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Contact time-dependency and stability of antibacterial efficacy on chitosan-treated fabrics

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I. Introduction

Fabrics and protective clothes used in schools, hotels, hospitals, nursing homes and crowded public areas can benefit from antimicrobial finishing. Antimicrobial finishes are an important market need to prevent a reduction of mechanical strength and the formation of unpleasant odors in athletic wear, intimate apparel, underwear, socks, upholstery and hospital linen [1]. Natural fibers, like cotton, are generally more susceptible to biodeterioration than synthetic fibers because their porous hydrophilic structure retains water, oxygen and nutrients, providing perfect environments for microbial growth [2].

In the last decades, there has been a focus on researching and developing new antimicrobial compounds of natural origin. Chitosan, 2-amino-2-deoxy-(1→4)-d-glucopyranan, is undoubtedly one of the more promising multifunctional polymers among textile finishing agents. Chitosan derives from the deacetylation process by chitin, which is the second most abundant biopolymer in the world after cellulose. Chitin is a component of the shells of crustaceans and constitutes the exoskeleton of insects, also the wall of fungi [3].

Chitosan shows antibacterial activity against both Gram-positive and Gram-negative bacteria thanks to its combined bactericidal and bacteriostatic action. Its property mainly includes four mechanisms [4]: it causes damage to microbial DNA, as a blocking agent of oxygen and nutrients in the bacteria cell, it can bind cationic metals (calcium, magnesium) and nutrients essential for the microorganism and also causes the loss of cytoplasmic intracellular components necessary for cell survival.

All antibacterial mechanisms described are due to chitosan molecules carrying positively charged amine groups that have an electrostatic interaction with negatively charged cell membranes of microorganisms [5]. This electrostatic interaction leads to bacterial death.

In the present work, cotton and polyamide 6,6 fabrics were coated with a solution of chitosan to be tested as antibacterial fabrics against Gram-negative and Gram-positive bacteria.

The novelty of the present research work is related to the study of the influence of the contact time between the bacterial inoculum and the treated fabrics on the antibacterial action of the chitosan-coated fibers, also after dyeing and washing.

Existing standard methods [6] suggested using contact times of 1 hour or more. This work aims to evaluate if shorter contact times can be more selective in evaluating antibacterial textiles' performances.

II. Materials and Methods

A. Bacteria strains and fabrics

The antibacterial activity of fabrics coated with a chitosan solution was evaluated using model strains: Gram-positive bacteria, *Staphylococcus aureus* ATCC 6538 and Gram-negative bacteria, *Escherichia coli* ATCC 11229. Adjacent cotton fabric (suitable for ISO 105-F02, mass per unit 110 g/m^2) and adjacent polyamide 6,6 fabric (suitable for ISO 105-F03, mass per unit 130 g/m^2) were chosen to be used as textile substrates.

B. Preparation of the chitosan solution

A 2% (w/w) low molecular weight chitosan in a 2 % (w/v) acetic acid solution was prepared. The solution was shaken for 7 h at room temperature. 10 g of cotton and polyamide 6,6 fabrics were dipped into the solution overnight to promote the adsorption of chitosan on the fabrics. The impregnated fabrics were manually padded to reach a 90 % wet pick-up. The fabrics were eventually placed in an oven at 95 °C for 3 min and at 150 °C for another 3 min to allow the reaction between chitosan and the fabrics.

C. Material characterization

The natural and synthetic fabrics coated with chitosan, dyed and washed were characterized by scanning electron microscopy (SEM), colorimetric analysis and by water contact angle. Characterizations were carried out on chitosan-treated fibers, dyed chitosan-treated fibers and dyed chitosan-treated fibers and subjected to washing cycles.

D. Antibacterial tests

The antimicrobial activity was evaluated on chitosan treated samples, according to ASTM E 2149-2013 "Standard test method for determining the antimicrobial activity of antimicrobial agents under dynamic contact conditions". This method employed Gram-negative *E. coli* and Gram-positive *S. aureus*. Antibacterial tests were performed by diluting the test culture incubated in a nutrient broth (the bacterial inoculum) in a buffer (pH 7.0) to give a concentration of 1.5-3.0 × 10^5 CFU/ ml (working dilution). For each test, a sample of 0.5 g of fabric was immersed into a flask containing 25 ml

of the working dilution. All flasks were shaken for different contact times (12 min, 30 min and 1 h) at 190 rpm at room temperature.

After a series of dilutions with buffer until a concentration of 150-300 CFU/ml, 1 ml of the liquid was plated in 15 ml of Yeast Extract Agar. The inoculated plates were incubated at 37 °C for 24 h, and surviving cells were counted by plate count method. The tests were conducted in duplicate.

III. Results and Discussion

First, it was necessary to verify that the untreated natural and synthetic fibers did not have any intrinsic antibacterial effects. Using the ASTM method with both Gram-positive and Gram-negative bacteria, untreated cotton and polyamide 6,6 resulted in a bacterial reduction of 0%.

After 12 minutes of contact between treated fibers and inoculum bacterium, *E. coli* (Gram-negative bacterium) is immediately more sensitive to the antibacterial action of the biopolymer. There is a bacterial reduction of 99.8 % compared to 96.6 % of *S. aureus*.

Another essential aspect to consider is the contact time. It was observed for both bacteria species a more significant bacterial reduction increasing the contact time between inoculum bacterial diluted and cotton fabric treated with chitosan (Table 1).

Table 1. Bacterial reductions of chitosan treated on cotton and polyamide 6,6 fabrics.

Fabric		**Bacterial reduction (%)**	
	Contact time	***E. coli***	***S. aureus***
Cotton	12 min	99.8	96.6
	30 min	99.8	100
	1 h	100	100
Polyamide 6,6	12 min	100	100
	30 min	100	100
	1 h	100	100

Since chitosan is an excellent cross-linker to improve the adhesion of natural dyes to fabrics, the antibacterial effect was also tested on fabrics treated with chitosan and dyed with *Carmine Red*. In this way, it was possible to understand if the dye had a minimal influence on the antimicrobial property conferred by chitosan. In the following Table 2 the results obtained considering the bacterial reduction after the dyeing are reported.

Table 2. Bacterial reductions of chitosan treated on cotton and polyamide 6,6 fabrics after dyeing.

Fabric		**Bacterial reduction (%)**	
	Contact time	***E. coli***	***S. aureus***
Cotton	12 min	94.2	97.1
	30 min	97.8	94.2
	1 h	98.5	98.9
Polyamide 6,6	12 min	90.9	70.6
	30 min	99.1	83.2
	1 h	99.7	95.2

The dyeing influenced the chitosan antibacterial activity only at shorter contact time with PA 6,6, observing a bacterial reduction of 70.6 % at 12 min. and 83.2 % at 30 min. of *S. aureus.*

Fabric samples were also tested after washing cycles to verify the resistance of the dye and if the antibacterial property was maintained. The results are reported in Table 3.

Table 3. Bacterial reductions of chitosan treated on cotton and polyamide 6,6 fabrics, dyed and washed.

Fabric		**Bacterial reduction (%)**	
	Contact time	***E. coli***	***S. aureus***
Cotton	12 min	59.0	64.4
	30 min	92.8	84.5
	1 h	98.5	97.7
Polyamide 6,6	12 min	61.4	36.5
	30 min	96.0	47.9
	1 h	95.5	90.4

The washing reduced the antibacterial activity only for short contact times (12 and 30 minutes), while at 1 hour the percentage bacterial reduction remained higher than 90 %, and in most cases higher than 95 %.

In conclusion, both natural and synthetic fibers treated with chitosan proved to be highly antibacterial afterward only 12 minutes of contact with the bacterial inoculum. The dye do not significantly affect the antibacterial activity of chitosan, in fact already after 30 minutes were obtained bacterial reductions nearly 100 %. Washing generally reduced the antibacterial effect but only for short contact time (12 and 30 minutes).

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