectrum and on the right is a graph of peak intesity vs. time. Peak intensity is also defined as the slope between the points between 335 nm and 375 nm. **Figures 10**, **11**, **12**, and **13** show the the plotted data for quercetin loaded micelles made with a 5:1, 1:1, 1:5 and 0:1 molar ratios of F68:F127. The graphs on the left each show the absorbance spectrums at each time point. The graph on the right shows peak intensity vs time, with peak intensity defined as the slope between the points at 335 nm and 375 nm.

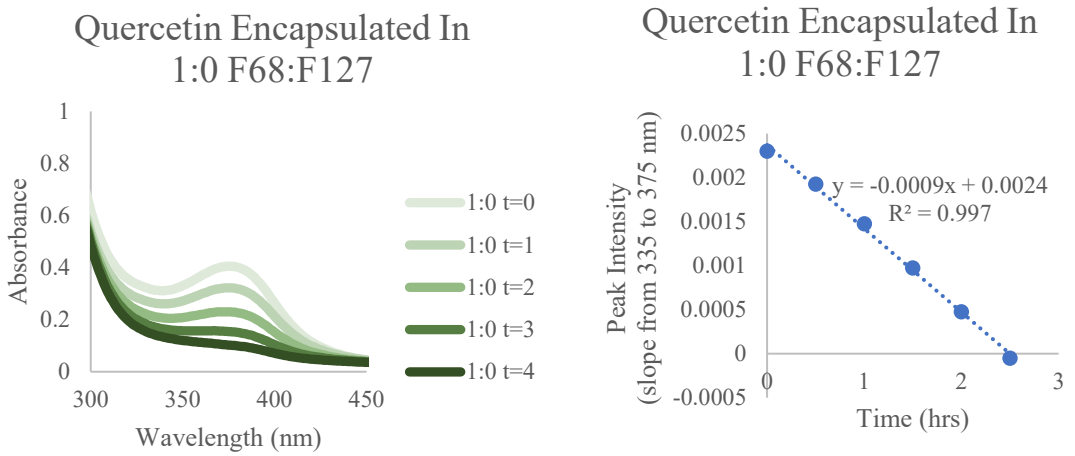

Figure 9: Left: plot of the absorbance spectrums at each time for quercetin that is encapsulated in pure F68. Right: graph of peak intensity measured as the slope between the points at 335 nm and 375 nm.

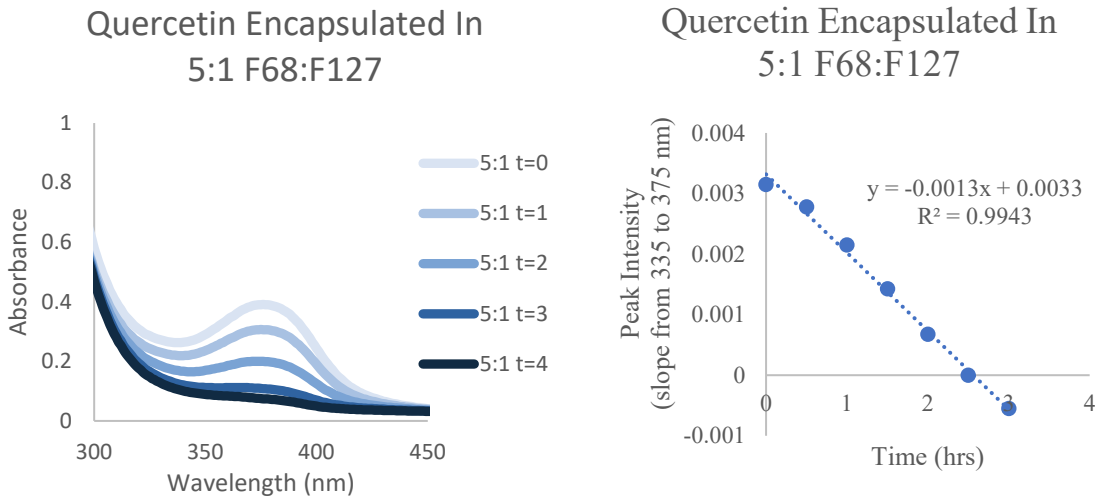

Figure 10: Left: plot of the absorbance spectrums at each time for quercetin that is encapsulated in a 5:1 molar ratio of F68:F127. Right: graph of peak intensity measured as the slope between the points at 335 nm and 375 nm.

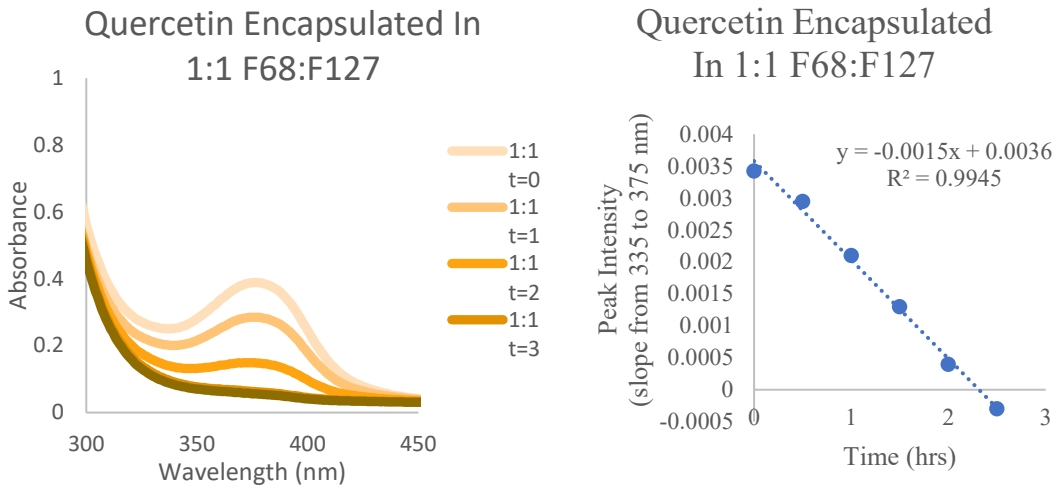

Figure 11: Left: plot of the absorbance spectrums at each time for quercetin that is encapsulated in a 1:1 molar ratio of F68:F127. Right: graph of peak intensity measured as the slope between the points at 335 nm and 375 nm.

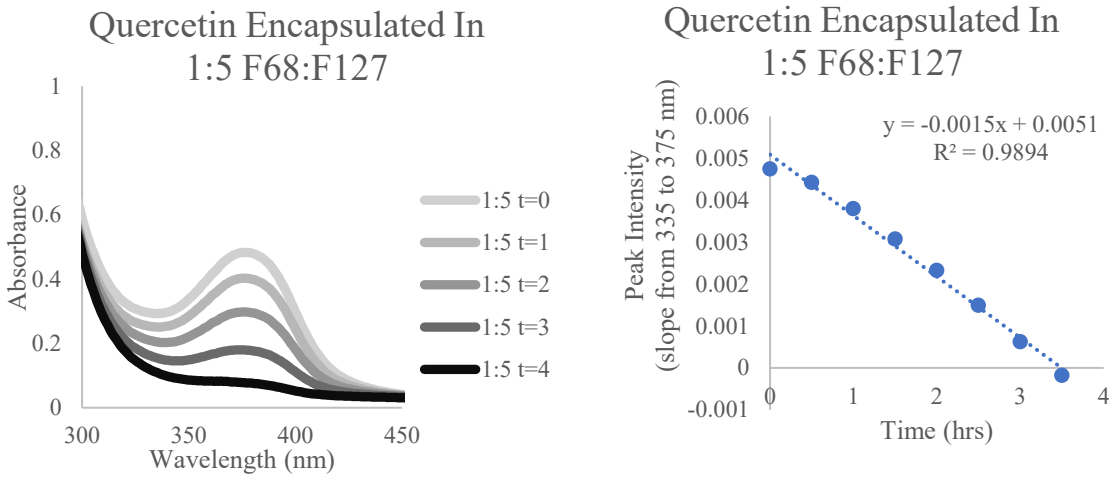

Figure 12: Left: plot of the absorbance spectrums at each time for quercetin that is encapsulated in a 1:5 molar ratio of F68:F127. Right: graph of peak intensity measured as the slope between the points at 335 nm and 375 nm.

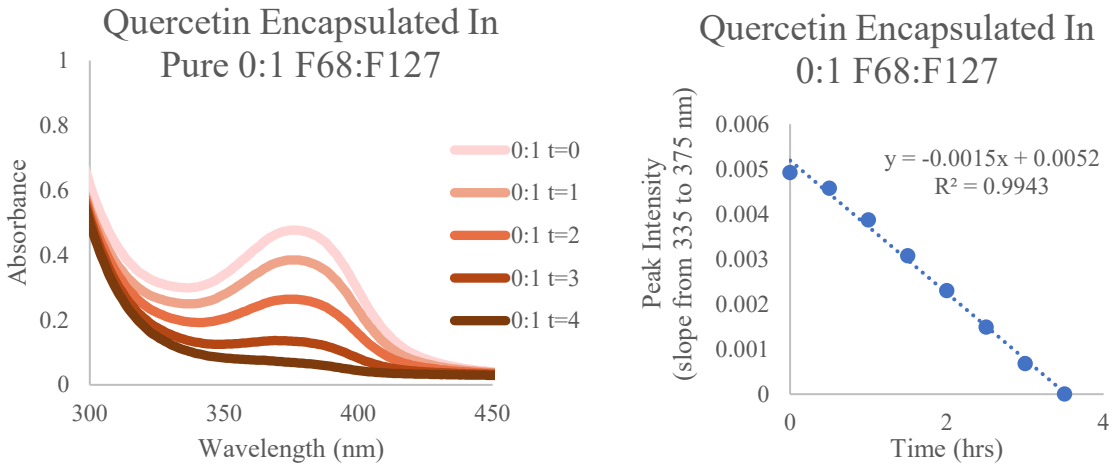

Figure 13: Left: plot of the absorbance spectrums at each time for quercetin that is encapsulated in pure F127. Right: graph of peak intensity measured as the slope between the points at 335 nm and 375 nm.

Visual analysis of the absorbance spectums in **Figures 9**, **10**, **11**, **12**, and **13** all show identical trends of decreasing absorbance at the 375 nm peak, which corresponds to the presence of non-oxidized quercetin. At 335 nm, absorbance also decreased. 335 nm cooresponds to the first oxidation product of quercetin o-quinone. Decrease at this point is thought to indicate further oxidation into more degraded products that produce absorbance at lower wavelengths. Due to interference from the addition of peroxide, this reasoning is only supported by the reaction chemistry of quercetin and reported literature values for these oxidation products. From the absorbance spectrums depicted on the left hand side of each of the figures, it is difficult to tell whether one particular micelle slowed oxidation more than another. However, the graphs on the right side of each of the figures show a clear decreasing trend of peak intensity. This trend closely fit a linear curve up until the the intensity dropped below zero, at which point the intensity stopped changing due to near complete oxidation of quercetin. The very first point on each of the peak intensity plots on the right of **Figures 9**, **10**, **11**, **12**, and **13** are each slightly lower than the line of best fit. Most likely this point fits the linear trend poorly due to the time it took to pipette peroxide into each of the samples and begin reading data. The time it took for peak intensity to reach 0 was of particular interest. A peak intensity of 0 means that the slope between 335 nm and 375 nm was equal to 0. If sampled at this point in time, the absorbance spectrums would be considered flat horizontal lines. The graphs on the right hand sides of **Figures 9**, **10**, **11**, **12**, and **13** do begin to show that some of the samples oxidized faster than others since peak intensity reached 0 faster.

In order to compare the time it took for each of the samples to reach 0 peak intensity, the line of best fit was used to calculate the point at which peak intensity crossed the x-axis. Then the time it took to reach this point was plotted for every sample and control on a bar graph for comparison, see **Figure 14**. When compared against unecapsulated quercetin (Q), all formulations of encapsulated quercetin does appear to offer some degree of oxidative protection. Micelle formulations with greater ratios of the polymer F27 appear to offer greater oxidative protection. These data may also give indication that somewhere between a 1:1 and 1:5 ratio of

F68:F127, there are changes in micelle properties that allow for better protection against oxidation.

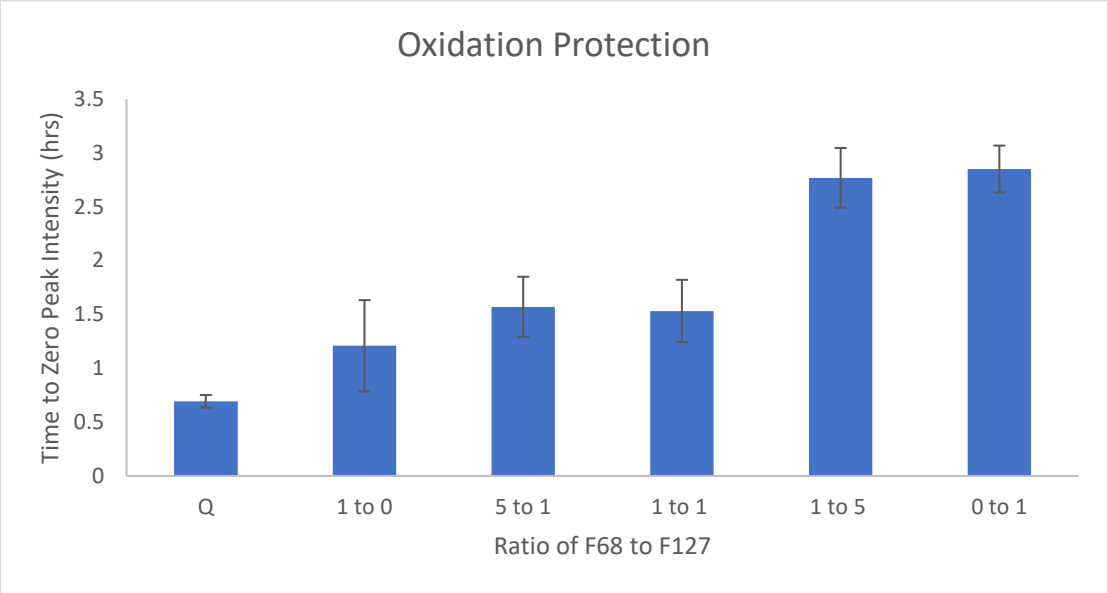

Figure 14: Comparison of oxidation protection offered by micelles made of differing molar ratios of F68:F127. Included is an unencapsulated quercetin control labeled 'Q'. On the y-axis is a measure of time it took for each sample to reach 0 peak intnsity, which has been defined as a slope of 0 between points at 335 nm and 375 nm. Standard error bars calculated using n = 3 and p = 0.05. Values for the bars labeled "Q" and "1 to 0" are statistically different.

It is likely that multiple factors could be contributing to faster oxidation times of non-encapsulated quercetin when compared to encapsulated quercetin. Differences in oxidation time among encapsulated samples are likely due to the properties of the copolymers that make up the micelles. Polymers F68 and F127 contain the same chemical subunits making it unlikely that chemical reactions between the polymer and quercetin are the cause for difference. Dynamic light scattering data indicates that particle size was about 20 nm. This suggests that micelles had not broken apart and were still intact. Although each sample has equivelant total amounts of quercetin, the loading efficiencies of each encapsulated sample differ. Samples with greater F127 content have fewer micelles but each contain more quercetin. Denser concentrations of quercetin would perhaps take longer for oxidative agents to penetrate. Greater loading efficiency is correlated to greater hydrophobic character or a decrease in HLB. Thus, further experimentation of pluronics of differing HLB could be done to further corroberate or disprove this hypothesis.

Future Work

In future work, processing time could be shortened by reducing the absorbance readings taken. Only collecting absorbance between wavelengths of 300nm and 500 nm would be necessary. Lower wavelengths have interference generated by the addition of peroxide and the absorbance curve flat lines just short of 500 nm. The testing period could have also been shortened by the addition of more concentrated peroxide, but it was desired to keep oxidation rates low enough that pipetting and testing delay would not generate significant error among samples. It may be

interesting to study molar ratios between 1:1 and 5:1 F68:F127 in order to better understand the point at which these two polymers begin to protect against oxidation better. Different testing methods of these samples would also be beneficial. Dynamic light scattering could be used to further study micelle size and presence. One chemical test that could be used to definitively prove oxidation was occurring would be to test for the release of a gaseous product using gas chromatography. Tests using copolymers of differing HLB would also be useful in further understanding the cause for differences in oxidation.

Conclusions

The data gathered in this experiment were very encouraging and gives reason to investigate further. The data that has been gathered suggest that quercetin encapsulated in Pluronic micelles made of either F68 or F127 has some degree of protection against oxidation. These data also suggest that there may be a F68:F127 molar ratio between 1:1 and 5:1 where micelle character is altered in a way to offer better oxidative protection. It is thought that HLB accounts for differences in oxidation time, because HLB determines the loading efficiency of a polymer's micelles. A denser and less distributed mass of quercetin in micelles could cause a greater amount of time to be needed for oxidative agents to penetrate and oxidize quercetin.

Reflective Writing / Word Count 1032

The opportunity to complete an honors capstone project is one I am very grateful for. When I was a freshman trying to decide if graduating with honors was worth my time, I remember reading capstone requirements and being filled with a sense of uncertainty. It seemed like a lot of work and I wasn't sure if it was going to be worth it to me. After fulfilling all the graduation requirements and completing a capstone project, I can say with certainty that the work has been worth the effort. My major is a demanding one that has required significant time and effort. Honors capstone has helped me convert that time and effort into several meaningful experiences that are a result of applying what I have learned while completing my degree. The great tragedy of education is the inevitable fading of information once learned and my honors capstone experience has cemented many concepts into my memory.

My honors capstone experience has doubled as my URCO grant experience. I have been lucky enough to receive funding for conducting research I was interested in. Thus, after some paperwork and signatures I have been able to also use the grant to complete capstone requirements. I would recommend other students to seek similar opportunities because motivating yourself to do research is much easier when you already have funding to back your efforts and value your time. I would caution that doing so requires a student to juggle differing requirements. Without detailed notes of specific requirements, it is easy to confuse what needs to be done for each experience.

When I began my work at the beginning of this school year, I had a lot planned out. I was able to acquire the lab materials needed, gain access to laboratory facilities and equipment, and begin experimentation. After initial experimentation, it became apparent that my experimental design would require changes. As with most research, unforeseen obstacles and problems began to take shape. For me, the polymers chosen for the experiment were a vital part of the experimental design. However, it was found that these polymers would be unsuitable for micelle formation without modification. Modification required changes to the original experimental design. Once a different design was determined, experimentation began anew. Refining an experimental design was one of the most important processes I learned while completing my capstone.

Once my experiment began generating data, it then became apparent that the methods I was using needed to be refined in order to generate more meaningful data. Measuring precisely, altering formulations, and perfecting analytical techniques all helped accomplished this. The optimization process was another valuable takeaway I received from my capstone experience. I hadn't realized how important it was to do research before I started my project. My data analysis and critical thinking skills also improved as a result of this project. I found that it is easier to work with data from class labs. The data is usually clean, you have classmates and TA's that can help you formulate conclusions, and the experiment is usually something that has already been done before. In contrast, my experience has shown me that real research is like solving a mystery game. There are many twists and turns, uncertainties, and unforeseen problems that need to be solved. A large part of my project was figuring out an effective way to analyze the data. I had to consider different testing methods and numerical analysis methods. Ultimately, I had to think critically and use basic logic to come up with a way to quantify differences I was seeing in the data. Then I had to apply mathematical knowledge I learned as a freshman to analyze the data. Such problem solving is simply not something needed in class guided lab work. The sheer

amount of data that can be generated in creative research is also a force to be reckoned with. I learned that a well-run experiment may generate data that has to be refined and replicated several times in order to be meaningful.

My major has been in biological engineering, but I had to explore many chemistry topics in greater depth than my major taught. At times, I had to resort to reading chemistry texts just to understand some of the procedures I was doing. I also had to develop a deeper vocabulary in chemistry in order to read applicable publications. These interdisciplinary efforts have proved to be very valuable and influenced my decision to finish a chemistry minor I was close to getting.

My current plan is to apply to medical school and become a doctor and my capstone experience has prepared me well. I believe that these experiences will help me gain acceptance to medical school and help me thrive while there. On the same note, my capstone has helped me develop a relationship with an academic mentor that is valuable to me personally and valuable in helping me achieve my future goals.

As COVID-19 began to make headlines, my work became challenged again. I suddenly felt a need to justify activity on campus and adapted to do as much as I could from home. Experimentation had to be conducted on campus, but I had to start communicating with my mentor through email. Virtual reports and presentations had to take the place of traditional meetings. I had planned to present at the undergraduate student research symposium, which turned out to be a drastically different experience. I still had to refine presentations skills but had to adapt and learn new methods of presenting research. On the positive side, COVID-19 did help give my work a sense of purpose in the larger community. The drug delivery method I had been researching has shown certain antiviral applications. Although not implicated in COVID-19 treatment, I began to realize that I was part of a greater whole, which was trying to solve the world's health challenges. I began to realize that the research process I had been learning is the same as the ones being used by those trying to find a treatment method for COVID-19. Realizing that I was not so different from these researchers is very comforting and has helped give me a sense of belonging among them.

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Author Biography

Mitchell Heap began his higher education at Northwest College where he pursued general studies from the year 2012 to 2013. After a year, he took a break from education to obtain a better understanding of what he wanted to do in life. In 2015, he continued his education at Utah State University and declared a major in Biological Engineering. While not an official designation on his transcript, he emphasized his study with classes that supported medical applications. This has led him to obtain a minor in chemistry along with his degree. He graduated from Utah State University with a B.S. in the spring of 2020. With degree in hand, he plans to take a gap year and attend medical school. While admission is not certain, he wishes to enter programs at either Texas A&M or Carle Illinois College of Medicine, which offer special programs for engineers seeking MD degrees. These programs emphasize the advance of technology in medicine and give students the tools to be both doctors and innovators.