



One-pot synthesis of cellulose-templated copper nanoparticles with antibacterial properties



Alireza Eivazihollagh^{a,*}, Joakim Bäckström^b, Christina Dahlström^a, Fredrik Carlsson^c,
Ismail Ibrahim^a, Björn Lindman^a, Håkan Edlund^a, Magnus Norgren^{a,*}

^a FSCN, Surface and Colloid Engineering, Mid Sweden University, SE-851 70 Sundsvall, Sweden

^b FSCN, Materials Physics, Mid Sweden University, SE-851 70 Sundsvall, Sweden

^c Department of Natural Science, Mid Sweden University, SE-851 70 Sundsvall, Sweden

ARTICLE INFO

Keywords:

One-pot synthesis
Copper nanoparticles
Cellulose
Hybrid material
Antibacterial properties

ABSTRACT

We report a facile *in situ* synthesis of spherical copper nanoparticles (NPs) templated by a gelled cellulose II matrix under alkaline aqueous reaction conditions. In under 20 min, the hybrid material could be obtained in a one-pot reaction. Field-emission scanning electron microscopy (FE-SEM) revealed that the polycrystalline NPs of 200–500 nm were well distributed in the regenerated cellulose matrix. The average Cu crystallite size was of the order of 20 nm, as estimated from both X-ray diffraction (XRD) and FE-SEM. XRD data also indicated that the composite contained up to approximately 20% Cu₂O. In suspensions containing the hybrid material, growth of *Escherichia coli* and *Staphylococcus aureus* strains was inhibited by 80% and 95%, respectively, after 72 h. The synthesis procedure offers a general approach to designing various low-cost hybrid materials of almost any shape, and the concept could be extended to utilization areas such as catalysis, functional textiles, and food packaging as well as to electronic applications.

1. Introduction

In situ synthesis of metal nanoparticles (NPs) in solid matrixes has attracted considerable attention for its practical applications and synthetic complications [1]. To be usable and manageable, metal NPs often need to be incorporated in a solid matrix forming hybrid material. Of several candidate matrix materials, cellulose is especially promising as it provides good mechanical performance and chemical resistivity in a wide range of solvents [1–8], while being an inexpensive and “green” material. The functionality of the particles in such hybrid material depends on the nanoparticle size, morphology, composition, crystallinity [9], and dispersion in the matrix, all of which require careful control of synthesis conditions [2].

Several methods have been proposed for preparing metal nanoparticle–cellulose hybrid materials with unique electronic, catalytic, magnetic, optical, biomedical and antibacterial properties [1–8]. Most such approaches involve the impregnation of solid cellulose materials with metal ions followed by reduction into metal and/or metal-oxide NPs by applying sodium borohydride [1–4] or hydrazine [4,5] has been investigated. However, although these routes are successful, they have drawbacks: for example, they can be time consuming due to multiple synthesis steps and complex protocols or entail difficulties controlling

the size and distribution of the NPs in the cellulose matrix. Simple preparation methods resulting in well-defined hybrid materials are certainly desirable.

In this study, we report on a rapid and scalable one-pot synthesis of spherical copper nanoparticles (Cu NPs) of a narrow size distribution templated by an *in situ*-regenerated cellulose network in a water-based solvent system. The synthesized hybrid material inhibited the growth of *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) strains by 80% and 95%, respectively, after 72 h. The work presents a general low-cost approach to designing various cellulose–nano copper hybrid materials of almost any shape, and the concept could be extended to other utilization areas, such as catalysis, functional textiles, and packaging, as well as to electronic applications.

2. Experimental

2.1. Materials

Nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) (purity ≥99%) were supplied by Sigma-Aldrich. CuSO₄·5H₂O, NaOH, urea, H₂SO₄ (purity 98%), and formaldehyde (36 wt% solution) were supplied by VWR International (Sweden) and were used without

* Corresponding authors.

E-mail addresses: alireza.eivazi@miun.se (A. Eivazihollagh), magnus.norgren@miun.se (M. Norgren).

further purification. Cellulose dissolving pulp (viscosity 450 mL g^{-1}) was supplied by Domsjö Fabriker, Örnsköldsvik, Sweden. The cellulose was dissolved directly in aqueous 7 wt% NaOH/12 wt% urea solution precooled to $-12 \text{ }^\circ\text{C}$ to prepare a transparent 0.5 wt% cellulose solution [10]. Bacterial strains from *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) were obtained from ATCC (LGC Standards GmbH, Germany).

2.2. Copper nanoparticle synthesis

First, 20 g of an aqueous NaOH/urea solution with a cellulose concentration of 0.5 wt% was added to 50 mL of a 0.01 M copper complex solution ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$):EDTA:NTA under magnetic stirring at ambient temperature. The highly alkaline mixed solution was stable without any indications of copper ion precipitation or cellulose coagulation. Thereafter, 12 mL of 1 M H_2SO_4 solution was injected into the mixed solution, lowering the pH to 13.3 and immediately regenerating the cellulose in a highly swollen state. Next, 30 mL of formaldehyde (CH_2O) and sodium hydroxide were added to reduce the chelated cupric ions *in situ* [11]. Continuous addition of NaOH in a solid form (pellets, 0.21 mol) that gradually dissolved was found to be the fastest and most successful protocol (under 20 min at ambient temperature). The hybrid material was separated by simple vacuum filtration, washed thoroughly with distilled water, and air dried at room temperature before further characterization. The sequential protocol of the proposed synthetic procedure is schematically depicted in Fig. 1.

3. Results and discussion

FE-SEM images and XRD pattern of the hybrid material are given in Fig. 2. The images show the fairly uniform dispersion of Cu NPs in the 200–500 nm size range distributed throughout the cellulose matrix (Fig. 2a). The XRD pattern in Fig. 2b has peaks corresponding closely to the known diffraction lines of Cu, cellulose II [12], and Cu_2O . The diffraction pattern is clearly dominated by metallic copper, while cuprous oxide is present as a minority phase. Based on the intensity of the Bragg peaks, we estimate that the ratio between the amounts of Cu and Cu_2O exceeds 80:20. The clear presence of cellulose II indicates that the cellulose has indeed been recrystallized in the process. Using the Scherrer equation, the average crystallite size of copper was estimated from the line broadening of diffraction peaks to be in the 15–30-nm range, assuming that the contributions of chemical disorder and mechanical strain to the line broadening of peaks are negligible. This crystallite size estimate is in agreement with the three-dimensional crystallites (TDCs) of the polycrystalline spherical particles observed in the FE-SEM images (see, e.g., insets of Fig. 2b).

The FE-SEM images in Fig. 2a show the morphological characteristics of the hybrid material, where Cu NPs are well dispersed throughout the cellulose matrix. Under the extremely alkaline conditions of the synthesis, dissociation of the few carboxyl acid groups

present in the cellulose polymer is obvious. More importantly, numerous hydroxyl groups in the glucose units of cellulose are also dissociated, especially at the C6 carbon [13]. The ionized hydroxyl groups keep the regenerated cellulose in a highly swollen state, act as effective nanoreactors, and provide improved conditions for the *in situ* synthesis of the NPs. Under the extreme alkaline conditions, the dissociated hydroxyl functions not only outcompete the complexing agent and anchor the copper ions tightly onto the cellulose via electrostatic and ion–dipole interactions/complexation, but also, after reduction, stabilize the prepared Cu NPs via surface interactions [14]. Compared with a simple mixture of the composite components, this process presents some advantages. As the host macromolecular chains play a templating role in the synthesis, the distribution of Cu NPs inside the cellulose matrix is improved and aggregate formation is prevented. At the same time, the polymer chains play an important role, leading to a narrow size distribution and well-defined shape of the metal NPs [15].

The mechanism of the autocatalytic reduction of chelated copper ions by CH_2O under alkaline conditions is derived from the mixed-potential theory applied in the interpretation of electroless copper plating [11,16–18]. In general, the oxidative reaction of CH_2O leads to the chemical reduction of (chelated) copper ions to Cu and Cu_2O under the consumption of hydroxide [19]. Thin-film formation in electroless deposition is characterized by three simultaneous crystal-building processes: nucleation, growth into TDCs, followed by coalescence [16,20]. Initially, nucleation proceeds from the copper single-crystal substrate, and the average density of the TDCs increases with deposition time. Later, the average TDC density reaches a maximum and then decreases with time. In the stage of decreasing TDC density, coalescence is the predominant crystal-building process. As can be seen from the inset in Fig. 2b, showing the Cu NPs in high resolution, the mechanism is probably applicable for the description of cellulose-templated copper nanoparticle formation as well.

Antibacterial properties of the cellulose-templated Cu NP material were tested with *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) bacterial strains [21]. As can be seen from Fig. 3, the growth of both strains of bacteria was clearly inhibited in the suspensions containing the hybrid material. After 72 h, the culture density remained the same in *E. coli* (0.32 OD), but continued to decline in *S. aureus* (0.08 OD). Controls remained roughly the same as at 48 h, with a small increase in *S. aureus* (Fig. 3).

The diverse utilization of copper-based hybrid materials and nanocomposites results in that copper ions and nanoparticles can be released into the environment. Recently, a review collected the nanoecotoxicity data on CuO NPs and Ag NPs for different organisms and showed that median LC50 values of CuO NPs were 2–3 mg/L for crustaceans and algae and 10–100 mg/L for fish and most of the organisms studied [22]. Copper ions showed higher toxicity than CuO NPs to all organisms (LC50: 0.024 mg/L for crustaceans and algae; 0.7–53 mg/L for the organisms studied). In comparison, Ag NPs and Ag ions were more toxic median LC50 values for most organisms studied was below 10 mg/L.

4. Conclusions

This communication presents a rapid and simple route for producing a novel and inexpensive hybrid material comprising well-dispersed spherical Cu NPs of a narrow size distribution anchored to cellulose. The hybrid material displays obvious antibacterial properties for both gram-negative and gram-positive bacteria. Our ongoing research is examining this material in catalytic and electronic applications in which the advantages of combining cellulose with metal NPs are of interest.

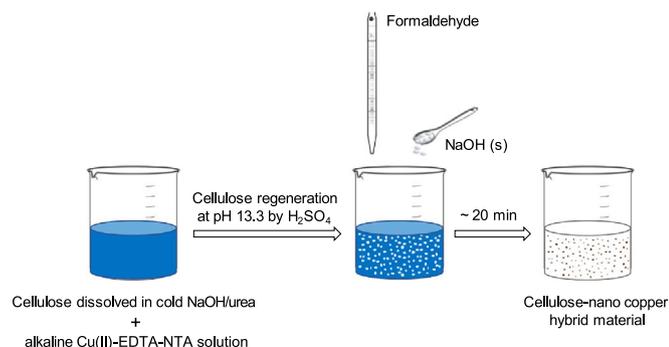


Fig. 1. Schematic of the facile preparation route for the *in situ* synthesis of Cu NPs in a matrix of regenerated cellulose at ambient temperature.

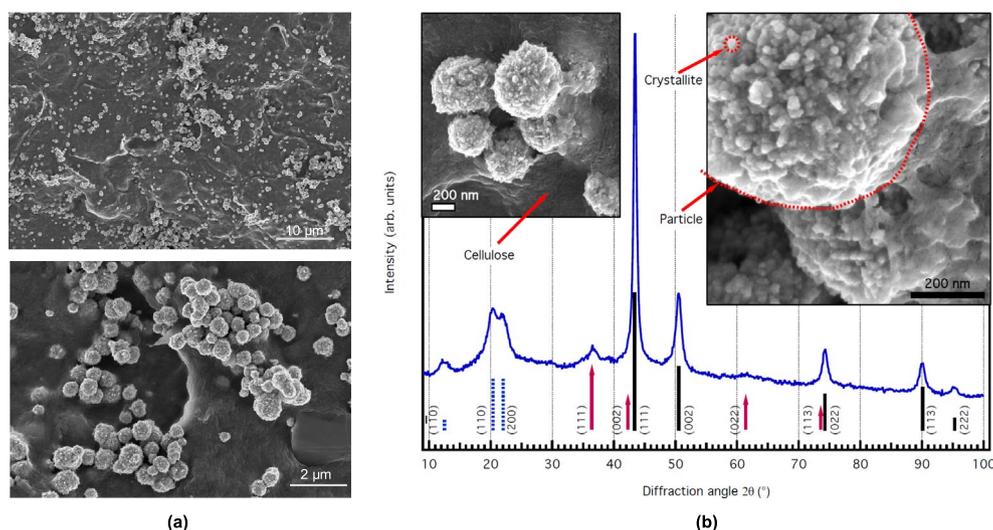


Fig. 2. (a) FE-SEM images of synthesized hybrid material, Cu NPs in a regenerated cellulose network, at two magnifications (b) XRD pattern of hybrid material. The positions of the expected Bragg peaks from copper (black lines), cellulose II (blue dotted lines), and cuprous oxide (red arrows) are marked and labeled with their respective Miller indices. The insets show FE-SEM images of the sample, highlighting the polycrystalline nature of the NPs in the cellulose matrix. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

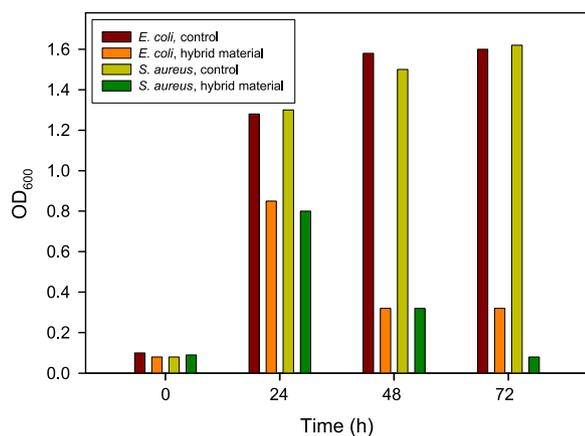


Fig. 3. Effect of the Cu NPs–cellulose hybrid material on bacterial growth in suspensions of *Escherichia coli* and *Staphylococcus aureus*. Cultures were inoculated at approximately 0.5 McFarland (OD 0.08–0.1). Absorbance at 600 nm was measured at the time of inoculation and after 24, 48, and 72 h.

Acknowledgments

Umeå Core Facility for Electron Microscopy (UCEM) is thanked for assistance with electron microscopy. Financing from the Swedish Research Council FORMAS, grant no. 942-2015-251, is gratefully acknowledged. II acknowledges Åforsk for financial support.

References

- [1] J. He, T. Kunitake, A. Nakao, *Chem. Mater.* 15 (2003) 4401–4406.
- [2] J. Cai, S. Kimura, M. Wada, S. Kuga, *Biomacromolecules* 10 (2009) 87–94.
- [3] B. Jia, Y. Mei, L. Cheng, J. Zhou, L. Zhang, *Appl. Mater. Interfaces* 4 (2012) 2897–2902.
- [4] U. Vainio, K. Pirkkalainen, K. Kisko, G. Goerigk, N.E. Kotelnikova, R. Serimaa, *Eur. Phys. J.* 42 (2007) 93–101.
- [5] K.R. Reddy, N.S. Kumar, B. Sreedhar, M.L. Kantam, *J. Mol. Catal. A* 252 (2006) 136–141.
- [6] R.T. Olsson, M.A.S. Azizi Samir, G. Salazar-Alvarez, L. Belova, V. Ström, L.A. Berglund, O. Ikkala, J. Noguez, U.W. Gedde, *Nat. Nanotechnol.* 5 (2010) 584–588.
- [7] S. Liu, J. Zhou, L. Zhang, J. Guan, J. Wang, *Macromol. Rapid Commun.* 27 (2006) 2084–2089.
- [8] L. Tamayo, M. Azócar, M. Kogan, A. Riveros, M. Páez, *Mater. Sci. Eng. C* 69 (2016) 1391–1409.
- [9] Y. Sun, Y. Xia, *Science* 298 (2002) 2176–2178.
- [10] J. Zhou, L. Zhang, J. Cai, H. Shu, *J. Membr. Sci.* 210 (2002) 77–90.
- [11] Y. Shacham Diamand, V. Dubln, M. Angyal, *Thin Solid Films* 262 (1995) 93–103.
- [12] E. Togawa, T. Kondo, *J. Polym. Sci.* 37 (1999) 451–459.
- [13] A. Isogai, *Cellulose* 4 (1997) 99–107.
- [14] J. He, T. Kunitake, A. Nakao, *Adv. Chem. Mater.* 15 (2003) 4401–4406.
- [15] P. Dallas, V.K. Sharma, R. Zboril, *Adv. Colloid Interface Sci.* 166 (2011) 119–135.
- [16] M. Paunovic, *Electroless deposition of copper*, in: M. Schlesinger, M. Paunovic (Eds.), *Modern Electroplating* 5th ed., John Wiley & Sons, Hoboken, NJ, 2010, pp. 433–446.
- [17] Z. Jusys, A. Vaškelis, *Langmuir* 8 (1992) 1230–1231.
- [18] F. Hanna, Z.A. Hamid, A.A. Aal, *Mater. Lett.* 58 (2003) 104–109.
- [19] R. Schumacher, J.J. Pesek, O.R. Melroy, *J. Phys. Chem.* 89 (1985) 4338–4342.
- [20] R. Sard, *J. Electrochem. Soc.* 117 (1970) 864–870.
- [21] R.J.B. Pinto, S. Daina, P. Sadocco, C.P. Neto, T. Trindade, *Biomed. Res. Int.* (2013), 280512.
- [22] O. Bondarenko, K. Juganson, A. Ivask, K. Kasemets, M. Mortimer, A. Kahru, *Arch. Toxicol.* 87 (2013) 1181–1200.