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Note

Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method

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Abstract—A simple, green method was developed for the synthesis of gold and silver nanoparticles by using polysaccharides as reducing/stabilizing agents. The obtained positively charged chitosan-stabilized gold nanoparticles and negatively charged heparin-stabilized silver nanoparticles were characterized with UV-vis spectroscopy and transmission electron microscopy. The results illustrated the formation of gold and silver nanoparticles inside the nanoscopic polysaccharide templates. Moreover, the morphology and size distribution of prepared gold and silver nanoparticles varied with the concentration of both the polysaccharides and the precursor metal salts.

Keywords: Polysaccharides; Synthesis; Nanoparticles; 'Green' method

1. Introduction

The preparation of metal nanoparticles is a major research area in nanoscale science and engineering given their unusual chemical and physical properties, such as catalytic activity, novel electronic, optic and magnetic properties, and their potential application in biotechnology.[1–6] A general method for the preparation of metal nanoparticles involves the treatment of metal salts with a chemical reducing agent, such as citrate acid, borohydride, or other organic compounds.[7–15] These reducing agents may have associated environmental toxicity or biological hazards. With the increasing interest in minimization or total elimination of waste and implementation of sustainable processes through the adoption of the 12 fundamental principles of green chemistry,[16] the development of biological and biomimetic approaches for the preparation of advanced materials is desirable.

In recent years, Mukherjee et al.[17,18] reported a novel biological method for the synthesis of silver and gold nanoparticles using the fungus *Verticillium*. The concept of green nanoparticle preparation was first developed by Raveendran et al.,[19] who used β-D-glucose as the reducing agent and starch as a capping agent to prepare starch silver nanoparticles. A green method for nanoparticle preparation should be evaluated from three aspects: the solvent, the reducing agent and the stabilizing agent. In our work, we present a simple and green method for the preparation of gold and silver nanoparticles using naturally-occurring polysaccharides as both the reducing and stabilizing agent. No other chemical reducing agent is needed. The reaction is carried out in an aqueous solution in a process that is benign to the environment.

2. Results and discussion

Chitosan, the structure of which is shown in Figure 1a, is a polysaccharide obtained by partial deacetylation of chitin. Recently, chitosan has been used as a protecting polymer for the preparation of gold nanoparticles,[20] but no reports have appeared describing the use of this polymer as a reducing agent in synthesis of metal nanoparticles. The method for the preparation of gold nanoparticles using chitosan as both a reducing agent

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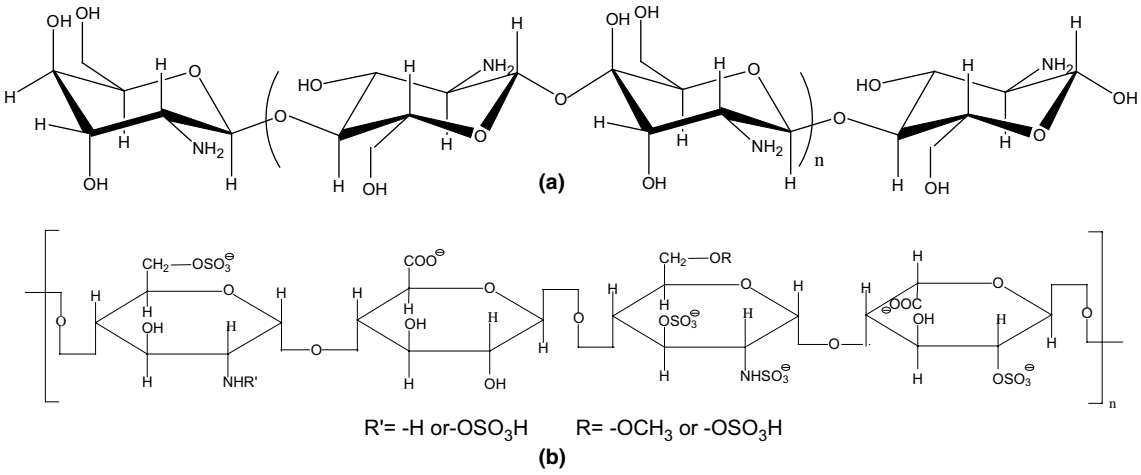

Figure 1. Structure of chitosan (a) and heparin (b).

and a protecting agent is quite simple. In a typical preparation, 100 μL of a 20 mM aqueous solution of $HAuCl_4$ was mixed with 3 mL of an aqueous solution of chitosan (2.0 mg/mL, 100% deacetylated, Fluka). The mixture was heated to 55 °C and kept at this temperature while stirring in a water bath. After approximately 2 h, a red solution was obtained, indicating the formation of gold nanoparticles. The heat source was then removed and the solution was allowed to cool to room temperature while stirring. The gold nanoparticles prepared using chitosan as a protecting agent were of high monodispersity, and are comparable to citrate-stabilized gold nanoparticles with regard to shape and stability.[21] A plasmon absorption band, characteristic of gold nanoparticles, was observed at 522 nm, as shown in Figure 2 (solid line).

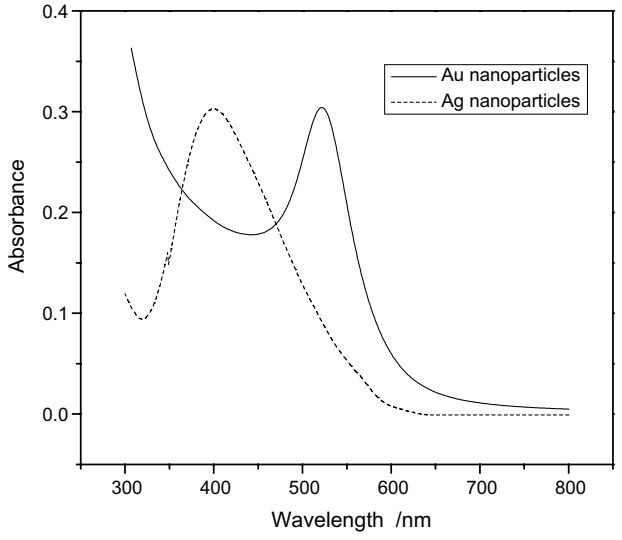

Figure 2. The plasmon absorbance of chitosan stabilized gold nanoparticles λ_{max} (λ_{max} = 522 nm) and heparin stabilized silver nanoparticles (λ_{max} = 401 nm).

Silver nanoparticles were obtained by using heparin as both the reducing and stabilizing agent. Despite heparin's widespread medical use as an anti-coagulant, its precise chemical structure, the range of its biological activities and the structure–activity relationship (SAR) for these activities is not yet well understood.[22] A partial proposed structure of heparin is presented in Figure 1b. The anionic property of heparin makes possible the preparation of silver nanoparticles using this polysaccharide as reducing and stabilizing agent. The abundance of sulfonate groups in heparin made Ag^+ ions in solution enrichment, thus facilitating the formation of silver nanoparticles. The preparation of heparin-stabilized silver nanoparticles is almost the same as synthesis of gold nanoparticles using chitosan, but the reaction time is much longer. Typically, 1 mL of a 2 mM aqueous solution $AgNO_3$ was mixed with 2 mL of an aqueous heparin solution (2.5 mg/mL, Sigma). The mixture was heated to 70 °C and was kept at this temperature while stirring. After 8 h, a yellow solution resulted, which indicated the formation of silver nanoparticles. The resulting solution was cooled to room temperature while stirring.

Silver nanoparticles absorb radiation in the visible region of the electromagnetic spectrum (ca. 380–450 nm) due to the excitation of surface plasmon vibrations, and this is responsible for the striking yellow-brown color of silver nanoparticles in various media.[23–25] The UV–vis absorption spectrum of the silver nanoparticle solution we prepared is shown in Figure 2 (dashed line). A plasmon absorbance of the silver nanoparticles was observed at 401 nm, and the plasmon band is symmetric, which indicates that the solution does not contain many aggregated particles, a conclusion that agrees with the electron micrograph observations (below).

Typical transmission electron microscope (TEM) images and the histograms of particle size distribution of the gold and silver nanoparticles are presented in

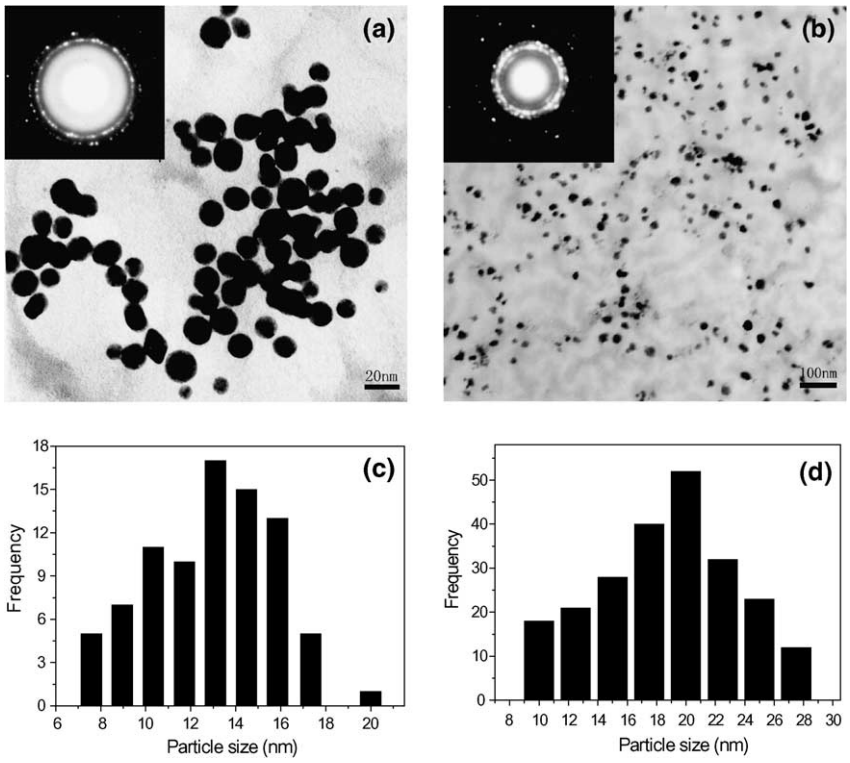

Figure 3. (a) TEM images of chitosan stabilized gold nanoparticles; inset electron diffraction pattern. (b) TEM images of heparin-stabilized silver nanoparticles; inset electron diffraction pattern. (c) Histogram of particle size distribution of chitosan stabilized gold nanoparticles. (d) Histogram of particle size distribution of heparin-stabilized silver nanoparticles.

Figure 3. In general, the particles are isotropic (i.e., low aspect ratio) in shape. These results illustrate the formation of gold and silver nanoparticles inside the nanoscopic polysaccharide templates. The electrostatic attractive forces between polysaccharide (amino groups in chitosan and sulfonic groups in heparin) and $AuCl_4^-$ or Ag^+ in solution provide an effective driving force for the formation and stabilization of the gold and silver nanoparticles. These nanoparticles were analyzed by electron diffraction directly on the microscope. The electron diffraction patterns (inset panels in Fig. 3a and b) showed concentric circles resulting from the random orientation of crystal planes.

We found that the morphology and size distribution of the gold or silver nanoparticles varied with the concentration of both the polysaccharides and the precursor metal salts. For example, when the concentration of heparin or Ag^+ ions was increased, the size of the silver nanoparticles also increased. Figure 4 shows the UV–vis absorption spectra of silver nanoparticles prepared with different concentrations of heparin and $AgNO_3$. All spectra exhibit an absorption band in the range of 400–450 nm, a typical plasmon resonance band of silver nanoparticles.[23–28] When the concentration of $AgNO_3$ is increased, the intensity of the absorption band increases, and the plasmon absorption shifts to 532 nm. When the concentration of $AgNO_3$ is fixed, and the concentration of heparin is increased, the intensity of the absorption band goes up again, and the plasmon absorption shifts to a longer wavelength (451 nm). Both the increases in absorption intensity and the shifts in plasmon absorption

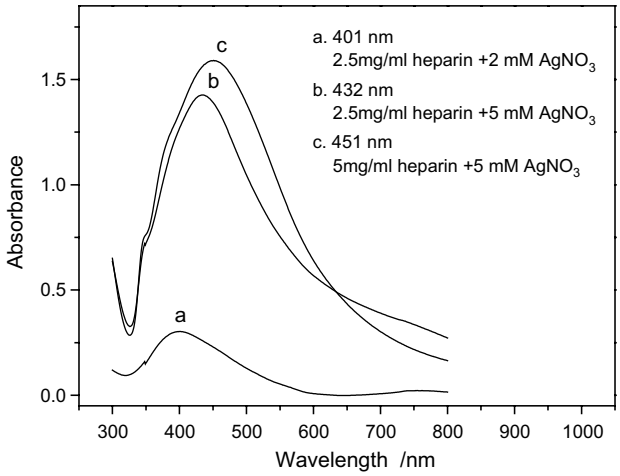

Figure 4. UV–vis absorption spectra of silver nanoparticles prepared with different concentrations of heparin and $AgNO_3$: (a) 2.5 mg/mL heparin + 2 mM $AgNO_3$ (λ_{max} = 401 nm, the same as Fig. 2 dotted line, illustrated here for the comparison); (b) 2.5 mg/mL heparin + 5 mM $AgNO_3$ (λ_{max} = 432 nm); (c) 5 mg/mL heparin + 5 mM $AgNO_3$ (λ_{max} = 451 nm).

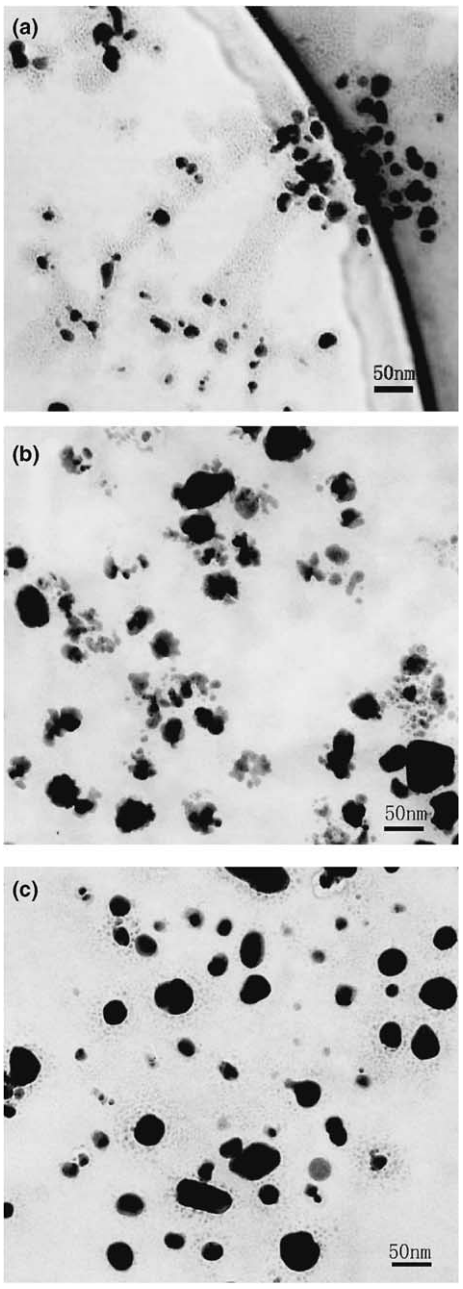

Figure 5. TEM images of silver nanoparticles prepared with different concentrations of heparin and $AgNO_3$: (a) 2.5 mg/mL heparin + 2 mM $AgNO_3$; (b) 2.5 mg/mL heparin + 5 mM $AgNO_3$; (c) 5 mg/mL heparin + 5 mM $AgNO_3$.

indicate the size of silver nanoparticles formed varies with the concentration of heparin and $AgNO_3$. This is further confirmed by transmission electron microscopy (TEM) experiments. TEM images of silver nanoparticles prepared with different concentrations of heparin and $AgNO_3$ are illustrated in Figure 5. When the concentration of $AgNO_3$ was increased from 2 to 5 mM, larger particles and Ag clusters were obtained while with a further increase in the concentration of heparin, larger spherical Ag nanoparticles were obtained. These changes in particle size and morphology may be controlled by the heparin as the controller of nucleation and stabilizer. Upon increasing the concentration of $AgNO_3$, more nuclei were formed, and few heparin molecules absorbed on the pre-formed particles resulting in the formation of large Ag clusters. The effect of chitosan concentrations on the gold nanoparticles will be discussed elsewhere.

In conclusion, we have developed a simple, green method for the synthesis of metal nanoparticles. The particles produced are highly stable and show no signs of aggregation after two months of storage. The use of these polysaccharides enables the preparation of positively charged gold nanoparticles and negatively charged silver nanoparticles, which may find applications in nanoscale superstructure fabrication. Moreover, the widespread occurrence of these naturally-occurring polysaccharides makes this process amenable to large scale industrial production.

3. Experimental

3.1. Materials

$HAuCl_4$ was purchased from Aldrich and used without further purification. Medium molecular weight chitosan (poly-(1,4-β-D-glucopyranosamine), 400,000 g/mol) with a degree of deacetylation of 100% was purchased from Fluka. Heparin from porcine intestinal mucosa (Sodium salt, 250,000 units) was obtained from Sigma. Both polysaccharides were used as received. Acetic acid was diluted to a 1% aqueous solution before use. All aqueous solutions were made using ultrahigh purity water purified using a Mill-Q Plus system (Millipore Co.).

3.2. Preparation of gold nanoparticles using chitosan

All glassware used was cleaned in a bath of freshly prepared aqua regia solution (HCl–HNO_3, 3:1), and rinsed thoroughly with H_2O prior to use. A stock solution of chitosan (2.0 mg/mL) was prepared by dissolving the chitosans in 1% acetic acid solution. Due to the poor solubility of chitosan, the mixture was vortexed and kept for about one week until a clear solution was obtained. An aqueous solution of $HAuCl_4$ (100 μL, 20 mM) was mixed with 3 mL of the chitosan solution and the mixture was heated at 55 °C on a water bath with magnetic stirring until a red solution was obtained.

3.3. Preparation of silver nanoparticles using heparin

An aqueous solution of $AgNO_3$ (1 mL, of variable concentration as described in Section 2) was added to a 2 mL aliquot of an aqueous solution of heparin (of variable concentration as described above) with magnetic

stirring in a 70 °C water bath. The reaction time depended upon the concentration of heparin and $AgNO_3$, although 8 h reaction time is sufficient.

Acknowledgements

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