Synthesis of Cysteine Capped Silver Nanoparticles by Electrochemically Active Biofilm and their Antibacterial Activities

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Received April 2, 2012, Accepted May 9, 2012

Cysteine capped silver nanoparticles (Cys-AgNPs) have been synthesized by employing electrochemically active biofilm (EAB), AgNO$_3$ as precursor and sodium acetate as electron donor in aqueous solution at 30 $^\circ$C. Cys-AgNPs of 5-10 nm were synthesized and characterized by UV-Vis, FT-IR, XRD and TEM. Capping of the silver nanoparticles with cysteine provides stability to nanoparticles by a thiolate bond between the amino acid and the nanoparticle surface and hydrogen bonding among the Cys-AgNPs. In addition, the antibacterial effects of as-synthesized Cys-AgNPs have been tested against two pathogenic bacteria Escherichia coli (O157:H7) and Pseudomonas aeruginosa (PAO1). The results demonstrate that the as-synthesized Cys-AgNPs can proficiently inhibit the growth and multiplication of E. coli and P. aeruginosa.

Key Words : Cys-AgNPs, Silver colloidal nanoparticles, (L)-Cysteine, Electrochemically active biofilm (EAB), Hydrogen bonding

Introduction

Nanoparticles are widely used for targeted drug delivery and for other biomedical application.$^{1,2}$ Among the metal nanoparticles, silver nanoparticles gaining much interest because of their antibacterial$^{1,3}$ and antifungal$^4$ response. In general, to synthesize AgNPs, metallic silver has been engineered into ultrafine particles by several methods, including wet chemical reaction, spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis.$^3,5$ Synthesis of reliable, green chemistry approach for nanoparticles is an important aspect of nanotechnology. Hence, there is a growing need to develop an environmentally benign nanoparticle synthetic procedure that does not use toxic chemicals and should be environment friendly.$^4$ Another important facet of nanotechnology is the development of new route to synthesize metal nanoparticles, especially AgNPs, which is toxicity free, is a great challenge. Several syntheses for AgNPs were reported, but in most of the cases chances of AgNPs contamination either with some chemicals$^{3,6}$ or with by-products of micro-organisms was involved.$^7,8$ Recently, few green synthesis for AgNPs have been reported but in most of these reports strong reducing agents such as NaBH$_4$ were used which further may contaminate the nanoparticles.$^3,9$ Another important factor related with the nanoparticles is the stability of the nanoparticles in the colloidal solution and after isolation of nanoparticles from colloidal solution. There are several reports to synthesize the naked AgNPs in colloidal solutions but stability and their interaction with biosystem remains the main concern.$^5,10$ In order to overcome these shortcomings, new methodologies were developed to synthesize AgNPs by employing different capping as well as stabilizing agents.$^{10,11}$ Among the capped/coated silver nanoparticles cysteine capped AgNPs is gaining much attention because of their selective biological and sensing activities towards microorganisms and amino acids.$^{10-12}$

Hence, there is a need to develop a protocol in which the approach should be green, nanoparticles should not get contaminated and the synthesized nanoparticles should be extracellular and stable. Recently, our group has reported the electrochemically active biofilm mediated synthesis of AgNPs in aqueous solution, in which the synthesized AgNPs were extracellular, stable and pure.$^{13}$ Inspiring with this work we extended our work to synthesize the cysteine capped AgNPs mediated by electrochemically active biofilm with enhanced activity and explore its antibacterial activity on Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa). To the best of our knowledge, synthesis of cysteine capped silver nanoparticles (Cys-AgNPs) by employing electrochemically active biofilm (EAB) is not yet documented and it is a novel and astute approach. However, synthesis of Cys-AgNPs through other approaches are well known and established.$^{10,11,14,15}$

Experimental

Materials. (L)-Cysteine, AgNO$_3$ and sodium acetate (Duksan Pure Chemicals Co. Ltd. South Korea) was used as received. Carbon paper (without wet proof, Fuel Cell Earth LLC), agar (Becton, Dickinson and Company, Sparks, USA) and paper disc (8 mm diameter, Tokyo Roshi Kaisha, Tokyo, Japan) were used as such. De-ionized (DI) water was prepared by using PURE ROUP 30 water purification system.

Methods. The as-synthesized Cys-AgNPs were characterized by using UV-Vis-NIR spectrophotometer (VARIAN, Cary 5000) and FT-IR (Excalibur Series, BIORAD, FTS 3000MX). X-ray diffraction (XRD) of powder was measured...
Synthesis of Cysteine Capped Silver Nanoparticles by EAB


Two pathogenic bacteria, *E. coli* (O157:H7) and *P. aeruginosa* (PAO1) of the sequenced strains were used in this study. Overnight cultures were re-grown to an optical density at 600 nm of 1.0 and about 10⁷ cells/plate were spread on LB agar. 20 µL of as-synthesized Cys-AgNPs colloidal solution was placed on the paper disc. Plates were incubated at 37 °C for 18 h and the antibacterial activities of samples were evaluated by measuring a clear zone where inhibition of bacterial growth takes place. Distilled water was used as a positive control. Three independent experiments were performed.

Results and Discussion

EAB is well known biogenic system that provide electrons by decomposing sodium acetate. After successful formation of EAB on carbon paper, we exploited it for the synthesis of cysteine capped silver nanoparticles. Figure 1 shows the overall mechanism for the synthesis of Cys-AgNPs by EAB. In the first step, Ag ions form complexes with the cysteine by thiolate bonding (Ag-SH) which arrest the fast reduction of Ag ions. It is well known that presence of cysteine decreases the rate of formation of silver nanoparticles. In the second step, EAB in presence of sodium acetate produces electrons, which were used for the slow reduction of Ag ions. It ultimately leads to the formation of cysteine capped silver nanoparticles which are apparent from the yellow color of the colloidal solution (Fig. 2).

UV-visible spectroscopic measurement of the dispersed aqueous solution was recorded (Fig. 2) and appearance of absorbance maxima at 397 nm, which is typically ascribed to the plasmon resonance band of AgNPs. Similar UV-vis spectra for Cys-AgNPs have been already reported. According to Mie theory, metal nanoparticles like Ag shows a surface plasmon band within the range of 400-450 nm. The absorbance spectra of as-synthesised Cys-AgNPs were measured after 5 days and the spectra remained same which indicates that the as-synthesized Cys-AgNPs were very stable for long time in the solution.

FT-IR spectra of the isolated Cys-AgNPs powder in KBr

Figure 1. Proposed scheme for the synthesis of cysteine capped silver nanoparticles by Electrochemically Active Biofilm.

Figure 2. UV-visible spectra of cysteine capped silver nanoparticles.
were recorded as shown in Figure 3. The prominent S-H vibrational band of free cysteine in general observed at ca. 2555 cm\(^{-1}\) which is not observed in the above spectra. It disappears on coordination of these molecules with colloidal silver. This is the strong evidence of surface binding of cysteine to the silver particles via a thiolate linkage. Murray and coworkers studied such behavior on alkanethiol modification of gold nanoparticles. Further indication for the presence of surface bound cysteine is provided by FT-IR measurements of the Cys-AgNPs in which carboxylate stretch vibration of the cysteine molecules is observed at ca. 1640 cm\(^{-1}\). It is well-known from studies on Langmuir-Blodgett films of metal salts of fatty acids that the position of this band is dependent on the nature of the bound metal cation. The position of the carboxylate stretch vibration at 1640 cm\(^{-1}\) in the silver colloidal solution suggests that some interaction of the acid group with other cysteine molecules, possibly through hydrogen bonding. This view is strengthened when one considers that the carbonyl stretch vibration of the acid group in free cysteine molecules occurs at 1650 cm\(^{-1}\).

The phase of the as-synthesized Cys-AgNPs was determined by X-ray diffraction with Cu K\(\alpha\) radiation. The X-ray diffraction pattern (Fig. 4) confirmed the formation of crystalline Cys-AgNPs. XRD pattern shows the four prominent diffraction peaks in the 2\(\theta\) range 30-80\(^{\circ}\) at 38\(^{\circ}\), 46.33\(^{\circ}\), 64.58\(^{\circ}\) and 77.24\(^{\circ}\) corresponding to 111, 200, 220 and 311 planes of polycrystalline face-centered cubic (fcc) silver, respectively (JCPDS file no. 04-0783). From the spectra it is also clear that no any impurity peaks were detected which reveals that the as-synthesized Cys-AgNPs were of high purity. The average of all the Cys-AgNPs peaks in the XRD spectra was used to calculate the average crystallite size by using the Scherrer equation and the full width at half maximum (fwhm). It shows that the average size of the Cys-AgNPs was ~10 nm which are concomitant with the TEM values.

Direct evidence for the state of aggregation of the Cys-AgNPs is determined by TEM at low magnification as shown in Figure 5(a). It illustrates that the size of as-synthesized Cys-AgNPs are in 5-10 nm range and monodispersed. Selected-area electron diffraction (SAED) is shown in Figure 5(b) which clearly shows well resolved lattice fringes and diffraction cycles and is indicative of a highly crystalline nature of Cys-AgNPs. It further confirmed the characteristic crystal planes of Cys-AgNPs which are concomitant with the XRD (Fig. 4) studies. Figure 5(c) shows the TEM image of naked AgNPs (without cap), determined at low magnification, synthesized by our group previously. In comparison to Figure 5(a), it shows that the AgNPs are monodispersed, discrete, in the range of 2-5 nm and are not aggregated. From Figure 5(a) it is clear that Cys-AgNPs were slightly aggregated in comparison to the naked AgNPs (Fig. 5(c)) where AgNPs were without capping. This slight aggregation is because of cross-linked structure via hydrogen bonding which provides stability to the AgNPs.

From the above scheme, characterization techniques and discussion, it was concluded that the as-synthesized Cys-AgNPs was well formed, crystalline in nature and pure. The amine and carboxylic acid functional groups of cysteine on the surface of the colloidal silver nanoparticles form hydrogen bonds which provide cross-linked structure to the colloidal silver nanoparticles which further stabilizes the AgNPs in aqueous solution. The AgNPs are well capped with cysteine as a capping and stabilizing agent. It further reveals that EAB might be a better tool to synthesize cysteine capped silver nanoparticles without any chemical contamination.

**Antibacterial Activity.** An antibacterial test against *E. coli* and *P. aeruginosa* was used to compare colonies on agar plates in the presence or absence of the as-synthesized Cys-AgNPs. Approximately 10\(^4\) colony-forming units (cfu)
of *E. coli* and *P. aeruginosa* were cultured on LB agar plates as control and LB agar plates supplemented with 20 μL of as-synthesized Cys-AgNP. After incubation at 37 °C for 18 h, bacterial colony crowding was observed on the control plates in the absence of any additives (Fig. 6(a) & 6(c)) whereas, growth of bacteria in the presence of Cys-AgNPs has inhibited (Fig. 6(b) & 6(d)). The presence of the Cys-AgNPs inhibited bacterial growth by approximately 80%, indicating that the as-synthesized Cys-AgNP has a good antibacterial effect (Fig. 6(b) & 6(d)). Zone of inhibition of as-synthesized Cys-AgNPs against pathogenic bacteria shows that in case of *P. aeruginosa*, it was ~4 mm and in case of *E. coli*, it was ~3 mm. The as-synthesized Cys-AgNPs shows much better bacterial inhibition as compared to without capped (naked) AgNPs.25

It is necessary to emphasize here that the as-synthesized Cys-AgNPs have bactericidal effects not only by inhibiting the bacterial growth but also by killing the bacteria. Cysteine enhances the role of antibacterial effect of AgNPs by interacting with the biosystems.11 Although the detailed and exact mechanism of the antibacterial effect of silver nanoparticles is still unclear, there are some clues in the literature that silver nanoparticles can interact with sulfur-containing proteins from cell membrane and phosphorus-containing compounds in cells, attacking the respiratory chain, with cell division leading to cell death.25,26

**Conclusion**

We have reported synthesis and surface modification of colloidal silver nanoparticles with the cysteine by EAB, which is a novel, simple, inexpensive, controlled and green protocol. EAB is used as a reducing agent. The presence of amine and carboxylic acid functional groups on the surface of the colloidal particles leads to formation of hydrogen bond and thereby cross-linking of the colloidal silver nanoparticles which further stabilizes the AgNPs in aqueous solution. Antibacterial experiments demonstrated that the as-synthesized Cys-AgNPs shows excellent antibacterial activity for *E. coli* and *P. aeruginosa* by inhibiting the growth and multiplication of the bacteria. The Cys-AgNPs synthesized by this clean biochemical route without using any additives are expected to find potential applications as antibacterial agents and in other medical and biotechnological fields. This novel approach could be used to synthesize other metal nanoparticles as well and it is under investigation in our laboratory.

**Acknowledgments.** Mohammad Mansoob Khan was supported by Brain Korea 21. S. Kalathil was supported by the Human Resources Development Program of Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant (No: 20104010100580) funded by the Korean Ministry of Knowledge Economy.

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