Seed-mediated grown silver nanoparticles as a colorimetric sensor for detection of ascorbic acid

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A simple and sensitive approach was demonstrated for detection of ascorbic acid (AA) based on seed-mediated growth of silver nanoparticles (Ag NPs). According to the seeding strategy, silver ions existing in the growth solution were reduced to silver atoms on the surface of silver seeds via redox reaction between silver ions and AA. This process led to appear an absorption band in near 420 nm owing to the localized surface plasmon resonance peak of the generated Ag NPs. This change in absorption spectra of Ag NPs caused a change in color of the mixture from colorless to yellow. It was found that the changes in absorption intensity at 420 nm have a good relationship with the concentration of AA. Also, detection of AA was achieved through the established colorimetric sensor in the range of 0.25–25 μM with detection limit of 0.054 μM. Moreover, the selectivity of the method was evaluated with considering potential interferences. The method showed high selectivity toward AA rather than potential interferences and coexisted molecules with AA. It was successfully applied for detection and determination of AA in pharmaceutical tablets and commercial lemonade.

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1. Introduction

Ascorbic acid (AA), an essential water-soluble nutrient, is a vital antioxidant which is important for health maintenance. AA is naturally occurring in fruits and vegetables. It involves in many physiological and biological processes, such as collagen cartilage, neurotransmitters formation, amino acid metabolism, ion absorption and so on, as an enzymatic cofactor [1]. Besides its existence in many biological fluids, it has been widely used in the chemical, cosmetic, pharmaceutical and food industry. It is a powerful antioxidant which efficiently scavenge toxic free radicals and other reactive oxygen species [2]. Consequently, it can protect human body from oxidative stress. In addition, it has a very important role in protection of the immune system. AA is also used for the prevention and treatment of scurvy, anemia, gingivitis, cancer, cardiovascular disease, gout, common cold, mental illness, infertility, drug poisoning, liver disease, allergic reactions, diabetes and AIDS [2, 3]. Despite the fact that AA is necessary for human body, excess AA levels in body is harmful for health and may cause to gastric irritation and renal disorders, so that it can result in diarrhea, hyperacidity and kidney calculi [4]. Therefore, rapid, sensitive, selective and accurate detection and quantification of AA level is considerably significant for pharmaceutical analysis, clinical diagnosis application and food industries.

Up to now, numerous analytical techniques have been reported for AA determination including titration [5], chromatographic [6–8], electrochemical [9,10], colorimetric [11–13], fluorescence [14,15] and chemiluminescence [16] techniques. However, these analytical approaches are quite complicated, expensive and time-consuming. Among these methods, colorimetric assay is more desirable method due to its simplicity, rapidity, low cost and easily read out by the naked eye without requirements of the sophisticated instrumentation [17].

Colorimetric plasmonic nanosensors have drawn enormous attention for detection of a wide range of analytes including organic [18–22], inorganic [23–26] and biomolecules [27–30]. In recent years, utilization of miniature colorimetric nanoprobes has been dramatically developed due to their remarkable advantages. Metal nanoparticles (NPs), mainly gold and silver nanoparticles with unique size, shape, distance dependence optical properties and also exhibiting extremely large molar extinction coefficients [31], have been known as powerful sensitive colorimetric sensors. Localized surface plasmon resonance (LSPR) of NPs can be changed by tuning their size, shape and aggregation state as they have interaction with target analytes [32]. Actually, metallic colorimetric sensors usually act based on three main strategies including aggregation [33,34], growth [35] and shape transformation of metal NPs [36]. Among the different strategies which have been widely used in the sensing approaches, the seed-mediated growth strategy is a simple and effective alternative LSPR-based approach for colorimetric sensing [37,38]. Accordingly, shape-dependent optical properties of
noble metal nanostructures encourage extensive investigations into developing sensitive colorimetric probes [39]. Recently, developments regarding the seed-mediated growth method have been widely used with the aim of introducing new strategies for highly sensitive sensing [38, 40]. A typical seed-mediated growth process involves the synthesis of nanocrystals as seeds, and subsequent growth in aqueous solutions containing metal ions, reducing reagents, and shape-directing reagents (i.e. surfactants) [32]. By adding the seeded particles to a growth solution, newly formed atoms are deposited onto the surfaces of pre-existing seeds through reduction of metal ions to metal NPs via the redox reaction between metal ion and reductant. In this approach, the easily oxidizing analytical targets, such as antioxidants, can be detected by color change [41]. Indeed, this change occurs according to nucleation and growth of NPs in solution phase which causes to change in the corresponding LSPR intensity and wavelength.

In the present study, seed-mediated growth of NPs based on the growth of citrate stabilized Ag nanoclusters as seed, was employed for detection and determination of AA. According to the redox reaction between silver ions and AA, as a mild reductant agent, newly produced Ag atoms deposited on the surfaces of Ag seeds in the presence of cetyltrimethylammonium bromide (CTAB). As a result, the LSPR peak of the generated Ag NPs shifted to a longer wavelength. This change which was related to AA concentration was successfully applied for the analysis of AA in a pharmaceutical product (vitamin C tablets) and a lemonade commercial drink.

2. Experimental

2.1. Reagents and Instruments

Silver nitrate (AgNO3), trisodium citrate (TSC) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (Shanghai, China). Sodium borohydride (NaBH4), AA and CTAB were obtained from Merck (Darmstadt, Germany). All the chemicals were of analytical grade without any further purification. All the solutions were prepared using deionized water and experiments were performed at ambient temperature. Two different vitamin C tablets groups produced by Daroo Salamat (vitamin C tablets of group 1) and Osvah (vitamin C tablets of group 2) Pharmaceutical companies (Tehran, Iran), were purchased from a local drugstore and commercial lemonade was obtained from a local market. Absorption spectra were recorded on a UV–Vis spectrophotometer (Agilent, USA) in the wavelength ranges of 300–700 nm. The images of Ag nanoclusters, as Ag seed, and Ag NPs were observed with a Zeiss EM10C high resolution transmission electron microscope (TEM, Zeiss, Germany). Dynamic light scattering (DLS) data were measured using Zetasizer ZEN3600 (Malvern, UK).

2.2. Synthesis of Silver Seeds

Silver seeds (Ag seed) were prepared based on the method reported in the literature [42]. Briefly, a 20 mL solution with final concentrations of 0.25 mM AgNO3 and 0.25 mM TSC was prepared in water. 0.6 mL of NaBH4 (0.01 M) was added drop wise to the solution while stirring vigorously. Immediately, the solution became bright yellow. This solution was used 2 h after preparation.

2.3. Colorimetric Detection of AA

For detection of AA, 100 μL of different concentrations of AA (0.1–100 μM, 100 μL) were added to a vial containing 50 μL of AgNO3 (10 mM). Then 750 μL of CTAB, 50 μL of Ag seed and 100 μL of NaOH 1 M were added to the mixture, respectively (total volume, 1100 μL). The solution was gently shaken after addition of NaOH. The color change was observed from colorless to yellow, indicating the growth of Ag NPs. Subsequently, the absorption spectra of the solution were measured by UV–Vis spectrophotometer in the range of 300–700 nm.

2.4. Preparation of Samples

For analysis of AA in vitamin C-tablets, at least 5 tablets from each batch of commercial tablet were ground and homogenized. Then a sample equal to the weight of one tablet was weighed and diluted to volume of 100 mL with water. Then the solution was sonicated for 15 min and filtered. An appropriate volume of the mixture was diluted 100 fold with water. Also, commercially lemonade was centrifuged and the supernatant was collected. Further the solutions were diluted by a factor of 10 with ultrapure water and were spiked with different concentration of AA. The spiked samples were introduced to the colorimetric system same as above described.

3. Results and Discussion

3.1. Spectral Features and Characterization of Silver Nanoparticles

Firstly, UV–Vis absorption spectra of the Ag seed and formed Ag NPs were monitored at wavelength range of 300–700 nm. As shown in Fig. 1a–(a), an obvious LSPR absorption peak of as-prepared Ag seed is observed at 400 nm. Also, the LSPR peak of Ag NPs in presence of AA can be observed in Fig. 1a–(b). As can be seen, the LSPR band shifted to longer wavelength (420 nm), generating Ag NPs through the seed-mediated growth approach of Ag seed based on redox reaction between Ag ion and AA in the presence of CTAB and NaOH. According to the observed shift in LSPR peak, the color of solution changed from colorless to yellow. Also, the images of the corresponding solution of Ag seed and Ag NPs were shown in Fig. 1b (shown in the inset). Moreover, the TEM was used to confirm the formation of Ag seed and growth of Ag NPs. In the TEM images, the Ag nanoclusters were found with the size less than 5 nm (Fig. 2a). Under this condition, TEM images of the mixtures in presence of AA indicated the growth of Ag seeds to Ag NPs with diameter larger than 10 nm (Fig. 2b). Also based on the DLS analysis (Fig. 2c), the average size of Ag nanoclusters was less than 5 nm and the average diameter of the generated Ag NPs (Fig. 2d) was about 50 nm.

3.2. Seed-mediated Growth of Ag NPs by Increasing AA Concentration

The Ag seeds were obtained by reduction of Ag ions with NaBH4 as reducing agent in the presence of TSC. The Ag seeds were grown through the generation of new Ag atoms on the surface of preformed Ag seeds with the aid of AA, as a mild reducing agent, and in the

![Fig. 1.](image-url)
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