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Degradation behavior of chitosan chains in the ‘green’ synthesis of gold nanoparticles

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ABSTRACT

The degradation behavior of chitosan chains in the synthesis of Au nanoparticles by a ‘green’ method was investigated in this paper for the first time. UV–vis absorption spectra suggested the formation of Au nanoparticles and TEM images showed that their sizes were between 10 and 50 nm. During the process of synthesis, the intrinsic viscosity $[\eta]$ of chitosan was observed to decrease gradually, implying that the chitosan chains degraded under the reaction conditions. Further studies showed that the degree of degradation of the chitosan chains was changed with different reaction temperatures, reactant ratios, and the molecular weights of chitosan.

1. Introduction

Metal nanoparticles have wide-ranging applications in a variety of areas, including physics, chemistry, materials science, and biomedical science. However, most of the synthetic methods reported to date rely heavily on organic solvents such as *N*,*N*-dimethylformamide and toxic reducing agents like sodium borohydride, which inevitably result in serious environmental issues for industrial production. In order to minimize or eliminate pollution to the environment, ‘green’ methods, which are carried out according to the 12 fundamental principles of green chemistry,[1] are required for preparing metal nanoparticles. 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. A green method for the preparation of nanoparticles should be evaluated from three aspects: the solvent, the reducing agent, and the stabilizing agent. Raveendran et al. first developed the concept of completely ‘green’ synthesis of metal nanoparticles. They used water as solvent, β-D-glucose as the reducing agent and starch as the stabilizing agent to prepare Au, Ag, and Au–Ag alloy nanoparticles.[2,3] Afterward, they presented another facile synthetic procedure by using the nontoxic and renewable biochemical, β-D-glucose, together with simply adjusting the pH in the aqueous medium. The β-D-glucose acted both as reducing agent and capping agent for the synthesis and stabilization of Au nanocrystals in the system.[4] In the meantime, Vidneshwaran et al. prepared stable Ag nanoparticles by only using soluble starch as both the reducing and stabilizing agents.[5] Besides starch, chitosan is another natural polymer which is a transformed polysaccharide obtained by the deacetylation of chitin. Because of the biocompatibility, biodegradability, nontoxicity, and adsorption properties of chitosan, Yang and co-workers used it as a stabilizing agent to prepare Ag, Au, Pt, and Pd nanoparticles.[6–9] These chitosan-protected nanoparticles can be easily integrated into systems relevant for pharmaceutical, biomedical, and biosensor applications. Accordingly, such a system is worthy of further exploitation.

In the process for the synthesis of metal nanoparticles, the high surface energy of these particles makes them extremely reactive and most systems undergo aggregation without protection or passivation of their surfaces. Therefore, a proper stabilizing agent is indispensable to the synthesis of nanoparticles. It is well known that solutions of polymeric materials, such as polyhydroxydroxylated macromolecules, contain size-confined, nanosized pools of inter- and intramolecular origin, which can be used to protect and stabilize the nanoparticles. The nature and properties (i.e., viscosity) of the polymer solutions can influence the size and stability of the nanoparticles significantly. However, most researchers have paid attention to the size, shape, and size distribution of nanoparticles, ignoring what happened to the polymer, which plays a very important role in the whole synthetic process.

For the preparation of Au nanoparticles stabilized by chitosan, Yang and co-workers suggested the same mechanism of reduction of metal salts as for glucose since chitosan is a derivative of glucose (a reducing sugar).[8] It is known that glucose is in open-chain form when it is oxidized by a reducing agent (the –CHO group can be oxidized into –COOH). For chitosan, only the glucosamine units at the end of chitosan chains can exist in open-chain form. If

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merely these end groups participate in the redox reaction, the molecular weight of chitosan will not change so much, considering the minute ratio of the end groups to the whole long-chain structure. And visually, the viscosity of the chitosan solution will keep almost the same before and after the reaction. But during our preparation of chitosan-stabilized Au nanoparticles, we found that the viscosity of the chitosan solution decreased noticeably, which indicated that the mechanism of the reaction of chitosan with gold ions was not quite the same as that of glucose. The chitosan chain was broken during the reaction, which results in a decrease in molecular weight and viscosity for the chitosan. In this paper, we prepare Au nanoparticles using chitosan as the reducing/stabilizing agent and systematically study the changes of viscosity of chitosan solution under different conditions, that is, different reaction times, temperatures, reactant ratios, pH values, and molecular weights of chitosan.

2. Results and discussion

The UV–vis absorption spectra of $HAuCl_4$/CTS solution at different time intervals are shown in Figure 1. All spectra exhibit an absorption band around 520 nm, suggesting the formation of gold nanoparticles.[3,4,6] Also it could be seen that the intensity of the absorption increased with time, indicating a continued reduction of the metal ions. Figure 2 was obtained by plotting the maximum absorbance (A_{max}) against time. The curve in Figure 2 clearly reveals that the A_{max} increased rapidly during the initial period of the reactions and became slower after about 10 h, indicating that the speed of formation of the Au nanoparticles was fast at the initial stage of the reaction because of the high concentration of $HAuCl_4$, becoming slower with the decrease in the concentration of $HAuCl_4$.

A typical TEM image of Au nanoparticles formed is displayed in Figure 3. In general, the particles were isotropic (i.e., low aspect ratio) in shape. Figure 3 also presents a histogram of Au particle size distribution with the mean particle diameter $D_n = 27.58$ nm and standard deviation SD = 12.27 nm. Another phenomenon that was noticed was that the sizes of prepared Au nanoparticles were not all the same. This was probably due to the fact that parts of the smaller Au nanoparticles clustered to form bigger ones during the very long reaction time (~30 h). According to the reports, an ordinary preparation time for such noble metal nanoparticles is much shorter (10 min–2 h), so that nanoparticles with equal size were easily obtained. However, a long reaction time was necessary in this paper in order to ensure that the chitosan reacted with the $HAuCl_4$.

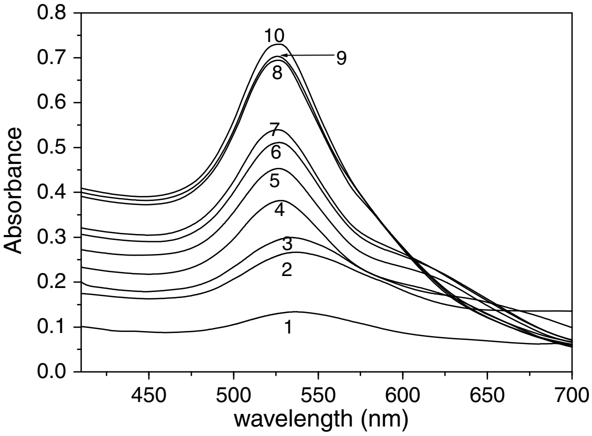


Figure 1. Time evolution of UV–vis absorbance spectra indicating the formation of gold nanoparticles. Conditions: 60 mL chitosan + 2 mL $HAuCl_4$, 55 °C. The time intervals are (1) 1.5 h; (2) 2.5 h; (3) 3.5 h; (4) 5 h; (5) 6.5 h; (6) 8.5 h; (7) 10.5 h; (8) 23.5 h; (9) 25.5 h; (10) 30.5 h.

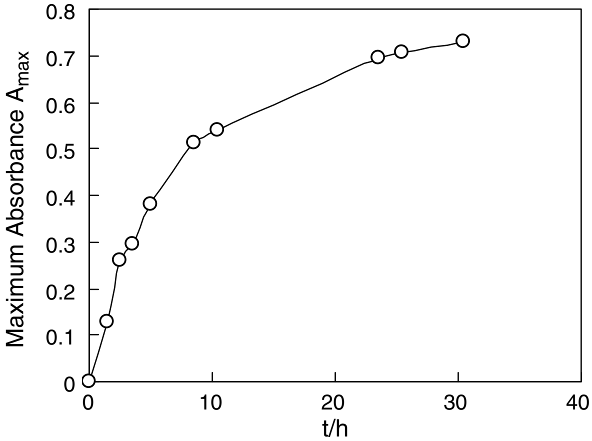


Figure 2. Plot of maximum absorbance (A_{max}) against time. Conditions: 60 mL chitosan + 2 mL $HAuCl_4$, 55 °C.

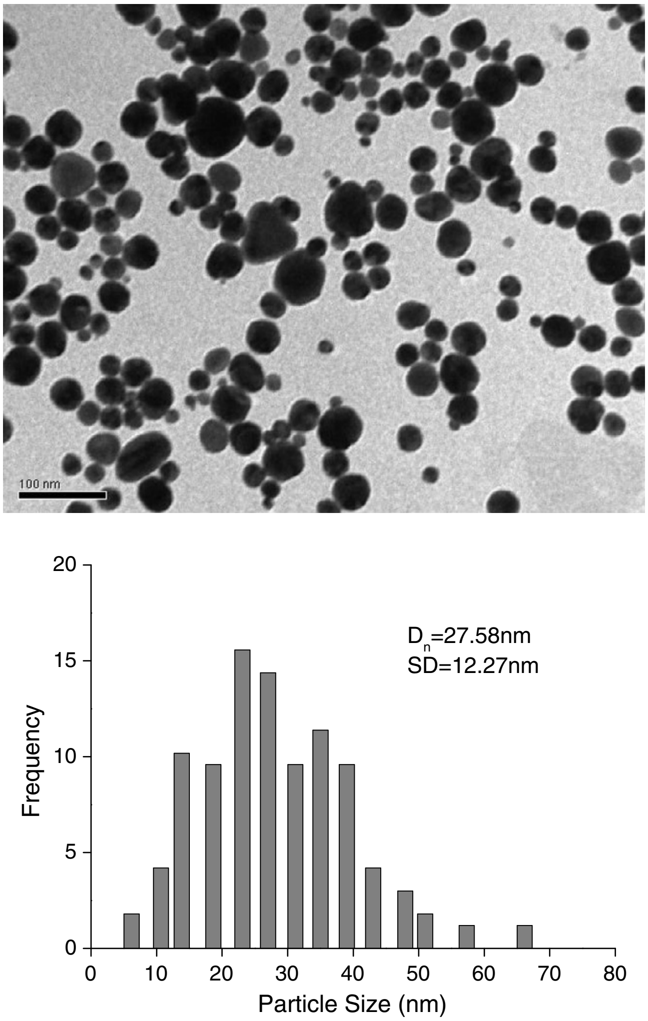


Figure 3. Representative TEM image of the Au nanoparticles (conditions: 60 mL chitosan + 4 mL $HAuCl_4$, 55 °C) together with a histogram for the size distribution of the Au nanoparticles.

It is well known that the viscosity of an acidic chitosan solution can decrease gradually during the period of storage because of the hydrolysis of chitosan itself.[10] So a blank experiment without the addition of $HAuCl_4$ was conducted for comparison. Simultaneously, another parallel experiment in the presence of nitrogen to eliminate oxygen was also carried out. All the results of [η] changes of chitosan during the reactions are presented in Figure 4. Obviously, the [η] value decreased dramatically during the initial period of the reactions of chitosan with $HAuCl_4$ in the absence or presence of nitrogen. About 10 h later, the decrease trend became gentle, implying that the reduction was almost finished. Since [η] can represent the value of the viscosity molecular weight of a polymer, it can be concluded that the chains of chitosan were broken in the reduction. Moreover, it can be seen from the 'CTS' curve in Figure 4 that the hydrolysis of chitosan itself made only a small contribution to the breaking of chitosan chains in the reaction. Furthermore, the oxygen effect on the reaction can be omitted since the two curves 'CTS + Au' and 'CTS + Au(nitrogen)' almost coincided with each other.

In order to explore the influence of the prepared Au nanoparticles on the viscosity of the chitosan, we designed another experiment as follows: excessive $NaBH_4$ was quickly added to the solution of chitosan/$HAuCl_4$ under stirring and the mixture was left stirring for 1 h at room temperature. Finally, the color of the solution turned red, indicating the formation of Au nanoparticles. Because of the much higher reducing power of $NaBH_4$ compared to that of chitosan, it was $NaBH_4$, not the chitosan, which reacted with $HAuCl_4$ at room temperature. In this experiment, chitosan only acted as a stabilizing agent, and the prepared Au nanoparticles were distributed among the polymer chains. The [η] values before and after the reaction were determined to be 1687 and 1690, respectively, implying that the prepared Au nanoparticles had little influence on the viscosity of the chitosan.

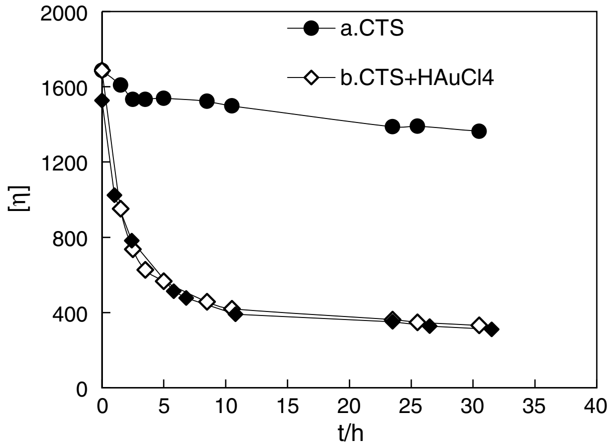
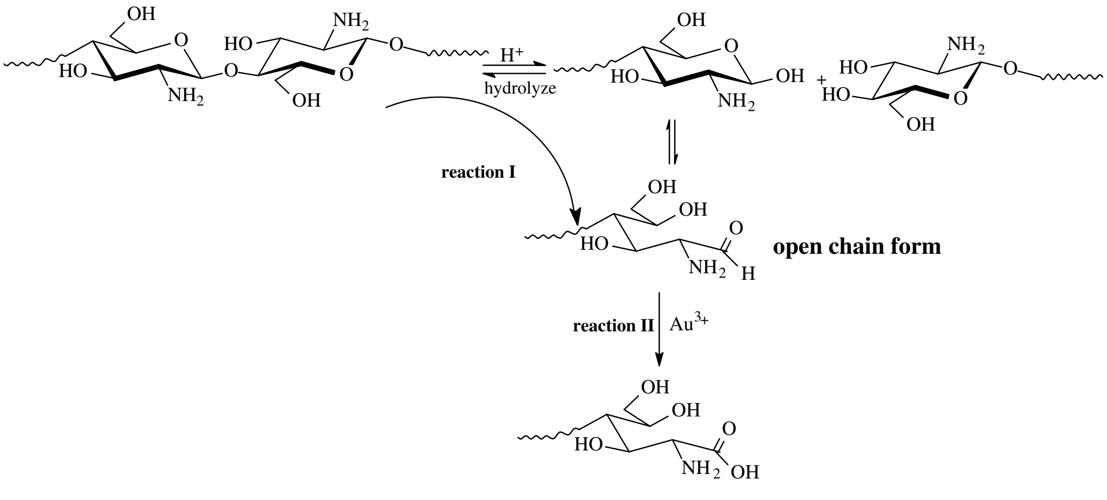


Figure 4. Changes of [η] in the reactions: (a) 60 mL chitosan, 55 °C; (b) 60 mL chitosan + 2 mL $HAuCl_4$, 55 °C; (c) 60 mL chitosan + 2 mL $HAuCl_4$, in nitrogen, 55 °C. The [η] was measured at 30 °C.

We have synthesized a chelating resin by anchoring glucosamine to a polystyrene matrix and studied its adsorption properties for Au^{3+}.[11] The SEM photos of the Au^{3+}-loading resin showed that there were a number of Au(0) particles on the surface. IR spectra of the resin after adsorption confirmed the presence of the characteristic peaks of carboxylic acid, C=O at 1720 cm^{-1} and O–H at 1400 cm^{-1}, implying that the glucosamine ring was opened and that the Au^{3+} ions oxidized the glucosamine to glucosamino acid. A similar conclusion was also obtained by Raveendran et al.[3,4] Since glucosamine is the monomeric unit of chitosan, the mechanism for the chain breaking of chitosan in this paper can be expressed by the reaction shown in Scheme 1. In acidic aqueous solution, the chitosan hydrolyzed (the chitosan chain was broken), and a part of the end groups existed in the open-chain form (see Reaction I). This process is reversible, and all the polymers are in dynamic equilibrium. Once Au^{3+} ions were added into the system, –CHO groups in the chain end would be oxidized to –COOH groups (see Reaction II). Since the oxidation reaction is irreversible, the dynamic equilibrium in Reaction I would be broken, and the reaction would proceed toward a positive reaction. Accordingly, the chitosan chain continually degraded and Au nanoparticles formed.

Figure 5 shows the FTIR spectra of chitosan oxidized by $HAuCl_4$. In order to be observed more clearly, the spectra between 1760 and 1580 cm^{-1} wavenumbers were treated by Fourier self-deconvolution program (see the small figure embedded in Fig. 5). It can be seen from the figure that two new peaks at 1740 and 1710 cm^{-1} appear, which correspond to both the stretching vibration of free –COOH groups and to those associated.[12] The appearance of the characteristic peak for the –COOH groups lends support that the mechanism of degradation of the chitosan chain shown in Scheme 1 is reasonable.

In order to verify the mechanism further, more reactions were conducted under different conditions. The results are shown in Table 1. Obviously, the [η] values all decreased under seven different kinds of conditions, implying that the chain breaking of chitosan was universal in the preparation of chitosan-stabilized Au nanoparticles. Another phenomenon that should be noted is



Scheme 1. Illustration of degradation of chitosan chain in the preparation of Au nanoparticles.

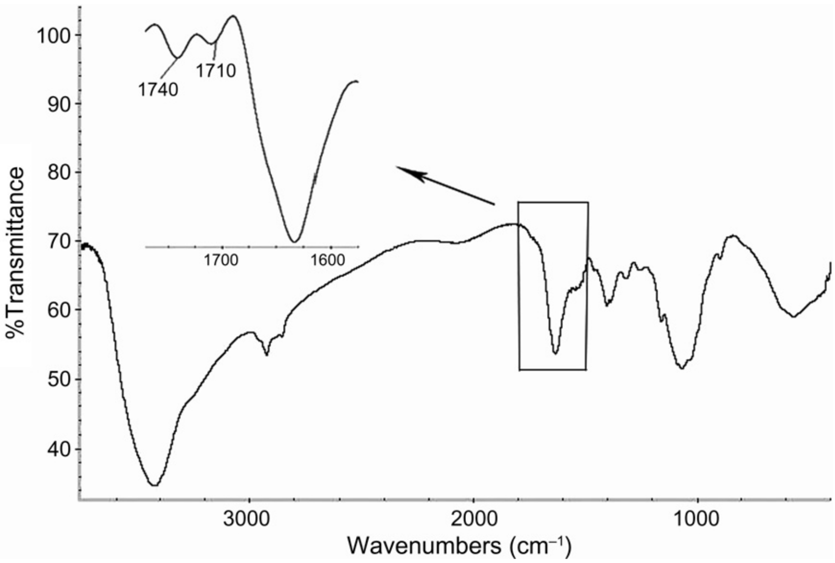


Figure 5. FTIR spectra of chitosan oxidized by $HAuCl_4$.

Table 1

Effect of reactant ratio, temperature, and chitosan molecular weight on $[\eta]$ (all the reaction times are 10 h)

Volume ratio of Au/CTS	$[\eta]_0$[a]	$[\eta]_F$[b]
1-3 (1) *Effect of reactant ratio, temperature is 55 °C*		
1:40	1687	375
1:30	1687	331
1:15	1687	219
Temperature/°C	$[\eta]_0$	$[\eta]_F$
(2) *Effect of temperature, the volume ratio of Au/CTS is 1:30*		
45	1687	378
55	1687	331
75	1687	231
Chitosan	$[\eta]_0$	$[\eta]_F$
(3) *Effect of chitosan molecular weight, temperature is 55 °C and the volume ratio of Au to CTS is 1:30*		
CTS-1	1687	331
CTS-2	2449	394
CTS-3	3129	419

[a] $[\eta]_0$ is the initial intrinsic viscosity of chitosan and $[\eta]_F$ is the intrinsic viscosity of chitosan after the reaction.

[b] $[\eta]_F$ and $[\eta]_0$ values were measured at 30 °C.

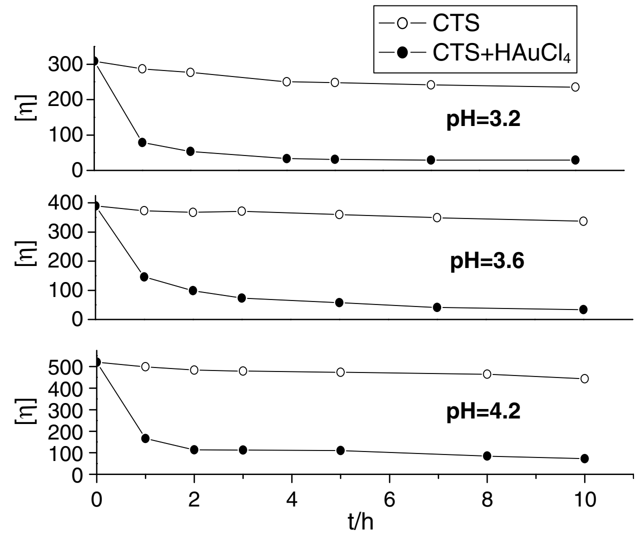


Figure 6. Changes of $[\eta]$ of chitosan and chitosan/$HAuCl_4$ solutions with different pH values at 55 °C.

that the final intrinsic viscosity $[\eta]_F$ varies with the conditions, that is, with the higher the temperature and greater the ratio of Au/CTS, the smaller $[\eta]_F$ value became. So it can be concluded that the chitosan chains degraded more with higher Au content and higher temperatures.

The influence of pH on chitosan hydrolyzation is well known. Figure 6 shows the changes of $[\eta]$ of chitosan and chitosan/$HAuCl_4$ solutions with different pH values. As can be seen from the figure, the change in trends of $[\eta]$ of chitosan and chitosan/$HAuCl_4$ at three different pH values is almost the same. The hydrolysis of chitosan itself at different pH values makes only a small contribution to the breaking of chitosan chains in the reactions. During the experiments, we also noticed that stable Au nanoparticles could be obtained in all the three systems with different pH values.

The polarized optical microscope images of chitosan and Au-chitosan nanocomposite film are presented in Figure 7. It can be seen that only small crystallites were formed in the film cast from the chitosan solution. But in the film cast from the Au/CTS nanocomposite, the morphology of crystalline chitosan is much different from that of those small crystallites; large branched-like structures appeared. Yang and co-workers found a similar phenomenon, and they speculated that gold nanoparticles may act as nuclei in the formation of those branched-like crystal structures.[7,13] In our opinion, the shortened chitosan chains may be another cause that results in the faster formation of branched-like crystal structures according to the theory of polymer crystallography, that is, for the same polymer, the shorter molecular chain leads to a faster crystalline growth velocity.[14]

3. Experimental

3.1. Materials and methods

$HAuCl_4$ was purchased from Aldrich Chemical Co. and used without further purification. Low, medium, and high molecular weight chitosans [poly-(1→4)-β-D-glcopyranosamine, CTS] were also purchased from Aldrich. Before being used, they were purified

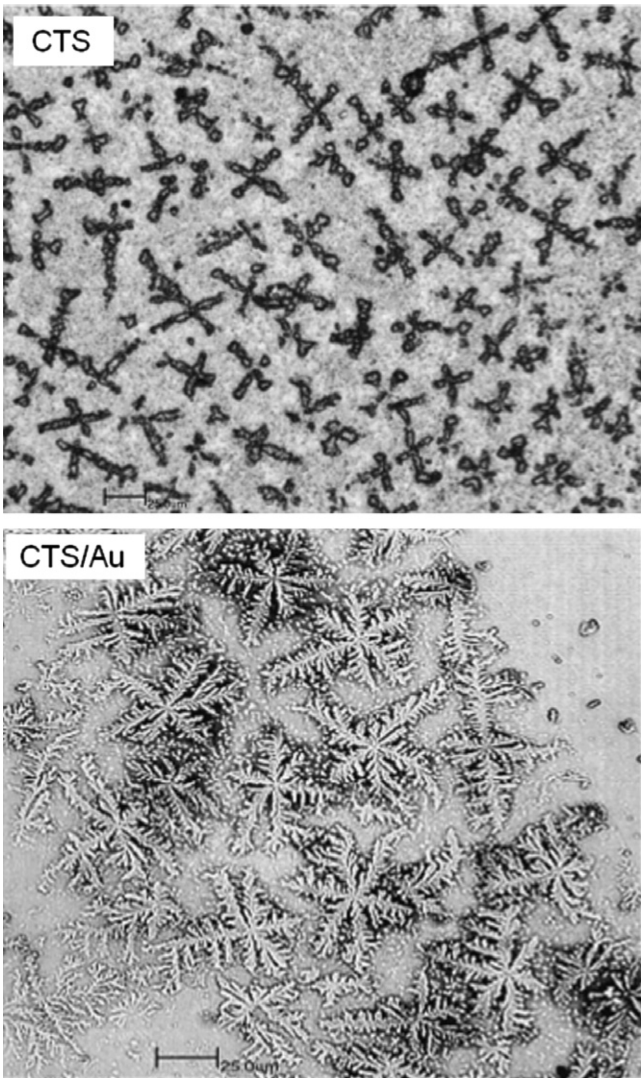


Figure 7. Representative POM images of crystal cast of chitosan and Au nanoparticles stabilized by chitosan.

by filtration. Acetic acid (A.R.) was diluted to 1% aqueous solution before use. The morphology of the Au nanoparticles was observed with a JEM1230 transmission electron microscope operating at 200 kV. UV–vis absorbance spectra of the gold nanoparticles collected using a Unico7200 UV–vis spectroscopy (Unico, Shanghai). Infrared spectra were recorded on a Nicolet MAGNAIR 550 (series II) spectrophotometer; test conditions: potassium bromide pellets, scanning 32 times, with a resolution of 4 cm^{-1}. The data were treated with Thermo Nicolet Corporation OMNIC32 software of version 6.0a. The morphology of the films cast from prepared nanocomposite was investigated by an Olympus, BX51 polarized optical microscope in conjunction with a Linkam hot stage.

3.2. Preparation of gold nanoparticles

The preparation of Au nanoparticles is quite straightforward. In a typical preparation, 2 mL of a 5 mM solution of $HAuCl_4$ was added to 60 mL of a 0.5 g/L solution of chitosan, which was prepared by dissolving the chitosan in 1% aq HOAc. The mixture was heated to 55 °C and was maintained at this temperature for 30 h. At predetermined time intervals, an appropriate amount of mixture was taken out, and the intrinsic viscosity $[\eta]$ of chitosan was measured by a Ubbelohde viscometer (ϕ = 0.6–0.7 mm). Finally, a red solution was obtained.

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