A Thesis Presented to the Faculty of Alfred University

Investigation of Using and Constructing Nanoparticles to Fight Tuberculosis

By Robert Giancarlo

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Under the Supervision of:

Chair: **Dr. John D'Angelo**

Committee Members:

Dr. Geoffrey Lippa

Dr. David Marsh

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Introduction

In the 21st century, tuberculosis is a largely treatable and curable disease. Despite this, the lengthy treatments involved in fully ridding the body of tuberculosis often are riddled with embarrassing and/or dangerous side effects. Complications that arise from these treatments include (but are not limited to): yellow skin, the yellowing of eyes, nausea, liver damage (especially in those who already suffer from liver problems or alcohol addiction), and a lowering of the effectiveness of birth control pills.[1] Individuals taking medicine to treat diabetes have reported that their tuberculosis treatment interferes with their diabetic medication.[1] The main drug behind these unpleasant side effects is Rifampicin, which is one of the most commonly used drugs in curing tuberculosis.[1]

To combat these negative side effects, we have been working to develop nanoparticles designed to deliver Rifampicin to affected areas (mainly the lungs and respiratory tracts) more selectively. Doing so could reduce the amount of time a person with tuberculosis needs to take it. As a result, the chances of people developing harmful side effects decreases while still allowing for a thorough treatment of the illness.[2] In this study, several variables were explored including: different concentrations of the drug, the polymer used to form the nanoparticle, the solvents used to dissolve the drug, and multiple methods of preparation to produce the highest encapsulation efficiency and drug loading efficiency. Encapsulation efficiency measures the amount of drug recovered against the total initial drug amount while drug loading efficiency compares the amount of drug recovered to the amount of polymer used. Certain measurements were the most successful in terms of encapsulation and drug loading efficiency.

I completed my research at the University at Buffalo's (UB) Clinical and Translational Research Center (CTRC) under the guidance of doctors Jessica Reynolds and Hill Kutscher during the summer of 2021. After being rendered unable to participate in any research during the summer of 2020 due to the coronavirus pandemic, I was eager to take advantage of any research opportunities available to me in 2021. Prior to this experience, I had never worked in a lab setting on my own nor did I work with hazardous materials outside of heavily supervised lab classes at Alfred University. Given that I am pursuing a career as an osteopathic physician, I found it absolutely necessary to acquire valuable lab experience where I could work under the tutelage of accomplished scientists while developing a sense of independence in the lab and office. As many doctors are, I am interested in pursuing research as part of my career in the future. Learning the ins and outs of successful lab work will help me with the research aspect of my career. In addition, developing the sense of independence that lab work requires will assist me in making important decisions about my future patients' health needs. Like lab work, these decisions often require quick yet critical thinking, and my experience in the lab will help me make them correctly.

When I was searching for research opportunities in the spring of 2021, I found Dr. Reynolds' work with tuberculosis, an infectious disease, to be incredibly interesting. Researching infectious diseases, especially treatable ones, is a passion that I hold. This passion is highly personal, as I had a near fatal experience with meningitis as a young child. I was only four, however, memories of the hospital and how ill I felt are still raw. I wanted to work in a lab

where my efforts could potentially lead to alleviating one's suffering from a disease or the strenuous treatments associated with knocking the disease out.

Working in a lab setting helped me fine tune my teamworking skills. The CTRC offered me the opportunity to work with a diverse group of lab mates of various ethnic and linguistic backgrounds. It helped me grow even more appreciative of how teammates from distinct cultural environments can work together as one unit. As an athlete and piano player, I already had experienced that sports and music are shared languages. This internship taught me that science is no different; it is also a shared language. Whenever an instrument stopped working, someone made a procedural error, or simply forgot to wash the lab tools at the end of the day, we always had each other's backs and never reviled one another. During times when my team and I were hot on a potential breakthrough in our research that ended up hitting a wall, we worked on various methods to smash through said walls and advance our research.

Fine tuning the numerous methods I utilized to develop the nanoparticles was one of the most enthralling aspects of the research. To increase the encapsulation and drug-loading efficiencies of our nanoparticle, we varied the methods of creating them. The process caused me to feel as if I was creating a secret recipe, adjusting various nuances to the procedure in hopes of achieving a functional nanoparticle in a similar way to a chef tinkering with their recipe to perfection.

All in all, my teammates and I did not create a fully functional nanoparticle that could be used to treat illness. Despite this, the work we accomplished very well may assist someone in their pursuit of creating these particles which could then lead to a decrease in suffering to

those being treated for tuberculosis. Our work led to improvements in creating a functional size of the particle while not compromising encapsulation or drug loading efficiencies. In the future, I hope to be able to work on developing these nanoparticles further, even possibly creating a functional particle that could enter into the clinical trial pipeline. Then, I would explore if the nanoparticles did indeed decrease side effects that come with normal treatment.

I chose Dr. John D'Angelo to head my thesis committee. Throughout the 2021-22 academic year, we met roughly twice a month to discuss my progress on the paper. Given Dr. D'Angelo's knowledge of chemistry and my previous experiences with him in organic chemistry and the associated lab, this was a fruitful decision. Dr. D'Angelo continually provides valuable assistance whenever I needed it. Not only has he been a great asset to the scientific side of the paper, but he has helped massively with my navigation of Microsoft Word and Endnote. I typed this thesis on Word and I utilized Endnote for the citations throughout the paper. Although I had used Word in the past, I am admittedly weak in using its more advanced features. On the other hand, I had never used Endnote in my life nor had I ever linked it to Word. Under Dr. D'Angelo's guidance, this thesis gave me an opportunity to not only learn how to use Endnote but to become more of an "expert" in using Word. Many professional positions in the science world require an extensive knowledge on how to use Word. Given that I am attending the Lake Erie College of Osteopathic Medicine (LECOM) starting in July 2022 with the goal of becoming an osteopathic physician, learning how to use Word in full is an important skill that I will use plenty of times in medical school and my early career.

The other two members of my committee included Drs. Geoffrey Lippa and David Marsh. Both also have extensive backgrounds in biology and chemistry. While I have not met

with them throughout the project with the same frequency as I met with Dr. D'Angelo, both provided valuable support from their positions on the committee. I have previously worked with Dr. Lippa in an advising role, however, I did not have him as a professor until the spring 2022 semester where he served as my biochemistry professor. I have never worked with Dr. Marsh in any capacity other than writing this thesis. My previously limited experiences with both men proved to be no obstacle, as both were extraordinarily helpful in providing me with clear and concise feedback and constructive criticism. Despite this, I found it worthwhile to work with someone whom I had no previous experience working with on such an important project.

First, I would like to extend my thanks to Drs. Reynolds and Hill. Both took time out of their extremely busy schedule to allow someone with no previous experience gain priceless time and training in their lab. I would like to thank the CTRC as a whole, as the entire staff was incredibly warm and welcoming towards me. I wish to thank every single lab mate I worked with over the summer. Whether we performed work together or just happened to be working in the same lab, it was all a pleasure. I'd like to thank Drs. Marsh, Lippa, and D'Angelo for the time they have put in making sure every detail is up to par and meeting with me whenever I needed their advice. I want to thank Dr. Julianna Gray and Ms. Laurie Lounsberry Meehan for granting me the opportunity to be a member of the Alfred University Honors Program and to complete this thesis. Most importantly, I must thank my family, especially my mom and dad, for their continual support in any endeavor I take on.

Nanoparticles

Nanotechnology refers to the use of materials in the "nano" range, which is between 1 and 100 nm. The prefix "nano-" speaks of a measurement in the 10^{-9} range (e.g. one meter is equivalent to 1,000,000,000 nanometers). While nanoparticles have been (largely unknowingly) used since ancient times in pottery and medicine, modern use of nanoparticles in medicine can be traced back to the work of Richard Feynman in 1959.[3] His research initiated much of the modern studies on nanoparticles and nanotechnology in general. Since the turn of the 21st century, nanoparticles have been studied extensively and looked upon as a potentially revolutionary treatment for diseases such as cancer, tuberculosis, and HIV/AIDS.[4]

The tiny size, upgraded solubility, and drug delivery functions of nanoparticles leave them with the ability to be applied in a wide variety of biomedical settings.[5] Use of nanoparticles is set to expand greatly in treating these devastating diseases throughout the current century. At a minimum, nanoparticles consist of two components with at least one of them being pharmaceutically active. In some cases, however, the active drug itself forms the particle.[6] During my research, my team and I formed the nanoparticle using Poly Lactic-co-Glycolic Acid (PLGA) as the polymer encapsulating the drug, Rifampicin. In the future, this could lead to more specific drug targeting and delivery, a reduction in the harmful side effects of Rifampicin while holding up its therapeutic uses, and greater safety to the patient receiving the treatment.[6] Nanoparticles use their specific drug targeting ability to reduce side effects, as free drug can reach and harm other organs.[7] For instance, Rifampicin is proven to occasionally damage the liver in tuberculosis patients.[1] Nanoparticle delivery would effectively eliminate the chance of this serious side effect of tuberculosis treatment occurring. The liver would still need

to metabolize and remove Rifampicin from the body, however, nanoparticles could allow the patient to take the lower dose of drug that he needs. As a result, there is a much smaller amount of Rifampicin that needs to be metabolized by the liver, leaving it less vulnerable to harm.

A major reason why nanoparticles show such promise for tuberculosis treatment is their precision targeting ability. Nanoparticles make the drugs more available at the sites of infection by protecting them from breakdown prior to reaching the site.[8] This results in a greater uptake of the nanoparticles into infected sites.[8]

PLGA

Since the 1990s, PLGA has emerged as one of the leading polymer candidates utilized in the formation of nanoparticles for drug delivery. PLGA is biocompatible and biodegradable, exhibits an ability to be finetuned for mechanical [medicinal] usage, and is an FDA approved polymer.[9] Fine tuning PLGA refers to its ability to form nanoparticles at different concentrations. Due to it not being toxic and its biodegradability, PLGA is already used in numerous FDA-approved treatments.[9] PLGA's favorable degradation allows for a sustained drug delivery release that does not require surgery.[9] Drug concentration can be manipulated when used with PLGA to attain a desired release interval of drug that will effectively combat the illness in the body while not dosing too much and causing injurious side effects.[10] The amount of drug released when entrapped by a PLGA polymer has yet to be fine-tuned to perfection, however, it shows great promise for future treatments.[9] For instance, PLGA is currently being used to form nanoparticles to deliver docetaxel more efficiently to tumors in cancer patients.[11] When I produced the nanoparticles, I mixed the polymer and dissolved drug with various

concentrations of Polyvinyl Alcohol (PVA) to prevent excessive clumping of drug that would otherwise be entrapped by the polymer. Excessive clumping would render the nanoparticle useless. Drug load plays a large role in its release rate with a PLGA polymer. Nanoparticles with a high amount of drug produce a large initial "burst" of drug release when activated due to the high drug to polymer ratio.[2]

Rifampicin

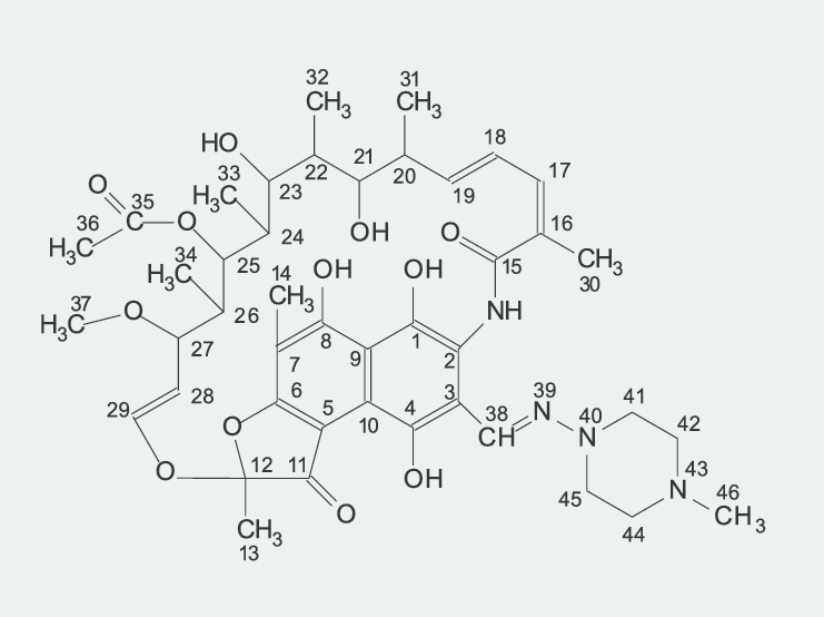

Figure 1: Chemical structure of Rifampicin[12]

Throughout my work at the lab, Rifampicin was the drug I encapsulated with PLGA, forming the nanoparticle. Rifampicin is the International Nonproprietary Name (INN) and the British Approved Name (BAN) of the drug while Rifampin is the United States Adopted Name (USAN). Its therapeutic effects against tuberculosis were discovered in the late 1950s. Rifampicin is a semi synthetic antibiotic that is derived from *Amycolatopsis rifamycinica*, a bacterium that can be found in soil.[7] By the late 1960s, Rifampicin was in full use treating patients suffering from drug resistant chronic pulmonary tuberculosis.[7] Rifampicin's mechanism

of action involves attacking transcription in tuberculosis bacteria.[13] The drug inhibits RNA polymerase in the bacteria.[13] Rifampicin binds to RNA polymerase inside the DNA/RNA channel, causing a direct blockage of the elongating RNA.[13] This ends up killing the bacteria, ridding patients of the disease.

Giving patients Rifampicin for tuberculosis does not come without risk for embarrassing, even dangerous side effects. Patients have experienced a yellowing of eyes and skin, large purple and/or red blotches on their skin, diarrhea, liver damage (exacerbated in those who suffer from alcohol addiction), and tooth discoloration.[1] Along with that, Rifampicin is known to interact with other medications negatively, decreasing the effectiveness of both birth control pills and diabetes medication.[1] In addition, the six month period a tuberculosis patient has to take Rifampicin and the fact that it has now been used in tuberculosis treatment for nearly six decades has allowed for tuberculosis bacteria to mutate into much more resistant strains.[7] These mutated versions of tuberculosis are a growing global health concern, especially in less developed countries. Stronger strains of tuberculosis allow it to efflux the medicine.[10] Drug efflux refers to the bacteria's, or any cell for that matter, ability to pump out unwanted substances. In the case of tuberculosis, the unwanted substance is Rifampicin meant to kill the bacterium. Nanoparticle systems enhance the therapeutic effectiveness and minimize the undesirable side effects that may come along with Rifampicin.[2]

Although it is commonly used to treat tuberculosis, Rifampicin is not the only drug used to treat tuberculosis. Rifapentine is another drug that is used in fighting tuberculosis. Studies have shown that once or twice weekly rifapentine treatment and daily rifampicin treatment have similar efficiency and safety for the treatment of HIV negative pulmonary tuberculosis

patients.[14] While cutting the treatment period of rifampicin to twice or thrice a week did not produce any negative effects, cutting the treatment of rifapentine to once a week or less increased the risk of bacteriological relapse.[14] Additionally, rifapentine may increase bacterial resistance to rifampicin in HIV positive tuberculosis patients.[14] Rifampicin can also be used in conjunction with other drugs to treat tuberculosis. Compared to a six-to-nine-month period treating patients under 15 solely with isoniazid, another prominent tuberculosis drug, an isoniazid and rifampicin treatment program over three months found more success in treating patients.[15] This success is in no small part due to increased compliance and completion rates of treatment when juxtaposed with the former treatment plan, as many patients tend to stop taking their drugs before the treatment period is complete.[15] Along with that, this conjoined treatment with isoniazid was not shown to produce a drug resistant form of tuberculosis bacteria.[15]

Tuberculosis

Even though treatment for tuberculosis has evolved significantly since the latter half of the 20th century, the disease is still a major public health crisis, especially in lower income countries. Treatments in the early to mid 20th century involved a variety of antibiotics that took up to 24 months to fully cure the disease.[4] Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis*.[16] It is an infectious disease, and the bacteria spread through the air when an infected individual coughs, speaks, or sings.[16] When a person is first infected, the bacteria settle in the lungs and start to reproduce rapidly.[16] Eventually, the illness can spread to the throat, kidneys, spine, and brain, and it can result in death if left untreated.[16] The main symptoms of tuberculosis infection include: a heavy cough lasting three or more weeks, chest

pains, and coughing up blood and/or phlegm.[16] Other less frequent yet possibly severe symptoms include: persistent fatigue, unexpected weight loss, decreased appetite, chills, fever, and night sweats.[16]

Certain diseases, specifically those that lead to one having a compromised immune system, increase the likelihood of one contracting tuberculosis and/or make it much harder to treat effectively. People who already suffer from either HIV or diabetes are at a much higher risk of serious illness from tuberculosis coinfection. HIV positive persons are 18 times more likely than people without the immunosuppressant disease to contract tuberculosis.[17] In 2019 alone, 1.4 million people worldwide died from tuberculosis, nearly 15% of which were coinfected with HIV.[17] Those who are coinfected with HIV and tuberculosis risk a much swifter progression of tuberculosis, often causing death much faster than someone without HIV.[17] Additionally, diabetes results in one having a compromised immune system, leading to more severe effects and an increased likelihood of death from tuberculosis.[17] Lifestyle factors can also increase the chance of tuberculosis infection and illness. Cigarette smoking and alcohol abuse were at least somewhat enhancing to 720,000 and 700,000 tuberculosis cases, respectively, worldwide in 2019.[17] While tobacco and alcohol misuse do not actually cause tuberculosis, they weaken one's lungs and immune systems, leaving them more vulnerable to infection. Smoking and excessive alcohol consumption increase tuberculosis disease risk by 1.6 and 3.3 times, respectively.[17]

While high and even some middle-income countries have made great strides in treating and containing tuberculosis spread, the developing countries sadly lag behind in their fight against the sickness. The vast majority of new tuberculosis cases occur in these oft marginalized

regions of the world. This disease is one of the top 10 leading causes of death in the world.[18] In 2019, the World Health Organization's (WHO) South-East Asian region contributed to 44% of the world's tuberculosis cases while the African region made up 28% of cases.[17] Both regions are developing areas. Overall, 87% of the planet's cases occurred in 30 high tuberculosis burdened countries, all of which are poor to middle income nations.[17] A wide number of aggravating factors contribute to this disproportionate level of infection and spread. Many residents of developing countries often struggle to find reliable access to enough food and clean water. As a result, they are chronically malnourished, leaving them much more vulnerable to tuberculosis.[17] Prisons in impoverished nations tend to face overcrowding, and prisoners generally do not receive adequate medical care, endangering them to tuberculosis at a greater rate than free men and women.[17] Refugees fleeing their home countries often get stuck in camps inside poorer nations. These camps are crowded and living conditions are unsatisfactory, leaving refugees more at risk to contracting tuberculosis.[18] People in these countries, especially in rural areas, tend to lack access to decent medical care, as treatments can be too expensive, hospitals too overcrowded, and/or are just too far away from a medical center with reasonable standards of care. Up to half of potential tuberculosis patients in developing countries do not come back to the health center after their initial visit due to economic hardship or transportation issues.[19]Tuberculosis infection follows one of two major patterns: primary tuberculosis and secondary tuberculosis.[20] Primary tuberculosis usually occurs in children, and the initial focus of infection is beneath the pleura of the lung.[20] Secondary tuberculosis generally is seen in adults, and it is often either a reactivation of a previous infection or a reinfection, mainly when the patient's health is declining.[20] Usually, the upper lung lobes are

most affected, and cavities in the lung can form as a result of the infection.[20] While the infection begins in the lungs, tuberculosis can spread throughout the body, wreaking havoc along the way. For instance, skeletal tuberculosis involves the degradation of the knees, hips, thoracic, and lumbar vertebrae, resulting in the weakening and necrosis of bone tissue.[20] Another major problem that arises from infection is genital tract tuberculosis. In women, the bacteria spreads through the fallopian tubes, causing inflammation, irregular menstrual bleeding, and even rendering the victim infertile.[20] Men whose tuberculosis progresses to this point face a hardening of the prostate and epididymis as well as infertility if it remains untreated.[20] One of the most dangerous places tuberculosis can spread to is the heart, causing cardiac tuberculosis. This development of the disease involves inflammation of the heart, causing hemorrhaging which can lead to death.[20] If tuberculosis patients do not receive treatment, around one third of those with the disease will die within two years while another third will die within five. Sadly, progression of untreated tuberculosis is a painful, drawn-out process. This process is known as caseation, a word that finds its roots in the Latin word for cheese.[21] Caseation was chosen because the slow yet painstaking progressive inflammation of tuberculosis destroys human tissue and leaves a thick, cheesy substance in its place.[21]

Advances in nanoparticles could revolutionize treatments, especially in impoverished areas and amongst those who are incarcerated. Nanoparticles have treated other bacterial infections with great success. For instance, nanoparticles are used in heart and dental implants to prevent *e Coli* and other harmful bacteria from forming on them.[22] Incorporating nanoparticles into tuberculosis treatment will likely find similar success.

Materials and Methods

Procedure

1. Decide which drug and polymer concentrations to test
2. Measure out appropriate amounts of drug and polymer into a 1.7 ml volume Eppendorf tube
3. Mix the drug and appropriate amount of solvent. Sonicate and vortex solution to ensure the solvent dissolves the drug
4. Mix this solution with the polymer. Sonicate and vortex solution to ensure the solvent dissolves the polymer.
5. Add 1 ml of PVA for every 500 ul of the previous solution. Concentration (%) of PVA relative to the water it was mixed in depended on the batch. Mix together
6. Spin solution in centrifuge for 10 minutes at 16,100 g
7. Take 100 ul sample from the supernatant after spinning. Mix with 1 ml of deionized water in a cuvette tube. Measure the average size of the nanoparticles using Horiba™ SZ-100
8. Record particle size. Remove remaining supernatant from Eppendorf tube leaving just the pellet.
9. Resuspend the pellet in deionized water.
10. Mix 1 part of the resuspended pellet with 3 parts acetonitrile
11. Take 10 ul of the mixture from step 10 and apply to Thermo Scientific™ NanoDrop™ 2000 to find the absorbance of the solution at a wavelength of 338 nm
12. Input absorbance to the standard curve's line of best fit (see image below) to find the concentration of drug per ml of solution

13. Use the concentration to measure drug loading and encapsulation efficiency. Record data

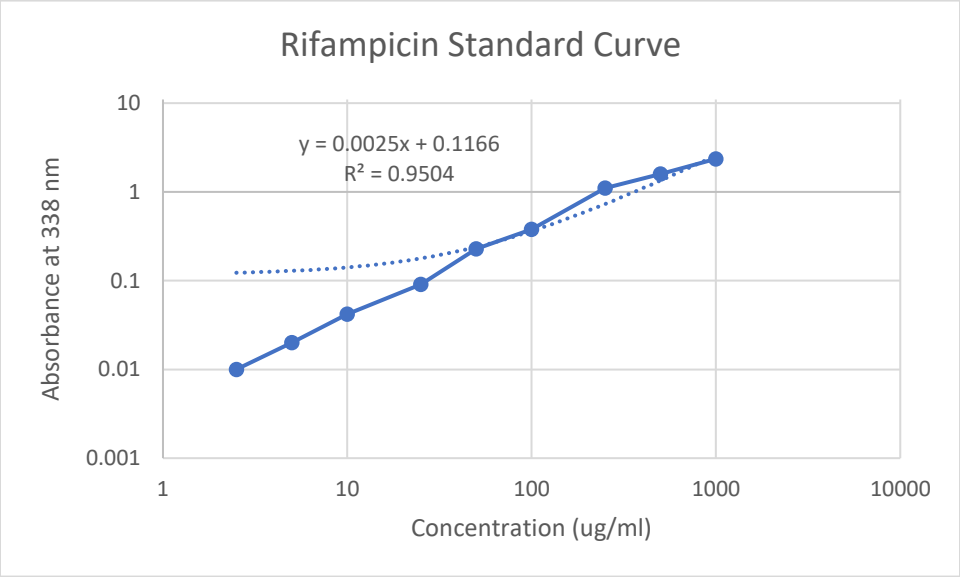

Figure 2: Standard curve

Deciding on a concentration for both the drug (Rifampicin) and the polymer (PLGA) was the main variable in the trials. These were the main factors in trying for a better drug loading and encapsulation efficiency. Another aspect of the procedure that my team and I worked with was the concentration of PVA. The purpose of adding PVA is to prevent excessive amounts of drug from clumping together while still allowing for sizeable particles to form. An ideal particle size is approximately 230 to 300 nm. The sizes of the particles in each batch varied, but the particle analyzer gave us an average of these sizes. Spinning the batch in the centrifuge allowed for most of the particles to congregate at the bottom of the Eppendorf tube which would then let me resuspend them in water for absorbance measurements. After that, I would use the standard curve line of best fit to find the final concentration of drug from the absorbance. The

line of best fit came from a standard curve that measured the concentration of Rifampicin in ug/ml against absorbance measurements. The absorbance was measured at 338 nm.

Data

Batch	Filter	Drug concentration	Polymer concentration	Amount of Final Solution	Drug Solvent	PVA %	Spin Setting/Stir Method
Batch 1	yes	15 mg/ml	25 mg/ml	500 ul	95% acetone/5% DMSO	0.5%	Added solution dropwise to vortexing PVA then spun for 10 minutes at 4,000 g
Batch 2	yes	15 mg/ml	25 mg/ml	500 ul	95% acetone/5% DMSO	1%	Added solution dropwise to vortexing PVA then spun for 10 minutes at 4,000 g
Batch 3	yes	15 mg/ml	25 mg/ml	500 ul	95% acetone/5% DMSO	4%	Added solution dropwise to vortexing PVA then spun for 10 minutes at 4,000 g
Batch 4	yes	30 mg/ml	50 mg/ml	500 ul	100% DMSO	4%	Added solution dropwise to vortexing PVA then spun for 10 minutes at 4,000 g

Batch 5	yes	20 mg/ml	30 mg/ml	500 ul	100% DMSO	4%	Added solution dropwise to vortexing PVA then spun for 10 minutes at 4,000 g
Batch 6	yes	20 mg/ml	30 mg/ml	500 ul	100% DMSO	4%	Spun solution for 10 minutes at 16,100 g and washed pellet with water
Batch 7	no	20 mg/ml	30 mg/ml	500 ul	100% DMSO	4%	Spun solution for 10 minutes at 16,100 g, didn't wash pellet

Figure 3: Compositions of Nanoparticle Batches

Results and Conclusion

Table I: Results of Each Batch

Batch	**Absorbance (wavelength 338 nm)**	**Concentration of the drug**	**Drug Loading Efficiency**	**Encapsulation Efficiency**	**Particle Size**
1	0.743	688.6 ug/ml	1.1%	1.8%	273.5 nm
2	0.795	736.2 ug/ml	1.2%	2.0%	273.5 nm
3	1.038	958.6 ug/ml	1.5%	2.6%	304.9 nm
4	1.058	977 ug/ml	0.8%	1.3%	262.1 nm
5	0.966	892.7 ug/ml	1.2%	1.8%	226.2 nm
6	negligible	N/A	N/A	N/A	260 nm
7	1.982	3,360 ug/ml	4.5%	6.7%	238.8 nm

Images

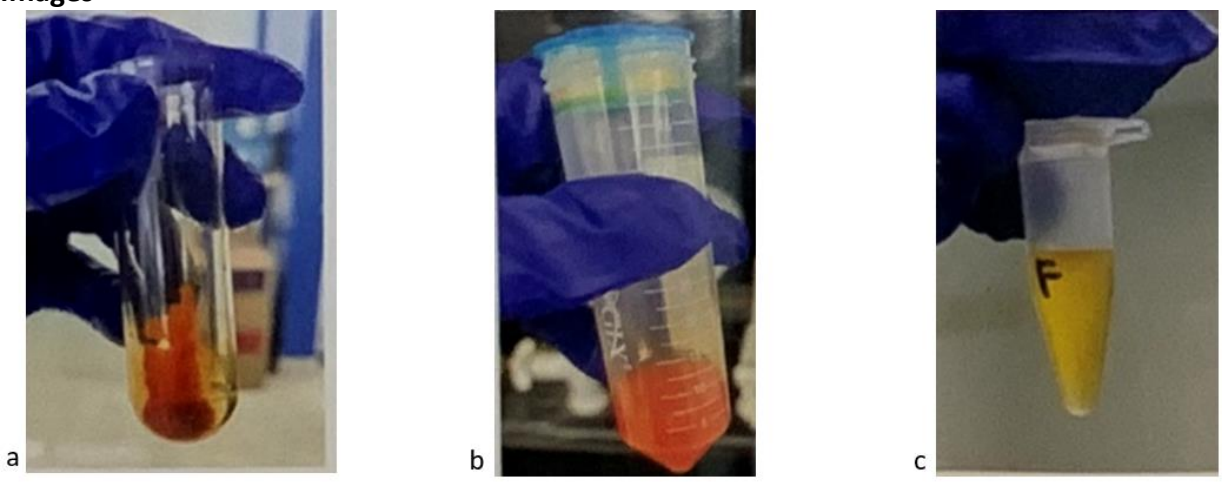

Figure 4: a. pellet after centrifuging; b. supernatant being filtered; c. resuspended pellet in deionized water and acetonitrile

In terms of drug-loading and encapsulation efficiency, batch 7 produced the best results at 4.5 and 6.7 percent, respectively. I found that a significant reason for this was the fact that I stopped using a filter for the supernatant during that batch. While the filter did block useless drug clumps from interfering with the nanoparticle, it seems to have filtered out drug from entering the particle in the first place. Along with that, combining the dissovled drug/polymer solution with a 4 percent PVA mixture proved to be effective at preventing drug from clumping up outside the particle while still allowing said particles to form. Utilizing a 100 percent DMSO solution to dissolve the PLGA polymer and Rifampicin drug played a great role in the success of the trial. Rather than using 95 percent acetone and only 5 percent DMSO, using only the latter solvent helped dissolve the particle effectively while allowing for it to reform during the centrifuging of the solution. It also allowed for the ability to dissolve a higher concentration of drug and polymer. For the first five batches, I tried to add the drug/polymer solution dropwise to the PVA while the PVA was being vortexed. Using this method proved to be ineffective at

increasing the drug-loading and encapsulation efficiencies. I then hypothesized that spinning the supernatant at a higher speed would help with this issue. The first time I tried this, in batch 6, I decided to watch the pellet with distilled water to remove any excessive solvent. This proved to be a faulty idea, as the procedure left the particles with negligible absorbance. In batch 7, I removed this step, and it produced the best results of any trial.

In the future, I believe my work could play a role in developing nanoparticles for tuberculosis treatment. Figuring out ways to improve the drug loading and encapsulation efficiencies in the nanoparticle are crucial in its development. Once these methods are found out, nanoparticles could be used to treat tuberculosis with greater efficiency. As for myself, this past summer I spent in the lab not only increased my scientific skills, but my critical thinking skills in general. Having to constantly make minute changes to procedures forced me to keep my mind open to new ideas. Additionally, the experience taught me that small victories in science should be enjoyed. Making small amounts of progress after trying several different methods were common over the summer. Even more common was these victories eventually hitting a wall and having to start again. These experiences improved my resiliency, and that although progress in science may be small and fleeting at times, eventually it may reap benefits in a significant way.

References

(1) https://www.webmd.com/drugs/2/drug-6049/rifampin/details/list-sideeffects **2021**.

(2) Jiří Trousil, Z. S., Nils-Jørgen K. Dal, Dmytro Rak, Rafał Konefał, Ewa Pavlova, Jana Matějková, Dušan Cmarko, Pavla Kubíčková, Oto Pavliš, Tomáš Urbánek, Marián Sedlák, Federico Fenaroli, Ivan Raška, Petr Štěpánek, and Martin Hrubý *Biomacromolecules* **2019**, *20*, 1798.

(3) Shanmugam, P. H. a. S. *International Journal of Current Microbiology and Applied Sciences* **2015**, *4*, 379.

(4) Iseman, M. D. *European Respiratory Journal* **2002**, *20*, 87.

(5) Jr., R. S. a. J. W. L. *Exp Mol Pathol* **2009**, *86*, 215.

(6) Jong, P. J. B. a. W. H. D. *Int J Nanomedicine* **2008**, *3*, 133.

(7) Melanie Grobbelaar, G. E. L., Samantha L. Sampson, Paul D. van Helden, Peter R. Donald, Robin M. Warren *Infection, Genetics, and Evolution* **2019**, *74*.

(8) D'Souza, S. D., Admire 2018; Vol. 2022.

(9) Makadia, H. K.; Siegel, S. J. *Polymers (Basel)* **2011**, *3*, 1377.

(10) Priyanka Padwal, R. B., Sarika Mehra *Langmuir* **2014**, *30*, 15.

(11) Pedram Rafiei, A. H. *International Journal of Nanomedicine* **2017**, *12*, 935.

(12) Oliveira, A. G. d. *ResearchGate* **2021**.

(13) Drew, R. H. *UpToDate* **2021**. https://www.uptodate.com/contents/rifamycins-rifampin-rifabutin-rifapentine#:~:text=Mechanism%20of%20action%20%E2%80%94%20Rifampin%20is%20thought%20to,thought%20to%20be%20concentration%20related%20%5B%204%20%5D.

(14) Gao, X.-F. L., J. Wang, Z-W. Li, Y-P. *The International Journal of Tuberculosis and Lung Disease* **2009**, *13*, 810.

(15) Assefa, Y. A., Yalemzewod. Woldeyohannes, Solomon. Hamada, Yohhei. Getahun, Haileyesus. *European Respiratory Journal* **2018**, *52*.

(16) https://www.cdc.gov/tb/topic/basics/default.htm, last accessed on 10/6/21.

(17) https://www.who.int/news-room/fact-sheets/detail/tuberculosis **2020**.

(18) https://www.international.gc.ca/world-monde/issues_development-enjeux_developpement/global_health-sante_mondiale/tuberculosis-tuberculose.aspx?lang=eng **2021**.

(19) Tokar, S. https://www.ucsf.edu/news/2012/10/104340/tracking-and-treating-tuberculosis-developing-countries **2012**.

(20) *University of Utah* **2021**. https://webpath.med.utah.edu/TUTORIAL/MTB/MTB.html

(21) *Medecins Sans Frontieres (Doctors Without Borders)* **2000**. https://www.msf.org/how-tb-kills

(22) Wang, L. H., Chen. Shao, Longquan. *Int J Nanomedicine* **2017**, *12*, 1227.