

Synthesis of silver nanoparticles stabilized with C-phycocyanin and for fluorimetric detection of copper ions

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Abstract. In this study, C-phycocyanin as protective agent, AgNO₃ as raw material and NaBH₄ as reducing agent synthesized C-phycocyanin-Ag nanoparticles (PC-AgNPs). The synthesis conditions of PC-AgNPs were determined by optimization. The maximum UV absorption peak of PC-AgNPs at 400 nm. The fluorescence excitation wavelength was 580 nm and the emission wavelength was 625 nm. PC-AgNPs was spherical in transmission electron microscope and the particles sizes were about 10-25 nm. In addition, fluorescence quenching was observed after adding copper ions to PC-AgNPs, which indicated that PC-AgNPs has potential applications in the detection of copper ions in diverse water environment.

1. Introduction

In recent years, precious metal nanomaterials have received increasing attention. Silver nanomaterials have significant differences from the macroscopic and small-molecular species because of their particle sizes. As silver nanomaterials show some excellent performances, they have extensive applications in chemical industry, medicine, biological, superconductivity and other aspects. A variety of synthetic silver nanoparticles (AgNPs) has been developed and can be synthesized using some substances as templates or protectors. Recently, it is popular to develop green methods to accomplish the synthesis of AgNPs for their clinical and biomedical application. The biomolecule-oriented synthesis of AgNPs has good water-soluble and high biocompatibility, which can be easily functionalized [1]. Therefore, proteins, peptides, DNA and other molecules will be as excellent templates for the synthesis of AgNPs [2].

Phycocyanin is an important pigment protein in cyanobacteria and plays a vital role in the photosynthesis. It is not only widely used in food, cosmetics and dyes, but also has important applications in improving human immunity, promoting animal blood cell regeneration, fluorescent labeling, etc [3,4]. The silver atom has a strong affinity for these functional groups, and the nanomaterials can be prepared stably [1,5]. Moreover, C-phycocyanin has some functional groups in the protein, such as amino, carboxyl and mercapto, etc, which can be as a protective agent for nanoparticles to participate in the synthesis of AgNPs. In this study, C-phycocyanin was as template and protective agent, AgNO₃ as silver source, NaBH₄ as reducing agent, to synthesize PC-AgNPs. The synthesized PC-AgNPs was characterized by spectroscopic and transmission electron microscopy, and their fluorescence responses to copper ions.



2. Materials and Methods

2.1. Materials

C-Phycocyanin was purified according to our previous work [6]. AgNO₃, NaBH₄, NaOH, and HCl of analytical grade were purchased from the Sinopharm Chemical Reagent Co.,Ltd (Beijing, China).

2.2. Synthesis of PC-AgNPs

For the synthesis of PC-AgNPs, a certain volume of 0.10 M AgNO₃ solution were added to the 1000 μ L 1.00 mg/mL of C-Phycocyanin solution under vigorous stirring for 2 hours at 30°C.

2.3. Characterization of PC-AgNPs

2.3.1. UV-vis spectra. The UV-vis absorbance spectra of synthesized PC-AgNPs were measured by UV-vis spectrophotometer at 220-700nm.

2.3.2. Excitation and Emission Spectra. Fluorescence excitation and emission spectra of PC-AgNPs was measured by fluorescence spectrophotometer (F-2700, Hitachi, Japan).

2.3.3. Transmission Electron Microscopy. TEM experiments of PC-AgNPs were performed with JEOL-2100 electron microscope working at an acceleration voltage of 200 kV.

2.4. Fluorimetric Response to Copper Ion

0.10 M CuSO₄ solution was added to 5 M in the PC-AgNPs solution, and the one without CuSO₄ was as control. The changes of fluorescence intensity were measured by fluorescence spectrophotometer.

3. Results and Discussion

3.1. Optimization of PC-AgNPs Synthesis

The synthesis conditions of PC-AgNPs were determined by optimization: 0.20 mg/mL C-phycocyanin was added to 0.35 mM AgNO₃ and 0.40 mg/mL NaBH₄ at pH 7.0, with a temperature of 30 °C and stirring for 2 hours (data not shown).

3.2. The UV-vis Absorption Spectra of PC-AgNPs

Figure 1 shows the UV-vis absorption spectrum of the synthesized PC-AgNPs. PC-AgNPs had two absorption peaks at 260 nm and 400 nm, respectively. Similar studies showed that Ag-LCDs prepared with long wavelengths of carbon had UV-vis absorption peak at 405 nm [7]. AgNPs synthesized by using dihydrolipoic acid as protective agent had a main absorption peak at 435 nm, and two shoulder peaks at 325 nm and 500 nm, respectively [8]. Besides, AgNPs were synthesized by TCTH as a reducing agent and stabilizer and had absorption peak at 425 nm [9]. Furthermore, AgNPs were synthesized with NaBH₄ and bovine serum albumin, and its UV-vis absorption peak was at 425 nm. In addition, AgNPs synthesized with oligonucleotides showed absorption peaks at 424 nm and 520 nm [10]. It clearly revealed that the absorption peaks of AgNPs with different templates, protective agents, method and even raw materials ratio would affect the UV-vis absorption spectra.

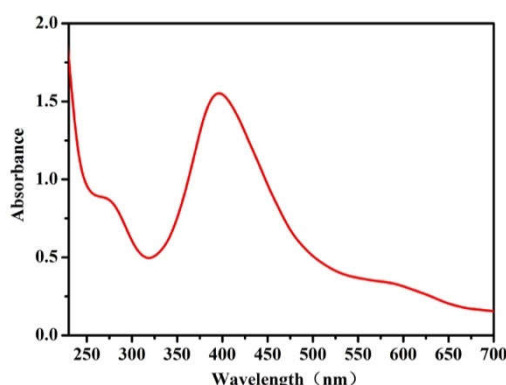


Figure 1. UV-vis absorption spectra of PC-AgNPs

3.3. Excitation and Emission Spectra of PC-AgNPs

The excitation and emission spectrum of PC-AgNPs are shown in Figure 2. The excitation wavelength of PC-AgNPs was at 580 nm and the maximum emission peak was at 625 nm. However, the fluorescence spectrum analysis of DNA-Ag nanoparticles synthesized showed that the maximum excitation wavelength was at 560 nm and the emission wavelength was at 650 nm [10]. BSA-Ag13 had excitation at 480 nm and emission at 625 nm [9]. Similar study showed that nano-silver synthesized with the hydrogel-based short peptides had the excitation wavelength at 530 nm and the emission wavelength at 634 nm [11].

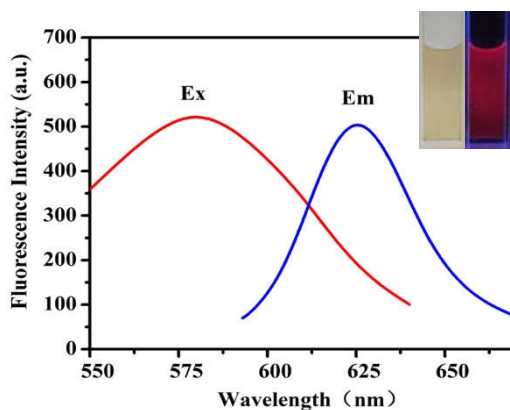


Figure 2. PC-AgNPs excitation and emission spectra. Inset shows PC-AgNPs under visible light (left) and UV light (right).

3.4. Transmission Electron Microscopy of PC-AgNPs

TEM of PC-AgNPs is shown in Figure 3. It can be clearly seen that PC-AgNPs were basically in the spherical distribution, and the particle size roughly were in about 10-25 nm with a small number of particle size of about 5 nm. The AgNPs with some proteins was in the size of about 20 nm. For example, the size of TCTH-AgNPs was about 20 nm [12], which was close to that of this study. AgNPs were synthesized with acetate as donor, polyvinyl butyral as dispersant, and the system dissolved in ethanol. In its best reaction system, the size of AgNPs was about 50 nm.

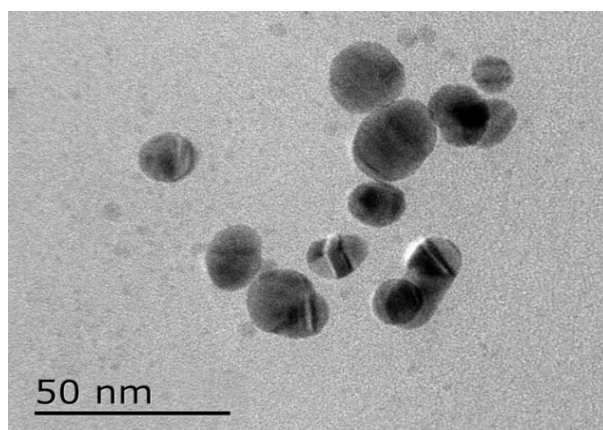


Figure 3. PC-AgNPs transmission electron microscopy images

3.5. Quenching Phenomenon of Copper Ions on PC-AgNPs

As shown in Figure 4, the fluorescence emission intensity was decreased with addition of copper ions at 625 nm. Meanwhile, the inset was taken to show the red fluorescent color changes of PC-AgNPs after the addition of copper ions, which could be obviously seen by the naked eye. This indicates that PC-AgNPs have potential applications in the detection of copper ions.

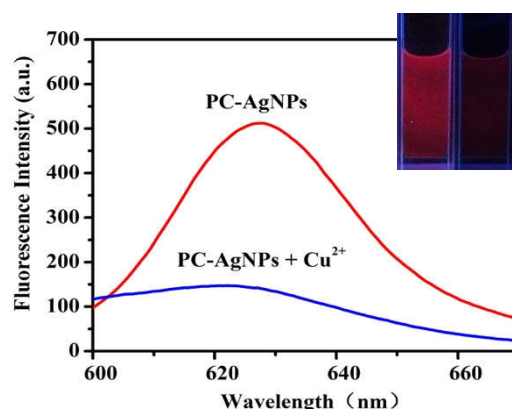


Figure 4. Fluorescence response of PC-AgNPs to copper ions. Inset shows the red fluorescent color changes of PC-AgNPs without the copper ions (left) and after the addition of copper ions (right).

4. Conclusion

C-phycocyanin as protective agent, AgNO_3 as raw material and NaBH_4 as reductant, the synthesis conditions of PC-AgNPs were studied. PC-AgNPs were characterized by UV-vis spectroscopy, fluorescence spectroscopy and TEM. Moreover, copper ions had obvious quenching effect on their fluorescence, which indicated that PC-AgNPs had potential applications in the detection of copper ions in diverse water environment.

Acknowledgments

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