A colorimetric assay for measuring iodide using Au@Ag core–shell nanoparticles coupled with Cu$^{2+}$

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HIGHLIGHTS

- Au@Ag core–shell NPs were synthesized and coupled with Cu$^{2+}$ for the colorimetric I$^-$/I$_2$ sensing.
- This assay is simple, rapid and selective.
- Au@Ag core–shell NPs-Cu$^{2+}$ were embedded into agarose gels as test strips.

ABSTRACT

Au@Ag core–shell nanoparticles (NPs) were synthesized and coupled with copper ion (Cu$^{2+}$) for the colorimetric sensing of iodide ion (I$^-$). This assay relies on the fact that the absorption spectra and the color of metallic core–shell NPs are sensitive to their chemical ingredient and dimensional core-to-shell ratio. When I$^-$ was added to the Au@Ag core–shell NPs-Cu$^{2+}$ system/solution, Cu$^{2+}$ can oxidize I$^-$ into iodine (I$_2$), which can further oxidize silver shells to form silver iodide (AgI). The generated Au@AgI core–shell NPs lead to color changes from yellow to purple, which was utilized for the colorimetric sensing of I$^-$. The assay only took 10 min with a lowest detectable concentration of 0.5 μM, and it exhibited excellent selectivity for I$^-$ over other common anions tested. Furthermore, Au@Ag core–shell NPs-Cu$^{2+}$ was embedded into agarose gels as inexpensive and portable "test strips", which were successfully used for the semi-quantitation of I$^-$ in dried kelps.

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1. Introduction

Iodine is an indispensable component of the thyroid hormone, which plays a crucial role in the regulation of cell metabolism, muscle tissue growth and neurological development [1]. Either deficiency or excess of iodine intake would cause major health problems. For example, iodine deficiency in pregnancy will cause fetal goiter, cretinism, intellectual impairment and neonatal hypothyroidism, while iodine excess will lead to hyperthyroidism [2]. In addition, iodide (I$^-$) has been used in various applications, such as organic synthesis, analytical chemistry and manufacture of dyes and drugs. Therefore, a variety of methods for detecting I$^-$ have been developed, such as electrochemical approaches [3,4], capillary electrophoresis [5], Surface-Enhanced Raman Scattering [6],
inductively coupled plasma-mass spectrometry [7] and atomic absorption spectrometry [8]. In spite of good reliability and accuracy, these approaches may suffer from several drawbacks, such as the need of expensive instruments, professional operators and complicated procedures. Hence, the development of new methods that meet the demands of simplicity, rapidity, sensitivity and selectivity is ongoing and fascinating.

Metal nanoparticles (NPs), such as Au NPs and Ag NPs, have been widely used as optical sensing nanomaterials due to their high extinction coefficients and distance-dependent optical properties in the visible-region [9]. Base on these properties, a series of metal NPs based colorimetric methods has been developed to detect I⁻ [10–18]. For example, Zhang et al. synthesized citrate-stabilized Cu@Au core–shell NPs and used them as colorimetric probes for the recognition of I⁻ through the I⁻-induced transformation of irregular shaped NPs to nearly spherical ones [10]. By this approach, the lowest detectable concentration of I⁻ observed by naked-eyes can be as low as 6 μM. Recently, Chen et al. have proposed a colorimetric assay for I⁻ measurement based on the anti-aggregation of thymine modified Au NPs in the presence of Hg²⁺ [11]. This assay is highly sensitive with a detection limit of 10 nM, but requires toxic Hg²⁺ in the chemical ingredient and dimensional shell-to-core ratio are changed by forming Au@Ag core–shell NPs, leading to observable color changes from yellow to purple (in the web version). Such color change is dependent on the concentration of I⁻, which can be used for the colorimetric detection of I⁻. In addition, this assay exhibits good selectivity towards I⁻, and other halide ions (Cl⁻, Br⁻ and F⁻) show negligible response as Cu²⁺ cannot oxidize these ions.

2. Experimental

2.1. Chemicals and solutions

Chloroauric acid (HAuCl₄·3H₂O), citrate trisodium, agar powder, KI, NaF, KCl, NaBr, NaNO₃, NaNO₂, Na₂SO₄, Na₂SO₃, Na₃PO₄, NaHCO₃, NaBO₃, NaClO₄, CH₃COONa, KSCN, Na₂B₄O₇, Na₂CO₃, KIO₃, AgNO₃, and CuCl₂ were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All of the reagents were of analytical grade and used without further purification. Ultrapure water was used throughout the experiments. The Tollens reagent was prepared by mixing AgNO₃ (0.5 M, 1 mL), NH₃·H₂O (25%–28%, 1.04 mL), NaOH (3 M, 0.65 mL) and water (17.3 mL).

2.2. Instrumentation

Ultrapure water (18.2 MΩ cm) was acquired from a Millipore Autopure WR600A system. UV–vis absorption spectra were measured on an UV-2450 spectrophotometer (Shimadzu). The photographs were taken using a Canon IXUS-125HS digital camera. Transmission electron microscopy (TEM) images were acquired using a JEM 1400 microscope (JEOL). Powder X-ray diffraction was performed using a X'Pert PRO MPD diffractometer (PANalytical).

2.3. Preparation of Au@Ag core–shell NPs-Cu²⁺ test solution

Citrated stabilized Au NPs (13 nm diameter) were prepared based on Frens’ method with little modification [23]. The concentration of the Au NPs was 12 nM as determined by UV–vis spectrometry. Au@Ag core–shell NPs were synthesized as follows based on our previous report [24]. An aliquot of 200 μL Au NPs (12 nM), 644 μL water, 60 μL Tollens reagent and 96 μL of aqueous HCHO (10 mM) were sequentially added into a sample vial. The mixed solution was incubated for 20 min and its color was changed from pink to deep yellow (in the web version), indicating the formation of Au@Ag core–shell NPs. The as-prepared Au@Ag core–shell NPs with CuCl₂ (0.01 M) in a volume ratio of 24:1 were mixed as the test solution.

Fig. 1. Schematic representation of the sensing mechanism using Au@Ag core/shell NPs-Cu²⁺.
2.4. Detection of $I^-$ using the Au@Ag core–shell NPs-$Cu^{2+}$

In brief, sample solution or standard solutions (500 µL) containing $I^-$ from 0 to 400 µM was added into the test solution (500 µL). The mixture was incubated at 20–25 °C for 15 min and then was used for photographing and UV–vis detection.

2.5. Immobilization of Au@Ag core–shell NPs-$Cu^{2+}$ into agarose gel

Agar powder (0.1 g) was added into a solution of CuCl$_2$ (0.002 M, 5 mL) under vigorous stirring, then heated to boil and kept for 30 s. The as-prepared agar solution (2 mL) was added into the Au@Ag core–shell NPs solution (3 mL), and the mixture was placed into a Petri dish (I.D. 3.5 cm). The Petri dish was then placed into a refrigerator at −18 °C for 2 min and cut into spherical shapes with 0.8 cm in diameter for further use.

2.6. Detection of $I^-$ using the Au@Ag core–shell NPs-$Cu^{2+}$

The as-prepared agarose gels were immersed into the standard or sample solution and incubated for 20 min. Then, the agarose gels were moved out and used for photographing.

2.7. Analysis of real sample

The assay was applied to the analysis of $I^-$ in drinking water and dried kelps. Drinking water samples were obtained from a local supermarket and used as received. Dried kelps were also purchased from the supermarket, and they were washed, dried and finally crushed into powders. Then, the powders (1.2 g) were put into a muffle furnace, gradually heated to 400 °C and maintained at this temperature for 3 h. After cooling to room temperature, the burned powders were dissolved in 50 mL ultrapure water, followed by a filtration procedure to remove residual solid particles. The detection procedure was the same with that of Sections 2.4 and 2.6.

3. Results and discussion

3.1. Sensing strategy of the colorimetric assay

The detection mechanism is primarily based on the following redox reactions:

\[ 2Cu^{2+} + 4I^- = 2CuI + I_2 \]  
\[ 2Ag + I_2 = 2AgI \]  

Au@Ag core–shell NPs-$Cu^{2+}$ detection system was selected based on following considerations. As listed in Table S1, the standard electrode potential of $Cu^{2+}/Cu$ is less than $Ag^+/Ag$, which means that $Cu^{2+}$ is difficult to oxidize Ag to disrupt the Au@Ag core–shell NPs system. Although $\phi(Cu^{2+}/Cu)$ is less than $\phi(I_2/I^-)$, it is believed that $Cu^{2+}$ can oxidize $I^-$ because the formation of the highly stable CuI results in a significant increase of $\phi(Cu^{2+}/Cu)$. More importantly, the generated $I_2$ is able to oxidize silver to form AgI, leading to the change of ingredient and dimensional shell-to-core ratio of the Au@Ag core–shell NPs, which induces color changes from deep yellow to purple. These color changes are depended on the concentration of $I^-$, and thus can be exploited for the colorimetric detection of $I^-$.  

3.2. Characterization of Au@Ag core–shell NPs-$Cu^{2+}$ probes

As reported previously by our group, Au@Ag core–shell NPs were synthesized using citrate-stabilized Au NPs as templates in the presence of Tollens reagents (the key component is $[Ag(NH_3)_2]^+$) and HCHO. The silver shells generated by the Tollens reaction were proved to deposit onto Au NPs to form core–shell structures. Fig. 2a–b shows that the majority of the as-synthesized NPs were sphere shapes, which displayed inhomogenous electronic
density with a dark core deposited by a lighter shell. The high-resolution transmission electron microscopy (HRTEM) (Fig. 2c) shows that the core and shell portion have values of lattice spacing of 0.235 and 0.205 nm, corresponding to the (111) and (200) planes of face centered cubic (fcc) Au and Ag, respectively. In addition, the UV–vis spectrum of the as-synthesized NPs exhibits two absorption peaks centering at 394 and 520 nm (Fig. S1 in Supporting information), which are considered to be the characteristic SPR peaks of Au@Ag core–shell NPs [19,22]. As shown in Fig. S1, neither the spectrum nor the color of the Au@Ag core–shell NPs solution was changed after the addition of Cu²⁺. In contrast, further addition of I⁻ into the mixture of Au@Ag core–shell NPs-Cu²⁺, the color of the solution turned from yellow to purple. Correspondingly, the intensity of the absorption peak at 394 nm decreased along with an emergence of an absorption peak centering at 422 nm, which was related to the SPR absorption of AgI [25–27]. TEM images (Fig. 2d–e) also indicate the original silver shells partly dissolved accompanied with the formation of thicker irregular shells. As can be seen from Fig. 2f, the previous lattice plane of fcc Ag disappeared, while a new one of 0.374 nm belonging to body centered cubic (bcc) AgI was found. To get better insight into the formation of Au@AgI core–shell NPs, X-ray diffraction (XRD) data were collected. Compared with the XRD pattern of the initial Au@Ag core–shell NPs (Fig. 3a), two new diffraction peaks belonging to the (110) and (200) lattice planes of the bcc AgI emerged. From the above results, it is reasonable to suppose that Au@AgI core–shell NPs were formed after the reaction between Au@Ag core–shell NPs-Cu²⁺ and I⁻.

3.3. Optimization of experimental conditions

Based on the detection mechanism mentioned above, the performance of the colorimetric assay may be strongly relevant to
the silver shell thickness of the Au@Ag core–shell NPs and the concentration of Cu²⁺. Our previous results indicate that different silver shell thickness of the core–shell NPs can be obtained by simply varying the concentration of [Ag(NH₃)₂]⁺ and HCHO [28]. We varied both the concentrations of [Ag(NH₃)₂]⁺ (from 0.08 to 0.4 mM) and HCHO (from 0.32 to 1.6 mM) to prepare core–shell NPs with different silver shell thickness, and the ratio of [Ag(NH₃)₂]⁺ to HCHO was set as 1:4. Fig. 4a shows that the core–shell NPs synthesized at the [Ag(NH₃)₂]⁺ concentration of 0.24 mM exhibited the best sensitivity for the I'/C₀ detection. The concentration of Cu²⁺ is also essential for this assay, because adequate concentration of Cu²⁺ will facilitate the iodide oxidation. However, excessive Cu²⁺ might induce the aggregation of Au@Ag core–shell NPs. Fig. 4b shows that the addition of Cu²⁺ from 0.05 to 0.3 mM barely affected the Au@Ag core–shell NPs system. Fig. 4c further indicated that the maximum sensitivity was obtained as the Cu²⁺ concentration reached 0.2 mM, which was selected for the following experiments. Fig. 4d reveals that the reaction requires 10 min to reach equilibrium. In order to make sure that the reaction is finished, the reaction time was fixed at 15 min. The pH effect was also investigated, and it was found that the assay exhibited no obvious difference in sensing efficiency at pH ranging from 5 to 9 (data not shown).

3.4. Analytical performances of the method

The optimized colorimetric assay was used for the detection of I⁻. As expected, with the addition of I⁻ (ranging from 0.5 to 400 μM) into the mixture of Au@Ag core–shell NPs-Cu²⁺, the color turned from deep yellow to purple (in the web version) (Fig. 5a), which enables the naked-eye detection of I⁻. The lowest concentration of I⁻ that can be detected by the naked-eyes is 5 μM. As a control, the same experiments were carried out using Cu²⁺ without Au@Ag core–shell NPs and using Au@Ag core–shell NPs without Cu²⁺. Fig. 5b and c shows that neither of them is capable for the colorimetric sensing of I⁻. Based on the above results, it can be concluded that both the Au@Ag core–shell NPs and the Cu²⁺ are responsible for the highly specific recognition of I⁻. UV–vis measurements were also performed to determine I⁻. Fig. 5e shows that, with the incremental concentration of I⁻ added into the

![Fig. 5. Photographs of (a) Au@Ag core–shell NPs-Cu²⁺+I⁻, (b) Cu²⁺+I⁻, (c) Au@Ag NPs + I⁻, (d) Ag NPs-Cu²⁺+I⁻, (e) corresponding absorption profiles of Au@Ag core–shell NPs-Cu²⁺ in the presence of different concentrations of I⁻ (0–400 μM) and (f) the relationship between the concentration of I⁻ and the ΔA₃94.](image-url)
mixture of Au@Ag core–shell NPs–Cu\textsuperscript{2+}, the absorbance at 394 nm decreased continuously and a new absorption band emerged at 422 nm, which corresponds to the formation of AgI as mentioned above. A linear relationship ($R^2 = 0.987, \Delta A_{394} = 0.0056[I^-] (\mu M) + 0.0032$) with the plot of the decreased absorbance at 394 nm ($\Delta A_{394}$) versus I\textsuperscript{-} concentration was found over the range of 0.5–80 \textmu M (Fig. 5f). The lowest detectable concentration of I\textsuperscript{-} was 0.5 \textmu M. In addition, monometallic Ag NPs without Au cores were synthesized and were also coupled with Cu\textsuperscript{2+} for the I\textsuperscript{-} sensing (Fig. S2). As shown in Fig. S2, the Ag NPs-Cu\textsuperscript{2+} system is also applicable for the naked-eye detection of I\textsuperscript{-}, but the color turned from yellow to colorless, which is visually insensitive to naked eyes. The absorbance at 400 nm decreased as the I\textsuperscript{-} concentration increased, but the $\Delta A_{400}$ was not linear with the incremental concentrations of I\textsuperscript{-}. The comparison of analytical performance among the proposed method and others is summarized in Table S2. To examine the reproducibility of this approach, analyses of standard solutions of I\textsuperscript{-} at 5, 50 and 100 \textmu M were all performed with six replicates. Fig. S3 shows that this approach exhibited good reproducibility with relative standard deviations of 0.62\% (5 \textmu M), 0.66\% (50 \textmu M) and 0.39\% (100 \textmu M).

3.5. Selectivity

The selectivity of this approach was examined by using the assay for the sensing of I\textsuperscript{-} as well as several common anions including F\textsuperscript{-}, Cl\textsuperscript{-}, Br\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, NO\textsubscript{2}\textsuperscript{-}, SO\textsubscript{4}\textsuperscript{2-}, PO\textsubscript{4}\textsuperscript{3-}, HCO\textsubscript{3}\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}, BO\textsubscript{3}\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, AcO\textsuperscript{-}, SCN\textsuperscript{-}, B\textsubscript{4}O\textsubscript{7}\textsuperscript{2-} and IO\textsubscript{3}\textsuperscript{-}. It can be clearly seen in Fig. 6 that none of the other anions, except I\textsuperscript{-}, induced an apparent color change from yellow to purple (in the web version) and a significant decrease of absorbance at 394 nm. The response for I\textsuperscript{-} is 9–153.5-fold to that of other anions tested. In addition, the coexistence of at least 10-fold in excess of other anions hardly affected the determination of I\textsuperscript{-}. Similarly, the effect of several

Table 1
Analytical results for the detection of I\textsuperscript{-} in drinking waters.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Detected (\textmu M)</th>
<th>Added (\textmu M)</th>
<th>Found (\textmu M)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried kelp</td>
<td>54.8</td>
<td>8</td>
<td>55.1</td>
<td>88.1</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>68.8</td>
<td>92.3</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>93.6</td>
<td>89.6</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6. (a) The photographs and (b) the $\Delta A_{394}$ of Au@Ag core–shell NPs–Cu\textsuperscript{2+} mixture in the presence of I\textsuperscript{-} and other anions. The concentration was 50 \textmu M for I\textsuperscript{-}, 500 \textmu M for BO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{3}\textsuperscript{2-}, 1.5 mM for F\textsuperscript{-}, Cl\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, NO\textsubscript{2}\textsuperscript{-}, PO\textsubscript{4}\textsuperscript{3-}, HCO\textsubscript{3}\textsuperscript{-} and CH\textsubscript{3}COO\textsuperscript{-}, and 1 mM for the other anions.
common metal ions was also investigated. Fig. S4 shows no obvious interfering effect was observed for all the cations tested.

3.6. Analysis of drinking water and dried kelp

The proposed method was applied to the determination of $I^{-}$ in drinking water and dried kelps to confirm its practical application capability. The content of $I^{-}$ in drinking water was found to be lower than the limit of quantification of the method, while the content of $I^{-}$ in the kelp extract was determined to be 54.8 $\mu$M, which was converted to be 0.29 mg g$^{-1}$ in dried kelps. To evaluate the reliability of the method, recovery tests were performed using the standard addition method. As listed in Table S3 and Table 1, the recoveries obtained for drinking water and dried kelp ranged from 87.6% - 126% to 88.1% - 92.3%, respectively with RSDs lower than 6.4%.

3.7. Embed Au@Ag core–shell NPs-Cu$^{2+}$ into agarose gels as test strips

To improve the applicability of the approach, we tried to immobilize the detection system into solid matrix as a test strip. Agarose gel has been proved to be a feasible matrix to embed colloidal NPs, since it is transparent, porous and contains a large amount of water [28]. Therefore, the mixture of Au@Ag core–shell NPs-Cu$^{2+}$ was embedded into agarose gels and used for the detection of $I^{-}$. Fig. 7 shows that, when exposed to the increasing concentration of $I^{-}$, the colors of the agarose gels changed from yellow to purple (in the web version). This color gradient is more obvious than that obtained in solutions. This is probably because that agarose gels can enrich $I^{-}$ from the aqueous sample, which might improve the method sensitivity. After incubation with $I^{-}$, the agarose gels were dried, powdered and used for the XRD characterization. Fig. S5 reveals the presence of (110) and (100) diffraction peaks, which belong to the hexagonal AgI. This result confirmed the formation of AgI after the incubation of agarose gel in the $I^{-}$ solution. To further demonstrate its practical applicability, we applied the test strip for the measurement of $I^{-}$ in the extract of dried kelp. The concentration of $I^{-}$ in the extract of dried kelp was found to be 54.8 $\mu$M as stated above. As shown in Fig. 7, the color of the test strip lies between those of 50 and 100 $\mu$M, indicating our test strips are applicable for the semi-quantitative determination of $I^{-}$ in real samples.

4. Conclusions

In summary, we have developed a simple, sensitive and selective colorimetric assay for $I^{-}$ sensing by coupling Au@Ag core–shell NPs with Cu$^{2+}$. It is well documented in text books that the oxidation ability of the Ag$^{+}$, I$_2$ and Cu$^{2+}$ decreased sequentially at their standard state, which implies that Cu$^{2+}$ is difficult to oxidize I$^{-}$ and I$_2$ difficult to oxidize Ag. However, the formation of stable Cun and AgI in the reaction processes makes possible the oxidation of I$^{-}$ into I$_2$ by Cu$^{2+}$. Also, the generated I$_2$ can oxidize the silver shell into AgI shell to change the ingredient and dimensional ratio of Au@Ag core–shell NPs, leading to the color change from yellow to purple. This assay allowed to detect $I^{-}$ with a detection limit as low as 5 $\mu$M using the naked eyes within 10 min, which is beneficial for rapid and sensitive detection of $I^{-}$. Moreover, most common anions and metal ions tested showed no significant interference for this assay. This assay was successfully applied to the analysis of $I^{-}$ in drinking water and dried kelps with good accuracy and precision. It is worth mentioning that Au@Ag core–shell NPs-Cu$^{2+}$ was embedded into agarose gels, which have found their application in the semi-quantitative determination of $I^{-}$ in dried kelps.

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Appendix A. Supporting information

Supporting information related to this article can be found at http://dx.doi.org/10.1016/j.aca.2015.06.043.

References


[26] D.R. Pedersen, S. Wang, F.S. Duncan, S.H. Liang, Adsorbate-induced diffusion of Ag and Au atoms out of the cores of Ag@Au, Au@Ag, and Ag@Ag core–shell nanoparticles, J. Phys. Chem. C 111 (2007) 13665–13672.
