THE KINETICS OF ANTIBACTERIAL EFFECT TESTED ON MODIFIED TEXTILE SUBSTRATES

I. Šlamborová[1], J. Wiener[2] and L. Martinková[3]

[1]*Department of Chemistry, Faculty of Science, Humanities and Education, Technical University of Liberec, Studentská 2, 461 17 Liberec, Czech Republic*
[2]*Faculty of Textile Engineering, Technical University of Liberec, Studentská 2, 461 17 Liberec, Czech Republic*
[3]*INOTEX spol. s.r.o., Dvůr Králové n. Labem*
irena.slamborova@tul.cz; jakub.wiener@tul.cz

Abstract: *The study of the kinetics of antibacterial effect was conducted on three antibacterial modified textile substrates. Two standard bacterial strains, Staphylococcus aureus (ATCC 1260) and Escherichia coli (ATCC 9637), were used for testing. The tests were performed according to internationally recognized norms AATCC Method 100 and AATCC Method 147. The results obtained are consistent with pilot experiments, which confirmed that the effects of antibacterial modifications of selected samples differ.*
Key words: *antibacterial, bacteriostatic, bactericidal, testing, Escherichia coli, Staphylococcus aureus, kinetics.*

1 INTRODUCTION

Compounds with antibiotic properties can be applied to modify the surface of textiles, which can be used as technical textiles in public health, clothing manufacturing (e. g. socks) and filtration systems.

When in contact with either pathogenic or non-pathogenic bacteria, antibacterial modified textiles may act bactericidally or bacteriostatically. *Bacteriostatic* finishes inhibit the growth of bacteria while *bactericidal* finishes kill them. The qualities of these finishes strongly depend on properties of antibacterial compounds used.

For bacteriostatic finishes, compounds gradually released into aqueous media are used. For example, cations of selected metals are able to penetrate into bacterial cells and bond to -SH groups of enzymes, which causes a decrease in activity and inhibition of growth of microorganisms. For these finishes, metals and metallic and phenolic compounds are commonly used [1].

For bactericidal finishes, compounds tightly fixed to fibres are used; for example, quaternary ammonium salts or chitosan [2]. When in contact with these compounds, bacterial metabolism is altered, which leads to cell wall damage and destruction of the cell [3]. At present, antibiotics immobilisation on textile substrates with either bactericidal or bacteriostatic effect has been taken advantage of [4]. Excellent results were achieved by immobilisation of antibiotics (tetracycline, penicillin) on silica nanofibres and chitosan nanofibres. The antibacterial effect was tested on a wide range of pathogenic bacteria that cause serious problems in wound healing in dermatology (decubitus and venous ulcers etc.) [5].

There are many various methods to detect bactericidal and bacteriostatic effects; AATCC Method 100 (a quantitative method) [6], AATCC Method 147 (a qualitative method – the size of the zone of inhibition is evaluated) [7] and ICS 59.080.01 norm [8] (analogous to the aforementioned methods) are commonly used. In addition to these international norms, it is possible to use a wide range of national norms (e. g. ČSN EN ISO 20645 Squared textiles – determination of antibacterial activity – Test of diffusion through the agar plate). For scientific studies that require testing of antibacterial effect on textile substrates, particularly the first two methods are used [9].

The main aim of this study was the assessment of the kinetics of three different antibacterial modified fabrics the antibacterial effect of which varied. The antibacterial effect was studied on two bacterial strains, Escherichia coli and Staphylococcus aureus. The key question was when the first signs of bacterial inhibition appear, or eventually, how many bacterial colonies survive.

2 EXPERIMENT

2.1 Materials

The aim of this study was to assess the kinetics of two selected bacterial strains on three antibacterial modified samples. The antibacterial modified textile substrates were provided by Inotex s.r.o. Dvůr Králové n. Labem.

The material composition of the samples consists of 50% cotton and 50% polyester of areic mass of 150 g/m^2 after a preliminary treatment. The products of Inotex spol. s.r.o. were used for antibacterial modifications. The first product, ULTRA FRESH NMV 2, is based on organochlorides. The second product, TENSILVERCAP Aloe Vera, is bifunctional microcapsules with nanoparticles of silver embedded on the outer side (antimicrobial effect) and encapsulated natural product of Aloe Vera (moisturising and well-being effect). Both products were applied by means of an impregnation process in a laboratory foulard and consequently utilized in drying and fixing apparatus Werner Mathis. To estimate the permanence of the modifications in repeated maintenance cycles, samples no. 1 underwent 75 washing cycles at 60°C and chemothermal disinfection.

The textile substrates tested were chosen on the basis of pilot experiments, which were conducted according to ČSN EN ISO 20645 [10] (the size of inhibition zones was evaluated). The samples varied in antibacterial activity: Sample No. 1 possessed low antibacterial activity, sample No. 2 high antibacterial activity and sample No.3 good antibacterial activity [Tables 1 and 2].

Sample No. 1 - a modified fabric after 75 washing cycles and chemothermal disinfection

Sample No. 2 - a fabric modified with TENSILVERCAP, not washed

Sample no. 3 - a fabric modified with Ultra Fresh NMV 2, not washed

Two bacterial strains were used for testing – Gram-negative, rod-shaped bacterium Escherichia coli – strain **CCM 2024 (ATCC 9637)** and Gram-positive, coccal bacterium Staphylococcus aureus – strain **CCM 299 (ATCC 1260)**. Both bacterial strains are reference cultures (according to ALE-G18, ČSNI) bought from the Czech Collection of Microorganisms of Masaryk University.in Brno.

For the cultivation of bacteria, blood agar (Columbia agar) obtained from Bio-Rad spol. s.r.o. Praha was used.

2.2 Experimental methods

AATCC Method 100 was used for testing.

Textile substrates (three samples of fabrics with different antibacterial finishes) of the size of 18 x 18 mm were placed into incubation containers. 0.1 ml of bacterial suspension (Escherichia coli, Staphylococcus aureus) at a concentration of 105 CFU/ml was added to each sample. The containers were placed in the thermostat set to 37°C. They were withdrawn after set time intervals and 10 ml of physiological solution was added to each sample (i. e. the suspension was diluted to a concentration of 103 CFU/ml). The samples were vortexed for 10 minutes. After that, 1 ml of each sample was taken and inoculated on blood agar in a Petri dish. The results were assessed after twenty-four hours' incubation in the thermostat at 37°C.

Table 1 Tests on Staphylococcus aureus (ČSN EN ISO 20645)

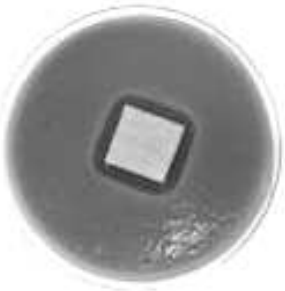
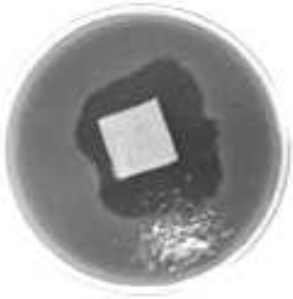
		
Sample No. 1 Inhibition zone 0 mm Insufficient antibacterial effect	Sample No. 2 Inhibition zone 8 mm High antibacterial effect	Sample No. 3 Inhibition zone 2 mm Good antibacterial effect

Table 2 Tests on Escherichia coli (ČSN EN ISO 20645)

		
Sample No. 1 Inhibition zone <1 mm Efficiency limit of the antibacterial effect	Sample No. 2 Inhibition zone 19 mm High antibacterial effect	Sample No. 3 Inhibition zone 3 mm Good antibacterial effect

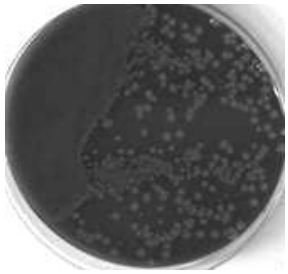
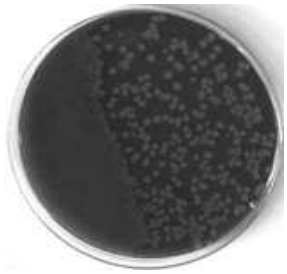
Samples were withdrawn after the following time intervals:
1. Sample – withdrawn after 1 hour
2. Sample – withdrawn after 2 hours
3. Sample – withdrawn after 3 hours
4. Sample – withdrawn after 4 hours
5. Sample – withdrawn after 5 hours
6. Sample – withdrawn after 5,5 hours
7. Sample – withdrawn after 6 hours

Based on these intervals, the kinetics of both bacterial strains tested was estimated.

3 RESULTS AND DISCUSSION

The primary aim of this study was to estimate the decrease in bacterial colonies related to time. Three textile samples with different antibacterial finishes were used for testing. Microbiological tests were performed on blood agar using two bacterial strains, Escherichia coli and Staphylococcus aureus. These bacterial strains are common microflora present in our surroundings. In case of skin damage, these pathogens are able to enter an organism through such a gate. If textiles (underwear, T-shirts, etc.) are finished with appropriate antibacterial agents, the finishes can vastly influence the number of bacteria, which are either inhibited (bacteriostatic effect) or killed (bactericidal effect) by the compounds immobilized on the surface of the textiles.

Table 3 Kinetic effect of E. coli (AATCC Method 100)

Sample	Inhibition effect of E. coli after 1 hour	Inhibition effect of E. coli after 3 hours	Inhibition effect of E. coli after 6 hours
1			
2			
3			

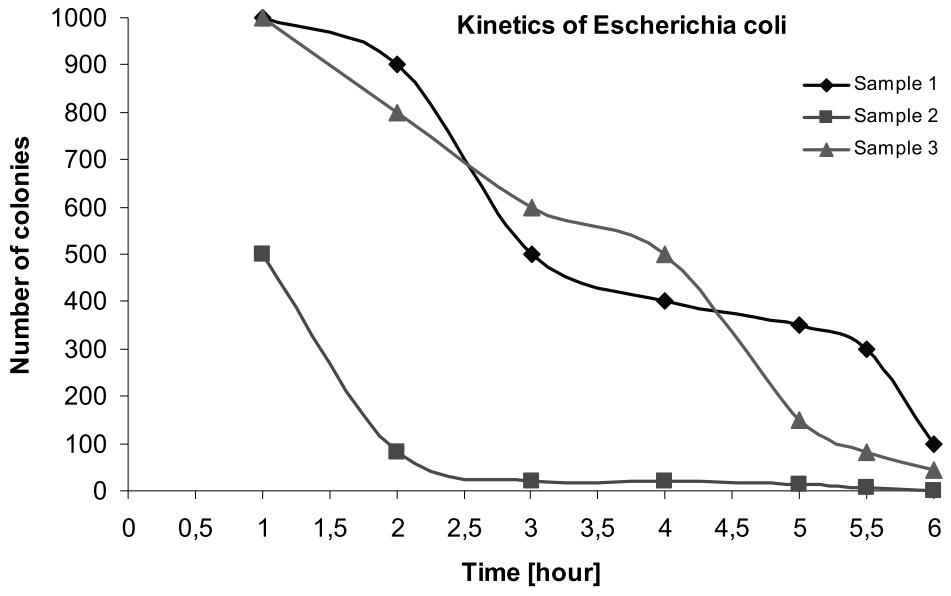


Figure 1 Decrease in colonies of Escherichia coli related to time when in contact with antibacterial modified textiles

Table 4 Numbers of recovered bacterial colonies (selected time intervals) - Escherichia coli

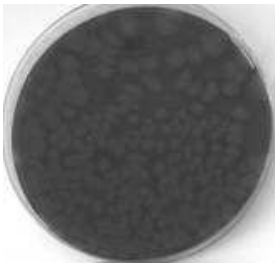
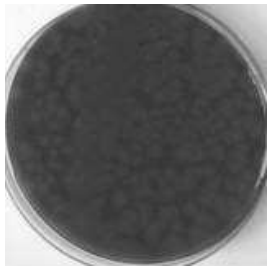
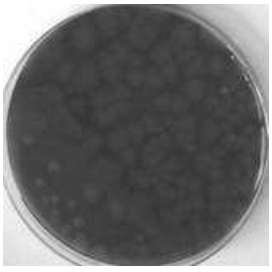
Sample	1 hour	3 hours	6 hours
1	1000	500	100
2	500	20	1
3	1000	600	45

The best results were obtained for sample no. 2. A strong inhibition was achieved between the 1st and the 2nd hour of incubation. The number of recovered colonies after six hours' incubation equalled 1.

For sample No. 3, a significant inhibition was achieved later, i. e. between the 4th and the 5th hour of incubation. After the last withdrawal (after six hours' incubation), the number of colonies equalled 45.

For sample No. 1, the inhibition appeared later in comparison to sample No. 3.

Table 5 Kinetic effect of St. aureus (AATCC Method 100)

Sample	Inhibition effect of St. aureus after 1 hour	Inhibition effect of St. aureus after 3 hours	Inhibition effect of St. aureus after 6 hours
1			
2			
3			

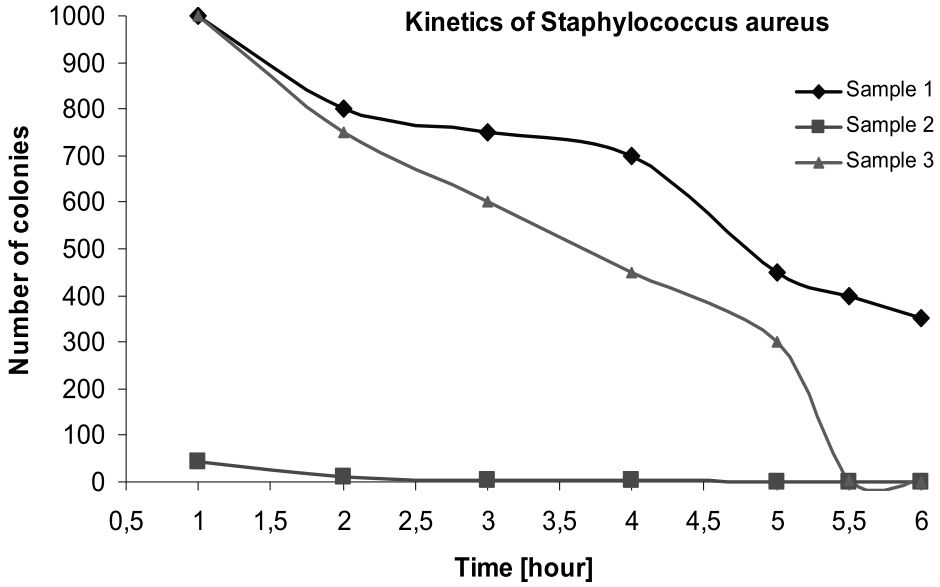


Figure 2 Decrease in colonies of Staphylococcus aureus related to time when in contact with antibacterial modified textiles

Table 6 Numbers of recovered bacterial colonies (selected time intervals) - Staphylococcus aureus

Sample	1 hour	3 hours	6 hours
1	1000	750	350
2	42	3	0
3	1000	600	0

The results of the kinetics of Staphylococcus aureus clearly showed that the highest inhibitory effect was achieved by sample No. 2. The inhibition started during the 1st hour of contact with the modified textile, the complete inhibition of bacterial growth being achieved after 5 hours.

For sample No. 3, the number of colonies plummeted between the 300th and the 330th minute. After the last withdrawal (after 360 minutes' incubation), the number of colonies equaled 0.

Although it is possible to detect some antibacterial effect for sample No. 1, it is insignificant in comparison to sample No. 2.

4 CONCLUSION

The results of the study unambiguously confirmed the differences among the antibacterial effect of the textiles tested (related to time the inoculum was in contact with the textiles). The inhibition of Gram-positive bacterium Staphylococcus aureus started much sooner (during the first hour of incubation) than the inhibition of Gram-negative bacterium Escherichia coli (between the 1st and the 2nd hour of incubation).

The other two samples tested (i. e. sample No. 1 and sample No. 3) showed similar antibacterial effects. The Gram-positive, coccal bacterium Staphylococcus aureus was proved to be more susceptible to the antibacterial compound used for the surface modification.

Appropriate antibacterial modifications of textiles result in inhibition of bacterial multiplication (bacteria can be a source of an unpleasant odour, for example when decomposing sweat). According to various types of surface finishes, textiles may be resistant to yeasts or fungi, and longer durability, colour stability and higher quality of such materials can therefore be guaranteed.

For surface finishes, it is desirable to be abrasion resistant and permanent after repeated washing.

Acknowledgements: *1M0553 – Research centre TEXTIL II.*

5 REFERENCES

1. Textilie v novém tisíciletí II; obrazová analýza v textile, sborník příspěvků – Vyd. Liberec : Technická univerzita v Liberci, 2004. Vysoce funkční vlákna, s. 8
2. Jayakumar J., Prabaharan M., Nair S.V., Tamura H.: Novel chitin and chitosan nanofibers in biomedical applications, Biotechnology Advances 28, 2010, 142-150
3. Alberts B., Bray D. et al.: Základy buněčné biologie – úvod do molekulární biologie buňky, Ústí n. Labem, Espero Publisching, 1998
4. Rong-Hua Yang, Ke-Min Wang, Dan Xiao, Xiao-Hai Yang, Li-De Zhang: A selective sensing membrane for the determination of tetracycline with heptakis (2,6-di-O-isobutyl) cyclodextrin as the substrate, Microchemical Journal 64, 2000, 213-220
5. Šlamborová I., Exnar P., Zajícová V.: Possibilities of immobilisation of selected bioactive compounds on nanofibres, Conference of Potential and Application of Nanotreatment of Medical Surfaces (PANMS), Hejnice - Liberec, Czech Republic, September 5-7, 2001, 42-44
6. AATCC Test method 100-1993 (2004), Antibacterial Finisches on Textile Materials, An American National Standars, AATCC Technical Manual/1995
7. AATCC Test Method 147-1993, Antibacterial Activity Assessment of textile Materials: Parallel Streak Method, An American National Standards, AATCC Technical Manual/1995
8. JISL 1902:1998 [E], Testing method for antibacterial of textiles, Japanese industrial standard, With ISO/ TC 38N182 (ICS 59.080.01)
9. Thuy Thi Thu Nguyen, Ok Hee Chung, Jun Seo Park: Carbohydrate Polymers 86, 2011, 1799-1806
10. ČSN EN ISO 20645 – (Plošné textilie – zjišťování antibakteriální aktivity – zkouška šíření agarovou destičkou)

KINETIKA ANTIBAKTERIÁLNÍHO ÚČINKU TESTOVANÁ NA UPRAVENÝCH TEXTILNÍCH SUBSTRÁTECH

Translation of the article

The kinetics of antibacterial effect tested on modified textile substrates

Studium kinetiky antibakteriálního účinku bylo uskutečněno na třech antibakteriálně upravených textilních substrátech. K pokusům byly použity dva standardní bakteriální kmeny Staphylococcus aureus (ATCC 1260) a Escherichia coli (ATCC 9637). Testování probíhalo podle mezinárodních testovacích norem AATCC Method 100 a AATCC Method 147. Výsledky studované kinetiky jsou v souladu s pilotními pokusy, které potvrdily různou účinnost antibakteriálních úprav vybraných vzorků.