Novel Infusion Procedure for Antimicrobial Fabric Generation

by

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A thesis

presented to the University of Waterloo

in fulfilment of the

thesis requirement for the degree of

Master of Applied Science

in

Mechanical and Mechatronics Engineering

Waterloo, Ontario, Canada, 2023

Author Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Antimicrobial fabrics are fabrics that can kill or slow down the growth of microorganisms that contact the fabric. It is a massive field that is developing due to the sheer variety of fabrics that are possible. Antimicrobial fabrics contain active (antimicrobial compounds) such as organic molecules, metals, or antimicrobial peptides. The variety in antimicrobial fabrics comes from the ability to combine and use the antimicrobial compounds to create a fabric finish with unique properties. Therefore, various properties can be explored to determine the best usage case for the fabric.

I created a novel antimicrobial infusion fabric nicknamed “Beryl” together with its production process. In collaboration with engineers Microbonds Inc., Markham, Canada. This fabric is based on a standard woven cotton subject to a pre-infusion (pretreatment) of a solution containing an organic acid, a polymer, and a surfactant. This fabric is then subject to a main infusion with various ppm levels of cupric (Cu^{2+}) ions. These successive infusions impart antimicrobial properties to the fabric.

Described in this thesis are the physical and antimicrobial properties of this new fabric. The fabric is compared to a previous proprietary fabric from Microbonds Inc, named AC5, as well as a control fabric treated with copper sulfate. Characterization begins with the fabric feel and odor, which remained unchanged. Color change was measured using a PICO paint matcher device, which showed that the Beryl fabric maintains the original color of the fabric better than the AC5 fabric. The copper content of the fabric was tested, showing that the Beryl had typically between 0.357 and 6.43 mg of copper per gram of fabric. Fabric morphology was determined using a scanning electron microscope, which showed the Beryl fabric contained a thin coating of copper on the surface of the cotton fibers, with only a few small copper deposits at some exposed locations on the fabric fibers. In contrast, the AC5 fabric showed a discontinuous, seemingly brittle but thick layer of copper, with larger deposits at multiple locations on the surface of the fabric. The thin layer of copper for the Beryl was confirmed using energy dispersive x-ray spectroscopy, despite it not being visible in the backscatter electron detector image. Antimicrobial efficiency tests were done before and after multiple wash cycles to show laundering resistance of the antimicrobial coatings. It was found that the fabric was perfectly efficient at 0 washes, while after 30 washes, the fabric’s efficiency dropped to 96 % for the 1000 ppm copper infused Beryl fabric. The antimicrobial properties of the pre-infusion were also tested showing the fabric was effective at 0 washes. Plates incubated for longer suggest Beryl is bacteriostatic at 30 washes rather than bactericidal. Contact time efficiency tests showed that the fabric reduced bacterial load within 45 seconds, with 100 % efficiency reached after 5 minutes of contact time with the fabric.

After laundering, the efficiency for the Beryl decreased below that of the AC5 fabric. However, by increasing the copper ion content of the cupric ion infusion bath, the wash resistance of the fabric can be expected to increase to maintain 100 % efficiency, so it retains effectiveness after larger numbers of washes. Furthermore, usage of the Beryl process is safer, making it more sustainable than the AC5 due to no hazardous gaseous products being created in the Beryl process.

Acknowledgements

I would like to thank Dr. Michael Mayer for his continued kindness and guidance throughout this entire project. Dr. William Anderson for his assistance in the biological aspect of the lab and giving access to lab equipment. I would like to thank Microbonds Inc., for providing the resources to develop this fabric. Robert Lyn and John Persic for access to resources for testing. Finally, I would like to thank the following colleagues: Nicholas Kam for continuous support and assistance in developing the testing protocols and troubleshooting, Cheryl Xing for helping develop this fabric and being the other namesake, Carson Huang, Alan Alicock, Jennise Luu, Jazmine Catly, Monica Kutnik, and Christina Zeng for assisting with testing and their encouragement.

Table of Contents

List of Figures

Figures marked with a * were created using the BioRender.com service.

List of Tables

List of Abbreviations

NPs – Nanoparticles

ROS – Reactive Oxygen Species

EDTA – Ethylenediaminetetraacetic acid

SEM – Scanning Electron Microscope

SE – Secondary Electron

BSE – Backscattered Electron

ESEM – Environmental Scanning Electron Microscope

GDD – Gaseous Detecting Device

EDS – Energy Dispersive X-Ray Spectroscopy

Chapter 1: Introduction

This chapter contains background information regarding antimicrobial fabrics, Beryl fabric creation process and microbiology background that is used throughout the thesis.

1.1 Motivation for Antimicrobial Coatings: Beryl and AC5

Antimicrobial fabrics are articles of cloth that can kill or slow down the growth of various microorganisms that come into contact with the surface of the cloth. These fabrics have gained a lot of traction in recent years due to the advent of the COVID-19 pandemic, with the most obvious usage being the elimination of pathogenic microorganisms, thus preventing the spread of disease. However, there are more usage cases even further beyond this. Antimicrobial fabrics can promote hygiene [1], where they can stop the onset of body odor or even acne through the antimicrobial action [2]. Furthermore, antimicrobial activity can also increase the lifespan of the fabric material [3]. These traits make antimicrobial fabrics highly desirable as they provide many benefits to the wearer. Presented within is the development of a novel antimicrobial fabric coating process nicknamed the Beryl process, and its comparison to a previous proprietary process named the AC5 procedure. These fabrics vary in their creation method, but both use cupric ions as the primary antimicrobial agent. An image of Beryl fabric before and after infusion is shown in Fig. 1. Beryl is the primary focus for this thesis, as its creation was driven by shortcomings of the AC5 fabric.

A) B)

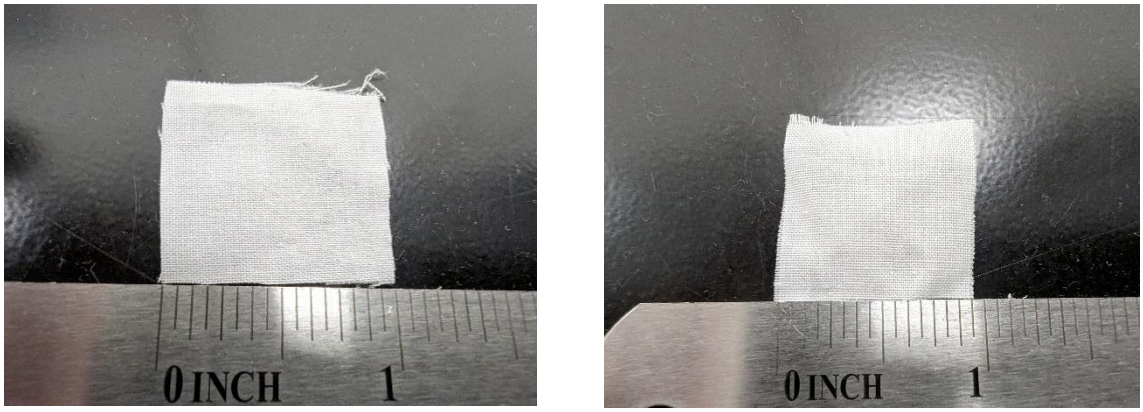


Figure #1: A) Pre-infusion fabric and B) post infusion Beryl fabric (1000 ppm copper infusion).

1.2 Diversity & Limitations of Antimicrobial Fabrics

One of the most useful aspects of antimicrobial fabrics is the diversity in the antimicrobial material used. Several fabrics have already been created that make use of antimicrobial agents such as: metals and metal nanoparticles (NPs), naturally derived organic compounds, and other miscellaneous treatments such as crosslinked antimicrobial proteins or hypochlorite [1]. This diversity provides countless possibilities for creating fabrics, as each of these materials can be used individually or combined with one another to alter the specific antimicrobial properties of a material. This diversity, however, is also a challenge, as each material can bring its own drawbacks. The drawbacks can range from severe, such as toxicity in the case of NPs [2] or can be minor such as a color change (discoloration). An example of color change is shown in Fig. 2, where a black adult sock infused with AC5 experienced an undesirable color change.

Figure #2: Discolored antimicrobial fabric that was black originally.

Another major issue relates to the actual antimicrobial activity, where these fabrics may be more efficient at killing one type of organism such as bacteria, while failing to be effective against another such as fungi. Furthermore, the fabric may also be more effective in inhibiting one type of bacteria versus another, such as the antimicrobial material chitosan, where the minimum inhibitory concentration is 100 ppm for *Escherichia coli* but 2000 ppm for *Salmonella enterica* [4]. Another consideration is the fabric base material. Materials such as polyester may not bind a specific antimicrobial material as well as cellulose can, due to the molecular structure of the polymer that makes up the fabric. Fabrics also vary in how they classify with the difference being the efficiency of a cidal or static fabric. Cidal fabrics are fabrics that kill the microorganisms, preventing their growth (bacteri**cidal**), while static fabrics inhibit the growth of the microorganism, meaning the organism can still be viable (bacterio**static**). Although both prevent the spread of infection, it is more desirable to eliminate the bacteria rather than slow the growth. A final major issue is washing. With antimicrobial fabrics the coatings may be sensitive to a typical laundering cycle, so the coatings can weaken over time, depending on how well they are integrated or bound to the fabric.

By combining different coating materials, these barriers may be overcome to provide a fabric with suitable antimicrobial properties while minimizing the drawbacks.

1.3 Current Antimicrobial Products Review

Since Beryl and AC5 are primarily copper based fabrics, comparisons can be made to other commercially available copper-based fabrics, which are widely available in many stores. Brands such as Tommie Copper™ already offer several copper infused fabrics, with the claims that they are anti odor. One of the clear benefits of infusion technology is the ability to apply to a multitude of fabrics. Tommie Copper™ offers a wide range of clothing options such as compression gear, shirts, pants, and arm sleeves that all contain copper to impart the antimicrobial activities. Other brands such as Swicofil™, a yarn and fiber manufacturer, have copper impregnated fabrics available for sale, for the same antimicrobial purposes. One issue does arise with the antimicrobial claims, which is that there is often no easily available published data to back up such claims. For instance, Tommie Copper™ claimed that their copper fabrics could reduce pain from conditions such as fibromyalgia and arthritis, but there was a lack of evidence for these claims, resulting in a settlement being paid [5]. In another case, Swicofil™ currently advertises their fabric with the ability to kill SARS-COV-2 viral particles [6] References to a peer-reviewed article are used, but the article involves usage of a pure copper surface, not a copper coated fabric [6]. This shows the distinct lack of clarity when advertising copper infused fabrics, which can tarnish consumer trust. Data is essential to prove the efficacy of the fabric and whether it is viable for use and can kill microorganisms.

Outside of commercial use, copper infusion fabric is commonly used to create antimicrobial fabrics. The most popular form of copper that is currently being implemented is copper oxide nanoparticles (CuO NPs), which are easily generated, while also being effective at killing microbes [7]. The creation process involves the generation of the CuO NPs through mixing a source of cupric ions such as copper sulfate or copper acetate, with a reducing agent such as sodium hydroxide. In addition, plant extracts have also been used as the reducing agent, which is a greener technique for the synthesis [8]. In one study, Sharma et al., used extracts from *Tinospora cardifolia* to act as the reducing agent for NP synthesis, as the plant was known to possess health benefits [8]. Other natural compounds have also combined with CuO NPs, such as alginates, which holds on to the CuO NPs to elicit antimicrobial activity [9]. Although NPs are widely used and efficient at killing microbes, they do have a drawback. Toxicity of NPs is a concern, especially in the face of safety and commercialization. Studies show that NPs are generally regarded as safe, because they cannot cross the skin barrier [7], however they do show slight cytotoxic capabilities in hepatocytes [7]. With respect to the skin the toxicity of NPs is unclear as some studies report no toxicity to human skin cells [7], while others report slight toxicity to skin cells after exposure [9]. Overall, CuO NP usage is common for imparting an antimicrobial effect, with various techniques used to embed the nanoparticles in the fabric. These methods can be altered to be greener, incorporating naturally derived compounds to add to the health benefits of the fabric. However, it is still unclear whether NP usage is completely safe for this application.

1.4 Goals

The first goal of this thesis is to develop an improved procedure for the infusion of cotton fabrics with a copper based antimicrobial coating. The second goal is to compare properties of the fabric generated by the novel procedure with fabric generated by pre-existing procedures. Comparisons were planned through the analysis of physical properties of the fabric, as well as the antimicrobial properties of the fabric.

Chapter 2: Experimental Methods and Backgrounds

This chapter contains the methods used to characterize the fabric as well as the background required for using these methods. The older infusion process is also described.

2.1 AC5 & Copper Sulfate Fabric Infusion Procedure

Unless otherwise specified, the fabric used in all the experiments is ISO adjacent cotton, purchased from Testfabrics.com (Product #1602001), which was shown in Fig. 1A. The fabric had various infusion procedures performed to impart antimicrobial activity to the fabric.

The control infusion procedure involves the usage of copper sulfate as the source of copper. The creation method is illustrated in Fig. 3.

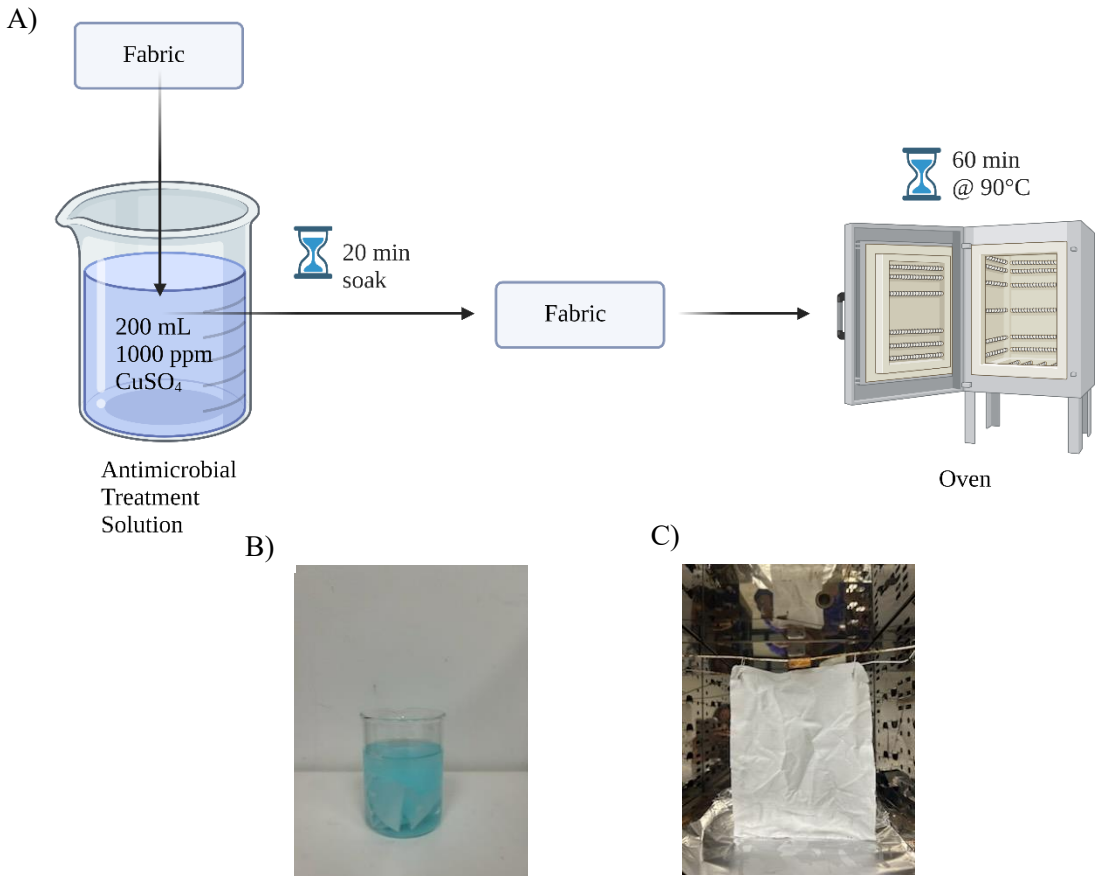


Figure #3: Illustration of A) copper sulfate infusion procedure and B) demonstration of fabric infusion and C) Fabric drying in the oven.

The copper sulfate bath was created using 11.79 g of copper sulfate pentahydrate dissolved in 300 mL of deionized water. This made a stock 10,000 ppm solution of copper sulfate. A volume of 20 mL of this solution was diluted in 180 mL of deionized water to create the treatment solution.

AC5 fabric uses a similar process but incorporates a proprietary source of cupric ions. This method involves the creation of a novel treatment bath. The process is outlined in Fig. 4.

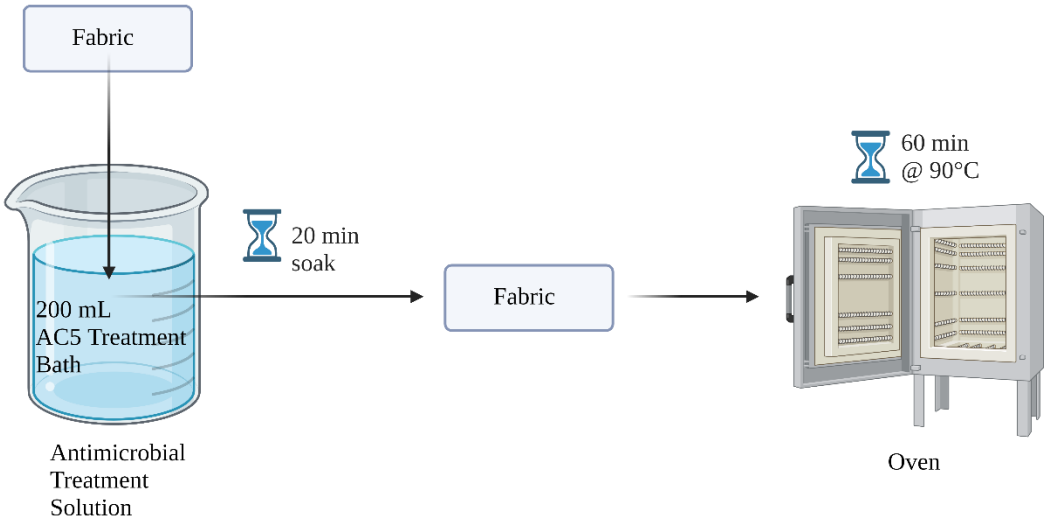


Figure #4: AC5 fabric creation procedure.

Additionally, the AC5 bath uses multiple concentrations of copper ions for the creation procedure. It uses 100 ppm, 1000 ppm and 10,000 ppm cupric ion concentrations. Creation of these solutions follow the same dilution scheme, where 10,000 ppm stock solution was created, and 20 mL was diluted in 180 mL of water to create the lower dilutions. A drawback of the AC5 process is the treatment creates hazardous vapors, which requires active ventilation to remove the vapors, reducing the sustainability of the production process.

The general fabric procedure was as follows:

1. Fabrics were cut to 17x21 cm rectangles using a rotary cutter.
2. Fabrics were fully submerged in the treatment bath and left to sit for 20 minutes.
3. Fabrics were suspended using pins on a rack within the oven and dried at 90 ºC for 1 hour.

2.2 Fabric Washing Procedure

Laundering resistance is an important characteristic of antimicrobial fabrics, as it is not uncommon for the fabrics to lose efficacy after being washed. Therefore, fabrics were tested at two different levels of laundering: 0 washes, directly after infusion, or 30 washes. To do this, fabrics were cut in half after infusion, where one half was kept as the 0-wash fabric, while the other half was subjected to washing. The washing cycle consisted of 30 cycles of the fabric being placed in a Haier HLP23E washer by itself. The fabric went through a standard cold water wash cycle with approximately 100 mL of 1% Tide™ original detergent being used each cycle. Cycles were run repeatedly. The fabrics were then dried in a standard dryer after going through the 30 wash cycles. To test laundering resistance, 30 washes was selected as other methods use it as a standard [7] and 30 washes represents approximately 9 months to a year of washing a fabric assuming washing every week to every other week.

2.3 Importance of Fabric Physical Characteristics

When creating any sort of fabric, considerations of its physical properties can be made. From the micro scale to the macro these properties are what dictate how the fabric may behave and how effective it is. Furthermore, physical properties play a role for any prospective buyer of the fabric. For example, if a fabric has an unpleasant odor, or feels coarse to the touch, a consumer would be hesitant to purchase it. These problems drive the creation of newer fabrics to meet the demands of consumers while still providing antimicrobial benefits.

2.4: Fabric Color Change

2.4.1: Introduction to Light and Color Perception

Color is an important characteristic for a consumer. Especially in the case of infusion, a customer would want to retain the color of the fabric they want infused. To analyze this, color theory is discussed. To understand color, the theory behind light is explained. Light is a form of electromagnetic radiation, which has one important property with relation to colour: the wavelength of the light. The wavelength is related to how much energy the light has. Humans can perceive a very small subsection of light dubbed the visible light spectrum, shown in Fig. 5.

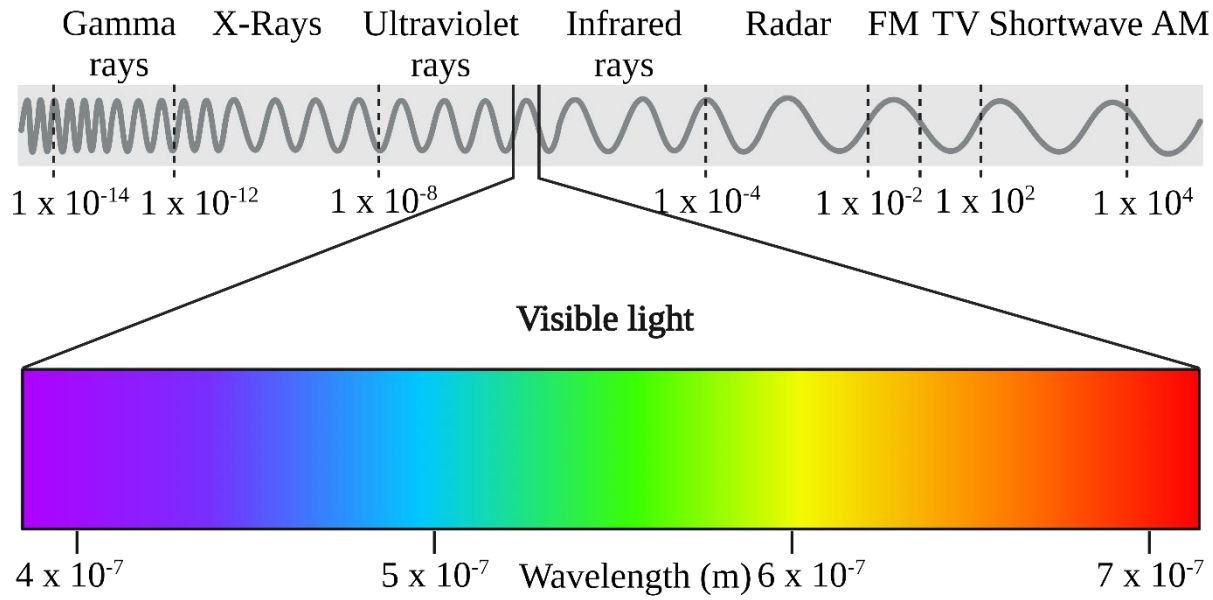


Figure #5: Visible light spectrum diagram.

Human eyes are organized such that interpretations of color are done using specialized cells called cone cells [10]. Cone cells have 3 different variations, which are specialized at absorbing certain wavelengths, specifically the red, green, and blue areas in the visible light spectrum [10]. Cone cells can transmit an electrical signal to the brain when the corresponding wavelength of light hits the cell, where this signal can vary in strength [10]. By having different intensities of signals from the different cone cells, the brain can interpret color beyond red, green, and blue based on the ratio between these signals. Absorption of blue, green, and red color by the three cone cell types is shown in Fig. 6.

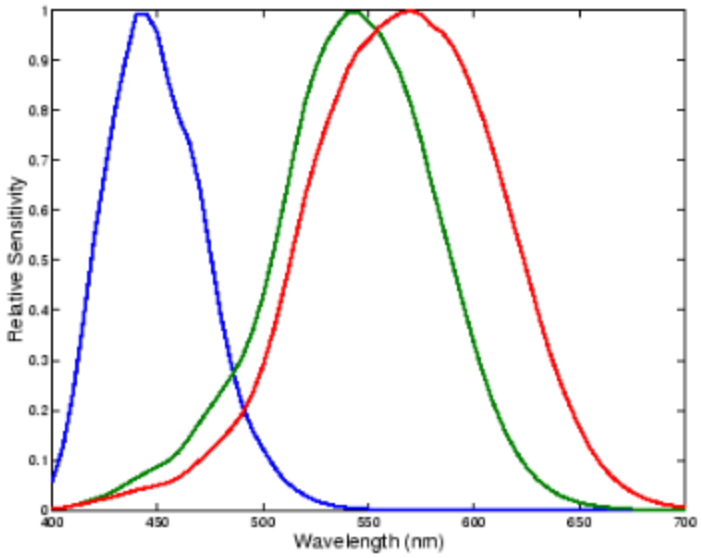


Figure #6: Cone cell light absorption wavelength diagram. Figure taken from [10].

To summarize, color is perceived through cells within the eyes absorbing light with a specific wavelength. This means color is determined through what wavelength of light hits the detecting cells within the eye. This can be applied to the fabric. In the case of color, what matters is the outbound ray of light that reflects off the fabric. So, depending on what wavelengths of light the fabrics absorb, color can shift; an example being absorbing red and blue light would reflect green light, making the object appear green.

2.4.2: RGB Color Space as a Quantifier

Now using the theory behind color, the method to quantify can be introduced. This is done using a color space called RGB. A color space is a 3D representation of a color using 3 values [11]. In this thesis RGB is used, a color space that has values for red, green, and blue ranging from 0 to 255 [12]. This method is commonly used for digital interpretation of color because it mimics that of the human eye described in Section 2.4.1. The RGB space is shown graphically in Fig. 7.

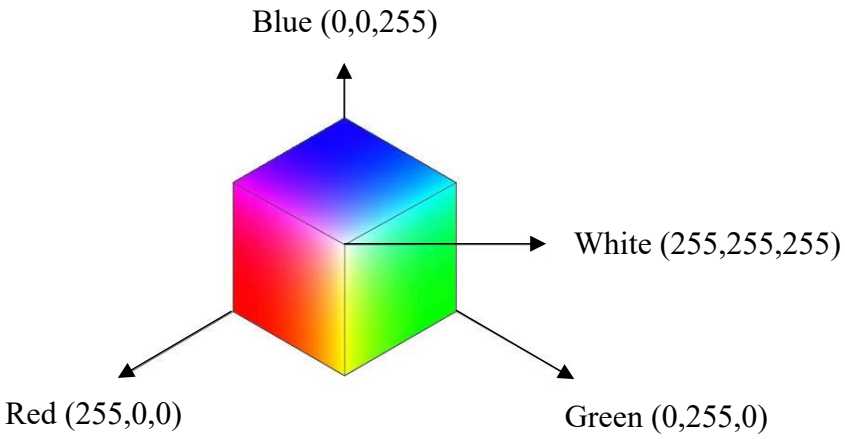


Figure #7: RGB color space diagram. Figure adapted from [13].

Using this color space, values can be assigned to a color for the purpose of visualizing colors and color changes.

2.4.3 Color Change Method

To obtain color measurements the Palette™ Pico Portable Colour Reader was used. This device allows the user to measure the color of a surface and gives the user the RGB values of the color. The device scanner is calibrated using a white surface that comes with the scanner. The device and its usage are shown in Fig. 8.

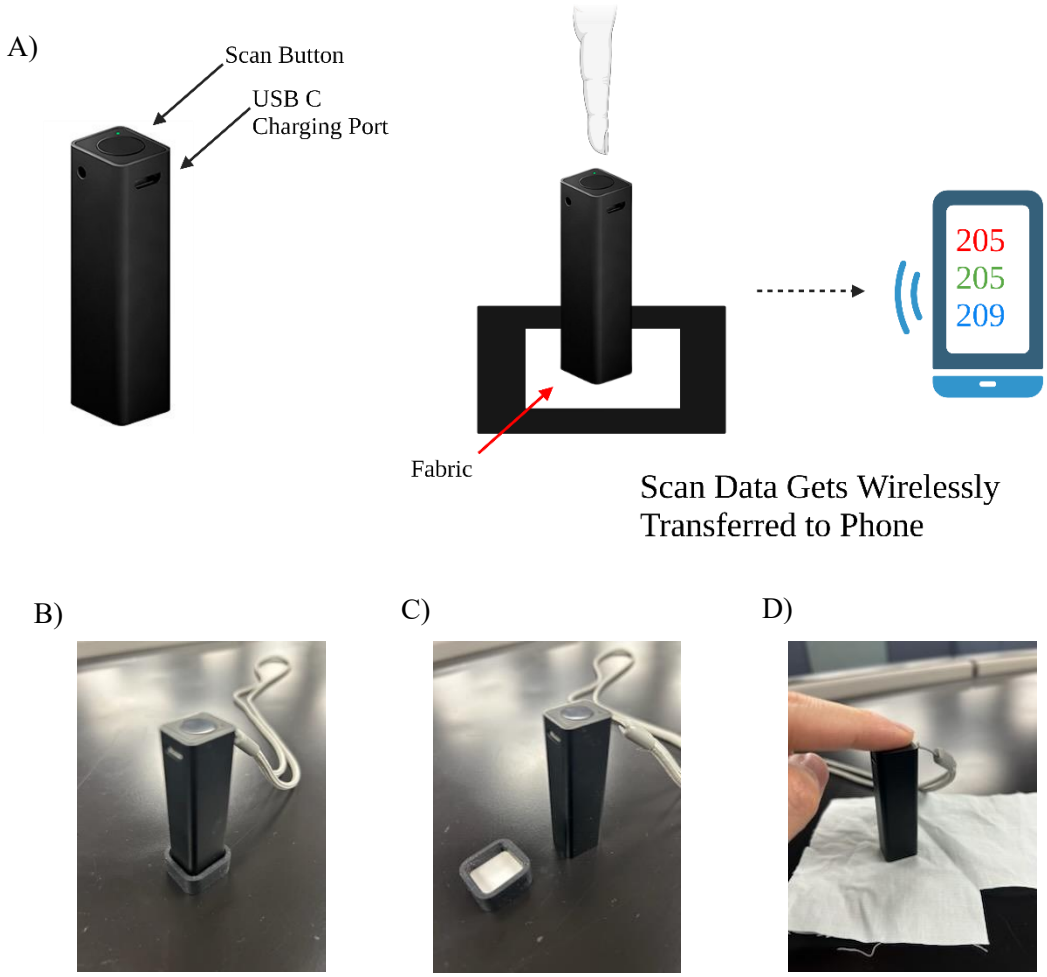


Figure #8: Illustration of A) Pico scanner schematic and usage diagram and B) scanner with calibration cap on and C) off, with D) usage of the device demonstrated.

Using this scanner, the RGB values of the antimicrobial fabrics were taken at 3 random spots. Fabrics were placed on a matte black surface for taking values.

2.5 Fabric Copper Content

2.5.1 Complexometric Titration Theory

To determine copper content within the fabric complexometric titration was used. This method uses a colored indicator called murexide [14]. This type of titration is called complexometric titration because it uses the ability of copper to complex both with an indicator that binds copper and a chemical that binds copper with a higher affinity than the indicator: ethylenediaminetetraacetic acid (EDTA), which is also referred to as the chelating agent [14]. How the titration works is that cupric ions are bound by an indicator molecule called murexide [14], shown in Fig. 9.

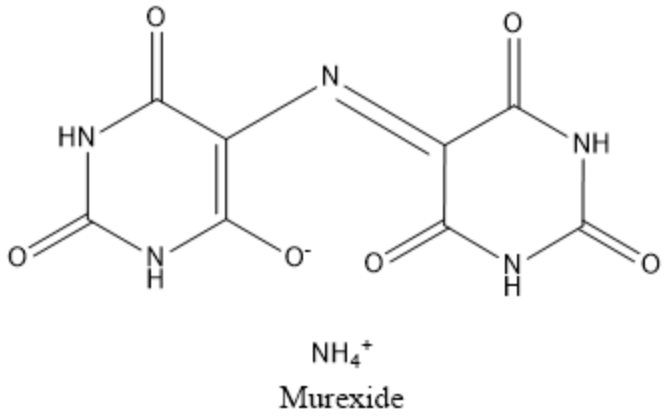


Figure #9: Murexide molecular structure.

Murexide on its own forms a purple solution, but in the presence of copper it binds to create a yellow solution. In a solution of copper and murexide, the solution color changes due to differential binding properties. EDTA forms a significantly more stable complex when bound to copper, meaning it takes the cupric ions from the murexide, causing the murexide's color to return to purple, indicating the endpoint. EDTA is a multidentate ligand meaning it has multiple "teeth" or functional groups that can bind the copper promoting the complex's stability, as shown in Fig. 10.

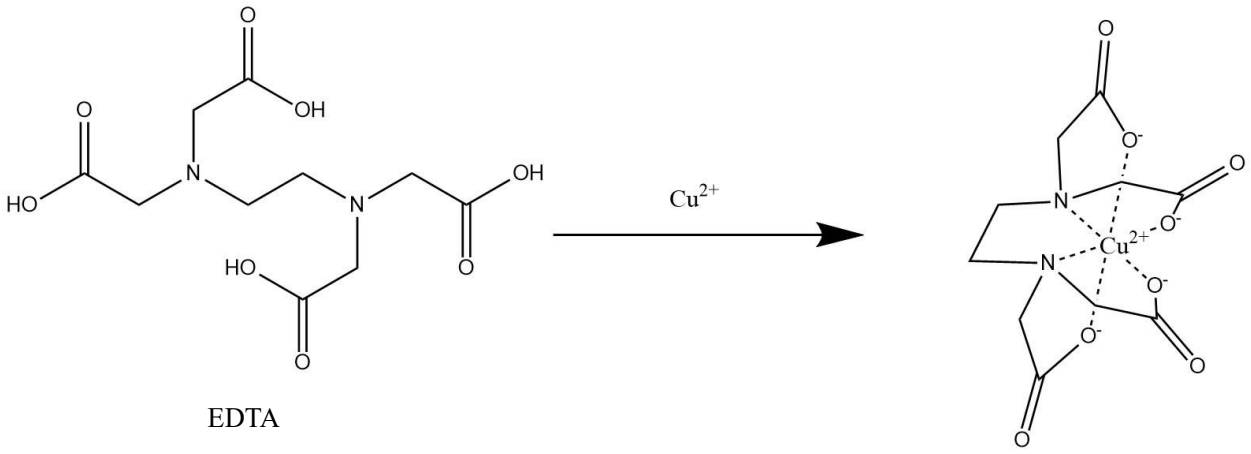


Figure #10: EDTA-copper complex molecular structure.

The equations needed to determine the amount of copper within a sample can be derived. Fig. 10 shows that one molecule of EDTA can bind one cupric ion, so the amount of moles of EDTA used are assumed to be equivalent to the moles of cupric ions. The amount of moles of EDTA can be calculated using

$$[EDTA] * Volume\ (L) = mol\ EDTA \tag{2.1}$$

By multiplying the resulting value by the molar mass of cupric ions, the amount of copper on the fabric sample is found. Dividing by the mass of the fabric leads to

$$\frac{g\ Cu}{g\ Fabric} = \frac{mol\ EDTA * Cu^{2+}\ molar\ mass\ Cu^{2+}\ (\frac{g}{mol})}{mass\ of\ fabric\ (g)} \tag{2.2}$$

which allows for the calculation of the amount of copper per gram of fabric.

2.5.2 Murexide EDTA Copper Complexometric Method

The titration method was as follows:

1. Approximately 1-inch squared fabrics were cut and weighed on a balance.
2. Fabrics were placed into a falcon tube and approximately 10 mL of 0.01M HCl was poured into the tube, submerging the fabric.
3. Tubes were sonicated in an ultrasonic bath for 1 hour.
4. A few crystals of murexide were added and stirred until the solution appeared orange yellow.
5. pH 10.5 ammonia buffer was added dropwise until the solution turned bright yellow (~5-6 drops).
6. The solution was titrated with either 0.01M or 0.001M EDTA until the solution became purple, indicating the endpoint. The concentration of the EDTA depends on the amount of copper on the fabric. Any fabric that reached the endpoint with 1 drop of 0.01M EDTA was titrated with 0.001 M EDTA.

The stages of the titration are shown in Fig. 11. Trials were run in duplicate for both 0 wash and 30 wash samples.

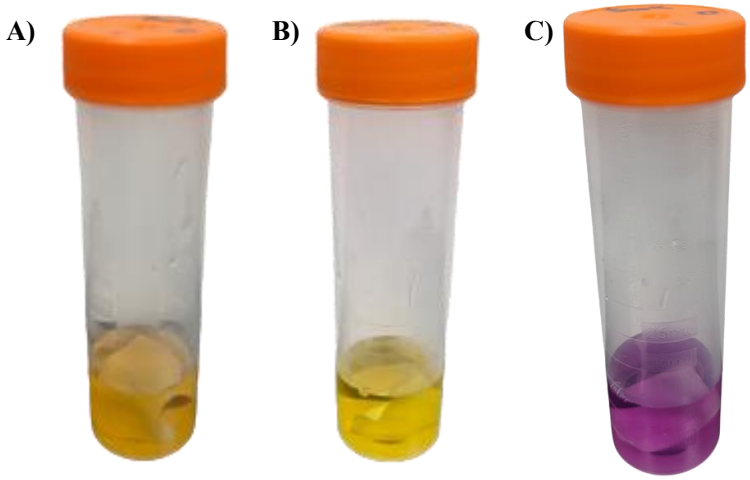


Figure #11: Stages of titration with A) start B) pH 10.5 ammonia buffer is added and C) endpoint.

2.6 Fabric Morphology

2.6.1 Scanning Electron Microscopy Introduction

To determine the presence of copper on the fabric, images can be taken using Scanning Electron Microscopy. Using this technique gives insight into the coating morphology of the fabric. The tool used is called a scanning electron microscope (SEM). To understand this tool, the mechanism by which the machine works is described. A traditional microscope uses light that passes through a condensing lens, which concentrates light on a specimen. The light then can pass through the specimen and through another lens to magnify the image for visualization. Rather than visible light, an SEM uses electrons [15]. By taking a beam of electrons and focusing it through magnetic lenses, the SEM can irradiate the surface of a sample with electrons [15]. A schematic of light microscope versus SEM can be shown in Fig. 12.

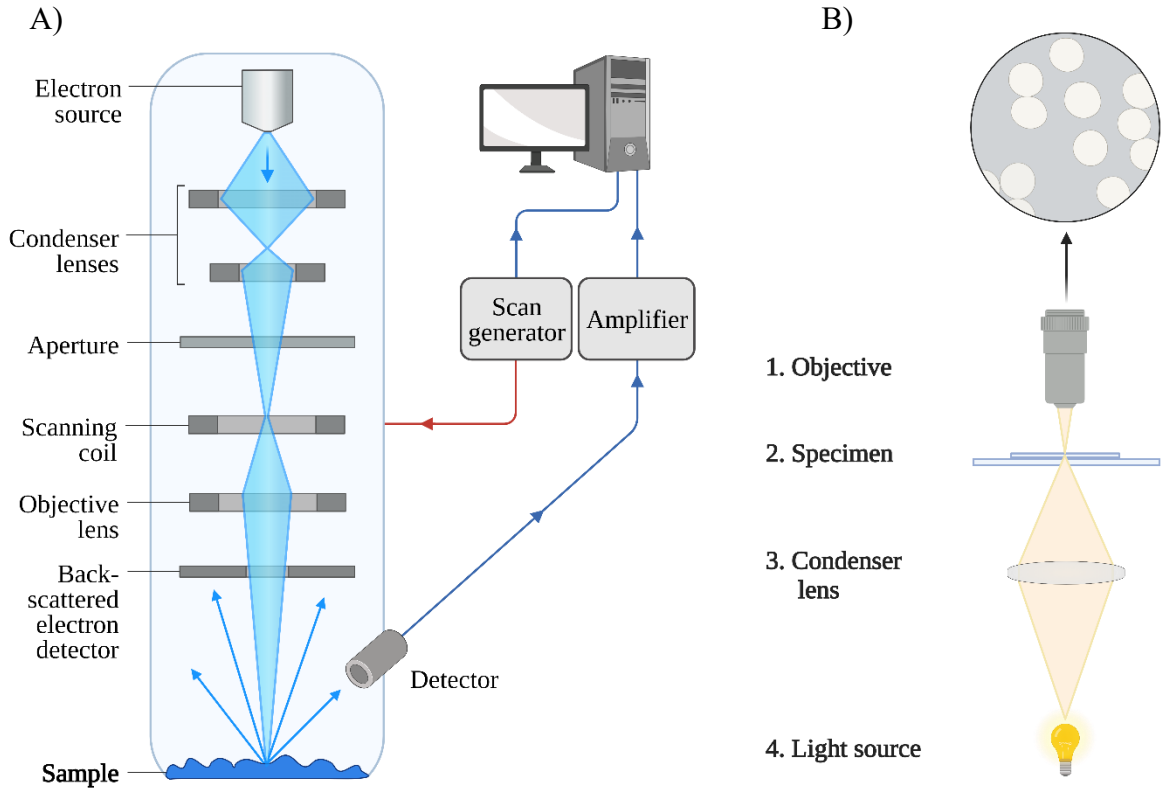


Figure #12: Schematic of A) scanning electron microscope and a B) brightfield light microscope.

The image from an SEM is produced through detection of electrons that are emitted from the surface. The beam scans across the sample from the top left towards the bottom right [15]. As it does this, the beam interacts with the sample and causes a multitude of interactions that kick a particle out of the sample. The type of particle which is kicked out is what allows for analysis of the specimen. Depending on what type of SEM is used, it has different types of detectors to analyze the different types of particles. For fabric analysis two primary types of electrons are used to analyze the fabrics.

The first type of particle is called a secondary electron (SE). This particle phenomenon is caused by an inelastic collision of an electron from the SEM beam and an electron from the nucleus of the sample's atoms [15]. This causes the electron from the atom to be launched out of the atom [15]. A diagram of this interaction is shown in Fig. 13 A). These particles have a lower relative energy than other particles, which means that they are unable to escape the sample from lower depths [15]. This means that secondary electrons are useful for observing the surface morphology of the sample. The next type of particle discussed is the back scattered electron (BSE). This particle phenomenon is the result of an elastic event where the electron interacts with the nuclei of multiple atoms, causing multiple redirections of the electron consequently launching it out of the sample [15]. A diagram of this is shown in Figure 13 B). What is important about this interaction is that it does not significantly decrease the energy of the electron that is navigating through the lattice of atoms [15]. This means that this particle can come from much deeper within the sample due to the higher energy of it. Using BSEs allows for analysis of deeper levels within the sample [15]. However, there is another characteristic of BSEs that is useful for fabric analysis, relating to contrast. The relative contrast between elements of different atomic number is different due to more BSEs being released from the sample surface, where higher atomic number elements have larger nuclei with more surface area, therefore reflecting more electrons, thus causing a brighter surface relative to a lower atomic number element [15]. Since copper is used on top of an organic fabric, this means the contrast coming from BSEs show to what extent copper is coating the fabric surface.

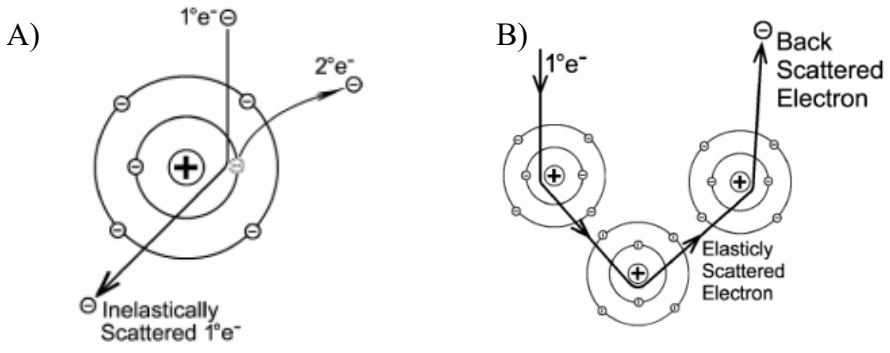


Figure #13: Visualization of A) secondary electron generation mechanism and B) backscattered electron generation mechanism. Figures taken and adapted from [15].

2.6.2 Fabric SEM Image Considerations

Using this technique, some additional considerations are discussed, due to sample characteristics. The major problem with fabric samples is they are not inherently conductive. What this causes is a phenomenon called charging. Because SEM relies on electrons to do visualization, if a non-conductive sample is irradiated for long enough, it begins to build a charge [15]. This interferes with the image, causing bright patches on the image, distorting it. This can be circumvented using a special type of SEM called an Environmental Scanning Electron Microscope (ESEM). The mechanism by which it circumvents this issue is the administration of gas into the sample chamber

[16]. Why introducing gas works relates to how the detector in an ESEM works. In an ESEM, rather than passively picking up electrons that happen to hit the detector, the ESEM can pick up many more electrons through its gaseous detecting device (GDD) [16]. The GDD applies an electric field from the detector to the sample, which interacts with the SEs that are ejected from the sample [16]. The field causes acceleration of the secondary electrons towards the detector. As mentioned, SEs have lower energy (<50 eV [15]) This field gives these electrons more energy, allowing them to ionize the gas within the chamber. This forms a cascade which gets picked up by the detector. More importantly, the resulting ionized gas can absorb the built-up charge on the surface of the sample, allowing for the imaging of non-conducting samples [16]. Traditionally, nonconductive samples are coated with a thin layer of metal, however for analysis, this is undesirable, as an uninfluenced sample surface is needed for further analysis techniques described in Section 2.6.3.

Another consideration for the fabric is going to be interaction depth and the edge effect. The edge effect is a result of the interplay of interaction depth, and geometry of the object being scanned. As mentioned in Section 2.6.1, different particles can interact at different depths within the sample. This interaction is summarized in Fig. 14.

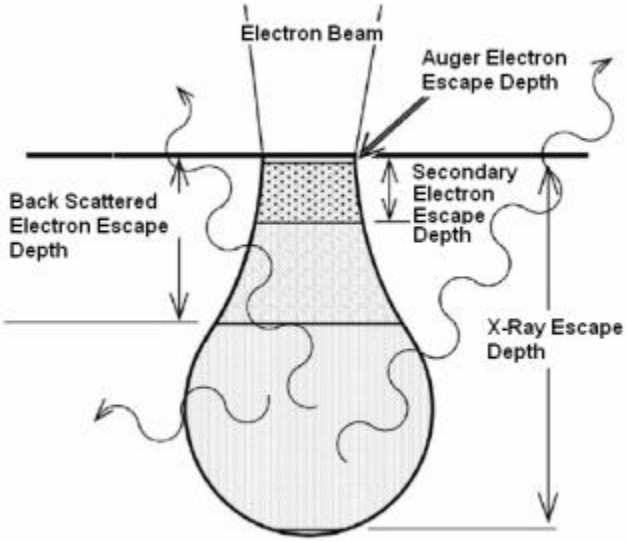


Figure #14: Scanning electron microscope interaction depth diagram. Figure taken from [15].

Issues appear when the geometry of the sample changes. On a flat surface, Fig. 14 represents what occurs; however, when varying geometry is involved, the edge effect becomes apparent [15]. With a cylindrical surface, if the incident electron beam hits the top, deeper regions of the sample are closer to the surface due to the curvature of the object, meaning electrons have a shorter path length to escape [15]. This causes the edges of the geometry within the sample to glow significantly brighter in the image. This can distort and cause the sharpness of the image to be decreased. A diagram of this interaction is shown in Fig. 15

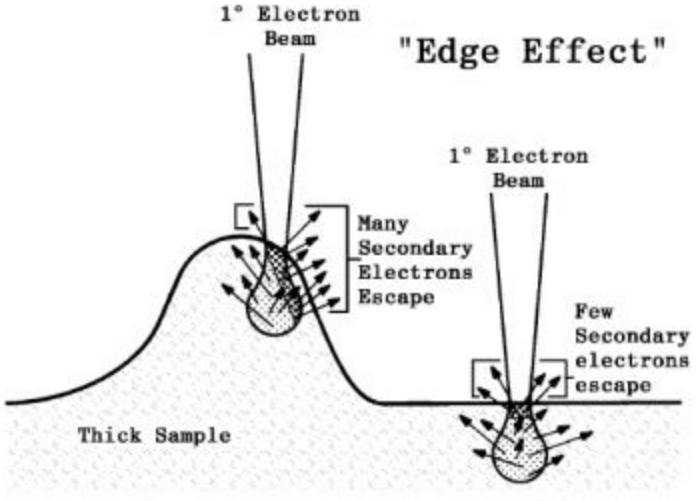


Figure #15: Edge effect diagram. Figure taken from [15].

2.6.3 SEM Micrograph Acquisition Method

The SEM used was the Quanta 250 FEG Scanning Electron Microscope using the environmental mode. The SEM was in the WATlab at the University of Waterloo in Ontario, Canada. Images were taken of the 1000 ppm samples of Beryl, AC5, copper sulfate and control fabrics. All images were taken using an accelerating voltage of 20.00 kV. For general morphology of the fabric, images were taken at 100x magnification. Fine morphology images were taken on individual strands at 2500x magnification.

2.6.4 Energy Dispersive X-Ray Spectroscopy Theory

Like the principle behind electron microscopy, EDS uses the x-rays that result from the sample being bombarded with electrons [17]. Each element has a characteristic energy associated with the release of x-rays from it [17]. This means inbound x-rays can have the energy levels quantified to determine the elemental composition of the scan area [17].

2.7 Microbiology Background

2.7.1 Bacterial Classification

A significant component of discussion relates to the fabric and its ability to kill microorganisms. To determine fabric properties, microbiological methods were used, thus requiring some background. One of the most basic classifications of bacteria is their cell wall morphology as well as their method for generating energy. The major cell wall morphologies outlined are Gram – and Gram + bacteria, where the morphologies are shown in Fig. 16. These morphologies are important as they are one of the inherent characteristics that can confer resistance to an antimicrobial agent.

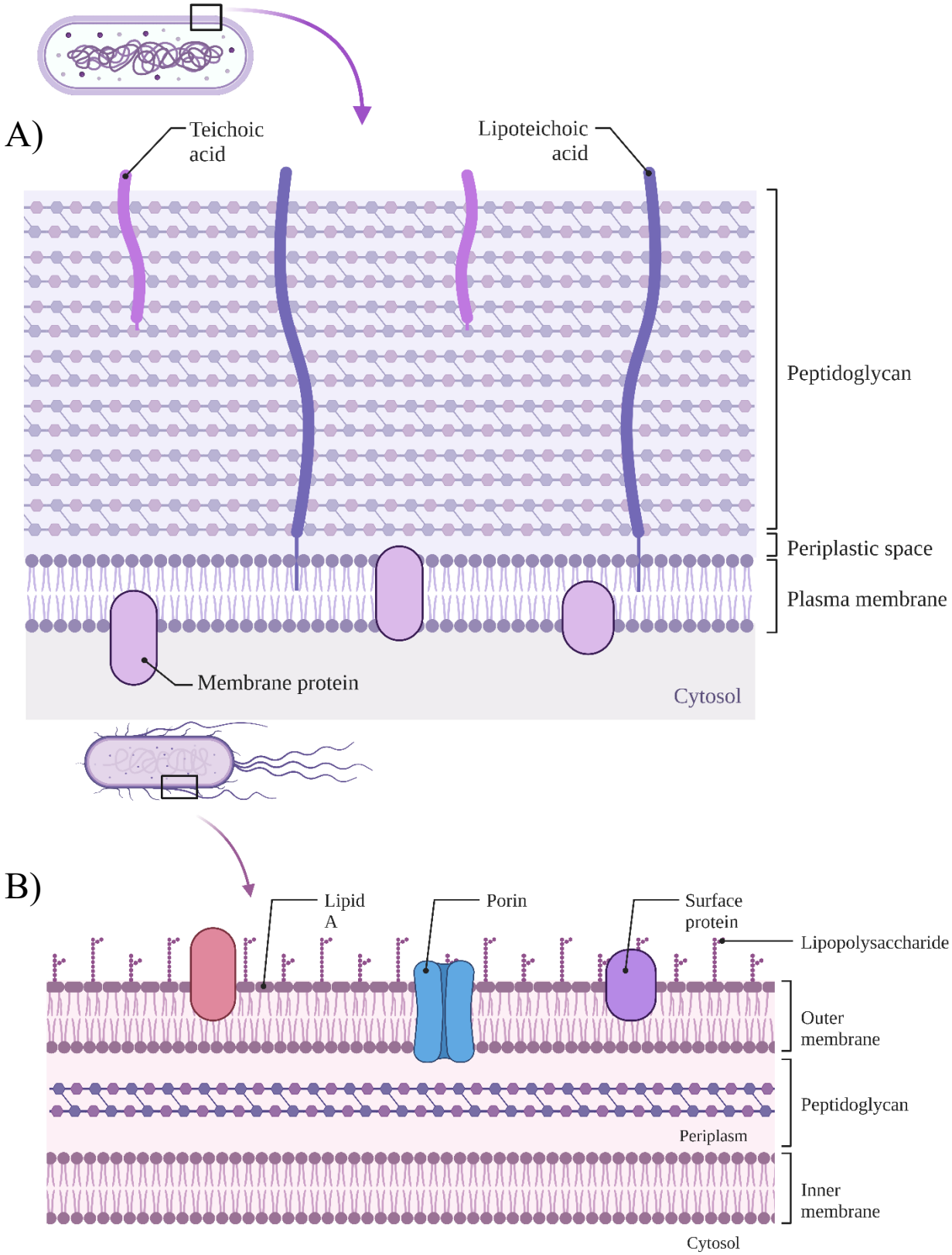


Figure #16: Depiction of A) Gram positive and B) Gram negative bacterial cell wall.

Bacterial metabolism is another important classification where bacteria are defined by aspects of their metabolism. This definition is incredibly important as it is the basis of how media can be developed for the growth of a target organism. The following characteristics are going to be primarily used for the growth procedure. The first characteristic is the energy production methods of bacteria. They are defined through multiple categories. The broadest classification is carbon source. Organisms that fix inorganic carbon are called autotrophs while ones that rely on organic carbon made by other organisms are called heterotrophs. Both can be further subdivided into where they acquire the energy from. This includes sources such as light (phototroph), organic carbon (organotroph) or even inorganic compounds (lithotroph). By utilizing the metabolic patterns of bacteria, specific culturing media can be created to properly grow them. Another important characteristic is the oxygen requirement. Bacteria can be aerobic, meaning they grow in the presence of oxygen, or anaerobic meaning they grow without oxygen. Bacteria can also be combination, where oxygen is not required, but the bacteria are still viable in its presence. Nutritional requirements of bacteria are also an important characteristic, where some bacteria are fastidious, meaning they need very specific conditions, while other bacteria may be able to grow in a wide range of conditions. A final condition to keep note of is the temperature at which the organism grows. The combination of all these factors is important when selecting a candidate microorganism for testing as it dictates the procedure of growth.

2.7.2 *Escherichia coli*: Growth of a Model Organism

The primary organism that was used in experiments is the bacterial species *Escherichia coli.* This bacterium is a model organism very commonly used in the microbiology field due to its ease of growth and because it is inexpensive [18]. It is also popular because of its safety, although pathogenic strains do exist; however nonpathogenic strains are mostly used in the laboratory [18]. *E. coli* are chemoheterotrophs that grow optimally at 37 ºC but can also grow under anaerobic conditions [19]. An important aspect for growth of microorganisms is the doubling time. This is the time it takes for the population of a microorganism to double through binary fission [19]. *E. coli* has a doubling time of approximately 20 minutes at ideal conditions [19]. Growth happens in multiple stages, outlined in Fig. 17.

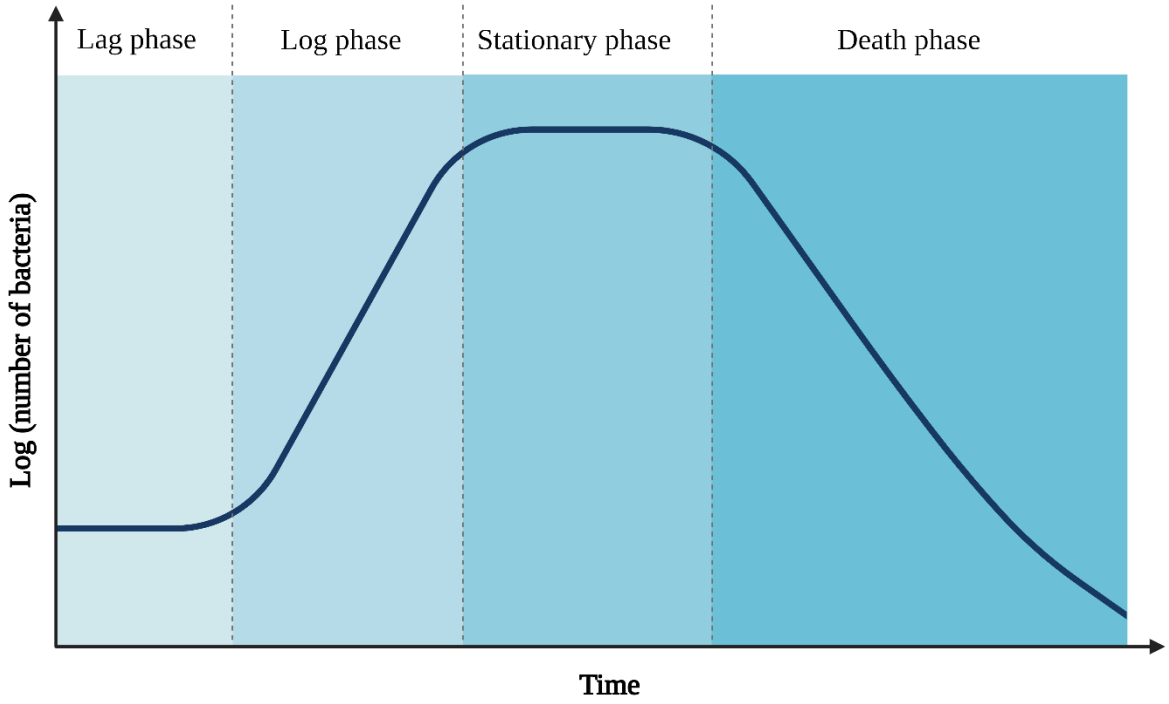


Figure #17: Bacterial growth curve graph.

When inoculating a bacterium into media, growth is initially inhibited as they acclimate to their surroundings [20]. Afterwards they start to exponentially double, following the approximate 20-minute doubling time; this phase being referred to as the log phase [20]. Afterwards the bacteria saturate, as the waste from their metabolism starts to inhibit growth and the nutrients are used up from the media, pushing them to the stationary phase [20]. Following this, the bacteria start to die as all the nutrients are depleted [20]. Because of this growth pattern, *E. coli* must be incubated for the right amount of time, which gives a healthy viable culture. Excessive incubation will lead to non-viable cultures. Further details with regards to timing are given in Section 2.9.2.

2.7.3 Influence of Metal Ions on Microbes

The ability of metals to kill microbes has been utilized far before the knowledge of microorganisms have existed [21]. In the past, it was found that by storing water in metal vessels, the water was sterilized. This process of metals eliminating microbes is called the oligodynamic effect [21]. The type of metal used is key as something that kills efficiently but is non-toxic is ideal. Copper fits these parameters because it is shown to kill quicker than other metals [21], while also remaining nontoxic [22]. This is due to the body being able to control the copper levels within, preventing buildup, although toxicity can happen through prolonged exposure to copper [22]. There have been some mechanisms that have been proposed for how metals can kill, shown in Fig. 18.

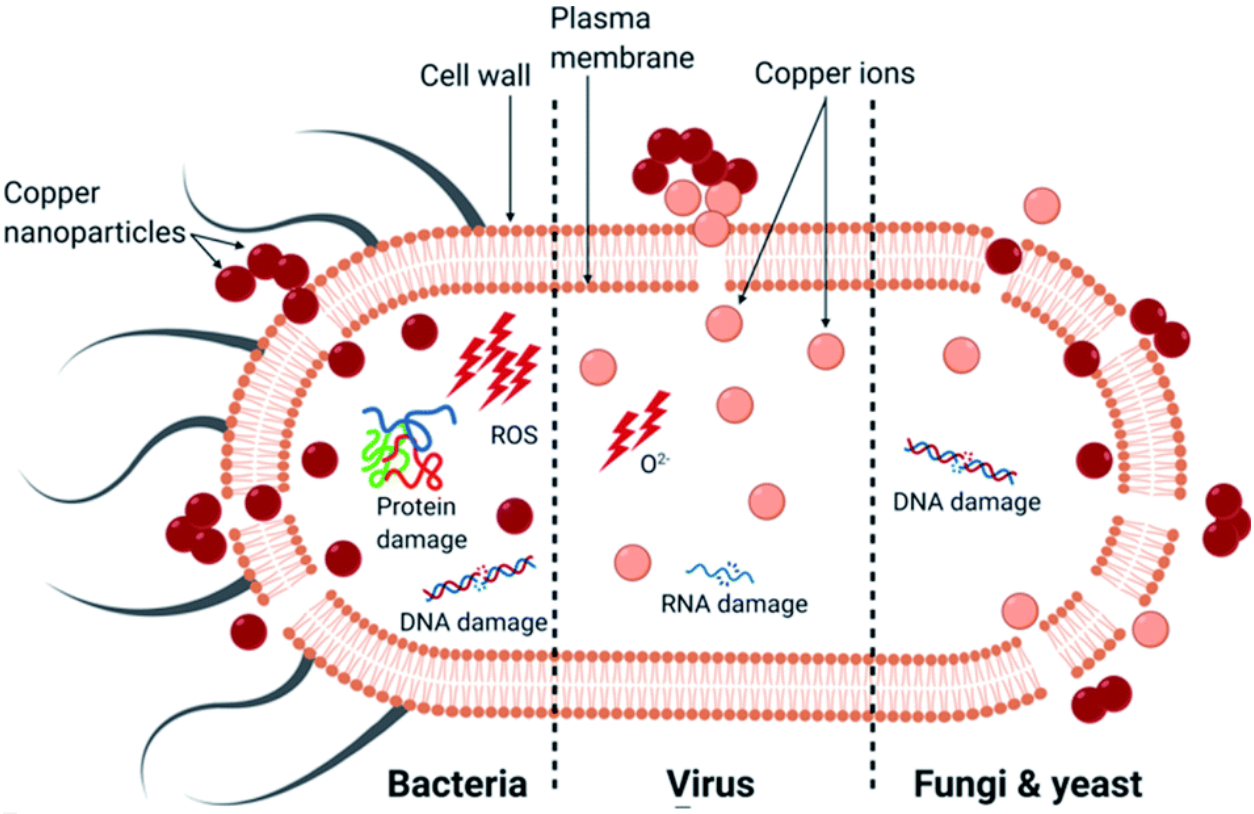


Figure #18: Copper killing mechanism of microorganisms. Figure taken from [23]

The first major mechanism relies on the ability of copper to bind and disrupt the cell membrane [23]. This causes holes in the membrane which let out internal cellular contents, killing the cell. The next mechanism is through binding proteins [23]. Metals bind certain groups on proteins such as sulfur containing groups, disabling protein function thus causing cellular death [21]. One final mechanism occurs through the generation of Reactive Oxygen Species (ROS) which damages the proteins and DNA of the cell [23]. This generation of ROS is one of the better-known mechanisms, as the chemical properties of copper are what allow it to form ROS. Copper can shift between the Cu^{2+} and Cu^{+} ion depending on the environment [22]. Both species have the potential to form ROS such as superoxide (O_2-) or hydroxide radicals ($OH\cdot$), which can cause intracellular damage [22]. These three mechanisms in combination are what is thought to be the killing mechanism of metals.

2.8 Culturing and Plating Microbes

2.8.1 Streaking and Inoculating

One of the largest problems faced by microbiologists is heterogeneity. When dealing with bacterial growth, since bacteria can evolve rapidly, inoculating a mix of bacteria onto a plate gives wildly varied results across experiments. To amend this, a widely used technique for isolating pure cultures called streak plating was used [24]. The method is outlined in Fig. 19.

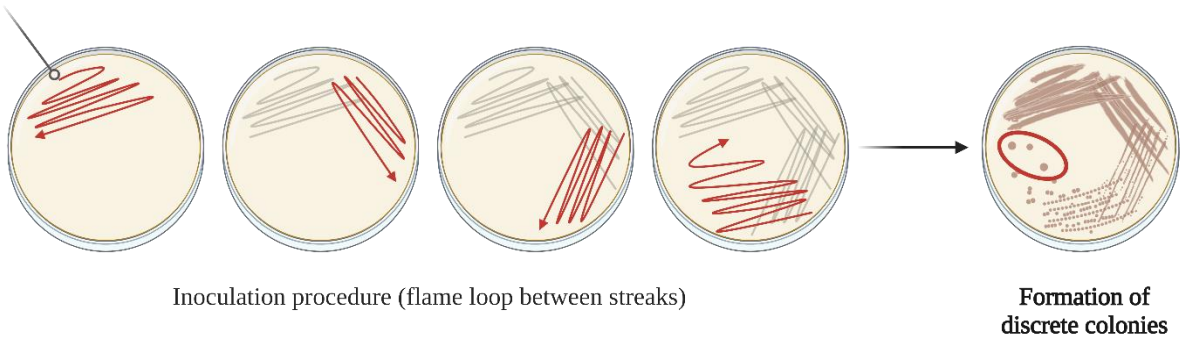


Figure #19: Visualization of the streak plate protocol.

This method involves taking a sample of bacteria from a culture and streaking it across a plate and diluting the culture mechanically [24]. Only the first streak contains fresh bacteria while the rest of the streaks continuously dilute the number of bacteria [24]. This allows for isolated colonies, which are groups of bacteria that are assumed to have risen from a single progenitor, thus making the culture pure. Afterwards an isolated colony can then be incubated in broth to grow the bacteria to the required density, still maintaining some purity. This culture can now be used for other testing.

2.8.2 Spread Plating

With the culture obtained from streaking, the spread plate method is used to enumerate bacteria. For usage in the thesis, it is primarily a gauge for how well the antimicrobial fabrics can kill. The method to do this is outlined in Fig. 20.

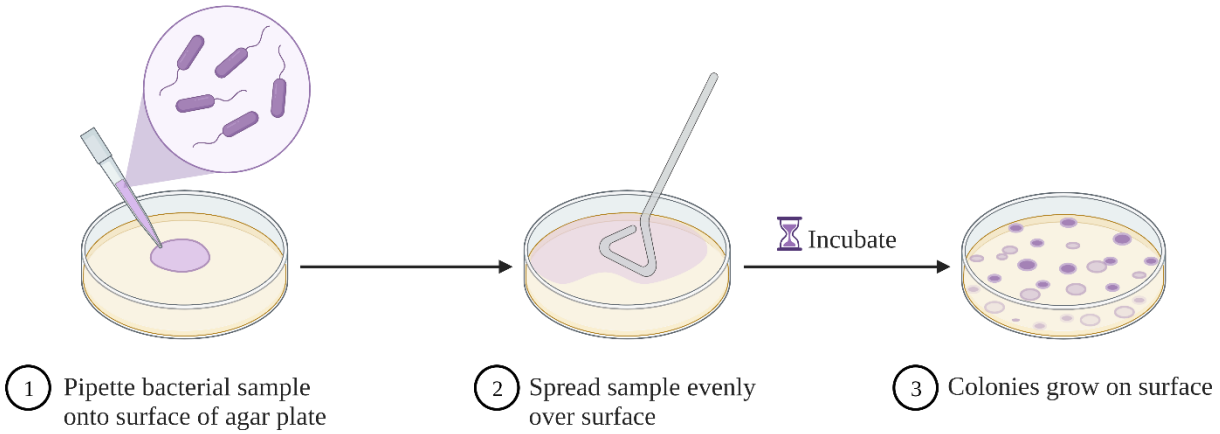


Figure #20: Visualization of the spread plate protocol.

By spreading the bacterial culture over the surface, colonies can be counted [24]. Every spot on a plate is a colony is assumed to have arisen from a single bacterium that divided to form the spot. These spots can be counted to determine the number of bacteria from the sample. By varying sample interactions, the effect of the test substrate can be determined on the viability of a bacterial culture. This technique's usage is outlined in Section 2.9.2.

2.9 Antimicrobial Efficiency

2.9.1 Introduction to Efficiency Testing

Covered next is the antimicrobial property of the fabric. If the needs of the consumer and the integrity of the fabric are balanced, the product can be expected to be marketable and effective. Antimicrobial kill efficiency is the method to determine whether the fabric is effective at killing microbes that land on it.

The spread plate method is used to do this. The idea of the antimicrobial efficiency test is to simulate what happens if bacteria land on top of the clothing, and how well they can survive. To evaluate this, the spread plate method was used in conjunction with a fixed contact time on the fabric. By allowing the bacteria to interact with the fabric, the ability of the fabric to kill the bacteria can be determined. The bacteria are in the form of a liquid drop, which simulates how they can travel from a sneeze.

The bacteria used was a strain of *E. coli* called K-12. This bacterium is one of the most popular in microbiology labs because of its ease of genetic transformation. However, for usage purposes the major advantage of the strain is its safety [25]. This ease of genetic transformation also allows for an especially important property to be installed into the bacteria: antibiotic resistance. Resistance allows for doped antibiotic plates that select for only the target bacteria. The bacteria used for the experiments was *E. coli* K-12 ER2738 purchased from New England BioLabs. This bacterium has a gene that codes for resistance to the antibiotic tetracycline.

To try and keep the quantity of bacteria similar, optical density (OD) was used. This technique is an estimate of bacterial concentration using the scattering of light within a sample [26]. Bacteria scatter light at a wavelength of 600 nm, which allows for a relationship between the amount of light scattered and the concentration of bacteria within the sample, that can be graphed [26]. Light scattering is proportional to the concentration of bacteria. Therefore, a spectrophotometer can be used to estimate bacterial concentration. Something to note is that this is an estimate, and the value can vary depending on the spectrophotometer, as well as the bacteria used as dead cells can scatter light too [26]. To get an accurate estimate of bacterial quantity using OD value, the bacteria were plated at different OD values to determine at what OD provides plate counts of 20-300 colonies. Despite these flaws, the inaccuracy in OD is less influential when using comparisons of the control plate to the plate that interacted with the treated fabric.

2.9.2 Antimicrobial Efficiency Method

For efficiency experiments, two types of media were used. Miller's Luria Broth (LB broth) was used for liquid culturing of the *E. coli* while Tryptic Soy Agar plates (TSA plates) doped with 10 μg/mL tetracycline antibiotic were used for the spread plates.

The following method was used for the efficiency experiments:

1. Generation of pure *E. coli* culture: E. *coli* was taken out of the stock culture tube and was streaked onto a TSA plate incubated for 24 h at 37 ºC. A single well isolated colony was taken and inoculated into a LB broth tube and incubated for 18 hours at 37 ºC.
2. Washing: A volume of 1 mL of the bacterial broth was pipetted aseptically into a microfuge tube and centrifuged for 1 minute to pellet the bacteria. The supernatant was discarded and sterile 0.9% saline was pipetted into the tube and vortexed until the pellet completely dissolved. This process was repeated twice to wash the bacteria.
3. Adjusting bacterial concentration: Sterile 0.9% saline was used as a blank for the spectrophotometer, and the solution was then diluted until a value of 0.02 was read on the spectrophotometer. This solution was then transferred to a microfuge tube.
4. Preparation of fabric specimens: The fabric was cut into 1-inch squares and pushed to the bottom of an empty flat-bottom test tube. Samples were run in duplicate.
5. A volume of 0.1 mL of bacterial solution was placed onto the fabric to create a wet spot in the middle of the fabric and incubated at room temperature for a contact time of 1 hour, during which the antimicrobial action takes place.
6. To quench the antimicrobial activity, 10 mL of neutralizing solution was added to the tube, so the fabric was completely submerged. The neutralizing solution consists of 0.1 mM EDTA in 0.9% saline. To release any bacteria that survived, the solution was vortexed for 5 seconds. The solution was then placed in an ultrasonic bath for 5 minutes to remove additional bacteria clinging to the surface of the fabric.
7. A volume of 0.1 mL was pipetted from the tubes to the center of a tet-HCL TSA plate and spread. After sitting for 10 minutes, plates were inverted and incubated at 37 ºC for 24 hours.
8. Plates were imaged and counted.

2.10 Microbial Contact Time Efficiency Experiments

2.10.1 Microbial Contact Time Efficiency Introduction

With any antimicrobial material, a property that is important in addition to efficiency, is the contact time efficiency, or how quickly the fabric can begin to effect viability of the microorganism. Different fabrics require different contact times to affect the bacteria, which influences the possible usage of the fabric. For example, in a hospital setting, having a one hour kill time means that the wearer may be more so protected from the pathogen rather than anyone interacting with the wearer. However, in a case where hospital staff move from room to room, the timing can be in the order of seconds to minutes. That means to prevent nosocomial infections, a very rapid kill time is imperative. This fast kill time provides the best chance at preventing the spread of harmful microorganisms.

2.10.2 Microbial Contact Time Efficiency Method

The method for testing the kill time is the same method as described in Section 2.9.2, except for changes to step 5 and 6. Contact time was varied for this experiment while the 1000 ppm Beryl fabric was used for every experiment. After the contact time, 10 mL of neutralizing solution was added to prevent further killing from the fabric and the bacteria were vortexed for 10 seconds to spread the solution. After all the samples were done, they were sonicated all together for 5 minutes then plated. Plates were done in singlicate.

Chapter 3: Results and Discussion

This chapter contains the novel infusion procedure and the results of the methods described in the previous chapter for the various fabrics.

3.1 Novel Infusion Procedure: Beryl

For applications there is a great need to balance two factors during infusion. The first factor is the color of the fabric. AC5 was primarily used due to its antimicrobial effectiveness, however there was a major issue. AC5 would discolor fabric very strongly, and not only white fabric. On some black fabrics, AC5 would impart a heterogeneous red color, shown in Fig. 2. On white fabrics AC5 can impart a strong blue color. This discoloration behavior was the primary drive to create a novel process, as some applications do not allow for discolored fabric. The newly developed process was found to be more sustainable than AC5 infusion due to no production of hazardous gases during the infusion process. This process was named the Beryl process. While the Beryl process remains largely proprietary to the industrial partner, my contribution was the process modification that provided the resistance to staining, while also imparting antimicrobial activity.

The Beryl process is a multi-step infusion process, which involves a pre-treatment bath followed by a cupric ion infusion process. The cupric ion source differs between AC5 and Beryl. The pre-treatment bath consists of a proprietary mixture of an organic acid, a polymer, and a surfactant. The process is outlined in Fig. 21.

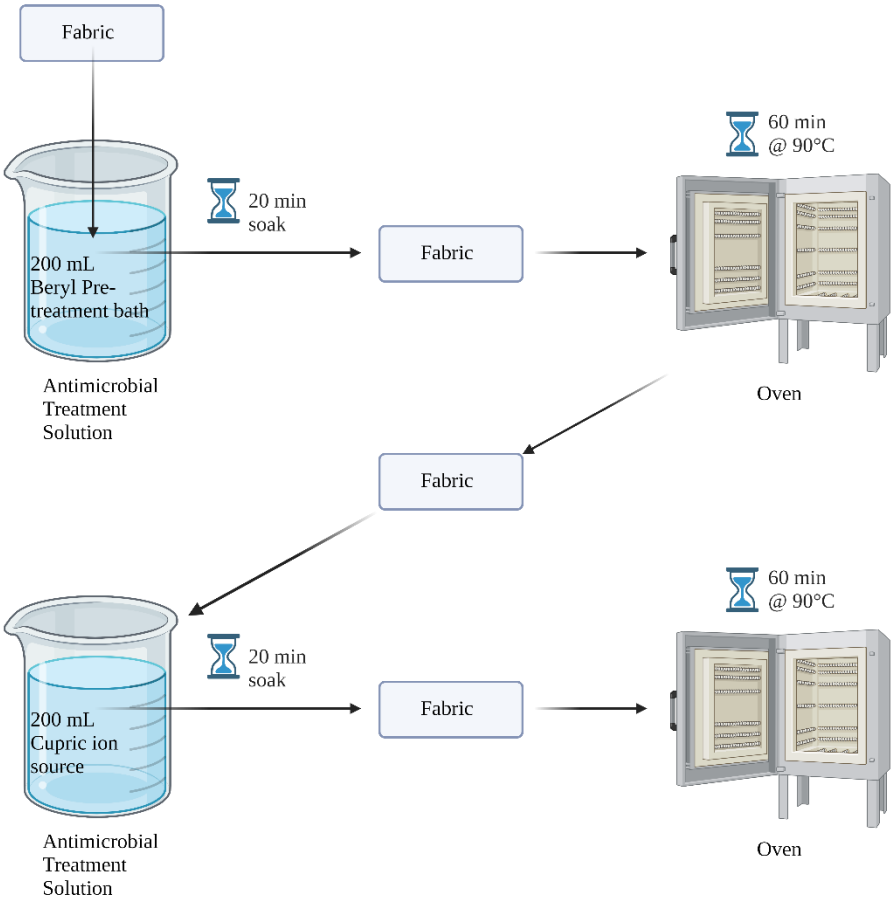


Figure #21: Beryl fabric infusion procedure.

3.2 Fabric Texture Quality

Fabric texture is one of the first qualities of the fabric that can be analyzed post infusion. This quality can be an indicator of coating thickness. Post-infusion, fabric may lose its elasticity, or the fabric itself may feel more stiff or coarse to the touch. It is desirable to conserve the original integrity of the fabric, that feels pleasant to touch. For the Beryl fabric, the pre-treatment caused the fabric to feel coarse, while also making the fabric very stiff. After the second infusion, the Beryl fabric softened up again and returned to the original texture of the control fabric. For the AC5 and copper sulfate fabric, the fabric feels stiff directly after curing, but the texture returns to normal over time. The elasticity of the fabric does not seem to be affected by the infusion. Despite the change in texture, the fabric returned to softness. Fabric can come in many forms, and in the case of testing, the ISO adjacent cotton is very thin. It was found that infusing fabrics with thicker fibers, such as those in socks, reduced the coarseness directly after infusion.

3.3 Fabric Odor

Another quality that is noticeable after post infusion is the fabric odor. Fabrics can be imparted with an unpleasant metallic smell after infusion, which can be detrimental to those with scent sensitivities and can be generally off putting to anyone purchasing the fabric. Therefore, not imparting odor is an important quality of the fabric. Beryl and copper sulfate fabric do not impart odor at all to the fabric directly after infusion and stay neutral over time. The AC5 fabric imparts a slight metallic odor to the fabric directly post infusion, but the scent dissipates over time.

3.4 Color Change

To analyze the color, the average RGB values of the fabrics at 0 washes and 30 washes are tabulated as shown in Table 1.

Table #1: Fabric 0 and 30 wash RGB values with control RGB values at 205, 205, 209.

	100 ppm 0 Wash	*1000 ppm 0 Wash*	*10,000 ppm 0 Wash*	*100 ppm 30 Wash*	*1000 ppm 30 Wash*	*10,000 ppm 30 Wash*
Beryl	202,206,207	197,203,204	191,201,201	200,205,206	199,206,208	195,203,208
AC5	196,201,204	186,201,204	172,199,207	200,206,207	197,205,200	183,202,207

The data in Table 1 can be transformed to a more visual medium, by filling table cells with the color coded by the RGB value to illustrate the differences as shown in Table 2.

Table #2: Fabric RGB visual color array.

	100 ppm 0 Wash	1000 ppm 0 Wash	10,000 ppm 0 Wash	100 ppm 30 Wash	1000 ppm 30 Wash	10,000 ppm 30 Wash
Control						
Beryl						
AC5						

From Table 2, the fabric is being discolored by both the Beryl and AC5 process. However, the degree at which it is discolored is much more severe in the AC5 fabric. The cupric ion used in the process is likely what is imparting blueish color. For the fabric to appear blue, blue light is reflected from the surface of the fabric [10]. This is shown by the red value for the RGB colors, where as the concentration of copper increases, the red value of RGB decreases. This is shown graphically in Fig. 22.

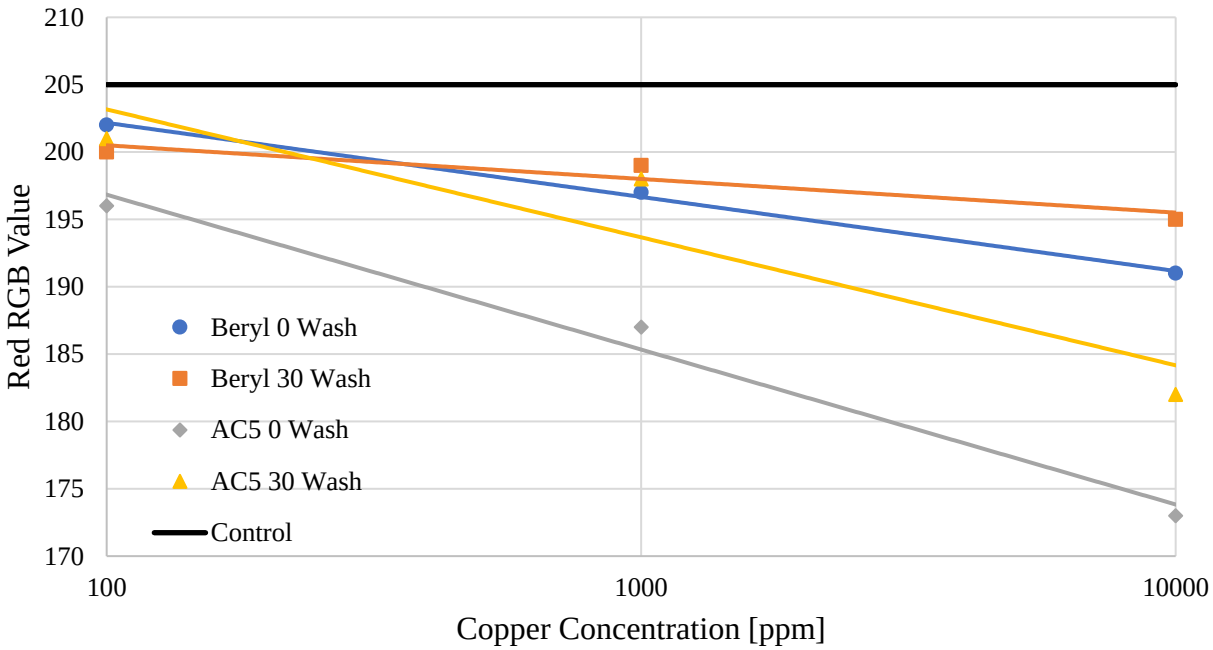


Figure #22 Copper concentration versus change in red RGB value for Beryl and AC5 fabrics at 0 and 30 washes.

From the data in Fig. 22, the Beryl process has overall less impact on the color change of the fabric while the AC5 fabric has a significantly larger impact on the color of the fabric. The Beryl fabric decreased the R value by 3 units at 100 ppm infusion compared to the AC5 fabric which decreased by 9 units at the same infusion concentration. This difference in color is the greatest at the 10,000-ppm copper infusion where the Beryl R value is decreased by 14 units while the R value for AC5 decreases by 33 units when compared to the control. This means the Bery still gets discolored post infusion, but it does not get affected as much as the AC5 fabric.

Other techniques have been developed to tell the scope of color difference between two values such as the pixel shift method, which uses grayscale transformations of colors to determine the differences between them [27]. This technique is more accurate in determining the shift of color, however for the purposes of this thesis, the RGB technique is more useful. This is because techniques such as pixel shift do not consider the human perception perspective, and for the cases of the color shift in the fabric, representing using RGB mirrors the structures within the eye [10]. This is important as the purpose of the Beryl fabric was to overcome the drastic color shifts that occurred with the infusion of copper on fabric, and visualization techniques that are natural to humans provide the best gauge of color shift.

Within the literature, there tends to be little emphasis on the color change aspect, which is an issue for the purposes of commercialization. Especially with NPs, discoloration of the fabric is very common, and shown through the before and after infusion images. For example, Sharma et al., used *Tinospora cardifolia* Cu NPs on white fabric, which caused a dark green discoloration [8]. In addition, Marković et al., used Cu NPs on fabric, which showed the color varying in stages [9]. First, infusion with copper sulfate creates the blue discoloration that was seen earlier [9]. Usage of

sodium borohydride causes this discoloration to turn dark blue, with this color shifting to dark grey after drying [9]. Finally, usage of ascorbic acid as the reductant of the cupric ions causes a yellow discoloration of the fabric [9]. In general, many modern techniques that involve antimicrobial techniques do not preserve the original color of the fabric very well, which bolsters the need to develop techniques that stain less.

3.4.1 Color Change Effect on Black Dyed Fabrics

Color change experiments were done on white fabric, but a different change occurs on black fabric. On the black fabric, the natural blue color of cupric ions are not present, but the fabric can be discolored. One theory for this change relates to dye structure.

As color depends on absorption and reflection on light, all different colors are absorbed to make something black; this is done by including multiple dyes that range the different wavelengths [28]. An example black dye composition can be seen in Fig. 23.

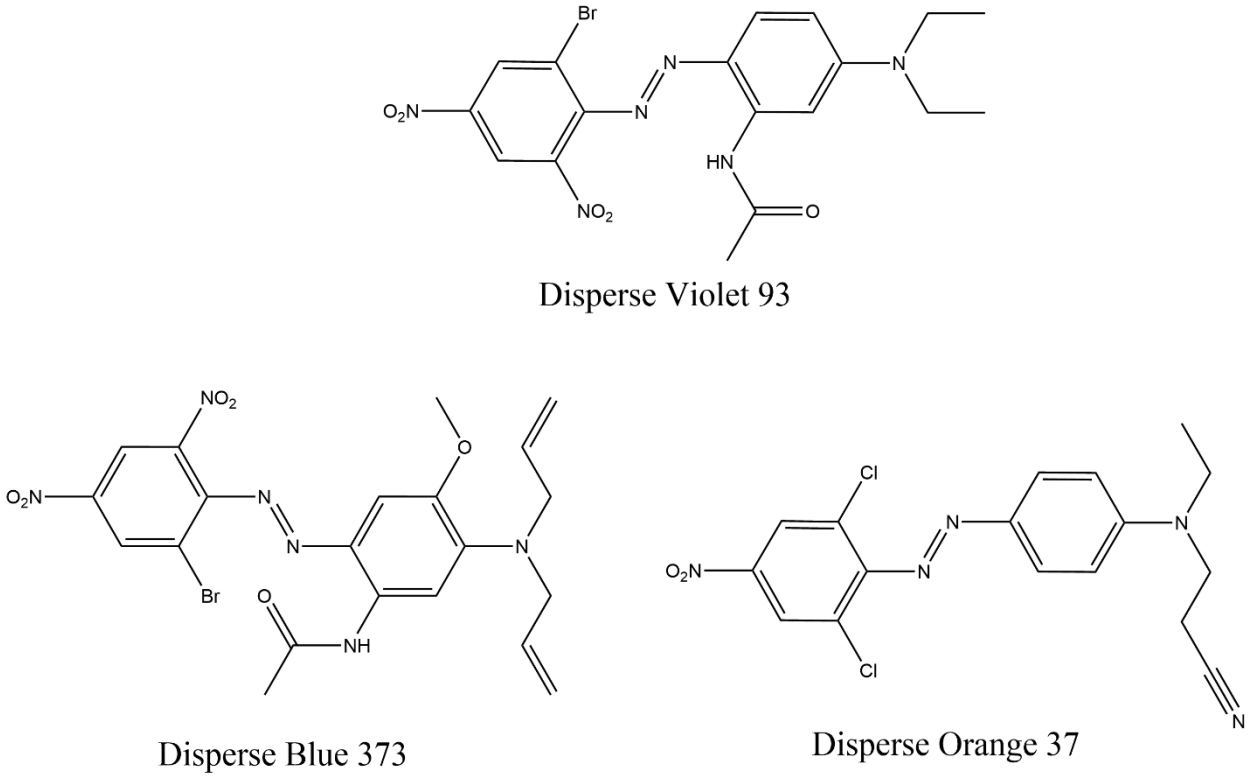


Figure #23: Molecular structures of dyes involved in a black dye solution. Figure adapted from [28].

The result of infusing black fabric using the AC5 procedure is shown in Fig. 24 B).

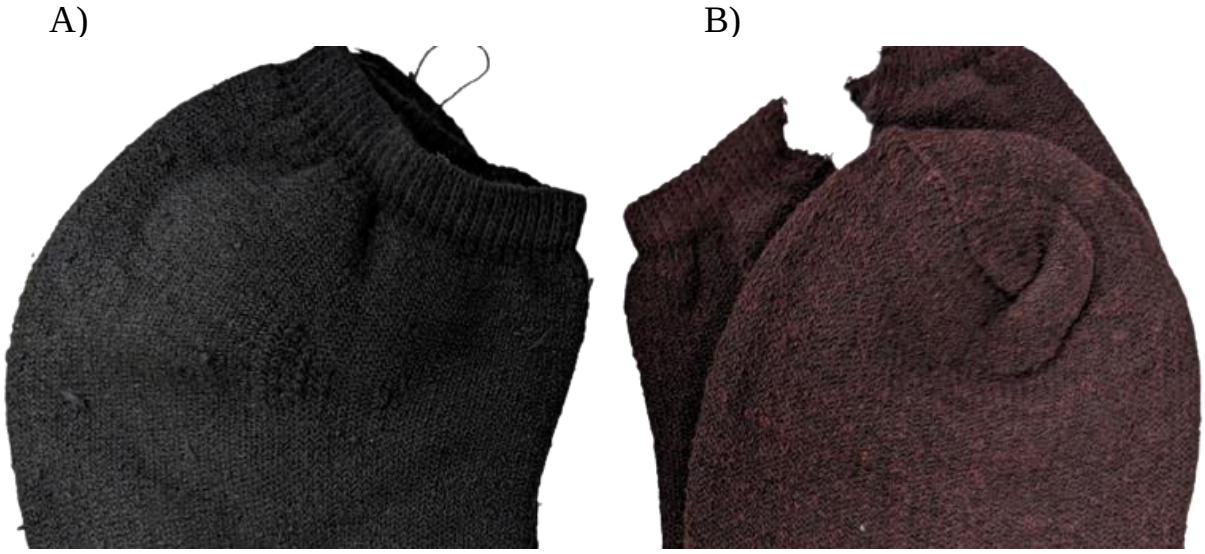


Figure #24: Side by side comparison of A) sock infused using the Beryl procedure and B) sock infused with the AC5 procedure.

Although the previous discoloration was related to the innate blue color of cupric ions, the color shift to red shown in Fig. 24 B) is much more drastic and visible. Furthermore, the red color is not only caused by AC5, but using the copper sulfate infusion procedure also causes this same color change after the fabric is dried or steamed. What is suspected to be causing this is that the cupric ions are binding to the dyes within the fabric and causing a color change. The hypothesized reason this occurs relates to color chemistry. Within color chemistry, different chemicals have specific structures called chromophores, which are responsible for absorbing specific wavelengths of light [29]. The property of a chromophore that allows it to absorb visible light so well is its ability to undergo resonance, meaning that electrons can flow through the structure of the chromophore, therefore stabilizing it [29]. Common groups found in dyes are shown in Fig. 25.

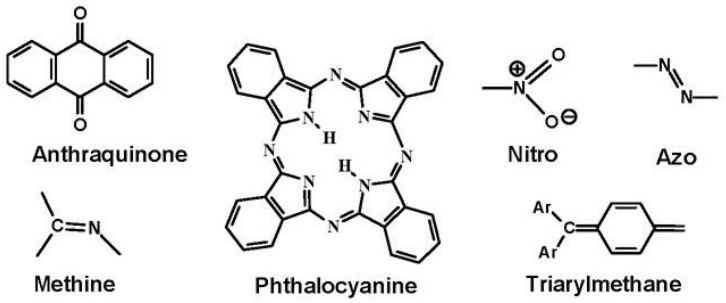


Figure #25: Common chromophore groups found in organic dye molecular structures. Figure taken from [29].

Because of the cupric cation, the copper can bind to some of these groups, causing the alteration of stability within the molecule's resonance structures, which alters the ability to absorb light at

the specific wavelength the dye typically absorbs at. Depending on which dye this occurs in, it can change the properties. What would elicit a red color is copper binding to a blue dye, causing it to not absorb red as well anymore, thereby reflecting more red light.

Through this color experimentation it is shown that the Beryl process can outperform the other methods as it does not stain as vibrantly as the AC5 fabrics.

3.5 Complexometric Titration

Raw data of all the fabrics was tabulated to show how much copper is on each of the types of fabric, to determine how well the fabrics can absorb copper. The analysis starts with the 0 wash fabrics: Beryl, AC5 and the control copper sulfate fabric, as shown in Table 3.

Table #3: Copper content of fabrics at 0 washes with trials run in duplicate.

0 Wash	*Trial #1* *mg Cu / g of Fabric*	*Trial #2* *mg Cu / g of Fabric*	*Average* *mg Cu / g of Fabric*
Beryl 100 ppm	0.443	0.271	0.357
Beryl 1000 ppm	0.803	0.982	0.892
Beryl 10,000 ppm	5.83	7.04	6.43
AC5 100 ppm	2.00	1.67	1.84
AC5 1000 ppm	3.50	3.60	3.55
AC5 10,000 ppm	10.6	11.3	10.9
$CuSO_4$ 1000 ppm	1.90	1.76	1.83

Next is the copper content at 30 washes for each of the fabrics, as shown in Table 4.

Table #4: Copper content of fabrics at 30 washes with trials run in duplicate.

30 Wash	*Trial #1* *mg Cu / g of Fabric*	*Trial #2* *mg Cu / g of Fabric*	*Average* *mg Cu / g of Fabric*
Beryl 100 ppm	0.671	0.628	0.650
Beryl 1000 ppm	2.13	2.29	2.21
Beryl 10,000 ppm	1.24	1.55	1.39
AC5 100 ppm	0.529	0.570	0.549
AC5 1000 ppm	1.84	1.80	1.82
AC5 10,000 ppm	5.18	5.23	5.21
$CuSO_4$ 1000 ppm	0.533	0.755	0.644

The percent change of the copper content value between washes can be determined by finding the difference between the washes and dividing by the original copper content per gram of fabric, which leads to

$$\%\ Change = \frac{(0\ Wash\ \frac{mg\ Cu}{g\ Fabric} - 30\ Wash\ \frac{mg\ Cu}{g\ Fabric})}{0\ Wash\ \frac{mg\ Cu}{g\ Fabric}} * 100 \qquad (3.1)$$

which is combined with the results before and after washing, shown in Table 5.

Table #5: Percent changes of copper content of fabrics between 0 washes and 30 washes.

Fabric	*0 Wash* *mg Cu / g Fabric*	*30 Wash* *mg Cu / g Fabric*	*% Change*
Beryl 100 ppm	0.357	0.650	+82%
Beryl 1000 ppm	0.892	2.21	+148%
Beryl 10,000 ppm	6.43	1.39	-78%
AC5 100 ppm	1.84	0.549	-70%
AC5 1000 ppm	3.55	1.82	-49%
AC5 10,000 ppm	10.94	5.21	-52%
Copper Sulfate	1.83	0.644	-65%

When analyzing the 0-wash data it is shown that the AC5 process can absorb the copper to the fabric more efficiently and can at most, pick up around 4.5 mg more of copper at 10,000 ppm infusion when compared to Beryl, as shown in Table 5. Both infusions use a source of cupric ions, but the reason for the Beryl process being less efficient is due to the pretreatment likely reducing the amount of areas copper can bind on the cellulose fiber. This means that Beryl is not able to hold on to as much copper as AC5, which could contribute to why it does not stain as intensely.

The deviation between samples can vary widely which can be attributed to uneven coating methods. Because the fabric is soaked within the treatment solution and dried, there may be uneven coating due to the treatment liquid pooling near the edges during curing. This would mean there could be a copper gradient at the edges of the fabric, leading to varying results. To alleviate this, the edges of the fabrics were cut off, as the edges would hold more copper as the drying procedure would cause the treatment solutions to well on edges of the fabric. This meant that the leftover fabric used would be a better representation of the copper content of the fabric.

When comparing the relative value of the copper content trends in how much difference there is in copper absorption to the fabric between Beryl and AC5 can be seen. To do this the copper content of the Beryl fabric is subtracted from the copper content of the AC5 fabric. This is shown in Fig. 26.

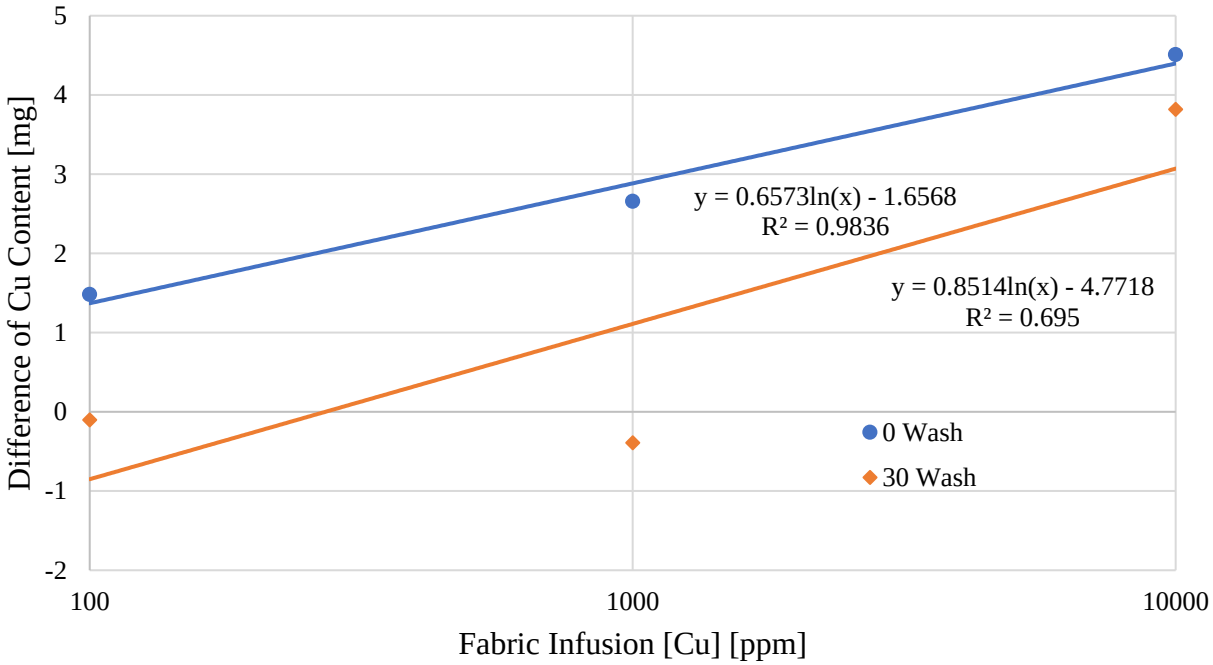


Figure #26: Difference in AC5 copper content compared to Beryl copper content at varying copper infusion concentrations.

From Fig 26, it is shown that there is a clear difference in the ability for the fabric to pick up copper. AC5 is generally more efficient and contains more copper per gram of fabric, but due to the issues with interfering ions, the 30 wash fabrics values vary much more. Table 5 shows that washing the fabric influences the copper content of the fabric, typically decreasing it by 50% or more. This presents an issue as if the active ingredient is being washed up in a laundering cycle the antimicrobial efficiency of the fabric may decrease. After washing, two of the fabrics increased in the amount of copper held onto the fabric. This is likely due to the type of water used in the washing procedure combined with the already low amounts of copper on the fabrics. Because the goal is to simulate a typical laundering cycle, tap water was used to see the effect on the fabric. This tap water can contain many other ions such as calcium or magnesium. These cations are also found as +2 ions which means that the EDTA solution used for titration would also be interacting with these cations [14]. This means that for all the 30 wash fabrics, each value is likely an overestimate of the actual copper content of the fabric, and the positive percent change results are artifacts from ions other than the antimicrobial copper. This effect is likely hidden at higher concentrations as the total amount of ions are unable to surpass the higher amount of copper on the unwashed fabrics. Another possible theory relates to the coatings. With washing, another possible artifact is that some copper may have a higher affinity to the fabric directly after infusion, where washing loosens this copper off the fabric, making it so the HCl can dissolve all the copper. Due to the presence of these artifacts, to improve the method, some possible changes are to use deionized water for the washing cycles to prevent interference from other ions, as well as to use a possible stronger acid to make sure everything is dissolved.

A major factor to note is the idea of diminishing returns with fabric infusion. Here the infusions were done by factors of 10. As fabric is infused, the higher the concentration of the initial infusion solution, the less overall copper binds. Linking to the binding site idea from earlier, if the fabric is over infused with higher and higher concentrations of copper, less binding occurs, as there are only so many spaces copper can bind. After saturating these spaces, the only way for more copper to absorb to the fabric is if it deposits on the surface of the fabric, or it can coat the fabric and bind to itself to form a thicker coating.

3.5.1 Copper Content Influence on Color Change

Now that the fabric's ability to absorb copper has been determined, the relationship between the amount of copper on the fabric and its color is explored. From this, the degree of color change can be predicted for an infusion based on the amount of copper used for infusion. This is demonstrated in Fig. 27. Fabrics at 30 washes are excluded due to the copper level artifacts.

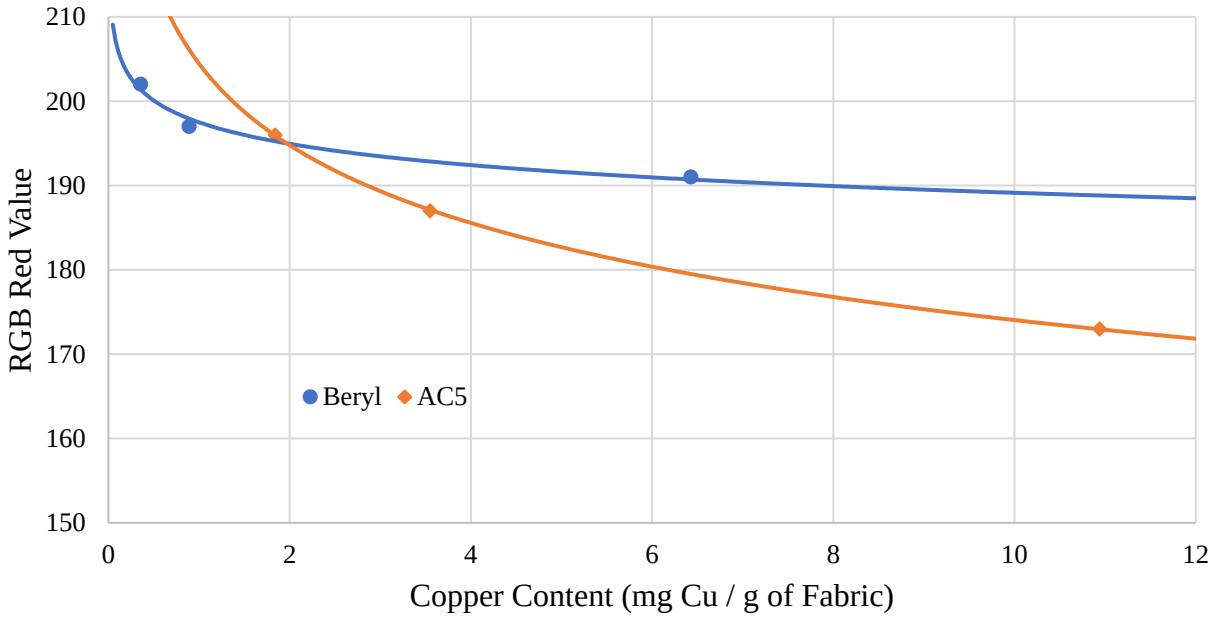


Figure #27: Relationship of copper content and red RGB value of the Beryl and AC5 fabric at 0 laundering cycles.

From this figure, it is shown that the copper content in the fabric is not the only determining factor for color. The Beryl and the AC5 fabrics have their own distinct curves for their relationship of copper content and color. Points on Fig. 27 start closer together but begin to diverge at higher copper content. This suggests that beyond just copper content, there is another factor that is causing the color change to be more drastic in the case of AC5. This is due to the differences in the infusion process, where the Beryl pre-treatment is causing the reduction in the color. Alternatively, both infusion methods use a source of cupric ions, but the difference in the source may also influence how intensely the fabric is colored.

3.6 Scanning Electron Microscopy

Images taken at 100x magnifications are outlined first, starting with the control fabric, shown in Fig. 28.

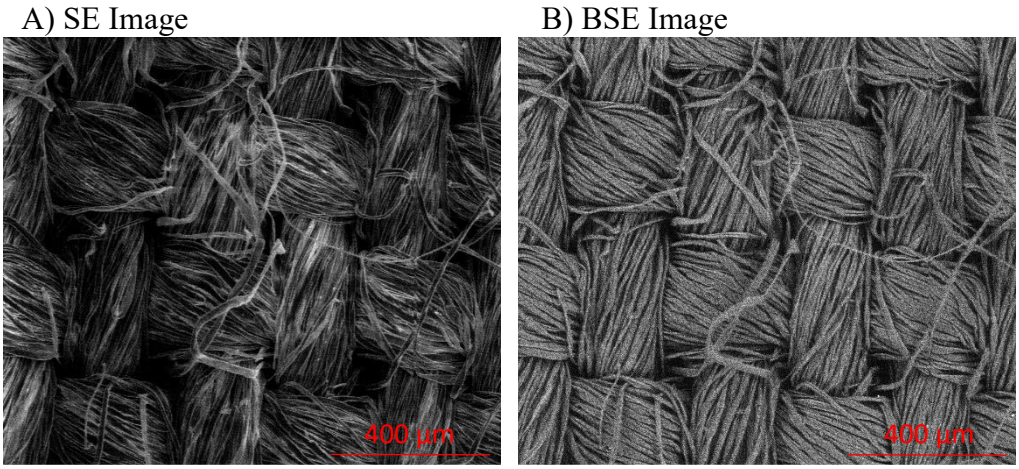


Figure #28: Control fabric 100x macro images.

Starting with the control fabric, the general structure of the fabric itself can be observed. Each of the threads is woven with other threads to form the lattice. There is no contrast due to there not being other elements besides what is present in cellulose. The bright spots on the SE image were artifacts due to the edge effect. Next, the Beryl fabric macro images are shown in Fig. 29.

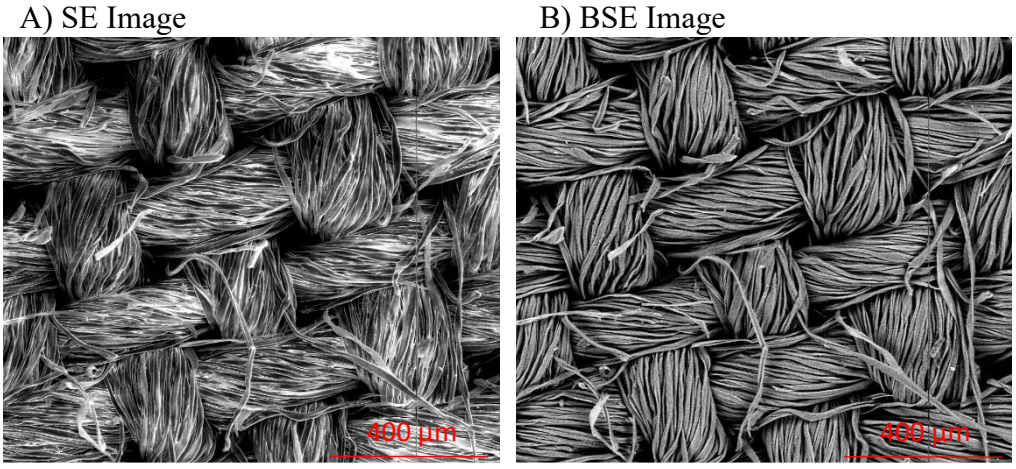


Figure #29: Beryl fabric 100x macro images.

The Beryl fabric from the macro point is like the control. The only difference is that within the BSE micrograph there seemed to be very small deposits of copper strewn about the fabric. These are likely artifacts of the copper infusion and are what likely is one of the first copper sources that

are washed away during a laundering cycle. Next the copper sulfate macro images are shown in Fig. 30.

A) SE Image B) BSE Image

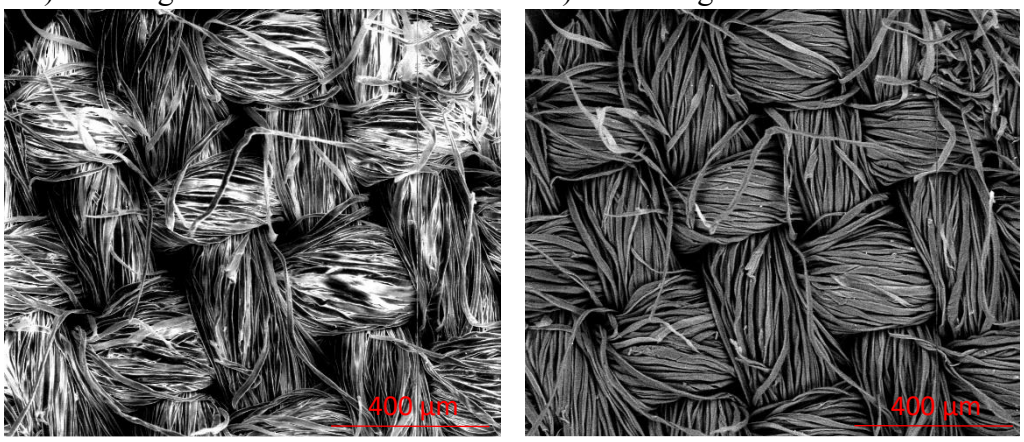


Figure #30: Copper sulfate fabric 100x macro images.

This fabric is like the Beryl images, where the common feature is small amounts of copper deposits within the fabric found in the in BSE image. It appeared that the copper sulfate fabric has a larger number of these copper deposits. Next the AC5 macro images are shown in Fig. 31.

A) SE Image B) BSE Image

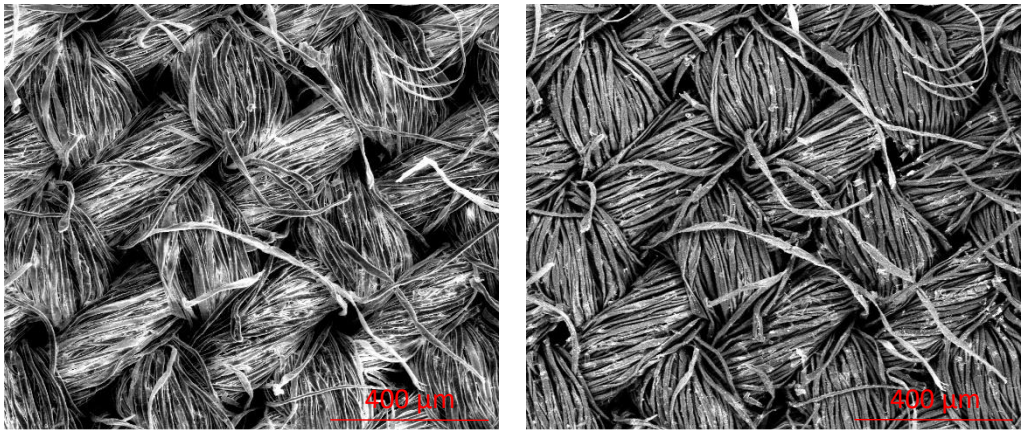


Figure #31: AC5 fabric 100x macro images.

AC5 provides the most contrast within the macro images with respect to the BSE image, here the abundant bright spots across the fabric suggested the ubiquitous presence of copper. This is consistent with the observations of copper content earlier where AC5 was able to absorb copper much more efficiently from the treatment baths.

Next, the individual fabrics are imaged to see the features of the coatings on the fiber scale, starting with the control fabric, shown in Fig. 32.

A) SE Image B) BSE Image

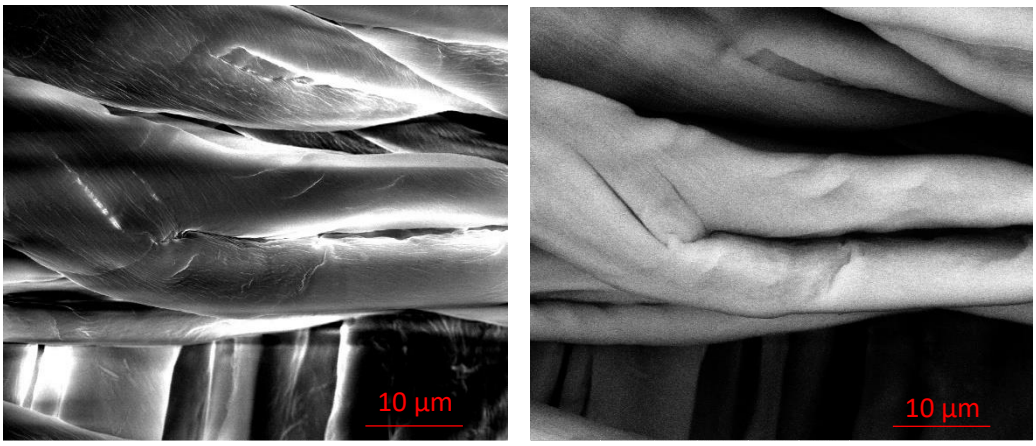


Figure #32: Control fabric 2500x micro images.

From the closeup images, rough surface features of the fabric such as small ridges were visible. Not much contrast is seen from the BSE micrograph as the fabric composition is uniform with very similar atomic mass atoms composing the fabric. Next, the Beryl fabric micrograph is shown in Fig. 33.

A) SE Image B) BSE Image

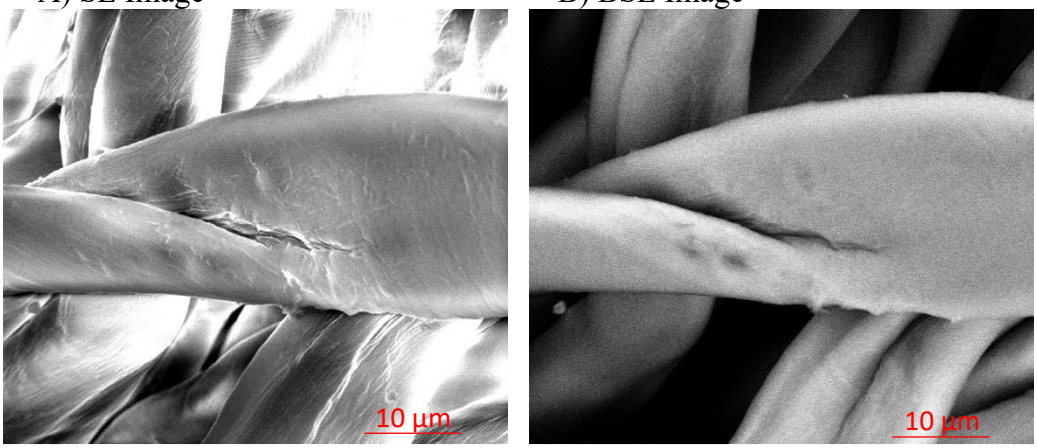


Figure #33: Beryl fabric 2500x micro images.

The Beryl fabric looks very similar to the control fabric in its morphology. The same ridges were present within both fabrics. Copper present on the fabric surface would contrast brightly on the fabric, but it looked uniform over the top of the fabric. There are two shaded areas below the crevasse area on both images but those are likely shadows formed from the complex structure of

the fabric. This suggests that there may be a very thin coating on the Beryl fabric. Next, shown in Fig. 34. are copper sulfate fabric micrographs.

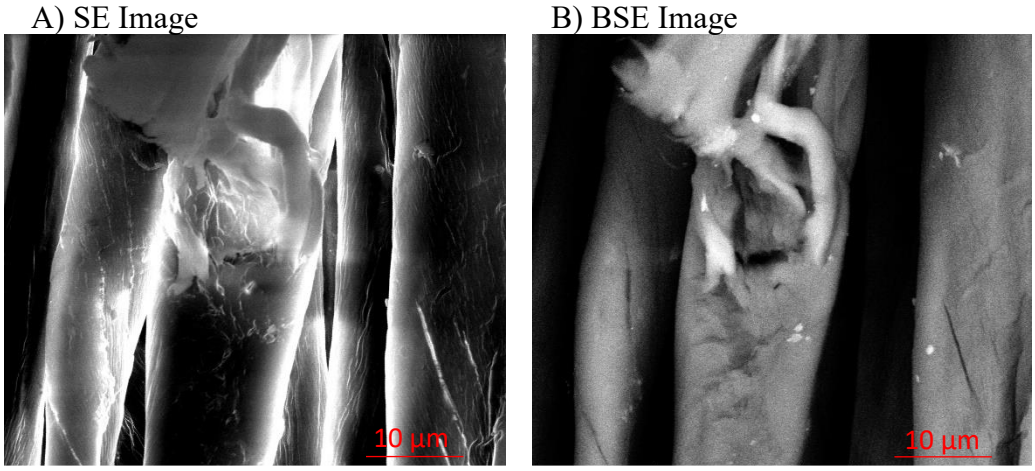


Figure #34: Copper sulfate fabric 2500x micro images.

On the copper sulfate fabric, The BSE image copper deposits are clearly visible, contrasting very strongly with the cellulose. Lastly, the AC5 micrograph is shown in Fig. 35.

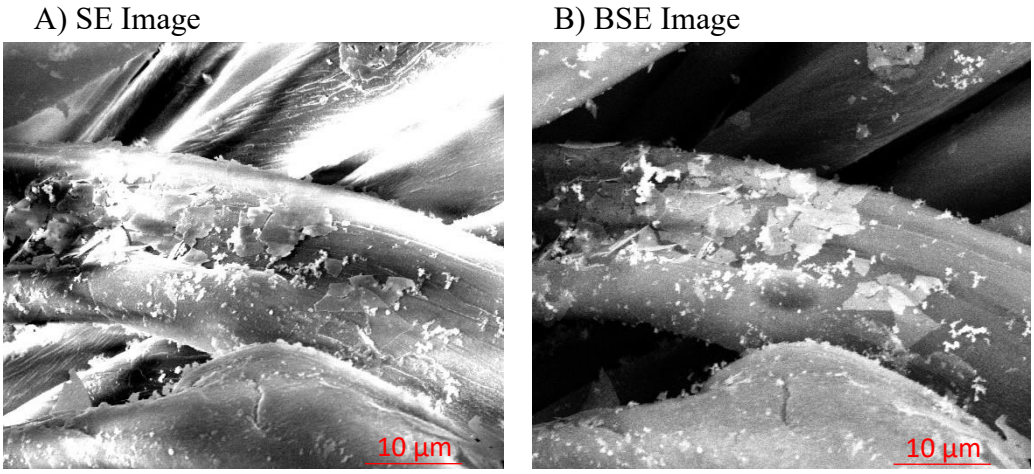


Figure #35: AC5 fabric 2500x micro images.

There is a clear difference in the amount of copper contained on the AC5 fabric. From the SE image there are clear plate like structures on the surface of the fabric. These seemed to be part of an outer coating that had broken off the fabric and deposited on it. There is a large mound on the bottom half of the middle fiber that seems to be part of the coating. The shattered plates appear to be approximately 5-10 µm across. The fiber on the bottom half of the image seemed to have a relatively intact surface coating of copper on top of it.

With all these micro images an insight into the properties of the coatings of each of the fabrics can be gained. The copper sulfate seemed to have a few copper deposits strewn around the fabric. The Beryl fabric was much more uniform with a lack of copper deposits. To determine the extent of deposits on the Beryl fabric the fabric had additional micrographs taken, with the only evidence of copper deposits on the head of a shredded Beryl fiber. This is shown in Fig. 36.

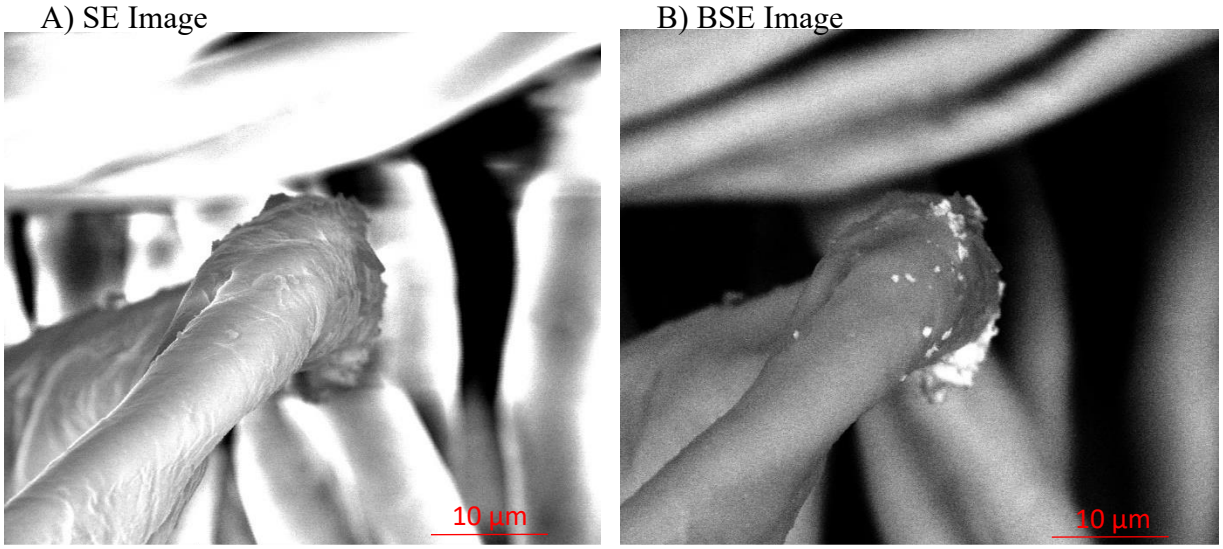


Figure #36: Beryl fabric 2500x evidence of copper deposit micrograph.

The deposits are likely CuO deposits. There is no reducing agent interacting with the fabrics, so formation of elemental copper is unlikely. The high heat during the drying process of the fabric likely caused a reaction between the cupric ions and the oxygen in the air forming the deposits. Presence of the deposits mean there is a possible thin coat of copper on top of the Beryl fabric. The pretreatment was designed to adhere to the cellulose fabric and provide a scaffold for the copper. This is evidenced by the fact that there is no visible copper besides a fractured fabric. This is due to a property of the SEM: the accelerating voltage. Higher voltages cause larger interaction depths (see Fig. 14.). The SEM was using an accelerating voltage of 20 kV, which means that the penetration depth is much greater, thus causing any thin surface level features to be unobservable on the BSE detector [15]. This still conforms with the theory of the thin coating on the Beryl fabric, but for the AC5 fabric this also means that the copper coating is significantly thicker compared to the Beryl fabric. This is seen through the countless layers and fractured plates that are ubiquitous on the AC5 fabric. The fracturing of the layers on the AC5 fabric also suggests that the coating found on it is quite brittle, as there were no mechanical tests done on top of the fabric, yet there is still a significant amount of fracturing. The copper content experiments can be linked to the SEM images. It was shown that there is much more copper on the AC5 fabric, but according to the Beryl

copper content tests there should be an adequate amount of copper infused at 1000 ppm infusion, but this copper does not seem to be visible through the eye of SEM. This can be confirmed through the usage of another technique called Energy Dispersive X-Ray Spectroscopy (EDS).

3.6.1 EDS

For taking EDS spectra, approximately 10 μm^2 areas were scanned to create the EDS spectra. Spectra were analyzed to determine the relative copper content of the fabric. Shown in Fig. 37 is the spectrum for the Beryl fabric.

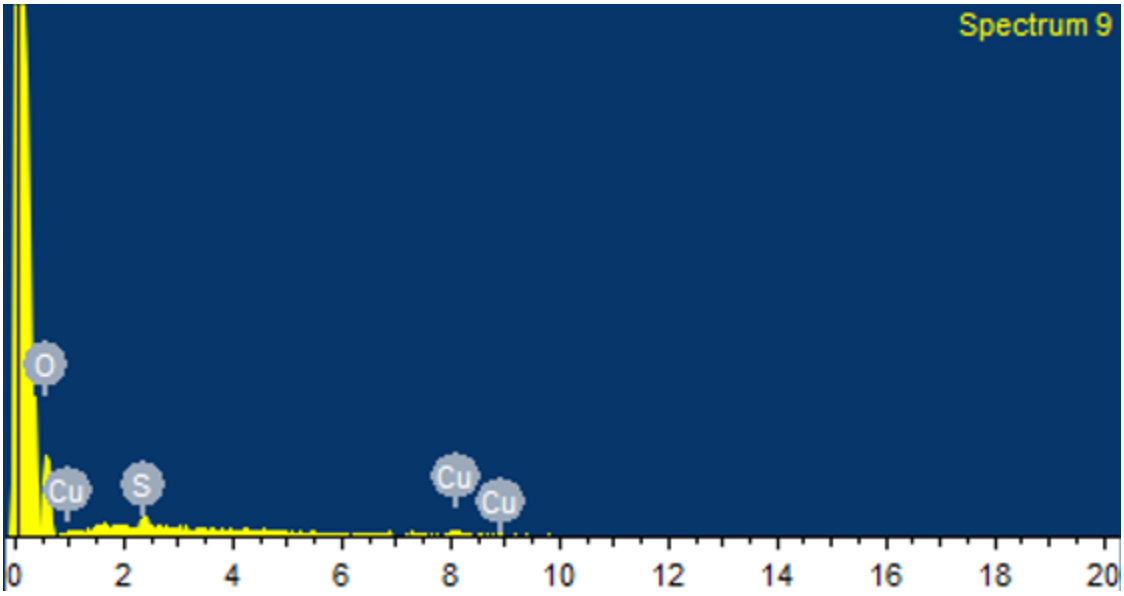


Figure #37: EDS spectrum of Beryl fabric.

This spectrum shows that there are relatively small amounts of copper located on the fabric, as indicated by the very small peak heights. The figure mostly contains oxygen and carbon, the latter having its peak likely hidden by the strong oxygen peak. The next spectrum shown is the AC5 fabric spectrum, shown in Fig. 38.

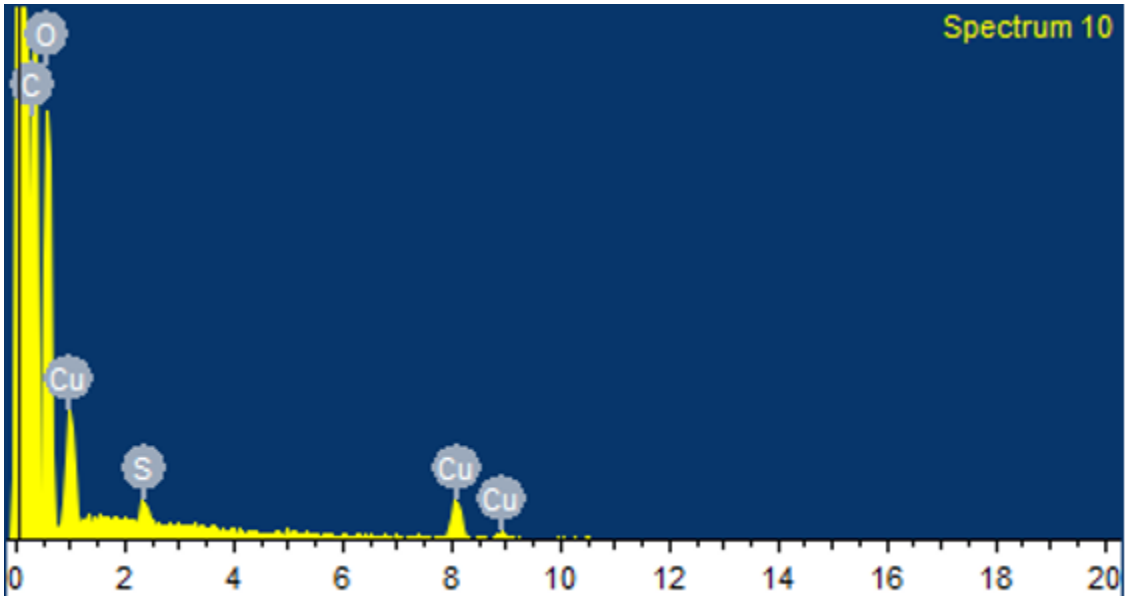


Figure #38: EDS spectrum of AC5 fabric.

Compared to the Beryl, there is a much greater peak of copper within the spectra. This is consistent with the images and the results of the copper content tests as a greater quantity of copper is visible on the surface of the fabric, as well as shown from the copper content tests, where 1000 ppm AC5 fabric contained around 2.7 mg/g more of copper per fabric. Here the carbon and oxygen peaks are also deconvoluted and visible.

From EDS the presence of small amounts of copper on the Beryl fabric can be confirmed. In general, maximizing the amount of copper on the fabric increases the antimicrobial activity, however, the Beryl process does not infuse the fabric with as much copper as AC5. This seems concerning, but this property of Beryl to hold on to less copper is what allows its color change resistance, an important characteristic for consumers. Although it is important for the fabric to be color change resistant, the most important property is the antimicrobial efficiency, discussed in the next section.

3.7 Antimicrobial Efficiency

3.7.1 Beryl Antimicrobial Efficiency

To determine the efficiency (antimicrobial efficiency in percent, or the % reduction / % efficiency), a total of 36 plates were prepared using the method as described in section 2.9.2. The number of colonies on a control plate with uninfused fabric are counted and compared to the colony counts of infused fabric. Efficiency is calculated using

$$\%Efficiency = \frac{Control\ Colony\ Count - Test\ Colony\ Count}{Control\ Colony\ Count} * 100\% \qquad (3.2)$$

Using the raw counts, the efficiency is calculated for all the tested fabrics. The efficiency experiments results are given in Tables 6-10 for various conditions for various conditions including control or treated fabric and washed or unwashed. Samples were plated in duplicate. Plates are shown in Appendix 1. The efficiency results of the 0 wash AC5 fabrics are shown in Table 6.

Table #6: Antimicrobial efficiency of 0 wash AC5 fabrics at varying copper infusion concentrations.

Sample Name	Colony Counts	Average Colonies	% Reduction
Negative Control 1	85	114	
Negative Control 2	142		
AC5 100 ppm 1	0	0	100
AC5 100 ppm 2	0		
AC5 1000 ppm 1	0	0	100
AC5 1000 ppm 2	0		
AC5 10,000 ppm 1	0	0	100
AC5 10,000 ppm 2	0		

The Beryl and copper sulfate fabrics at 0 washes are shown in Table 7.

Table #7: Antimicrobial efficiency of 0 wash Beryl and copper sulfate fabrics at 1000 ppm infusion concentration for copper sulfate and varying copper infusion concentrations for Beryl.

Sample Name	Colony Counts	Average Colonies	% Reduction
Negative Control 1	168	184	
Negative Control 2	199		
Copper Sulfate 1	0	0	100
Copper Sulfate 2	0		
Beryl 100 ppm 1	0	8	96
Beryl 100 ppm 2	15		
Beryl 1000 ppm 1	0	0	100
Beryl 1000 ppm 2	0		
Beryl 10,000 ppm 1	0	0	100
Beryl 10,000 ppm 2	0	0	100

The AC5 fabrics after 30 washes are shown in Table 8.

Table #8: Antimicrobial efficiency of 30 wash AC5 fabrics at varying copper infusion concentrations.

Sample Name	Colony Counts	Average Colonies	% Reduction
Negative Control 1	80	82	
Negative Control 2	84		
AC5 100 ppm 1	0	0	100
AC5 100 ppm 2	0		
AC5 1000 ppm 1	0	0	100
AC5 1000 ppm 2	0		
AC5 10,000 ppm 1	0	0	100
AC5 10,000 ppm 2	0		

The Beryl and copper sulfate fabrics at 30 washes are shown in Table 9.

Table #9: Antimicrobial efficiency of 30 wash Beryl and copper sulfate fabrics at 1000 ppm infusion concentration for copper sulfate and varying copper infusion concentrations for Beryl.

Sample Name	Colony Counts	Average Colonies	% Reduction
Negative Control 1	102	117	
Negative Control 2	131		
Copper Sulfate 1	2	1	99
Copper Sulfate 2	0		
Beryl 100 ppm 1	14	12	90
Beryl 100 ppm 2	9		
Beryl 1000 ppm 1	1	5	96
Beryl 1000 ppm 2	9		
Beryl 10,000 ppm 1	0	0	100
Beryl 10,000 ppm 2	0		

The results are summarized in Table 10.

Table #10: Antimicrobial efficiencies pre and post wash.

Sample Name	0 Wash % Reduction	30 Wash % Reduction
Copper Sulfate 1000 ppm	100	99
AC5 100 ppm	100	100
AC5 1000 ppm	100	100
AC5 10,000 ppm	100	100
Beryl 100 ppm	96	90
Beryl 1000 ppm	100	96
Beryl 10,000 ppm	100	100

With plating, the efficiency of the Beryl fabric is determined. At 0 washes Beryl can retain around 100% efficiency on all the trials, except for one. This exception is likely an error of contamination, as evidenced by lower colony counts in the 30-wash fabric, shown in Table 9. Furthermore, the other 100 ppm Beryl fabric also tested at 100% efficiency, meaning that the 15 colonies were likely contamination. The AC5 fabric was able to test at 100% efficiency for all the trials.

For laundering resistance, Beryl maintains a greater than 90% efficiency at 30 washes, which remains a high antimicrobial efficiency. Fabrics that used a similar dip coating method for infusion comparatively had lower laundering resistances than the Beryl method, where dip coating ranged in resistance from 5-30 laundering cycles [7]. Despite this, more robust infusion methods allowed for resistance of 65 cycles for the fabric [7].

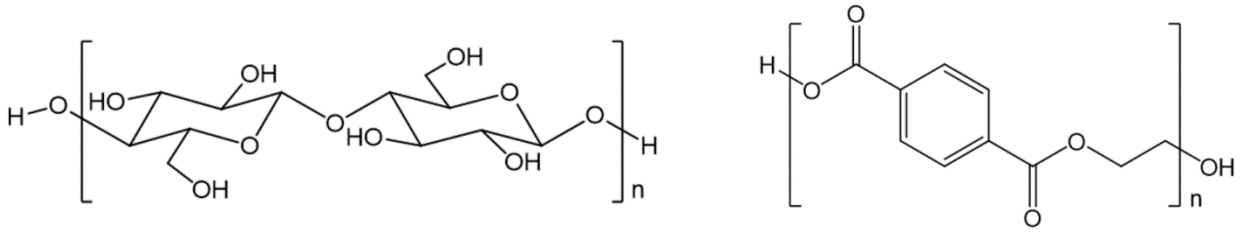
From the copper content tests and SEM micrographs, this is consistent with what is expected in the trials. From Table 3, it is shown that the amount of copper on the Beryl fabric falls between the AC5 100 ppm and 1000 ppm fabrics, so it is valid that it is the only fabric to survive with 100% efficiency. Using this information, the amount of copper on the Beryl fabric can be fine-tuned based on the concentration of the copper infusion bath. By increasing the copper infusion bath to a value between 10,000 and 1000, copper levels like the 100 ppm AC5 fabric can be achieved, which would provide 100% efficacy.

The copper sulfate fabric was able to maintain a high efficiency throughout the trials, only dropping 1% efficiency in the 30-wash fabric. Despite the high efficiency, the copper sulfate fabric does not cling to copper as efficiently as AC5 can. Copper sulfate was infused at 1000 ppm, but the amount of copper on the fabric was very close to the AC5 fabric, where the difference was .01 mg/g between the fabrics. Therefore, it should perform similarly at 0 washes. After washing, however, the copper sulfate failed to achieve 100% efficiency, while the AC5 fabric maintained 100% efficiency, which suggests the source of cupric ions from AC5 can kill more efficiently.

A variable that can influence results shown in Table 9 and 7 relates to the Beryl process itself. As mentioned, there is a pretreatment for the Beryl fabric, and this pretreatment can imbue the fabric with antimicrobial properties. This is further compounded through the addition of copper which adds additional amounts of antimicrobial activity to the fabric. This is further described in Section 3.1.4. What this means is similar amounts of antimicrobial activity can be obtained in the Beryl, with 0 wash efficiencies comparable to the AC5 process, despite the lower copper content on the fabric. This pretreatment coating can also act as a downside to the Beryl fabric. Because this pre-treatment prevents as much binding of copper, by washing the fabric, you lose copper, as well as you lose some of the pretreatment coating from the fabric. Since there was already less copper to begin with, this can detriment the fabric, as shown in the 30 wash results, where Beryl lost its 100% effectiveness against the bacteria.

Another factor to consider is the fabric itself. For the experiments ISO adjacent cotton was used, which is a thin sheet of cotton. Typical clothes that are worn can contain multiple layers of fabric, which can influence the amount of coating that is on the fabric. Not only the fabric thickness, but the composition of the fabric can influence the coating strength. Something like cellulose contains plenty of hydroxyl groups, which can bind easily with the coatings, due to the group reactivity

[30]. Polyester on the other hand contains many more stable ester groups which are not as easily bound by the coating. An example of cellulose and an example polyester are shown in Fig. 39.



Cellulose Polyester

Figure #39: Cellulose and polyester polymer molecular structure. Figure taken and adapted from [30].

Another factor that compounds with this is dyes. In dyeing a fabric, one important consideration to make is the type of fabric that is being used. This alters the types of dye that can be used. In the case of cellulose fibers, reactive dyes are often used to bind to the cellulose fiber to impart a color [29]. This means that the dyes themselves take up binding spots on the cellulose fabric causing there to be less copper load on the fabric, as the copper cannot bind. This can be shown in Fig. 40, where a micrograph of black AC5 fabric shows it is not coating the fabric the same as in Fig. 35.

A) SE Image B) BSE Image

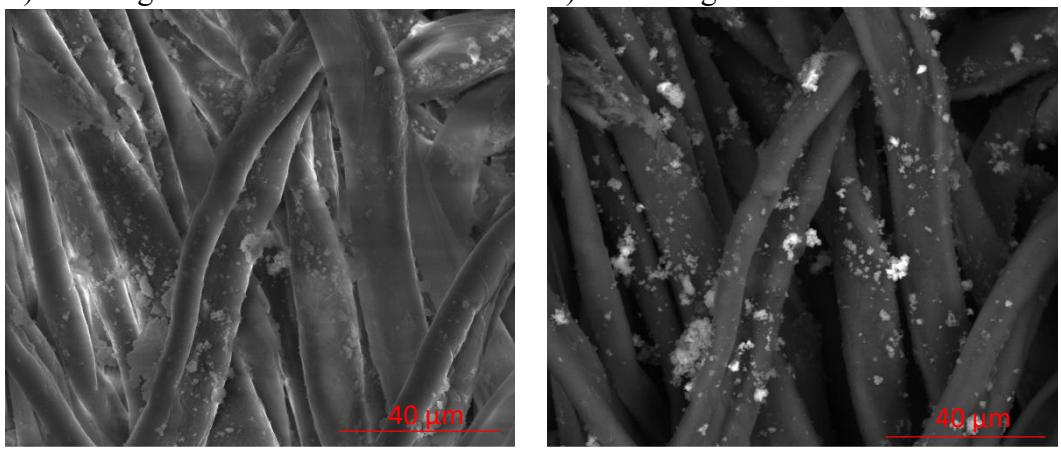


Figure #40: SEM micrographs of black AC5 fabric.

The copper appears to not have a thick coating as in Fig. 35, where there are clear fractured coating structures. Rather, the copper has deposited on the surface of the fabric causing large clumps of copper to be strewn about the fabric, making an uneven coating.

In general, all fabrics tested have shown a 90% or greater antimicrobial efficiency at the different infusion concentrations. This efficiency, however, can depend on the type of fabric being used, with Beryl fabric having its efficiency drop a maximum of 10%

3.7.2 Effect of Pre-Treatment

To determine the effect of the Beryl pre-treatment, the same antimicrobial efficiency test was run, with one difference being the plate incubation time. Plates were incubated at room temperature for 72 hours over the weekend. Shown in Fig. 41 is a plate of a fabric with only the pre-treatment done, no copper added and tested at 0 washes, showing no colonies, resulting in a 100% reduction result.

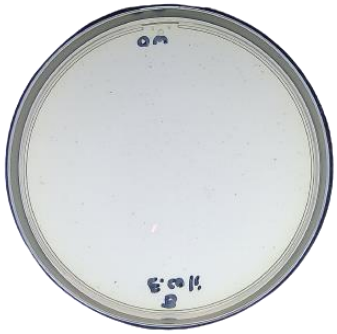


Figure #41: Spread plate using fabric only treated with Beryl pre-treatment and no copper.

The plate in Fig. 41 has 100% kill efficiency. This means that the Beryl pre-treatment is very effective at killing bacteria. However, the efficacy reduced greatly after 30 washes, and the fabric became ineffective at killing microbes. Furthermore, this was tested alongside normal Beryl with a 10,000-ppm copper infusion. Both fabrics failed completely with regards to efficiency. The plates are shown in Fig. 42.

A) B)

Figure #42: Spread plates of 30 wash A) 10,000 ppm Beryl fabric and B) pre-treatment only Beryl fabric incubated at a plate incubation time of 72 hours.

The antimicrobial activity of the Beryl coating was assessed to determine whether the fabric is bactericidal or bacteriostatic. According to the plates in Fig. 42, Beryl is bactericidal when at 0 wash and reverts to bacteriostatic after 30 washes. From Table 9 it was shown that the efficiency decreased at 30 washes for all Beryl fabrics except for the 10,000-ppm fabric. However, this contradicts what is shown in Fig. 42. This means Beryl is likely a bacteriostatic fabric after washing, as the bacteria grew over the 72 hours. Contamination from other bacteria can be eliminated as a

factor in this as tetracycline plates are used, and the colonies appear rough which is in line with the characteristics of K-12 *E. coli* [25].

Bacteriostatic materials can be useful for the prevention of illness, even if they do not stop microbial growth completely. For example, a major usage can be for clothing. In the case of clothing the contact times could be longer. For example, medical gowns being worn for an operation would have a contact time spanning all the wear time, constantly stopping or slowing the growth of bacteria.

3.8 Microbial Contact Time Efficiency

The microbial count data of 1000 ppm Beryl was determined experimentally for various contact times. For this study, 26 plates were used, and samples were run in singlicate. Formula 3.2 was used to determine the % Reduction for the various contact times, and the results are shown in Table 11. Images of all the plates with their corresponding control plates can be found in Appendix 2.

Table #11: Contact time efficiency of Beryl 1000 ppm fabric.

Time (s)	*Control Plate Count*	*Beryl Plate Count*	*% Efficiency*
5	490*	172	65
15	550*	450*	18
30	428*	244	43
45	450*	75	83
60	584*	281	52
120	350*	73	79
180	440*	12	97
240	375	48	87
300	319	0	100
900	288	0	100
1800	271	0	100
2700	269	0	100
3600	215	0	100

Please note values marked * were estimates, as the plates were overgrown, so a representative half of the plate was counted, and that value multiplied by 2. The data is represented in Fig. 43. to show how efficiency changes over time.

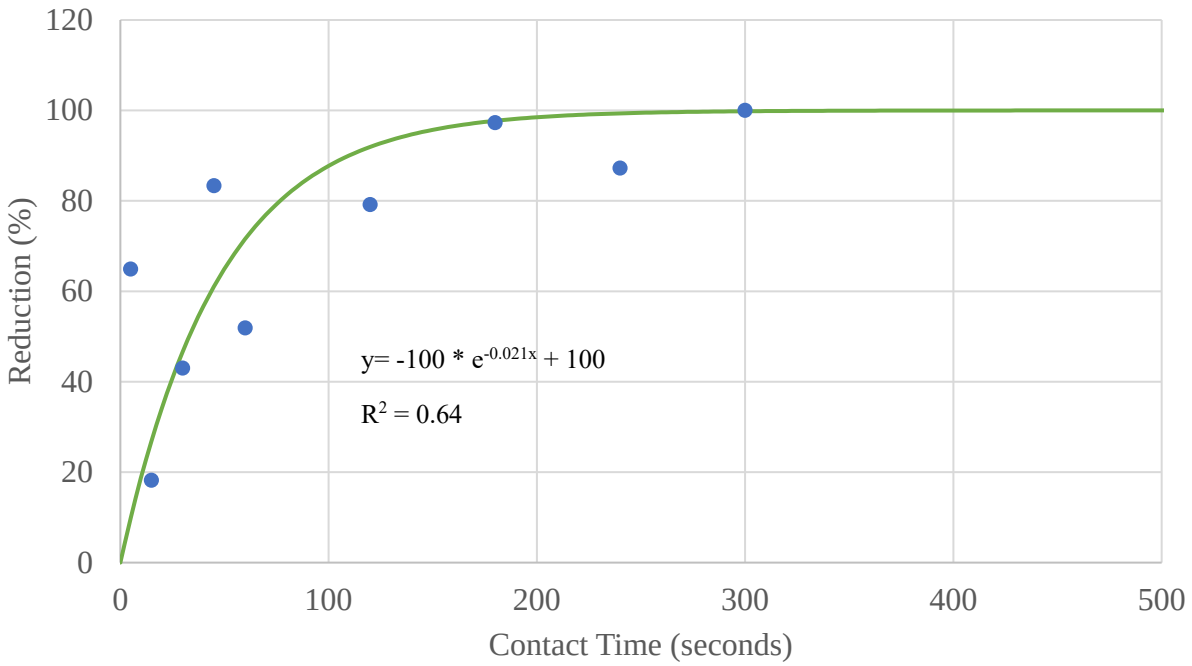


Figure #43: Beryl 1000 ppm % reduction vs. contact time. Solid line is fit to points with function and correlation value given in plot area.

To create the line of best fit certain constraints are set. The first is the assumption that at t=0 the fabric is ineffective. To fit the data curve, an exponential function is used that is reflected over the x and y axis. Lastly, the horizontal asymptote occurs at y=100 to represent the max possible efficiency. These parameters lead to the base function of

$$y = -b * e^{(-ax)} + 100 \quad (3.3)$$

Now constants are needed. For b, since the function must go through the origin, b is set to 100, which leads to

$$y = -100 * e^{(-ax)} + 100 \quad (3.4)$$

Last, the contact time coefficient a is calculated. This variable determines how fast the fabric reduces bacterial viability, where the value is proportional to the rate of efficiency. To determine the value Microsoft Excel and solver are used to fit Equation 3.3 to the data from Table 11. Solver is a program for Excel that allows for analysis by varying a value to determine maximums or minimums. The parameters for solver were minimizing the standard error by varying the a value. This gives an a value of 0.02098 s^{-1} or approximately 0.021 s^{-1}, which leads to

$$y = -100 * e^{(-0.021x)} + 100 \quad (3.5)$$

This equation has a correlation coefficient of approximately 0.64 for this model, as well as a standard error of 18.94 for the value of a in this equation.

The amount the efficiency value moves around influences the error of the parameter value in this model. A certain error is bacteria always vary from sample to sample, as well as due to the large amounts of growth on the plates causing estimates to be used rather than exact counts. Rather the trends present within the model are useful for analysis. First, the onset of high antimicrobial efficiency begins at 45 seconds, while complete efficiency begins at around 300 seconds or 5 minutes. This is valuable as the 1000 ppm Beryl can begin disinfection within a minute and can completely inactivate the bacteria within 5 minutes of contact. This can be compared with copper surfaces, where it was found that with wet contact, it could take 65 minutes to completely inactivate the bacteria [31]. This is due to the time it takes for the liquid to evaporate on the metal surface [31]. However, since its surface is hydrophilic and can absorb the liquid, the fabric is able to interact with the bacteria suspended in the water droplet more efficiently. Comparing to wet disinfectants, such as chlorine, the Beryl fabric can still retain a similar disinfection time [32]. Chlorine also disinfects most of the bacteria within one minute of contact time [32]. This means dry Beryl hosts a rapid disinfection time, comparable to that of a liquid disinfectant. This liquid method was used to simulate a sneeze where bacteria may be suspended in tiny water droplets, which can land on top of clothes and be absorbed.

Furthermore, the pretreatment for Beryl is also imparting antimicrobial properties on the fabric. This also causes faster kill times when compared to bacteria on a pure copper surface.

Chapter 4: Conclusion

4.1 Conclusions

An improved process for the infusion of cupric ions into fabric was successfully developed, and the resulting fabric was called Beryl. Comparison between the Beryl process and the pre-existing process was partially successful, with complete results available concerning the characteristics of Beryl about color change resistance which make Beryl more appealing compared to the AC5 fabric. More work is suggested, described in Section 4.2, to add rigor to the comparisons for laundered fabrics. With additional machinery a full comparison of Beryl to other infusion procedures could be elucidated.

4.2 Recommendations

The Beryl process does require more changes in the infusion procedure. Dipping the fabric is a rapid method for infusion, but it can cause unequal distribution and is not controllable for consistency. To alleviate this, implementation of standard pad-dry-cure methods could be used to have a uniform coating on the fabric, where liquid does not well on the edges of the fabric.

Methods used in this thesis can be improved. To improve results of the copper content tests, addition of more samples would allow for more validity by determining whether the variation is due to uneven coating. If uneven coating is not a factor, improvements such as using stronger acids for complete dissolution of the fabric and copper ions would provide more accurate results. To eliminate any error from the interfering ion effect, usage of an Inductively Coupled Plasma machine would allow for elucidation of specific ions. For taking fabric images, decreasing the accelerating voltage of the SEM would possibly allow for better visualization of thin coatings on the fabric, and for estimates of fabric thickness. For the antimicrobial testing, usage of standard methods such as zone of inhibition plates would allow for direct comparisons to many other fabrics. Usage of different bacteria would also provide more insight into the antimicrobial capabilities of the fabric, as well as see what characteristics of bacteria may provide resistance to the fabric coating. For the contact efficiency time experiments, usage of lower dilutions would allow for more accurate counts instead of estimation if required.

Due to the presence of metal ions, Beryl has the potential to conduct electricity along the fabric, which would allow for the integration of sensor components within the fabric. This could lead to the generation of antimicrobial “smart” fabrics. Further comparisons could be made between the fabrics with respect to the effects on the skin as well as any possible toxicity that the fabrics may have.

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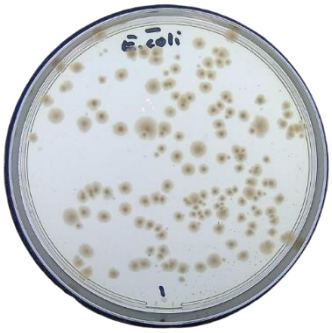
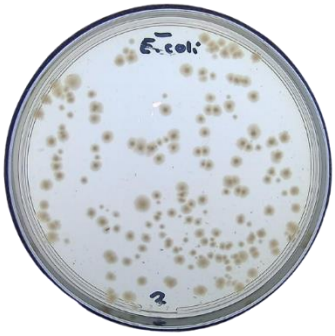
Appendix 1: Efficiency Experiment Plate Images

1.1 Beryl and copper sulfate fabric 0 wash plate images



Negative Control 1



Negative Control 2

$CuSO_4$ 1

$CuSO_4$ 2

Beryl 100 ppm 1

Beryl 100 ppm 2

Beryl 1000 ppm 1

Beryl 1000 ppm 2

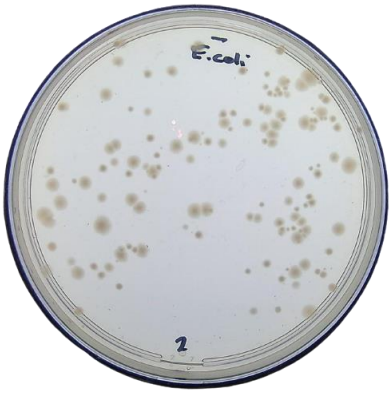
Beryl 10,000 ppm 1

Beryl 10,000 ppm 2

Please note for this set of plates, due to inclement weather, plates were left out for 24 hours at room temperature before incubation, which is the reason for the darker center dots and larger colonies.

1.2 AC5 fabric 0 wash plate images

Negative Control 1



Negative Control 2

AC5 100 ppm 1

AC5 100 ppm 2

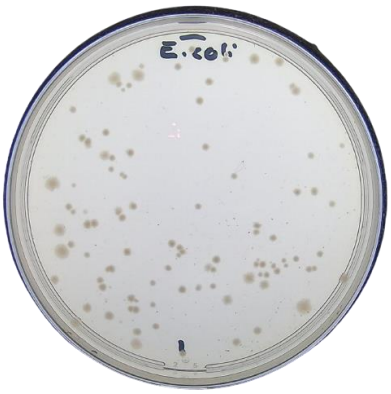
AC5 1000 ppm 1

AC5 1000 ppm 2

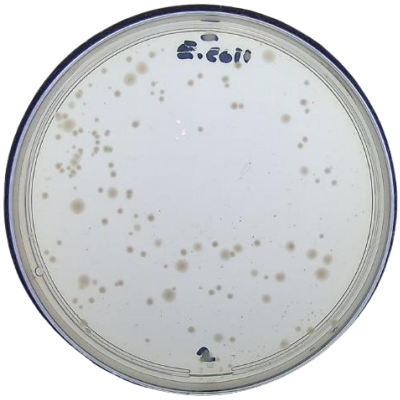
AC5 10,000 ppm 1

AC5 10,000 ppm 2

1.3 Beryl and copper sulfate fabric 30 wash plate images



Negative Control 1



Negative Control 2

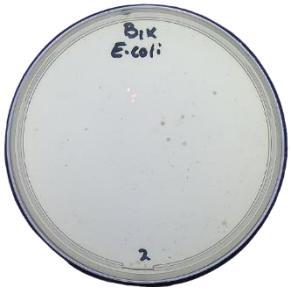
$CuSO_4$ 1

$CuSO_4$ 2

Beryl 100 ppm 1

Beryl 100 ppm 2

Beryl 1000 ppm 1

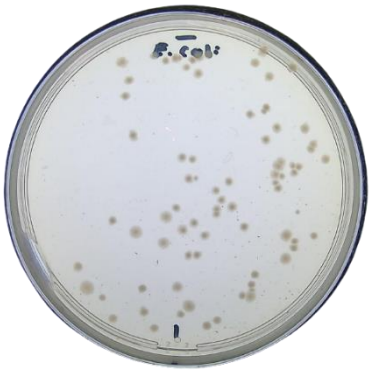


Beryl 1000 ppm 2

Beryl 10,000 ppm 1

Beryl 10,000 ppm 2

1.4: AC5 fabric 30 wash plate images



Negative Control 1

Negative Control 2

AC5 100 ppm 1

AC5 100 ppm 2

AC5 1000 ppm 1

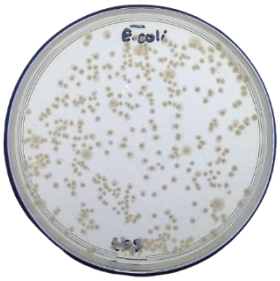
AC5 1000 ppm 2

AC5 10,000 ppm 1

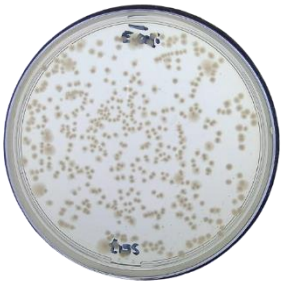
AC5 10,000 ppm 2

Appendix 2: Contact Time Efficiency Plate Images

Negative Controls



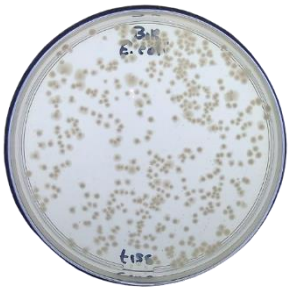
t = 5 seconds



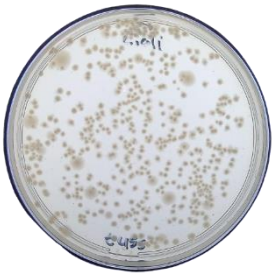
t = 15 seconds

t = 30 seconds

Beryl Plates



Negative Controls



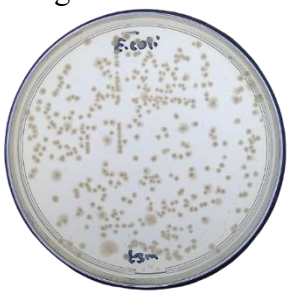
t = 45 seconds

t = 1 minute

t = 2 minutes

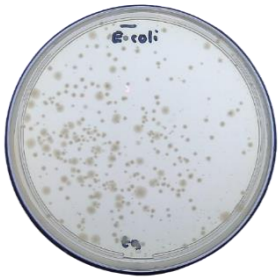
Beryl Plates

Negative Controls



t = 3 minutes

t = 4 minutes



t = 5 minutes

Beryl Plates

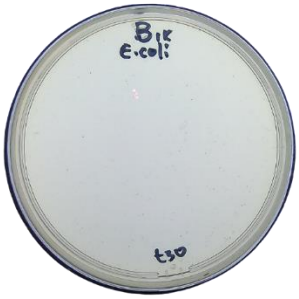
Negative Controls

t = 15 minutes

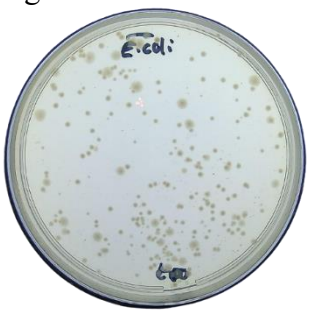
t = 30 minutes

t = 45 minutes

Beryl Plates



Negative Controls



t = 60 minutes

Beryl Plates