Detection of tetracycline in water using glutathione-protected fluorescent gold nanoclusters

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Abstract

Tetracycline(Tc), a widely used antibiotic, is one of the major pollutants in water. Herein, glutathione (GSH)-protected Au nanoclusters (GSH-AuNCs) were prepared to detect Tc. The fluorescence quenching ratio of GSH-AuNCs shows a excellent linear response against tetracycline with the concentration of 50μg/L~50mg/L with the detection limit of 5.31μg/L. For the test paper prepared by GSH-AuNCs, 1 mg/L Tc caused significant difference recognized by naked eyes. The method have a good selectivity and have an excellent recovery for a tap water sample. The method has potential in real sample Tc detection.

Keywords: Au nanocluster, tetracycline detection, quenching ratio, glutathione, recovery experiment
Introduction

Tetracycline (Tc) has been widely used as antibiotics in the field of veterinary and aquaculture. As a byproduct, it also caused serious environmental problems. It is known that oxytetracycline, a kind of Tcs, can not degrade even after ten months in marine sediments and soils. More than half of the applied drugs were not consumed and kept in the environment, which explains the presence of high amount of veterinary drugs and their metabolites surrounding aquaculture areas. Therefore, a rapid method to detect drug residues in water was needed. Although the detection methods on methyl viologen, organophosphorus and pesticides are relatively mature, the rapid detection method of tetracycline was still challenging.

Nowadays, there are so many analytical methods has been developed to detect Tc, such as high performance liquid chromatography (HPLC), LC with tandem mass spectrometry (LC–MS), enzyme-linked immunoassay, colorimetric analysis method and electrochemical detection. In general, the method of LC–MS or HPLC have good sensitivity to identify Tc, but these method need complicated operation and the pretreatment of samples takes time. In the field of analysis, people are increasingly concerned with the fluorescence detection method in recent years because of its easy operation, low cost, real-time detection, high sensitivity.

Tc, with no fluorescence, was detected by a series of fluorescence sensors in the past few decades with the help of Eu-based nanoparticles, metal–organic coordination polymers, CdTe quantum dots, and carbon quantum dots, etc. Compared with these fluorescent probes, gold nanoclusters (AuNCs) has low toxicity with a ultra-small size (~2nm). Among many protecting agents, glutathione (GSH) is a tripeptide with a good biocompatible and water-soluble properties. Therefore, more and more people begin trying to use glutathione as a protecting agent to synthesize metal nanoclusters.

In this work, gold nanoclusters (GSH-AuNCs), reduction from hydrogen tetrachloroaurate by glutathione, were used as fluorescence probes to detect Tc. Meanwhile AuNCs-based test papers
were also developed for detecting Tc using naked eyes.

**Experimental**

Materials and apparatus

Hydrogen tetrachloroaurate (HAuCl\(_4\cdot3\)H\(_2\)O) was bought from Sigma Aldrich (China). Glutathione (GSH) was obtained from Aladdin. Tetracycline was purchased from Macklin (China). During the whole experiment, dionized water (DI water) was used. Dopamine was purchased from Sigma (USA), Pb(NO\(_3\))\(_2\), MnCl\(_2\cdot4\)H\(_2\)O, Hg(NO\(_3\))\(_2\cdot2\)H\(_2\)O, Ni(NO\(_3\))\(_2\cdot6\)H\(_2\)O, CaCl\(_2\cdot2\)H\(_2\)O, Cd(NO\(_3\))\(_2\cdot4\)H\(_2\)O, Mg(NO\(_3\))\(_2\cdot6\)H\(_2\)O, KCl, Ni(NO\(_3\))\(_2\cdot6\)H\(_2\)O, Na\(_2\)SO\(_4\), ZnSO\(_4\), citric acid, NH\(_4\)Cl, glucose and EDTA-2Na were all provided from Tianjin Fengchuan Chemical Reagent Company (Tianjin, China).

Measurements

The high-resolution transmission electron microscope (HR-TEM) characterization was measured by a JEM-2100 transmission electron microscope under 200 kV accelerating voltage. Fluorescence spectra was obtained on a LS55 luminescence spectrometer (PerkinElmer, U.K.). Ultraviolet-Visible (UV–vis) absorption spectroscopy was measured with Cary 60 UV/Vis (Agilent Technologies, U.S.). Zeta potential was recorded on Zetasizer Nano (Malvern Instruments Ltd., U.K.).

Synthesis of GSH-AuNCs

GSH functionalized AuNCs was synthesized following previous paper\(^27\). 1) 2.0 mL HAuCl\(_4\) with the concentration of 20 mM was added to 17.4 mL DI water. 2) 0.0184 g GSH was added to 0.60 mL DI water. 3) The solution abovementioned were mixed together at 25°C for 5 min. 4) After gentle stirring for 24h under 70 °C, the solution of GSH-AuNCs were obtained with orange-emitting. The prepared GSH-AuNCs was kept at around 4 °C.

Determination of Tc in aqueous solution by AuNCs

In order to measure the concentration of Tc in aqueous solution, the aliquots of 1mL of
GSH-AuNCs(0.5mM) were added into several tubes. 1mL Tc solution with different concentrations in the range of 50 μg/L~50 mg/L were added to each tube. All the solution was diluted by buffer solution, meanwhile, to avoid the effect of pH change. The control experiment were carried out by adding certain amount of dopamine, Ni^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, citric acid, glucose, NH_4^+, K^+, Na^+, EDTA-2Na.

Detection of Tc based on AuNCs-modified test papers

The filter paper was cut into circles with a diameter of 1cm, and immersed into previously GSH-AuNCs solution for 2 hours, then dried in a closed environment. A certain amount of Tc sample (20 μL) was dropped on the filter paper modified with AuNCs. From the change of the fluorescence intensity of the test paper, the concentration of tetracycline can be roughly determined.

Results and Discussion

Characterization of GSH-AuNCs

The black dots in Fig. 1A are gold nanoclusters. From high resolution images in Fig.1B, the lattice spacing can be easily discerned. A single GSH-AuNC was showed in the inset of Fig.1B, in which the lattice spacing of GSH-AuNC is 0.240nm. The data is consistent with the primary reflection of the (111) lattice of Au NCs and confirms the crystalline nature of Au^{28}. The inset of Fig.1A shows the size distributions of GSH-AuNCs. the majority of AuNCs is in the range of 1 to 3 nm with an average diameter of 2.3 nm.

Fig.1C is the photoluminescent (PL) spectrum and UV–vis absorption spectrum of the GSH-AuNCs. In absorption spectrum, there is a shoulder peak at ~ 400 nm, which means the prepared AuNCs has the size of ~ 2.3 nm since neither surface plasmon resonance (SPR) nor molecular absorption was detected. The main emission peak of GSH-AuNCs is about 590 nm with 365 nm excitation wavelength (blue curve in Fig. 1C). The synthesized GSH-AuNCs solution is light yellow under natural light and orange under UV light (the inset in Fig.1C).
above data confirm that the GSH-AuNCs have been successfully synthesized.

Tc detection

The prepared AuNCs were then used for Tc detection. To investigate the effect of pH on TC detection, pH titration of GSH-AuNCs was performed. The value of F/ F0 is used to indicate the fluorescence quenching degree of GSH-AuNCs, where F0 is the initial fluorescence intensity of GSH-AuNCs, and F means the fluorescence intensity of GSH-AuNCs after adding and reacting with a certain concentration of Tc. The value of F/ F0 has no significant change with the pH between 1~8 (black curve in Fig.2), which implies pH has no obvious effect on the fluorescence intensity of GSH-AuNCs. The zeta potential of GSH-AuNCs was measured to determine the isoelectric point of GSH-AuNCs. From blue curve in Fig. 2, it is noted that the isoelectric point of GSH-AuNCs is between pH3~4. Based on the isoelectric point of Tc of pH=5.5\(^{30}\), we decided to use pH of 5 in order to enable the electrostatic interaction between AuNCs and Tc molecules for Tc detection.

The same amount, different concentrations of tetracycline was added into gold nanocluster solution to observe the fluorescence quenching effect, as shown in Fig. 3. The fluorescent spectra of the samples (Fig.3A) show that the fluorescence intensity at 590 nm decreased with the Tc concentration increasing from 0.05mg/L to 50mg/L, which means that the fluorescence intensity of GSH-AuNCs is highly correlated with the concentration of Tc. A good linear relationship was fitted between the F/F0 and Tc concentration (the fitting follows y = 0.98-4.72e\(^{-5}\)x with R\(^2\) of 0.99) in the range of 50 μg/L~10mg/L (as shown in Fig. 3B) with the detection limit of 5.31 μg/L with a signal-to-noise ratio of 3. Table.1 shows a comparison of the fluorescence detection of tetracycline reported in papers published in recent years, which means our method has a good sensitivity of Tc detection.

The mechanism of how GSH-AuNCs response to Tc has been investigated. The zeta potential of GSH-AuNCs before and after addition of Tc was compared. An obvious change (from -6.27 to
-5.40) of the zeta potential was occurred when pH=5, which indicated the strong electrostatic interaction between AuNCs and Tc. Therefore, we make a hypothesis about the mechanism for the fluorescence quenching phenomenon. It is more likely that the electron transfer occurs between the tetracycline and gold nanoclusters due to electrostatic interactions.

AuNCs probes were also studied for the selectivity for Tc detection. Different kinds of organics and metal ions was added into the GSH-AuNCs, such as dopamine, Ni$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, EDTA-2Na etc., to evaluate the selectivity. Here 50 mg/L Tc solution was added into the solution of GSH-AuNCs, while the concentration of interfering substances are 100 times of Tc. As shown in Fig.4, the corresponding metal ions and organics F/F0 value are all about equal to 1, which means these substances do not interfere the detection and affect the fluorescence intensity of GSH-AuNCs. GSH-AuNCs have excellent selectivity for tetracycline among those interfering substances. These results clearly show that AuNCs is capable of specifically identifying Tc.

Detection of Tc in real samples

In order to evaluate real application ability of this method, it was used to the analysis of TC in pure water and tap water samples. In short, different amounts of Tc were added to the same samples of pure water and tap water, respectively. The TC concentrations detected in the tap water samples were calculated using standard curves and regression equations to obtain the corresponding recoveries and RSD. Importantly, as listed in Table S1, the average recovery in pure water samples and tap water sample performed by the standard addition method and the RSD were generally satisfactory.

Also, the GSH-AuNCs based test papers was prepared to detect Tc. The samples contained different concentrations of Tc were dropped on the test paper and the images were taken under the UV lamp. (Fig.5). As seen in Fig.5, different concentrations of Tc from 50mg/L to 1mg/L(Fig.5 B–E) result in a clear visible fluorescent quenching effect, which can be observed by naked eyes. Meanwhile, the same amount of DI water was dropped on test paper as a control.
The test paper can detect as low as 1mg/L Tc by naked eyes. Therefore, the present method has potential of practical applications of detecting Tc in real water samples.

Conclusions

In this study, GSH-AuNCs were synthesized as fluorescent probes to detect tetracycline by the quenching effect of tetracycline to the GSH-AuNCs. The detection limit was determined to be 5.31 μg/L. A portable AuNCs-based filter paper was also prepared by soaking filter paper in AuNCs solution. The difference can be easily distinguished by naked eyes when 1mg/L tetracycline was dropped on the test paper. With the good sensitivity and selectivity, our method holds potential to detect Tc in real samples.

Acknowledgements

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Table 1 The comparison of the proposed method with other reported methods of Tc fluorescence detection in recent years

| Analytical method       | Reaction   | Detection limit (μg/L) | Enhancement reagent | Refs. |
|-------------------------|------------|------------------------|---------------------|-------|
| Flow injection analysis | Aqueous solution | 0.03 | No | 31 |
| Chemiluminescence       | Aqueous solution | 22.2 | No | 32 |
| Fluorescence            | Aqueous solution | 8.88 | AgNPs+Eu³⁺ | 18 |
| Fluorescence            | Aqueous solution | 20  | CdTe | 33 |
| Fluorescence            | Aqueous solution | 5.31 | AuNCs | This work |
Figure Captions

Fig. 1 HR-TEM images (A, B) of GSH-AuNCs and single AuNC. The inset of A is the size distributions of GSH-AuNCs. (C) (a) UV–vis absorption spectrum and (b) photoluminescent spectrum (ex = 365 nm) of prepared AuNCs:. The inset is the photographs of AuNCs solution under room light (left) and UV-lamp (right), respectively.

Fig. 2 Effect of pH on fluorescence intensity of GSH-AuNCs

Fig. 3 (A) Fluorescence emission spectra of the GSH-AuNCs in the presence of increasing Tc concentrations (0.05-50mg/L). (B) the linear plot of the $F/F_0$ at 590 nm as a function of the Tc concentration (0.05–10 mg/L).

Fig. 4 Selectivity of the GSH-AuNCs in the presence of 50 mg/L Tc or 5g/L other analytes. $F_0$ and F are the fluorescence intensity of GSH-AuNCs in the absence and presence of Tc and the other analytes, respectively.

Fig. 5 The GSH-AuNCs based test papers dropped with different concentration of Tc. Dropped with water(A). Dropped with water(A), dropped with 1mg/L Tc(B), 2.5mg/L Tc (C) 5mg/L Tc(D), 50mg/L Tc (E).
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