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Size-Separation of Silver Nanoparticles Using Sucrose Gradient Centrifugation

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Abstract

Size and shape distributions of nanoparticles can drastically contribute to the overall properties of nanoparticles, thereby influencing their interaction with different chemotherapeutic molecules, biological organisms and or materials and cell types. Therefore, to exploit the proper use of nanoparticles for various biomedical and biosensor applications, it is important to obtain well-separated monodispersed nanoparticles. However, gaining precise control over the morphological characteristics of nanoparticles during their synthesis is often a challenging task. Consequently, post-synthesis separation of nanoparticles is necessary. In the present study, demonstration on the successful one-pot post-synthesis separation of anisotropic silver nanoparticles to near monodispersities using sucrose density gradient sedimentation. The separation of the nanoparticles was evidenced based on optical confirmation, and spectrophotometric and transmission electron microscopy measurements. Our results clearly demonstrate the facile separation of anisotropic silver nanoparticles using sucrose density gradient sedimentation and can enable the use of nanoparticles for various labeling, detection and biomedical applications.

Keywords: Anisotropic; Density gradient; Fractionation; Sucrose; Separation; Nanoparticles

Introduction

Nanoparticles (NPs) possess unique physico-chemical characteristics, which are dependent on their size and shape distributions [1-5]. Consequently, selection of morphological and structural properties of NPs is critical for their applications in drug and gene delivery, sensors, solar systems, storage and photovoltaic devices etc. [6-10]. Therefore, obtaining monodispersed NPs, in order to better define and exploit their characteristic properties is imperative [11,12]. Even though tremendous efforts are directed towards optimizing synthesis strategies, typical, as-synthesized suspensions still tend to include polydispersed sizes and shapes [5,13]. With an aim towards achieving monodispersed nanoparticles, significant efforts are focused on the post-synthesis separation of nanoparticles [5,13,14]. Various separation techniques including chromatography, magnetic separation, electrophoresis, selective precipitation, membrane filtration, solvent or solution-based extractions and density gradient centrifugation have been used for isolating nanoparticles [5,13-16]. Density-based centrifugation is considered as a particularly convenient method as it is economic, non-laborious, and does not involve the use of liquid-solid phase interactions and/or hazardous chemical reactions. This technique makes use of the sedimentation coefficients of nanoparticles in the surrounding medium, which can vary with nanoparticles form and mass [5], and has been successfully implemented to separate a wide range of nanoparticle using density gradient centrifugation rate [5,13]. For example, the separation of various sizes of FeCo@C nanocomposites to defined size distributions was demonstrated using iodixanol gradient solution and by varying step-gradient density parameters and centrifugation duration [14]. Likewise, dimers and trimers of gold nanoparticles were isolated using high-density cesium chloride ($CsCl_2$) suspensions [5,10]. Similarly, rapid separation of metal and quantum dot (CdSe) nanoparticles was demonstrated using non-hydroxylic organic as a density gradient [5].

Silver nanoparticles are widely used as antibacterial agents against diverse microorganisms due to their potent anti-microbial properties [17-20]. Additionally, they have been exploited in biomedical applications for treating burns and wounds, in dental materials, as coatings for stainless steel and textiles, for water treatment, and sunscreen lotions [20]. AgNPs can possess low cytotoxicity, high thermal stability and low volatility [20]. Therefore, uniform monodispersed silver nanoparticles are highly desirable, especially for meeting the needs of these various applications [11,20]. Facile separation of mixed shapes (rods, spheres, triangles pyramids, stars and pentagons) of gold nanoparticles, synthesized using fungus, to discrete shapes using sucrose density gradient using a tabletop centrifuge was demonstrated [7]. Using similar technique, the separation of gold and silver nanoparticles synthesized using a plant extract was also demonstrated recently [21].

The present investigation demonstrates the separation of anisotropic AgNPs using a simple one-step sucrose density gradient sedimentation. This separation technique is commonly used for isolating cellular organelles and viruses by high-speed ultracentrifugation. Our results permitted the separation of highly dense silver nanoparticles at low speed (4000 g) using table top-centrifugation. The sucrose density gradient method has been previously used to separate polymeric nanoparticles [7]. The ease and facile nature of the adopted methodology offers new insights for sustainable separation and can be extended to separate other forms of biologically relevant metallic nanoparticles.

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Materials and Methods

The bacterium *Pseudomonas* sp. strain GM102 used to produce anisotropic AgNPs was isolated from surface sterilized root tissue collected from native *Populus deltoids* [21]. All other chemicals and reagents were from standard commercial sources and of the highest quality available.

Preparation of density gradient sucrose suspension

A discontinuous gradient of sucrose was prepared by successive layering of dilute sucrose solution upon one another as described earlier with slight modifications [22]. Briefly, 5 mL each of 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5% and 20% sucrose were layered on top of each other in a 40 mL polystyrene centrifuge tube. The tube was marked in increments of single percentage points within each region.

Synthesis and fractionation of anisotropic silver nanoparticles

For the synthesis of anisotropic AgNPs, a single *Pseudomonas* sp. bacterial colony from a 12 h cultured R2A agar Petri dish served as inoculum for 100 mL of R2A broth in a 500 mL Erlenmeyer flask, followed by incubation at 25°C on a shaker (200 rpm) for 24 h. The bacteria were collected by centrifugation (5000 × g, 25°C, 30 min) and washed with sterile distilled water under sterile conditions. In a 500 mL Erlenmeyer flask, ~3–5 g wet bacterial biomass was suspended in 100 mL of 1 mM $AgNO_3$ solution and incubated at 25°C under shaking (200 rpm). Synthesis of AgNPs was monitored by UV-vis spectra (200-700 nm). After completion of the reaction (48 h), the reaction mixture was centrifuged (5000 × g, 30 min) to remove the bacteria, filtered using a sterile 0.1 µm syringe filter and the AgNPs were collected by performing ultra-centrifugation (100,000 × g, 1 h). After washing twice with Milli Q water the anisotropic AgNPs were used for further characterization and separation.

Separation was accomplished by forming a discontinuous gradient of sucrose by successive layering of dilute sucrose solution upon one another as described above. Then, 5 mL of the above-prepared anisotropic AgNPs suspension was loaded on top of the sucrose gradient and centrifuged at 4000 g for 1 h using a tabletop centrifuge (Spinchtron™ R, Beckman Coulter, CA). Fractions of 2 mL were carefully collected using a micropipettor and further purified by dialysis using a molecular weight cut-off of 1000 Da (Spectrum Laboratory Inc.) against Milli Q water. These samples were concentrated to half of their original volumes using a Speed Vac SC 100 (Savant Instruments Inc., NY) and used for further analysis.

Physical Characterization of Nanoparticles

UV-vis absorbance was recorded on a CARY 100 Bio spectrophotometer (Varian Instruments, CA) operated at a resolution of 1 nm. Fourier transform infrared (FTIR) analysis of the samples deposited on a ZnSe window was performed on a Nicolet Magna-IR 760 spectrophotometer at a resolution of 4 cm^{-1}. X-ray diffraction (XRD) of the dried AgNPs powder was performed on a Discover D8 X-ray diffractometer with a Xe/Ar gas–filled Hi-Star area detector and an XYZ platform, operated at 40 kV and at a current of 40 mA. Transmission electron microscopy (TEM) measurements for the NPs samples prepared on carbon-coated copper grids were performed on a Hitachi HD-2000 STEM operated at an accelerating voltage of 200 kV. For Atomic Force Microscopy (AFM), nanoparticle samples were imaged in either contact mode or intermittent contact mode with a PicoPlus AFM (Aligent Technologies, Tempe, AZ) using a 100 µm scanning head at 128–512 pixels per line scan and a scan speed of 0.5 line/s. The cantilevers used were Veeco silicon nitride probes (MLCT-AUHW, Veeco, Santa Barbara, CA).

Results and Discussion

Anisotropic AgNPs with a wide range of size and shape distributions were synthesized using a previously reported bacterial-based biosynthesis method [20,23]. This technique results in a mixture of diverse particle sizes and shape distributions and illustrates the diverse NP mixtures that can result from biological and chemical based synthesis methods [11,20-24]. Therefore this material was used to illustrate the size/shape separations that can be accomplished using simple sucrose density gradient sedimentation. Figure 1 shows transmission electron microscopy and atomic force microscopy measurements of the as-prepared parent AgNP suspension; the diversity of AgNP sizes and shapes can be seen in TEM images of the particles taken from different areas of the grid and at increasing magnifications. These images reveal a heterogeneous distribution of AgNPs sizes and shapes. The particles included polydispersed spherical (55%), polygonal and hexagonal (24%), parallelograms (8%) and triangular (13%) shaped nanoparticles (Figure 1 and Supplementary Figure 2). A particle size histogram plot, obtained by counting ~100 particles from the TEM images, showed that AgNPs varied in size and ranged from ~2 to 70 nm (Figure 1B and Supplementary Figure 1). The presence of polydispersed AgNPs was further confirmed based on AFM imaging, which revealed well-separated heterogeneous nanoparticles with a particle height ranging from ~2 to 70 nm (Figure 1C and 1D), which correlates well to the size and shape distributions observed using TEM (Figure 1A).

The anisotropic nature of the AgNPs was also evident from UV-vis spectroscopy measurements where a broad surface plasmon resonance (SPR) peak (315-600 nm) was observed, confirming the polydisperse nature of the AgNPs. Also, based on the intensity of the transverse SPR peak, it can be inferred that the majority of the particles are spherical (Figure 2A).

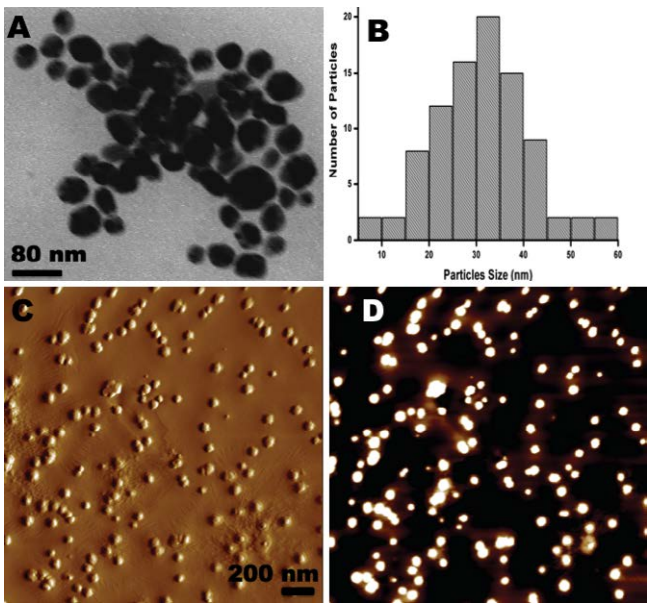

Figure 1: Transmission electron microscopy (A), and atomic force deflective (C) and topographical (D) images of the as-prepared parent silver nanoparticles suspension showing the anisotropic size and shape distributions of nanoparticles. (B) Surface height measurements obtained from the TEM image by counting ~100 particles.

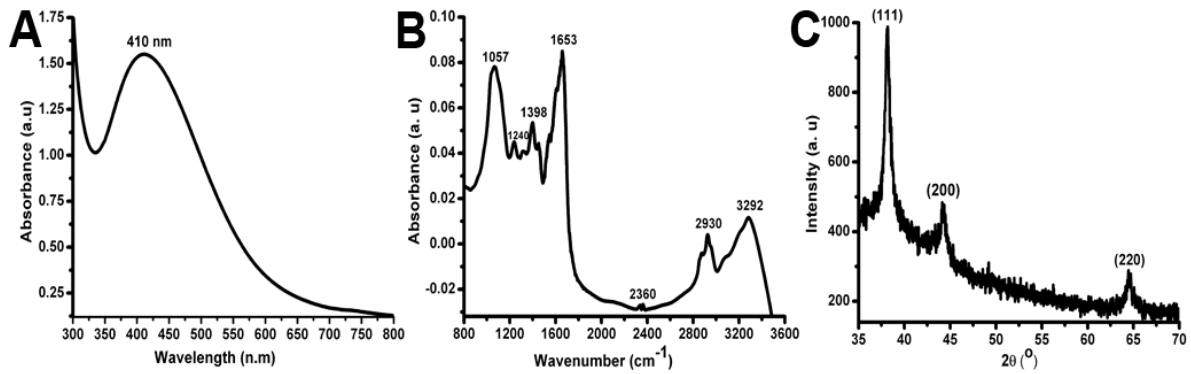

Figure 2: UV-Vis, FTIR and XRD measurements of the as-prepared parent silver nanoparticle suspension. (A) UV-Vis spectrum of the as-prepared parent silver nanoparticles showing a broad peak indicating the presence of anisotropic silver nanoparticles. (B) Fourier transform spectra of the as-prepared silver nanoparticles, significant vibration bands are labeled. (C) XRD analysis of the as-prepared silver nanoparticle powder.

To understand the stabilizing agent that might be associated with the AgNPs [20], and which might influence the separation of the particles, FTIR spectroscopy was performed. FTIR spectra revealed bands at 1057, 1240, 1398, 1653, 2360 and 2930 along with an intense, broad band at 3292 cm^{-1} (Figure 2B). The bands at 1057 and 1653 cm^{-1} can be attributed to the–N-H and carbonyl (-C-O-C- or –C-O-) stretch vibrations in the primary and secondary amide linkages. A peak for tertiary amide was also observed at 1398 cm^{-1}. Collectively these peaks are consistent with the presence of protein or peptide on the NP surfaces those likely acts as a capping molecule [11,25].

The crystalline nature of the AgNPs was evaluated by performing XRD analysis. Intense peaks at 111, 200 and 220 are observed, confirming the crystalline nature of the AgNPs (Figure 2C). These peaks are due to the line broadening of Bragg reflections in the 2θ range of 35°–70° at 38.1, 44.8 and 64.9 respectively based on the face-centered cubic structure of silver and agree with reported values [20].

Size fractionation of the AgNPs could be achieved by creating a discontinuous density gradient of 2.5% to 20% sucrose by layering successively dilute sucrose solutions upon one another, and centrifugation at 4000 g for 1 h (Figure 3). It was observed that the nanoparticles, initially brown and at the top of the sucrose gradient, form a gradient across the length of the centrifuge tube upon centrifugation (Figure 3B), indicating a density-based separation.

The purity and separation of individual fractions collected from the gradient was confirmed by UV–vis spectroscopy (200-900 nm) and TEM measurements. The fractions that showed prominent peak shifts; hence forth designated as fractions a, b, c, d, e and f, occurring at density gradient points of 2.5%, 4.8%, 5.3%, 7.1%, 10.4% and 16.2% respectively, were subjected to further characterization. As seen in the UV-vis spectra of these fractions, blue shift for the fractions a and b, and red shift for the fractions d, e and f in the surface plasmon peak was observed when compared to that of the as-prepared parent suspension (Figures 2A and 4). The absorbance of the isolated sample fractions displayed peaks at 403 nm, 405 nm, 410 nm, 416 nm, 420 nm and 425 nm, respectively for the fractions a, b, c, d, e and f (Figure 4). The differences in the observed SPR peaks show differences in NP size distribution [26]. The size-dependent absorbance of light by metal nanoparticles is a well-known phenomenon that occurs due to the coherent oscillations of the conductance band electrons generated by interaction with the electromagnetic field [27].

Transmission electron microscopy confirmed distinct morphological characteristics for the various fractions. Fraction a, concentrated in the 2.5% gradient, consisted of the smallest spherical shaped particles of 4.5 ± 0.5 nm (Figure 5A); fractions b and c, separated in the 4.8% and 5.3% gradient points, respectively, included monodispersed spheres of 15 ± 3 nm (Figure 5B and 5C); fraction d, contained spheres and triangles of 28 ± 4 nm and eluted at the 7.1% gradient point (Figure 5D); fraction e, included pentagonal and hexagonal shaped particles of 45 ± 5 nm and partitioned at the 10.4% gradient point (Figure 5E); whereas the largest spheres and largest triangles 60 ± 10 nm and above, were collected in fraction f, at the 17.3% gradient point (Figure 5F).

Overall, our results demonstrate the utility of density gradient techniques to separate mixtures of different size and shape distributions of nanoparticles to near monodispersities, illustrating the separation of AgNPs using sucrose density gradient sedimentation. The separation of gold nanoparticles has been previously reported [7]. However, in that study, the authors used a gradient of sucrose with higher percentages 30-70%, compared to the 2-20% gradient used here. These differences may be the result of differences in the average sizes of the nanoparticles being separated as well as the core material of the nanoparticle. Nanoparticle density may depend on nanoparticle type, and consequently require different percentages of sucrose gradients for their separation [28]. Alternatively, hydrodynamic characteristics of the nanoparticle may contribute to their separation in density gradients. Ongoing experiments to further characterize the bases for nanoparticle separation in density gradients and assess if this simple technique can be extended to separate, a mixture of diverse types of nanoparticles is under investigation.

Conclusions

In summary, sucrose density gradient have been successfully used as a separation method to fractionate anisotropic AgNPs containing particles of different sedimentation rates. Compared to the existing conventional methods our described sucrose density based separation methodology is scalable, economic, avoids the use of corrosive and toxic organic gradient solutions, and can be achieved with commonly accessible laboratory equipment, a tabletop centrifuge. The ease and facile nature of the adopted methodology offers new insights for sustainable separations, and can be extended to other types of metallic nanoparticles.

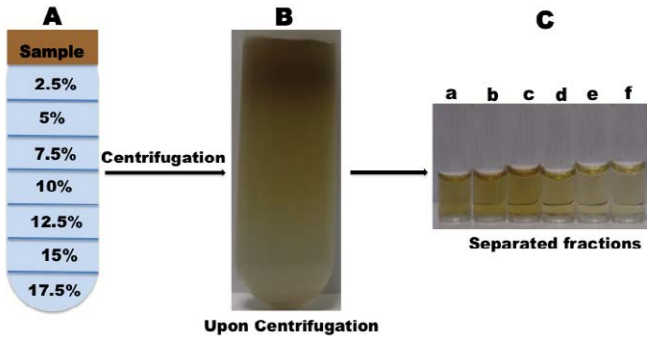

Figure 3: Optical images showing the sucrose density gradient separation of anisotropic silver nanoparticles. Images of the as-prepared parent silver nanoparticle mixture (left); anisotropic silver nanoparticles in sucrose gradient after fractionation (center); and the color images (right) of the individual fractions upon successful size fractionation.

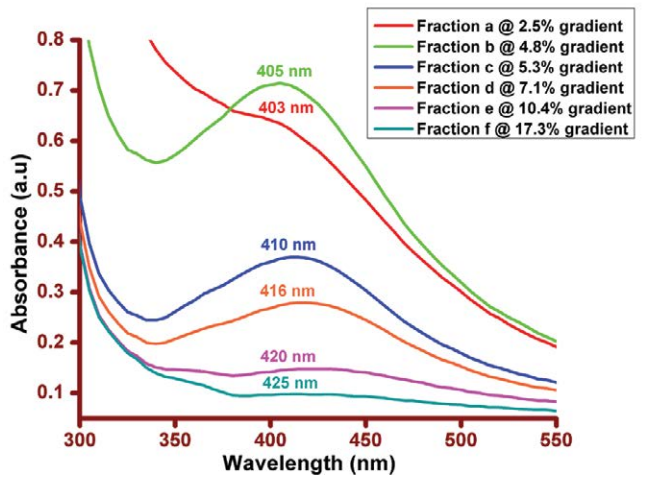

Figure 4: UV-Vis spectroscopy measurements of the various pooled fractions a, b, c, d, e and f sedimented at sucrose gradients of 2.5%, 4.8%, 5.3%, 7.1%, 10.4% and 17.3% respectively. The individual samples and their surface plasmon peak are labeled.

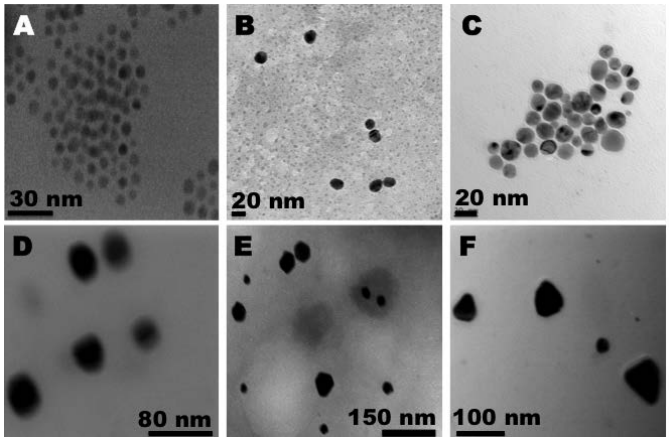

Figure 5: Transmission electron microscopy images of the various pooled fractions; A, B, C, D, E and F upon sedimentation using sucrose density gradient at 2.5%, 4.8%, 5.3%, 7.1%, 10.4% and 17.3% respectively, following separation of the anisotropic silver nanoparticles.

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