ANTIFUNGAL PROPERTIES OF COTTON FABRIC WITH COPPER (I) OXIDE NANOPARTICLES PREPARED VIA GREEN SYNTHESIS

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Abstract

Two antioxidants with relatively strong reducing properties, tannic acid and ascorbic acid, were used for the preparation of the cotton fabric with copper (I) oxide (Cu_2O) nanoparticles from cupric ions sorbed into the cotton fabric. Different reducing power of tannic acid and ascorbic acid had an influence on the size of resulting nanoparticles and also on the resulting color of the fabric. The antifungal properties were investigated with the use of mixture of several fungi (genus *Penicillium*, *Rhizopus*, *Cladosporium* and *Chaetomium*).

Keywords: Tannic acid, ascorbic acid, copper oxide nanoparticles, antifungal properties, molds

1. INTRODUCTION

The technical textiles are subject to different requirements according to their applications. E.g. fabric used as building insulation materials or packaging materials for transporting and storage of crops should resist the mildew and rot besides having good mechanical properties. This is one of the reasons why the packaging from natural materials used in the food and agriculture (mainly cellulosic fibers, such as jute, flax or cotton) are being replaced by synthetic ones (polypropylene, polyester, polyamide). The production of synthetic fibers, however, is largely dependent on the oil and brings with it a number of adverse environmental impacts - such as secondary effluents, and especially, the worsening of the global problem of poorly degradable waste. Return to natural materials in connection with practices of the so-called green chemistry is a good way to minimize the impact of civilization on the environment.

The aim of this study was to design a simple way of antifungal treatment of plant (cellulose) fibers with copper oxide nanoparticles and then test their ability to resist the growth of mildew.

Metal nanoparticles can be prepared from precursor metal salts by different chemical and physical methods, but the green synthesis of nanoparticles has several advantages over the chemical or physical synthesis, such as th simplicity and eco-friendliness. Cuprous oxide nanoparticles on the cotton fabric were synthesized from precursor (cupric ions in the aqueous solution) and reduced on the fibers using two natural antioxidants - tannic acid and ascorbic acid.

1.1. Tannic acid

Tannic acid (TA) is a natural oligomeric polyphenolic substance with astringent effects (tannin). It is usually formed by 2 to 12 monomer units of gallic acid or quinic acid connected by ester bonds that are subject to relatively easy hydrolysis. The chemical formula for commercial tannic acid is often given as $C_{76}H_{52}O_{46}$, with molar mass 1701.1 g·mol, which corresponds to decagalloyl glucose, but in fact, it is a mixture of polygalloyl glucoses or polygalloyl quinic acid esters. The tannic acid is present especially in wood, bark, leaves and gall nuts and it is usually extracted from Aleppo oak (*Quercus infectoria*) or Sicilian Sumac (*Rhus coriaria*). The tannic acid is soluble in water, is moderately acidic, and forms numerous hydrogen bonds with many substances due to the large amount of phenol groups. This is e.g. the cause of protein denaturation, astringent properties or cross-linking of polymers. Additionally, the tannic acid forms complexes with metal ions. It acts as an antioxidant and scavenger of free radicals when the original polyphenol is oxidized to the different quinoid or semiquinoid structures.

1.2. Ascorbic acid

Ascorbic acid (AA) is a water-soluble organic compound with strong antioxidant properties. It is present in plants, especially in fruits and vegetables. Its regular dietary intake is vital for humans and some animals whose bodies are unable to synthesize it. It plays an important role in number of tasks in the organism: the ascorbic acid acts as cofactor for the biosynthesis of collagen, catecholamines, amino acids, and various peptide hormones [1]. As a strong antioxidant it is able to deactivate the reactive oxygen species (ROS) that attack nucleic acids, proteins, lipids, and cell structures at the molecular level. The ascorbic acid is oxidized by the loss of one electron to form a radical cation and then by the loss of a second electron to form the dehydroascorbic acid. [2]

1.3. Copper (I) oxide

Copper (I) oxide (cuprous oxide) is found in nature as a cuprite. It is commonly used as an inorganic red pigment. More precisely, depending on the size of pigment particles, its color appears in a range from yellow, yellow-green, orange to red. In contrast to the copper (II) oxide nanoparticles, which are widely studied, used, and described in the scientific literature, the copper (I) oxide is studied less, although some properties of both copper oxides are similar. The biological properties of copper compounds play an important role as fungicides in agriculture and biocides in antifouling paints for ships and wood preservations [3] [4] [5]. Copper (I) oxide is used as an anti fungal agent for marine paints as an alternative of Tributyltin compounds (toxic organic tin compounds that threaten the water ecosystems) and Cu2O nanoparticles has been widely utilized in anti fungal coatings [6] or for the fabrication of nanocomposites with strong antimicrobial activity [7].

2. EXPERIMENTAL PART

2.1. Material and chemicals

Cotton fabric Sara (Licolor), area weight 135 g·m^{-2}; suspension of four kinds of fungi in water of concentration 10^7 CFU/ml: *Penicillium chrysogenum* (CCM F-362), *Rhizopus stolonifer* (CCM F-445), *Cladosporium sphaerospermum* (CCM F-351), *Chaetomium globosum* (CCM F-275); ascorbic acid, tannic acid, copper(II) sulfate pentahydrate (blue vitriol), potassium sodium tartrate tetrahydrate, sodium hydroxide (all chemicals from Lach-Ner, Czech); trypto-casein soya agar (TSA) (Biovendor, Czech), laboratory-prepared sucrose agar (SA); water soluble glue Planaxol (Planatol Wetzel, Germany) based on PVA; cardboard, area weight 200 g·m^{2}.

2.2. Preparation of textile with copper (I) oxide nanoparticles

Fehling´s reagent was prepared by mixing solutions of Fehling I (6.9 g of copper sulfate pentahydrate in 100 ml of distilled water) and Fehling II (34.5 g of potassium sodium tartrate and 12.0 g of NaOH in 100 ml of distilled water). The solutions of AA and TA were prepared, both in a concentration of 1.5 g·l^{-1}. Four grams of cotton fabric was immersed in 200 ml of Fehling's reagent for 10 minutes. After removal from the solution, the fabric was wrung and dried at 50 ºC. Subsequently, these fabrics were inserted into solutions of AA or TA at 50 ºC (with the bath ratio of 1 g cotton to 50 ml of solution) while gently stirring for 15 minutes. The formation of nanoparticles of copper (I) oxide from Cu^{+2} ions is carried out in the alkaline environment of Fehling reagent in the presence of these reducing agents spontaneously: nanoparticles are formed on, or in, the fiber of the fabric „in situ“. Afterwards, the fabrics were gently washed in the distilled water, wrung out and dried again at 50 ºC. As blanks were prepared the samples of cotton fabrics that were dipped in a solution of AA or TA at 50 ºC for 15 minutes and then wrung out and dried.

2.3. Tests of the antifungal effect

Samples of fabrics of size 6 cm^2 were dipped in the test tubes each with 5 ml of mixture of four fungi with concentrations of 10^4 CFU·ml^{-1} for 8 hours constantly shaking lightly. A half ml of each suspension of fungi was inoculated to half of the Petri dish with TSA. A half of samples were then removed from test tubes, placed directly on the plates with SA and incubated at 22 °C for 9 days. The second half of the samples was left in dynamic contact with the suspension of fungi for 3 days. Then samples were removed, and each was strongly agitated in 10 ml of sterilized distilled water. These solutions were plated onto SA and incubated at 22 °C for 9 days. Other samples (cotton fabric, fabric with AA or TA only, fabric with Cu_2O nanoparticles) were fixed on a cardboard with PVA based adhesive and subsequently stitched on. The mixture of fungi was applied to them by spraying. Their incubation took place in a glass box with a high relative humidity for 6 weeks. After incubation times, the colonies of fungi grown on agar and fabrics were counted and compared. The relatively large colonies of fungi on TSA were counted macroscopically; the very small colonies on the fabric put on SA were counted by use of a photographic zoom (10x).

3. RESULTS AND DISCUSSION

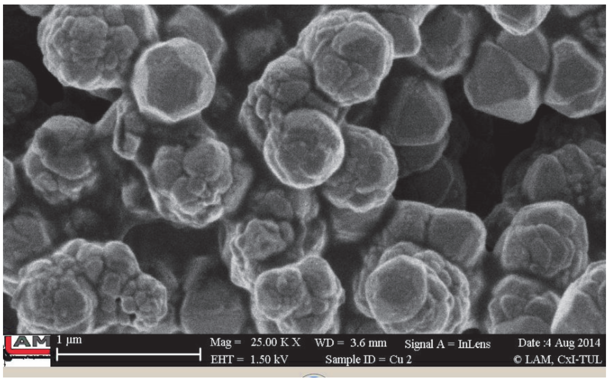

Figure 1 Cu_2O nanoparticles reduced from Fehling´s reagent using ascorbic acid (25 000x)

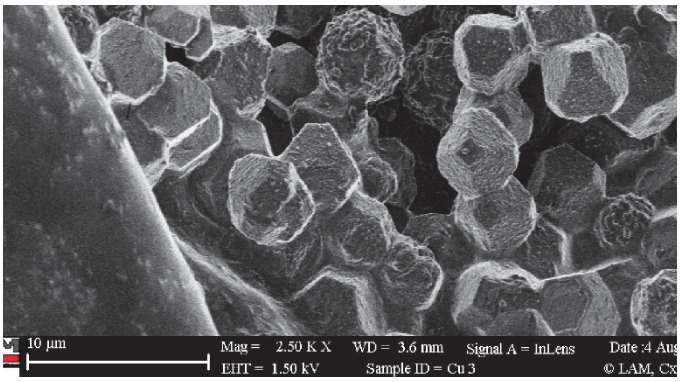

Figure 2 Cu_2O nanoparticles reduced from Fehling´s reagent using tannic acid (2 500x)

The SEM images in **Figures 1** and **2** show Cu_2O nanoparticles on cotton fibers that originated from the Cu^{+2} ions by the reduction effect of AA or TA. A different reducing power of these two substances is reflected in the size of resulting nanoparticles: AA works as a very strong and rapid antioxidant and the resulting particles have a diameter of about 400-500 nm. On the contrary, even though TA has an equivalent antioxidant capacity, its reducing power is manifested by a much lower acceleration and for a longer period of time. In this case, it led the slow growth of Cu_2O crystals. Cu_2O particles had a diameter of about 3-4 microns, which is about 10 times larger than the particles made by AA. The cubic crystalline structure of Cu_2O and the layered growth of crystals are clearly seen on these particles. The size ratio of the particles formed by the reduction of AA and TA correlates well with the antioxidant properties of both substances. Previously, we completed their comparison with the finding that the reduction (antioxidant) capacity of TA is about 6 times higher than the capacity of AA (at the same molecular ratio of both compounds) [8]. A lot of small Cu_2O nanoparticles with reducing effect of TA are visible on cotton fibers, but there is also a significant number of crystals of size up to 10 microns (**Figure 2**). Also, the variety of particles sizes on cotton fibers resulted in different colors of fabrics: bright orange (fabric CO/AA/Cu) and yellowish with a slight green tinge (fabric CO/TA/Cu).

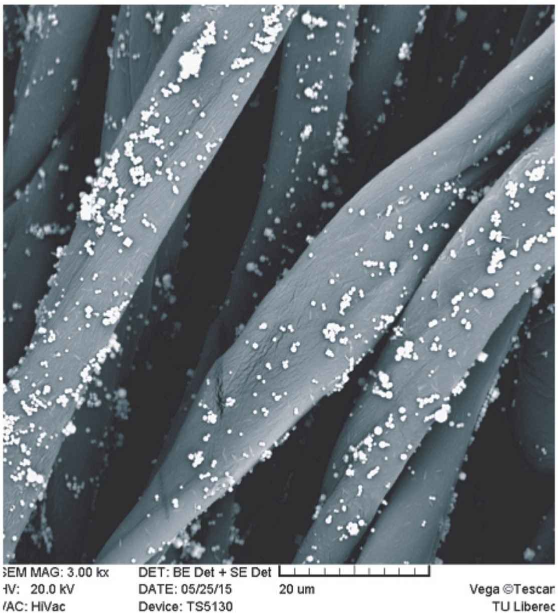

Figure 3 Cotton fibers with Cu_2O nanoparticles prepared with ascorbic acid

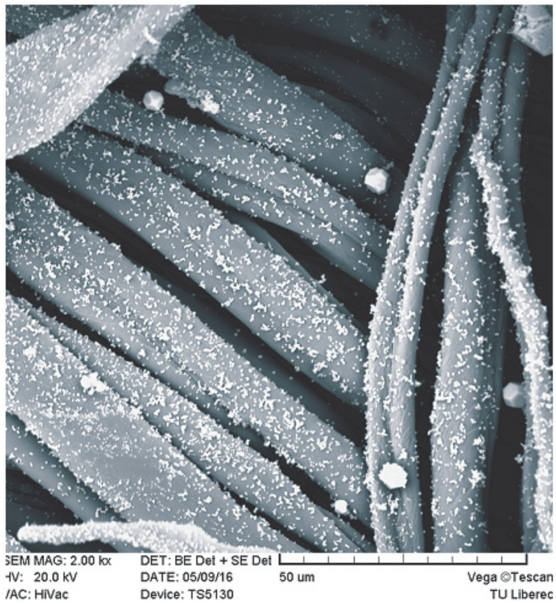

Figure 4 Cotton fibers with Cu_2O nanoparticles prepared with tannic acid

Table 1 provides an overview of the number of grown fungal colonies (colony forming units, CFU) on TSA (suspension of fungi after 8 hours of dynamic contact with treated fabrics), and on SA (colonies grown on fabrics after 8 hours of the dynamic contact with fungi suspension): CO is untreated cotton fabric; CO/AA and CO/TA are cotton fabrics that contain only ascorbic acid or tannic acid; CO/AA/Cu and CO/TA/Cu are cotton fabrics with particles of cuprous oxide prepared by reduction of ascorbic acid or tannic acid.

Table 1 Number of CFU of fungi grown on agars and fabrics with or without effect of Cu_2O

	Suspension of fungi	**CO**	**CO/AA**	**CO/TA**	**CO/AA/Cu**	**CO/TA/Cu**
CFU in 1 ml of suspension	$5 \cdot 10^3$	$4 \cdot 10^3$	$6 \cdot 10^2$	$4 \cdot 10^2$	50	sporadic
CFU on 1 cm^2 of fabric	-	140	250	160	65	0

It is evident from the **Table 1** that both fabrics with Cu_2O nanoparticles (CO/AA/Cu and CO/TA/Cu) exhibited a significant antifungal effect, while the fabric with Cu_2O particles prepared by the reduction of TA was more effective than fabric with particles prepared by reduction with AA. This is interesting, because considering the small size of particles formed in the presence of AA we would expect the opposite result. The assumption is that if two fabrics contain at the beginning the same amount of Cu^{+2} ions from Fehling's solution, then a large number of small nanoparticles represents a greater surface area that comes into contact with fungi and thus higher antifungal efficiency, rather than the fabric on whose surface of the part of copper is bound in the form of larger microcrystals! The reason could be the presence of residual AA, which increased the grow of fungi in fabric CO/AA a it can be assumed that ascorbic acid supports the growth of mold.

This assumption corresponds to the growth of fungi sprayed on fabrics fixed on cardboard. **Figure 5** shows that after 3 weeks the fabric with the content of AA (far left) starts to get moldy first, while the untreated cotton fabric Sara (far right) does not have, macroscopically, any mildew. After 6 weeks of incubation (**Figure 6**), the most molds are evident on the fabrics with AA, TA (the first two from left), besides that on also on the cotton fabric Sara (far right). At the same time a slight grow of mold startedm on the sample with lower (approximately half) concentration of Cu_2O, which was prepared by reduction with AA from the fabric immersed in the Fehling's solution of about a half initial concentration (third from right). It is therefore evident that the antifungal activity of cotton fabric with Cu_2O nanoparticles is also influenced by the concentration of particles on the fabric surface. Fabrics on cardboard that contained nano- and microparticles of Cu_2O prepared by reduction with AA and TA from Fehling's reagent of the original concentration were not apparently attacked by fungi neither after 6 weeks of incubation in a wet environment at 22 °C (second from right and the third from left).

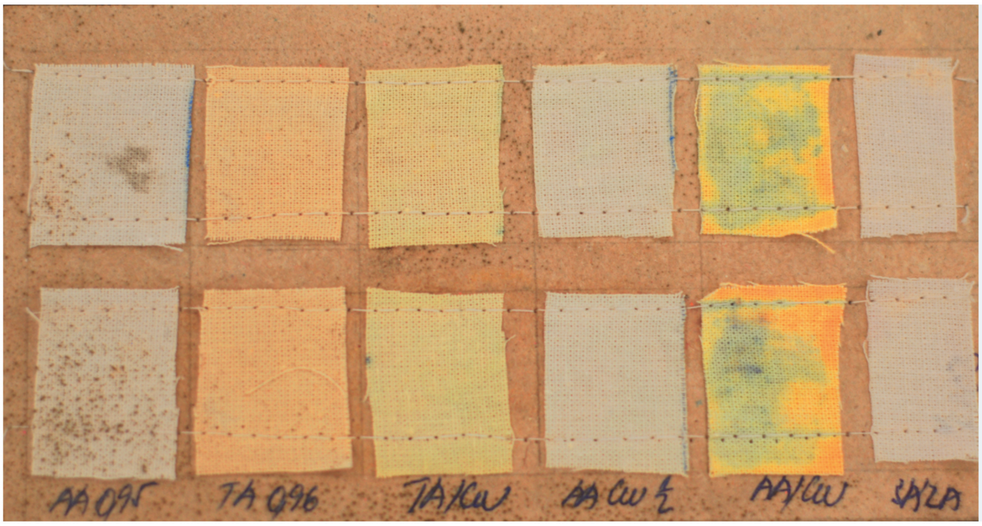

Figure 5 Cardboard with samples sprayed with suspension of fungi after 3 weeks of incubation

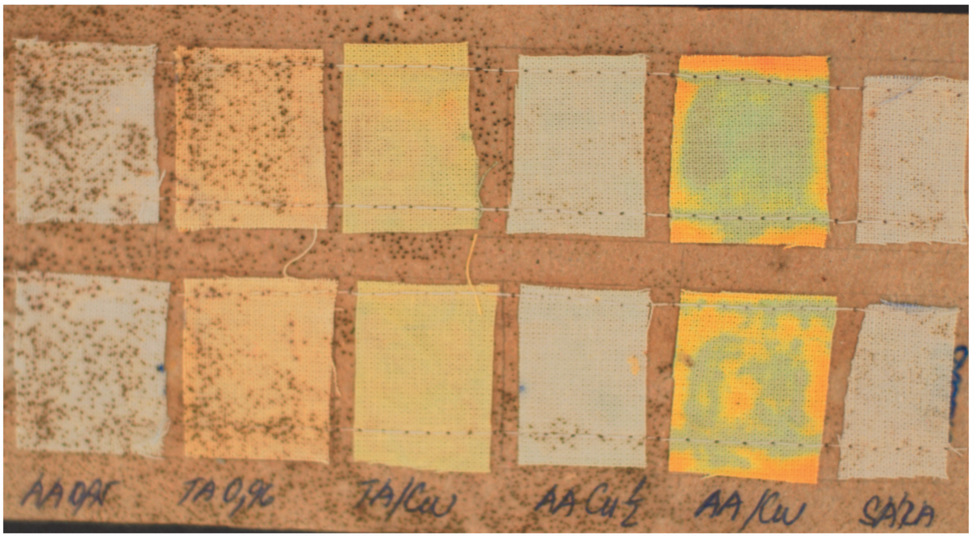

Figure 6 Cardboard with samples sprayed with suspension of fungi after 6 weeks of incubation (from left to right: CO/AA - CO/TA - CO/TA/Cu - CO/AA/Cu (half concentration) - CO/AA/Cu - CO)

4. CONCLUSION

We have demonstrated in several ways that cotton fabric with Cu_2O nanoparticles on the surface, which were prepared by the reduction of Cu^{+2} ions with the ascorbic acid or the tannic acid, exhibited an excellent resistance to the mixture of four fungi (*Penicillium chrysogenum*, *Rhizopus stolonifer*, *Cladosporium sphaerospermum* and *Chaetomium globosum*). Nanoparticles of copper (I) oxide on the fabric surface prepared with the tannic acid showed better antifungal effect than nanoparticles prepared with the ascorbic acid, since tests suggested that the presence of ascorbic acid stimulates the growth of fungi. The cotton fabric with Cu_2O nanoparticles prepared by use of the tannic acid showed the excellent resistance to mold growth in the direct contact with nutrient medium (sucrose agar), simulating the presence of moisture and nutrients (sugar). Hence, it could be used e.g. as packaging, shipping and storage material for fruit or other agricultural crops.

In regards to the fabrics fastened with glue and stitched to the cardboard, there was no visible growth of mold on the surface of either fabric with the Cu_2O nanoparticles, not even after 6 weeks in the environment with high relative humidity. Therefore, those could be appropriate to use, for example, as bookbinding canvasses resistant to the fungi in library archives.

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