

Another possibility of carboxymethyl chitosan: a ‘sunshine’ reductant for the gold nanoparticles with high stability

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In this research, carboxymethyl chitosan and solar light were used to prepare gold nanoparticles. After being irradiated by solar light, a characteristic peak of gold nanoparticle can be found in UV-visible absorption spectrum of the aqueous solution of carboxymethyl chitosan and HAuCl₄. Most particles prepared by this method were around 20 nm in size. It was proved that the resultants were polycrystalline nanoparticles rather than amorphous nanocomposites by X-ray diffraction analyse and selected area electron diffraction experiment. The gold nanoparticles prepared by this method were stable even in high salinity solution. It was conjectured that the carboxyl group of carboxymethyl chitosan may be the reason of reducibility.

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1. Introduction

Metallic nanoparticles have attracted great attention because of their size-dependent physical and chemical properties [1]. The conventional method for preparing metallic nanoparticles, which is based on the chemical reduction, is simple and effective, but removal of the agents used is both cost- and time-intensive; furthermore, the residual agents show biological toxicity [2]. Thus, there is an increased interest in using dispersing or reducing agents based on biomaterial systems [3]. Over the last decade, Polysaccharide based biopolymers such as, alginate [4], starch [5, 6], and chitosan [7, 8] have been used as “green” alternatives [9] to conventional agents due to their low cost, nontoxicity and environment-friendly processing.

In most cases, the chemical dispersants for controlling growth of metallic nanoparticles was replaced by these biopolymers and toxic reductants were still used. Some researchers had reported that some polysaccharides or their derivatives can be used both as dispersant and reductant [10, 11], but the reducing reaction must be initiated by γ -rays [11], UV-rays [12], or heat [13]. A “greener” and handier method for preparing silver nanoparticles had been created by our group [14, 15]. Following this method, silver nanoparticles can be prepared by using silver chloride and carboxymethyl chitosan (CMCS) under solar irradiation. This method

was called as “solar reduction in CMCS solution” by us.

CMCS is one of important derivatives of chitosan and shows good solubility in water over a wide range of pH. It had been shown to be non-toxic both in vitro and in vivo [16]. Because of the biocompatibility of this biopolymer, it had been used widely in medicine.

Recently, we found that gold nanoparticles can be produced from Au³⁺ using only CMCS and solar light. That is to say, “solar reduction in CMCS solution” method can also be used for the preparation of gold nanoparticles. To the best of our knowledge, this is the first time that gold nanoparticle have been synthesized using this method. The gold nanoparticles prepared by this method were prevented from aggregating even in high salinity condition. The carboxyl group of CMCS was due to the reason of reducibility. These nanoparticles were examined by UV-visible (UV-vis) spectroscopy, transmission electron microscopy (TEM), and X-ray diffractometer (XRD). All these works were presented in this paper.

2. Materials and methods

2.1. Materials

All compounds were used as received. Pharmaceutical grade carboxymethyl chitosan (95.1%

substituted ratio) was obtained from Honghai Biotechnology Co. Ltd. (Qingdao, China). Gold (III) chloride hydrate (47.8% Au) was obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). Trisodium citrate was obtained from Kelong Chemical Reagent Factory (Chengdu, China). Ultrapure water was obtained from a Millipore Milli-Q Plus filtration system.

2.2. Preparation of gold nanoparticles solution

A stock aqueous solution of CMCS (0.1%, w/v) was prepared and stirred overnight. 0.5 mL HAuCl_4 stock solution (1%, w/v) was drop-wise added into 50 mL CMCS stock solution under magnetic stirring. The reaction solutions were then irradiated by sunlight for 7 hours at room temperature. The mixtures were stirred with a magnetic stir bar during the irradiation. Control experiments in the absence of solar light (Control A) and CMCS (Control B) were carried out under the same conditions.

2.3. Characterization

Before some characterizations, the nanoparticles were purified from the reaction solution by centrifugation. The precipitates were rinsed three times and re-suspended in ultrapure water.

The reaction solution was examined by a UV-3600 UV-vis spectrophotometer (Shimadzu, Japan). TEM and selected area electron diffraction (SAED) experiments were conducted on a LIBRA 200 TEM (ZEISS, Germany) at an accelerating voltage of 200 kV. The TEM samples were prepared by slowly evaporating a drop of the nanoparticle solutions on a copper grid covered by a carbon-supported film at room temperature. XRD experiments were performed on a D/MAX-2500PC X-ray diffractometer (Rigaku, Japan) using $\text{Cu K}\alpha$ radiation. The tube voltage and current were 40 kV and 150 mA, respectively. The purified nanoparticle suspensions were coated on glass plates and dried. The obtained films were used for the XRD measurements.

2.4. Preparation of regular gold nanoparticles

Regular gold nanoparticles were prepared by a method modified from the procedure described in Storhoff's paper [17]: 100 ml aqueous solution of HAuCl_4 (0.01%, w/v) was brought to a reflux while stirring, and then 10 ml trisodium citrate solution (0.3%, w/v) was added quickly, which resulted in a change in solution color from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min and allowed to cool to room temperature.

3. Results and discussion

3.1. UV-vis analysis

After being irradiated by solar light for 7 hours, the solution of CMCS and HAuCl_4 turned to purple from pale yellow (Fig. 1, inset). A characteristic peak of gold nanoparticle at 545 nm can be found in UV-vis absorption spectra of the reaction solution (Fig. 1, black line). Control experiments carried out in the absence of solar irradiation or CMCS (C.A. and C.B.) do not show any extinction peak in the UV-vis spectra (Fig. 1, blue and red lines), suggesting that solar irradiation and CMCS was essential to the preparation of gold nanoparticles using this method.

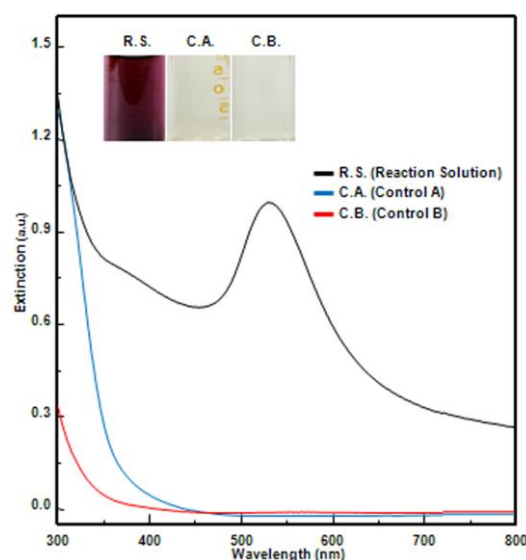


Fig. 1. UV-vis spectra of the reaction mixture. The reaction was carried out using CMCS (0.1%, w/v) and HAuCl_4 (0.01%, w/v) in the presence of sunlight for 7 hours. In first control experiment, the aqueous solution of CMCS (0.1%, w/v) and HAuCl_4 (0.01%, w/v) was kept in the dark for 7 hours (C.A.). In second control experiment, an aqueous solution containing only HAuCl_4 (0.01%, w/v) was irradiated by solar light for 7 hours (C.B.). The inset shows the photograph of the solutions

3.2. XRD analysis

The XRD patterns of the nanoparticles purified by centrifugation (Fig. 2) showed five peaks ($2\theta = 38.2^\circ$, 44.6° , 64.9° , 77.8° , and 82.1°) corresponding to the diffraction lines of Au^0 (111, 200, 220, 311 and 222).

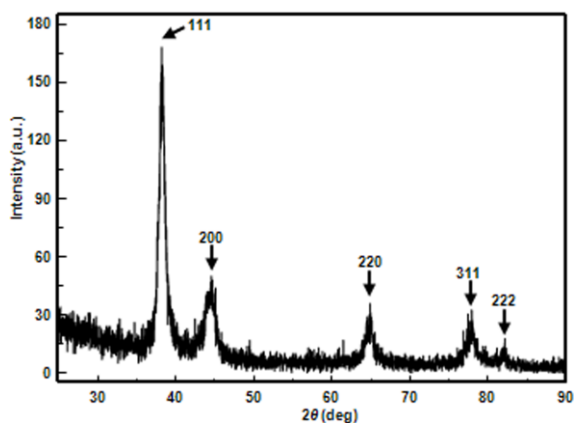


Fig. 2. XRD spectra of the gold nanoparticles purified from the reaction mixture containing CMCS (0.1%, w/v) and HAuCl_4 (0.01%, w/v) after irradiating with sunlight for 7 hours

3.3. TEM and SAED experiments

The morphology of the nanoparticles could be easily observed by TEM. Most particles were around 20 nm in size (Fig. 3, a, and b). Comparing the TEM images of unpurified gold nanoparticles in reaction solution containing CMCS (Fig. 3, a) and purified gold nanoparticles in ultrapure water without CMCS (Fig. 3, b), it can be found that CMCS was necessary to preventing nanoparticles from aggregating. The five rings observed in the electron diffraction pattern (Fig. 3, c) correspond to the five crystallographic planes of the gold nanoparticles, thus proving the existence of polycrystalline metallic nanoparticles rather than amorphous nanocomposites.

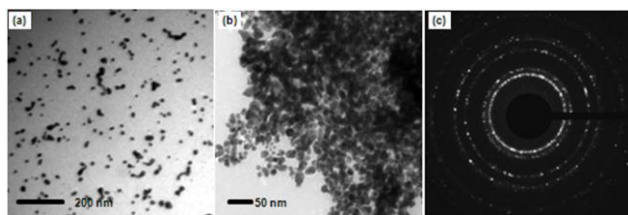


Fig. 3. (a) TEM images of the particles in the unpurified reaction mixture containing CMCS (0.1%, w/v) and HAuCl_4 (0.01%, w/v) after 7 hours of solar irradiation; (b) and (c) TEM images and electron diffraction pattern of the particles purified from the reaction mixture containing CMCS (0.1%, w/v) and HAuCl_4 (0.01%, w/v) after 7 hours of solar irradiation

3.4. Stability experiment of gold nanoparticles

In this work, the stabilities of the gold nanoparticles prepared by this method and regular gold nanoparticles

were compared in NaCl aqueous solution (100 mM) which is similar to physiological environment. After standing for 16 hours, the mixture of the gold nanoparticles prepared by this method and NaCl was just darker slightly (Fig. 4, b), although regular gold nanoparticles almost were precipitated completely in same condition (Fig. 4, d). In many cases, the gold nanoparticles must be used in physiological environment, such as molecular beacon or probe of gold nanomaterials. So, the stable property of gold nanoparticles in high salinity solution is very important.

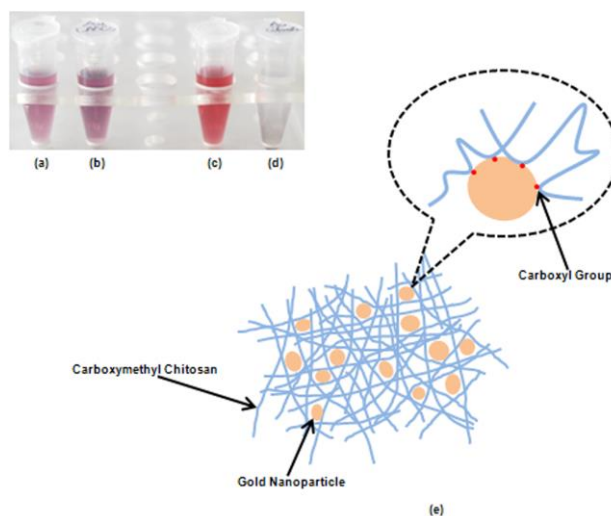


Fig. 4. (a) Photograph of 1ml the reaction solution containing CMCS (0.1%, w/v) and HAuCl_4 (0.01%, w/v) after 7 hours of solar irradiation; (b) Photograph of the mixture of 900 μl solution (a) and 100 μl NaCl aqueous solution (1M); (c) Photograph of 1 ml the gold nanoparticle solution prepared by the method in Storhoff's paper (the detailed procedure can be found in "Experimental"); (d) Photograph of the mixture of 900 μl solution (c) and 100 μl NaCl aqueous solution (1M). (e) Schematic diagram showing gold nanoparticles stabilized in CMCS matrix. All solutions stood for 16 hours

It had been found that polyelectrolytes can be used for dispersing metallic nanoparticles by the chelation effect between these polymers and metal ions [18]. CMCS shows higher chelating ability because of carboxyl groups [19]. In other words, the stability of gold nanoparticle prepared by using the method described in this paper may be attributed to the carboxyl groups of CMCS (Fig. 4, e).

3.5. Discussion

Obviously, compared to other methods, solar photolysis is more suited for use in the industry as it does

not involve the use of any complex apparatus and low energy consumption. Just in one pot, by one step, gold nanoparticles were assembled. To the best of our knowledge, this is the first time that gold nanoparticles have been synthesized using so handy method. The gold nanoparticles prepared by this method is "high-stable" even in high salinity solution. All above characters just meet the needs of realistic application for gold nanoparticles.

Photoreduction of Au^{3+} ion induced by solar light in CMCS solution is a promising method, but the reaction mechanism is not well known. It has long been known that irradiation of silver salts of low molecular weight carboxylic acids with UV light forms metallic silver [20]. In the research of Miyama and coworkers [12], irradiation of the silver salt of carboxymethylcellulose film with UV light ($\lambda = 253.7$ nm) caused photoreduction of Ag^+ ions, yielding silver nanoparticles. Thus, we conjectured, the carboxyl group of CMCS may be the reason of reducibility.

4. Conclusions

In summary, the discovery that the photoreduction of Au^{3+} ion can be induced by solar light in CMCS solution was presented in this paper. It was attempted to prepare gold nanoparticles using this method. The formations of nanoparticles were confirmed by UV-vis absorption and TEM experiments. XRD analyse and SAED experiment proved that the resultants were polycrystalline nanoparticles rather than amorphous nanocomposites. The mechanism of this method was also discussed, and the carboxyl group of CMCS was conjectured to be the reason of reducibility. The gold nanoparticles prepared by this method were stable even in high salinity solution. We envisage that this method will find widespread use in many realistic applications.

Acknowledgments

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