One-pot synthesis of gold nanocubes

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Abstract. Precious metal nanomaterials have the advantages of low toxicity, optical stability, water-solubility, good biocompatibility and strong photoluminescence. In this paper, by controlling the proportion of the reaction substance, the amount of reducing agent, pH value, reaction temperature and reaction time, we synthesized the fluorescent gold nanocubes with uniform size and morphology, good fluorescence signal and excellent biocompatibility. It provides some guidance for the shape control of nanocubes synthesis.

1. Introduction
With the development of science and technology and the progress of society, more and more studies have been done on the fluorescence properties, and the application range of fluorescent materials has become more and more extensive. It is necessary to develop fluorescent materials with a simple preparation method, good stability and excellent fluorescence properties. In the research field of fluorescent materials, gold nanomaterials have attracted the attention of many researchers because of their unique optical, electrical, magnetic, thermal, mechanical and catalytic properties and their applications in the fields of new energy materials, optoelectronics, information storage, biomedical and surface enhancement [1]. Relevant studies show that the special properties of gold nanoparticles are determined by their size, shape, composition, crystal form and structure [2, 3]. Due to the small size effect, surface effect, quantum size effect and macroscopic quantum tunneling effect, gold nanoparticles can produce special physical and chemical properties different from bulk gold, such as surface plasmon resonance property, fluorescence property, electrochemical property, molecular recognition property [4]. Therefore, the synthesis of gold nanoparticles with a single shape, controllable size, clear crystal shape and structure is a crucial step for the study and application of their properties.

There are many synthetic methods of gold nanoparticles. At present, a variety of anisotropic gold nanoparticles have been prepared, such as nanorods, nanotubes, nanowires, nanocages, nanoshells, triangles, hexagons, regular octahedrons, nanocubes [5-8]. Although the preparation methods of gold nanoparticles have been basically mature, how to reduce the size of gold nanoparticles, improve their morphology, and prepare gold nanoparticles with uniform size and controllable shape is still a difficult point in synthesis. Gold nanocubes (AuNC) is a kind of gold nanostructure with great development potential. Compared with other precious metal nanoparticles, AuNC has good chemical stability, surface modification, good biocompatibility and excellent optical and catalytic properties. The flat surface of AuNC is also often used as the component of the assembly structure, such as the cubic dimer assembled face to face [9], or as a template for further synthesis of more complex structures [10]. It can be widely used in many fields, but the lack of high yield, high precision, simple synthesis
strategy restricts its practical application. At present, the synthesis methods of AuNC include electrochemical method [11], biosynthesis method [12], wet chemical reduction method [13], etc. However, some of these methods require longer synthesis time, some require special equipment, and some of the synthesized AuNC have an uneven size and poor reproducibility. Therefore, the synthesis of AuNC with high reproducibility and controllability by a simple one-pot method is of great significance in the field of materials science and fluorescent materials.

Most proteins contain active sites for aggregation and reduction of metal ions, Bovine serum albumin (BSA) is one of the proteins commonly used in the synthesis of metal nanomaterials. BSA contains 35 potential sulfhydryl groups, 17 disulfide bonds and a free cysteine, which can be used as a reducing agent to reduce metal ions, a polyvalent ligand to passivate metal surfaces, and a stabilizer to stabilize metal materials [14]. In this paper, we adopt the following synthesis method: First, BSA is mixed with HAuCl₄, and then the reducing agent ascorbic acid (AA) is added. Under alkaline conditions, BSA configuration changes, reducing capacity is enhanced. Together, BSA and AA reduce Au³⁺ to gold atoms. Under heating conditions, BSA will undergo irreversible thermal denaturing during the heating process, forming cube-like scaffolding, which provides a template for the formation of nanocubes [15]. Gold nanoparticles are coated in the bovine serum albumin cube scaffold and reacted to grow into AuNC.

2. Experimental

2.1. Reagents and instruments

The following reagents were used: Bovine Serum Albumin (99%, Biofroxx), HAuCl₄·3H₂O (99.9%, Adamas), ascorbic acid (99.7%, Shanghai Sinopharm Reagent Co., Ltd), NaOH (96%, Sinopharm Chemical Reagent Co., Ltd), Cell Counting Kit-8 (Biosharp), all other reagents were commercial analytical pure.

Precision balance (Beijing Sartorius Scales Co., Ltd), F-7000 fluorospectro photometer (Hitachi Ltd), Magnetic stirrers (DragonLab), Malvern Zeta Sizer-Nano Z analyzer (Malvern Instruments Ltd), Microplate system (Thermo Fisher Scientific, USA), HT7700 transmission electron microscope (Hitachi Ltd), Ultrapure water system (Millipore Corporation, USA).

2.2. Optimization of synthesis conditions

The factors affecting the synthesis of gold cube mainly include reaction temperature, pH value, reaction time, the ratio of reactants and the amount of reducing agent. In this paper, the following aspects will be investigated to determine the optimal synthesis conditions:

First of all, standard solution was prepared: BSA (20 mg/mL), HAuCl₄ (10 mM), AA (2 mM), NaOH (1 M). All reactions were carried out under stirring conditions.

2.2.1. Effect of reaction temperature on the synthesis of AuNC. Measure 5 mL of BSA and HAuCl₄ standard solution respectively, mix well under the condition of violent stirring, then add 100 μL of AA standard solution. After a period of reaction, 0.4mL of NaOH standard solution was added to adjust the pH to 12.10. Prepare 5 parts of the same volume of the mixed solution as above. Finally, the reaction temperature was adjusted to 37 °C, 60 °C, 80 °C, 100 °C, 120 °C, respectively, and the reaction was heated for 2 h.

2.2.2. Effect of pH value on the synthesis of AuNC. Measure 5 mL of BSA and HAuCl₄ standard solution respectively, mix well under the condition of violent stirring, then add 100 μL of AA standard solution. Prepare 9 parts of the same volume of the mixed solution as above. Add different volumes (0 mL, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL, 0.7 mL, 0.8 mL) of NaOH standard solution to the mixed solution, adjust the pH value of solution to 2.50, 4.44, 7.12, 10.88, 12.10, 12.64, 12.83, 12.94, 13.03, respectively. Finally, the reaction temperature was adjusted to 100 °C for 2 h.
2.2.3. Effect of reaction time on the synthesis of AuNC. Measure 5 mL of BSA and HAuCl₄ standard solution respectively, mix well under the condition of violent stirring, then add 100 μL of AA standard solution. After a period of reaction, 0.4mL of NaOH standard solution was added to adjust the pH to 12.10. Prepare 8 parts of the same volume of the mixed solution as above. Finally, the reaction temperature was adjusted to 100 °C for 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 6 h and 12 h, respectively. Finally, the reaction temperature was adjusted to 100 °C and the reaction was heated for 2 h.

2.2.4. Effect of the Ratio of BSA to HAuCl₄ on the synthesis of AuNC. First, prepare standard HAuCl₄ solution with a concentration of 100mM, absorb 0.01 mL, 0.05 mL, 0.1 mL, 0.3 mL, 0.5 mL, 0.7 mL and 1 mL of standard solution and dilute them to 5mL. Then mixed with 5mL of BSA standard solution to prepare 7 parts of mixed solution. Then add 100 μL of AA standard solution. After a period of reaction, 0.4mL of NaOH standard solution was added to adjust the pH to 12.10. Finally, the reaction temperature was adjusted to 100 °C and the reaction was heated for 2 h.

2.2.5. Effect of AA dosage on the synthesis of AuNC. Measure 5 mL of BSA and HAuCl₄ standard solution respectively, mix well under the condition of violent stirring. Add different volumes (5 μL, 25 μL, 50 μL, 100 μL, 200 μL, 300 μL, 400 μL, 500 μL) of AA standard solution to the mixed solution, adjust the concentration of AA to 1 μM, 5 μM, 10 μM, 20 μM, 40 μM, 60 μM, 80 μM, 100 μM, respectively. After a period of reaction, 0.4mL of NaOH standard solution was added to adjust the pH to 12.10. Finally, the reaction temperature was adjusted to 100 °C for 2 h.

2.3. Biocompatibility of gold AuNC

Good biocompatibility is the premise of the clinical transformation of biomaterials. In this paper, Cell Counting Kit-8 (CCK-8) analysis and hemolysis test were used to evaluate the biocompatibility of AuNC.

2.3.1. Cytotoxicity. Before CCK-8 analysis, AML-12, HK-2, and MCF-7 cells were first cultured. When the cell growth density was 80-90%, the cells were digested with trypsin containing EDTA from the culture dish, then the cells were collected by centrifugation and inoculated into 96-well plates. Serum-free DMEM medium containing AuNC at concentrations of 10, 20, 40, 80, 160, 250 and 400 μg/mL were added and incubated for 24 h. Then 10 μL CCK-8 solution was added to each well for further incubation for 3 h. Finally, the absorbance of each well was determined at 450 nm.

2.3.2. Hemolytic test. First, the whole blood of mice was prepared into 2% erythrocyte suspension, which was divided into different EP tubes. AuNC PBS solutions with concentrations of 10, 20, 40, 80, 160 and 320 μg/mL were added as the experimental group, PBS solution as the negative control group and H2O as the positive control group. The EP tubes in each group were incubated in a water bath at 37°C for 2 h, followed by centrifugation at 3000 rpm for 10 min. The supernatant was taken to measure the absorbance of hemoglobin at 540 nm to calculate the hemolysis ratio. The formula is as follows:

\[
\text{Hemolysis ratio} = \left( \frac{A_{\text{experimental group}} - A_{\text{negative control}}}{A_{\text{positive control}} - A_{\text{negative control}}} \right) \times 100\%
\]

3. Results and Discussion

3.1. Effect of synthesis conditions on AuNC

As shown in Figure 1a, the reaction temperature affects the fluorescence intensity of the AuNC. With the increase of temperature, the fluorescence intensity of the AuNC also increases, and reaches the peak value at 100 °C. Then, the fluorescence intensity decreases with the increase of temperature. The reaction temperature of 37 °C does not have the characteristic absorption peak of the AuNC, indicating that it is necessary to make BSA irreversible thermal denaturation in the heating process to form cube-shaped scaffolding, in order to provide a template for the subsequent cube formation. The electron
The microscope (TEM) image in Figure 1b also proves that there is no nanocubes formed at 37 °C, but a spherical structure.

![Figure 1. (a) Fluorescence excitation spectra of gold nanoparticles synthesized at different temperatures, (b) TEM image of gold nanoparticles synthesized at 37 °C.](image)

From Figure 2a, it can be seen the fluorescence intensity of the AuNC increases with the increase of alkalinity and reaches the peak at pH 12.10. Then, the fluorescence intensity decreases slowly with the increase of alkalinity. As shown in Figure 2b, within the reaction period of 0-2 h, the fluorescence intensity of the AuNC increases with the increase of the reaction time. The peak value of the reaction is reached at 2 h, and the fluorescence intensity will decrease as the reaction continues. From Figure 2c, it can be seen when the concentration of BSA in the mixed solution is 10 mg/mL and the HAuCl₄ is 5 mM, the fluorescence intensity of the AuNC is the strongest. As shown in Figure 2d, the fluorescence intensity of the AuNC increased with the increase of the reducing agent concentration, and the fluorescence intensity was the strongest at 20 μM.

![Figure 2. (a) Fluorescence excitation spectra of AuNC synthesized at different pH values, (b) Fluorescence excitation spectra of AuNC synthesized at different reaction time, (c) Fluorescence](image)
intensity of AuNC synthesized at different Ratios of BSA to HAuCl4, (d) Fluorescence intensity of AuNC synthesized at different concentrations of AA

We prepared AuNC according to the optimal conditions investigated. As shown in Figure 3, the excitation wavelength of the AuNC was 495 nm and the emission wavelength was 650 nm (Figure 3a). Regular cubes can be seen in the TEM image (Figure 3b). The particle size was about 110 nm (Figure 3c), and the Zeta potential was -22.2 mV (Figure 3d).

3.2. Biocompatibility of gold AuNC

BSA molecules, which were covered on the surface of AuNC nanocomposites during the preparation process, are beneficial for improving the biocompatibility of the nanocomposite. The cytotoxicity of AuNC was determined by CCK-8 method, as shown in Figure 4a, there was no significant decrease in cell viability after incubation for 24 h. The Hemolytic test also showed that the hemolysis ratio was less than 2% at 2 h, which proved that AuNC had good biocompatibility.
4. Conclusions
The AuNC was successfully prepared by the one-pot method. The optimal preparation conditions were determined as follows: under stirring condition, the concentration of BSA in the mixed solution was 10 mg/mL and the concentration of HAuCl₄ was 5 mM in the reaction system of 10 mL. Then 20 μM of reducing agent AA was added. After a period of reaction, 0.4 mL of NaOH was added to adjust the pH to 12.10. The reaction temperature was adjusted to 100 °C and the reaction was heated for 2 h. According to the above operation, the AuNC with strong fluorescence signal and good biocompatibility can be obtained.

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