Colorimetric Sensor for Thiocyanate Based on Anti-aggregation of Gold Nanoparticles in the Presence of 2-aminopyridine

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Abstract

Based on the anti-aggregation mechanism of citrate stabilized gold nanoparticles (AuNPs), a new specific and sensitive colorimetric sensor for thiocyanate (SCN\textsuperscript{−}) was developed. In this scheme, the AuNPs were aggregated in the presence of the aggregating agent 2-aminopyridine (2-AP) due to electrostatic attraction. The solution color changed from red to blue. When SCN\textsuperscript{−} was present, SCN\textsuperscript{−} formed sulfur-gold bond with the AuNPs to protect the AuNPs from aggregation. Thiocyanate can be detected by the color change of the solution from blue to red. The results showed that the absorbance ratio $A_{675}/A_{520}$ was linear with the concentration of SCN\textsuperscript{−} in the range of 0.4 - 1.2 μmol L\textsuperscript{−1} by UV-Vis spectroscopy. The limit of detection (LOD) of this assay was 0.37 μmol L\textsuperscript{−1}. The system also had excellent selectivity and anti-interference ability. In addition, this method had been successfully used for the detection of SCN\textsuperscript{−} in actual water samples and had achieved good results.

**Keywords:** Gold nanoparticles, Thiocyanate, Colorimetric sensor, Anti-aggregation, 2-aminopyridine
Introduction

As an important chemical substance, thiocyanate (SCN⁻) is widely used in medicine, dying, photography and other fields. Thiocyanate can be converted to cyanide, which is harmful to humans and the environment. Thiocyanate also affects the absorption of iodine by the human thyroid gland. Therefore, the detection of SCN⁻ is of great significance. At present, conventional methods, such as colorimetry¹, atomic absorption spectrometry², flow injection analysis³, electrochemistry⁴-⁶, chromatography/mass spectrometry⁷, ion mobility spectrometry⁸, electrophoresis⁹, fluorimetry¹⁰, double-valve sequential injection analysis¹¹ and Surface Enhanced Raman Scattering (SERS)¹², have been used to measure SCN⁻ with high sensitivity and accuracy. However, these measures have many problems such as complicated instruments, difficult operation and long time. It is still relevant to find a quick and easy method to detect SCN⁻.

In recent years, colorimetric sensors have received more and more attention due to their simplicity, low cost and high sensitivity¹³-¹⁵. The gold nanoparticles (AuNPs) sensors are an important colorimetric analysis method, which can be visually observed and stabilized due to changes in its color¹⁶-²⁰. The AuNPs in the dispersed state are wine red, but blue in the aggregate state. So far, some articles based on the AuNPs colorimetric sensors for SCN⁻ detection have been reported²¹-²⁵. However, these methods have relatively long reaction time or complex for the functionalization of the AuNPs²⁶. Therefore, it is necessary to develop a simple and convenient the AuNPs colorimetric sensor to detect SCN⁻.

Here, we proposed a simple and stable detection system. The sensor was based on the anti-aggregation mechanism of citrate stabilized AuNPs to detect SCN⁻²⁷-²⁸. It was found that many substances can interact with AuNPs through electrostatic interactions²¹-²². But it will take a lot of time²⁴-²⁵. In our experiments, it was found that when 2-AP is used, SCN⁻ can be detected.
in a quick time. Therefore, 2-AP was used as the aggregating agent on the colorimetric sensor. In the absence of SCN⁻, 2-aminopyridine (2-AP) was added to the AuNPs solution, and the AuNPs were aggregated due to their electrostatic action. However, when SCN⁻ was present, the formation of sulfur-gold bonds protected the AuNPs from aggregation\textsuperscript{21, 24}. Therefore, the AuNPs was restored in a stable state. The detection mechanism of colorimetric sensor was shown in Scheme 1. The absorbance of the sample was measured by UV-Vis spectroscopy. In addition, we have confirmed the potential application value of this sensor in actual water samples.

**Experimental**

**Reagents and chemicals**
Trisodium citrate dihydrate (C\textsubscript{6}H\textsubscript{5}Na\textsubscript{3}O\textsubscript{7}·2H\textsubscript{2}O) was purchased from Tianjin guangfu fine chemical research institute. Tris (hydroxymethyl) aminomethane was bought from Sinopharm chemical reagent Co. Ltd (Beijing, China). Chloroauric acid (HAuCl\textsubscript{4}) was obtained from Sak chemical technology Shanghai Co. Ltd. All other reagents were of analytical grade and were used without further purification.

**Apparatus**
Absorption spectra were recorded using an UV-3100 UV-VISNIR (Shimadzu, Japan). Zeta potential and particle size were analyzed using a Nano ZS90 laser particle analyzer (Malvern Instruments, UK).

**Fabrication of AuNPs**
The AuNPs were prepared according to the well-known citrate reduction of HAuCl\textsubscript{4}\textsuperscript{29-31}. 
The glass instruments in the synthesis process were washed with aqua regia (HCl/HNO₃ = 3:1(v/v)), and then were washed with deionized water. In a round bottle equipped with a reflux condenser, 100 mL of aqueous HAuCl₄ (1.0 mmol L⁻¹) was heated to boiling and then 10 mL trisodium citrate (38.8 mmol L⁻¹) was added to the solution and was stirred for 30 minutes. During this process, the color of the solution changed from yellow to black and then to wine red. The solution was cooled to room temperature, passed a 0.22 µm filter and stored at 4 °C. The concentration of AuNPs is approximately 8.3 nmol L⁻¹, which was calculated by Lambert Beer’s law (extinction coefficient is 2.78 × 10⁻⁸ (mol L⁻¹)⁻¹ cm⁻¹)³²⁻³³. The average diameter of AuNPs prepared was about 21 nm, which was further confirmed by DLS.

Colorimetric sensing of SCN⁻

All the substances involved in the test are in aqueous solution. 200 µL of 0.04 mol L⁻¹ (pH 7.0) Tris-HCl buffer solution containing different concentrations of SCN⁻ and 50 µL of 3 mmol L⁻¹ 2-AP were added into deionized water to volume of 2800 µL. Then 200 µL AuNPs solution were added into the above solution. Finally, the absorbance at 520 nm and 675 nm were measured.

Thiocyanate detection in tap water

In order to detect SCN⁻ in tap water sample, we obtained tap water from the laboratory. After the tap water was passed through a 0.22 µm filter, different concentrations of SCN⁻ were added, followed by 200 µL of 0.04 mol L⁻¹ (pH 7.0) Tris-HCl buffer solution and 50 µL of 3 mmol L⁻¹ 2-AP. Then tap water to volume of 2800 µL and 200 µL AuNPs solution were added into the above solution.
Results and Discussion

Mechanism of the sensor

The mechanism of the colorimetric sensor for detecting SCN⁻ was showed in Scheme 1. In the aqueous solution, the AuNPs were well dispersed due to strong electrostatic repulsion between the negatively charged citrate ions on the surface of the AuNPs. The color of the solution was red. In the presence of aggregating agent 2-AP, which had a positive charge due to the protonation of 2-AP, the AuNPs were severely aggregated due to the action of static electricity. The color of the solution changed from red to blue. 2-AP and SCN⁻ were in a competitive relationship to AuNPs, and SCN⁻ exhibits a stronger binding force than 2-AP. With both of them presented in the solution, SCN⁻ can protect the AuNPs from being aggregated by 2-AP. Therefore, we provided a colorimetric sensor that can detect SCN⁻ specifically and quickly based on the anti-aggregation mechanism of AuNPs.

To further verify the reaction mechanism, TEM and DLS were explored. Fig. 1A-(1) was TEM of the AuNPs reduced by citrate, and it can be seen that the AuNPs with dispersed state was successfully synthesized. After the addition of 2-AP, the AuNPs exhibited aggregate state in Fig. 1A-(2), demonstrating that the aggregation of the AuNPs was due to 2-AP. When SCN⁻ was present, SCN⁻ inhibits the aggregation of AuNPs by 2-AP in Fig. 1A-(3). In addition, DLS gave specific particle size values in Fig. 1B.

Fig. 2 displayed the UV-Vis absorption spectra of AuNPs in the presence or absence of SCN⁻. The UV-Vis absorption spectra of the AuNPs solution had a maximum absorbance at 520 nm. After the addition of 2-AP, the absorption peak of AuNPs was red-shifted. The absorbance at 520 nm decreased, and a new absorption peak at 675 nm indicated that AuNPs were heavily aggregated. When SCN⁻ is present, the UV-Vis absorption spectra were restored because that the formation of the sulfur-gold bond protected the stable state of the AuNPs.
Therefore, it can be concluded that the probe can specifically detect SCN⁻.

**Optimum assay conditions**

The concentration of 2-AP

It is significant to find the optimal 2-AP concentration on the AuNPs aggregation. The concentration of 2-AP was optimized from 0 to 100 µmol L⁻¹. As shown in Fig. 3, the absorbance ratio A₆₇₅/A₅₂₀ increased with the increasing of 2-AP concentration, indexing that the addition of 2-AP promoted AuNPs aggregation. When 1 µmol L⁻¹ SCN⁻ is present, the absorbance ratio does not change much with the amount of 2-AP. This phenomenon indicates that SCN⁻ can inhibit the aggregation of AuNPs. To obtain high sensitivity, the concentration of 2-AP was selected as 50 µmol L⁻¹ for further investigations.

The effect of pH and buffer solutions

The effect of pH on the absorbance ratio of A₆₇₅/A₅₂₀ was studied by adding different pH buffers. As shown in Fig. 4A, it was observed that when SCN⁻ is absent, the pH has little effect on the AuNPs aggregation by 2-AP in the range of 3-11. When 1 µmol L⁻¹ SCN⁻ was present, the AuNPs were still in a stable state. The response was the most pronounced under acidic, neutral and weak basic conditions, so pH 7.0 was chosen as the condition for this study.

The effects of different buffer solutions to the color sensor are explored. Fig. 4B shown that the absorbance ratio of A₆₇₅/A₅₂₀ was hardly changed with various buffer solutions in pH 7.00. However, it can be seen that the use of Tris-HCL is slightly better than other buffering reagents. Therefore, the experiment used Tris-HCl buffer solution throughout.

Stability

The stability of the colorimetric sensor is very important in its application. As shown in Fig. S1, the values of A₆₇₅/A₅₂₀ for both AuNPs + 2-AP and AuNPs + 2-AP + SCN⁻ are almost constant after three months. Therefore, the colorimetric sensor has good stability within three
months at least.

Selectivity

The selectivity of this assay was studied toward SCN\(^-\) by adding environmentally relevant anions and metal ions (SO\(_4^{2-}\), Ac\(^-\), NO\(_2^-\), NO\(_3^-\), F, Cl\(^-\), I, ClO\(_4^-\), HPO\(_4^{2-}\), HCO\(_3^-\), PO\(_4^{3-}\), H\(_2\)PO\(_4^-\), K\(^+\), Ca\(^{2+}\), Na\(^+\), Mg\(^{2+}\), Mn\(^{2+}\) and Cr\(^{3+}\)). It can be seen from Fig. 5A that other ions have no effect on the detection of SCN\(^-\). When SCN\(^-\) is absent, the solution is blue. When SCN\(^-\) is present, the color of the solution is red (Fig. 5B). It presented that other ions have no ability to resist the AuNPs aggregation by 2-AP except for SCN\(^-\). Therefore, the colorimetric sensor has good selectivity and anti-interference ability.

Linearity in detecting SCN\(^-\)

Under the optimum experimental conditions, the sensitivity of colorimetric sensors for SCN\(^-\) is explored. As shown in Fig. 6A, with the concentration of SCN\(^-\) gradually increased, the absorbance at 520 nm increased, and the absorbance at 675 nm decreased. These results displayed that the presence of SCN\(^-\) can effectively inhibit the aggregation of AuNPs by 2-AP. In addition, when the concentration of SCN\(^-\) increased from 0.4 to 1.2 µmol L\(^{-1}\), the absorbance ratio \(A_{675}/A_{520}\) decreased linearly, and the regression equation was \(y = -1.49604x + 2.00212\), where \(y\) was the absorbance ratio \(A_{675}/A_{520}\) and \(x\) was the SCN\(^-\) concentration, \(R^2 = 0.994\) (Fig. 6B). The LOD calculated in this experiment was 0.37 µmol L\(^{-1}\) (S/N = 3) (calculation process is in supporting information)\(^34\). The results illustrated that the \(A_{675}/A_{520}\) ratio was linearly related to the SCN\(^-\) concentration within the range of 0.4-1.2 µmol L\(^{-1}\). Therefore, the AuNPs anti-aggregation colorimetric sensors can be used to detect SCN\(^-\) quantitively.

In addition, this method was also compared with other AuNPs-based colorimetric sensors for SCN\(^-\). As shown in Table S2, the LOD and the linear range of this sensor are as good as
those of other sensors. Compared with other colorimetric sensors, the biggest advantage of this sensor is time saving. The short-time detection will make this colorimetric sensor more widely used.

**Thiocyanate detection in tap water**

The detection of SCN− in tap water was researched and it was found that the concentration of SCN− was too low to be detected. The tap water samples were spiked with 0.4 μmol L\(^{-1}\), 0.5 μmol L\(^{-1}\) and 0.6 μmol L\(^{-1}\) SCN−. The results were summarized in Table 1 and Table S3. The range of recovery rates was between 98.52% and 105.48%. The information showed the applicability and reliability of the proposed method.

**Conclusions**

In summary, a simple, short-time, high-sensitivity and high-selectivity colorimetric sensor was developed for the detection of SCN−. The AuNPs are aggregated by electrostatic attraction under the existence of 2-AP. The presence of SCN− protected the AuNPs from the effects of the aggregating agent. The detection range of the colorimetric sensor was between 0.4 μmol L\(^{-1}\) and 1.2 μmol L\(^{-1}\), and the limit of detection was 0.37 μmol L\(^{-1}\) by UV-Vis spectroscopy. In addition, it was successfully verified that the sensor can be applied to the detection of actual water samples.

**Supporting Information**

Supporting Information includes stability of the sensor on the ratio of \(A_{675}/A_{520}\) (Fig. S1), calculation of the limit of detection, comparison with other AuNPs-based colorimetric sensors for SCN− detection and \(t\) values of AuNPs-based colorimetric sensors for SCN− detection (Table
S2 and Table S3). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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Table 1  Analytical results of the determination of SCN⁻ in tap water samples (n=3)

| Sample   | Added/（μmol/L） | Found/（μmol/L） | Recovery(%) | RSD(%) |
|----------|----------------|----------------|-------------|-------|
| Tap water| 0.40           | 0.42           | 105.48      | 4.13  |
|          | 0.50           | 0.49           | 98.52       | 4.60  |
|          | 0.60           | 0.62           | 102.90      | 2.57  |
Figure Captions

Scheme 1  Mechanism of the colorimetric assay for SCN⁻ based on anti-aggregation of AuNPs.

Fig. 1  TEM (A) and DLS (B) of AuNPs solution (1), and 2-AP+AuNPs solution in the absence (2) and presence (3) of 1 µmol L⁻¹ SCN⁻.

Fig. 2  UV–Vis absorption spectra of AuNPs (black), in the presence of 2-AP (red), in the presence of both 2-AP and 1 µmol L⁻¹ SCN⁻ (blue).

Fig. 3  Effects of different concentrations (0-100 µmol L⁻¹) of aggregating agents on the AuNPs absorbance ratio (A₆₇₅/A₅₂₀).

Fig. 4  Effects of pH (A) (buffer solutions: Britton-Robinson buffer solution) and buffer solutions (B) (concentration: 0.04 mol L⁻¹; pH: 7.0) on the ratio of A₆₇₅/A₅₂₀.

Fig. 5  Absorbance ratios A₆₇₅/A₅₂₀ of AuNPs (A) and color of solution (B) containing 1 µmol L⁻¹ different ions (SO₄²⁻, Ac⁻, NO₃⁻, NO₂⁻, F⁻, Cl⁻, I⁻, ClO⁻, HPO₄²⁻, HCO₃⁻, PO₄³⁻, H₂PO₄⁻, K⁺, Ca²⁺, Na⁺, Mg²⁺, Mn²⁺, and Cr³⁺) in pH 7.0 Tris-HCl buffer solution. “blk” stands for “AuNPs+2-AP”. “SCN⁻” stands for “AuNPs+2-AP+1 µmol L⁻¹ SCN⁻”.

Fig. 6  Absorption spectra (A) and the ratio A₆₇₅/A₅₂₀ (B) of the AuNPs-based detection systems with different concentrations (0.4-1.2 µmol L⁻¹) of SCN⁻.
Graphical Index

Scheme 1

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Fig. 5

Fig. 6