Effect of capping ligand on serum protein adsorption and cell uptake of gold nanoparticles

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Abstract:

Gold (Au) nanoparticles (NP) exhibit plasmonic properties, which make them ideal for biosensing, imaging, and drug delivery. Due to the range of applications for Au NPs, it is necessary to evaluate their interactions in biologically relevant media. The objective of this study is to investigate protein adsorption and uptake trends for 60 nm Au NPs capped with citrate (CA) versus tannic acid (TA). To approach this objective, Au NPs capped in CA versus TA were dispersed in water or media supplemented with increasing amounts of serum protein (0-10%). The resulting dispersions were characterized using ultraviolet-visible spectroscopy, hyperspectral imaging, dynamic light scattering and laser Doppler electrophoresis. Significant agglomeration in media without serum and complete re-stabilization at 2% serum in media was observed for both CA or TA capped Au NPs, with no statistically significant change for increasing concentrations of serum (0-10%). NP characterization techniques indicated a statistically significant difference in size increase of about 21.4±1.8 nm and 27.2±1.8 nm for CA and TA capped NPs, respectively when dispersed in 10% serum. Cellular uptake into a lung epithelial cell-line (A549) was investigated qualitatively with darkfield microscopy and quantitatively via inductively coupled plasma-mass spectrometry. Uptake studies did not reveal a difference between the Au NPs capped with different ligands dispersed in 10% serum.

Introduction

Gold (Au) nanoparticles (NPs) exhibit plasmonic properties, which make them ideal for biosensing, imaging, and drug delivery.[1,2] Due to the range of applications for Au NPs, it is necessary to evaluate their interactions in biologically relevant media and with cells. Au NPs agglomerate in the presence of the salts found in cell culture media and are readily stabilized by the addition of serum proteins.[3] The agglomeration state for Au NPs has been shown to affect cell uptake, where the relative uptake of either single or agglomerated particles was unique for different cell types.[4]

It was previously shown that the capping agent can also play a role in cell uptake of 10 nm Au NPs.[3] Au NPs are often stabilized using electrostatically bound capping ligands, such as citric acid (CA) and tannic acid (TA). Additionally, many studies have demonstrated that regardless of size and capping agent, Au NPs adsorb serum proteins, which stabilize them in high conductivity media (i.e. cell culture media).[3,5] The adsorbed proteins serve as the primary interface for interaction with cells.[6,7,8,9]

In this study, the role of 2 different capping agents on protein adsorption for 60 nm Au NPs was systematically investigated for serum concentrations ranging from 0-10% in non-conductive (water) and conductive media (RPMI media). Cell uptake was then quantified for CA-Au NP and TA-Au NP dispersed in RPMI media containing 10 % serum.

Approach

Au NPs (60 nm) capped in CA or TA were used in this study. CA capped Au NPs (60 nm) were purchased from the National Institute of Standards and Technology (NIST) and TA capped Au NPs (60 nm) were purchased from nanoComposix. NPs were characterized using transmission electron microscopy (TEM) to determine size and morphology.

Au NPs were diluted to 10 µg/mL in either water or RPMI-1640 media (ATCC) supplemented with 1% penicillin/streptomycin and increasing concentrations of fetal bovine serum (0- 10%). Solutions were made by adding in order, serum, NPs, and water or media to prevent the aggregation of Au NPs in media without serum proteins. The resulting dispersions were characterized using ultraviolet-visible spectroscopy (UV-vis; Varian Cary 5000 UV-VIS-NIR Spectrophotometer), hyperspectral imaging (HSI; CytoViva, Inc.), dynamic light scattering (DLS) and laser Doppler

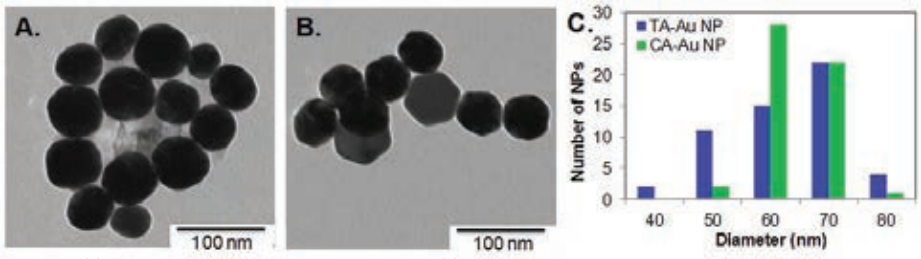

Figure 1: TEM data for 60 nm Au NPs. A. Image of TA capped Au NPs; B. Image of CA capped Au NPs; C. Histogram showing the size distribution for each NP.

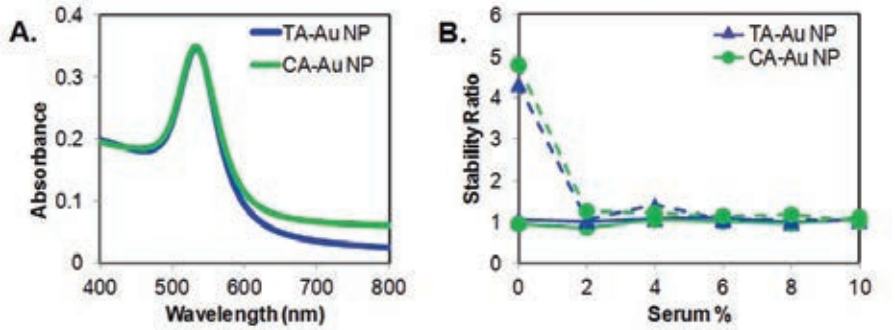

Figure 2: UV-vis data for 60 nm Au NPs. A. TA and CA capped Au NPs in water; B. Stability ratio for TA and CA capped Au NPs in water (solid lines) or media (dashed lines) with varying concentration of serum (0-10%).

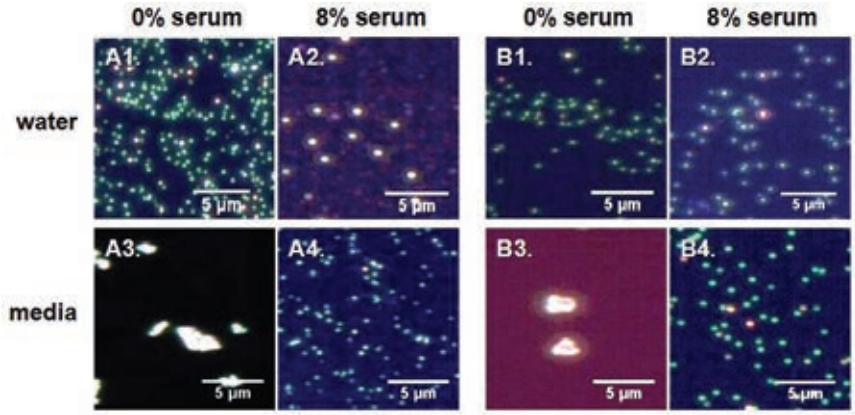

Figure 3: Darkfield images of Au NPs functionalized with tannic acid (A1-4) or citrate (B1-4) after dispersion in (1) water, (2) water + 8% serum, (3) media, or (4) media plus 8% serum.

electrophoresis (Malvern Zetasizer nano ZS). Due to the plasmonic properties of Au NPs, many characterization techniques can be used to investigate their interaction with serum proteins. Using more than one characterization technique is useful to validate and clarify results.

In order to compare the curves acquired by UV-vis, a stability ratio was calculated by dividing the area under the curve by the magnitude of the peak wavelength. The stability ratio was normalized to 1.0 based on the average stability ratio value for each sample. A drawback to the UV-vis technique is that the final curve is an average of the entire solution. This can be complimented by analyzing individual particles and clusters using HSI, which can measure the scattering spectrum from individual pixels in an image of NPs. Theoretical data for surface plasmon resonance was calculated based on an in-house program written in Matlab based on Mie theory.[10]

Optical microscopy is a useful tool in characterizing cell morphology and NP interactions with cells *in vitro*. Ultra resolution darkfield microscopy can be used to illuminate dense structures, such as cell membranes, organelles, and NPs without fluorescent tagging, but the identity of the dense structure cannot be confirmed using this technique alone.[11] Hyperspectral Imaging (HSI) is a spectroscopy technique that can be employed with both fluorescence and darkfield microscopy to confirm the 2-dimensional location of inorganic optically active NPs based on their spectral signatures. When HSI is employed in conjunction with ultra resolution darkfield microscopy, an image resolution of less than 150 nm and detection of less than 30 nm can be achieved.[11,12,13]

Hydrodynamic diameter and zeta potential data are useful for assessing changes in properties of NPs in different media. However, dynamic light scattering is limited to monodisperse spherical particles. Also, this technique is optimal within a specific concentration range and is not applicable for sub-10 nm size particles.

A human lung epithelial cell-line (A549) was used for uptake studies. Cells were cultured in RPMI-1640 media (ATCC) supplemented with 1% penicillin/streptomycin and 10% heat inactivated fetal bovine serum. Cellular interaction was studied qualitative using hyperspectral imaging, while quantitative Au NP uptake was analyzed using total metal analysis by inductively coupled Plasmon mass spectrometry (ICP-MS).

Results and Discussion

Au NPs were characterized using TEM (Figure 1). The Au NPs exhibited a mostly spherical morphology, with an average diameter of 58.1 ± 9.5 nm and 59.2 ± 6.5 nm for TA and CA, respectively, corroborating the values provided by the manufacturers. The average diameter was measured as described in materials and methods and a histogram for the size distribution is shown in figure 1C.

Au NP dispersions were characterized spectrally using UV-vis (Figure 2). The spectra shows slight variation in intensity for samples diluted to equal concentration (Figure 2A), but this difference is not expected to affect the results for stability ratios or dynamic light scattering. The peak absorption wavelength was 534 and 533 nm for TA- and CA-Au NPs, respectively, which correlates with the theoretical value for uncoated 60 nm Au NPs in water (536 nm).[14] The reason for the slight discrepancy is unclear, but may be related to the local refractive index variation caused by the presence of citric acid and tannic acid in solution or the slight size deviations observed by TEM.

The stability ratio is an analysis of the peak intensity and curve shape from the UV-vis data for TA- and CA- Au NPs in water or media with increasing concentration of serum protein. The results show that the samples exhibit reduced stability in exposure media without serum and that the samples are stabilized after the addition of 2% serum, remaining stable for increasing concentrations of serum in media and water (Figure 2B). There is no statistically significant difference for the stability ratio between samples from 2-10% FBS in either particle type.

Darkfield images are displayed in Figure 3. TA-Au NPs can be seen in Figure 3A1-A4, while CA-Au NPs are shown in Figure 3B1-B4. For each capping ligand, the trends remain consistent with results observed via UV-vis. When dispersed in water without serum or media supplemented with 8% serum, Au NPs appear to be green and are well-dispersed on the slide (Figure 3A1, 3A4, 3B1, 3B4). For Au NPs in water supplemented with 8% serum, TA-Au NPs appear yellow, and CA-Au NPs appear green (Figure 3A2

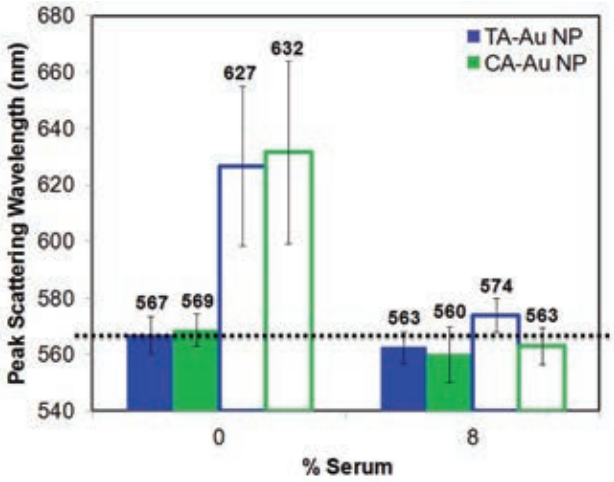

Figure 4: Peak scattering wavelength for TA- and CA- NPs in water (solid fill) and media (white fill) for 0% and 8% serum. Dotted line indicates theoretical plasmon resonance peak for 60 nm Au NPs with a local refractive index of glass or immersion oil (1.515).

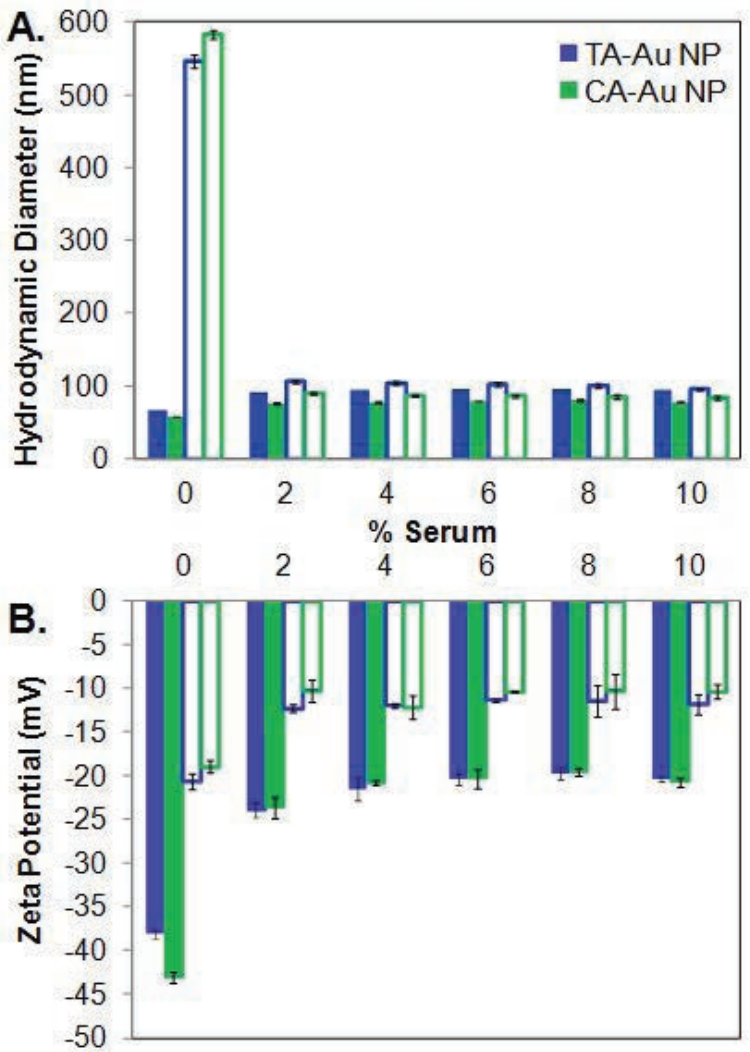

Figure 5: Hydrodynamic diameter (A) and zeta potential data (B) for increasing serum concentration in water (solid fill) and media (white fill).

and 3B2). The yellow appearance of the TA-NPs in water supplemented with serum may be caused by experimental error, as this indicates the formation of agglomerates, which is not supported by the UV-vis or HSI data. The significant decrease in stability observed via UV-vis is supported by visual observation using darkfield microscopy for both TA- and CA-Au NPs (Figure 3A3 and 3B3).

The scattering wavelengths collected from the darkfield images shown in Figure 3 are expressed in the bar graph in Figure 4. When dispersed in water without serum, the NPs scatter light with a peak wavelength of 567 and 569 nm for TA- and CA-Au NPs, respectively. This correlates well with theoretical data for the plasmon resonance peak of 60 nm Au NPs with a local refractive index of 1.515 (silica glass slide; immersion oil), which is 567 nm. The peak wavelengths are 563 and 574 for TA-Au NPs, and 560 and 563 for CA-Au NPs dispersed in serum supplemented water and media, respectively. These values are also close to the theoretical estimations. Wavelength values for the NPs dispersed in media correlate with the agglomeration observed in darkfield images. The peak wavelength for Au matches closely to the theoretical value, which is 545 nm in water.[10] The shift of about 10 nm is due the higher refractive index of glass and microscope oil, which must be accounted for when using HSI.[15,16]

Hydrodynamic diameter (A) and zeta potential data (B) for increasing serum concentration in water (solid fill) and media (white fill) are shown in Figure 5. DLS characterization data indicate a statistically significant difference in size increase of about 27.2±1.4 nm and 21.4±1.8 for TA- and CA- Au NPs, respectively when dispersed in serum. When dispersed in water, the difference in size increase is not statistically significant for any serum concentration (2-10%). Significant agglomeration in media without serum and complete re-stabilizatxion at 2% serum in media was observed for both NPs.

The zeta potential for TA-Au NP is about -38 mV versus -43.1 mV for CA-Au NP (Figure 5B). This is likely due to innate differences in the capping ligand chemistry. Once 2% serum is added, the value for zeta potential increases by 14.1 mV to -24.0 mV for TA-Au NPs. The value for CA-Au NPs increases by 19.4 mV for a zeta potential of -23.7 mV. The change in zeta potential with the addition of serum indicates protein adsorption; the similarity between the final values indicates the outer layer of proteins adsorbed to the Au NPs is similar regardless

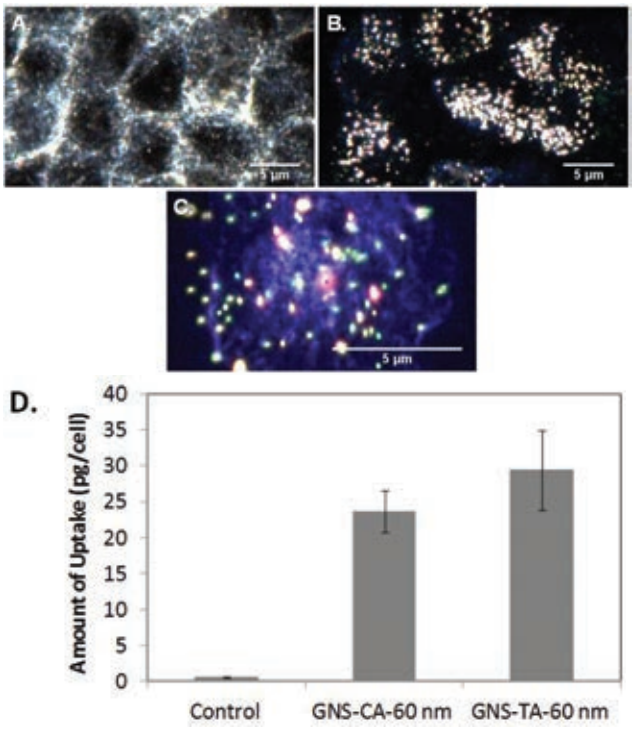

Figure 6: Interaction of Au NPs with A549 cells. A-C. Darkfield images; A. Control; B. Cells exposed to TA-Au NPs; C. Close-up image of cell exposed to CA-Au NPs; D. ICP-MS results.

of capping ligand. For Au NPs with both capping ligands, the change from 2% serum is statistically significant from 6, 8, and 10% serum conditions. The final value for these conditions is -20.2 mV for both TA- and CA-Au NPs, further indicating the similarity in protein adsorption trends for the different capping ligands.

The zeta potential data for conditions in media indicate a decrease in value for all samples compared to the 0% serum in water condition. The value for zeta potential in media with 0% serum is -20.7 and 19.0 mV for TA- and CA- Au NPs, respectively. This increase is likely related to the adsorption of Na+ to carboxylic acid groups on citric acid and tannic acid.4 After addition of serum, the value further increases to -11.8 mV and -10.7 mV, respectively, with no significant difference for the 2-10% serum concentration in either sample. This indicates that proteins may adsorb onto the NPs in a unique manner in media versus water.

NP uptake was investigated in cells using darkfield microscopy (Figure 6). The images show strong association of NPs will cells, and when compared to darkfield images of NPs in Figure 3, demonstrates the presence of primary particles and agglomerates. ICP-MS was used to quantify the particle uptake in the A549 cell-line. Results show that there is not statistically significant difference in cell uptake for CA- or TA-Au NPs (Figure 6D). This result is interesting because it has been previously shown that the capping agent can affect cell uptake for 10 nm Au NPs.[3]

Conclusion

The results of this work indicate that ≤ 2% serum is needed for stabilizing Au NPs in cell culture media. The adsorbed protein layer does not appear to change as the concentration of serum is increased; however, the composition of the adsorbed proteins is unknown. Based on preliminary research, questions arise, including: what concentration of serum will not stabilize the particles and does penicillin/streptomycin affect the stability. In conclusion, the capping ligand does not seem to strongly influence the adsorption of proteins or cell uptake of Au NPs.

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