

Rapid and ultrasensitive colorimetric detection of mercury(II) by chemically initiated aggregation of gold nanoparticles

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Abstract The article describes a method for rapid and visual determination of Hg(II) ion using unmodified gold nanoparticles (Au-NPs). It involves the addition of Au-NPs to a solution containing Hg(II) ions which, however, does not induce a color change. Next, a solution of lysine is added which induces the aggregation of the Au-NPs and causes the color of the solution to change from wine-red to purple. The whole on-site detection process can be executed in less than 15 min. Other amines (ethylenediamine, arginine, and melamine) were also investigated with respect to their capability to induce aggregation. Notably, only amines containing more than one amino group were found to be effective, but a 0.4 μM and pH 8 solution of lysine was found to give the best results. The detection limits for Hg (II) are 8.4 pM (for instrumental read-

out) and 10 pM (for visual read-out). To the best of our knowledge, this LOD is better than those reported for any other existing rapid screening methods. The assay is not interfered by the presence of other common metal ions even if present in 1000-fold excess over Hg(II) concentration. It was successfully applied to the determination of Hg(II) in spiked tap water samples. We perceive that this method provides an excellent tool for rapid and ultrasensitive on-site determination of Hg(II) ions at low cost, with relative ease and minimal operation.

Keywords Colorimetry · Gold nanoparticles · Visual inspection · Lysine-induced aggregation · Mercury-induced aggregation · Sensing mechanism investigation

Yinji Chen and Li Yao contributed equally to this work.

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Introduction

Global heavy metal contamination has continued to increase considerably with increasing industrial developments. Consequently, the high toxicity and the likely adverse effects of heavy metals on human health have been raising serious concern. Among the common heavy metals, mercury, especially the water soluble divalent mercuric ion (Hg(II)), has continued to receive considerable attention. Of the estimated 7500 t of mercury released into the environment, the aqueous mercury ion (Hg(II)) is the most common and stable form of mercury pollution [1, 2]. Furthermore, extensive studies have reported that trace mercury intake can induce both acute and chronic damages to human health [3]. More significantly, the bi-methylation of mercury ion by the microorganisms to form methylmercury (CH_3Hg^+) leads to bioaccumulation in fish tissues and lead to significant human exposure with dire health consequences as has been previously reported in Japan [4–6]. There has, therefore, been an increasing concern about the

potential toxicity of free and methylated mercury. This concern has, in turn, stimulated considerable interest in the reliable determination of mercury in various sample materials. In particular, there is a growing interest in the development of highly sensitive, selective and inexpensive analytical method for real-time and rapid monitoring of Hg levels in water, soil, plant and food samples without the need for advanced or costly instruments. The use of a colorimetric method offers one of the options for achieving this goal.

Classic colorimetric detection methods have been widely adopted for pH titration reaction between acidic and basic solutions, the identification of Fe(III) [7], and several other substrates. Colorimetric methods are also increasingly gaining use for routine bioassays with comparable or even higher sensitivity and better selectivity than conventional methods [8].

Recent developments have led to the use of metallic nanoparticles, such as gold and silver nanoparticles, as indicators for colorimetric methods and have attracted much interest in developing on-site detection methods because of their unique surface plasmon resonance properties [9–12]. In previous studies, we have successfully used AuNPs-based colorimetric strategies to develop rapid detection methods for melamine and bisphenol in food samples, respectively [13, 14]. Also, the use of chemical initiated aggregation of AuNPs has attracted considerable interest for colorimetric detection of mercury ions [15–18]. Usually, a specific T-rich ssDNA probe is employed for inducing aggregation of AuNPs and subsequent recognition [19–22]. However, the achievable sensitivity for the detection of mercury by this approach is still not sufficient for on-site or in-field monitoring of mercury. Further development of this strategy is therefore still necessary. In this regard, the activity of the chosen recognition probe is very crucial and it is known to be influenced by the chemical reaction and spatial hindrance [23]. Mirkin and co-researchers [24] have previously reported on the specific binding between mercury ions and cysteine which was used to accomplish the detection of cysteine. Recently, Denizli and co-researchers [11] reported a method for mercury ion detection based on the use of lysine as the “bridge molecule” to bind with mercury ions and induce the aggregation of AuNPs. However, the sensitivity and the sensing mechanism of the method were not investigated in sufficient details to permit a good understanding of the factors responsible for and/or influencing the detection of mercury ions by this approach. A more complete understanding of the mechanism of this approach is of paramount importance in fully exploiting the analytical capabilities it offers, particularly with respect to sensitivity, selectivity and detection limit.

In this study, we have carried out a detailed investigation of the broad mechanism involved in the chemical initiated aggregation of gold nanoparticles (AuNPs) in the presence of mercury as a basis for further improving its performance.

The selection of the desired recognition probes for ultrasensitive determination of mercury ion involved comparison of a number of amine-based chemicals to identify the most efficient for this purpose and for gaining some understanding of the underlying mechanism. The influence of solution pH on the effectiveness of the selected recognition probe was carefully investigated to determine its optimum concentration. The addition of the chosen amine-based chemical as the recognition probes in the presence of colloidal AuNPs was initially explored for visual detection of mercury ions based on induced color change. The detection of mercury by this approach was further extended to permit substantial improvement in sensitivity and detection limit by the use of a UV–vis spectrometer. Furthermore, the selectivity of our method was investigated by exposure to some common metal ions. Also, the application of the method to the determination of mercury in tap water and tea water samples was considered.

Experimental

Materials

Chloroauric acid (HAuCl_4) and sodium citrate were purchased from J & K Scientific Ltd (Shanghai, China, www.jkchemical.com). The standard mercury and lead samples were ordered from National Center of Standard Substrate, Beijing, China and treated according to the instructions. Lysine was obtained from Sangon Biotech (Shanghai, www.sangon.com) Co., Ltd. while arginine, ethylenediamine and melamine were from Siopharm Chemical Reagent Beijing Co., Ltd (Beijing, China, <http://www.reagent.com.cn>). Other reagents including nickel(II) chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), silver nitrate, calcium chloride and zinc sulfate were all purchased from Siopharm Chemical Reagent Beijing Co., Ltd (Beijing, China, <http://www.reagent.com.cn>). All other chemical reagents were of analytical grade and used directly without any further purifications. Millipore Milli-Q ultrapure ($>18 \text{ M}\Omega \text{ cm}$) water was used throughout the whole research.

Preparation of gold nanoparticles (AuNPs)

The AuNPs adopted for sensing were prepared in the laboratory according to our previously reported methods [13, 25]. Briefly, 1 % (w/w) sodium citrate solution was added rapidly to a boiling 0.1 % (w/w) chloroauric acid solution with vigorous stirring. After the color changes from transparent to dark blue and final red wine color, the reaction mixture was heated for another 10 min and cooled to room temperature without stirring. The concentration of sodium citrate was also varied to obtain the AuNPs with different diameters for optimal sensing

performance of mercury ions. The prepared AuNPs were characterized by the UV–vis spectrometry and dynamic light scattering (DLS).

Colorimetric detection of aqueous mercury ions and instrumental confirmation

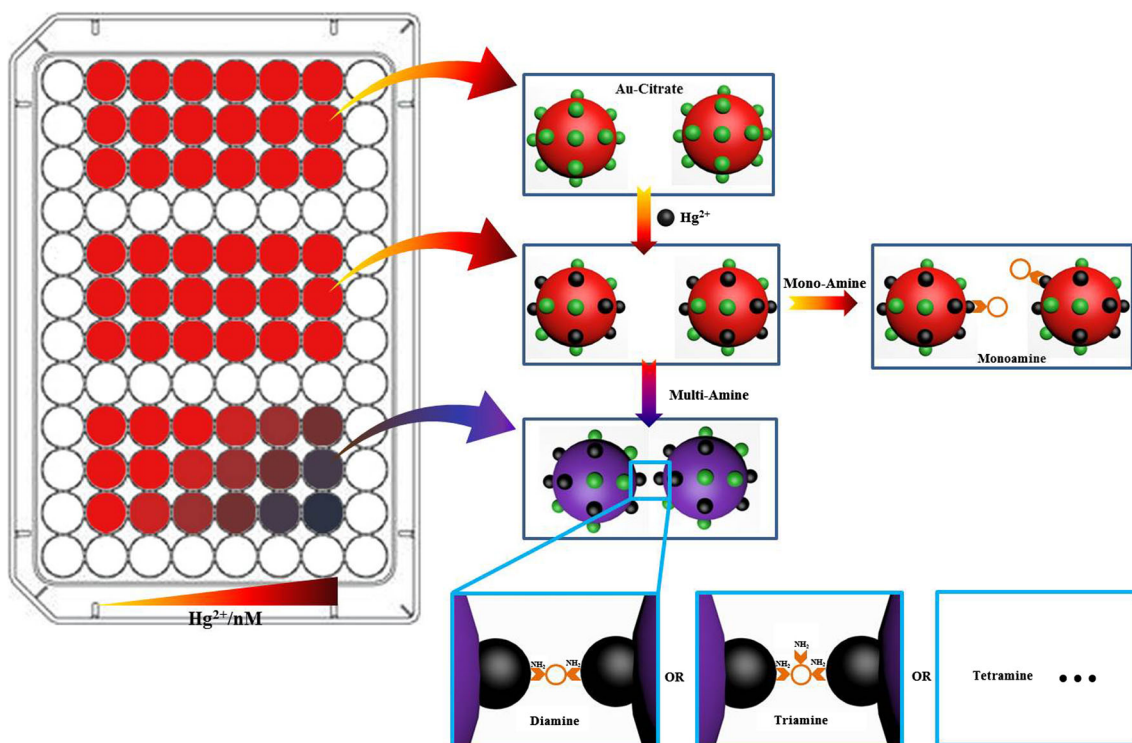
The AuNPs solutions were added into target mercury ion solution at different concentration and mixed by shaking for one minute. Then, the 10 μL 0.4 μM lysine solution was added to the mixture and further mixed by shaking for another one minute. The resulting color signal was observed visually or recorded by UV–vis spectrometry. Mercury concentrations in real spiked tap water and tea water samples were detected directly by this colorimetric method without any pretreatments. The spiked samples were transferred into the flask and diluted to 50 mL with 1 % HNO_3 solution and finally filtered with a membrane of 0.45 μm pore size before ICP-MS measurement.

Results and discussion

The sensing of mercury ions by the proposed strategy used in this study is based on a color change induced by an amine-assisted aggregation of AuNPs. As shown in scheme 1, the first step involves the addition of AuNPs to the solution containing mercury ions, which of its own does not induce a color

change of the AuNPs. This was followed by the addition of an amine-based chemical into the mixture to promote the aggregation of AuNPs which induces a color or signal change when mercury ions are present. The change in intensity of the color is dependent on the mercury ion concentration in the solution and, thus, provides a useful basis for qualitative and quantitative analysis. Consequently, the concentration of mercury ions can be determined by relating it to the signal intensity.

Firstly, the prepared AuNPs of different diameters were compared to choose the best one with optimum optical property for sensing. The AuNPs with different diameters have almost equal optical strength except the wavelength (See results in Electronic Supplementary Material, ESM). Considering the stability of the AuNPs, a diameter of 25 nm was adopted as the best AuNPs size for subsequent sensing investigations. To gain a better understanding of the sensing mechanism, we started with the structural analysis of lysine. With this, we established that the two amino groups in lysine play a key role in the aggregation induced colorimetric method. As discussed earlier, under the specific conditions, the amino group will be in the form of ammonium which will adsorb onto the surface of AuNPs by electrostatic attraction. However, the bridge effect between the amino group and mercury ion is the driving force of the aggregation of AuNPs. Therefore, it was expected that the number of amino group in the chosen chemicals will have an influence on the aggregation-based sensing. To test this hypothesis, we chose some chemicals containing different amino groups as the “bridge molecule”



Scheme 1 Microplate based detection scheme for rapid colorimetric detection of mercury ions

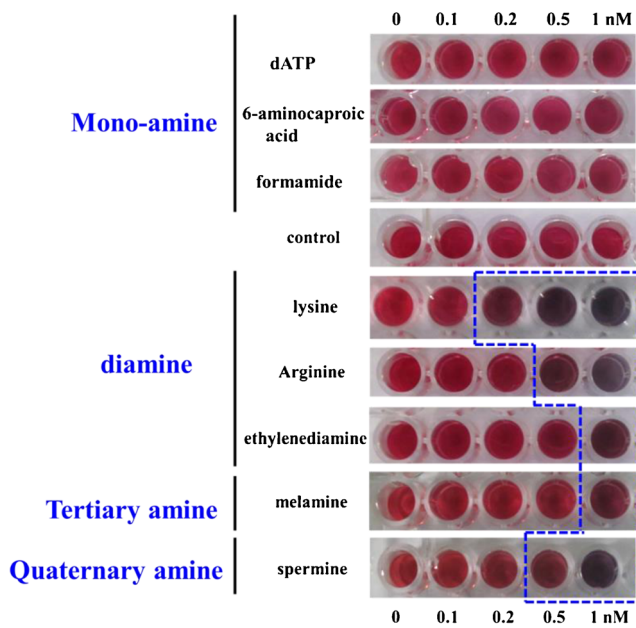


Fig. 1 Sensing mechanism study of the colorimetric protocol

for the sensing of the mercury ions. From the results shown in Fig. 1, it was obvious that only chemicals containing more than one amino group was effective as the “bridge molecule” for the AuNPs-based sensing of mercury ions. The protonated amino group of the chosen chemical would preferably bind with the negatively charged AuNPs, while the amino group would react with target mercury ions, which is the main basis of the bridge effect for the aggregation of AuNPs (See the chemical structures of lysine at different pH in Supporting Information Figure S12). Therefore, it can be concluded that only chemicals with more than one amino group can be used as the “bridge molecule”. Based on the comparison of the performance of the different amine-based chemicals and as illustrated in Scheme 1 and Fig. 1, compared with other bridge molecules, both the sensitivity and sensing range of lysine-assisted system are better than those of other molecules even under non-optimized conditions. Therefore, in this research for mercury(II) determination, lysine was chosen as the model recognition probe for ultrasensitive detection of mercury ions. In order to achieve optimal sensing performance with this

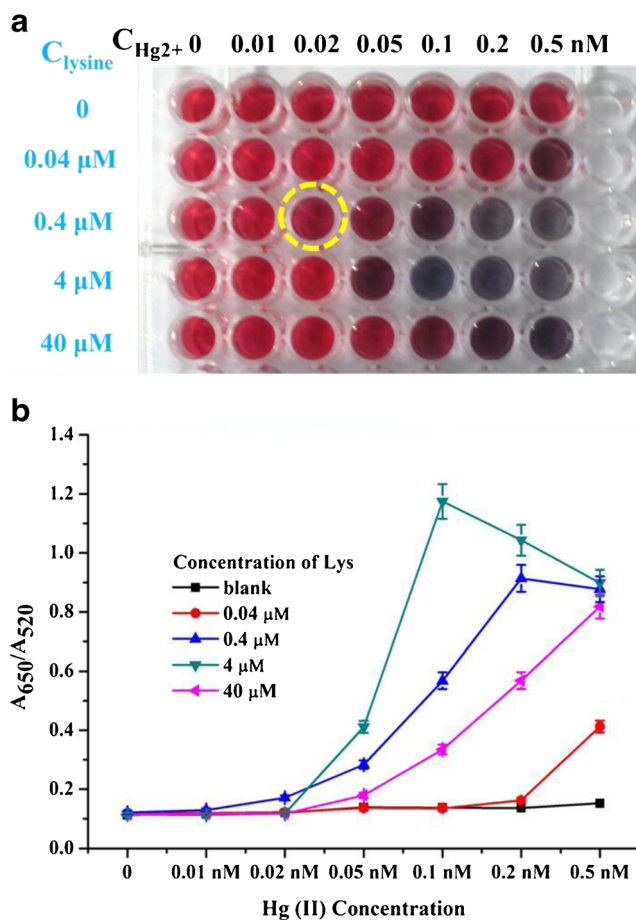


Fig. 2 Influence of lysine concentration on the sensing performance of mercury ions. **a** Colorimetric sensing results of mercury ion at different lysine concentrations; **b** Quantitative relationship between lysine concentrations and sensing performance

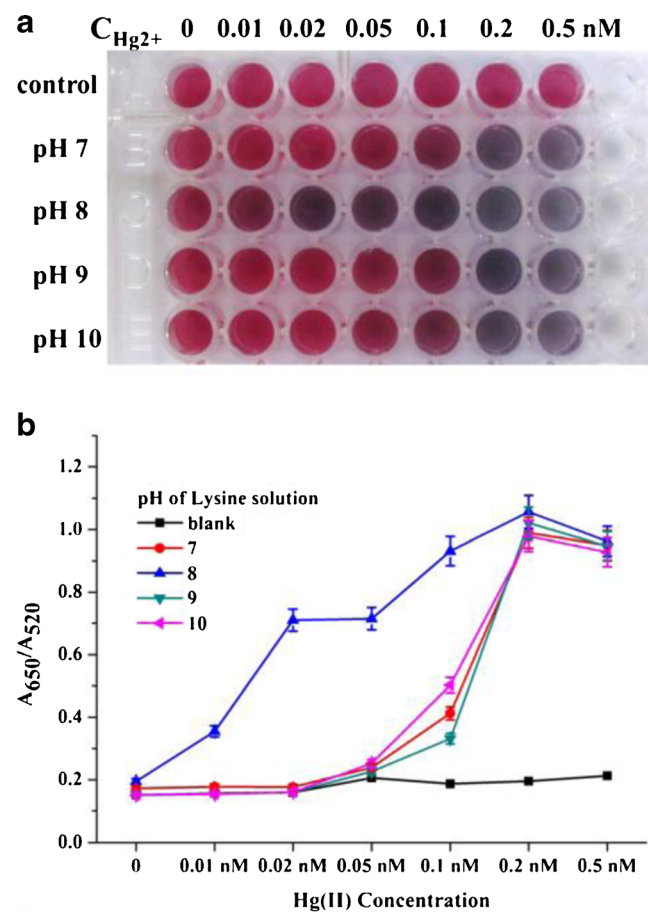


Fig. 3 Influence of pH of lysine solution on the detection of mercury ions. **a** Colorimetric mercury(II) sensing results of lysine-assisted method at different pH conditions; **b** Quantitative relationship between pH values and sensing performance

probe, the influence of several key factors were investigated and optimized. The results of these investigations are discussed in the following paragraphs.

The optimization of the required lysine concentration, as the chosen recognition probe for mercury ion sensing, is one of the key considerations. Figure 2a shows that no color change was observed with increasing mercury ion concentration when lysine was absent (line 1, control group). Thus, indicating that the aggregation of AuNPs in the presence of mercury ions was not possible without the addition of lysine. However, with the addition of lysine at different concentrations, a notable color change caused by the aggregation of AuNPs was clearly evident. More importantly, it was found that, at a lysine concentration of 0.4 μM , the presence of 0.02 nM mercury ions can be detected (yellow dashed circle in the third line). It is important to note that this lysine concentration was 1000-fold lower than used recently in another study [11]. The benefit of using the much lower lysine concentration was even more evident in Fig. 2b. Comparatively, the sensing ranges achieved in the presence of 4 and 40 μM lysine were narrower than that obtained with 0.4 μM lysine (See detailed UV-vis results at different concentrations of lysine in Supporting Information). For this reason, 0.4 μM lysine was employed for optimal mercury ion detection in all other investigations.

As the detection mechanism is based on the “bridge effect” of the amino group (from lysine) induced aggregation of AuNPs, it is likely that, as an amphipathic molecule, the effectiveness of lysine will be influenced by the pH of the

solution. Figure 3 shows that the sensitivity obtained for the detection of mercury ions varied considerably with increasing solution pH. More notably, the sensitivity obtained for mercury at very low concentrations (<0.1 nM) and the sensing range were distinctly higher and wider at pH 8 than at all other pH (See the detailed UV-vis spectrum at various pH in Supporting Information). This is due to the attachment of lysine (or other amino-contained chemicals) to AuNPs by an electrostatic effect. Under acidic conditions (pH<7), the amino group is present in the form of ammonia, which is good for the attachment of the bridge molecule on the AuNPs surface rather than by binding with mercury ions. When pH>pI=9.74, the net charge on the bridge molecule is negative and, hence, hinders the attachment to AuNPs. Therefore, at around pH 8, the attachment equilibrium and the competitive recognition with mercury ions gave the optimum response. Therefore, a solution pH of 8 was adopted for other investigations. Of note, considering above discussed optimization of bridge molecules, the pH of the sensing system and the pI of adopted different potential bridge molecules should be first optimized to achieve the best sensing performance.

Under the optimized detection conditions, the addition of different mercury ion concentrations was detected rapidly by the colorimetric method. As shown in Fig. 4a, in the control group (without “bridge molecule” lysine), there was no color change in the AuNPs solution in presence of different concentrations of mercury ions. While in the detection group, a color change in the AuNPs solution was observed from the original wine red color to a dark purple color, even in the presence of

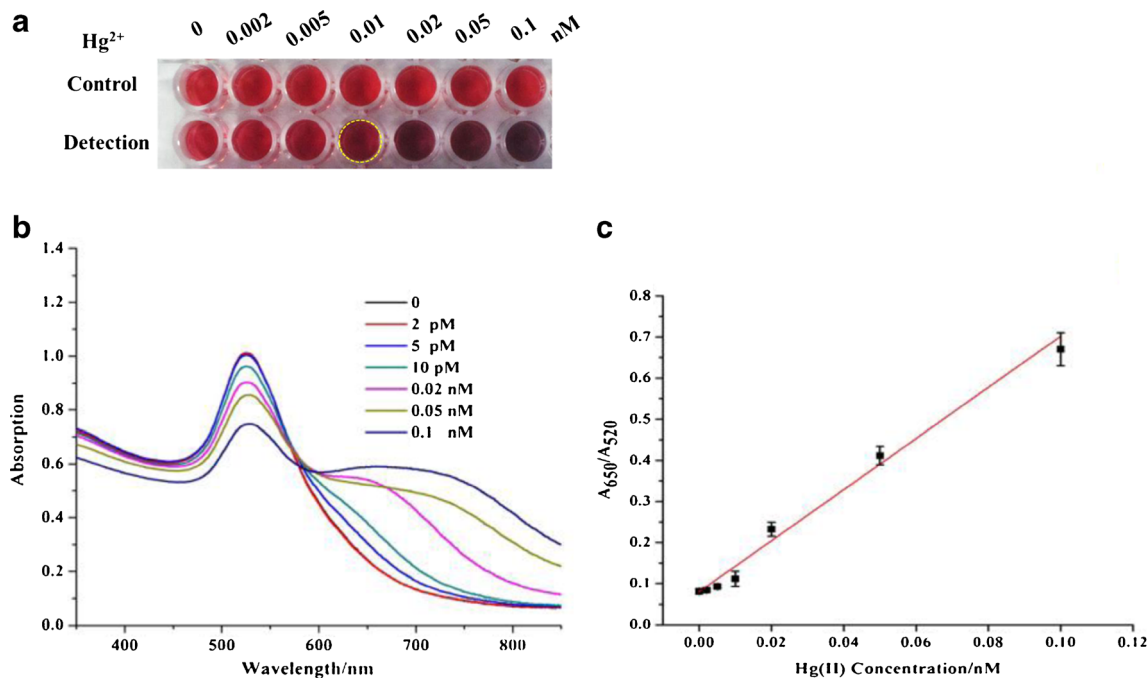


Fig. 4 Quantitative determination of mercury ions by the lysine-assisted method. **a** Colorimetric mercury(II) detection results under optimized conditions; **b** UV-vis results of the mercury(II) determination under optimized conditions; **c** Calibration curve for lysine-assisted colorimetric method

0.01 nM (0.002 ppb) mercury ions. Thus, this concentration is the visual limit of detection (v-LOD) of this method for mercury ion determination. The semi-quantitative determination results were further verified and analyzed by UV–vis spectroscopy, as shown in Fig. 4b. The characteristic peak of AuNPs solution is evident at 520 nm and the intensity of this peak decreased with increasing mercury ion concentration. However, a new characteristic peak associated with AuNPs was also observed initially at 650 nm and then further shifted to about 700 nm and the intensity of this new peak increased with increasing mercury ion concentration due to the aggregation of AuNPs in the sensing solution. The absorption intensity ratio of these two peaks (A_{650}/A_{520}) can be used to construct a calibration curve for aqueous mercury ion detection, as shown in Fig. 4c. Evidently, a linear relationship between the absorption intensity ratio and mercury concentration was observed from 0.002 to 0.1 nM with a linear regression correlation coefficient of 0.984. The LOD (3σ) achieved by this colorimetric sensing method was calculated to be 0.0084 nM (8.4 pM or 1.68 ppt). To the best of our knowledge, this LOD is better than those reported for any other existing rapid screening methods for mercury ion and related comparison results were summarized in Table S1 (See details in ESM). It is also comparable to our previously reported signal amplified dual-colorimetric method and some instrument-based methods [6]. Evidently, this method can readily detect the presence of aqueous mercury ions at ultra-low concentrations in an “Add-Read” one-step model without the need for any instruments or just by use of a conventional portable UV–vis monitor. Thus, it has significant potential for wide applications for on-site rapid screening of aqueous mercury ion concentrations.

Fig. 5 Selectivity test for various kinds of cations results of the developed amino-assisted method. **a** Effects of various cations. **b** Spectral changes caused by these ions. **c** Effect of ions on the ratio of absorbance at 650 and 520 nm

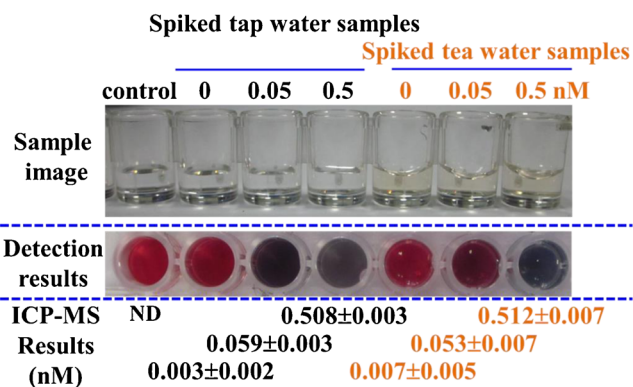
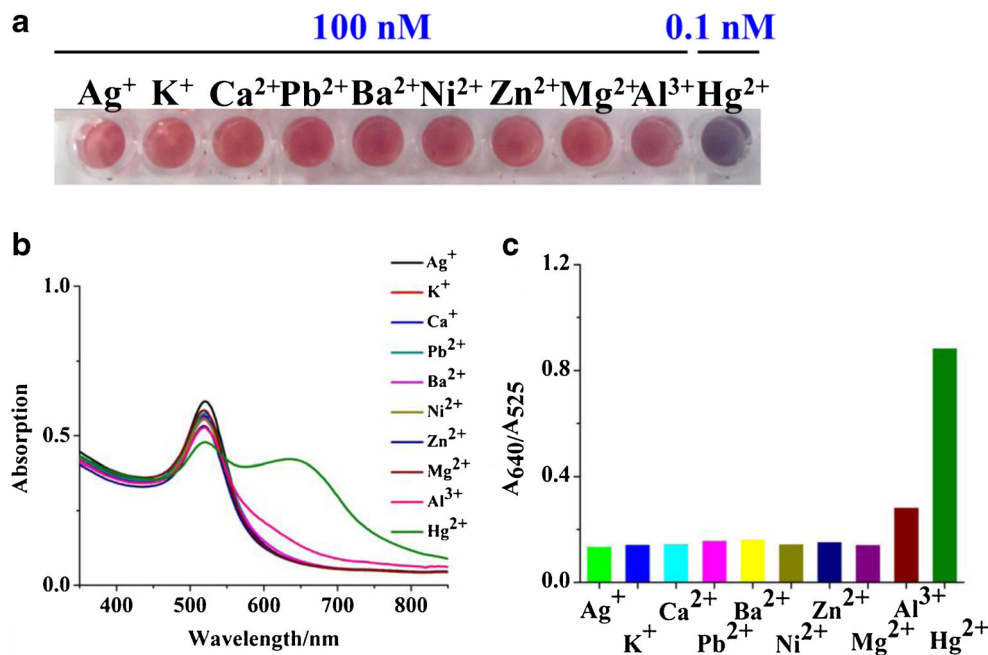


Fig. 6 Detection of mercury(II) ions in real spiked samples. Sample image shown the color of the real spiked tap water samples and spiked tea water samples; Middle detection results show the colorimetric results of spiked samples with the lysine-assisted method; ICP-MS results indicate the exact amount of mercury(II) in the spiked samples; (ND means not detected)

The selectivity of our method is also very good when tested in the presence of some common metal ions. As demonstrated in Fig. 5, most of the common metal ions including the mono-, di- and trivalent ions did not induce any signal (color) change of the AuNPs sensing solution even at 100 nM (1000 folds higher than mercury ion concentration). The UV–vis results in Fig. 5b further indicated that only the presence of mercury ions gave the additional characteristic peak of AuNPs solution at the higher wavelength. However, Al(III) ions also induced a slight shift of the original spectrum of AuNPs solution. This small variation of color signal did not interfere with the target mercury ion detection for two reasons. Firstly, the small color variation induced by Al(III) ions cannot be distinguished from the blank or control groups and, therefore, can be ignored.

Secondly, this small spectrum shift was induced in the presence of 100 nM Al(III) ions, which is much higher than the concentration range of this colorimetric method. It can, thus, be concluded that the selectivity of the colorimetric method is good enough for rapid and ultrasensitive detection of target mercury ions.

The application of the simple method to the determination of mercury ions in spiked tap water and tea water samples without any pretreatments is demonstrated in Fig. 6. In all cases, for the spiked tap water samples or spiked tea water samples, the results obtained with the colorimetric method are all highly in accordance with the classic instrumental method, demonstrating that this colorimetric method can be successfully applied for the detection of mercury ions in real samples.

Conclusions

Detailed and careful investigation of the broad mechanism involved in the chemical initiated aggregation of gold nanoparticles (AuNPs) in the presence of mercury has demonstrated that only amine-based chemicals containing more than one amino group are effective as the “bridge molecule” for the AuNPs-based sensing of mercury ions. The optimization of the solution pH enabled reduction of the required lysine concentration by 1000-fold at pH 8, while enabling the achievement of detection limits as low as 8.4 pM and 0.01 nM for quantitative and qualitative analysis, respectively. Excellent selectivity of this colorimetric method was clearly demonstrated in the presence of some common heavy metal ions at 1000-fold increase in concentration beyond that of mercury ions. The ultrasensitive colorimetric method was successfully applied to the determination of mercury ion in tap and tea water samples and the results agreed reasonably well with those obtained by ICP-MS measurements. Thus, this proposed method will provide an ideal approach for a simple and rapid on-site or in-field screening of mercury ions in aqueous samples with an extraordinary sensing performance and relatively low sensing cost, without the need for any expensive and/or complicated instruments.

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