

Development and Characterization of an Antimicrobial Coating for Medical Textiles

by

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Author's 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.

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Abstract

This study investigates the antimicrobial capabilities of Ever-gel (EG) and its derivatives, which are enhanced with additives like silver nitrate, zinc chloride, and copper sulfide. This antimicrobial coating is to employ both contact-active and release-active mechanisms to effectively prevent the transmission of pathogens. From this thesis study, it was determined that the Ever-gel itself and the addition of zinc chloride did not prove to be statistically significant for a test, in which an EG coated medical textile (melt blown polypropylene) was subjected to an *E. coli* inoculum. Silver nitrate and copper sulfide both proved effective as antimicrobial additives in the same assessment and was found to produce statistically significant results in greater concentrations of both additives in EG. These samples had achieved a log reduction of 0.92 of *E. coli* in two hours using 0.5 wt% AgNO₃ in EG and 2.95 for samples containing 0.75 wt% of CuS. The mechanical, thermal, and chemical characterization of the EG containing the successful antimicrobial additive, CuS, was studied in varying concentrations. From this assessment, it was determined that increasing the concentration of CuS in EG increased the adhesive strength as concluded in the T-Peel test. In the peel adhesion assessment, it was found that the adhesive strength increased from 0.024 N for samples containing no CuS to 0.057 N for samples containing 0.75 wt% CuS. The Youngs modulus was seen to decrease from 44.3 kPa to 1.19 kPa with the increase of CuS in EG (from no CuS to 0.75 wt% CuS), indicating that the sample had gotten softer due to the inclusion of CuS. The chemical basis of EG in comparison to EG-CuS remains similar throughout all samples tested. It is recommended that future research should prioritize a comprehensive analysis of Ever-gel's contact-active antimicrobial capabilities possibly using fluorescence dye techniques. Further studies on the feasibility of EG coated fabrics, including reusable textiles, should investigate air permeability across coated and uncoated substrates, conduct wick tests with and without the coating, and assess the laundering

effectiveness of coated fabrics. Additionally, exploring the application of the coating on various substrate compositions and fiber diameters would provide valuable insights into its broad-spectrum effectiveness.

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List of Abbreviations

TNTC	Too Numerous to Count
TFTC	Too Few to Count
EG	Ever-gel
EG-CuS	Ever-gel containing Copper Sulfide
MB	Melt-blown
PP	Polypropylene
METAC	[2-(methacryloyloxy)ethyl]trimethyl Ammonium Chloride
GlyDMA	Glycerol dimethacrylate
SB	Spun bond
DEAP	2,2-Diethoxy Acetophenone
QAC	Quaternary Ammonium Compounds/cations (depending on context)
PA	Polyacrylamide
MBA	N,N'-Methylenebisacrylamide
AAMI	Association for the Advancement of Medical Instrumentation
AATCC	American Association of Textile Chemists and Colorists

Chapter 1 Introduction

Personal protective equipment (PPE) is the first line of defense to prevent transmission of said pathogens. Transmission of pathogens occurs from surfaces of contacted regions; while pathogens need an organism to perform their metabolic functions, residual pathogens remain on contact surfaces for extended periods of time. To address this issue, this thesis assessed the antimicrobial properties of a novel coating Ever-gel (EG) developed by EverBond Interface Technologies Inc. and then formulated for its potential use of coating healthcare-based PPE textiles. EG is synthesized according to the proprietary information provided by EverBond, where the polymer network is made of poly[2-(methacryloyloxy)ethyl]trimethyl ammonium chloride (METAC) and the liquid phase is a glycerol aqueous solution. METAC has previously demonstrated antimicrobial feasibility in applications involving co-polymerization of the material with another quaternary ammonium compound (QAC), grafting of the material, or the use of the monomer on its own as an antimicrobial surfactant. Glycerol and water combinations were previously studied to be chemically stable and capable of interstitial spaces of polymeric matrix. This characteristic is desirable for biomedical applications as it increases the biocompatibility of the coating. For this study, EG and its derivatives are evaluated for their antimicrobial, mechanical, and thermal properties as coatings for commonly used healthcare gowns.

This thesis consists of five Chapters. Following the introduction presented in Chapter 1. Chapter 2 provides a literature review on textiles and their properties, antimicrobial additives used in this study, and the components of EG. Chapter 3 discusses the findings and results for the antimicrobial feasibility of EG and its derivatives. Mechanical and thermal testing of the successful additive and EG will be discussed in Chapter 4. Chapter 5 will summarize the findings from this project and as to provide prospective goals for future experimentation.

Chapter 2 Literature Review

2.1 Medical Textiles

2.1.1 Existing textiles and applications within the healthcare industry

Healthcare based textiles refer to a specialized category of textiles designed and manufactured to specifically aid professionals and personnel within the healthcare setting. These textiles include a wide range of products including surgical gowns, bandages, linens, towels, exam table covers, and drapes. These textiles must also meet a specific criterion of standard for safety, quality, and functionality to ensure their compliance in a medical environment [1, 2, 3]. Disposable textiles were first marketed to have increased protective capabilities, cost-effective qualities, and more accessible for healthcare workers [4].

In recent years, progress in textile technology product design has resulted in the creation of synthetic and natural material-based textile methods. These methods enable the production of textiles with unique qualities and applications. Performance characteristics of such textiles include increased durability and strength, improved resistance against pathogens, and improved comfort for the user. As an example of product design, incontinence products, such as wearable pads and bed sheets, can remove liquid contaminants from the environment and the user [4]. These products are designed to keep skin dry, prevent seepage of bodily excrements, and to be of low irritability for the user. An example of this type of product is disposable face masks, which follow a design that allows for an outer protective layer and inner absorbent layer, combined to create a composite non-woven piece.

Non-woven and woven textiles are the two classifications of fabric structure commonly used in healthcare applications. These two types of textiles differ mainly from their most elementary components, the fibre or thread used in production. Non-woven textiles can be distinguished through fibres while woven textiles are distinguished through either thread or yarn [5]. Fibres are produced by

the melting of polymers then extruding of the melted polymer through small nozzles to produce filaments of a desired diameter [6]. The fibres are then bonded together through further mechanical and/or chemical processes. Woven textiles are produced through a process known as weaving and can be described as either single layer or multilayer textile sheets.

The process of manufacturing woven textiles involves two basic components known as the weft and warp, to turn yarn or thread into fabric. The warp component of the textile is held vertically in place while the weft is run horizontally across the loom to create an interlocking pattern along the woven textile. These properties of these elementary components influence the integrity, moisture permeation, comfort, and breathability of textiles [7].

2.2 Disposable and Reusable Textiles in Healthcare

Traditionally, textiles in healthcare settings were composed of reusable woven natural fibres. In the early 1900's the pore size of cotton textiles was substantially large thus susceptible to pathogens. More than one layer of sterile woven cotton was used to provide an aseptic barrier; however, more recent studies have shown that moisture on the fabric creates a passage for bacteria to enter between the layers of textiles [8]. As a result, there was an incentive to design water-repellent, hydrophobic structures in the fabric and to create fabrics of smaller pore sizes more modernly this can be seen in electrospun textiles for instance [9]. Figure 1 depicts possible textile manufacturing pathways of various types of materials including fibres that are manufactured from synthetic materials, metallic materials used in producing ropes and wires, and natural fibres. Non-woven textile performance are highly influenced by the web-formation, fibre selection, bonding, and finish techniques used in production. Manufacturers may use any type of available commercial fibre in its production. This includes, natural fibres, synthetic fibres, and any combination fiber [10].

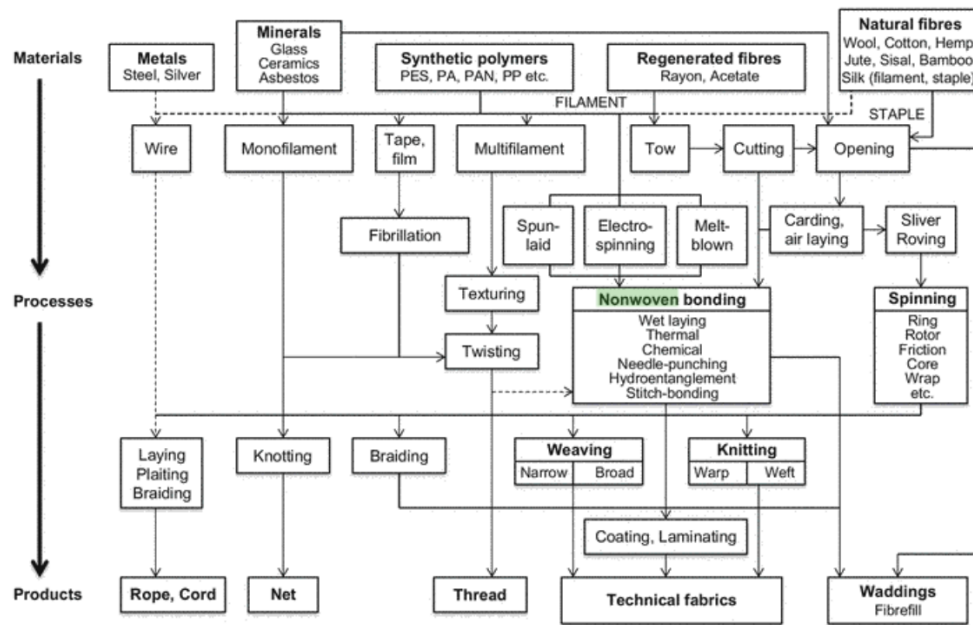


Figure 1: Textile Processing Pathways [6] Reproduced with permission from Elsevier

Medical gowns are rated on according to the Association for the Advancement of Medical Instrumentation (AAMI) standard, which consists of four levels (L1, L2, L3, and L4). Each performance level has a set of requirements that must be met to qualify as such. For textiles of L1, L2, and L3 classification, an impact penetration (determined through the American Association of Textile Chemists and Colorists [AATCC 42] protocol) must be less than 4.5 g, 1.0 g, and 1.0 g respectively. As well, textiles with the classification of L2 and L3 require hydrostatic pressures greater than 20 cmH₂O and 50 cmH₂O respectively (assessed using the AATCC 127 protocol) [4, 11].

2.2.1 Production of Disposable Textiles

Specific classifications of manufacturing techniques include spun bonding, melt blown, and more recently, electrospinning. Spun bond textiles are manufactured using the melt spinning technique through four phases of a production process, this being: spinning, drawing, web formation, and web bonding. The polymer of choice is produced in chips and is then transferred to an extruder. The chips

are then fed to the extruder to be melted through the mechanical and thermal energy transferred to the heated screw which then forces the material through a screen. Additives are incorporated during the extruder step to combine stabilizers, modifiers, and colours to the final product. The filtered molten polymer is then transferred to a metering pump, which is used to sustain a specified flow rate of operation.

Following steps utilize a spin pack, which is a die block assembly composed of a polymer feed distribution and another component known as the spinneret. The spinneret is a unit containing thousands of drilled holes, which generates fibres through gravitational drainage from the drilled holes. The molten material is then transferred from the feed distributor into one of the many spinnerets, a web of fibres is then created from this block assembly. During this process, air is used to quench the fibres from one of the many air quenching units. High velocity currents of air are used to assist in the stretching of the filaments as well. This aspect modifies the characteristic molecular orientation of the filament. Depending on the spinning speed of the unit, diameter of the filament will vary. Filaments are then placed on a moving belt and to obtain optimal textile coverage. Depending on the method used, these filaments are separated mechanically, aerodynamically, or electrostatically. The web is then bonded through mechanical, chemical, or thermal bonding means. Overall, properties of these produced fibres have factors of mechanical fortitude attributed to the polymer's crystallinity [8, 12].

As with spun-bond production, melt-blown fibres are produced through the extrusion of polymer melts through small holes, similar to spun-bond fibres. The primary contrast between the two manufacturing techniques is that, in the case of the polymer melt, a molten thermoplastic resin is blown from the tip of an extruder die using high-velocity air [13].

Electrospinning is another method used in production of nanofibers. The process involves feeding a polymer melt through a syringe using a needle and a pump, followed by the application of an

electrostatic force to the tip of the syringe. This process allows for very low diameter fibres to be drawn out of the apparatus. This is due to the surface properties of the polymer melt, when the force from the tension is greater than that of the surface energies of the polymer droplet, a precursor filament is projected onto the grounded collector. As this process occurs, solvent evaporates as the filament travels to the collector [14, 15].

2.3 Properties of Disposable Textiles

Polypropylene (PP) is commonly used for healthcare-based textiles as the material itself is lightweight, strong, and durable. It is also inexpensive in comparison to alternative polymer fibres. However, there is a trade-off in the comfort of the material in comparison [13, 16]. Of the commonly used textile materials, cotton, silk, viscose, polyester, the least transfer of bacteria was found in PP, making it an excellent candidate for PPE [17]. Regardless of whether the textile is reusable or disposable, both types of textiles are subject to contamination upon contact with moisture [18]. For non-woven textiles, the fabric itself has comparatively high strength to weight ratios in comparison to other non-woven, woven, and knitted textiles. These fibres most often range between 15-35 μm in diameter but may range from 1 to 50 μm , their web thickness may range from 0.1 – 4.0 mm but most likely within the 0.2 – 1.5 mm range [12].

Spun-bond (SB) fibres often use polymers of high molecular weight and broad molecular distribution (PP and PET are common examples). There are several reasons PP is commonly used: its low molecular weight, low density, hydrophobicity, high yield, low cost of production, and strength. Crystallinity of the polymer influences the pre-drying, excludability, and filament orientation of the fibre. The fibre size of the polymer also greatly influences the surface energetics of the material. This is due to the available surface area of contact on the outer layer of the fibre [12].

To gain insight into the fluid dynamics within textiles, an analysis of the filtration performance of woven filter cloth could be carried out by evaluating factors such as fabric tightness, yarn twist, and textile weave [8]. Air permeability within textiles is another factor attributed by the porosity of textiles as well. This factor is often considered through the measurement of the materials mesh sizing [19, 20]. Flow rate through the textile can also be studied to assess the textile's pore size.

Equation 1 below relates the volumetric flow rate (Q) of a humid vapor entering a porous textile to the time of water permeation (T), the water permeability mass at specific pressure point (W), the area of the pressure point (A), and density of the water (ρ) under specified conditions.

$$Q = \frac{W}{TA\rho} \quad \text{Equation (1)}$$

As for the surface properties of polymeric textiles, not all polymers behave similarly due to the polymer's non-polar or polar behavior. Polar plastics have an imbalance of electric charge due to the distribution of electrons. When an electric field is present, the imbalance will cause the polar plastics to align in accordance with the direction of the electric field. Examples of this include nylon, polyvinyl chloride (PVC), and polymethyl methacrylate (PMMA). Dielectric properties of textiles are important to recognize as textile fibres are mainly composed of polymers with large length to diameter ratio [21, 22]; the dimension and composition of the fibre effects the outcome of the fabric quality since the dielectric property of the textile is related to the moisture content, drying, and static properties of the product.

Measurements of the moisture content of fibres can be associated to the material's capacitance. The thermo-physiological property of the textile is related to the thermal insulation capabilities of the fabric. A set temperature difference can be used to calculate this property based on the heat flow on an area of interest. By measuring the changing temperature of both samples, the cooling function of the fabric can

be determined since the heat capacity of the textile could then be determined for both dry fabric and wet fabric. In literature, it was also observed that finer fibres had an improvement on the thermal and moisture transport capabilities of the textile. Increase of thermal resistance means that there is an inverse correlation to the thermal transfer ability of the fabric [23, 24]. In general, bulk polymers possess lower range thermal conductivities ($0.1 - 0.5 \text{ K}^{-1}$) [25].

Another factor is the moisture absorption of the textile. The wicking height of the fabric may provide insight on the water uptake and the fluid transport properties of the material. Fabric samples were tested for their wicking abilities using the vertical wick test. In general, there is a positive correlation between the presence of pores, moisture adsorption of a textile, and its wicking ability. Fibres may also be modified to contain adsorbing groups, which have a high affinity for water molecules [9, 16].

Nonwoven textiles have characteristic properties that lie between woven textiles and paper, being that the fabric is very lightweight and flexible but may also be produced to be heavy and stiff depending on the attenuation and density of product during manufacturing and design.

2.4 Coating Adhesion

There are several common industrially recognized coating methods used for textile coating applications. These methods include but are not limited to direct coating, transfer coating, lamination coating, and foam coating. Coating adhesion may also vary depending on the application method, these methods include brushing, spraying, or roll coating [26, 27].

Coating adhesion of textile substrates often use the T-peel testing method (see Figure 2). In Figure 2, the clamp holds both ends of the substrate prior to pulling the sides apart to determine the peel force required for either cohesive or adhesive failure. This is a method used for flexible substrates to assess a coating's adhesive and or cohesive characteristics. Adhesive failure in coating adhesion is described as the interfacial bond failure between the coating/adhesive and the substrate applied to the substrate.

Cohesive failure occurs such that a fracture forms within the adhesive itself, thus allowing the layer of adhesive to remain on the adherend. Substrate failure may occur due to high adhesive or cohesive forces [27, 28].

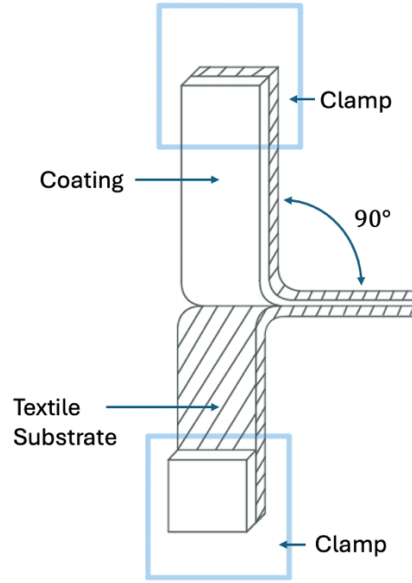


Figure 2: An Example of a T-Peel Test

There are numerical models developed to explain the actions of a thin, flexible film separating from a substrate. The first adhesion peel tests were conducted by Rivlin in 1944 [30]. These tests were conducted under the condition that any deformations occurring within the sample adhered to the substrate were ignored. A more commonly known model is Kendall's model, which is displayed as Equation 2 below:

$$\left(\frac{F}{b}\right)^2 \frac{1}{2tE} + \left(\frac{F}{b}\right)(1 - \cos\theta) - R = 0 \quad \text{Equation (2)}$$

Kendall's model is used to describe the phenomenon between an adhesive and a solid substrate. F in the above equation defines the peel force, t describes depth of the sample, b is the width, R is the

adhesive energy, and θ is the angle of the peel. There are also more recent models established under unsteady state-peeling conditions which may be used to describe peeling from flexible substrates [30, 31].

2.5 Microbes in Healthcare

Microbial pathogens including *P. aeruginosa*, *S. aureus*, *Enterococcus* spp., *Candida* spp., and *E. coli* are among some types of healthcare related infections. Bogusz et al. in their 2013 paper studied the effect of detergent-based cleaning of *S. aureus* in pre-screened near-patient sites. For all sites tested, the growth of *S. aureus* decreased in the first two to four hours post cleaning followed by growth after this period. Thus, further establishing the need for continuous residual cleaning within healthcare settings [32, 33, 34, 35, 36].

Bacteria persist more prevalently on rougher surfaces as they are more likely to deposit themselves along creases or depressions along these surfaces. Dyes and finishes of the textile also influence the ability of the textile to prevent onset of biofilm formation. This is due to hydrophobicity from the material and the textile dye and finish [38, 39, 40, 17]. Bacteria were also commonly observed to form biofilms that were unevenly distributed across surfaces. Contact time emerged as another critical factor; longer exposure periods were found to significantly enhance the likelihood of adhesion to both polyester and cotton substrates [39, 41]. In consideration of the transfer of bacteria through human and textile contact, the transfer of bacteria to and from the wearer promotes a certain selective pressure for certain microbes to persist depending on their own affinity to the wearer [42].

Depending on the textile, some textiles have a native microbiome. A microbiome is the native community of microorganisms present on textiles such as cotton and while cotton has a microbiome of its own, polyester for instance, was not seen to contain a native microbiome [43, 44]. The microbiome existing on the human skin and the textile of contact may be different due to different

bacterial enrichments found in the two environments [39, 41]. This is an important factor to consider since the microorganisms on a textile may exhibit certain traits that can either prevent or encourage the fouling of the material.

2.5.1 Biofilms and Cellular Interactions

Planktonic cells are free-floating, while bacterial cells within a biofilm are known as biofilm cells. Microorganisms rarely exist as planktonic cells because they prefer to aggregate with other cells. According to Sauer et al., bacteria undergo changes in their phenotypic expression when transitioning from planktonic, free-floating cells to stationary biofilm cells. This transition partially explains why the composition of extracellular polymeric substances varies depending on the environment the cells inhabit [45, 15].

Microbial adhesive interactions with the surface can be analyzed using two main physicochemical approaches. The first approach assumes that the cellular surface is in thermodynamic equilibrium with the contacting surface, utilizing concepts from surface thermodynamics. The second approach is based on the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory. Since bacteria are within the size range of 0.2-0.5 μm , which is similar to that of colloidal particles, the initial planktonic cellular adhesion can be described by the DLVO theory [46]. The DLVO theory describes the interaction forces between charged surfaces in a liquid medium, combining electrostatic and van der Waals forces. According to the DLVO theory, the adhesive interactions between the cell surface and the material surface can be explained by considering their respective surface energies and the intervening medium. [27].

Biofilms can be regarded as agglomerated masses of single cellular organisms co-adhered to one another through their surface of contact. Biofilms refer more specifically to bacteria but not limited to describing only behavior between bacteria, this term refers to a collective mass of single cellular

organisms and their wastes. The steps involved in biofilm formation begin with the initial planktonic cell adhesion, in which, single cells undergo adsorption onto the surface of contact. At this phase, the planktonic organism may still undergo reversible attachment from the surface of contact. After multiple cells adhere to the surface, the aggregated mass of adhered cells will become irreversibly attached to it; this mass will then mature into a cell cluster. Extracellular polymeric substances (EPS) such as polysaccharides, proteins, and lipids, surround the cell cluster, which can be as thin as a few cells thick. This mass then begins to form microcolonies, which may disperse when the EPS begins to degrade [45].

Note that this model of biofilm formation is a conceptually created using results gathered from a study on *P. aeruginosa* biofilm formation within a laboratory flow cell; it does not incorporate the complexities of biofilm formation in real-world applications but provides great visual construction as to depict the concepts of the mechanism. Examples of observations outside laboratory observation include the introduction of new species to the mass or the formation of microbial mats (cell masses that range from a few millimeters to a centimeter thick). Newer models have been developed to discuss the behavior of biofilm behavior in non-ideal situations, Figure 3 depicts different possible ways for a biofilm to form under varying environmental circumstances [47]. It can be observed from Figure 3 that cells are also capable of aggregating within the liquid phase without the presence of an observable surface. Aggregation may also occur from existing EPS in solution, single planktonic aggregates, and processes that involve the use of polymers as hosts.

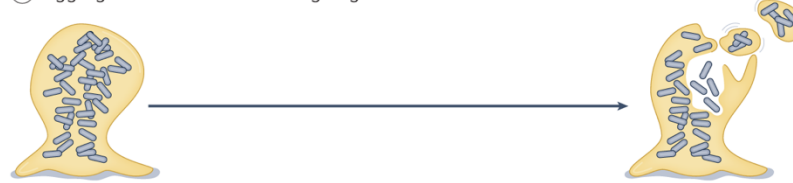
a Surface-associated biofilm formation

Attachment followed by clonal growth and EPS production

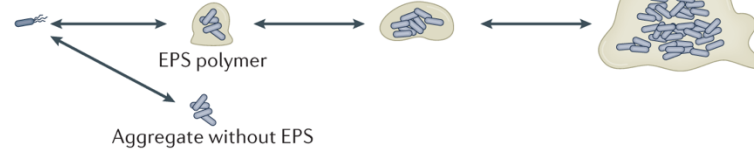


b Biofilm-like aggregate formation in the liquid phase

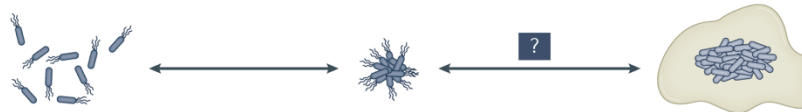
① Aggregates formed due to sloughing from surface-attached biofilm



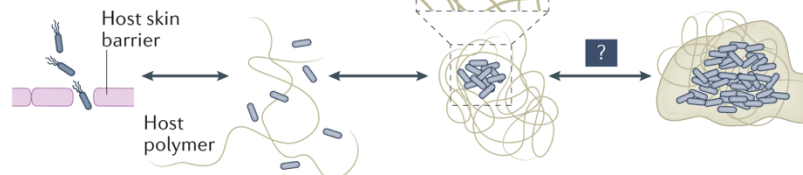
② Aggregates grown in planktonic phase



③ Aggregate formation initiated through cell surface components of single cells



④ Polymer depletion



⑤ Polymer bridging

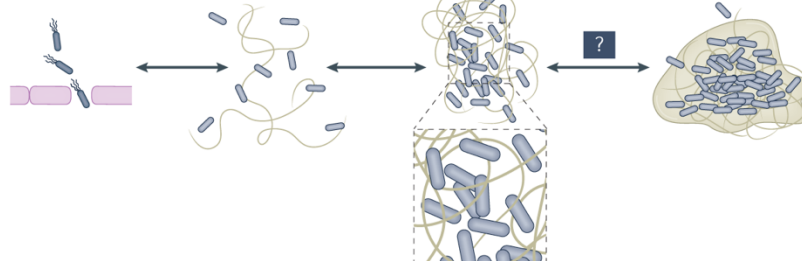


Figure 3: Mechanisms of Biofilm Formation [47]

The textile choice will inevitably demonstrate varying degrees of fouling results from the laundering model. Depending on the surface properties the textile exhibits. Figure 4 provides an illustrative cycle of a biofilm formation as the microbes continue to go to cycles of adhesion, growth, drying, and washing of the textile before it becomes exposed to adhering bacteria once again [50]. Without the laundering of the textile, bacteria will proliferate in subjectively ideal conditions depending on the biofilm and textile surface properties. Møllebjerg et al. has shown that increasing the hydrophobicity of the textile was favorable for bacterial adhesion. This justifies an earlier study by Krasowska et al. which also concluded that bacterial cells have an affinity towards hydrophobic material [51, 48]. Fouling will also depend on the polar groups present on the cellular membrane or wall and such so that the degree of fouling will also depend on the surface interaction of the textile with the cellular membrane. In summary, the colonization of bacteria on and within textiles is of concern as these biofilms are difficult to remove post adhesion [48].

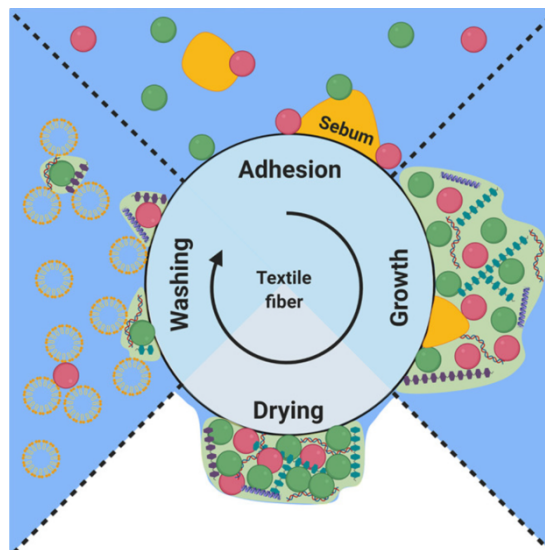


Figure 4: Adhesion of Bacteria and Formation of Biofilms Through Laundering Cycles [48]

2.6 Defining Antimicrobial and Antimicrobial Substances

An antimicrobial is defined as a material that kills, slows, or prevents the growth of microorganisms. It is a broader term encompassing a subset of classifications to describe antimicrobial action on certain groups of organisms, including antibiotics, antifungals, antivirals, antiprotozoals, and antiparasitic. Materials that claim antimicrobial efficacy are widely utilized in multiple sectors of industry; this included but is not limited to healthcare, food packaging, and consumer-based products. In the United States, the Environmental Protection Agency (EPA) holds the responsibility of registering products that claim antimicrobial efficacy. According to the EPA, these substances may be classified as disinfectants, sanitizers, and antibiotics (the EPA does not regulate the use of antibiotics in human medicine). To be sold and marketed in the United States, products in each of these categories must meet a specific set of criteria including antimicrobial efficacy and safety requirements [52].

Regulating the safety and effectiveness of antimicrobial drugs, medical devices, and food products falls under the purview of the Food and Drug Administration (FDA). This is similar to and overlaps with the regulatory responsibilities of the EPA; however, these two entities have distinct areas of focus under different mandates. The FDA focuses mainly on human and animal health while the EPA has a focus on environmental protection and public health concerns [52].

Polymeric antimicrobials are a class of antimicrobial agents that incorporated in polymer materials. These materials in general do not volatilize and are chemically stable and are not permeable to the skin. The production process of these materials is economically favorable [53]. These materials have had a relatively recent history as their advent of significant attention did not occur until the 1980s. During this time, the development of polymeric antimicrobials garnered significant attention due to the rise of antibiotic-resistant bacteria and the increasing demand for antimicrobials amongst the public [54, 55, 56]. As a result, a wide range of polymeric antimicrobials have been developed using antimicrobial

agents such as: silver nanoparticles, QACs, chitosan derived materials, and more [57, 58, 59]. These agents can be incorporated through processes such as melt blending, surface coating, and grafting.

2.6.1 Biocidal and Biostatic Materials

There are two terms to describe the population state upon antimicrobial substance testing, being biostatic and biocidal. Biostatic materials can prevent the additional growth of a microbial population in which the population which remains stagnant upon extended exposure time. Biocidal materials can provide protection from residual pathogens by depleting bacterial populations. For healthcare applications this function would be effective through the residual killing of species on the surfaces [60]. The inhibition of microbial growth is described to be biostatic in nature, the rapid depletion of microbial population is biocidal. Depending on function and purpose of the textile, either description may be used.

2.6.2 Modes of Antimicrobial Action

To describe the mechanism of antimicrobial behavior, literature reports cytotoxic activity as active or passive. Active antimicrobials refer to antimicrobial agents that are embedded into a material. These materials are capable of releasing or contact exposure killing. Passive antimicrobials are distinctive from active antimicrobials such that antimicrobials under this category do not destroy or disrupt the cellular membrane [61, 60]. As the name suggests, antimicrobial agents are not released but rather the mechanism is due to the nature of the material. These materials are distinguished by their ability to either repel or kill the organism through repulsive interactions of the cellular membrane and/or through the texture of the antimicrobial material. An illustration of these modes are depicted in Figure 5 in which the green microbes are repelled passively away from the surface while the active surface polymer is shown to be in contact with the microbes in grey. These materials can be categorized into three main types: polymeric biocides, which have inherent antimicrobial properties; biocidal polymers, which are

modified or functionalized to include antimicrobial capabilities; and biocide-releasing polymers, which release biocides into their surrounding environment.

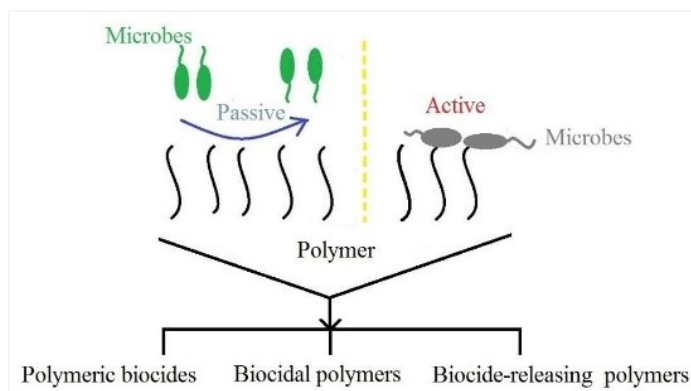


Figure 5: Classification of Antimicrobials [62]

2.6.2.1 Contact-active and Release-active antimicrobials

Contact-active antimicrobials can be distinguished by their ability to destroy bacteria upon contact through mechanisms involving cell membrane disruption, enzymatic inhibition, protein denaturation, and oxidative stress. Leaching of biocides and direct contact biocides are difficult to distinguish but can be characterized by the materials ability to induce cell lysis [63]. This phenomenon was demonstrated in Jenkins et al.'s study in which TiO_2 nanopillars were used to rupture cells and thus prevent the formation of biofilms on the surface. From their study, the oxidative stress was seen to generate much more ROS than compared to control samples used in the study. TEM imaging did show proof of cell penetration and deformation, but this was not enough evidence to conclude sufficient cell lysis as the frequency of such events were low [64].

Electrostatic properties may also contribute to active antimicrobial action such that the positive charge of ions or cationic surfactants rupture the cellular membrane upon exposure. An example of this is polyethyleneimine, which disrupts the cellular membrane through electrostatic interactions [62, 65].

Release-active antimicrobials rely on leaching of materials to effectively disrupt metabolic functions of a microorganism. Metallic coatings have been discussed by Kwon et al. who used liquid metal copper nanoparticles to produce nanoparticles of similar size. In this test antiviral, antifungal and antibacterial capabilities of the LMCu were assessed for *S.aureus*, *E.coli*, and *Candida* spp. All samples shown antimicrobial capabilities against the three strains. These metallic coatings rely on the release of ions to produce antimicrobial results [66, 67, 68].

2.6.2.2 Passive antimicrobials

Cell surface attachment behavior is not only determined by its hydrophobicity, but also by gravitational force, van der Waals attraction, electrostatic forces, and Brownian motion [69, 51, 70]. Cellular membranes are composed of a double layer structure known as a lipid bilayer. This bilayer is composed of a phosphate head and adjacent hydrophobic heads of a two fatty acid chains. Both sides of the membrane are negatively charged due to the existence of the phosphate head, which is then stabilized by magnesium and calcium. Thus, the cellular membrane itself is hydrophobic in nature and contains electrostatically charged membrane proteins that contribute to its characteristic surface.

Some polymeric materials may have been tuned or inherently contain properties that contribute to an overall lower surface energy level to prevent the onset of planktonic cellular adhesion. These characteristics provide a surface that makes it difficult for microbes to adhere onto. This includes polymers of higher hydrophilicity and negative charge. Poly(ethylene glycol) (PEG), for instance, is a commonly studied polymer capable of reducing protein adsorption and bacterial adhesion [62]. Table 1 provides a summary of existing polymeric passive antimicrobials [62].

Table 1: Passive Antimicrobials [62]

Polymer	Target	Remark
Poly(ethylene glycol)	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	Used as neutral polymer brush systems to prevent protein and cell adhesion
Poly(sulfobetaine methacrylate)	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus epidermidis</i>	Resist protein adsorption, cell attachment, and bacterial adhesion
Poly[3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate- <i>b</i> -2-(diisopropylamino)ethyl methacrylate]	<i>Staphylococcus aureus</i>	Zwitterionic coronae and pH-responsive cores can impart bacterial anti-adhesive properties
Poly(2-methyl-2-oxazoline)	<i>Escherichia coli</i>	Dual-functional antimicrobial surface of poly(L-lysine)-graft-poly(2-methyl-2-oxazoline)-quaternary ammonium
Albumin, whey	<i>Bacillus subtilis</i> , <i>Escherichia coli</i>	No bacterial growth was observed on albumin-glycerol and whey-glycerol after 24 h inoculation
Polyphenols	<i>Streptococcus mitis</i> , <i>Fusobacterium nucleatu</i> , <i>Porphyromonas gingivalis</i>	Effective against periodontal bacteria

2.6.3 Methods of evaluation

Minimum inhibitory concentration (MIC) is used to describe the amount of biocides needed to inhibit, prevent growth of a certain microorganism observed. Usually this term encompasses bacteria; it is also used to describe other similar criteria of evaluation such as: Minimum fungicidal concentration (MFC) and Minimum virucidal concentration (MVC). A common method of evaluation used to test for this is the Kirby Brauer method or the inhibitor disk method in which relative antimicrobial efficacy can be compared by evaluating the inhibition zone diameter [57]. Other commonly used methods of antimicrobial viability assessments involve the use of a known concentration of pathogenic cells in a liquid suspension being subjected to the antimicrobial tested followed by further bacterial count. These methods are often limited since as they particularly assess active antimicrobials effectively but not

contact active antimicrobials. Microscopic dead/live stains may be used in compensation to evaluate the contact active property of some polymeric materials [60, 63].

2.6.4 Quaternary ammonium compounds (QACs)

Quaternary ammonium compounds (QACs) are a category of cationic surfactants frequently utilized in sanitation applications (i.e. sanitization and food processing). These substances are well-known for their ability to contact kill and thus in turn, prevent secondary spread of pathogens. QACs are composed of a nitrogen atom surrounded by four other organic substituents such as alkyl or aryl groups. These compounds are often categorized into three specific groups: linear alkylammonium, imidazole, and pyridinium compounds. The nitrogen group is the surface-active property, it is capable of antimicrobial activity due to its positively charged nature. QACs are industrially high production volume chemicals that may be a potential alternative to traditional sanitizers [53].

Recent advances in academia explore the use of polymeric QACS, poly(QACs), as another method of contact-killing, yet limiting cytotoxicity and while keeping structural integrity of the polymer, these compounds are effective against viral strains [63, 62]. In a study produced by Schrank et al., a series of QACs were evaluated based on their antiviral efficacy to inactivate a series of different coronaviruses. This assessment concluded that the antimicrobial efficacy varies depending on the coronavirus, with no QAC providing sterilization for all tested strains [71].

2.6.5 Detecting QACs

QACs could be detected through using dyes, examples of existing methods including fluorescence-based titration through the use of fluorescent markers as indicators and methylene blue [72]. Some of these methods for instance can be used to determine the concentration of amine groups present. As mentioned in Blazheyskiy et al's study, "Titrimetric methods for determining cationic surfactants",

a method commonly used in the pharmaceutical industry is the biphasic method titration, which uses the presence of chloroform to confirm the concentration of QAC amine groups present [73].

The feasibility of possible titration methods had been well studied in literature. In a study produced by Metcalfe et al., sodium tetraphenylboron was used as the titrant using dichlorofluorescein as the indicator. The mixture initially turns pink upon the presence of the QAC. With the maximum addition of the sodium tetraphenylboron, the solution turns yellow from the complete reaction of the QAC to the titrant [74]. Cationic surfactants are different from their polymeric counterparts, and due to this reason, the traditional methods for surfactant detection may not be feasible for polymeric materials as the penetration timing into the polymeric matrix would not be accounted for. Potentiometric titration could be a viable method for quantifying the tertiary amine groups present in QACs and poly(QAC)s. Price & Wan in their 2010 study used an automated titrator coupled by an ionic surfactant electrode and silver/silver chloride reference electrode. This method proved to be more favorable as it does not require the use of chloroform [75]. Zhang et al. also used potentiometric titration with hydroxylamine hydrochloride as a method used to determine the presence of the QAC content level in modified APA-TFC membranes [76]. Wang et al. also used potentiometric titration; their method uses tetraphenylborate as the titrant coupled with a platinum electrode to determine the end point. This system provided an accurate quantifiable result as previous methods required the visual (colorimetric) detection of the titrant end point [77].

2.6.6 Properties of METAC and its polymeric derivatives

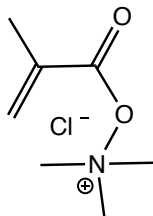


Figure 6 : [2-(methacryloyloxy)ethyl]trimethyl Ammonium Chloride (METAC)

As shown above in Figure 6, [2-(methacryloyloxy)ethyl]trimethyl Ammonium Chloride (METAC) is a QAC that has been seen in studies involving mucoadhesive materials, polymeric grafting, anionic dye removal, minimum fungicidal concentration studies, and other related antimicrobial tests. A polymer with a cationic charge can be formed using METAC, which is a type of methacrylate monomer. From the structure of METAC, the biocidal component is due to the presence of its amine group. The monomer consists of an ester group attached to the tertiary amine group (see Figure 8). Polymeric METAC, or poly(METAC) may have a chemical structure as shown in Figure 7.

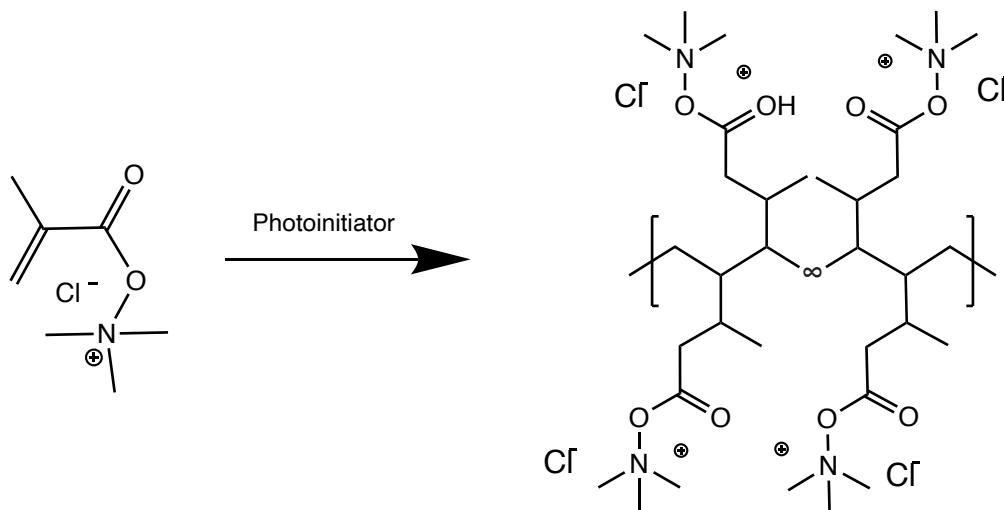


Figure 7: Synthesis of Poly(METAC)

The monomeric METAC (methacryloxyethyltrimethyl ammonium chloride) is soluble in water due to its polar nature. Many other QAC (quaternary ammonium compound)-based polymers also exhibit water solubility because they contain positively charged groups that promote hydrogen bonding. The solubility of some QACs can be controlled by the nonpolar nature of the material's polymer backbone [78, 79].

Based on the results collected from Stopiliga et al., the monomer in varying concentrations was found to be biocidal against *Candida* spp, a fungal species often found in dental carries [78]. In a separate study conducted by Goel et al., when the monomer is grafted onto cotton, it was concluded to be effective against *E. coli* and *S. aureus* however, both studies conclude that these METAC based polymeric derivatives are not as effective as existing antibiotics in market currently [80].

Noting that hydrogels exhibit behavior influenced by the pH of their environment, attributed to ionizable functional groups. In the case of METAC based hydrogel, this behavior may arise from the presence of tertiary amine groups on its surface. The degree of swelling in hydrogel samples varies with pH, as functional groups ionize or repel each other. Altering pH levels can also disturb existing bonds thus impacting the structural integrity of the sample. Shiga et al.'s 2018 study on polymerized METAC based copolymers and hydrogel found that both the polymer and the hydrogel forms are both antimicrobial against MSSA, *B. subtilis*, *C. albicans*, and MRSA [79].

Additionally, METAC has been shown to exhibit low cytotoxicity. In Wang et al.'s study, a packaging barrier film composed of METAC-functionalized graphene oxide sheets was evaluated for cytotoxicity to human HeLa cells. The study found that at a concentration of 50 µg/mL, the cell viability remained at 90% [81].

2.6.7 Copper Compounds

Copper and its related compounds have been utilized as disinfectants throughout human history. The antimicrobial mechanism is due to the release of copper ions, which causes oxidative stress facilitated through the production of reactive oxygen species (ROS) [83, 84, 85, 86]. This includes direct contact killing through damage of DNA, membrane damage causing lysis of the cell, and interference to vital viral proteins. Depending on the type of compound and the rate at which copper ions are soluble in solution, the membrane is ruptured by direct contact to copper, thus the flux of copper ions increases the amount of oxidative stress within the cell and subsequently initiate DNA degradation [87, 88]. The introduction of copper compounds and ions to the cell may also take place in a different manner, Karlsson et al. in their study reported that their copper oxide nanoparticles undergo endocytosis into the cell as opposed to rupture of the cellular membrane as observed in copper tested cells [89, 90].

For textile-based applications, previous literature has reported the use of copper-based nanoparticles for the purposes of impregnating the fibres of textiles to provide an antimicrobial effect. An instance of this was demonstrated in Chowdhury et al.'s 2013 paper on the use of copper nanoparticles with an average diameter of 3.5 nm in empty fruit bunch fibres. In this paper the antimicrobial capabilities and the mechanical behavior of the fibres were tested for, and biocidal properties were generally found to be associated with the material [91].

2.6.8 Copper sulfide antimicrobial properties and cytotoxic compatibility

Copper sulfide (CuS) is a recent compound of interest as it may be relatively less cytotoxic in comparison with other copper compounds due to its lower water solubility (which has a solubility product constant of 7.9×10^{-37}) [92, 93, 94, 95, 96]. Li et al. (2010) conducted a comparative study on the cytotoxicity of CuS nanoparticles (CuSNPs) and gold nanoparticles (AuNPs). Using human HEK 293 cells, this assessment found CuSNPs and AuNPs conclusive of similar low cytotoxicity [97]. By

administering the tested nanoparticles intravenously to mice, Guo et al. (2013) obtained comparable findings while comparing pegylated CuS and gold nanoparticles [98]. Recent advancements in developing adequate face mask PPE for the prevention of HAI onset have found that the incorporation of CuS NPs to the fibre matrix of a mask to be feasible for public use [99]. Feng et al further concluded that CuS nanoplates are not as cytotoxic as compared to two separate lines of cancerous cells, HeLA and KB, cell viability begins to decrease when the concentration reached 400 g/mL [100].

2.6.9 Zinc Compounds

ZnCl, ZnO, and ZnNO₃ are commonly used zinc compounds in antimicrobial applications. The antimicrobial activity of zinc oxide has been assessed in recent literature; it was found that the efficacy may be attributed to the pH of the environment. The pH of the environment influences the ion release from hydrogels through mechanisms such as ionization of functional groups, swelling behavior, ionic crosslinking, diffusion dynamics, and chemical stability. Understanding these interactions is crucial for designing hydrogels with controlled ion release properties for applications in drug delivery, wound healing, and other biomedical fields [101, 102, 103, 104, 105, 106]. Optimal conditions for cytotoxic effects were observed when ZnO NPs and nanoplates were in conditions of high pH using the disk diffusion method [107]. In contrary, another study observed the effects of ZnO nanofluid to *E. coli* and *S. aureus* in glycerol. The optimal effects were observed at a lower pH range (4 for *E. coli* and 5 for *S. aureus*) at a temperature of 42 °C [108].

Studies also claim that ZnCl is capable of preventing the onset of infectious diseases found in tissues [109]. In Guo et al.'s 2012 study, the group determined that the substance is capable of deteriorating plaque growth using ZnCl. Choi et al. from their 2010 study found that the inclusion of Pyrrolidine Dithiocarbonate to increase the killing efficacy by a 100-fold viability reduction as compared to the substance alone [110]. In Yakoob et al.'s comparative study, ZnCl was found to be more effective at

eliminating *Helicobacter pylori* in comparison to bismuth subsalicylate and was comparable to that of clarithromycin [111].

2.6.10 Silver Compounds

Like copper, silver is an antimicrobial metal due to similar mechanisms of cytotoxic activity. Starting in the 1800s it was used as an antiseptic for surgical applications, wound treatment, medical devices, and dentistry. The exact mechanism of silver antimicrobial efficacy is not well understood. Silver ions are known to inhibit enzymes containing thiol groups which in turn increases the production of ROS. DNA becomes fragmented upon the cellular apoptosis, which can be characterized by the cell's shrunken body [112, 113, 114].

In terms of recent advancements, currently silver NPs are of high interest and have been proved effective as an antimicrobial additive in the recent years [115]. Use of silver NPs have been seen in the incorporation of NP to textile fibres directly then further produced through extrusion methods of production. Montes-Hernandez et al.'s 2021 paper "In Situ Formation of Silver Nanoparticles (Ag-NPs) onto Textile Fibers" reported that Ag-NPs were formed in-situ onto textile fibres through the chemical reduction of silver ions. This method allows for the Ag-NPs to adhere directly to the textile without any outside interference. Overall, Ag-NPs synthesized were of less than 20 nm in size and were found to induce cell death in *Bacillus subtilis* using the inhibition zone method of evaluation indicating the release of silver ions to be the mode of action [116].

Silver nitrate is also a silver compound of interest in inducing antimicrobial activity. The concentration silver nitrate may be a factor to varying cellular apoptosis mechanistic models. In Palantoken et al.'s 2010 paper, introducing low concentrations (1mM) was reported to cause certain bacteria species and in this case, *Bacillus licheniformis*, to synthesize silver NPs ranging around 50nm whilst, higher concentrations (5mM) rendered the cells dead [117].

With the increase in concern of metallurgical waste, silver flakes have also been considered an option; however, these materials require further processing to increase their antimicrobial efficacy. Unless ionized, silver flakes are inert and do not induce cytotoxicity until corroded. These two characteristics prevent the metal from exhibiting active antimicrobial properties. In their 2011 paper, Anzano et al. discussed that these flakes required thermal activation in a reduced atmosphere or chemical activation with hydrogen peroxide [112].

2.7 Properties of Glycerol and Water

Glycerol is a colorless, viscous, and odourless fluid with a high boiling point of 290 °C at 1 atm, characterizing its strong intramolecular attractions between the hydroxyl groups [118]. These hydroxyl groups allow the molecule to demonstrate hygroscopic behavior and miscibility in water. This affinity to water is due to the hydrogen bonding capabilities of glycerol-water in solution.

In Dashau et al.'s study, which researched the characteristics of varying volume ratios of glycerol and water, it was concluded that as the concentration of glycerol increased, the solvation shell of the glycerol molecule, known as the hydration shell, begins to collect water around the hydroxyl groups of the glycerol. The hydration shell describes a layer of water molecules surrounding the molecule of interest. The intermolecular interactions of highly concentrated glycerol and water solutions were found to be like that of ice, this was determined through the hydrogen bond angle distribution and average bond number. As well, there was a shift in bond frequencies seen in the FTIR analysis. Thus, from this study, glycerol was seen to improve the hydrogen bond network of the solution [119, 118].

Previous studies using glycerol have shown the substance is mildly antibacterial [120]. In Saegeman et al.'s study assessing bacterial viability in glycerol, it was concluded that using an 85% glycerol in water solution produced consistently antimicrobial results. This was confirmed using TEM to determine the

number of cells void of cytoplasmic content, thus characterizing the lack of cellular content to conclude that the cell could no longer metabolize [121].

Glycerol has also been seen in nature to have cryoprotective properties. Alone, glycerol is not an effective cryoprotectant however, optimal cryoprotective properties can be observed in mixtures of glycerol and water containing 66.7 wt% glycerol with a freezing point of -46 °C. In recent studies, the use of glycerol-based hydrogels extends its use in below freezing conditions. Dai et al. in their 2021 study discuss that the addition of glycerol to water prevents freezing and preserves the structural aspects of the hydrogel [122].

Vlacha et al. studied glycerol as a plasticizer in comparison with oleic acid, the increase of glycerol allowed for greater plasticization of chitosan films, as it allowed for greater elongation at break and a lower glass transition temperature (T_g). Compared to oleic acid, glycerol was observed to exhibit reduced water retention due to its ability to decrease the number of hydrated crystals that could potentially interact with the water in the system [123].

Pure glycerol and water and their mixtures were studied for their thermodynamic properties, combustion, and ignition. Table 2 lists the properties of water and glycerol mixtures.

Table 2: Thermal and Physical Properties of Glycerol and Water Solutions [124]

Property	Pure glycerol	Water	10% of water in glycerol	20% of water in glycerol
Density (kg m^{-3})	1261	1000	1235	1208
Heating value (MJ kg^{-1})	18	0	16.2	14.4
Boiling point (K)	563	373	411	394
Heat of vaporization (kJ mol^{-1})	91.7	43.99	n/a	n/a
Specific heat capacity ($\text{J mol}^{-1}\text{K}^{-1}$)	221.9	4.18	n/a	n/a
Thermal conductivity ($\text{mW m}^{-1}\text{K}^{-1}$)	289	608	292	326

Chapter 3 Antibacterial efficacy of EG and EG Derivatives

In this section, the antimicrobial properties of EG and its additives (ZnCl, AgNO₃, and CuS) were studied through a series of experiments aimed at assessing the statistical significance of their antibacterial effects. The method involved coating one side of melt-blown polypropylene (MB-PP) with a solution of EG precursor, either with no additive or with a selected additive, followed by UV curing before testing. Circular samples with a diameter of 13.1 mm were cut from the coated MB-PP sheets. These samples were then immersed in a PBS solution containing 10⁸ *E. coli* cells/mL. After a specified immersion period, a portion of the solution was extracted and sequentially diluted tenfold in PBS, then plated onto agar plates. The plates were subsequently incubated at 37°C for 24 hours. This process was designed to evaluate the antimicrobial efficacy using comparative plate counts, and statistical analysis was performed using ANOVA to determine any significant antimicrobial activity.

3.1 Experimental Design

3.1.1 Synthesis of Ever-gel (EG)

EG was synthesized using a basis of 80 wt% glycerol and 20 wt% deionized water. Since the monomer contains 25 wt% in water, the water content was considered when synthesizing different amounts of the solution. The addition of monomer was 20 wt% of the total weight of glycerol and water. With respect to the monomer, the amount of crosslinker (GlyDMA) and photoinitiator (DEAP) added was 5 wt% and 2 wt% respectively. All components were then added to a 20 mL glass scintillation vial and were vortexed until homogenous.

For experimentation, 4 g of glycerol and 1 g of water were used as the basis. From this, the amount of METAC that was used in solution was 1.33 g to account for water present in the METAC solution. The amount of DEAP normally used was 0.02 g and the amount of GlyDMA used was 0.05 g. DI water

(0.67 g) was added to account for the required water needed for the solution. Figure 8 presents the chemical reaction involved in the synthesis of EG.

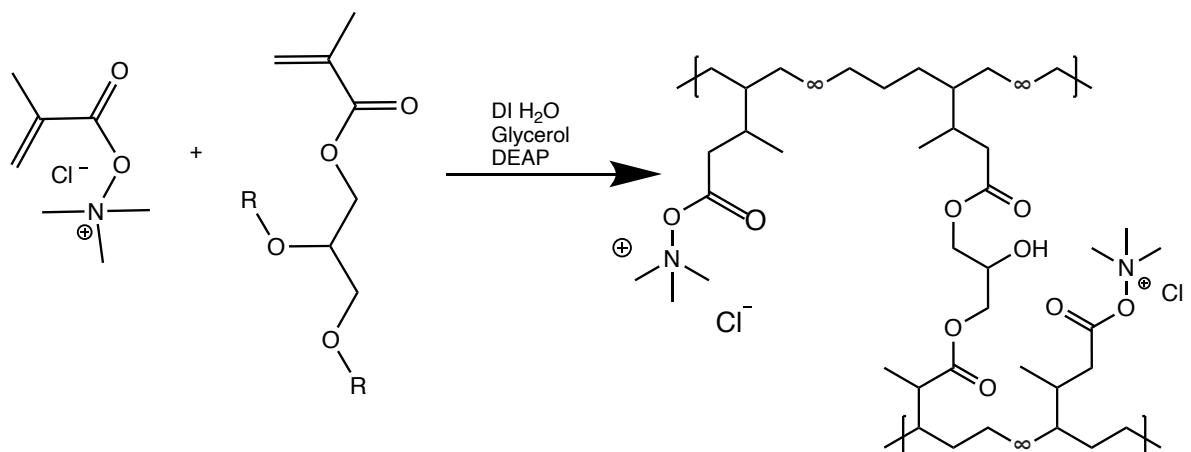


Figure 8: Synthesis of EG

3.1.2 Preparation of control hydrogel

The control sample used was synthesized using 10 wt% acrylamide, 1 wt% N,N'-Methylenebisacrylamide (MBA), 0.1 wt% DEAP, and the same ratios of DI water to glycerol as EG. For experimentation, 4 g of glycerol and 1 g of water are used as the basis. The amount of DEAP normally used was 0.005 g and the amount of MBA used is 0.05 g. Water (1 g) was added to account for the required leftover water needed for the solution.

3.2 Materials and Method

3.2.1 Materials

For all experiments conducted, all materials were used as received unless otherwise stated. For the synthesis of EG, [2-(methacryloyloxy) ethyl] trimethylammonium chloride solution (METAC) 75 wt.% in H₂O, Glycerol dimethacrylate (GlyDMA), 2,2-Diethoxy Acetophenone (DEAP), Silver nitrate, Zinc

chloride and glycerol were purchased from Sigma Aldrich. Copper sulfide was purchased from Alfa Aesar.

The chosen organism for this study was *E.coli*, the culture was grown using Nutrient broth (purchased from Sigma Aldrich). Phosphate buffered saline (10X) from Sigma Aldrich was the buffer of choice used in this test, this solution was further diluted to 1X for use. Difco™ Plate Count Agar powder was purchased from Fischer Scientific.

3.2.2 Instruments

All glassware was sterilized in an autoclave unit at 121 °C prior to testing and use. Analog vortex mixer from VWR and centrifuge unit was used to produce the *E.coli* solution. The cell concentration of the solution was found using the Diode Array Spectrometer HP 8452A. To grow the culture, a bacterial broth consisting of nutrient broth (from Sigma Aldrich) and the culture was grown in an Isotemp incubator from Fisher Scientific. All samples were sterilized in a UV-C device (from UV-Pod) for 3 minutes prior to experimentation.

3.3 Assessment of Antibacterial Activity of EG and EG additives

3.3.1 Preparation of culture

E.coli was cultivated in Nutrient Broth at 37 °C for a duration of 20 to 24 hours. This solution was then centrifuged for 2 minutes at 4600 RPM followed by the removal of the supernatant. 1X PBS was then used to suspend the centrifuged cells using a VWR vortex mixer, the solution was then centrifuged once more to remove impurities left in the supernatant. This process was then repeated two more times to achieve sufficient removal of the nutrient broth. With the nutrient broth removed from the PBS-*E.coli* suspension, the optical density (OD) was achieved at $OD_{600} = 0.125$ (specifies a concentration

of 10^8 cells/mL) using the Diode Array Spectrometer HP 8452A and adjustment of the supernatant concentration.

3.3.2 Sample Synthesis and Preparation

Samples containing EG, varying amounts of crosslinker (GlyDMA), and additives were assessed for antimicrobial efficacy. Samples containing additives were then synthesized by incorporating varying amounts of ZnCl, AgNO₃, and CuS into the EG solution and vortexed for 1 minute. The coated substrates were then UV cured for 15 minutes to assess each additives potential to be used as an effective antimicrobial additive.

Each EG coated sample of MB-PP fabric was immersed in 2 mL of *E.coli*-PBS suspension for a set amount of time depending on the condition to be met. After the set time of contact, the solution was vortexed and 100 μ L of the suspension was taken from the bulk solution of each test and diluted in separate vials. 50 μ L of this solution was then taken and plated onto a single agar plate specifying the dilution concentration. This was done for all tests at all feasible dilutions. The plate colonies were then cultivated at 37 °C for 24 hours. This methodology was adapted from the AATCC 100 Protocol, a quantitative test method used for antimicrobial finishes applied on textiles.

The numbers presented are the plate count numbers and corresponding dilution factors. For example, if a plate count were to be 100 at a dilution factor of 10^{-7} and the initial sample taken was 50 μ L, the number of cells present in one milliliter would be approximately:

$$\frac{100}{0.05 \text{ mL} \times 10^{-7}} = 2 \times 10^{10} \text{ cells/mL}$$

The calculation conducted to determine the log reduction was as followed through the below equation:

$$\text{Log Reduction} = \log_{10} \left(\frac{N_0}{N_1} \right) \quad \text{Equation (3)}$$

Where N_0 is the initial plate count that has a viable number of colonies to be compared and N_1 is the final plate count for a sample that has been treated.

3.4 Results and Discussion

3.4.1 Antimicrobial Assessment of EG compared to MB-PP

The antimicrobial assessment was conducted to evaluate the antimicrobial efficacy of EG in comparison to a sample of MB-PP coated with PAM crosslinked with MBA (the control sample). Samples were prepared such that EG coats the substrate prior to curing, this is the same with the control sample, each sample was then exposed to the same but separate 2 mL of *E.coli*-PBS suspension for a set amount of time (2 hours in this case). This experiment was conducted to evaluate existing antimicrobial capabilities of EG in comparison to the similar control sample (PAM+MBA). Table 3 below summarizes the findings from this analysis. The percent weight given on the furthest right column labelled “Coating Mass Increase (%)”, is recorded to investigate the effect of the weight of the coating in comparison to the amount of antimicrobial additive included. The calculated coating mass increase was determined using the following equation:

$$\begin{aligned} &\text{Coating Mass Increase (\%)} \quad \text{Equation (4)} \\ &= \frac{\text{Coated Substrate Mass (g)} - \text{Mass of Substrate (g)}}{\text{Mass of Substrate (g)}} \times 100\% \end{aligned}$$

Equation 4 describes the percent increase of mass for each sample in comparison to the substrate mass prior to coating.

The ANOVA analysis used in this investigation serves to identify the contributing factors of the antimicrobial efficacy of the material.

Table 3: Antimicrobial efficacy of EG coated samples and control samples.

<i>Type of Coating</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>	<i>Plate Count (Dilution factor: 10^{-4})</i>	<i>Plate count (Dilution factor: 10^{-3})</i>	<i>Coating Mass Increase (%)</i>
PAM+MBA	TNTC	77	7	124
PAM+MBA	TNTC	91	7	121
PAM+MBA	TNTC	122	9	108
EG	TNTC	81	0	103
EG	TNTC	223	10	88
EG	TNTC	216	23	126

***Note TNTC denotes colonies on the plate exceeds a countable limit.**

A single factor ANOVA analysis was conducted to examine the relation between the coating and the control with results presented in Table 4. The following null hypothesis was established to evaluate the relation between the plate count and the mass of coating present.

$$H_0 = \text{The presence of EG does not contribute to a change in the colony count}$$

Table 4: One-Way ANOVA Analysis of EG coated and Control Samples $\alpha=0.05$

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	11469.93	5	2293.99	0.21	0.95	4.39
Within Groups	65470.36	6	10911.73			
Total	76940.29	11				

Based on the results presented in the one-way ANOVA analysis in Table 4, we fail to reject the null hypothesis. The presence of EG does not contribute to a change in the colony count since the critical value is less than the F value determined from this assessment. Thus, the presence of EG does not significantly relate to the plate count recorded. It is suspected that this result is because the method of assessment is more effective for active-release biocides rather than contact-active biocides. Contact biocides work by disrupting the cellular membrane of the pathogen upon contact, but this is not detected through the adjusted AATCC 100 protocol. If a bacterium were to penetrate the polymeric matrix of the coating, this method would not be able to determine the biocidal activity of the bacterium within the matrix.

3.4.2 Contribution of Crosslinker Concentration as an Antimicrobial Additive

To understand the contribution of crosslinker, GlyDMA, to the antimicrobial efficacy of METAC, varying concentrations of crosslinker were studied using an ANOVA two-factor analysis. The two factors in consideration are the increase in mass of the coating on the MB-PP substrate and the crosslinker concentration (determined from Equation 4). The interaction term tests for the effect of the two factors and whether one of these factors influences the other, in this case this is the interaction between the coating applied and the amount of crosslinker present. Table 5 presents the results gathered from this assessment.

Table 5: Antimicrobial Testing of Varying Concentration of GlyDMA (0.025 g and 0.05 g) as an Additive to EG

<i>Mass of Crosslinker (GlyDMA)</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>	<i>Plate Count (Dilution factor: 10^{-4})</i>	<i>Plate Count (Dilution factor: 10^{-3})</i>	<i>Coating Mass Increase(%)</i>
0.05 (original)	44	16	14	86
0.05 (original)	91	21	1	79
0.05 (original)	213	1	0	95
0.025	112	6	0	76
0.025	241	51	1	69
0.025	89	67	12	71
0.01	37	19	0	115
0.01	122	21	2	82
0.01	TNTC	23	6	89

***Note TNTC denotes colonies on the plate exceeds a countable limit.**

A two-way ANOVA analysis was performed on plate count 10^{-5} to determine if the concentration of crosslinker shows statistical significance in relation to the plate counts obtained. The following null hypotheses statements were established:

$H_{0,1}$ = *The crosslinker concentration does not contribute
to a change in the colony count*

$H_{0,2}$ = *Colony counts are not affected by the weight change caused by adding EG*

$H_{0,interaction}$ = *Colony counts are not affected by an interaction between
the crosslinker concentration and the amount of EG*

Table 6: Two-Way ANOVA Analysis of Different Concentrations of GlyDMA $\alpha=0.05$

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Crosslinker WT %	1379.25	2	689.62	0.12	0.89	3.89
Increase in Mass	87277.34	1	87277.34	15.34	2.05E-03	4.75
Interaction	1377.06	2	688.53	0.12	0.89	3.89
Within	68292.74	12	5691.06			
Total	158326.38	17				

Based on the results of the ANOVA analysis from Table 6, we fail to reject the null hypothesis for both $H_{0,1}$ and $H_{0,interaction}$. Thus, the crosslinker concentration and the interaction of the crosslinker concentration with percent mass did not contribute to the antimicrobial efficacy of EG.

As part of a separate experiment (Figure 9), poly(METAC) and GlyDMA crosslinked poly(METAC) samples of similar sizes were placed on an agar plate coated with *E. coli* biofilm. Over the timespan of twelve hours, the crosslinked sample had become swollen but did not demonstrate any leaching nor have any clear indication of antimicrobial activity. The sample containing no crosslinker was found to have partially dissolved in the presence of moisture and the region around the sample was also cleared of biofilm.

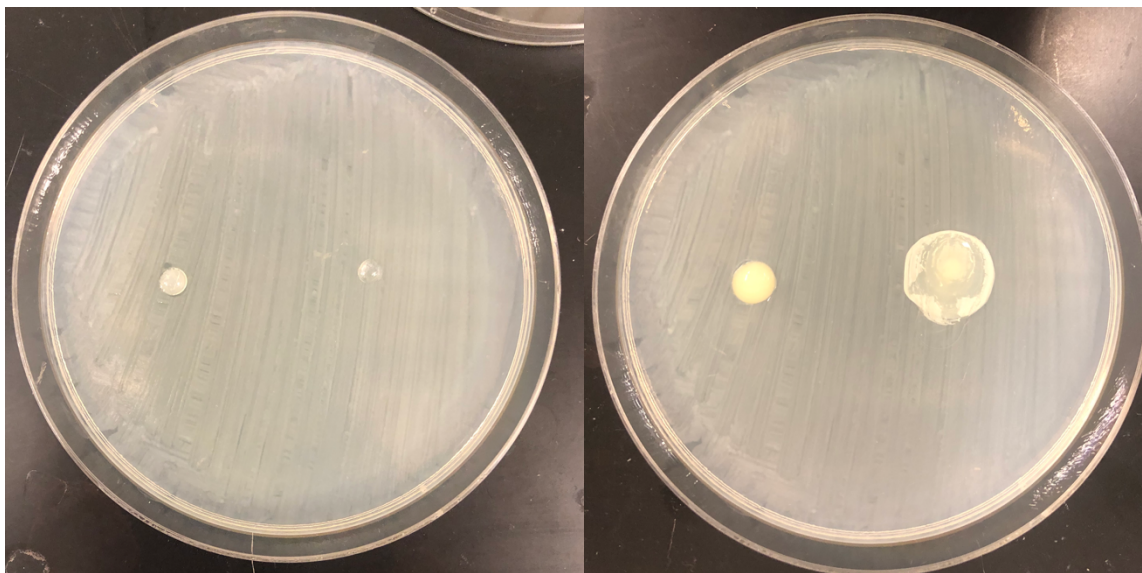


Figure 9: GlyDMA crosslinked Poly(METAC) and Poly(METAC) (Left to right: sample upon placement and sample after 12 hours)

In a separate experiment to determine the solubility of poly(METAC) in water it was observed that when poly(METAC) was left in DI solution over time, the polymer solubilized completely after 1 minute.

3.4.3 Zinc Chloride as an Antimicrobial Additive

In this assessment the contribution of ZnCl as an antimicrobial additive was assessed using an ANOVA two-way method of analysis with plate counts presented in Table 7. This test was performed to determine if varying the ZnCl concentration in EG would cause a change in antimicrobial activity in comparison to the EG control sample.

For samples containing ZnCl, ZnCl is added as a solid powder to the EG solution then vortexed prior to coating the MB-PP. Each sample was then exposed to the same but separate 2 mL of *E.coli*-PBS suspension for 2 hours. The percent weight given on the furthest right column labelled “Coating Mass Increase (%)”, is recorded to investigate the effect of the weight of the coating in comparison to the amount of antimicrobial additive included, see Equation 4 for this calculation.

The ANOVA analysis used in this investigation serves to identify the contributing factors of the antimicrobial efficacy of the material.

Table 7: Antimicrobial Testing of Varying Concentration of ZnCl (0.0025 g and 0.005 g) as an Additive to EG

<i>Mass of ZnCl (g)</i>	<i>Plate Count (Dilution factor: 10^{-4})</i>	<i>Plate Count (Dilution factor: 10^{-3})</i>	<i>Coating Mass Increase(%)</i>
0.0025	121	12	113
0.0025	44	2	121
0.0025	198	40	63
0.005	12	0	104
0.005	67	6	104
0.005	68	2	121
0	49	7	94
0	81	1	123
0	102	23	106

The following null hypotheses statements were established:

$H_{0,1}$ = *The ZnCl concentration does not contribute to a change in the colony count*

$H_{0,2}$ = *Colony counts are not affected by the weight change caused by adding EG*

$H_{0,interaction}$

= *Colony counts are not affected by an interaction between the ZnCl concentration and the amount of EG*

Table 8: ANOVA For Zinc Chloride Sample comparison $\alpha=0.05$

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
ZnCl WT %	3934.41	2	1967.21	1.54	0.25	3.89
Increase in Mass	29809.05	1	29809.05	23.32	4.12E-04	4.75
Interaction	3959.16	2	1979.58	1.55	0.25	3.89
Within	15336.93	12	1278.08			
Total	53039.56	17				

From the results of the ANOVA test presented in Table 8, ZnCl was found to be statistically insignificant in reducing the number of colonies present after testing. The interaction of ZnCl and increase in EG mass, however, is significant as we fail to reject the null hypothesis for the increase of EG. Thus, the sample's colony count was not related to the interaction of the mass of EG-ZnCl and concentration of ZnCl. Since these results may be due to minimal amounts of ZnCl present, further evaluation using greater amounts of ZnCl was conducted and presented in Table 9.

Table 9: Antimicrobial Testing of ZnCl (0.025 g and 0.0375 g) as an Additive to EG

<i>Mass of ZnCl (g)</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>	<i>Plate Count (Dilution factor: 10^{-4})</i>	<i>Coating Mass Increase (%)</i>
0.025	TNTC	102	136
0.025	TNTC	296	127
0.025	TNTC	119	139
0.0375	TNTC	113	86
0.0375	TNTC	97	142
0.0375	TNTC	182	82
0	TNTC	210	61
0	TNTC	132	167
0	TNTC	167	119

* **Note TNTC denotes colonies on the plate exceeds a countable limit.**

Using the same stated null hypothesis as in the test involving lower concentrations of ZnCl, a two-way ANOVA analysis was conducted for samples of a greater concentration as presented in Table 10.

**Table 10: Two-Way ANOVA Assessment for ZnCl of (0.025 g and 0.0375 g) concentrations
 $\alpha=0.05$**

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
ZnCl WT %	1650.20	2	825.10	0.33	0.73	3.89
Increase in Mass	110045.03	1	110045.03	43.70	2.50E-05	4.75
Interaction	1614.16	2	807.08	0.32	0.73	3.89
Within	30218.79	12	2518.23			
Total	143528.18	17				

Based on the ANOVA assessment for antimicrobial efficacy of ZnCl in greater concentrations, it was determined that increasing the concentration did not prove to be statistically significant for the resulting plate count, since we reject the null hypothesis in this case. This is hypothesized to be due to the pH of

the EG, which is more acidic in the presence of METAC as ZnCl is less readily dissociated in acidic conditions as opposed to basic conditions.

3.4.4 Silver Nitrate as an Antimicrobial Additive

In this assessment, EG containing AgNO₃ was evaluated to determine the antimicrobial efficacy in comparison to EG. Using the same synthesis methodology, EG containing AgNO₃ was prepared with solid powder AgNO₃ particles that are added to the solution then vortexed to mix. The MB-PP textile is then coated and UV cured.

Plate count test was conducted on samples with varying concentrations of AgNO₃ to EG as shown in Table 11. The column titled “Coating Mass Increase (%)” (right-most column) is the percent increase of the coating in comparison to the uncoated MB-PP see Equation 4 for details on this calculation. This is to determine (in a 2 factor ANOVA analysis) if there is a significance in coating quantity in comparison to the amount of antimicrobial additive included.

Table 11: EG and AgNO₃ at varying concentrations (0.025 g and 0.01 g)

<i>Mass of AgNO₃</i> <i>(g)</i>	<i>Plate Count</i> <i>(Dilution factor:10⁻⁵)</i>	<i>Plate Count</i> <i>(Dilution factor:10⁻⁴)</i>	<i>Coating Mass</i> <i>Increase (%)</i>
0.01	57	9	63
0.01	23	1	124
0.01	129	12	93
0.025	17	0	92
0.025	39	14	128
0.025	21	0	138
0	209	51	98
0	TNTC	81	82
0	TNTC	102	129

* Note TNTC denotes colonies on the plate exceeds a countable limit.

**Table 12: Two-Way ANOVA Assessment for AgNO₃ of (0.025 g and 0.0375 g) concentrations
α=0.05**

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
AgNO ₃ WT %	5183.27	2	2591.64	20.60	1.32E-04	3.89
Increase in Mass	3770.89	1	3770.89	29.97	1.42E-04	4.75
Interaction	5195.50	2	2597.75	20.65	1.30E-04	3.89
Within	1509.75	12	125.81			
Total	15659.41	17				

Based on the information provided, the following null hypotheses statements were established:

$H_{0,1}$ = *The AgNO₃ concentration does not contribute to a change in the colony count*

$H_{0,2}$ = *Colony counts are not affected by the weight change caused by adding EG*

$H_{0,interaction}$

= *Colony counts are not affected by an interaction between the AgNO₃ concentration
and the amount of EG*

From the results obtained from the ANOVA test as presented in Table 12, we reject the null hypothesis in all cases. Thus, AgNO₃ is concluded to have statistical significance in the number of colonies present. From the interaction test there is a correlation between the mass of the coating and the AgNO₃. The EG sample containing 0.5 wt% AgNO₃ achieved a log reduction of 0.92 in two hours while 0.2 wt% AgNO₃ achieved a 0.48 log reduction of *E.coli*.

Thus, silver nitrate was overall found to be an effective antimicrobial, but samples were seen to become discolored upon UV curing as can be seen in Figure 10.



Figure 10: Comparison of EG samples Left to right, EG CuS, EG alone, and AgNO₃

In Figure 11, an inhibition zone test was conducted to observe the presence of leaching antimicrobial activity. This method proved inconclusive because the gel swelled in the moisture of the agar plate. Consequently, the coated MB-PP also yielded inconclusive results, performing similarly.

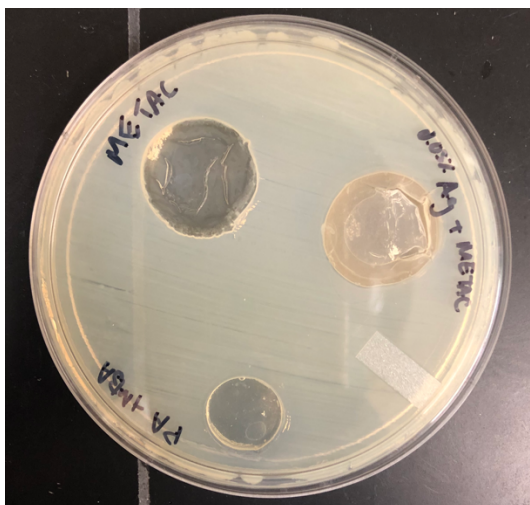


Figure 11: Inhibition Zone Test of EG, PAM crosslinked with MBA, and 0.05 wt% AgNO₃ in EG

3.4.5 Copper Sulfide as an Antimicrobial Additive

The antimicrobial properties of EG-CuS were tested with the results summarized in Table 13. These samples were prepared the same way as to other additive based samples in which EG solution is mixed with the additive for the selected mass, then coated onto the MB-PP textile substrate followed by sample curing. The Coating Mass Increase (%) on the right-most column is the percent increase of the EG-CuS coating in comparison to the uncoated MB-PP see Equation 4 for details on this calculation. The purpose of this assessment is to determine if there is a significance in coating quantity in comparison to the amount of antimicrobial additive included.

Table 13: EG and CuS at varying concentrations (0.0025 g and 0.005 g)

<i>Mass of CuS (g)</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>	<i>Plate Count (Dilution factor: 10^{-4})</i>	<i>Plate Count (Dilution factor: 10^{-3})</i>	<i>Coating Mass Increase(%)</i>
0	198	34	0	117
0	257	14	1	102
0	293	21	0	93
0.0025	258	23	0	98
0.0025	277	31	0	137
0.0025	210	7	0	112
0.005	117	20	0	117
0.005	213	9	0	112
0.005	87	13	0	112

Lower quantities of CuS were observed to not have significant antimicrobial activity as can be seen in the two-factor ANOVA analysis in Table 14, in which greater amounts of CuS in EG were tested for significance.

Table 14: Two-Way ANOVA Assessment for ZnCl of (0.025 g and 0.0375 g) concentrations

$\alpha=0.05$

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
CuS WT %	12055.39	2	6027.70	4.62	0.03	3.89
Increase in Mass	200556.83	1	200556.83	153.79	3.35E-08	4.75
Interaction	12072.86	2	6036.43	4.63	0.03	3.89
Within	15649.44	12	1304.12			
Total	240334.522	17				

$H_{0,1}$

= *The concentration of CuS (0 to 0.1 wt%) does not contribute to a change in the colony count*

$H_{0,2}$

= *Colony counts are not affected by the weight change caused by adding EG*

$H_{0,interaction}$

= *Colony counts are not affected by an interaction between the CuS concentration*

and the amount of EG

From Table 14, it was observed that greater amounts of CuS proved to be antimicrobial, having an average log reduction of 2.95 for samples containing 0.0375 g of CuS (or 0.75 wt% CuS). These results are presented in Table 15.

Table 15: EG and CuS at greater varying concentrations Antimicrobial Test for a 2-hour test

<i>Mass of CuS (g)</i>	<i>Plate Count (Dilution factor: 10^{-7})</i>	<i>Plate Count (Dilution factor: 10^{-6})</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>	<i>Coating Mass Increase(%)</i>
0	TNTC	104	7	202
0.01	0	0	0	224
0.01	78	7	0	157
0.01	0	0	0	267
0.0375	1	0	0	285
0.0375	0	0	0	288
0.0375	1	0	0	137

*** Note TNTC denotes colonies on the plate exceeds a countable limit.**

Using the data produced, it was concluded that within 8 hours, the log reduction of *E.coli* had increased to 3.12. The log reduction would signify a further reduction in the *E.coli* population indicating that the sample has continued antimicrobial efficacy. This would be an additional 32 % reduction of *E.coli* present.

Table 16: EG and 0.0375 g of CuS Antimicrobial Test (8 hours)

<i>Mass of CuS (g)</i>	<i>Plate Count (Dilution factor: 10^{-7})</i>	<i>Plate Count (Dilution factor: 10^{-6})</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>
0	TNTC	TNTC	TNTC
0.0375	71	9	0
0.0375	120	13	1
0.0375	94	16	0

*** Note TNTC denotes colonies on the plate exceeds a countable limit.**

3.4.6 EG-CuS Antimicrobial Feasibility over time

Fresh samples (samples synthesized the day of) and older samples (samples made 31 days prior to testing) were evaluated under the same criteria of testing (modified AATCC 100 protocol). All samples

were made using 0.2 wt% CuS in EG. It was speculated that the antimicrobial activity of EG-CuS would change over time due to the slower release capabilities of CuS. Old and new samples were compared using the adjusted AATCC 100 protocol, the results from this analysis are presented in Table 17. The Coating Mass Increase (%) was determined using Equation 4 to determine the interaction between the age and the mass of the coating applied to the MB-PP substrate, the results from this analysis are presented in Table 18.

Table 17: EG-CuS Sample Age Antimicrobial Test

<i>Sample Age</i>	<i>Plate Count (Dilution factor: 10^{-6})</i>	<i>Plate Count (Dilution factor: 10^{-5})</i>	<i>Plate Count (Dilution factor: 10^{-4})</i>	<i>Coating Mass Increase(%)</i>
New	37	1	0	43
New	109	21	1	98
New	31	0	0	75
Old	44	2	1	71
Old	39	1	0	114
Old	28	0	0	76

Table 18: Two-way ANOVA analysis for EG-CuS Sample Age comparison

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Sample Age	357.99	1	357.99	0.73	0.42	5.32
Increase in Mass	6684.73	1	6684.73	13.70	0.01	5.32
Interaction	368.05	1	368.05	0.75	0.41	5.32
Within	3902.26	8	487.78			
Total	11313.02	11				

$H_{0,1}$ = The sample's age does not contribute to a change in the colony count

$H_{0,2}$ = Colony counts are not affected by the weight change caused by adding EG

$H_{0,interaction}$ = Colony counts are not affected by an interaction between the sample's age
and the amount of EG

From Table 18, the interaction between older and newer samples were found to be not significant. The result from the $H_{0,1}$ and $H_{0,interaction}$ null hypothesis is that did not have statistical significance to the plate count results. The mass of the sample has shown to be statistically significant much like previous ANOVA assessments have concluded.

3.5 Summary

The initial assessment of the antimicrobial efficacy of EG shows that when compared to an uncoated sample, the sample did not show statistical significance as to whether there was a distinguishable difference between uncoated and coated MB-PP substrates. It was also concluded that there is no statistically significant contribution of the crosslinker concentration through ANOVA testing of variables that may allude to being associated with the antimicrobial behavior of EG. Further testing involving the use of ZnCl and AgNO₃ has shown that ZnCl was found to be statistically insignificant. This was concluded in the ANOVA two-way analysis using varying concentrations of the additive as there was no significant relation between the plate count and the amount ZnCl added to EG. This may be due to the effect pH in which the presence of hydronium ions in solution will increase the solubility of the compound, thus impacting the hydrogel's swelling, ionization, crosslinking, and diffusion dynamics.

Copper sulfide was observed to have antimicrobial properties upon testing and had produced log reductions of 2.15 and 3.12 in 2 hour and 8 hour sample contact times respectively. Lower concentrations did not produce the same result and we fail to reject the null hypothesis in these conditions. AgNO₃ was found to be effective, however, EG-AgNO₃ was noted also to be more difficult

to cure, and often had a tackier surface in comparison to the copper sulfide samples. Due to the material tinting upon UV exposure, EG-AgNO₃ was observed to stain clothing and skin. Therefore, was not an ideal additive for the objectives of this project.

In discussion of the control sample, PAM crosslinked with methylbisacrylamide, there was no considerable difference between the control sample and EG. This was determined using 2-way ANOVA table of variance, considering variables of whether there was any considerable difference between the two groups that may allude to antimicrobial efficacy of the material. This was due to the water-soluble and hygroscopic nature of poly(METAC) as the biofilm and the agar plate both contained moisture. To confirm this, poly(METAC) was left in DI solution over time, it was observed that the polymer solubilized completely after 1 minute. It can be confirmed from this observation that the material is antimicrobial through contact, but only to the extent that it is capable of contacting surrounding microorganisms. In summary, the antimicrobial efficacy was evaluated based on the industrial standard, the AATCC 100 protocol, which is commonly used to assess a coated textiles capability to release active antimicrobial agents into the surrounding environment. Thus, contact enabled antimicrobials would not be effectively detectable using the method.

Chapter 4 Characteristics of EG and EG-CuS as Textile Coating Materials

The chemical and physical characteristics of EG were assessed to determine the material's feasibility as a coating. In this chapter, EG and EG-CuS were tested to compare their adhesive strength, material softness, wettability, physical properties such as swelling, and thermal properties through a series of tests.

4.1 Experimental Design

4.1.1 Materials and Method

EG and EG-CuS samples were synthesized according to the method described in Chapter 3.1. For the preparation of coated samples in the assessment of material adhesion (T-Peel load testing), two sections of MB-PP fabric were cut into 1 in x 6 in. Two pieces (equivalent to one sample) are then sandwiched together in parallel. A 500g rolling device was then used to press out the excess by rolling out the sample. The sample was then hung on a hanging apparatus and cured for 15 minutes in the UV curing chamber.

4.1.2 Characterization

The mechanical characterization of EG and EG-CuS was conducted using UMT (CETR/Bruker UMT-Tribometer). Indentation testing was done to determine the Young's modulus; for soft materials such as hydrogels, the methodology follows that of Hertz Law. The indenter used was comprised of a spherical tip made of glass mounted onto a 1kg load. Deionized water was used to prevent adhesion of EG to the tip of the indenter. Each sample had three tests each in different regions of the gel. For the adhesion of EG coating on a fabric substrate, the testing protocol was based on "ASTM D751 – Standard Test Methods for Coated Fabrics" and "IS 7016 part 5-2003 (RA2011)" test methods. T-Peel

testing was conducted using the UMT with the program specification set to 5.00 ± 0.2 mm/s (12 ± 0.5 in./min). Water contact angle measurements were conducted using a custom-built sessile drop apparatus. Each measurement was conducted at room temperature. A syringe pump (New Era Pump System Inc.) is utilized to dispense a single droplet onto the surface of the coated fabric.

Six samples were created for the purpose of evaluating EG alone and EG coated on textile. The textiles were prepared such that one was not coated, one was dip-coated and pressed using a 500g roller then cured for 15 minutes, and the final sample has a layer patted on then cured for 15 minutes. Three EG samples, each of these samples had a different specified amount of time left in the environment (1 month, 1 week, and 1 day).

The chemical fingerprinting of EG and EG-CuS samples was achieved using ATR-FTIR. ATR-FTIR spectral measurements were conducted on the Bruker Hyperion 3000 FTIR Microscope using the ATR settings. The thermal properties of the gel were assessed using TGA Q500, TA instrument for Thermogravimetric Analysis and the TA Instruments, DSC Q2000 for DSC characterization. Smaller samples of EG were prepared in both the TGA and DSC characterization of EG and EG-CuS, each sample is approximately 15 mg in mass.

4.2 Results and Discussion

4.2.1 T-Peel Adhesion

Peeling force or loading is defined as the force required to lift an adhesive material from its substrate. T-Peel adhesion testing is used to assess adhesive and/or cohesive loads between two flexible substrates. This testing may also provide insight on other characteristic properties such as material rheology and removability. From the peel test, it was observed that the samples containing higher amounts of copper sulfide had experienced a cohesive failure when pulled apart. Only one sample was

observed to experience adhesive failure. Figure 16 below is an image of cohesive and adhesive failure for sheets of EG coated MB-PP.

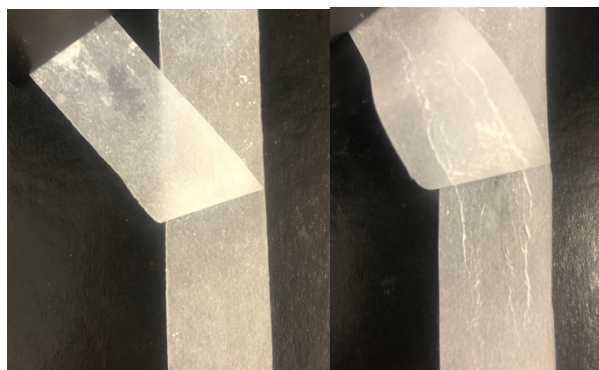


Figure 12: Example of Cohesive (Left) and Adhesive (Right)) Failure on MB-PP

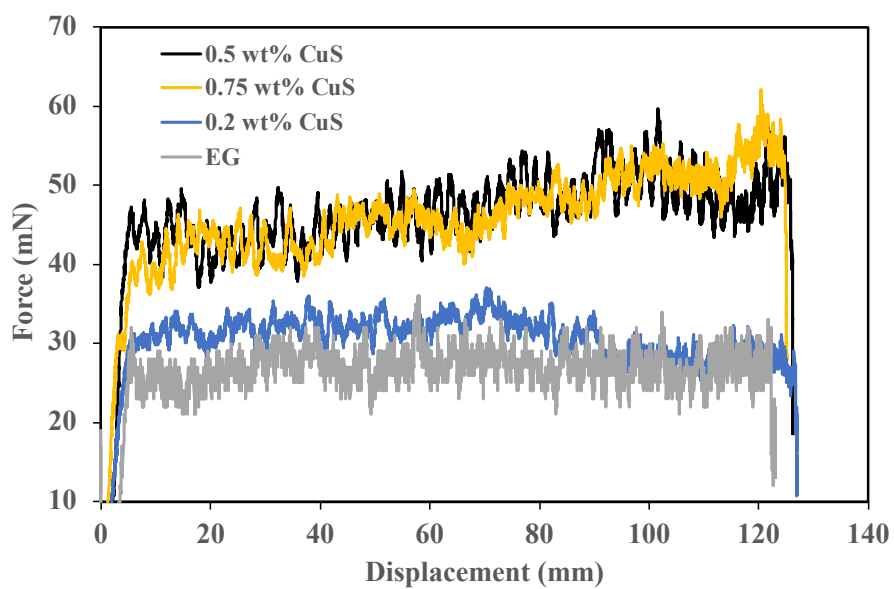


Figure 13: T-Peel Force Test Results as a Function of Vertical Displacement

Figure 13 shows the T-Peel test results comparison with increased amounts of CuS to each sample subset. For each sample subset (samples of varying amounts of CuS concentration), four repeat tests were conducted to achieve the average peel force and maximum peel force. From the results presented in Figure 13, there was a noticeably greater load seen in samples of greater CuS concentration. Overall, the increased concentration of CuS is related to the greater peel force of the material. Figure 14 and Figure 15 depict the trends in both the average and maximum peeling force changes with varying CuS content, respectively.

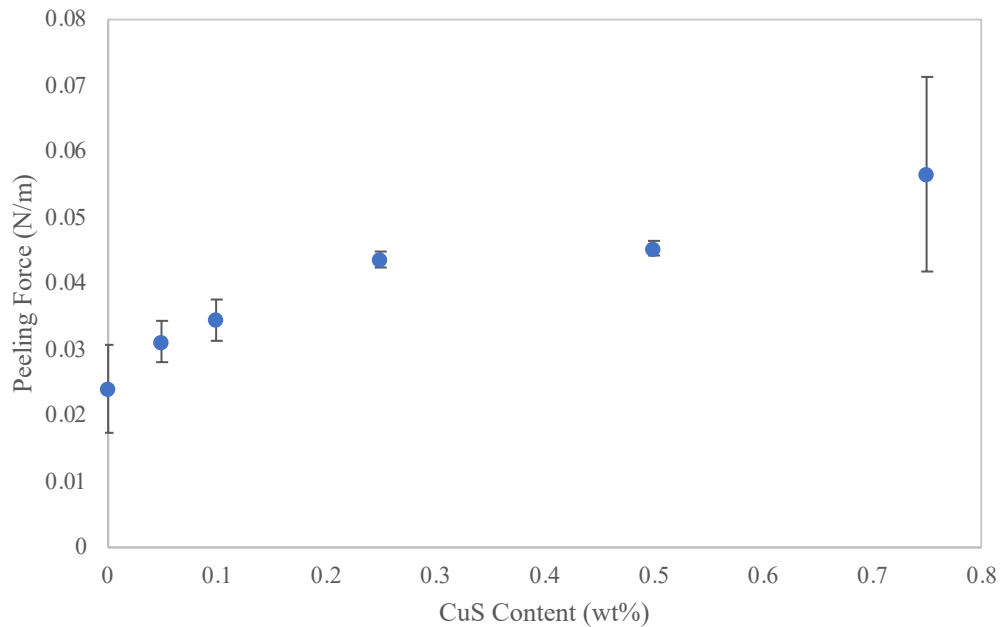


Figure 14: Average T-Peeling Force of EG and EG-CuS on MB-PP Substrate

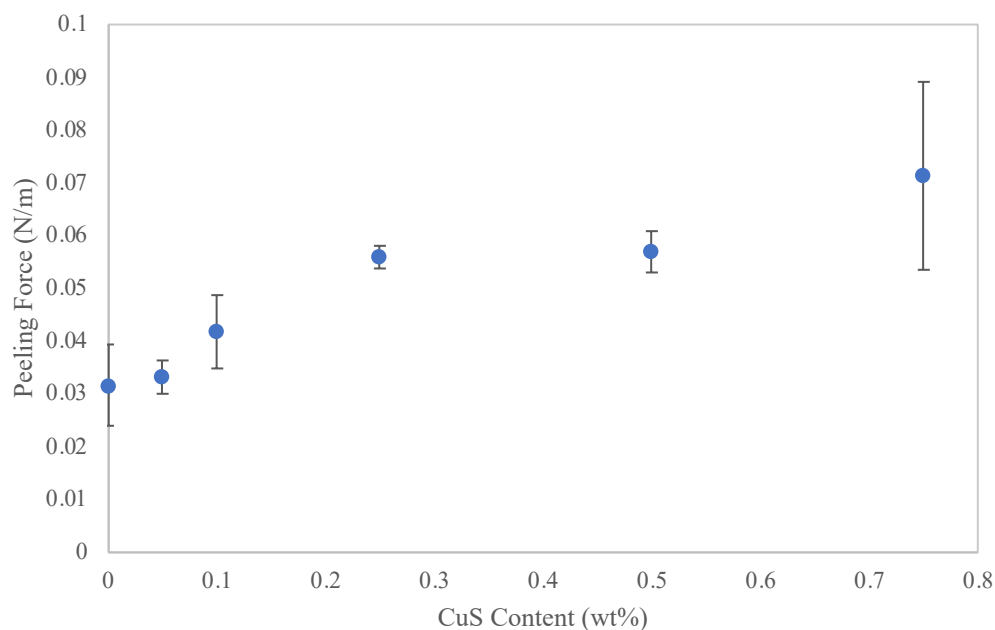


Figure 15: Maximum T-Peeling Force of EG and EG-CuS on MB-PP Substrate

Out of the samples tested for EG, 1 sample out of the 3 tested had failed adhesively rather than cohesively. The cohesive failure of samples containing greater amounts of CuS were seen to also have greater adhesive strength to the fabric substrate. The average load was found to be between 0.024 N (EG alone) to 0.057 N for samples containing 0.75 wt% CuS. Higher deviation was found in samples containing 0.75 wt% CuS as the sample had more instances of substrate failure prior to end of testing. The measured peel forces for these samples shows an overall general trend of increasing adhesive strength.

4.2.2 Water Contact Angle on Coated MB-PP

Water contact angle tests were conducted to observe the wettability of the EG coated samples. These tests were done for the coated MB-PP samples. Each sample was subject to 5 seconds of contact time for each test.

The increased amount of EG coating to the substrate was seen to have increased the wettability of the surface. This was expected as the hydrogel and its components are hygroscopic in nature. It was observed that the samples containing CuS did not drastically change the water contact angle, although it was expected that the inclusion of CuS was to have some effect to the wettability considering how the increase of ions to the system would provide a greater polar charge for the adjacent water molecules. It could be that the solubility of CuS is not as great and as a result the dissociation may not have been great enough for there to be as much of a difference. In summary, the contact angles recorded are within the range of 85° to 52° and with addition of EG as a coating to the textile substrate, the hydrophobicity increases as greater amounts are applied as a coating. Figure 16 and Figure 17 present examples of contact angle measurements conducted on both coated and uncoated MB-PP fabric. SB-PP alone did not have any changes in contact angle, it was seen that the sample remained non-wetting during the 5 minutes of contact time with the fabric. With no coating, the MB-PP sample was non-wettable for 6 hours. Figure 18 presents the water contact angle as a function of CuS content present in EG. Wettability does not seem to vary in comparison to samples containing greater concentrations of CuS.

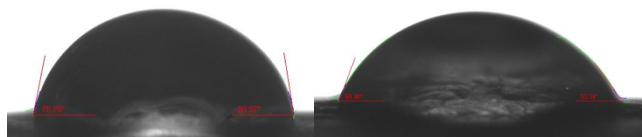


Figure 16: Left to right: Contact Angle Measurement Results for Coated MB-PP with EG 20 wt% CuS (80°) and 0.75 wt% CuS (61°)

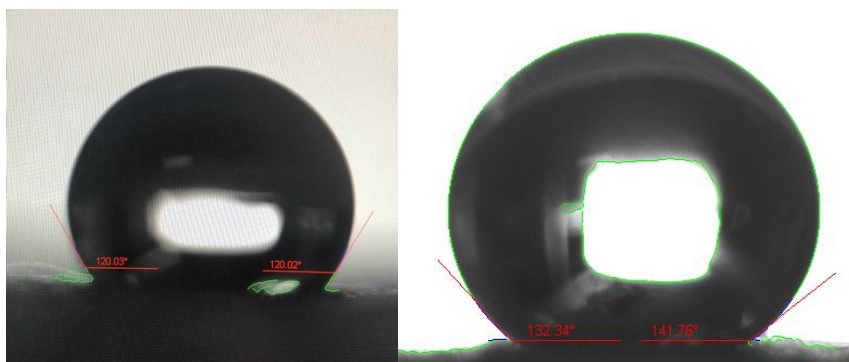


Figure 17: Left to right: Comparison of SB-PP Fabric Contact Angle (120° and 120°) and MB-PP (132° and 141°)

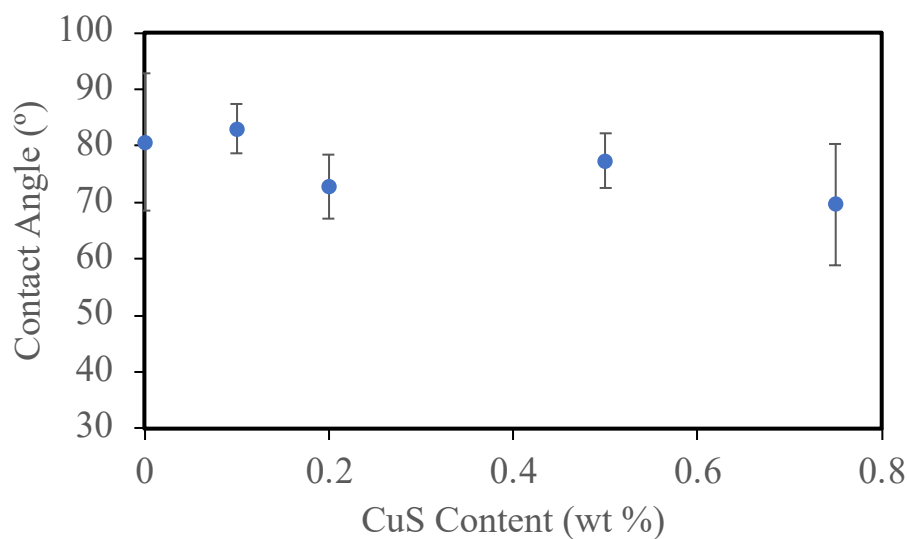


Figure 18: Water Contact Angle as a Function of CuS Content in EG Coated on MB-PP Fabric

4.2.3 Attenuated Total Reflection (ATR) FTIR

The structural and chemical properties of each EG sample were evaluated through attenuated total reflection (ATR) FTIR characterization; EG and EG-CuS samples are compared to observe if there are any significant changes.

Figure 19 depicts the comparative results gathered from this analysis. Fresh samples of EG, 0.01 wt% CuS in EG, and 0.75 wt% CuS in EG were synthesized the day prior while older samples were synthesized a week prior.

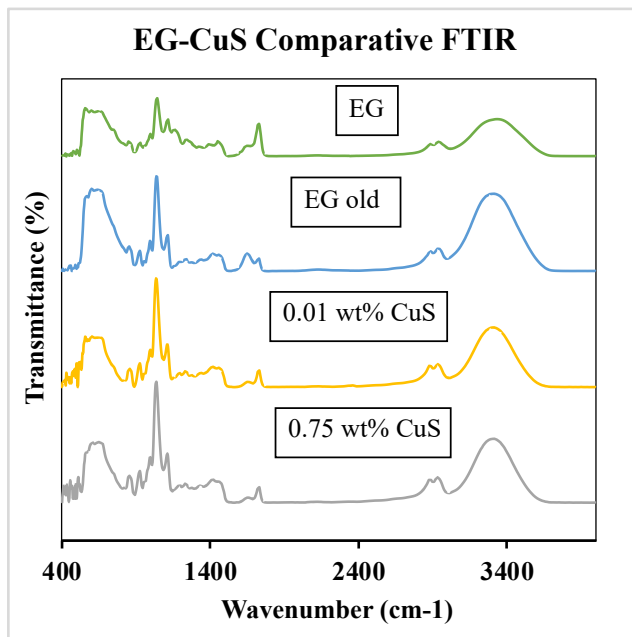


Figure 19: EG-CuS Comparative FTIR

From the plot shown in Figure 19, the broadest band on the range of 3299-3400 cm^{-1} , which indicates the existence of -OH bonds occurring predominately in glycerol. Further past this range, there are two visible but broad peaks within the range of 2800-2900 cm^{-1} , these are the stretching vibrations commonly found in C-H. Further past this point, there are consistent peaks within the 1430-1490 cm^{-1} range, indicating the existence of O-H in the material. The stretching vibrations from C-O bonds are visible as a sharp peak showing between 1600-1800 cm^{-1} . Stretching of C=O bond adsorption can be visible at 1737 cm^{-1} . Past this point, the C-N stretch was seen at 1500 cm^{-1} . The strong peak within the 1030-1130 cm^{-1} range indicates the presence of C-O stretching found in secondary alcohols.

The increase in concentration of CuS did not change the wavelength of the peaks and did not alter much in terms of the broadness and strength of the peaks, this indicates that the samples were consistent in chemical structure. Variation between sample peak strength is primarily prominent between the older and newer samples, this is due to the change in amount of water retained within the sample as the sample ages or due to the way the sample is placed on the apparatus prior to FTIR use. Variation between peak height is normally seen and is often due to the way the sample is placed during testing. Overall, there are no new chemical alterations discovered between samples in this analysis.

4.2.4 Environmental scanning electron microscopy (ESEM)

Environmental scanning electron microscopy (ESEM) analysis was conducted to reveal possible presence of a distribution from the CuS particles in EG-CuS. Figure 20 presents EG-CuS with 1 wt% CuS; through backscattered electron detection imaging (BSD) it can be seen that the CuS in EG is well distributed amongst the sample. The reason for this distribution may be due to the electrostatically charged particle interaction between CuS particles and the sample as well.

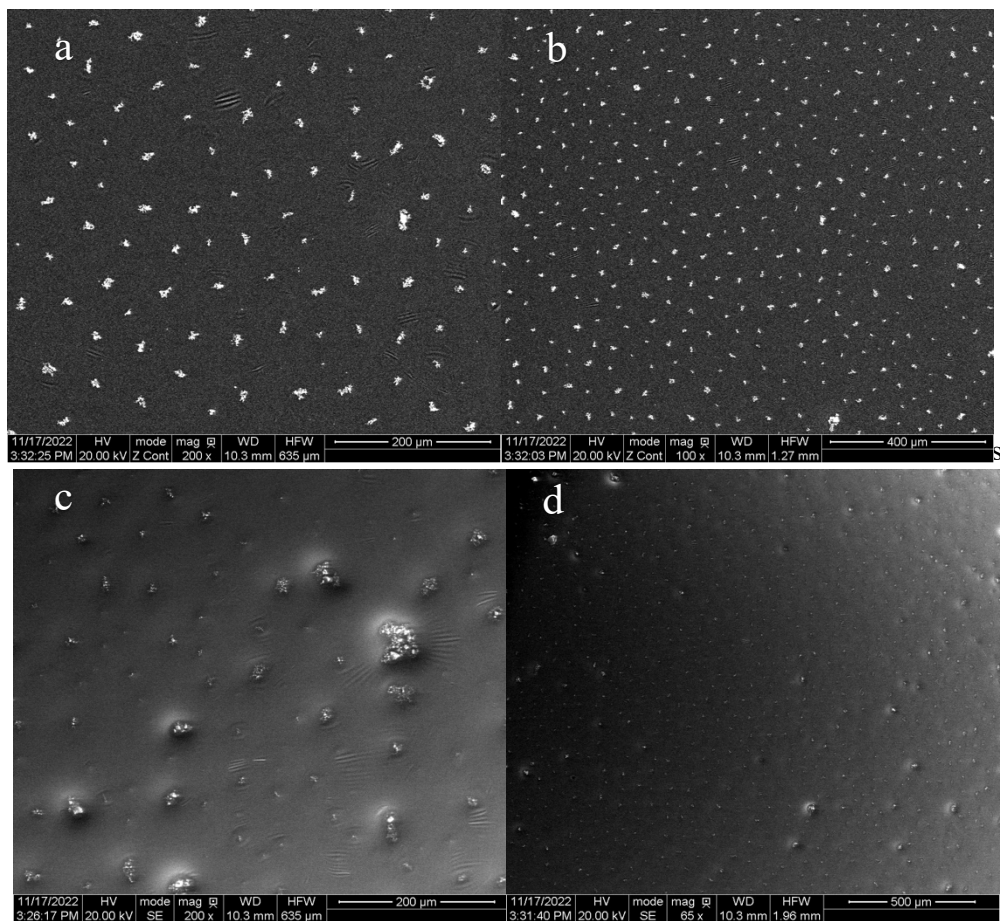


Figure 20: 1 wt% CuS in EG (a) Backscattered Electron Detector (BSD) of 1 wt% CuS in EG 200X; b) BSD of 1 wt% CuS in EG 100X magnification; c) ESEM of 1 wt% CuS in EG 200X; d) ESEM of 1 wt% CuS in EG 65X

Dimpling of the gel surface was observed in all samples but most predominantly in samples containing larger aggregates (30 to 50 μm) likely due to the interfacial energy of the CuS in contact with the surface of EG when curing.

Figure 21 is a close-up image of an aggregate. The aggregate is seen to have areas of higher CuS densities rather than a much more homogenous structure.

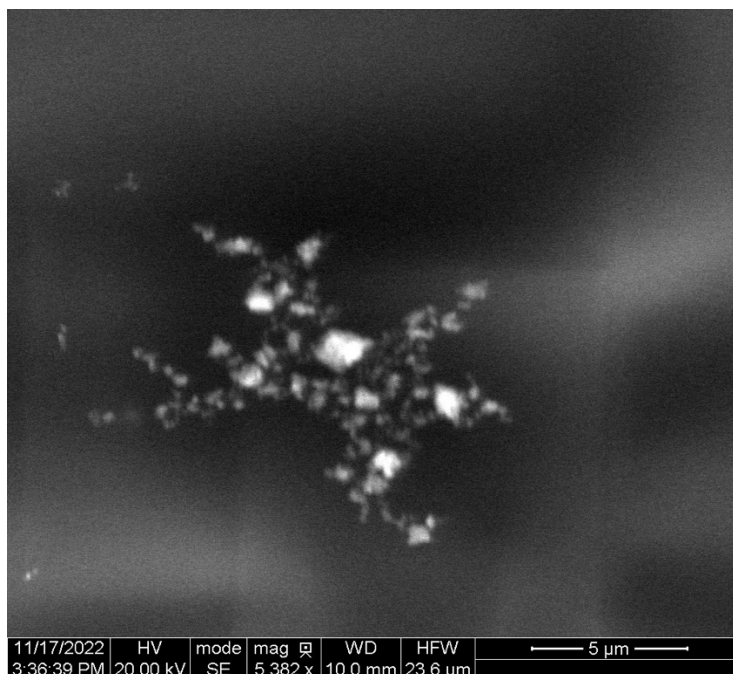


Figure 21: BSD Close up to a CuS Aggregate in 1 wt% CuS in EG

Other observations show that the age of the EG pre-gel solution did have an impact on the solutions ability to cure. Samples of greater age (1 week old) containing 1 wt% CuS were found to not cure despite being exposed to UV light for extended amounts of time. This is hypothesized to be due to the increase in copper ions present in the solution due to the time elapsed as the solubility of CuS is not as great in water.

In older pre-synthesized samples (samples that were synthesized 28 days prior, UV cured the day of ESEM assessment), it was observed that the distribution of CuS in EG was not as prevalent as in newer synthesized samples. Images having the backscattered electron detector (BSD) were seen to be brighter in older samples (0.5 wt% and 0.25 wt%), possibly alluding to increase in distribution of metallic ions since the BSD imaging becomes brighter with increased metallic content. In images of higher magnification, the distribution of CuS in EG can be observed less readily (see Figure 22).

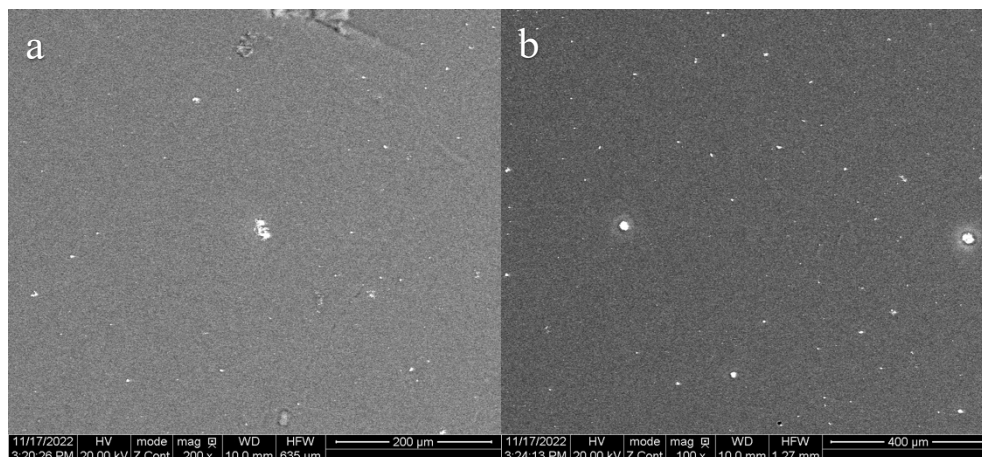


Figure 22: BSD of 0.5 WT% CuS in EG synthesized a month prior, a) 200X; b) 100X

From Figure 23, the average size of visible aggregates was found to be within a similar size range of the 0.1 wt% sample this sample was seen to have more of a spread-out distribution of particles.

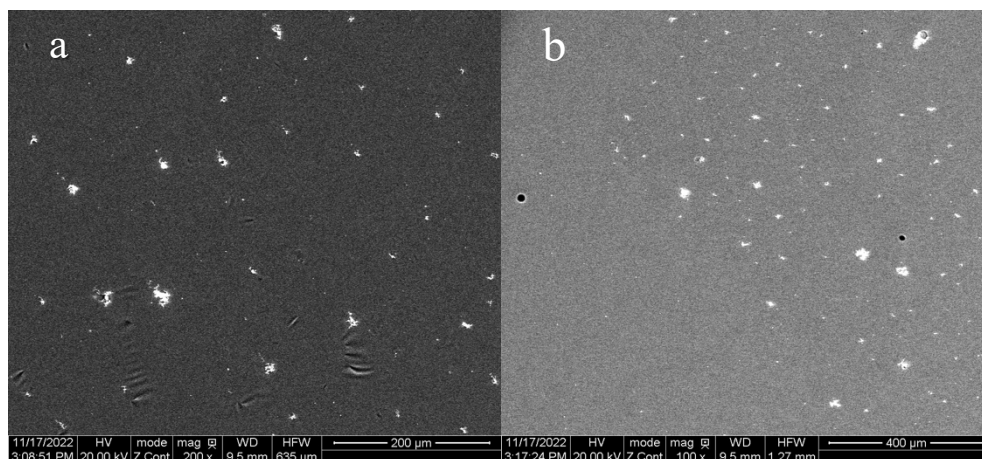


Figure 23: BSD of 0.1 wt% CuS, a) 200X magnification; b) 100X magnification

In these older samples, the visible aggregates ranged from 50 to 5 μm, indicating the dissolution of these particles over time. Figure 24 presents the horizontal and vertical cross-sectional imaging of EG-CuS. The purpose of this test was to observe if the CuS particles were evenly suspended throughout the matrix. These particles overall were observed to be consistently well distributed

throughout the gel matrix. These samples were cured 1 hour before undergoing evaluation using the ESEM. From Figure 24, imaging from the BSD and the SEM shows that the distribution of CuS within EG is not condensed in one region but is well distributed throughout.

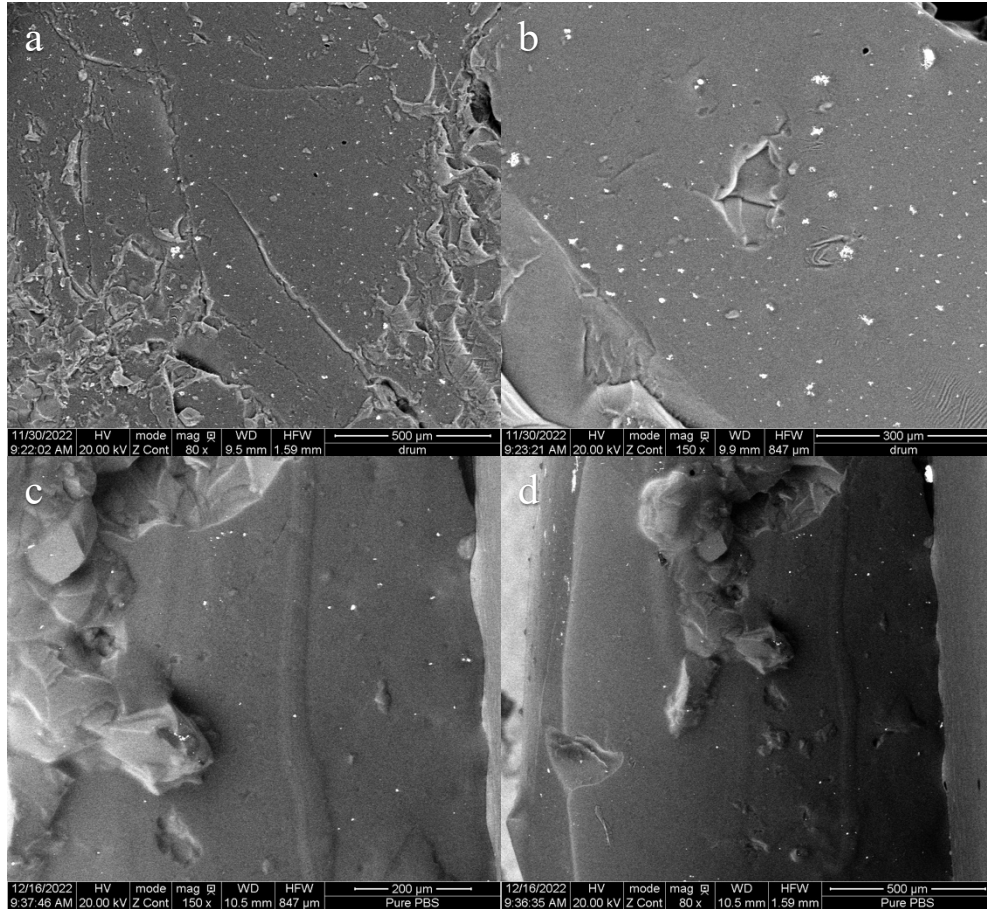


Figure 24: 0.75 wt% CuS in EG Cross-sectional image, a) 80X BSD horizontal cross section; b) 150X BSD horizontal cross section ; c) 150X BSD vertical cross section ; d) 80X BSD vertical cross section

4.2.5 Energy dispersive X-ray spectroscopy (EDS)

Energy dispersive X-ray spectroscopy (EDS) was used to analyze the contents of EG-CuS for a sample containing 0.1 wt% and 0.2 wt% of CuS. The purpose of this analysis is to evaluate the dispersal of lower concentrations of CuS in EG-CuS and evaluate these findings to determine if

there are groups tertiary nitrogen groups actively present that may indicate a contributing factor of contact active antimicrobial behavior.

Figure 25 present an example of the EDS analysis result in a tested region in 0.1 wt% CuS in EG-CuS as showed in the SEM image. Table 19 presents the numerical values of weight and atomic percent distribution of the same analysis.

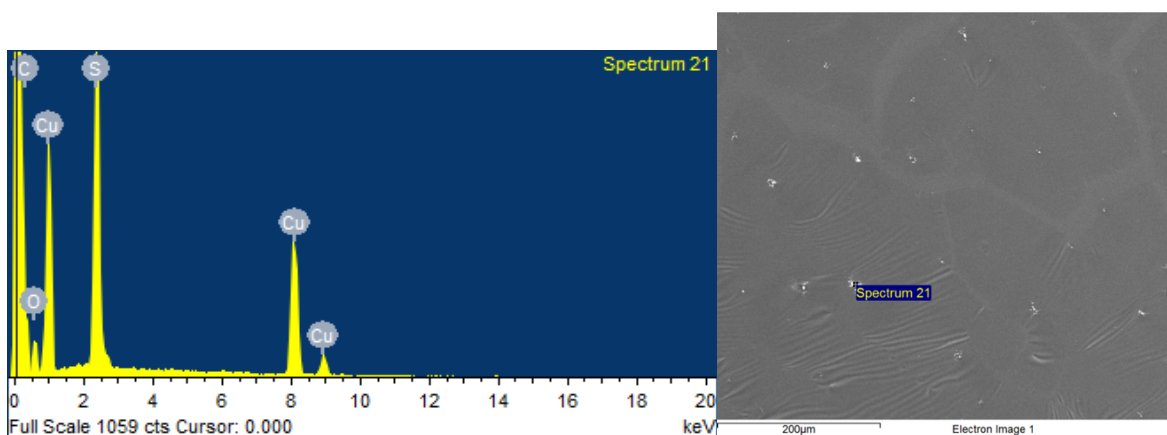


Figure 25: EDS plot of 0.1 wt% CuS in EG for Spectrum 21

Table 19: Weight and Atomic Distribution for Spectrum 21, CuS dispersed in EG

Element	Weight (%)	Atomic (%)
C	26.31	51.91
O	12.49	18.50
S	18.46	13.65
Cu	42.74	15.94

Spectrum 21 as shown in Figure 25, is specifically targeted at a point in which the BSD shows brightness, this being a most likely spot for where CuS could be detected. In this analysis it was found that most expected components were detected except for nitrogen and chlorine. What this may

indicate is that the antimicrobial feasibility of METAC is not as sufficient as the tertiary nitrogen group is not as readily exposed to the surface. For the spot tested, all elements expected were determined to be present in the sample. The use of EDS cannot establish the overall distribution of CuS within EG-CuS. In other regions of the same sample, the presence of copper could not be detected.

Figure 26 presents the EDS for the same sample with a different testing region. The weight and atomic distribution did not indicate of the presence of copper or sulfur. Chlorine, however, was detected in trace amounts. This region has much more oxygen in comparison to Spectrum 21 due to the higher glycerol component since the metallic component is absent. There was no trace of nitrogen.

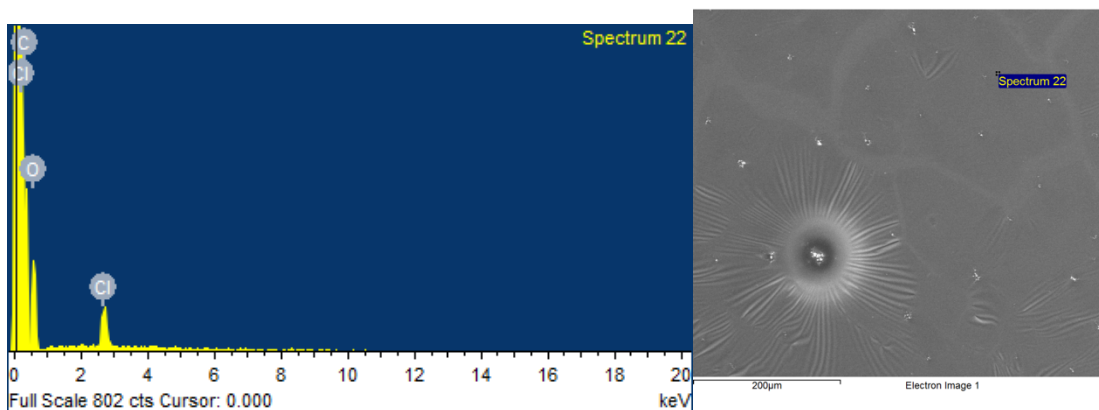


Figure 26: EDS plot of CuS in EG for Spectrum 22

Table 20: Weight and Atomic Distribution for Spectrum 22, CuS dispersed in EG

Element	Weight (%)	Atomic (%)
C	38.69	46.63
O	57.05	51.63
Cl	4.26	1.74

Figure 27 presents the EDS spectrum for a separate region of interest (Spectrum 23) within the same sample. In another region of the sample, the brightness of the BSD imaging region may indicate there to be a presence of CuS.

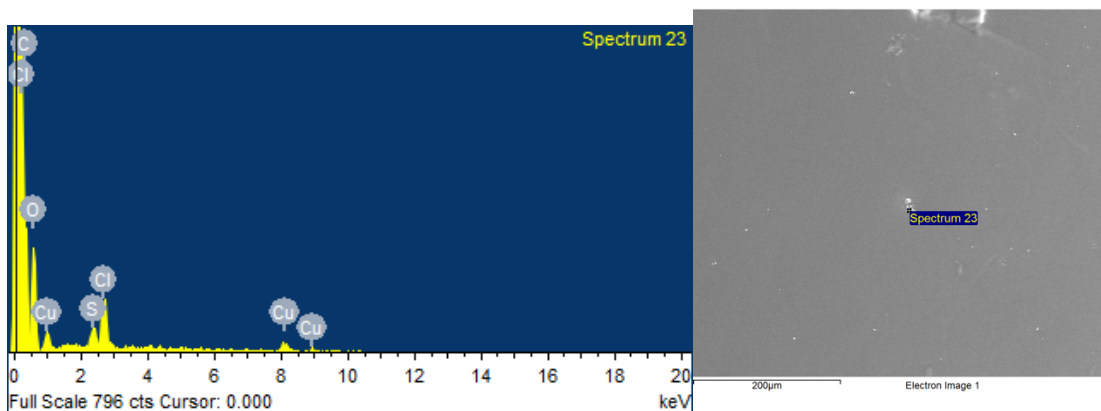


Figure 27: EDS plot of 0.1 wt% CuS in EG for Spectrum 23

Table 21: Weight and Atomic Distribution for Spectrum 23, CuS dispersed in EG

Element	Weight (%)	Atomic (%)
C	33.18	42.60
O	55.15	53.15
S	1.84	0.88
Cu	4.69	1.14
Cl	5.13	2.23

As presented in Table 21, sulfur and copper were both detected in trace amounts that being 1.84 wt% and 4.69 wt% respectively. This is much less than the amount detected in Spectrum 21. Copper, oxygen, and chlorine were all consistent with the results gathered from the analysis done in Spectrum 22 in terms of their atomic weight percentage.

Similar results were also found in separate samples such as 0.2 wt% CuS in EG-CuS (presented in Figure 28 and Table 22). In these tests it was concluded that nitrogen was not a trace substance.

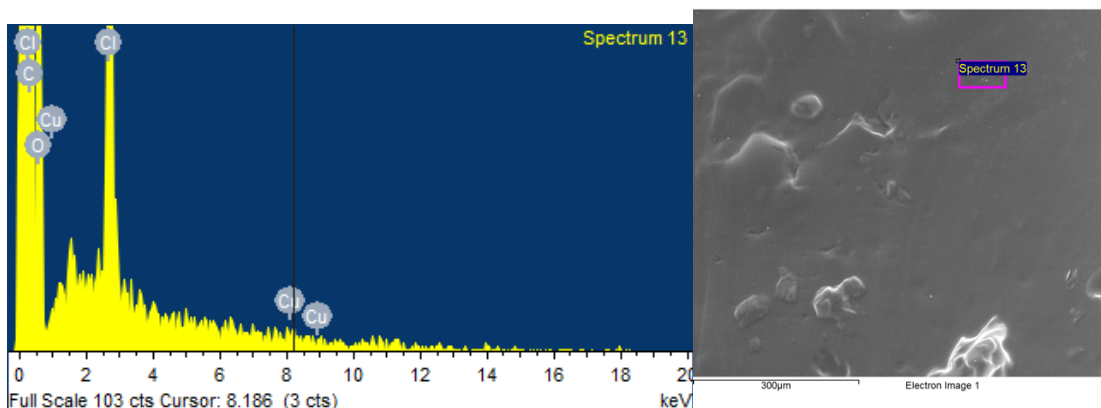


Figure 28:EDS plot of 0.2 wt% CuS in EG for Spectrum 13

Table 22: Weight and Atomic Distribution for Spectrum 23, CuS dispersed in EG

Element	Weight (%)	Atomic (%)
C	43.80	52.76
O	49.17	44.47
Cu	0.51	0.12
Cl	6.52	2.66

Based on the EDS analyses, it is noticed that the distribution of Cu within the EG matrix varies, and the ions exhibit reduced solubility after extended pre-cursor solution settling times. While chlorine was detected, the tertiary nitrogen group responsible for contact-active antimicrobial behavior was not found in the samples tested.

4.2.6 Thermogravimetry Analysis (TGA)

Thermogravimetry analysis (TGA) was used to characterize the material through the analysis of characteristic decomposition. Thermal degradation of synthesized EG, water with glycerol (1:4), EG-CuS (of varying concentrations), METAC polymer crosslinked with GlyDMA, EG with water only, and EG with glycerol only were analyzed by TGA. Particularly this TGA assessment was used to analyze the effect of the CuS and contribution of interstitial fluid to the EG components established prior to the addition of the additive.

Figure 29 and Figure 30 present Thermogravimetric Analysis (TGA) and Differential Thermogravimetric Analysis (DTG) curves for a comparison between samples with and without CuS and a comparison of samples of EG containing varying amounts of CuS respectively. This was done to examine the effect of CuS to EG under high temperatures. As seen in Figure 29, the thermal degradation of EG and EG derivatives can be described by four steps, each denoting either the evaporation or degradation of EG components. The first stage occurs at an onset temperature of 25°C; volatile components were seen to have evaporated within the first stage, at a loss of 5 to 10%, the temperature range for this component is synonymous to the vaporization behavior of water. The next stage indicated is of the greatest weight loss ranging from 60 to 85%; this was speculated to the vaporization of glycerol from 158°C to 245°C. The third degradation stage is speculated to be the loss of the polymeric matrix of METAC crosslinked with GlyDMA. The final stage is the degradation of the rest of the highly crosslinked polymeric contents.

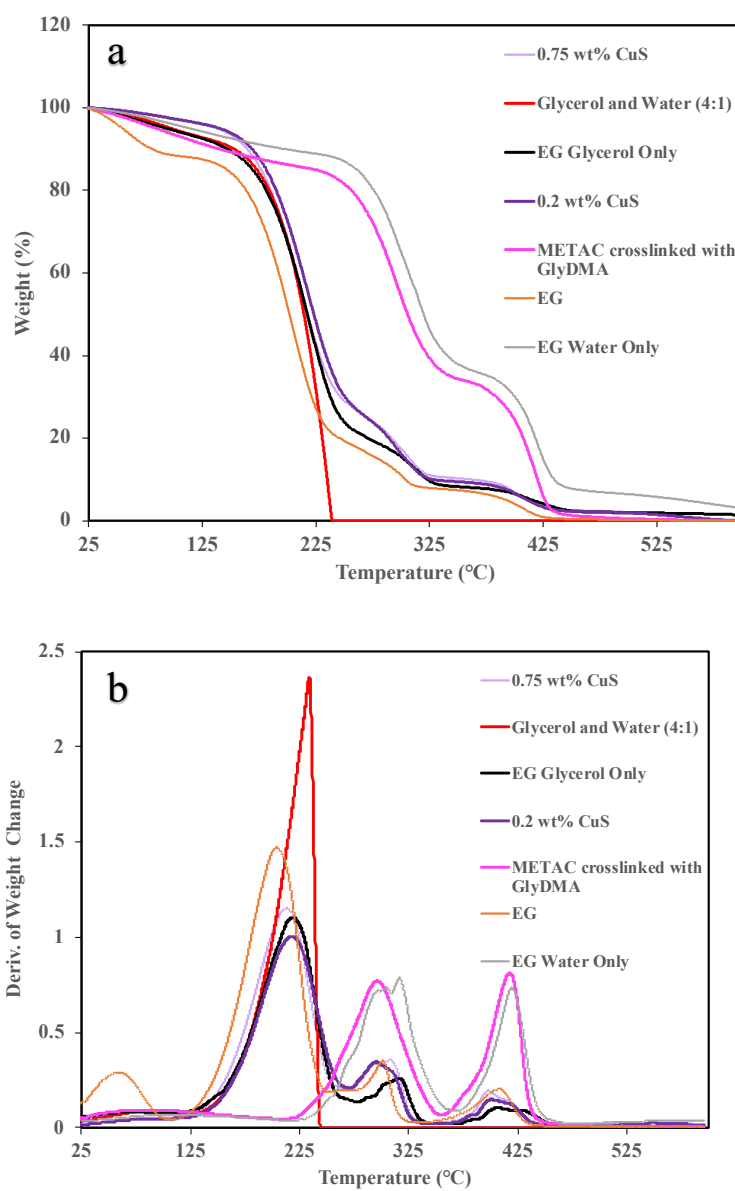


Figure 29: a) TGA Curves of EG, METAC Crosslinked with GlyDMA, EG containing glycerol only, EG containing water only, and EG containing varying CuS concentrations; b) DTG Curves of EG, METAC Crosslinked with GlyDMA, EG containing glycerol only, EG containing water only, and EG containing varying CuS concentrations

For samples of varying CuS content, compared to samples containing only water or glycerol, the same evaporation-based mass loss of water was observed at 155 °C and 243°C. In the EG sample containing only glycerol and samples the same mass loss was observed as in previous samples. Other trends show that onset temperature remained the same for all stages, thus the increase of CuS in EG did not induce any new notable intermolecular interactions. The EG samples that only contained water or were just GlyDMA crosslinked METAC were observed to only have three distinct loss phases. The first being that of the water content, the next drop in mass is that of the polymeric material that has an onset temperature around 275°C and is potentially the glycerol leaving the system through evaporation, finally followed by another drop in polymeric mass at an onset temperature of 375°C. The two phases of polymeric degradation are consistent in all samples as the onset temperature remains the same, the main difference is that glycerol is not present within these samples. Table 23 below summarizes the deviations in weight for all samples.

Table 23: Summary for weight loss at each stage of degradation for each tested sample

Sample	First Deviation in weight (%)	Second Deviation in weight (%)	Third Deviation in weight (%)	Fourth Deviation in weight (%)
EG	8.49 ± 6.26	84.07 ± 11.30	13.47 ± 3.81	8.21 ± 1.46
EG CuS 0.01 wt%	5.88 ± 1.14	69.67 ± 7.36	15.93 ± 1.98	8.45 ± 1.38
EG CuS 0.2 wt%	5.10 ± 0.59	70.21 ± 2.24	14.63 ± 0.53	7.89 ± 0.74
EG CuS 0.5 wt%	3.99 ± 0.22	62.62 ± 0.93	14.25 ± 1.85	7.70 ± 0.83
EG CuS 0.75 wt%	4.40 ± 0.58	67.26 ± 9.18	17.03 ± 0.33	8.35 ± 0.76
PG(M)	11.83 ± 2.49	44.35 ± 5.61	28.78 ± 3.23	-

From Table 23, the percent weight deviation is consistent with the components in EG, these results are presented in Figure 30. In addition to TGA, the derivative thermogravimetric curve (DTG), which shows the rate of mass change with respect to temperature, is also depicted in (b) of below. The

addition of CuS to the EG hydrogel matrix was shown to produce similar results. This may indicate that the presence of CuS overall does not contribute to the interactions between the interstitial fluids and the polymeric matrix. The variation in the first drop was due to sample variations in terms of water containment.

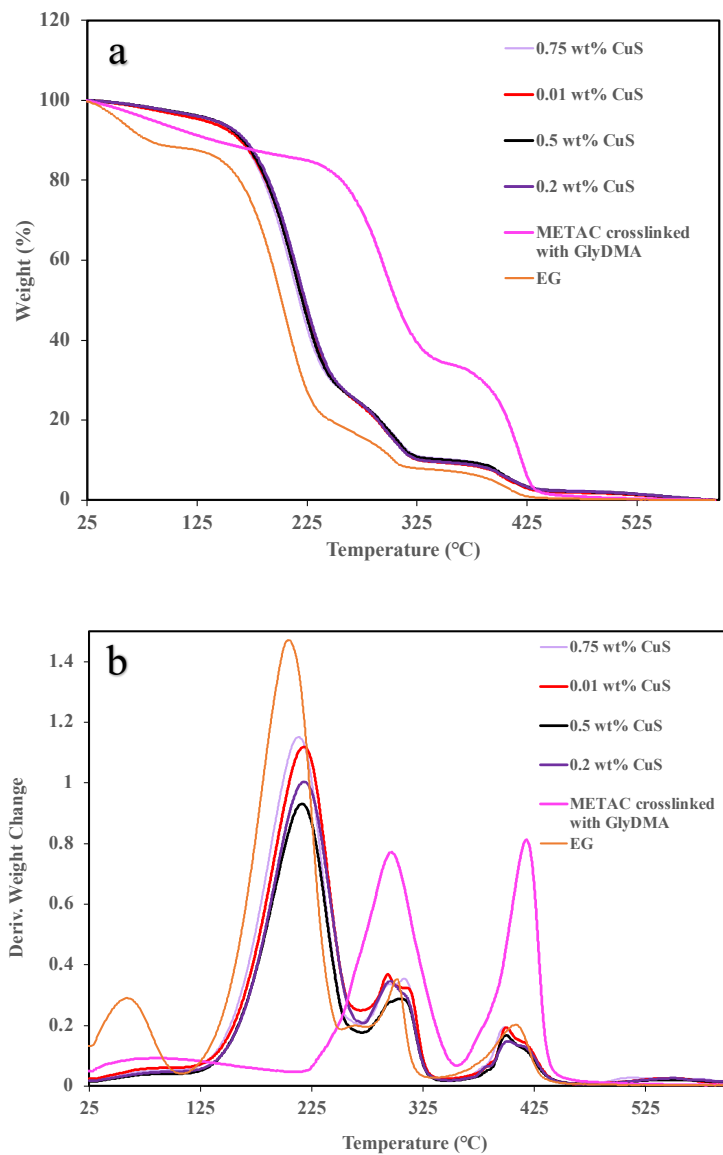


Figure 30: a) TGA Curves of EG, METAC Crosslinked with GlyDMA, and EG-CuS at different CuS Concentrations; b) DTG Curves of EG, METAC Crosslinked with GlyDMA, and EG-CuS at different CuS Concentrations

4.2.7 Differential Scanning Calorimetry (DSC)

In this section, differential scanning calorimetry (DSC) was utilized to assess EG, METAC, and various concentrations of EG-CuS for each sample's characteristic thermal properties. A single cyclic scan was performed from -90°C to 400°C at a ramp of 10 °C/min to observe all changes upon heating. For EG alone, it was found that the heat of vaporization (ΔH_v) was within a range of 110 °C to 160 °C and the thermal degradation (T_D) was observed as a peak within a range of 280 °C to 330 °C. A comparative plot is as depicted in Figure 31 below.

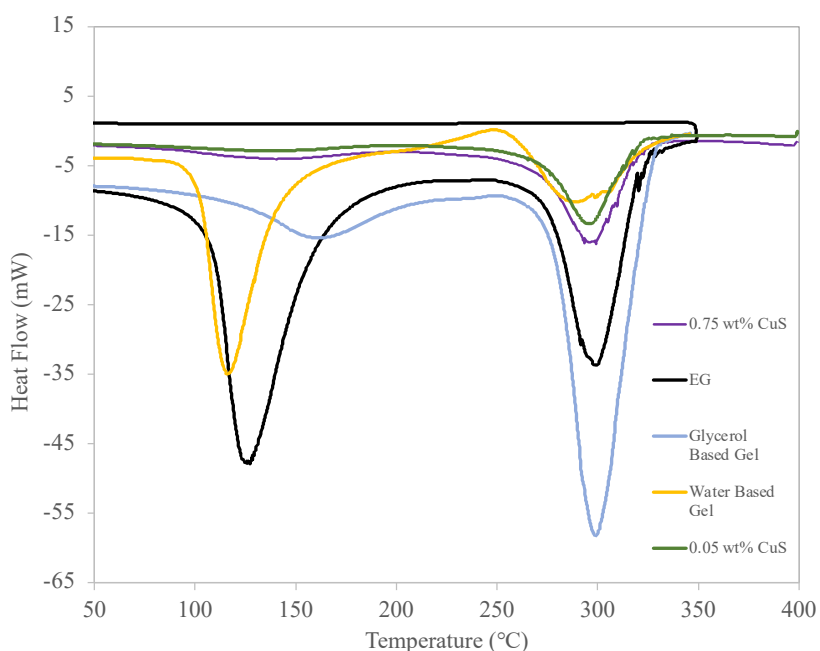


Figure 31: DSC Comparison of Peaks between Different Samples

For all samples of EG, the temperature of degradation (T_D) was present at 299.2 °C. Evaporation of the interstitial fluid was also seen consistently within the range of 90 °C to 152 °C; however, depending on the sample, the peak and energy required to vaporize the fluid varied. The evaporation peaks were also consistent with the amount of glycerol to water ratio. Trends indicate that for samples containing more water, this vaporization peak would be stronger and skewed towards lower temperatures. For samples

that contain more glycerol the opposite was observed. This may be due to the hydration shell effect as discussed in Chapter 2.

A separate assessment demonstrated that copper sulfide interactions with hydrogel resulted in vaporization at 150 °C to 160 °C. In all tested samples, this was not seen to have a drastic difference in heat of vaporization. These results are presented in Figure 32 within the range of -100 °C to 160 °C, where a close-up of the T_g transition is indicated by a circle. An amorphous phase transition can be observed in all samples at 37 °C. This phase transition can later be seen in the same temperature upon cooling. Consistently, it was observed that the T_g occurred at the same point for both heating and cooling curves of the same cycle. This means that the amorphous phase can be reversible such that it will return to its original state without variation in its amorphous characteristics.

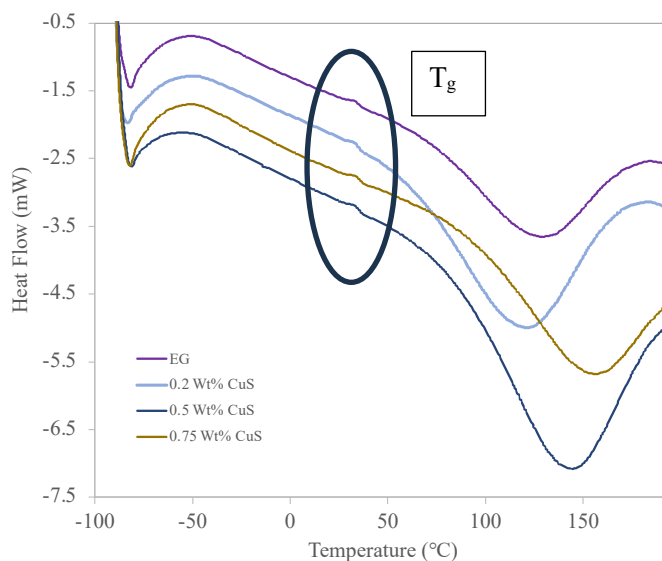


Figure 32: DSC Plot of EG and Eg-CuS in Varying Concentrations

Table 24 summarizes each sample's heat of vaporization, ΔH_v , from this assessment. For each sample, 3 tests were performed.

Table 24: DSC Heat of Vaporization Data of EG and EG-CuS Samples

Sample	ΔH_v (J/°C)	T_v (°C)
EG	120.57 $\pm$ 19.07	122.69 $\pm$ 27.89
EG CuS 0.01 wt%	140.37 $\pm$ 9.89	125.20 $\pm$ 1.97
EG CuS 0.2 wt%	141.8 $\pm$ 35.45	122.24 $\pm$ 8.78
EG CuS 0.5 wt%	121.40 $\pm$ 22.98	139.78 $\pm$ 4.81
EG CuS 0.75 wt%	92.19 $\pm$ 28.38	126.44 $\pm$ 31.39

Figure 33 shows a DSC plot of EG when multiple runs are performed on the same sample. From Figure 33, the vaporization peak was confirmed to be that of vaporization in a 4-cycle run ranging from -10 °C to 210 °C with a ramp of 10 °C/min. The phase transition occurred at the same point despite the loss of the volatile component meaning that the amorphous transition does not rely on the presence of the interstitial fluids. This may mean that the contribution of the fluidic/volatile component does not contribute much to the intermolecular interactions present in the polymeric matrix.

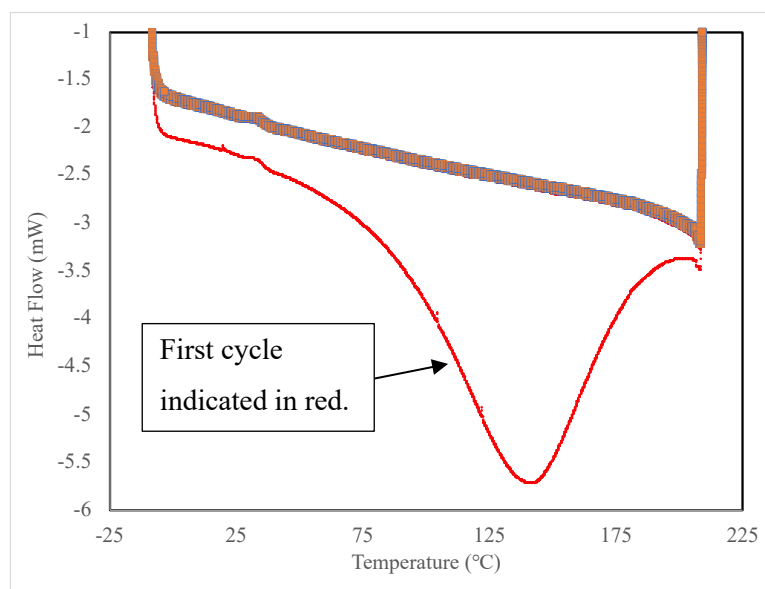


Figure 33: DSC Plot of EG 4 times cyclic run -10°C to 210°C, 10°C/min ramp

4.2.8 Swelling Tests

Swelling tests of EG were conducted using deionized (DI) water. Figure 34 a shows EG prior to water exposure. When exposed to deionized water, the gel immediately deviated in shape as can be seen in b). This irregular shape remains a part of the gel after 30 seconds of exposure as can be seen in Figure 34b. It was observed that after 5 minutes of exposure (image e), the irregular protrusions on the surface of the gel become very apparent. This kind of formation was seen in all samples at this time interval, it was seemingly more uniform and symmetrical in distribution of bulges as compared to earlier observations. In Figure 34f, it was observed that the bulges had become swollen to the extent that the material folds onto itself. At this point, EG becomes extremely fragile and prone to breakage. In Figure 35, it was observed that the folding and irregularities of EG had smoothed out over time; however, the edge was still swollen. After 12 hours of exposure to deionized water, the structure became completely smoothed out.

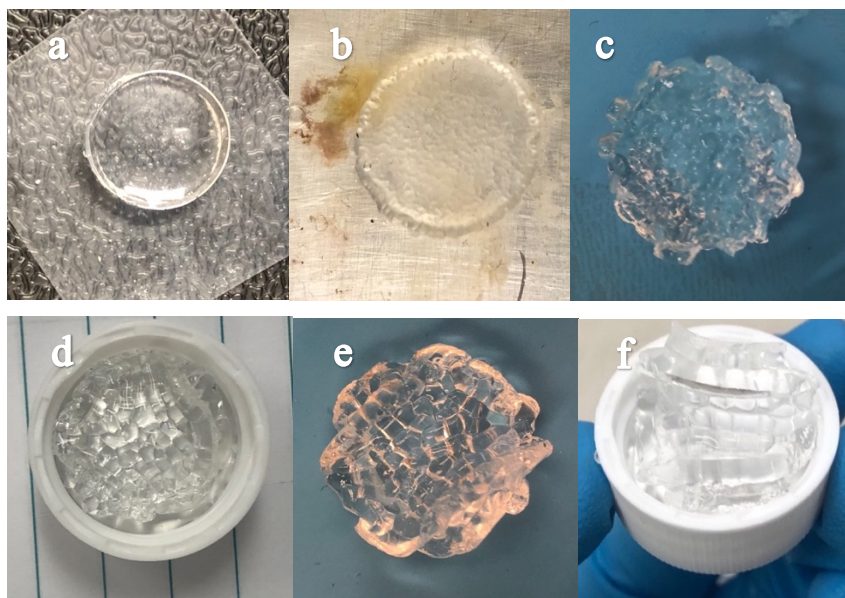


Figure 34: EG after exposure to water: a) before water exposure; b) 30 seconds in water; c) 1 minute after water exposure; d) 2 minutes after water exposure; e) 5 minutes after water exposure; f) 10 minutes after water exposure

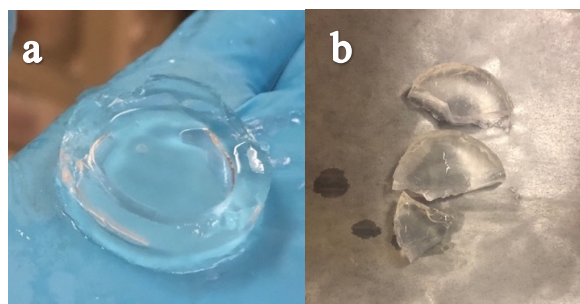


Figure 35: Left to right, EG in water exposure time after: a) 30 minutes; b) 12 hours

The same observation was made for all EG samples containing CuS. CuS particles would accumulate towards the grooves of the swollen gel (see Figure 36, c). This feature disappeared upon further

expansion and water exposure time. It is hypothesized that the flowrate of DI water to and from the matrix contributes to this redistribution of CuS particles.

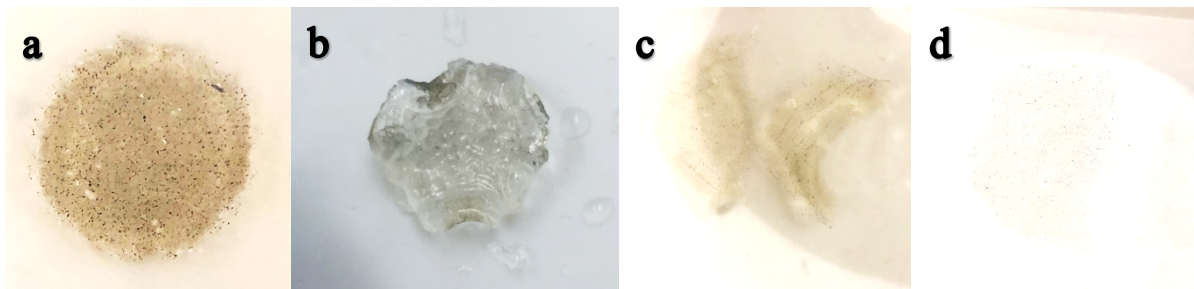


Figure 36: EG containing 0.5 wt% CuS in water: a) 30 seconds of water exposure; b) 2 minutes of water exposure; c) 10 minutes in water; d) 12 hours in water

Figure 37 shows the weight percentage of EG only swollen in DI water at certain points in time. To show consistency between samples of the same formulation, each sample presented in Figure 37 are repeated measurements on EG in which Samples 1, 2, 3, 4, and 6 are separate samples of EG with the same composition and synthesis method. For Sample 5 presented in Figure 38, EG was left without disturbance for hours prior to measurement. After 12 hours, the diameter of EG was seen to have swollen to a maximum of 26.2 mm from the original 13.2 mm and the height of the sample grew to 7.4 mm from 2.9 mm.

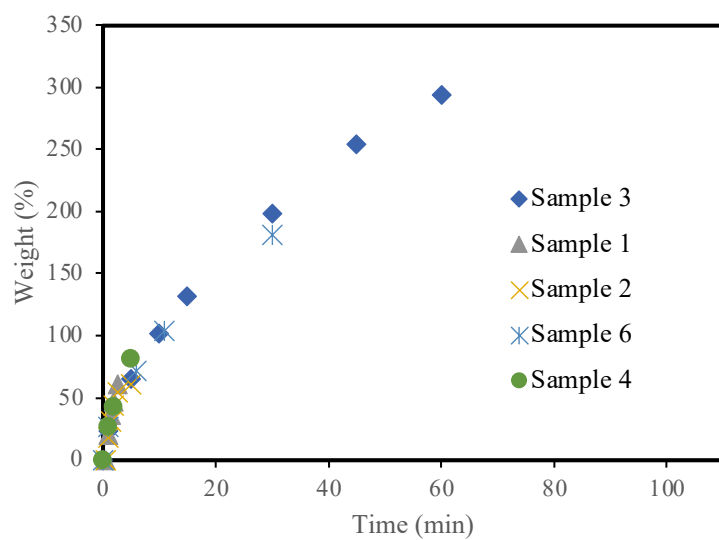


Figure 37: EG Weight (%) as a Function of Time (min) (Samples 1, 2, 3, 4, and 6)

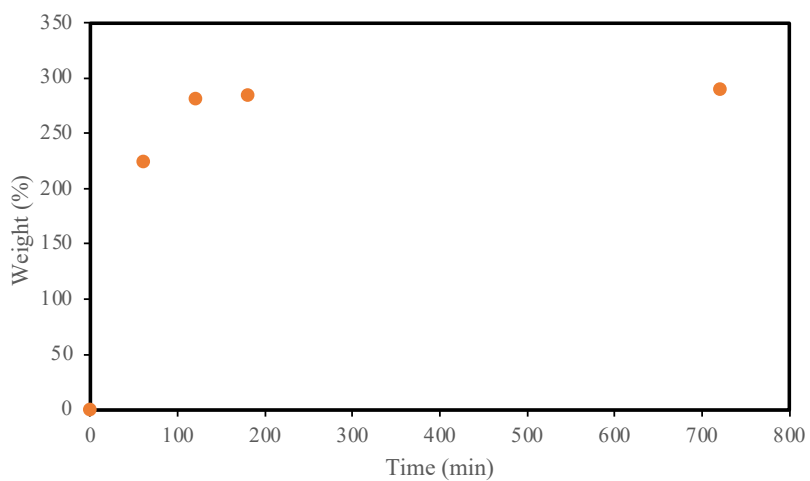


Figure 38: Sample 5 EG Weight (%) as a Function of Time (min)

Table 25 below summarizes initial and final conditions of each EG only sample. Swelling was observed to be consistent for these samples.

Table 25: Initial and Final Conditions of EG Swelling Test Summary

Sample	Initial Mass (g)	Final Mass at breakage (g)	Time at Breakage (min)
Sample 1	0.5567	1.164	2
Sample 2	0.4970	1.110	2.5
Sample 3	0.5529	3.494	60
Sample 4	0.5569	1.370	5
Sample 5	0.5110	3.413	720
Sample 6	0.5344	2.346	30

As can be seen in Table 25, a similar mass change was observed for samples with shorter time intervals of mass recording. Sample 5 is an outlier. Samples 3 and 5 have longer times until breakage, these samples may have not been as brittle as the other tested samples and thus were able to hold its shape.

Figure 39 and Figure 40 show a comparison between separate EG-CuS samples undergoing a swelling test. Separate samples are assessed to compare swelling capabilities within the same sample set.

For Figure 39 and Figure 40 a similar swelling effect was observed for both samples, these samples were observed to increase in brittleness as water retention increased.

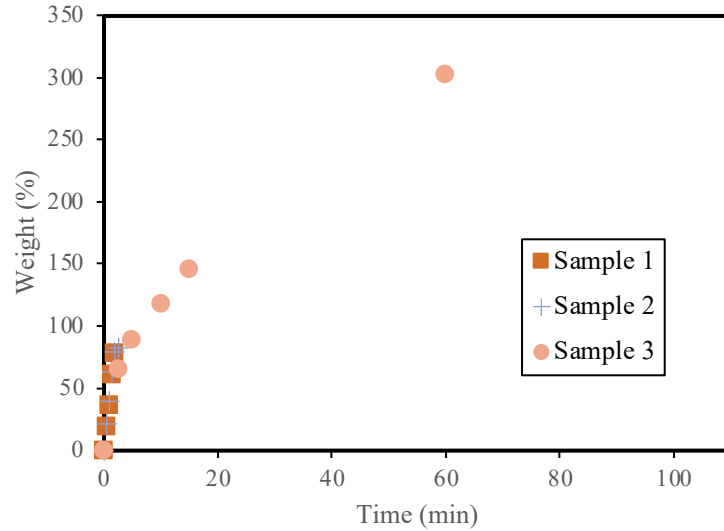


Figure 39: EG-CuS (0.2 wt%) Weight (%) as a Function of Time (min)

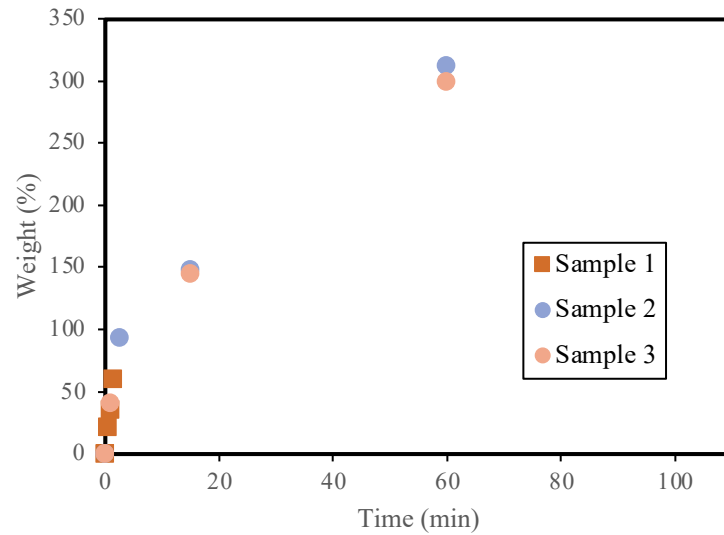


Figure 40: EG-CuS (0.5 wt%) Weight (%) as a Function of Time (min)

In a separate assessment, three samples of varying concentrations of CuS in EG were left to sit over an extended amount of time, all three separate samples containing 0.75 wt% CuS in EG were observed to undergo structural failure after 2 months (see Figure 41 below). This was not observed in samples containing lower concentrations of CuS such as 0.2 wt% CuS in EG as can be seen in Figure 46. The

structural failure may be due to the gravitational force of CuS on the polymeric matrix. A darker gradient was also observed in both images presented in Figure 41 and Figure 42. Thus, the concentration of the CuS does account for the structural integrity of the hydrogel. As well, the dispersion is trapped within the polymeric matrix itself.

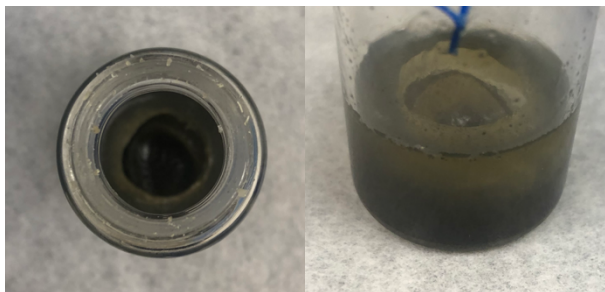


Figure 41: 0.75 wt% CuS in EG (left image is top view of the sample, right is side view of the sample)

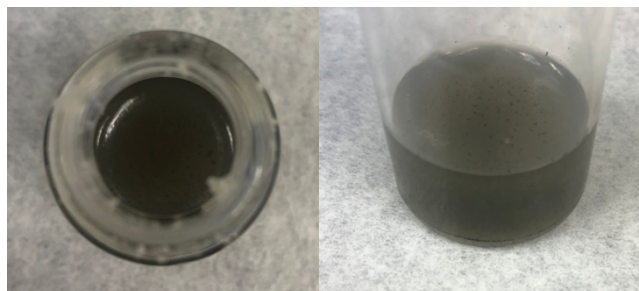


Figure 42: 0.20 wt% CuS in EG (left image is top view of the sample, right is side view of the sample)

4.2.9 Determining the Young's Modulus

Young's modulus is a characteristic property of a material that can be used to describe the materials behavior when subjected to a force. For EG and EG-CuS, the mean Young's Modulus values are determined assuming the Hertz model. The Hertz model assumes that the indentation is negligible in comparison to the thickness of the tested sample, this implies that the sample is an elastic solid which

occupies an infinitely extending region. Other assumptions state that the indenter is not deformed under contact and that there are no additional surface/energetic interactions between the surface of the tested material and the indenter. Under the Hertzian model, each sample produces a characteristic initial slope region, which is used to calculate the Young's modulus of the sample.

$$Slope = F = \frac{4}{3} \cdot \frac{E_s}{1 - \nu_s^2} \cdot \sqrt{r} \cdot \delta^{\frac{3}{2}} \quad \text{Equation (5)}$$

$$Slope\ factor = \frac{4}{3} \cdot \frac{\sqrt{r} \cdot \delta^{\frac{3}{2}}}{1 - \nu_s^2} \quad \text{Equation (6)}$$

$$E_s = \frac{Experimental\ Slope}{Slope\ factor} \quad \text{Equation (7)}$$

The formulas as shown above are used for indenters with spherical probes. E_s is the Young's Modulus, ν_s is the Poisson's ratio (0.47 is used for this study, this value is chosen based on existing hydrogels), the radius of the indenter is denoted as the variable r , and δ is the distance travelled by the indenter. F is the indentation force using a silicon-based hemisphere geometry. The radius, r , describes the radius of the indenter and δ is the distance indented by the indenter.

Figure 43 illustrates the general trend of Young's modulus with varying CuS amounts. Each sample had a replicate of 3.

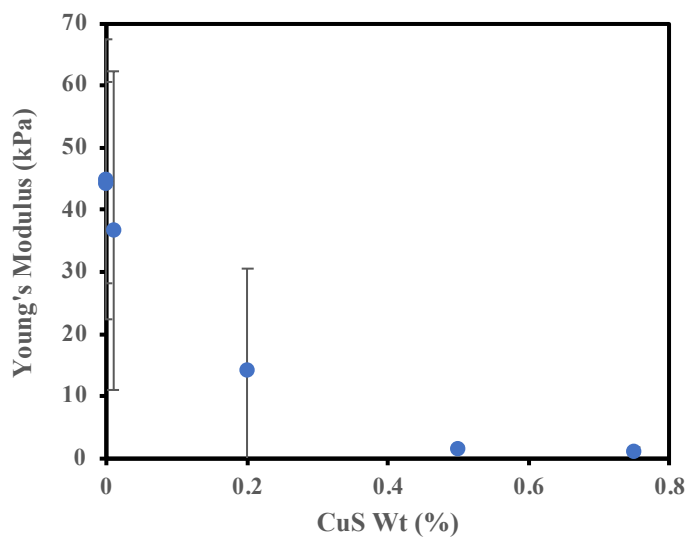


Figure 43: Young's Modulus of EG and EG-CuS Samples of Varying Concentration

From Figure 43, samples containing greater concentrations of CuS (0.5 and 0.75 wt%) found to have similar Young's Moduli of 1.62 kPa and 1.19 kPa respectively. For samples containing less CuS, the Young's Moduli was found to be 14.20 kPa, 36.69 kPa, and 44.3 kPa for 0.2, 0.01, and 0 wt% CuS respectively. This trend indicates that there is a progressing softening of material with the inclusion of CuS. From the FTIR results, it was concluded that there were no additional bonds formed by increasing the concentration of CuS in EG, thus it can be concluded that the addition of CuS softened the EG. It is hypothesized that CuS particles may hinder the EG crosslinking.

4.2.10 Summary

In this Chapter, the chemical, thermal, and physical properties of EG and EG-CuS were evaluated to reveal differences in attributes upon the inclusion of CuS. EG itself has good mechanical properties, with a Young's modulus of 44.3kPa. The addition of CuS to EG resulted in an increased adhesion peel force as concluded from the T-peel test. The adhesive strength of two coated MB-PP substrates increased from 0.024 N (EG alone) to 0.057 N for samples containing 0.75 wt% CuS. It was observed

that EG horizontally swells to twice the original diameter in length and vertically 2.5 times that of its original height. The inclusion of CuS to EG did not change any aspect of these findings although the distribution of CuS particles upon swelling did provide further insight onto the distribution of CuS particles within the polymeric matrix. DSC analysis showed that there was an observed amorphous glass phase transition occurring consistently at 37°C. Aside from the transition phase, the degradation was seen consistently at 288°C for all EG samples tested. Between all the samples tested, the heat of vaporization varied the most and was consistently found within the temperatures of 150°C to 160°C. TGA further confirmed the degradation temperature as the temperature for polymeric degradation was consistently found at around 288°C. Varying the concentration of CuS in EG did not show a presence of new interactions through bond formation as concluded in the FTIR analysis. The EDS assessments showed that copper, sulfide, and chlorine are detectable within the sample however, nitrogen was not detected at all, indicating that the tertiary amine group may not occur frequently enough for the group to be an effective antimicrobial through contact-active antimicrobial action. The hydrophobicity of EG was only slightly influenced by the addition of CuS if there is any. In summary, lower concentrations of EG-CuS (0.01 - 0.5 wt%) was found to display similar attributes to EG, being overall a feasible coating without discoloration or undesirable characteristics.

Chapter 5 Conclusions and Future Work

In this study, the antimicrobial efficacy of EG and its additive incorporated derivatives were first tested. It was found through an EDS analysis that the tertiary amine group that is responsible for the contact active antimicrobial behavior was not found in multiple samples. This may be a reason as to why the polymeric material is ineffective without the additive. Poly(METAC) without crosslinker, was found to be effective when displaced on an agar plate containing *E. coli*. Overtime the polymer would become a viscous fluid on the agar plate containing the biofilm and the biofilm would begin to disappear upon contact. Therefore the main mode of antimicrobial activity was through the soluble nature of its polymer counterpart; the ability of the polymer (not crosslinked) to leach and dissolve in water was a necessary component for antimicrobial efficacy. Out of the antimicrobial additives tested, AgNO₃ and CuS demonstrated evidence of antimicrobial activity. The results show that CuS was the most feasible option to increase the antimicrobial efficacy of the material as AgNO₃ produced a discoloration of EG and the fabric substrate. The chemical, thermal, and physical properties of EG and varying concentrations of CuS in EG were assessed for changes in the material's adhesive strength, structure, thermal characteristics, and degradation. From these analyses, it was determined that the inclusion of CuS did not change the existing chemistry as concluded from the FTIR results. The inclusion of CuS did however increase the peel adhesion strength and lower the Young's modulus. Increasingly greater concentrations of CuS were seen to settle and cause structural failure to the hydrogel matrix after long periods of time and become more difficult to UV cure.

To enhance and better understand the characteristics of EG and its derivatives, future work should be centered around tests involving placing the material under humidity tests in commonly occurring healthcare conditions. The characterization tests were conducted on disposable medical PPE; however, assessment of the material's attributes should be conducted on that of reusable textiles as

well. FTIR does give indication of present hydrogen bond intermolecular interactions but does not highlight any present dipole-dipole interactions for instance, NMR is recommended for further evaluation of the intermolecular interactions present in EG and EG-CuS. It is also recommended that the gel-point shall be investigated through the testing of its rheology and most especially the G'' (loss modulus, viscous behavior of material) and G' (storage modulus, elastic behavior of material). The determination of gel point through these plots can serve as an indicator of consistent and effective curing time for each compound and specified additive amounts.

Currently, there are no existing industrial protocols to effectively validate the efficacy of contact active antimicrobials; fluorescence microscopy of the material may confirm the existence of the property. Outcome from E.coli experimentation has been investigated; however, other types of gram-negative bacteria or gram-positive bacteria have not been tested for, these should be investigated to further prove the antimicrobial efficacy of the material. Future experimentation may include the assessment of efficacy against pathogens adsorbed within the hydrogel's polymeric matrix as well as the determination of the amount of unused photoinitiator since the photoinitiator may have been a biocidal component if the sample was not cured to the gel-point. The adjusted version of the AATCC100 protocol used in the assessment of the antimicrobial efficacy does not mimic the realistic conditions of how bacterial colonies proliferate in public spaces. Further testing in conditions that imitate airborne and dry surface transmission of bacteria is recommended. Another limitation is the dispersion of each additive as no means had been employed in this study to ensure good dispersion; CuS particle might have aggregated into larger cluster. Future work may need to evaluate the dispersion and find ways to ensure good dispersion of the additive particles and assess how the state of dispersion may affect the antimicrobial efficacy.

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