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# Antimicrobial activity of AgCl embedded in a silica matrix on cotton fabric

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ABSTRACT

An antimicrobial finishing for cotton fabric was prepared from commercial (iSys AG, Germany) silver chloride (Ag) dispersed at different concentrations in a reactive organic–inorganic binder (RB) (iSys MTX (CHT, Germany). Pad-dry-cure and exhaustion methods were used for the sols application, giving Ag-RB coating with Ag concentration from ca. 48 to ca. 290 ppm on the cotton fabric. The presence of silver on the cotton finishes was confirmed by measuring its concentration in the fabrics with the help of inductively coupled plasma mass spectroscopy (ICP-MS). The morphology of the finished fabrics was investigated by SEM, while their composition was established from EDXS measurements combined with the results of FT-IR spectral analysis. The antimicrobial activity of variously treated cotton fabrics was assessed before and after repetitive (up to 10×) washing by the application of standard tests: for the fungi *Aspergillus niger* (ATCC 6275) and *Chaetomium globosum* (ATCC 6205) by the modified DIN 53931 standard method, while the presence of Gram-negative bacterium *Escherichia coli* (ATCC 25922) was followed by using ISO 20645:2004 (E) and AATCC 100-1999 standard methods. Results revealed that the antimicrobial activity of the coatings strongly depended on the concentration of Ag in the corresponding Ag-RB dispersions, indirectly depending on the preparation method (pad-dry-cure vs. exhaustion) and that the Ag-RB coatings were more effective for bacteria than for fungi. The Ag concentrations on the cotton fabrics achieved by the pad-dry-cure method (48 and 52 ppm) were not sufficient to impart satisfactory antifungal activity to the cotton fabrics, though they assured excellent reduction of the bacterium *E. coli* (98–100%). A minimal inhibitory concentration of Ag in the coating providing a sufficient bacterial reduction of 60% was ca. 24 ppm. Effective antifungal activity was achieved only by applying the exhaustion method, enabling high initial Ag concentration in the Ag-RB coating (>100 ppm). The antibacterial activity depended on the washing treatment. No antifungal activity was noted for washed cotton fabric, even those with highly concentrated Ag (290 ppm) in the Ag-RB coating, but a 94% bacterial reduction was obtained for the corresponding cotton fabric, after 10 repetitive washings, corroborated by the Ag concentration on washed fabric of about 65 ppm.



## 1. Introduction

Antimicrobial finishing for textile fibres has attracted much attention in recent years (Haufe, Thron, Fiedler, Mahltig, & Böttcher, 2005; Holme, 2007; Mahltig, Fiedler, & Böttcher, 2004; Mahltig, Haufe, & Böttcher, 2005; Purwar & Joshi, 2004). It has become necessary in the production of protective, decorative and technical textiles. As a consequence of its importance, the number of different antimicrobial agents suitable for textile application on the market increased drastically. Selection of an antimicrobial agent depends on several criteria, such as the mechanism of antimicrobial activity, its effectiveness for bacteria and fungi, toxicity, application method, washing fastness and cost ( Heywood, 2003; Schindler & Hauser, 2004).

Silver and silver-based compounds are well-investigated antimicrobial agents (Dubas, Kumlangdudsana, & Potiyraj, 2006; Gorenšek & Recelj, 2007; Jiang, Newton, Yuen, & Kan, 2007; Kulpinski, 2007; Lee, Cohen, & Rubner, 2005; Lee & Jeong, 2004; Lee, Park, Lee, Kim, & Park, 2007; Maneerung, Tokura, & Rujiravanit, 2008; Ortiz-Ibarra et al., 2007; Parkih et al., 2005; Son, Youk, & Park, 2006; Sondi & Salopek-Sondi, 2004) being biocompatible and non-toxic to human cells at concentrations effective against microorganisms (Lee & Jeong, 2005) when in the form of non-agglomerated and well dispersed nanoparticles. Silver-based agents are not chemically bonded to the textile fibres and their antimicrobial activity is attributed to their gradual and persistent release from the textile into the surroundings, where they act as a poison to a

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wide spectrum of bacteria and fungi. It is believed that silver binds with protein molecules, causing inhibition of cellular metabolism and final eradication of microorganisms. The antimicrobial efficiency of silver depends directly on its concentration, which should not drop under the limit value required for minimal inhibition. The release of Ag from the fibre surface into its surroundings leads to problems related to their uniform dispersion and embedment in the polymer host, rarely achieved by nanometallic particles incorporated into organic polymers. Sol–gel finishing based on either single-capped (R′—Si(OR)$_3$, R′ = organic group (acryl, epoxy, isocyanato, isooctyl perfluoro ...) or bis end-capped ((RO)$_3$ Si—R″—Si(OR)$_3$ , R″ = organic polymers) silane precursors are a good alternative to organic networks, enabling the uniform dispersion, embedment and controlled release of antibacterial agents such as silver nanoparticles (Akkopru & Durucan, 2007; Haufe et al., 2005; Jeon, Yi, & Oh, 2003; Kim, Lee, Cha, Kim, & Kang, 2007; Mahltig et al., 2004; Mahltig et al., 2005; Tarimala et al., 2006; Tomšič et al., 2008; Xing, Yang, & Dai, 2007).

The gradual release of the silver particles from a modified organic–inorganic silica matrix bonded onto the textile surface allows the effective antimicrobial properties of textiles to be sustained over a long time and even after several washings. Such sol–gel finishing, however, has mostly been carried out on the laboratory level and has not yet been widely used in industrial processes because of its cost and the additional processing equipment required. Namely, most commercially available silicon alkoxyde precursors are soluble in alcohol, which requires special safety precautions. This has led to increasing interest on the development of water-based silica sol solutions simplifying their industrial applications. Xing et al. (2007) recommended water glass as a precursor for treatment of cotton fabrics followed by impregnation of the fabric in an $AgNO_3$ solution. In order to simplify the application procedure, it was also suggested to impregnate the fabric in a mixture of silver salt and silica sol at the same time. Accordingly, stimulated by the need for industrial applications requiring water-born alkoxysilane-based finishes, in this study we used an antimicrobial agent iSys AG in combination with iSys MTX (CHT, Germany) in order to impart antibacterial and antifungal properties to cotton fabrics. The former is a dispersion containing AgCl and the latter is a reactive organic–inorganic binder (RB) having an undisclosed structure and composition. Both precursors can be mixed with water to any desired concentration, enabling the simulation of actual industrial processing. The corresponding precursors have been already used in combination with commercial fluoroalkoxysilane for making hydro- and oleophobic- coating for cotton fibres with high washing fastness (Tomšič et al., 2008).

This work consisted of two parts. In the first, we focused on the assessment of the Ag concentration on the cotton samples treated by the pad-dry-cure (P for short) and exhaustion (E for short) methods before and after repetitive washing. In order to determine the concentration of Ag on the cotton samples before and after repetitive washing, inductively coupled plasma mass spectroscopy (ICP-MS) was used, while the presence of the coating and its structural features was assessed from SEM and EDXS measurements followed by analysis of vibrational spectra obtained from IR ATR spectra measurements of the finished cotton fabrics.

The second part of this work was devoted to the assessment of the antimicrobial properties of non-washed and washed finished cotton fabrics, treated by P and E methods, against the fungi *Aspergillus niger* and *Chaetomium globosum* and the bacterium *Escherichia coli*. The results of the antimicrobial test were correlated with the Ag concentrations derived from ICS-MS measurements, showing that beside the initial concentrations in the Ag in Ag-RB dispersions, the application method also affected the fungicidal and bactericidal properties of Ag-RB treated cotton fabrics. The significance of this research was also the assessment of fungicidal activity of the cotton fabric, treated by the Ag based antimicrobial agent, which has been very rare in the literature up to now.

## 2. Experimental

### 2.1. Materials

Plain-weave 100% cotton woven fabric with a mass of 164 g/m$^2$ was used in the experiments. In a pre-treatment process the fabric was bleached in an $H_2O_2$ bath, mercerised in a NaOH solution and neutralized in a diluted $CH_3COOH$ solution.

As an antimicrobial agent, iSys AG was used in combination with iSys MTX (CHT, Germany). The former is a dispersion containing AgCl (Ag) and the latter is a reactive organic–inorganic binder (RB). All products can be mixed with water to any desired concentration.

### 2.2. Finishing of cotton fabric

The Ag-RB finish was applied to cotton fabric by the pad-dry-cure and by the exhaustion method. For the pad-dry-cure method, a sol consisting of 3.0 g/l of Ag and 15.0 g/l of RB was used. This method included full immersion at 20 °C, wet-pick-up of 80 ± 1% at 20 °C, drying at 120 °C and 1 min of curing at two different temperatures, 150 °C (sample P1) and 170 °C (sample P2). The finish concentrations and the application conditions were used as recommended by the producer.

For the exhaustion method, two different concentrations of Ag were used; 3.0 g/l, which is equivalent to 0.15% o.w.f. (sample E1), and 6 g/l, which is equivalent to 0.30% o.w.f. (sample E2), in combination with either 15 g/l or 30 g/l of RB, respectively. The samples were immersed in Ag-RB sol–gel solutions with a ratio of 1:50 and left at room temperature with occasional stirring until equilibrium was achieved. Afterwards, the samples were wrung to a wet-pick-up of 80 ± 1%, dried at 120 °C and cured at 150 °C for 1 min.

After the application of the sols, the samples were left for 14 days to complete network formation of the applied finishes. Each sol was applied to 4 fabric samples (20 × 30 cm) in order to provide a sufficient number of replicate samples for carrying out measurements and statistical analysis.

### 2.3. Washing procedure

The washing fastness of the Ag-RB coatings was determined after repetitive washing in an AATCC Atlas Launder-O-Meter Standard Instrument, which is widely used for evaluating laundry results on a laboratory scale. One wash in a Launder-O-Meter (ISO 105-C01:1989(E) standard method) provides an accelerated washing treatment corresponding to five home washings. The finished fabric samples were washed repetitively up to 10 times; the duration of the washing cycles was 30 min and was carried out in a solution of SDC standard detergent with a concentration of 5 g/l, previously heated to 40 °C, to give a liquid ratio of 50:1.

After washing, the samples were rinsed in cold distilled water, held under a cold tap water for 10 min and squeezed and dried at room temperature. The quality of the coatings was assessed after the first and tenth washing cycles.

### 2.4. Analyses and measurements

#### 2.4.1. Fungicidal activity

The fungicidal activity of the Ag-RB-treated cotton samples was estimated for the fungi *A. niger* (ATCC 6275) and *C. globosum* (ATCC 6205) according to the modified DIN 53931 standard method,

where synthetic nutrient-poor agar (SNA) (Nirenberg, 1976), consisting of 1 g of $KH_2PO_4$, 1 g of $KNO_3$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.2 g of glucose, 0.2 g of saccharose and 15 g of technical agar per 1 l distilled water was used instead of the prescribed malt-extract agar (MEA). SNA is a less nutritious cultural medium, allowing a more delicate colony growth and easier evaluation of the antifungal activity of Ag. Thirty μl of a spore-suspension ($10^5$/ml were spread on each SNA plate. The inoculated plates were incubated at 29 °C for 24 h. Afterwards, samples of cotton fibres 5 × 5 cm were placed on the medium and incubated at 29 °C for 7 and 14 days. After incubation, the fungicidal activity was determined in terms of mycelial growth on and below the surface of the cotton fibres and the intensity of sporulation. To determine fungal development below the finished samples, the cotton samples were removed from the agar plate and the medium was examined microscopically. A droplet of cotton blue dissolved in lactic acid was placed on the medium and covered with a glass slip, allowing the detection of the stained fungal mycelium at low magnification. The degree of fungal growth was ordered in eight grades from 00 to 5, where 00 indicates no growth, 0 fungal growth outside an inhibition zone surrounding the cotton specimen, (0) fungal growth up to the specimen's edge, (1) fungal growth only on and below the specimen's edge, (2) fungal growth on and below less than 25% of the specimen, (3) fungal growth on and below 25–75% of the specimen, (4) fungal growth on and below more than 75% of the specimen and (5) 100% overgrowth of the specimen. The intensity of sporulation was assessed using the following symbols: – means clear, without mycelium; + weak, only mycelium; ++ noticeable growth, partly spores; and +++ strong overgrowth, extensive spore formation.

*2.4.2. Bactericidal activity*

The antimicrobial activity of the Ag-RB treated samples was estimated for the Gram-negative bacterium *E. coli* (ATCC 25922) using two different standard methods, i.e. ISO 20645:2004 (E) and AATCC 100-1999.

By following ISO 20645:2004 (E) standard method, known as the Agar diffusion plate test, two-layered agar plates were prepared. The lower agar layer consisted of 10 ml of ordinary agar; the upper layer consisted of 5 ± 1 ml agar inoculated with bacteria, whereby 1 ml of bacteria working solution with a concentration of 1–5 × $10^8$ CFU was added per 150 ml of agar. Circular pieces of cotton, 25 ± 5 mm in diameter, were uniformly pressed on the agar and incubated for 24 h at 37 ± 1 °C. After incubation, assessment was 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:

$$H = \frac{D - d}{2} \quad (1)$$

where $H$ is the inhibition zone in mm, $D$ is the total diameter of the cotton specimen and inhibition zone in mm, and $d$ is the diameter of specimen in mm. For bacterial growth, the contact zone under the samples was also determined with a microscope at 20-times magnification. Following the standard method, the inhibition zone was measured in mm and the degree of bacterial growth was estimated in the nutrient medium under the specimen. The antibacterial effect of the studied samples was described either "good", "limited" or "insufficient". All tests were performed in duplicate.

According to the AATCC 100-1999 standard method, circular swatches of finished cotton samples, 4.8 cm in diameter, were put into a 250 ml Erlenmeyer flask and inoculated with 1.0 ml of a nutrient broth culture containing 1–2 × $10^5$ CFU of bacteria. An unfinished cotton sample was used as a control. After incubation at 37 °C for 24 h, the bacteria were eluted from the swatches by shaking them in 100 ml of neutralizing solution for 1 min. After making serial dilutions with sterilized water, the suspensions were plated on nutrient agar and incubated at 37 °C for 24 h. Afterwards, the number of bacteria forming units (CFU) was counted, and the reduction of bacteria, $R$, was calculated from:

$$R = \frac{(B - A)}{B} 100(\%) \quad (2)$$

where $A$ is the CFU recovered from the inoculated cotton sample swatch in the jar incubated over the desired contact period (24 h), and $B$ is the CFU recovered from the inoculated cotton sample swatch in the jar immediately after inoculation (at "0" contact time). For each finished cotton fabric, three treatments were performed on two samples.

*2.4.3. Fourier transform infrared (FT-IR) spectroscopy*

FT-IR spectra were obtained on a Bruker IFS 66/S spectrophotometer, equipped with an attenuated total reflection (ATR) cell (SpectraTech) with a Ge crystal ($n = 4.0$). The spectra were recorded over the range 4000–600 $cm^{-1}$, with a resolution of 4 $cm^{-1}$ and averaged over 128 spectra. Before measurement, the studied samples were dried to constant mass.

*2.4.4. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDXS)*

The morphology and the composition of the coatings on cotton fabrics were investigated in a JEOL JSM 5800 scanning electron microscope (SEM) equipped with an Oxford-Link ISIS 300 EDXS system with an ultra-thin window Si(Li) detector. The samples for SEM and EDXS analyses were coated with ≈30-nm-thick carbon layer to ensure sufficient electrical conductivity and to avoid charging effects. Analyses were performed using a 10-keV electron beam, 200–500 pA beam current and X-ray spectra acquisition under a 35° take-off angle. SEM micrographs were recorded using both secondary electron (SE) and backscattered electron (BSE) imaging modes. BSE compositional (Z-contrast) imaging was applied to emphasize and expose the difference between the added particles and the cotton fibre-matrix.

*2.4.5. Inductively coupled plasma mass spectroscopy (ICP-MS)*

The concentration of Ag in the finished cotton bulk samples was determined by ICP-MS on a Perkin Elmer SCIED Elan DRC spectrophotometer. A sample of 0.5 g was prepared in a Milestone microwave system by acid decomposition using 65% $HNO_3$ and 30% $H_2O_2$. For each sample, three measurements were made and the Ag concentration was given as a mean value.

*2.4.6. Illumination with artificial light*

The coloration of the finished cotton samples under artificial light was determined using an air-cooled xenon arc lamp apparatus Xenotest 150 (Original Hanau, Germany) according to the ISO 105-B02: 1994(E) standard method. After the illumination the value of CIE Whiteness Index, *WI*, was calculated for each sample from the spectrophotometric measurements using a Datacolor Spectraflash SF 600 Plus-CT spectrophotometer (Datacolor International) according to the EN ISO 105-J02: 1997(E) standard.

## 3. Results and discussion

### 3.1. Characterisation of the Ag-RB coating

The ATR technique (Vince et al., 2006) was used to provide valuable information about the molecular groups and species present in the Ag-RB coating. Because of the very strong bands ascribed to cotton in the 1150–900 $cm^{-1}$ spectral region which could blur the detailed absorption of the finish in this region (Fir et al.,

2007; Tomšič, Simončič, Orel, Vilčnik, & Spreizer, 2007; Vince et al., 2006), Ag-RB and RB were deposited on a Si wafer. Inspection of the coating spectra in Fig. 1 revealed bands of Si—O—Si linkages at 1130, 1075 and 1025 $cm^{-1}$ (shoulder), indicating that the silica network was formed during the RB condensation process, and capable of incorporation of Ag particles (Haufe et al., 2005). Since no sign of the Ag salt was detected in the ATR spectrum of the Ag-RB coating (Fig. 1), SEM, EDXS and ICP-MS analyses were performed to confirm the presence of Ag particles in the coating and to determine the size and the shape of the particles and their concentration in the finished cotton fabric samples before and after repetitive washing.

The SEM micrographs in Fig. 2 show the morphological changes and the distribution of Ag particles induced by the application of Ag-RB to the cotton fabric. Spherically shaped silver particles were observed in samples P1 and E1 with a particle grain size ranging from 100 to 500 nm. From comparison of the SEM/BSE micrographs it was evident that the concentration of Ag particles in sample E1 is much higher than in the sample P1, as expected. This was attributed to the longer contact time between the sample and the sol during the exhaustion process, which enabled higher concentration of Ag particles to be adsorbed by the fibres. Qualitative EDXS microanalysis was performed on several larger individual particles and/or on particle agglomerates. Typical EDXS spectra (Fig. 3) of P1 and E1 samples are equivalent and clearly reveal characteristic peaks which belong to the family of Ag-Lα spectral lines. The presence of the Cl-Kα peak was consistent in all spectra and additionally indicated that the particles in the coating did not consist of pure metallic silver, but their chemical composition corresponded to silver-chloride (AgCl) salt. The peaks of C-Kα and O-Kα originated mainly from the carbon and oxygen that were present in the cotton fibre and partially from the applied carbon coating.

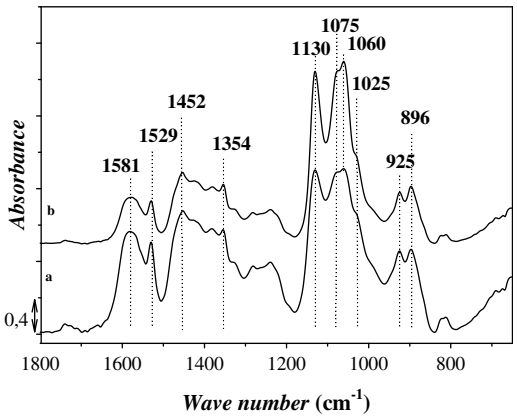


**Fig. 1.** ATR IR spectra of RB (a) and Ag-RB (b) coatings on a Si wafer in the 1800-650 $cm^{-1}$ spectral region.

SEM micrographs of the 10× washed samples P1 and E1 (Fig. 4a,b) showed that Ag (AgCl) particles are no longer present and were removed from the cotton fabric during the washing process. In addition, the BSE micrographs revealed some bright inclusions/particles that remained embedded in the fabric after washing. EDXS analysis of such an inclusion marked in Fig. 4b, is shown in Fig. 4c where the dominant Si-Kα, Zr-Lα and O-Kα peaks indicate that the silica matrix, which was formed by RB in the presence of the catalyst, is still present on the fibres.

An ICP-MS analysis was performed in order to determine the bulk concentration of Ag particles on the cotton samples. The variation of Ag concentration on the cotton samples caused by the different finish application methods and the repetitive washing is presented in Table 1. The results revealed that the application method strongly influenced the concentration of Ag on the cotton

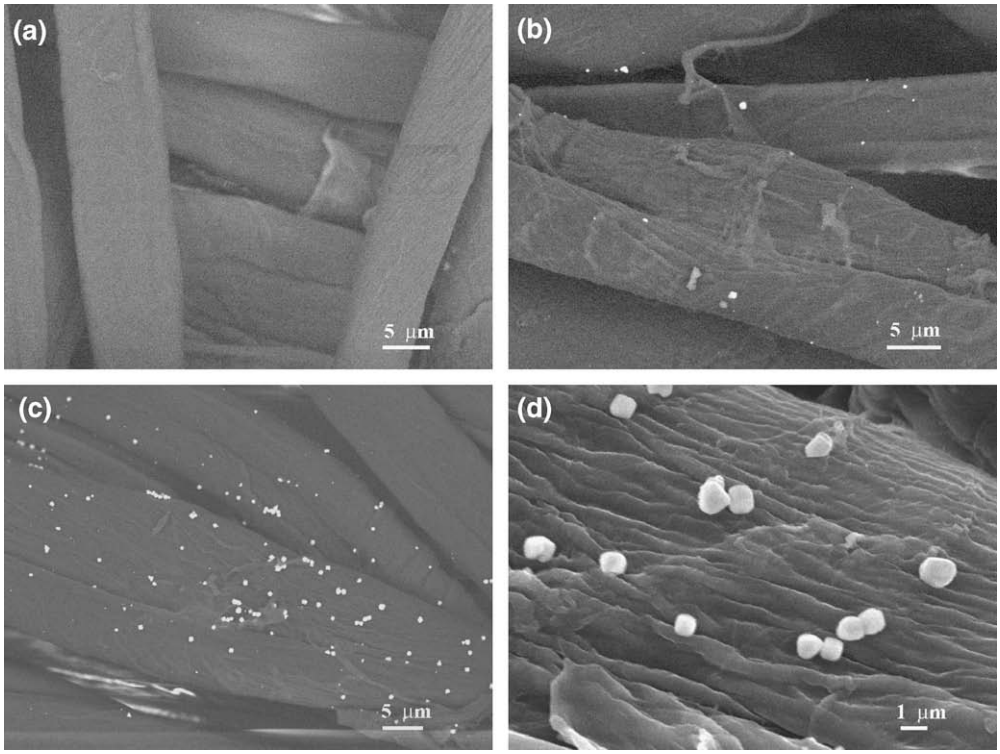


**Fig. 2.** SEM/BSE micrographs of cotton fabric: (a) unfinished sample, (b) unwashed sample P1, (c) unwashed sample E1 (d) SEM/SE detailed image of Ag-particles in sample E1.

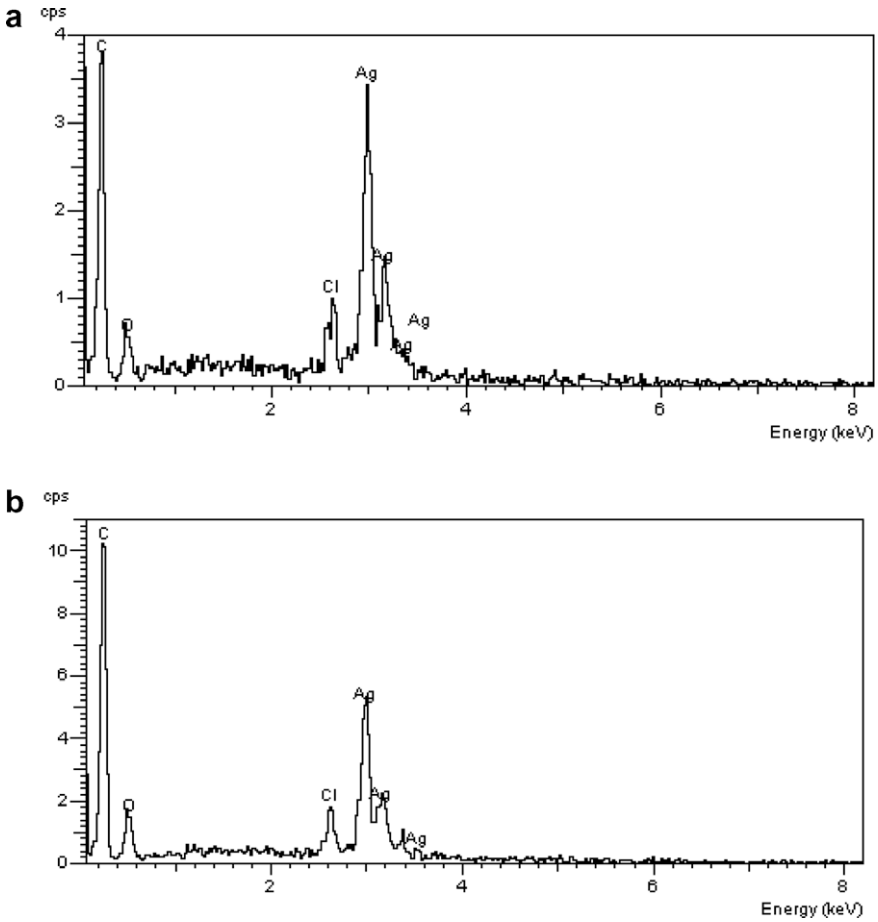


**Fig. 3.** EDXS spectra acquired from coating particles in the samples P1 (a) and E1 (b).

fabric. Thus, using the same initial concentration of Ag in the sol, the pad-dry-cure application method resulted in a much lower concentration of adsorbed Ag particles (samples P1 and P2) in comparison to the exhaustion method (sample E1). As expected, an increase in the Ag concentration in the sol led to an increased concentration of Ag particles in the fibres by the exhaustion method (sample E2). The results also showed that the Ag concentration dropped over the course of repetitive washings due to the leaching of Ag particles, irrespective to the application method. The increase in the curing temperature from 150 to 170 °C for the pad-dry-cure method did not influence the washing fastness of the coating. The gradual decrease of the Ag concentration by repetitive washing suggested that this phenomenon could certainly affect the antimicrobial properties of the coating.

Application of finishes decreased the whiteness of samples (Fig. 5). It was expected, since the application of AgCl as well as the silica matrix resulted in slight yellowness of the samples. Illumination of samples additionally decreased the *WI* value, which was related to the Ag concentration in the sample. The higher the Ag concentration, the higher the drop of whiteness. These results indicated that coloration of the finished samples changed in sun-light due to formation of metalline silver particles.

### 3.2. Antimicrobial properties of the Ag-RB coating

Up to now, there have been only a few reports about the antifungal activity of Ag on cotton fabric, showing good growth reduction of various *Candida* and *Aspergillus* species (Hipler, Elsner, & Fluhr, 2006a; Hipler, Elsner, & Fluhr, 2006b; Jung et al., 2007). Surprisingly, there are no studies on the antifungal activity of silver doped cotton after repetitive washings, although the washing stability of the silver coating is of special importance for the textile. Therefore, the washing resistance of the antimicrobial activity of Ag-RB finished samples against the fungi *C. globosum* and *A. niger* and the bacterium *E. coli* was determined, while the correlation between the concentration of Ag on the cotton fibres and its antimicrobial properties was investigated. The results on the antifungal activity of Ag-RB coating (Table 2) confirmed its direct dependence on the Ag concentration on the cotton samples. A large surface area of P1 and P2 cotton samples overgrown with both fungi, indicated that an insufficient amount of Ag was applied on the fibres by the pad-dry-cure method in attempting to achieve effective antifungal activity. The Ag concentration on the unwashed finished samples was lower than 60 ppm and rapidly decreased after repetitive washing. Compared to the unfinished samples, the growth of both fungi on samples P1 and P2 was only slightly reduced. In this case, rich mycelium development and strong sporulation were observed on the sample surfaces.

Contrary to the samples P1 and P2, much better antifungal activity of Ag was seen on samples E1 and E2 samples, where the finish was applied by the exhaustion method. In this application method, the concentration of Ag reached 130 ppm for sample E1 and 290 ppm for sample E2, measured by the ICP-MS method. These concentrations were high enough to obtain sufficient antifungal activity (Table 2). However, the degree of growth inhibition differed for the two fungal species tested. The Ag finish in the unwashed samples E1 and E2 fully suppressed growth of *C. globosum* on and below the cotton but mycelium and ascomata developed well on the area of the nutrient medium surrounding the samples (Figs. 6Aa, Ba, and 7a). No inhibition zone was observed surround-

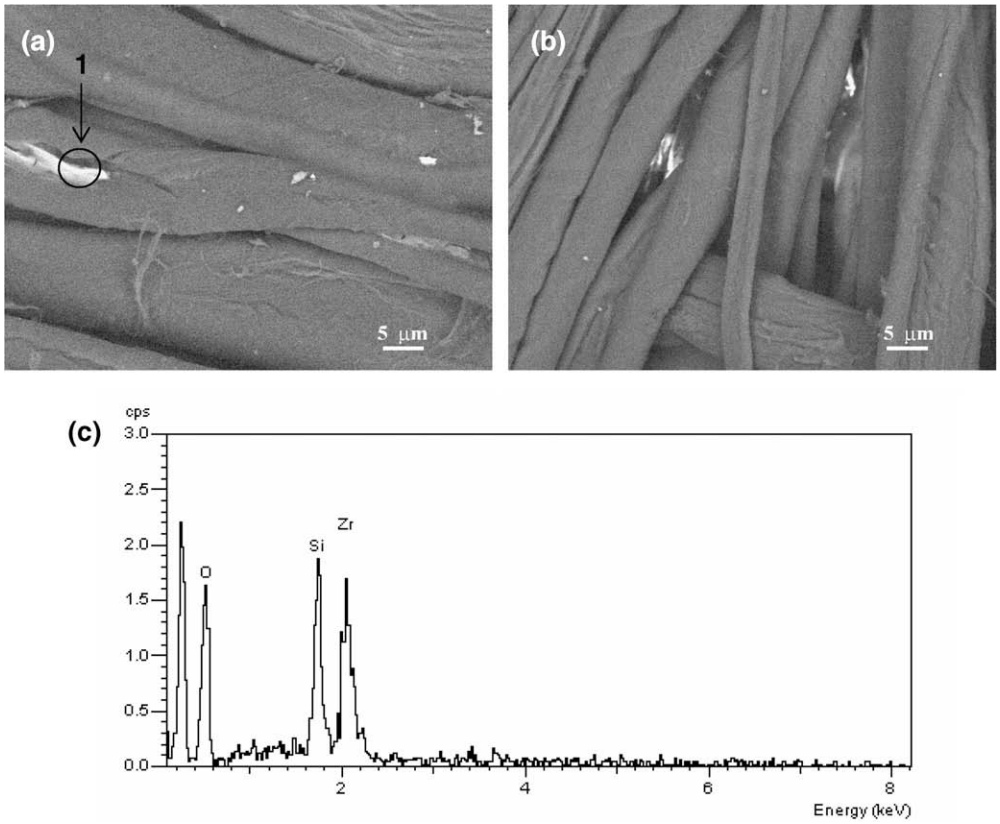


**Fig. 4.** SEM/BSE micrographs of cotton fabrics washed ten times for sample P1 (a) and sample E1 (b). (c) EDXS spectrum acquired from the typical inclusion "1" in the sample P1.

**Table 1**
Concentration of Ag in finished cotton samples using different application methods before (0 W), after a single washing (1 W) and after 10 repetitive washings (10 W) determined with ICP-MS

| Application method/sample | Concentration of iSys Ag in sol (% o.w.f.) | Concentration of Ag on cotton sample (ppm) | | |
|---|---|---|---|---|
| | | 0 W | 1 W | 10 W |
| P1 | 0.15 | 48 | 28 | 2.9 |
| P2 | 0.15 | 52 | 28 | 3.2 |
| E1 | 0.15 | 130 | 74 | 18 |
| E2 | 0.30 | 290 | 98 | 65 |

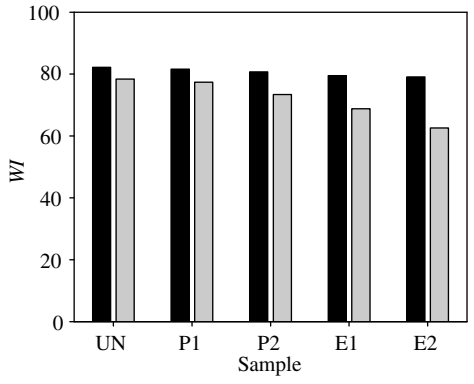


**Fig. 5.** CIE Whiteness Index, *WI*, for untreated (UN) and finished cotton samples P1, P2, E1 and E2 before (■) and after (■) illumination.

ing the samples. It seems that the Ag released did not dissolve effectively in the surrounding solid medium. A similar result was obtained after the cotton samples E1 and E2 were washed once (Figs. 6Aa, Bb, and 7b) but repeated washings apparently removed the antimicrobial agent Ag significantly, allowing growth of *C. globosum* on and below the cotton samples (Figs. 6Ac, Bc, and 7c).

The antifungal activity Ag was lower for *A. niger* than for *C. globosum*. While the sample E2 was totally unaffected by *C. globosum* (Figs. 6A, B, and 7a), an inspection of the sample E2 in contact with the medium previously inoculated by *A. niger* revealed that, in spite of the fact that there was no fungal growth present on the sample, a restricted mycelium with chains of candidia was found on some spots of the medium under the sample. These results indicated that the fungal growth was attacked by the silver ions but not completely inhibited. This was confirmed by the totally different fungal growth in the nutrient medium surrounding the sample.

In addition, the antibacterial activity of the Ag-RB finished samples was estimated for the bacteria *E. coli* by the Agar diffusion plate test. As expected, an insufficient antibacterial effect was obtained for the untreated cotton sample (Table 3), caused by a lack of an inhibition zone and a heavy bacterial growth on the medium under the sample (Fig. 8).

The bactericidal activity of Ag particles was much better than the fungicidal activity (Tables 2 and 3). Obviously, the amounts of Ag on the unwashed finished samples P1 (containing 48 ppm Ag) and P2 (containing 52 ppm Ag) were high enough for good antibacterial activity against *E. coli* (Table 3), but these concentrations did not affect growth of *C. globosum* and *A. niger* sufficiently (Table 2). While the antifungal activity of sample E2 was insufficient after the tenth repetitive washing (resulting in a Ag concentration of 65 ppm), its antibacterial properties were still effective. While an inhibition zone was not observed in tests against the two fungal species, Ag particles apparently diffused into the medium surrounding the finished cotton specimens in such a concentration that was high enough to suppress growth of *E. coli*

**Table 2**
Antifungal activity of Ag applied to cotton samples for *C. globosum* and *A. niger* after 7 days of incubation at 29 °C, according to the modified DIN 53931 standard method (for code explanation, see Section 2)

| Sample | Washing cycles | Growth | | Intensity of sporulation | |
|---|---|---|---|---|---|
| | | *C. globosum* | *A. niger* | *C. globosum* | *A. niger* |
| Untreated | / | 4 (>75) | 5 (100) | +++ | +++ |
| P1 | 0 | 3 (60) | 4 (>75) | +++ | +++ |
| | 1 | 3 (40) | 4 (65) | +++ | +++ |
| | 10 | 3 (45) | 4 (>75) | +++ | +++ |
| P2 | 0 | 3 (70) | 5 (100) | +++ | +++ |
| | 1 | 3 (30) | 4 (>75) | ++ | +++ |
| | 10 | 3 (45) | 5 (100) | +++ | +++ |
| E1 | 0 | [0] (0) | 1 (5) | / | + |
| | 1 | [0] (0) | 2 (<25) | + | ++ |
| | 10 | 3 (30) | 3 (60) | + | +++ |
| E2 | 0 | [0] (0) | 1 (5) | / | + |
| | 1 | [0] (0) | 1 (10) | / | + |
| | 10 | 2/3 (25) | 3 (50) | + | +++ |

sufficiently (Fig. 8). These results suggested that, in general, a lower concentration of Ag is required for effective antibacterial activity than for antifungal properties.

To determine the antibacterial activity of Ag present on the cotton fabric in a more quantitative way, the reduction of bacteria, *R*, in contact with the finished textile samples was calculated according to the AATCC 100-1999 standard method (Table 4). As expected, no reduction of *E. coli* bacteria was found on the unfinished cotton sample. Moreover, there was actually an increase in the number of bacteria recovered from the inoculated sample after 24 h of incubation compared to the sample at "0" contact time, indicating that *E. coli* can use pure cotton as a substrate. For all of the cotton samples P1, P2, E1 and E2, a high reduction of bacteria *E. coli* occurred on the unwashed finished cotton fabric. Obviously, the antibacterial activity dropped over the course of repetitive washing due to the leaching of Ag particles. As a result of this gradual leaching, no reduction of bacteria was noted after the tenth washing cycle for samples P1 and P2, while only a low and insufficient reduction was obtained for sample E1. The only exception was sample E2, where the Ag concentration dropped during the 10 washings from 290 ppm to 65 ppm, which resulted in a bacterial reduction of more than 90%. These results clearly show that the degree of bacterial reduction is directly related to the Ag concentration on the cotton fabric. A sufficient antibacterial activity of $R > 60\%$ was obtained at an Ag concentration of ca. 24 ppm (Fig. 9). It is important to note that this concentration was much higher than that reported by Xing et al. (2007), where only 5 ppm of $AgNO_3$ solution embedded in a $SiO_2$-fibre matrix reduced bacterial growth by 98.13%. Similar results were also ob-

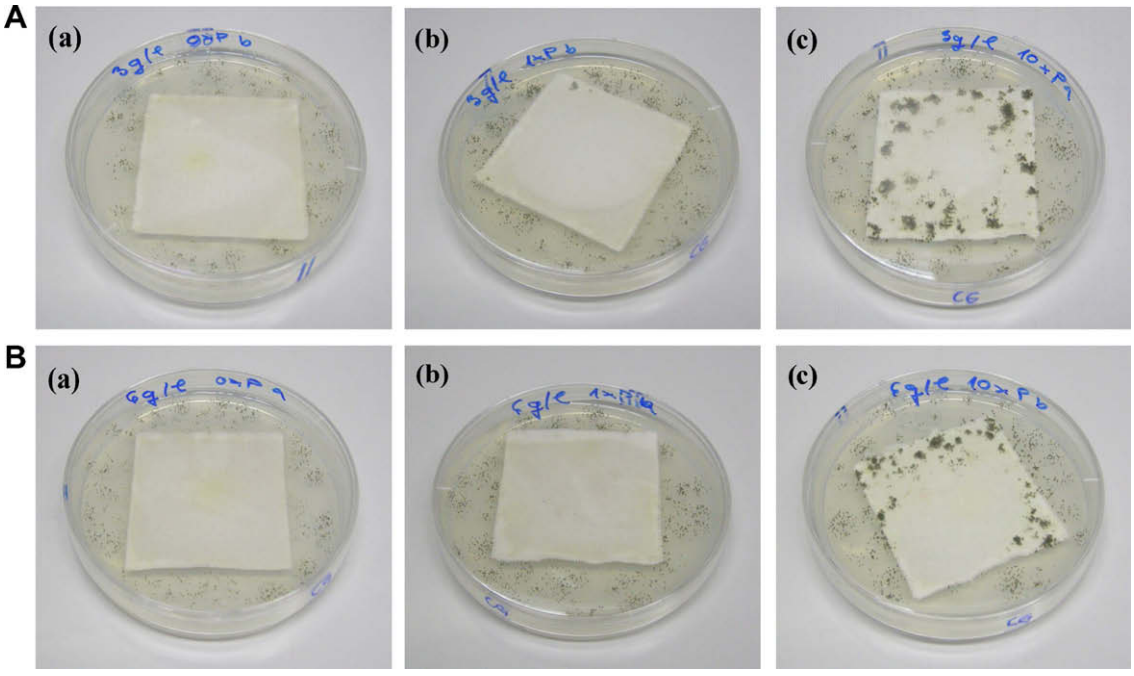


**Fig. 6.** Growth of *C. globosum* on SNA covered with finished samples E1 (A) and E2 (B); unwashed (a), washed once (b), washed ten times (c).


**Fig. 7.** Microscopic observation of submersed mycelial growth on SNA of *C. globosum* near the edge of the removed cotton specimen E1; unwashed (a), washed once (b), washed 10 times (c).

**Table 3**
The antibacterial effect of variously treated cotton samples against *E. coli* obtained by the agar diffusion tests

| Sample | Washing | Size of inhibition zone (mm) | Growth of bacteria in the medium under the specimen | Antibacterial effect |
|---|---|---|---|---|
| Untreated | 0 | 0 | Heavy | Insufficient |
| P1 | 0 | 0 | None | Good |
| | 1 | 0 | Slight, only some restricted colonies, growth nearly totally suppressed | Limited |
| | 10 | 0 | Heavy, compared to the control no growth reduction | Insufficient |
| P2 | 0 | 0 | None | Good |
| | 1 | 0 | Slight, only some restricted colonies, growth nearly totally suppressed | Limited |
| | 10 | 0 | Heavy, compared to the control no growth reduction | Insufficient |
| E1 | 0 | >1 | None | Good |
| | 1 | 1–0 | None | Good |
| | 10 | 0 | Heavy, compared to the control only slightly reduced growth | Insufficient |
| E2 | 0 | >1 | None | Good |
| | 1 | >1 | None | Good |
| | 10 | 1–0 | None | Good |

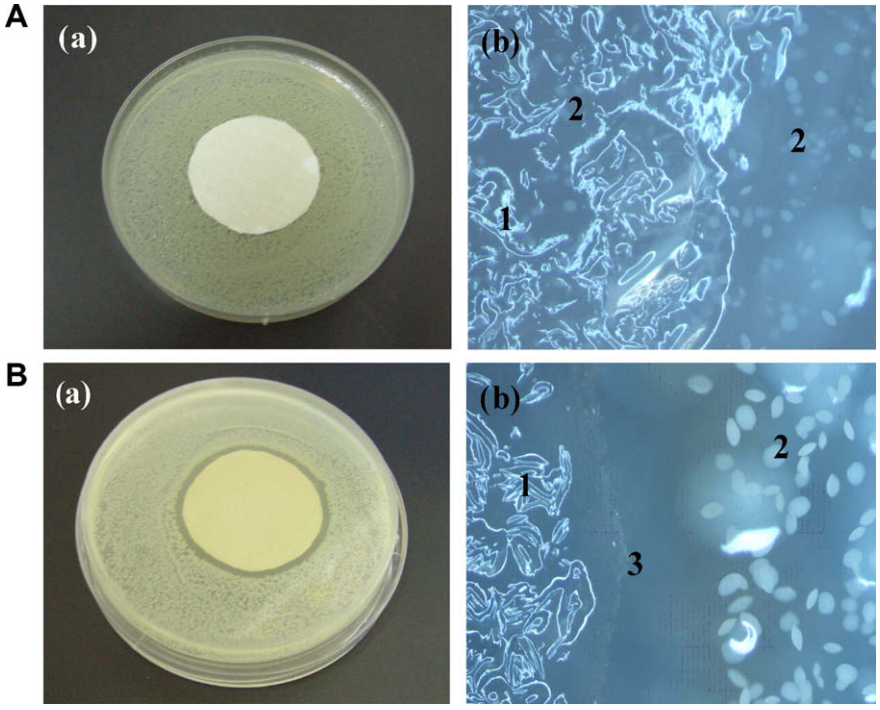


**Fig. 8.** Growth of *E. coli* on the nutrient medium covered with an untreated sample (A) and unwashed finished sample E2 (B). (a) photography, (b) microscopic observation of bacterial growth on the nutrient medium under the sample: (1) the bright pattern belongs to the print of the fabric sample; (2) bacterial colonies seen as small shadowed spots; (3) inhibition zone exceeding 1 mm.

**Table 4**
Antibacterial activity of finished cotton samples against *E. coli* according to the AATCC 100-1999 standard method

| Application method/sample | *R* (%) | | |
|---|---|---|---|
| | 0 W | 1 W | 10 W |
| P1 | 98 ± 2 | 89 ± 3 | –[a] |
| P2 | 100 | 87 ± 2 | –[a] |
| E1 | 100 | 100 | 16 ± 8 |
| E2 | 100 | 100 | 94 ± 9 |

[a] No reduction of bacteria was noted after the tenth washing cycle for samples P1 and P2.

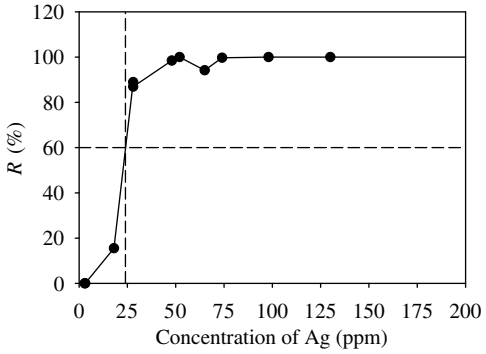


**Fig. 9.** Plot of the bacterial reduction *R*, against *E. coli* versus the concentration of Ag on the finished cotton fabric. The broken line (——) denotes the limit value for antibacterial efficiency.

tained by Lee and Jeong (2005), who reported a reduction of *Staphyloccocus aureus* and *Klebsiella pneumoniae* bacteria of 99.99% with only 10 ppm of colloidal silver on cotton fabric. In the letter case the diameter of the Ag particles used was ca. 2–3 nm, while the diameter of Ag particles in our study was between 100 and 300 nm. This confirmed the finding that smaller-sized silver particles have a better antibacterial efficiency in comparison to larger-sized ones (Lee & Jeong, 2005). However, the commercial availabil-

ity of iSys AG in the form of a user-friendly aqueous dispersion, represents a major advantage over the alcoholic solutions handled by Lee and Jeong (2005).

## 4. Conclusions

In this study we demonstrated that aqueous AgCl (supplied as the commercially available iSys Ag) posses antibacterial and antifungal properties on cotton fabrics when applied together with a reactive organic–inorganic binder matrix (iSys MTX). According to the application procedure the concentration of Ag on the cotton samples was from 48 to 290 ppm derived from ICS-MS measurements and the diameter of Ag particles was between 100 and 500 nm assessed from the SEM and EDXS measurements. The antibacterial activity of the Ag particles containing finishes was higher than their antifungal activity. The latter became effective when the Ag concentration on the cotton sample was higher than 100 ppm. Results showed that the aqueous solution of AgCl could provide long lasting antibacterial activity of cotton fabrics only if applied at a concentration of ca. 290 ppm, resulting in a sufficient concentration (ca. 65 ppm) for effective antibacterial reduction (ca. 94%) even after 10 repetitive washings. In this case, its application to cotton fabric by the exhaustion method is much more suitable over the pad-dry cure method.

## Acknowledgments

Names of products are necessary to report factually on available data but we neither guarantee nor warrant the standard of any product, and the use of any name implies no approval of the product to the exclusion of others that also may be suitable.

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