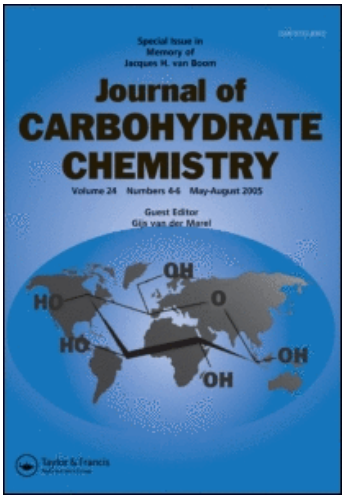

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Guo, Yanli and Yan, Hongtao(2008) 'Preparation and Characterization of Heparin-Stabilized Gold Nanoparticles', Journal of Carbohydrate Chemistry, 27: 5, 309 — 319
To link to this Article: DOI: 10.1080/07328300802158752
URL: http://dx.doi.org/10.1080/07328300802158752

Journal of Carbohydrate Chemistry, 27:309–319, 2008

ISSN: 0732-8303 print 1532-2327 online
DOI: 10.1080/07328300802158752

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Preparation and Characterization of Heparin-Stabilized Gold Nanoparticles

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A simple method for preparing gold nanoparticles in aqueous solution has been developed by using glycosaminoglycan-heparin as reducing and stabilizing agent and $HAuCl_4$ as precursor. The obtained gold nanoparticles were characterized by UV-vis spectroscopy, resonance light scattering spectroscopy (RLS), transmission electron microscopy (TEM) and electrophoresis technology. The influence of reactant concentration for the preparation of gold nanoparticles was investigated. The results indicated that the gold nanoparticles carried negative charges in the aqueous solution and the size and shape of the gold nanoparticles could be controlled by changing the concentration of the heparin. Moreover, the gold nanoparticles obtained with relatively high concentration of heparin were very stable and had relative narrow size distribution.

Keywords Gold nanoparticles, Preparation, Glycosaminoglycan, Heparin

INTRODUCTION

Gold nanoparticles have shown promising applications in the areas of optics, electronics, catalysis, and chemical/biochemical sensors because of their attractive optical and electronic properties related to the quantum size effect.[1–5] Therefore, the synthesis of gold nanoparticles with different chemical composition, size distribution, and controlled monodispersity has attracted considerable attention from a fundamental and practical point of view. To date, many methods have been developed to prepare gold

Received June 28, 2007; accepted April 24, 2008.
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nanoparticles.[6,7] Chemical reduction of gold slats is still a general route, where reducing agents, such as citrate acid, borohydride, formaldehyde, polyaniline, or other organic compounds, and a stabilizing agent, such as thiol-functionalized organic, surfactant, or polymer, were used. However, these reducing and stabilizing agents may have associated environmental toxicity or biological hazards. Recently, there has been an increased interest on "green" chemical processes. The green chemical processes are characterized by the total elimination or the minimization of generated waste. Utilization of nontoxic chemicals, environmentally benign solvents, and renewable materials are some of the key issues that merit important consideration in a green synthetic strategy.[8] As reducing or stabilizing agents, saccharides have been used for the preparation of metal nanoparticles because of their favorable biocompatibility and environmentally friendly characteristic. Liu et al.[9] have developed a straightforward strategy to create aqueous gold nanoparticle dispersions using small amounts of β-D-glucose as a "green" alternative to the conventional phase-transfer catalyst approach. Ag@C nanospheres were synthesized from aqueous glucose solution through coupled reduction of $AgNO_3$ and catalyzed carbonization.[10] A method for synthesizing Au nanoparticles using sucrose as the raw material based on gold seeding formation in the solid phase and seeding growth in aqueous solution has been reported.[11] Panacek et al.[12] have prepared size-controlled silver colloid nanoparticles using monosaccharides and disaccharides. Huang et al.[13] have reported the synthesis of chitosan-stabilized gold nanoparticles in the absence/presence of tripolyphosphate.

Heparin is a linear sulfated polysaccharide belonging to the family of glycosaminoglycans. It is widely distributed in a variety of animal tissues and is often used as a medical anticoagulant. We developed a straightforward method to create aqueous gold nanoparticle dispersions using heparin as both reducing and stabilizing agents. To the best of our knowledge, this is the first time a heparin has been used in the preparation of gold nanoparticles. The properties of gold nanoparticles obtained were characterized by UV-vis spectroscopy, resonance light scattering spectroscopy (RLS), transmission electron microscopy (TEM), and electrophoresis technology. The influence of reactant concentrations for the preparation of gold nanoparticles was investigated. In addition, the growth of gold nanoparticles was monitored by UV-vis spectroscopy.

EXPERIMENTAL

Reagents

Chloroauric acid ($HAuCl_4$) and heparin (sodium salt, 140 IU mg^{-1}) were all obtained from Sinopharm Chemical Reagent Ltd Company (China) and used as received without further purification.

Preparation of Gold Nanoparticles

Into refluxing water in a 100-mL round-bottom was added chloroauric acid ($HAuCl_4$, 0.01%, 25 mL) and the solution was heated to boiling. Then, a solution of heparin (1%) was injected under stirring and the mixture was heated for about 3 h. The total volume of reaction was 50 mL.

All glassware used was cleaned in a bath of freshly prepared aqua regia solution (HCl:HNO_3 3:1) and then rinsed thoroughly with H_2O prior to use.

Characterization of Gold Nanoparticles

Samples for TEM analysis were prepared by placing drops of the gold nanoparticle solutions on carbon-coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min, following which the extra solution was removed using a blotting paper and the grid allowed to dry prior to measurement. TEM measurements were performed using a JEOL, JEM-3010 high-resolution transmission electron microscope at an accelerating voltage of 300 kV.

Particle sizes were determined from an arbitrarily chosen area of enlarged photographs. Histograms of the particle size distribution and the average diameter were obtained from the measurements of about 100 particles.

UV-vis spectra were measured at 20°C with a Shimadzu dual-beam spectrophotometer (Model UV-1700) equipped with a 10-mm quartz cell.

The resonance light-scattering spectra (RLS) were obtained by synchronously scanning the excitation and emission monochromators (namely, $\Delta\lambda = 0.0$ nm) of 970 CRT fluorescence spectrophotometer (SPSIC, China) in the wavelength region from 300 to 800 nm.

RESULTS AND DISCUSSION

Absorption Spectra of Gold Nanoparticles

Nine samples with corresponding concentrations 0.01, 0.025, 0.1, 0.3, 0.5, 1, 2, 3, and 4 mg/mL of heparin were prepared to study the influence of heparin concentration on the gold nanoparticles. When the concentration of heparin increased from 0.025 mg/mL to 4 mg/mL, the color of the samples gradually changed from purple to red, giving evidence for the formation of dispersed gold nanoparticles.[14] However, bulk gray metal deposits that precipitated from solution due to the coagulation of as-prepared particles were observed when heparin concentration was 0.01 mg/mL.

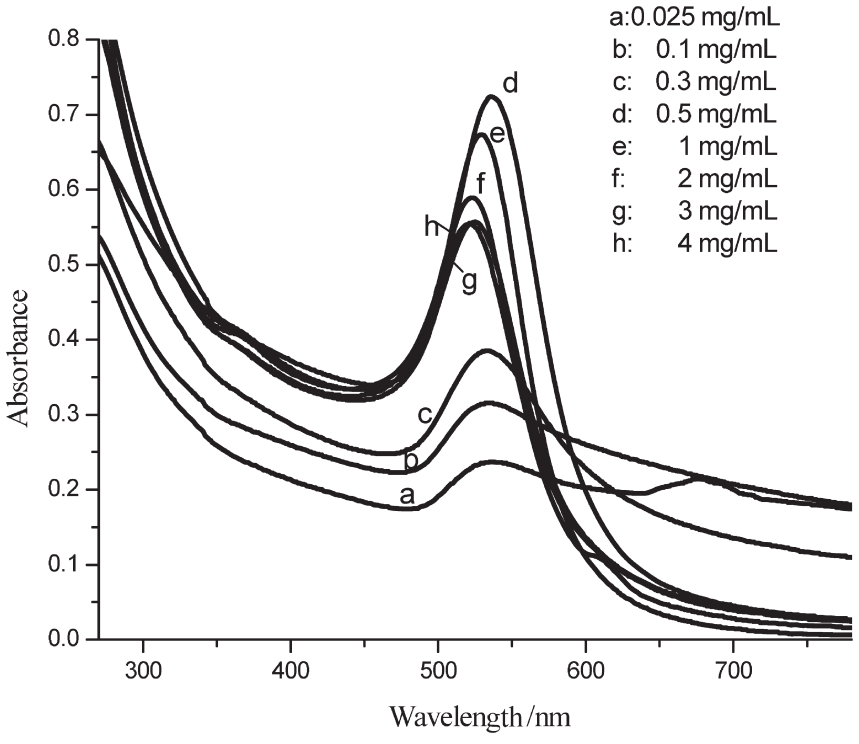

Figure 1: UV-vis absorption spectra of gold nanoparticles prepared with different concentration of heparin.

In Figure 1, the UV-vis absorption spectra is shown for gold nanoparticles prepared with different concentrations of heparin. The λ_{max} values have been observed in the range 520 to 540 nm, which are the typical plasmon resonance band for gold nanoparticles.[14] When the concentration of heparin was 0.025 mg/mL, another absorbance around 600 nm was also observed, which may be due to the congregation or deviation from spherical geometry of gold nanoparticles.[15,16] As the concentration of heparin increased from 0.1 to 0.5 mg/mL, the plasmon absorption band gradually became narrow and symmetric and the intensity of the absorption band markedly increased, whereas the λ_{max} values were unchanged and located at 535 nm. When the concentration of heparin was higher than 0.5 mg/mL, the intensity of the absorption band decreased, and the plasmon absorption blue shifted to 524 nm. The position and shape of the plasmon absorption band are closely related to the size, shape, and dispersion appearance of the gold nanoparticles.[17] Therefore, the changes of the plasmon absorption of gold nanoparticles indicated that the size of the obtained gold nanoparticles was altered with the concentration of heparin, which acted as a controller of nucleation as well as a stabilizer. This has been authenticated from TEM measurements (Fig. 3).

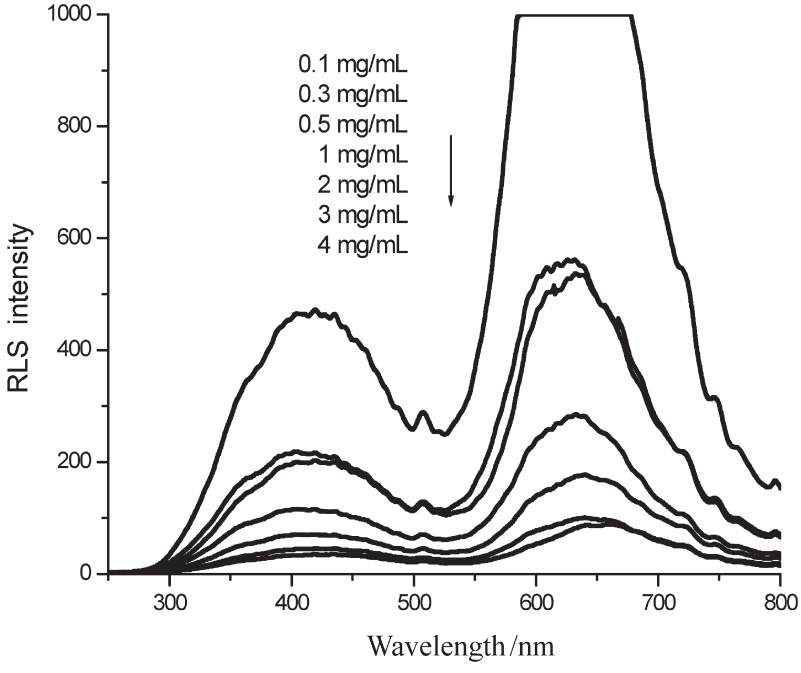

Figure 2: Resonance light-scattering spectra of gold nanoparticles with different concentration of heparin.

Resonance Light-Scattering Spectra of Gold Nanoparticles

Figure 2 shows the resonance light-scattering spectra of gold nanoparticles prepared with different concentrations of heparin. It can be seen that there is a strong maximum resonance light-scattering peak at 630 nm and a smaller RLS peak at 435 nm, and the peak intensity of resonance light-scattering spectra decreases with the concentration of heparin from 0.1 to 4 mg/mL. According to Rayleigh theory, the increase of the volume of the particles would lead to the enhancement of the scattering intensity; therefore, it indicated that the size of the resulting gold nanoparticles decreased when the heparin concentrations increased.

TEM Measurements of Gold Nanoparticles

The gold nanoparticles obtained with various heparin concentrations were examined by TEM. Some of the TEM pictures and the particle size distribution histograms are collected in Figure 3. TEM observation indicated that the size of product nanoparticles could be altered by changing the concentration of

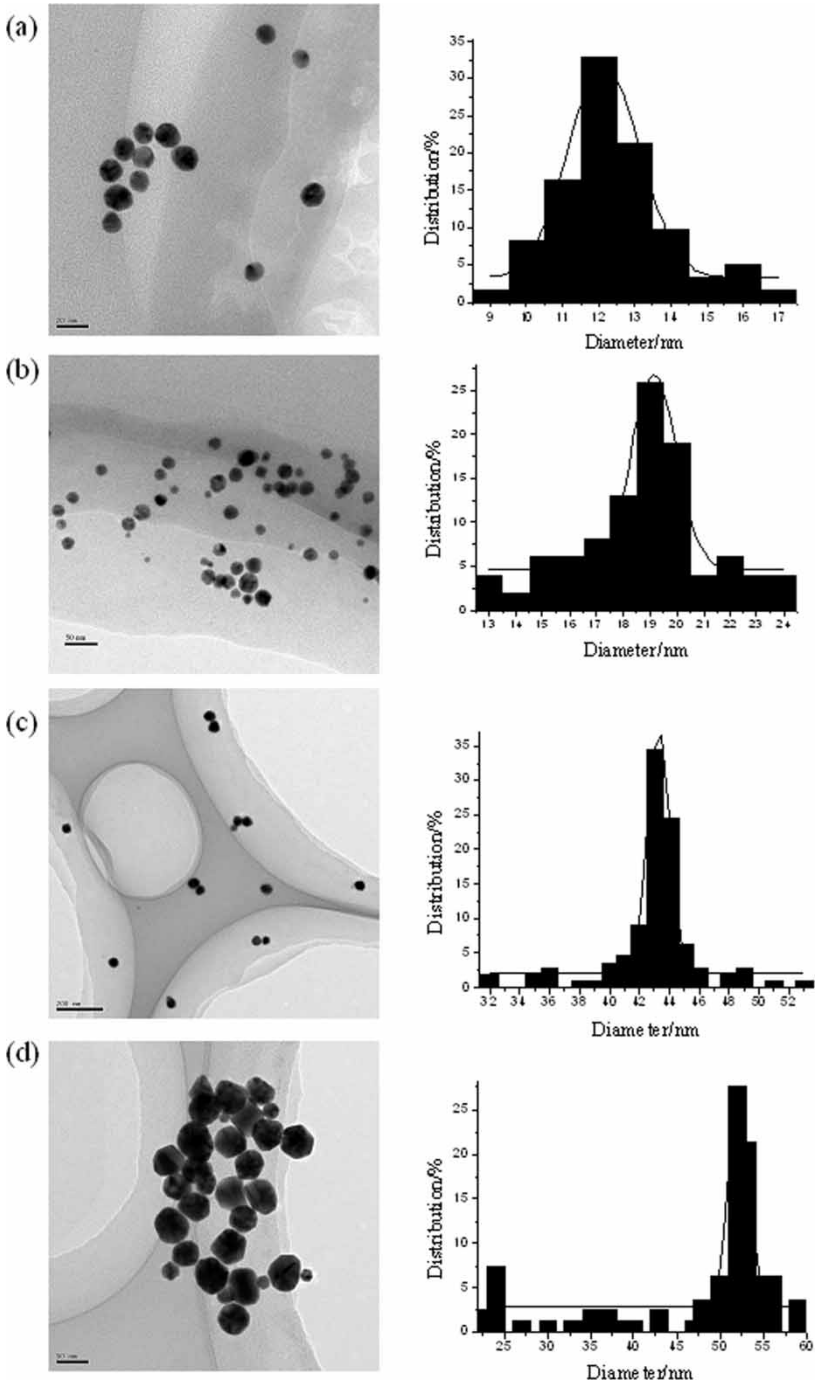

Figure 3: Transmission electron micrographs and size distributions of gold nanoparticles with different concentration of heparin (heparin) = (a) 3 mg/mL; (b) 2 mg/mL; (c) 0.5 mg/mL; (d) 0.3 mg/mL.

heparin while keeping the gold concentration fixed. Taking the concentration of heparin as 0.3, 0.5, 2, and 3 mg/mL, the average sizes of gold nanoparticles obtained were 52, 43, 19, and 12 nm, respectively. It is apparent that the average particle size of gold nanoparticles prepared with higher concentration of heparin was much smaller. The particles were monodisperse and had a spherical form; moreover, a narrow size distribution was observed for their photograph. This might be due to a protective action by heparin: a higher concentration of heparin acted as an effective capping agent to prevent the growth of gold particles by adsorbing on the surface of the already produced gold nanoparticles.[18] With decreasing concentration of heparin (3 mg/mL), the size of particles increased and it showed some signs of aggregation of gold nanoparticles. In addition, polygonal particles were also formed besides spherical particles, and the particle size distribution was wider (as shown in Fig. 3d). This clearly tells that the particle growth becomes facile in the presence of dilute heparin solutions when heparin molecules adsorbing on the surface of gold nanoparticles are very few, but the growth of gold nanoparticles has a natural tendency to be haphazard, and thus the larger polygonal particles were obtained.[19]

Charges of Gold Nanoparticles

To determine the surface charges of gold nanoparticles, an electrophoresis experiment was carried on. A glass U tube was filled with the gold nanoparticle colloidal solution, and two Pt electrodes were inserted into the solution at both ends of the tube. A DC voltage of 110 V was applied between the electrodes to start the electrophoresis. After 5 min, the color of solution became deep on the side of the anode, whereas the color became thin on the side of the cathode. Such findings indubitably revealed that gold nanoparticles prepared by heparin are negatively charged.

Stability of Gold Nanoparticles

Gold nanoparticles prepared with relatively high concentration of heparin ($\geq$0.3 mg/mL) had higher stability. The solutions of gold nanoparticles showed no signs of aggregation and the intensity and position of plasmon absorption peak were unchanged even after 2 months of storage in the dark at 4°C. However, under the same preserving condition, gold nanoparticles prepared with relatively lower concentration of heparin, such as 0.025 and 0.1 mg/mL, precipitated after 2 weeks. This further indicated that a higher concentration of heparin acted as an effective capping agent for the preparation of gold nanoparticles, which made the gold nanoparticles separate mutually, and thus kept the gold nanoparticles from aggregating.

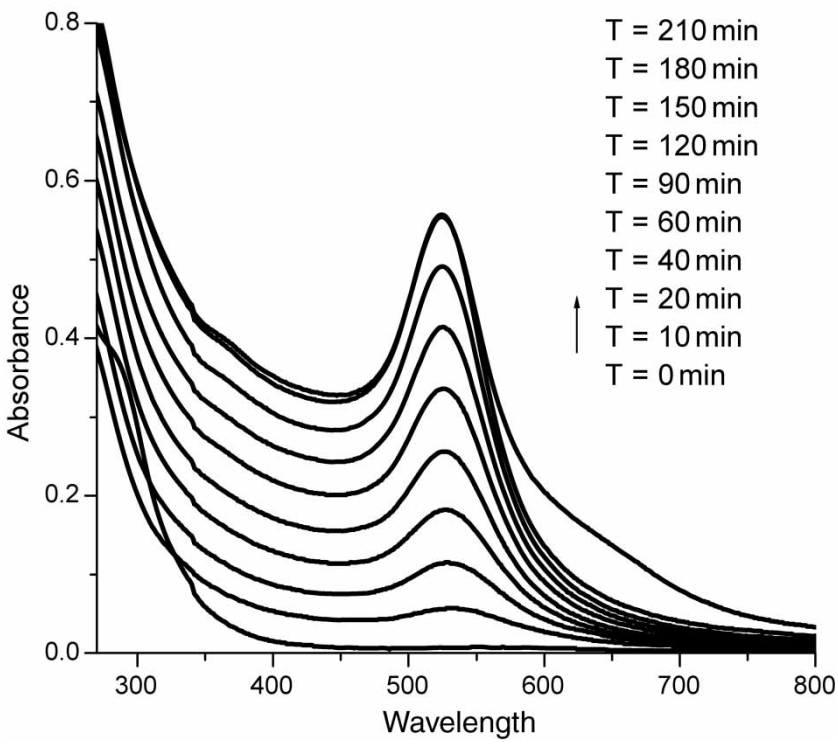

Figure 4: Time-dependent UV-vis absorption spectra of solution containing 0.005% $HAuCl_4$ and 2 mg/mL heparin.

Real-time UV-vis Absorption Spectra of Gold Nanoparticles

To get further insight into the formation of gold nanoparticles by heparin, we also recorded UV-vis spectra at different times of reaction of a 0.005% solution of chloroauric acid with 2 mg/mL heparin (Fig. 4). When the reaction started (0 min), it was observed that there was an absorbance band around 285 nm, which is assigned to metal to ligand charge-transfer transition (MLCT).[20] With the process of the reaction (10 min), the absorption band around 285 nm disappeared and another absorption band around 530 nm appeared, corresponding to the surface plasmon absorption of gold nanoparticles. After 20 min, a slight blue-shift of the surface plasmon absorption was observed and the absorption peak was at 524 nm. Subsequently, with the elapsed time, the peak position almost fixed at 524 nm, and the surface plasmon absorption grew in intensity, narrowed, and was symmetric gradually. The absorption intensity at 524 nm no longer increased after 3 h, whereas the absorption band intensity in the range of 580 to 800 nm

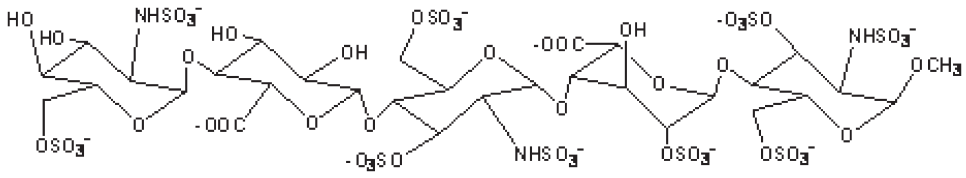

Figure 5: Structure of heparin.

increased. This change of UV-vis spectra indicated that the large aggregation of gold nanoparticles may be formed at the beginning of reaction. Then, owing to the repulsion among particles, the unsteady aggregation decomposed, recombined, and finally formed inerratic spherical gold nanoparticles with the eventual narrowing of the size distribution as the reaction reached an equilibrium. In addition, the longer heating time led to some aggregation of gold nanoparticles in the aqueous medium, which caused the increase the absorption band intensity of in the range of 580 to 800 nm.[15]

The results above indicated that heparin plays a role as a reducing and stabilizing agent for the preparation of the gold nanoparticles, but the exact mechanism is not clear. Heparin consists of repeating disaccharide units of uronic/glucuronic acid and glucosamine residues, and had plenty of ether oxygen and the hydroxyl group (Fig. 5), which may anchor metal ions tightly onto heparin via ion-dipole interactions, and act as passivation contacts for the stabilization of the nanoparticles by strong bonding interaction with their surface metal atom.[8,21]

CONCLUSION

We have proposed and demonstrated a facile green method for the preparation of gold nanoparticles in aqueous solution by using glycosaminoglycan-heparin as a reducing agent and stabilizing agent. The properties of gold nanoparticles were characterized by UV-vis spectroscopy, resonance light scattering spectroscopy (RLS), transmission electron microscopy (TEM), and electrophoresis technology. We also investigated the effects of heparin concentration for the preparation of gold nanoparticles. The results showed that the gold nanoparticles carried negative charges in the aqueous solution and the size and shape of the gold nanoparticles could be controlled by changing the concentration of the heparin. In addition, the gold nanoparticles obtained with relatively high concentration of heparin were very stable and had relative narrow size distribution. The present approach to prepare gold nanoparticles is simple, safe, and convenient and had environmentally friendly characteristics.

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