Use of seed-mediated growth of bimetallic nanorods as a knob for antioxidant assay

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ABSTRACT

Seed-mediated growth has been proved to be a powerful means in the controlled synthesis of nanomaterials. In this work, using Au nanorods (AuNRs) as seeds, we successfully apply the principle of seed-mediated growth of bimetallic NRs in antioxidant assay. Specifically, evolution of the analytical probe from AuNRs to Au@Ag core-shell NRs is well regulated by seed-mediated growth, where trace amount of antioxidants play important roles in this process. Based on the localized surface plasmon resonance (LSPR) shift that directed by this evolution, a simple, reliable, highly sensitive and selective analytical method for antioxidant is developed. According to the linear relationship between LSPR peak shift and the concentration of antioxidant, the present method achieves a detectable range of 0.01–30 μM, with a limit of detection as low as 6.4 nM. Moreover, it has also been successfully applied in the detection of antioxidants in green tea. This work provides new insights into establishing sensitive sensing methods through precisely manipulating the evolution of analytical probe by seed-mediated growth.

1. Introduction

Colloidal metal nanocrystals with different architectures have found wide use as optical probes in areas ranging from disease diagnosis and treatment [1,2] to pharmaceutical analysis [3], food inspection [4], and environmental pollutants monitoring [5]. The merit of these applications mainly arises from the expression of remarkable localized surface plasmon resonance (LSPR) properties with strong correlations to their individual or combined physical parameters, including composition, size, geometric shape, structure, and surface chemistry. All these parameters can be well controlled during or after the synthesis to engineer the LSPR properties of metal nanocrystals and thereby enhance their performance in various applications. For instance, the LSPR wavelength and intensity are able to be tuned through obtaining different sizes and particle numbers of the metal nanocrystals, which can be manipulated by introducing different amounts or types of reductant during the synthesis [6]. For another example, different LSPR signal can also be obtained via manipulating the inter-particle distance of metal nanocrystals after the synthesis, which has been achieved by solvent effect, covalent coupling, hydrogen bonding, and ligand-target recognition [7–10].

The concept about manipulation of LSPR properties of colloidal metal nanocrystals is able to be used for sensing purpose. In other words, a variety of different analytical methodologies and sensing techniques can be developed based on the variation of LSPR of metal nanocrystals that directed by any parameter such as pH, temperature, solvent, reductant, or a certain molecule. Previous reports have confirmed the feasibility of this strategy, which thereby has been successfully applied in analytical chemistry. For example, Xia et al. [11] developed time and temperature indicators based on the LSPR variation of Ag nanoplates. Huang et al. [12] achieved the visual detection of Sudan red in food by monitoring the LSPR evolution of the in-situ generated Ag nanoparticles. Long et al. [13] established a colorimetric method to detect iodide through the shape change-induced LSPR variation of Ag nanoplates. Our group [14] also achieved sensitive detection of vitamin C in pharmaceutical products based on the LSPR shift of Au nanocages.

Antioxidants are the molecules that inhibit the oxidation of other molecules. They can counter act free radicals, terminate chain reactions and thus prevent the damage of cells. Therefore, antioxidants play important roles in protection of human health (e.g., prevention of oxidative stress related diseases such as cancers, cardiovascular and neurodegenerative diseases) [15], anti-aging [16], as well as nutrition and food safety [17–19]. Detection of antioxidant content or evaluation of the antioxidant capacity of any product or natural substance is

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indispensable. To this end, a wide range of nanomaterials including those made of noble metals (e.g., Au, Ag and Rh), metal oxides (e.g., Fe_2O_3, TiO_2 and CeO_2), carbon materials (e.g., graphene quantum dots and carbon nanotubes), and hybrid materials have been reported for antioxidant assay [20]. Owing to the strong reducing power, antioxidant is able to act as an ideal reductant in the formation of metal nanoparticles, where the metal precursor can be transformed into metal atoms by the reduction of antioxidant. Thus, antioxidants assay have been achieved by several groups through the monitoring of LSPR absorption or scattering signal that derived from the generated metal nanoparticles [21–30]. However, homogeneous nucleation that occurred in one-pot synthesis, a pathway that formation of metal nanoparticles through aggregating metal atoms once their concentration reach the point of supersaturation, needs to step over relatively high energy barrier [31]. In this case, the concentration of a certain antioxidant should be increased in the reaction system to enhance its reducing performance, which will greatly limit the sensitivity of the sensors for antioxidant detection. In addition, compared with the reduction of Au^{3+} to Au atoms, Ag^+ is more easily to be reduced by the same reductant (i.e., a certain antioxidant) since the redox potential of Ag^+/Ag (0.80 V) is much lower that Au^{3+}/Au (1.50 V) [32].

In this work, seed-mediated growth of Au@Ag bimetallic nanorods is employed as a powerful means for sensitive detection of antioxidants. Different from the one-pot synthesis method, newly formed Ag atoms are deposited onto the surfaces of preformed seeds via heterogeneous nucleation in the seed-mediated growth [33,34]. According to the knowledge of thermodynamics, heterogeneous nucleation only needs to step over much lower energy barrier than the homogeneous nucleation when the formation of metal nanocrystals [31]. Thus, only trace amount of antioxidant in the reaction system can achieve the formation of metal atoms via reduction and then deposition of them on the surface of seeds, resulting in the shape or composition variation of the metal nanocrystals and their corresponding LSPR signal. As such, sensitivity of the analytical method for antioxidant assay is able to be largely improved. Herein, Au nanorods (AuNRs) are used as seeds for the epitaxial growth of the second metal, Ag, which can be generated from the redox reaction between antioxidants and silver nitrate (Scheme 1). Due to the formation of bimetallic Au@Ag NRs, the longitudinal LSPR peak of the nanocrystals blue shifts. Based on the quantitative relationship between the concentration of antioxidant and LSPR shift, a nanomaterial-based antioxidant sensor is develop. Gallic acid (GA), a polyphenol compound that commonly used as a standard to evaluate total antioxidant capacity, is used as a representative antioxidant to establish the calibration curve for quantification. Namely, the final amount of antioxidants in the test sample is expressed in term of GA equivalents. This analytical method is simple, sensitive, and reliable, which has been successfully applied in the detection of total antioxidants in green tea.

2. Experimental

2.1. Chemicals and materials

Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4\cdot3H_2O$) and silver nitrate ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Gallic acid (GA), L-ascorbic acid (AA), tannic acid (TA), catechol, hexadecyltrimethylammonium bromide (CTAB), 5-bromosalicylic acid (5-BrSA), and sodium borohydride ($NaBH_4$) were purchased from Aladdin Industrial Corporation (Shanghai, China). All the chemicals were used as received. Britton-Robison (BR) buffer was used to control the acidity of the solution in detection. Milli-Q purified water (18.2 MΩ cm) was used throughout the experiments.

2.2. Instrumentations

Transmission electron microscopy (TEM) images of the nanocrystals were taken using a Tecnai G2 F20 transmission electron microscopy (USA) operated at 200 kV. Extinction spectra of the nanocrystals were measured using a UV-2550 UV–vis spectrophotometer (Shimadzu, Japan). A numerical controlled KH-2200DE ultrasonic cleaner (Shandong, China) was used to disperse nanocrystals in solution. A high-speed TGL-16 M centrifuge (Hunan, China) was used in the purification of samples.

2.3. Synthesis of AuNRs

AuNRs were synthesized by a modified seed-mediated growth method [35]. Firstly, Au seeds were prepared as reported previously [36]. In detail, 5 mL of $HAuCl_4$ (0.5 mM) and 5 mL of CTAB (0.2 M) solution were mixed. Then, another solution (1 mL) containing 0.6 mL of freshly prepared $NaBH_4$ (0.01 M) and 0.4 mL water was rapidly injected under vigorous stirring. Color of the solution changed from yellow to brownish-yellow, and the stirring was stopped after 2 min. This seed solution was aged at room temperature for 30 min prior to use in order to consume excess $NaBH_4$.

To prepare the growth solution, 9 g of CTAB together with 1.1 g of 5-BrSA were dissolved in 250 mL of warm water (60 °C) in a flask. After the solution was cooled to 30 °C, 12 mL of $AgNO_3$ (4 mM) was added. The mixture was kept undisturbed at 30 °C for 15 min, after which 250 mL of $HAuCl_4$ (1 mM) was added. After 15 min of slow stirring, 2 mL of AA (0.064 M) was added, and the mixture was vigorously stirred for 30 s until it became colorless. Finally, 0.4 mL of Au seeds was injected into the above growth solution. The resultant mixture was stirred for 30 s and left undisturbed at 30 °C. After 12 h, the AuNRs were centrifuged at 8500 rpm for 25 min and then redispersed in 10 mL of water.
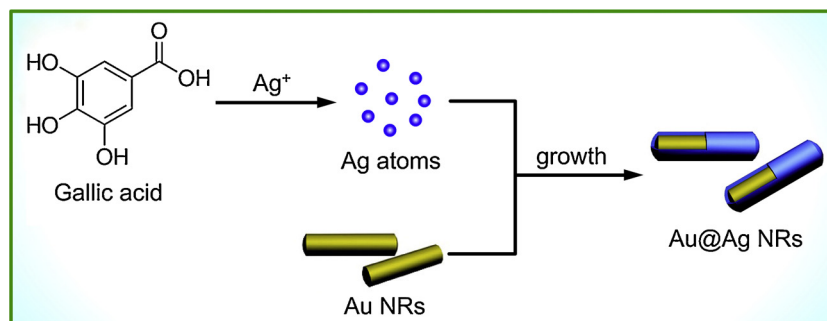
Scheme 1. Schematic illustration showing the strategy for the detection of GA based on the seed-mediated growth, using AuNRs and Ag^+ as the seeds and growth solution, respectively.