Colorimetric Detection of Ascorbic Acid Based on the Trigger of Gold Nanoparticles Aggregation by Cr(III) Reduced from Cr(VI)

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Au nanoparticles (AuNPs) dispersed in water were stabilized by tripolyphosphate (P₃O₁₀⁵⁻). When Cr₂O₇²⁻ (Cr(VI)) was present, the AuNPs did not change; when Cr³⁺ (Cr(III)) was present, the AuNPs would aggregate because of cooperative metal-ligand reaction. Aggregated AuNPs showed different color from the non-aggregated ones. Thus, a simple colorimetric assay was made using AuNPs-Cr(VI) to detect ascorbic acid (AA). Upon introducing AA to the AuNPs-Cr(VI) system, Cr(VI) was reduced to Cr(III), and the aggregation of AuNPs occurred. This colorimetric assay performed high selectivity and a linear concentration response in the range of 0.2 to 10 μM; its limit of detection was 0.15 μM.

**Keywords** Gold nanoparticles, aggregation, Cr(VI), Cr(III), ascorbic acid

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Introduction

Ascorbic acid (AA, also known as vitamin C), widely used as an antioxidant, nutritional factor or enzyme, extensively exists in biological fluids, foodstuffs and pharmaceuticals. The quantitative detection of AA is obviously important. Common analysis methods for AA detection include fluorescent spectrophotometry,1,2 electrochemistry,3 chromatogram,4 colorimetric analysis.5–9 Some of these techniques require complicated sample preparation or sophisticated instrumentation, but the colorimetric method has great potential for AA detection owing to its simple, rapid operation and cost-effectiveness.10,11 Noble metal nanoparticles, such as gold or silver nanoparticles, have unique surface plasmon resonance (SPR) properties; they are ideal chromogenic agents for colorimetric analysis with color changes arising from SPR during aggregation.12–17 In this case, a surface modifying agent (such as small molecules, polymers, aptamers) usually interacts with the target in ways of cross-linking or electrostatic attraction, which leads to the aggregation of metal nanoparticles.8 The resulting changes in both color and absorption spectra can be quantified for target detection. Wang et al. designed DNA functionalized Fe₃O₄@Au nanoparticles and used them as colorimetric probes for Hg²⁺ detection while relying on thymidine-Hg²⁺-thymidine coordination.18 Zhang et al. designed two modifying agents having azide and alkyne terminal groups separately. After functionalized Au nanoparticles (AuNPs), these colorimetric probes were used for AA detection via “click-chemistry”.6 However, there are still issues existing in this kind of colorimetric probes, such as complicated surface modification, expensive agents, being susceptible to the metal ion strength and interference, and poor reproducibility. The development of simple and feasible colorimetric methods is still a practical need.

P₃O₁₀⁵⁻-AuNPs interacted with Cr(III) through a cooperative metal-ligand reaction, which leads to the aggregation of AuNPs quickly, following by obvious color changes from red to purple.19 AA can reduce Cr(VI) to Cr(III). Here, we report on a colorimetric assay for the detection of AA based on it. The sensitivity and selectivity of this AuNP-Cr(VI) assay were investigated.

Experimental

Reagents and chemicals

Chlorauric acid hydrate (HAuCl₄·4H₂O), sodium borohydride (NaBH₄), ascorbic acid (AA) and sodium tripolyphosphate (Na₅P₃O₁₀), potassium dichromate (K₂Cr₂O₇) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cr(NO₃)₃ was purchased from Aladdin Industrial Corporation. All of the materials were of analytical grade without further purification. The solution of AA was freshly prepared using deoxygenated deionized ultrapure water (18.2 MΩ·cm) before each experiment.

Apparatus

A Hitachi S-2500 transmission electron microscope (TEM) was employed to observe the morphology of AuNPs. UV-visible
adsorption spectra were recorded on a Hitachi U-3900H UV-vis spectrophotometer at room temperature.

**Procedure**

Preparation of AuNPs stabilized with P₃O₁₀⁵⁻. All of the glasswares were washed with aqua regia, and then thoroughly rinsed with ultrapure water. The AuNPs were prepared by sodium borohydride reduction according to published literature with some modification. Typically, a HAuCl₄·4H₂O solution (25 mL, 5 mM) was added to a Na₅P₃O₁₀ solution (100 mL, 1 mM) in a flask, and stirred vigorously at room temperature. Then, a NaBH₄ solution (4 mL, 0.1 mM) was added dropwise, and the mixed solution color changed from pale-yellow to wine-red. The obtained solution was stored at 4°C until future use.

For the detection of AA, Cr(VI) was firstly added to the prepared AuNPs, and the pH of the mixed solution was adjusted to 4.0. Then, various amounts of AA were added into the above-mentioned solution. The mixtures were maintained at room temperature for 10.5 min. UV-vis absorption spectra of the solutions were recorded in the range of 300 – 800 nm. The selectivity for AA was performed by adding other molecules or metal ions in the assay solution.

**Results and Discussion**

**Principle of AA detection based on aggregation of AuNPs**

Figure 1 depicts the mechanism of colorimetric detection of AA based on the aggregation of AuNPs. Cr(VI) exists in the form of HCrO₄⁻, CrO₄²⁻, or Cr₂O₇²⁻, which leads to electrical repulsion to P₃O₁₀⁵⁻, which will not induce the aggregation of AuNPs. Cr(III), acts as a hard Lewis acid, that strongly prefers coordinating to negatively charged oxygen groups, which can form six-coordinate complexes with many molecules that contain O, N, or S atoms. Upon interactions with P₃O₁₀⁵⁻ AuNPs, and Cr(III) complexes with P₃O₁₀⁵⁻ through the cooperative metal-ligand reaction, the aggregation of AuNPs is triggered, followed by an obvious color changes from red to purple, which is consistented with Ref. 19. When AA is present, Cr(VI) can be quickly reduced to Cr(III) by AA, resulting in the aggregation of AuNPs. The reaction can be described by

To investigate the feasibility of this method for AA detection, the color responses of the AuNPs to AA, Cr(III), Cr(VI) and the mixed solution of AA and Cr(VI) are shown in Fig. 2. It can be seen that AA or Cr(VI) do not induce the aggregation of AuNPs, while Cr(III) and the mixed solution of AA and Cr(VI) can trigger such aggregation. It is Cr(III) that plays the key role for triggering AuNPs aggregation, which cannot be observed for other di- and trivalent metal ions (see Supporting Information).

The morphology of AuNPs was observed under TEM (Fig. 3a). It showed that the mean size of AuNPs is 3.70 ± 0.77 nm. The solution was red, as shown in Fig. 2a, which indicated well-dispersed AuNPs. It can be seen from Fig. 3b that AuNPs-

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**Fig. 1** Procedure for the preparation of a simple column electrode, S-CE. Schematic illustration of the analytical process for detecting AA based on the aggregation of AuNPs.

**Fig. 2** Colors of a, AuNPs; b, AuNPs + AA; c, AuNPs + Cr(III); d, AuNPs + Cr(VI) + AA; e, AuNPs + Cr(VI). Experimental conditions: AA, 12.5 µM; Cr(III), 5 µM; Cr(VI), 5 µM. The pH was 4.0.

**Fig. 3** a, TEM images of AuNPs (inset is the particle size distribution histogram); b, TEM images of AuNPs-Cr(VI) after adding AA.
Cr(VI) aggregated after the addition of AA; correspondingly, the color changed to purple as shown in Fig. 2d.

**Optimization of the sensing conditions**

The analytical performance of this AA assay was evaluated concerning the pH value and response time. The effect of the pH value is shown in Fig. 4. It can be seen that in the presence of Cr(III), AuNPs aggregated at below pH 6.0 due to the reaction between Cr(III) and AuNPs. However, the AuNPs-Cr(VI) system was stable when the pH was above 3.5, due to the repulsive force between Cr(VI) and P3O105–; Cr(VI) is expected to exist in the form of HCrO4– in the pH region 3 – 5.21,22 Therefore, the suitable pH value for the assay was in the range of 3.5 – 6.0. From Fig. 4, it can be seen that the color change is clear when pH is 4.0, which is convenient for colorimetric detection. Thus, pH 4.0 was chosen for the detection experiment.

The aggregation of AuNPs induced their plasmon peak to decrease at about 528 nm, and increase at about 670 nm; then the absorption ratios at 670 and 528 nm (i.e., A670/A528) were used to represent the relative amounts of dispersed and aggregated AuNPs. Figure 5 shows the influence of the response time; it can be seen that the ratio A670/A528 increases progressively as the response time increased from 1.5 to 10.5 min. But, with the response time being longer than 9 min, the ratio did not change much; the optimum response time was 9 – 12 min for the detection of AA. Finally, 10.5 min was selected as the best response time in our experiment.

**Colorimetric detection of AA**

Under the optimal conditions, AA with different concentrations was added into the assay, respectively. As shown in Fig. 6a, in the presence of Cr(VI), the assay showed a clear red color; the assay gradually became purple with an increase of AA. Correspondingly, the ratio of A670/A528 increased with an increase of AA from 0.2 to 12.5 μM. The inset in Fig. 6b shows a linear correlation between the value of A670/A528 and the AA concentration, ranging from 0.2 to 10 μM, with a correlation coefficient of 0.9967. The limit of detection (LOD) was found to be low as 0.15 μM (based on 3σ/k, where σ was the standard deviation of blank measurements and k was the slope of the calibration curve). Table 1 lists comparisons of our method and other approaches for AA detection. It is to be noticed that our method has a comparable LOD and detection range. Besides, the assay possesses the merits of easy obtaining in material, short response time, and being stable during long-time storage.

**Selectivity study**

To evaluate the specificity of the assay, the influence of potentially interfering species were investigated to verify the applicability of this assay. We tested the selectivity of the assay by carrying out several control experiments using PO43–, Zn2+, Cu2+, SO42–, Ni2+, Li+, Na+, Mg2+, Br–, NO3–, glucose (Glu), citric...
acid (CA), oxalic acid (OA) as interferences. No obvious changes were observed, as shown in Fig. 7, which clearly reveals that the proposed method is suitable for AA detection.

Pharmaceutical product detection

To investigate the possibility of actual applications, the present approach was applied to AA detection in vitamin C tablets and nestle fruit C+. For vitamin-C tablets, several tablets were powdered to be dissolved in deoxygenated deionized water; after centrifuging (8000 r/min for 5 min), the supernatant was diluted in a 500-mL volumetric flask. This solution was measured by the proposed assay. To ensure the reliability of the results, AA standard solution addition was performed to estimate the recovery. All of the data for the real samples are summarized in Table 2. The recoveries ranged from 96.1 to 98.9%. For nestle fruit C+, powders were dissolved in deoxygenated deionized water; it was detected by the proposed assay. The responding data are summarized in Table 3. The detected merchandise description versus virtual merchandise description ranged from 93.3 to 101.7%; indicating test results that were consistent with merchandise description. These results indicate that our proposed assay is suitable for determining the AA concentration in various samples.

Conclusions

In summary, we have demonstrated a simple colorimetric method for detecting AA using AuNPs-Cr(VI). AA could be rapidly detected with the assay at room temperature within 10.5 min. The colorimetric assay exhibited a linear response ranging from 0.2 to 10 μM, and the LOD was 0.15 μM. Besides, this assay showed high selectivity. In practical tests, it showed high effectiveness and reliability.

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Supporting Information

It is Cr(III) that plays the key role for triggering AuNPs aggregation, which cannot be observed for other di- and trivalent metal ions. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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