

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

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Based on the anti-aggregation mechanism of citrate stabilized gold nanoparticle (AuNPs), a new specific and sensitive colorimetric sensor for thiocyanate (SCN^-) 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^- was present, SCN^- formed a 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^- in the range of 0.4 – 1.2 $\mu\text{mol L}^{-1}$ by UV-Vis spectroscopy. The limit of detection (LOD) of this assay was 0.37 $\mu\text{mol L}^{-1}$. The system also had excellent selectivity and anti-interference ability. In addition, this method was successfully used for the detection of SCN^- in actual water samples and achieved good results.

Keywords Gold nanoparticles, thiocyanate, colorimetric sensor, anti-aggregation, 2-aminopyridine

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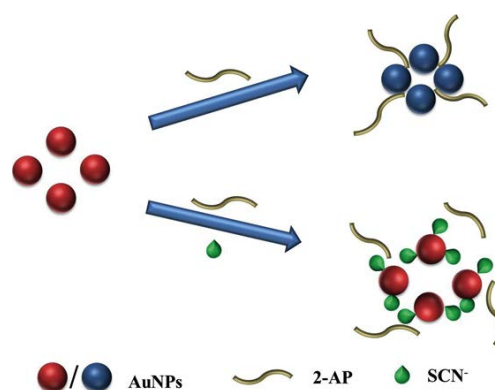
Introduction

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 colorimetry,¹ atomic absorption spectrometry,² flow injection analysis,³ electrochemistry,⁴⁻⁶ chromatography/mass spectrometry,⁷ ion mobility spectrometry,⁸ electrophoresis,⁹ fluorimetry,¹⁰ 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 procedural 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 sensitivity.¹³⁻¹⁵ The gold nanoparticle (AuNP) sensors are an important colorimetric analysis method, which can be visually observed and stabilized due to changes in its color.¹⁶⁻²⁰ The AuNPs in the dispersed state are wine red, but blue in the aggregate state. So far, some articles based on AuNP colorimetric sensors for SCN^- detection have been reported.²¹⁻²⁵ However, these methods require a relatively long reaction time

or complex processes for the functionalization of the AuNPs.²⁶ Therefore, it is necessary to develop a simple and convenient AuNP 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 interactions.^{21,22} But it will take a lot of time.^{24,25} In our experiments, it was found that when 2-AP is used, SCN^- can be detected in a short 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 AuNP solution, and the AuNPs were aggregated due to their electrostatic action.



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

However, when SCN^- was present, the formation of sulfur-gold bonds protected the AuNPs from aggregation.^{21,24} Therefore, the AuNPs were restored to a stable state. The detection mechanism of the colorimetric sensor is 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 ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$) 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_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). The particle size was analyzed using a Nano ZS90 laser particle analyzer (Malvern Instruments, UK).

Fabrication of AuNPs

The AuNPs were prepared according to the well-known method of citrate reduction of HAuCl_4 .²⁹⁻³¹ The glass instruments in the synthesis process were washed with aqua regia ($\text{HCl}/\text{HNO}_3 = 3:1$ (v/v)), and then were washed with deionized water. In a round bottle equipped with a reflux condenser, 100 mL of aqueous HAuCl_4 (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 min. 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 through a $0.22\text{-}\mu\text{m}$ filter and stored at 4°C . The concentration of AuNPs was approximately 8.3 nmol L^{-1} , which was calculated by Lambert Beer's law (extinction coefficient is $2.78 \times 10^8 (\text{mol L}^{-1})^{-1} \text{ cm}^{-1}$).^{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 were in aqueous solution. First, 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 and make up to a volume of 2800 μL . Then 200 μL AuNPs solution was added to the above solution. Finally, the absorbance at 520 and 675 nm were measured.

Thiocyanate detection in tap water

In order to detect SCN^- in tap water samples, we obtained tap water from the laboratory. After the tap water was passed through a $0.22\text{-}\mu\text{m}$ 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 the tap water was made up to a volume of 2800 and 200 μL AuNP solution was added to the above solution.

Results and Discussion

Mechanism of the sensor

The mechanism of the colorimetric sensor for detecting SCN^-

is shown 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 strongly aggregated due to the action of static electricity. The color of the solution changed from red to blue. It was noted that 2-AP and SCN^- exhibited a competitive relationship to the 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. Figure 1A-(1) shows a TEM of the AuNPs reduced by citrate, and it can be seen that the AuNPs with dispersed state were successfully synthesized. After the addition of 2-AP, the AuNPs exhibited an aggregate state, as shown 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, as shown in Fig. 1A-(3). In addition, DLS provided specific particle size values, as shown in Fig. 1B.

Figure 2 displays the UV-Vis absorption spectra of AuNPs in the presence or absence of SCN^- . The UV-Vis absorption spectra of the AuNP 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 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

2-AP concentration. It is important to find the optimal 2-AP concentration for AuNP aggregation. The concentration of 2-AP was optimized from 0 to $100 \mu\text{mol L}^{-1}$. As shown in Fig. 3, the absorbance ratio A_{675}/A_{520} increased with the increasing of 2-AP concentration, indexing that the addition of 2-AP promoted AuNP aggregation. When $1 \mu\text{mol L}^{-1}$ 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 2-AP concentration of $50 \mu\text{mol L}^{-1}$ was selected for further investigations.

Effect of pH and buffer solutions. The effect of pH on the absorbance ratio of A_{675}/A_{520} 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 AuNP aggregation by 2-AP in the range of 3–11. When $1 \mu\text{mol L}^{-1}$ 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 on the color sensor were explored. Figure 4B shows that the absorbance ratio of A_{675}/A_{520} 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 (Supporting Information), the values of A_{675}/A_{520} for both AuNPs + 2-AP and AuNPs + 2-AP + SCN^- were almost constant after 3 months.

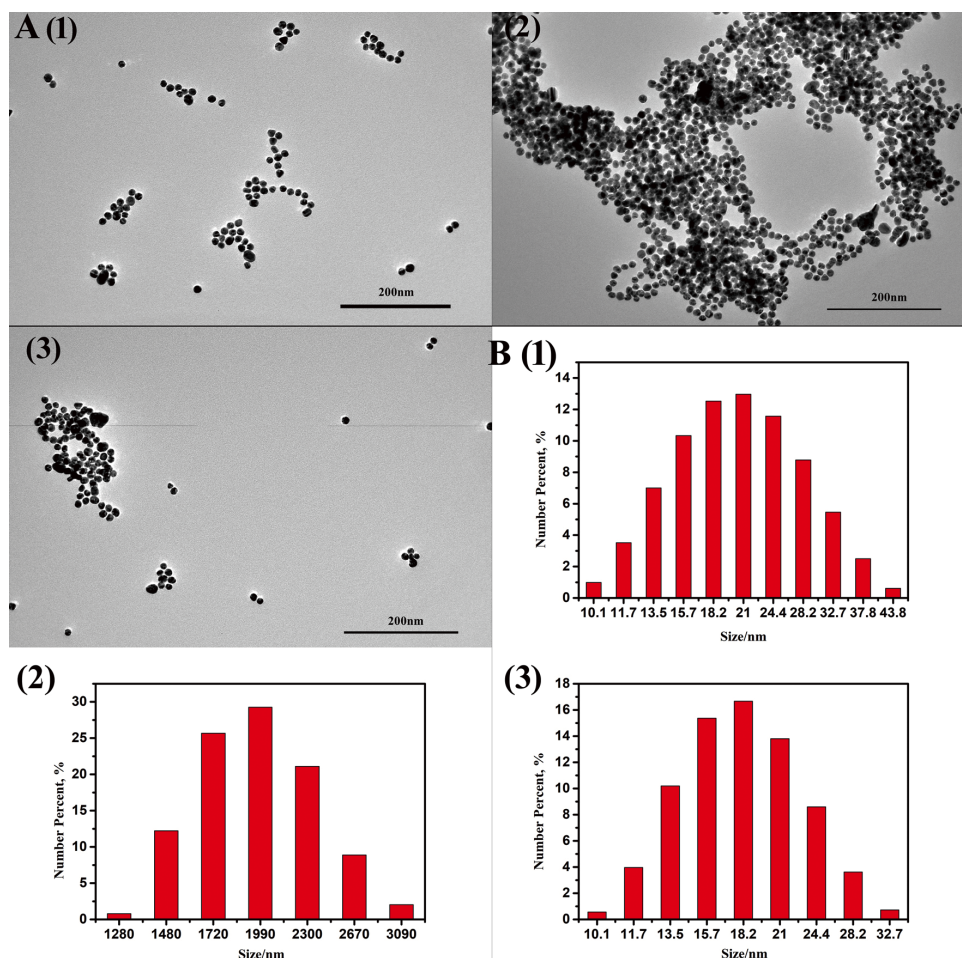


Fig. 1 TEM (A) and DLS (B) of AuNP solution (1), and 2-AP + AuNP solution in the absence (2) and presence (3) of $1 \mu\text{mol L}^{-1} \text{SCN}^-$.

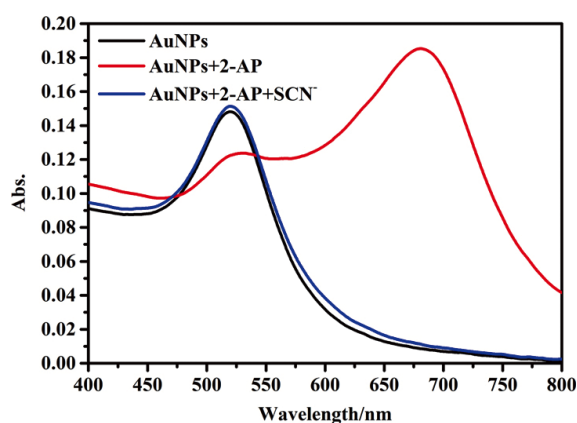


Fig. 2 UV-Vis absorption spectra of AuNPs (black), in the presence of 2-AP (red), in the presence of both 2-AP and $1 \mu\text{mol L}^{-1} \text{SCN}^-$ (blue).

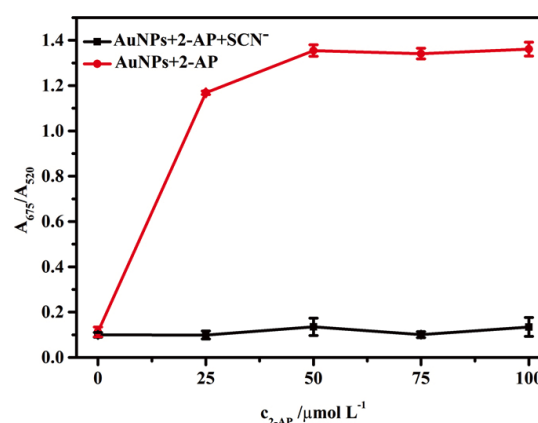


Fig. 3 Effects of different concentrations (0 – $100 \mu\text{mol L}^{-1}$) of aggregating agents on the AuNP absorbance ratio (A_{675}/A_{520}).

Therefore, it was determined that the colorimetric sensor has good stability for at least three months.

Selectivity. The selectivity of this assay toward SCN^- was studied by adding environmentally relevant anions and metal ions (SO_4^{2-} , Ac^- , NO_3^- , NO_2^- , F^- , Cl^- , I^- , ClO^- , HPO_4^{2-} , HCO_3^- , PO_4^{3-} , H_2PO_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). This indicated that other ions have no ability to restrict the AuNP aggregation by 2-AP except for SCN^- . Therefore, it was deemed that the colorimetric sensor has good selectivity and anti-interference ability.

Linearity in detecting SCN^-

Under the optimum experimental conditions, the sensitivity of

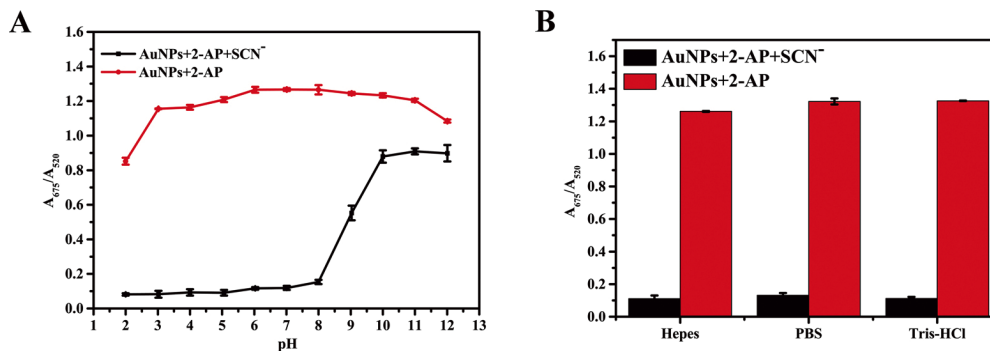


Fig. 4 Effects of pH (A) (buffer solutions: Britton-Robinson buffer solution) and buffer solutions (B) (concentration, 0.04 mol L⁻¹; pH, 7.0) on the ratio of A_{675}/A_{520} .

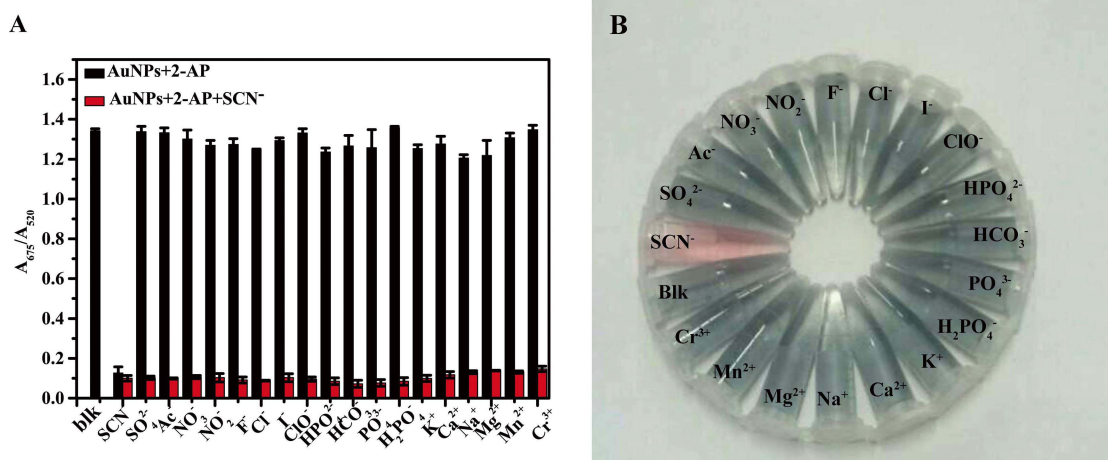


Fig. 5 Absorbance ratios A_{675}/A_{520} of AuNPs (A) and color of solution (B) containing 1 $\mu\text{mol L}^{-1}$ different ions (SO₄²⁻, Ac⁻, NO₃⁻, NO₂⁻, F⁻, Cl⁻, I⁻, ClO⁻, HPO₄²⁻, HCO₃⁻, PO₄³⁻, H₂PO₄⁻, K⁺, Ca²⁺, Na⁺, Mg²⁺, Mn²⁺, and Cr³⁺) in pH 7.0 Tris-HCl buffer solution. "blk" stands for "AuNPs + 2-AP". "SCN⁻" stands for "AuNPs + 2-AP + 1 $\mu\text{mol L}^{-1}$ SCN⁻".

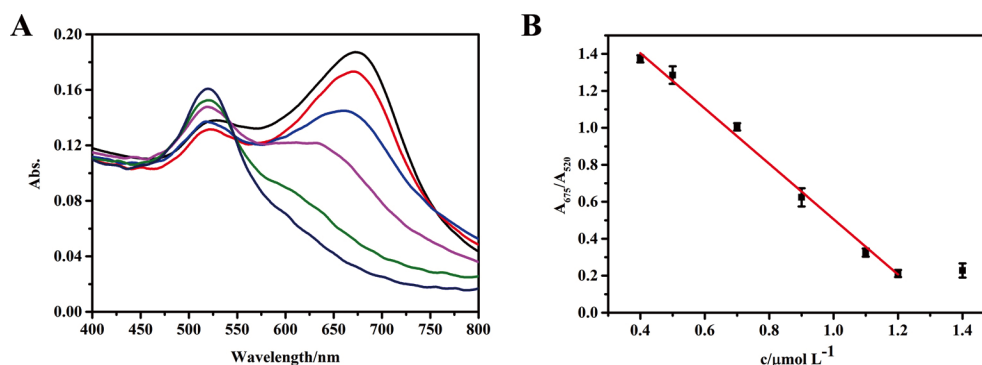


Fig. 6 Absorption spectra (A) and the ratio A_{675}/A_{520} (B) of the AuNP-based detection systems with different concentrations (0.4 – 1.2 $\mu\text{mol L}^{-1}$) of SCN⁻.

colorimetric sensors for SCN⁻ was explored. As shown in Fig. 6A, with gradually increasing concentrations of SCN⁻, the absorbance at 520 nm increased, and the absorbance at 675 nm decreased. These results showed 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 $\mu\text{mol L}^{-1}$, 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 $\mu\text{mol L}^{-1}$ ($S/N = 3$) (calculation process is described 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 $\mu\text{mol L}^{-1}$.

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

Sample	Added/ μmol L ⁻¹	Found/ μmol L ⁻¹	Recovery, %	RSD, %
Tap water	0.40	0.42	105.48	
	0.50	0.49	98.52	4.60
	0.60	0.62	102.90	2.57

Therefore, the AuNP anti-aggregation colorimetric sensors can be used to detect SCN⁻ quantitatively.

In addition, this method was also compared with other AuNP-based colorimetric sensors for SCN⁻. As shown in Table S2 (Supporting Information), 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 needed for detection will allow for wider use of this colorimetric sensor.

Thiocyanate detection in tap water

The detection of SCN⁻ in tap water was researched. The tap water samples were spiked with 0.4, 0.5 and 0.6 μmol L⁻¹ SCN⁻. The results are summarized in Table 1 and Table S3 (Supporting Information). 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, fast, highly sensitive and highly selective colorimetric sensor was developed for the detection of SCN⁻. The AuNPs were aggregated by electrostatic attraction under the presence 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 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 SCN⁻ in actual water samples.

Acknowledgements

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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 AuNP-based colorimetric sensors for SCN⁻ detection and *t* values of AuNP-based colorimetric sensors for SCN⁻ detection (Tables S2 and S3). This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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