(REFEREED RESEARCH)

# EFFECTS OF NOVEL ANTIBACTERIAL CHEMICALS ON LOW TEMPERATURE PLASMA FUNCTIONALIZED COTTON SURFACE

# DÜŞÜK SICAKLIKTA PLASMA İLE İŞLEM GÖRMÜŞ PAMUK YÜZEYİNDE YENİ ANTİBAKTERİYAL KİMYASALLARIN ETKİLERİ

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## ABSTRACT

Since natural fibers are very susceptible to bacterial attacks, various antibacterial finishes have been developed to control the bacterial growth. Metal and metal salts, quaternary ammonium compounds, N-halamines, chitosan, polybiguanids and halogenated phenols can be used for antibacterial finishes. On the other hand, plasma treatment has become very popular for improved antibacterial activity in recent times. In this study, the effect of argon plasma treatment on cotton fabrics before antibacterial finishing process was investigated. After antibacterial finish process, they were washed up to ten cycles. The antibacterial activities of the washed and unwashed samples were evaluated according to the AATCC Test Method 147-1998. The surface morphology was characterized by SEM and FTIR analysis. From the results, it's observed that plasma application clearly increased the antibacterial activity of the samples and the most efficient and washing durable antibacterial agent was non-ionic diphenylalkane based chemical agent. From the SEM images, it's seen that after argon plasma treatment, the cotton surface has become rougher and more silver particles have been attached to the surface. This fact was also confirmed by EDX analysis.

**Key Words:** Argon plasma, FT-IR, Surface modification, Antibacterial, Cotton.

## ÖZET

Doğal lifler, bakteri etkilerine karşı oldukça duyarlı olduklarından, bu liflerden elde edilen kumaşlarda bakteriyal gelişimi kontrol etmek için çeşitli antibakteriyal bitim işlemleri uygulanmaktadır. Metaller ve metal tuzları, kuarterner amonyum bileşikleri, N-halaminler, kitosan, polibiguanidler ve halojenlenmiş fenoller bu amaçla kullanılabilen maddelerdir. Diğer taraftan, son zamanlarda plasma işlemi de gelişmiş antibakteriyal aktivite sağlamak için oldukça popüler hale gelmiştir. Bu çalışmada, antibakteriyal bitim işlemi öncesi pamuklu kumaşlar üzerinde argon plasma işleminin etkileri incelenmiştir. Antibakteriyal bitim işleminden sonra numuneler on defaya kadar yıkanmıştır. Yıkanmış ve yıkanmamış numunelerin antibakteriyal aktiviteleri AATCC Test Metot 147-1998'e göre değerlendirilmiştir. Numunelerin yüzey morfolojileri, SEM ve FTIR analizleri ile karakterize edilmiştir. Çalışmada, plasma işleminin antibakteriyal aktiviteyi belirgin şekilde arttırdığı ve yıkamaya en dayanıklı antibakteriyal maddenin noniyonik difenilalken esaslı kimyasal olduğu gözlenmiştir. SEM görüntülerinden, argon plasma işlemi sonrası pamuk yüzeyinin daha pürüzlü hale geldiği ve daha fazla gümüş partikülünün yüzeye tutunduğu anlaşılmıştır. Bu durum, EDX analizi ile de doğrulanmıştır.

**Anahtar Kelimeler:** Argon plasma, FT-IR, Yüzey modifikasyonu, Antibakteriyal, Pamuk.

## 1. INTRODUCTION

Natural fibers such as cotton, viscose and flax are very susceptible to bacterial attacks because their porous hydrophilic structures retain water, oxygen and nutrients, which provide a perfect media for the growth of microorganisms. Therefore, various antibacterial finishes have been developed. In addition to the effective control of bacteria, molds and fungi, such finishes must also meet the requirements such as; durability to laundering, dry cleaning or leaching, selective activity towards undesirable microorganisms, acceptable moisture transport properties, compatibility with other finishing agents, absence of toxic effects for both manufacturer and consumer, ease of application, applicable with no adverse effect on the fabric, enviromental safety, and cost-effectiveness (1, 2).

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Antimicrobial agents either inhibit the growth (-static) or kill (-cidal) the microorganisms. The mode of action is directly related to the concentration of the active substance in the textile material. Almost all antimicrobial agents used in commercial textiles damage the cell wall or alter cell membrane permeability, denature proteins, inhibit enzyme activity or inhibit lipid synthesis. Since all these facts are essential for cell survival, the microorganisms can't grow (2, 3).

Metal and metal salts, quaternary ammonium compounds, N-halamines, chitosan, polybiguanids and halogenated phenols can be used for antibacterial finishes (3).

Some heavy metals such as mercury, silver, zinc, cobalt and copper act as antimicrobial agents by combining with cellular proteins and inactivating them. The most common used metal salt is silver based, silver has antimicrobial activity against gram-positive and gram negative bacteria, fungi, protozoa and certain viruses. Silver can be used to reduce infections in the treatment of burned areas, to prevent bacterial colonization on medical devices as well as in textile fabrics. But, on the other hand, high concentrations of heavy metal salts will coagulate cytoplasmic proteins, resulting in cell damage or death. (1-5).

Triclosan, or 2,4,4'-trichloro-2'-hydroxydiphenyl ether is a broad-spectrum antibacterial agent that can be found in toothpaste, soap, cosmetics, and many other consumer products. (1, 2, 3, 6, 7). Triclosan inhibits the growth of microorganisms by an electrochemical mode of action. The biocidal agent blocks the active site of the protein reductase enzyme (ENR), which is an essential enzyme for fatty acid synthesis in bacteria (1, 6, 7). By blocking this enzyme, the bacteria can't synthesize fatty acid, which is necessary for building cell membranes and reproducing. Since humans don't have the ENR enzyme, it is considered harmless. In addition to this, since this agent is not water-soluble, it doesn't leach out, and it continuously inhibits the growth of bacteria in contact with the surface (1, 2). But when triclosan is exposed to sunlight in the environment, it breaks down into 2,8-dichlorodibenzo-p-dioxin which is chemically related other toxic polychlorinated dioxins (2).

Dichlorophenol is a degradation product of triclosan (8) and diphenylalkane is a triclosan derivative (6, 7). They are used as alternative to triclosan products.

Plasma based antimicrobial treatments of sensitive products are in the interest of several research. There is a huge potential to use plasma for effective antimicrobial treatment (9).

Plasma has been defined as an ionized gas with mixture of charged particles, excited atoms or molecules, neutral particles, free radicals and photons produced by discharges at atmospheric pressure or low pressure. Plasma treatments have been used to induce both surface modifications and bulky property enhancements of textile materials. These treatments can improve dyeing rates of polymers, develop color fastness and wash resistance, increase adhesion of coatings and modify wettability of fibers and fabrics (1, 10, 11, 12). However, the most desired function of the plasma treatments is to improve the durability of the finishing rather than increase water, dyestuff or chemical uptake (13).

The general reactions occurred by plasma treatment are the oxidation of the surface of a material, the generation of radicals and the edging of the surface. In general, three main effects (etching or cleaning, surface chemical modification and plasma polymerization) can be obtained. Etching is associated with changes in surface texture and wetting properties (i.e. changes in surface roughness). Surface chemical modification involves the introduction of particular chemical functional groups, depending on the nature of the gas plasma. These groups may improve wettability, biocompatibility and adhesion of the textile materials (1, 10). As a consequence of the reaction of the plasma species with the material, contaminants or even thin layers of the material are removed and surface is cleaned. At the same time, the adhesion of the coating is strengthened by the increase in the surface area (13).

Plasma treatments offer some advantages like being dry and clean technology operating in a closed system, providing textile surface properties unobtainable by most conventional techniques and reducing degradation of surface morphology (1, 13, 14, 15). In addition, plasma treatments are also better than wet-chemical based finishing methods in terms of energy consumption and running costs (12).

Plasma treatment may be performed at either low pressure (vacuum) or atmospheric pressures. Low pressure systems require a continuous flow of gas into the plasma chamber and operation at sub-atmospheric pressure. The fact that vacuum conditions are necessary for low-pressure plasma treatments makes this process impractical to use in industries requiring high rates of throughput such as textile. On the other hand atmospheric plasma treatment is well suited for continuous processing (1, 10, 16).

Atmospheric plasma treatment is a useful technique to modify polymer surfaces and leads to polymerization, grafting and crosslinking of chemical inclusion. In atmospheric plasma, helium or argon gases which have low dielectric breakdown voltages are generally used (1, 17).

In this study, the effects of argon plasma treatment on cotton fabrics finished with silver, triclosan, dichlorophenol and diphenylalkane based antibacterial chemicals were investigated. For this aim, some of the fabric samples were exposed to argon gas and then treated with antibacterial chemical agents. On the other hand, the others were only treated with antibacterial chemical agents. After antibacterial finish process, they were washed up to ten cycles. The antibacterial activities of the washed and unwashed samples were evaluated against both *S.aureus* and *K.pneumoniae*. In addition to these, the antibacterial activities of the untreated and only argon plasma treated fabrics were also assessed.

## 2. MATERIAL AND METHOD

### 2.1 Materials

100% scoured and bleached woven cotton fabric with a weight of 153 g/m$^2$ was used in the experiments. The cotton fabrics were cut in the size of 10 cm x 50 cm. For the antibacterial processes, silver, dichlorophenol, triclosan and diphenylalkane derivative based antibacterial chemicals in recommended concentrations by the supplier were applied to the fabrics. Antibacterial chemicals were gently supplied from Gemsan, Setas, Rudolf Duraner and Cognis.

## 2.2. Methods

### 2.2.1 Plasma Treatment

A hand-made dielectric barrier discharge (DBD) atmospheric plasma device was used. The distance between the electrodes was 0.2 cm. The samples were placed between the electrodes and passed continuously with the speed of 0.45 m/min. In all treatments, argon gas was used under a constant power of 130W. The samples were treated for 40 sec in the plasma.

### 2.2.2 Antibacterial Treatment

10 cm x 50 cm cotton samples were dipped in the antibacterial treatment solutions given in the Table 1 for 30 sec and then padded with a wet-pick-up of 80±1% at room temperature. The padded materials were then dried at $100^0$C for 2 min and cured at $130^0$C for 3 min.

To evaluate the washing durability, the treated samples were washed in Linitest Plus (Atlas), with a liquor ratio of 50:1. Washing process was carried out at $60^0$C for 30 min. In order to prevent any effect of detergent, washings were carried out in a soap solution with a concentration of 5 g/l. After washing, the samples were rinsed in cold pure water, squeezed and dried at room temperature. The antimicrobial activity was assessed after the first, fifth and tenth washing cycles.

### 2.2.3 Antibacterial Assessment

AATCC Test Method 147-1998 was used to test the antimicrobial activity of the untreated and all treated fabrics. Two different kinds of bacteria, *Staphylococcus aureus* (ATCC 6538) as Gram positive bacteria and *Klebsiella pneumoniae* (ATCC 4352) as Gram negative bacteria were studied. The medium was Trypticase Soy Agar which was prepared by heating 40 g of agar powder in 1000 ml distilled water for 25 minutes at 1.2 atm and $121^0$C. Test samples were cut by hand in rectangular shape (25 mm x 50 mm); they were uniformly pressed on the agar and incubated for 24 h at 37±1 $^0$C. After incubation, assessment based on the absence or presence of bacterial growth in the contact zone between the agar and the sample and on the eventual appearance of an inhibition zone which was calculated from:

$$W = (T-D) / 2$$

where W is the width of clear zone of inhibition in mm, T is the total diameter of test specimen and clear zone in mm, and D is the diameter of the test specimen in mm was made. Following the standard method, the inhibition zone was measured in mm and the degree of bacterial growth in the nutrient medium under the specimen was assessed. In this method, the higher W values mean better antibacterial activity. All tests were performed in duplicate.

### 2.2.4 Characterization

The structure of the treated and untreated cotton fabrics were studied by scanning electron microscopy (SEM-EDX) using Phillips XL-30S FEG device. The characteristic properties were analyzed using a Fourier Transform Infrared Spectrophotometer (FT-IR), Perkin Elmer, in the region from 4000 to 800 $cm^{-1}$.

## 3. RESULTS AND DISCUSSION

### 3.1 The antibacterial activity results of the samples treated only with antibacterial agents

The antibacterial activity results of the cotton samples treated only with antibacterial agents are given in Table 2. To the test results, it was observed that triclosan and triclosan derivative based chemicals had perfect antibacterial activity. These results were consistent with the other studies (6, 8). The order of the clear zones was non-ionic diphenylalkane > dichlorophenol > triclosan based chemical. So, it was concluded that the derivatives of the triclosan were more effective than triclosan and they didn't require any auxiliary agents as well. On the other hand, the silver based chemicals were found to be less effective, since they didn't have clear zones. But under the samples, no bacterial growth was observed. Hence, silver was proved to be non-diffusible antibacterial agent and have bacteriostatic activity rather than bactericidal activity. After first washing, the activities of the silver based chemicals were considerably changed, so they weren't exposed to further washing processes.

According to the clear zone diameters, it was also observed that the antibacterial activity was better against *S.aureus* (gram positive bacteria) than *K.pneumoniae* (gram negative bacteria). This result has confirmed the previous study of Orhan et al (6).

After washing process, the antibacterial activity decreased. The clear zone diameters of the washed samples (after first cycle) are given in Table 3. It was found that non-ionic diphenylalkane based chemical was the most washing durable agent (24.2% and 25.9% decrease in zone diameter). After fifth washing cycle, the clear zone was considerably changed and there was only some activity under the samples. However, after tenth wash cycle, none of them showed activity. It's concluded that for excellent washing durability results antibacterial agents must be used with auxiliary agents like crosslinking chemicals (7).

**Table 1.** The antibacterial treatment recipes

| Antibacterial Chemicals | Concentration | pH |
|---|---|---|
| Silver based chemical (1) (ionic silver) | 15 g/l | 5 |
| Silver based chemical (2) (inorganic silver salt) | 3.5 g/l | 5 |
| Dichlorophenol based chemical | 30 g/l | 7 |
| Triclosan based chemical | 2 g/l antibacterial chemical 3 g/l auxiliary chemical | 5 |
| Non-ionic diphenylalkane based chemical | 45 g/l | 5 |

**Table 2.** The antibacterial activity results of the cotton samples treated only with antibacterial agents

| Antibacterial Agents | Clear zones in mm | |
|---|---|---|
| | ***S.aureus*** | ***K.pneumoniae*** |
| Non-ionic diphenylalkane based chemical | 16.50 | 6.75 |
| Dichlorophenol based chemical | 12.50 | 5.25 |
| Triclosan based chemical | 9.75 | 2.25 |
| Silver based chemical (1) | Contact zone | Contact zone |
| Silver based chemical (2) | Contact zone | Contact zone |

**Table 3.** The clear zone diameters of the washed cotton samples treated only with antibacterial agents (after first cycle)

| Antibacterial Agents | Clear zones in mm | | | | | |
|---|---|---|---|---|---|---|
| | ***S.aureus*** | | | ***K.pneumoniae*** | | |
| | ***Before Washing*** | ***After Washing*** | ***Decrease in zone diameter*** | ***Before Washing*** | ***After Washing*** | ***Decrease in zone diameter*** |
| Diphenylalkane based chemical | 16.50 | 12.50 | 24.2% decrease | 6.75 | 5.00 | 25.9% decrease |
| Dichlorophenol based chemical | 12.50 | 9.00 | 28.0% decrease | 5.25 | 3.50 | 33.3% decrease |
| Triclosan based chemical | 9.75 | 7.00 | 28.2% decrease | 2.25 | 1.50 | 33.3% decrease |

The untreated sample and only argon plasma treated sample were also tested against *S.aureus* and *K.pneumoniae*, but they showed no activity before or after washing.

### 3.2 The activity results of the samples treated with argon plasma before antibacterial finish

The antibacterial activity results of the cotton samples treated with argon plasma before antibacterial finish are given in Table 4. To the test results, it was observed that argon plasma application clearly increased the antibacterial activity of the samples. The order of the clear zones was non-ionic diphenylalkane > dichlorophenol > triclosan based chemical and silver based chemicals showed contact zones again.

After washing process, the antibacterial activity decreased. The clear zone diameters of the washed samples (after first cycle) are given in Table 5. It was found that non-ionic diphenylalkane based chemical was the most washing durable agent (25.6% and 24.1% decrease in zone diameter).

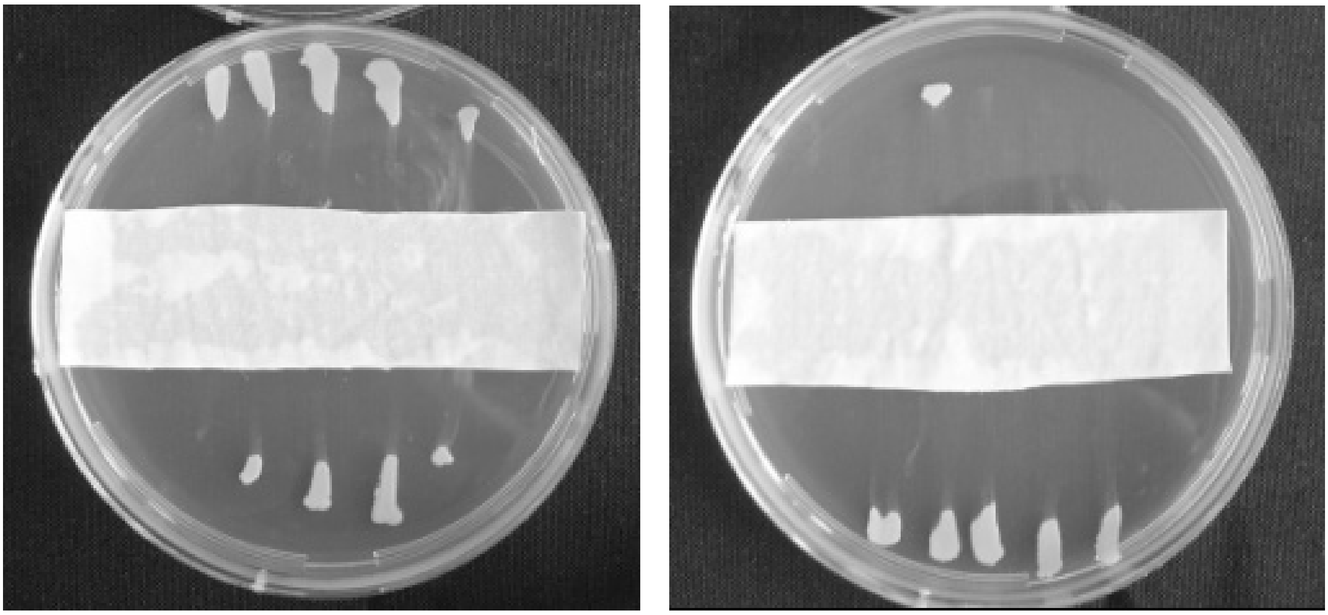
**Table 4.** The antibacterial activity results of the cotton samples treated with argon plasma before antibacterial finish

| Antibacterial Agents | Clear zones in mm | | | |
|---|---|---|---|---|
| | ***S.aureus*** | | ***K.pneumoniae*** | |
| | ***Before plasma*** | ***After plasma*** | ***Before plasma*** | ***After plasma*** |
| Non-ionic diphenylalkane based chemical | 16.50 | 19.50 | 6.75 | 7.25 |
| Dichlorophenol based chemical | 12.50 | 15.50 | 5.25 | 5.75 |
| Triclosan based chemical | 9.75 | 10.25 | 2.25 | 2.50 |
| Silver based chemical (1) | Contact zone | | Contact zone | |
| Silver based chemical (2) | Contact zone | | Contact zone | |

**Table 5.** The clear zone diameters of the washed cotton samples treated with argon plasma before antibacterial finish (after first cycle)

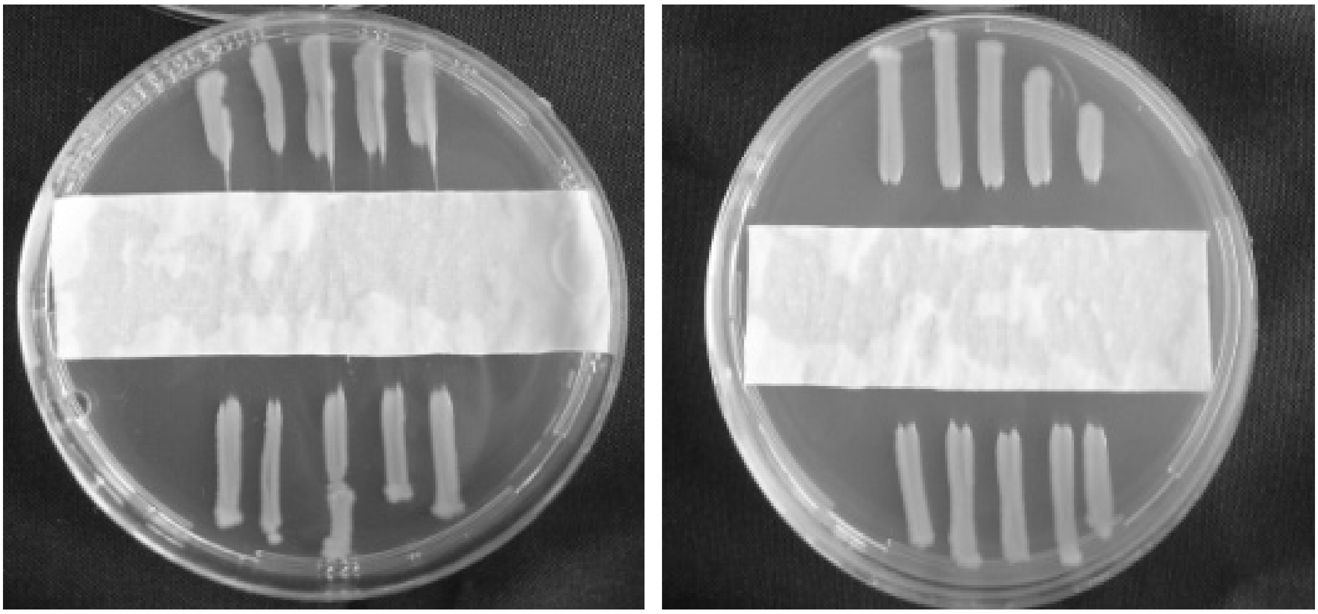
| Antibacterial Agents | Clear zones in mm | | | | | |
|---|---|---|---|---|---|---|
| | ***S.aureus*** | | | ***K.pneumoniae*** | | |
| | **Before Washing** | **After Washing** | ***Decrease in zone diameter*** | **Before Washing** | **After Washing** | ***Decrease in zone diameter*** |
| Diphenylalkane based chemical | 19.50 | 14.50 | 25.6% decrease | 7.25 | 5.50 | 24.1% decrease |
| Dichlorophenol based chemical | 15.50 | 10.50 | 32.2% decrease | 5.75 | 3.70 | 35.6% decrease |
| Triclosan based chemical | 10.25 | 5.75 | 43.9% decrease | 2.50 | 1.50 | 40.0% decrease |

In Figures 1 and 2, the antibacterial activities of the most efficient and washing durable antibacterial agent (non-ionic diphenylalkane based chemical) before and after plasma application are shown.



(a) (b)

**Figure 1.** The activity of the diphenylalkane based chemical agent against *S.aureus*; (a) before plasma application (b) after plasma application

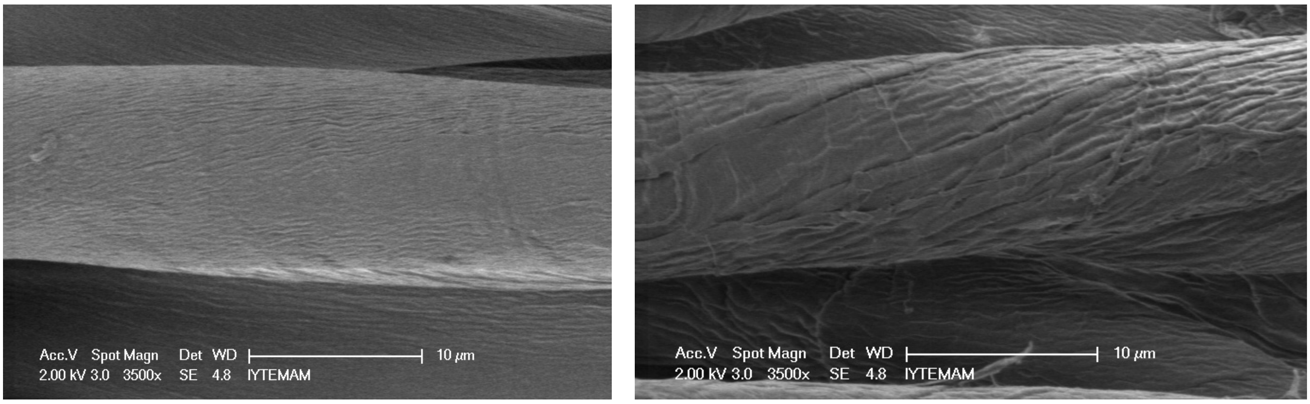


(a) (b)

**Figure 2.** The activity of the diphenylalkane based chemical agent against *K. pneumonia*; (a) before plasma application (b) after plasma application

### 3.3 SEM-EDX Analysis

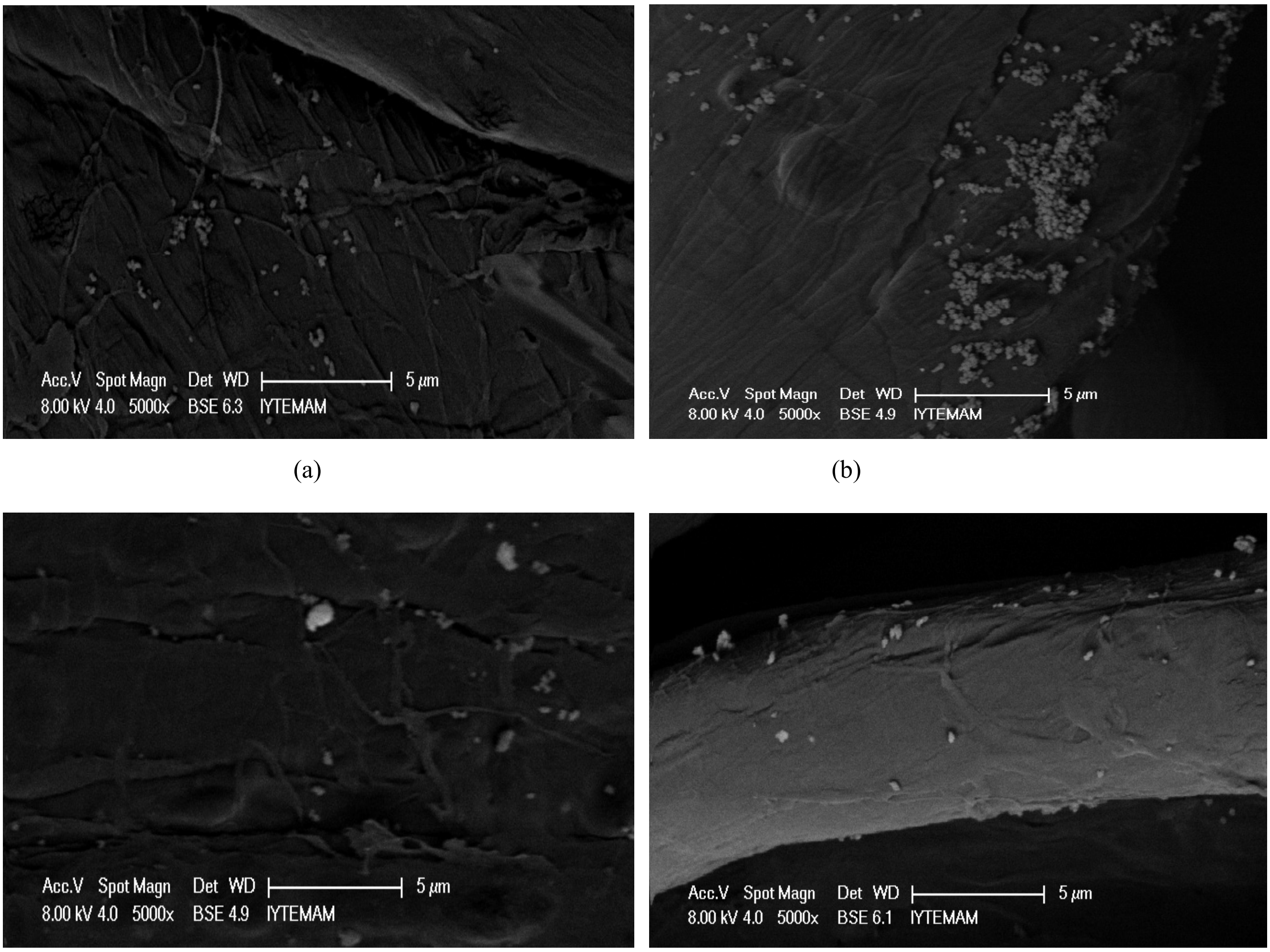
In Figure 3, the SEM images of the untreated and plasma treated cotton are shown. As seen, after argon plasma treatment, the cotton surface has become rougher and ripple-like patterns, small pores and cracks developed along the fiber axis. These results verify the evaluations of the previous studies (15, 18, 19).



(a) (b)

**Figure 3.** SEM images of the samples (a) untreated cotton and (b) plasma treated cotton

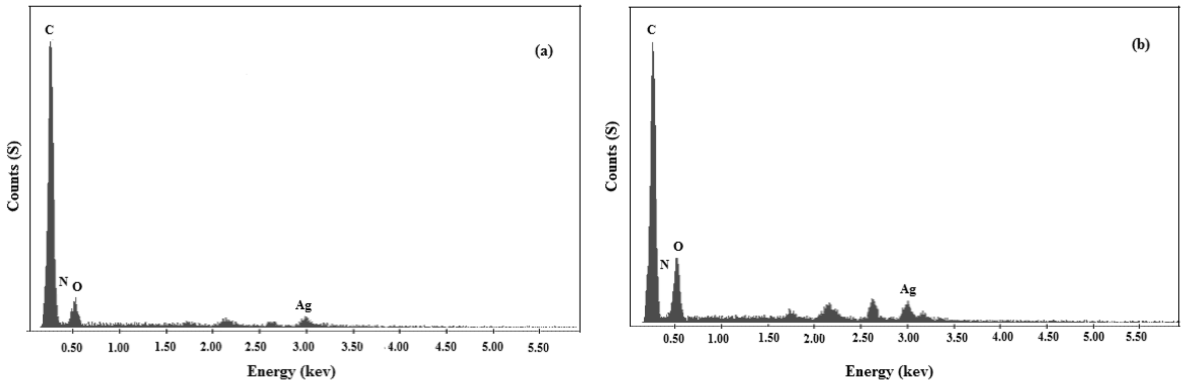
Figure 4 shows the SEM images of the silver treated samples before and after plasma modification. As shown, more silver particles have been attached to the surface of the samples pretreated by plasma. To enrich the results, EDX analysis was made to these samples as well as SEM analysis. The EDX results (Figure 5) showed that the silver content on the cotton plasma-treated before antibacterial finish was more than the silver content on the cotton treated only with antibacterial agent.



(a) (b)

(c) (d)

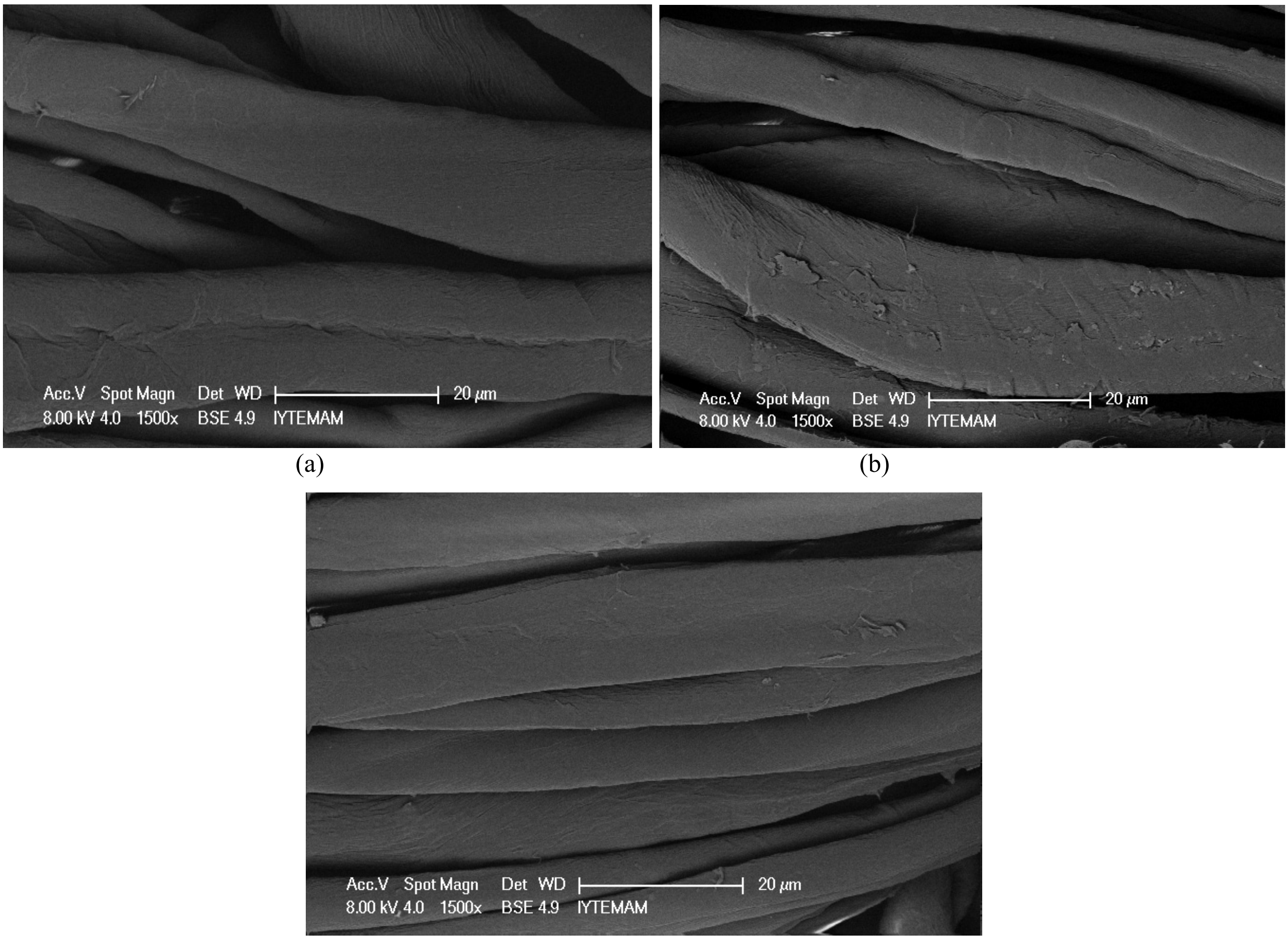
**Figure 4.** SEM images of the silver treated samples before and after plasma modification, (a) Silver based chemical (1), (b) Plasma+silver based chemical (1), (c) Silver based chemical (2), (d) Plasma+silver based chemical (2)



(a) (b)

**Figure 5.** EDX analysis of silver based chemical (1), (a) before plasma modification, (b) after plasma modification

Figure 6 shows the SEM images of the samples treated by triclosan and its derivatives. From the images, it's seen that they formed uniform and compatible antibacterial coating film on the cotton surface.



(a) (b)

(c)

**Figure 6.** SEM images of the samples treated by triclosan and its derivatives; (a) triclosan, (b) dichlorophenol, (c) diphenylalkane

### 3.4 FTIR Analysis

Figure 7 shows the FTIR spectra of the untreated and plasma treated cotton. For plasma treated cotton, a decrease of absorbance in the band at 3331 cm$^{-1}$ (–OH and –NH bonding) and in the bands at 1200, 1314 and 1427 cm$^{-1}$ (-CO-NH- bonding) was observed. This result was consistent with the study of Luciu et al (15). In addition to these results, it was also seen that the band at 2895 cm$^{-1}$ shifted to 2890 cm$^{-1}$ and there was considerable change in the range of 800 and 1200 cm$^{-1}$ (cellulosic bonds) after argon plasma modification.

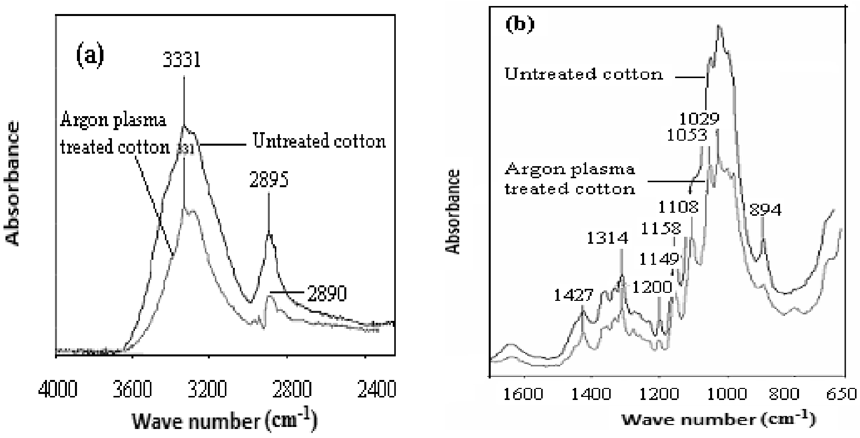


**Figure 7.** FTIR spectra of the untreated and argon plasma treated cotton (a) 4000-2400 $cm^{-1}$, (b) 1600-650 $cm^{-1}$

## 4. CONCLUSION

Antibacterial treatments are very important for natural fibers especially used for medical purposes. In this study, plasma treatment was used to modify cotton surface and to increase adhesion of antibacterial coatings.

Results confirmed that plasma application clearly increased the antibacterial activity of the samples and adhesion of the antibacterial coating. It's found that the most efficient and washing durable antibacterial agent was non-ionic diphenylalkane based chemical. According to the clear zone diameters, it was also observed that the antibacterial activity was better against *S.aureus* (gram positive bacteria) than *K.pneumoniae* (gram negative bacteria). From the SEM images, it's seen that plasma treatment made the cotton surface rougher and ripple-like patterns, small pores and cracks developed along the fiber axis. Also, it's observed that plasma increased the adhesion of antibacterial coatings and agents. The EDX results showed that the silver content on the cotton plasma-treated before antibacterial finish was more than the silver content on the cotton treated only with antibacterial agent.

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