Colorimetric detection of Cr\(^{3+}\) based on gold nanoparticles functionalized with 4-mercaptobenzoic acid

Zhikun Zhang*, Xiaojie Ye, Qingqing Liu, Yumin Liu* and Runjing Liu*

Abstract

Herein, we report the construction of a colorimetric probe used to detecting Cr\(^{3+}\) ions in aqueous solution based on functionalized gold nanoparticles. We investigated 4-mercaptobenzoic acid, 4-nitrobenzenethiol, and a mixture of 4-mercaptobenzoic acid and 4-nitrobenzenethiol as ligands for Cr\(^{3+}\) ions to functionalize the gold nanoparticles, respectively. The results showed that the three probes were all aggregated in the presence of Cr\(^{3+}\) ions, which induces a color change from ruby to violet. Moreover, gold nanoparticles modified with 4-mercaptobenzoic acid exhibit a higher response toward Cr\(^{3+}\) than the two other probes, which can be detected by the naked eye and UV-vis absorption spectroscopy. The detection time was rapid (within 25 min). A linear relationship was obtained from 20 to 25 \(\mu\)M between the ratio of the absorbance observed at 635 nm and 520 nm (A\(_{635}\) nm/A\(_{520}\) nm) with the limit of detection was 5 \(\times\) 10\(^{-6}\) M. This method exhibited excellent selectivity for Cr\(^{3+}\) ions over other tested heavy metal ions, anions, and organic molecules in the absence of another shielding reagent of metal ion. The system was successfully utilized to detect Cr\(^{3+}\) ions in simulated samples.

Keywords: Chromium III, Gold nanoparticles, 4-Mercaptobenzoic acid, Colorimetric detection

Introduction

Chromium is extensively applied in various fields, including steel works (Gomez et al. 2006; Parlayici, et al. 2019), electroplating, tanning industry, and chemical industries. This extensive use has resulted in environmental pollution (Shuang et al. 2017; Wei, et al. 2014). Cr\(^{3+}\) is an essential trace nutrient that is regulated at normal levels for human’s health (Gómez et al. 2006). An abnormal level of Cr\(^{3+}\) affects DNA’s function for protein synthesis and damages biomacromolecules (Glimsman et al. 1966; Wei et al. 2014); it can also induce diabetes and cardiovascular disease (Anderson 1986; Chen et al. 2011). Therefore, the detection of Cr\(^{3+}\) ions in drinking water is of great significance to ensure human health. Traditional analytical technologies mainly utilize atomic absorption spectrometry (Mashhadizadeh et al. 2013), chemiluminescence (Yang et al. 2003), high-performance liquid chromatography (Cathum et al. 2002), fluorescence spectroscopy (Chen et al. 2014), and electrochemical methods (Wei et al. 2007). These are expensive, time-consuming, and require complicated instrumentation. Therefore, the development of a simply and selective methods for the detection Cr\(^{3+}\) ions is urgently required. Metal nanoparticles have been widely utilized for biosensing due to their excellent optical properties (Guo et al. 2011; Du, et al. 2013). Gold nanoparticles (AuNPs) functionalized with organic molecules exhibit a colorimetric change according to their aggregation and dispersion (Anderson 2014; Upadhyay, et al. 2018). Herein, we have investigated the single/collaborative behavior of 4-mercaptobenzoic acid (4-MBA) and 4-nitrobenzenethiol (4-NPT) for functionalizing AuNPs in Cr\(^{3+}\) ions detection. 4-MBA and 4-NBT contained a -SH group, which interacts with AuNPs via a covalent bond.
Additionally, the -NO_2 group in 4-NBT and -COOH group in 4-MBA are both modified on the surface of the AuNPs together. These groups have a strong affinity to metal ions (Hemmateenejad et al. 2015; Lin, et al. 2002). Functionalized AuNPs are aggregated in the presence of Cr^{3+} ion via the ion-templated chelation, resulting in an appreciable color changes that can be detected by the color by the naked eyes and UV-vis spectroscopy.

**Experimental**

**Materials**

All chemicals used were of analytical grade. Hydrogen tetrachloroauroate hydrate (HAuCl_4·4H_2O) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai China). 4-Mercaptobenzoic acid was purchased from Tokyo Chemical Industry Co., Ltd (Japan). 4-Nitrobenzenethiol and melamine were obtained from Sigma-Aldrich (USA). Cr(NO_3)_3·9H_2O and other metal ions were purchased from Beijing Chemical Company (Beijing, China). All the solutions were prepared using purified water with a resistance of 18 MΩ·cm. UV–Vis absorption spectroscopy was recorded on a UV-2550 spectrophotometer (Shimadzu, Japan), using a 1-cm pathlength quartz cuvettes for measurements. Transmission electron microscopy (TEM) was performed on an H-7500 instrument (Hitachi, Japan) operated at 80 kV.

**Preparation of AuNPs functionalized with 4-MBA and 4-NPT**

We utilized the method of the chemical reduction of HAuCl_4 to prepare citrate-capped AuNPs (Ji, et al. 2007). Briefly, 15 mL of 38.8 × 10^{-3} mol L^{-1} solution of trisodium citrate was rapidly added into 150 mL 1.0 × 10^{-3} mol L^{-1} solution of HAuCl_4 heated at reflux under vigorously magnetic stirring. The mixed solution was heated under reflux with stirring for another 30 min to produce a ruby red colored solution. Then, we used 4-MBA, 4-NTP, and a 4-MBA/4-NTP mixture were used as capping agents to functionalize the AuNPs. Typically, stock solutions containing 1 × 10^{-4} M 4-MBA, 4-NTP, and 4-MBA/4-NTP were prepared using purified water. 1.0 mL of 10^{-4} M the above-mentioned solutions was added into 100 mL of the AuNPs solution, and the resulting mixture was equilibrated at the room temperature for 30 min to ensure the self-assembly of 4-MBA and 4-NBT on the surface of the AuNPs.

**Detection of Cr^{3+} ions**

The colorimetric detection of Cr^{3+} was carried out using functionalized AuNPs. Typically, 300 μL of an aqueous solution of Cr^{3+} prepared at different concentrations was mixed with 2.7 mL of the AuNPs solution. Subsequently, the mixture was incubated for 10 min at the room temperature, and the absorbance and absorbance ratio (A_{635 nm}/A_{520 nm}) were recorded. Meanwhile, the effect of Cr^{3+} on the absorbance of 4-MBA and 4-NPT was observed.
of pH value on detection of Cr$^{3+}$ was investigated. We employed 0.1-M sodium hydroxide (NaOH) or 0.1-M hydrochloric acid (HCl) to adjust pH value of the solution of 4-MBA modified AuNPs from 6 to 10. Then, 300 μL of aqueous solutions of Cr$^{3+}$ ions were added into 2.7 mL of the 4-MBA-AuNPs solution with the various pH value, respectively. Finally, the process of the mixture was the same as detection of Cr$^{3+}$ ions. The final concentration of Cr$^{3+}$ ions were all 20 μM.

Selectivity Cr$^{3+}$ ions
The selectivity of detection for Cr$^{3+}$ was investigated. 150 μL of an aqueous solution of Cr$^{3+}$ and 150 μL of an aqueous solution of interferents were mixed with 2.7 mL of the 4-MBA-AuNPs solution. The final concentration of Cr$^{3+}$ and interferents were all 1 × 10$^{-4}$ M. Then, the process of the mixture was the same as detection of Cr$^{3+}$ ions.

Result and discussion
Sensing mechanism
Our detecting system contained three key parts: The target ion (Cr$^{3+}$), probe (functionalized Au NPs), and signal readout (UV-vis absorption and colorimetric reaction). The probe is aggregated in the presence of Cr$^{3+}$ ions, which is accompanied by a color change. We have proposed a mechanism for this phenomenon in Fig. 1. The surface of the AuNPs linked with 4-MBA and 4-NPT, containing the carboxyl group and nitro group. The two groups have a strong affinity to Cr$^{3+}$ ions (Hemmateenejad et al. 2015; Lin et al. 2002), which induced the neighboring AuNPs to become closer and finally aggregated with one another. This results in an appreciable change in their color and absorption properties.

Optimal ligand for the functionalized AuNPs
According to the previous studies (Zhang et al. 2015), 4-MBA and 4-NTP have higher coordination properties with Cr$^{3+}$ ions. To choose the optimal ligand in our study, we also utilized 4-MBA and 4-NTP to functionalize the AuNPs for the sensitively detecting of Cr$^{3+}$ ions. The Fig. 2 shows that the 4-MBA-AuNPs exhibit a higher response than 4-NTP-AuNPs and 4-MBA/4-NTP-AuNPs. The 4-NTP and the mixture of 4-MBA and 4-NTP modified AuNPs have lower sensitivity than...
that of 4-MBA-AuNPs. This result indicates that 4-NTP decreases the sensitivity of the probe toward the detection of Cr$^{3+}$ ions. Therefore, we chose 4-MBA as the ligand to functionalize the AuNPs for the detection of Cr$^{3+}$. Meanwhile, the FT-IR spectra recorded for pure 4-MBA and the 4-MBA modified AuNPs (4-MBA-AuNPs) are shown in Fig. 3a. When comparing these FT-IR spectra, the characteristic absorption peak of –SH at 2524 cm$^{-1}$ in pure 4-MBA had disappears in the FT-IR spectrum of 4-MBA-AuNPs. This indicates that 4-MBA had been successfully modified onto the surface of the AuNPs via the –SH group in 4-MBA, which is similar to that previously report in the literature (Zhou et al. 2011). Besides, the 4-MBA-AuNPs has the stable optical properties (Fig. 3b), which is conducive to analysis for Cr$^{3+}$.

The 4-MBA modified AuNPs are stable and well-dispersed (Fig. 4a). The mean size of 4-MBA-AuNPs was estimated to be 20 nm from their size distribution using TEM image (Fig. 4). Direct evidence for the Cr$^{3+}$-induced aggregation of the 4-MBA-AuNPs could be further supported by TEM measurements. Figure 3 shows the TEM images of the 4-MBA-AuNPs in the absence and presence of a 4 $\times$ 10$^{-6}$ M aqueous solution of Cr$^{3+}$ ions. In the absence of Cr$^{3+}$, the 4-MBA-AuNPs were well-dispersed in the aqueous solution. On the other hand, 4-MBA-AuNPs were aggregated when added to an aqueous solution of Cr$^{3+}$ ions (Fig. 4b). These results clearly indicate that the addition of trace amounts Cr$^{3+}$ ions can readily lead to the aggregation of the modified AuNPs.

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![Fig. 5 UV-vis spectra of 4-MBA-AuNPs solutions in the presence of Cr$^{3+}$ with different pH ranging from 6.0 to 10](image-url)

![Fig. 6 The detection of Cr$^{3+}$ using the modified AuNP. a The changing in the UV-vis spectra recorded for the 4-MBA modified AuNPs upon increasing of the Cr$^{3+}$ concentration. b The relationship between $A_{635\text{nm}}$/$A_{520\text{nm}}$ and the Cr$^{3+}$ concentration, Inset: the linear calibration curve](image-url)
Effect of pH
The pH condition for colorimetric detection of Cr\(^{3+}\) was optimized over the range from 6.0 to 10.0. As shown in Fig. 5, when the concentration of Cr\(^{3+}\) was 20 μM, the modified AuNPs show obvious UV–vis spectroscopy absorption changes at pH 6. However, at the higher pH, the absorption showed a little change. Therefore, pH 6 was selected for further experiments considering the preferable sensitivity.

Limit of detection
The color change of the 4-MBA modified AuNPs induced by Cr\(^{3+}\) can be detected using UV-vis absorption spectroscopy. Upon the addition of Cr\(^{3+}\), the absorbance of the 4-MBA-AuNPs observed at 520 nm decreased, and a new absorption band appears at 635 nm appeared. Increasing the concentrations of Cr\(^{3+}\) led to the absorbance at 635 nm increasing and a concomitant decrease in the SPR peak observed at 520 nm (Fig. 6). Accordingly, the color of the AuNPs progressively changes from ruby red to purple and finally to blue. The ratio of the absorbance observed at \(A_{635}\) and \(A_{520}\) nm was used for the quantitative analysis of Cr\(^{3+}\). A linear correlation was observed between the absorption ratio (\(A_{635}\) nm/\(A_{520}\) nm), and the Cr\(^{3+}\) concentration within the range of 20–25 μM. The limit of detection was 5 × 10\(^{-6}\) M (S/N).

To further find the performance of the proposed method, a comparison with other methods is shown in Table 1. Table 1 indicated that the proposed method exhibited lower detection limit.

The optimal of reaction time
The detection time is a key factor that needs to be determined. We studied the kinetics of the detection step (Fig. 7). From 0 to 10 min, the \(A_{635\text{nm}}/A_{520\text{nm}}\) value increased with a steep slope; after 10 min, the value reached a plateau. Thus, our detection can be completed in less than 30 min, indicating it is a rapid probe.

Selective detection of Cr\(^{3+}\) using 4-MBA modified AuNPs and simulated samples detection
In comparison with the laboratory-made samples, real aqueous solution contains more and real interferents, which affect the detection results. Therefore, the selectivity of the sensor is important towards the detection Cr\(^{3+}\). We studied the selectivity of the sensor against several interferents in an aqueous solution, including Al\(^{3+}\), Ca\(^{2+}\), Fe\(^{2+}\), K\(^+\), Mg\(^{2+}\), Mn\(^{2+}\), Na\(^+\), Zn\(^{2+}\), NO\(_3\)\(^-\), SO\(_4\)\(^{2-}\), carbamide, and glucose (Fig. 8). Upon the addition of the interferents, the absorbance at 520 nm showed a

### Table 1: Comparison of other methods for the detection of Cr\(^{3+}\)

| Methods                        | Limit of detection (nM) | Detection range (nM) | References                      |
|--------------------------------|------------------------|----------------------|---------------------------------|
| Flame atomic absorption spectrometry | 12                     | 38 – 1.1 × 10\(^3\)  | Chwastowska et al. 2005         |
| Fluorescence                   | 1.7 × 10\(^3\)         | -                    | Panda et al. 2011               |
| Pyridoxal conjugated AuNPs assay | 11.5 × 10\(^3\)       | 7.5 – 1.3 × 10\(^{-5}\) M | Bothra et al. 2017              |
| Rhodamine capped AuNPs assay   | 9.3 × 10\(^3\)         | -                    | Manjubaashini et al. 2018       |
| This colorimetric assay        | 5.0 × 10\(^3\)         | 20 – 25 × 10\(^3\)   | This work                       |

Fig. 7 The effect of the reaction time on the detection of Cr\(^{3+}\). a The UV-Vis spectra of 4-MBA modified AuNPs solutions with reaction time from 0 to 30 min. b The plot of the absorption ratio (\(A_{635\text{nm}}\)/\(A_{520\text{nm}}\)) versus reaction time. The Cr\(^{3+}\) concentration was 1 × 10\(^{-6}\) M.
slight change; however, a new absorption band did not appear. Therefore, no obvious effect on our sensor system was observed. Clearly, 4-MBA-AuNPs showed high selectivity for Cr$^{3+}$ over the interferents studied. The spectral and color changes observed for 4-MBA-AuNPs upon the addition of Cr$^{3+}$ can be explained well by the aggregation of the AuNPs via the coordination interaction between Cr$^{3+}$ and 4-MBA. The 4-MBA-AuNPs were stabilized in the solution because the 4-MBA ligands on the surface of the AuNPs protected them from aggregation. 4-MBA has $–$COOH groups, which can be used to bind with the metal ions.

To assess the applicability of this colorimetric sensor for the analysis of real samples, an aqueous solution of Cr$^{3+}$ was added into the interferents solution to prepare simulated samples that were then detected utilizing our Cr$^{3+}$-induced colorimetric method. The results were shown that the simulated samples induced the colorimetric reaction that can be quantified by using UV-vis absorption spectroscopy (Fig. 9). This indicates that the colorimetric
detection of Cr$^{3+}$ is a practical tool for the determination of Cr$^{3+}$ ions in real samples.

Conclusions
In conclusions, we studied 4-MBA, 4-NTP, and a mixture of 4-MBA and 4-NTP as ligand to modify the AuNPs for detection of Cr$^{3+}$ ions. The results showed that 4-MBA modified AuNPs exhibit a higher response and selectivity toward Cr$^{3+}$ via the carboxyl group in 4-MBA. The detection can be observed by the naked eye or the UV–Vis absorption spectroscopy. The detection can be completed within 25 min over a linear range from 20 to 25 μM with the detection of limit of 5 μM. Therefore, our probe achieved rapid and sensitivity detection of Cr$^{3+}$.

Abbreviations
4-MBA: 4-Mercaptobenzoic acid; 4-NTP: 4-Nitrothiophenol; AuNPs: Gold nanoparticles

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Not applicable.

Authors’ contributions
ZKZ, YML, and RJL designed the experiment. ZKZ and YXJ carried out the experimental studies and collection, analysis, and interpretation of data. ZKZ wrote the manuscript. QQL helped to draft and revise the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Research data have been provided in the manuscript.

Competing interests
The authors declare that they have no competing interests.

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