COMMUNICATION

High-throughput colorimetric assays for mercury(II) in blood and wastewater based on the mercury-stimulated catalytic activity of small silver nanoparticles in a temperature-switchable gelatin matrix†

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A catalysis-based, label-free, and high-throughput colorimetric protocol has been initially proposed for detecting mercury(II) in blood and wastewater with 96-cell plates, based on the mercury-enhanced catalytic activity of small silver nanoparticles synthesized in a gelatin matrix with unique temperature switchable sol–gel transition.

Recent years have witnessed the rapid development of nanoscale materials of noble metals (i.e., Au, Ag, Pt, and Pd) with many fantastic physicochemical properties. Especially, their unique enzyme-like catalysis has attracted considerable interest in catalysis and biochemical analysis applications. For example, gold nanoparticles (NPs) could present peroxidase-like catalysis activity for sensing H2O2 and glucose. Catalytic Pt NPs have also been utilized for the detection of H2O2 and scavenging superoxide free radicals. Moreover, the catalytic activity of noble metal nanomaterials can substantially depend on their sizes, known as the “size effect”, for example, high catalytic activity can be observed for gold NPs with sizes smaller than 5.0 nm. As a result, many efforts have been devoted to the synthesis of size-reducing gold NPs or nanoclusters. The preparation of cheap and catalytic small silver NPs (AgNPs), however, still remains a challenging but abstractive issue, due to the difficult synthesis, low stability, and the poor catalytic performance of small AgNPs.

As the most hazardous heavy metal ions, mercury ions were targeted by many modern detection methods such as DNA folding-based electrochemical detection, fluorescence quenching-based analysis, and gold or silver NPs-based colorimetric assay. However, these methods might suffer from either low detection sensitivity and throughput, or poor analysis stability and abilities against background interferences. Recently, some catalytic noble metal nanomaterials have been utilized for probing heavy metal ions like Hg2+ ions that could inhibit and stimulate their catalytic activity through a specific Au+--Hg2+ interaction and the change in the surface properties of AuNPs or AgNPs by forming Ag–Hg alloys, respectively. Such a catalysis-based analysis methodology may possess some advantages over the traditional methods like fluorescence-based ones in terms of the detection stability and targeting abilities against interferences from the complicated media (i.e., blood).

Gelatin, as a natural biopolymer derived from collagen widely used in food products and medicines, is nontoxic, non-immunogenic, biodegradable, and especially with unique sol–gel transition (critical transition at 35 °C). Such a biomass was introduced for the synthesis of AgNPs but entailing additional reductants like maltose and citrate. Also, small AgNPs with Hg2+-enhanced catalysis activity are rare. In this work, the gelatin matrix was employed to act as the reducing agent and the stabilizing matrix for the one-pot synthesis of small AgNPs at physiological temperature (37 °C) without adding any reducing agents. Unexpectedly, the prepared gel-AgNPs could display Hg2+-stimulated powerful catalytic activities in catalyzing 3,3′,5,5′-tetramethylbenzidine (TMB)–H2O2 reactions.

The special phenomenon of mercury(II)-enhanced catalysis of small gel-AgNPs was explored using comparable tests in the absence and presence of Hg2+ ions (Fig. 1A). It was found that gel-AgNPs showed a lower catalytic activity in the TMB–H2O2 reaction at a meaningfully low concentration (Fig. 1A(c)). However, their catalytic activity could be significantly enhanced by Hg2+ ions, showing a deep blue reaction product in the photograph (Fig. 1A(d)). Furthermore, effects of some other ions on stimulating the catalytic activity of gel-AgNPs were investigated (Fig. 1B and Fig. S1, ESI†). One can observe that common inorganic ions do not have the ability to stimulate the catalytic activity of gel-AgNPs, even at concentrations of about 100-fold higher than that of Hg2+ ions. Based on the specific mercury-stimulated catalytic activity of gel-AgNPs, a label-free colorimetric protocol has been thus proposed for the detection of Hg2+ ions. Scheme 1 illustrates the catalysis-enhanced analysis.
protocol using 96-well plates towards high-throughput colorimetric assays for Hg^{2+} ions in water, blood, and wastewater. As described in Scheme 1, gel-AgNPs (thawed at 37°C) alone (left top) exhibited no significant catalysis in TMB–H_{2}O_{2} reactions at low concentration. In contrast, the catalysis activity of gel-AgNPs could be greatly enhanced by Hg^{2+} ions to yield blue reaction products.

A transmission electron microscope (TEM) and a UV-vis spectrophotometer were separately utilized to characterize gel-AgNPs in the absence and presence of mercury(II) (Fig. 2). As shown in Fig. 2A, original gel-AgNPs were dense and well dispersed with the average particle diameter of about 5.0 nm (Fig. 2A, left). When Hg^{2+} ions were introduced, AgNPs in the suspension became sparser and smaller (Fig. 2A, right), as more apparently shown in the amplified view of one particle (inset, right top). Also, the hydrodynamic diameters of gel-AgNPs before and after Hg^{2+} treatment were comparably examined by dynamic light scattering (Fig. S2, ESI†), showing that their average sizes decreased from about 13.0 nm to 8.5 nm. Furthermore, UV-vis spectra were also recorded for gel-AgNPs with and without Hg^{2+} ions (Fig. 2B(a)). It was observed that Hg^{2+} ions could increase the UV-vis absorbance of gel-AgNPs; yet, they could cause the yellowish gel-AgNPs to fade out, as evidenced from their photographs (inset, Fig. 2B(b) and (c)). The above results indicate that the mercury(II) etching of AgNPs in gel-AgNPs could occur when they were treated with Hg^{2+} ions.

Furthermore, the enhanced catalysis mechanism for the Hg^{2+}-stimulated catalytic activity of gel-AgNPs was verified by essential experiments to make sure whether mercury(II) could etch gel-AgNPs. Herein, gel-AgNPs were deliberately treated under UV lamp so as to grow AgNPs till their yellowish color changed to deep yellow. Fig. S3A (ESI†) shows TEM images of the UV-treated gel-AgNPs before and after the Hg^{2+} addition. A size-reducing change of AgNPs was observed for the latter. It could also be verified by the UV-vis spectra showing that UV-treated gel-AgNPs could decrease in size after Hg^{2+} etching, as shown in the corresponding photographs (inset) (Fig. S3B, ESI†). On the basis of the above evidence, the mercury(II)-stimulated enhancement of the peroxidase-like catalytic activity of gel-AgNPs should result mainly from the decreased size of AgNPs by Hg^{2+} etching, of which the size-reducing AgNPs might achieve much higher catalysis activity, known as the “size effect”, as also evidenced elsewhere for gold and Fe_{3}O_{4} nanoparticles.³ Also, the special interaction between Hg^{2+} and AgNPs and the formation of Ag–Hg alloys changing the surface properties of AgNPs might be involved,⁶ which remains to be further investigated in the future.

Moreover, the mercury(II)-enhanced catalytic dynamics of gel-AgNPs was investigated in the absence and presence of mercury(II) (Fig. S4, ESI†). Here, colorimetric measurements were performed alternatively for different concentrations of TMB and H_{2}O_{2} to obtain the Michaelis–Menten curves⁸ (Fig. S4B and D, ESI†). It was found that Hg^{2+}-stimulated gel-AgNPs presented a much lower Michaelis constant (K_{m}) than gel-AgNPs alone for TMB and H_{2}O_{2}, together with larger maximal
reaction velocity ($V_{\text{max}}$). The data indicate that Hg$^{2+}$-stimulated gel-AgNPs could possess stronger catalytic activity especially higher affinity to TMB and H$_2$O$_2$. Colorimetric analysis of Hg$^{2+}$ ions could thereby be achieved based on the Hg$^{2+}$-enhanced catalysis of gel-AgNPs.

Moreover, the unique property of sol–gel transition of the gelatin matrix (critical temperature at 35 °C) was investigated in switching the catalysis of gel-AgNPs (Fig. 3). It was noted that gel-AgNPs in the gel phase (at 4 °C) with Hg$^{2+}$ ions could not catalyze the TMB–H$_2$O$_2$ reactions, where the catalytic reactions occurred only on the gel surface (Fig. 3A(a)). When gel-AgNPs were thawed at 37 °C and diluted to be further mixed with Hg$^{2+}$ ions, they could exert strong catalysis for the colorimetric reaction (Fig. 3A(b)), with the Hg$^{2+}$-stimulation time of about 5.0 min (Fig. S5, ESI†). Particularly, such a temperature-switching catalysis of gel-AgNPs offered by the gelatin matrix could allow for high catalysis stability of gel-AgNPs, remaining up to one year without a significant change (Fig. 3B). Importantly, gel-AgNPs could be stably immobilized onto 96-well plates for long-term storage at 4 °C to facilitate colorimetric Hg$^{2+}$ assays in a label-free and high-throughput way. An advantage over other mercury($n$) sensing assays, including those based on mercury-stimulated catalysis activity reported elsewhere, could thus be expected.

Moreover, the dosage of gelatin for the synthesis of gel-AgNPs was optimized as 4.0% (Fig. S6A, ESI†). Furthermore, the reaction conditions for the colorimetric Hg$^{2+}$ analysis were optimized as 7.2 μM gel-AgNPs (Fig. S6B, ESI†), ionic strengths of 5.0 mM KNO$_3$, a color reaction time of 20 min, neutral solution (pH 6.0–8.0) at room temperature (10–25 °C) (Fig. S7, ESI†), with 0.25 mM TMB and 10 mM H$_2$O$_2$ (Fig. S4A and C, ESI†).

Finally, calibration detection curves were obtained for Hg$^{2+}$ ions with different concentrations in water, blood, and wastewater samples using 96-well plates (Fig. 4), corresponding to photographic results shown in Scheme 1. It was found that the typical UV-vis absorbance values could increase with increasing Hg$^{2+}$ concentrations (Fig. 4A). A linear detection range of Hg$^{2+}$ concentrations was obtained from 0.50 to 800 nM with the limit of detection (LOD) being 0.125 nM in water (Fig. 4B), which is lower than those of most other detection methods (Table S1, ESI†). Moreover, the colorimetric detection of Hg$^{2+}$ ions in blood and wastewater was conducted, with linear Hg$^{2+}$ concentrations ranging from 5.0 nM to 700 nM and 1.0 nM to 800 nM, respectively. High detection selectivity for Hg$^{2+}$ ions could also be achieved with no significant interference from other metal ions, as shown in Fig. 1A. Therefore, the developed catalysis-based colorimetric assays could allow for the high-throughput Hg$^{2+}$ detection with pretty high sensitivity and selectivity.

In summary, a temperature-switchable gelatin matrix has been successfully employed for the one-pot synthesis of catalytic small AgNPs without any reductants. The resulting gel-AgNPs could display mercury-stimulated catalysis activity toward a simple, rapid, label-free, and high-throughput colorimetric protocol for probing mercury($n$) in blood and wastewater using 96-cell plates. The catalysis-based detection mechanism involved was thought to result mainly from the mercury etching of AgNPs in the gelatin matrix leading to smaller sizes of AgNPs with greatly enhanced catalysis activity for TMB–H$_2$O$_2$ reactions. The unique property of sol–gel transition of the gelatin matrix could not only facilitate the temperature-switchable catalysis of gel-AgNPs, but also allow for high catalysis stability of gel-AgNPs. Remarkably, the catalysis-based method could detect Hg$^{2+}$ ions in complicated wastewater and blood with high sensitivity, selectivity, and throughput. It might also circumvent some disadvantages of traditional detection approaches like fluorescence assays in terms of the detection stability and abilities against interferences from the complicated media. Such a colorimetric mercury assay promises huge potential applications in the clinical diagnosis, environmental monitoring, and pharmaceutical analysis fields.

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Notes and references


