Label-free colorimetric sensing of copper(II) ions based on accelerating decomposition of H$_2$O$_2$ using gold nanorods as an indicator$^\dagger$

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A novel label-free colorimetric strategy was reported for sensitive detection of copper ions (Cu$^{2+}$) by using the decelerating etching of gold nanorods (GNRs). H$_2$O$_2$ was employed as the oxidant for corrosion of GNRs, leading to the decrease of the aspect ratio of GNRs. In the absence of Cu$^{2+}$, the redox corrosion of GNRs by H$_2$O$_2$ occurred rapidly, causing the distinct color change of GNRs from bluish green to purplish red. By virtue of the strong and specific catalysis by Cu$^{2+}$ of the decomposition of H$_2$O$_2$, the rate of redox corrosion can be decelerated. Relevant experimental parameters, including pH value, concentrations of NaSCN and H$_2$O$_2$, incubation temperature and time were evaluated. Under optimal conditions, our method gave a good linear range of 10–300 nM ($R = 0.9985$) for Cu$^{2+}$ and the detection limit with the naked eye is as low as 10 nM. Thus, the proposed colorimetric sensor is simple, sensitive (4.96 nM) and selective, and it has been successfully applied to detect Cu$^{2+}$ in shellfish samples. Moreover, the potential mechanism was also discussed.

Introduction

Colorimetric detection methods are extremely attractive in chemical and biological analysis, as they require no sophisticated instrumentation and signal recognition can be achieved by the naked eye in the form of a color change. Compared with other detection methods, colorimetric detection also offers advantages of simplicity, rapidity, and cost-effectiveness. It is reported that a variety of signal reagents have been used in colorimetric assays, such as organic dyes,$^1$ polymers,$^2$ enzymes$^3$ and nanoparticles.$^4$ Among different kinds of signal reagents, gold nanorods (GNRs), which possess distinctive optical properties as a typical elongated metal nanostructure, have been proved to be of great use in colorimetric detections.$^5$ The UV-IR absorption spectrum of GNRs displays two peaks, one is the transverse surface plasmon resonance (TSPR) band and the other is the longitudinal surface plasmon resonance (LSPR) band. The LSPR band is so sensitive that a tiny change in surface binding environment will lead to an obvious wavelength shift.$^6$ Additionally, the extinction coefficient of GNRs ($\sim 10^9$ M$^{-1}$ cm$^{-1}$) is larger than that of gold nanoparticles ($2.7 \times 10^8$ M$^{-1}$ cm$^{-1}$), and several orders of magnitude higher than those of traditional organic chromophores.$^7$ It is also important to mention that elongated nanomaterials are inherently more sensitive to the local dielectric environment, when compared to similar-sized spherical nanoparticles.$^8$ By virtue of the above advantages, GNRs have become a good candidate for sensor research.

The past few years have witnessed great progress in colorimetric detection based on GNRs.$^9$ One of the strategies is based on the aggregation of specific ligand modified GNRs. A variety of molecules (polymers, small molecules, DNA, etc.) can be joined to the surface of nanorods and the modifying molecules would be combined to different parts (end or side) of the surface due to the anisotropy of nanorods.$^{10}$ When a specific object is added, an obvious target induced aggregation of GNRs occurs by end-to-end linkages or side-by-side assembly via covalent bonding, hydrogen bonding or electrostatic interaction, along with the color change of colloid. For example, cysteine-modified GNRs were reported for the colorimetric detection of Cu$^{2+}$. In the presence of Cu$^{2+}$, the nanorods aggregated through longitudinal assembly due to the strong coordination between Cu$^{2+}$ and cysteine (Cys–Cu–Cys), accompanied by a visible color change from blue-green to dark gray.$^{11}$ However, most aggregation based colorimetric strategies display some drawbacks, such as the complex synthetic procedures of crosslinkers and strong non-specific interactions. The other colorimetric strategy is based on the redox-regulated surface chemistry on GNRs. It is well known that the optical properties of GNRs are greatly affected by a small change in the shape and size.$^{12}$ Taking
advantage of this property, a variety of colorimetric methods have been presented involving wavelength changes in the absorption spectra of GNRs. For instance, in the presence of Br\(^-\) and Cl\(^-\) ions, the standard electron potential of Au(0)/Au(I) is lower than that of Cr(III)/Cr(VI), which enables GNRs to be oxidized by Cr(VI).\(^{13}\) As a result, the redox etching caused an obvious decrease in length but only a slight change in diameter, presenting distinct color changes. A similar method was reported involving oxidation etching of GNRs induced by ferric chloride at room temperature.\(^{14}\)

Recently, Fang et al. developed a colorimetric method for visual detection of Cu\(^{2+}\) based on the oxidative corrosion of gold nanoparticles.\(^{15}\) In their presented method, H\(_2\)O\(_2\) and SCN\(^-\) were employed as the oxidant and the ligand, respectively. Due to the catalysis by Cu(NH\(_3\))\(_2\)Cl\(_2\) of the decomposition of H\(_2\)O\(_2\) in NH\(_3\)/NH\(_4\)Cl buffer, the oxidative corrosion of gold nanoparticles can be inhibited. The detection limit with the naked eye was down to 50 nM.

Inspired by this study, we designed a highly sensitive and selective colorimetric strategy based on the strong and specific inhibition of Cu\(^{2+}\) on the corrosion of GNRs in the absence of NH\(_3\). Our proposed colorimetric method displays high sensitivity and selectivity and the detection limit by the naked eye is down to 10 nM. The sensing mechanism and practical application of the method in shellfish samples were also investigated and satisfactory results were obtained.

## Experimental section

### Chemicals

Cetyltrimethylammonium bromide (CTAB) and tris(hydroxymethyl) aminomethane (Tris) were purchased from Sigma. CuSO\(_4\)-5H\(_2\)O was obtained from Aladdin Industrial Incorporation. Hydrogen tetrachloroaurate(III) dehydrate (HAuCl\(_4\)-4H\(_2\)O), sodium borohydride, sodium thiocyanate, hydrogen peroxide (~30%), ascorbic acid, HCl (36–38%), HNO\(_3\) (65–68%) and other metal salts used were obtained from Sinopharm Group Chemical Reagent Co. Ltd (Beijing, China). All the reagents were of analytical grade and used without any further purification.

### Apparatus

Solutions were prepared with deionized water (18.2 M\(\Omega\)-cm specific resistance) purified by a Cascada LS Ultrapure water system (Pall Corp., USA). Transmission electron microscope (TEM) images were taken on a JEM-1230 electron microscope (JEOL Ltd, Japan) operating at 100 kV. UV-Vis absorption spectra were recorded on a Thermo Scientific NanoDrop 2000C spectrophotometer (Gene Company Ltd, USA).

### Synthesis of GNRs

For preparation of GNRs a typical seed-mediated and CTAB surfactant-directed method was adopted with some necessary modification.\(^{16}\) Briefly, 50 \(\mu\)L of 50 mM HAuCl\(_4\) was added to 7.7 mL of 0.10 M CTAB solution upon stirring, and 0.30 mL of fresh, ice-cold 0.01 M NaBH\(_4\) solution was put into the mixed solution, which resulted in the change of solution color from bright brown-yellow to pale brown. The obtained solution was stirred for another 2 minutes and kept at 28 °C as the seed solution for the next procedure. Then, 50 mL of 0.10 M CTAB solution was mixed with 600 \(\mu\)L of 50 mM HAuCl\(_4\) with constant stirring. To this solution, 150 \(\mu\)L of 0.01 M AgNO\(_3\) solution was added and 480 \(\mu\)L of 0.1 M ascorbic acid (AA) was added with gentle stirring, leading to the change of solution color from dark yellow to colorless. Subsequently, 100 \(\mu\)L of the seed solution was injected into the colorless solution at 28 °C. The mixture was stirred for another 20 minutes and the resultant colloidal of GNRs was stored for future use.

### Colorimetric assay process for Cu\(^{2+}\)

After optimization of the experimental conditions, the colorimetric detection of Cu\(^{2+}\) was tested under the selected conditions. The measurement was carried out in 50 mM Tris–HCl buffer solution (pH 8.7). 10 \(\mu\)L of 0.1 M NaSCN and 10 \(\mu\)L of 3 M \(\mathrm{H}_2\mathrm{O}_2\) were subsequently dissolved in 800 \(\mu\)L of buffer solution containing 200 \(\mu\)L of the GNRs, and then 10 \(\mu\)L of different concentrations of Cu\(^{2+}\) were added to each of the mixture solutions. Finally, the mixture solutions were incubated in a 60 °C water bath for 17 minutes and the UV-Vis absorption spectra were recorded.

In the selectivity experiments, all samples were tested in a similar way. We investigated the selectivity of our approach for Cu\(^{2+}\) over other metal ions (Li\(^+\), Na\(^+\), K\(^+\), Zn\(^{2+}\), Al\(^{3+}\), Mg\(^{2+}\), Fe\(^{3+}\), Mn\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), Ni\(^{2+}\), Cr\(^{3+}\), Ag\(^+\), As\(^{3+}\), Hg\(^{2+}\)) under the same optimized conditions.

### Analysis of real samples

Shellfish samples were digested with concentrated nitric acid.\(^{17}\) Typically, by removing the outer shells, the obtained samples were washed with deionized water and then put into the refrigerator. Once frozen, the materials were placed in a freeze-dryer to remove water. After drying, the materials were ground to powder by mortars. To 0.3 g of powder sample, 10 mL concentrated nitric acid were added, and the mixtures were incubated in a high pressure digestion tank at 150 °C for 6 h. Finally, the obtained colorless solution was diluted to 50 mL.

To detect the concentration of Cu\(^{2+}\) in the real samples, a similar test to the above assay process was adopted, just using samples directly to replace copper solution.

### Results and discussion

#### Sensing mechanism

The sensing mechanism for colorimetric detection of Cu\(^{2+}\) based on decelerating etching of GNRs is shown in Scheme 1. The UV-Vis absorption spectrum of the initial GNRs (length/diameter ratio about 2 : 1) exhibits two peaks located at 520 nm and 650 nm (Fig. 1(A), curve a), corresponding to the SPR band and the LSPR band, respectively. When 1 mM NaSCN and 30 mM \(\mathrm{H}_2\mathrm{O}_2\) were added to the colloidal solution, the LSPR band weakened and shifted to a shorter wavelength (Fig. 1(A), curve c) after incubation in a 60 °C water bath for 17 minutes with a color change from bluish green to purplish red. The
obvious color change resulted from the oxidation of GNRs by \( \text{H}_2\text{O}_2 \) in the presence of \( \text{SCN}^-/\text{C}_0 \). Because of the strength of the \( \text{Au}^-\text{S} \) bond, \( \text{SCN}^-/\text{C}_0 \) containing a thio-group can strongly coordinate with gold ions, replacing part of the CTAB on the surfaces of GNRs. The standard potential of \( \text{Au(SCN)}_2^-/\text{C}_0 \) is 0.69 V in eqn (1), and the conditional potential of \( \text{H}_2\text{O}_2/\text{H}_2\text{O} \) is 1.24 V, calculated according to Nernst equation at pH 9.0 at room temperature. So gold can be easily oxidized by \( \text{H}_2\text{O}_2 \) and form \( \text{Au(SCN)}_2^- \) complexes in solution. The total redox reaction can be described as:

\[
\text{Au} + 2\text{SCN}^- + \text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow \text{Au(SCN)}_2^- + 2\text{H}_2\text{O}.
\]

On the contrary, the presence of 50 nM \( \text{Cu}^{2+} \) resulted in a slight blue shift of the LSPR band (Fig. 1(A), curve b) and the color of the solution was pale blue. According to the phenomenon we suggested that \( \text{Cu}^{2+} \) can catalyse the decomposition of \( \text{H}_2\text{O}_2 \) to inhibit the oxidative corrosion of GNRs. The differences among TEM images also indicated the inhibition of \( \text{Cu}^{2+} \) towards the etching of GNRs (Fig. 1(C)). The mechanism we put forward is different from Fang’s \textit{et al.} In their report, it was \( \text{Cu(NH}_3)_6^{2+} \) that could efficiently catalyse the decomposition of \( \text{H}_2\text{O}_2 \) in alkaline media to inhibit the etching of gold nanoparticles. In the absence of \( \text{NH}_3 \), the bare \( \text{Cu}^{2+} \) at low concentration had no significant inhibition.

\[
\text{Au(SCN)}_2^- + \text{e}^- \rightarrow \text{Au} + 2\text{SCN}^- \quad E^\circ = 0.69 \text{ V} \quad (1)
\]

\[
\text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow 2\text{H}_2\text{O} \quad E^\circ = 1.77 \text{ V} \quad (2)
\]

In order to further explore the potential mechanism of our proposed method, the residual \( \text{H}_2\text{O}_2 \) was finally estimated according to the intensity of the maximum absorption peak of potassium permanganate in 526 nm. As is shown in Fig. 1(B), when 10 \( \mu \text{l} \) of 3 M \( \text{H}_2\text{O}_2 \) was dissolved in 1 mL of buffer solution in the presence or absence of 1 mM NaSCN, the potassium permanganate was reacted completely (curve d and e), illustrating the catalytic decomposition of \( \text{H}_2\text{O}_2 \) did not occur. However, when 1 mM \( \text{Cu}^{2+} \) was added to the above two mixture solutions, the maximum absorption peaks of potassium permanganate in the absence of \( \text{SCN}^-/\text{C}_0 \) (curve b) were a little higher than in the presence of \( \text{SCN}^-/\text{C}_0 \) (curve c), showing the catalysis by \( \text{Cu}^{2+} \) of the decomposition of \( \text{H}_2\text{O}_2 \) was more remarkable in the presence of \( \text{SCN}^-/\text{C}_0 \). The major mechanism and the basic steps of the catalytic reaction have been shown on the basis of previous work.\textsuperscript{18} Eqn (3) and (4) are fast and \( \text{Cu}^{2+} \) was reduced in the form of \( [\text{CuI(SCN)}_n]^{+ n} \) and \( \text{HO}_2^-/\text{CuI} \). Eqn (5)–(7) result in \( \text{OSCN}^-/\text{C}_0 \) autocatalysis, which is of great importance in

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**Scheme 1** Schematic mechanism for \( \text{Cu}^{2+} \) sensing based on decelerating etching of GNRs.

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**Fig. 1** (A) UV-Vis absorption spectra and (C) TEM images of before incubation (a) and after incubation in the presence (b) and absence (c) of 50 nM \( \text{Cu}^{2+} \) solution; (B) UV-Vis absorption spectra of potassium permanganate reacted with 1 mM NaSCN (a), 30 mM \( \text{H}_2\text{O}_2 \) + 1 mM NaSCN + 1 mM \( \text{Cu}^{2+} \) (b), 30 mM \( \text{H}_2\text{O}_2 \) + 1 mM \( \text{Cu}^{2+} \) (c), 30 mM \( \text{H}_2\text{O}_2 \) + 1 mM NaSCN (d), 30 mM \( \text{H}_2\text{O}_2 \) (e) after incubation, respectively.
the regeneration of SCN− and [CuII(SCN)2]− (eqn (8)). Among
these reaction steps, the rate of oxidation of stable
[CuI(SCN)n]− was the rate-determining step of the reaction,
deciding the main length of the catalytic reaction. Thus, H2O2
could be efficiently catalyzed in alkaline media in the absence
of NH3. What’s more, SCN− could not only reduce the oxidation
reduction potential of GNRs, but also take part in the catalytic
process of H2O2.

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{Cu}^{2+} + \text{OH}^- & \rightarrow \text{HO}_2^- \cdot \text{Cu}^+ + \text{H}_2\text{O} \\
\text{HO}_2^- \cdot \text{Cu}^+ + n\text{SCN}^- & \rightarrow \text{[CuI(SCN)n]}^{-n} + \text{H}_2\text{O} \\
\text{H}_2\text{O}_2 + \text{SCN}^- & \rightarrow \text{OSCN}^- + \text{H}_2\text{O} \\
\text{H}_2\text{O}_2 + \text{OSCN}^- & \rightarrow \text{OOSCN}^- + \text{H}_2\text{O} \\
\text{OOSCN}^- + \text{SCN}^- & \rightarrow 2\text{OSCN}^-
\end{align*}
\]

\[
\text{[CuI(SCN)n]}^{-n} + \text{H}_2\text{O} + \text{OSC}^{-} \rightarrow \text{[CuI(SCN)n]}^{2-n} + \text{OH}^- + \text{SCN}^- + \text{OH}^-
\]

Optimization of experimental conditions

For optimization of the colorimetric strategy, relevant experimental
parameters, including pH value, concentrations of NaSCN and H2O2, incubation temperature and time were evaluated
by the colloidal color.

The influence of pH value on the performance of the GNRs
colorimetric probe was carried out in a range from 7.5 to 8.9,
obtained by adjusting the ratio of Tris to HCl. In the pH range
7.5–8.5, little oxidation of GNRs occurred because of the high
rate of catalytic decomposition of H2O2, along with no obvious
color change. With increasing pH value, the rate of catalytic
reaction became slower, leading to the more extensive oxidation
of GNRs (Fig. S1†). Thus, we chose 50 mM Tris–HCl buffer
solution (pH 8.7) as the reaction media.

The effect of concentration of NaSCN was investigated over
the range from 0.6 to 1.4 mM, as shown in Fig. S2. With
increasing concentration of NaSCN, the rate of the total redox
reaction became faster. However, despite higher concentrations
of NaSCN forming more stable complexes [CuI(SCN)n]−, the rate
of oxidation of stable [CuI(SCN)n]− became slower. Hence, we chose 1 mM NaSCN in the following studies.

The concentration of H2O2 was changed from 10 to 50 mM.
The rate of catalytic reaction became faster with increasing
concentration of H2O2. However, the redox corrosion of GNRs
by H2O2 was also increased at the same time (Fig. S3†). In order
to meet the needs of colorimetric detection, 30 mM H2O2 was
chosen as a compromise.

Finally, the effect of incubation temperature and time was
taken into consideration. As is observed in Fig. S4 and S5† the
etching of GNRs was dependent on both incubation temperature
and time. The oxidative corrosion of GNRs increased with
the increase of temperature at a fixed reaction time of 17
minutes. By setting the reaction temperature at 60 ℃, the
selective etching caused an obvious color difference in the
absence and presence of 50 nM Cu2+ after reacting for 17
minutes. Thus, the reaction was performed by incubating the
system for 17 minutes in the following studies.

Sensitivity and selectivity for Cu2+

Fig. 2 shows the analytical performance of this method for the
determination of Cu2+. Under optimal detection conditions, a
good linear relationship between the maximum absorbance of
LSMR band and the concentrations of Cu2+ could be obtained
in the range of 10 nM to 300 nM. The detection limit was 4.96 nM
calculated by 3σ/S.

To further realize the selectivity of our developed method,
other metal ions, including Li+, Na+, K+, Zn2+, Al3+, Mg2+, Fe3+,
Mn2+, Cd2+, Pb2+, Ni2+, Cr3+, Ag+, As3+, Hg2+ were examined
under optimum conditions. As illustrated in Fig. 3, the presence
of 100-fold Li+, Na+, K+, Zn2+, Al3+, Mg2+, Fe3+, Mn2+, 10-fold
Cd2+, Pb2+, Ni2+, As3+ and 1-fold Cr3+, Ag+, Hg2+ cannot catalyze
the decomposition of H2O2 to inhibit the oxidative corrosion
of GNRs; this can only occur with Cu2+. In addition, the increase of
Hg2+ concentration would affect the results because of the formation of Hg(OH)2 → HgO capped on the rod, inhibiting
the etching of gold. However, as a common metal ion in environ-
mental samples, Cu2+ is normally present in greater concen-
trations than other ions, such as Cd2+, Pb2+, Ni2+, As3+, Cr3+, Ag+
and Hg2+. As a consequence, the color of solution with Cu2+ was
blue, while others are pale red. These results indicate the high
selectivity of the proposed method.

Detection of Cu2+ in real samples

To evaluate the potential application of the proposed method,
the detection of Cu2+ in shellfish samples was further carried
out. As shown in Table 1, the recoveries ranged from 72.11% to
102.8%, which indicates the results obtained by this assay
match well with those by ICP-MS, showing the high potential
of the colorimetric method for Cu2+ quantification in real samples.

![Fig. 2. UV-Vis absorption spectra of GNRs after incubation with different concentrations of Cu2+ for 17 minutes. Insets are a plot of the maximum absorbance of LSPR band versus the concentration of Cu2+ and the color changes with the increasing concentrations of Cu2+ from left to right, respectively.](image-url)
**Table 1** Determination of Cu$^{2+}$ in shellfish samples

<table>
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<tr>
<th>Sample</th>
<th>Found by ICP-MS/μM</th>
<th>Detected/μM</th>
<th>Recovery (%)</th>
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**Conclusions**

In conclusion, we have reported a highly sensitive and selective colorimetric method for the detection of Cu$^{2+}$ based on GNRs. The present work takes advantage of catalysis by Cu$^{2+}$ of the decomposition of H$_2$O$_2$ which could specifically inhibit the corrosion of GNRs, and we employ this system as the colorimetric probe. Additionally, our method shows sensitive and selective response towards Cu$^{2+}$ ions without any other labelling or modification steps. Furthermore, the proposed approach exhibits great practicality for the detection of Cu$^{2+}$ in real samples, which might present a promising potential for application in environmental monitoring.

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**Notes and references**