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Preliminary Investigation into the Antimicrobial Activity of an Electrospun Polyamide Nanofibrous Web with Micro Particles of Baltic Amber

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Abstract

Antimicrobial textile is a very important field for new investigations. The two aspects need be taken into account at the time of such investigations into the protection of the textile itself from damage caused by microorganisms, and that of the textile user against pathogenic or odour causing microorganisms. However, it is known that some materials which have really good antimicrobial activity are harmful or toxic and cannot be used for health care or medical application, due to which the necessity to find new natural and human friendly antimicrobial active materials and methods of how to increase the antimicrobial activity of textile is still open. One of the ways to solve this problem is usage of natural antimicrobial agents such as chitosan, plant extracts and others. Investigations on the usage of amber micro particles in the formation of a polyamide 6 nanoweb via electrospinning and on the antimicrobial activity thereof is analysed in this paper. The results show the antimicrobial activity of the material with Baltic amber investigated and the possibility of developing functional antimicrobial textile with amber micro particles via electrospinning.

Key words: antimicrobial activity, amber, electrospinning, nanofibers, textile.

Introduction

Antimicrobial textile is one of the fastest growing areas of multifunctional textile. Two different aspects of antimicrobial protection of textiles can be discussed. The first aspect is the protection of the textile itself from damage caused by microorganisms, and the second one is the protection of the textile user against pathogenic or odour causing microorganisms. Antimicrobial activity is a very important functional property of textile used for various applications, not only for medical but also for health care, hygienic, sports and leisure, and even for ordinary textile goods such as socks, underwear, etc. Textiles for outdoor use are constantly exposed to the influence of microorganisms. A lot of articles about investigations on antimicrobial activity and antimicrobial agents used in special finishing have been published in the last few decades. Different types of substances are used in the textile industry, for example metallic salts, nano-sized metals, and metal oxides, mainly silver, titanium dioxide, zinc oxide and copper oxide, the majority of which are for textile finishing, using treatment with antimicrobial compounds such as metallic silver and fungicide copper nanoprecipitates (SiO₂/Ag+Cu), zinc oxide, titanium dioxide, Triclosan, and others [1 - 5]. Silver nanoparticles (AgNPs), silver nitrate (AgNO₃), and silver chloride AgCl compounds are the best known and most commonly used inorganic antimicrobial

finishing agents/materials [6, 7]. Such materials have really good antimicrobial activity, but on the other hand, some of them could be harmful or toxic and cannot be used for health care or medical application [8]. Due to that the necessity to find new natural and human friendly antimicrobial active materials and methods of how to increase the antimicrobial activity of textile is still open. Some authors have presented investigations with different natural antimicrobial active fibres, such as natural bamboo, hemp, flax, or natural antimicrobial agents such as chitosan (polysaccharide obtained from the shells of crabs, shrimps and other crustaceans), and plant extracts (*Terminalia chebula*, *Curcumin*, citric acid, etc.) [9 - 11].

Antimicrobial agents can be applied to a textile substrate by the exhaust, pad-dry-cure, coating, sol-gel, spray and foam techniques [12]. One more possibility of covering a textile with antimicrobial agents is electrospinning, which is a well-known process used for the formation of a web from nano- and micro fibres due to electrostatic forces between two electrodes. Usually the diameters of such fibres are from 10 to 1000 nm [13]. Electrospun webs can be used for various applications, including medical and health care. In recent years, some investigations have been made on the usage of natural additives which increase the antimicrobial activity of an electrospun nanoweb or support the absorbance of

iodine to increase the antimicrobial activity [14, 15]. One of the natural organic materials is amber. Amber is a natural material about whose therapeutic properties have been known since prehistoric times. The ancient Greek physician Hippocrates (460 - 377 BC) in his works described the therapeutic properties of amber. The positive properties of amber in healthcare have also been stated in some papers of the last decades [16, 17]. In [18 - 20], micro particles of amber were used in classical chemical spinning, in the process of which the diameter of fibres are given in microns (usually no less than 10 µm) and the smallest micro particles of amber are incorporated into the inside of fibres. However, for the retention of amber activity, micro particles of amber cannot be covered by the polymer (polymer of fibre). Fibres with a diameter less than 1 µm are possible to form via electrospinning.

In an earlier work made by the authors [21], the possibility to manufacture electrospun webs from poly(vinyl alcohol) micro- or nanofibres with micro particles of natural Baltic amber is presented. The possibilities of creating an electrospun web with amber was analysed in this paper, but due to the solubility of the poly(vinyl alcohol), the antimicrobial activity of this web was not investigated. A web from stable in normal environment fibres needed to be developed for such investigations.

The main goal of this work was to develop a PA6 electrospun nanoweb with micro particles of Baltic amber and to investigate the antimicrobial activity of the web.

Materials and methods

Manufacturing of electrospun web with Baltic amber

A web from polyamide (PA6) nanofibres was created by means of “Nanospider™” equipment (Elmarco, Czech Republic) using a rotational electrode with tines [22]. A 15 wt.% concentration PA6 solution in 85% formic acid was prepared by magnetic stirring for 6 hours. 2 wt.% of amber micro particles was added into the solution, which was mixed again by magnetic stirring for 2 hours. Natural Baltic amber gathered from the sea coast of Palanga (Lithuania) was used in the experimental work. The natural amber was milled with a Laboratory Vibrating Disc Mill “pulverisette 9” (Fritsch GmbH, Germany) and next sifted with a Sonic Sifter Separator L3P (QAQC LAB, USA). A sieve of 63 µm size was used. The electrospun web was formed on polypropylene spunbond nonwoven (with 21.5 g/m² weight mass and 20 µm diameter of fibres). A 13 cm distance between electrodes, an applied voltage of 70 kV, temperature of the environment 20 ± 2 °C and air humidity 50 ± 2% were used at the time of electrospinning.

The structure of the electrospun web received as well as the amber micro particles were analysed by Scanning Electron Microscopy (SEM) with the use of a Quanta 200 FEG (the Netherlands) and LUCIA Image 5.0 programme.

Assessment of antimicrobial activity

The antimicrobial activity of samples was studied using the standard agar diffusion plate method according to a modified version of ISO 20645-2004. The samples were sterilised using UV radiation for 15 min. Standard overnight cultures for textile antimicrobial testing: Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and Gram-negative bacteria *Escherichia coli* DH5α cells were grown at 37 °C with aeration in Luria-Bertani broth (LB; Roth, Germany), containing 0.5% NaCl. To make bacterial lawns, the night cultures were added to 20 ml of melted LB agar (Roth, Germany) at a cell concentration of 4.5 × 10⁶ cfu/ml, and then poured into a Petri dish. Then the solidified agar on the plates was covered

with nanoweb samples or pieces of amber and incubated at 37 °C for 24 h. After incubation the samples on plates were examined under a microscope a Motic AE31 (Wetzlar, Germany). Photographs were taken by a digital imaging system consisting of a digital camera (Moticam Pro 285B; Wetzlar, Germany) and image software (Motic Images Advanced 3.2; Wetzlar, Germany).

After growth of the night culture with amber in the medium, we saw that the optical density of *S. aureus* was decreased in the probe studied comparing with the control (results not shown). As a result of that, growth curves of *S. aureus* suspensions were obtained monitoring the turbidity of fresh medium-diluted to OD₆₀₀ 0.04 over-night culture grown with aeration at 37 °C for 2.5 h. Samples were taken every 30 min to measure the optical density. The assays were repeated three times and the results demonstrated as mean values.

Results and discussions

The Baltic amber used in our investigations was milled and sieved out using a 63 µm size sieve mesh. A view of the milled amber particles is presented in **Figure 1**. As can be seen in **Figure 1**, amber particles vary in size and form, some of which are very small and some can have a maximum diameter even higher than 63 µm because of the oblong form of the particle.

A polyamide 6 nanofibrous web with Baltic amber micro particles was electrospun on polypropylene spunbond nonwoven. An SEM panoramic view (with resolution 200 µm) of the electrospun PA6 nanoweb with amber micro particles is presented in **Figure 2**. The PA6 nanoweb consists of PA6 fibres with a diameter of 20 - 160 nm (average diameter value 50 nm), due to which the web is not clearly visible in this image. Analysis of the PA6 nanoweb showed that amber micro particles are incorporated in all the surface of the nanofibrous web, and many of amber microparticles are not only stocked by polyamide to polypropylene spunbond but also are covered by polyamide nanofibres. Similar results have been obtained in previous studies with poly(vinyl alcohol) [21]. Results show that PA6 can be used for formation of electrospun nanoweb with amber micro particles. A very low diameter of PA6 nanofibres, in comparison with am-

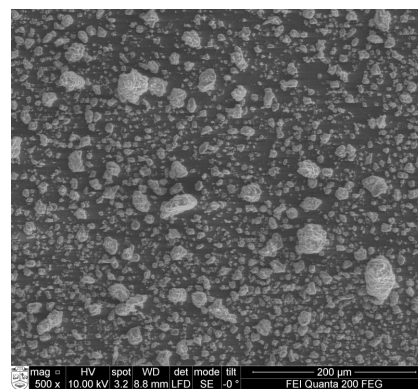


Figure 1. SEM image of milled Baltic amber micro particles [21].

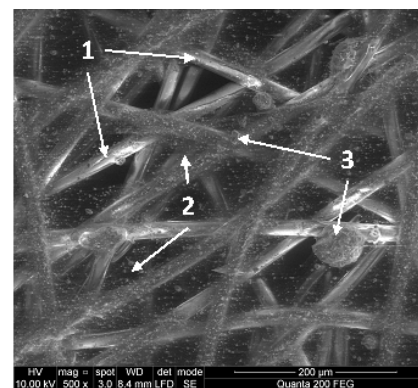


Figure 2. SEM panoramic view of PA6 nanofibrous web with Baltic amber micro particles: 1 – polypropylene fibres, 2 – polyamide nanoweb, 3 – microparticles of amber.

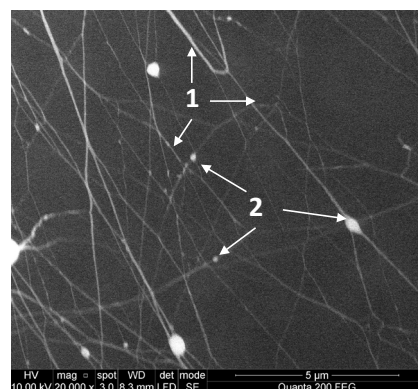


Figure 3. SEM image of the smallest amber particles in PA6 nanofibrous web: 1 – PA6 nanofibres, 2 – particles of amber.

ber particles and PVA nanofibres [21], is not a problem for much higher amber particles transfer from the lower electrode (bath with solution) to the upper electrode (covered by nonwoven spunbond). Amber particles of very various diameter – from very small (≤ 50 nm) up to the maximum (≥ 60 µm) – have been inserted in to the PA6 nanoweb as is seen in **Figure 3**.

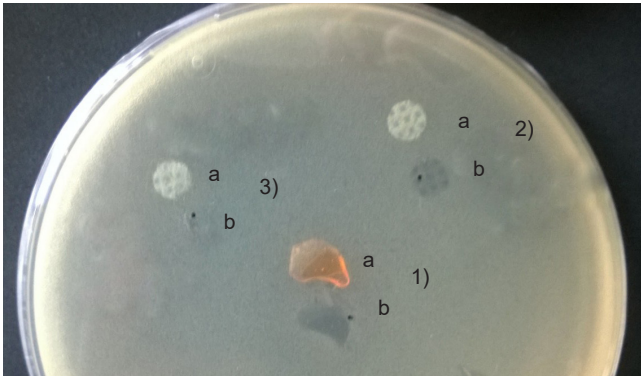


Figure 4. Tentative test of antimicrobial activity of amber 1), polypropylene nonwoven with PA6 nanofibrous web with amber micro particles 2) and web without amber particles 3) (a – after *S. aureus* growth moved tested specimen, b – initial area under specimen at the time of *S. aureus* growth).

The therapeutic properties of amber have been known a long time [16]. For the tentative antimicrobial testing of pure Baltic amber, a specimen of polypropylene nonwoven with a PA6 nanofibrous web with amber micro particles and the same nonwoven with a PA6 nanofibrous web but without amber particles were used. Results presented in **Figure 4** demonstrate that under the amber brick *S. aureus* bacteria did not grow, although an inhibition zone round the amber was not observed. Similar results were obtained for *E. coli* bacteria as well. Nevertheless the antimicrobial activity of Baltic amber was proven. Polypropylene nonwoven with a PA6 nanoweb but without amber particles has no influence on the growth of bacteria. However, the growth of bacteria under the specimen of polypropylene nonwoven with a PA6 nanoweb with amber particles was stopped. Particles of Baltic amber from less than 50 nm up to 63 mm have antimicrobial activity.

The results of *S. aureus* and *E. coli* bacteria growth under the Baltic amber and zones of free bacteria growth at a higher resolution are presented in **Figure 5**. As is seen in the images of **Figure 5**, the differences and dividing line between zones is clearly visible in all cases of bacteria investigated – colonies of bacteria under the Baltic amber are much lower than in those of free bacteria growth.

We registered the growth of *S. aureus* cultures in a liquid LB medium containing small pieces of amber (see **Figure 6**). Starting from the 90th min of cultivation, the amber-containing culture started to grow more slowly. After 150 min of cultivation, the OD₆₀₀ of the amber-containing *S. aureus* culture was ~15% lower compared to the control suspension's growth without amber. It was only a preliminary experiment, but positive results are evident even after 150 min of observation.

The antimicrobial activity of samples with a nanofibrous web using Gram-positive *S. aureus* and Gram-negative *E. coli* cell lawns is shown in **Figure 7**. Microcolonies of *S. aureus* and *E. coli* cell growth on LB agar under amber-containing nanoweb samples (**Figure 7.a** and **7.c**) were considerably smaller compared to the control samples without amber in the nanoweb (**Figure 7.b** and **7.d**). Thus analysis of the PA6 nanofibrous web with Baltic amber micro particles shows that the material developed has evident antimicrobial activity.

It is the first paper in which the authors would like to show their achievements in the development of such material and to state their preference in the development of an electrospun nanofibrous web with amber micro particles. Analysis of

the main technological and structural parameters' influence on the antimicrobial activity of a nanoweb with amber will be the further investigation of the authors.

Conclusion

The possibility to develop a nanofibrous polyamide 6 web with microparticles of Baltic amber was stated. The electrospinning process using Nanospider™ equipment is suitable for the development of such materials. Particles of Baltic amber varying in size from less than 50 nm up to 63 μm, were transferred from the lower solution bath to the upper spunbond. A low diameter of PA6 nanofibres did not have a negative influence on the formation of a nanoweb with amber particles. The antimicrobial activity of a PA6 nanofibrous web with amber particles was confirmed in all cases of bacteria investigated - Gram-positive *S. aureus* bacteria and Gram-negative *E. coli* bacteria. In both cases, the differences between areas covered by the web with amber and free zones after bacteria growth are clearly visible, which means that textile with even very small sizes and quantity of amber show antimicrobial properties. The development of such materials with amber particles could increase the area of application of such natural material as amber as an antimicrobial active agent.

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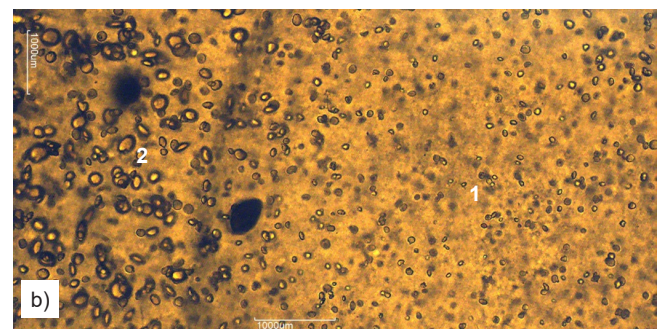
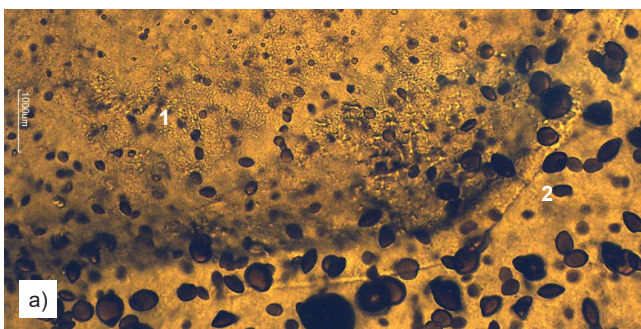


Figure 5. Antibacterial activity of amber against: a) Gram-positive *S. aureus*, b) Gram-negative *E. coli* (1 - zone under the amber brick and 2 – zone of free bacteria growth).

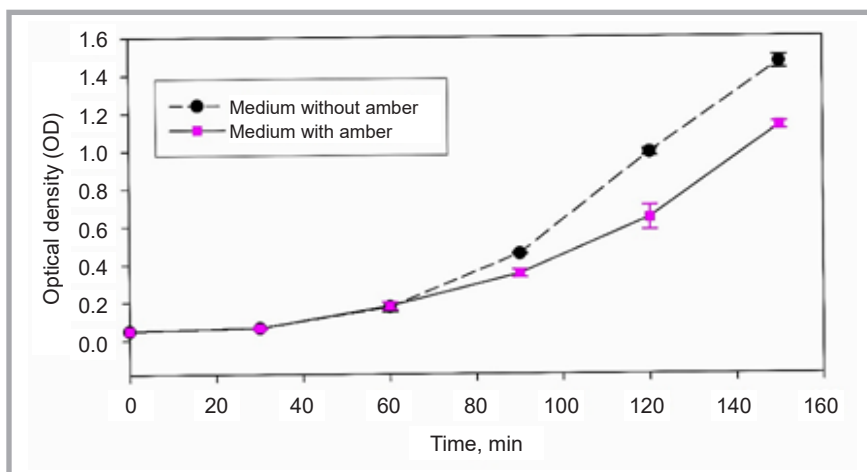


Figure 6. Growth curves of *S. aureus* cell cultures in liquid LB medium: without amber and in the presence of 6.5 g of amber in 100 mL of the medium.

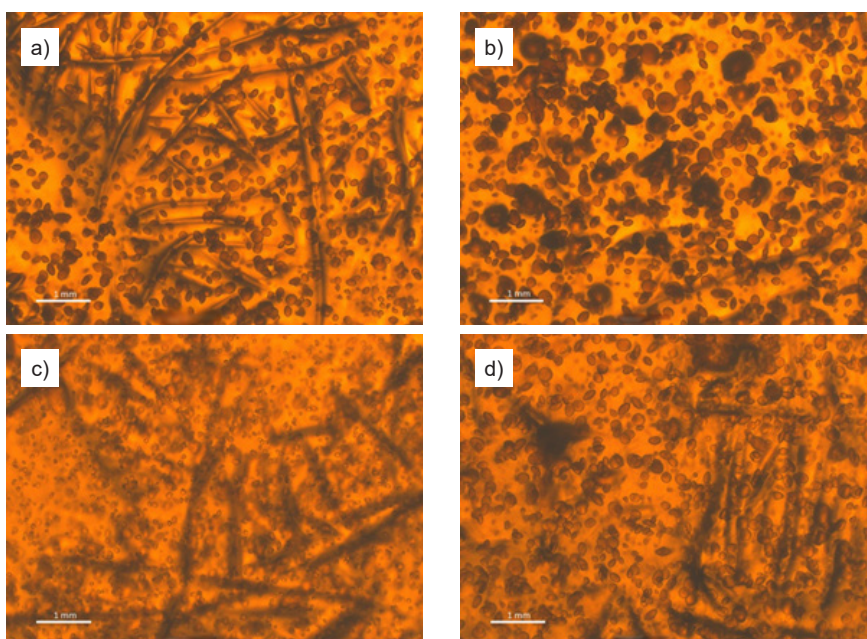


Figure 7. Growth of microcolonies: a) of *S. aureus* cells on nanoweb samples containing amber; b) of *S. aureus* cells on nanoweb samples without amber; c) of *E. coli* cells on nanoweb samples containing amber; d) of *E. coli* cells on nanoweb samples without amber.

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