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A New Antimicrobial ALCERU® Fibre with Silver Nanoparticles

Abstract
Antimicrobial fibres were produced by the implementation of nanoscaled silver particles into a solution of cellulose and N-methylmorpholine-N-oxide (NMMO). Usage of particles with 80 – 140 nm and 5 - 20 nm grain size, which have a large surface, resulted in a much greater release of silver ions. Moreover, Lyocell fibre is favoured for its permeability of ions. Atomic absorption spectrometric and x-ray analysis confirmed sufficient recovery of the incorporated silver. Thermostability of the cellulose/NMMO solutions with a concentration of silver up to 500 mg/kg and textile processability is warranted. These items resulted in a low-loaded fibre ensuring the stability of the spinning process and typical Lyocell tensile strength. Finenesses below 0.2 tex are achieved without any problems. The bactericidal status of the fibre was determined by a new, more sensitive test assay (NUMETRIKA™) with a wide spectrum of activity. Less than 100 mg/kg of silver in the fibre give a pronounced bactericidal effect. The blending of higher loaded fibres with pure unmodified fibres is possible while maintaining antimicrobial activity.

Key words: *cellulose, N-methylmorpholine-N-oxide, Lyocell process, nanoscaled silver particles, antimicrobial fibres.*

Introduction

The modification, shaping and analytical characterisation of native polymers, particularly cellulose, is a research topic of the Thuringian Institute for Textile and Plastics Research (TITK). Already scaled up world-wide and successfully commercialised, the Lyocell process [1, 2] utilises the physical solubility of cellulose in N-methylmorpholine-N-oxide (NMMO). Special features - the dissolving of cellulose without chemical modification and nearly complete recovering of the solvent - establish this technology as an economically and environmentally alternative to conventional processes of fibre-making. Especially ALCERU technology is increasingly used beyond the textile sector in order to create novel innovative fibre materials, using its high receiving capacity for preferably insoluble secondary components [3]. Namely, the physical dissolving process of cellulose without derivatisation and the high loading capacity of the solution enable the incorporation of different chemically inert compounds, e.g. ceramics, carbon black, activated charcoal, ion exchange resins, super absorbing polymers etc. [4]. Up to now, the particle sizes of additives in the lower micrometer range have generally been utilised. The fact that decreasing particle sizes are expected to overlay the chemical properties of the resulting cellulose composite creates very interesting opportunities for the development of shaped cellulosic bodies possessing improved product characteristics result. Knowledge of incorporating spherical nano-additives has been elaborated in detail by Melle et al. [5].

Recently, silver is of special interest with regard to materials maintaining bactericidal effects. With its wide effect spectrum for bacteria, and contrary to other heavy metals, its toxicological safety to the human organism, silver exhibits an alternative to antibiotics [6]. Galvanic deposition of silver on the surfaces of polymers and the incorporation of different silver compounds into melt spun fibres, e.g. polypropylene, polyester or polyamide, are the main features to produce bactericidal fibres. Application of silver nitrate on the surface of Lyocell fibre modified with an ion exchange resin is currently manufactured in a semi-technical scale by Smartfiber AG [7]. All of the approaches mentioned lack the application of high concentrations of silver, time and energy consuming process steps or post dyeing of the fibre.

The aim of the paper is to discuss the influences of nanoscaled silver particles on the spinning process, the detection of silver in the fibre and the measurement of the bactericidal effect of the fibres produced.

Experimental

Reagents

Chemicals were obtained from Merck, Darmstadt, Germany and were of the highest purity available. N-Methylmorpholine-N-oxide (NMMO; BASF, Ludwigshafen, Germany) was used as 50% (v/v) aqueous solution. The cellulose used was a bleached spruce sulfite pulp, characterised by the following data: content of α-cellulose = 90.6%, degree of polymerisation (DP) = 495, carboxyl groups = 6.9 μmol/g, carbonyl groups = 48.3 μmol/g, moisture = 7.5%. NaOH, NH_2OH, propyl gallate (PG) [8], iminodiacetic acid sodium salt covalently bound to a styrene/divinyl benzene copolymer - TP207 (abbr. ISDB, Bayer AG, Leverkusen, Germany) and benzyl amine covalently bound to a styrene/divinyl benzene copolymer - VPOC1065 (abbr. BSDB, Bayer AG, Leverkusen, Germany) [9] were applied as stabilisers. Modification was carried out using nanoscaled silver particles (the product is distributed via CIBA under the brand name HYGENTIC™ [10]), namely with porous MicroSilver BG™ (80 - 140 nm, Figure 1.a) and colloidal NanoSilver BG™ (5 - 20 nm, Figure 1.b).

Preparation of cellulose solutions

5720 g of NMMO as 50% (v/v) aqueous solution and 570 g of cellulose were weighed in a kneader. Stabilisation and modification was carried out by the admixture of the above-mentioned additives/modifiers with varying concentrations as specified in the text. After stirring the mixture for 15 min at room temperature, the reactor was connected to a Rotavapor (approx. 30 mbar) and the temperature of the reactor was gradually raised up to 90 °C.

The shaping of cellulose solutions

The solutions prepared were processed on a laboratory spinning set-up. It consists of a dope storage tank, a spinning pump, a thermo-controlled spinning head and a multi-step washing bath. The spun fibre bunches were wrapped around a

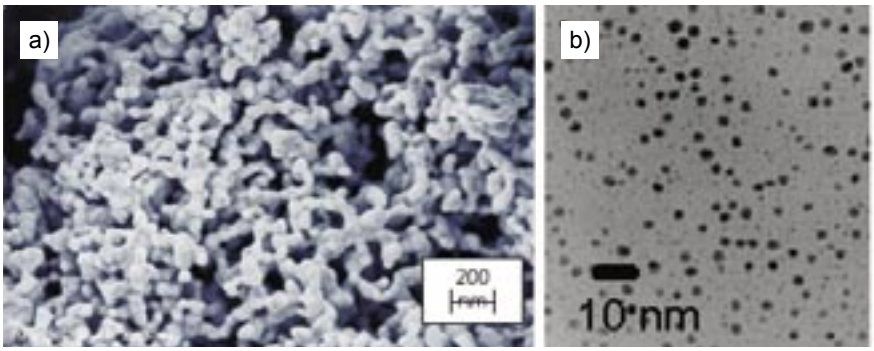

Figure 1. *Nano scaled silver particles distributed via CIBA under the brand name HYGENTIC™ [10]; a) porous MicroSilver BG™ and b) colloidal NanoSilver BG™.*

bobbin and manually cut into staples of 60 mm length prior to drying. Depending on the fineness intended, the spinning velocity was adjusted between 20 and 30 m/min. The fibre were dried at 85 °C in a hot-air circulation cabinet drier.

Methods of measurements

Reaction calorimetry

Thermal investigations were undertaken with a Systag RADEX calorimeter. Approximately 2 g of the cellulose/NMMO solution were used in the steel vessel (design pressure: 100 bar) equipped with a bursting disk and an internal sensor device for temperature determination. Dynamic measurements (screening) were provided by heating the vessel with a heating rate of 0.75 K/min from room temperature up to 300 °C, followed by holding this temperature for 1 h. The first thermal activity of the solution indicated the onset temperature (T_{on}), which was determined by plotting the deviation of pressure with respect to time (dp/dt) versus temperature. For the isoperibolic long time mode, the surrounding temperature was kept at 140 °C for 24 h. Temperature and the deviation of pressure with respect to time (dp/dt) were investigated [11].

High Performance Liquid Chromatography (HPLC)

A DIONEX HPLC system with diode array detector UV 340 was used for the determination of aldehydes and amines [12]. Aldehydes: 30 ml of pure water was added to 10 g of a cellulose/NMMO solution and left for 48 h. 5 ml of the filtrated extract was mixed with 4 ml of acetonitrile and 0.5 ml of 2.4-dinitrophenylhydrazine solution and filled up to 10 ml with pure water. After 1 h measurements were carried out in the following conditions: an injection volume of 20 µl, Eurospher C18 RP100-5/150. 4 column (Knauer), eluent acetonitrile/water; 60 min, 60% acetonitrile, isocratic; a flow rate of 1 ml/min, a temperature of 25 °C, a wave length of 350 nm. Amines: Aliquots of distillates collected in the Rotavapor during the preparation of cellulose/NMMO solutions were subjected to measurements. After filtration (0.45 µm) the distillates were measured with the following parameters: an injection volume of 100 µl, Nucleogel RP100 - 8/150 column (Macherey-Nagel), eluent methanol/water (0,05 m sodium(meta)borate tetrahydrate), a flow rate of 1 ml/min, a temperature of 35 °C and a wave length of 200 nm.

X-ray fluorescence spectroscopy (XRF)

A fluorescence spectrometer Oxford ED 2000, Oxford Instruments (K_α. Line, Current 10, read time 60 s) was applied to register silver in the fibre directly.

Flame atomic absorption spectrometry (F-AAS)

To measure the concentration of 0.5 g of silver, the fibre was weighed into a quartz crucible and reduced to ash at 500 °C in a muffle oven. The residue was dissolved in nitric acid and filled with distilled water to a volume of 100 ml. An AAnalyst 800, PERKIN ELMER was used to measure silver content in the solutions.

Whiteness

Determination of the whiteness was carried out by remission measurement with a Datacolor SF 600 according to the German standard DIN 5033 (CIE). Light exposure was proceeded by a xenon beamer over 62 hours.

Coloration

Aqueous extracts of cellulose/NMMO solutions were used for measuring the coloration with a Shimadzu UV- 2401 spectrometer. 30 ml of pure water was mixed with 10 g of the cellulose/NMMO solution and allowed to stand for 48 h. The extinction of the filtrated extracts was measured at a wave length of 470 nm.

Rheological analysis

The degree of polymerisation (DP) of cellulose and cellulose/NMMO solutions was measured by the Cuoxam method, CFK standard CFS-7-2053, 1980. DP degradation is defined as a factor of the original DP and DP immediately after preparing the solution.

Determination of fibre properties

For fibre characterisation the fibre fineness (German standard DIN EN ISO 1973), conditioned tenacity, elongation at break (DIN EN ISO 5079) as well as loop tenacity (DIN 53843, Part 2) were measured [13].

Antimicrobial testing

The antimicrobial activity of the fibres was monitored using the Bio-Gate assay (NUMETRIKA™) described in ref. [14]. In a microtitre plate with up to 96 samples, the optical density (OD) of the medium (*Staphylococcus epidermis*) in which the material sample is placed is measured over time (48 hours). Optical density correlates with the number of bacteria released from the biofilm on the material (proliferation). Bacteriostatic and bactericidal behaviour can be detected in comparison to an untreated material.

Results and discussion

Preparation of Lyocell solutions with silver nanoparticles

In principle, the dissolution of cellulose in NMMO and fibre spinning are entirely physical processes; chemical alterations may appear under industrial conditions. Higher temperatures for cellulose dissolving and longer residence times of the solutions result in the discoloration and degradation of NMMO and cellulose. These reactions arise from the thermal instability and reactivity of the NMMO solvent, as well as its reactions with cellulose and redox reactions of heavy metals [15 - 17]. Additives with functional groups or reactive surfaces influence the reaction mechanisms, change their directions or cause subsequent reactions [11]. Therefore, stabilisation of the cellulose/NMMO system is necessary, especially in the case of modified solutions [8, 9].

The incorporation of nanoscaled particles into cellulose/NMMO solutions exhibits

Table 1. *Comparison of onset temperatures (T_{on}), coloration and DP for 12% cellulose/NMMO solutions added with to nano-scaled silver particles stabilized with different substances.*

Concentration of nano-scaled silver particles in the solution, mg/kg	Stabilization	T_{on}, °C	Coloration	DP reduction original/solution
0	-	149	0.048	1.25
0	0.06% PG, 0.1% NH_2OH, 0.04% NaOH	158	0.030	1.02
100	-	148	0.050	1.60
500	-	148	0.052	1.64
1000	-	145	0.084	1.74
500	0.21% ISDB, 0.21% BSDB	155	0.044	1.13
500	0.06% PG, 0.1% NH_2OH, 0.04% NaOH	152	0.497	1.03

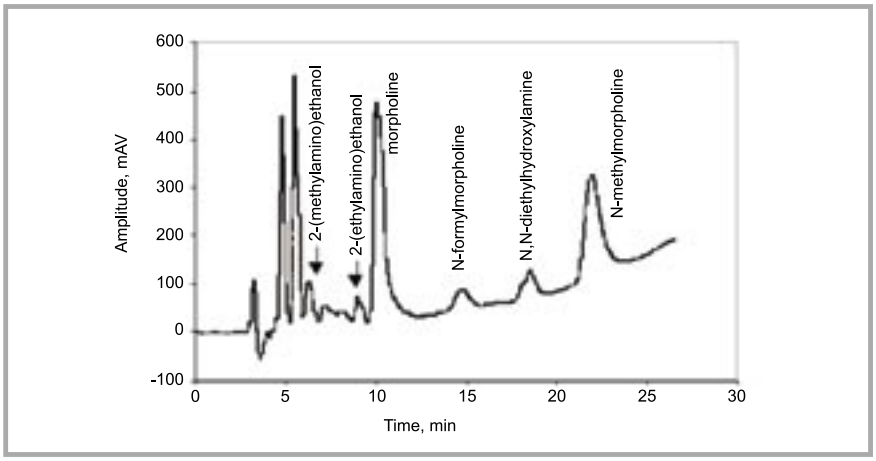

Figure 2. *Chromatogram of a distillate of a cellulose/NMMO solution.*

an extraordinary challenge concerning thermostability. Evidently, antimicrobial activity, due to a much greater surface, goes with enhanced catalytic reactivity. To produce fibres containing 0.01 – 1% of silver, cellulose/NMMO solutions were prepared possessing silver in the range of 5 - 1000 mg/kg. Thermal stability was evaluated by comparison of solutions with/without silver and with/without stabilizers. The onset temperature (T_{on}) is denoted as the most distinctive parameter describing thermo - stability. Application of a stabiliser increases the Ton of an unmodified solution from 149 to 158°C; however, higher amounts of silver decrease T_{on} to 145 °C (Table 1). These results are supported by DP measurements of the cellulose in solutions showing a reduction in DP for solutions with higher concentrations of silver. Again, the efficiency of the stabiliser systems is demonstrated by minimising cellulose decomposition. Chromophoric by-products are indicated by measurement of the coloration. Here it can be concluded that nanoscaled particles catalyse degradation reactions of cellulose. Formation of monomeric units like ketoses and aldoses contribute to additional discoloration due to their carbonyl or carboxyl groups [18].

Moreover, HPLC, as a convenient tool to measure degradation products, mainly amines and aldehydes, revealed that increasing silver amounts enrich those compounds arising from NMMO decomposition or the reaction of NMMO with cellulose (Figure 2). Concentration of N-methylmorpholine is enhanced four times compared to the unmodified solution (Figure 3). By-products like morpholine and formaldehyde were denoted as initiators for subsequent reactions [17].

Comparison of both stabiliser systems with regard to a solution with 500 mg/kg of silver, delivered a slight advantage for the PG-NaOH-NH_2OH system, providing an enhanced onset temperature and evidently maintaining DP. However, pronounced coloration can be ascribed to the reactions of propyl gallate with cellulose or silver [8].

Isoperibolic measurements at 140°C over 24 h were applied to investigate long term thermal behaviour. As depicted in Figure 4 the modified solutions show pressure slope maxima at a time of 5 hours, compared to 7 h for the un-

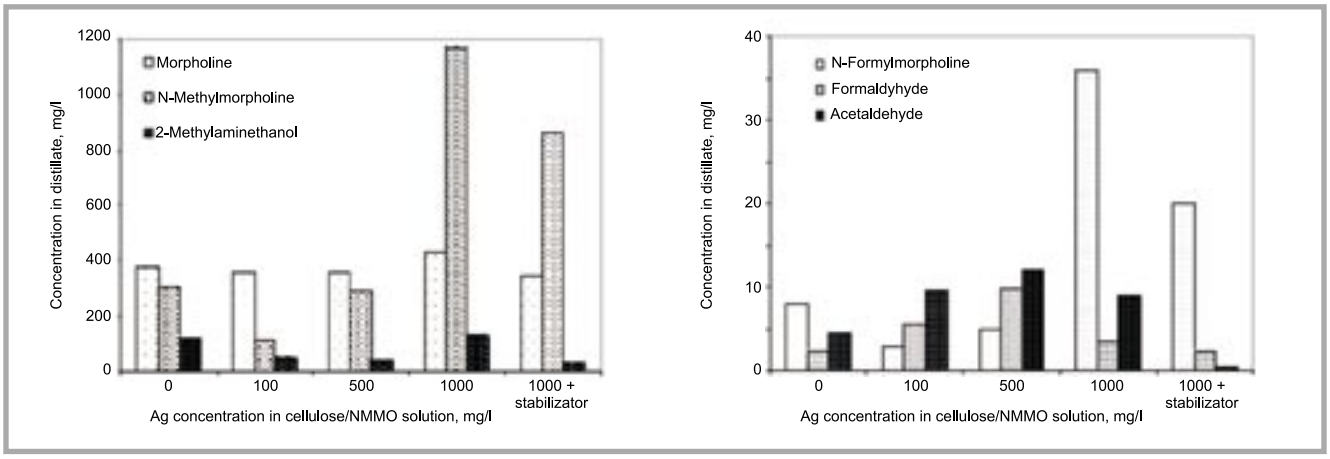

Figure 3. *By-products measured by HPLC in distillates of cellulose/NMMO solutions modified with varying silver concentrations and stabiliser (ISDB/BSDB).*

modified solution. More pregnant is the enhancement of the pressure slope with rising silver concentration, referring to the formation of gaseous by-products. In addition, solutions with 500 and 1000 mg/kg of silver show after 22 and 18 h, respectively a second pressure maximum confirming the occurrence of auto catalytic reactions [11]. It is obvious that particles at nm scale influence catalytic efficiency.

Stabilised silver solutions exhibit decreased pressure maxima and no second maximum compared to unstabilised solutions. Again, the PG-NaOH-NH_2OH system represents the more effective stabilisation system up to a concentration of 500 mg/kg silver. Solutions with 1000 mg/kg can not be stabilised successfully.

Production of Lyocell fibres with silver nanoparticles

The spinning process of Lyocell fibres with the application of nanoscaled silver particles up to 0.5% (approx. 600 mg/kg in the solution) proceeds without any impact on textile physical parameters (Table 2). Tenacity decreases, but at a low level. A crucial issue concerning the functional effect of the fibre is the homogeneity of silver particles in the spinning solution. The addition of silver as a suspension in NMMO assures sufficient distribution in the fibre. Desired silver content is adjustable with a recovery rate of approx. 70%.

Incorporation of colloidal silver particles (5 - 20 nm) occurred without any problems concerning the stabilisation of the cellulose/NMMO solutions and the spinning process. Here, the concentrations of silver do not exceed 200 mg/kg in the fibre, respectively and 24 mg/kg in the solution. What is noteworthy in the application of colloidal silver in those concentration ranges is that it results in fibres of very high whiteness (approx. 35%).

The measurement of silver was accomplished by x-ray fluorescence spectroscopy (XRF) and flame atomic absorption spectrometry (F-AAS). XRF represents a solid method with the benefit of a direct measurement of the fibre without any sample preparation. However, only a detection limit of approx. 100 mg/kg is achievable. Prior to measurement F- AAS requires sample digestion, nevertheless a detection limit of 5 mg/kg is realistic. Figure 5 shows the excellent correlation of both methods.

Antimicrobial screening

The number of new biomaterials in medicine is steadily growing. Highly efficient *in vitro* methods are required for quality control, screening and product improvement. For precise testing of antimicrobial efficacy, three independent aspects must be considered: adhesion (detect and quantify adherent microorganisms), proliferation (assay the potential of adherent bacteria for proliferation), and the detection of bactericidal and bacteriostatic activity. Here, a technique for testing the antimicrobial properties of biomaterials using a micro plate system was applied. It can be used to investigate the influences of the geometry and microstructure of surfaces as well as the binding behaviour of surfaces in response to cells, agents, coatings and surface-active compounds [14, 19].

Table 3 summarises the antimicrobial activity of fibres modified with nanoscaled silver particles. Fibres with porous

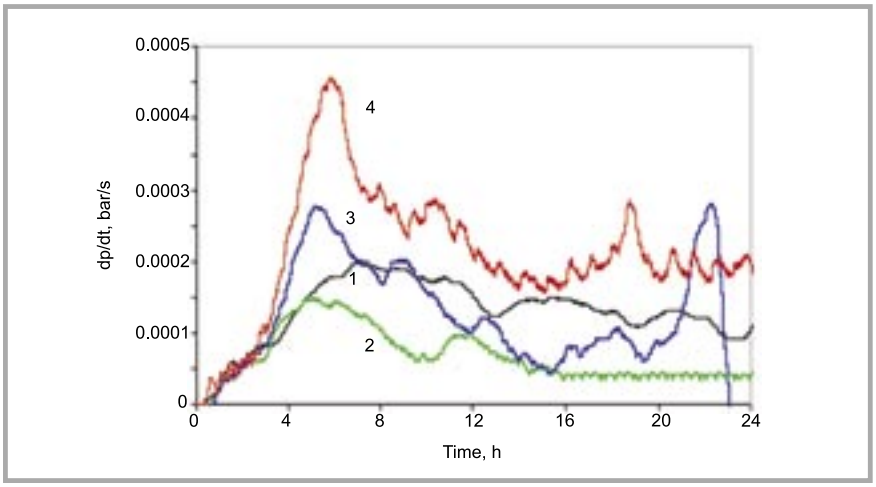

Figure 4. *Relationships between the heating time and pressure slope (dp/dt) of cellulose/NMMO solutions modified with varying silver concentrations at 140 °C; 1 - without, 2 - 100 mg/kg, 3 - 500 mg/kg, 4 - 1000 mg/kg.*

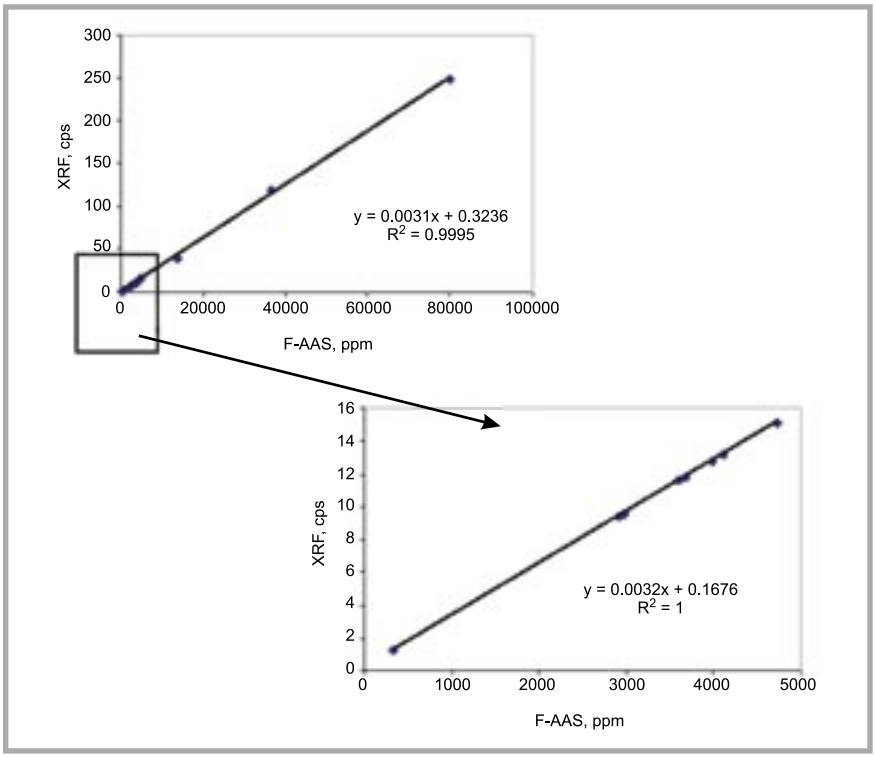

Figure 5. *Correlation of x-ray fluorescence spectroscopy (XRF) and flame atomic absorption spectrometry (F-AAS) for Lyocell fibres modified with different silver concentrations.*

Table 2. *Textile-physical parameters of fibres with nanoscaled silver particles (MicroSilver BG™).*

Parameter	Fiber 1	Fiber 2	Fiber 3	Fiber 4
Ag concentration, theoretical, mg/kg	500	2500	5000	5000
Ag concentration in the fiber, mg/kg	336	1936	3500	3990
Recovery rate, %	67.2	77.4	70.0	79.8
Fineness, dtex	2.01	2.05	1.9	1.95
Tenacity, dry, cN/tex	39.6	35.4	37.5	34.7
Elongation, dry, %	12.3	13.6	12.3	13.7
Loop tenacity, cN/tex	8.74	7.95	10.1	10.7

Table 3. *Test results of antimicrobial activity of fibres modified with MicroSilver (1 - 4) and NanoSilver (5 - 7).*

Sample	Ag concentration, mg/kg	Onset OD, brutto, h	Onset OD, netto, h	Test result
0 (reference)	0	6.1	-	
1	336	5.2	-	non antimicrobial
2	1936	33.5	27.4	good antimicrobial
3	3500	19.2	13.1	pronounced antimicrobial
4	3990	Limits	> 48	bactericide
5	24	28.2	8.3	antimicrobial
6	47	Limits	> 48	bactericide
7	76	Limits	> 48	bactericide

MicroSilver (sample 1 - 4) show antimicrobial effects only for higher concentrations; fibre 1 with 336 mg/kg possesses no effect. Because of the formation of aggregates, the antimicrobial performance of nanoscaled particles is achievable only by increasing the concentration. Furthermore, there is no correlation between silver concentration and antimicrobial effect. On the other hand, fibres with colloidal NanoSilver (sample 5 - 7) exhibit distinguished results. Even from 24 mg/kg of silver antimicrobial activity was staked whereas, from approx. 50 mg/kg, a bactericide effect is accessible. As mentioned above these fibres exhibit a permanent whiteness, and hence comply with the aim of this study. Higher loaded fibres (100 or 200 mg/kg) can be blended with unmodified fibres maintaining antimicrobial activity.

Conclusions

The objective of this study was to investigate the incorporation of nanoscaled silver particles into cellulose/NMMO solutions in order to manufacture bioactive fibres. With regard to thermostability solutions with concentration of porous silver up to 500 mg/kg can be successfully stabilised. Stability of the spinning process is warranted. Textile processability and textile-physical parameters remain unaffected. Atomic absorption spectrometric and x-ray analysis confirmed a sufficient recovery of the incorporated silver. Both methods revealed a pronounced correlation with an advantage to F-AAS due to a lower detection limit. Fibres of high fineness (B - type 0.2 tex) and high tensile strength are producible. By application of colloidal NanoSilver, a bactericide effect at very low silver concentration (50 mg/kg) is available. Consequently, higher loaded fibres blended with unmodified fibres can easily be processed to get yarns maintaining antimicrobial activity. These features firstly enable the production of silver containing fibres with a high degree of white and light dyeing. White shirts and underclothes as well as white paper for long term storage are new applications.

Acknowledgments

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