Danaja Štular[1], Barbara Simončič[1], Ivan Jerman[2], Brigita Tomšič[1]
[1]University of Ljubljana, Faculty of Natural Sciences and Engineering, Department of Textiles, Graphic Arts and Design, Snežniška 5, 1000 Ljubljana, Slovenia
[2]National Institute of Chemistry, Hajdrihova19, 1000 Ljubljana, Slovenia

Application of Stimuli Responsive Microgel for Creation of Smart Cotton Fabric with Antibacterial Properties

Original Scientific Article
Received 03-2016 • Accepted 04-2016

Abstract
Temperature and pH responsive microgel, based on poly-N-isopropylacrylamide and chitosan (PNCS) was studied, as possible carrier for antimicrobials on cotton fabric, to create smart stimuli responsive antimicrobial active textiles. Among antimicrobials 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC) and silver nanoparticles (AgNP) were chosen and applied to cotton fabric subsequently after the deposition of PNCS microgel. Influence of Si-QAC and AgNP on the swelling/deswelling activity of the PNCS microgel was obtained by temperature and pH responsiveness, along with determination of antimicrobial activity against Staphylococcus aureus and Escherichia coli. Even though Si-QAC slightly influenced swelling ability of the PNCS microgel and incorporation of AgNP reduced its amount on the surface of the fibres, PNCS microgel proved to be a suitable carrier of antimicrobial agents, thus imparting moisture management properties with effective controlled release of Si-QAC and AgNP. This provided excellent antimicrobial activity against tested bacteria, triggered by the change of temperature and pH of the surroundings.
Keywords: smart textiles, stimuli-responsive microgel, poly-NiPAAm, chitosan, antimicrobial activity

1 Introduction

Surface application of stimuli responsive microgels offers a great possibility for the creation of smart textiles, which can in addition to special functional properties, form a variety of responsive interaction with the user. Namely, stimuli responsive microgels have the ability of reverse swelling and de-swelling, which is triggered by various stimuli coming from the environment. When in their swollen phase, microgels show extraordinary absorptivity, absorbing more than 20% of water based by their dry weight. Applied on textiles, microgels influence moisture content as well as air permeability of the fabric, thus granting controlled thermoregulation properties. Poly-(N-isopropylacrylamide) (poly-NiPAAm) is the most extensively investigated synthetic temperature responsive polymer, which displays reversible phase transition (hydration-dehydration) at lower critical solution temperature (LCST) in aqueous solution at ~32 °C. When temperature is below LCST, the polymer is in coil conformation and is soluble in water. With raising temperature, sharp coil-to-globule transition occurs at the LCST, due to the rather complex polarity of the molecule, hence hydrophobic association is made. Chitosan is polysaccharide, known by its biocompatibility, non-toxicity and pH responsiveness. The latter is based on weak basic moieties (amino groups), attached to a hydrophobic backbone. Upon ionization at pH below ~6.5, amino group protonate and the charge is imparted over the molecule. Coiled chains extend, responding to electrostatic repulsion of the generated charges and increase the volume of the polymer. With copolymerisation of poly-NiPAAm and chitosan, PNCS hydrogel can be synthesized, obtaining both pH and temperature responsiveness [1–3].

Corresponding author:
Assist. Prof. DrSc Brigita Tomšič
Phone: ++386 1 200 3233
E-mail: brigita.tomsic@ntf.uni-lj.si

*Tekstilec, 2016, **59**(2), 142-148*
DOI: 10.14502/Tekstilec2016.59.142-148

Stimuli responsive microgels show great potential as carriers of different active substances, such as antimicrobial agents, which can be released from the microgel structure into surroundings only at required controlled conditions [1]. Therefore, the aim of the research was to study the possibility of using dual temperature and pH responsive microgel poly(N-isopropylacrylamide)/chitosan (PNCS), applied on cotton fabric as a carrier of different antimicrobials, i.e. silver nanoparticles (AgNP) which act upon controlled release mechanism and 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC), which forms biobarrier on the surface of the fibres. Such novel textile composites would reflect controlled thermo-regulation ability along with controlled antimicrobial activity, both triggered by the change of temperature and pH of the surroundings.

2 Experimental

2.1 Materials

In this study, 100 % cotton fabric with 165 g/m^2 mass area was used. For preparation of the microgel, chitosan (Chitoclear, Primex (Iceland); DD = 95%, η = 159 mPa) and Poly-NiPAAm, N,N-metylenebisacrylamide and ammonium persulfate were used purchased by Sigma Aldrich. Two different antimicrobial agents were selected: 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC) (ABCR, Germany), which is an organosilicon polymer with alkyldimethylammonium side groups and AgNP nanoparticles, synthesised by using $AgNO_3$ and NaCl (Sigma Aldrich).

2.2 Functional finishing of cotton fabric

PNCS microgel was prepared according to the procedure described by Lee [4]. Pad-dry-cure procedure was used for the incorporation of PNCS microgel onto cotton fabric, with 80% wet pick-up. Samples were dried for 5 min at 80 °C. Using the same procedure, Si-QAC was subsequently coated (PNCS+SiQ sample) and cured for 5 minutes at 150 °C. Additionally, in situ synthesis of AgCl nanoparticles was obtained on the hydrogel coated cotton (PNCS+Ag sample), as described by Klemenčič [5]. For comparison PNCS microgel was applied individually (PNCS sample), as well as Si-QAC (sample SiQ) and silver nanoparticles (sample Ag).

2.3 Analysis and measurements

Morphological changes of unfinished and finished cotton samples (PNCS, Ag, SiQ, PNCS+SiQ and PNCS+Ag) were determined by microscopic evaluation using SEM – 6060 LV (JEOL, Japan).

FT-IR spectra were obtained from Spectrum GXT spectrometer (Perkin Elmer, GB), with ATR cell (reflection technique) with diamond crystal. The spectra were recorded over the range 4,000–600 cm^{-1} and averaged over 28 spectra.

Moisture content measurements was performed on Moisture analyser MLB-C (Kern, Germany). Samples were conditioned at 65 % R.H. at two different temperatures, 25 °C and 40 °C for 24 h, put in the analyser and dried at temperature of 105 °C until the constant mass. Moisture content (MC) was determined by following equation 1:

$$MC = \left(\frac{m_o - m_f}{m_o}\right) \cdot 100\ [\%] \quad (1)$$

where m_o [g] is the weight of the sample before drying and mf [g] is dry weight of the sample. Three measurements were taken for each sample.

Dual temperature and pH responsiveness of the studied samples was determined by water uptake measurements, obtained at three different buffer solutions with pH 3, 6.5 and 10 at two different temperatures of 20 °C and 40 °C. Water uptake (WU) was determined by equation 2:

$$WU = \left(\frac{m_w - m_d}{m_d}\right) \cdot 100\ [\%] \quad (2)$$

where m_w [g] is the weight of the sample after 30 minutes of soaking in buffer solution and md [g] is the weight of dry sample conditioned for 24 hours at 20 °C and 65% relative humidity. For each sample ten measurements were taken.

Antibacterial activity of finished samples was estimated according to ASTM E 2149-01 standard method against Gram-positive bacteria Staphylococcus aureus (ATCC 6538) and Gram-negative bacteria Escherichia coli (ATCC 25922). Unfinished cotton fabric was used as a control.

Test with bromophenol blue (BPB) reagent was performed to qualitative evaluate the presence of Si-QAC antibacterial agent on cotton samples. BPB is an alkaline dilution of the sodium salt 3'-3"-5'-5"-tetrabromophenolsulfonphtalein. For the BPB test,

1 g of sample was immersed in 50 ml of 0.005% BPB reagent diluted in water and slightly alkalised with a few drops of a Na_2CO_3 solution. The samples were stirred vigorously for 20 min, rinsed with cold tap water and dried at room temperature. The BPB test was performed on the samples preconditioned for one hour at 20 °C and 40 °C. Assessment of the intensity of blue coloration on the samples was performed by the reflectance, R, measurements of the dyed samples on a Datacolor Spectraflash SF 600 Spectrophotometer using D 65/10° light. Before these measurements, the samples were conditioned at 65 ± 2% relative humidity and incubated at 20 ± 1 °C for 24 hours. Corresponding K/S values were calculated according to the Kubelka-Munk equation 3:

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (1)$$

where *K*/*S* is the ratio of the coefficient of light absorption (K) to the coefficient of light scattering (*S*) and *R* the reflectance at the maximum absorption wavelength determined at 610 nm.

3 Results and discussion

3.1 Morphological and chemical properties

Morphological changes on finished samples are shown in Figure 1. PNCS microgel particles appeared in the form of unevenly scattered bulges on the fibre surface up to ~2 μm in size. In the case of SiQ and Ag samples it can be seen that application of the Si-QAC smoothened the fibres surface, whereas the Ag nanoparticles could be observed as bright spots. In SEM pictures of the samples coated with two-component finish PNCS+SiQ, microgel in the form of lumps was noticed. Comparing with PNCS microgel applied to the fabric alone, the lumps were more evenly distributed, hence the bubbles are in more geometrical round shapes. Besides, sol-gel film partially closed the area between the fibres. Both PNCS bulges and AgNP could be observed on the fibre surface of PNCS+Ag coated sample. In this case, the microgel particles were more concentrated on the area between the fibres, thus implying that some of the microgel particles were removed during in situ synthesis of Ag nanoparticles, which included subsequent immersion of the fabric into $AgNO_3$ and NaCl solutions. Nevertheless, presence of PNCS microgel particles seemed to contribute to more even deposition and less size fluctuating of the AgNP.

Chemical properties of studied cotton fabric samples were studied by IR ATR spectroscopy. The presence of PNCS microgel in the IR ATR spectra of the PNCS and PNCS+SiQ samples was confirmed by appearance of the absorption band at 1450 cm^{-1}, belonging to the N–H vibration of both poly-NiPAAm and chitosan, as well as by the absorption bands of

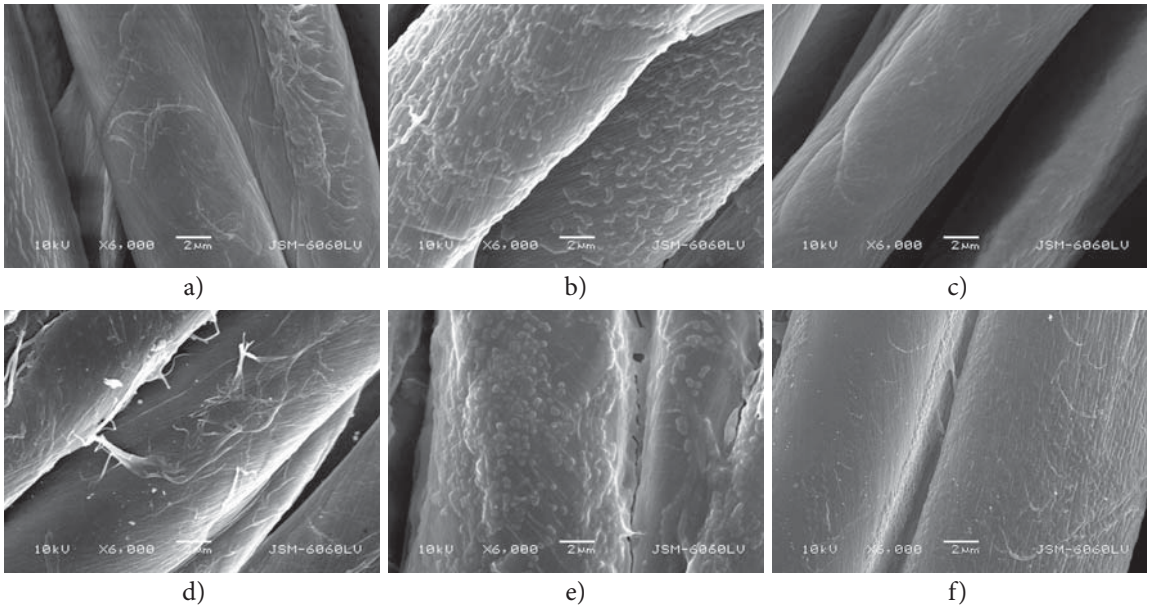

Figure 1: Scanning electron microscope images of unfinished cotton (a) and finished cotton samples PNCS (b), SiQ (c), Ag (d), PNCS+SiQ (e) and PNCS+Ag (f)

amide I and amide II at 1645 and 1535 cm^{-1}, manifested by C=O stretching vibration of poly-NiPAAm [6, 7]. The intensity of aforementioned absorption bands was slightly lower in the case of the PNCS+Ag sample, validating our predictions on partial removal of the PNCS microgel particles during in situ synthesis of AgNP. While the presence of AgNP had no influence on IR ATR spectra of the studied samples, which proves that nano sized particles are not chemically bounded to the fibres and therefore cannot be detected by FT-IR analysis [5, 6, 8], the presence of the Si-QAC in the IR ATR spectra of SiQ and PNCS+SiQ samples could be confirmed from the absorption bands at 2920 and 2850 cm^{-1}, attributed to C–H stretching vibrations of long alkyl moieties of the antibacterial agent. Moreover, from the comparison of these IR ATR spectra, higher intensities of both absorption bands, i.e. 2920 and 2850 cm^{-1}, could be observed in the case of the PNCS+SiQ sample, implying that PNCS microgel influenced higher absorption of the Si-QAC on the cotton fibres.

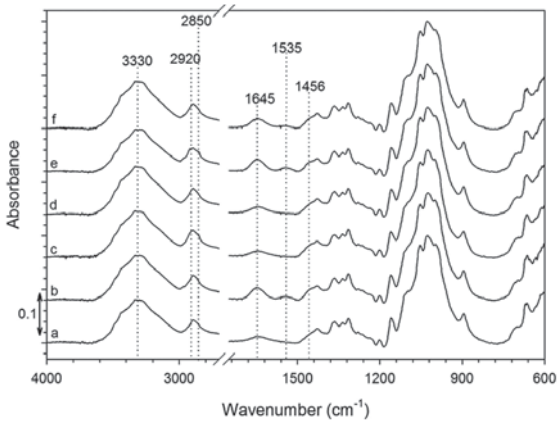

Figure 2: IR ATR spectra of studied cotton samples in the 4,000–600 cm^{-1} spectral region
a – unfinished cotton sample, b – PNCS, c – SiQ, d – Ag, e – PNCS+SiQ, f – PNCS+Ag

3.2 Temperature responsiveness

For studying temperature responsiveness of PNCS microgel, contributed by poly-NiPAAm, moisture content (MC) and water vapour transmission rate (WVTR) were measured. All the samples show greater MC at 25 °C in comparison to that at 40 °C (Figure 3). Such behaviour was expected, since poly-NiPAAm is in hydrophilic state at 25 °C and microgel particles bound molecules of water, thus 16.5% higher MC of the PNCS sample was obtained as for the unfinished cotton. At 40 °C, microgel particles were shrunken and water was extracted into the surroundings, e.g. 2.2% lower MC was determined. Both antimicrobial agents limited the degree of swelling of the PNCS microgel, whereas water absorption was the most effected in the presence of Si-QAC. The reason lies in hydrophobic behaviour of Si-QAC, due to the long alkyl chain in its structure, which can be confirmed by the lowest MC of the SiQ sample. At 40 °C, PNCS microgel and Si-QAC worked simultaneously, whereas hydrophobic nature of Si-QAC influenced on increased elimination of water in comparison to other studied samples. Therefore, when compared to the unfinished sample, 6.0% lower MC was determined.

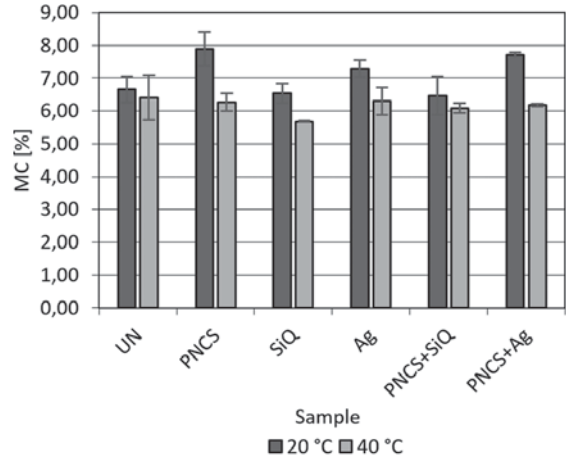

Figure 3: Moisture content, MC, of the unfinished cotton (UN) and finished studied samples obtained after preconditioning at 65% R.H. and 25 °C (darker grey bar) and 40 °C (lighter grey bar)

3.3 Dual temperature- and pH-responsiveness

By measuring water uptake (WU), dual temperature and pH responsiveness from poly-NiPAAm and chitosan was studied. At this point, it has to be mentioned that a broad range of pH between 3 to 10 was taken into observation to emphasise the pH responsiveness of the PNCS microgel, thus including variable pH values of human skin surface or sweat, ranging mostly from pH of 4.0 to 7.0, but can increase up to 8.0 [9, 10]. It can be seen in Figure 4 that simultaneous rising the temperature and pH, caused owerall decrease in water absorbance. Thus, at pH 3 and 20 °C, when both poly-NiPAAm and chitosan were

hydrophilic, allowing microgel particles to swell without restraint, the highest *WU* was observed in the case of PNCS sample. On the other hand, the lowest *WU* was found at 40 °C and pH 10, when both components of the PNCS microgel were in their hydrophobic state. Comparison of *WU* obtained at 20 °C and pH 10 (i.e. poly-NiPAAm is in hydrophilic and chitosan in hydrophobic state) with *WU* determined at 40 °C and pH 3 (i.e poly-NiPAAm is shrunken and expels water and chitosan is swelled and bounds water), has implied that poly-NiPAAm dominated within the microgel structure and had greater effect on hydrogel performance than chitosan. Namely, higher *WU* of the finished samples was obtained in conditions which dictates hydrophilic nature of poly-NiPAAm and vice versa, lower *WU* was determined in conditions which dictates its hydrophobic nature.

As for the temperature responsiveness, presence of both antimicrobial agents also influenced on dual-temperature and pH responsiveness of the microgel. Incorporation of Si-QAC into the PNCS microgel particles hindered swelling properties of the chitosan. Namely, PNCS+SiQ sample showed opposite pH responsiveness as predicted, since smaller *WU* was determined at pH 3 when chitosan is hydrophilic (room temperature), while the highest absorption was obtained at pH 10 (elevated temperature), when chitosan is in hydrophobic state. Such

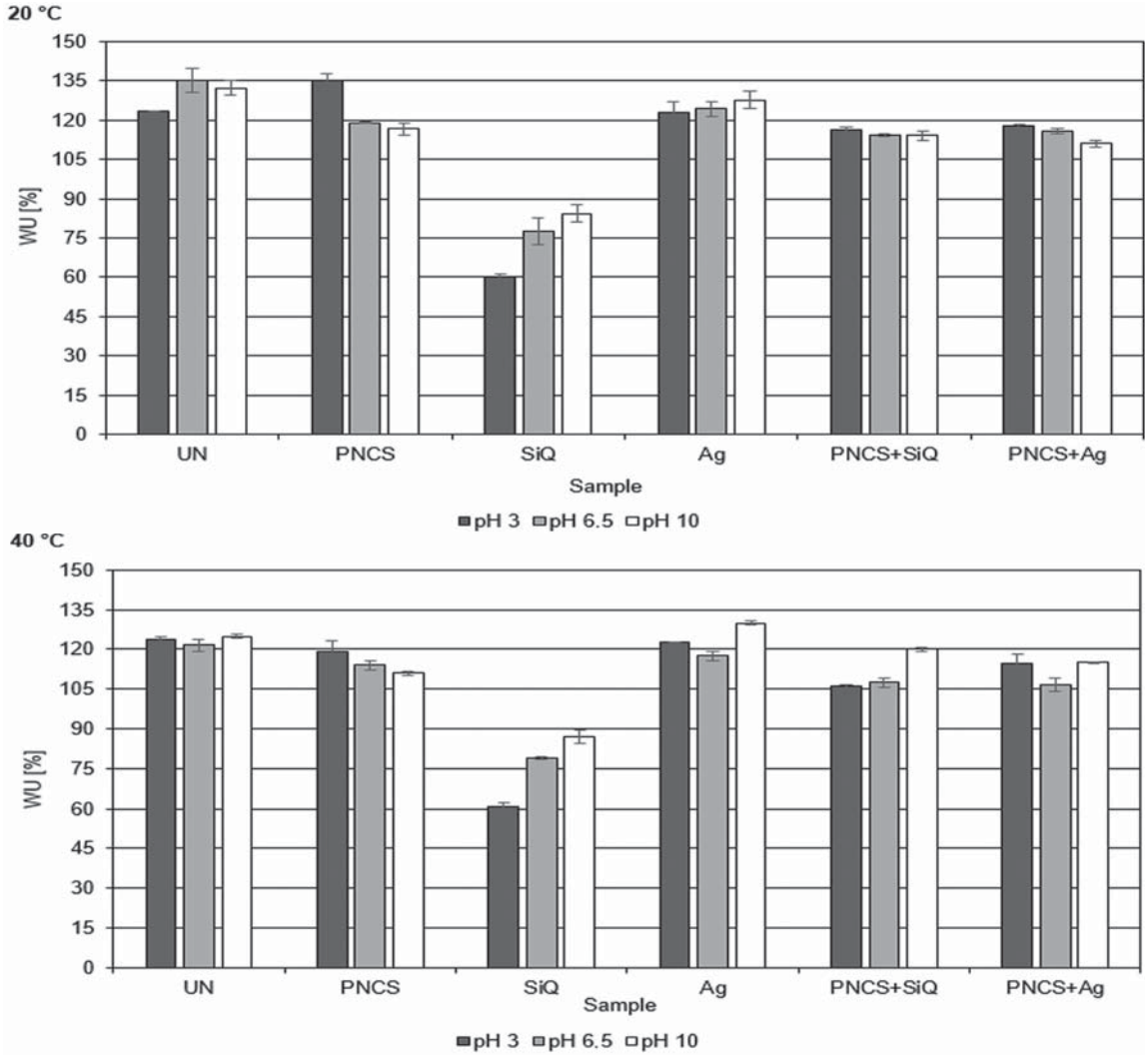

Figure 4: Water uptake, WU, of the unfinished (UN) sample and studied finished samples obtained after being immersed in different buffer solutions: pH 3 (darker grey bar), pH 6.5 (lighter grey bar) and pH 10 (white bar) at 20 °C (upper graph) and 40 °C (bottom graph) for 30 min

behaviour was also obtained in the case of SiQ sample at both studied temperatures 20 and 40 °C. According to Jones [11] smaller *WU* can be solely ascribed to water-repealing long alkyl moieties of Si-QAC, restricting the swelling ability of chitosan within the PNCS microgel particles. However, in basic media the quaternary ammonium salts are generally more susceptible to degradation, reflecting in increased hydrophilicity of the Si-QAC agent and consequently in increased *WU* of the SiQ and PNCS+SiQ samples at pH 10. However, this cannot be considered as disadvantage, meaning that PNCS microgel when loaded with Si-QAC has the ability also to absorb alkaline sweat, which is rare, but arises as a result of disease and thus by administration of drugs or loss of acidic trough vomiting [10]. Thus, increased *WU* of PNCS+SiQ sample could show its suitability for use for medical textiles. As predicted, in the case of PNCS+Ag sample less intensive dual temperature and pH responsivness was observed as for PNCS and PNCS+SiQ sample, due to lower concentration of the microgel particles left on the surface of the fibers after *in situ* synthesis of the Ag nanoparticles.

3.4 Antimicrobial activity

Results of antibacterial properties of the studied samples are shown in Figure 5. Synergistic activity between both antimicrobial agents and PNCS microgel was obtained, since both, PNCS+SiQ and PNCS+Ag samples showed excellent >99% growth reduction of Gram negative bacteria *E. coli,* which was comparable to bacterial reduction obtained for SiQ and Ag samples. PNCS+SiQ and SiQ samples exhibited also excellent >99% bacterial reduction of Gram positive *S. aureus* while PNCS+Ag sample demonstrated slightly reduced antibacterial activity, which was in accordance to that obtain for one-component Ag sample. This implies that higher concentration of $AgNO_3$ and NaCl reagents should be used for *in situ* synthesis of AgNP in order to obtain its biocidal activity against both types of tested bacteria.

Successful release of the Si-QAC antimicrobial agent from the PNCS microgel particles at temperatures above the LCST of the poly-NiPAAm was further confirmed with the BPB test, which is based on the formation of a complex between the BPB reagent anion and the quaternary ammonium group of Si-QAC on the surface of the fabric; the formation of this complex dyes the fabric blue. Therefore, from

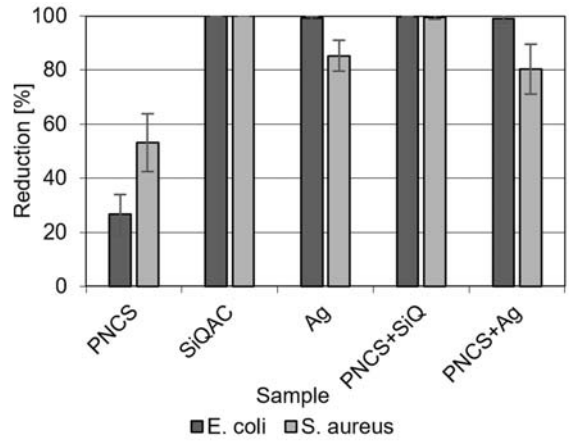

Figure 5: Growth reduction, R, of bacteria E. coli and S. aureus determined on the studied samples according to the ASTM E 2149 – 01 standard method

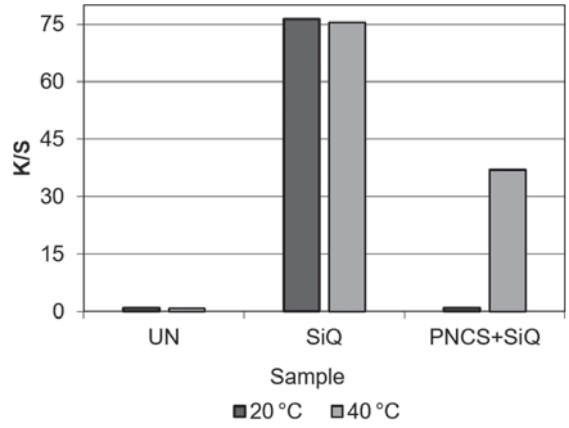

Figure 6: K/S values of BPB test performed on the unfinished (UN) and SiQ and PNCS+SiQ samples preconditioned at 20 °C and 40 °C

the *K/S* values the information about the amount of active quaternary ammonium groups of Si-QAC present on the coated samples can be obtained [12]. From the results in Figure 6 extremely low *K/S* value of the PNCS+SiQ sample can be seen, comparable to that of untreated sample. However, when the studied samples were exposed to elevated temperature for one hour, strong increase of the *K/S* value of the PNCS+SiQ sample was obtained, indicating the presence of certain amount of the Si-QAC agent on the surface of the cotton fabric. Namely at 40 °C (temperatures above the LCST of poly-NiPAAm) the PNCS microgel collapsed, thus releasing certain amount of the Si-QAC into the sourroundings. Since this test is appropriate only for determination

of Si-QAC, we assumed that the same behaviour could be obtained in the case of PNCS+Ag sample.

4 Conclusion

By applying temperature and pH responsive PNCS microgel in combination with antimicrobial agents Si-QAC and AgNP, cotton with simultaneous moisture management and antibacterial activity was obtained. Due to its hydrophobic nature, the presence of Si-QAC restrained swelling of the PNCS microgel particles at room temperature, but contributed to higher water extraction at 40 °C. On the other hand, *in situ* synthesis of AgNP reduced the amount of PNCS microgel on the fibre surface, but did not affect swelling ability of the remained PNCS microgel particles. Studied cotton samples exhibited sufficient antibacterial activity, acting through the release of Si-QAC and AgNP only at controlled and required conditions, i.e. at elevated temperature, thus confirming the suitability of the PNCS microgel as a carrier of antimicrobial agents.

Acknowledgements

This work was supported by the Slovenian Research Agency (Program P2-0213, Infrastructural Centre RIC UL-NTF and a Grant for the doctoral student D.Š.)

References

1. HU, Jinlian, MENG, Harper, LI, Guoqiang, IBEKWE, Samuel I. A review of stimuli-responsive polymers for smart textile applications. *Smart Materials and Structures*, 2012, **21**(5), 053001, doi: 10.1088/0964-1726/21/5/053001.
2. KULKAMI, Amit, TOURRETTE, Audrey, WARMOESKERKEN, Marijn M.C.G., JOCIĆ, Dragan. Microgel-based surface modifying system for stimuli-responsive functional finishing of cotton. *Carbohydrate Polymers*, 2010, **82**(4), 1306–1314, doi: 10.1016/j.carbpol.2010.07.011.
3. TOURRETTE, Audrey, DE GEYTER, Nathalie, JOCIĆ, Dragan, MORENT, Rino, WARMOESKERKEN, Marijn M. C. G., LEYS, Christophe. Incorporation of poly(N-isopropylacrylamide)/chitosan microgel onto plasma functionalized cotton fibre surface. *Colloids and Surfaces A: Physicochemical Engineering Aspects*, 2009, **352**(1–3), 126–135, doi: 10.1016/j.colsurfa.2009.10.014.
4. LEE, Chia-Fen, WEN, Chia-Jen, CHIU, Wen-Yen. Synthesis of poly(chitosan-N-isopropylacrylamide) complex particles with the method of soapless dispersion polymerization. *Journal of Polymer Science Part A: Polymer Chemistry*, 2003, **41**(13), 2053–2063, doi: 10.1002/pola.10733.
5. KLEMENČIČ, Danijela, TOMŠIČ, Brigita, KOVAČ, Franci, SIMONČIČ, Barbara. Antimicrobial cotton fibres prepared by in situ synthesis of AgCl into a silica matrix. *Cellulose*, 2012, **19**(5), 1715–1729, doi: 10.1007/s10570-012-9735-z.
6. SOCRATES, George. *Infrared and Raman characteristic group frequencies*. New York : John Wiley & Sons, 2001, 347.
7. TOMŠIČ, Brigita, KRIŽMAN LAVRIČ, Pavla, SIMONČIČ, Barbara, OREL, Boris, JOCIĆ, Dragan. Sol-gel technology for functional finishing of PES fabric by stimuli-responsive microgel. *Sol-Gel Science and Technology*, 2011, **61**(1), 463–476, doi.org/10.1007/s10971-011-2647-9.
8. ZAFAR, Muhammad, SHAH, Tahir, RAWAL, Amit, SIORES, Elias. Preparation and characterisation of thermoresponsive nanogels for smart antibacterial fabrics, *Materials Science and Engineering C*, 2014, **40**, 135–141, doi: 10.1016/j.msec.2014.03.033.
9. LAMBERS, Hans, PIESSENS Sofie, BLOEM Audrey, PRONK, Helena, FINKEL, Peter. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *International Journal of Cosmetic Science*, 2006, **28**, 359–70, doi: 10.1111/j.1467-2494.2006.00344.x.
10. BURRY, S. Jason, COULSON, Henry Frederick, ESSER Ingrid, MARTI Vargas, MELLING, S. J., RAWLINGS, V. Anthony, ROBERTS Graham, MILLS A. K. Erroneous gender differences in axillary skin surface/sweat pH. *International Journal of Cosmetic Science, 2001,* **23**, 99–107, doi: 10.1046/j.1467-2494.2001.00085.x.
11. JONES, Richard Alan. *Quaternary ammonium salts: Their use in phase-transfer catalysis*. London : Academic Press, 2001.
12. TOMŠIČ, Brigita, ILEC, Eva, ŽERJAV, Metka, HLADNIK, Aleš, SIMONČIČ, Andrej, SIMONČIČ, Barbara. Characterisation and functional properties of antimicrobial bio-barriers formed by natural fibres. *Colloids and surfaces. B, Biointerfaces, 2014,* **122**, 72–78, doi: 10.1016/j.colsurfb.2014.06.047.