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The Antimicrobial Effect of Benzalkonium Chloride on Some Pathogenic Microbes Observed on Fibers of Acrylic Carpet

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Abstract: In the presented research, the main aim is to investigate the antibacterial effectiveness of BAC solutions on acrylic fibers used in machinery carpet. An acrylic pile carpet laid in a public place for 30 days and the existence of some microbes were investigated on it. The antimicrobial effect of different BAC solutions for identified microbes was studied *in vitro*. The acrylic fibers were treated with the same different solutions of BAC as before and the antibacterial effectiveness was assessed by the zone of inhibition method in different times. Two pieces of carpet untreated and treated with BAC solution sewed together and laid for one week in the public place and the amounts of bacterial growth determined by colony count method and the results compared. Finally some mechanical properties of treated acrylic fibers measured after 30 days and compared with untreated one. The results showed the presence of some pathogenic microbes on the laid carpet such as *Escherichia coli* and *Staphylococcus aureus*. The inhalation time for treated acrylic fibers improved. The amount of colony growth on treated carpet reduced considerably and besides the mechanical tests results showed no significant deterioration effect of studied properties in comparing with untreated yarn.

Key words: Carpet, benzalkonium chloride, acrylic, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Textile goods provide an excellent environment for microorganisms to grow. As Schindler and Hauser (2004) mentioned, two different aspects of antimicrobial protection provided by chemical finishes can be distinguished. The first is the protection of the textile user against pathogenic or odor causing microorganisms (Hygiene finishes). The second aspect is the protection of the textile itself from damage caused by mould, mildew or rot producing microorganisms that it can be called antibacterial protection finish. According to Schindler and Hauser (2004) and Vigo (1997) many agents including inorganic salts, organometallics, iodophors (substances that slowly release iodine), phenols and thiophenols, onium salts, antibiotics, heterocyclics with anionic groups, nitro compounds, urea and related compounds, formaldehyde derivatives, biopolymers and amines have been used as antibacterial agent for hygiene finish or antibacterial protection finish. There are many studies in this field using these compounds (Anand *et al*., 2006; Diz *et al*., 2001; Han and Yang, 2005; Lim and Hundson, 2004; Nakashima *et al*., 2001; Young *et al*., 2003).

Between these antimicrobial agents quaternary ammonium salts exhibit marked antimicrobial activity against a wide range of bacteria, fungi and viruses. These compounds are any of a group of ammonium salts in which organic radicals have been substituted for all four hydrogen's of the original ammonium cation. They have a central nitrogen atom which is joined to four organic radicals and one acid radical. The organic radicals may be alkyl, aryl, or aralkyl and the nitrogen can part of a ring system. They are prepared by treatment of an amine with an alkylating agent. Quaternary ammonium halide cationic surfactants are widely used in antimicrobial finishing of textiles, given their additional surface-active and detergent properties (Shao *et al*., 2003; Huang *et al*., 2004; Tatsuo *et al*., 1989).

Benzalkonium Chloride BAC (Fig. 1) is one of the conventional quanternary ammonium salt. Its solutions are rapidly acting anti-infective agents with a moderately long duration of action. They are active against bacteria and some viruses, fungi and protozoa. Bacterial spores are considered to be resistant. Solutions are bacteriostatic or bactericidal according to their concentration. The exact mechanism of bacterial action is unknown but it is thought to be due to enzyme inactivation. Activity

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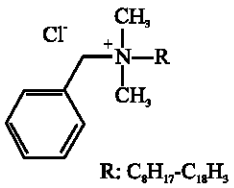

Fig. 1: Molecular structure of Benzalkonium Chloride (BAC)

generally increases with increasing temperature and pH. Gram positive bacteria are more susceptible than gram-negative (Bahgat *et al*., 1985; Marple *et al*., 2003).

BAC has a wide range of applications; as Marple *et al*. (2003) mentioned it has been in clinical use since 1935 as an antibacterial agent. It is used as a surface disinfectant and detergent-primarily as hand scrub or face wash. It can be used as a disinfecting agent for instruments and in low concentration as a preservative for drugs including ophthalmic solutions (Gloor *et al*., 1979). It is also a cationic germicide. It has been used in textile industry too, such as an insecticidal, or antimicrobial (Kim and Sun, 2001; Tatsuo *et al*., 1989).

Floor covering textiles such as carpet, due to their pile nature and long washing periods, prepare a suitable medium for growing microorganisms, some of which not only produce putrid and mildew odors, but also contribute to unsightly defacement and deterioration of carpet components (White *et al*., 1984). The other important factor which is in our consideration in the presented study is that some of these microbes may also produce infections and/or allergenic conditions in humans.

Acrylic fibers are one of the most conventional fibers used in carpet producing. Many studies have done for giving antimicrobial effect to these fibers (Wang *et al*., 2006; Cai and Sun, 2004). In the presented study a conventional antiseptic agent; Benzalkonium Chloride (BAC) was applied through immersion method for improving acrylic fibers inhibition against some pathogenic microorganisms and the antibacterial effectiveness of it was evaluated by standard tests.

MATERIALS AND METHODS

Materials: Benzalkonium chloride was prepared from Asalib Co. (Table 1). The carpets were purchased from Neghin-e-Mashhad Co. (Table 2). Acrylic yarns were prepared from Neghin-e-mashhad (10/2 Nm).

The pure microbes were supplied from the Bouali Hospital, Tehran, Iran and all tests were done in the Laboratories of Tarbiat Modarres University, Islamic Azad University Science and Research Campus Branch and Islamic Azad University of Shahr-e Ray in 2005-2006.

Table 1: Technical data of the applied Benzalkonium Chloride (BAC)

Trade name	Benzalib 50
Product	A 50 % w/v solution of alkyl benzyl dimethyl ammonium chloride, complying with BP 2003 and USP 26-NK 21 monograph
Appearance at 20°C	Clear liquid
Color	Colorless to pale yellow
Density at 20°C	0.99 G cm^{-3}
Viscosity at 20°C	120 CS
Assay (mmw = 349.8)	50±1
Non-quaternished amine (mmw = 223.3)	0.5% max
Sulphated ash	0.2% max
pH (5% in water)	6.5-8.5

Table 2: The specification of the applied carpets

Color	Ivory (mostly)
Pile yarn	100% Acrylic
Pile length	11±1 mm
Warp yarn	Cotton/Polyester 35/65%
Weft yarn	100% Jute

Methods: For investigating the kind of present microbes on carpet, (especially pathogenic ones) carpets were laid in a public place (in the entrance of the Mosque of Islamic Azad University Shahr-e Ray) for 30 days (everyday approximately 200 people were stepped on it), thereafter some fibers were cut of the carpets randomly and immersed in Thioglycolate and Nutrient broth mediums. After incubating for 24 h at 37°C, the solutions of each media sub-cultured in Nutrient and Blood agar mediums and after incubating for 48 h at 37°C the colonies of microbes were cultured by streak test method. For identifying the kind of cultured microbes the gram stain, catalase, oxidase, citrate agar, Christensen's urea broth and TSI agar tests were done. The cultured microbes kept in skimmed milk for next step.

Ditch plates method was used for evaluating the antibacterial effectiveness of BAC against found microbes on carpet. Ditch plates were prepared by allowing the Mueller Hinton Agar to solidify in a Petri dish and ditches (with diameter of approximately 4 mm) produced on it by removing the agar. Ditches were inoculated by different BAC solutions (0.5, 0.1, 0.05 and 0.025% w/v solutions of BAC). The dishes incubated for 18 h in 37°C to let the BAC solutions penetrate to the agar medium. Microbes (stored in skim milk) were mixed with a semi liquid Mueller Hinton Agar (Agar conc.<1%) and added to the inoculated plates. The plates were incubated at 37°C and the zone of inhibition in different time intervals (12, 24, 48, 72, 96, 120, 148, 172, 196, 220, 244 and 268 h) determined. The positive results repeated three times and the mean of zone of inhibition reported for 120 h.

Acrylic fibers were immersed for 10 min in different solutions of BAC (0.5, 0.1, 0.05 and 0.025% w/v solutions of BAC) and after drying they entered in plates containing the pure microbes and the zone of inhibition were

observed until the zone of inhibition disappeared. Every 24 h the plates were replaced with new plates of pure microbes.

For comparing the antibacterial effectiveness of BAC in practice one carpet splitted into two parts, one remained untreated and the other treated with BAC solution (10 min in 0.1% w/v solution of BAC). Two pieces sewed together and laid in the public place (in the entrance of the Mosque of Islamic Azad University Shahr-e Ray). Some fibers cut of two samples and the previously described methods use for culturing and separating of microbes and the antibacterial effectiveness of BAC on carpet was measured by colony count method.

Some mechanical properties of untreated and treated (10 min in 0.1% w/v solution of BAC) acrylic yarns were measured by Tensorapid (SDL Co.) after 30 days. The length for every sample was 300 mm and the speed of test was 999.9 mm/min.

RESULTS AND DISCUSSION

The presence of some bacteria and fungi were proved in the studied carpet laid for 30 days, including *Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Penicillum*, *Fusarium*, *Aspergillus* and *Mucor*. (The presence and kind of fungus were determined by observation and experimentally and no particular test has done).

The antimicrobial effectiveness of BAC solutions on the found microbes after 120 h is shown in Table 3. According to the results the 0.025% w/v solution of BAC shows no insignificant antimicrobial effect.

Antibacterial effects of treated acrylic fibers with different solutions of BAC assessed by the remaining time of the zone of inhibition are shown in Table 4.

Comparing results of the treated and untreated two pieces of carpet after laying for on week in a public place is shown in Table 5. It can be seen that the number of colonies growth were decreased 50, 92, 90 and 90% for *Staphylococcus*, *Bacillus*, *Pseudomonas* and *Escherichia coli* orderly.

The effect of 0.5% w/v solution of BAC on some mechanical properties of treated carpet in compare with untreated one is shown in Table 6. There is no significant deterioration effect on the studied mechanical properties mechanic (in the significant level of $\alpha = 0.05$).

CONCLUSIONS

Benzalkonium chloride was chosen for this study because it is a common antiseptic and it belongs to the group of cationic surface active agents. Considering its charge it can act like a cationic dye and tend to take up and hold on the surface of anionic substrate such as acrylic.

According to the results the presence of some pathogenic microbe on the laid carpet confirmed including *Escherichia coli* and *Staphylococcus* which can be causing many infections. So it is worthy to enhance the antimicrobial activity of the carpet with a proper antimicrobial finishing. Although the kind of microbes on

Table 3: Antimicrobial effect of BAC solutions on the found microbes on the carpet

	The zone of inhibition (mm) of BAC solution			
Microbe	0.5% BAC (w/v)	0.1% BAC (w/v)	0.05% BAC (v/w)	0.025% BAC (v/w)
Escherichia coli	18	14	12	*-
Enterobacter	18	14	12	-
Staphylococcus aureus	16	14	12	-
Pseudomonas	14	12	12	-
Penicillum	14	12	Less than 12	-
Aspergillus	14	12	-	-
Fusarium	14	12	-	-
Mucor	14	12	-	-
Bacillus	12	12	-	-

*- Showed no zone of inhibition

Table 4: Antimicrobial effect of treated acrylic fibers with BAC

	Maximum time of inhibition of treated fibers with BAC solution (h)			
Microbe	0.5% BAC (w/v)	0.1% BAC (w/v)	0.05% BAC (v/w)	0.025% BAC (v/w)
Escherichia coli	264	168	72	24
Enterobacter	264	168	72	24
Staphylococcus	240	96	72	24
Pseudomonas	168	48	24	*-
Penicillum	240	72	-	-
Aspergillus	240	96	-	-
Fusarium	240	96	-	-
Mucor	216	72	-	-
Bacillus	48	24	12	-

*-Showed no inhibition time

Table 5: Number of colonies growth on untreated and treated carpet with 0.1% BAC (w/v) after 7 days

	No. of colonies	
Bacteria	Untreated carpet	Treated carpet
Staphylococcus	10	5.0
Bacillus	100	8.0
Pseudomonas	15	1.5
Escherichia coli	15	1.5

Table 6: The effect of treating acrylic yarn with BAC on some mechanical properties after 30 days

Mechanical property	Elongation at break (%)	Work of rupture (Nm)	Initial modulus (cN/Tex)	Tenacity (cN/Tex)
Untreated	58.243	0.9542	157.48	11.550
Co-Eff Var.	12.990	17.3300	6.74	4.070
Treated	59.199	1.0128	161.57	11.859
Co-Eff Var.	10.100	11.5700	3.99	2.470

the carpet depends considerably to the environment of course, but it was shown that treating acrylic fabric with Benzalkonium chloride inhibits considerably the growth amount of studied microbes and in some cases up to 100%.

The wash fastness or durability of the effect against washing of the treated carpet in the study was not under attention because the interval of washing periods for textile floor covering is not short and during these intervals usually the activity of the antimicrobial agent vanishes, as it was seen in the case of BAC, which its maximum inhibition time with a high concentration (0.5% w/v) was just 264 h. So the treatment must be renewed after a time and using methods like spraying the solutions of Benzalkonium chloride is suggested.

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