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Colorimetric determination of mercury(II) using gold nanoparticles and double ligand exchange

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Abstract

A colorimetric assay is described for highly selective and sensitive determination of Hg(II) ions by using gold nanoparticles (AuNPs) functionalized with dithioerythritol (DETL). This method relies on the unique optical properties of DETL-functionalized AuNPs as well as the thiophilicity of both AuNPs and Hg(II). In the presence of DETL, the AuNPs aggregate due to ligand exchange between thiol groups of DETL and the citrate ions on the surface of AuNPs. This induces a color change from red to blue. On addition of Hg(II), the thiol groups preferably interact with Hg(II) rather than with AuNPs. Thus, the DETL is released from the surface of the AuNPs and binds to Hg(II). This triggers the redispersion of the AuNPs. The ratio of absorbances at 650 and 525 nm drops linearly in two Hg(II) concentration ranges (viz. from 0.1 to 0.5 μM, and from 0.5 to 5 μM). The ions Cu(II), Pb(II), and Cd(II) do not interfere even in the absence of masking agents. The detection limit is as low as 24 nM.

Introduction

Mercury (Hg^{2+}) is one of the most stable forms of mercury contamination [1]. Hg^{2+} displays strong affinity for the ligands containing sulfur atoms and induces the obstructing of sulfhydryl groups of membranes, enzymes, and proteins [2]. Hg^{2+} is also related to the abnormal functions of gastrointestinal tract, brain, kidney, and liver [3]. Owing to its serious hazardous effects to human safety and health, there is an urgent demand to develop a highly selective and sensitive method for determination of Hg^{2+} in aqueous media [4].

Methods such as inductively coupled plasma mass spectrometry [5], atomic absorption spectroscopy [6], and atomic fluorescence spectroscopy [7] etc., have been found to detect Hg^{2+}. Moreover, a great effort has been exerted to develop Hg^{2+} detection methods using fluorophores [8], organic chromophores [9], DNAzymes [10], oligonucleotides [11], and colorimetric methods [3, 4]. But there are still major disadvantages associated with some of these methods regarding their practical application: poor repeatability, sophisticated synthesis of probe materials and solubility in aqueous media.

Because their absorption coefficients are several orders of magnitude higher than those of common organic dyes in the visible region, gold nanoparticals (AuNPs) have been reported as a class of important colorimetric materials [12]. Simultaneously, the surface of AuNPs is easily modified by some ligands such as -SH, $-NH_2$ etc.. This can enhance the selectivity and sensitivity of AuNP-based colorimetric methods, and expands its application [13]. When the aggregation or redispersion of AuNPs occurs, the color turns blue or purple or returns red. This is because the nanoparticles coming in close proximity or separating to each other inducing the coupling of the plasmon absorbance.

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Employing this concept, researchers reported AuNP-based colorimetric assays for the determination of DNA [14], antibiotics [7, 13], and metal ions [15]. As for metal ion assays, the functional groups on AuNPs shows a critical role in the recognition properties. The one of the important tools to introduce the custom functionality on AuNPs is ligand exchange reaction. 4-mercaptophenylboronic acid [16], 4-amino-3-hydrazino-5-mercapto-1,2,4-triazol [17], and 6-Thioguanine [18] are used as capping reagents of AuNPs through ligand exchange reactions for the detection of Hg^{2+}, dopamine, and Hg^{2+}, respectively.

Comparing with major competing heavy metal ions such as Cd^{2+} and Pb^{2+}, Hg^{2+} shows stronger thiophilic tendency [18]. Therefore, many sulfur-based functional groups of chromophores and fluorescent receptors have selected for the selective determination of Hg^{2+} [19]. Inspired by these observations, Kim et al. designed a highly sensitive AuNP-based colorimetric detecting of Hg^{2+} through ligand exchange reaction [20]. With the addition of Hg^{2+}, the DETL modified AuNPs can quickly aggregate through specific sulfur-Hg^{2+}-sulfur interaction. However, this novel method is interfered by other metal ions including Cu^{2+} and Ba^{2+}. It needs masking agent ethylenediamine tetraacetic acid (EDTA) to eliminate interference. Moreover, Chen et al. designed a non-aggregation colorimetric detection for Hg^{2+} based on AuNPs and thiocyanuric acid (TGA) [21]. The AuNPs occurs aggregation due to the formation of Au-S bonds between three thiol groups of TGA and AuNPs. With the addition of Hg^{2+}, thiol group is more tend to interact with Hg^{2+} than AuNPs which induces AuNPs redispersion again. This non-aggregation method shows highly selectivity toward Hg^{2+} in the absence of masking agents [18]. According to those observations, we guess whether DETL might be used for the modification of AuNPs just like the TGA, and thus improve the selectivity. Fortunately, Tsai et al. reported a research about the controlled formation and characterization of DETL conjugated AuNPs [22]. The DETL conjugate with AuNPs can form cross-linking mode by control the concentration of DETL and AuNPs, and thus induce the aggregation of AuNPs. Based on the previous researches, we herein report an attractive highly selective nanoprobe for Hg^{2+} detection through twice ligand exchange reaction: (i) Au-S reaction exchange the citrate ions of surface of AuNPs; and (ii) specific Hg^{2+}-sulfur reaction exchange the thiol groups on the surface of dithioerythritol-modified AuNPs (DETL-AuNPs). This work is based on the formation of Au-S bonds between DETL and AuNPs, and higher affinity of DETL toward Hg^{2+} over AuNPs; such an idea has been attempted in this work for the development of Hg^{2+} nanoprobe in aqueous media. The designed colorimetric method shows highly selectivity toward Hg^{2+} in the absence of masking agents compared with previous works [20]. Hg^{2+} results in colorimetric change because of the change in SPR absorption.

Experimental section

Chemicals

Chloroauric acid hydrated ($HAuCl_4 \cdot 4H_2O$), trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (www.sinopharmholding.com, Shanghai, China). Dithioerythritol was purchased from TCL (www.macklin.cn, Shanghai, China). Mercuric nitrate was purchased from Xiya Chemical Industry Co., Ltd. (www.xiyashiji.com, Shandong, China). All the other chemicals were of analytical grade and used without further purification. Each experimental process was using ultrapure water (18.2 MΩ·cm, Milli-Q Millipore). All of the aqueous solutions were prepared with ultrapure water.

Apparatus

Colorimetric measurement was recorded by a UV–2700 spectrophotometer (www.shimadzu.com, Shimadzu corporation, Kyoto, Japan) to obtain the absorption spectra of reaction solution using a quartz cuvette (1-cm pathlength), and the transmission electron microscopy (TEM) images were performed on a TECNAI T20 G^2 electron microscope instrument operated at an accelerating voltage of 200 KV (www.antpedia.com, FEI, Netherland). The concentration of Hg^{2+} in the real samples was measured using atomic absorption spectroscopy (AAS).

Preparation of functionalized AuNPs with DETL

All glassware was soaked in a bath of freshly prepared aqua regia for 30 min, thoroughly washed with ultrapure water (18.2 MΩ·cm, Milli-Q Millipore), and finally dried in constant temperature oven before use.

AuNPs were prepared by citrate-mediated reduction of $HAuCl_4$ according to reported method with a bit modification [7, 15] (the detailed information please see in Electronic Supporting Material). The concentration of the prepared AuNPs was about 8.22 nM according to Beer-Lambert's low with an extinction coefficient of 2.78×10^8 M^{-1} cm^{-1} at 520 nm for the 13-nm AuNPs [7].

DETL-AuNPs were prepared by adding a certain amount of DETL into AuNPs solution [22] (the detailed information please see in Electronic Supporting Material).

Scheme 1 Colorimetric detecting mechanism of Hg^{2+} in the presence of AuNPs and dithioerythritol based on double ligand exchange reaction
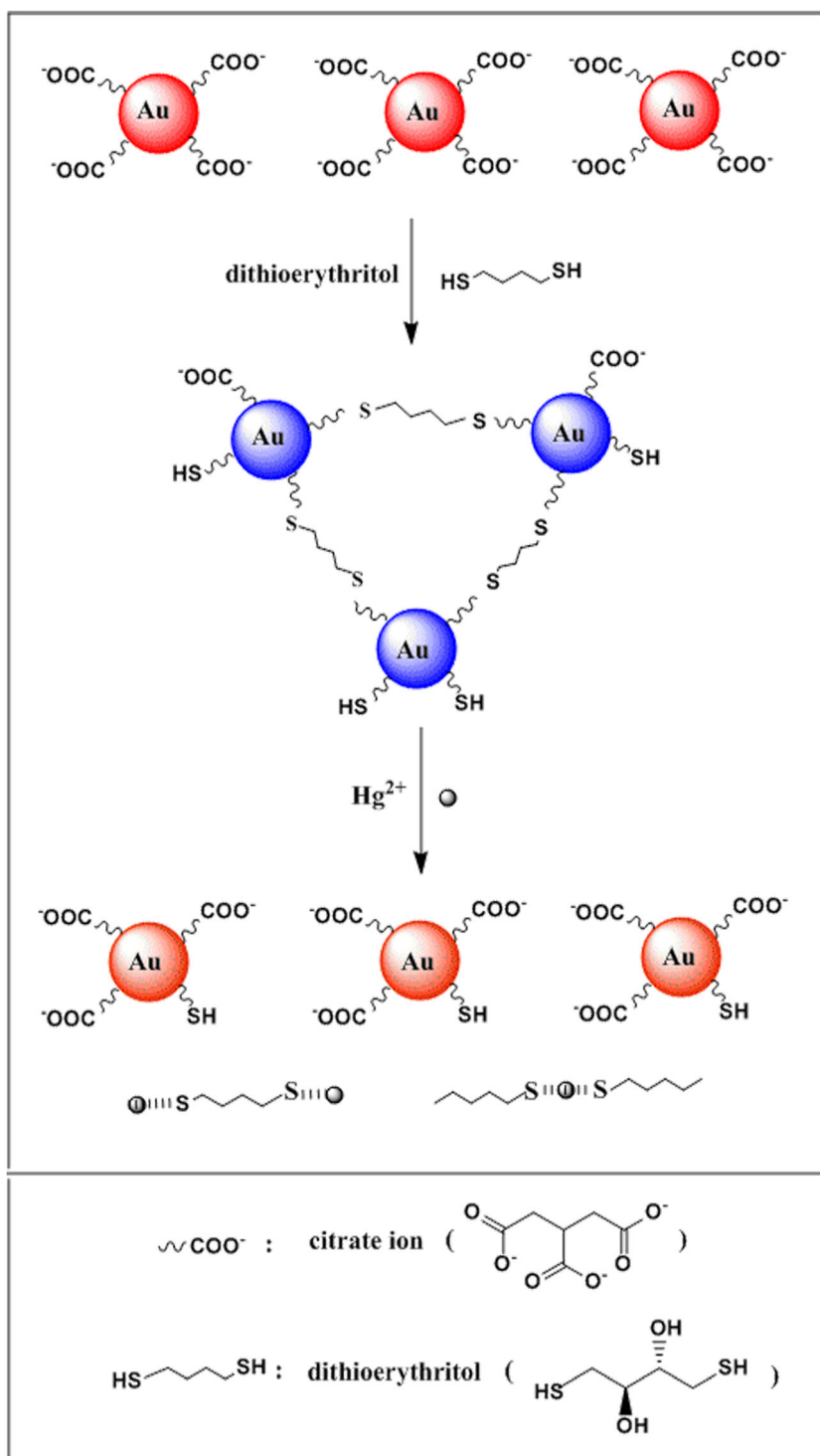
Colorimetric detection of Hg^{2+}

Colorimetric detection of Hg^{2+} is evaluated by detecting a range of concentration of Hg^{2+} with the DETL-AuNPs system. The pH value was adjusted to 6.6, and then a series of Hg^{2+} solutions (0.01 mL) with different concentrations (0, 0.1, 0.25, 0.35, 0.45, 0.5, 1, 2, 3, 5, 10 μM) was added into the DETL-AuNPs solution. After thorough shaking, the color change of the mixture was observed and recorded. 15 min later, the UV-vis absorption spectra of resulting solution was measured by the UV-vis spectrophotometer over the wavelength range from 200 nm to 800 nm. During the detection process, the concentration of AuNPs was 2.12 nM. All assays were performed at 30 °C.

Analysis of real samples

Analysis of real samples is used to test the applicability of DETL-AuNPs in detecting of Hg^{2+}. Samples of river water, tap water and pond water were collected from the Hsiang River (Changsha, China), our laboratory and Lake of Peach (Changsha, China), respectively. Firstly, the water samples were filtered through a 0.22 μm syringe filter. And then, we adjusted the pH of the samples to 6.6. Finally, the samples were prepared by spiking with the standard solutions of Hg^{2+}. The final concentration was 0.2, 0.5 and 4 μM, respectively.

Results and discussion

Mechanism of the colorimetric assay

Scheme 1 shows the mechanism for the determination of Hg^{2+}. The as-prepared AuNPs are stable in aqueous solution, due to the electrostatic repulsion of the negative charge of citrate ions prevents the aggregation of AuNPs. Moreover, the AuNPs solution shows wine-red color, because suitable visible light induces the collective oscillations of the surface electrons that is highly depended on the interparticle distance. With the addition of DETL, the thiol group of DETL can be quickly and easily attached to the surface of the AuNPs through ligand exchange of thiol group and citrate ions, and formation of Au-S bonds. Accordingly, the AuNPs can be cross-linked together because of the two thiol groups of DETL, and the solution color changes to blue [22]. While the DETL-AuNPs solution are added with Hg^{2+}, the thiol groups leave the surface of AuNPs and combine with Hg^{2+} formation of Hg^{2+}-sulfur bond because thiol groups are more tend to interact with Hg^{2+} than AuNPs. When thiol groups leave AuNPs, the citrate ions ligand exchange with thiol groups, then citrate ions attach to the surface of the AuNPs again. Because the electrostatic repulsion of the negative charge of citrate ions induces the redispersion of AuNPs, a significant color change from blue to purple or even red is observed. The changes in the UV-vis spectra and solution color, induced by ligand exchange reaction, allowed the colorimetric determination of Hg^{2+} by UV-vis spectroscopy or unaided eye in aqueous solution.

It is well-known that AuNPs display a maximum absorption band at 520 nm. When AuNPs are modified by DETL, the maximum absorption band appears at 650 nm, and absorption band shifts from 520 to 525 nm. The tremendous shift of wavelength may be related to the ligand exchange of thiol group and citrate ions which induces the aggregation of AuNPs. Fig. 1 (A, a) shows the UV-vis spectra of DETL-AuNPs. After the addition of Hg^{2+}, the
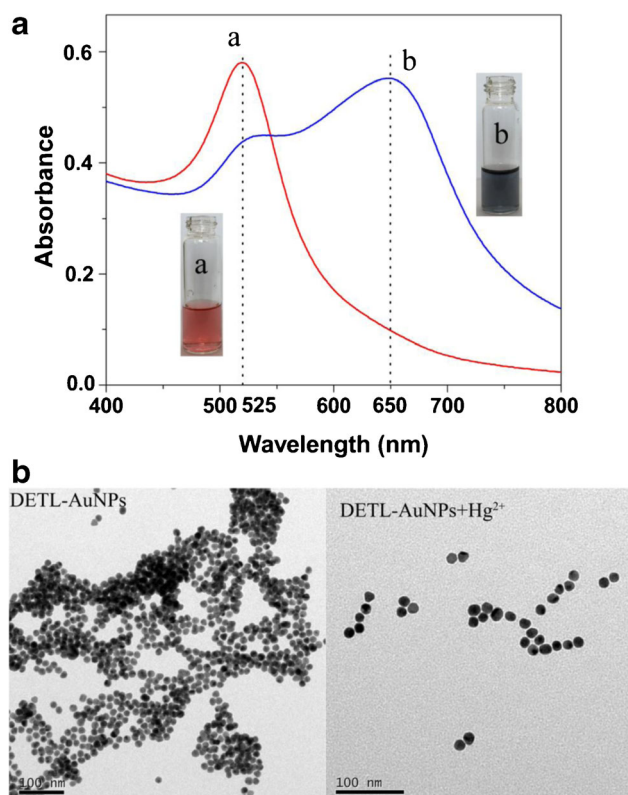

Fig. 1 (A) UV-vis absorption spectra and photographic images of DETL-AuNPs in the (a) presence (absorption peak occurs at 525 nm) and (b) absence (absorption peak occurs at 650 nm) of Hg^{2+} (10 μM). (B) TEM images of DETL-AuNPs in the (left) absence and (right) presence of Hg^{2+} (10 μM)

spectrum displays a red shift, the absorption peak decreases at 650 nm and increase at 525 nm (Fig. 1 (A, b)) because of the redispersion of AuNPs. These results are in agreement with those works [7, 13, 18]. We confirm that color change of DETL-AuNPs solution is induced by the addition of Hg^{2+}. The color changes immediately from blue to red upon the addition of Hg^{2+} (Fig. 1 (A, b)). TEM images also show that the redispersion of AuNPs occurs after the addition of Hg^{2+} (Fig. 1 (B)).

Optimization of method

The performance of this Hg^{2+} method is strongly influenced by the experimental parameters such as reaction time, temperature, and pH in the colloidal solution. Therefore, each detection parameter was optimized in our study, while keeping the other parameters constant. The following parameters were optimized: (a) Sample pH value; (b) Reaction temperature; (c) Reaction time. Respective data and Figures are given in the Electronic Supporting Material. The following experimental conditions were found to give best results: (a) Best sample pH value: 6.6; (b) Optimal temperature: 30 °C; (c) Optimal reaction time: 20 min.

Sensitivity of the assay

Figure 2 shows the colorimetric responses of the DTET-AuNPs upon the addition of various concentrations of Hg^{2+}. As we can see, with the increase of Hg^{2+} concentrations, an color change from blue to red is displayed in Fig. 2 (B). It is easy to observe the detection effect from the change of color by naked-eye. Besides, the absorbance peaks at 650 nm decrease gradually, for the corresponding increasing at 525 nm. Furthermore, the absorption ratio (A_{650}/A_{525}) linearly grows with the increase of Hg^{2+} in the range from 0.1 to 0.5 μM and from 0.5 μM to 5 μM, with the correlation coefficients of 0.9845 and 0.9986,
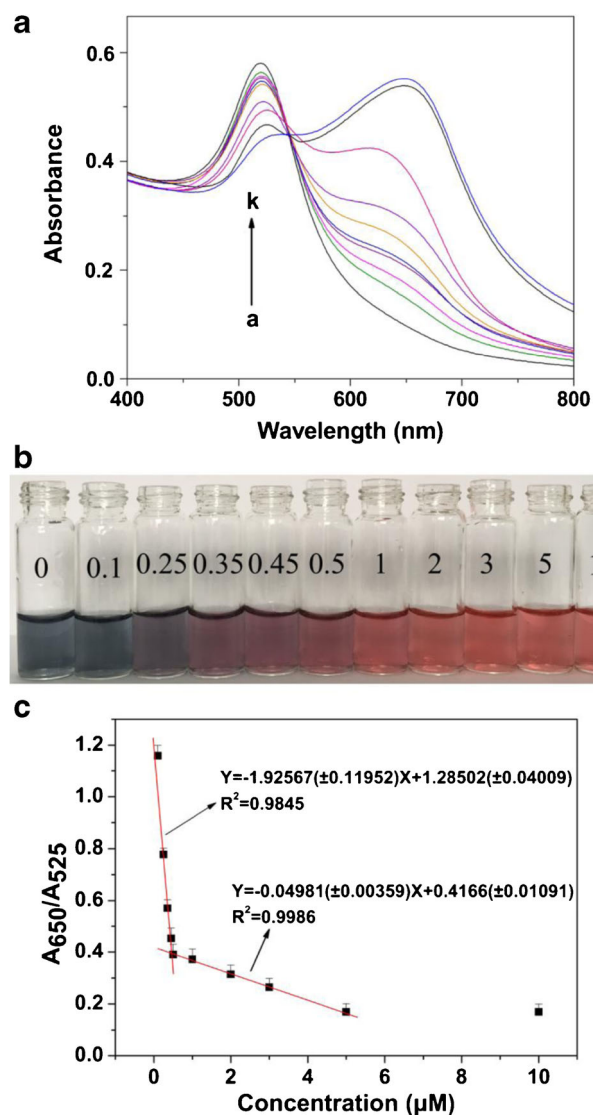

Fig. 2 (A) UV-vis absorption spectra of DETL-AuNPs in the presence of different concentrations of Hg^{2+} (a to k express 0, 0.1, 0.25, 0.35, 0.45, 0.5, 1, 2, 3, 5, 10 μM). (B) Images of DETL-AuNPs containing different concentrations of Hg^{2+} (0, 0.1, 0.25, 0.35, 0.45, 0.5, 1, 2, 3, 5, 10 μM). (C) Absorption ratio (A_{650}/A_{525}) of DETL-AuNPs versus the concentrations of Hg^{2+} (0, 0.1, 0.25, 0.35, 0.45, 0.5, 1, 2, 3, 5, 10 μM). Error bars were calculated from three experiments

respectively. The calibration equation is calculated by fitting the experimental data:

$$y = (-1.92567 \pm 0.11952)x + (1.28502 \pm 0.04009) \quad (1)$$

$$y = (-0.04981 \pm 0.00359)x + (0.4166 \pm 0.01091) \quad (2)$$

Where y is the ratio of spectral absorbance A_{650}/A_{525}, and x is the concentration of Hg^{2+}. The detection limit is 0.024 μM ($S/N = 3$), and the relative standard derivations of triplicate measurements are 1.4%. These results show that this assay has a satisfied sensitivity towards the determination of Hg^{2+}. Table 1 exhibits the different methods for the detection of Hg^{2+}. As shows in Table 1, in comparison with methods in literatures, the linear range of our method is wider than those of other methods including the AuNPs as fluorescent method [24] and 3-mercaptopropionate acid-AuNPs colorimetric method [29]. In addition, the detection limit is lower than that of the SPR [25] and FRET [26] methods.

Selective detection of Hg^{2+}

The above results indicate that the colorimetric method is sensitive to the detection of Hg^{2+}. In this case, the selectivity, the most important factor for a method, needs to be explored. To evaluate the selectivity of the method for Hg^{2+}, various competitors such as Cr^{3+}, Mn^{2+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Cd^{2+}, and Pb^{2+} were examined. As shows in Fig. 3, except for Hg^{2+}, none of these metal ions can prevent the DETL-induced aggregation of AuNPs, because the sulfydryl in DETL preferentially bind to Hg^{2+} compared to other metal ions in chemical affinity. Compared with those works [30, 32–34], this method do not need to add other masking agent such as ethylenediamine tetraacetic acid (EDTA), and pyridinedicarboxylic acid (PDCA), which shows effective masking ability toward Ba^{2+}, Pb^{2+} or Cu^{2+}. The concentration of other ions used in this experiment is 100 times higher than that of Hg^{2+}.

Determination of Hg^{2+} in real water samples

To evaluate our method for the detection of Hg^{2+} in real samples, tap water samples from our laboratory, pond water samples from the Lake of Peach (Changsha, China) and river water samples from Hsiang River (Changsha, China) are used for this method. Firstly, we filtered the water samples through 0.22 μm membrane. Then, river water, tap water and pond water were spiked with standard Hg^{2+} solutions (0.2 μM, 0.5 μM and 4 μM, respectively). The results are summarized in Table. S1 (see in Electronic Supporting Material). As can be observed in Table. S1, the recoveries are within the range from

Table 1 Comparison of AuNP-based methods for the determination of Hg^{2+}

| Techniques	Fabrication of methods	Masking agents	LOD (nM)	Linear range	references
Fluorescence	RB-AuNPs	PDCA	10	0.015–0.25 μM	[23]
	BAS@R6G/MPA-AuNPs	EDTA	20	0.1–2.5 μM	[24]
SPR	DNA-AuNPs	PDCA	300	0.3–10 μM	[25]
FRET	R6G/MPA/AMP-AuNPs	no	50	0.05–1 μM	[26]
	Rh6G@TCA-AuNPs	PDCA	0.06	0.5–35.5 nM	[27]
Colorimetry	TCA-AuNPs	no	0.5	0.5–300 nM	[21]
	OPD-AuNPs	no	5	0.01–2 μM	[28]
	MPA-AuNPs	PDCA	100	0.2–0.5 μM	[29]
	MPA/AMP-AuNPs	no	500	0.5–3.5 μM	[26]
	Cysteine-AuNPs	EDTA	25	0.05–1, 1–10 μM	[30]
	8-HQ-AuNPs	no	10	0.01–100 μM	[31]
	Peptite-AuNPs	EDTA	20	10–45 μM	[32]
	Tween 20-AuNPs/AA	EDTA	5	0.5–10 μM	[33]
	T_{33}-AuNPs/Mn^{2+}	PDCA	10	10–250 nM	[34]
	DETL-AuNPs	no	24	0.1–0.5, 0.5–5 μM	This work

SPR: surface plasmon resonance; FRET: fluorescence resonance energy transfer; 8-HQ: 8-hydroxy-quinoline; PDCA: pyridinedicarboxylic acid; EDTA: ethylenediamine tetraacetic acid; RB: rhodamine B; BSA: bovine serum albumin; R6G, rhodamine 6G; MPA, 3-mercaptopropionate acid; TCA: thiocyanuric acid; OPD: O-phenylenediamine; DETL: dithioerythritol

100.40 to 104.50%. The data are then compared by the AAS measurement. The results are still satisfactory. Therefore, the method has promising feasibility for rapid detection of Hg^{2+} in real water samples.

Conclusion

We describe a highly selective colorimetric Hg^{2+} detecting system using DTET-AuNPs in aqueous media. This rapid

Fig. 3 (A) Absorption ratio (A_{650}/A_{525}) of the DETL-AuNPs dispersion in the presence of different metal ions (1 mM). (B) Photographic image of DETL-AuNPs dispersion in the presence of different metal ions (1 mM). M^{n+} denotes the mixture of all above ions except Hg^{2+}, and M^{n+1} denotes the mixture of all above ions including Hg^{2+} (10 μM)
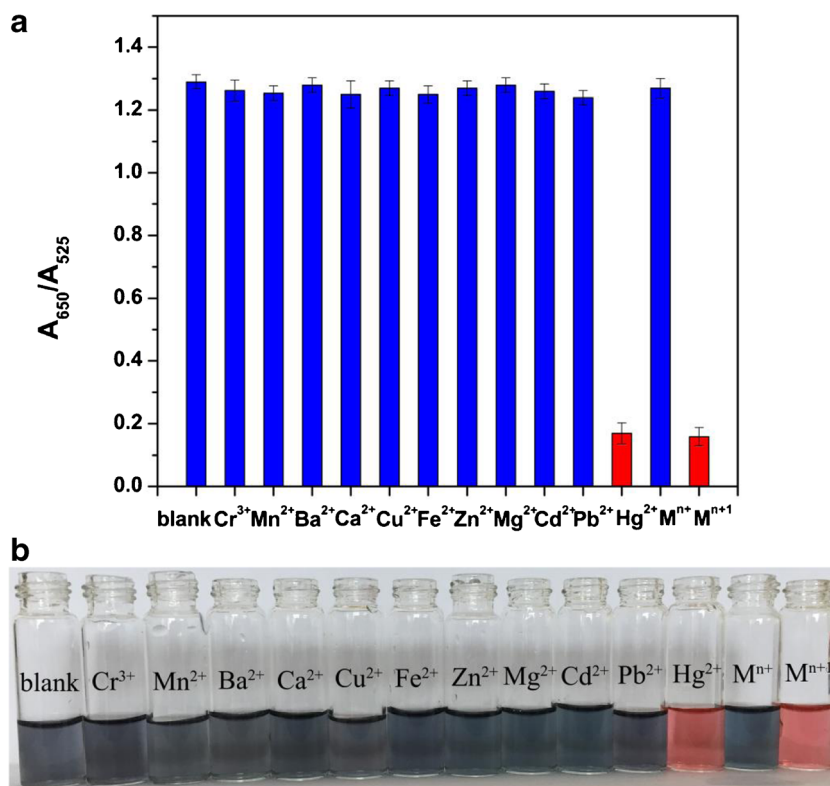
and reliable detecting system provides additional advantages: (i) high selectivity for Hg^{2+} over other major interfering metal ions such as Cu^{2+}, Cd^{2+}, and Pb^{2+} without using any masking agents compared with previous works; (ii) short assay time and lab-friendly condition (aqueous solution, room temperature); (iii) it does not require a fluorescence dye, and simplicity of the use of AuNPs and commercially available DETL as the probe without any modification, design, and complex synthesis; (iv) direct visualization of end result with color change; and (v) low cost due to use of inexpensive instruments. Finally, we hope this nanoprobe can contribute to valuable development of Hg^{2+} detection strategy.

Compliance with ethical standards The author(s) declare that they have no competing interests.

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