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Grancarić, Ana Marija; Tarbuk, Anita; Kovaček, Ivančica

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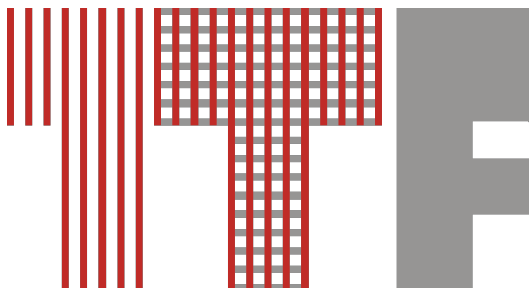
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ANA MARIJA GRANCARIĆ¹
 ANITA TARBUK¹
 IVANČICA KOVAČEK²

¹Faculty of Textile Technology,
 University of Zagreb, Croatia
²Zagreb Institute of Public Health,
 Zagreb, Croatia

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NANOPARTICLES OF ACTIVATED NATURAL ZEOLITE ON TEXTILES FOR PROTECTION AND THERAPY*

Activated natural zeolite clinoptilolite is microporous hydrated aluminosilicates crystals with well-defined structures containing AlO_4 and SiO_4 tetrahedral linked through the common oxygen atoms. It is to point out that zeolites act as strong adsorbents and ion-exchangers but having many other useful properties. Due to its cationexchange ability, zeolites have catalytic properties and, for that, multiple uses in medicine and industry, agriculture, water purification and detergents. Zeolites are nontoxic substance, excellent for UVR and microbes protection, for proteins and small molecules such as glucose adsorption. In this paper its positive effect on the metabolism of living organisms and its anticancerogenic, antiviral, antimetastatic and antioxidant effect. The activity of natural zeolite as natural immunostimulator was presented as well as its help in healing wounds. Therefore, the present paper is an attempt to modify cotton (by mercerization) and polyester (by alkaline hydrolysis) fabrics for summer clothing with addition of natural zeolite nanoparticles for achieving UV and antibacterial protective textiles.

Key words: zeolite; cotton; polyester; summer cloths; UVR protection; antibacterial protection.

Zeolites are hydrated natural or synthetic microporous crystals with defined structures based on AlO_4 and SiO_4 tetrahedral building blocks connected through oxygen atoms. Zeolites have a variety of uses. They are employed as adsorbents, ionic exchangers, catalysts and detergent builders in industry, agriculture, veterinary medicine, health care and environmental protection [1,2]. Natural zeolites are rock-forming, microporous silicate minerals. A mineral of the natural zeolite, clinoptilolite, has a crystalline configuration of a tetrahedron structure. It has a lattice structure with long channels comprising water molecules and alkaline earth ions. As these do not occupy fixed positions, these ions may shift within the lattice. They can be easily released and exchanged without changing the character of crystal lattice, enabling clinoptilolite to have strong ion exchange properties, as example clinoptilolite bonds heavy metals. Many re-

searchers report that clinoptilolite absorbs toxins and mould [3].

Clinoptilolite can be ground by a certain tribomechanical processing in a patented machine (patent: PCT/1B99/00757) yielding particles on a submicron level (micro and nanoparticles) and this process leads to an increased specific surface which amongst others yields a higher activity with respect to the above mentioned properties (Fig. 1). Nanoparticles of one gram of micronized clinoptilolite have an inner surface of 50000 m^2 - 50 million microchannels representing exceptionally powerful microfilters [4].

Due to the increased total surface area and an increased accessibility of the micro-channels the activity of the zeolite is increased several hundred times. The activated natural zeolite has multiple uses in industry and agriculture, and there is a broad spectrum of medical uses [3].

Its positive effect on the metabolism of living organisms has been confirmed in case of human application. Most recent research conducted basically over the last ten years proves its anticancerogenic and antimetastatic effect [5,6], its strong antiviral activity and an assistance of metabolic processes. Scientists define it as the most powerful natural immunostimulator and antioxidant [7]. Zeolites could be

Corresponding author: A.M. Grancarić, Faculty of Textile Technology, University of Zagreb, Prilaz baruna Filipovića 28a, Croatia.
 E-mail: amgranca@ttf.hr

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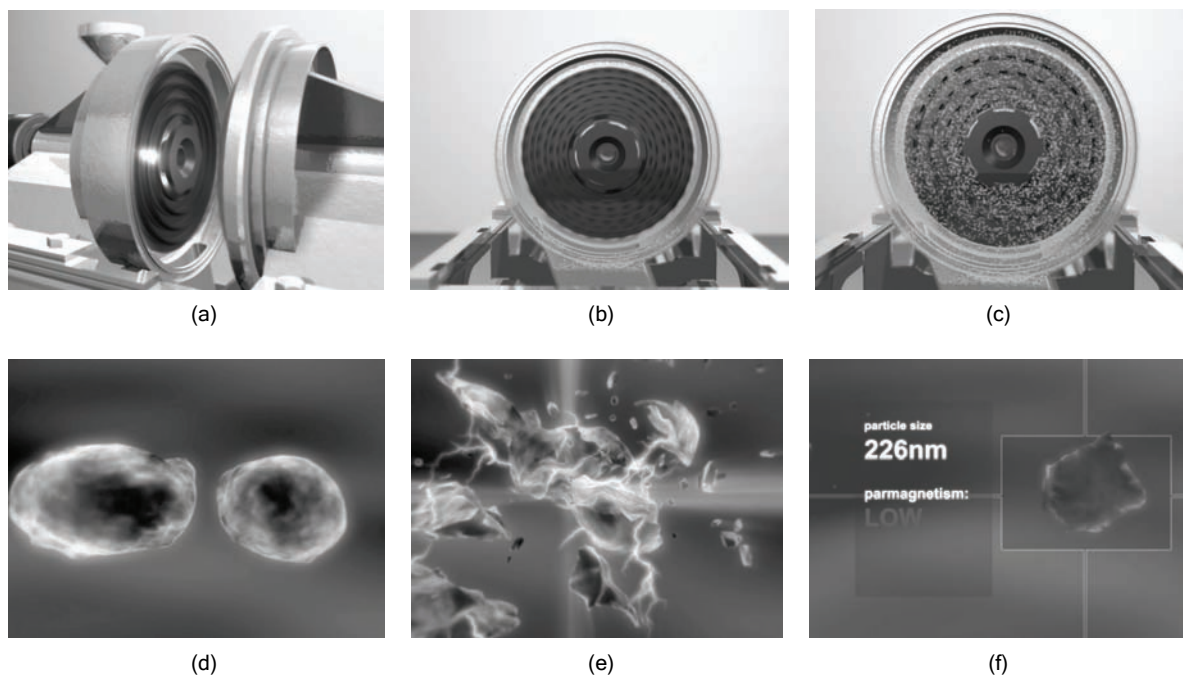


Figure 1. Tribomechanical activation (TMA) - a) TMA machine, b) TMA machine before input of particles, c) TMA machine with particles, d) input of clinoptilolite particles, e) crash of zeolite particles which results with micro and nanoparticles and f) nanoparticles of clinoptilolite [4].

now considered to be full-fledged mineral commodities, the use of which promises to expand even more in the near future [1,2].

When applied externally in powder form, it has been found to quicken the healing of wounds and surgical incisions, and act as proven bactericides and fungicides as well.

In this paper a positive effect of activated natural zeolite in diverse biological activities such as immunostimulator and antioxidant, and help in healing wounds is presented. The paper is also an attempt to modify cotton (by mercerization) and polyester (by alkaline hydrolysis) fabrics for summer clothing with addition of natural zeolite nanoparticles for achieving UV and antibacterial protective textiles.

UV Protection

A good fabric UV protection is a guarantee that clothing will have the ability to protect the skin from incident solar energy.

In addition to some beneficial effects of UV radiation (UV-R, from 100 to 400 nm) on skin it may cause skin damage such as sunburn, allergies, skin aging and even skin cancer especially during the summer time. The UV-C radiation (from 100 to 280 nm) get absorbed by atmosphere but UV-B (from 280 to 320 nm) and UV-A (from 320 to 400 nm) rays reach the Earth and cause well-known skin aging and recently the formation of skin malignant neoplasm. Di-

minishing of the ozone layer raised this risk what have resulted in large investigation of fabric UV protection.

It is well known that garment provides some UV protection. Fabric can reflect, absorb and scatter solar wavelengths, but in most cases it does not provide full sun screening properties. UV protection highly depends on a large number of factors, such as a type of fiber, porosity, density, moisture content, type and concentration of dye, UV-B protective agents, and fluorescence whitening agents (FWA) in the case of white textiles, if applied [4,8,9].

Nanoparticles of a natural zeolite (clinoptilolite) applied on textile material scatters the light, resulting in UV protection [4,10].

Antimicrobial protection

Clothing and textile materials are carriers of microorganisms such as pathogenic and odor-generating bacteria, and mold fungi because of the adhesion of these organisms on the fabric surface. In the last few years a current vogue that promotes a healthier and active lifestyle has led to a rapid growth of antimicrobial finishes which results in textile material imparting durable freshness and a feeling of safety and well-being of consumers.

Azalides are a subclass of macrolide antibiotics. Azithromycin, the most famous azalide with brand names Zithromax® (Pfizer) and Sumamed® (Pliva), is

derived from erythromycin by adding a nitrogen atom into the lactone ring of erythromycin A, thus making the lactone ring 15-membered.

Azithromycin is used for the treatment of the respiratory-tract, soft-tissue and genitourinary infections. It prevents bacteria from growing by interfering with their protein synthesis [11,12].

Therefore it is of great interest to research its antibacterial ability on textiles - non-woven that can be used as protection masks and clothing in hospitals, woven for laundry and sheets in hotels and hospitals and knitted for summer clothing, socks and underwear. Although its mechanism of action and susceptibility to resistance are similar to those of the macrolide antibiotics, the extended spectrum of azithromycin activity includes Gram positive and Gram negative organisms, as well as atypical pathogens [4].

EXPERIMENTAL PART

The activated natural zeolite (Z) is clinoptilolite particles made by tribomechanical activation on the patented instrument manufactured by Tribomin d.o.o., Osijek. The origin of clinoptilolite used in this paper is Konica, Slovakia. By X-ray diffractometry it was found

that the sample consists of about 80% clinoptilolite and the rest are clay minerals montmorillonite and mordenite. The moisture content was investigated by heating to 105 °C the detected amount was maximum 6% [6]. The particle size is around 200 nm. The composition and physical chemical properties according to the analysis of ISEGA Forschungs und Untersuchungsgesellschaft mbH, Aschaffenburg, Germany [6] are given in Table 1.

For the sun screening properties of cotton (C) and polyester (poly(ethylene-terephthalate), PET) the fabrics were modified by standard surface modifications - cotton mercerization and PET alkaline hydrolysis and then treated with zeolite nanoparticles and FWA's. Additionally, other set of cotton fabrics was antimicrobially treated with azlide. Antimicrobial treatment was performed by the exhaustion method in Linitest (Original, Hanau) at pH 5.5, $t = 60$ °C for $\tau = 20$ min with 3% of the AZI 25% water solution. Fabric labels and treatments are collected in Table 2.

UV protection was determined through Ultraviolet protection factor (UPF) using a transmission spectrophotometer Cary 50 Solar-screen (Varian) according to AS/NZS 4399:1996 Sun Protective Clothing: evaluation and classification [13]. Fabric antimicrobial

Table 1. Composition and physicochemical properties of TMAZ analyzed by ISEGA Forschungs und Untersuchungsgesellschaft GmbH, Aschaffenburg, Germany [6]

| Component | Chemical composition, % |
|--|---------------------------------|
| SiO ₂ | 65.0-71.3 |
| Al ₂ O ₃ | 11.5-13.1 |
| CaO | 2.7-5.2 |
| K ₂ O | 2.2-3.4 |
| Fe ₂ O ₃ | 0.7-1.9 |
| MgO | 0.6-1.2 |
| Na ₂ O | 0.2-1.3 |
| TiO ₂ | 0.1-0.3 |
| Si/Al ratio | 4.8-5.4 |
| Empirical formulae: (Ca,K ₂ ,Na ₂ ,Mg) ₄ Al ₈ Si ₄₀ O ₉₆ ·24H ₂ O | |
| Physicomechanical property | Value |
| Specific mass, g/cm ² | 2.2-2.5 |
| Porosity, % | 32-40 |
| Effective pore diameter, nm | 0.4 |
| Ion | Ion-exchanging capacity, mol/kg |
| Total | 1.2-1.5 |
| Ca ²⁺ | 0.64-0.98 |
| Mg ²⁺ | 0.06-0.19 |
| K ⁺ | 0.22-0.45 |
| Na ⁺ | 0.01-0.19 |
| Ion-exchanging selectivity: Cs>NH ₄ ⁺ >Pb ²⁺ >K ⁺ >Na ⁺ >Mg ²⁺ >Ba ²⁺ >Cu ²⁺ >Zn ²⁺ | |
| Chemicals absorbed: NH ₃ , hydrocarbons C ₁ -C ₄ , CO ₂ , H ₂ S, SO ₂ , NO _x , aldehydes | |
| Toxicity: nontoxic according to US Code of Federal Regulations (21 CFR 82, Subpart C) | |

activity was determined according to EN ISO 20645 Textile fabrics - Determination of antibacterial activity - Agar diffusion plate test [14].

Table 2. Labels and treatments of cotton and polyester fabrics

| Label | Treatment |
|-------|--|
| CB | Bleached cotton fabric with H ₂ O ₂ |
| CBM | Bleached mercerized cotton fabric in 24 % NaOH |
| CBZ | Bleached cotton fabric impregnated with 5/g l zeolite |
| CBMZ | Bleached mercerized cotton fabric impregnated with 5 g/l zeolite |
| PET | Untreated polyester fabric |
| PETH | Hydrolized polyester fabric in 1,5 mol/l NaOH |
| PETZ | Polyester fabric impregnated with 5 g/l zeolite |
| PETHZ | Hydrolized polyester fabric impregnated with 5 g/l zeolite |
| A | AZI by exhaustion method |

RESULTS AND DISCUSSION

Activated zeolite in diverse biological activities

Amongst other useful properties and application the activated zeolite possesses diverse biological activities successfully used previously as a vaccine adjuvant and for the treatment of diarrhea. The activated zeolite plays an important role in regulation of the immune system containing a high portion of silica, silicates and aluminosilicates act as non-specific immunostimulators like superantigens [2].

Superantigens (SAG) are a class of immunostimulators and disease causing proteins of bacterial and viral origin with the ability to activate a relatively large fraction of T cell population. Where immunity is reduced, zeolite activates B and T cells populations. In the disease of autoimmune system, it lowers the cancer marker CD 56 [15].

It is well known that protein kinase stimulates oncogenes which results in the mutation of DNA and creation of malignant cells. The activated zeolite inhibits protein kinase B (c-Act) to other kinase included in the apoptosis process and provokes cancer. In this way it prevents the creation of malignant cells [5].

Zeolite has antibacterial, antioxidative and immunostimulatory property.

The local application of such elite to animal skin cancers effectively reduced tumor formation and growth. It was shown that it might affect the cancer growth by attenuating survival signals and inducing tumor suppressor genes in treated cells.

Having antiviral properties, the activated zeolite used locally, on the skin, has a therapeuticall appli-

cation against the herpes virus infection or orally in the cases of adenovirus or enter virus infections.

It is to point out that viruses are very small and on nonsocial level, from 65-80 nm for adenoviruses and enter viruses to 100-200 nm for herpes viruses. Antiviral effects of the activated zeolite can be explained by incorporation of virus particles into the pores of such crystals [10].

Antioxidative effects

The activated zeolite has very strong antioxidative properties that could affect an oxidative stress in cancer and diabetes patients. Additionally, *diabetes mellitus* patients can use zeolite for the reason of its glucose high absorbance.

Free radicals are the main factors of many pathological changes in organisms. It was found that around 90% of various diseases are results of disturbances in a cell function or cell damage caused directly or indirectly by the activity of oxygen free radicals.

Free radicals derived from oxygen have been implicated in numerous pathological processes including inflammation, reperfusion injury, hemorrhagic shock, autoimmune diseases, neurological disorders, *diabetes mellitus* and cancer [16].

It is confirmed that activated zeolite acts against disease *Psoriasis vulgaris* on the base of antioxidative activity [17].

Total antioxidant status (TAS) measures the concentration of antioxidants in the organisms. From many results it is shown that the activated zeolite influenced an increase in TAS value for more than 26% which is in comparison to known antioxidants (A, C, and E vitamins, flavonoids, melatonin etc.) ten time more effective [7].

Currently, various antioxidants are under clinical investigation as an adjunct to standard and experimental cancer therapies. Anticancer activity of antioxidants may not only be attributed to their radical scavenger properties, but also to direct modulation of cellular signal transduction pathways, resulting in growth arrest and apoptosis of cancer cells.

Recently, it has been shown that 4 weeks of oral supplementation with activated zeolite resulted in restoration of previously increased antioxidant levels (Randox Total Antioxidant Status) and decreases of free radicals (d-ROM-s Free Radical Analytical System) in plasma of cancer patients.

Beside its antioxidant capacity, activated zeolite demonstrated anticancer activity *in vitro* tissue cultures by inhibition of protein kinase B (c-Act) and the induction of expression of tumor suppressor proteins, independently from p53 protein. The blockade of the

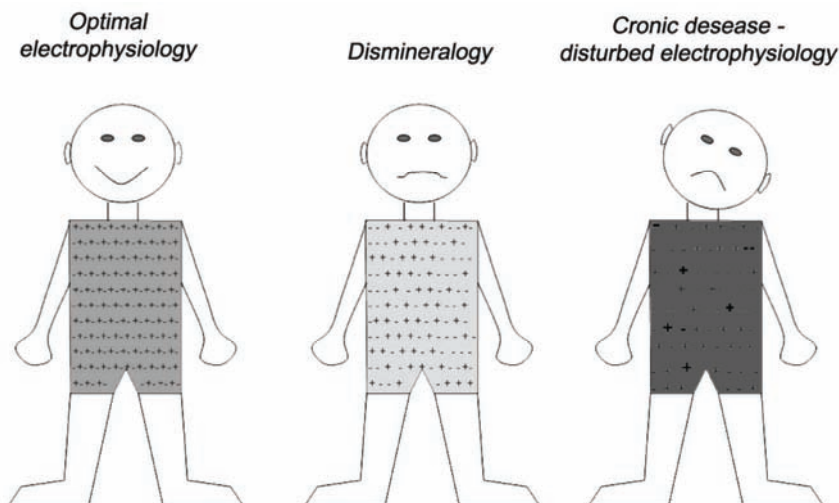


Figure 2. Human body is electric charged with examples of models for optimal electrolytic regulation and its disorders [2].

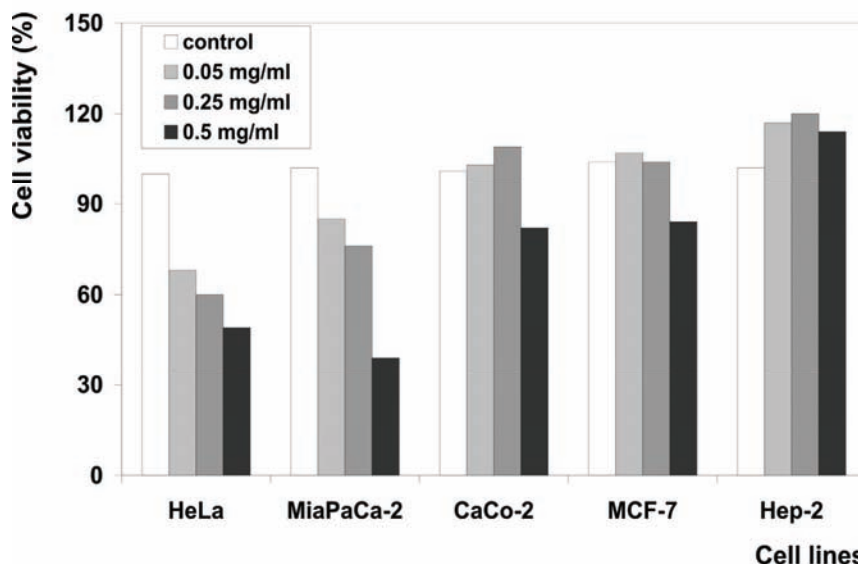


Figure 3. The effect of activated zeolite treatment on growth of different human cell lines. The results are represented as a percentage of growth control cells. Legend: HeLa - cervical carcinoma; MiaPa-Ca-2 - pancreatic carcinoma; CaCo-2 - colon carcinoma; MCF-7 - breast cancer; Hep-2 - laryngeal carcinoma.

cell growth has been shown in several cancer cell lines | Figure 3.

The effect of the activated zeolite on cell proliferation *in vitro* was studied on several human cell lines.

The cell viability was determined using MTT assay (from Sigma) which detects dehydrogenase activity in viable cells.

The effect of this zeolite on the growth of next human cell lines is shown in Fig. 3: HeLa-cervical carcinoma; MiaPaCa-2-pancreatic carcinoma; CaCo-2 - colon carcinoma; MCF-7 - breast cancer; Hep-2 - laryngeal carcinoma. The high inhibitory effects of zeolite are obtained with two cell lines, HeLa and

MiaPaCa-2 what confirmed the recent results on the zeolite inhibition of protein kinase B (c-Act).

Additionally, the combination of activated zeolite with doxorubicin for the treatment of mammary carcinoma-bearing mice was significantly more effective in reducing the number pulmonary metastases when compared to doxorubicin mono-therapy [7,18].

TMAZ in healing wounds

In the wound healing there are five important requirements: moist environment, antimicrobial, non-toxic to human cells, hypoallergenic and continuous debridement. Activated zeolite just follows these requirements having for such a reason favorable effects

on the healing process in the next superficial skin wounds:

Acute skin problems - mechanical skin damage, thermal burns, abrasions, insect bites, post-operative wound treatment, herpes simplex and herpes zoster.

Chronic skin problems - various skin infections, allergic reaction, degenerative diseases, neurodermatitis, ulcer cruris, decubitus ulcers, etc.

Nanoparticles of a natural zeolite (clinoptilolite) applied on textiles

Nanoparticles of a natural zeolite (clinoptilolite) applied on textiles are following the tendency for new textile applications as materials for human performance, as are medical, protective and sports application. During the last few years these tendencies led to rapid growth of different protective finishes, material modification and application of nanoparticles, as well. Textile and clothing is a human's second skin, therefore it is the most suitable interface between the environment and a human body. It is an ideal tool for personal protection and safety [19].

UV protection is expressed *via* ultraviolet protection factor (UPF) indicating the ability of protection by textile materials to prevent erythem. From the results shown in Figure 4 it is evident that polyester fabric gives off better UV protection than cotton fabric after surface modification and treatment with nanoparticles of zeolite due to polyester chemical constitution.

A polyester fiber has double bonds in a polymer chain which can absorb small amounts of UV-R. Nevertheless, small amounts of UV-R are reflected from polyester multifilament. After FWA treatment it is evident that cotton fabric absorbs higher amounts of FWA. Bleached cotton is non-rateable for UV protection. Mercerization, as standard cotton modification,

results in higher cotton absorbability and little increment of UPF, because fabric shrinks.

Nanoparticles of zeolite on the fabric surface scatter UV-R resulting in a better UV protection. Additionally, zeolite increases the fabric surface area resulting in higher adsorption of FWA. Therefore, mercerized and zeolite treated cotton fabrics give off an excellent UV protection after FWA.

UPF values for polyester fabric show similar behavior as for cotton fabric. Modification of polyester fabric results in little better UV protection, while treatment with nanoparticles of zeolite results in good UV protection. FWA treatment of polyester results in good protection as well. Meanwhile, FWA treatment to polyester fabric with zeolite results in very good UV protection.

Fabric antimicrobial activity was determined according to EN ISO 20645. The results are shown in Table 3.

It is clearly shown in Table 3 that traditionally pretreated cotton fabrics - bleached and mercerized - have no antimicrobial activity. Impregnation with zeolite (BMZ) results in an antimicrobial activity to both, Gram positive as well as Gram negative *Klebsiella pneumoniae* bacteria.

Azalide compound showed an excellent antimicrobial activity on Gram positive as well as Gram negative bacteria. It is evident that zeolite treated fabrics, and treated with AZI show off the best results. Therefore, synergism between zeolite and azalide is confirmed.

CONCLUSION

It was shown that when applied on textile, clinoptilolite increases the active surface area. If added

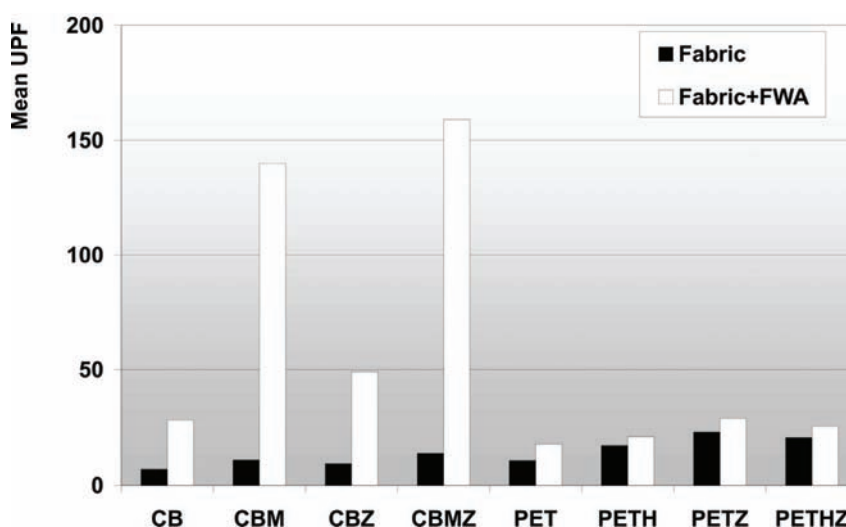


Figure 4. Mean UPF values of cotton and polyester fabrics after surface modification and treatment with zeolite and FWA's.

Table 3. Antimicrobial activity of treated woven fabrics on Gram positive *Staphylococcus aureus* and Gram negative *Klebsiella pneumoniae*

| Sample | <i>Staphylococcus aureus</i> | | <i>Klebsiella pneumoniae</i> | |
|--------|------------------------------|----------|------------------------------|----------|
| | D / mm | Activity | D / mm | Activity |
| CB | 24 | no | 24 | no |
| CBA | 42 | high | 30 | high |
| CBM | 24 | no | 24 | no |
| CBMA | 44.5 | high | 39.5 | high |
| CBZ | 25 | small | 24 | no |
| CBZA | 42 | high | 37 | high |
| CBMZ | 34.5 | high | 29 | small |
| CBMZA | 48 | high | 42 | high |

to azalides in textile finishing, they increase the efficiency of antimicrobial action to both - Gram positive as well as Gram negative bacteria. It is to point out that there is a very high and unexpected synergistic effect of activated zeolite and azalide compound for achieving UV protection. It is to conclude that cotton fabrics treated with nanoparticles of zeolite show high synergistic protection of cotton fabrics even after washing.

Zeolite gives a contribution to UV protection by scattering UV radiation unlike other agents which absorb this radiation preventing their transmission. Treated textiles achieved a high level of UV protection. FWA treatment leads to excellent UV protection for cotton and very good protection for polyester. Even though natural zeolites are yellowish they do not affect fabric whiteness significantly. It is to point out that nanoparticles of zeolite and FWA give off a synergistic effect in fabric UV protection.

Based on a small part of presented biological properties, it is to conclude that textile and clothing with activated zeolite can protect the skin, showing effects on the healing process in the skin wounds and therapy effect against different skin diseases like, psoriasis and cancer.

On the base of wide described zeolite properties as its high adsorbance, microporous structures, microclimate condition, antioxidative effects, UV protection, antimicrobial and antiviral protection, it is to conclude that activated zeolite:

1. adsorbs sweat and stops unpleasant odors,
2. cleans the skin from oxidants and free radicals,
3. protects the body against harmful UV emission,
4. protects the body from microorganisms and prevents its access and activity,
5. constantly holds the body temperature,
6. increase energy levels of entire body,

7. protects of rheumatic problems,
8. enhances exchanges of substances in the body and helps the skin "breath",
9. has favorable effects on the healing process in the skin wounds,
10. regulates the pH values of skin and
11. is very comfortable to wear.

Tribomechanical activated zeolite on textiles promises multifunctional properties and different biological effects in the near future what requires further research work.

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