Antibacterial properties of wool fabrics treated with 8-hydroxyquinoline and boron compounds

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ABSTRACT – REZUMAT

Antibacterial properties of wool fabrics treated with 8-hydroxyquinoline and boron compounds

In this study, antibacterial properties of wool fabrics treated with 8-hydroxyquinoline (8HQ) and boron compounds were investigated against E. coli, B. subtilis, S. aureus and P. aeruginosa. Experimental group consisted of wool fabrics treated with 8HQ, boric acid (BA) and borax (BX) at 10 g/L concentration for each using the exhaust process. Control group consisted of untreated and silver nitrate treated wool fabrics. Treatments were performed using meta- and pre-mordanting methods and homogenous distribution of treatment chemicals were confirmed with SEM and EDX images. Wool samples treated with a solution containing 8HQ+BX (10 g/L each) and 8HQ+BA+BX (10 g/L each) showed the highest level of antibacterial activity. Antibacterial activity decreased analogous with the decrease in 8HQ concentration however it was still present at the lowest concentration of 1 g/L for E. coli, B. subtilis and S. aureus. But at least 5g/L concentration of 8HQ was required for antibacterial activity against all four tested bacteria. Antibacterial properties decreased after 5 cycles of washing but did not completely disappear.

These antibacterial wool products look promising for the medical fields because of their strong effects against bacteria which grow in wounds and cause nosocomial infections. In addition, using the meta-mordanting method was more effective in terms of saving energy, water and time compared to the pre- mordanting method.

Keywords: *antibacterial, boric acid, borax, wool, 8-hydroxyquinoline*

Proprietăţile antibacteriene ale ţesăturilor din lână tratate cu 8-hidroxichinolină şi compuși ai borului

În acest studiu, proprietățile antibacteriene ale țesăturilor din lână tratate cu 8-hidroxichinolină (8HQ) și compuși ai borului au fost investigate împotriva E. coli, B. subtilis, S. aureus și P. aeruginosa. Grupul experimental a constat în țesături din lână tratate cu 8HQ, acid boric (BA) și borax (BX) la o concentrație de 10 g/L, pentru fiecare folosindu-se procesul de epuizare. Grupul de control a fost format din țesături din lână netratate și, respectiv, tratate cu nitrat de argint. Tratamentele au fost efectuate folosind metode de meta- şi pre-mordansare, iar distribuția omogenă a substanțelor chimice de tratament a fost confirmată cu imagini SEM și EDX.

Epruvetele din lână tratate cu o soluție conținând 8HQ + BX (10 g/L fiecare) și 8HQ + BA + BX (10 g/L fiecare) au prezentat cel mai înalt nivel de activitate antibacteriană. Activitatea antibacteriană a scăzut odată cu scăderea concentrației de 8HQ, cu toate acestea a fost prezentă și la cea mai mică concentrație de 1 g/L pentru E. coli, B. subtilis și S. aureus. Dar, concentrația de cel puțin 5 g/L 8HQ a fost necesară pentru activitatea antibacteriană împotriva tuturor celor patru bacterii testate. Proprietățile antibacteriene au scăzut după 5 cicluri de spălare, dar nu au dispărut complet. Produsele din lână cu proprietăți antibacteriene sunt promițătoare pentru domeniul medical datorită efectelor lor puternice împotriva bacteriilor, care se găsesc în răni și cauzează infecții nosocomiale. În plus, utilizarea metodei de meta-mordansare a fost mai eficientă în ceea ce privește economisirea de energie, apă și timp, comparativ cu metoda de pre-mordansare.

Cuvinte-cheie*: antibacterian, acid boric, borax, lână, 8-hidroxichinolina*

INTRODUCTION

8-Hydroxyquinoline is a small planar molecule with a lipophilic effect and a metal chelating ability. 8HQ is used in textile, wood, and paper industries because of its fungicide, insecticides, antibacterial and antimicrobial properties [1]. It is very effective against infection-causing pathogens and several bacteria [2]. 8HQ and its derivatives also have important properties that is useful in pharmacological and medicinal field acting as anti-neurodegenerative, anticancer, antioxidant, anti-inflammatory and anti-HIV agents.

Because of the proximity of the hydroxyl group to the heterocyclic nitrogen, 8HQ forms stable chelate complexes with various metal cations such as Cu^{2+}, Mn^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+} [3–4]. Antibacterial effect of 8HQ enhances when it makes complex with metal ions [5–6]. However, there are concerns about heavy metal ions associated with environment and health, so there is a need for more eco-friendly alternative for chelation with 8HQ.

There are about 230 varieties of boron compounds in nature and they are being used for many years [7–8]. Some boron compounds show antifungal and antibacterial activities [9–10]. In addition, due to the presence of reduction, bleaching and flammability properties, they are frequently used in glass, ceramics, agriculture and textile sectors. Boron compounds are used in industry instead of many harmful substances because of their environmentally friendly characteristics [11–12]. Therefore, usage and importance of boron compounds in high-tech production are increasing day by day.

Wool fiber has a similar protein composition of the human skin surface [13]. It has been estimated that wool contains more than 170 different proteins. The proteins in wool are composed of amino acids, which show similar chemical properties to 8HQ, especially in terms of chelate formation. Therefore, wool fiber, one of the natural textile fibers, was preferred for this research to be complexed with 8HQ.

Aim of this research was to investigate the antibacterial properties of 8HQ treated wool fabrics for medical textile industry. Boric acid and borax were chosen as eco-friendly complexation agents to increase the antibacterial effect of the 8HQ.

EXPERIMENTAL WORK

Materials

For this study, silver nitrate, 8-hydroxyquinoline (8HQ), boric acid (BA), borax (BX) and nitric acid were supplied from Merck, Germany. 100% wool fabric was purchased from Yunsa, Turkey (166 g/m^2, 23 warp ends/cm, 24 weft ends/cm).

Biosan DEN1 model McFarland densitometer was used for estimating bacterial concentration. TERMAL B21606E model machine was used for washing of the samples. Morphological measurements and determination of elemental composition of the materials' surfaces were examined using FEI QUANTA FEG 250 model scanning electron microscope with EDX module.

Bacterial strains and culture media

Main cultures of *Escherichia coli* ATCC 35218, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25293 and *Pseudomonas aeruginosa* ATCC 27853 were supplied from Microbiologics.

Nutrient broth and nutrient agar (for *E. coli* and *B. subtilis* strains) and Trypticase Soy Broth and Trypticase Soy Agar (used for *S. aureus* and *P. aeruginosa* strains) culture media were obtained from Hi-media.

Methods

Application of antibacterial agents on wool samples

After scouring with non-ionic detergent (0.5%, w/v) in the bath with F:L (fabric to liquor ratio, w/v) 1:15 for 30 min at 60°C, the wool fabric samples were treated with various chemicals (table 1) in accordance with the mordanting methods below. Several treatment solutions (baths) were prepared by dissolution of chemicals (table 1) in deionized water under magnetic stirring at room temperature. Silver treated, 8HQ treated and untreated wool samples were prepared for comparison.

"Mordanting" is a term commonly used in textile dyeing. A mordant is a chemical binding agent that adheres well to both fibers and dyes. The substances used for this purpose are called mordant substances. Classically defined, mordants are usually water-soluble metal salts and materials exhibiting weak acid or base properties. Wool is an example of natural textile materials that can form insoluble chelate complexes with various mordants including heavy metals [14].

Antimicrobial agents were applied on the fabrics using mordanting methods. Two baths were used in the pre-mordanting method [15], where fabrics were treated with 8HQ solution (1–10 g/L) at the first bath, then with boron compounds (10 g/L) at the second bath. At the meta-mordanting method, samples were treated in a single bath containing all the chemicals simultaneously. For each bath, the treatments were performed at 60°C for 30 minutes with a *F:L* (weight of Fabric to volume of Liquor) of 1:20.

After the mordanting operations, treated woollen samples were washed with tap water in order to remove non-bonded chemical particles.

Determination of antibacterial activity

ISO 20645:2004 method was mimicked to determine the antibacterial activity of wool samples [16]. Two-layered soft agar plates were prepared by adding 7.5 g/L Agar into the broth media, which were Nutrient Broth for *E. coli* and *B. subtilis* strains, Trypticase Soy Broth for *S. aureus* and *P. aeruginosa*. The lower layer was 10 ml without any bacteria. The upper layer was 5 ml and contained 100 μl of the bacterial culture containing 10^8 CFU/ml which was estimated from McFarland measurement [15, 17].

Later, each wool sample to be tested were prepared in 30 mm × 30 mm size and placed on the soft agar medium. Petri dishes were incubated at 37±1°C for 24 hours. For accuracy, the experiments were performed and repeated three times. Average diameters of inhibition zones were calculated using equation (1) [18]:

$$A = (B - C) / 2 \quad (1)$$

where A is the average diameter of clear inhibition zone in mm, B – the total diameter of inhibition zone

Table 1

STYPES AND CONCENTRATIONS (G/L) OF INGREDIENTS IN THE TREATMENT SOLUTIONS

Samples	8HQ*	Silver nitrate	Borax decahydrate	Boric acid
W8	10	-	-	-
W8A	10	10	-	-
WA	-	10	-	-
W8B10	10	-	10	-
W8B5	5	-	10	-
W8B2.5	2.5	-	10	-
W8B1	1	-	10	-
W8BB10	10	-	10	10
W8BB5	5	-	10	10
W8BB2.5	2.5	-	10	10
W8BB1	1	-	10	10

* 8HQ containing solutions were prepared by dissolution of 8HQ and other chemicals in deionized water acidified with nitric acid under magnetic stirring at room temperature.

including wool sample and clear zone in mm, and C – the length of the tested wool sample.

Antibacterial activities of the samples were tested before and after washing (up to 5 times) by using Standard Test Method ISO 105-C06 A1S [19]. After washing, the samples were rinsed in pure cold water, dried in the open air, and then tested for antibacterial activity as mentioned above.

RESULTS AND DISCUSSIONS

Surface morphology and elemental composition of the wool fibers

Surface morphologies and the elemental composition of the wool samples were investigated by SEM-EDX (table 2). Untreated wool (W0) surfaces had mole ratios of N/C, S/C and O/C which corresponded to 0.94, 0.08 and 1.98 respectively.

Treatment with 10 g/L 8HQ solution (W8) resulted in a small decrease in N/C ratios while changes in S/C mole ratios were insignificant. It was concluded that the 8HQ compound did not bind to the Sulphur in the 8HQ treated fabric (W8) due to the unchanged S/C ratio. Furthermore, EDX results showed that the oxygen content of the surfaces increased after 8HQ treatment (W8).

Samples treated with 8HQ and boron compounds (W8B1-10 and W8BB1-10) had homogenous boron distributions, which were confirmed by SEM-EDX mapping (figure 1). These samples had significantly decreased S/C mole ratios (from 0.08 to 0.02–0.04), probably due to the homogenous distribution of boron compounds which covered surface of the 3Sulphur elements (figure 1). The change in 8HQ concentration of the treatment solutions from 10 g/L to 1 g/L decreased N/C ratios proportionally while it did not have any significant effect on B/C and O/C ratios (table 2).

When SEM/EDX images of the wool fabric samples were examined (table 2 and figure 2), 8HQ treatment showed decrease in fiber diameter from 21.4 μm (W0) to 17.7 μm (W8). Fiber diameters were protected in the presence of boric acid in addition to 8HQ bath (W8B1-10). The highest fiber diameter was observed when

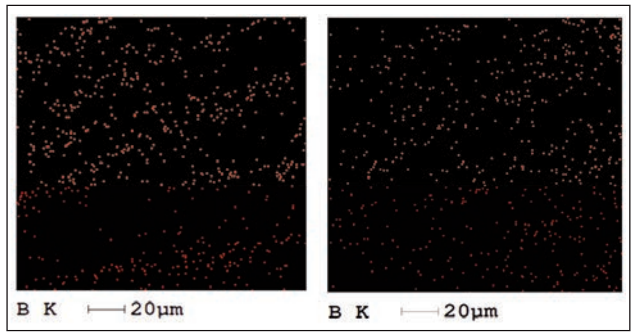

Fig. 1. SEM-EDX mapping images showing boron distribution of W8BB1 and W8BB10 samples under 2000× magnification and 20 kV setup conditions

the treatment solution also included borax in addition to 8HQ and boric acid (W8BB1-10). Although 8HQ and boron treated samples had little micro cracks and rough structures, the wool fibers were not damaged significantly.

Antibacterial activity of the samples

Antibacterial properties of the samples against two gram-positive (*B. subtilis* and *S. aureus*) and two gram-negative (*E. coli* and *P. aeruginosa*) bacteria were measured as presented in table 3. As seen in table 3, the meta-mordanting method, in general, provided more effective antibacterial properties than the

Table 2

MORPHOLOGICAL AND ELEMENTAL ANALYSES OF THE SAMPLES

Sample name	Fiber diameter (μm)	N/C (mol/mol)	S/C (mol/mol)	B/C (mol/mol)	O/C (mol/mol)
W0	21.40	0.94	0.08	0.00	1.98
W8	17.70	0.86	0.08	0.00	2.02
W8B10	19.90	0.66	0.03	1.01	1.11
W8BB10	22.20	0.65	0.03	0.94	1.06
W8B5	18.80	0.63	0.02	0.96	1.04
W8BB5	21.15	0.62	0.02	1.01	0.96
W8B2.5	18.20	0.58	0.02	0.97	0.99
W8BB2.5	21.05	0.56	0.03	0.98	1.13
W8B1	18.90	0.54	0.03	0.97	1.14
W8BB1	21.00	0.53	0.04	0.96	1.13

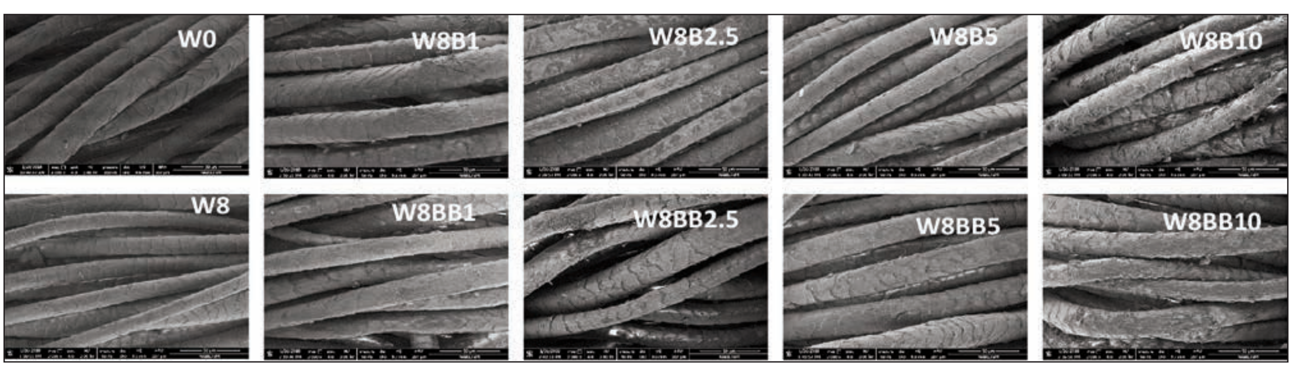

Fig. 2. SEM images of treated and untreated wool samples under 2000× magnification

pre-mordanting method did probably due to dissolution of some 8HQ during the second bath process.

Examining the results shown in table 3 it can be concluded that the untreated wool sample (W0) was not effective against any of the bacteria tested. 8HQ by its own and 8HQ treated sample (W8) showed antibacterial activity against all the tested bacteria. The silver treated wool sample (WA) was effective only against gram-negative strains.

Wool fabric samples treated with 8HQ using the meta-mordanting method produced a 12 mm zone against *E. coli*. It was observed that while 8HQ concentration remained constant, addition of silver ions reduced the antibacterial effect of 8HQ by 40%, whereas this effect increased when borax (W8B10) was used alone or together with borax/boric acid (W8BB10). For *P. aeruginosa*, the use of Ag, borax and boric acid + borax did not significantly change the antibacterial effect of 8HQ. Taking all samples into consideration, it was determined that *P. aeruginosa* was the most resistant bacteria among the tested strains and the smallest zone diameters were observed at *P. aeruginosa*. These results are similar to the work of Srisung et al. (2013), who investigated the antibacterial effect of 8HQ on several bacteria and found that the most resistant bacteria is *P. aeruginosa* [2].

In the case of *B. subtilis and S. aureus*, the use of Ag generally decreased the antibacterial effect of 8HQ while the use of boron compounds with 8HQ increased this effect.

The highest antibacterial activity was observed at W8B10 and W8BB10 samples. Later, 8HQ concentration was decreased from 10 g/L (W8B10 and W8BB10) to 1 g/L (W8B1 and W8BB1) step by step in order to reduce production costs and observe the effects. At minimum 5 g/L 8HQ concentration was required for antibacterial activity against *P. aeruginosa* while 1 g/L 8HQ was enough against *E. coli, B. subtilis* and *S. aureus* (table 4).

The effect of 5-cycles of washing on the antibacterial activity was investigated (table 5). As seen in table 5, antibacterial effect decreased about 40–50% after 5 cycles of washing. Samples treated only with 8HQ (W8) were effective against all the bacteria except *P. aeruginosa* after washing.

It was also observed that all washed wool samples treated with 8HQ (W8, W8A, W8B and W8BB) were still more antibacterial than the non-washed silver treated samples (WA). This result shows that 8HQ has a critical role for antibacterial activity.

CONCLUSIONS

Aim of this study was to present antibacterial properties to wool samples by applying

Table 3

INHIBITION ZONE DIAMETERS (MM) CREATED BY THE TREATED SAMPLES USING META-MORDANTING AND PRE-MORDANTING (IN PARENTHESIS) METHODS

Sample	Gram-Negative Bacteria		Gram-Positive Bacteria	
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*
W0	0	0	0	0
8HQ*	35	3	39	18
W8	12	2	22	14
WA	2	1	0	0
W8A	7 (5)	4 (3)	8 (6)	4 (3)
W8B10	15 (11)	4 (2)	≥30** (25)	8 (6)
W8BB10	18 (13)	4 (2)	≥30** (22)	19 (14)

* Zone diameter created by 10 µl of 10g/L 8HQ solution.
** Full inhibition (no bacterial growth) was observed.

Table 4

INHIBITION ZONE DIAMETERS (MM) OF THE SAMPLES TREATED WITH VARIOUS CONCENTRATIONS OF 8HQ*

Sample	Gram-Negative Bacteria		Gram-Positive Bacteria	
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*
W8B10	15 (11)	4 (2)	≥30* (25)	17 (12)
W8B5	9 (7)	3 (2)	≥30* (25)	13 (10)
W8B2.5	7 (5)	0 (0)	22 (16)	8 (6)
W8B1	2 (1)	0 (0)	1 (<1)	8 (6)
W8BB10	18 (13)	4 (2)	≥30** (22)	19 (14)
W8BB5	11 (8)	3 (2)	≥30** (20)	17 (13)
W8BB2.5	6 (4)	0 (0)	29 (21)	13 (10)
W8BB1	2 (1)	0 (0)	1 (<1)	7 (5)

* Used meta-mordanting (bold) and pre-mordanting (in parenthesis) methods.
** Full inhibition (no bacterial growth) was observed.

Table 5

INHIBITION ZONE DIAMETERS (MM) OF 5-CYCLES WASHED WOOL SAMPLES*

Sample	Gram-Negative Bacteria		Gram-Positive Bacteria	
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*
W8	4	0	9	10
WA	2	0	0	0
W8A	3	2	3	2
W8B10	9	2	18	11
W8B5	5	1	20	7
W8B2.5	3	0	12	5
W8B1	1	0	0	4
W8BB10	11	2	22	12
W8BB5	5	1	19	10
W8BB2.5	2	0	12	7
W8BB1	1	0	0	3

* Metamordanting method was used for 8HQ treatment (bold).

8hydroxyquinoline (8HQ) alone or together with boric acid and borax chemicals (experimental group). 8HQ and boron compounds were applied by using the pre-mordanting (the application of boric acid + borax solutions to 8HQ applied wool fabric specimens) and the -meta-mordanting (the application of all chemicals at the same time) methods. Untreated- and silver nitrate treated-wool fabrics were used as the control group. Antibacterial activities of the samples against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*B. subtilis* and *S. aureus)* bacteria were investigated.

Untreated wool samples showed no antibacterial properties against any tested bacteria while Silver treated wool samples showed low levels of antibacterial activity against Gram (–) bacteria and no antibacterial effect against Gram (+) bacteria.

Among the samples in the experimental group, it was determined that the sample showing the highest antibacterial effect against all tested bacteria was W8BB10. When 8HQ concentration was reduced from 10 g/L (W8BB10) until 1 g/L (W8BB1), antibacterial activity decreased in a dose-dependent manner, while maintaining antibacterial activity in all strains except for *P. aeruginosa*. After 5 cycles of washing, the antibacterial activities decreased about 40–50%, but the samples treated with 5 g/L and higher concentration of 8HQ were still effective against all tested bacteria.

It was estimated that the industrial application of 8HQ as a mordanting agent on wool fabrics would acquire superior antibacterial properties than existing silver solutions in the market even after washing process. Silver treated wool samples were less effective against *P. aeruginosa*, which causes nosocomial infection, than 8HQ treated ones. Therefore, it was considered that 8HQ and boron containing formulations can be good candidates for application in the medical textiles sector. In addition, the meta-mordanting method required 50% less water and time for application in comparison to the pre-mordanting method thus it was shown that the meta-mordanting method is a more feasible and environmentally friendly process due to saving of water and energy.

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