1. Introduction

Silver salts and silver compounds have been extensively utilized in batteries catalysts, photography, the semiconductor industry and biomedical field. Owing to antibacterial abilities, silver-based salts and nanoparticles have been employed in the disinfection of drinking water and in the production of medical materials such as bandages, prostheses and catheters. Silver from industrial wastes and emissions reaches up to approximately 2500 tons annually, and about 80 tons are released to the surface water. **Ag**⁺ is liable to coordinate with amine, imidazole, carboxylate and thiol groups of proteins, resulting in their biological toxicity. Therefore, the U.S. Environmental Protection Agency reported that **Ag**⁺ causes toxicity to fish and microorganisms at a concentration higher than 1.6 nM, and has defined the maximum permissible limit of 0.9 μM in drinking water.

Developing fluorescent and colorimetric **Ag**⁺-sensing systems, based on the selective interactions of **Ag**⁺ with organic molecules,**⁵,⁶** nucleic acids,**⁷,⁸** and metal nanoclusters,⁹ has shown the advantage of portable monitoring with no need of sophisticated techniques such as atomic absorption spectrometry,¹⁰ inductively coupled plasma-atomic emission spectrometry,¹¹ and voltammetry.¹² For instance, Sung et al. developed a colorimetric **Ag**⁺ detection method by using N-1-(2-mercaptoethyl) adenine functionalized gold nanoparticles (MEA-AuNPs), resulting in the limit of detection (LOD) of 3.3 nM. In this sensing system, addition of **Ag**⁺ causes significant aggregation of MEA-AuNPs, yielding a color change from red to blue.¹³ Thus, we are inspired to develop colorimetric detection of **Ag**⁺ that produces high sensitivity and selectivity.

Palladium nanomaterials have been recognized as a kind of highly desirable catalysts, of which their catalytic activities are greatly associated with surface geometric effects, electronic properties and quantum size effects.¹⁴ So far, water-soluble Pd nanocatalysts with excellent stabilities and desirable sizes have been successfully synthesized by using nucleation templates such as polymers, dendrimers, peptides, proteins and nucleic acids.¹⁵–²¹ Recently, Pd nanoparticles or Pd-based hybrid nanostructures have been demonstrated to possess intrinsic peroxidase mimicking activities, which have been successfully used in colorimetric assays for Fe(II),²² hydrogen peroxide,²³ glucose,²⁴ xanthine,²⁵ sarcosine,²⁶ prostate-specific antigen,²⁷ etc. Importantly, the most common metallophilic interactions are observed in the d¹⁰ configuration including **Hg**²⁺, **Au**⁺, **Ag**⁺, **Cu**⁺, **Pd**⁰ and **Pt**⁰.²⁸–³¹ The metallophilic interactions between **Hg**²⁺ (4f¹⁰5d¹⁰) and **Pt**⁰ (4f¹²5d¹⁰) have been employed to develop ultrasensitive detection of **Hg**²⁺ in aqueous solution, on the basis of **Hg**²⁺-induced inhibition of peroxidase mimicking activity of Pt nanoparticles.³²–³⁵ This inspires us to employ Pd-based nanozyme for colorimetric detection of hazardous metal ions.

Reduced glutathione (GSH, γ-Glu-Cys-Gly) has functional groups including –SH, –NH₂ and –COOH, which exhibit high affinities to transition metal ions. Therefore, GSH has been
reported to be successfully used as a stabilizing agent for the environmentally benign synthesis of noble metal nanoparticles with desirable physicochemical properties.\textsuperscript{36–39} Moreover, the whole synthesis process of metal nanoparticles using GSH as the capping agent is eco-friendly, with little consumption of chemical reagents. Owing to the relatively low molecular weight of GSH molecules, other metal ions can easily interact with the metal surfaces of GSH-capped nanoparticles. In this paper, GSH was chosen as a nucleation template to synthesize Pd-based peroxidase nanomimetics. By using characterization of enzyme kinetics, TEM and XPS, it is demonstrated that the peroxidase-like activity of Pd-based nanzyme is greatly associated with the proportion of metallic Pd atoms. This is the first report that Ag\textsuperscript{+} inhibits the peroxidase-like activity of Pd-based nanzyme, totally opposite to the effect of Hg\textsuperscript{2+} on the enzymatic activity. GSH-Pd is employed to explore colorimetric detection of Ag\textsuperscript{+} and Ag nanoparticles in aqueous solution with high sensitivity.

## 2. Experimental section

### 2.1 Chemicals

Reduced GSH was purchased from J\&K Scientific. Na\textsubscript{2}PdCl\textsubscript{4} (99.9%), CuCl\textsubscript{2}-2H\textsubscript{2}O, Pb(CH\textsubscript{3}COO)\textsubscript{2}-3H\textsubscript{2}O, Hg(ClO\textsubscript{4})\textsubscript{2}-3H\textsubscript{2}O, and H\textsubscript{2}O\textsubscript{2} (30 wt%) were purchased from Alfa Aesar. 3,3-Dimethylbenzidine (TMB) was purchased from Heowns. Dimethylamine borane (DMAB) was purchased from Sigma-Aldrich. Fetal bovine serum was purchased from Lanzhou Bailing Biotechnology. Other reagents such as AgNO\textsubscript{3}, Na\textsubscript{2}HPO\textsubscript{4}, NaH\textsubscript{2}PO\textsubscript{4}, NaCl, MgCl\textsubscript{2}-6H\textsubscript{2}O, MnCl\textsubscript{2}-4H\textsubscript{2}O, CdCl\textsubscript{2}-5/2H\textsubscript{2}O, ZnCl\textsubscript{2}, FeCl\textsubscript{3}-6H\textsubscript{2}O, CoCl\textsubscript{2}-6H\textsubscript{2}O, and Ni(NO\textsubscript{3})\textsubscript{2}-6H\textsubscript{2}O were purchased from Tianjin Kermel Chemical Reagent Company.

### 2.2 Synthesis of GSH-Pd nanoparticles

Pd nanoparticles were synthesized through the reduction of Na\textsubscript{2}PdCl\textsubscript{4} by DMAB. Firstly, different concentrations of GSH (2, 5, 6.75, 12.5, 25, 50, 100, 200 \(\mu\)M) were added to 10 mL NaH\textsubscript{2}PO\textsubscript{4}-NaH\textsubscript{2}PO\textsubscript{4} buffer (pH 5.0) which were incubated with 300 \(\mu\)M Na\textsubscript{2}PdCl\textsubscript{4} for 2 h at 25 °C. Then freshly prepared DMAB aqueous solution was added to the above mixture to the [DMAB]/[Na\textsubscript{2}PdCl\textsubscript{4}] ratio of 5. GSH-Pd was prepared after 12 h-reduction at 25 °C.

### 2.3 Reaction kinetics

Enzyme kinetics was monitored at 652 nm at 20 °C. In the reaction system, the concentration of TMB and H\textsubscript{2}O\textsubscript{2} was fixed at 0.125 mM and 125 mM respectively, in NaH\textsubscript{2}PO\textsubscript{4}-H\textsubscript{3}PO\textsubscript{4} buffer (pH 4.0). Then the as-prepared GSH-Pd was added into the above solution to a final concentration of 900 nM (calculated from the precursor). All the experiments were repeated thrice. The initial velocities (\(v\)) were calculated according to eqn (1) and (2):

\[
C_p = \frac{A_{52}}{\varepsilon L}
\]

where \(C_p\) is the concentration of oxTMB, \(\varepsilon\) is the extinction coefficient of oxTMB, and \(L\) is the optical path length of 1 cm.

In order to calculate the kinetic parameters of GSH-Pd, a series of solutions were prepared at various concentrations of TMB or H\textsubscript{2}O\textsubscript{2}. The kinetic parameters were calculated using the Michaelis–Menten eqn (3):

\[
v = \frac{V_{max}[S]}{K_m + [S]}
\]

where \(v\) is the initial velocity of the reaction, \(V_{max}\) is the maximal velocity of reaction, \([S]\) is the substrate concentration, and \(K_m\) is the Michaelis–Menten constant.

\[
\frac{1}{v} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}}
\]

### 2.4 Colorimetric detection of Ag\textsuperscript{+} in aqueous solution

The sensing system contains 0.125 mM TMB, 125 mM H\textsubscript{2}O\textsubscript{2}, 900 nM GSH-Pd (calculated from the precursor) and different concentrations of Ag\textsuperscript{+}. Firstly, 9 \(\mu\)L GSH-Pd solution (precursor concentration of 300 \(\mu\)M) was added into 1.5 mL NaH\textsubscript{2}PO\textsubscript{4}-H\textsubscript{3}PO\textsubscript{4} buffer (pH 4.0). After adding certain volume of AgNO\textsubscript{3} stock solution, the mixtures were diluted to 2.93 mL using triple distilled water. Finally, 15 \(\mu\)L of TMB stock solution (25 mM dissolved in ethanol) and 38.5 \(\mu\)L of H\textsubscript{2}O\textsubscript{2} stock solution (30 wt% aqueous solution) were added. The A\textsubscript{522} was recorded after 10 min-reaction at 25 °C. The LOD defined as 3\(\sigma\)/slope was calculated from the standard deviation of the blank (\(\sigma\)) and the slope in the linear region.

To investigate the effects of other metal ions including Na\textsuperscript{+}, Mg\textsuperscript{2+}, Cu\textsuperscript{2+}, Pb\textsuperscript{2+}, Cd\textsuperscript{2+}, Zn\textsuperscript{2+}, Ni\textsuperscript{2+}, Mn\textsuperscript{2+}, Fe\textsuperscript{3+}, Co\textsuperscript{2+}, and Hg\textsuperscript{2+}, respectively, 9 \(\mu\)L GSH-Pd solution was added into 1.5 mL NaH\textsubscript{2}PO\textsubscript{4}-H\textsubscript{3}PO\textsubscript{4} buffer (pH 4.0). After adding certain volume of stock solution of different metal ions, the mixtures were diluted to 2.93 mL using triple distilled water. Finally, 15 \(\mu\)L of TMB stock solution (25 mM dissolved in ethanol) and 38.5 \(\mu\)L of H\textsubscript{2}O\textsubscript{2} stock solution (30 wt% aqueous solution) were added. The A\textsubscript{522} was recorded after 10 min-reaction at 25 °C.

### 2.5 Detection of Ag\textsuperscript{+} in real samples

We carried out the spiked-recovery experiments and ICP-MS using the lake water collected from Jingyi Lake, Tianjin Medical University. In the sensing system, the lake water was filtered with a pore size of 0.2 \(\mu\)m and diluted 30-times to reach an appropriate concentration of Ag\textsuperscript{+}. The detection system was spiked with 20, 50 and 80 nM Ag\textsuperscript{+}, respectively. The detection was performed in a 3 mL-system. Firstly, certain volume of AgNO\textsubscript{3} stock solution was mixed with 100 \(\mu\)L pre-treated water samples. Then the above mixtures were diluted to 2.93 mL by using NaH\textsubscript{2}PO\textsubscript{4}-H\textsubscript{3}PO\textsubscript{4} buffer (pH 4.0) and triple distilled water. Finally, 9 \(\mu\)L GSH-Pd solution, 15 \(\mu\)L of NaH\textsubscript{2}PO\textsubscript{4}-H\textsubscript{3}PO\textsubscript{4} buffer (pH 4.0) and 15 \(\mu\)L of TMB stock solution were added. The detection was performed after 30 min-reaction at 25 °C.
TMB stock solution (25 mM dissolved in ethanol) and 38.5 μL of H₂O₂ stock solution (30 wt% aqueous solution) were successively added. The A₆₅₂ was recorded after 10 min-reaction at 25 °C.

2.6 Detection of Ag nanoparticles
Citrate-capped Ag nanoparticles were synthesized according to the previously reported method. Firstly, different concentrations of the as-prepared Ag nanoparticles (calculated from the Ag⁺ precursor) were incubated with H₂O₂ at 25 °C for 30 min, at the molar ratio of [H₂O₂]/[Ag⁺] of 400. Then the above mixtures were diluted to 2.93 mL by using NaH₂PO₄–H₃PO₄ buffer (pH 4.0) and triple distilled water. Finally, 9 μL GSH-Pd solution, 15 μL of TMB stock solution (25 mM dissolved in ethanol) and 38.5 μL of H₂O₂ stock solution (30 wt% aqueous solution) were successively added. The A₆₅₂ signal was recorded after 10 min-reaction at 25 °C.

2.7 Characterization
UV-vis spectroscopy was performed on a Varian Cary300 spectrophotometer using a quartz glass cuvette with 1 cm path length. Transmission Electron microscopy (TEM) was carried out on a JEM-2010FFEF equipment (JEOL, Japan). All the average sizes were obtained by measuring more than 100 dispersive nanoparticles. X-ray Photoelectron Spectroscopy (XPS) was performed on the PHI5000 Versaprobe. In the sample preparation, 30 μL of the as-prepared GSH-Pd solution was dropped onto a clean silicon wafer, and then dried under a nitrogen gas atmosphere. This process was repeated thrice. Inductively coupled plasma mass spectrometry (ICPMS) was carried out on an Agilent 7700CE to determine the concentration of Ag⁺ in the lake water.

3. Results and discussion
3.1 Synthesis of GSH-capped Pd nanoparticles
GSH-capped Pd nanoparticles were prepared by using Na₂PdCl₄ as the precursor, and DMAB as the reducing agent. The physicochemical properties of GSH-Pd were investigated by varying the molar ratio of [Na₂PdCl₄]/[GSH] in the synthesis process. As shown in Fig. 1a, GSH-Pd prepared at the [Na₂PdCl₄]/[GSH] ratio of 3 exhibits an average diameter of 1.4 nm. The particle size gradually increases up to 3.5 nm upon increasing the [Na₂PdCl₄]/[GSH] ratio from 3 to 60 (Fig. 1b and c). Therefore, we denote GSH-Pd prepared at different molar ratios of [Na₂PdCl₄]/[GSH] as GSH-Pd1.4 ([Na₂PdCl₄]/[GSH] = 3), GSH-Pd2.6 ([Na₂PdCl₄]/[GSH] = 6) and GSH-Pd3.5 ([Na₂PdCl₄]/[GSH] = 60), respectively. In comparison, GSH-Pd nanoparticles reduced by NaBH₄ ([Na₂PdCl₄]/[GSH] = 6, [NaBH₄]/[Na₂PdCl₄] = 2) exhibit an average diameter of 3.8 nm (Fig. S1 in the ESI†).

As shown in Fig. 2, the deconvolution spectra of Pd 3d orbitals display two typical bands: the band at the binding energy of 336.9 eV corresponding to Pd²⁺ species and that at 334.9 eV corresponding to metallic Pd⁰. It is demonstrated that...
GSH-Pd1.4 contains 70% Pd$^{2+}$ and 30% Pd$^{0}$ species, respectively. With the particle size increasing from 1.4 to 3.5 nm, the proportion of Pd$^{0}$ species increases significantly from 30% to 72%, whereas the fraction of Pd$^{2+}$ species decreases from 70% to 28%. It is demonstrated that the proportion of Pd$^{2+}$ increases with the concentration of the GSH template. Previously, the reduction of Pt$^{2+}$ precursors was reported to be hindered by the coordination of Pt$^{2+}$ with amide groups of the dendrimer template. Moreover, $p$-mercaptobenzoic acid-stabilized Ag nanoclusters exhibited a shell of the protecting layer through coordination between Ag and thiolate. Lysozyme-stabilized Au nanoclusters was a mixture of Au$^{0}$ and Au$^{+}$, of which Au$^{+}$ species (∼24%) could be assigned to Au atoms ligated with lysozyme. Therefore, it is reasonable to conclude that Pd$^{2+}$ species exist on the surface of the particles through the ligation with GSH templates.

### 3.2 GSH-Pd nanoparticles for peroxidase mimetics

For testing the peroxidase-like activities of GSH-Pd, steady-state kinetics was performed adopting the oxidation reaction of TMB in the presence of H$_2$O$_2$.

The initial reaction rate increases by approximately 100-times as the [Na$_2$PdCl$_4$]/[GSH] increases from 1.5 to 12, and then it decreases by more than 5-times as the [Na$_2$PdCl$_4$]/[GSH] ratio increases to 150 (Fig. S2†). Among these GSH-Pd nanoparticles, the GSH-Pd nanoparticles prepared with the [Na$_2$PdCl$_4$]/[GSH] ratio of 12 exhibit the highest peroxidase mimicking activity with the initial $v$ of 0.35 μM s$^{-1}$.

Michaelis–Menten curves were obtained for GSH-Pd1.4, GSH-Pd2.6 and GSH-Pd3.5 respectively, by varying the concentration of TMB or H$_2$O$_2$ (Fig. 3, S3 and S4†). Upon increasing the size from 1.4 to 3.5 nm, the $K_m$ with TMB increases from 0.045 to 0.08 mM. Oppositely, the $K_m$ with H$_2$O$_2$ decreases from 254 to 137 mM (listed in Table 1). It is suggested that the affinity of GSH-Pd toward TMB increases with the fraction of Pd$^{2+}$ species, whereas the affinity toward H$_2$O$_2$ increases with the proportion of metallic Pd$^{0}$ species. GSH-Pd2.6 exhibits the highest enzymatic activity with the $K_m$ of 0.068 mM toward TMB and 156 mM toward H$_2$O$_2$ at 20 °C, which possesses activities comparable with previously reported peroxidase nanomimetics for colorimetric detection such as Pd nanostructures, Pt nanoparticles, Au nanoclusters, Cu nanoclusters, Fe$_3$O$_4$ nanoparticles and Co$_3$O$_4$ nanotubes (as listed in Table 1). For example, Yan and coworkers reported that 30 nm Fe$_3$O$_4$ nanoparticles exhibited the $K_m$ value of 0.098 mM toward TMB and 154 mM toward H$_2$O$_2$ at 40 °C. Moreover, our previous studies have employed GSH molecules as the nucleation templates to synthesize 3.3 nm Pt nanzyme, which possessed highly peroxidase-like activity with the $K_m$ of 0.03 mM and 88.7 mM toward TMB and H$_2$O$_2$ at 20 °C, respectively. Therefore, it is demonstrated that GSH-Pd can be recognized as a promising candidate for biological detection.

### 3.3 Colorimetric assay of Ag$^+$ in aqueous solution

To test the ability of GSH-Pd on the colorimetric detection of Ag$^+$, the sensing system contains 900 nM Pd (calculated from the precursor), 0.125 mM TMB and 125 mM H$_2$O$_2$, respectively. According to the absorption spectra of H$_2$O$_2$-mediated oxidation of TMB catalyzed GSH-Pd2.6, the absorption peak at 652 nm gradually decreases with the concentration of Ag$^+$ (Fig. S5†). According to reaction kinetics, the equilibrium can be reached within 10 min in the absence or the presence of Ag$^+$ (Fig. S6†). As shown in Fig. 4a, the A$_{652}$ collected at 10 min gradually decreases with the [Ag$^+$] increasing to 300 nM. The reaction rate gradually decreases with the concentration of Ag$^+$, suggesting that Ag$^+$ inhibits the peroxidase-like activity of GSH-Pd (Fig. S7†). A pseudo-linear relationship can be obtained between the A$_{652}$ and the [Ag$^+$] in the concentration range of 2–100 nM, with the calibration curve of A$_{652}$ = 1.28548–0.00725[Ag$^+$]. The LOD is determined to be 1.14 ± 0.003 nM from three independent experiments. In order to test the influence of Cl$^-$ ions (from precursor Na$_2$PdCl$_4$) on the detection of Ag$^+$, 5 μM NaCl is additionally added into the TMB–H$_2$O$_2$ reaction system. With the concentration of 80 nM Ag$^+$, the A$_{652}$ signal in the sensing system containing Cl$^-$ is comparable to that generated in the system without Cl$^-$ (Fig. S8†). Therefore, free Cl$^-$ ions have a slight influence on the detection of Ag$^+$.

The selectivity of this detection method was evaluated by different interfering ions. As shown in Fig. 4b, addition of Na$^+$, Mg$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Fe$^{3+}$ and Co$^{2+}$ individually into the detection system at 10-fold concentration excess contributes to a small interference on the final A$_{652}$.
signal (<3%). However, the Az signal is intensified by 15% in the presence of 10-fold excess of Hg²⁺. Therefore, we employed 1 μM EDTA into this sensing system. Consequently, addition of low concentration of EDTA can mask the interference of Hg²⁺, while it shows little effect on the signal generated by Ag⁺ (Fig. 4c). The LOD is calculated to be 1.27 nM with 1 μM EDTA added, and 1.23 nM upon addition of 500 nM Hg²⁺ and 1 μM EDTA (Fig. S9 and S10†). Therefore, it is demonstrated that GSH-Pd nanozyme possesses high feasibility for Ag⁺ sensing in aqueous solution.

The mechanism of the Ag⁺-induced inhibition of peroxidase mimicking activity was further investigated by XPS, TEM as well as enzyme kinetics. After adding equivalent molar Ag⁺ into GSH-Pd2.6, the binding energy of Pd⁰ shifted from 334.6 to 334.9 eV, which could be attributed to aurophilic attraction between d¹⁰ shells of Pd⁰ and Ag⁺ (Fig. 5a). The Pd⁰ → Ag⁺ donor–acceptor bond is susceptible to reduce the electron density of Pd⁰ and to cause an observed shift of the binding energy. According to kinetic studies, the affinities toward both TMB and H₂O₂ decrease in the presence of Ag⁺ (listed Table 1). According to the TEM image, GSH-Pd2.6 tends to aggregate upon addition of equivalent molar of Ag⁺ (Fig. 5b). It is suggested that Ag⁺ can interact with Pd⁰ species from different GSH-Pd, resulting in the observed aggregation of Pd nanoparticles. The apparent decrease of the GSH-Pd peroxidase-like activity should attribute to the Ag⁺-induced reduction of Pd⁰ electron density as well as the Ag⁺-induced aggregation of GSH-Pd. In contrast, a slight shift to lower binding energy is monitored for Pd⁰ species in the presence of Hg²⁺. Addition of Hg²⁺ (10-fold concentration excess) causes an increase of the affinity toward H₂O₂ while a decrease of the affinity toward TMB. Although aggregation is also detected for GSH-Pd2.6

<table>
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<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Kₘ (mM)</th>
<th>vₘₐₓ × 10⁻⁸ (Ms⁻¹)</th>
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³⁷[Ag⁺] = 100 nM. ³⁸[Hg²⁺] = 1 μM.
Fig. 4 (a) Plots of the $A_{652}$ with the concentration of $\text{Ag}^+$, and the inset is the corresponding calibration curve. (b) The $A_{652}$ in the TMB–$\text{H}_2\text{O}_2$ reaction catalyzed by GSH-Pd2.6 in the presence of 0.1 $\mu\text{M}$ $\text{Ag}^+$, 1 $\mu\text{M}$ metal ions including $\text{Na}^+$, $\text{Mg}^{2+}$, $\text{Cu}^{2+}$, $\text{Pb}^{2+}$, $\text{Cd}^{2+}$, $\text{Zn}^{2+}$, $\text{Ni}^{2+}$, $\text{Mn}^{2+}$, $\text{Fe}^{3+}$, $\text{Co}^{2+}$ and $\text{Hg}^{2+}$, respectively. (c) Effect of 1 $\mu$M EDTA on the output signal generated by GSH-Pd2.6 in the presence of $\text{Ag}^+$ and $\text{Hg}^{2+}$ respectively; The $A_0$ and $A$ represent the $A_{652}$ produced by GSH-Pd2.6 and GSH-Pd2.6-ion, respectively. The reaction system contains 0.125 mM TMB, 125 mM $\text{H}_2\text{O}_2$ and 900 nM Pd. The $A_{652}$ is collected at 10 min after initiation.

Fig. 5 (a) Pd 3d XPS spectra of GSH-Pd2.6 incubated with equivalent molar of $\text{Ag}^+$ and $\text{Hg}^{2+}$, respectively. TEM image of GSH-Pd2.6 upon addition of equivalent molar of $\text{Ag}^+$ (b) and $\text{Hg}^{2+}$ (c).
(Fig. 5c), the $v_{\text{max}}$ value shows that Hg$^{2+}$ facilitates the peroxidase-like activities of GSH-Pd. Different metalophilic interactions have been reported to exhibit distinct effects on the peroxidase-like activity of metal nanoparticles. For example, Hg$^{2+}$ significantly enhances the peroxidase-like activity of Au nanoparticles while it inhibits the related activity of Pt nanoparticles. Therefore, it is reasonable to conclude that Hg$^{2+}$ and Ag$^{+}$ show the opposite influence on the peroxidase-like activity of Pd-based nanzyme.

To validate the sensing application of GSH-Pd in real samples, spiked-recovery experiments were carried out in the lake water upon addition of 1 $\mu$M EDTA. The recovery rates are calculated as 121%, 111% and 92% for 20, 50 and 80 nM spiked Ag$^{+}$, respectively (Table S1$^\ddagger$). It is suggested that the concentration of Ag$^{+}$ detected by ICP-MS is lower than the concentration determined by the colorimetric method proposed in this study. Compared with other detection methods listed in Table 2, GSH-Pd nanzyme can be recognized as a promising candidate for quantitative determination of Ag$^{+}$ in drinking water, owing to several advantages such as low LOD, low material and energy consumption, as well as green synthesis.

Further, we tested the potential application of this sensing system on the detection of Ag nanoparticles. UV-vis spectra of as-prepared citrate-capped Ag nanoparticles with the size distribution of 5–20 nm, exhibit a maximum absorption peak at 412 nm (Fig. S13 and S14$^\ddagger$). Upon addition of H$_2$O$_2$, the absorption band gradually decreases within 30 min, suggesting the H$_2$O$_2$-mediated oxidation of Ag nanoparticles. In the TMB-H$_2$O$_2$ reaction catalyzed by GSH-Pd2.6, the A$_{652}$ signal gradually decreases with the concentration of Ag nanoparticles (Fig. S15$^\ddagger$). A good linear relationship can be obtained in the concentration range of 10–100 nM, with the LOD of 6.1 nM. It is demonstrated that GSH-Pd nanzyme can be potentially utilized for colorimetric detection of Ag nanoparticles.

### 4. Conclusion

Pd nanoparticles are facilely synthesized by using GSH as the nucleation template, showing highly peroxidase-like activity. The molar ratio of [Na$_2$PdCl$_4$]/[GSH] significantly affects the physicochemical properties of GSH-Pd including particle sizes, surface charge states and enzymatic activities. The most efficient Pd nanzyme consisting of approximately 57% Pd$^0$ species with an average diameter of 2.6 nm, exhibit the $K_m$ value of 0.068 mM toward TMB and 156 mM toward H$_2$O$_2$. Ag$^{+}$ selectively binds to Pd$^0$ species through metalophilic interactions and inhibits the peroxidase-like activity of Pd nanoparticles. GSH-Pd is employed to explore colorimetric detection of Ag$^{+}$ in aqueous solution, resulting in the LOD of 1.2 nM. This developed sensing system is potentially applicable for quantitative detection of Ag$^{+}$ in drinking water samples as well as Ag nanoparticles in the aqueous solution.

### References


**Table 2** Comparisons of different Ag$^{+}$-sensing platforms

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<tr>
<th>Method</th>
<th>System</th>
<th>LOD/nM</th>
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<tr>
<td>Electrochemistry$^5$</td>
<td>C-rich DNA-modified electrode</td>
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<td>Electrochemistry$^6$</td>
<td>Fe$_3$O$_4$@Au nanoparticles</td>
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<td>Electrochemistry$^7$</td>
<td>Alkanethiol-carbon nanotube-oligonucleotide electrodes</td>
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<td>Fluorescence$^8$</td>
<td>CH$_3$CN-MOPS</td>
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<td>Fluorescence$^9$</td>
<td>FAM-ssDNA/GO</td>
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<td>DNA-Ag nanoclusters</td>
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<td>Fluorescence$^{11}$</td>
<td>CdS quantum dots</td>
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<td>Colorimetry$^{12}$</td>
<td>G-quadruplex-capped gold nanoparticles</td>
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<td>G-quadruplex-hemin</td>
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<td>Colorimetry</td>
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**Acknowledgements**