Carbohydrate Polymers 95 (2013) 214–219

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Facile synthesis of tunable silver nanostructures for antibacterial application using cellulose nanocrystals

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ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 19 February 2013
Accepted 28 February 2013
Available online 7 March 2013

Keywords:
Cellulose nanocrystals
Silver nanostructure
Antibacterial activity
Shape control
Environmentally friendly synthesis

ABSTRACT

In this study, we report a facile and environmentally friendly strategy for synthesis of well dispersed and stable silver nanostructures using cellulose nanocrystals in aqueous solution without employing any other reductants, capping or dispersing agents. Importantly, it is feasible to adjust the morphology of the silver nanostructures by varying the precursor $AgNO_3$ concentration. Silver nanospheres were formed when the $AgNO_3$ concentration was 0.4 mM, while the dendritic nanostructures predominated when the $AgNO_3$ concentration was increased to 250 mM. The antibacterial activity of the two different silver nanostructures against *Escherichia coli* and *Staphylococcus aureus* was characterized. Dendritic nanostructure showed a better antibacterial activity than that of silver nanosphere. The approach presented in this paper offers a very promising route to noble metal nanoparticles using renewable reducing agents.

1. Introduction

Noble metal nanoparticles, especially silver nanoparticles (Ag NPs) have attracted considerable attention due to their potential applications as electronic, optical, sensing, catalytic and importantly antibacterial materials. Silver has been utilized in antibacterial applications for thousands of years due to their broad-spectrum antimicrobial activities and high toxicity to different type of microorganisms (Kamel, 2012; Nassar & Youssef, 2012). However, among the conventional preparative methods, most of the reducing and stabilizing reagents used for the synthesis of Ag NPs are not environmentally benign. In view of the awareness toward green chemistry and sustainable strategy, the development of a simple and environment-friendly method for the synthesis of Ag NPs is necessary.

Biological synthesis of metal NPs using microorganisms, enzymes, and carbohydrate polymers have been suggested as possible eco-friendly alternatives to conventional petrochemical reductants-based methods (Iravani, 2011; Shaligram et al., 2009). A number of researchers have described the synthesis of various metal NPs using carbohydrate polymers, such as leaf broth (Shankar, Ahmad, Pasrichaa, & Sastry, 2003), hydroxypropyl starch (Hebeish et al., 2011), hydroxypropyl cellulose (Abdel-Halim & Al-Deyab, 2011) and bacterial cellulose (Yang, Xie, Deng, Bian, & Hong, 2012). Using renewable carbohydrate- or cellulose-based materials for the synthesis of NPs can be advantageous over microorganisms process because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale NPs synthesis (Song, Jang, & Kim, 2009). Moreover, NPs produced by carbohydrate- or cellulose-based materials are more stable and the rate of synthesis is faster than that in the case of microorganisms (Iravani, 2011).

Cellulose nanocrystals (CNs), which can be isolated from a variety of natural sources such as cotton, tunicate, bacteria and wood pulp, has emerged as a new class of renewable carbohydrate polymers owing to its high stiffness, low density, well-defined size and morphology, controlled surface chemistry, environmental sustainability and anticipated low cost (Lam, Hrapovic, Majid, Chong, & Luong, 2012). The electron-rich feature of hydroxyl and sulfate ester groups on its surface endow CNs a good colloidal stability in water, which makes it well suitable for the preparation of metal NPs (Benaissi, Johnson, Walsh, & Thielemans, 2010; Hirai, Nakao, & Toshima, 1979; Lin et al., 2011). However, to date, the use of CNs in the biosynthesis of metal NPs is very limited. A small number of reports (Cirtiu, Dunlop-Briere, & Moores, 2011; Lam et al., 2012; Shin, Bae, Arey, & Exarhos, 2008) describe the formation of metal NPs using CNs as a stabilizing template, which inevitably involved the use of a chemical reducing agent. Recently, synthesis of platinum NPs using CNs as a reducing agent was reported by Benaissi et al. (2010). However, their results indicated that the reaction only proceeds when the water is in contact with supercritical carbon dioxide. The strict reaction condition hindered the

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http://dx.doi.org/10.1016/j.carbpol.2013.02.077

large-scale application of this approach. To the best knowledge of the authors, no reports on the facile synthesis of metal NPs using CNs as reductant and stabilizer under mild conditions have been published.

Herein we present the successful synthesis of Ag NPs using CNs in aqueous solution without employing any other reductants, capping or dispersing agents under mild conditions. Furthermore, it is feasible to adjust the morphology of the Ag nanostructures by varying the precursor $AgNO_3$ concentration. We report for the first time that the dendritic Ag nanostructures can be obtained using CNs as a reducing agent. The antibacterial activity of Ag NPs with different nanostructures against *Escherichia coli* and *Staphylococcus aureus* was investigated. The green and environmentally benign approach developed in this paper offers a very promising route to the synthesis of other metal NPs, especially those to be used for antibacterial applications.

2. Experimental

2.1. Materials

All chemical materials and solvents used in the experiments were analytical grade reagents, and were used without further purification. Waste cotton fabrics were obtained commercially from the surplus of textile industries. Silver nitrate ($AgNO_3$), hydrochloric acid, nitric acid and sulfuric acid were purchased from Kelong Chemical Regent Co., Ltd. (Chengdu, China). All solutions were prepared with deionized water.

2.2. Preparation of CNs

The CNs was obtained by acid hydrolyzing the microcrystalline cellulose (MCC). The method of preparing MCC has been reported in our previous work (Xiong, Zhang, Tian, Zhou, & Lu, 2012), and the procedure of preparing CNs is based on the report of Bondeson, Mathew, and Oksman, 2006). The MCC and sulfuric acid (64 wt%) suspension were heated with stirring at 45 °C for 130 min. The suspensions were then washed with deionized water using repeated centrifuge cycles (10 min at 12,000 rpm for a cycle). The last wash was conducted using dialysis with deionized water until the wash water maintained a constant pH.

2.3. Hydrothermal synthesis of silver nanosphere and dendritic nanostructure

In a typical experiment, the desired amount of the metal precursor, $AgNO_3$ (0.4 and 250 mM), was added to 30 ml aq. 0.1 wt% CNs suspension in a hydrothermal synthesis reaction kettle for 12 h at 100 °C. Then the suspensions were washed with deionized water using repeated centrifuge cycles (10 min at 12,000 rpm for a cycle) to remove the unreacted $AgNO_3$.

2.4. Characterization

The transmission electron microscopy (TEM, JEOL JEM-100CX, Japan) and atomic force microscopy (AFM, Nanoscope Multimode & Exoplore, Vecco Instruments Inc., USA) was carried out to observe the morphology of the obtained CNs. The morphology and structure of the obtained CNs and Ag nanostructures were measured by TEM (JEOL JEM-100CX, Japan), high resolution transmission electron microscopy (HRTEM), selected area electron diffraction (SAED) and energy dispersive X-ray spectroscopy (EDS). The particle sizes of the Ag NPs were measured using ImageJ software. In order to measure the particle size accurately, those uncovered particles by the cellulose microfibrils were chose for measurement, and at least 100 particles of the sample from different TEM images were analyzed. The UV–vis spectra were taken at room temperature on a Mapada UV-1800 spectrophotometer using a quartz cuvette with an optical path of 1 cm. The XRD patterns were collected on a Philips Analytical X'Pert X-diffractometer (Philips Co., Netherlands), using Cu-Kα radiation ($k = 0.1540$ nm) at an accelerating voltage of 40 kV and the current of 40 mA. The data were collected from $2\theta = 35–80°$. The contents of Ag in the nanocomposites were determined by atomic absorption spectroscopy (AAS).

2.5. Antibacterial assays

E. coli (ATCC 25922) and *S. aureus* (ATCC 6538) were used for antibacterial activity assessments by using the minimum inhibitory concentrations (MIC) method. The MICs were quantified using a broth microdilution method according to National committee for clinical laboratory (NCCLS). For the tests of the ability of nanohybrids to inhibit bacterial growth, concentrated aqueous dispersions of CNs/Ag nanosphere and CNs/Ag dendritic nanostructre hybrids at concentrations of 1000 mg/L were prepared. For determination of the MIC values, the concentrations of CNs/Ag nanosphere and CNs/Ag dendritic nanostructre hybrids were adjusted by 2–128 times dilution using geometrical progression. The testing of CNs/Ag nanosphere and CNs/Ag dendritic nanostructre hybrids and determination of MIC values were independently repeated at least twice.

3. Results and discussion

3.1. Morphological observation of CNs

Fig. 1a shows a TEM image of CNs, which has been isolated from waste cotton fabrics by acid hydrolysis. CNs suspension contains rod-like crystalline celluloses with 10–20 nm in diameter and 100–200 nm in length. In order to further observe the morphology of the CNs, AFM analysis was carried out. Fig. 1b shows the AFM micrograph of the obtained CNs in suspension. Aggregations are observed in CNs, which might be ascribed to the water evaporation. Meanwhile, some isolated individual particles are clearly observed especially in the AFM image (Fig. 1b). The individual particles clearly demonstrate that the CNs indeed are rod-like particles. The dimension of the CNs corresponds well with the TEM images. The CNs suspension has a good stability, because the incorporation of sulfate groups on the cellulose surface creates a strong electrostatic repulsion between the anionic sulfate ester groups at the surface. (Klemm et al., 2011). Therefore, CNs exposed a larger specific surface area, which is expected to possess a better reducing and stabilizing ability.

3.2. UV–vis analysis

Fig. 2a shows the digital camera picture of the CNs, CNs/Ag nanospheres and CNs/Ag dendritic nanostructure suspensions. It is well known that Ag NPs exhibit yellow colors, these colors arising due to excitation of surface plasmon vibrations in the Ag NPs (Shankar, Rai, Ahmad, & Sastry, 2004). Both the CNs/Ag nanospheres and dendritic nanostructure suspension shows a good stability due to stabilization of the CNs. Fig. 2b shows the UV–vis absorption spectrum of Ag nanospheres and Ag dendritic nanostructure, which is obtained by dispersing the CNs/Ag particles in water under sonication. The spectrum of Ag nanospheres shows the characteristic surface plasmon resonance (SPR) absorption band at 426 nm. In contrast, the SPR adsorption of Ag dendritic nanostructure is at 500 nm. It is consistent with the SPR absorption band of the Ag NPs in literature (Song & Kim, 2009), which indicates