(REFEREED RESEARCH)

EVALUATION OF SILVER CONTENT AND ANTIBACTERIAL ACTIVITIES OF SILVER LOADED FIBER/COTTON BLENDED TEXTILE FABRICS

GÜMÜŞ KATKILI LİF/PAMUK KARIŞIMINDAN ÜRETİLEN KUMAŞLARIN GÜMÜŞ İÇERİKLERİNİN VE ANTİBAKTERİYEL AKTİVİTELERİNİN BELİRLENMESİ

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ABSTRACT

The aims of this work were to analyze the antibacterial activity and laundering durability of the silver loaded cellulosic fiber/cotton blended antibacterial textile fabrics, to quantify the silver content present in fabrics, and to examine the relationship between the antibacterial activity and silver content. For this aims knitting fabrics consisting of SeaCell® Active, which is the cellulosic fiber incorporated with seaweed and silver ions, and cotton fibers blended with five different ratios were produced. All fabric samples bleached and washed 60 times. The antibacterial efficiency was evaluated according to AATCC 100-1999 method after each ten laundry cycles. Silver content of the fabrics were determined by atomic absorption spectroscopy (AAS). Fiber and fabric surfaces were investigated using Scanning Electron Microscopy (SEM) and by X-Ray Photoelectron Spectroscopy (XPS) views. Antibacterial tests showed that good antibacterial activity can be achieved after several washings even with 3% of SeaCell ® Active fibers in blended fabrics. Significant correlation was found between silver content and bacterial reduction.

Key Words: Antibacterial fibers, Antibacterial activity, Cotton, Silver, AAS, XPS.

ÖZET

Bu çalışmanın amacı gümüş katkılı selülozik lif/pamuk karışımından üretilen kumaşların antibakteriyel aktivitelerini ve yıkama dayanımlarını analiz etmek, kumaşlarda bulunan gümüş miktarını belirlemek ve antibakteriyel aktivite ile gümüş içeriği arasındaki ilişkiyi araştırmaktır. Bu amaçlar doğrultusunda deniz yosunu ve gümüş iyonu içeren SeaCell® Active lifleri ile pamuk lifleri, beş farklı oranda karıştırılarak örme kumaşlar üretilmiştir. Tüm kumaş numuneleri ağartılmış ve 60 defa yıkanmıştır. Antibakteriyel aktivite AATCC 100-1999 test metoduna göre her on yıkama sonrasında test edilmiştir. Kumaşların gümüş içeriği Atomik absorpsiyon spektrofotometresi (AAS) ile belirlenmiştir. Lif ve kumaş yüzeyleri Taramalı Elektron Mikroskobu (SEM) ve X Işını Fotoelektron Spektroskopisi (XPS) ile incelenmiştir. Antibakteriyel testler %3 SeaCell ® Active lifi içeren kumaşlarda dahi çok sayıda yıkama sonrasında antibakteriyel etkinin elde edilebildiğini göstermiştir. Gümüş içeriği ile bakteri sayısındaki azalma arasında önemli korelasyon bulunmuştur. ster kumaşların aksine, selülozik/poliester karışımı kumaşların etkili bir şekilde sıvı absorbsiyonu ve iletimini sağladığı bulunmuştur.

Anahtar Kelimeler: Antibakteriyel lif, Antibakteriyel aktivite, Pamuk, Gümüş, AAS, XPS.

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Introduction

Microorganisms can cause functional, hygienic and aesthetic problems on textile products. Therefore, antibacterial materials have been developed to prevent the textiles from the harmful effects of microorganisms. Desirable features for an antimicrobial textile include durability of activity after laundering, selective activity against undesirable microorganisms, acceptable moisture transport properties, especially for the controlled releasing agents, compatibility with other finishing agents, absence of any toxic effects to the user, and commercial availability (1). There are many antimicrobial agents used in textile applications

such as aromatic halogen compounds, benzoic esters, organometalics and metal salts, quaternary ammonium salts, and chitosan. Among these, silver is widely preferred as an agent for antibacterial fibers and finishing chemicals. The first reason of this popularity is that it has long been known to be a potent antibacterial agent with a very broad spectrum of activity and has been used safely in medicine for many decades (2). In addition to its antibacterial effect, silver does not have any negative side effects like skin irritation (3).

Antibacterial and antifungal activities of silver based antimicrobial fibers and finishing agents have been reported in various papers (4-16). In addition to the microbiological analysis, determination of the silver content in the antimicrobial textiles by the instrumental analyses can provide supplemental information about the antimicrobial activity of the products. Released silver to the laundry water can also be estimated by this way. Gorensec and Recelj (17) applied silver nanoparticles on polyamide knitted fabrics and detected 48 mg silver per kg of fabric by inductively coupled plasma atomic emission spectroscopy (ICP AES). Perkas et al. (18) after deposition of silver nanoparticles on porous polypropylene beads, analyzed and characterized silver particles using TEM, SEM, EDX, XPS, and Raman spectroscopy. Threshold of the biocidal activity was achieved with as low as 0.5 wt % silver. Benn and Westerhoff (19) investigated silver released from commercial socks into water, and its fate in wastewater treatment plants. SEM and TEM were used to confirm the presence of silver nanoparticles in the sock and in the wash water samples. Silver was quantified by ICP-OES. Xu *et al.* (20) prepared silver loaded SiO_2 nano antibacterial agent and applied to the wool fibers. Fibers were characterized by SEM, TEM and FTIR and the silver content was measured by ICPS. Silver content of Ag-loading SiO_2 nano-antibacterial agent was found as 0.69 wt% from atomic absorption spectrum. Although the loss of silver was detected to be about 33 wt%, good antibacterial activity was recorded even after 30 times of washing. Yu *et al.* (21) produced 0.5 wt% silver nitrate doped polyacrylonitrile hollow fibers and examined the morphology of the resulting hollow fibers by SEM and the Ag content of the fiber was measured using an ICP-AES (bulk) and XPS (surface). It was found that after flushing with water for 60 days, the silver content in the hollow fibers decreased to 0.1 wt%, and still showed antibacterial activity against *E. coli* and *S. aureus*. Chou *et al.* (22) produced silver loaded asymmetric cellulose acetate (CA) hollow fiber. The morphology of the hollow fibers was examined using SEM and the silver content in the fiber was measured using ICP-AES. After immersing in water bath for 180 days, the silver content in the bulk of the hollow fibers decreased to 60% and the silver content on the surface reduced to 10%, yet still showed antibacterial activity against *E. coli* and *S. aureus*. It was also pointed out that the proper range of $AgNO_3$ in the spinning solution for CA hollow fiber should be about 100– 1000 ppm. Üreyen *et al* (23) reported silver content of SeaCell ®Active fiber cotton blended fabrics by ICP-OES. Rezic and Steffan (24), Pranaitytė *et al.* (25) tested analytical methods for determination of heavy metals for their application on textiles.

The main aim of this work is to examine the antibacterial performance and laundering durability of the silver loaded antibacterial fiber (SeaCell ® Active) and cotton blended fabrics. Silver content and the released silver from the fabrics after laundry were tried to determine by instrumental analysis to understand the relationship between the antibacterial activity and silver content. For this aim three different analytical methods (atomic absorption spectroscopy (AAS), X-ray Photoelectron Spectroscopy (XPS) and Scanning Electron Microscopy (SEM) were used.

MATERIALS AND METHODS

2.1. Fibers and Fabric Preparation

Within the scope of this work SeaCell® Active (SCA) fiber which is produced by Lyocell processes was selected as an antibacterial fiber. The SeaCell® fibers are manufactured by adding finely ground seaweed, mainly from the family of brown, red, green and blue algae to the spinning solution (26). The algae are added either as a powder or as a suspension in one of the process steps preceding the spinning of the cellulose solution. The SCA fibers are produced by loading the SeaCell® fiber with a diluted silver solution within an intermediate step known as activation. Chemical composition and antimicrobial properties of SCA fibers are extensively analyzed and reported by Zikeli (27). Fluhr *et al.* (4) and Hipler *et al.* (5) also examined antifungal and antibacterial properties of SCA fibers. Üreyen (6) has recently investigated the spinning performance of SCA/cotton blended open end rotor yarns and antibacterial activities of fabrics produced by these yarns.

SCA fibers purchased from Topkapi İplik Sanayi ve Ticaret A.Ş./Türkiye. SCA/cotton blended slivers were prepared on drawframe stages with five different blending ratios (3/97% SCA/cotton, 5/95% SCA/cotton, 14/86% SCA/cotton, 27/73%SCA/cotton, 53/47%SCA/cotton). All slivers were spun into yarns on open end rotor spinning machine (Rieter R 40) at a yarn count of Ne 20 (30 tex). Twist multiplier was selected as α_e3.7.

All cotton/SCA blended yarns were knitted on Fouquet circular knitting machine having 36 knitting systems and 30 needles per inch. The pattern of the fabrics produced were interlock having the average fabric weight of 250 g m^{-2}. All the fabrics were bleached by hydrogen peroxide in the

same bath. Similarly, all fabric samples were washed at the wascator machine 60 times according to BS EN ISO 26330 standard (5a program at 40 °C) and after each ten washing cycles antibacterial properties of the fabrics were tested according to ATCC 100-1999 method. Because of the fact that some detergents themselves have antibacterial effects to some extent, the fabric samples had been washed by soap.

2.2. Atomic Absorption Spectroscopy (AAS) Analyses

Silver content of the SCA/cotton blended fabrics were examined by means of atomic absorption spectroscopy (Perkin Elmer Analyst 800). All materials were dried in the oven at 105 ^{o}C, 2 hours in order to remove moisture from the samples. The percentage of humidity each sample contains was determined by weighing before and after it was dried. Accurately, 1.00 g of dried sample was weighed into pre-weighed clean dish. The sample was placed in the electric furnace at 600 ±5 ^{o}C until the carbon has been totally burned away. The sample, then, was removed from the furnace and cooled in a desiccator. 15 ml of 6M HNO_3 was added slowly and mixed thoroughly. Finally, the solution was filtered for removal of undissolved particles. The filtered solution was diluted to 100 ml in a volumetric flask with the deionized water. This procedure was applied to all samples.

The solutions were analyzed by using atomic absorption spectrophotometer with air-acetylene flame. Deuterium background correction was used and spectral slid width used was 0.7 nm. The working current and wavelength were 15 mA and 328.1 nm, respectively. The calibration curve was acquired by using the standard solutions which contain known concentration of Ag^+ ions. The instrument calibration was periodically checked using standard Ag^+ solutions after every 10 measurements.

2.3. X-Ray photoelectron spectroscopy (XPS) Analysis

X-Ray photoelectron spectroscopy (XPS) is the analytical technique for characterizing various chemical/ physical forms of elements in surface structures. It is a powerful tool for extracting chemical and structural information from surface structures in the nanometer scale. The characterization is solely based on associating the changes in measured binding energies of certain atomic levels with chemical or physical forms of the corresponding elements (27).

In this work 14% SCA and 86% cotton blended unbleached fabric sample was analyzed by XPS. KRATOS ES300 spectrometer with non-monochromatic Mg Kα X-ray source is used to record the XPS data.

2.4. Scanning Electron Microscope (SEM) Analyses

The scanning electron microscope (SEM) is a versatile instrument for examination and analysis of micro-structure morphology and characterization of textile products. Therefore, size and distribution of the silver particles and fibers were examined by SEM.

Sample preparation for SEM analysis is quite simple but the conductivity of the sample is very important for successful analysis. Poor conductivity causes variations in surface potential or charging. Positive charges cause brightness and negative charges create the darker areas on the image (28). Poor conductivity also causes heat damages on the sample. Charging and heat damage of sample can be prevented by metal coating of test samples such as gold (29). Because of the low density of textile polymers, penetration of electron beam is higher. If a high beam voltage is used, penetration of beam produces signals from the below the surface of sample. It usually restricts the examination of the textile materials.

A Zeiss Supra 50 VP with Oxford Instruments Inca Energy EDX module was used for SEM analyses. Firstly fiber and fabric samples were dried and mounted on the stub by double coated adhesive tape. Then, samples were placed in the vacuum system for the preparation and analysis. Some fiber and fabric samples were coated by gold. Both coated and uncoated fabric samples were analyzed. Initial tests by uncoated samples showed that 5 kV gives the best results for the SCA fibers and SCA/cotton blended fabrics. Working distance was shortened to 12 mm for increasing the resolution. Analyses could be done by coated samples up to 20 kV. Energy dispersive X-Ray analysis (EDX) was used to confirm the elemental presence of silver on the fiber and fabric surfaces.

2.5. Antibacterial Analyses

The antibacterial activities of the fabric samples were quantitatively evaluated against *Staphylococcus aureus,* (ATCC 6538) a Gram positive bacterium and *Klebsiella pneumoniae*, (ATCC 4352) a Gram negative bacterium by the AATCC 100-1999 test method. Anti-bacterial efficiencies of textile products expressed as general and specific antibacterial activity. In this work general and specific activities of the fabrics were calculated and compared.

General activity was calculated by the following formula:

$$R_G = 100\ (B-A)/B \qquad (1)$$

where:

R_G = % reduction (general activity)

A = the number of bacteria recovered from the inoculated antibacterial test specimen swatches in the jar incubated over the “24 h” contact period.

B = the number of bacteria recovered from the inoculated antibacterial test specimen swatches in the jar immediately after inoculation (at “0 h” contact time).

Table 1. Determined silver content of fabric samples by AAS

Laundry cycles	Silver (mg/kg)				
	Blending ratio of SeaCell Active fiber (%)				
	3% SCA*	5% SCA	14% SCA	27% SCA	53% SCA
0**	78.286	118.441	231.500	288.254	960.643
0	59.716	89.948	152.804	253.749	855.803
20	45.473	66.100	148.551	200.073	380.952
40	32.834	62.800	122.453	133.022	201.800
60	22.327	29.785	64.166	104.879	122.239

* SeaCell Active ** Unbleached

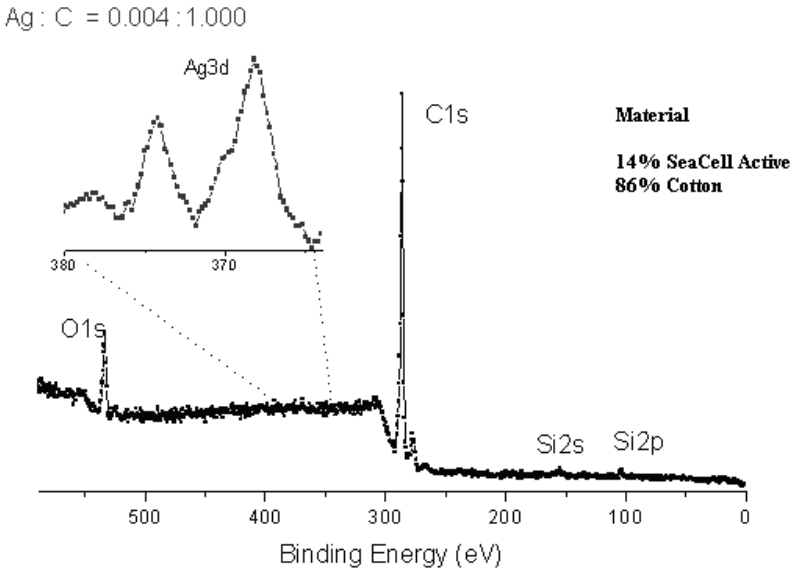


Figure 1. XPS result of 14% SeaCell Active/86% cotton blended fabric

And specific activity was calculated by the following formula:

$R_S = 100((D-C)/D-(B-A)/B)/(D-C)/D$ (2)

where:

R_S = % reduction (specific activity)

A, B = see Eq. (1)

C = the number of bacteria recovered from the inoculated control test specimen (100% cotton) swatches in the jar incubated over the "24 h" contact period.

D = the number of bacteria recovered from the inoculated control test specimen (100% cotton) swatches in the jar immediately after inoculation (at "0 h" contact time).

RESULTS AND DISCUSSION

AAS Results

SCA fibers and SCA/cotton blended fabrics were analyzed by AAS separately. Silver content of SCA fiber was determined as 3944.71 mg kg^{-1}. Test results of the each type of blended fabric samples are shown in Table 1. It can be seen that bleaching by hydrogen peroxide decreases the silver content of the fabrics significantly. The amount of silver lost after bleaching was higher than the released silver after 20 washing cycles for almost all samples. Moreover silver amount of the 53/47 % SCA/cotton blended fabrics decreases dramatically depending on number of washing cycles. Mechanical forces generated during washing damage the surface of SCA fibers and cause the loss of silver particles.

XPS result

Figure 1 shows the XPS spectra of C1s, O1s, Si and Ag regions. Silver content was found as 0.04 wt%. This result indicated that SCA fibers include approximately 2,860 mg/kg silver particle.

SEM results

SEM images of SCA fibers are shown in Figure 2. Silver particles could be easily observed on the fiber surfaces. The mean diameter of silver particles was measured as approximately 200 nm.

Figure 3 shows the SEM image of SCA fiber and EDX mapping result of silver particles on the surface of the fiber. It can be ascertained that the small particles on the surface are Ag. Mapping result in Figure 3 (b) show the distribution of Ag on fiber is good. Table 2 shows the EDX results of the SCA fiber. Ca is also detected on the surface.

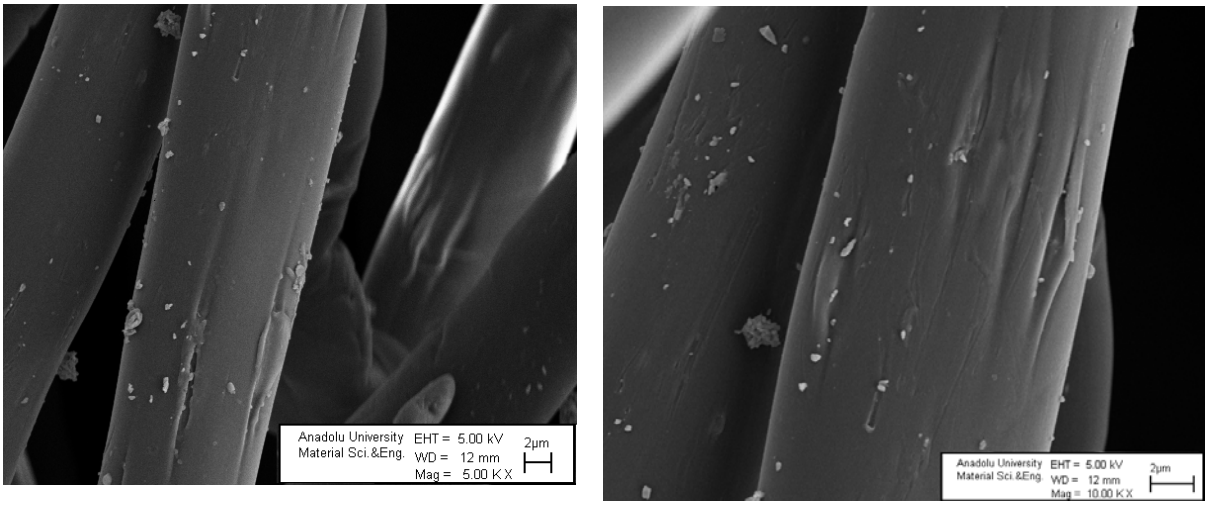
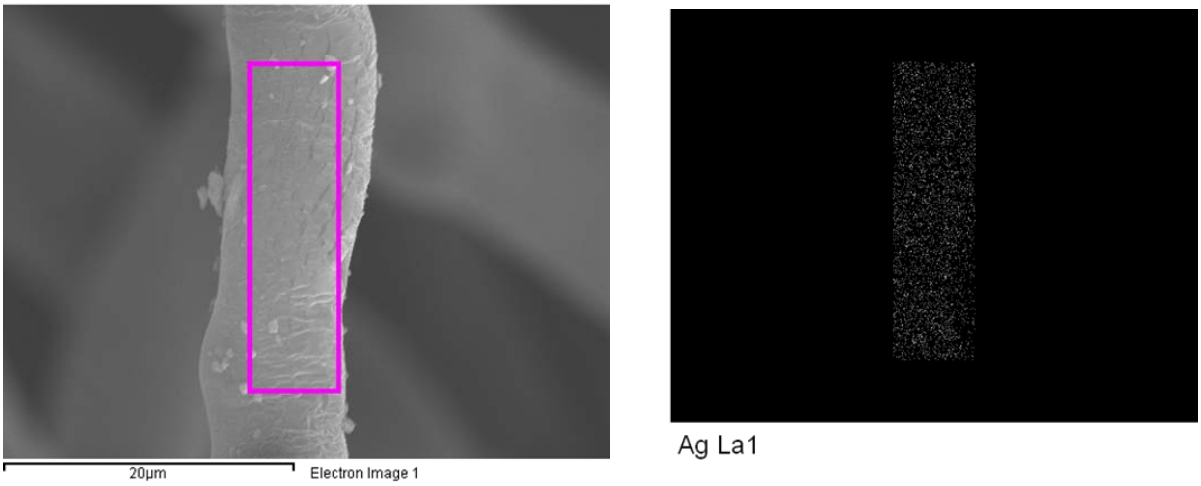


Figure 2. SEM images of SeaCell Active fibers (magnified 5,000 and 10,000 times)



(a) **(b)**

Figure 3. a) SEM image of the SCA fiber; b) EDX mapping result of Ag La1 corresponding to selected area of (a) (SEM accelerating voltage = 20 kV)

Table 2. EDX results of the SeaCell Active fiber

Element	Weight%	Atomic%	Compd%	Formula
C K	27.23	33.30	99.76	CO2
Ca K	0.11	0.04	0.16	CaO
Ag L	0.08	0.01	0.08	Ag2O
O	72.58	66.65		
Totals	100.00			

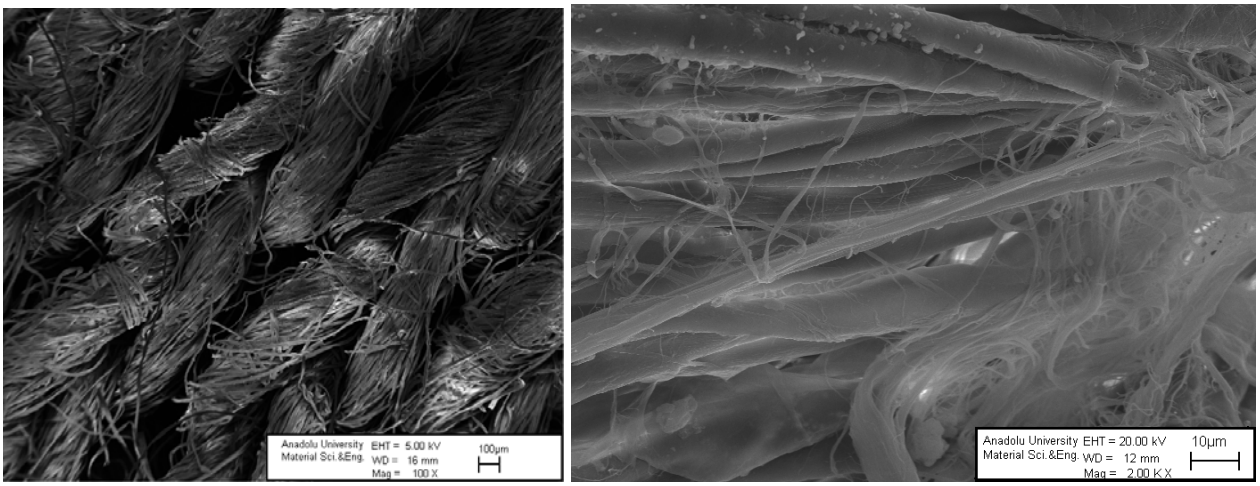
SEM images of 14% SeaCell Active and 86% cotton blended fabric are shown in Figure 4. SCA fibers were fibrillated after laundering as shown in Figure 4b. Silver couldn't be detected on the blended fabric samples by EDX.

Antibacterial test results

Table 3 and Table 4 show the antibacterial activities i.e., R_S, R_G against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

The lowest antibacterial activity was observed by the fabrics containing 3% SCA. As expected the highest antibacterial activity was observed by the fabrics containing 53% SCA.

General antibacterial activity was found even after 60 washing cycles by the fabrics containing 27% and 53% SCA.



(a) **(b)**

Figure 4. SEM images of 14% SeaCell Active/86% cotton blended fabric. a) unwashed; b) 20 times washed

Table 3. Antibacterial activities of the fabrics against *Staphylococcus aureus*

Laundry cycles	Control Fabric (100% Cotton)	3% SCA		5% SCA		14% SCA		27% SCA		53% SCA	
	R (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)
0	-	99.99	65.79	99.99	77.69	99.99	89.34	99.99	98.78	99.99	98.83
10	-	99.99	63.06	99.99	54.82	99.99	62.37	99.99	92.78	99.99	93.75
20	-	99.99	59.09	99.99	47.62	99.99	53.12	99.99	88.31	99.99	87.80
30	-	99.99	31.45	99.99	24.31	99.99	34.19	99.99	68.02	99.99	68.81
40	-	50.00	-	99.99	13.95	99.99	24.80	99.99	64.51	99.99	67.03
50	-	33.33	-	99.99	3.53	78.25	-	99.99	37.72	99.99	39.72
60	-	10.52	-	36.36	-	90.87	-	99.99	30.81	99.99	24.57

Table 4. Antibacterial activities of the fabrics against *Klebsiella pneumoniae*

Laundry cycles	ControlFabric (100% Cotton)	3% SCA		5% SCA		14% SCA		27% SCA		53% SCA	
	R (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)	R_S (%)	R_G (%)
0	-	99.99	62.57	99.99	66.23	99.99	79.93	99.99	98.52	99.99	90.04
10	-	99.99	59.14	99.99	49.72	99.99	59.88	99.99	80.25	99.99	85.00
20	-	99.99	52.33	99.99	42.71	99.99	49.81	99.99	74.70	99.99	79.75
30	-	99.99	27.50	99.99	20.12	99.99	30.11	99.99	67.76	99.99	69.88
40	-	33.33	-	99.99	10.23	99.99	20.11	99.99	60.17	99.99	66.38
50	-	16.32	-	99.99	2.70	71.03	-	99.99	34.78	99.99	38.50
60	-	-	-	39.49	-	85.10	-	99.99	20.21	99.99	23.68

Table 5. Results of correlation analyses

		Staphylococcus aureus			*Klebsiella pneumoniae*		
	silver content	R_G	logR_G	logR_S	R_G	logR_G	logR_S
Pearson correlation	1	0,600	0,782	0,781	0,600	0,753	0,748
Sig. (1-tailed)		0,005	0,000	0,000	0,003	0,000	0,000

Antibacterial activity can also be calculated by common logarithm. Both the general and specific antibacterial activity of test samples was also calculated by the following formulas to compare the silver content and antibacterial efficiency:

$LogR_G = logB-logA$ (3)

$logR_S = (logA-logB)-(logD-logC)$ (4)

Finally Pearson correlation coefficients were calculated between silver content measured by AAS and R_G, $logR_G$ and $logR_S$ respectively. Table 5 shows the correlation coefficients. Results showed that there is a significant relationship between silver content and bacterial reduction.

CONCLUSION

The results of the research work can be summarized as follows:

- In this work silver content of the antibacterial fiber/cotton blended fabric samples were measured by Atomic Absorption Spectroscopy. The relationship between determined silver content and blending ratio of SCA fibers was not linear. It means that blending of SCA and cotton on drawframe machine was not uniform.
- AAS test results also showed that bleaching by hydrogen peroxide decreases the silver content of the fabrics significantly. Washing process also decreased the silver content of fabrics.
- Silver particles on SCA fiber surface could also be detected by SEM. The mean diameter of silver particles was measured as approximately 200 nm. Presence and distribution of silver particles on fiber and fabric surface were confirmed by EDX analysis. But the quantity of the silver on the fabric surface couldn't be determined by EDX module because of the low amount of silver particles.
- Silver content on the surface of the antibacterial fabric could be determined by XPS successfully. But the reported silver content of SCA fiber by the Zikeli (26) was higher than the determined silver content in this work by XPS. This result is logical, because XPS device can analyze only the surface of the sample. On the other hand analyzed surface area was too small. Highly uniform blending of different kinds of fibers can not be obtained by drawframe blending. Therefore the reliable analysis of SCA/cotton blended samples by XPS is not possible. It is believed that XPS device can be used for the analysis of fabrics treated by finishing antibacterial agents or coated fabrics more successfully than fabrics produced by antibacterial fibers.
- Antibacterial tests showed that good antibacterial activity can be achieved after several washings even with 3% of SeaCell ® Active fibers in blended fabrics. Significant correlation was found between silver content and bacterial reduction. Higher linear relationship was found between logarithmic values and silver content. It means that the calculation of bacterial reduction in logarithm gives more reliable results related to antibacterial efficiency of textile materials.
- It should be noted that Antibacterial activity of the agent depends on the minimum inhibitory concentration (MIC) which is the lowest concentration of an antibacterial agent that will inhibit the visible growth of the bacteria (30). Significant relationship between antibacterial activity and the silver content can be found over MIC value.
- Antibacterial test results and AAS test results showed that more than 50 mg/kg silver is needed for strong antibacterial activity.

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ABBREVIATIONS

Table 6 lists the abbreviations in the paper.

Table 6: List of abbreviations.

SCA	SeaCell® Active fibre
AAS	Atomic Absorption Spectroscopy
SEM	Scanning Electron Microscopy
EDX	Energy Dispersive X-Ray Analysis
XPS	X-Ray Photoelectron Spectroscopy
TEM	Transmission Electron Microscopy
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICP-OES	Inductively Coupled Plasma -Optical Emission Spectroscopy
ICPS	Inductively Coupled Plasma Spectroscopy
FTIR	Fourier Transformation Infrared Spectra
wt %	Weight (%)
CA	Cellulose Acetate
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus,* (ATCC 6538)
K.pneumoniae	*Klebsiella pneumoniae*, (ATCC 4352)
Ag	Silver
Ca	Calcium
C1s	***Carbon 1s*** photoelectron peak
O1s	***Oxygen 1s*** photoelectron peak
Si 2s	Silisium 2s photoelectron peak
Si 2p	Silisium 2p photoelectron peak

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