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# An Evaluation of the Relationship Between Application Method, Concentration, and Antimicrobial Efficacy of an Antimicrobial Finish After Accelerated Laundering

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AN EVALUATION OF THE RELATIONSHIP BETWEEN  
APPLICATION METHOD, CONCENTRATION, AND  
ANTIMICROBIAL EFFICACY OF AN ANTIMICROBIAL  
FINISH AFTER ACCELERATED LAUNDERING

BY

ELISE DESBONNET

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
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IN  
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2016

MASTER OF SCIENCE IN TEXTILE SCIENCE  
OF  
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## ABSTRACT

This study addresses the need for an effective and durable antimicrobial finish for performance textiles using silver (Ag) as the antimicrobial agent on plain-weave nylon fabric. Three different concentrations of AgCl + TiO<sub>2</sub> (500ppm, 2500ppm, and 5000ppm) were applied using pad and exhaust application methods. Exhaust bath samples were taken at 0, 15, 30, 45, and 60 minute time points. Ag concentration analysis provided insight on exhaustion behavior. Samples from each treatment group underwent 0, 1, 5, and 10 accelerated laundering cycles and were tested against *Escherichia coli* and *Staphylococcus aureus*. Exhaustion in all three fabric bath concentrations occurred rapidly in the first 15 minutes, continuing to exhaust at a slower rate throughout the sample time frame. Samples treated with the exhaust application method produced larger zones of inhibition and retained antimicrobial effectiveness through more launderings than those treated with the pad application against both bacterial species. All fabric samples lost antimicrobial effectiveness after 5 accelerated launderings, well below the industry standard of 50. A statistically significant difference in the mean zones of inhibition existed between pad and exhaust application methods after 0 wash cycles for *E. coli*. After 0 accelerated laundering cycles, *S. aureus* exhibited a mean zone of inhibition on the statistical cusp of significance between application methods. A statistically significant difference existed in the mean zones of inhibition between Ag concentrations after 0 accelerated laundering cycles for both bacterial species.

While results of this experimentation show Ag does exhaust onto nylon fabric, a bath treatment or alteration in exhaustion conditions, such as addition of a binding

agent, may yield complete exhaustion, leaving minimal Ag in wastewater. While the exhaust application method provides greater antimicrobial action at low washings than the pad method, it does not improve the longevity of antimicrobial properties after extended washing. Addition of an auxiliary to the exhaustion bath could improve durability to laundering, reducing Ag in wash effluent, and perhaps cost effectiveness of treatment. This study provides future researchers with a foundation for research that focuses on improving the exhaust application method.

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# CHAPTER 1

## INTRODUCTION

Clothing subjected to heavy perspiration often develops an unpleasant odor due to the degradation of sweat by certain bacteria, such as *Staphylococcus* species.<sup>15</sup> Bacterial attack on textiles also can lead to a loss of tensile strength and discoloration.<sup>25</sup> Textiles used in hospitals often become contaminated with a multitude of bacterial species, developing resistance to routine cleaning agents and disinfectants and promoting pathogen transfer.<sup>4</sup>

While antimicrobial textiles and devices intended for medical use aim to reduce the risk of infection and pathogen transfer, a successful antimicrobial finish intended for consumer goods aims to significantly limit the incidence of bacteria growth, therefore reducing the formation of unpleasant odors, as well as performance loss due to microbial-induced fiber degradation.<sup>28</sup> Antimicrobial textile finishes commonly are applied by the “pad-dry-cure” method, which requires minimal water and processing energy. Fabric is dipped into the antimicrobial solution or suspension, squeezed between two rollers to reduce excess liquid, dried, and cured. Although effective, this method can yield an end product with decreased tensile strength, poor abrasion resistance, and increased stiffness due to the use of auxiliary chemicals like binders and cross-linking agents.

As antimicrobial suspensions possess an ionic charge, they also can be applied to fabric the same way a dye is exhausted. The active antimicrobial agent does not

penetrate the fiber like a dye, but it does adhere to the fiber's surface through ionic bonding. This method of application can potentially avoid the aforementioned strength, abrasion resistance, and stiffness mechanical problems.

Currently used antimicrobial agents include metal compounds (notably copper and silver), chitosan, triclosan, quaternary ammonium compounds, antibiotics (a class of antimicrobials produced from microorganisms that act against one another), and N-halamines.<sup>3</sup> The possibility of bacterial resistance limits antibiotic use to specific medical applications, and triclosan is currently under scrutiny for possible toxicity to aquatic organisms. Although quaternary ammonium compounds are stable and easily manufactured, resistance is also a concern.<sup>27</sup> The bulk of current research focuses on metals, including silver, as it provides excellent antimicrobial action.<sup>13</sup>

This study explores the application of silver as an antimicrobial agent employed in action-wear textile manufacture. The following is a review of the literature as it pertains to the application of silver in general as an antimicrobial agent, with emphasis on its use in the textiles industry.

## CHAPTER 2

### REVIEW OF LITERATURE

Silver has been used throughout history in healthcare for water purification, wound care, cardiac devices, and surgical appliances. This metal is less toxic in the human body than other heavy elements with a smaller risk for exposure through inhalation, ingestion, or dermatological exposure.<sup>29</sup>

The antibacterial efficacy of silver is directly proportional to the amount of bioactive silver ions released in the presence of moisture, as well as its ability to penetrate bacterial cell membranes.<sup>29</sup> Silver can be applied in various forms: silver ion exchangers, silver salts, and silver metals. Silver zirconium phosphate and silver zeolites are examples of ion exchangers. Silver chloride (AgCl), nanosilver chloride, and AgCl microcomposites (AgCl nanoparticles attached to titanium dioxide as a carrier material) are types of silver salts. Silver metal can be used in the form of filaments and silver metal composites.<sup>17</sup> All silver-based antimicrobials generate and release different amounts of silver ions, with silver metals releasing the least, silver ion exchangers releasing the most, and silver salts somewhere in between.<sup>23</sup> Although silver's exact bactericidal mechanism is not completely understood, generally accepted theories include: <sup>17,19,20</sup>

1. Binding silver to thiol groups on bacterial enzymes responsible for respiration, as well as portions of the electron transport system, leading to enzyme inactivation.

2. Interaction with fimbriae (negatively charged peptidoglycan structures on the cell surface), which are responsible for the adhesion of bacteria to a surface. This occurs through extracellular binding of the silver ion to the bacterial cell wall.
3. Silver accumulation inside bacterial cells, which disrupts DNA and RNA synthesis.

Evidence presented in the literature suggests that bacterial resistance to silver is rare and can only occur if the cellular efflux system (responsible for flushing antibiotics and other toxins out of the cell) and outer membrane protein synthesis mechanisms are decreased, binding the silver somewhere outside of the cell.<sup>20</sup>

Silver is a leaching antimicrobial agent, which means that its effectiveness relies on the release of silver ions to the local environment. The antimicrobial agent applied to textiles thus affects bacteria both on the material and in its adjacent environment, with the effect gradually weakening over time as the leaching depletes the silver.<sup>25</sup>

While silver has excellent antimicrobial activity, leaching from treated textiles into laundering effluent is problematic. Ionic silver is highly toxic to aquatic organisms, with the EPA setting water quality criteria at 1.9 ppb in salt water and 3.4 ppb in fresh.<sup>2</sup> Effluent from both home laundering and industrial application can transfer silver into sewage treatment facilities, depleting necessary bacterial communities.

Research conducted by Geranio et al. found that fabric treated with AgCl released only 2.7 ppb (2.4 ppb for AgCl plus a binder) of total silver per gram of textile after the first wash cycle.<sup>6</sup> As the effectiveness of silver depends on the release of silver ions, too few ions result in a lack of antimicrobial action, and too many yield an excess leading to pollution and waste. Success depends on finding the balance between minimum antimicrobial concentration and effectiveness.

A successful antimicrobial finish applied to a fabric must be durable, surviving multiple launderings. Ideally, antimicrobial testing would be performed after a number (1, 10, 50, etc.) of laundering cycles in a conventional home washing machine. Although this method would be the most accurate representation of how an antimicrobial textile performs over time, testing agencies are not able to use this method due to financial and time-based restraints. To compensate for this, the industry utilizes AATCC Test Method 61: Colorfastness to Laundering as a proxy for conventional home laundering to test antimicrobial finish durability. In this method, one 45 minute accelerated laundering cycle is equal to five typical home laundering cycles.<sup>26</sup>

The correlation between the two laundering methods however, is only approximate. The industry considers an antimicrobial apparel item to be successful if it continues to have good antimicrobial activity after 50 home launderings.<sup>31</sup> This number of laboratory-based launderings would take seven to eight days to complete in a conventional washing machine, versus two days using an accelerated laundering procedure.

The research presented here explores the exhaustion behavior of a silver-based antimicrobial, defining a minimum concentration of the finish needed to provide antimicrobial effectiveness and approximating the extent to which that effectiveness is maintained over extended laundering cycles.

## CHAPTER 3

### METHODOLOGY

The following section describes the methods used in the application (both pad and exhaust) of silver to nylon fabric, chemical analysis of silver in the exhaust bath, accelerated laundering, antimicrobial testing using AATCC Test Method 147, and two-way ANOVA statistical analysis using Minitab software.

#### Materials

- 70 denier plain-weave nylon 6,6 fabric
- AgCl + TiO<sub>2</sub> (Supplied by Rudolf Chemie)
- 0.1M HCl
- Deionized water
- Rustol ACA defoaming agent
- AATCC 1993 Standard Reference Detergent Without Optical Brightener
- Nutrient agar (containing beef extract, peptone, agar)
- 90x15mm sterile petri dishes
- Inoculating loop

#### Equipment

- Mathis JFO Venturri Jet Dyer
- Mathis Padder Type HVF
- Mathis Continuous Coating Range Type KTF-S



- Atlas Launder-Ometer with corresponding canisters and steel balls
- Shimadzu ICPE 9000
- Circulating oven

#### Application of the Antimicrobial Agent by Padding and Exhausting

A 9L bath containing 500ppm of AgCl/TiO<sub>2</sub> was added into the jet dyer and agitated at room temperature for five minutes to evenly disperse the ingredients. A 25mL bath sample was taken to conduct silver concentration analysis. 500g of nylon fabric was added into the jet dyer. Once the bath was heated to 30°C, the jet was run at the default setting of 50% pump pressure and a speed of 5 revolutions per minute for 60 minutes. Two mL of defoamer was added after 30 minutes to reduce excess foam due to the high liquor turbulence of the dyeing machine. Twenty-five mL bath samples were collected at 15, 30, 45, and 60 minute time points. The bath was drained, refilled with 9L of deionized water, and run for 5 minutes at 30°C to rinse the fabric. The fabric sample was removed from the jet and dried at 68°C for 30 minutes in a conventional home tumble clothing dryer. This procedure was repeated for bath concentrations of 2500ppm and 5000ppm Ag, the highest recommended concentration by the product manufacturer.

To provide a treated fabric control, nylon fabric was padded, dried, and cured, using 500ppm, 2500ppm, and 5000ppm concentrations at 70% pickup.

## Chemical Analysis of Silver Concentration in the Exhaust Bath

The 25mL bath samples collected during the jet dyeing application of 500ppm, 2500ppm, and 5000ppm silver concentrations were collected and evaporated to dryness overnight in an oven set at 70°C. Next, 12.5mL of 0.1M hydrochloric acid was added to each sample, which was then agitated under a fume hood for 18-24 hours, precipitating out the silver chloride from the titanium dioxide. Deionized water was added to each sample to bring the volume back to the original 25mL.

For ICPE analysis, four calibration curves were made by making dilutions of the stock solution; 200ppm (100%), 100ppm (50%), 50ppm (25%), and 4ppm (2%). These calibration curves provide the machine with the necessary reference points to measure silver concentration in each bath sample. Bath samples from each time point were run in a Shimadzu ICPE 9000 with a normal globe nebulizer to obtain the concentration of silver in the bath, yielding information on the amount of exhaustion occurring throughout the 60-minute application period.

## Durability of Antimicrobial Finish to Washing

Fabric samples (15x5cm) from each concentration group (both pad and exhaust) underwent accelerated laundering in an Atlas Launder-Ometer in 150mL of a 0.15% solution of AATCC 1993 Standard Reference Detergent (without optical brightener) in a 2L canister with 50 steel balls for 45 minutes at 120°C, according to AATCC Test Method 61 (2015): Colorfastness to Laundering (option 2A).<sup>21</sup> Separate fabric samples of each application method and silver concentration underwent 1, 5, and 10 accelerated wash cycles. After the samples were removed from the cannisters

and rinsed, they were laid flat to air dry for at least 24 hours. A total of three replications of this accelerated laundering procedure from each application method and silver concentration were completed. See Table 1: Experimental Variables Applied to both *E. coli* and *S. aureus* for all experimental variables. A 25x50mm portion of each sample was then cut for antimicrobial testing.

Table 1. Experimental Variables Applied to both *E. coli* and *S. aureus*

Application Method	Ag (ppm)	Accelerated Laundering Cycles
PAD	500	0
		1
		5
		10
	2500	0
		1
		5
		10
	5000	0
		1
		5
		10
EXHAUST	500	0
		1
		5
		10
	2500	0
		1
		5
		10
	5000	0
		1
		5
		10

#### Antimicrobial Analysis

AATCC Test Method 147, Antimicrobial Activity Assessment of Textile Materials: Parallel Streak Method was conducted on each concentration of initial and laundered samples using *Staphylococcus aureus* and *Escherichia coli*.<sup>22</sup>.

### Preparation of Culture Medium

Pre-mixed 1000mL jars of sterile peptone and beef extract nutrient agar<sup>22</sup> were heated gradually on a hot plate to 100°C in a hot water bath until it reached a liquid state. The nutrient agar was allowed to cool to approximately 60°C and then was dispersed in 25 x 100mm polystyrene petri dishes in approximately 15mL quantities. The petri dishes were capped, allowed to cool to room temperature to solidify, subsequently inverted, and placed into a refrigerator at 4°C overnight to finish solidifying.

### Preparation of Diluted Bacterial Cultures

*E. coli* from a concentrated culture was transferred into 9mL of sterile premade peptone and beef nutrient broth with a sterile inoculation loop using aseptic technique (flame the inoculation loop until red hot between each inoculation). A test tube holding the inoculated nutrient broth was placed into a 37°C incubator for 24 hours with the caps on, as *E. coli* is a facultative anaerobe. Bacterial growth was observed by noting that the nutrient broth had a cloudy appearance. The inoculated test tube was removed from the incubator and allowed to cool to room temperature, where a loopful of this diluted bacterial culture was streaked out five times onto a previously prepared agar plate (warmed to room temperature) using aseptic technique. The inoculated agar was then placed into a 37°C incubator for 24 hours. One loopful of this stock culture was transferred into another 9mL tube of sterile nutrient agar and placed in a 37°C

incubator for 24 hours, creating a diluted subculture. This procedure was repeated for *S. aureus*.

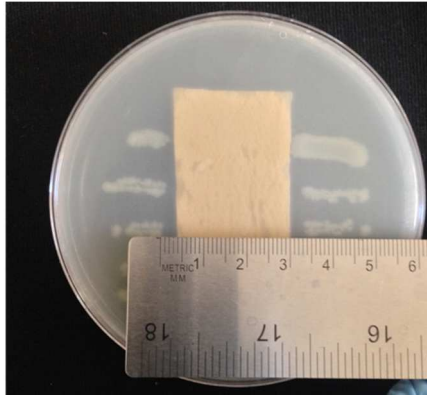
#### Preparation of Test Specimens

25 x 50mm samples of treated nylon fabric were used as stated in AATCC Test Method 147. This length of fabric allows the specimen to lie across five parallel inoculum streaks, placed approximately 10mm apart.<sup>22</sup> Three replications of each silver concentration, wash cycle, and application method were prepared and labeled. A control of unfinished, unwashed fabric was also tested.

#### Antimicrobial Testing

Sterile agar plates were removed from the refrigerator and allowed to warm to room temperature. Using aseptic technique, one loopful of the diluted subculture was transferred to the agar plates by making five streaks from left to right, approximately 60mm in length and 10mm apart without refilling the loop in between streaks. The 25 x 50mm test specimens were gently placed across the inoculum streaks on each agar plate and were pressed down using a sterile applicator. Once covered, the inoculated agar plates were placed in a 37°C incubator for 24 hours. Following incubation, bacterial growth was observed under and around each fabric sample. Zones of inhibition, or the clear halo around the fabric samples was measured in millimeters. Figure 1 shows the zone of inhibition being measured.

Figure 1: Measuring the Zone of Inhibition



### Data Analysis

Statistical analysis of the data was performed using two-way ANOVA in Minitab 17 software.

## CHAPTER 4

### FINDINGS

The ICPE analysis for silver concentration (in mg/L) in the exhaust bath at 0, 15, 30, 45, and 60 minute time points in the exhaust application method for 500ppm, 2500ppm, and 5000ppm concentrations is shown in Table 2.

Table 2: ICPE Analysis of Silver in Exhaust Bath

<b>ICPE Analysis of Silver in Exhaust Bath (mg/L)</b>			
	500ppm	2500ppm	5000ppm
Initial	9.86	47.2	107
15 minutes	4.58	10.5	25.7
30 minutes	4.31	6.09	20.9
45 minutes	4.23	5.73	16.4
60 minutes	4.22	4.31	16.2

Exhaustion of the silver from the 500ppm bath occurred quickly in the first 15 minutes after the fabric was added, then continued to exhaust slowly for the rest of the application process. The exhaustion of the silver in both the 2500ppm and 5000ppm baths also exhausted quickly in the first 15 minutes after the fabric was added, then continued to exhaust slowly for the rest of the application process. The total silver in the 500ppm bath after the 60 minute application period was 4.22mg/L, 4.31mg/L for

the 2500ppm bath, and 16.2mg/L in the 5000ppm bath, suggesting that the majority of the silver exhausted onto the fabric.

The mean zone of inhibition for each sample treated with the antimicrobial agent is shown in Tables 3 and 4.

Table 3. Mean zones of inhibition expressed against *E. coli* for pad and exhaust application methods, at Ag concentrations of 500, 2500, and 5000 ppm, after 0, 1, 5, and 10 accelerated wash cycles

***E. coli***

<b>Accelerated Wash Cycles</b>	<b>[Ag] ppm Applied</b>	<b>Average ZOI Pad (mm)</b>	<b>Average ZOI Exhaust (mm)</b>
0	500	5.4	7.8
	2500	8.3	10.2
	5000	8.6	9.9
1	500	0	0
	2500	0	3.9
	5000	1.4	5.9
5	500	0	0
	2500	0	0
	5000	0	0
10	500	0	0
	2500	0	0
	5000	0	0



Table 4. Mean zones of inhibition expressed against *S. aureus* for pad and exhaust application methods, Ag concentrations of 500, 2500, and 5000ppm after 0, 1, 5, and 10 accelerated wash cycles

***S. aureus***

<b>Accelerated Wash Cycles</b>	<b>[Ag] ppm Applied</b>	<b>Average ZOI (3 replications) Pad (mm)</b>	<b>Average ZOI (3 replications) Exhaust (mm)</b>
0	500	1.4	3.5
	2500	3.1	4.1
	5000	4.6	4.1
1	500	0	0
	2500	0	1.5
	5000	0	3.5
5	500	0	0
	2500	0	0
	5000	0	0
10	500	0	0
	2500	0	0
	5000	0	0

All applied concentrations of silver effectively produced a zone of inhibition around samples from both application methods against *E. coli* before undergoing laundering. The effectiveness of the silver as an antimicrobial agent diminished after 1

accelerated laundering cycle for the padded sample, but less so for the exhausted sample (Table 3). Compared to *E. coli*, a smaller zone of inhibition in unlaundered samples was created by *S. aureus*, with the antimicrobial effect completely diminished after one accelerated laundering cycle for the padded sample, though the exhausted sample retained some antimicrobial effect (Table 4). After undergoing five and ten accelerated laundering cycles, neither the padded nor exhausted samples at any concentration demonstrated any antimicrobial effect against either bacteria.

#### Results of Two-Way ANOVA

Results of two-way ANOVA testing for differences in mean zone of inhibition measurements according to tested application method and Ag concentration for 0 and 1 wash cycles for each bacteria are displayed in tables 5 and 6. Although the data from 1 wash cycle was statistically significant, but there was also significant interaction, so the main effects could not be examined. Statistical analysis could not be performed on data collected from the samples that underwent 5 and 10 accelerated laundering cycles, as no zone of inhibition was formed, due to loss of antimicrobial effectiveness after laundering.

Table 5. Two-way ANOVA of interaction and main effects for application method and silver concentration for all accelerated laundering cycles against *E. coli*

<b>Bacteria 1 (<i>E. coli</i>) P Values</b>		
	0 Wash Cycles	1 Wash Cycle
Interaction	0.7482	0.0015
Application Method	0.0013	<0.0001
[Ag]	0.0005	<0.0001

Table 6. Two-way ANOVA of interaction and main effects for application method and silver concentration for all accelerated laundering cycles against *S. aureus*

<b>Bacteria 2 (<i>S. Aureus</i>) P Values</b>		
	0 Wash Cycles	1 Wash Cycle
Interaction	0.0646	0.0075
Application Method	0.0587	0.0007
[Ag]	0.0093	0.0043

Application of a two-way ANOVA to examine the influence of application method and silver concentration on the mean zone of inhibition against each bacteria (or antimicrobial efficacy) showed no statistically significant interaction at 5% significance level between application method and silver concentration after zero accelerated wash cycles for either bacteria (Tables 5 and 6, Figures 1 and 2). Both the application method and silver concentration were found to be statistically significant against *E. coli* in their main effects after zero accelerated laundering cycles (Table 5).

For *S. aureus*, the main effect of application method was on the cusp of statistical significance while silver concentration showed a significant difference (Table 6).

Analysis of data from one accelerated laundering cycle showed significant interaction between application method and silver concentration against both bacteria so comparisons of main effects levels cannot be performed.

The mean zone of inhibition of the exhaust application samples was statistically significantly higher than the pad application samples after zero accelerated launderings against *E. coli* (Figure 2). The mean zone of inhibition at 500ppm was significantly lower than concentrations 2500ppm and 5000ppm. There was no significant difference between the mean zone of inhibition between 2500ppm and 5000ppm against *E. coli*. The mean zone of inhibition for of the exhaust application samples was not statistically significantly higher than the pad application samples after zero accelerated launderings against *S. aureus* (Figure 3). The mean zones of inhibition between 500ppm, 2500ppm, and 5000ppm concentrations were all statistically significantly different (Figure 3).

The mean zones of inhibition from the exhaust and pad application methods were significantly different against *E. coli* before laundering (Figure 4). The exhaust application method showed a higher mean zone of inhibition than the pad application method. While there was a significant difference between 500ppm and 2500ppm silver concentrations, there was no significant difference between 2500ppm and 5000ppm silver concentrations (Figure 4).

The mean zones of inhibition from the exhaust and pad application methods were significantly different against *S. aureus* before laundering (Figure 5). The

exhaust application method showed a higher mean zone of inhibition than the pad application method. There was a significant difference between 500ppm and 2500ppm silver concentrations, 500ppm and 5000ppm concentrations, and 2500ppm and 5000ppm silver concentrations (Figure 5).

Figure 2. Interaction plot of application method and silver concentration for mean zones of inhibition in mm after 0 accelerated laundering cycles against *E. coli*.

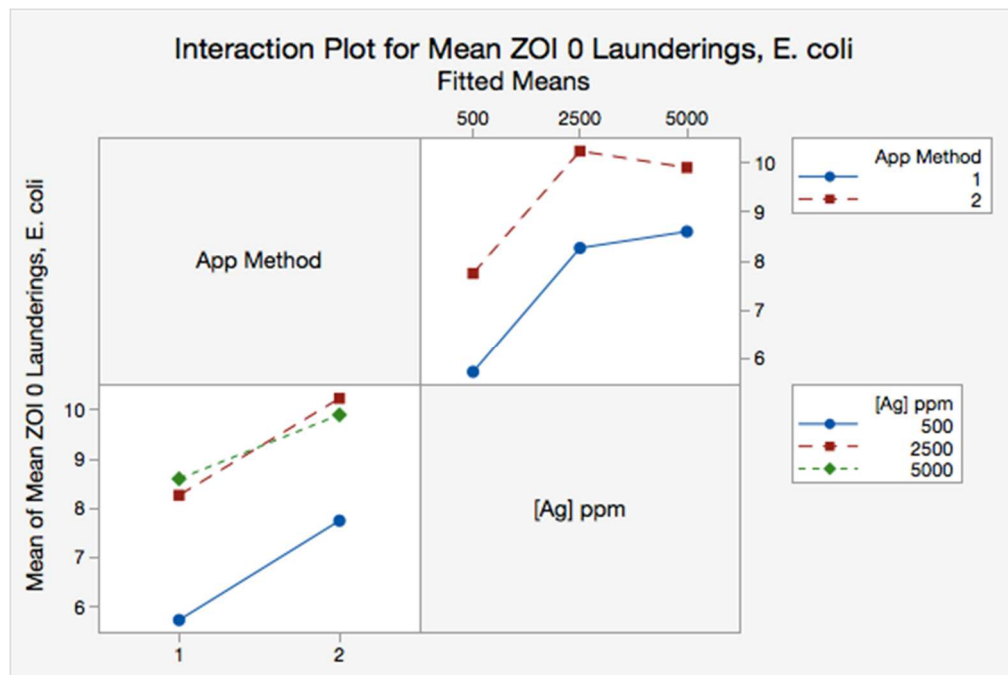


Figure 3: Interaction plot of application method and silver concentration for mean zones of inhibition in mm after 0 accelerated laundering cycles against *S. aureus*.

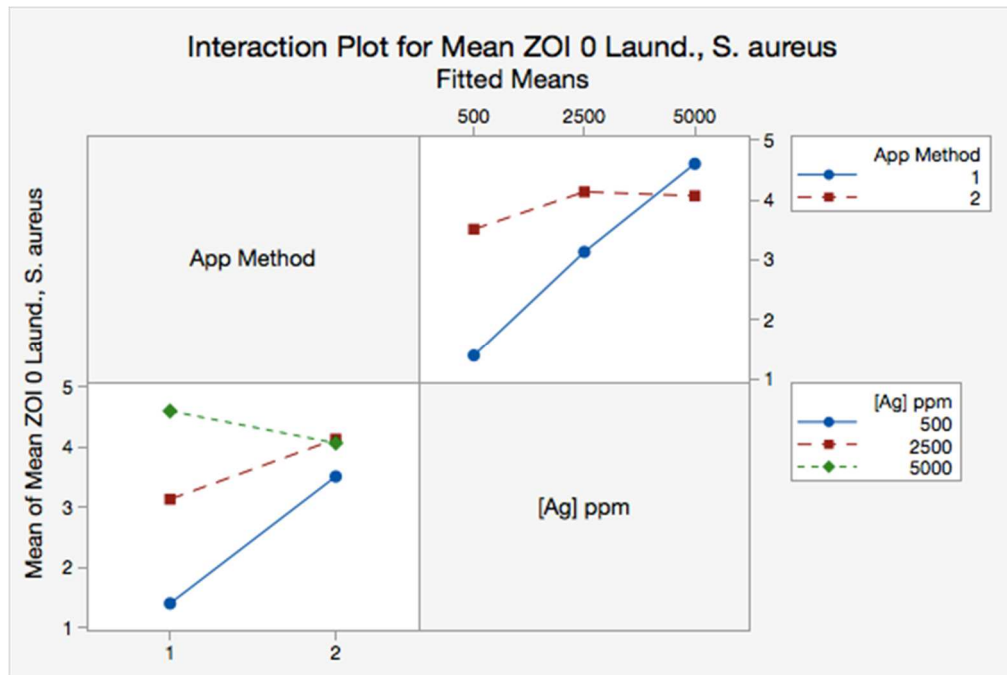


Figure 4: Main effect plot of application method and silver concentration for mean zones of inhibition in mm after 0 accelerated laundering cycles against *E. coli*.

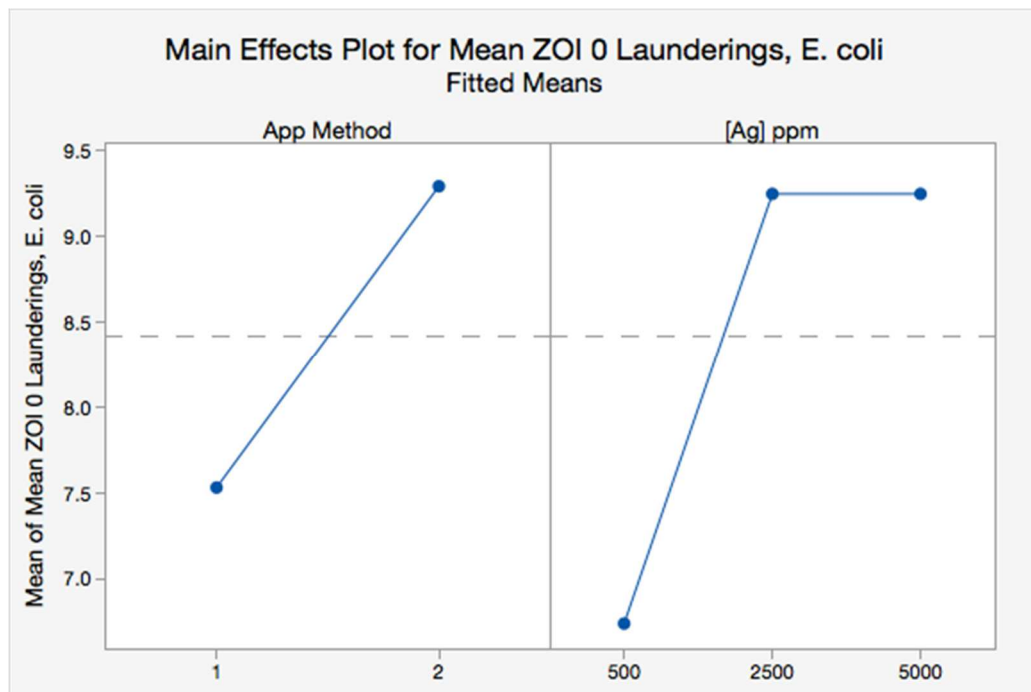
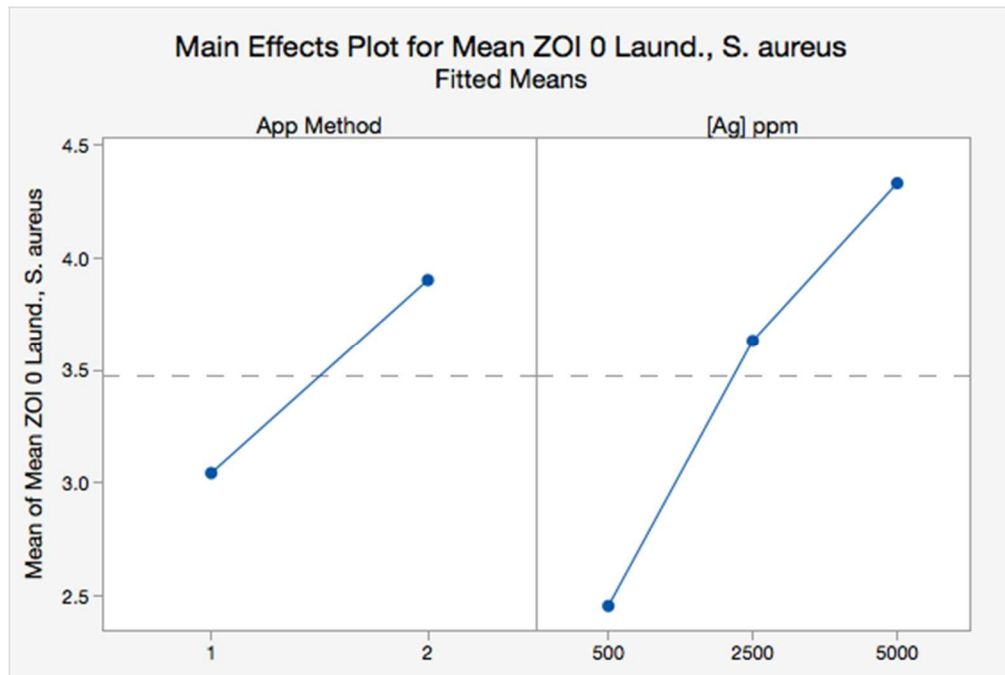


Figure 5: Main effect plot of application method and silver concentration for mean zones of inhibition in mm after 0 accelerated laundering cycles against *S. aureus*.



In summary, the samples treated with the exhaust application method consistently produced larger zones of inhibition, compared to the samples treated with the pad application method. The size of the zone of inhibition offers some indication of the potency of the silver, or its release rate over 24 hours.<sup>30</sup> The silver in the exhaust application samples may have diffused more readily than the pad samples, potentially due to the fact that the exhaust samples were immersed in the antimicrobial solution for a greater length of time. Against *E. coli*, there was a significant difference between the 500ppm and both 2500ppm and 5000ppm silver concentrations. There was no significant difference between 2500ppm and 5000ppm silver concentrations. Against *S. aureus*, there was a significant difference between all three silver concentrations.

## CHAPTER 5

### CONCLUSION

This research offers an initial study of the exhaustion behavior of a silver-based antimicrobial finish on nylon fabric. It also identifies a minimum concentration of the finish needed to provide antimicrobial effectiveness and approximates the extent to which that effectiveness is maintained over extended laundering. This research showed the better of two methods of application.

The ICPE findings show that the silver exhausted down to 4.22mg/L, 4.31mg/L, and 16.2mg/L for the 500ppm, 2500ppm, and 5000ppm baths (respectively) over a 60 minute exhaust application process (Table 1). Although these bath concentrations are low, the silver did not fully exhaust onto the fabric. The aim was to get the silver to fully exhaust, and this could be carried out in a future study by adding an auxiliary to alter the pH of the water, which could make the affinity between the silver and the nylon fabric stronger.

Against *E. coli*, the exhaust application samples demonstrated better antimicrobial action than the pad application samples before accelerated laundering cycles at all silver concentrations. The data suggests that the exhausted samples took up more of the silver from each concentration group, possibly due to the fact that it was immersed in the silver bath for 60 minutes, whereas the pad samples passed through it quickly. Only the 5000ppm sample from the pad application produced a zone of inhibition after one accelerated laundering cycle, while the 2500ppm and 5000ppm samples from the



exhaust application group produced measurable zones of inhibition. This could be attributed to either the possibility that more of the silver depositing on the fabric during the exhaust application than the pad application, or that the silver was incorporated more thoroughly into the material after the exhaust application, while it only coated the fabric surface in the pad application. Both treatment groups lost their antimicrobial action by the fifth accelerated laundering cycle. Since neither treatment group withstood 5 accelerated laundering cycles, this exhibits a lack of durability to laundering for this antimicrobial finish applied by these two methods at these three concentrations.

Against *S. aureus*, the samples from the pad application treatment group only produced zones of inhibition at all silver concentrations before accelerated laundering cycles and lost all antimicrobial action after the first accelerated laundering cycle. The exhaust application samples produced larger zones of inhibition than the pad application samples at all silver concentrations after zero accelerated laundering cycles, and at 2500ppm and 5000ppm concentrations after one accelerated laundering cycle. The data indicates that the exhaust application method provided a stronger antimicrobial response, as shown by the larger zone of inhibition at before and after one accelerated laundering cycle. A lack of a zone of inhibition after five accelerated laundering cycles can be attributed to the loss of silver from the material during laundering. Although the majority of the silver successfully exhausted onto the material, leaving minimal amounts in the dye bath effluent, those silver ions would be in the home washing machine effluent and could be harmful to the microbes needed to process wastewater. Increasing the durability of the exhausted antimicrobial finish to

withstand 50 launderings (or more) would reduce the risk of silver in consumer wash effluent.

After zero accelerated laundering cycles for both bacteria, there was a statistically significant difference in the mean zones of inhibition between the two application methods, as well as among the three silver concentrations against *E. coli* (Table 5). After zero accelerated laundering cycles, the exhaust application method (method 2) showed greater antimicrobial efficacy against *E. coli* and *S. aureus* (Figures 2-5). More specifically, there was a significant difference between samples treated with 500ppm and 2500ppm, and 500ppm and 5000ppm of silver against *E. coli* (Table 5, Figures 2 and 4). There was no significant difference between samples treated with 2500ppm and 5000ppm of silver (Tables 3 and 5, Figures 2 and 4). The lack of a difference in mean zones of inhibition between samples treated with 2500ppm and 5000ppm of silver shows that using a higher concentration does not necessarily equal greater antimicrobial activity. Using the lower of the two aforementioned concentrations of silver yields less of the metal in wash effluent. Against *S. aureus*, there was a significant difference between all silver concentrations before laundering, as well as between application methods (Figures 3 and 5).

After one accelerated laundering cycle, the exhaust application method showed greater antimicrobial efficacy against *E. coli* than the pad method (Table 3), but the statistical analysis revealed significant interaction between the main variables so their main effects cannot be examined statistically.

The finished fabrics from either application method were able to withstand 5 accelerated laundering cycles. While the exhaust application method provides greater

antimicrobial properties at low washing usage than the pad method, that application does not improve the overall longevity of antimicrobial properties. Future research should include the use of some type of auxiliary, either a binder or fixing agent, to improve the durability to laundering of the lower silver concentrations during the exhaust application method. Improving this durability to laundering would reduce the amount of silver ions escaping into water treatment facilities from household washing machines. As using the exhaust application method requires more water than the pad-dry-cure method, future studies could combine the antimicrobial finish with the desired dye in the bath, so an additional exhaustion cycle is not needed.

This research provides a baseline comparison of two application methods for silver as an antimicrobial agent on nylon fabric, allowing future researchers to forego the initial research to focus on improving the exhaust application method. This preliminary study shows that the exhaust application method has the potential to be a preferred method to focus future research on the lack of durability to laundering. Future studies should aim to carry out the laundering procedure using a home laundering test method, such as AATCC 135, to yield more refined results that would offer a more direct correlation between the testing and what would occur in a consumer's home washing cycles. Focusing on a concentration around 2500ppm of silver would be best, as this concentration demonstrates greater antimicrobial efficacy than 500ppm of silver and equal antimicrobial efficacy to 5000ppm of silver, after zero and 1 accelerated laundering cycle. Future studies should focus on adding a fixing agent to the exhaust application process to increase the durability of the silver

antimicrobial agent. The use of silver nanoparticles should also be explored, as they may offer greater durability to laundering.

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