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Synthesis of Silver Mono- and Di-Carboxylates and Investigation of their Usage Possibility in Textiles as an Antibacterial Agent

Abstract
The study aims to examine the antibacterial efficiency of cotton fabrics loaded with silver cyclo hexane mono or di carboxylates (silver naphthenates). After the synthesis of silver naphthenates, their chemical structures were analysed with spectrophotometric methods (IR and NMR). Then the usage possibility of Ag naphthenates as an antibacterial agent in the finishing of cotton fabrics was investigated. Their antimicrobial activity against three gram-negative (Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa) and three gram-positive (Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis) bacteria were studied. The stability of the antibacterial effect after repeated washings (1-5-10-20) was also tested.

Key words: *silver, naphthenic acid, cotton, antibacterial, washing.*

Introduction

It has been known that silver ions have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities for bacteria, fungi and viruses since ancient times [1 - 3]. The minimisation of microbial growth on textile materials is of great importance since microbes can harm not only the material itself, but they can also affect the comfort of the wearer. In other words, they may cause plenty of negative effects such as the generation of unpleasant odour, stains, depolarisation of the material, and a decrease in fabric mechanical strength [4, 5].

Antibacterial agents are very important in the textile industry, water disinfection, medicine, and food packaging. Antibacterial activity is related to compounds which locally kill bacteria or slow down their growth without being in general toxic to surrounding tissue. The bacterial cell wall is designed to provide strength, rigidity and shape, and to protect the cell from osmotic rupture and mechanical damage [6]. According to their structure, components and functions, the bacteria cell wall can be divided into two main categories: Gram-positive (+) and Gram-negative (–).

Cotton fibers are particularly suitable for the manufacturing of textiles for sports, leisure, medical non-implantables (different bandages, plasters, gauze dressings, lint, wadding, adsorbent pads) and healthcare/ hygiene products (surgical gowns and hosieries, sheets, pillowcases, uniforms, blankets) [7]. However, the ability of cotton to absorb a huge amount of moisture makes this fiber more prone to microbial attack. Under certain conditions of humidity and temperature, cotton may act as a nutrient, becoming a suitable medium for bacterial and fungal growth [4, 5].

The rising problem of microbes resistant to multiple antibiotics has renewed interest in silver and silver compounds, which are historically recognised as powerful biocides for more than 650 various microbes [8, 9]. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, and catalytic and antibacterial activity, and is used in industrial applications [10]. The development of new resistant strains of bacteria acting against current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [11]. Different forms of silver products used in various studies are metalic silver [12], silver chloride [13, 14], silver zeolite [15], silver dendrimer and composites [16], polymer-silver nano particules [17, 18], silver nano particules [19 - 21], PU coated silver nano particles [22] and Ag/TiO_2 composite nano powders [23]. Although in the literature there are many studies about the use of silver as an antibacterial agent for cotton, to the best of the authors' knowledge, there is not any study in which a complex compound of silver was synthesised and used for the antibacterial finishing of cotton.

In our previous study, we examined the usage possibility of various metal (copper, zinc, cobalt, nickel, potassium and sodium) mono carboxylates (metal naphthenates) as antibacterial agents in textile applications [24]. In the current study, it was aimed to determine the antibacterial efficiency of cotton fabrics loaded with silver cyclohexane mono or di carboxylates (silver naphthenates). After the synthesis of silver naphthenates, their chemical structures were analysed with spectrophotometric methods (IR and NMR). Then the usage possibility of Ag naphthenates as an antibacterial agent in the finishing of cotton fabrics was investigated. Their antimicrobial activity against three gram-negative (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa)* and three gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis)* bacteria were studied. The stability of the antibacterial effect after repeated washings (1-5-10-20) was also tested.

Experiment

In the study, the usage possibility of naphthenates, which are known as saturated hydrocarbons present in the chemical composition of grease obtained from the handling of petrol, as an antibacterial

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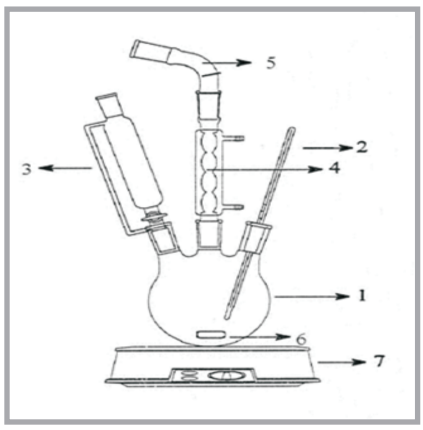


Figure 1. *Mechanism used in the synthesis of antibacterial agents; 1) three necked flask, 2) thermometer, 3) dropping funnel, 4) condenser, 5) tube of $CaCl_2$, 6) magnet, 7) magnetic stirrer).*

agent in the textile industry was investigated. All experiments were carried out using pure water with desized and bleached 100% cotton woven fabric. The study consists of two main stages:

- synthesis and characterisation of antibacterial agents,
- investigation of the usage possibility of synthesised antibacterial agents in textile applications.

Synthesis and characterisation of antibacterial agents

In this part of the study, silver cyclohexane mono carboxylate and di carboxylate compounds (silver naphthenates) of white color were obtained from the reaction of naphthenic acid and silver salt. The synthesis reaction of silver cyclohexane mono carboxylate and di carboxylate compounds are as follows;

$$2RCOOH + 2NaOH + Ag(NO_3)_2 \rightarrow \rightarrow Ag(RCOO)_2 + 2NaNO_3 + 2H_2O$$

R - cyclohexane

$$2YCOOH + 2NaOH + Ag(NO_3)_2 \rightarrow \rightarrow Ag(YCOO)_2 + 2NaNO_3 + 2H_2O$$

Y - cyclohexane mono carboxylate

For actualisation of the reaction, firstly cyclohexane mono or di carboxylic acid was dissolved in an organic solvent and sodium salt of naphthenic acid was constituted by adding caustic soda [25 - 28]. Then silver cyclohexane mono or di carboxylate was obtained by adding silver nitrate. The synthesis of the agent is described below.

Synthesis of agent: A thermometer, condenser and dropping funnel were placed at the necks of a three necked flask, as shown in ***Figure 1***. Then a solution of cyclohexane mono or di carboxylic acid (Merc) in 10% (v) diethyl ether (Merc), which is calculated stoichiometrically, was put into the flask, and a solution of 10% (v) NaOH (Merc) was put into the dropping funnel. The temperature was raised to 40 - 45 °C by running the magnetic stirrer, and the solution was stirred while dropping NaOH from the dropping funnel for 60 minutes. The pH of the medium was adjusted to between 7 and 8. Afterwards the amount of 10% (v) solution of $AgNO_3$ (Sigma Aldrich) calculated was added to this mixture. The heater was turned on, the dropping funnel opened and the mixture stirred by running an electromagnetic stirrer (Hot-Plate 300 °C 15 cm circular M15 type) at room temperature for 1 hour. Then this solution was held for 24 hours. Afterwards the solution obtained was put into an extraction flask and the liquid phase was separated from the organic phase. After removal of the solvent from the organic phase, silver cyclohexane mono or di carboxylate compound was obtained.

After the agents were synthesised, their chemical structures were analysed with spectrophotometric methods (IR and NMR). IR spectra were taken using the KBr table with a Shimatsu (Japan) IR-470 model Infrared Spectrophotometer. In order to determine the ^1H NMR spectra, Varian (USA) brand 300 MHz model equipment was used. For NMR analysis deuterated chloroform ($CDCl_3$) was used as a solvent.

Investigation of the usage possibility of synthesised antibacterial agents in textile applications

In this part of the study, liquor containing 40 g/l of the silver mono or di carboxylate compound, 1 g/l of a dispersing agent, and 1 g/l of a wetting agent and acetic acid (to adjust pH to 5) was prepared by stirring at 40 - 45 °C for 10 - 15 minutes in an ultrasonic bath (Baysonic). The application recipe was determined by taking the pre-test results into consideration. Then cotton fabrics were impregnated with these liquors in a foulard with a pick-up value of 80% and dried with a tenter frame dryer at 85 °C for 4 minutes.

Then antibacterial tests were applied to the fabrics before washing and after 1-5-10-20 washes according to the JIS L 1902 (2008) protocol. Test and control specimens (non-sterile) were cut to 25 × 50 mm, as recommended, and sterilised by autoclaving (at 120 °C for 15 min.). Inoculum was prepared as follows: the bacteria was incubated for 24 h at 37 ± 2 °C. in nutrient broth (NB). Then, 1.0 ± 0.1 ml of inoculum with 1×10^7 cells/ml was added to 15 ml of nutrient agar (NA) warmed to 45 - 46 °C. This solution was placd on a sterilised Petri dish. After agar solidification, the sterilised textile samples were placed over the agar and incubated for 24 h at 37 ± 2 °C.

As gram-negative bacteria *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC13883) and *Pseudomonas aeruginosa* (ATCC27853), and as gram-positive bacteria *Staphylococcus aureus* (ATCC29213), *Bacillus subtilis* (NRRL NRS-744) and *Enterococcus faecalis* (ATCC29212) were used. The stability of the antibacterial effect after repeated washings (1-5-10-20) was also tested according to the BS EN ISO 26330 standard at 30 °C, where ECE standard detergent of SDC Enterprises Ltd. was applied.

Furthermore SEM analysis was carried out for the cyclohexane mono carboxylate compound applied to cotton fabrics. A JMS 5910-LV scanning electron microscope (JEOL, Japan) set at an accelerating voltage of 20 kV was employed for imaging fabric samples at X100, X1000 and X5000 magnifications. Samples were coated with gold in a SC7620 Sputter Coater Unit (Polaron, UK) prior to SEM analysis.

Results and discussion

Results related to the characterization of silver cyclohexane mono and di carboxylate compounds (silver naphthenates)

The IR spectrum of silver cyclohexane mono and di carboxylate compounds are given in ***Figures 2*** and ***3***, respectively.

As can be seen in ***Figures 2*** and ***3***, carbonyl groups (peak at 1680 - 1730 cm^{-1}), C-O stretching (peak at 1000 - 1250 cm^{-1}) and methylene groups (peaks at 1400 - 1450 cm^{-1}) of cyclohexane ring were detected in the structures of synthesised agents [29].

The NMR spectrum of silver cyclohexane mono and di carboxylate compounds are given in ***Figures 4*** and ***5***, respectively.

As can be seen in ***Figures 4*** and ***5***, methylene protons of cyclohexane mono and di carboxylate rings (multiplet) were observed at 1.12 - 1.86 ppm and 3.71 - 4.56 ppm, respectively. On the other hand, methine protons were detected at 2.26 ppm and 5.06 ppm for cyclohexane mono and di carboxylate rings (multiplet), respectively.

In ***Figure 6***, molecular structures of synthesised antimicrobial agents are given. From the chemical structure of the synthesised agents, it is very clear that they contain carbonyl, C-O and methylene groups. This situation confirms IR and NMR results.

Results related to the investigation of the usage possibility of synthesised antibacterial agents in textile applications

Antibacterial test results according to the JIS L 1902 protocol of silver cyclohexane mono or di carboxylate compound applied to cotton fabrics before washing and after 1-5-10-20 washes are presented in ***Table 1***.

From ***Table 1***, it can be seen that the silver cyclohexane mono carboxylate compound gave better results both before and after washing compared to the di carboxylate compound. The reason for this could be easily understood when their chemical structures (see ***Figure 6***) are investigated. In the molecular structure of the silver cyclohexane di carboxylate compound, the silver atom completed its maximum ability of coordination bond formation, while in the silver cyclohexane mono carboxylate the compound silver atom still has the possibility to form a coordination bond.

In literature there are different explanations related to the action mechanism of metal ions. A brief explanation of their anti-microbial mechanism can be as follows: Generally metal ions destroy or pass through the cell membrane and bond to the -SH group of cellular enzymes. The consequent critical decrease in enzymatic activity causes micro-organism metabolisms to change and inhibits their growth, leading to the cell's death. Metal ions also catalyse the production of oxygen radicals that oxidise the molecular structure of bacteria. Such a mechanism does not need any direct contact between the anti-microbial agent and bacteria, because the active oxygen produced diffus-

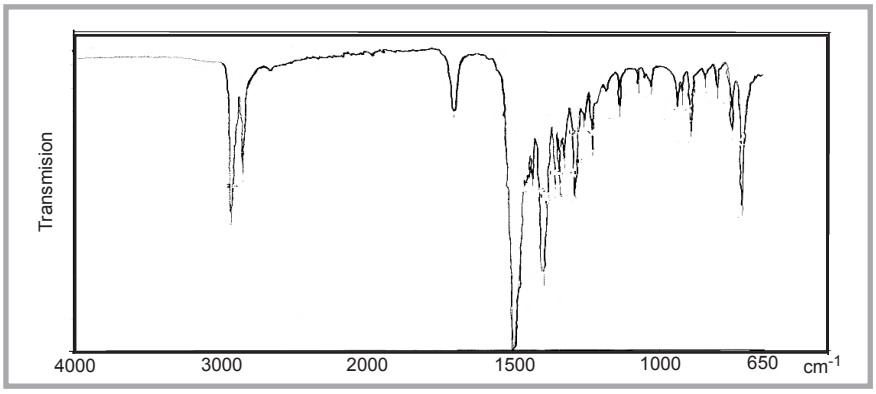


Figure 2. *IR spectrum of silver cyclohexane mono carboxylate.*

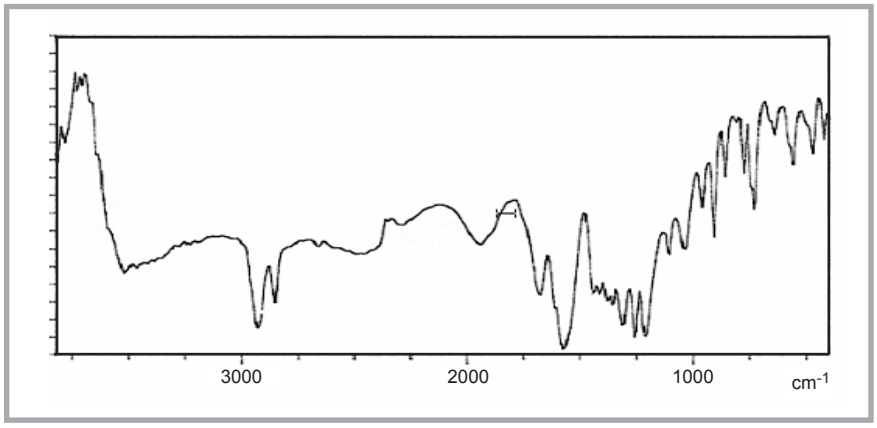


Figure 3. *IR spectrum of silver cyclohexane di carboxylate.*

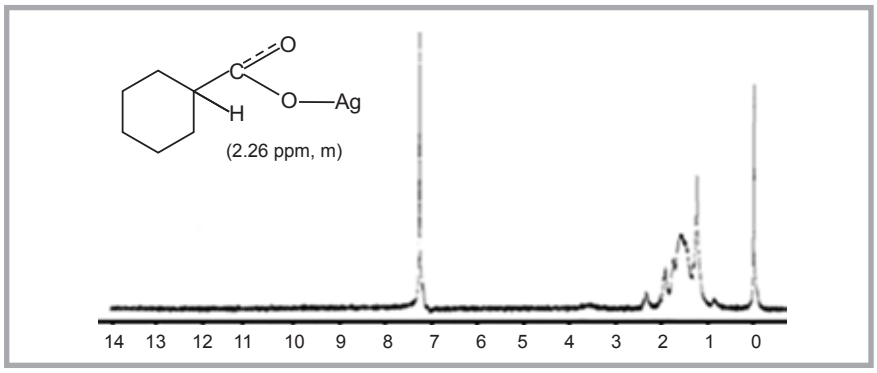


Figure 4. *NMR spectrum of silver cyclohexane mono carboxylate.*

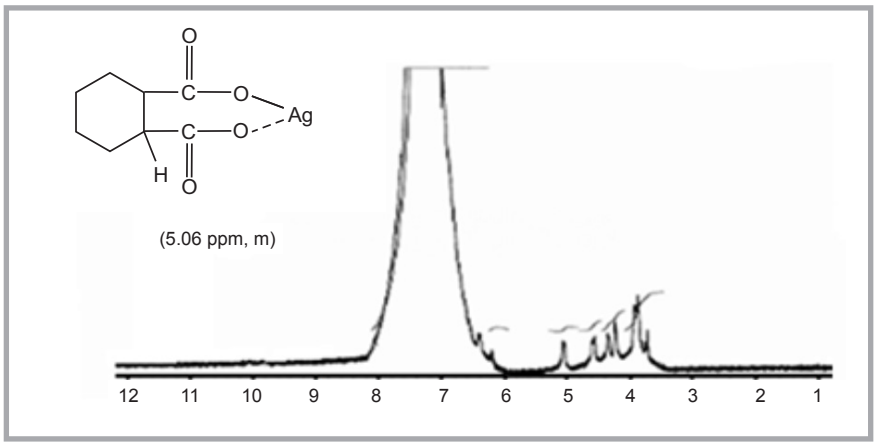


Figure 5. *NMR spectrum of silver cyclohexane di-carboxylate.*

Table 1. *Inhibition zones of silver cyclohexane mono and di carboxylate applied fabrics after 1, 5, 10 and 20 washes against 6 different bacteria.*

Bacteria types	Inhibition zones in mm after washing									
	-		1st		5th		10th		20th	
	mono	di	mono	di	mono	di	mono	di	mono	di
E. coli	7	3	7	2	6	2	5	2	4	2
K. pneumonie	5	4	5	4	5	4	4	4	2	3
P. aeruginosa	8	4	8	4	8	4	6	4	4	3
S. aureus	4	2	4	2	3	2	3	2	2	1
B. subtilis	6	2	6	2	5	2	5	2	2	2
E. faecalis	3	2	3	1	3	1	3	1	2	0.5

Table 2. *Inhibition zones of silver cyclohexane mono carboxylate applied fabric after 20 washes against 6 different bacteria; a) E. coli, b) K. pneumonie, c) P. aeruginosa, d) S. aureus, e) B. subtilis, f) E. faecalis.*

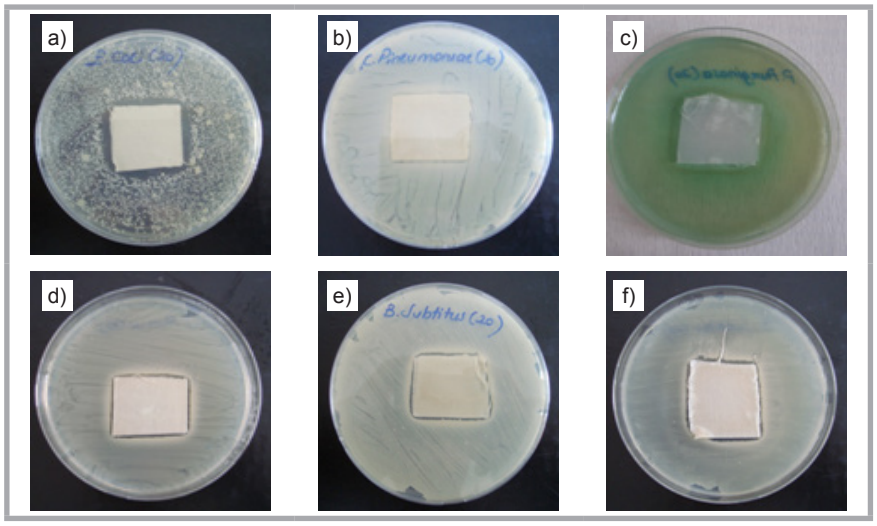
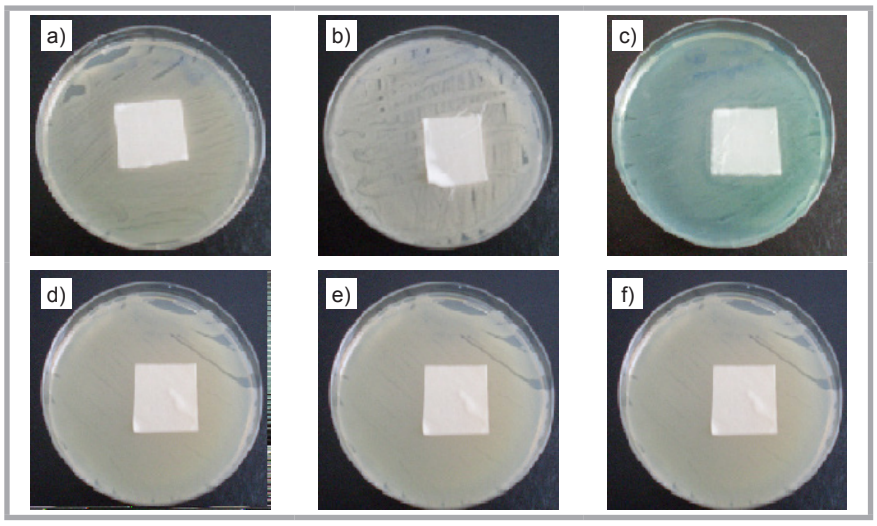


Table 3. *Inhibition zones of untreated cotton fabric before washing against 6 different bacteria; a) E. coli, b) K. pneumonie, c) P. aeruginosa, d) S. aureus, e) B. subtilis, f) E. faecalis.*



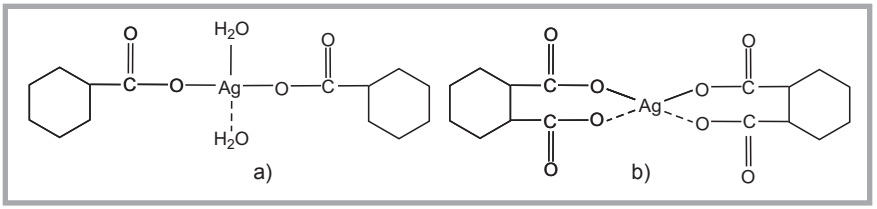


Figure 6. *Molecular structures of silver cyclohexane mono (a) and di (b) carboxylates.*

es from the fiber to the surrounding environment. Therefore metal ions inhibit the multiplication of micro-organisms [30].

The silver atom present in the structure of the compound synthesised in this study is bound with coordinative bonds in the structure, as distinct from silver salts where free metal atom (silver) exists in the medium. For this reason, the action mechanism of this compound is thought to be as explained in the second part. For this reason the catalysation of oxygen radical formation by silver will be easier for cyclohexane mono carboxylate, because the silver atom in this compound will complete its coordination number with water (coming from humidity present in the medium) (see ***Figure 6.a***), and it will be able to catalyse the transformation of this water firstly to hydrogen peroxide by the action of atmospheric oxygen and then to active oxygen, as shown in the reaction given below [30].

$$H_2O + 1/2\ O_2 \xrightarrow{\text{Metal ion}} H_2O_2 \rightarrow \rightarrow H_2O + (O) \quad (1)$$

All these results explain why the efficiency of the mono carboxylate compound is substantially (approx. double) higher compared to the di carboxylate compound.

When the washing stability of these two compounds is compared, it can be said that in the case of the mono carboxylate compound good antibacterial effects were obtained even after 20 washes. Inhibition zones against 6 different bacteria of silver cyclohexane mono carboxylate applied fabric after 20 washes and untreated cotton fabric before washing (negative control) are given in ***Tables 2*** & ***3***, respectively. As can be seen from ***Table 3***, in the case of untreated fabric, although no bacterial growth was detected on the fabric (as it is known, textile processing such as bleaching renders fabrics some antibacterial activity), no inhibition zone was observed even before washing. But fabric treated with the silver cyclohexane mono carboxylate compound showed good inhibition to bacterial growth even after 20 washes.

The reason for the washing stability differences between mono and di carboxylate compounds can be understood when their interaction with cotton fiber is investigated. As can be seen in ***Figure 7***, the silver atom present in the mono carboxylate compound did not complete its

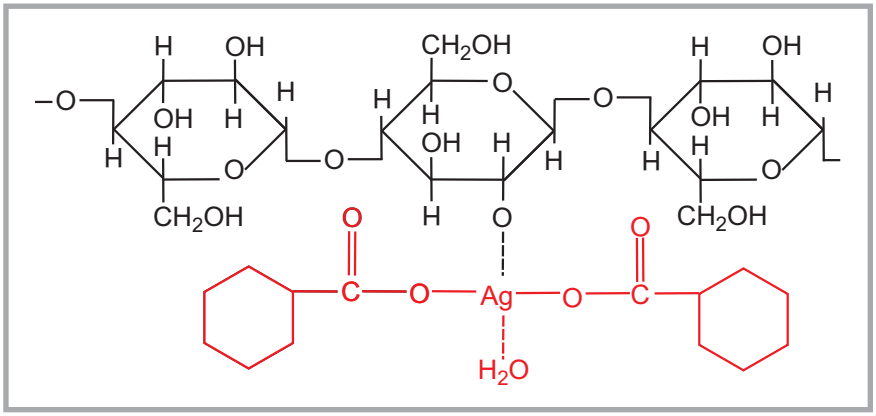


Figure 7. *Binding mechanism of cyclohexane mono carboxylate to cotton fibres.*

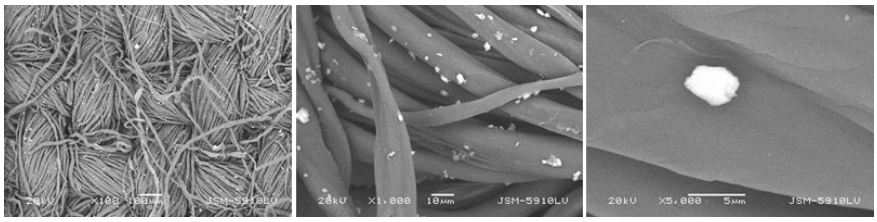


Figure 8. *SEM photos of silver cyclohexane mono carboxylate applied cotton fabrics (100×, 1000×, 5000×).*

coordination number, and for this reason it still has the ability to form coordinative bonds with alcohol groups of cotton fibers. On the other hand, the silver atom in the di carboxylate compound completed its coordination number, consequently it will just be able to form hydrogen bonds and secondary attraction forces. When the strength difference among the aforementioned bonds is taken into consideration, the reason for the mono carboxylate compound's washing stability being higher is well understood.

From results above it can be stated that the cyclohexane mono carboxylate compound will be a better alternative. For this reason SEM photos of only the cyclohexane mono carboxylate compound applied cotton fabrics were taken, the results of which are given in ***Figure 8***.

From ***Figure 8***, silver naphthenate compounds can be clearly seen on cotton fabrics.

Conclusions

The study aimed to examine the usage possibility of naphthenates (cyclohexane mono and di carboxylates), which are obtained from side fractions of petrol, as a new antibacterial agent in the textile sector. Considering that recycling from waste and using it in various areas is going to be more important in the future when restrictions increase, it can be concluded that the antibacterial agent synthesized in the study is beneficial in today's conditions, where environmental consciousness is increasing. Another advantage of the study is that the antibacterial agent obtained is classified as a complex compound. Such a silver ion in a complex compound is not possible to get onto the skin, as a result of which, it can be stated that the synthesised silver cyclohexane mono carboxylate compound can be used as a new antibacterial agent in the textile sector.

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