Carbohydrate Polymers 97 (2013) 391–397

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Green synthesis of silver nanoparticles using 4-acetamido-TEMPO-oxidized curdlan

Jing-Kun Yan[a,b,*], Pan-Fu Cai[a], Xiao-Qiang Cao[a], Hai-Le Ma[a,b], Qi Zhang[a], Nan-Zi Hu[a], Yi-Zhang Zhao[a]

[a] *School of Food & Biological Engineering, Jiangsu University, Zhenjiang 212013, Jiangsu, China*
[b] *Physical Processing of Agricultural Products Key Lab of Jiangsu Province, Zhenjiang 212013, Jiangsu, China*

ARTICLE INFO

Article history:
Received 10 March 2013
Received in revised form 24 April 2013
Accepted 20 May 2013
Available online 27 May 2013

Keywords:
Curdlan
4-Acetamido-TEMPO
Green synthesis
Silver nanoparticles

ABSTRACT

A facile, simple, and eco-friendly method using 4-acetamido-2,2,6,6-tetramethypiperidine-1-oxyl radical-oxidized curdlan (Oc) as both reducing and stabilizing agents was developed for the fabrication of silver nanoparticles (AgNPs) from silver nitrate ($AgNO_3$). The structure, morphology, and particle size of the as-prepared AgNPs were investigated by ultraviolet-visible spectroscopy, transmission electron microscopy, energy dispersive X-ray spectrometry, X-ray diffraction, and dynamic laser light scattering. The well-dispersed AgNPs were sphere like with a mean diameter of 15 nm. Their formation was dependent on reaction duration, reaction temperature, Oc concentration, and $AgNO_3$ concentration. Fourier transform-infrared and Raman spectra demonstrated that the as-prepared AgNPs can readily bind covalently with the carboxylate groups of Oc through the strong monodentate interaction in the reaction medium.

1. Introduction

Nanotechnology is considered as the most advanced technology in the 21st century. It is closely related to physics, chemistry, biology, material science, and medicine. It has been widely used in biomedicine for the development of nanoparticle (NP)-based drug delivery systems, rapid pathogen detection, biomolecular sensing, and NP-based cancer diagnosis and therapy (Daaboul et al., 2010; Hallaj-Nezhadi, Lotfipour, & Dass, 2010; Miranda, Creran, & Rotello, 2010; Portney & Ozkan, 2006). In particular, silver (Ag) NPs are preferred over bulk metals because of their distinctive electrical, electronic, thermal, optical, magnetic, catalytic, sensing, and antimicrobial functionalities (Lara, Garza-Treviño, Ixtepan-Turrent, & Singh, 2011; Ravindran, Chandran, & Khan, 2012). Numerous methods, including chemical reduction (Lee & Meisel, 1982), thermal decomposition (Yang, Matsubara, & Xiong, 2007), laser ablation (Simakin, Voronov, Kirichenko, & Shafeev, 2004), and sonochemical synthesis (Salkar, Jeevanandam, Aruna, Koltypin, & Gedanken, 1999), have been utilized for the synthesis of AgNPs. Among these methods, chemical reduction using sodium borohydride and citrate as reducing and protecting agents, respectively, is the most common approach to prepare AgNPs (Evanoff & Chumanov, 2005).

The preparation of AgNPs using biopolymers has drawn considerable attention (Eby, Schaeublin, Farrington, Hussain, & Johnson, 2009; Matthew, Dickerson, Sandhage, & Naik, 2008; Xia, Monterio-Riviere, & Riviere, 2010). Under relatively mild conditions, biomacromolecules can be used as both reducing and stabilizing agents to synthesize high-yield and environment-friendly AgNPs in aqueous solution. As the most abundant polymers in the biosphere, natural polysaccharides are potential candidates for the preparation of AgNPs. Raveendran, Fu, and Wallen (2003) was the first to report the complete green synthesis of AgNPs using β-D-glucose and starch as reducing and stabilizing agents, respectively (Raveendran et al., 2003). Several polysaccharides, such as starch, cellulose, chitosan, dextran, heparin, and hyaluronan fibers, have been widely used as both reducing and stabilizing agents to fabricate AgNPs (Abdel-Mohsen et al., 2012; Bankura et al., 2012; Chen, Wang, Zhang, & Jin, 2008; Kemp & Linhardt, 2010; Raveendran et al., 2003; Wei & Qian, 2008).

Curdlan is a neutral bacterial exopolysaccharide composed primarily of linear β-(1,3)-glycosidic linkages. It has been widely applied in the food and pharmaceutical industries because of its unique gelling properties and important bioactivities. However, the water-insolubility of curdlan limits its practical applications. Carboxymethylation has become one of the most important and effective methods used to improve the functional properties of curdlan (Jin, Zhang, Yin, & Nishinari, 2006). Carboxymethyl curdlan (CMc) can act as both reducing and stabilizing agents for the synthesis of AgNPs because of its hydroxyl and carboxylic groups

---

[*] Corresponding author at: School of Food & Biological Engineering, Jiangsu University, Zhenjiang 212013, Jiangsu, China. Tel.: +86 15952819661.
E-mail address: jkyan_27@163.com (J.-K. Yan).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.05.049

(Leung, Wong, & Xie, 2010; Wu, Zhang, & Zhang, 2012). However, the preparation of CMc usually depends on the use of the esterification reagent chloroacetic acid, which is potentially toxic to the environment. Recent studies have reported the regioselective oxidation of the C6 primary hydroxyl groups of curdlan using 2,2,6,6-tetramethypiperidine-1-oxyl radical (TEMPO) or its analog as a catalyst under mild conditions (Tamura, Wada, & Isogai, 2009; Tamura, Hitora, Saito, & Isogai, 2010; Watanabe, Tamura, Saito, Habu, & Isogai, 2012). TEMPO- or 4-acetamido-TEMPO-oxidized curdlan (Oc) contains numerous hydroxyl and carboxylic groups. Thus, similar to CMc, Oc can also be used as both reducing and stabilizing agents for the green synthesis of AgNPs.

This study aims to develop a novel approach for the green synthesis of AgNPs using 4-acetamido-TEMPO-Oc as both reducing and stabilizing agents under various reaction conditions. The structure, particle size, and morphology of the as-prepared AgNPs were investigated by ultraviolet-visible (UV-vis) spectroscopy, transmission electron microscopy (TEM), energy dispersive X-ray spectrometry (EDX), X-ray diffraction (XRD), dynamic light scattering (DLS), Fourier transform-infrared spectroscopy (FT-IR), and Raman spectroscopy. The formation mechanism of the AgNPs was also discussed in this study.

2. Materials and methods

2.1. Materials

Commercial curdlan (DP$_w$ 6790) was obtained from Wako Pure Chemical Corporation (Osaka, Japan). 4-Acetamido-TEMPO-Oc was synthesized following the method described by Tamura et al. (Tamura et al., 2010). The carboxylate (COO$^-$) content of Oc was determined to be 4.87 mmol/g using electric conductimetry titration (Saito, & Isogai, 2004). 4-Acetamido-TEMPO and silver nitrate (AgNO$_3$) were purchased from Sigma–Aldrich Chemical Corporation (St. Louis, MO, USA). All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. In situ synthesis of AgNPs

AgNPs were synthesized by direct mixture of AgNO$_3$ and 4-acetamido-TEMPO-Oc in aqueous solution. Briefly, 1.0 mL each of different concentrations of AgNO$_3$ solution (1.0–100 mM) and Oc (0.1–2.0 mg/mL) were mixed under continuous stirring at different temperatures (70–100 °C) and durations (30–120 min) in the dark. The progression of the reaction was monitored by UV-vis absorption.

2.3. Size-exclusion chromatography with multi-angle laser-light scattering (SEC-MALLS) analysis

The weight-average molecular weight (M_w) and radius of gyration ($\langle S^2 \rangle z^{1/2}$) of the Oc in 0.1 M NaCl were determined by SEC-MALLS (DAWN HELEOS II, $\lambda = 658$ nm: Wyatt Technologies Corporation, USA). A differential refractive index detector (RI, 2414, Waters Corporation, USA) and a 515 pump (Waters Corporation, USA) with a SEC column (OHpak SB-806 M HQ, 8 mm $\Phi \times 30$ cm, Shodex, Japan) in 0.1 M NaCl at 25 °C were used. The eluent was 0.1 M aqueous NaCl at a flow rate of 0.5 mL/min. The polysaccharide solution and solvent were purified by a 0.45 μm filter and then degassed before use. The injection volume was 100 μL with a concentration of 1.0 mg/mL for the sample. The specific RI increment (dn/dc) of Oc in 0.1 M NaCl was 0.125 mL/g (Shibata, Yanagisawa, Saito, & Isogai, 2006). The Astra software was utilized for data acquisition and analysis.

2.4. Characterization

The UV-vis spectra of the as-prepared AgNPs using Oc were recorded in a Varian cary 100 spectrophotometer (Varian Co., USA) from 300 nm to 600 nm. FT-IR spectra were recorded from 500 cm^{-1} to 4000 cm^{-1} using a Nexus 670 FT-IR instrument (Thermo Nicolet Co., USA). Raman spectra were recorded from 500 cm^{-1} to 3500 cm^{-1} using a DXR Raman microscope (Thermo-electron Co., USA) equipped with He–Ne laser (532 nm, 10 mW). The as-prepared AgNPs were observed using a Malvern Zetasizer Nano (3000 SHA Malvern Instruments Ltd., UK) at 632.8 nm and 90° scatterring angle. The average particle size was measured based on the DLS analysis. TEM (JEM-2100, JEOL Ltd., Japan, 200 kV) was used to characterize the size and morphology of the as-prepared AgNPs. For the preparation of test samples, a drop of the sample solution was placed on 300 mesh carbon-coated copper grids and then dried at room temperature for 30 min. An EDX spectroscope (Inca Energy-350, Oxford Co., UK) attached to an SEM (JSM-7100F, JEOL Ltd., Japan) was used to determine the elemental composition of the AgNPs. A wide-angle XRD instrument (D8-Advance, Bruker Co., Germany) was used to characterize the critical structure of the AgNPs. XRD patterns with Cu K$_\alpha$ radiation ($\lambda = 0.15406$ nm) at 40 kV and 40 mA were recorded in the region of 2θ from 30° to 80° with a step speed of 4 °/min.

3. Results and discussion

3.1. Chain conformation of Oc

SEC combined with MALLS is a convenient approach for the determination of M_w, $\langle S^2 \rangle z^{1/2}$, and the polydispersity (M_w/M_n) of polymers in solution. Fig. 1a shows the SEC chromatogram of Oc in 0.1 M aqueous NaCl at 25 °C. Based on the results of SEC-MALLS, the values of M_w, $\langle S^2 \rangle z^{1/2}$, and M_w/M_n for Oc were calculated to be 1.2×10^5 g/mol, 59.8 nm, and 1.38, respectively. The single peak suggested the absence of aggregation or polysaccharide of different structures in the aqueous solution. The SEC-MALLS chromatogram can produce the function of $\langle S^2 \rangle z^{1/2} = f \cdot M_w^\alpha$. The power law of the former can be estimated from many experimental points in the SEC chromatogram (Mendichi, Giammonab, Cavallarob, & Giacometti Schieronia, 2000). The exponent may provide additional insights into the conformation of the polymer solution. The exponents of 0.33, 0.50 to 0.60, and 1.0 often reflect the chain shape for the adaptation of sphere, random coil, and rigid rod, respectively (Kato, Okamoto, Tokuya, & Takahashi, 1982; Picton, Bataille, & Muller, 2000). Fig. 1b shows the log-log plot of $\langle S^2 \rangle z^{1/2}$ against M_w for the Oc in 0.1 M aqueous NaCl at 25 °C. The obtained α value of 0.71 suggested that the Oc in 0.1 M NaCl aqueous solution was in the state between random coil and rigid rod, which was exhibited as a semi-flexible chain conformation. The conversion of C6-CH$_2$OH groups to C6-COO$^-$ groups significantly destroyed the intra- and inter-molecular hydrogen bonds and reduced the main-chain hydrophobicity that was indispensable to stabilize the triple strand of curdlan. Furthermore, the introduction of C6-COO$^-$ groups enhanced the steric hindrance between the polymer chains, leading to the relatively expanded random coil conformation of Oc.

3.2. Preparation of AgNPs

AgNPs were prepared using Oc as both reducing and stabilizing agents. The abundance of COO$^-$ groups in Oc enriched the Ag$^+$ ions in solution, thus facilitating the formation of AgNPs. The preparation of Oc-stabilized AgNPs is almost similar to that of AgNPs using CMc (Leung et al., 2010). The different factors affecting the