

**A Direct and Facile Approach to the Fabrication of Chitosan-Gold Hybrid  
Nanospheres**

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## 1. Size control of the low molecular weight chitosan (LWCS)-EDTA composite nanospheres.

Size control is of great importance for a colloidal system for biomedical related applications. In our case, we used a nonsolvent aided counter-ion complexation method, in which ethanol was added to facilitate the counter-ion condensation between LWCS and EDTA, to prepare LWCS-EDTA composite nanospheres. Since the counter-ion condensation between LWCS and EDTA is a process determined by the electrostatic interaction between two oppositely charged substances, the ratio of the positively charged LWCS to the negatively charged EDTA may play a key role in the formation of LWCS-EDTA composite nanospheres. Actually, we found that when the ratio of LWCS to EDTA in the initial aqueous solution was increased, the concentration of ethanol required to induce the counter-ion condensation between LWCS and EDTA also increased whereas the diameter of the resultant LWCS-EDTA composite nanospheres decreased (see Table S1).

**Table S1. Ethanol concentration required to induce the counter-ion condensation and the resultant particle size with different ratios of LWCS and EDTA.**

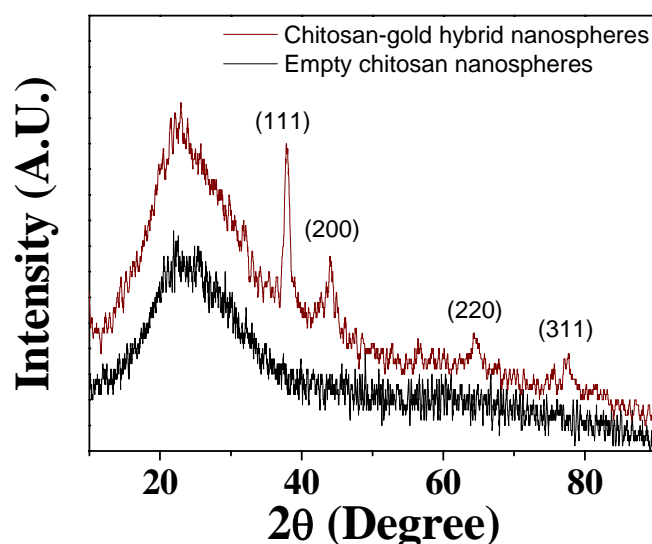
LWCS:EDTA (w/w)	Ethanol concentration % (v/v) <sup>a</sup>	Diameter (nm)
3:1	37	230
5:1	43	126
10:1	50	80

a. Ethanol concentration required to induce the counter-ion condensation between LWCS and EDTA.

EDTA also serves as the reactant in our system, so a low ratio of LWCS:EDTA is ideal in order to better demonstrate the effectiveness of our strategy. Unfortunately, EDTA alone is poorly soluble in pure water. Yet with the help of the basic chitosan, EDTA becomes soluble due to deprotonation. When LWCS:EDTA is less than 3:1 (w/w), there would be some fraction of EDTA that can not be dissolved because of insufficient deprotonation. Therefore, a LWCS:EDTA ratio of 3:1 was used in our investigation unless otherwise stated.

## 2. XRD analysis.

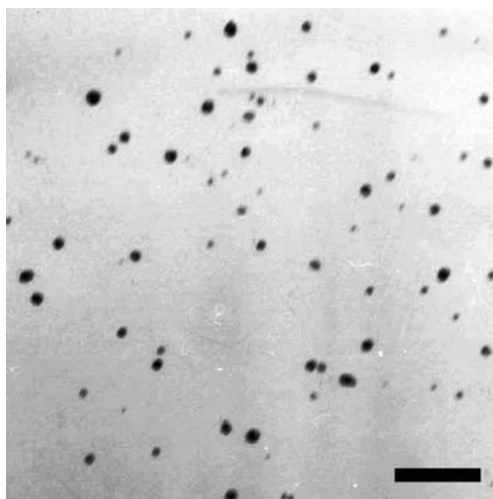
XRD analysis were performed on a Philips diffractometer using Cu- $\alpha$  radiation (40 KV, 30 mA). The XRD spectra shown in Figure S1 verified the existence of gold nanoparticles in the system based on the characteristic diffraction peaks of gold.



**Figure S1.** XRD spectra of chitosan-gold hybrid nanospheres and empty chitosan nanospheres.

### **3. TEM observation of the gold nanoparticles formed by in situ reduction of gold salt within the LWCS-EDTA composite nanospheres.**

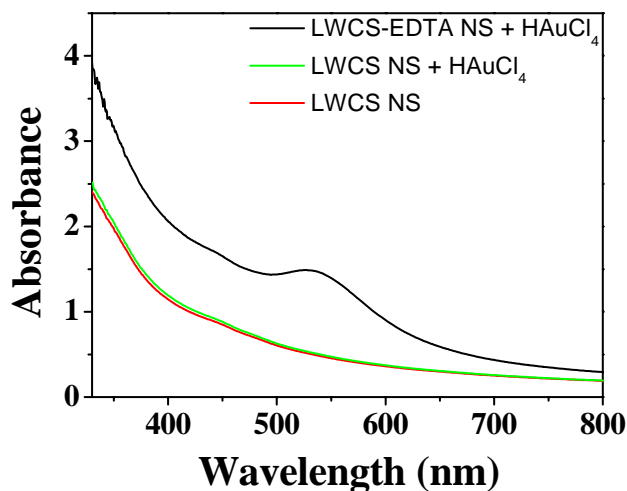
In order to get a better view of the gold nanoparticles residing within the interior of chitosan nanospheres, a sample of uncrosslinked hybrid nanospheres was prepared (10% HAuCl<sub>4</sub> feeding). After dialysis, the uncrosslinked chitosan-gold hybrid nanospheres were disintegrated, while the gold nanoparticles that originally existed in the polymeric nanospheres remained. The isolated gold nanoparticles had a polydispersity of 0.129 determined by DLS, while the LWCS-EDTA nanospheres ( $d = 230$  nm) is 0.070. Figure S2 shows the TEM micrograph of the gold nanoparticles obtained within uncrosslinked chitosan-EDTA composite nanospheres which were disintegrated by dialysis against water. As opposed to the severely aggregated gold nanoparticles obtained with pure EDTA solution, the gold nanoparticles obtained here were well dispersed. This strongly suggests that the gold nanoparticles were formed within the discrete chitosan-EDTA composite nanospheres and the chitosan matrix could effectively prevent the already-formed gold nanoparticles from mutual contact.



**Figure S2.** TEM micrograph of the gold nanoparticles obtained within uncrosslinked chitosan-EDTA composite nanospheres which were disintegrated by dialysis against water. Bar corresponds to 200 nm.

#### 4. Reductive ability of LWCS-EDTA composite nanospheres in comparison with that of pure LWCS nanospheres.

Chitosan has been reported to be able to reduce gold salt under certain conditions. So will chitosan be also responsible for the fast reduction of  $\text{HAuCl}_4$  in our approach? To answer this question, parallel experiments have been conducted. Figure S3 shows the results of our parallel experiment.

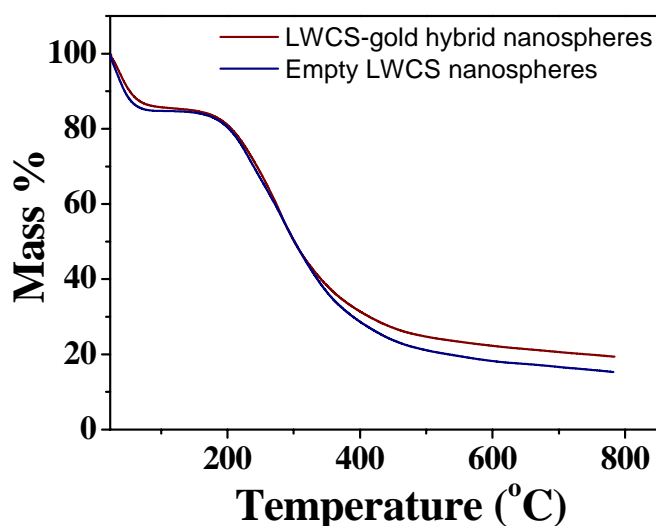


**Figure S3.** (a) UV-Vis spectra of different solutions that were heated using a 70 °C water bath for 10 minutes. Black line: solution containing LWCS-EDTA composite nanospheres added with 5%  $\text{HAuCl}_4$  (chitosan weight based); Green line: LWCS nanospheres obtained by removal of EDTA from crosslinked LWCS-EDTA nanospheres, with 5%  $\text{HAuCl}_4$  feeding; Red line: Empty LWCS nanospheres.

It is very obvious that the entrapment of EDTA molecules play key role for the fast *in-situ* reduction of the added  $\text{HAuCl}_4$ , and chitosan's reducing ability is too limited and can be ignored in our case.

## 5. Determination of gold loading content of the resulting chitosan-gold hybrid nanospheres.

To determine the ratio of Au to LWCS of the resulting chitosan-gold hybrid nanospheres, thermogravimetric analysis (TGA) was carried out on a Pyris 1 TGA (Perkin Elmer, USA). Freeze-dried powders of purified LWCS-gold hybrid nanospheres (prepared with 10%  $\text{HAuCl}_4$  feeding) were heated up to 800 °C at a heating rate of 10K/min under nitrogen atmosphere. Empty LWCS nanospheres were also treated in the same way as a control to calibrate the residue mass that should be attributed to carbonization of LWCS under  $\text{N}_2$  atmosphere. Figure S4 shows the TGA results.



**Figure S4.** TG curves of LWCS-gold hybrid nanospheres in comparison with empty LWCS nanospheres.

It can be seen from Figure S4, carbonization results in 15.5% residue mass in the case of empty LWCS nanospheres. For chitosan-gold hybrid nanospheres, however, the residue mass is 19.5%, among which the extra 4% should be attributed to the indecomposable gold. Considering the bounded water of about 15%, the actual gold to chitosan ratio is calculated to be 5.84% (weight based), correlating very well with the theoretical value (5.79%). This result suggests that all the added gold salt has been effectively reduced into gold nanoparticles in the approach reported in the present article.

## 6. Removal of EDTA in gold-LWCS-EDTA hybrid nanospheres

In order to characterize the residue amount of EDTA in the LWCS nanoparticles after dialysis and to elucidate whether the dialysis against water can really remove the remaining EDTA from the crosslinked chitosan nanospheres, we carried out the following experiment. The obtained solution (40% ethanol) containing LWCS-EDTA composite nanospheres without gold-loading was centrifuged at 15,000 rpm for 10 min. Then the sediment nanospheres were redispersed in distilled water and the resultant dispersion was centrifuged again. The supernatant was collected into a beaker and this redispersing/centrifugation cycle was repeated for 3 times, which would have a very similar effect as extensive dialysis against water. The supernatants of all cycles were collected. The amount of EDTA in the supernatant was subsequently titrated by standard  $\text{Zn}^{2+}$  solution with eriochrome black T (EBT) as the indicator. It was found that more than 96% of the theoretical amount of EDTA could be detected in the supernatant. Similarly, LWCS-EDTA-Au hybrid nanospheres with 20% (chitosan weight based) of  $\text{HAuCl}_4$  feeding were also treated in the same way. Titration result revealed that more than 90% of the added EDTA could be found in the collected supernatant. Taking into consideration that a certain fraction of EDTA may be consumed due to reaction with the oxidative  $\text{HAuCl}_4$  and the redispersing/centrifugation cycle was repeated for only 3 times, we conclude that the amount of EDTA in the LWCS-EDTA nanospheres or gold-LWCS-EDTA hybrid nanospheres is in good agreement with the feeding amount of EDTA, and the remaining EDTA could be effectively removed by extensive dialysis against water despite the crosslinked chitosan matrix, because of the small molecule nature of EDTA. Thus, there would be only a very small and negligible amount of EDTA in the obtained final hybrid nanospheres. Actually this is in good accordance with our FT-IR results, and also correlates well with the fact that uncrosslinked LWCS-EDTA composite nanospheres will be disintegrated quickly by dilution with or dialysis against water.