Chicken Egg White-stabilized Au Nanoclusters for Selective and Sensitive Detection of Hg(II)

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In this paper, chicken egg white purchased from a local market without further purification was directly used to prepare fluorescent gold nanoclusters through a one-step, simple, fast and green synthesis approach for analytical purposes. The as-prepared chicken egg white stabilized gold nanocluster probe has strong red fluorescence emission, which can be quenched by mercury ions and copper ions sensitively. By using an ethylenediaminetetraacetate (EDTA) masking method, mercury ions in the range from 0.60 to 10 μM can be linearly detected with the limit of detection (LOD, 3σ) of 0.510 μM in the presence of equivalent copper ions. Since the preparation of a chicken egg white stabilized gold nanocluster probe is fast, easy and cheap, this selective analytical method for mercury pollution monitoring in environmental waters may be widely used in daily life by ordinary people.

Keywords Chicken egg white, gold nanocluster, fluorescence quenching, mercury ions

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Introduction

Mercury is widespread in our daily life and can affect humans, for example, through contact with inferior cosmetics and when breaking a medical thermometer. Mercury is a severe environmental pollutant to human beings because its accumulation in organisms through the food chain would cause acute and permanent damage to the brain, liver and the central nervous system.1–4 The detection and monitoring of mercury pollutants have been studied for tens of years and countless methods for mercury detection have been established in the past several decades. However, traditional and reliable methods for the detection of metal ions are time-consuming or require large equipment such as for atomic absorption/emission spectrometry.5 In consideration of increasing concerns over the potential threat of mercury, the development of a simple and rapid mercury detection method for daily use by the public is still of interest to scientific researchers.

Noble metal nanoclusters (NCs) are a new type of fluorescent nanomaterial with excellent fluorescence stability and biocompatibility compared to organic fluorescence dyes. Many methods for the synthesis of noble metal nanoclusters, including chemical reduction,6–7 chemical etching,1 phase transfer method,8 microwave-assisted method9 and photo-reduction,10,11 have been reported in recent years. The applications of noble metal nanocluster probes have been studied widely in biological sensing,12–16 cellular imaging17,18 and disease diagnosis.19 Noble metal nanocluster probes have also been used for heavy metal ion detection in environmental and biological systems.20 Various heavy metal ions, including mercury, can be selectively detected by a variety of fluorescent nanoclusters.21–34

Besides developing a novel synthesis method and expanding the application field for fluorescent nanoclusters, studies for a cheaper and more readily available reagent for the simple preparation of fluorescent nanoclusters and their further application to heavy metal ion monitoring and detection by ordinary people are also important for fluorescence nanocluster research. In the present work, a cheap and easily obtained chicken egg white (CEW) was selected as a template for the synthesis of fluorescent gold nanoclusters.35,36 By studying the interaction between the as-prepared chicken egg white stabilized gold nanoclusters (CEW-Au NCs) and environmental metal ions, we found that mercury and copper could effectively quench the fluorescence of CEW-Au NCs. Considering that mercury is a widely presented environmental metal pollution, we introduced an ethylenediaminetetraacetate (EDTA) masking method to exclude the interference of copper ion in mercury ion detection. As a result, we established a cheap, rapid and selective analytical method for environmental mercury detection. We believe that the studies of using readily available reagents for fluorescent nanocluster preparation would be useful for the further application of fluorescent nanoclusters in daily life.

Experimental

Reagents and chemicals

Chloroauric acid (AR, HAuCl4·3H2O) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); sodium hydroxide (NaOH, AR) was purchased from Tianjin Fengchuan Chemical Reagent Co., Ltd.; EDTA (AR) was purchased from China Shanghai Reagent Factory; FeCl3, CuCl2, CaCl2, ZnSO4, MgSO4, CoSO4, Pb(NO3)2, SnCl2, CdSO4, CrCl3 and HgCl2 were analytical grade and used without further
purification. Ultra-purified water (18.25 MΩ) from the Millipore Milli-Q system was used throughout the experiments. Britton-Robinson (BR) buffer solution (40 mM, pH value ranges from 2 to 12) was prepared by mixing 0.2 M NaOH and an acid mixture of 0.04 M H₂PO₄, H₂BO₃, and CH₃COOH in suitable proportions.

**Apparatus**

An F-7000 fluorescence spectrophotometer (Hitachi, Japan) was used for measuring the fluorescence spectra of the gold nanoclusters solution; a QL-901 vortex mixer (Haimen Qilinbeier Instrument Manufacturing Co., Ltd., Haimen, China) was used for mixing the solution; and a PHS-25 pH meter (Shanghai Electronics Science Instrument Co., Ltd., Shanghai, China) was used for monitoring the pH value of the buffer solution.

**Synthesis of chicken egg white stabilized gold nanoclusters**

As a typical experiment, the CEW was prepared by carefully separating the white portion of the chicken egg from the whole fresh egg, followed by freeze drying; the obtained solid dry white powder was used as such without any further purification. The CEW-Au NCs were synthesized based on a reported method with slight modification. Briefly, 10 mL of HAuCl₄(10 mM) solution was added to 10 mL CEW (50 mg/mL) with stirring. Two minutes later, 1.4 mL of 1 M NaOH was added to the mixture of HAuCl₄ and CEW, and then, the mixture was placed in 37°C water bath for 20 h with continuous stirring. The change of the mixed solution color to yellow-brown indicated the formation of CEW-Au NCs, and then the as-prepared CEW-Au NCs were stored in a refrigerator at 4°C before use. As a simple description, the concentration of CEW-Au NCs is directly calculated and expressed by the concentration of gold atoms in a CEW-Au NC solution.

**General procedures for studying interaction between Au NCs and metal ions**

Briefly, 20.0 μL of the as-prepared CEW-Au NCs was added to several 1.5 mL cuvettes, and subsequently mixed with 200 μL of BR buffer. Then, a series of metal ion solutions, such as HgCl₂, CuSO₄, FeCl₃, CaCl₂, ZnSO₄, MgSO₄, CoSO₄, Pb(NO₃)₂, SnCl₂, CdSO₄ and CrCl₃, were added to the cuvettes and mixed vigorously. After waiting for 5 min, the mixtures were transferred to spectrophotometers for absorbance and fluorescence measurements. For an EDTA masking method, additional EDTA with a definite concentration was pre-mixed with CEW-Au NC solution before the addition of metal ions.

**Detection of Hg²⁺ in the presence of Cu²⁺**

For detection of Hg²⁺ in the presence of Cu²⁺, an EDTA masking method was used according to the reported paper. Briefly, 20 μL of CEW-Au NC solution, 200 μL of BR buffer and a certain amount of EDTA was firstly mixed together, and then a certain concentration of Cu²⁺ and a series of different volumes of Hg²⁺ were added to the CEW-Au NC mixture solution. After waiting for 5 min, the mixtures were transferred to spectrophotometers for absorbance and fluorescence measurements.

**Results and Discussion**

**Fluorescence of the chicken egg white stabilized gold nanoclusters**

After a simple pretreatment of a chicken egg, the obtained CEW was directly used for Au NC preparation without further purification. Under the excitation at 490 nm, the as-prepared CEW-Au NCs have a fluorescence emission with maximum wavelength at 620 nm as measured by a fluorescence spectrometer. The orange-red fluorescence of CEW-Au NCs can also be seen under a 365 nm UV light illumination (Fig. 1). Since the synthesis method of the CEW-Au NCs was according to the classical Xie’s method with little modification and also the fluorescence spectral feature is also similar to that described in the punished paper, no further characterization of the CEW-Au NCs was carried out for verifying the of nanoclusters.

**Fluorescence response of CEW-Au NCs toward metal ions**

Many reports have been reported that protein stabilized gold nanoclusters could be used to detect heavy metal ion such as Hg²⁺, Pb²⁺ and Cu²⁺. However, Au NCs stabilized with different kinds of proteins would have different fluorescence responses to metal ions. In this paper, we tested a number of metal ions for their effect on CEW-Au NC emission by comparing their fluorescence quenching ratio, I₀/I, the ratio of CEW-Au NCs fluorescence intensity at 620 nm before (I₀) and after (I) interaction with metal ions. The results shown in Fig. 2A indicated that only two metals, Hg²⁺ and Cu²⁺, could effectively quench the emission of the CEW-Au NCs. This phenomenon was different from the report that the fluorescence of some proteins stabilized Au NCs could be highly selectively quenched by only one metal ion. The reason may be due to structure and purity differences of proteins.

Considering mercury pollution in the natural environment is dangerous to human beings while copper is not as harmful, we decided to establish a selective method for mercury detection here. An EDTA masking method was used to eliminate the interference of copper in the detection of mercury. As shown in Fig. 2B, after the addition of an appropriate concentration of EDTA to the Au NC solution, copper ions would not affect the detection of mercury. And thus, the EDTA masking method was used for mercury detection in this study.

**Optimization of pH on detection of Hg²⁺**

The pH value of the medium is a most important factor for fluorescence emission of a fluorophore and its interaction with a quencher/receptor. As an EDTA masking method was used to
mask the interference of copper, we studied the effect of pH value on the fluorescence of the CEW-Au NCs/EDTA/Cu²⁺ mixture before and after the addition of mercury. As shown in Fig. 3A, the fluorescence of the CEW-Au NCs/EDTA/Cu²⁺ mixture varied with pH value (solid line) and decreased with the addition of mercury with a similar variation trend to pH changes (dashed line). However, we could still determine an optimal experimental condition at pH of 5, at which the fluorescence quenching ratio ($I_0/I$) reaches maximum (as shown in Fig. 3B). It must be pointed out that, the pH value of the system should not be adjusted to the isoelectric point (near 4) of CEW, as it may induce an aggregation of CEW-Au NCs and produce a large amount of precipitates.

**Effect of reaction time for Hg²⁺ detection**

Studying dynamic process of a fluorescence quenching is important for establishing a fluorescent analytical method. As the EDTA masking method was used for eliminating the effect of copper ions, we monitored the fluorescence of the CEW-Au NCs/EDTA/Cu²⁺ mixture before and after addition of Hg²⁺. As shown in Fig. 4A, in the presence of EDTA and Cu²⁺, the fluorescence of CEW-Au NCs is stable, however, the addition of Hg²⁺ could quench the fluorescence immediately. We also measured the fluorescence intensity for stability over a long time and the results (Fig. 4B) showed that the fluorescence would be stable for around 30 min.
Fluorescence detection of Hg²⁺ in the presence of Cu²⁺

Under the optimal experimental condition, CEW-Au NCs were used for quantitative detection of Hg²⁺ in the presence of Cu²⁺ by EDTA masking method. As shown in Fig. 5A, in the presence of 7.5 μM of EDTA and 5.0 μM of Cu²⁺, the fluorescence of CEW-Au NCs was quenched gradually with an increase of Hg²⁺. There is a good linear relationship between the quenching ratio (I₀/I) and mercury ions in the range from 0.6 to 10 μM (Fig. 5B). The linear regression equation of the relationship is I₀/I = 0.840 + 0.423 c_Hg (R² = 0.984), with a detection limit of (3σ) 0.510 μM.

The detection of Hg²⁺ can also be visualized by watching the orange-red fluorescence quenching image of CEW-Au NCs under ultraviolet analyzer. As shown in Fig. 6, the orange-red color of the CEW-Au NCs is dismissed with the addition of Hg²⁺. We believe that it will be easy to establish a handheld mercury analyzer using a common ultraviolet LED laser by comparing the fluorescence emission of CEW-Au NCs between a sample and control.

Conclusions

In summary, we prepared cheap, easily available and biocompatible chicken egg white stabilized gold nanoclusters (CEW-Au NCs) through a simple, green and one-step synthesis approach in aqueous solution and established a selective fluorescence analytical method for the detection of environmental Hg²⁺. We found that fluorescence of the as-prepared CEW-Au NCs could be quenched by both mercury and copper ions. However, by using an EDTA masking method, we achieved a selective detection of Hg²⁺ in the presence of Cu²⁺ using CEW-Au NCs as a probe. Mercury ions in the range from 0.6 to 10.0 μM can be linearly detected with the detection limit of 0.510 μM. We also established visual detection by taking pictures of the CEW-Au NCs under ultraviolet illumination. We believe that the established simple, easy and selective analytical method for mercury pollution monitoring may be significant for environmental monitoring and may also be widely used for daily life by ordinary people.

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