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Positively Charged Gold Nanoparticles Synthesized by Electrochemically Active Biofilm—A Biogenic Approach

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Positively charged gold nanoparticles [(+AuNPs) of 5–20 nm were synthesized by using electrochemically active biofilm (EAB) formed on a stainless steel mesh, within 30 minutes, in aqueous solution containing HAuCl4 as a precursor and sodium acetate as an electron donor. Electrochemically active bacteria present on biofilm oxidize the sodium acetate by producing electrons. Simultaneously, stainless steel also provides electrons because of the Cl− ions penetration into the stainless steel. Combined effect of both the EAB and stainless steel mesh enhances the availability of electrons for the reduction of Au3+ in the solution, which makes this synthesis efficient and fast. Therefore, small size, positively charged (+32.72 mV), monodispersed, controlled, easy separation and extracellular synthesis of (+AuNPs makes this protocol highly significant. As-synthesized AuNPs were characterized by UV-vis, DLS, XRD, TEM, HRTEM, EDX and SAED. (+AuNPs shows remarkable enhancement in the rate of reduction of methyl orange by NaBH4 because of the electron relay effect.

Keywords: Gold Nanoparticles, Positively Charged Gold Nanoparticles, Electrochemically Active Biofilm, Electron Relay Effect, Dye Degradation.

1. INTRODUCTION

Gold nanoparticles (AuNPs) are in prodigious demand by scientist because of its intriguing properties such as strong surface plasmon,1 catalytic,2–4 redox behavior5 and applications in medical diagnostics,6–8 dye-sensitized solar cells,7 optical9 and forensic.9 Typically, gold nanoparticles are obtained by chemical reduction of tetrachloroauric acid by sodium citrate or NaBH4.3–6 However, these approaches were based on the use of external chemical reductants that often produce undesirable side products and contaminates the nanoparticles. Therefore, a series of functionalizing agents for AuNPs has recently been developed that display a dual role for effective reducing agents for gold precursor and stabilizers, by providing a capping to metal nanoparticles.12–15 However, these syntheses involved large amounts of chemicals, and in addition, boron can contaminate the nanoparticles.4,12,16 Hence, employing microorganisms for nanoparticle synthesis seems to have better choice and benefits. Several microorganisms have been exploited to synthesize metal nanoparticles such as bacteria,17 fungi,16,18 and yeast.19 Bacteria are favored for the synthesis of nanoparticles over eukaryotic microorganisms due to its easy handling, genetic manipulation and the fact that the findings of one bacterium can be easily induced to others.

In many of the reported microbial synthesis, the nanoparticles were synthesized intracellularly i.e., within the biomatrix.20 In general, the intracellular synthesized nanoparticles are not monodispersed, get contaminated, far substandard to that isolated from conventional chemical methods.17–21 However, extracellular syntheses of nanoparticles are easy, size and morphology of nanoparticles can be controlled, easy separation and nanoparticles will not get contaminated.21,22 Therefore, extracellular nanoparticle synthesis has broader applications than intracellular synthesis.22 Until now biofilm (bacteria) formed on solid surfaces have been considered as detrimental to environment, human health and industrial products.23,24 However, recent research suggests that biofilm have properties that can be used to catalyze and control electrochemical reactions such as biological synthesis, bio-corrosion, bio-energy, bio-remediation and biosensors.25–27 These characteristics has open up new horizons for electrochemically active biofilm (EAB).25–27

Electrochemically active bacteria found on EAB are well known in microbial fuel cells (MFCs) because of their high oxidation power and ability to produce electrons.25,28
We have chosen an EAB on stainless steel mesh for the extracellular synthesis of (+) AuNPs. In general, EAB was made on carbon paper and our research group has recently reported it for the synthesis of silver nanoparticles. But in this report, we developed EAB on stainless steel mesh as a support because it is easily available, not expensive, provide more surface area for EAB in comparison to carbon paper and enhances the availability of electrons to reduce Au+ ions in solution. The combined effect of EAB and stainless steel mesh enhances the availability of electrons and makes the overall process very fast for the reduction of Au+.

During recent years (+) AuNPs are attracting much attention because of their use in biological mimics, biotechnology and catalysis. But in most of the cases positively charged gold nanoparticles were synthesized by coating/capping. Therefore, there is a need to synthesize the positively charged gold nanoparticles without using any capping agent. Metal nanoparticles especially gold nanoparticles are well known catalysts. Xiao et al. has shown that AuNPs can act as “electrical nanoplug” and enhance the electron transfer. In redox reaction, there may be large redox potential difference between electron donor and acceptor species which restrict the transfer of electrons. A suitable catalyst with intermediate redox potential may help electron transfer and acts as an electron relay system. Recently, it was found that metal nanoparticles could enhance the rate of degradation of organic dyes by 

Herein, we report an efficient extracellular synthesis of highly monodispersed and positively charged gold nanoparticles [(+)/AuNPs] using an EAB on stainless steel mesh. The entire synthesis was carried out in water at 30 °C without using any toxic chemicals. To the best of our knowledge, this is the first protocol to use an EAB formed on stainless steel mesh as a support for the extracellular synthesis of (+) AuNPs in water without using any capping and stabilizing agent. The synthesis is environment friendly and it is not yet documented. The as-synthesized (+) AuNPs acquired high positive charge which plays important role in catalytic activity, hence, we employed it as a catalyst for methyl orange reduction by NaBH4.

2. EXPERIMENTAL DETAILS

2.1. Materials

Chloroauric acid (HAuCl4 · nH2O) (n = 3.6; Kojima Chemical, Japan). Sodium citrate (Na3C6H5O7 · 2H2O), sodium acetate, methyl orange, extra pure NaOH, and NaBH4 (Duksan Pure Chemicals Co. Ltd. South Korea) was used as received. Stainless steel of type SUS 304 (Ildong Wire Cloth, Korea) which is a commercial alloy containing 68–72% Fe, 18–20% Cr, 8–10% Ni and very small amount of C, Mn and Si was used. De-ionized water was prepared by using PURE ROUP 30 water purification system.

2.2. Methods

The as-synthesized (+) AuNPs were characterized by using UV-Vis-NIR spectrophotometer (VARIAN, Cary 5000) and powder X-ray diffraction (XRD) was measured on diffractometer (PANalytical, XPert-PRO MPD) with Cu Kα radiation (λ = 0.15405 nm). Diffraction peaks of crystalline phases were compared with those of standard compounds reported in the JCPDS data file. The gold particle size of the sample was measured by TEM and HRTEM (Tecnai G2 F20, FEI, USA) operating at an accelerating voltage of 200 kV equipped with energy-dispersive X-ray (EDX) analysis system. (+) AuNPs were dispersed in ethanol and were sonicated for 10 minutes by sonicator (BRANSON 5510) for TEM studies. Selected-area electron diffraction (SAED) images were recorded with the TEM instrument. The zeta potential and particle size of the (+) AuNPs in aqueous solution were measured by using a Delsa Nano zeta potential (Beckman Coulter, USA).

2.3. Preparation of Electrochemically Active Biofilm (EAB)

EAB on stainless steel mesh was made as reported earlier. In short, stainless steel mesh (2.5 cm × 4.5 cm) were dipped into a mineral salt medium containing sodium acetate as substrate. 10 mL of anaerobic sludge (Biogas plant in Paju, Republic of South Korea) was added under strictly anaerobic conditions by sparging N2 gas for 5 min. All media, including the bacterial inoculum, were changed at every two days under strict anaerobic conditions. This was repeated for two weeks. The living electrochemically active biofilm formed on the stainless steel mesh was employed for the synthesis of (+) AuNPs.

2.4. Synthesis of (+) AuNPs by Electrochemically Active Biofilm

In a 250 mL bottle, 200 mL, 1 mM HAuCl4 aqueous solution was prepared. 0.2 gm sodium acetate was added as an electron donor and pH 4 was maintained by using 0.1 M NaOH. N2 gas was sparged for 5 min to keep the anaerobic conditions. EAB on stainless steel mesh was hanged and the system was sealed. The whole reaction mixture was left for magnetic stirring at 30 °C. Within 15 minutes the initial golden yellow color changed to ruby red color. It shows the formation of gold nanoparticles. The reaction mixture was further stirred for 15 minutes to complete and stabilize the reaction. Henceforth, (+) AuNPs were synthesized within 30 minutes. Finally, UV-visible spectra were recorded and absorbance maxima were observed at 535 nm (Fig. 2) which is the characteristics of AuNPs.
Finally, the reaction mixture was centrifuged and powder AuNPs was isolated for further characterization.

Synthesis of (+) AuNPs at pH 4 was opted because it was the optimum condition. We tried the synthesis of (+) AuNPs at pH 4, 7 and 9. But our observation was that the synthesis of (+) AuNPs at pH 4 provides better results than the synthesis of (+) AuNPs at pH 7 and 9 as it was evident from progress of the reaction i.e., change in color of the reaction mixture and UV-visible spectra.

2.5. Synthesis of Citrate Capped Gold Nanoparticles

Citrate capped AuNPs were prepared as reported earlier. Briefly, in a 500 mL two-neck round-bottom flask, 300 mL of a 0.01% solution of HAuCl₄ was brought to boiling under refluxing with stirring and then 10.5 mL of 1% trisodium citrate in water was added quickly. After the color change finished in the first 3–5 min, the mixture was kept boiling for another 20 min. The heating source was removed, and stirring was kept until the solution cooled to room temperature. The suspension was stored in the refrigerator at 4 °C until needed.

2.6. Reduction of Methyl Orange by NaBH₄ in Presence of (+) AuNPs

Three set of experiments were performed. For set A, 15 mL methyl orange solution (13 mg/lit), 2 mL of sodium borohydride (0.1 M) solution and 1 mL aqueous colloidal solution of as-synthesized (+) AuNPs were added. For set B, instead of (+) AuNPs, 1 mL of citrate capped AuNPs colloidal solution were added, whereas, in set C 15 mL methyl orange and 2 mL of sodium borohydride solutions were added only. For the uncatalyzed reaction (set C) 1 mL of double distilled water was added instead of AuNPs to have the same volume. The AuNPs catalyzed reactions were performed at 25 °C and absorption for each set was measured by a spectrophotometer at every 2 minutes. The reaction was followed by monitoring the time-dependent absorbance (A) by a UV-visible spectrophotometer at 464 nm and the fall in A with time was recorded.

3. RESULTS AND DISCUSSION

EAB is a well known biogenic system that provides electrons and protons by decomposing sodium acetate. Stainless steel is also known for reduction behavior through corrosion of its surface by Cl⁻ ions penetration which provide electrons. These generated electrons could be used for reduction of Au³⁺ ions. Taking the advantage of these two phenomena shown by EAB and stainless steel, we combined these two principles into one as an electrochemically active biofilm on stainless steel mesh. After successful formation of EAB on stainless steel mesh, we exploited it for the synthesis of gold nanoparticles. The above scheme (Fig. 1) shows the overall mechanism for the synthesis of (+) AuNPs by EAB.

UV-visible spectroscopic measurement of the dispersed colloidal aqueous solution was recorded (Fig. 2) and appearance of absorbance maxima at 535 nm was observed, which is typically ascribed to the surface plasmon band of spherical AuNPs. Similar UV-vis spectra for AuNPs have been already reported. According to Mie theory, metal nanoparticles like Au shows a surface plasmon band within the range of 500–550 nm. The absorbance spectra of as-synthesized (+) AuNPs solution was measured after 3 hours, one week and later after two months. Its spectra remained same, it shows that there is no any ageing effect or ostwald ripening of (+) AuNPs. It indicates that as-synthesized (+) AuNPs were very stable for long time even without any capping and stabilizing agent in the solution.

The phase of the as-synthesized (+) AuNPs were determined by X-ray diffraction with Cu Ka radiation. The X-ray diffraction pattern (Fig. 3) confirmed the formation of crystalline AuNPs. XRD pattern shows the five
prominent diffraction peaks in the 2θ range 30–90° for planes of polycrystalline gold. The peaks at 2θ values of 38°, 46.33°, 64.58°, 77.24° and 81.72° corresponds to the 111, 200, 220, 311 and 222 planes of face-centered cubic (fcc) gold, respectively (JCPDS powder diffraction file no. 4-0784). It indicates that the AuNPs were dominated by [111] facets. From the diffractogram, it is also clear that no any impurity peaks were detected which reveals that the as-synthesized (+) AuNPs were of high purity. The average of all the (+) AuNPs peaks and the full width at half maximum (fwhm) in the XRD pattern was used to calculate the average crystallite size by using the scherrer equation, it shows that the average size of the AuNPs were ~25 nm which is very close to the TEM values.

The morphology of the (+) AuNPs were determined by TEM at low magnification as shown in Figure 4(a). It illustrates that the size of as-synthesized (+) AuNPs are in 5–20 nm range, highly monodispersed and discrete. Figure 4(b) shows the representative HRTEM image of individual (+) AuNPs. It also shows lattice fringes which suggest that the (+) AuNPs are polycrystalline in nature. Selected-area electron diffraction (SAED) is shown in Figure 4(c) which clearly show well resolved lattice fringes and diffraction rings for face-centered cubic (fcc) gold (space group Fm3m) and are indicative of highly crystalline nature of (+) AuNPs. It further confirmed the characteristic crystal planes of (+) AuNPs which are concomitant with the XRD studies (Fig. 3).

The electron diffraction rings, which were indexed to...
the (111), (200), (220), (311) and (222) planes of fcc gold (JCPDS No. 4-0784) indicated the small size of the particles and the polycrystalline nature. To identify the element composition, an energy-dispersive X-ray (EDX) was employed with an accelerating voltage of 200 kV. Figure 4(d) shows typical EDX spectrum of as-synthesized (+) AuNPs. It shows strong and weak signals at about 23 kV, 10 kV, 12 kV and 14 kV respectively. It also show signals for carbon and copper which were from the carbon coated copper grid. This spectrum again confirmed the presence of element gold, hence the formation of (+) AuNPs.

The average size and hydrodynamic zeta potential of the (+) AuNPs in aqueous solution were measured by electrophoretic movement and dynamic light scattering (DLS) using a Delsa™ Nano zeta potential and submicron particle size analyzer respectively. Average size of (+) AuNPs was ≈ 25 nm, which is in the close agreement with the TEM. Zeta potential measurement of colloidal solution revealed a surface potential of +32.72 mV implying that (+) AuNPs are highly positively charged. Subsequently, the gold colloidal solution was centrifuged, washed and redispersed in DI water and zeta potential was measured. It was repeated three times. Following plot (zeta potential vs. pH Fig. 5) shows the decrease in the zeta potential after each washing and redispersing in DI water, which could be because of increase in pH. It suggest that the (+) AuNPs were surrounded by some ions, mainly positive ions (such as H\(^+\) and Na\(^+\)), which were removed during centrifuge and washing, which lead to the decrease in zeta potential.\(^{35}\) These H\(^+\) ions were generated \textit{in-situ} by the EAB as shown in the proposed mechanism.\(^{29,38,40}\)

At low pH (+) AuNPs were well dispersed and stable, but as the pH starts increasing, the (+) AuNPs were less dispersed and starts aggregating because of decrease in the concentration of positive ions (H\(^+\) and Na\(^+\)) or in other words, the charge on the (+) AuNPs. This suggests that high positive charge provides dispersion and stability to (+) AuNPs. It was observed that (+) AuNPs were stable in low pH i.e., in acidic condition and become unstable as pH increases i.e., neutral or basic condition.

From the above scheme, characterization techniques and discussion, it was concluded that, the (+) AuNPs were well synthesized, pure and crystalline in nature. It was also found that the as-synthesized (+) AuNPs were highly positively charged in comparison to the previous reports.\(^{2,3,32}\) In general, the nanoparticles acquired positive charge because of some capping.\(^{32}\) But in this case, the as-synthesized (+) AuNPs were without capping and have acquired high positive charge which may be because of the surrounding environment of (+) AuNPs which has plenty of positive ions (i.e., H\(^+\) and Na\(^+\)) from the oxidation of sodium acetate. It further reveals that EAB on stainless steel mesh could be a suitable tool to synthesize highly positively charged (+) AuNPs without any chemical contamination and capping agent.

Taking the advantage of the high positive charge (+32.72 mV) of the as-synthesized (+) AuNPs we performed a comparative study for the reduction of methyl orange by NaBH\(_4\) in presence of as-synthesized (+) AuNPs and citrate-capped AuNPs which are negatively charged and in the absence of AuNPs i.e., NaBH\(_4\) only. Figure 6 shows fall in absorbance (A) at 464 nm with time (min). This plot suggest that (+) AuNPs has catalyzed the methyl orange reduction much faster than citrate capped AuNPs and NaBH\(_4\) alone. The uncatalyzed reaction where AuNPs were not used shows very slow reaction because of large kinetic barrier, i.e., high activation energy. It was observed that it takes about 24 min for set A to completely decolorize the original color of the solution, 42 min for set B to decolorize about 80% of the original color of the solution, whereas, for set C, in 42 min, only 64% decolorization of the original color was observed.

The catalyzed reduction of methyl orange essentially follows Langmuir–Hinshelwood kinetics, which can be
simplified to apparent first-order kinetics at low initial dye concentrations.\textsuperscript{46,47} The catalytic activity for all the samples was quantitatively evaluated by calculating the respective first-order rate constants ($k$) according to the following equation:

$$\ln \left( \frac{C_0}{C} \right) = kt$$  \hspace{1cm} (1)

where $c_0$ and $C$ are the initial concentration and the reaction concentration of methyl orange at time $t$ respectively.\textsuperscript{46,47} Rate constants ($k$) was calculated from Figure 7 which shows that $k_{M.O.-AuNPs} = 2.245 \times 10^{-3}$ sec$^{-1}$, $k_{M.O.-cit.AuNPs} = 1.063 \times 10^{-3}$ sec$^{-1}$ and $k_{NaBH_4} = 4.783 \times 10^{-4}$ sec$^{-1}$. Rate shown by (+) AuNPs is about 2.2 times higher than that of citrate capped AuNPs and 4.7 times higher than the NaBH$_4$. The higher the rate constant value, the faster the reduction of methyl orange and thus better the catalytic activity of the catalyst. The reduction of methyl orange by NaBH$_4$ in absence of catalyst is thermodynamically favorable, but it is kinetically unfavorable. As it was observed that (+) AuNPs provides an alternative path for the reaction by decreasing the activation energy, hence reducing the kinetic barrier, thus making it thermodynamically and kinetically favorable. It was observed that in presence of NaBH$_4$, (+) AuNPs shows much faster reduction of methyl orange than negatively charged citrate capped AuNPs as well as NaBH$_4$ alone. It is because of the electron relay effect shown by the (+) AuNPs.\textsuperscript{36,37} It was also observed that the rate of reduction of methyl orange by NaBH$_4$ in presence (+) AuNPs was much faster in comparison to the previous report.\textsuperscript{37} The possible reason of this may be the high positive charge acquired by as-synthesized (+) AuNPs.

4. CONCLUSIONS

EAB on stainless steel mesh was used as a reducing tool to synthesize positively charged gold nanoparticles without using any capping or stabilizing agent. It provides (+) AuNPs which were stable and free from any chemical contamination. The synthesis of (+) AuNPs by EAB was novel, easy, fast, controlled, cost effective and environment friendly. During methyl orange reduction, we found that (+) AuNPs acts as a much better catalyst as compared to citrate-capped AuNPs which were negatively charged. Sodium borohydride being such a strong reducing agent was not able to reduce methyl orange completely in absence of (+) AuNPs, indicates the catalytic ability of (+) AuNPs. Further investigations of (+) AuNPs are under progress in our laboratory. Our outcomes open up wide variety of new prospective applications for (+) AuNPs in particular and other metal nanoparticles in general, in biotechnology, environmental chemistry and medical sciences. EAB on stainless steel mesh could be used to synthesize other metal nanoparticles.

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References and Notes

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