Letters to Analytical Chemistry

Highly Sensitive, Colorimetric Detection of Mercury(II) in Aqueous Media by Quaternary Ammonium Group-Capped Gold Nanoparticles at Room Temperature

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We provide a highly sensitive and selective assay to detect Hg\textsuperscript{2+} in aqueous solutions using gold nanoparticles modified with quaternary ammonium group-terminated thiols at room temperature. The mechanism is the abstraction of thiols by Hg\textsuperscript{2+} that led to the aggregation of nanoparticles. With the assistance of solar light irradiation, the detection limit can be as low as 30 nM, which satisfies the guideline concentration for mercury in drinking water set by the WHO. In addition, the dynamic range of detection is wide (3 × 10\textsuperscript{-8}–1 × 10\textsuperscript{-2} M). This range, to our best knowledge, is the widest one that has been reported so far in gold nanoparticle (AuNP)-based assays for Hg\textsuperscript{2+}.

We report a simple method to detect Hg\textsuperscript{2+} in aqueous media by quaternary ammonium group-capped gold nanoparticles (QA-AuNPs). Hg\textsuperscript{2+} poses severe threats to both human health and the environment.\textsuperscript{1} Long-term exposure to high levels of Hg\textsuperscript{2+}-based toxins leads to serious and permanent damage of the central nervous system and other organs.\textsuperscript{2} Many of the settings required for such assays lack advanced resources, such as electricity. Highly sensitive and selective assays for Hg\textsuperscript{2+}, without resorting to advanced instruments are urgently needed. Researchers have published a number of methods for detecting Hg\textsuperscript{2+}, based on chemical sensors using small organic molecules,\textsuperscript{3} thin films,\textsuperscript{4,5} electrochemistry methods,\textsuperscript{6,7} polymeric materials,\textsuperscript{8} oligonucleotides,\textsuperscript{9,10} proteins,\textsuperscript{11} inductively coupled plasma-atomic emission spectrometry,\textsuperscript{12} and atomic absorption spectroscopy.\textsuperscript{13} Most of these methods, however, have limitations with respect to sensitivity and selectivity or require complex instrumentation or at least electricity. In particular, methods that require no sophisticated starting materials and allow visual readout might be very useful for detecting Hg\textsuperscript{2+} in resource-poor settings. AuNPs are increasingly employed for a wide spectrum of biological and biomedical applications.\textsuperscript{14–18} Colorimetric assays based on AuNPs have attracted increasing consideration on account of their unique and size-dependent optical and electronic properties. Recently, DNA-functionalized AuNPs have been widely used as colorimetric sensors for a variety of targets, including metallic ions.\textsuperscript{19–23} The thymine (T) bases in DNA sequences endow DNA–AuNP assays excellent selectivity for Hg\textsuperscript{2+} that can interact with T–T mismatches to form T–Hg\textsuperscript{2+}–T complexes. However, most DNA–AuNP assays rely on accurate control of the detection conditions, such as temperature. In addition, DNA can be costly and difficult to handle. Another kind of

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colorimetric assay for Hg^{2+} is based on acid-capped AuNPs.\textsuperscript{24} The ion-templated chelation between the acid groups on AuNPs and Hg^{2+} can induce the aggregation of AuNPs. Acid-capped AuNPs are simple but often lack sufficient selectivity for Hg^{2+}, because other kinds of metallic ions such as Cd^{2+} and Pb^{2+} can readily interact with acid groups to cause the aggregation of acid-capped AuNPs. To improve the selectivity, it is indispensable to add 2,6-pyridinedicarboxylic acid to mask Cd^{2+} and Pb^{2+}.\textsuperscript{25} Tween 20-modified AuNPs have been reported as another simple colorimetric assay for Hg^{2+} by the reduction of Hg^{2+} to form Hg–Au alloys, but Ag^{+} also can be reduced and absorbed on the surface of the AuNPs to cause AuNP aggregate in high-ionic-strength solutions.\textsuperscript{26} Thus, the above-discussed AuNPs may not be convenient enough for detecting Hg^{2+}. More details about the AuNPs-based assays for Hg^{2+} are summarized in Table S1, Supporting Information. The limitations of the classical AuNP-based assays for Hg^{2+} encourage us to develop a simpler approach for detecting Hg^{2+} with better sensitivity and selectivity.

In this study, we demonstrate that QA-AuNPs can detect Hg^{2+} in aqueous solutions at room temperature without the addition of any masking agents. The excellent selectivity of this system can be expected by comparing the stability constant (log K) of metallic ions with a model small molecule thiol. We note that the log K of Hg(cysteine) is ca. 43.5, whereas those of Co^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, Cu^{2+}, and Au^{+} are ca. 16, 18, 17, 19, 12, and 4, respectively.\textsuperscript{27} Additionally, the log K of Hg(SCN)\textsubscript{n} is ca. 21.8, whereas those of Co^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, Cu^{2+}, and Au^{+} are ca. 1.72, 2.0, 2.8, 1.76, 1.48, 1.23, 1.31, 4.64, 3.08, 10.4, and 16.98, respectively.\textsuperscript{28} The log K of Hg(SCN)\textsubscript{n} is the only one that is larger than that of Au(SCN)\textsubscript{n}, –SCN is similar to –SH that can bind strongly onto the surfaces of AuNPs,\textsuperscript{29,30} so among the metallic ions, we expect only Hg^{2+} is capable of removing thiolates chemisorbed on Au surface, thus destabilizing a well-dispersed aqueous solution of AuNPs results in a naked eye-based assay for Hg^{2+} (Scheme 1).

We used the hydrophilic (11-mercapto-undecyl)-trimethylammonium (MTA) as the QA-terminated thiols in this study because it is water-soluble and positive in charge and acts as a stabilizing agent to disperse AuNPs in aqueous solutions.\textsuperscript{31–33} MTA capped onto Au surfaces via Au–S bonds to form QA-AuNPs by means of ligand exchange.\textsuperscript{24–27} Compared with AuNPs modified with ligands terminated in other types of functional groups such as oligo(ethylene glycol),\textsuperscript{38,39} zwitterion,\textsuperscript{40,41} and negatively charged groups,\textsuperscript{42,43} QA group-terminated thiols can stabilize the dispersity of AuNPs only in acidic aqueous solution due to the electrostatic repulsion between the QA cations and positively charged H^{+}. While in basic conditions, the QA-AuNPs aggregate rapidly because the QA cations on surfaces of AuNPs can bind with anions (OH\textsuperscript{–}) via dipole–dipole interactions,\textsuperscript{44} which decrease the electrostatic repulsion among AuNPs and, thus, cause the aggregation, resulting in the color change from red to blue immediately along with the appearance of the new absorption band at 600 nm (Figure S1, Supporting Information). Therefore, we choose acidic conditions for the following detection experiments.

As expected, QA-AuNPs quickly aggregated after the addition of Hg^{2+} (Scheme 1) in acidic solution. We reasoned that the electrostatic repulsion between the QA cations and the positively charged H^{+} and Hg^{2+} can accelerate the displacement reaction of Hg^{2+} with the thiols chemisorbed on the surfaces of AuNPs,

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making the abstraction of ligands from Au surfaces easy. The lack of sufficient charges or stabilizing agents on Au surfaces would induce the aggregation of AuNPs.\(^{(45)}\) The red color of AuNPs well-dispersed in aqueous solution was due to a plasmon peak around 520 nm with extremely high extinction coefficients \((2.7 \times 10^7 \text{ M}^{-1}\text{cm}^{-1})\), \(\times 1000\) times higher than those of organic dyes.\(^{(46)}\) In the presence of \(\text{Hg}^{2+}\), the color of the solution turned from red to blue immediately because of the red-shifted absorption band with decreased extinction coefficients.\(^{(47)}\) The extremely high affinity of thiols toward \(\text{Hg}^{2+}\) triggered the breakage of \(\text{Au}–\text{S}\) bonds on surfaces of AuNPs, causing \(\text{QA}-\text{terminated thiols to dissociate from Au surfaces.}\(^{(48)}\) Electrospray ionization mass spectrometry (ESI-MS; Figure S2, Supporting Information) of the products confirmed this abstraction mechanism. We compared samples before (Figure S2b, Supporting Information) and after (Figure S2a, Supporting Information) the addition of \(\text{Hg}^{2+}\) into the QA-AuNPs solutions, the distinct difference was the prominent peak at \(m/z = 245.2\) in Figure S2a (Supporting Information), which was attributed to the QA-terminated ligands dissociated from surfaces of AuNPs. X-ray photoelectron spectroscopy (XPS) data of QA-AuNPs also confirmed the abstraction mechanism: the ratios between \(\text{Au}/\text{S}, \text{Au}/\text{C},\) and \(\text{Au}/\text{N}\) before and after adding the \(\text{Hg}^{2+}\) match our expectations (Table S2, Supporting Information). With the addition of \(\text{Hg}^{2+}\), ligands left the surfaces of AuNPs, while the amount of AuNPs was unchanged; hence, the ratios of \(\text{Au}/\text{S}, \text{Au}/\text{C},\) and \(\text{Au}/\text{N}\) increased as the amount of ligands decreased on Au surfaces.

Additional analysis of the mass and charge on surfaces of AuNPs further confirm a mechanism of the abstraction of thiols from AuNPs. We used thermogravimetric analysis (TGA), which allowed for direct measurement of the weight content of organic layer on AuNPs,\(^{(34–36)}\) to investigate the amount of ligands before and after the addition of \(\text{Hg}^{2+}\). The TGA of well-dispersed QA-AuNPs revealed that the ligands on AuNPs accounted for approximately 7.06% of the mass of AuNPs, from which we can calculate the amount of ligands to be about 4500 molecules per AuNP. After adding \(\text{Hg}^{2+}\), the composition of ligands on AuNPs was determined to be 4.12% (Figure S3, Supporting Information). The remaining ligands on the aggregates of QA-AuNPs were calculated to be approximately 3000 molecules per AuNP, indicating the loss of 1500 molecules of ligands per AuNP. We used zeta potential measurements to compare the surface charge on AuNPs before and after the addition of \(\text{Hg}^{2+}\). Zeta potential correlated with the surface charge and the local environment of AuNPs. The zeta potential of well-dispersed QA-AuNPs was about 26 mV, while that of \(\text{Hg}^{2+}\)-induced aggregates of QA-AuNPs decreased to be approximately 20 mV (Figure S4, Supporting Information). The decrease of positive charges on QA-AuNPs displayed the \(\text{Hg}^{2+}\)-induced loss of ligands, which consistently agreed with the results that came from ESI-MS, XPS, and TGA.


We confirm that the color change of the solution of QA-AuNPs in the presence of \(\text{Hg}^{2+}\) is caused by the aggregation of AuNPs. In the UV–vis spectra of an aqueous solution of QA-AuNPs (1.5 mM, pH 1.0), there is an extinction band at 520 nm (Figure S5, Supporting Information). Upon the addition of 100 \(\mu\text{M}\) \(\text{Hg}^{2+}\), QA-AuNPs aggregate with a color change of the solution from red to blue within several seconds, while the absorption band red-shifts to about 600 nm with a broad peak. The intensity of the broad absorption band decreases gradually; a precipitate appears after 8 h, and the solution becomes nearly colorless with precipitates at the bottom of the bottle.

Next, we investigate the selectivity of this assay for \(\text{Hg}^{2+}\) by testing the response of the assay to other environmentally relevant metallic ions, including \(\text{Al}^{3+}, \text{Ba}^{2+}, \text{Ca}^{2+}, \text{Cd}^{2+}, \text{Co}^{2+}, \text{Cr}^{3+}, \text{Cu}^{2+}, \text{Fe}^{3+}, \text{Fe}^{3+}, \text{Hg}^{2+}, \text{K}^{+}, \text{Mg}^{2+}, \text{Mn}^{2+}, \text{Na}^{+}, \text{Ni}^{2+}, \text{Pb}^{2+},\) and \(\text{Zn}^{2+}\) (Figure 1b), each with a concentration of 100 \(\mu\text{M}\). Only \(\text{Hg}^{2+}\) causes the aggregation of QA-AuNPs, resulting in a color change from red to blue within several seconds. This selectivity can be visualized with the naked eye (Figure 1a). Figure 1a covers only a small fraction of the results: other samples show similar responses, and all are summarized in Figure 1b. In addition, organic mercury such as methyl mercury cannot induce aggregation. To further confirm that these AuNPs aggregate, we compare the TEM images of QA-AuNPs before and after incubation with \(\text{Hg}^{2+}\) (Figure 1c). The selectivity can be guaranteed when the concentrations of other metallic ions reach as high as 10 mM. None of the other ions seem to induce such aggregation as observed under TEM. This observation is also supported by the dynamic light scattering (DLS) data (Figure S6, Supporting Information).

The average hydrodynamic diameter of well-dispersed QA-AuNPs is 23.9 nm, while that of the \(\text{Hg}^{2+}\)-induced

![Figure 1](image-url)

**Figure 1.** Selectivity of this assay. (a) Color change of the solution in the presence of various representative metallic ions at concentrations of 100 \(\mu\text{M}\). (b) The change of the absorption bands at 520 nm for different metallic ions; (c) TEM images of QA-AuNPs before (c1) and after incubation with \(\text{Hg}^{2+}\) (c2).
with the increase of the concentration of Hg$^{2+}$, the detection limit can be significantly improved. With the assistance of solar light irradiation, the color changed to purple; a result also confirmed by the UV-vis spectra (Figure S8b, Supporting Information). We conclude that solar light irradiation has the capacity to accelerate the Hg$^{2+}$-induced dissociation of ligands from the surfaces of QA-AuNPs, thus causing the aggregation of QA-AuNPs.

The selectivity of the system was not influenced by solar light irradiation for 30 s. The selectivity was evaluated by testing the response of the same set of other metallic ions as those discussed in Figure 1. The concentrations of all ions were 500 nM, only Hg$^{2+}$ caused the color change of the AuNP solution from red to purple (Figure S9, Supporting Information).

To further investigate the potential practical application of this colorimetric assay, we tried to detect Hg$^{2+}$ in simulated polluted samples (by adding Hg$^{2+}$ into drinking water). Using drinking water to dissolve QA-AuNPs resulting in a red solution, upon the addition of Hg$^{2+}$ (at a final concentration of 10 µM) into drinking water (pH 1.0), the color of the solution turned from red to blue within several seconds. When 30 nM Hg$^{2+}$ is added, the guideline value set by the WHO, followed with 30 s of solar light irradiation, the color changed to purple; a result also confirmed by the UV-vis spectra (Figure S10, Supporting Information).

In conclusion, we designed a colorimetric method to detect Hg$^{2+}$ that offered advantages of simplicity, rapidity, high sensitivity, and selectivity compared with many reported AuNP-based approaches (Table S1, Supporting Information). Most of the materials used in this assay are inexpensive and available commercially, making this assay particularly useful for resource-poor settings. The lowest detectable concentration by the naked-eye was 30 nM, which satisfies the guidelines of drinking water set by the WHO. The dynamic range of detection is wide (3 × 10$^{-8}$–1 × 10$^{-2}$ M, Figure S11, Supporting Information). This range, to our knowledge, is the widest one that has been reported so far in AuNP-based assays for Hg$^{2+}$. Interestingly, biothiols such as cysteine can redisperse the Hg$^{2+}$-induced aggregates of QA-AuNPs, which can be potentially used to detect biothiols for the diagnosis of related diseases. We will investigate the

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detection of biothiols by the Hg$^{2+}$-induced aggregates of QA-AuNPs in future work. We hope that this type of assay will be useful for many settings, including assays based on lab-on-chip format, where highly sensitive assays requiring no advanced instrumentation are highly desired.$^{54-57}$

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**SUPPORTING INFORMATION AVAILABLE**

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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