



Synthesis and characterization of Au:Ag core-shell nanoparticles with 4-aminothiophenol Surface Enhanced Raman Spectroscopy (SERS) tag

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Introduction

Overview. Noble metal nanoparticles have become increasingly important over recent years, specifically in the pharmaceutical sector for drug delivery. By taking advantage of their unique surface properties antibodies and therapeutic agents can be attached to the particles allowing targeted drug delivery, particularly in cancer therapy¹. By using targeted drug delivery, active ingredients can be released directly at the site of the tumor allowing for a decreased dosage to be employed and thus reduced side effects². For nanoparticles to be accepted into cells and their uptake monitored, they must be appropriately sized (<100 nm), their surface modified with a lipid bilayer, and bound with chemical tag. Surface Enhanced Raman Spectroscopy (SERS) tags enhance the Raman signal of SERS active molecules bound to noble metal surfaces, giving a much stronger peak and characteristic peak for the tag. Although many have studied the effectiveness of different SERS tags, few have yet to investigate retention of Raman signal enhancement and stability upon the addition of a lipid bilayer with core-shell nanoparticles.

This Work. In this study, we investigated the utility of 4-aminothiophenol as a SERS tag and its effect on the stability on lipid-coated gold core, silver shell nanoparticles. A gold core synthesis method was employed, which allowed for better regulation of particle size, followed by incorporating a silver shell around the gold core³ that generates the Raman signal that 4-aminothiophenol amplifies, and finally coated with a POPS:LPC bilayer⁴. We monitored aggregation, the success of 4-aminothiophenol incorporation, and nanoparticle size using UV-Vis spectroscopy, Raman spectroscopy, and Nanosight LM-10 using particle light scattering. Prior to the lipid coating being applied the SERS signal proved stable over the course of several weeks with a small size increase in the nanoparticle size. Upon coating with the lipid layer a significant increase in nanoparticle size was observed, however the SERS signal was successfully retained.

Methods

Chemicals

- 4-aminothiophenol solution prepared in ethanol (5mM)
- 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (20mM, pH=7) and poly(allylamine hydrochloride) (PAH) (10mg/mL) prepared in water
- 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) and lysophosphatidylcholine (LPC) solution prepared 1:1 in HEPES
- Chloroauric acid (0.01M) and silver nitrate (10mM) prepared in water
- Sodium borohydride (0.075% wt./vol.), trisodium citrate (38.8 mM) and sodium chloride (0.1M) prepared in water

Gold core nanoparticles (Au₁₀₀-NP) Synthesis

Citrate capped and reduced gold cores

- Trisodium citrate (10 mL) and chloroauric acid (10 mL in 90 mL dH₂O) refluxed (15min)

Citrate capped gold cores with sodium borohydride reducer

- Sodium borohydride (1mL), chloroauric acid (3 mL), and trisodium citrate (2 mL in 88 mL dH₂O) mixed at room temperature (7min)

Core-shell nanoparticles (Au_x-Ag_y-NP) Synthesis (y=5-50%)

- Silver nitrate (0.2 mL every 10 min) added with trisodium citrate (1 drop every 10min) to boiling Au₁₀₀-NP in reflux until desired amount of silver added
- Citrate and sodium borohydride Au₁₀₀-NP were coated with silver comparing their stability
- Citrated particles were used for the remainder of the experiments.

SERS Tagging

- Au₅₀-Ag₅₀-NP purified using Beckman Coulter Algebra 6R centrifuge (hr at 3600 rpm) and suspended in water
- Purified Au₅₀-Ag₅₀-NP were mixed with 4-aminothiophenol in a 5:4 ratio and purified

Lipid coating

- Tagged Au₅₀-Ag₅₀-NP (1 mL) were mixed with PAH (0.2 mL) and sodium chloride (0.1 mL)
- Purified 24hr later via Eppendorf centrifuge 5424R (1100 rpm for 90 min)
- Purified PAH particles suspended in HEPES buffer (0.5 mL) and added to POPS:LPC solution (0.5 mL)

Methods Continued

UV-vis spectrophotometer

- Au₁₀₀-NP synthesis was confirmed through a full spectrum of 200-800 nm at medium scan speed with a slit width of 2 nm
- Silver coating was verified through an additional scan of the same parameters.

Nanosight LM10 HS

- Particle size determined with 200:1 dilutions and recording of 10 s with camera level between 5 and 16
- Measurements were made initially after synthesis and over 7 days so ensure stability and minimal aggregation

Raman spectrophotometer.

- Au₅₀-Ag₅₀-NP and 4-aminothiophenol mixed 1:1 to ensure a viable signal with Raman excitation at 532 nm, using a 0.25 m spectrometer (600 gr/mm grating) with an electrothermally cooled CCD.
- Stability of the tagged Au₅₀-Ag₅₀-NP were tested over a period of a week

Results

Table 1. Sizing after each layer addition for purified nanoparticles, unpurified particles are denoted by a *.

	Mode size (nm)	Mean size (nm)	SD (nm)	Monodispersion
Au ₁₀₀ -NP citrate*	56	68	36	yes
Au ₁₀₀ -NP borohydride*	29	49	31	yes
citrate*	12	31	28	yes
borohydride*	72	113	84	yes
citrate	27	43	27	yes
SERS tag	113	71	71	yes
PAH	105	152	92	no
lipid	131	213	99	no

Note: All nanoparticles are Au₅₀-Ag₅₀-NP unless otherwise denoted.

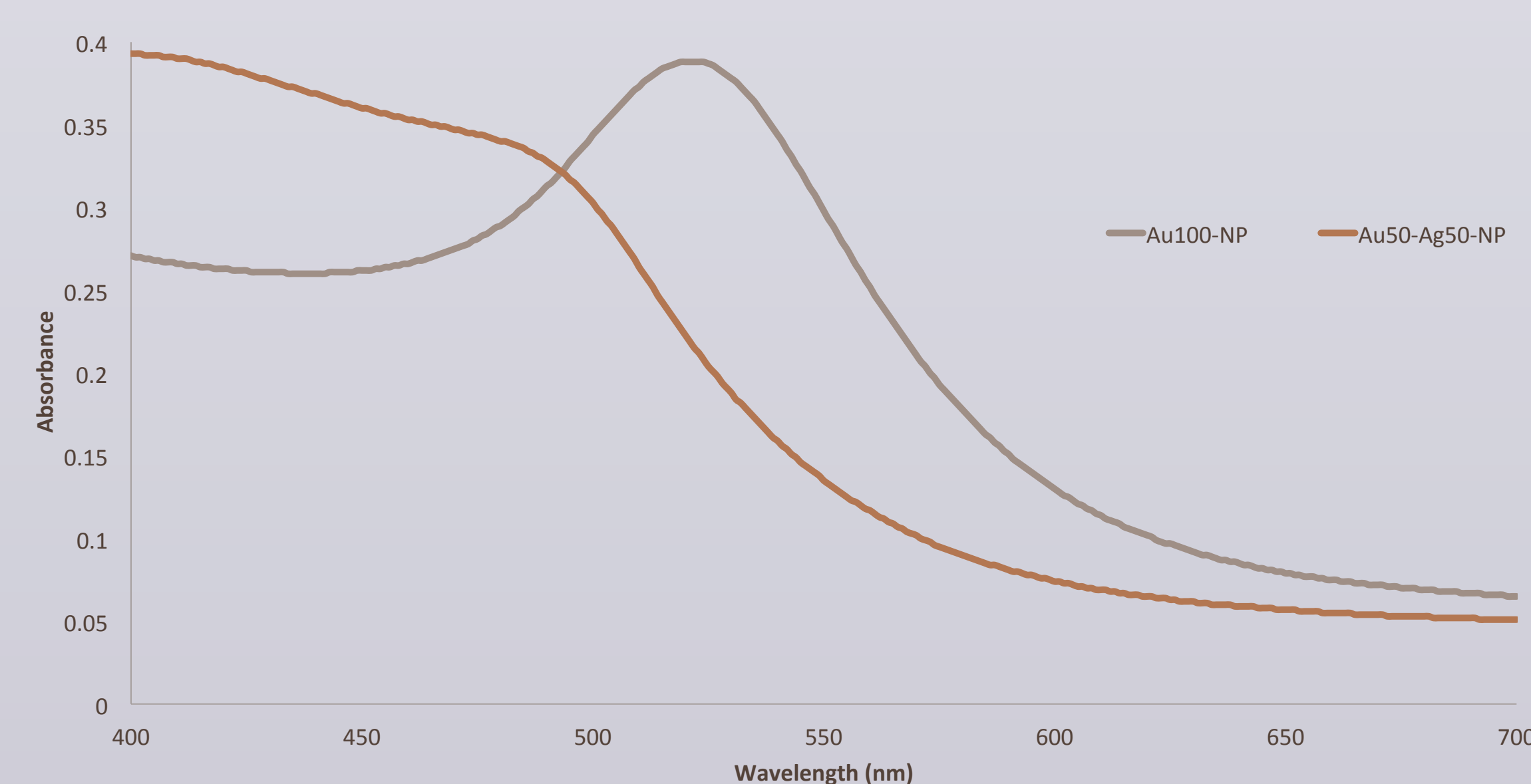


Figure 1. Absorbance over wavelength of citrate capped Au₁₀₀-NP (λ_{max} =521.5nm) and Au₅₀-Ag₅₀-NP (λ_{max} =398.5nm).

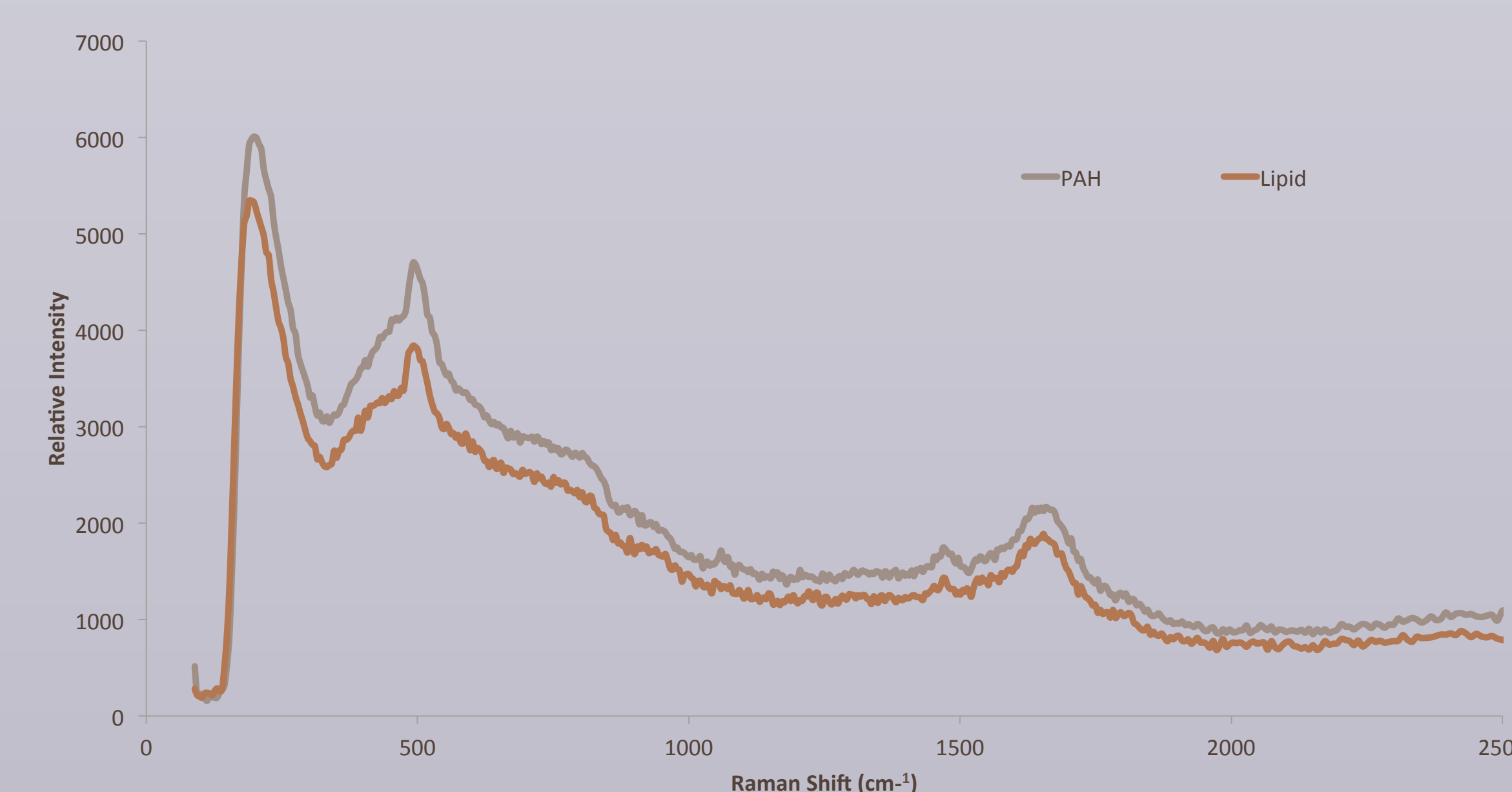


Figure 2. SERS spectra for purified PAH coated and lipid coated Au₅₀-Ag₅₀-NP with SERS peaks at 483 and 1633cm⁻¹. Initial SERS concentration was 2.2mM in Au₅₀-Ag₅₀-NP solution but was further purified, removing any unbound tag molecules.

Results

Nanosight Sizing

The citrate reduced particles were larger than the borohydride reduced particles. Silver coated borohydride particles were larger and less stable than citrate particles. (table 1).

UV-Vis Spectroscopy

Au₅₀-Ag₅₀-NP λ_{max} shifted left, but remained one peak when scanned at a slower speed (figure 1).

Characterization of SERS

Particle size increased by about 85nm (table 1) but Raman signal was retained after purification.

Characterization of Lipids

PAH gave a slight decrease in particle size while lipids increased, both became polydispersed (table 1). PAH and lipids retained SERS peaks at 1633 from a C-C stretching vibrational mode and 483 cm⁻¹ from a C-S bend mode⁵ (figure 2).

Discussion

Characterization of Au₅₀-Ag₅₀-NP

UV-vis spectrum for Au₅₀-Ag₅₀-NP showed a left shift in the maximum wavelength with the retention of a single peak, indicating a successful silver coating. Although broadened, the presence of one peak implies a successful coating with no Ag₁₀₀-NP formation. Particles sized decreased relative to Au₁₀₀-NP, most likely due to increased reflux time.

Characterization of SERS tagged Au₅₀-Ag₅₀-NP

Particle size increased and remained monodispersed, indicating addition of another chemical layer and even layer addition. Raman signal confirmed addition layer was due to SERS tag.

Characterization of Lipids

PAH particles were found to decrease in mode size but an increase in mean size and become polydisperse. Lipid particles were found to increase in size, as expected, and became polydisperse. Polyspersion implies the particles were no longer evenly coated upon addition of chemical layers. Standard deviations for SERS, PAH, and lipid particles overlap making the difference insignificant. It was found PAH and lipid produced the SERS Raman signal, confirming 4-aminothiophenol was still associated with the particle surface.

Conclusions

The nanoparticles were successfully coated with silver, tagged with 4-aminothiophenol, and coated with PAH and lipids while retaining their SERS tag signal. Since the SERS tags were retained and still gave a Raman signal after PAH and lipid coatings the tags can be used with lipids. The authors intended on introducing lipid-coated nanoparticles to live cells in an attempt to achieve cellular uptake of the particles. 4-aminothiophenol allows for the nanoparticles to be located in the cells using SERS. Challenges we face are ensuring complete lipid coating and their size being sufficiently small enough to enter the cell. To that end, parameters that affect lipid size and aggregation will need to be investigated.

References

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