Materials Letters 138 (2015) 154–157

Contents lists available at ScienceDirect

Materials Letters

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

Preparation of gold nanoparticles using chitosan oligosaccharide as a reducing and capping reagent and their in vitro cytotoxic effect on Human fibroblasts cells

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ARTICLE INFO

Article history:
Received 27 July 2014
Accepted 20 September 2014
Available online 28 September 2014

Keywords:
Gold
Nanoparticles
Chitosan oligosaccharide
Biosynthesis
FTIR
Cytotoxicity

ABSTRACT

Gold nanoparticles (GNPs) were successfully synthesized by use of chitosan oligosaccharide (COS) as reducing agent and capping agent. The results revealed that the average particle size was 115.21 ± 16.87 nm and were crystallized in the face centered cubic symmetry. The possible synthesis mechanisms were elucidated through FTIR. In vitro cytotoxicity of the GNPs against Human fibroblasts cells showed a dose–response activity at concentration higher than 62.5 μg/ml.

Introduction

Gold nanoparticles (GNPs) are biocompatible materials and they have various biological applications including labeling, drug-delivery, photothermal therapy, and tissue/tumor imaging [1,2]. To achieve biocompatibility and biodegradability, different biopolymers such as alginate, starch, and chitosan have been used as alternatives to the synthesis of GNPs [3,4]. Among the biopolymers, chitosan is one of the prominent natural biomaterial for reducing and stabilizing the metal nanoparticles [5]. However, poor solubility and high viscosity limit its application. Unlike chitosan, its hydrolyzed products chitosan oligosaccharides (COS) are readily soluble in water due to their shorter chain lengths and free amino groups in D-glucosamine units.

An extensive literature survey revealed that there is no report on the exploitation of COS as both reducing agent and capping agent for GNPs synthesis. In this paper, a novel and simple route for the synthesis of GNPs using COS as reducing and capping agent was demonstrated. Meanwhile, the cytotoxic effect of the COS-mediated GNPs against Human fibroblasts cells was investigated in vitro.

Experimental

COS was obtained by enzymatic degradation of chitosan according to Ying's method [6]. The molecular weight (MW) of COS was controlled by reaction time of hydrolysis, which was determined by gel permeation chromatography (GPC) [7]. The COS of approximately 5000 Da was prepared. Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, $> 99.9\%$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The reduction was initiated by adding 0.5 g COS powder to 20 ml $HAuCl_4 \cdot 3H_2O$ solution (1.0 mM) and stirred with a magnetic stir bar for 5 min at 25 °C. The effect of pH on nanoparticles synthesis was determined by adjusting the pH to 2.90, 7.31 and 11.15. The experiments were also carried out by varying the initial $HAuCl_4 \cdot 3H_2O$ concentrations and the quantities of the COS. The stability of nanoparticles was examined by exposing them to ambient condition for 3 months.

The optical absorption spectra of the nanoparticles were obtained by UV-2450 Shimadzu UV–vis spectrometer. The morphology and particles size of the GNPs were evaluated using a High resolution transmission electronmicroscopy (HRTEM) (Jeol JEM100SX). The purified and freeze dried GNPs were subjected to Fourier transform infrared spectroscopy (FTIR) analysis (Shimadzu FTIR spectrophotometer 8400). X-ray diffraction (XRD) of the GNPs was obtained using XPERT-PRO diffractometer using Cu $K\alpha$ radiation ($\lambda = 0.1542$ nm). The concentration of the final GNPs was

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http://dx.doi.org/10.1016/j.matlet.2014.09.078
0167-577X/

determined by Inductively coupled plasma atomic emission spectrometry (ICP-AES).

The cytotoxicity was assessed by cell viability assay MTT carried on Human fibroblasts cells. In brief, the cells (1×10^5) grown in 96-well plates were treated with GNPs in concentrations from 15.6 to 500.0 μg/ml for 24 h at 37 °C. The cells were further incubated with MTT (0.5 mg/ml) at 37 °C for 3 h. The resulting formazan was dissolved in 100 ml of dimethyl sulphoxide with gentle shaking at 37 °C. The color intensity was measured at 570 nm (reference wavelength 620 nm) using an enzyme linked immunosorbent assay (ELISA) reader.

Result and discussion

Fig. 1a shows UV–vis spectra of the GNPs at initial pH values 2.90, 7.31, and 11.15. In the pH range from 2.90 to 11.15, the absorbance peaks shifted from 542 nm to 566 nm. The red shifts at higher pH are attributed to the increased size and/or increasing aggregation of the GNPs. Similar pH effect on Au particle size was also reported by Gan et al.'s work [8]. At pH 2.90, gold is present in solution in anionic form $[AuCl_4]^-$ and the functional groups of the COS such as amino groups tend to undergo protonation and become positively charged. The overall positively charged surface could promote the interaction between the reduction sites and the negatively charged $[AuCl_4]^-$ through electrostatic attraction [9]. Thus, the reduction rate increases and most $[AuCl_4]^-$ ions are consumed in the initial reduction process, blocking the secondary reduction process on the surface of the preformed Au nanoparticles to form larger particles. On the other hand, better dissolution and uniform distribution of COS at lower pH, which helps the formation of dispersible gold particles rather than aggregated ones.

The synthetic yields of GNPs were 55%, 40% and 35% for pH 2.90, 11.15 and 7.31. The pH value of 2.90 is preferred for the formation of dispersed and high yield of nanoparticles.

Fig. 1b shows the UV–vis spectra for the GNPs obtained at various COS quantities ($HAuCl_4 \cdot 3H_2O$ concentration: 1.0 mM; temperature: 25 °C; pH: 2.90). The absorbance peaks occurred at about 550 nm and increased in intensity with little shift while increasing COS quantities. The most synthetic yield 75% of GNPs was obtained at the COS quantity of 1.000 g. This phenomenon implies that increasing the COS quantity favors the increasing of the GNPs yield.

The influence of $HAuCl_4 \cdot 3H_2O$ concentration was investigated by varying the concentration from 0.5 mM to 5.0 mM (COS: 0.5 g; temperature: 25 °C; pH: 2.90), Fig. 1c shows the effect of the $HAuCl_4 \cdot 3H_2O$ concentration on the formation of GNPs. It was found that the absorbance peaks shifted from 544 nm to 596 nm as the $HAuCl_4 \cdot 3H_2O$ concentration increased. Similar $HAuCl_4 \cdot 3H_2O$ concentration effect on Au particle size was also reported by Shashi et al.'s work [10]. The highest GNPs yield 70% was obtained at 1.0 mM for $HAuCl_4 \cdot 3H_2O$.

Fig. 2 shows the TEM images of the GNPs. It is elucidated that the GNPs formed were hexagonal, triangular, rodlike and predominantly spherical in shape. It is noted that the GNPs are surrounded by a distinct vacuole-shape layer of organic material, which is supposed to be the capping agent from COS. The average particle size was determined to be 115.21 ± 16.87 nm on a representative TEM micrograph. The fringe spacing measured from the HRTEM image is 0.25 nm for the GNPs which corresponds to the spacing between the (1 1 1) plane of FCC lattice of gold.

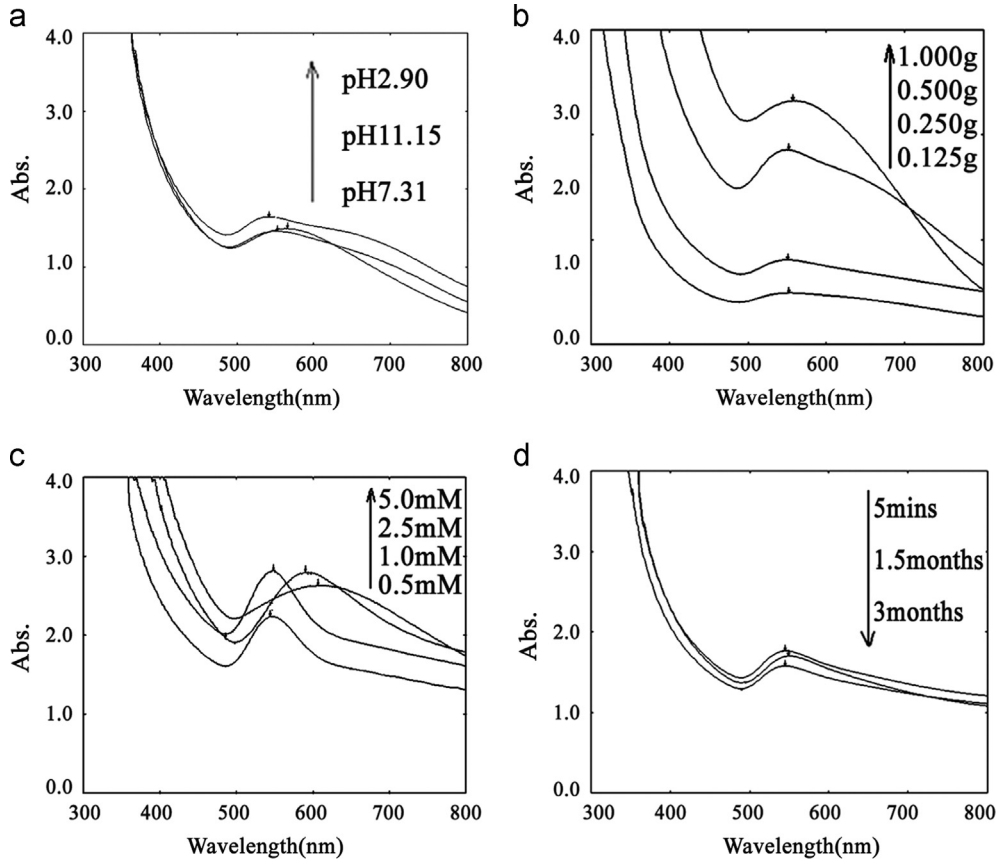

Fig. 1. (a) UV–vis spectra of GNPs synthesized at different pH values [2.90, 7.31, and 11.15]; (b) UV–vis spectra of the GNPs synthesized at different COS quantities; (c) UV–vis spectra of the GNPs synthesized at different $HAuCl_4 \cdot 3H_2O$ concentrations; d Stability of the synthesized GNPs monitored by UV–vis spectra.