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Investigation on the Antimicrobial Effect of Ammonyx on Some Pathogenic Microbes Observed on Sweatshirt Sport

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Abstract**—**In this research, the main aim is to investigate the antimicrobial effectiveness of ammonyx solutions finishing on Sweatshirt Sport with immersion method. 60 Male healthy subjects (football player) participated in this study. They were dressed in a Sweatshirt for 14 days and some microbes found on them were investigated. The antimicrobial effect of different ammonyx solutions(1/100, 1/500, 1/1000, 1/2000 v/v solutions of Ammonyx) on the identified microbes was studied by the zone inhabitation method in vitro. In the next step the Sweatshirt Sports were treated with the same different solutions of ammonyx and the antimicrobial effectiveness was assessed by colony count method in different times and the results were compared whit untreated ones. Some mechanical properties of treated cotton/polyester yarn that used in Sweatshirt Sport were measured after 30 days and were compared with untreated one. Finally after finishing, scanning electron microscopy (SEM) was used to compare the surfaces of the finished and unfinished specimens. The results showed the presence of five pathogenic microbes on Sweatshirt Sports such as Escherichia coli, Staphylococcus aureus, Aspergillus, Mucor and Candida. The inhalation time for treated on Sweatshirt Sports improved. The amount of colony growth on treated clothes reduced considerably and moreover the mechanical tests results showed no significant deterioration effect of studies properties in comparison to the untreated yarn. The visual examination of the SEM indicated that the antimicrobial treatments were applied usefully to fabrics.

Keywords**—**Pathogenic microbes, Sweatshirt Sports, Ammonyx, antimicrobial treatment

I. Introduction

THE growth of microorganisms on textiles inflicts a range of unwanted effects not only on the textile itself but also on the wearer. These effects include the generation of unpleasant odor, stains and discoloration in the fabric, a reduction in fabric mechanical strength and an increased likelihood of contamination [1],[18]. For these reasons, it is highly desirable that the growth of microbes on textiles be minimized during their use and storage [3].

In order to obtain the greatest benefit, an ideal antimicrobial treatment of textiles should satisfy a number of requirements [3],[5]. Firstly, it should be effective against a broad spectrum of bacterial and fungal species, but at the same time exhibit low toxicity to consumers, e.g. not cause toxicity, allergy or irritation to the user. Antimicrobial-treated textiles have to meet standards in compatibility tests (cytotoxicity, irritation and sensitization) before marketing. Secondly, the finishing should be durable to laundering, dry cleaning and hot pressing. This is the greatest challenge as textile products are subjected to repeated washing during their life. Thirdly, the finishing should not negatively affect the quality (e.g. physical strength and handle) or appearance of the textile. Finally, the finishing should preferably be compatible with textile chemical processes such as dyeing, be cost effective and not produce harmful substances to the manufacturer and the environment[2],[4],[11].

Several major classes of antimicrobial agents are used in the textile industry. They are generally not new per se and have been in use in other industries, e.g. as food preservatives, disinfectants, swimming pool sanitizers or in wound dressings. These agents are potent in their bactericidal activity, as indicated by their Minimal Inhibitory Concentration (MIC) values [2],[9],[10].

Quaternary ammonium compounds, particularly those containing chains of 12–18 carbon atoms, have been widely used as disinfectants [6],[22]. These compounds carry a positive charge at the N atom in solution and inflict a variety of detrimental effects on microbes, including damage to cell membranes, denaturation of proteins and disruption of the cell structure [2],[7],[14],[19]. During inactivation of bacterial cells, the quaternary ammonium group remains intact and retains its antimicrobial ability as long as the compound is attached to textiles [2],[3],[20].

Quaternary ammonium halide cationic surfactants are widely used for antibacterial surface-active and detergent properties [15],[21].

Ammonyx is one of the conventional quaternary ammonium salts. Its solutions are rapidly actas anti-infective agents with a moderately long duration of action. They are active against bacteria some viruses, fungi and protozoa. Solutions are bacteriostatic or bactericidal according to their concentration [3],[4],[16].

The exact mechanism of bacterial action is unknown but it

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is thought to be due to enzyme inactivation. Activity generally increases with increasing temperature and PH. It has been used in textile industry, as an insecticidal or antimicrobial agent [6],[7],[17].

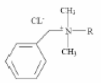
In this study a conventional antiseptic agent, Ammonyx was applied through immersing method for improving clothes's inhibition against some pathogenic microbes and the antibacterial effectiveness of the clothes was evaluated by standard test methods.

II. Materials and Methods

A. Materials

Ammonyx was purchased from Asalib Co. Table I. The clothes were purchased from Poyan Co. Polyester/Cotton blend yarns were prepared from Poyan Co. (20/2 Nm). The pure bacteria were supplied by 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 Shahre-Rey in 2009.

TABLE I
MOLECULAR STRUCTURE AND TECHNICAL DATA OF THE APPLIED AMMONYX

Trade name	Ammonyx 50
Product	A 50% v/v solution of alkyl benzyl dimethyl ammonium chloride, complying with BP 2003 and USP 26-NK 21 monograph.
 R: $C_{14}H_{29}$	
Appearance at 20^0 C	Clear liquid
Color	Colorless to pale yellow
Density at 20^0 C	099G .cm^{-3}
Viscosity at 20^0 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

B. Methods

Immersing method was used for adding antibacterial finishes on the test clothes. A polyester/cotton blend fabric was used in this study because it is one of the most frequently used fabric for scrub suits, lab coats and uniforms [1].The test fabric, supplied by the Test fabrics Inc. with a code #7409, was 65% Dacron polyester/35% cotton.

60 Male healthy Building Workers (age 21±3 years, stature 175±3 cm, and weight74±10 kg) participated in this study. The subjects were dressed in a cotton/polyester sweatshirt sport for 14 days. For investigating the kind of bacteria on clothes, (especially pathogenic ones), some fibers were cut from the clothes randomly and immersed in Thioglycolate and Nutrient broth mediums. After incubating for 24 hrs at 370C, the solutions of each media were sub-cultured in Nutrient and Blood agar mediums and after incubating in Nutrient and Blood agar mediums for 48 h at 370C 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 were kept in skimmed milk as the next step. Ditch plates method was used for evaluating the antibacterial effectiveness of Ammonyx against the detected bacteria on the clothes. 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 Ammonyx solutions (1/100, 1/500, 1/1000 and 1/2000 v/v solutions of Ammonyx). The dishes were incubated for 18 hrs at 370C to let the Ammonyx solutions penetrate into 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 370C and the zone of inhibition at different time intervals (12, 24, 48, 72, 96, 120, 148, 172, 196, 220, 244 and 268 hrs) were determined. The positive results were repeated three times and the mean of the zone of inhibition was reported for 120hrs. fibers were sprayed with in different solutions of Ammonyx (1/100, 1/500, 1/1000, 1/2000 v/v solutions of Ammonyx) and after drying they entered in plates containing the pure microbes and the zone of inhibition was observed until the zone of inhibition disappeared. Every 24 hrs the plates were replaced with new plates of pure Microbes. For comparing the antibacterial effectiveness of Ammonyx on clothes, the subjects were dressed in a new treated cotton/polyester sweatshirt sport for 14 days and some microbes found on them were investigated again. First remained untreated and the second treated with Ammonyx solution (1/500 v/v solution of Ammonyx by immersing method).

After the using clothes by subjects, some fibers cut out of two samples and the previously described methods were use for culturing and separating the microbes and the antimicrobial effectiveness of Ammonyx on clothes was measured by colony count method.

Some mechanical properties of untreated and treated (1/500 % v/v solution of Ammonyx) polyester/cotton blend 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. An International Standards Instrument ISX-430 SEM was used to compare the surfaces of the finished and unfinished specimens.

III. Results and Discussion

The presences of some microbes were proved in the experimental clothes, including Escherichia coli, Staphylococcus aureus, Aspergillus, Candida and Mucor.

The antimicrobial effectiveness of Ammonyx l solutions on the detected microbes after 120hr is shown in Table II. According to the results the 1/2000 v/v solution of Ammonyx shows no significant antimicrobial effect.

Antibacterial effects of treated fibers with different solutions of Ammonyx assessed for the remaining time in the zone of inhibition are shown in Table III. Comparing results

of the treated and untreated clothes is shown in Table IV. It can be seen that the number of colonies growth were decreased about 70 and 90% for Stapylococcus and Escherichia coli respectively.

The effect of 1/100 v/v solution of Ammonyx on some mechanical properties of treated clothes in comparison with untreated one is shown in Table V. There is no significant deterioration effect on the studied mechanical properties (e.g. the significant level of α =0.05, about Table V). A successfully finished specimen should look smoother and more sweatshirt sport compared to the unfinished specimen because the finish improves the surface properties fibers and yarns. At 1,000 times magnification, the swatch with no treatment showed an unevenness on the fiber surfaces (see fig. 1). Fiber surfaces with Ammonyx treatments looked smooth (see fig. 2). The visual examination of the SEM indicated that the antibacterial treatments were applied successfully to second swatch.

TABLE II
ANTIMICROBIAL EFFECT OF AMMONYX SOLUTIONS ON THE FOUND MICROBES ON THE CLOTHES

	The zone of inhibition (mm)of Ammonyx solution			
Microbe	1/100 (v/v)	1/500(v/v)	1/1000 (v/v)	1/ 2000 (v/v)
Escherichia coli	1 8	15	12	*
Staphylococcus	1 6	14	*	*
Candida	14	12	*	*
Aspergillus	13	12	*	*
Mucor	13	12	*	*

*: Showed no zone of inhibition

TABLE III
ANTIMICROBIAL EFFECT OF TREATED POLYESTER/COTTON BLEND FIBERS WITH AMMONYX

	Maximum time of inhibition of treated fibers with Ammonyx solution (hr)			
Microbe	1/100 (v/v)	1/500(v/v)	1/1000 (v/v)	1/ 2000 (v/v)
Escherichia coli	265	165	67	2 8
Staphylococcus	240	95	68	31
Candida	246	76	*	*
Aspergillus	245	99	*	*
Mucor	222	76	*	*

*: Showed no inhibition time

TABLE IV
THE NUMBER OF COLONIES GROWTH ON UNTREATED AND TREATED CLOTHES WITH 1/100 AMMONYX (V/V) AFTER 14 DAYS

	No. of colonies	
Bacteria	Untreated carpet	Treated carpet
Staphylococcus	9	7. 2
Escherichia coli	20	0.9

TABLE V
EFFECT OF TREATING COTTON/POLYESTER YARNS WITH AMMONYX ON SOME MECHANICAL PROPERTIES AFTER 30 DAYS

Mechanical property	Elongation at break (%)	Work of rupture (N.m)	Initial modulus (cN/Tex)	Tenacity (cN/Tex)
Untreated	6.3	6	550 27	37.620
CV	9.344	10.1250	6.69	5.215
Treated	6.103	6.1640	548 50	39.129
CV	9.238	9.265	3.17	2.283

Fig. 1 Scanning electron microscopy no treatment

Fig. 2 Scanning electron microscopy of Ammonyx with treatment

IV. CONCLUSIONS

Ammonyx 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 link a cationic dye and tend to take up and hold on the surface of natural substrate such as polyester/cotton blend. According to the results the presence of some pathogenic microbes on the cliothes confirmed including Escherichia coli and Staphylococcus which can be causing many infections. So it is worthy to enhance the antimicrobial activity of the clothest with a proper antimicrobial finishing. Although the kind of microbes on the clothes depends considerably to the environment of course, but it was shown that treating fabric with Ammonyx inhibits considerably the growth amount of studied bacteria and in some cases up to 90%.

The wash fastness or durability of the effect against washing of the treated clothes in the study was not under attention because the interval of washing periods for militarism textile clothes are not short and during these intervals usually the activity of the antbacterial agent vanishes, as it was seen in the case of Ammonyx Which its maximum inhibition time with a high concentration (1/100 v/v) was just

265 hrs. Fiber surfaces with Ammonyx treatments looked smooth and the antibacterial treatments were applied successfully to improving surface clothes.

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