



Green synthesis of curcumin conjugated nanosilver for the applications in nucleic acid sensing and anti-bacterial activity



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ABSTRACT

Silver nanoparticles (Ag NPs) are often synthesized by chemical and physical methods. Natural and non-toxic molecules are recently being replaced for nanoparticles preparation. In this paper we have used curcumin, which interacts with Ag⁺ and subsequently synthesizes silver nanoparticles. Further continuation of the reaction often makes aggregation and forms dark brown/black silver oxide. Presence of glycerol in the reaction mixture gives mono-disperse curcumin conjugated Ag NPs, which can be made stable by capping with polyvinylpyrrolidone (PVP). XRD data confirm that curcumin conjugated Ag NPs are crystalline in nature with a mean crystalline size of 13.27 nm. The Ag NPs are spherical and in the range of 10–50 nm though their hydrodynamic radius is found to be higher, ~294 nm, due to polyvinylpyrrolidone capping and aggregation of nanoparticles in solution. The production of curcumin conjugated Ag NPs follows first order kinetics and the effect of curcumin concentration during formation of Ag NPs indicates a linear enhancement in the production of Ag NPs with an increase in concentration of curcumin. These curcumin conjugated silver nanoparticles show anti-bacterial activity and can successfully determine nucleic acid (DNA and RNA) in the concentration range 100–1000 ng/mL with a linear regression coefficient >0.997 using Resonance Rayleigh Scattering spectra.

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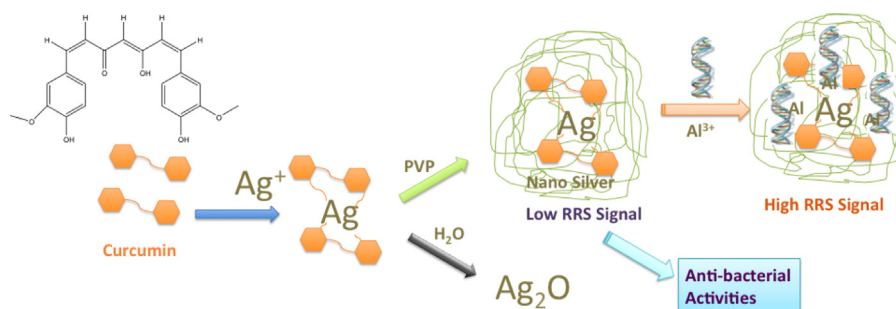
1. Introduction

Understanding physical and chemical properties of nanoparticles has drawn intense interest in the past two decades because of their scientific use and potential application in technology, medicine and other fields. Metallic nanoparticles exhibit extraordinary optical, chemical, thermal and physical behavior. The pronounced effects on the physical properties of metal nanoparticles are due to their reduced dimension compared to bulk material. As a consequence, they display a large surface atom, a large surface energy, a spatial confinement and reduced imperfections. Therefore, development of a fast and consistent experimental synthetic procedure for the preparation of metal nanoparticles has been a key aspect of research. These metal nanoparticles are mostly prepared from noble metals like palladium, silver, gold, and platinum. Silver nanoparticles (Ag NPs) particularly have drawn intense research interest because of their potential applications in biomedical science and sensor development [1]. Silver is also relatively more affordable than gold, palladium and platinum.

Commercially colloidal silver has been used as a biocide for almost a century. Over the last ten years, use of nanosilver has dramatically increased, thanks to the advancement in nanoscience and engineering. Properties of Ag NPs can be modified by coating shell with functional groups, such as, modulating their solubility [2], self-assembly [3] or, allowing selective interactions [4]. Different chromophores have been coupled to Ag NPs for the design of sensors [5] and as vehicles for tracers and drugs [6]. Interaction of Ag NPs with DNA has been extensively studied for potential applications as smart bio-based nanoparticle assemblies [3], new intercalating agents [7] or gene delivery vectors [8].

Although preparation of Ag nanoparticles could be straight forward, the size distribution of synthesized Ag NPs and their application can be challenging. A variety of techniques have been developed to synthesize Ag NPs, which include laser irradiation technique [9], photochemical reduction [10], electrochemical or sonochemical deposition [11,12], aerosol technique [13] etc. The chemical reduction process using a number of chemical reductants has been another popular method. These reductants are NaBH₄, N₂H₄, NH₂OH, ethanol, ethylene glycol and *N,N*-dimethylformamide (DMF) [14–18]. The chemical techniques also include sol process, micelle, chemical precipitation and hydrothermal methods [19]. Few of these available methods are easy

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Scheme 1. Illustration of nanosilver synthesis from Ag⁺ using curcumin.

and give good control of crystallite size by restoring the reaction environment. However, using such methods, achieving the mono-disperse nano-size and stable Ag NP products are still challenging [20]. In addition, capital intensive and inefficiency in material and energy use of these methods need to be addressed [21]. Another emerging field to synthesize Ag NPs has been green synthetic routes [1,22,23]. Advantages of green synthetic routes are: (i) environmental friendly methods to reduce waste, and (ii) minimum possible toxicity of the materials formed.

Curcumin is a natural diphenolic yellow orange pigment extracted from the dried rhizome of turmeric *Curcuma longa* [24]. It has effective therapeutic properties not only as an anti-inflammatory drug [25], but also as a chemo-preventive [26], chemotherapeutic [27], anti-oxidant [28], anti-amyloid [29], antiarthritic [30], anti-HIV [31], antimicrobial [32,33] and thrombosuppressive [34] agent. In addition, curcumin is being used in the treatment of cystic fibrosis [35] and Alzheimer disease [36]. Curcumin is not toxic and safe, thus, it can be a very good candidate to prepare nanoparticles through green synthetic route [37,38]. Here, we report that Ag NPs can be prepared using curcumin in buffer solution at pH 5. Formation of Ag NPs continues to proceed for a long time until silver nanoparticles are converted to silver oxide. Adding PVP/glycerol to the system can cease the formation of silver oxide. These curcumin conjugated Ag NPs are crystalline in nature with a mean crystallite size of 13.27 nm. The Ag NPs are spherical and in the range of 10–50 nm. Due to PVP capping and aggregation of nanoparticles in solution, their hydrodynamic radius is higher, ~294 nm. Effect of curcumin concentration during formation of Ag NPs are investigated, the production of curcumin conjugated Ag NPs follows first order kinetics. Similarly, we show that curcumin conjugated nanosilver has anti-bacterial properties and can sense nucleic acid by applying Resonance Rayleigh Scattering measured by synchronous fluorescence scan.

2. Materials and methods

2.1. Materials

Curcumin, silver nitrate, polyvinylpyrrolidone (PVP), deoxyribonucleic acid (DNA) from fish sperm, and ribonucleic acid (RNA) from torula yeast were obtained from Sigma–Aldrich and directly used without further purification. Potassium dihydrogen phosphate used was received from SOLAR LABORATORIES and its stock solution was prepared in de-ionized water. Aluminum nitrate was from Baker Chemical. All the solvents were HPLC or spectroscopic grade obtained from Sigma–Aldrich. For sensing experiment, 1 mg/mL stock of DNA/RNA was prepared by dissolving DNA/RNA in double distilled water. Aluminum nitrate (0.1 M) was also prepared by dissolving Al(NO₃)₃ in double distilled water.

2.2. Synthesis of curcumin conjugated Ag NPs

Synthesis of silver nanoparticles was done by dissolving silver nitrate of desired concentration (about 1 mM) in KH₂PO₄ buffer of pH 5 at 90 °C mixed with or without 3 g of glycerol. The stirring was maintained at 90 °C and curcumin (0.1 mM) was added to the solution, and then sonicated. When the color changed from yellow to olive green, 3 g of PVP were introduced, and the solution was kept stirring for 5 min. Finally, the solution was centrifuged at 20,000 rpm to separate the silver particles. It was washed with Millipore water and centrifuged for as many cycles as needed to remove the curcumin from the sample.

2.3. Characterization

The particle size distribution was analyzed using DLS (Brookhaven Instruments Corps) technique with a laser source of 658 nm and a PMT detector (HAMAMATSU, HC120-30). The software used was 90Plus Particle Sizing Software Ver. 5.23 and the dust was set at 40. The X-Ray diffraction (XRD) data were recorded using a Bruker d8 discover X-Ray diffractometer equipped with Cu-K α radiation ($\lambda = 1.5405 \text{ \AA}$). The monochromator used was Johansson Type. The step size was 0.02 s and the scan rate was 20 s per step. Scanning electron microscopy (SEM) analysis was done using Tescan, Vega 3 LMU with Oxford EDX detector (Inca XmaW20). For SEM measurement, nanoparticles were dissolved in 5 ml of de-ionized water, and few drops of the nanoparticles suspension were mounted on an aluminum stub, coated with carbon adhesive. After being dried the sample was ready for the SEM analyses. Transmission electron microscopy (TEM) measurement was carried out with a JEOL JEM-1400Plus, operating at 120 kV. TEM samples were prepared by casting a drop of the nanoparticle suspension onto copper grids covered with carbon films.

2.4. Spectroscopic measurement

The absorption spectra were recorded using a JASCO-570 UV–vis–NIR Spectrophotometer at room temperature. The steady-state fluorescence spectra (excitation and emission) were recorded at room temperature using Jobin–Yvon–Horiba Fluorolog III fluorometer and the FluorEssence program where the excitation and emission slits width were 5 nm. The source of excitation was a 100 W Xenon lamp, and the used detector was R-928 operating at a voltage of 950 V. Synchronous fluorescence scan was measured using the same instrument by keeping the excitation and emission slits width at 5 nm. Resonance Rayleigh Scattering spectra were obtained by simultaneously scanning excitation and emission monochromators over the range 250–600 nm with zero wavelength interval ($\Delta\lambda = 0 \text{ nm}$) in synchronous fluorescence scan mode. The resonance light scattering intensity was measured in the form of synchronous fluorescence scan (SFS) intensity.

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