Positively Charged Ag-dendron Conjugates: Stability Enhanced AgNPs for Biomedical Applications

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ABSTRACT

We developed positively charged silver nanoparticles ([Ag-Ds]$^+$), nominally 20 nm in diameter, using dendron chemistry combined with reduction of silver nitrate in the presence of sodium borohydride. The conjugate was developed within the context of potential biomedical applications. Rational design was applied to yield a dendron capping agent that enhances surface anchoring, hydrophilicity, and cationic surface charge. The colloidal stability and physico-chemical properties of the conjugates were evaluated under physiologically relevant conditions using dynamic light scattering, zeta potential, UV-vis absorbance, and transmission electron microscopy. Properties evaluated include size, size distribution, shape and uniformity, positive surface charge, and surface plasmon response. Colloidal stability was extensively investigated with respect to shelf-life over 6 months, temperature variation, pH, and interaction with proteins in cell culture media. Overall, the investigation confirmed the successful development of a stable positive complex with remarkable stability in biologically relevant test media containing proteins and electrolytes, and with a shelf-life exceeding 6 months. The excellent aqueous stability for this conjugate enhances its potential use as a test material for investigating interactions between positively charged NPs and bio-entities, as an antibacterial agent or a vehicle for drug delivery.

Keywords: silver nanoparticles, dendrons, positively charged, characterization, stability

1 INTRODUCTION

Among the many engineered nanomaterials, silver nanoparticles (AgNPs) are most widely utilized in commercial products [1 - 4] due to their (1) antimicrobial activities (*silver*) and (2) unique physico-chemical properties (*nano-scale structure*). In the past decades, preparation methods for AgNPs have been developed [2, 3] using different approaches to acquire successful (ideal) nanoparticles exhibiting properties such as good dispersion, uniformity, and stability (free from agglomeration or aggregation, etc.) for their biological applications. Herein, we describe the development of dendron-stabilized silver nanoparticles by modification of gold-dendron conjugate synthesis used in our previous study [5] to yield enhanced colloidal stability. Moreover, by introducing a trimethyl ammonium end group on the dendron structure, the resulting silver-dendron conjugates (hereafter abbreviated [Ag-Ds]$^+$) possess positive charges on the surface. In addition to general antimicrobial activity of AgNPs, the cationic AgNPs could potentially provide additional biological activities such as cellular uptake [6] or transfection efficiency [7] (like positively charged gold nanoparticles) that are induced by electrostatic interaction with negatively charged cell surfaces, however, there are only a few reported materials [8, 9] for this purpose. The [Ag-Ds]$^+$ developed in this report were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), UV-Vis absorbance, and inductively coupled plasma mass spectrometry (ICP-MS) for investigation of size distribution, shape uniformity, surface plasmon resonance (SPR) behavior, and for quantifying nanoparticles mass, respectively. Also, very importantly, we systematically examined the colloidal stability of the [Ag-Ds]$^+$, including long-term shelf life, in physiological media (e.g., phosphate buffered saline (PBS), and Dulbecco's modified Eagle's medium (DMEM)), as a function of pH and temperature. Additionally, we evaluated ion release rates for Ag^+ ions from the [Ag-Ds]$^+$, and other conjugates including citrate- and polyvinylpyrrolidone (PVP) coated AgNPs by ICP-MS, to determine potential relative antibacterial activity of the [Ag-Ds]$^+$ by comparison to known AgNPs.

2 EXPERIMENTS

2.1 Materials and Instruments[1]

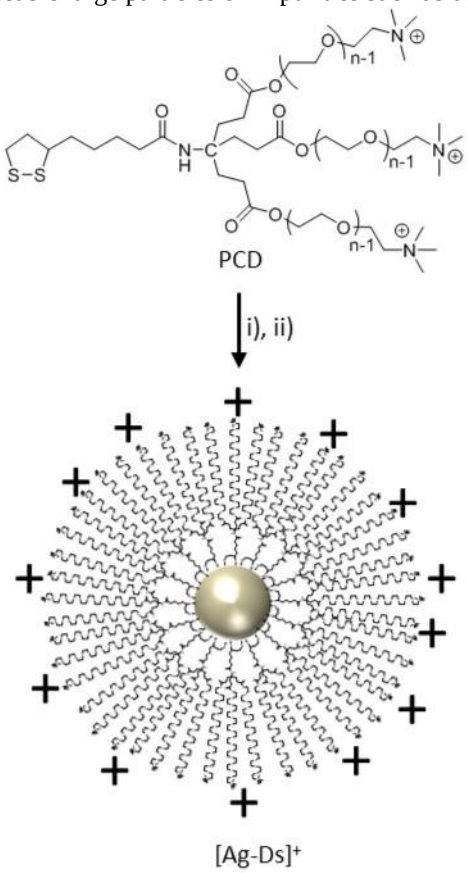
AgNPs (nominal 20 nm) were purchased from Ted Pella, Inc. (Redding, CA). Reference Material (RM) 8017 (PVP coated AgNPs nominally 75 nm) was obtained from NIST. [11] Other specific reagents used in this study are identified in the reference [5]. All chemicals were used without further purification. A quadrupole ICP-MS (X

[1] The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

series[II], ThermoFisher Scientific, Waltham, MA, USE) with a PFA-ST nebulizer (Elemental Scientific, Omaha, NE, USA) and an impact bead spray chamber was used for single particle analysis. The instrument was tuned daily for maximum ^{115}In sensitivity and minimum $^{156}CeO/^{140}Ce$ oxide level. The sample uptake rate was measured every day in triplicate by weighing a vial containing DI water before and after 5 min of aspiration, and was relatively constant at (0.18 to 0.19) mL/min. Details regarding other instruments (DLS, UV-Vis, and TEM) and methodology are also provided in reference [5]. The uncertainty of size and zeta potential represent the mean and one standard deviation of at least three measurements under repeatability conditions.

2.2 Preparation of $[Ag\text{-}Ds]^+$

To an aqueous solution of $AgNO_3$ (10 mL, 2.5 mmol/L, 99.9 %, Aldrich), 1 mL of aqueous positively charged dendron (PCD, 2.5 mmol/L) and 1 mL of freshly prepared $NaBH_4$ (50 mmol/L in H_2O) were added sequentially at room temperature. The color of the reaction mixture changed from pale yellow to brown immediately after the addition was completed. After stirring for 2 h, the crude colloidal silver solution (reddish brown) was dialyzed against DI water (MWCO = 10kD, cellulose ester membrane) for 2 d and passed thru a 0.1 µm filter to remove any traceable large particles or impurities such as dust.



Scheme 1. Synthesis of positively charged dendron stabilized AgNP ($[Au\text{-}Ds]^+$); i) AgNOs, ii) $NaBH_4$, r.t., 2 h

3 RESULTS AND DISCUSSIONS

3.1 Synthesis of $[Ag\text{-}Ds]^+$

As shown in scheme 1, the designed 1→3 branched cationic dendron (PCD) [5] is composed of a thioctic acid moiety, polyethylene glycol (PEG, $M_r \approx 600$) chains, and quaternary ammonium terminal groups to provide reactivity, hydrophilicity/aqueous stability and pH-independent cationic sites. The $[Ag\text{-}Ds]^+$ were prepared from $AgNO_3$ with PCD in the presence of sodium borohydride ($NaBH_4$) as a reducing agent (Scheme 1) to yield a translucent solution with a reddish brown color. This product was purified by dialysis against deionized water.

3.2 Characterization of $[Ag\text{-}Ds]^+$

The physico-chemical properties of $[Ag\text{-}Ds]^+$ were determined by a combination of complementary and orthogonal measurement techniques including DLS, UV-Vis, TEM, and ICP-MS. The concentration of silver mass in initially purified $[Ag\text{-}Ds]^+$ was determined to be (335 ± 1.94) µg/mL by ICP-MS. The z-average diameter of the $[Ag\text{-}Ds]^+$, obtained by DLS, was (19.1 ± 0.1) nm with a monomodal size distribution (polydispersity index = 0.17, Figure 1a) and the calculated hydrodynamic size distribution gave no indication of significant aggregation between the particles. The measured zeta potential was (+24.0 ± 0.4) mV at pH 8.1, which confirmed a positively charged corona surrounding the silver core. The uncertainty of z-average diameter and zeta potential represent the mean and one standard deviation of at least three measurements under repeatability conditions. UV-Vis measurements reveal an SPR band near 408 nm (Figure 1b), a slightly red-shifted value compared to citrate-stabilized AgNPs (at 390 nm) in this size range.

TEM yields a mean diameter of (6.8 ± 2.1, figure 1c) nm for the silver core of $[Ag\text{-}Ds]^+$, and indicates a spherical uniformity in particle shape. As expected through previous work, [5] the TEM diameter of the $[Ag\text{-}Ds]^+$ is smaller than the diameter obtained by DLS due to the presence of the dendron corona that contributes to the hydrodynamic envelope of the particles but is transparent to TEM.

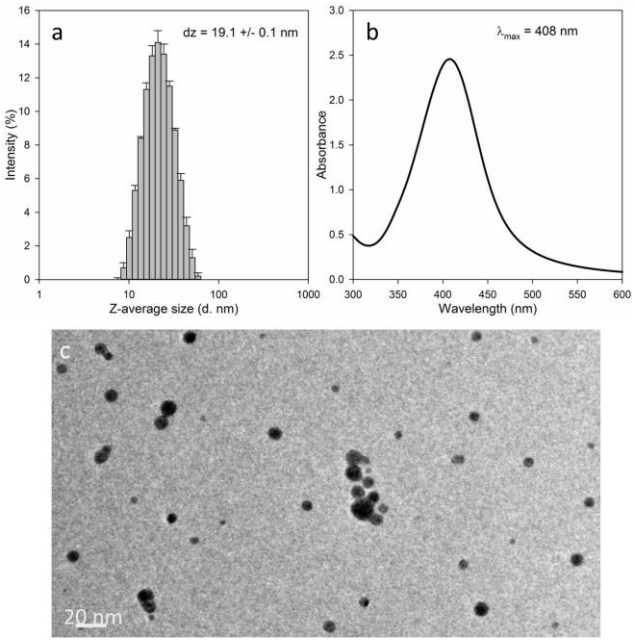


Figure 1. Characterization of $[Ag\text{-}Ds]^+$: (a) DLS size distribution. Error bars represent standard deviation, (b) SPR band by UV-Vis absorbance for initial purified sample; dilution factor is 10 for UV-vis measurements, and (c) TEM image; scale bar is 20 nm.

3.3 Stability study of $[Ag\text{-}Ds]^+$

Colloidal stability is an important issue for any commercial application of AgNPs. We evaluated the stability of the $[Ag\text{-}Ds]^+$ over a range of relevant conditions utilizing previously established protocols. [10] Native $[Ag\text{-}Ds]^+$ aged for 6 months under ambient laboratory conditions yielded a size distribution and SPR band (Figure 2a, b) that were almost identical to the freshly prepared and purified product. These results suggest that there is no significant change in the physico-chemical properties with respect to at least a 6-month shelf life under ambient conditions.

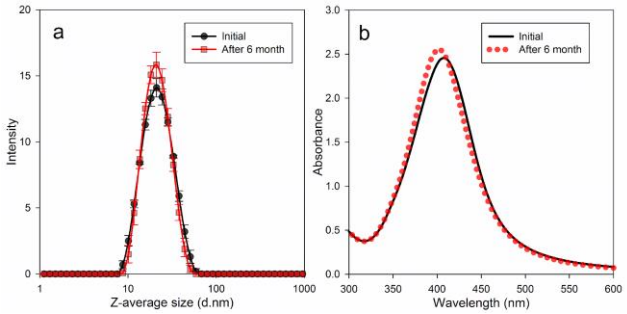


Figure 2. Shelf-life test of $[Ag\text{-}Ds]^+$; (a) DLS size distributions for the initial product (black line) and after 6 months (red line). Error bars represent standard deviation, (b) UV-Vis absorbance showing SPR band for the initial product (black line) and after 6 months (red line).

For biological application, in particular, stability in physiological media is critical. Based on UV-vis, citrate-stabilized AgNPs showed immediate instability in PBS (Figure 3a), otherwise $[Ag\text{-}Ds]^+$ exhibited excellent stability in PBS over a 48 h period relevant to cell exposure assays (figure 3b). We attribute this stability to the hydrophilicity of the inserted PEG chains and dendritic steric repulsions that substantially reduce the charge screening effect. In addition, $[Ag\text{-}Ds]^+$ were tested in DMEM, which is another common biological test medium for cell assays. The results for DMEM (Figure 3c) show the SPR band intensity reduced by about 50 % over 48 h, and the reduction in the SPR absorbance can be attributed to removal of material, perhaps by agglomeration followed by rapid sedimentation. A similar observation was reported in a previous study [5] on cationic Au-dendron conjugates. Furthermore, we evaluated the colloidal behavior of $[Ag\text{-}Ds]^+$ with protein in physiological medium, such as 10 % bovine serum albumin (BSA) in DMEM, and it appeared that similar result was observed (Figure 3d) compared to that in BSA free DMEM, over the same time period. This indicates that there is no significant interaction between $[Ag\text{-}Ds]^+$ and BSA to induce any distinct behaviors of $[Ag\text{-}Ds]^+$ in protein abundant medium.

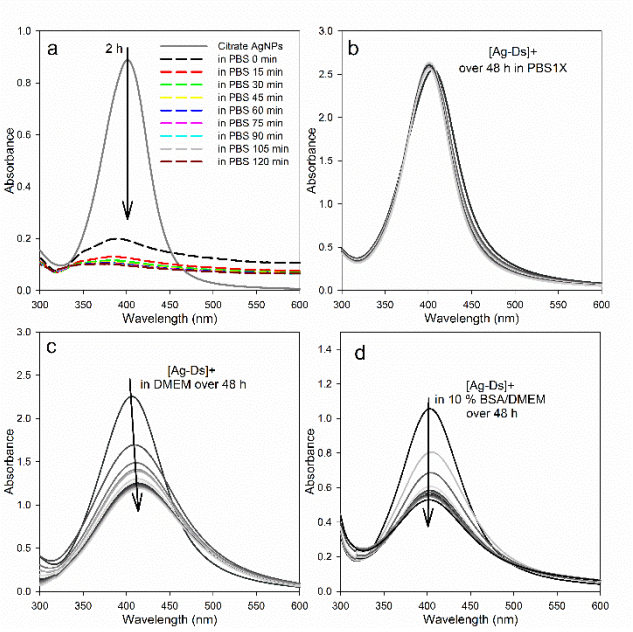


Figure 3. Stability of $[Ag\text{-}Ds]^+$ in biological test media over time, as monitored by UV-Vis: (a) citrate AgNPs, (b) PBS, (c) DMEM, and (d) 10 % BSA in DMEM.

Stability over a wide range of pH values was also investigated. From mildly acidic to basic conditions (pH 3 ~ 10), $[Ag\text{-}Ds]^+$ showed remarkable stability. Under harsher pH conditions, they exhibit gradually decreasing absorbance (45 % and 20 % reductions for 50 mmol/L HCl or 50 mmol/L NaOH, respectively; data omitted) over 12 h. Overall, the resistance against acid destabilization is greatly

improved relative to citrate AgNPs. [12]

Thermal stability of $[Ag\text{-}Ds]^+$ was evaluated by UV-Vis over the range from (20 to 60) °C, which covers the relevant range for most biological assays. Samples were incubated for 30 min at each temperature before measurements were conducted. The constancy of the SPR band (from UV-Vis spectra, data omitted) confirm that the $[Ag\text{-}Ds]^+$ are stable with respect to temperature variations over the tested range.

3.4 Release of Ag^+ ions from AgNPs

The antimicrobial activity of AgNPs is closely related to the oxidative dissolution process that releases bioactive Ag^+ ions. [13] Briefly, the total silver mass in $[Ag\text{-}Ds]^+$ was determined with ICP-MS after sample digestion using 70 % HNO_3. To examine the Ag^+ release process, $[Ag\text{-}Ds]^+$, citrate AgNPs, and PVP AgNPs were diluted in 5 mmol/L of acetate buffer (pH 4) to Ag mass concentration of 5 µg/mL, which were then incubated at room temperature in the dark. Aliquots of AgNP suspensions were taken at desired time points and were subjected to centrifugal ultrafiltration (Amicon-0.5 filter, 3 kDa), followed by quantification of the dissolved fraction in the filtrates by ICP-MS.

The ion release behavior of $[Ag\text{-}Ds]^+$ was compared with that of citrate and PVP coated AgNPs at pH 4. As shown in Figure 4, a continuous increase of dissolved Ag species was observed regardless of surface functionalization. The $[Ag\text{-}Ds]^+$ exhibit a release profile comparable to citrate AgNPs, suggesting possible use of this novel positively charged AgNP for antimicrobial applications.

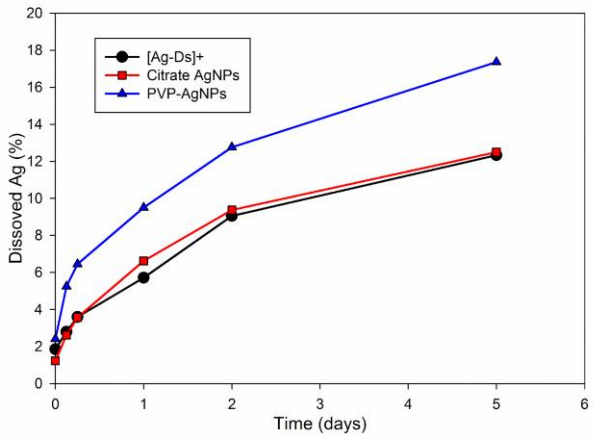


Figure 4. Time resolved Ag^+ release from AgNPs of different functionality. The release experiment was conducted in 5 mmol/L and pH 4 acetate buffer at AgNP concentration of 5 µg/mL.

4 CONCLUSION

The positively charged silver nanoparticles, $[Ag\text{-}Ds]^+$ were developed as a candidate for a nanoscale test material for biological application such as cellular assays, antimicrobial agent and/or a drug carrier in nanomedicines. In summary, we recently created a novel 1→3 directional first generation cationic dendron (PCD) and successfully developed its silver conjugate. The critical physico-chemical properties including size and size distribution, optical property, shapes, and surface charge of the $[Ag\text{-}Ds]^+$ were fully characterized by DLS, UV-Vis, TEM, and zeta potential measurements. Very importantly, especially for its biological application, the colloidal behaviors of the $[Ag\text{-}Ds]^+$ were investigated under physiologically relevant conditions, and exhibited remarkably enhanced stability relative to the control citrate AgNPs and therefore satisfactory for consideration in applications requiring long shelf-life, good dispersion in physiological media, wide range of pHs and temperatures. The ion release behavior of $[Ag\text{-}Ds]^+$ was evaluated by ICP-MS, and showed a similar release profile as citrate AgNPs suggesting possible use as a antibacterial agent.

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