Visual and colorimetric detection of Hg^{2+} by cloud point extraction with functionalized gold nanoparticles as a probe†

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Association with Hg^{2+} enhances the hydrophobicity and triggers the cloud point extraction of ~4 nm-diameter gold nanoparticle probes functionalized with mercaptopropionic acid and homocysteine, which results in the color change of the TX-114-rich phase from colorless to red, and therefore provides a novel approach for visual and colorimetric detection of Hg^{2+} with ultrahigh sensitivity and selectivity.

The high toxicity of Hg^{2+} has resulted in ever-growing demand for sensitive detection methods, especially rapid and field detection of Hg^{2+} in environmental samples.¹ In recent years, gold nanoparticles (AuNPs) functionalized with DNA,² alkylthiol ligand,³ and other species⁴ have emerged as a new generation of probes for Hg^{2+} determination.⁵ AuNPs-based methods for visual or colorimetric sensing of Hg^{2+} have become of great interest because the intense red color arising from surface plasmon absorption should allow highly sensitive detection, and the simple colorimetric approach, eliminating the usage of complex and expensive instruments, could facilitate rapid field detection. Most of these methods involve a color change from red to blue through the analyte-triggered aggregation of AuNPs, giving rise to the reduction of sensitivity due to the interference from background colors.⁶ Another restriction of these methods is the limited tolerance to the interference of coexisting heavy metals, which was reported to be between 1 and 150-fold of the Hg^{2+} concentration.^{2b,e,3c} To overcome these drawbacks, we demonstrate here, for the first time, that cloud point extraction (CPE) of small sized AuNPs can be used for sensing trace Hg^{2+} in water with excellent selectivity and sensitivity.

Scheme 1 illustrates the concept of this proposed procedure, which was based on the Hg^{2+} -triggered CPE of the AuNP probe. In the absence of Hg^{2+} , the functionalized ~4 nm-diameter AuNP probe were hardly extracted into the Triton X-114 (TX-114)-rich phase, which remained colorless after CPE. In the presence of Hg^{2+} , however, the TX-114-rich phase changed from colorless to red due to the enrichment of the AuNP probe from the aqueous solution. Therefore, upon the completion of the CPE process, visual and colorimetric detection of Hg^{2+} can be conducted by sensing the color of the TX-114-rich phase with the naked eye and measuring the absorbance of the TX-114-rich phase at 520 nm, respectively.

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It is noteworthy that the CPE procedure can also serve to enrich the Hg^{2+} associated AuNPs probe and eliminate the interference of coexisting metals, which markedly improves the sensitivity and selectivity, relative to other methods. The preparation of the AuNP probe and CPE procedure are detailed in ESI.†

In order to improve the method selectivity toward Hg^{2+} , the 4 nm-diameter AuNPs, prepared by NaBH_4 reduction of HAuCl_4 ,^{3c} were modified with mercaptopropionic acid (MPA) and homocysteine (HCys), and 2,6-pyridinedicarboxylic acid (PDCA) was introduced into the system. The larger stability constants of MPA and PDCA with Hg^{2+} in comparison with other heavy metals enable the MPA and HCys modified AuNPs (MPA-HCys-AuNPs) to show a high selectivity toward Hg^{2+} .³

For reducing the background absorbance of the reagent blank and thus enhancing the method sensitivity, the as-prepared MPA-HCys-AuNPs were purified by CPE to remove large AuNP particles that can be extracted into the TX-114-rich phase in the absence of Hg^{2+} . The CPE procedure was conducted 2–3 times until the surfactant-rich phase was nearly colorless. After this procedure, the aqueous-rich phase contained only small sized MPA-HCys-AuNPs that was ready for use as a probe for detection of Hg^{2+} by CPE.

Using the purified MPA-HCys-AuNPs probe, we first studied the mechanism for the Hg^{2+} induced CPE of the small sized AuNPs. UV-Vis spectra showed that λ_{max} of the TX-114-rich phase with enriched MPA-HCys-AuNPs was at 520 nm (Fig. 1), suggesting the AuNPs were not aggregated. This was confirmed by the TEM image of the obtained TX-114-rich phase (Fig. 1) in which no significant aggregation of AuNPs was observed. The hydrodynamic diameter of the MPA-HCys-AuNPs in the mixture before CPE was determined to be 4.0 and 3.8 nm with and without the presence of Hg^{2+} , respectively. This almost constant hydrodynamic diameter suggests that the MPA-HCys-AuNPs were not aggregated.

Scheme 1 Schematic representation of the Hg^{2+} -triggered cloud point extraction of MPA-HCys-AuNPs for Hg^{2+} detection.

Fig. 1 Description of visual and colorimetric detection of Hg^{2+} by MPA-HCys-AuNPs using cloud point extraction. TEM images of MPA-HCys-AuNPs in the TX-114-rich phase with (A) absence and (B) presence of 20 ppb Hg^{2+} , and (C) UV-vis spectra of the TX-114-rich phase.

Experiments also revealed that with the addition of Hg^{2+} the zeta potential of the MPA-HCys-AuNPs was reduced from -34 to -9 mV. Therefore, it was the association of Hg^{2+} with MPA-HCys-AuNPs that changed their protecting layer from hydrophilic to hydrophobic and thus facilitated the transfer of the probe to the hydrophobic TX-114-rich phase. This is in accord with literature⁷ in which it was reported that the addition of cationic tetraoctylammonium into negatively charged water-based AuNPs resulted in ion-pair formation on the particle surface, which induced the transfer of small sized AuNPs to the hydrophobic organic phase. Our previous study also demonstrated that in the CPE of nanoparticles the highest extraction efficiency was obtained at around their zero point charge.⁸

Parameters that affect the CPE of MPA-HCys-AuNPs were optimized to improve the method sensitivity and selectivity. Results (for details see ESI†) showed that the optimum conditions were as follows: in the samples adjusted to pH 7.5 were added 0.06% NaCl (w/v), 1 μM PDCA, 0.18% (w/v) TX-114 and 0.3 nM MPA-HCys-AuNPs, which were then incubated at 60 $^{\circ}\text{C}$ for 30 min. Under the optimized condition, 5 ppb Hg^{2+} in aqueous solution can be easily detected by the naked eye (Fig. 2). To the best of our knowledge, this is the lowest visual test limit for Hg^{2+} that have been observed by simple visual detection with AuNPs as a probe. At least two factors contributed to this low detection limit. At first, the CPE procedure concentrated the Hg^{2+} associated probe with a factor of ~ 100 . Furthermore, unlike most visual methods that are based on color fading or change from one color to another, the present procedure was based on the color transformation from colorless to red which provides a higher sensitivity for visual observation. By measuring the absorbance

Fig. 2 (A) Photographic image of the TX-114-rich phase obtained at different concentrations of Hg^{2+} (0–100 ppb); (B) Plot of absorbance of the TX-114-rich phase at 520 nm vs. Hg^{2+} concentration, note the reagent blank was subtracted from the absorbance of Hg^{2+} solutions.

of the TX-114 rich phase at 520 nm with a microplate spectrophotometer, Hg^{2+} was determined with a linear range of 2–100 ppb and a correlation coefficient (R) of 0.9975 (Fig. 2). This proposed method has a relative standard deviation (RSD) of 6% ($n = 5$) at 20 ppb Hg^{2+} level, and a detection limit of 2 ppb Hg^{2+} ($S/N = 3$), enabling its application in the analysis of Hg^{2+} in drinking water which has a maximum contaminant level of 2 ppb.

The specificity of the proposed method for Hg^{2+} was evaluated by testing the response of the assay to other environmentally relevant metal ions at a concentration of 20 ppb. Results demonstrated that only the Hg^{2+} sample gave rise to a significantly red TX-114-rich phase. Since in environmental water samples the concentration of foreign metal ions might be much higher than that of Hg^{2+} , it is of importance to study the tolerance ratio of coexistent metals to Hg^{2+} ($C_{\text{M}}/C_{\text{Hg}}$) for application in real sample analysis. The highest tolerant $C_{\text{M}}/C_{\text{Hg}}$ was determined by using solutions with constant concentration of Hg^{2+} (20 ppb) and varied concentrations of foreign metal ions, and adopting a criteria of 90–110% recovery of Hg^{2+} as non-interference. Fig. 3 shows that this proposed procedure can tolerate 10–500 $\times$ excess of foreign metal ions, which is impressive comparing to many relevant assays that are usually evaluated by using foreign metals with the same concentration as that of Hg^{2+} .

In order to further improve the tolerance to interference, a dual CPE procedure was developed. The first CPE step was conducted under the above optimized conditions but without the addition of MPA-HCys-AuNPs. After centrifugation, the upper aqueous-rich phase with most of the foreign ions were discarded, whereas into the lower TX-114-rich phase with the concentrated Hg^{2+} were added 9 mL of ultra pure water, 0.3 nM MPA-HCys-AuNPs and 0.06% NaCl (w/v). With this mixture a second CPE step was performed, and the TX-114-rich phase was separated for Hg^{2+} test. Results shown in Fig. 3 demonstrate that the dual CPE procedure can significantly enhance the tolerance to foreign metal ions with $C_{\text{M}}/C_{\text{Hg}}$ values in the range of 200–2000.

Fig. 3 Tolerance to the interference of foreign heavy metals shown as the ratio of coexistent metals to Hg^{2+} (C_M/C_{Hg}).

In summary, we have demonstrated that CPE of MPA and HCys modified AuNPs in the presence of PDCA provides a novel approach for visual and colorimetric detection of Hg^{2+} . Unlike most AuNP-based methods for sensing Hg^{2+} that involve a color change from red to blue through the analyte-triggered aggregation of AuNPs, this proposed method is based on the color change from colorless to red caused by the transfer of Hg^{2+} associated AuNPs into the TX-114-rich phase. The association of Hg^{2+} with AuNPs enhanced the hydrophobicity of the probe and thus triggered the transfer of the probe into the hydrophobic TX-114-rich phase. Taking advantage of the selective enrichment function of CPE and the color change of the TX-114-rich phase from colorless to red, this novel method provides ultrahigh sensitivity. This methodology can be generalized for the detection of other metals by using suitably functionalized AuNP as probes.

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