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 and therapeutic agents can be attached to the particles allowing targeted drug delivery, particularly in cancer therapy. 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 effects. 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 core that generates the Raman signal that 4-aminothiophenol amplifies, and finally coated with a POPS-LPC bilayer. 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) (10mM, pH = 7) and poly (allylamine hydrochloride) (PAH) (10mg/mL) prepared in water
- 1-palmitoyl-2-oleoyl-sn-glycerol-3-phospho-L-serine (POPS) and lysophosphatidylcholine (LPC) solution prepared 1:1 in HEPES
- Chlorouric 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 \(\text{Au}_{250}\text{-NP}\) Synthesis

Citrate capped and reduced gold cores

- Trisodium citrate (10 mL) and chlorouric acid (10 mL in 90 mL \(\text{NH}_3\) reffluxed (15min)
- Citrate capped gold cores with sodium borohydride reducer
- Sodium borohydride (1mL), chlorouric acid (3 mL), and trisodium citrate (2 mL in 88 mL \(\text{NH}_3\)) mixed at room temperature (7min)

Core–shell nanoparticles \(\text{Au}_{50}\text{-Ag}_{250}\text{-NP}\) Synthesis (5–50%) (nm)

- Silver nitrate (0.2 mL every 10 min) added with trisodium citrate (1drop every 10min) to boiling \(\text{Au}_{250}\text{-NP}\) in reflux until desired amount of silver added
- Citrate and sodium borohydride \(\text{Au}_{250}\text{-NP}\) were coated with silver comparing their stability
- Citrate particles were used for the remainder of the experiments.

SERS Tagging

- \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) purified using Beckman Coulter Algebra 6R centrifuge (hr at 3600 rpm) and suspended in water
- Purified \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) were mixed with 4-aminothiophenol in a 5:5 ratio and purified

Lipid coating

- Tagged \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) (1 mL) were mixed with PAH (0.2 mL) and sodium chloride (0.1 mL)
- Purified 24hr later by 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

- \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) synthesis was confirmed through a full spectrum of 200-800 nm at medium scan speed with a silt width of 2 nm
- Silver coating was verified through an additional scan of the same parameters.

Nanosight LM-10 BS

- Particle size determined between 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

- \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) 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 \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) were tested over a period of a week

Results

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

<table>
<thead>
<tr>
<th>Mode size (nm)</th>
<th>Mean size (nm)</th>
<th>SD (nm)</th>
<th>Monodispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Au}_{250}\text{-NP}) citrate*</td>
<td>56</td>
<td>68</td>
<td>36</td>
</tr>
<tr>
<td>(\text{Au}_{250}\text{-NP}) borohydriddre*</td>
<td>29</td>
<td>49</td>
<td>31</td>
</tr>
<tr>
<td>citrate*</td>
<td>12</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>borohydriddre*</td>
<td>72</td>
<td>113</td>
<td>84</td>
</tr>
<tr>
<td>citrate</td>
<td>27</td>
<td>43</td>
<td>27</td>
</tr>
<tr>
<td>SERS tag</td>
<td>113</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>PAH</td>
<td>105</td>
<td>152</td>
<td>92</td>
</tr>
<tr>
<td>lipid</td>
<td>131</td>
<td>213</td>
<td>99</td>
</tr>
</tbody>
</table>

Note: All nanoparticles are \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) unless otherwise denoted.

Figure 1. Absorbance over wavelength of citrate capped \(\text{Au}_{250}\text{-NP}\) \((\lambda_{\text{max}}=521.5\text{nm})\) and \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) \((\lambda_{\text{max}}=398.5\text{nm})\).

Figure 2. SERS spectra for purified PAH coated and lipid coated \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) with SERS peaks at 483 and 1633cm\(^{-1}\). Initial SERS concentration was 2.2mM in \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) solution but was further purified, removing any unbound tag molecules.

Discussion

Characterization of \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\)

UV-vis spectrum for \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\) 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 \(\text{Ag}_{250}\text{-NP}\) formation. Particle sized decreased relative to \(\text{Au}_{250}\text{-NP}\), most likely due to increased reflux time.

Characterization of SERS tagged \(\text{Au}_{250}\text{-Ag}_{250}\text{-NP}\)

Particle size increased and remained monodispersible, 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. Polysperism 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


Acknowledgements

We would like to thank the Linfield Chemistry Department, with special thanks to Dr. Elizabeth J. O. Atkinson and Dr. Thomas Reinert. Additional thanks to fellow researchers Chris Munjaz, Allison Smith, and Victoria Wood.