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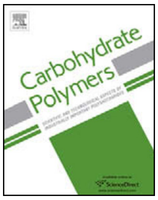

Antibacterial activity of silver nanoparticles synthesized In-situ by solution spraying onto cellulose

Jinhua Yan[a], Abdelrahman M. Abdelgawad[b,c], Mehrez E. El-Naggar[b,c,*], Orlando J. Rojas[b,d]

[a] Guangdong Industry Technical College, Guangzhou, China
[b] Biobased Colloids and Materials Group (BiCMat), Departments of Forest Biomaterials, North Carolina State University, Campus Box 8005, NC, USA
[c] Textile Research Division, National Research Centre (Affiliation ID: 60014618), Dokki, Cairo, Egypt
[d] Department of Forest Products Technology, Aalto University, P.O. Box 16300, FIN-00076 Aalto, Finland

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ABSTRACT

Spray technique was used for the adsorption of *in-situ* silver nanoparticles (AgNPs) onto and inside the surface of nano- and micro- fibrillar cellulose (NFC and MFC) as well as filter paper. The abundance of hydroxyl and carboxyl groups located in NFC and MFC are used to stabilize Ag ions (Ag^+) which were then *in-situ* reduced to (AgNPs) by chemical or UV reduction. The surface characteristic features, elemental analysis, particle size as well as size distribution of the obtained MFC, NFC and filter paper loaded with AgNPs were characterized via field emission scanning electron microscopy connected to energy dispersive X-ray spectroscopy (FESEM- EDX) and transmission electron microscopy (TEM). The associated chemical changes after growth of AgNPs onto the cellulose substrates were assessed by fourier transform infra-red (FT-IR) while the thermal stability of such systems were investigated by thermogravimetrical analyses (TGA). The antibacterial properties of AgNPs loaded NFC, MFC and filter paper as well was investigated against *Escherichia Coli*. The resulted data indicate that the particle size was found to be 11 and 26 nm for AgNPs nucleated on NFC and MFC—based papers respectively. The antibacterial activity of AgNPs loaded MFC exhibited higher antibacterial activity than that of AgNPs loaded NFC. Overall, the present research demonstrates facile and fast method for in-situ antibacterial AgNPs loading on cellulose substrates.

1. Introduction

Metal particles in the nanometer size have attracted considerable interest as they find many applications in several fields (Abdel-Mohsen et al., 2014), (Hebeish, El-Rafie, El-Sheikh, & El-Naggar, 2013b; Hebeish, El-Rafie, El-Sheikh, Seleem, & El-Naggar, 2014b) for their optical, electrical, magnetic, and antimicrobial properties (Kim, Johnson, & Hupp, 2001; Zhong & Maye, 2001; Zhu, Liu, Palchik, Koltypin, & Gedanken, 2000). Silver nanoparticles (AgNPs) has been considered as one of the most metal nanoparticles which can be used as antimicrobial agent for water treatments (Dankovich & Gray, 2011), painting (Kumar, Vemula, Ajayan, & John, 2008) and textile finishing (Hebeish, El-Naggar, Fouda, Ramadan, Al-Deyab, & El-Rafie, 2011; Ravindra, Mohan, Reddy, & Raju, 2010; Zhang, Wu, Chen, & Lin, 2009). It well known that the antimicrobial effect AgNPs correlates inversely with its particle size (Rai, Yadav, & Gade, 2009). This is explained by the larger surface area that is available for contact with bacterial cells and hence the number of interactions compared to that for relatively larger particles (Jiang, Mashayekhi, & Xing, 2009; Morones et al., 2005; Raimondi, Scherer, Kötz, & Wokaun, 2005). Aggregation of AgNPs is noted to drastically decrease the accessibility to the surfaces, resulting in a low activity (Cai et al., 2012). Therefore, AgNPs dispersed in liquid media demands proper colloidal stabilization to avoid the aggregation that is otherwise triggered by the high surface energy of the particles.

Barud et al. (2011) outlined that the bacterial cellulose (BC) can act as a good host for loading AgNPs. It was also observed that TEMPO-oxidized cellulose nanocrystals (TEMPO-CNC) prevented the aggregation of AgNPs mainly by electrostatic effects associated with carboxyl groups located on the surface of CNC (Uddin et al., 2014). Yin, Wang, Shen, Zhang, and Galib, 2013 applied cationic sols to treat cellulose substrates to form a dense and firm cationic

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* Corresponding author at: Textile Research Division, National Research Centre, Dokki, Cairo, Egypt.
E-mail address: mehrez_chem@yahoo.com (M.E. El-Naggar).

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hybrid film on the surface of the substrate, which was found to be beneficial for transferring printing dye.

The great challenge in the current research is to synthesize the well stabilized and morphologically-controlled AgNPs using Microfibrillar and nanofibrillar cellulose (MFC and NFC) as a host for the in situ formed AgNPs on their surface in short time and without using extra chemical substances.

MFC and NFC can be isolated from cellulose fibers by mechanical action after enzyme or chemical pretreatments. MFC and NFC have been widely investigated recently due to its sustainable, renewable, and nontoxic properties while they can be produced at relatively low cost and with high physical-chemical stability (Dong, Snyder, Tran, & Leadore, 2013; Eichhorn et al., 2010; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011). Moreover, MFC and NFC are considered as porous materials and can be used as hosts for nucleation and growth of nanoparticles, thus restricting their size. Therefore, the resulting hybrid materials may have some advantages if given functionalities of the matrix are added to those of the nanoparticles. The addition of pre-formed nanoparticles onto NFC/MFC, the nanoparticles may affect the strength of the substrate material due to reduced hydrogen bonding between fibrils, as is the case of typical mineral fillers (Luong et al., 2013).

Up to our knowledge, there no report is available related to the functionalization with nanoparticles of pre-formed MFC and NFC papers. This work focuses on the installation of AgNPs on cellulose fibers and fibrils by simple surface treatment followed by particle growth via chemical as well as UV reduction without using any extra stabilizer or binder. The morphological surface, shape and diameter of the resultant systems was characterized by using SEM and TEM techniques. The nature of the binding of AgNPs to MFC and NFC was assessed by FT-IR. Finally, the antibacterial activity was determined against *Escherichia coli*.

2. Materials and methods

2.1. Materials

Silver nitrate ($AgNO_3$, purity >99%) was purchased from Sigma-Aldrich Co, USA, Sodium borohydride (99%) was purchased from Across Co., USA. Fully bleached hardwood (eucalyptus) microfibrils (microfibrillar cellulose, MFC) were prepared through microgrinding (6 passes) an aqueous fiber dispersion (2.3% solids) with a Masuko Supermass Colloider (Model MKCAG-2). Deionized water was used for all experimental. All chemicals are of analytical grade and used as received without purification.

2.2. Methods

2.2.1. Preparation of nanofibrillar cellulose (NFC)

NFC was produced by treatment of the MFC by TEMPO oxidation. First, 120 mg of TEMPO was combined with 750 mg of NaBr dissolved in 300 mL of deionized water. The TEMPO/NaBr mixture was added to 325 g of MFC suspension (7.5 g dry weight). Secondly, The TEMPO-mediated oxidation was initiated by adding drop by drop 16.3 mL of 12% NaClO aqueous solution at ambient temperature. The reaction was kept at a constant pH value of 10 using 0.5 M NaOH as pH controller followed by adding 0.5 M HCl to reach pH 7 and to stop the reaction. Finally, the final product was completely washed by DI water for 10 times before passing the dispersion in a microfluidizer (M-110EH, Microfluidics Ind., USA). The TEMPO oxidation was performed according to the literature (Lasseuguette, Roux & Nishiyama, 2008) by using sodium hypochlorite as the terminal oxidant. Dialysis was applied to remove excess salts from the NFC dispersion by using dialysis membranes (Fisherbrand regenerated cellulose) with nominal MWCO of 3500 Da for 48 hrs and using Milli-Q water exchange every 5 hrs. The resultant NFC aqueous dispersion was kept in a fridge until use.

2.2.2. Preparation MFC and NFC paper preparation

Given amounts of MFC and NFC aqueous dispersion were stirred with a magnetic mixer for 30 min and a Whatman Nuclepore Track-Etch film (0.5 μm pore size) was used in an AAGWR water retention test device operated at 1.8 bar. Over pressure was applied to drain water and to allow the formation of the MFC and NFC paper on the film. The wet webs were carefully peeled off the film and placed between two polymer films during oven drying at 40 °C at low load (500 g) pressure.

2.2.3. In situ silver NPs loaded MFC and NFC using chemical and UV-techniques

$AgNO_3$ solution was sprayed on the respective porous web (MFC and NFC papers as well as filter paper) at ambient temperature to fully wet the substrate until saturation. This method is an alternative to traditional soaking or solution immersion, which typically require large solution volumes. Since the webs are hydrophilic, the aqueous salt solution effectively penetrated and saturated the substrate. The in situ growth of AgNPs onto the porous webs was carried out through chemical reduction of $AgNO_3$ solution at given concentrations (10, 50, 100, and 500 mM) by using sodium borohydride as a very strong reductant.

Air drying during given times produced a partially dehydrated system that was then immersed in ethanol for 5 min and then in aqueous solution of $NaBH_4$ (50 mM) for 20 min. As a result, the color of the samples turned yellow and dark brown depending of the concentration of the reduced Ag^+ which converts to clusters of AgNPs. The resultant hybrid paper was rinsed with Milli-Q water three times to remove water-soluble substances and any unbound AgNPs. Finally, the hybrid paper was dried in air.

MFC was selected as a host for loading AgNPs under UV reduction. To this end, $AgNO_3$ (100 mM) was sprayed on the substrate followed by application of the same procedures except that the wet substrate was subjected to UV irradiation (100 W, 365 nm, (k5, 4800 lw/cm^2 @15″, Spectroline Corporation) for 20 min to accelerate the formation of AgNPs).

2.3. Characterization

The porous MFC and NFC papers were examined using a field emission scanning electron microscope (FESEM) at an accelerating voltage of 20 kV (a high resolution JEOL 6400F cold field emission SEM). The MFC and NFC paper loaded with AgNPs were also characterized using VPSEM (Variable Pressure Scanning Electron Microscope – Hitachi S3200 N with an energy dispersive X-ray spectrometer (EDX) with a voltage of 20 kV.

20 mL MFC and NFC dispersion (0.08%) was stirred for 45 min under magnetic stirrer followed by sonication for 30 s (Branson Digital Sonifier Model 102C with Microtip for volumes 1–10 mL). A silica wafer was used as support of the suspended MFC and NFC. Firstly, it was cut into small pieces, which were soaked in 10% NaOH solution for 30 s and then in Milli-Q water. The silica surfaces were dried by nitrogen blowing, followed by UV exposure for 20 min. A small drop of MFC and NFC dispersion was placed on the surface of the clean silica support. The samples were coated with gold before SEM imaging and air drying overnight was applied.

A JEOL 2000FX transmission electron microscope (TEM) operating at 20.0 kV was utilized to image the AgNPs in the MFC and NFC papers. TEM specimens were prepared by cutting the sample into thin slices using a diamond cutter, then by cutting 3-mm-diameter discs from the slice, thinning the disc on a grinding wheel, dimpling the thinned disc, then using ion milling.FT-IR spectroscopy was performed on a PerkinElmer Spectrum (Version 10.03.09). Spectra was