# Antimicrobial Polymeric Materials; Cellulose and m-Aramid Composite Fibers

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## ABSTRACT
Cellulose and m-aramid were dissolved in an ionic liquid, and dry-jet wet spinning was employed to prepare composite fibers which could be rendered antimicrobial through exposure to chlorine bleach. The small domains of the m-aramid allowed a much higher accessibility and degree of chlorination than has been reported even for 100% m-aramid fibers. The mechanical properties including denier, tenacity, and strain at break were evaluated. The chlorinated composite fiber inactivated both Gram-positive and Gram-negative bacteria. The antimicrobial activity was retained after repeated washing and recharging.

## INTRODUCTION
N-halamine materials have been developed as biocides during the last few decades[1-4]. N-halamines refer to compounds that have at least one covalent bond between nitrogen and halogen. Due to dehydrohalogenation which can occur if an α-hydrogen is adjacent to a nitrogen-halogen bond, nitrogen in a heterocyclic structure with no α-hydrogens is preferred for N-halamine biocides. Of the three common N-halamine structures – imide, amide, and amine – the amine has the lowest dissociation constant and is most stable. Since, two adjacent carbonyl groups result in electron delocalization, the imide halamine has the highest dissociation constant and is the least stable. The amide halamine has intermediate stability and perhaps the best balance of longevity and reactivity[5]. Because they are rechargeable and are active against a spectrum of microorganisms with rapid kill, N-halamines have received considerable attention[6].

To date, most work for N-halamine antimicrobials has been devoted to water disinfection[1-3]; however, the potential applications of N-halamines have led some researchers recently to attach those biocides to polymers and fibers [5, 7, 8]. In general, N-halamine antimicrobial polymers or fibers have been prepared in three different ways.

1. N-halamine precursors are bound to polymers or fibers with physical/chemical bonding [5, 7, 8],
2. An N-halamine precursor is mixed with a fiber forming polymer[9], and
3. Fiber-forming polymers are N-halamine precursors[10].

Specifically, fiber-forming polymers, which are also N-halamine precursors, have advantages such as higher durability and no additional finishing. Indeed, aromatic polyamides, which consist of an amide group between two aromatic rings, are a representative of fiber-forming N-halamine precursors. Particularly, poly(m-phenylene isophthalamide), m-aramid (known in the industry as Nomex®) has meta substituted rings in the molecular chain unlike Kevlar®, which is para substituted. It is generally recognized that p-aramid fiber (Kevlar®) suffers serious degradation of physical properties after chlorination in hypochlorite bleach; whereas, the m-aramid does not suffer this problem. Work in this laboratory has explained the mechanism responsible for this difference[11]. The oxidative chlorine that can be placed on the m-aramid fiber (Nomex®) is limited[10]. Perhaps it is significant that the relatively high glass transition temperature (~275 ºC) of the m-aramid fiber induces poor accessibility to dyes as well[12]. The amide nitrogens in m-aramid have no α-hydrogens; thus, if the accessibility of m-aramid could be enhanced, m-aramid might be converted to an N-halamine having increased oxidative chlorine content and better antimicrobial properties.

Ionic liquids (IL) are solvents for chemical reactions and for polymers during wet extrusion of fibers and films. Certain ionic liquids can dissolve cellulose to form stable solutions at reasonable temperatures. The ionic solutions are relatively non-toxic and easy to handle making the IL's candidates for extruding regenerated cellulose. Especially, the ionic liquid (1-

butyl-3-methylimidazolium chloride) dissolves both cellulose[13] and poly (*m*-phenylene isophthalamide).

*Figure 1* shows the cycle of disinfection by the chlorinated *m*-aramid, and recharging with diluted-chlorine bleach. In this study, the composite fiber of cellulose (a natural polymer) and *m*-aramid (a synthetic polymer) will be discussed. The physical properties, antibacterial efficacy, stability, durability and rechargeability after repeated laundering, will be described.

It was reasoned that a composite fiber of both cellulose and *m*-aramid might provide desirable properties of cellulose, as well as the ability to create an antimicrobial *N*-halamine in the fiber.
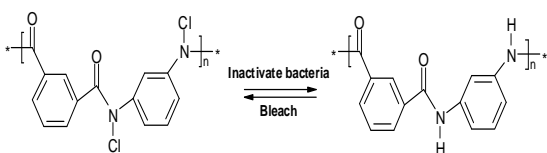


FIGURE 1. Scheme of the function of *m*-aramid disinfection

## EXPERIMENTAL
### Materials
Bleached cotton cellulose, with a degree of polymerization (DP) of 1440, was used. The *m*-aramid was a DuPont fiber product. The ionic liquid, 1-butyl-3-methylimidazolium chloride, was purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. Bacterial cultures of *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* O157:H7 ATCC 43895 employed were from the American Type Culture Collection (Rockville, MD), and Trypticase soy agar from Difco Laboratories (Detroit, MI).

### Instrument and Measurement of Physical Properties
The scanning electron microscope investigation was performed with a Jeol JSM-7000F (SEM). Samples were coated with gold under argon purge before observation.

Tensile properties were investigated with a Universal Materials Testing Machine (Instron Model 1122) at 22 ºC and 65% of humidity. The data were obtained by averages of 10 tests. The length between upper and lower jaws was 25.4 mm, and the crosshead speed was 20 mm/min.

### Preparation of Cellulose/*m*-Aramid Solution
Bleached cotton was ground and dried at 70 ºC under vacuum for 8 h. 3.5 g of the cotton cellulose was put in 100 g of 1-butyl-3-methylimidazolium chloride to produce a solution of 3.5% cellulose. The solution was then stirred for 72 h at 80 ºC. Concurrently, *m*-aramid was pre-washed with water (~ 60 ºC), ethanol and acetone, followed by heating at 65 ºC for drying. Once dried, *m*-aramid was added to the ionic liquid/cellulose solution. This mixture was then stirred for 24 h at 80 ºC. A total of six solutions were made with the following ratios of cotton/*m*-aramid by weight: 100/0, 100/2, 100/6, 100/10, 100/20 and 100/50. The solution was then degassed under vacuum for at least 2 h at 70 ºC without stirring to remove air bubbles, after which, it was poured into the extruder.

### Wet Spinning Process
The wet spinning apparatus (ISCO Series D, piston pump) was fitted with 0.45 mm diameter single-hole spinneret. A tap water coagulating bath and a stepped godet with four levels and a take-up winder were used. Dry-jet wet spinning was used at 50~65 ºC, with a 0.2 mL/min extrusion velocity, and an appropriate godet speed was maintained to provide minimum tension on the filament. The solution was forced through a spin pack with a 325 mesh screen and the single hole spinneret. Extrusion conditions were as follows:

| | |
|---|---|
| Process: | dry-jet wet spinning |
| Extrusion temperature: | 50 – 65 ºC |
| Throughput: | 0.2 mL/min |
| Air gap: | 20 mm |
| Coagulation bath: | tap water |
| Godet speed: | 25 RPM |
| Drawing: | 3 stage, steps 1, 2, 3, and 4 |
| Draw ratio: | 2.95 – 3.89 |
| Take-up speed: | 3.09 – 4.07 m/min |

After dry-jet wet spinning, the yarns were soaked in tap water at ambient temperature for 24 h to extract the solvent and then dried.

### Chlorination and Titration
The fibers were chlorinated using a commercial 6% sodium hypochlorite solution (diluted to 10% of the commercial strength with distilled water) at pH 7 to produce biocidal materials. After soaking the fiber in the solution at ambient temperature for 60 min, and rinsing with distilled water, the samples were then dried at 45 ºC for 2 h to remove any unbonded chlorine.

An iodometric/thiosulfate titration was used to analyze and to calculate oxidative chlorine content according to the equation:

$$[Cl^+]\% = (V \times N \times 35.45) / (W \times 2 \times 10) \qquad (1)$$

where $[Cl^+]\%$ is the wt% of oxidative chlorine on the sample, V is equal to the volume of the titrant (sodium thiosulfate solution) in mL, N is equal to the normality of the titrant, and W is the weight of the sample (g). The constants are 35.45 (molecular weight of Cl), 2 (the change in oxidation state of Cl during titration), and 10 to normalize the units in numerator and denominator to give % Cl.

**Laundering Test**
AATCC Test Method 61-2001 was used to investigate the stability of chlorinated *m*-aramid in/on the fibers after home laundering. A Launder-Ometer was fitted with stainless steel cylinders (3 x 5 inch). A volume of 150 mL of water, 0.15% AATCC detergent, and 50 stainless steel balls were added to the cylinder which was rotated for 45 min at 42± 0.5 RPM and 49 ºC. These conditions are estimated to be equivalent to 5 washing cycles in a home laundry. The fibers within the cylinder were rinsed with three 300 mL portions of distilled water, then air dried at ambient temperature, and either titrated directly, or recharged with chlorine bleach.

**Antimicrobial Test**
After the fibers were made into a crude nonwoven fabric by rubbing the filaments, both control and chlorinated samples were challenged with *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895) using a modified AATCC Test Method 100-1999. A total of 25 µL bacterial suspensions made with pH 7 phosphate buffer were added to each fabric swatch, and a second swatch was sandwiched over it to ensure contact between the suspension and the fabric. After contact times of 5 min and 30 min, the samples were transferred to 5.0 mL of sterile 0.02 N sodium thiosulfate solution to quench the chlorine residue and rinse off survived bacteria. Serial dilutions of the quenched samples were prepared using pH 7 phosphate buffer and plated on Trypticase soy agar. The plates were incubated at 37 ºC for 24 h and then counted to determine the numbers of viable bacteria.

**RESULTS AND DISCUSSION**

**Coagulation of the Polymers**
The dissolution mechanism of cellulose in ionic liquids is known to be breaking hydrogen bonds between chains of cellulose and reformation of the bonds with the ionic liquid[14]. The major bonding force among *m*-aramid chains is also hydrogen bonding, which means that the possible dissolution mechanism is similar to that of cellulose. However, the cellulose film, which was coagulated in water, was transparent; whereas, *m*-aramid film, which was coagulated in water, was translucent or opaque. It is presumed that the lack of transparency is due to small voids within the structure formed during coagulation. The SEM images of coagulated *m*-aramid film (*Figure 2*) reveal an aggregate of particles about 0.8~1.0 µm in size. This structure would have voids between the particles and is a likely explanation for the opaqueness of the coagulated *m*-aramid film.
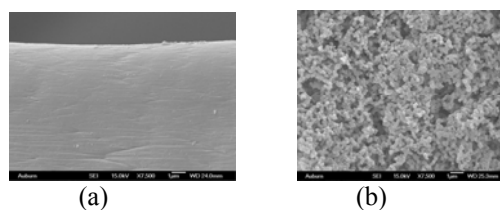


FIGURE 2. SEM traces of (a) surface of original *m*-aramid fiber, (b) surface of coagulated *m*-aramid film.

**Physical Properties and Chlorination of the Composite Polymer**
The results in *Table I* show mechanical properties of the fiber in terms of denier, tenacity, and strain at break. At lower *m*-aramid content, even up to 10% (based on weight of cellulose), denier and tenacity of the fiber are similar; however, in 100/50 (cellulose/*m*-aramid), tenacity decreases. Meanwhile, strain at break tends to be enhanced with increased portion of *m*-aramid. In case of the composite of cellulose and polyamide 66 (PA66), a partial miscibility has been reported between cellulose in amorphous regions and PA66[15]. Hydrogen bonds would be expected to form between hydroxyl groups in cellulose and amide groups in *m*-aramid which are available. In addition, bonds may also occur between proton donors and π-electrons in the cellulose/*m*-aramid composite and could enhance the miscibility between cellulose and *m*-aramid (*Figure 3*).

Secondary bonds have been reported between active hydrogens and aromatic rings in phenol-benzene and ammonia-benzene mixtures. The proton donors normally associated with hydrogen bonds are connected to π-electrons in benzene with a bond energy of 2~4 kcal/mol[16]. Therefore, it could be explained that some portion of the *m*-aramid mixed

with cellulose might remain molecularly dispersed in the coagulated fiber or film. Evidence of significant phase separation in the cellulose/*m*-aramid composite fibers was not evident under polarized light microscopy.
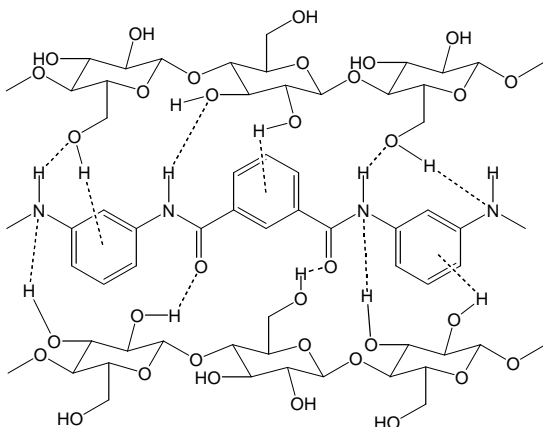


FIGURE 3. Intermolecular bonds between cellulose and *m*-aramid

A *m*-aramid film coagulated in water is shown along-side of the original *m*-aramid fiber (*Figure 2*). The coagulated film exhibits a very porous structure that was shown to accept 2.7% of oxidative chlorine (22.9% of theoretical). The rough, porous surface would have a marked increase in surface area and accessibility and is consistent with the increase in reaction with chlorine (compared to original fiber – *Table II*). The weakness of the film produced indicates that water is not a good coagulation medium, and this is bourne out by attempts to extrude the *m*-aramid into water, and perhaps by the lowered strength of the 100/50 (cellulose/*m*-aramid) composite fiber.

Oxidative chlorine loading onto the fibers varied with the concentration of *m*-aramid (*Table I*). It can be seen that original *m*-aramid fibers have very poor chlorine loading even in high concentration of chlorine solution at high temperature (80 °C); however, coagulated *m*-aramid shows considerably enhanced chlorine loading (*Table II*). In fact, the high glass transition temperature of commercially available *m*-aramid fiber limits dyeability[12] and might be expected to limit chlorination as well. The extended surface area of *m*-aramid fiber, after a solvent treatment, improved the dyeing properties in basic dyeing[17]. Similarly, *m*-aramid in cellulose should have increased surface area due to dispersion within the cellulose, even if phase separation occurred. Whatever the mechanism, the coagulated *m*-aramid in the cellulose/*m*-aramid composite fibers exhibited an increased oxidative-chlorine content.

## Durability and Rechargeability of the Antimicrobial Fibers

Rechargebility is necessary for *N*-halamines to retain their antimicrobial functions after repeated uses. The results in *Table III* indicate that partial recharging is possible even after 50 wash cycles. The fibers recovered their original antimicrobial functions to around 70%. Therefore, it must be presumed that *m*-aramid does not readily escape through the swollen cellulose matrix during washing. Durability of chlorine on the fibers also appeared quite stable. After 50 cycles, 0.14% of oxidative chlorine was retained by 6% *m*-aramid blend, and 0.27% of oxidative chlorine was retained by 10% *m*-aramid blend, which is easily sufficient to inactivate bacteria[10].

TABLE I.
Mechanical properties and chlorine content of the composite fibers (0.2 mL/min of extrusion velocity,
4.07 m/min of take-up speed and 3.89 of draw ratio)

| Ratio (Cellulose/ *m*-aramid) | 100/0 | 100/2 | 100/6 | 100/10 | 100/50 [c] | 0/100 [d] |
|---|---|---|---|---|---|---|
| [Cl+] % [a] | 0 | 0.23 | 0.70 | 1.14 | 3.77 | 0.02 |
| [Cl+] % [b] | 0 | 0.05 | 0.15 | 0.17 | 0.56 | 0 |
| Denier (g/9000m) | 21.9±1.8 | 24.2±2.6 | 21.9±1.7 | 24.0±1.5 | 27.1±1.1 | 2.1±0.4 |
| Tenacity (g/den) | 2.45±0.54 | 2.48±0.46 | 2.82±0.34 | 2.58±0.73 | 1.90±0.14 | 3.68±0.58 |
| Strain at break (%) | 4.7±1.7 | 6.0±1.7 | 5.8±1.9 | 7.6±1.6 | 16.2±2.2 | 52.6±26.4 |

[a] Chlorine concentration and condition of chlorination: 3300 ppm, pH 7.0 and ambient temperature for 60 min.
[b] Chlorine concentration and condition of chlorination: 330 ppm, pH 10.2 and ambient temperature for 60 min.
[c] Take-up speed; 3.09 and draw ratio; 2.95.
[d] Original *m*-aramid fibers, no additional wet-spinning added.

TABLE II.
Oxidative chlorine content of *m*-aramid fibers and coagulated *m*-aramid film after chlorination

Unit: [Cl⁺]%

| Chlorinated temperature [a] | Original *m*-aramid fiber | Coagulated *m*-aramid film |
|---|---|---|
| 25 °C | 0.02 | 2.73 |
| 40 °C | 0.05 | 3.71 |
| 60 °C | 0.04 | 3.63 |
| 80 °C | 0.09 | 3.95 |

[a] Chlorination condition: 3300 ppm of chlorine, pH 7 for 60 min.

The shelf-live of the antimicrobial fibers is illustrated in *Figure 4*. About 90% of the oxidative chlorine is available even after 100 days storage.

In general, the stability of nitrogen and halogen covalent bonding in *m*-aramid is dominated by the stability of negative charge on nitrogen after releasing the halogen. In the *m*-aramid formula, the nitrogen in the amide group attracts the electrons from the phenyl ring inductively; however, by resonance, the amide group is the electron-donating group in the $\pi$-system. In reality, the whole situation is dominated by the resonance effect. However, since the amide groups are in the meta-position, the resonance cannot be extended to other amide groups − unlike *p*-aramid fiber (Kevlar®). Consequently, chlorine on *m*-aramid maintains antimicrobial function with quite stable durability.
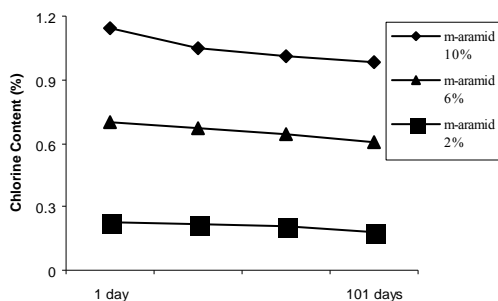


FIGURE 4. Shelf-lives of the composite fibers

TABLE III.
Durability and rechargeability of composite fibers. (0.2 mL/min of extrusion velocity and 1.04 m/min of a take-up speed)

Unit: [Cl⁺]%

| Washing cycles | Group 1 [a] | | | Group 2 [b] | | |
|---|---|---|---|---|---|---|
| | *m*-aramid 2% [c] | *m*-aramid 6% [c] | *m*-aramid 10% [c] | *m*-aramid 2% [c] | *m*-aramid 6% [c] | *m*-aramid 10% [c] |
| 0 | 0.23 | 0.70 | 1.14 | 0.23 | 0.70 | 1.14 |
| 5 | 0.06 | 0.32 | 0.55 | 0.22 | 0.68 | 1.07 |
| 10 | 0.05 | 0.26 | 0.45 | 0.22 | 0.66 | 1.02 |
| 50 | 0.01 | 0.15 | 0.28 | 0.18 | 0.50 | 0.85 |

[a] Group 1: Prechlorinated samples.
[b] Group 2: Prechlorinated samples were rechlorinated after each cycle.
[c] Weight based on cellulose.

As might be expected, the oxidative chlorine content on cellulose/*m*-aramid composite fiber after chlorination increased with the amount of *m*-aramid. The results in *Figure 5* show that 100% *m*-aramid fiber, which was coagulated in water, released less oxidative chlorine than other portions of *m*-aramid in the composite fibers. Perhaps the aggregation of *m*-aramid particles in a coagulated 100% *m*-aramid (see *Figure 2*, b) reduces the accessibility of the structure to the chlorinating species.
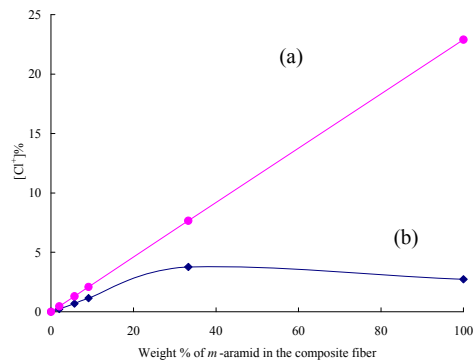


FIGURE 5. Oxidative chlorine content of (a) theoretical and (b) actual composite fiber (Cellulose/*m*-aramid)

## Antibacterial Efficacy against Gram-positive and Gram-negative Microorganisms

The results in *Table IV* indicate that all chlorinated composite fibers inactivated both Gram-positive and Gram-negative bacteria at a contact time of 30 min. All samples except the 2% of m-aramid produced a 6 log reduction of both pathogens within 5 min contact; the reduction was 3.9 log for the Gram-negative bacterium at 5 min contact for this sample. It is presumed that a biocide, which has lipophilicity, has

difficulty in penetration of the water occupied porins, which are water-filled tubes in the outer membrane of Gram-negative bacteria, unlike hydrophilic biocides[18]. Therefore, due to increased hydrophobicity of the *N*-halamine after rendering of biocidal properties (i.e. chlorination), chlorinated samples result in slightly less effective *m*-aramid antimicrobial function on *E. coli* (Gram-negative) than *S. aureus* (Gram-positive).

TABLE IV.
Biocidal efficacy against microorganisms

| Samples [a] (% *m*-aramid) | Contact time | *E. coli O157:H7* [b] Bacterial reduction | | *Staphylococcus aureus* [c] Bacterial reduction | |
|---|---|---|---|---|---|
| | | % reduction | Log reduction | % reduction | Log reduction |
| **Control** 0 % | 5 min | 0.74 | 0 | 15.44 | 0.1 |
| | 30 min | 31.07 | 0.2 | 71.60 | 0.5 |
| 2 % | 5 min | 9.01 | 0 | 53.95 | 0.3 |
| | 30 min | 42.10 | 0.2 | 70.77 | 0.5 |
| 6 % | 5 min | 14.53 | 0.1 | 62.50 | 0.4 |
| | 30 min | 20.04 | 0.1 | 68.84 | 0.5 |
| 10 % | 5 min | 11.77 | 0.1 | 63.60 | 0.4 |
| | 30 min | 44.86 | 0.3 | 65.26 | 0.5 |
| **Chlorinated** 0 % | 5 min | 31.07 | 0.4 | 55.88 | 0.4 |
| | 30 min | 78.49 | 0.7 | 92.28 | 1.1 |
| 2 % | 5 min | 99.99 | 3.9 | 100 | 6.3 |
| | 30 min | 100 | 6.4 | 100 | 6.3 |
| 6 % | 5 min | 100 | 6.4 | 100 | 6.3 |
| | 30 min | 100 | 6.4 | 100 | 6.3 |
| 10 % | 5 min | 100 | 6.4 | 100 | 6.3 |
| | 30 min | 100 | 6.4 | 100 | 6.3 |

[a] *m*-aramid content in the composite polymer (based on weight of cellulose).
[b] Total bacteria: $2.43 \times 10^6$ cfu/sample.
[c] Total bacteria: $2.06 \times 10^6$ cfu/sample.

## CONCLUSIONS

An ionic liquid, 1-butyl-3-methylimidazolium chloride, has allowed the production of cellulose/*m*-aramid fibers. Physical and antimicrobial properties were investigated. Stable and rechargeable cellulose/*m*-aramid composite fibers were prepared by dry-jet wet spinning of a composite polymer solution. Chlorination of the aramid nitrogen produced antimicrobial properties which were retained over 50 standard-washing cycles. Cellulose/*m*-aramid blends show a much higher chlorination level than the pure *m*-aramid fiber. Up to 10% (based on weight of cellulose) *m*-aramid composite fibers did not produce any decline in mechanical properties. The chlorinated fibers inactivated both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria, generally within 5 min with 6 log reductions.

## ACKNOWLEDGEMENT

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