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Full Length Article

Rapid colorimetric and spectroscopy based sensing of heavy metal and cellular free oxygen radical by surface functionalized silver nanoparticles

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ABSTRACT

Here, starch functionalized silver nanoparticles ($AgNP_{ST}$) with a size range of 2–10 nm were synthesized using starch as a reducing as well as surface capping agent. Nanoparticles were characterized for shape, hydrodynamic size, and stability by standard techniques such as electron microscopy, dynamic light scattering as well as zeta potential analysis. Nanoparticles exhibited localized surface plasmon resonance at 402 nm and fluorescence peaks in the range of 400–680 nm, when excited at 350 nm. We demonstrated that when $AgNP_{ST}$ interacted with six different heavy metals (Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+}, and As^{5+}), it exhibited different extent of surface plsamon resonance shift. However, Hg^{2+} showed the maximum SPR shift as well as concentration dependent visible yellow color in a concentration range of 1–10 ppm. The analysis of reactive oxygen species (ROS) by fluorescence, localized surface plasmon resonance, and colorimetric assay revealed that $AgNP_{ST}$ were able to detect and quantify cellular (*E. coli*) ROS as well as *in vitro* H_2O_2. Our results, thus evidenced that $AgNP_{ST}$ might be used as a multifunctional sensor to detect toxic metal such as Hg^{2+} and free oxygen radical in biological milieu as well as H_2O_2 *in vitro* condition.

1. Introduction

Free radical generation in biological system has been a great concern for maintaining cellular homeostasis. Both inorganic and organic molecular contamination perturbs various metabolic processes via causing oxidative stress [1–3]. Organic molecules or their metabolic intermediates [1,4], and heavy metals such as Hg, As, Pb, Zn [5–7] when uptake by biological systems, generate reactive species of O_2^-, NO and H_2O_2 [8,9], which are responsible for free radical mediated stress in biological systems. Even trace of heavy metals such as Hg, As, Pb is toxic and carcinogenic [10,11]. Moreover, bio-accumulation and non-biodegradable nature of heavy metals pose a severe threat to the ecosystem as well as human health. Similarly, organic molecules and their metabolic intermediates also cause cellular toxicity via generation of oxidative stress in biological system [1]. Therefore, it is essential to detect/measure these contaminations such as heavy metals in water, and reactive oxygen species (ROS) in biological fluids at a rapid and cheap mode.

Conventional techniques for heavy metal measurement include atomic absorption spectroscopy, inductively coupled plasma/mass spectrometry (ICPS), and plasma/atomic emission spectrometry (ICPES), UV–Vis spectroscopy, etc. [12,13]. Although, these techniques are highly specific but require tedious sample processing, analysis and highly skilled professional [14,15]. Moreover, non-portability of these instruments is also an issue. In contrast, sensors have great potential due to its portability, sensitivity and specificity in assistance of lab on chip (LOC) technology [16]. On the other hand, fluorescence molecule and ROS interaction based probes such as most commonly used dyes dihydroethidium (DHE) [17] and 2′,7′-dichlorodihydrofluorescein (DCF) [18] are available for ROS quantification. H_2O_2 specific boronate deprotection probe [19], Nitric oxide (NO) specific diamine linked probe [20] and fluorescent protein tagged probe [21] are also available for specific molecules detection. However, these probes are costly, photo sensitive, and lack robustness. To avoid any health hazard, it is required to develop multifunctional sensor with high sensitivity for on-site measurement and thus, it has become essential to develop on field and laboratory scale multimode sensor for the detection and quantification of heavy metal contamination and oxidative stress.

Plasmonic and photoluminescence based nanostructures showed great hope and opportunity. The synthesized nanoparticles can be used in heavy metal and toxic chemical sensing and removal [22]. Gold nanoparticles were used for various heavy metals sensing by colorimetric and SPR based methods [23–25]. Silver nanoparticles (AgNP) were recently demonstrated application in heavy metal, organic

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molecules, H_2O_2 sensing [26–28] and showed a property of broad spectrum antimicrobial agent [29]. Moreover, optical properties of AgNP found application in SPR based sensor [30–32], drug delivery [33], biomedical devices [34], and consumer products [35]. Such properties can be manipulated by varying size, shape, as well as surface capping agents [36–40]. Moreover, it is highly desirable to use non-toxic materials in these manipulations. Infact, the synthesis of AgNP by green/semi-green method and using cheap and abundant biomolecules have been a prime attention of many groups. The use of abundant carbohydrate molecules such as starch has been utilized by few groups for metal nanoparticle synthesis. Starch can be modified by high pressure heating, alkali treatment [41] to generate reducing agent. These modifications make starch as a reducing as well as capping agent and can be used for silver nanoparticle synthesis [42,43].

Here, we synthesized silver nanoparticles (AgNP) by controlled modification of starch using sodium hydroxide (NaOH) of 6 mM with heating. Starch was used as capping as well as reducing agent and NaOH was used as a catalyst for the reaction. We used silver nitrate (0.5 mM), NaOH (6 mM), starch (1% w/v) as a starting materials and reaction temperature by boiling the solution. Further, the synthesized starch capped silver nanoparticles ($AgNP_{ST}$) was characterized using standard techniques such as UV–Vis, XDR (X-ray diffraction), electron microscopy, zeta potential, and dynamic light scattering (DLS) analysis. Moreover, the heavy metal (Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+}, and As^{5+}) and reactive oxygen species (ROS) sensing study *in-vitro* was performed with H_2O_2 and *E. coli* cellular extract. For the study, colorimetric, localized surface plasmon resonance (LSPR), and fluorescence were measured and analyzed.

2. Materials and methods

2.1. Materials

Analytical grade chemicals were used without purification. $AgNO_3$, NaOH, and pure starch were purchased from Himedia pvt. Ltd., Mumbai, India. All heavy metal compounds such as $Pb(CH_3COO)_2{\cdot}3H_2O$, $HgSO_4$, $Na_2HAsO_4{\cdot}7H_2O$, $CuSO_4$, $Ni(CH_3COO)_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$ were purchased from Merck pvt. Ltd. Glassware was cleaned using aqua regia and rinsed with Milli-Q water.

2.2. Synthesis of AgNPs by chemical reduction method using starch

Silver nanoparticle ($AgNP_{ST}$) was synthesized by using $AgNO_3$, starch and sodium hydroxide (NaOH) as starting materials. The reaction was optimized using different concentration of starting materials and temperature. $AgNP_{ST}$ used in our sensing study was synthesized by boiling of 0.5 mM of $AgNO_3$, and 1% (w/v) starch. In boiling solution, 6 mM of NaOH was added. The solution turns transparent to brown and finally to yellow color during 30 min of reaction.

2.3. UV–VIS spectroscopic analysis

The surface plasmon resonance of silver nanoparticle (AgNP) was analyzed by UV–Vis spectrophotometer (λ-35, Perkin Elmer Pvt. Ltd.). The samples were diluted in Milli-Q water (18 mΩ) and were scanned in the range of 200–700 nm.

2.4. Analyzing the hydrodynamic size and stability of AgNP

The hydrodynamic size with respect to Mean number percent and zeta potential of nanoparticles were analyzed using zeta sizer (Nano-ZS, Malvern pvt. Ltd.). For analysis, transparent samples were prepared in Milli-Q water and DLS analysis was performed using laser light (λ is 633 nm), at 90° scattering. For data analysis, DTS 7.0 software provided by Malvern was used.

2.5. X-ray diffraction analysis

The $AgNP_{ST}$ sample was deposited on glass slide and dried in vacuum. The samples were analyzed in the range of 25–90° using XRD (ULTIMA-IV, Rigaku, Japan), with 2θ/min scanning speed. During data analysis, base line correction was performed using glass slide scanned in working range. Further, data were smoothen using Savitzky-golay method and peaks were fitted using Gauss fitting and crystallite size of nanoparticle calculated using Scherre equation given below:

$$D = \frac{0.94\lambda}{\beta(1/2Cos\theta)} \quad (1)$$

Here, λ is wavelength of X-ray (1.54 A), β is line broadening, θ is Bragg's angle

2.6. Electron microscopy imaging

For scanning electron microscopy (FE-SEM, JSM- 7600F, Jeol) imaging, the samples were diluted and deposited on silicon wafer and dried in vacuum drier. The dried samples were gold coated for 30 s and observed for shape and size. Further, AgNP sample was deposited on copper grid (300 mesh) by drop casting method and dried sample was observed using TEM (Jeol, JEM-2100F).

2.7. Fluorescence analysis

All the fluorescence experiments were analyzed using a Cary eclipse, Agilent made spectrofluorimeter. For fluorescence analysis, $AgNP_{ST}$ samples were excited at 350 nm at an excitation and emission slit width of 10 and 20 nm, respectively. The emission spectra were recorded in the range of 400–700 nm.

2.8. Sensing of heavy metals and H_2O_2

To analyze the heavy metal sensing potential of $AgNP_{ST}$, stock solution (200 ppm) of six different sulfate and nitrate of heavy metals (Pb, Hg, As, Cu, Ni, and Zn) were used. The experiment was performed using different concentration of heavy metals (1–50 ppm) with 100 μM of $AgNP_{ST}$. The samples were incubated for 10 min at room temperature. *In vitro* detection of reactive oxygen species (ROS) was performed using different concentration of H_2O_2. The sensing was also performed using different concentration (1–10% v/v) of H_2O_2. To detect the heavy metals and H_2O_2, the gradient of color change, and SPR shift were monitored. Moreover, different concentration of $AgNP_{ST}$ was also used for optimization of the sensing potential.

2.9. Sensing of oxidative stress in E. coli

E. coli (DH5α) culture was incubated in Luria-Bertani (LB) broth media and incubated at 37 °C and 120 rpm for 6 h. Brennan and Schiest earlier showed that administration of aniline in yeast culture caused production of reactive oxygen species (ROS) [1]. Hence, we administered different concentrations (0, 5, 10, 20 v/v) of aniline in *E. coli* (DH5α) during exponential phase (after 6 h of growth) and incubated the culture for 12 h. The *E. coli* culture was lysed by 5 min of sonication. The culture was centrifuged at 7000 rpm for 10 min, supernatant was used for the estimation of free radical using $AgNP_{ST}$, and data were compared using standard ROS quantification dye, H_2DCFDA. Testing samples (with $AgNP_{ST}$) were excited at 350 nm and emissions were recorded in a range of 400–680 nm when excitation and emission slit width was fixed at 10 and 20 nm, respectively. Samples with H_2DCFDA were excited at 495 nm and emission was measured between 500 and 600 nm. The excitation and emission slit width were fixed at 5 and 10 nm, respectively. The data were analyzed after normalized the curve. Further, we have also analyzed the surface plasmon resonance shift of $AgNP_{ST}$ due to interaction with free radicals using cell extract in