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Detection of thiocyanate through limiting growth of AuNPs with C-dots act as reductant

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We have found that hydroxyl-rich carbon dots (C-dots) has ability to reduce Au^{3+} to form gold nanoparticles (AuNPs). Thiocyanate (SCN^-) can be absorbed on AuNPs surface due to its high affinity toward AuNPs, which inhibit the growth of AuNPs. Meanwhile, SCN^- has ability to etch the as-synthesized big AuNPs to small AuNPs, which can also cause the absorption peak of AuNPs decrease. Therefore, an optical sensor is developed for detection of SCN^- based on measuring the plasmon resonance absorption peak change of AuNPs. Under the optimal conditions, this method yields excellent sensitivity (the limit of detection is 0.16 μM) and selectivity toward SCN^-. This method can detect SCN^- in raw milk with satisfactory result. This work gives a new sight for the monitoring the quality of milk.

1. Introduction

Thiocyanate (SCN^-) exists in many kinds of foods, including milk, and is one of the major metabolic detoxification products in the liver of human beings.[1, 2] SCN^- also is one of the three components of lactoperoxidase system for increasing storage stability of milk. But the low concentration of SCN^- in milk is the main limiting factor for the activation of the lactoperoxidase system.[3] Therefore, it is necessary to add the SCN^- for activating the bacteriostatic effects and extending the storage life. In order to extend the shelf life of milk, some unprincipled people add SCN^- unscrupulous into milk. However, SCN^- as a potent anti-thyroid substance can lead to inhibit the iodine uptake of the thyroid gland and induce goiter when ingested at higher levels.[4-6] And it is more serious for infants, pregnant women and populations of iodine deficiency areas.[6] Apparently, the high concentration level of SCN^- in milk product will increase the risk of goiter. Nowadays, many analytical methods have been proposed for the detection of SCN^-, including gas chromatography/mass spectrometry,[7-9] high performance liquid chromatography,[10] electrochemistry,[11, 12] electrophoresis,[13, 14] surface-enhanced Raman scattering[15, 16] and so on. However, some of these methods are time-consuming and costly or require sophisticated instrumentation and professional staff. Therefore, developing a simple, rapid, reliable, and sensitive method for the detection of SCN^- is meaningful.

Carbon nanodots (C-dots), as a new class of carbon nanomaterials, has recently garnered a number of interests due to their outstanding physicochemical and photochemical properties.[17] Based on the properties of high fluorescence, robust chemical inertness, low photobleaching, good water solubility, low toxicity, good biocompatibility and easy synthesis, C-dots is promising to replace highly toxic semiconductor quantum dots and organic dyes in optical sensors and bioimaging.[18-22] So far, many sensors based on fluorescence intensity change of C-dots caused by analytes are developed.[17, 18] However, it is difficult to eliminate the background interference in complex samples due to the shorter wavelength excitation (commonly utilize ultraviolet as the excitation light). Therefore, it is necessary to explore the potential of C-dots for construction of sensors with excellent performance

It has been demonstrated that C-dots can act as an excellent electron acceptor and electron donor, and it has promising potential to be an oxidizing or reducing agent.[23-25] Our group has successfully synthesized AuNPs by using hydroxyl-rich C-dots as the reductant and stabilizing agent.[26] Enlightened by Li *et al*. reported a self-limiting growth of nanoparticles system.[27] Herein, based on the strongly coordination and etching effects between AuNPs and SCN^-, we proposed a selective and sensitive method to detect SCN^- by monitoring the change of absorption spectrum of AuNPs. It was successfully applied to detect SCN^- in raw milk sample, and satisfactory results were achieved.

2. Experimental

2.1 Materials

Chloroauric acid ($HAuCl_4$) was purchased form Sigma (Shanghai, China), all other chemicals were purchased form Shanghai Reagent (Shanghai, China). All chemicals were used as received without any further purification. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiments.

2.2 Apparatus

The UV-vis spectra were obtained by a UV-2450 UV-vis spectrophotometer (Shimazu Co., Japan). Transmission electron microscope (TEM) images were collected from a JEOL-1230 transmission electronic microscope (JEOL, Japan).

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2.3 Synthesis of C-dots

C-dots was electrochemically synthesized based on our previous work.[26] Briefly, ethylene glycol and sodium hydroxide mix solution was used as the electrolytes solution, platinum sheets as the positive and the negative electrodes. The C-dots was synthesized by electrolyzing electrolyte solution under 30 V (DC) for 40 min. After electrolysis, the electrolyte solution was neutralized with hydrochloric acid and then dialyzed (Mw 1000) against water for over two days. Then, the product was centrifuged at a speed of 10000 rpm for 15 min to remove the large size particles.

2.4 Synthesis of AuNPs by using C-dots as reducing and stabilizing agent

The AuNPs was synthesized as follows. A certain amount of $HAuCl_4$ aqueous solution was added to 1 mL aqueous solution of as-synthesized C-dots (the final concentration of $HAuCl_4$ and C-dots are 0.05 mM and 0.06 mg mL^{-1}, respectively). Then the mixture was incubated at room temperature for 20 min. After that, the UV-vis spectra were recorded immediately.

2.5 The detection procedure of SCN^-

100 µL of different concentrations of SCN^- were added into 900 µL aqueous solution of C-dots (the final concentration was 0.06 mg mL^{-1}), followed by adding 1.55 µL $HAuCl_4$ solution (the final concentration was 0.05 mM). After incubated at room temperature for 20 min, the UV-vis spectra were recorded immediately.

2.6 Sample pretreatment

The raw milk was purchased from local supermarket. The pretreatment of raw milk samples was carried out following the literature.[28-30] Briefly, 15 mL of 1 % CCl_3COOH, 5 mL of CH_3CN were added into 2.0 mL of the raw milk. Then the mixture was ultrasonically treated for 15 min and centrifuged at 10000 rpm for 10 min. After that, the supernatant was filtered through a 0.22 µm membrane filter. Then the pH of filtrate was adjusted to 6.8 and the filter was filtered through 0.22 µm membrane filter again after centrifugation. The filtered liquid was diluted 10-fold with water for further analysis.

3 Results and discussion

3.1 Sensing mechanism

According to previous reports, C-dots can reduce $HAuCl_4$ to form AuNPs and act as the stabilizing agent to prevent the as-synthesized AuNPs from aggregating at the same time.[26] As shown in Fig. 1, as-synthesized AuNPs shows an observed absorption peak centered at 530 nm, which matches well with the typical plasmon resonance absorption peak of AuNPs. However, when SCN^- was introduced, the absorption peak of AuNPs is obvious decreased with a slight blue shift. The effect of SCN^- on the synthesis system was investigated. As shown in Fig. S1, C-dots shows strong fluorescence emission peak at 466 nm when excited at 364 nm. After the addition of SCN^-, the fluorescence emission spectrum of mixture solution shows no difference with C-dots. This observation indicates that there is no interaction between SCN^- and C-dots. With the addition of SCN^- (Fig. 1), the synthesis of AuNPs by C-dots is obviously influenced due to the covalent binding of the sulfur of SCN^- and AuNPs.[31] The SCN^- can act as capping agent and prevent AuNPs from growing, resulting an obvious decrease of absorbance intensity and a slight blue shift of the absorption peak of as-synthesized AuNPs. Meanwhile, SCN^- can etch as-synthesized big AuNPs to small AuNPs,[32] leading to the absorbance change of AuNPs As shown in Fig. S2, when addition of SCN- into as-synthesized AuNPs solution, the absorption intensity of as-synthesized AuNPs obvious decreased and the absorption peak blue shift. The experiments prove that the SCN- can decompose the as-synthesized AuNPs to smaller AuNPs. It was further supported by the TEM images (Fig. S3). This observation indicates that the SCN^- has both inhibition effect and etching effect to the AuNPs. These synergistic effects lead to the change of absorption peak of AuNPs. As shown in Fig. 1, the absorbance decreases largely with the increase in SCN^- concentration, indicated that there should be some relationship between the SCN^- concentration and the decreased absorbance. Based on above results, quantitative detection of SCN^- is feasible by measuring the plasmon resonance absorption peak change of AuNPs. The proposed detection mechanism is depicted in Fig. 2, and summarized as follows: C-dots reduces $HAuCl_4$ to AuNPs, which results in an absorption peak at 530 nm. Upon addition of SCN^-, the growth of AuNPs is prevented due to the covalent binding between the sulfur of SCN^- and AuNPs. At the same time, the SCN^- can also decompose the as-synthesized AuNPs to smaller AuNPs. As a result, the absorption peak of AuNPs is obviously decreased. By measuring the absorption peak of as-synthesized AuNPs, an optical sensor is developed for the detection of SCN^-. Meanwhile, due to the high extinction coefficient of AuNPs, the proposed method may achieve high detection sensitivity.[33, 34]

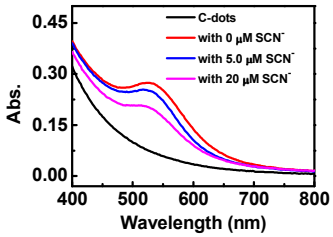

Fig. 1 UV-vis spectra of C-dots and as-synthesized AuNPs in the absence and presence different concentration of SCN^-, respectively. (The concentration of C-dots is 0.06 mg mL^{-1}, and the incubation time is 20 min).

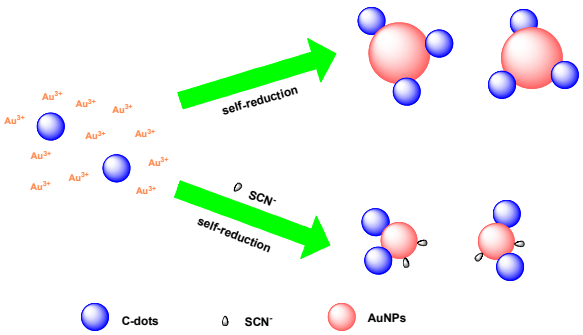

Fig. 2 Proposed mechanism for the detection of SCN^-.

3.2 Optimization of the detection conditions

As mentioned above, the Au^{3+} was reduced by C-dots to form

AuNPs. Apparently, the concentration of AuNPs will directly affect the sensitivity of the proposed method, while the concentration of C-dots and $HAuCl_4$ are the key factors affecting the concentration of AuNPs. Therefore, the concentration of C-dots and $HAuCl_4$ should be optimized to improve the detection sensitivity. From the relationship between the absorbance of AuNPs and the concentration of C-dots (Fig. S4), it can be known that the rate of synthesis of AuNPs is enhanced with the increase of the concentration of C-dots. However, the excessive high AuNPs generation rate is not conducive to SCN^- to adsorb on the surface of AuNPs and etch AuNPs. What's more, the signal-to-noise ratio (*S*/*N*) would be decreased when the concentration of C-dots is low. As shown in Fig. 3A, the absorbance decrease rates ($(A_0\text{-}A)/A_0$) increase with the increase of the concentration of C-dots and reach the highest value at the C-dots concentration of 0.06 mg mL^{-1}. Therefore, the C-dots concentration of 0.06 mg mL^{-1} is the most suitable and adopted in detection experiments. As shown in Fig. 3B, it is observed that the maximum absorbance decrease rate is obtained at the $HAuCl_4$ concentration of 0.05 mM. With the increasing of $HAuCl_4$, the number of as-synthesized AuNPs is also increased, which makes the SCN^- can not be absorbed on the surface of AuNPs completely, and the etching effect also decrease. Therefore, the $HAuCl_4$ concentration of 0.05 mM is used in this method.

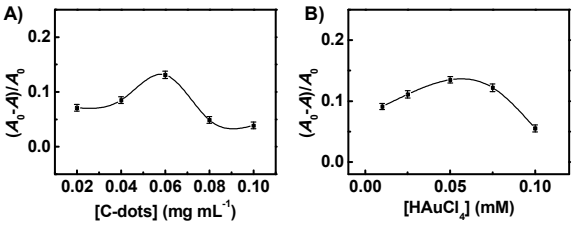

Fig. 3 Effects of (A) C-dots concentration and (B) $HAuCl_4$ concentration on the absorbance decrease rate of the proposed method for SCN^- detection. (A_0 and *A* correspond to the absorbance of AuNPs at 530 nm in the absence and presence of SCN^-, respectively.)

3.3 The analytical performance

Under the optimal assay conditions, the analytical performance of the proposed method for SCN^- detection was evaluated. The UV-vis spectra of as-synthesized AuNPs in the presence of various concentrations of SCN^- are shown in Fig. 4A. The increase of SCN^- concentration results in an obvious decrease of absorbance intensity of AuNPs, and a slight blue shift of its absorption peak. As shown in Fig. 4B, a good linear relationship is observed at the concentration of SCN^- range of 7 μM - 50 μM, and the corresponding calibration equation was $y = 0.0137x + 0.0382$ ($R^2 = 0.9908$). However, at the concentration range of 0.2 μM - 7 μM, the absorbance decrease rate undergoes logarithmic change with respect to concentration of SCN^- (Fig. 4B inset),[35] and the square root calibration equation is $y=0.0401\sqrt{x} + 0.0390$ (R^2=0.9929). The limit of detection (LOD) is the concentration of analyte which produces an analytical signal equal to 3 times the standard deviation of the blank measurements,[36] which is calculated to be 0.16 μM. The comparison of different methods for SCN^- detection is shown in Table S1, suggesting that the proposed method exhibits superior sensitivity and a wider linear response range for SCN^- detection.

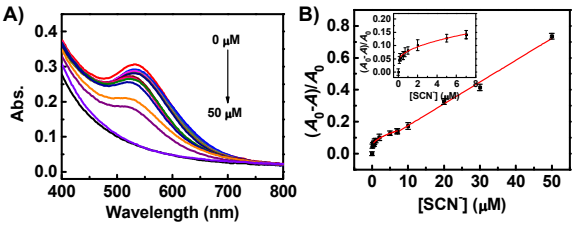

Fig. 4 (A) UV-vis spectra of the AuNPs with different concentration of SCN-. (B) The linear relationship between the absorbance decrease rate and the concentration of SCN^-. Inset shows the calibration cure at the low concentration. The error bars were magnified by a factor of ten.

In order to determine SCN^- in practical samples such as raw milk, the potential interfering substances including common ions and amino acids were investigated to evaluate the selectivity of the proposed method. As shown in Fig. 5, the absorbance change rate caused by SCN^- is larger than the other compounds, which indicates that the method shows excellent selectivity toward SCN^- over the other compounds. Since the amino group in amino acids can interact with AuNPs,[37, 38] amino acids especially cysteine and glutathione show an interference, but the interference of cysteine and glutathione is not a fatal defect to the proposed method which was applied to the detection of SCN^- in milk. There is not cysteine and glutathione in milk. Therefore, the interference from cysteine and glutathione can be ignored in the practical application. In practical assays, combining appropriate pretreatment technology (such as adjusting the pH value to make amino acid flocculation) the potential interference from other amino acids may be insignificant and this method will be a promising method to detect SCN^- in practical samples.

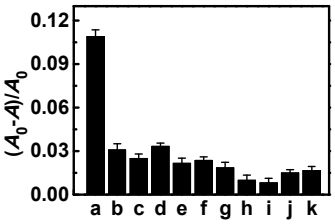

Fig. 5 The absorbance change rate of the AuNPs in the presence of SCN^- and other compounds. (a) SCN-, (b) glycine, (c) lysine, (d) threonine, (e) cysteine, (f) glutathione, (g) glucose, (h) Na^+, (i) K^+, (j) Ca^{2+}, (k) Mg^{2+}. (The concentration of SCN^- is 2.0 μM, cysteine is 50 μM, glutathione is 50 μM, and other compounds concentration is 100 μM)

3.4 Determination of SCN^- in raw milk

In order to validate the feasibility of proposed method for selectively detection of SCN^- in practical sample, a recovery experiment was conducted by adding known amounts of SCN^- to the raw milk. Certain amounts of SCN^- were directly spiked into the raw milk before sample pretreatment. Then the raw milk was treated and determined according to the procedures described in Sections 2.5 and 2.6. The results are summarized in Table 1. As shown in Table 1, excellent recovery in the range from 95.50 % to 107.33 % is obtained, indicating the proposed method is reliable and suitable for practical applications.

Table 1.Results of the determination of SCN^- in raw milk.

Sample	Determination (μM)	Added (μM)	Detected (μM)	Recovery (%)	RSD (%)
Raw milk	24.63	10.00	34.93	103.00	2.43
		15.00	40.73	107.33	2.81
		20.00	43.63	95.50	3.17

4 Conclusions

In contrast to the conventional sensors based on the fluorescence intensity change of C-dots, the present study proposes an AuNPs plasmon resonance absorption-based sensor for sensitive and selective detection of SCN^-. The AuNPs is synthesized by C-dots acting as reductant. The strongly coordination and etching effects between AuNPs and SCN^-, which can lead to the change of absorption peak of as-synthesized AuNPs. By monitoring the change of absorption spectrum of AuNPs, an optical sensor is developed for detection of SCN^-. The proposed method displays high sensitivity and excellent selectivity for the determination of SCN^- and has successfully applied to practical sample detection with satisfactory result. We provide a new approach for the sensing application of C-dots and a new analytical method for SCN^- detection.

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