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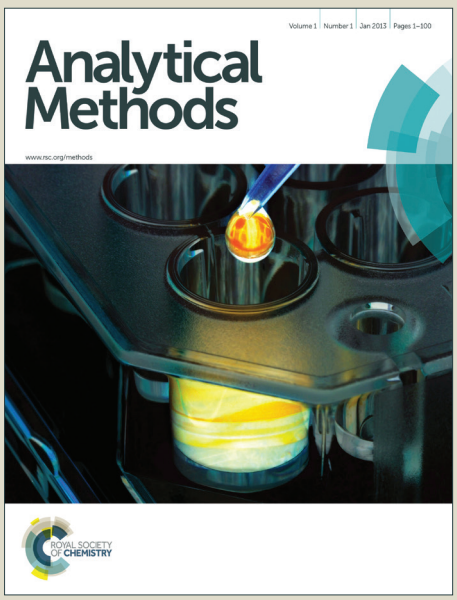

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ARTICLE

A colorimetric approach for measuring mercuric ion with high selectivity using label-free gold nanoparticles and thiourea

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We report a simple colorimetric assay for Hg^{2+} measurement using label-free gold nanoparticles (Au NPs, 13.0±1.6 nm) with the aid of thiourea (TU). The addition of TU into Au NPs resulted in aggregation of Au NPs with a red-to-blue color change. While in the presence of Hg^{2+}, it inhibited the aggregation of Au NPs induced by TU, resulting in a reverse color change from blue to purple and finally to wine red. These color variations are dependent on the Hg^{2+} concentration and can be utilized for the colorimetric sensing of Hg^{2+}. This assay requires only 3 min to reach a naked-eye detection limit of 100 nM, which satisfies the guideline concentration of Hg^{2+} (250 nM) in industrial water regulated by the EPA. And the concentration of Hg^{2+} is quantitatively determined using a UV-Vis spectrophotometer with a limit of detection (LOD) of 40 nM. The response of the assay caused by Hg^{2+} (2 μM) was 6.0 to 9.1 times higher than those induced by other metal ions (100 μM), indicating its high specificity towards Hg^{2+}. The assay has been applied for the analysis of Hg^{2+} in drinking and lake water samples with recoveries in the range of 98.3-120.0%.

Introduction

The level of bivalent mercuric ion (Hg^{2+}) in water (drinking, sea and lake water) has to be strictly monitored on account of its hazardous effect to both human health and environment[1,2]. Traditional analytical methods for Hg^{2+} measurement including atomic absorption/emission spectrometry[3], inductively coupled plasma mass spectrometry[4] and electrochemistry[5,6] offer high sensitivity and selectivity, but these techniques generally require sophisticated instruments, time-consuming pretreatment steps and professional operators.

Over the past decades, gold nanoparticles (Au NPs) based colorimetric probes have emerged as good alternatives for Hg^{2+} sensing, in view of their intrinsically exploitable properties of high extinction coefficient and of distance-dependent variation in color[7-9]. Most of these probes are based on the rational modification of Au NPs with specific binding ligands such as aptamers, peptides, proteins, small thiol ligands, etc[3,10-12]. The interaction between target analytes and ligands changes the dispersion/aggregation state of the Au NPs, resulting in visible color changes. To overcome tedious synthesis of special ligands and complicated modification of Au NPs, much attention has been paid to developing label-free Au NPs based colorimetric probes in order to simplify the detection process[13-17]. Recently. the colorimetric probes based on label-free Au NPs with sensing elements such as o-phenylenediamine[18], 4-mercaptophenylboronic acid[19], alkanethiols[14], thymine[20], 4,4'-dipyridy[21] and cysteine[22] have been designed for Hg^{2+} detection. Although these assays have made great contributions toward Hg^{2+} detection, some limitations including interference by other metal ions[22], use of poor water-soluble or highly toxic reagents[14,18-21], adoption of masking agents[22] and relatively long reaction time[18-21], still exist.

With these insights, here we provide a simple colorimetric assay for Hg^{2+} measurement using label-free gold nanoparticles (Au NPs) and thiourea (TU). This colorimetric assay is based on the fact that TU had stronger affinity with Hg^{2+} than that with Au NPs. Thus, the presence of Hg^{2+} inhibited the TU-induced aggregation of Au NPs accompanying with a color change from blue to red. The colorimetric assay was successfully applied to the analysis of Hg^{2+} in drinking and river water samples.

Experimental

Chemicals

$HAuCl_4 \cdot 3H_2O$, TU, and the metal salts, including $HgCl_2$, NaCl, KCl, $CaCl_2$, $MgCl_2 \cdot 6H_2O$, $AlCl_3 \cdot 6H_2O$, $BaCl_2$, $CdCl_2$, $Pb(NO_3)_2$, $FeCl_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $ZnCl_2$, $CrCl_3 \cdot 6H_2O$, $NaNO_3$, Na_2SO_4, Na_3PO_4, NaF, NaBr, $NaNO_2$, $NaHCO_3$, Na_2SO_3,CHCOONa, $Na_2B_4O_7$, $NaBO_3$ and $NaClO_4$ were of analytical grade and purchased from Sinopharm Chemical Reagent Co.,Ltd (Shanghai, China). All other reagents were of analytical grade and used as received.

Instruments

Ultrapure water (18.2 MΩ·cm) was obtained from a Millipore Autopure WR600A system and used throughout. The UV-vis spectra were recorded using a UV-2450 spectrophotometer (Shimadzu). A Canon IXUS-125HS digital camera was used for photographing.

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† Electronic Supplementary Information (ESI) available: Figure S1-S7and Table S1-2 See DOI: 10.1039/c000000x/

Transmission electron microscopy (TEM) images were obtained with a JEM 1400 microscope (JEOL).

Synthesis of gold colloidal solution

Citrate-stabilized Au NPs at two different sizes were prepared by reducing $HAuCl_4$ with citrate[23]. 13-nm Au NPs were synthesized as follows. Briefly, 100 mL of $HAuCl_4$ solution (1 mM) was heated to boiling under vigorous stirring, followed by a quick injection of 10 mL of trisodium citrate (38.8 mM). Heating and stirring under reflux were carried out for another 15 min, during which time the color of the mixture changed from pale yellow to wine red. Finally, the solution was cooled to room temperature and stored in a refrigerator at 4 ^{o}C for further use. The particle concentration of the as-synthesized Au NPs was calculated to be 12 nM using Beer's law. The synthesis of 37-nm Au NPs was carried out using the above mentioned procedures except the use of $HAuCl_4$ solution (0.3 mM).

Detection of Hg^{2+} using the colorimetric assay

100 µL of 26 µM thiourea, 600 µL of ultrapure water, 100 µL of Hg^{2+} with different concentrations and 200 µL of Au NPs were sequentially added into a glass sample vial. The mixture was incubated at ambient temperature (25 ^{o}C) for 5 min. After incubation, the UV-vis spectra of the solution were recorded using a spectrophotometer and the corresponding colors of the solution were captured using the digital camera.

Analysis of real water samples

Drinking water samples were obtained from supermarket and used without any pretreatment. Lake water samples were collected from a lake nearby the campus and filtered using filter paper (0.25 mm in pore size) before use. For recovery experiments, these samples were spiked using Hg^{2+} (100-1000 nM) with various concentrations and were analyzed using the same procedures mentioned in the section of "Detection of Hg^{2+} using the colorimetric assay".

Results and discussion

Sensing mechanism of the assay

TU, a kind of low-cost and water-soluble reagent, is often used for metal electro-deposition and gold leaching instead of highly toxic cyanide[24]. TU can form complex with metal ions through strong coordination interactions, and we note that the $lg\beta_x$ of $Hg(TU)_n^{2+}$ is 26.3, whereas those of $M(TU)_n^{x+}$ (Cu^{2+}, Bi^{3+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, and Au^{+}) are 15.40, 11.90, 6.44, 3.55, 2.04, 1.77, and 21.50, respectively[25]. In other words, the $lg\beta_x$ of $Hg(TU)_n^{2+}$ is the only value which is larger than that of $Au(TU)_n^{+}$. Herein, the sensing mechanism of the anti-aggregation of the Au NPs probes for Hg^{2+} is hypothesized in Fig. 1. In the absence of Hg^{2+}, the addition of TU to the citrate-stabilized Au NPs led to the aggregation of Au NPs via the possible aurophilic[26] and coordination interaction between the electron-rich sulfur/nitrogen atoms of TU and the electron-deficient surface of Au NPs[27-29]. Meanwhile, the color of the solution changed from original wine red to violet blue. In contrast, in the presence of Hg^{2+}, TU was prior to chelating with Hg^{2+} by forming a more stable coordination compound $Hg(TU)_n^{2+}$, resulting in inhibition of the color change mentioned above. With the increase of Hg^{2+} concentration, the color of Au NPs-TU system changed from blue to purple, and finally to wine red, and thus can be utilized for colorimetric sensing of Hg^{2+}.

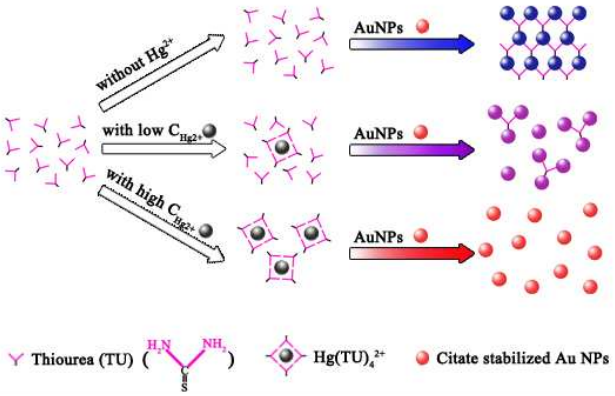

Fig. 1 Schematic illustration of the sensing mechanism.

As shown in Fig. 2, the citrate-stabilized Au NPs were red in color and exhibited a surface plasmon resonance (SPR) band at 520 nm (curve a). With the addition of TU, a new SPR band emerged at about 700 nm (curve b), and the solution color changed to blue owing to the aggregation induced by TU. In contrast, when TU was first treated with Hg^{2+} followed by mixing with Au NPs solution, the maximal SPR band of Au NPs went through a reverse process (curve c), and the color changed back to red. This can be explained by the fact that TU preferentially chelates with Hg^{2+} due to the high $lg\beta_x$ of $Hg(TU)_n^{2+}$. TEM measurement further confirmed this dramatic anti-aggregation induced by Hg^{2+}. As can be observed in Fig. 3a, the Au NPs incubated with TU aggregated severely with a blue color. The introduction of Hg^{2+} can inhibit the aggregation and the inhibition effect is positively related to the Hg^{2+} concentration accompanied with color variations from blue to purple and then to red (Fig. 3b-c). For comparison, we used urea, a structural analogue of TU, to replace TU and repeated the same experiment. The only difference between TU and urea is that TU contains carbon-sulfur double bond while urea contains carbon-oxygen double bond. Fig. S1 shows that, unlike TU, urea was not able to cause the aggregation of Au NPs. The UV-vis spectra and solution color exhibited almost no change before and after the addition of Hg^{2+} into the Au NPs-urea system. In other words, the Au NPs-urea system cannot serve as a colorimetric approach for measuring Hg^{2+}. These results also implied that the carbon-sulfur double bond of the TU plays a crucial role for this Hg^{2+} colorimetric assay.

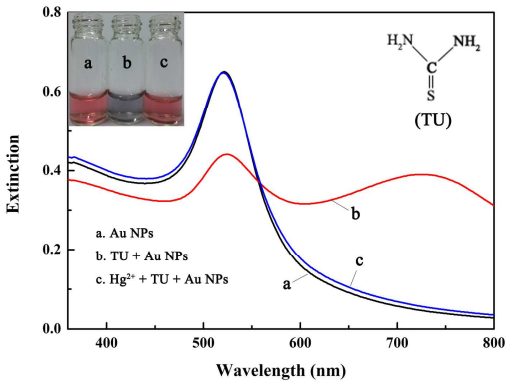

Fig. 2 Photograph and UV-vis spectra of (a) the Au NPs solution (2.4 nM), (b) the Au NPs in the presence of 2.6 μM TU, (c) the Au NPs in the presence of 2.5 μM Hg^{2+} and 2.6 μM TU, inset image is the structure of TU.

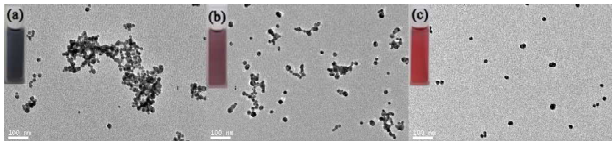

Fig. 3 TEM images of (a) Au NPs with 2.6 μM of TU, (b) Au NPs with 2.6 μM of TU and 0.7 μM of Hg^{2+}, (c) Au NPs with 2.6 μM of TU and 100 μM of Hg^{2+}, inset images are the corresponding photographs.

Optimization of the assay

To obtain the best performance of the colorimetric assay, several important parameters including the size of Au NPs, the concentration of TU and Au NPs, solution pH and reaction time were investigated. Two types of citrate stabilized Au NPs with average size at 13 nm (13.0±1.6 nm, see Fig. S2) and 37 nm (36.8 ± 4.1 nm, see Fig. S3) were synthesized and used for the detection of Hg^{2+}. Both types of Au NPs are applicable for the detection of Hg^{2+}. However, the sensitivity of the assay using 13-nm Au NPs was approximately one magnitude higher than that using 37-nm Au NPs (data not shown). Therefore, 13-nm Au NPs were adopted for the remaining experiments. The UV-vis spectra of the Au NPs in the presence of different concentrations of TU were recorded (Fig. 4(a)). We noted that TU concentration higher than 2.6 μM was harmful to method sensitivity because the excessive TU did not complex with Hg^{2+} and induced aggregation of Au NPs. However, the concentration of TU lower than 2.6 μM cannot induce the complete aggregation of Au NPs. Hence, 2.6 μM of TU was selected for subsequent experiments. We then explored the effect of pH over a range of 3-7 on account of the decomposition of TU in alkaline conditions[31]. Fig. 4(b) shows that the value of $Ex_{520\ nm}/Ex_{700\ nm}$ reached maximum in the pH range of 5.5-7. No pH adjustment was carried out for the following experiments because the pH of the original system lies in this range. Experiments on the effect of the concentration of Au NPs were also performed. Fig. 4(c) shows that the value of $Ex_{520\ nm}/Ex_{700\ nm}$ attained its maximum at 2.4 nM, which was selected for the following experiments. The effect of response time as shown in Fig. 4(d) indicates that the response time of this assay attained equilibrium within 3 min. To ensure the complete reaction, 5 min of reaction time was chosen for further studies. We also investigated the effect of ionic strength on the detection system. As shown in Fig. S4, the ionic strength exhibited little effect on the assay as the sodium chloride increased from 0-3 mM. However, as the concentration of sodium chloride increased to 5 mM, the ionic strength exhibited some negative effect on the sensing of Hg^{2+}. As the concentration of sodium chloride reached 48 mM, the Au NPs tended to aggregate since the repulsion between the unmodified negative-charged Au NPs is screened by strong ionic effect[30].

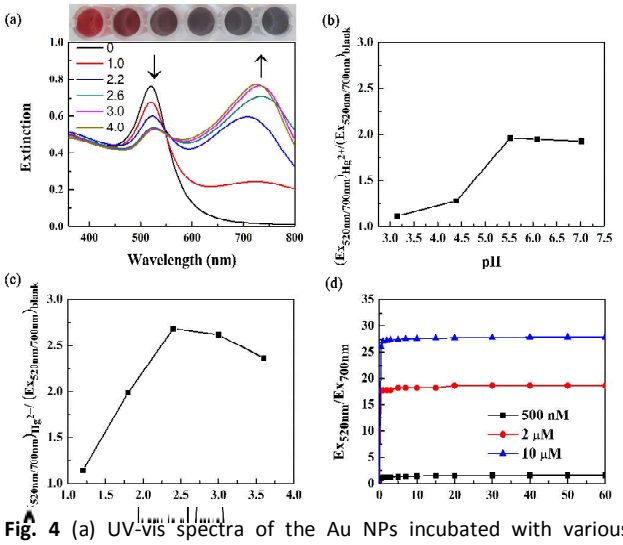

Fig. 4 (a) UV-vis spectra of the Au NPs incubated with various concentrations of TU ranging from 1.0 to 4.0 μM, inset images are the corresponding photographs. (b) Effect of pH of the Au NPs-TU detection system on the value of $Ex_{520\ nm}/Ex_{700\ nm}$ in the presence and absence of Hg^{2+} (1 μM). (c) Effect of Au NPs concentration of the Au NPs-TU detection system on the value of $Ex_{520\ nm}/Ex_{700\ nm}$ in the presence and absence of Hg^{2+} (1 μM). (d) The plot of $Ex_{520\ nm}/Ex_{700\ nm}$ of Au NPs-TU mixture versus reaction time at different concentrations of Hg^{2+}.

Selectivity of the assay

In order to investigate the selectivity of this probe, the effect of some co-existing metal ions such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Ba^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+} and Cr^{3+} was evaluated by the naked eyes and UV-vis spectra. Fig. 5(a) shows the photograph of the Au NPs-TU detection system incubated with Hg^{2+} or other ions. It was found that only Hg^{2+} can inhibit the aggregation of the Au NPs induced by TU, resulting in a color change from blue to red. Meanwhile, as shown in Fig. 5(b), the response induced by Hg^{2+} was 6.0-9.1-fold higher than that caused by other metal ions (50 equiv. of Hg^{2+}). It is of particular importance to note that our probe

could discriminate Hg^{2+} from Cd^{2+} and Pb^{2+}, which exhibited similar responses to Hg^{2+} of some Au NPs-based colorimetric assays[32,33]. Simultaneously, the effects of some anion species, including CO_3^{2-}, Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, F^-, Br^-, NO_2^-, HCO_3^-, SO_3^{2-}, AcO^-, $B_4O_7^-$, BO_3^- and ClO_4^- have also been investigated. Compared with the control, both the color and the response did not undergo apparent changes after the addition of these anions (Fig. S5). In addition, the tolerance limits of the assay for the common cations and anions are listed in Table S2.On the basis of these results, it is clear that the colorimetric assay is highly selective for Hg^{2+} analysis, which is attributed to the superior affinity of TU towards Hg^{2+} as stated above.

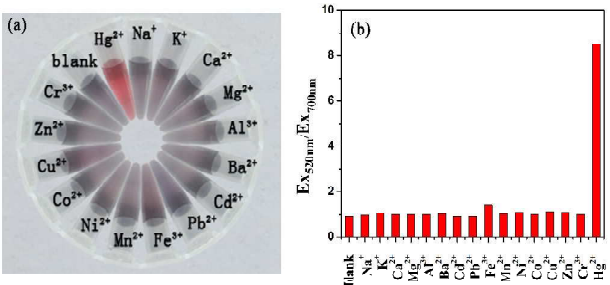

Fig. 5 Selectivity of the Au NPs-TU detection system for Hg^{2+} versus other cations. Photograph (a) and the value of $Ex_{520\ nm}/Ex_{700\ nm}$ (b) of the detection system incubated with Hg^{2+} (2.0 μM) or other cations (100 μM).

Sensitivity of the assay

To evaluate the sensitivity of this method, different concentrations of Hg^{2+} were tested under the optimized conditions. As shown in Fig. 6(a), with the increase of Hg^{2+} concentration, the color of the solution gradually changed from blue to red. The lowest detectable concentration using the naked eyes was 100 nM due to the obvious color change compared with the control. And the limit of detection (LOD) of the method is 40 nM which was calculated by the equation LOD=3σ/k, where σ is the standard deviation of the control groups and k is the slope of the calibration graph. As can be seen in Fig. 6(b) and (c), the intensity of SPR peak at 520 nm increased gradually with the decreased intensity at 700 nm, and there was a linear relationship (R^2=0.9866) of $Ex_{520\ nm}/Ex_{700\ nm}$ versus Hg^{2+} concentration in the range from 100 to 1000 nM, which comfortably covers the US Environmental Protection Agency (EPA) standard for industrial waste water of 250 nM (50 ppb). Compared to the existing label-free Au NPs based colorimetric method (Table S1), this method features the characteristic of rapidity, high sensitivity and selectivity.

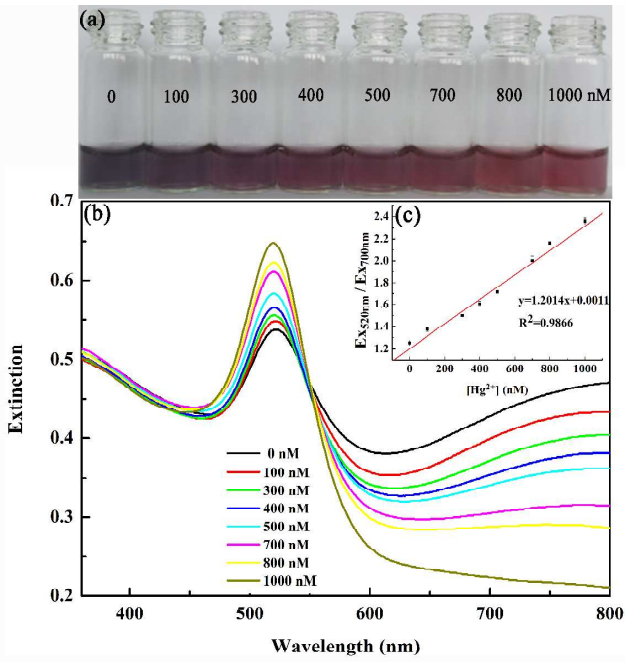

Fig. 6 Photographic image (a) and UV-vis spectra (b) of the Au NPs-TU detection system (containing 2.6 μM of TU) incubated with various concentrations of Hg^{2+} and (c) a plot of $Ex_{520\ nm}/Ex_{700\ nm}$ versus Hg^{2+} concentration in the range of 100-1000 nM.

Real water sample analysis

For the purpose of practical application, the colorimetric assay was tested using drinking and lake water samples. According to Fig. S6 and Fig. S7, $Ex_{700\ nm}/Ex_{520\ nm}$ increases linearly upon increasing the spiked concentration of Hg^{2+} in drinking water over the range of 100-1000 nM (R^2=0.9980) and in lake water over the range of 100-1000 nM (R^2=0.9914), confirming that the proposed method is applicable to the quantitative analysis of Hg^{2+} in real water samples. Furthermore, we performed recovery experiments using spiked water samples with different concentration of Hg^{2+}. As listed in Table 1, all the recovery values in drinking and lake water are between 98.3 and 120.0%, indicating the reliability of our Hg^{2+} detection system in real water samples. Lake water generally contains dissolved organic matters which may bind to both Au NPs and Hg^{2+}. This is probably the reason why recoveries increase to 120%. In view of complex matrix of biological samples, appropriate sample pretreatment is necessary if the proposed assay is used for the detection of Hg^{2+} in biological samples.

Table 1 Determination of Hg^{2+} in the blank and spiked drinking and lake water samples using the colorimetric assay.

Real water samples	Detected (nM)	Added (nM)	Found (nM)	Recovery (%)
Drinking water	ND[a]	100	105.0	105.0
	ND[a]	1000	982.8	98.3
Lake water	ND[a]	200	240.0	120.0
	ND[a]	1000	986.1	98.6

ND[a]. Not detected

Conclusions

In summary, we have developed a rapid, simple, sensitive and selective colorimetric assay to detect Hg^{2+}. Under optimized conditions, the detection limit toward Hg^{2+} is 40 nM. This assay showed excellent selectivity towards Hg^{2+} over other metal ions including Pb^{2+} and Cd^{2+}, which is ascribed to the fact that none of the other metal ions except Hg^{2+} exhibits better affinity towards TU than that of Au NPs. In addition, it can be easily constructed in aqueous solution avoiding any other labeling or modification steps. The developed method was applied to the determination of Hg^{2+} in drinking and lake water samples with satisfactory results. Most of the materials required in this assay are commercially available with low cost, which makes this assay particularly attractive in remote areas.

Acknowledgements

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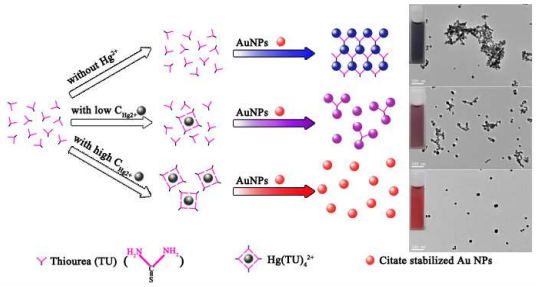

A simple, sensitive and selective colorimetric assay was developed for detecting Hg^{2+} by coupling Au NPs with thiourea.