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Viscoelastic properties and antimicrobial activity of cellulose fiber sheets impregnated with Ag nanoparticles

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ABSTRACT

A silver nanoparticle colloid was prepared by a modified Tollens method using D-glucose as the reduction agent. The obtained nanoparticles were used for the modification of pine, linter and recycled cellulose fibers. Although the silver contents were relatively low (0.05–0.13 wt.%), the cellulose-sheets prepared from the modified fibers show improved mechanical and viscoelastic properties. The tensile index (strength) increased with up to 30% in comparison to the index of the sheets obtained from the untreated fibers. The influence of the nanoparticles on the viscoelastic properties of the cellulose sheets was investigated by dynamic mechanical analysis (DMA) in the temperature range from −120 to 20 °C and with a force frequency of 100 Hz. A broad relaxation transition positioned at −80 °C was observed in the loss modulus spectrum of all the cellulose sheets, while the Ag-modified sheets exhibited higher storage moduli values in the whole temperature range. The antimicrobial activity tests show that the pine, silver and recycled cellulose fiber sheets with silver nanoparticles can be successfully employed to prevent the viability and growth of the common pathogens *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

1. Introduction

Paper is a relatively inhomogeneous material, which on a structural level can be described as a dense network of cellulosic wood pulp fibers. The essential feature of this network is that the fibers are bonded, i.e. the cohesion in paper is achieved rather by hydrogen bonding at the fiber–fiber contact points and not by their simple mechanical entanglement (Dodson, 1970; Haslach, 2000). The mechanical properties of paper depend on different factors but mainly on the corresponding fiber properties, the network structure and drying conditions. Since the hydrogen bonding strongly affects the behavior of paper under mechanical loading, different bonding agents are usually employed to increase the number of bonds between the surfaces of adjacent fibers. On the other hand, the infra-red spectrum analysis of cellulose showed that almost all of the hydroxyl groups present are hydrogen bonded among themselves, i.e. very few free hydroxyl groups are available to participate in the inter-fiber bonding during the network formation (Dodson, 1970). One of the methods for improving the fiber–fiber bonding could be incorporation of the fibrillated cellulose fibers and nano-cellulose, because they have much higher surface areas than conventional fibers. Recently, Henriksson, Berglund, Isaksson, Lindstrom, and Nishino (2008) used cellulose nanofibrils to prepare nanopaper structures with extremely high toughness. In the present study, we exploited a tendency of silver nanoparticles to interact with OH groups (Mbhele et al., 2003) in order to modify cellulose fibers and to use them for the preparation of sheets with enhanced mechanical and viscoelastic properties.

The biomineralization and biotemplating methods have evolved into an important research area of nanoscience and nanotechnology since they offer a possibility for the preparation functional nanostructures and their integration into technologically useful forms. Cellulose and its derivatives have been proved to be good materials for functionalization with metallic nanoparticles since they have a large number of hydroxyl groups that are accessible for chemical modification (Cai, Kimura, Wada, & Kuga, 2009; Diez et al., 2011; Drogat et al., 2011; Shin, Bae, Arey, & Exarhos, 2007, 2008; Son, Youk, & Park, 2006; Pinto et al., 2009). Several groups exploited this property in order to stabilize silver nanoparticles on the cellulose nanocrystals/fibers and investigated the antimicrobial activity

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of the obtained nanostructures (Diez et al., 2011; Drogat et al., 2011; Pinto et al., 2009). On the other hand, in a recent study, Gottesman et al. (2011) showed that paper with microbiocidal properties can be obtained by sonochemical coating of silver nanoparticles onto its surface. Here we decided to combine these two approaches and prepare paper sheets starting from silver nanoparticle modified cellulose fibers. The silver nanoparticles were synthesized by a green procedure using D-glucose as reducing agent and after that mixed with three types of cellulose fibers: pine, linter and recycled. As will be seen, the incorporation of the modified fibers into the network structure significantly increase the strength of the obtained sheets while at the same time they exhibit a strong activity against the common pathogens *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The observed effects are especially important in the case of sheets prepared from recycled fibers. It is well known that recycled paper usually has poor mechanical properties because the recycled fibers are short, damaged and hornificated. Also, these fibers might originate from sources that are more exposed to different microorganisms. The modification of recycled fibers by silver nanoparticles can address both these problems. Besides the activity against possible pathogens, the silver nanoparticles attached on the surface of the fibers can facilitate fiber–fiber bonding and consequently improve the web formation.

Another, equally important, part of this study concerns the dynamic mechanical behavior of the obtained fiber sheets as function of temperature. Paper is the viscoelastic material but the methods usually employed to study its viscoelastic properties are creep and stress relaxation (Alfthan, 2004, 2010; DeMaio & Patterson, 2006, 2007; Mustalahti, Rosti, Koivisto, & Alava, 2010). To our best knowledge, there is only one article that reports on the mechanical relaxation processes observed in the dynamical mechanical spectrum of paper. In the early eighties, Roylance, McElroy, and McGarry (1980) from MIT investigated the viscoelastic properties of paper used for preparation of cones for loudspeakers. It was found that paper has a broad mechanical relaxation transition positioned at approximately −40 °C. Unfortunately, their work stayed widely unnoticed probably due to rather complicated experimental procedure at that time which included dynamical mechanical analysis at an extremely high force frequency of 110 Hz in a wide temperature range from −120 °C to room temperature. We believe that the mentioned procedure, easily accessible with modern DMA instruments, could provide additional information about the changes in the viscoelastic properties of the fiber sheets induced by modification with silver nanoparticles but also by some other factors such as density, bonding agents, and length of the fibers. In that sense, our attempt to draw attention on the mentioned method might also be useful to the researchers from the other fields of the paper science and technology.

2. Materials and methods

2.1. Materials

2.1.1. Cellulose fibers

Three types of cellulose fibers were used in this study: pine, linter and recycled. Bleached linter and pine fibers were received from Buckeye Technologies Inc. and Robert Placzek GmbH., respectively. The recycled fibers were collected from Hartmann Hungary Ltd., a recovery paper processing company.

2.1.2. Preparation of silver nanoparticle colloid

Silver nanoparticles were prepared by a "green" synthetic approach using D-glucose as reducing agent. Silver nitrate ($AgNO_3$) and D-glucose were purchased from Sigma Aldrich and used as received. In a typical procedure, 5 ml of 0.1 M solution of D-glucose was added to 100 ml of a 2 mM aqueous solution of $AgNO_3$ and the mixture was stirred and purged with argon for 20 min at room temperature. After that, 5 ml of the mixture was transferred to a separate vial and the pH of the solution was set to 8.5 by adding a few drops of 10% NH_4OH. Finally, the obtained solution was treated in microwave oven at 750 W for 30 s.

Table 1
List of samples, preparation conditions and silver content.

Sample[a]	Fibers (g)	2 mM Ag colloid (ml)	wt.% of Ag[b]
P-Ag-0	3	0	0
P-Ag-5	3	15	0.07
P-Ag-10	3	30	0.13
L-Ag-0	3	0	0
L-Ag-5	3	15	0.07
L-Ag-10	3	30	0.12
R-Ag-0	3	0	0
R-Ag-5	3	15	0.05
R-Ag-10	3	30	0.10

[a] L, linter; P, pine; and R, recycled fibers.
[b] wt.% of Ag denotes the weight percentage of silver in the cellulose sheets obtained by ICP-OES.

2.1.3. Modification of cellulose fibers with silver nanoparticles

The silver colloid solution was added to a water suspension of pine, linter or recycled cellulose fibers (3 g of fibers in 300 ml of water). In order to check whether the concentration of the nanoparticles affects the viscoelastic properties of the obtained fiber sheets (see the procedure below), the amount of silver colloid used in the modification procedure was varied to 15 ml and 30 ml (or 5 and 10 ml of colloid per gram of fibers). Denotation of the samples and the preparation conditions are given in Table 1.

2.1.4. Preparation of the fiber sheets

Hand-sheets of as received (untreated) and modified pulps were made with a basis weight of 100 g m^{-2} by using HAAGE D-4330 Systems laboratory sheet former according to DIN EN ISO 5269-2. After drying, all the samples were conditioned at 50% relative humidity and a temperature of 23 °C.

2.2. Characterization

2.2.1. Scanning electron microscopy (SEM)

Scanning electron microscopy of untreated and silver nanoparticle modified fibers was carried out using a JEOL JSM-6610LV instrument at an accelerating voltage of 30 kV. Prior to observation, the samples were covered with a thin layer of gold.

2.2.2. UV–vis absorption spectroscopy

The UV–vis absorption measurements of the Ag-colloid solution as well as the water suspensions of Ag-modified pine, linter or recycled cellulose fibers were carried out on a Thermo Evolution 600 spectrophotometer.

2.2.3. Tensile tests

Tensile tests of unmodified (P-Ag-0, L-Ag-0 and R-Ag-0) and Ag nanoparticle modified (P-Ag-10, L-Ag-10 and R-Ag-10) cellulose shits were performed on a FRANK type Tensile Tester machine according to EN ISO 1924-2 standard. The cross head speed was 20 mm min^{-1} and the distance between the clamps 180 mm. The samples were rectangular in shape with a size width of 15 mm and an approximate thickness of ~0.7 mm.

2.2.4. Dynamic mechanical analysis (DMA)

Dynamic mechanical analyses of the fiber sheets were carried out in tensile mode in the temperature range from −120 to 20 °C on

a Perkin Elmer Diamond DMA with a 10 μm strain amplitude and a 50 mN initial force amplitude. The measuring frequency was 100 Hz and the heating rate was 2 °C min^{-1}. The specimens were in a rectangular shape (20 mm × 10 mm), with an approximate thickness of ~0.7 mm. The distance between the clamps was 10 mm.

2.2.5. Inductively coupled plasma-atomic spectrometry

The atomic spectrometry (ICP-OES method) was used to determine the amount of silver nanoparticles in the cellulose sheets as well as the amount of silver released from the sheets during the antimicrobial activity tests. In order to find the silver content in the cellulose sheets, the digestion of the samples was performed on an Advanced Microwave Digestion System (ETHOS 1, Milestone, Italy) using HPR-1000/10S high pressure segmented rotor. In the digestion procedure, about 0.25 g of a precisely weighed sample was mixed in a clean vessel with a mixture of 10 ml HNO_3 (65%) and 1 ml H_2O_2 (30%), and then heated with microwave energy for 30 min. The temperature was controlled with a predetermined power program. The temperature was typically raised to 200 °C in the first 15 min, kept constant at peak temperature of 200 °C in the next 15 min, and then cooled down rapidly. After cooling and without filtration, the solution was diluted to a fixed volume (25 ml). The obtained solutions are subsequently investigated on a Thermo Scientific iCAP 6500 Duo ICP spectrometer (Thermo Fisher Scientific, Cambridge, United Kingdom) equipped with RACID86 Charge Injector Device (CID) detector. The weight percentage of silver was stated in Table 1 (last column).

To establish the amount of silver release, the Ag cellulose sheet (approx. 0.01 g) was immersed in 10 ml of buffer solution and kept at 37 °C (the same procedure carried out during antimicrobial testing). After 1 h of release, 1 ml aliquots were taken from the solution and diluted by concentrated solutions of HCl and HNO_3 in order to disintegrate any remaining nanoparticles in the samples. The release contents are presented in Table 2. All the reported results of ICP-OES measurements are the average values from three identically prepared samples.

2.3. Microorganisms and antimicrobial activity

2.3.1. Microorganisms and culture conditions

The antimicrobial activity tests were carried out using common pathogens as indicator strains: gram-negative bacteria *E. coli* ATCC 25923, gram-positive bacteria *S. aureus* ATCC 25922 and yeast *C. albicans* ATCC 24433. The maintenance and growth of microorganisms was carried out in a trypton soy broth supplemented with 0.6% (v/v) yeast extract (TSYB Institute of Immunology and Virology, Torlak, Belgrade). The microorganisms were cultivated in 3 ml TSBY at 37 °C and left overnight (late exponential stage of growth). The initial numbers of *S. aureus*, *E. coli* and *C. albicans* in the testing medium were 3.52×10^5 CFU ml^{-1}, 3.74×10^5 CFU ml^{-1}, and 8.12×10^5 CFU ml^{-1}, respectively.

2.3.2. Antimicrobial activity testing

The antimicrobial activity of the silver-modified paper sheets was quantitatively assessed in a potassium hydrogen phosphate buffer solution according to the ASTM E 2149-01 standard. Cellulose sheet samples 0.01 g in weight (approximately 1 cm × 1 cm in size) obtained from untreated and silver modified fibers (pine, linter and recycled) were placed in glass tubes containing 9.9 ml of testing solution followed by addition of 0.1 ml of microbial inoculum (prepared by mixing 1 ml of initial cultures with 9 ml of saline). The resulting mixture with samples was vortexed for 10 s and incubated at 37 °C in a waterbath shaker. After 1 h of exposure, 0.1 ml aliquots were removed and further diluted with sterile physiological saline solution (8.5 g NaCl in 1 l of water). From all dilutions 0.1 ml aliquots were placed in Petri dishes, covered with trypton soy agar (Torlak, Serbia) and after 24 h of incubation at 37 °C, the counts of viable bacteria were made.

The percentage of bacterial reduction (R, %) was calculated using the following equation:

$$R = \frac{C_0 - C}{C_0} \times 100, \quad (1)$$

where C_0 (CFU – colony forming units) is the number of bacterial colonies from the control saline and C (CFU) is the number of bacterial colonies from the samples.

3. Results and discussion

3.1. Silver colloid and modification of the fibers

The silver hydrocolloid was synthesized by a modified Tollens procedure, in which Ag^+ ions were reduced by saccharides in the presence of ammonia, yielding Ag nanoparticles with sizes from 50 to 200 nm (Panacek et al., 2006). Fig. 1 shows the UV–vis absorption spectrum of the as-prepared colloid. The spectrum shows a dominant absorption at ~410 nm, which is typical for nanostructured silver.

The SEM images of the untreated and modified fibers are shown in Fig. 2. It can be seen in Fig. 2b, d, and f that agglomeration of the particles at the fiber surfaces takes place. It should be mentioned that EDX spectra (not shown) confirmed that the bright formation in Figs. 2b, d, and f originates from the silver. However, the EDX spectrum of the recycled fibers also showed traces of impurities such as Ni and Al.

3.2. Mechanical and viscoelastic properties

Mechanical tensile tests were performed to investigate the effect of silver nanoparticles on the strength of the obtained cellulose sheets. The tensile index values of the sheets prepared using as received and modified fibers are shown in Fig. 3. As can be seen, the tensile indices of the sheets made from untreated fibers vary

Table 2
Viable cells reduction activity of silver–cellulose fiber sheets on *E. coli*, *S. aureus* and *C. albicans* after 1 h of exposure.

Sample	Ag release after 1 h (μg/ml)	*S. aureus*		*E. coli*		*C. albicans*	
		CFU (ml)	R (%)	CFU (ml)	R (%)	CFU (ml)	R (%)
P-Ag-0	–	2.90×10^5	–	3.36×10^5	–	8.50×10^5	–
P-Ag-5	0.36	7.10×10^3	97.55	6.00×10^3	98.21	–	100
P-Ag-10	0.42	1.56×10^3	99.46	1.00×10^3	99.7	–	100
L-Ag-0	–	1.90×10^5	–	4.96×10^5	–	5.10×10^5	–
L-Ag-5	0.31	6.90×10^3	96.37	1.10×10^4	97.78	3.60×10^3	99.29
L-Ag-10	0.34	4.60×10^3	97.58	9.30×10^3	98.13	2.40×10^3	99.53
R-Ag-0	–	1.90×10^5	–	1.25×10^5	–	6.00×10^4	–
R-Ag-5	0.27	1.60×10^4	91.58	8.00×10^3	93.60	1.50×10^3	97.5
R-Ag-10	0.30	1.10×10^4	94.21	5.00×10^3	96.00	1.13×10^3	98.12

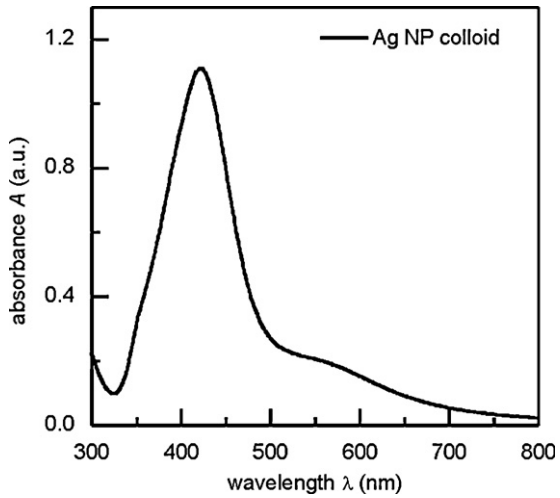

Fig. 1. UV–vis absorption spectra of a silver nanoparticle water colloid.

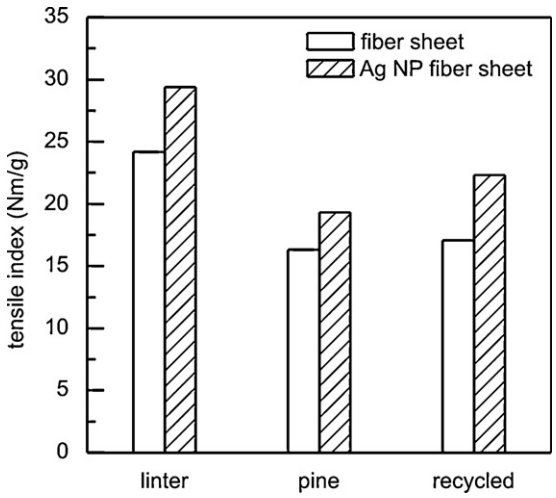

Fig. 3. Tensile index of the cellulose sheets obtained from untreated and silver nanoparticle modified linter, pine and recycled fibers.

from 16.3 N mg^{-1} for pine up to 24.1 N mg^{-1} in the case of the linter. The tensile strength of the paper depends on several factors that include: (1) the average load-bearing ability of the individual fibers, (2) the number of fibers at any given cross-section available for load transfer, and (3) the uniformity of the load transfer that is allowed by the network structure (Cowan, 1995). On the other hand, inhomogeneity is a fundamental feature of the paper network, meaning that non-uniformity of the load transfer cannot be avoided. It is also obvious that improving fiber bonding can improve the ability of network to support load. Fig. 3 shows that all three sheets prepared from the silver-modified fibers show much higher tensile indices than their unmodified counterparts. The highest increase of about 30% was noticed in the case of the recycled fiber sheet, while in the case of the pine and linter sheets the index increases by about 20%. The observed effect could be a direct consequence of a stronger fiber–fiber interaction in the presence of silver nanoparticles. Another possibility might be that the presence of glucose and ammonia can affect the fiber–fiber bonding. However, when the pine sheets were prepared from fibers previously mixed with glucose only and with glucose/ammonia solutions instead of water, no significant changes in the mechanical properties were observed. It should also be emphasized that strength of the paper can

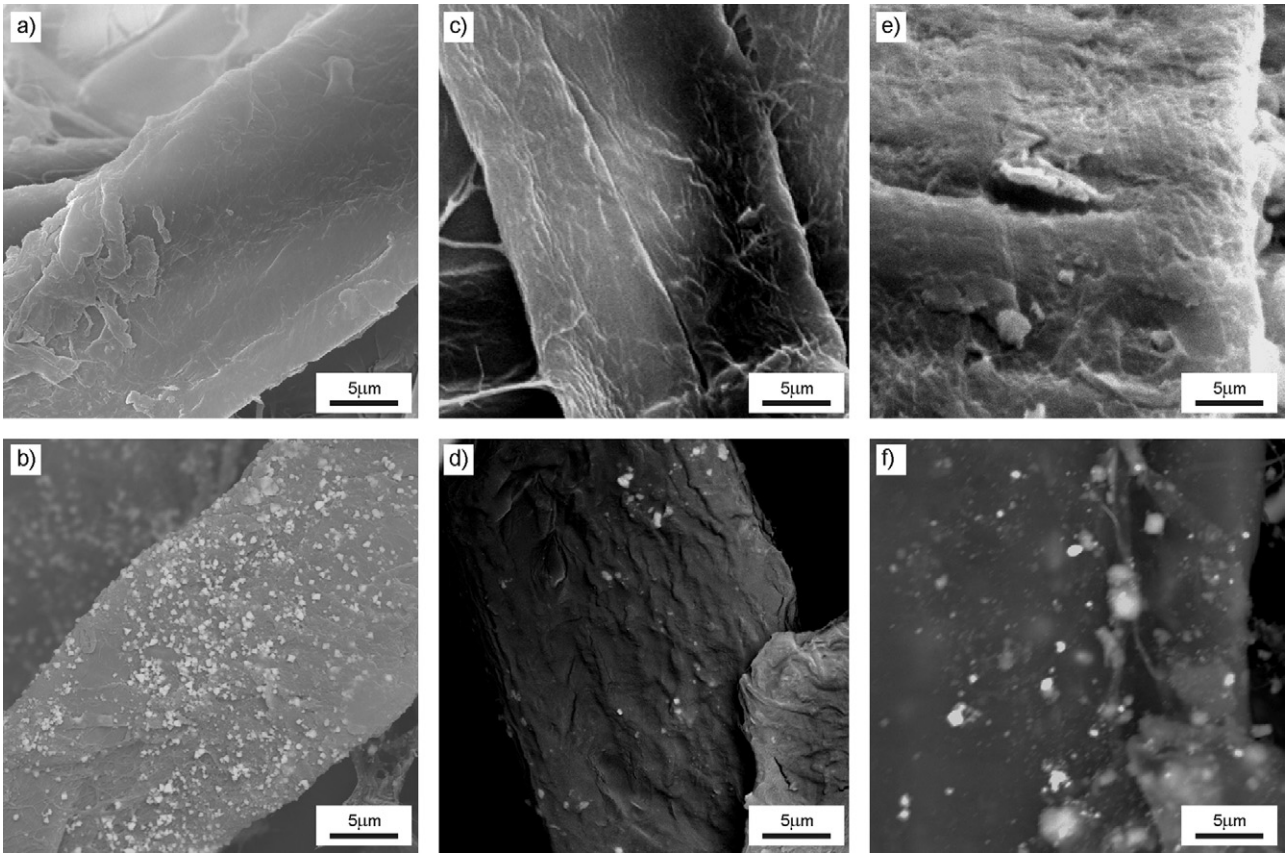

Fig. 2. SEM micrographs of as received and silver nanoparticle modified linter (a and b), pine (c and d) and recycled (e and f) fibers.

sometimes be affected by formation, grammage, pulping process, or fiber orientation and length (Considine, Scott, Gleisner, & Zhu, 2005). In the present study, the laboratory sheet former produced the sheets with random orientation of the fibers, which resulted with isotropic mechanical properties. Prior to measurements the samples were conditioned in order to minimize the effects of moisture and temperature. Finally, tensile index of the sheets is reported instead of tensile strength since it enables comparison of the strength of the sheets regardless of their grammage.

Paper is an important engineering material and its time-dependent properties are also of importance. Application of load to paper sheets will result in a combination of elastic and viscous responses and various theoretical approaches were suggested to model this behavior. Researchers usually employ linear and non-linear continuum models in order to explain stress relaxation and creep, since these are the standard methods for investigation of paper viscoelastic properties (Alfthan, 2004, 2010; DeMaio & Patterson, 2006, 2007; Mustalahti et al., 2010). In the present study, we investigated possible changes in viscoelastic properties of the cellulose sheets after modification with silver nanoparticles using dynamic mechanical analysis (DMA). Figs. 4–6 show the storage modulus (E'), loss modulus (E'') and loss tangent ($\tan\delta$) of the sheets obtained from as received and modified linter, pine and recycled cellulose fibers. Figs. 4a, 5a and 6a show that the storage modulus of all three materials exhibit similar functional dependence from temperature, i.e. it gradually decreases with increasing of temperature from −120 to 20 °C. The magnitude of the storage modulus obviously depends on the type of fiber and it is the highest in the case of the L-Ag-0 sample and it is the lowest for R-Ag-0. However, in all three cases, the modification of fibers with silver nanoparticles induces an increase in storage modulus of the obtained sheets in the whole temperature range (Figs. 4a, 5a and 6a). The observed effect is not linear. The modification with lower amounts of silver colloid has a much higher impact on the increase in the elasticity of the material (P-Ag-5, L-Ag-5 and R-Ag-5 samples). Further increase of the amount of colloid reversed the effect and in the case of linter fibers the magnitude of E' even falls below the value obtained for the unmodified fibers. Besides positive effects on the strength of fiber–fiber interactions, it seems that at higher concentrations, the silver nanoparticles also affect the free OH groups that participate in inter-fiber hydrogen bonding which in turn reduces the ability of the network to react elastically to an applied load. On the other hand, in addition to the elastic response to frequency dependent stress, an irreversible plastic deformation in the fiber network will take place and it is associated with microscopic fracture damage and energy dissipation. Of course, the energy dissipation is related to the obtained loss moduli (E''), which are shown in Figs. 4b, 5b and 6b. The E'' curves of all three materials are little bit 'noisy' due to the high measurement frequency (100 Hz), but they clearly show the presence of a broad relaxation transition positioned around −80 °C. The fact that the transition occurs over a broad temperature interval suggests that the fibrous network is highly inhomogenious. The results presented here are in agreement with those obtained by Roylance et al. (1980). They observed a broad transition at −40 °C in the DMTA spectrum of the paper used for the cones of loud speakers (force frequency was 110 Hz). In spite of the inhomogeneous microstructure of the paper, they also found that the dynamic mechanical behavior can be modeled by using the continuum three arms Wiechert model (Roylance et al., 1980).

It should be emphasized that the cellulose sheets were conditioned at 50%RH prior to DMTA measurements and that the water present in the sample can affect the shape of the curves at temperatures close to 0 °C. Having no other experimental data to compare with, we decided to strictly follow the procedure suggested by Roylance et al. (1980) and we did not dry the samples before the measurements. The low intensity transitions in the E'' curves at

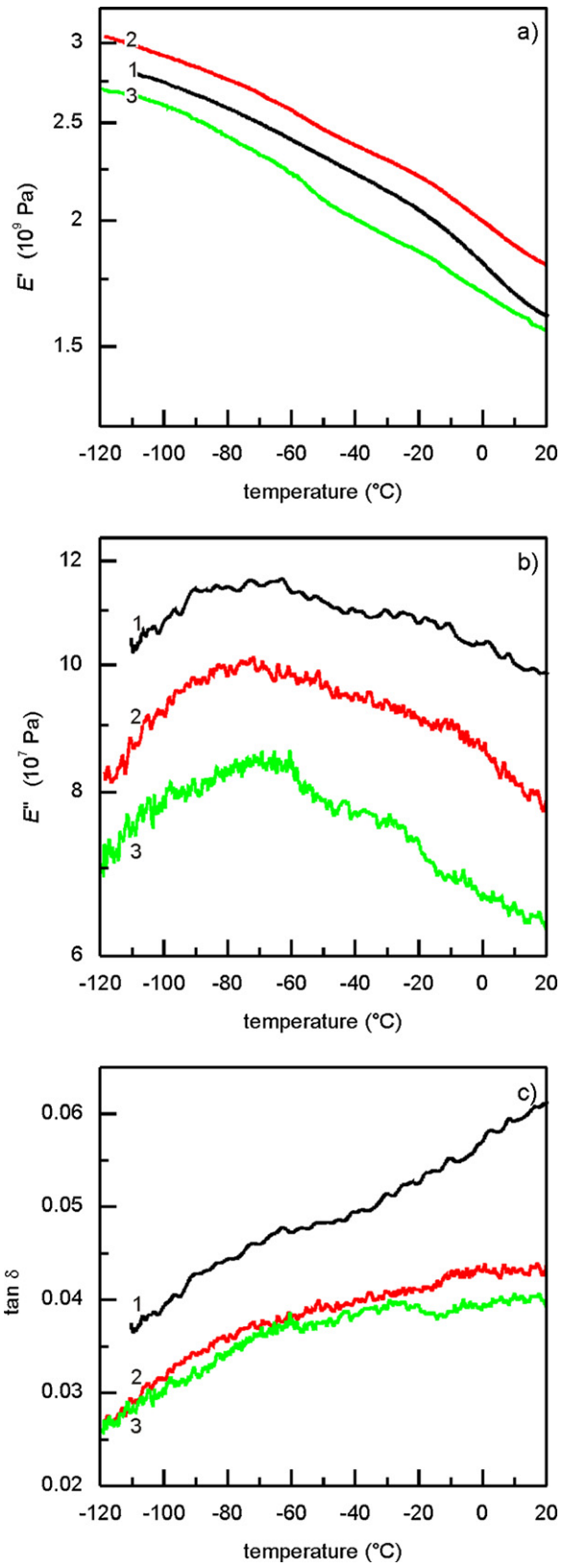

Fig. 4. (a) Storage tensile modulus (E'), (b) loss modulus (E'') and (c) loss angle tangent ($\tan\delta$) versus temperature for the L-Ag-0 (curve 1), L-Ag-5 (curve 2) and L-Ag-10 (curve 3) samples. The measurements were carried out at 100 Hz.

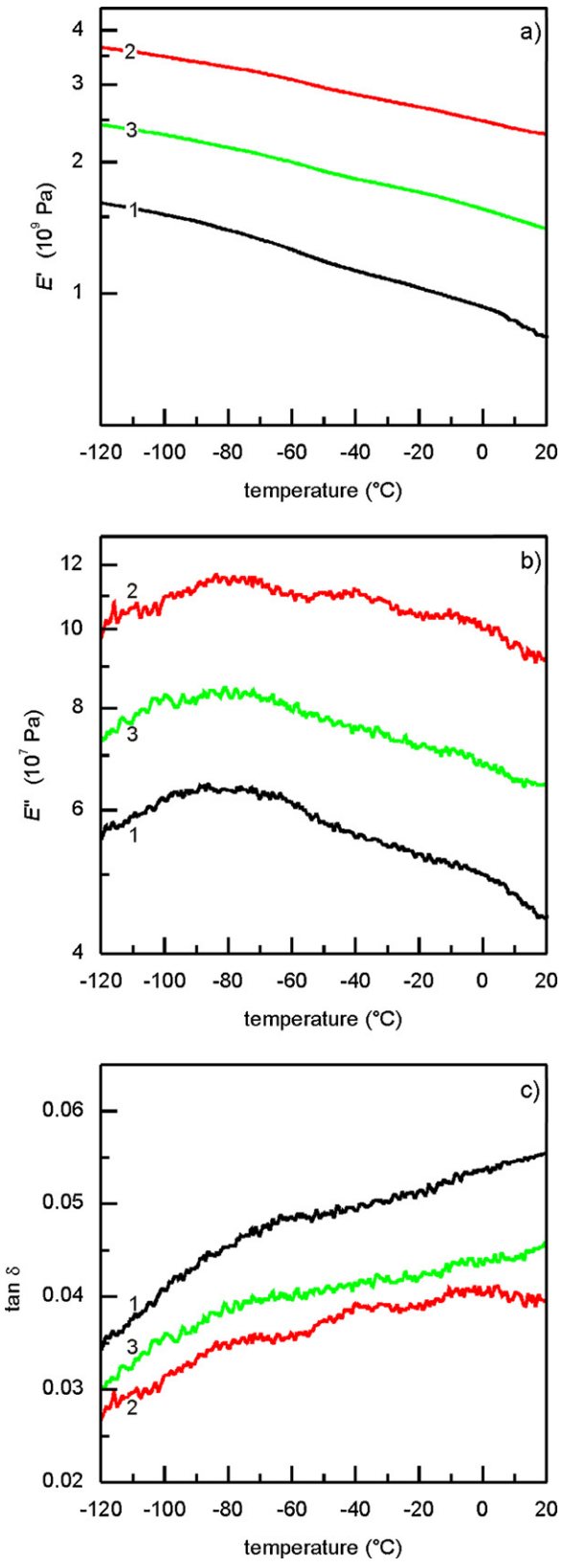

Fig. 5. (a) Storage tensile modulus (E'), (b) loss modulus (E'') and (c) loss angle tangent ($\tan\delta$) versus temperature for the P-Ag-0 (curve 1), P-Ag-5 (curve 2) and P-Ag-10 (curve 3) samples. The measurements were carried out at 100 Hz.

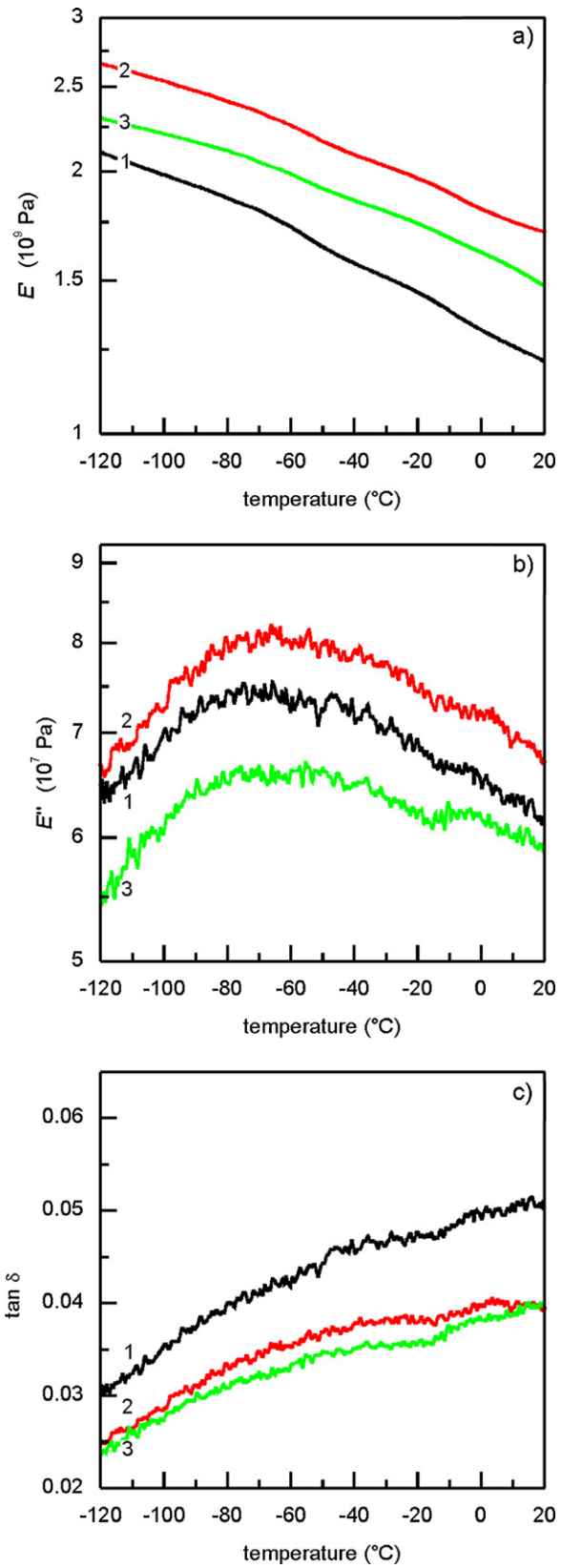

Fig. 6. (a) Storage tensile modulus (E'), (b) loss modulus (E'') and (c) loss angle tangent ($\tan\delta$) versus temperature for the R-Ag-0 (curve 1), R-Ag-5 (curve 2) and R-Ag-10 (curve 3) samples. The measurements were carried out at 100 Hz.

temperatures ~0 °C are probably the result of the activation of water molecules. However, the loss modulus of the P-Ag-5 sample (Fig. 4b) evidently shows the presence of another transition at ~40 °C (far below the freezing temperature of water), meaning that the silver nanoparticles might induce additional heterogeneity into the fiber network.

Figs. 4c, 5c and 6c show the loss tangents of the pine, linter and recycled fiber sheets. In all three cases, the sheets made of unmodified fibers exhibit higher tan δ values in the temperature interval investigated here. The modification with silver colloid clearly improves the elasticity of the materials and reduces the energy losses. Nevertheless, as noticed above, better results were obtained with lower amounts of colloid. The fiber bonding can improve viscoelastic properties only up to a certain extent. DeMaio and Patterson (2006), who studied the influence of bonding on constant humidity tensile creep behavior, showed that either decreasing specific bond strength (with a debonder) or increasing specific bond strength (with a bonder) had no effect on the creep behavior as long as there was an adequate level of bonding to maintain an efficiently loaded structure. Once paper reached a fully efficient loaded state, changes in bonding would no longer influence creep deformation but only creep failure. It is worth emphasizing that individual cellulose fibers themselves show viscoelastic properties. However, since the typical creep behavior of the network does not coincide to that of a single fiber, it was concluded that the viscoelastic properties of paper should be treated from a coarse-grained point of view (Mustalahti et al., 2010). For this reason, we consider that changes in the overall viscoelastic properties of the modified sheets are governed rather by improved fiber–fiber interaction than by possible changes in the viscoelastic properties of the individual fibers after modification with silver nanoparticles.

3.3. Antimicrobial activity

Silver is one of the most powerful antimicrobial agents that shows a strong activity toward a broad range of microorganisms and simultaneously a remarkably low human toxicity (Chaloupka, Malam, Sefalian, 2010). The antimicrobial activity of ionic silver from the silver salts and complexes is generally governed by its strong affinity toward a number of electron donor functional groups (thiols, phosphates, imidazoles, indoles and amines) from the biomacromolecular components of the cells (McDonnell & Russell, 1999). However, although silver complexes are effective antimicrobial agents, they are prone to neutralization by the anions from the environment and their use may also result in unwanted adsorption of ions in epidermis cells and sweat glands (Russell & Hugo, 1994; Silver, 2003). This is why researchers turned to silver nanoparticles and especially to their composites with synthetic and biopolymers. The results on the antimicrobial activity of silver–polymer nanocomposites were summarized in recent review by Dallas, Sharma, and Zboril (2011). The mechanism of the antimicrobial activity of silver nanoparticles is still a matter of dispute. According to Dallas et al. (2011), the three most common mechanisms proposed are: (i) gradual release of free silver ions, followed by disruption of ATP production and DNA replication, (ii) silver nanoparticle direct damage to cell membranes (Morones et al., 2005), and (iii) silver nanoparticle and silver ion generation of reactive oxygen species (AshaRani, Mun, Hande, & Valiyaveettil, 2009; Banerjee, Mallick, Paul, Chattopadhyay, & Ghosh, 2010; Kumar & Munstedt, 2005; Lok et al., 2007; Smetana, Klabunde, Marchin, & Sorensen, 2008). In a study on polyamide–silver microcomposites, Kumar and Munstedt (2005) reported that the oxidation of metallic silver to an active Ag^+ species can be achieved through an interaction of the silver with the water molecules. It seems that this is also an important condition for the silver nanoparticles to be fully efficient, since Lok et al. (2007) showed that partially oxidized silver nanoparticles exhibit antimicrobial properties, whereas zero-valent nanoparticles do not. On the other hand, electron microscopy analyses gave evidence that the silver nanoparticles can attach to the surface of the cell membrane and thus impair its proper functions or to penetrate inside the bacteria (whether by diffusion or by endocytosis processes) and cause additional cytotoxic and genotoxic effects (Silver, 2003).

The results of the antimicrobial activity of cellulose sheets obtained from unmodified and silver nanoparticle modified fibers are presented in Table 2. The cellulose sheets prepared from unmodified fibers showed virtually no activity against the tested strains. On the other hand, the sheets prepared from silver modified fibers exhibited strong antimicrobial activity with a percentage of reduction which is higher than 90% for all three microorganisms. The highest activity was noticed against *C. albicans* with more than 98% of reduction, while pine sheets even induced total reduction of this microbial fungus. Silver modified pine sheets were also the most effective in the case of *S. aureus* and *E. coli* bacteria with more than 99% of reduction observed for the P-Ag-10 sample. The results in Table 2 further show that the sheets made from silver nanoparticle modified recycled fibers exhibited lower activity than those obtained from pine and linter fibers. Nevertheless, the presented results suggest that highly effective antimicrobial activity of the sheets prepared from silver modified fibers can prevent growth of pathogens on their surfaces. This is particularly important in the case of the sheets made of recycled fibers, since they come from various sources and they can be exposed to different pathogens in the first place. Also, trapping of silver nanoparticles in the cellulose sheets enables slow release of the ionic silver and/or silver nanoparticles into the pathogen medium. The ability of cellulose to absorb water can facilitate oxidation of the nanoparticles present in the sheets and the slow release of Ag^+ active species (Kumar & Munstedt, 2005), which could be important for possible applications of these materials. On the other hand, ICP testing showed that the concentration of the silver released from the sheets in the testing solution was extremely low and of the order of ~0.3–0.4 μg/ml (Table 2). The fact that only small amounts of silver contributed to the antimicrobial activity is a great advantage bearing in mind that at high concentrations silver nanoparticles might exhibit cytotoxic and genotoxic effects on human cells (AshaRani et al., 2009).

Although all three materials showed more than 90% of pathogen reduction, it is interesting to notice that *S. aureus* was slightly more resistant in the presence of all three silver modified sheets than *E. coli*. We believe that this might be related to the cell wall properties, which differ in Gram-negative and Gram-positive bacteria (Heijenoort, 2001). A higher resistance of *S. aureus* to the action of silver has also been noticed by Gottesman et al. (2011) in a study on a paper that is sonochemically coated with silver nanoparticles.

4. Conclusions

The present study shows that the mechanical and viscoelastic properties of cellulose sheets can be significantly improved through modification of the cellulose fibers with low concentrations (~0.1 wt.%) of silver nanoparticles. The sheets made of pine, linter and recycled modified fibers exhibited higher tensile indices (strengths) compared to their unmodified counterparts. It was demonstrated for the first time that dynamical mechanical thermal analysis (DMTA) at 100 Hz can be successfully used for studying the changes in the viscoelastic properties of the sheets induced by silver nanoparticle modification. The sheets that contained silver showed higher storage moduli and lower loss tangent (tan δ)

values over the whole temperature range of the measurement (from −120 to 20 °C). The obtained results were discussed in terms of the effects of the nanoparticles on the inter-fiber bonding in the cellulose web. We believe that the present approach can also be applied in studying the influence of other factors such as density, bonding agents, and length of the fibers on the viscoelastic properties of paper.

The pine, linter and recycled sheets made of silver nanoparticle modified fibers showed strong activity against gram-negative bacteria (*E. coli*), gram-positive bacteria (*S. aureus*) and pathogen fungus (*C. albicans*). The antimicrobial activity increased with increasing silver concentration, while the pine sheets were the most effective with more than 99% reduction of all three pathogens after 1 h of exposure. The strong antimicrobial activity makes these sheets promising materials for food packing, i.e. the prevention of contamination as well as food preservation. Also, the fact that the preparation with modified cellulose fibers had a positive effect on the mechanical and viscoelastic properties of the sheets could be a great advantage during the paper making process.

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