Ligand Effects on Gold and Silver Nanoparticle Stability

A UV-vis Spectroscopic Study

Nicholas Mak Hoi Hean

Victoria Junior College
Singapore, Singapore
Email: nicholas.mak.hoi.hean.2014@vjc.sg

Low Jia Li, Cheryl

Victoria Junior College
Singapore, Singapore
Email: low.jia.li.cheryl.2014@vjc.sg

Abstract— **Gold and silver nanoparticles can be employed in a wide range of light-related applications, but silver nanoparticles are less commonly used due to greater instability, albeit being better absorbers of light. To increase stability, aggregation of nanoparticles has to be prevented or at least reduced. Nanoparticle aggregation can be prevented by either giving nanoparticles a surface charge to repel other nanoparticles, or using large flexible ligands to sterically hinder aggregation. This project serves to explore the use of ligands for capping to improve nanoparticle stability. Nanoparticles were capped with peptide ligands and observed for aggregation by comparing absorbance spectras. Suitable ligands were selected for testing with salt, to further investigate its stability. From the results, it can be seen that ligands employed in this project had different effectiveness in stabilizing nanoparticles. Furthermore, certain ligands worked on only certain sizes of nanoparticles. However, it can be seen that certain peptides used were more effective than the traditional PEG ligand. Hence, from this project, PEG, CALNN, and CALNN-ol are identified as potential ligands for stabilizing silver nanoparticles, laying the groundwork for future research.**

I. Introduction

Gold and silver nanoparticles are highly versatile materials with a broad range of real world applications. This is due to their many desirable physical properties, predominant of which is their interaction with light. Gold and silver nanoparticles are highly efficient at both absorbing and scattering light in the ultraviolet (UV) and visible light range; moreover, some nanoparticles such as gold nano-rods, interact with infrared (IR) light. As such, they can be used in a wide range of light-related applications such as the use in light-harvesting materials (solar cells), and colour-based biological applications to detect DNA or protein interactions.

While gold and silver nanoparticles both absorb light very well (considered the highest amongst nanoparticles), silver is a greater absorber than gold, due to higher extinction values (Haiss, Nguyen, Aveyard, Fernig, 2007 and Paramelle et al, 2014). However, silver is less commonly used as a sensor, as it is generally accepted that silver nanoparticles are more difficult to stabilize than gold nanoparticles (Gould. J, 2013). Stabilizing nanoparticles involve keeping them discrete. The traditional method to stabilize a nanoparticle is to form a dense self-assembled monolayer around the surface, using chemicals called capping ligands. These capping ligands either cause a surface charge to electrostatically repel other nanoparticles, or are large and flexible to sterically hinder aggregation.

This project focuses on increasing the stability of silver nanoparticles through the use of capping ligands on the surface, in order to make them a more viable alternative over gold nanoparticles. During the course of the project, focus was placed on hydrophilic ligands due to their ease of use and subsequent water stability to the particles. Further exploration of other peptides was also done. By using these peptides, observations were made on the impacts each type of ligand has on the stability of different gold and silver nanoparticles.

II. Experimental - Methods

A. Materials

Silver and gold nanoparticles are spherical and in aqueous solution, unless stated otherwise (e.g. Silver Nanocubes, Gold Nanorods). Gold Nanorods used measure 100x25mm. Silver nanocubes measure 100nm. Capping ligand [HS-(CH2)11-(OCH2CH2)4-OH] (known as PEG ligand) was used. Capping ligand Pep1 is the C-terminal charged peptide H2N-CALNN-OH, referred to as CALNN. Capping ligand Pep3 is the C-terminal uncharged peptide H2N-CALNN-CH2-OH. Note that the capping ligands stick to the nanoparticle surface via the HS- (or peptide cysteine) end.

B. Dilution of nanoparticle solutions

Each sample of nanoparticle solution was diluted to obtain an optimum maximum absorbance (Abs) value. For each nanoparticle sample, an absorbance spectra was measured, from which the maximum absorbance value was determined. The concentration of nanoparticles was adjusted until a desired maximum absorbance value (Abs) was obtained. The dilution criteria was to have an absorbance range of approximately 0.5 Abs for gold, 0.4 Abs for silver nanoparticles equal or below 50 nm in size, 0.3 Abs for silver nanoparticles larger than 50 nm, and 0.3 Abs for silver nanocubes and gold nanorods. These absorbance values were measured in a 100 μl well. The 100 μl well included the appropriate volume of nanoparticles for the desired absorbance value, with water making up the rest of the 100 μl. The dilution factors for all nanoparticle solutions were recorded. All the following tests involving nanoparticles used

the same concentration found here to maintain consistency, unless stated otherwise.

C. Solvent stability of gold and silver nanoparticles

Solvents (Ethanol, THF, DMF, DMSO) were tested for suitability of use with nanoparticles. Solvents were tested at 20% and 50% volume. 20% DMSO was selected as our solvent for capping as it had minimal effect on the nanoparticles. This allows for consistent solvent conditions.

D. UV-visible Spectroscopy

UV-visible spectra of nanoparticles was recorded at room temperature using a UV-visible light spectrometer. Samples were placed in a plastic 384-well cuvette. Spectra were taken of nanoparticles from a wavelength of 350 nm to 1000 nm, at 5nm intervals. A blank for each test was defined as the appropriate solvent alone. Capping ligands alone do not contribute significantly to the absorbance spectra.

E. Stabilisation of gold and silver nanoparticles by capping peptide ligands

100μl samples for recording UV-vis spectra were prepared. In the sample, diluted nanoparticles and ligands were mixed with 20 μl DMSO. 20 μl of 1 mM capping ligands were added, resulting in a final concentration of 0.2 mM. The remaining portion was made up to 100 μl with water. Absorbance spectra of solutions were taken of all the samples (8 different mixes of peptides were used). The absorbance spectra graphs were compared to deduce the effectiveness of the peptide in stabilizing the nanoparticle. The graphs were compared based on whether the maximum absorbance value decreased drastically, or whether there was an increase in absorbance in the longer wavelength region. Such effects usually indicate aggregation as occurred.

F. Salt-mediated aggregation of stock or capped gold and silver nanoparticles

Different sized gold and silver nanoparticles were left for 4 hours with 20 μl of 1mM capping ligands (P4, Pep1 to Pep8), or in the case of stock nanoparticles, added to water alone, to a total volume of 90 μl. 10 μl of 2M NaCl was then added and left for 4 hours. These samples were then subjected to UV-visible spectroscopy. Some of these samples also had the capping added overnight (16 hrs) and salt added again for 4 hrs, before recording the UV-vis spectra.

III. EXPERIMENTAL – RESULTS AND DISCUSSION

A. Comparing light-interacting gold and silver nanoparticles

UV-visible spectroscopy was used to determine the stability of nanoparticles. Nanoparticle stability can be seen by changes in the absorbance spectra (to be discussed later). The relationship between absorbance and the concentration of these particles is described by the Beer-Lambert Law *(Clark. J, 2007)*. The Beer-Lambert Law equation is as follows:

$$A = \log_{10}\frac{I_0}{I} = \varepsilon\, l\, c$$

The UV-visible light spectra of different sized gold and silver nanoparticles without capping were recorded. From the spectra, a clear, defined trend is seen. As the size of the silver nanoparticles increase, the maximum absorbance wavelength (L_{max}) also increases. For gold nanoparticles (except nanorods), L_{max} relatively constant.
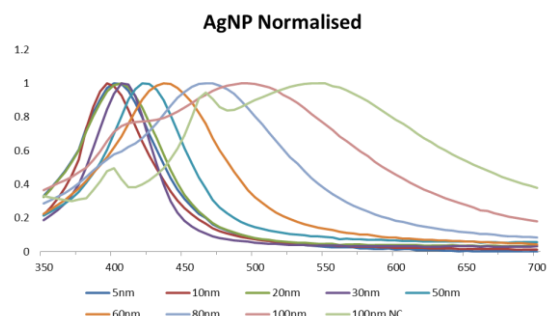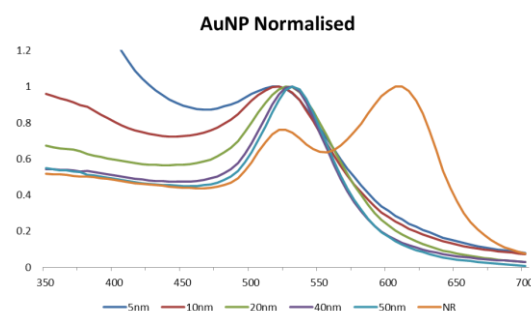

Figure 1: Normalised Spectra of Silver Nanoparticles

Figure 2: Normalised Spectra of Gold Nanoparticles

As a result of this trend, an aggregation parameter *(Zhang. F et al, 2008)* cannot be used to determine whether the nanoparticles have aggregated. The aggregation parameter is the ratio of absorbance values at 2 different wavelengths (For gold nanoparticles, it is usually taken at 650nm and 520nm). The parameter can be fixed for gold nanoparticles as L_{max} remains relatively constant. The same cannot be applied to silver nanoparticles; as L_{max} increases with size, it is much more difficult to define a constant aggregation parameter.

Hence, to determine whether the nanoparticles have aggregated, the absorbance spectras of nanoparticles were compared qualitatively based on two criteria. Firstly, the maximum absorbance was checked as a drop in maximum absorbance indicates that the concentration of nanoparticles has decreased, hence indicating aggregation. Secondly, Lmax was checked as aggregated nanoparticles absorb longer wavelengths of light. Thus, an increase in Lmax would show that the nanoparticles aggregated. Aggregation has most probably occurred when any one of these criteria is met; to confirm it, other methods of analysis such as NanoSight microscopy would have to be used.

Stock nanoparticles are loosely stabilized with citrate molecules. When we add our capping ligands (peptides), the citrate molecules are displaced in favour of the peptides.

Nanoparticles are capped with nine capping ligands, labeled P4, and Pep1 to Pep8 (Pep1 to Pep8 are all peptide mixes). After capping, the spectra of all the capped nanoparticles are taken. Spectra are analysed based on the two criteria mentioned earlier. Out of the nine different capping ligands, only three (P4, Pep1, Pep3) were successful as the rest of the ligands induced aggregation in the nanoparticles, based on the criteria set in the previous paragraph. For example, for the silver nanocubes with Pep5 and Pep7, the maximum absorbance has dropped by around 50%, showing that the silver nanoparticles have aggregated. In addition, the 50nm silver nanoparticles showed signs of aggregation with P4, Pep2, Pep4, Pep6 and Pep8, as shown by the depressed maximum absorbance compared to the stock nanoparticles.
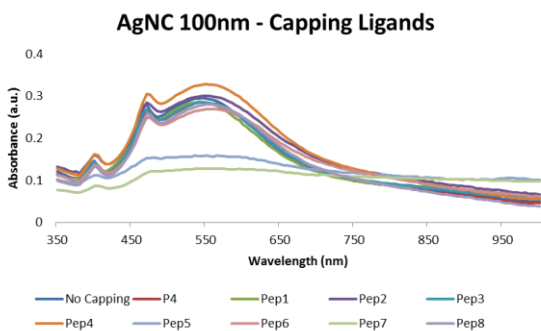

Figure 3: Spectra of 100nm Silver Nanocubes with different capping ligands
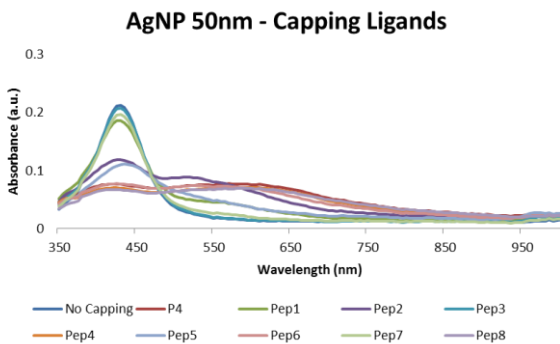

Figure 4: Spectra of 50nm Silver Nanoparticles with different capping ligands

While P4 showed aggregation with most of the silver nanoparticles, they were considered successful as they managed to successfully stabilize most of the gold nanoparticles (A comprehensive list of results for all nanoparticles tested can be found in Figure 5).

(Green)	Nanoparticles Stable - Does not aggregate (No change in L_{max} or maximum absorbance)
(Yellow)	Nanoparticles Mostly Stable - Some aggregation (Slight increase in L_{max} and/or slight change in maximum absorbance)
(Red)	Nanoparticles Unstable - Most nanoparticles aggregate (Large increase in L_{max} and/or large change in maximum absorbance)

NP Size (nm)/Shape	P4	Pep1	Pep2	Pep3	Pep4	Pep5	Pep6	Pep7	Pep8
Ag 5	Yellow	Green	Green	Green	Green	Red	Yellow		
Ag 10	Green	Green	Green	Green*	Green	Green	Green	Red	Green
Ag 20	Red*	Green*	Red	Green*	Red	Red	Red	Red	Red
Ag 30	Red*	Green*	Yellow	Green*	Red	Red	Red	Red	Yellow
Ag 50	Red*	Green*	Red	Green*	Red	Red	Red	Red	Red
Ag 60	Red*	Green*	Yellow	Green*	Yellow	Red	Red	Red	Red
Ag 70	Green	Yellow*	Yellow	Green*		Red	Red	Red	Red
Ag 80	Red*	Green*	Green	Green*		Red	Red	Red	Red
Ag 100	Red*	Green*		Green*		Red	Yellow	Red	Red
Ag Cubes	Green*	Green*		Green*		Red	Red	Red	Red
Au 5	Green	Green		Green*		Green	Green	Green*	Green
Au 10	Green	Green		Green		Red	Green	Green	Green
Au 20	Green	Green		Yellow		Red	Green	Green	Red
Au 40	Yellow*	Green*		Green*		Red	Yellow*	Green	Green
Au 50	Yellow	Yellow		Yellow		Red	Red	Red	Red
Au Rod	Yellow*	Green*		Green*		Green*	Green*	Green*	Green

* - Less stable in presence of 200mM NaCl. 4 hours

Figure 5: Table compiling results of ligand test with nanoparticles

In addition, in figures 6 and 7, a comprehensive analysis on the effectiveness of CALNN-capped (Pep1) silver nanoparticles is presented, with the change in maximum absorbance and change in L_{max} being compared. From this comparison, it is seen that CALNN is more effective in capping larger-sized silver nanoparticles as compared to the smaller sized ones (Le'vy. R, 2004).
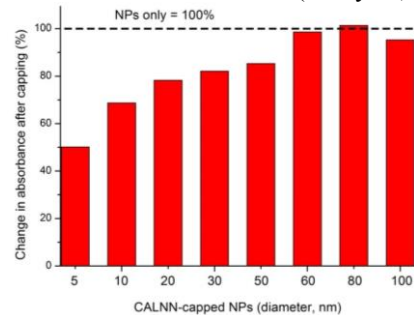

Figure 6: Graph of Change in Maximum Absorbance after capping for CALNN-capped silver nanoparticles
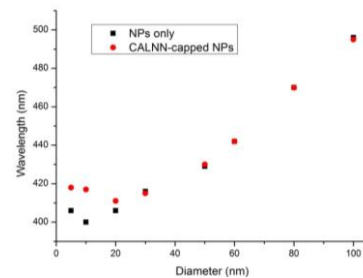

Figure 7: Graph of Change in L_{max} after capping for CALNN-capped silver nanoparticles

C. Salt-mediated aggregation of capped gold and silver nanoparticles

To further test the stability of a nanoparticle, there are three different common methods: Addition of Salt, Ligand Exchange, and Cyanide Etching *(Schulz, Vossmeyer, Bastús, Weller, 2013)*. For this project, only addition of salt was used due to the ease in experimentation. Uncapped gold and silver nanoparticles were also tested as controls. Salt was added to gold and silver nanoparticles capped with P4, Pep1, and Pep3, in order to further test the effectiveness of the capping ligands mentioned. For the purpose of this report, only the 100nm silver nanocubes (AgNC) will be discussed.

Upon addition of salt (NaCl), the weakly capped silver nanocubes aggregated (labeled as no capping), as seen in the large decrease in the maximum absorbance. The P4-capped nanoparticles are mostly stable in salt solution, but there was a slight aggregation, as seen in the small increase of Lmax¬. The CALNN-capped (Pep1) and CALNN-ol capped (Pep3) AgNCs are also seen to have aggregated, due to the sizable decrease in maximum absorbance at the same wavelength.
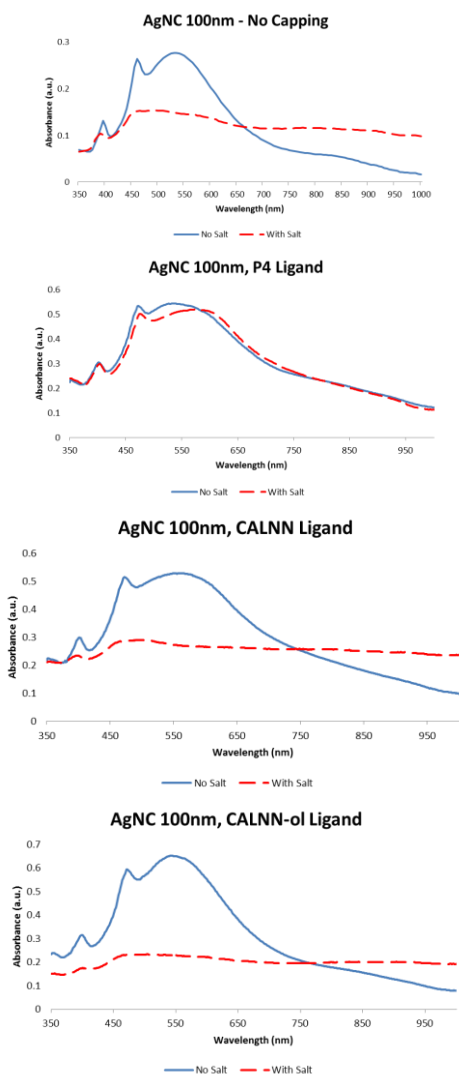

Figure 8: 4 different spectras of 100nm Silver Nanocubes with and without salt in different capping ligands

From this salt-mediated aggregation, it can be deduced that while the 3 ligands (P4, CALNN, CALNN-ol) were effective in capping and stabilizing nanoparticles. However, they were rendered ineffective when exposed to a salt solution. Hence, more work would have to be done in order to fully stabilize these nanoparticles before more widespread use is possible.

D. NanoSight microscopy of capped silver nanoparticles

Nanosight microscopy is a technique used to measure the dispersion of particles in a solution. It works by recording a video of the motion of the particles in a solution (Brownian Motion) The software calculates the size of each particles it sees by tracking its speed of movement (Figure 9).
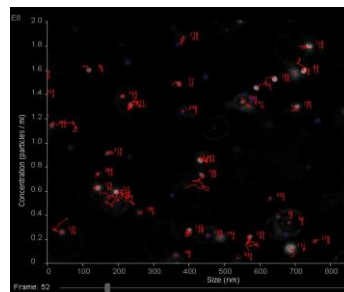

Figure 9: NanoSight tracking of individual nanoparticles in solution

Cumulation of the data from several different videos and thousands of particles allows for a histogram of the size data to be generated (Figure 10).
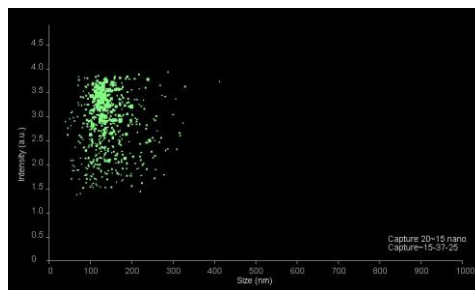

Figure 10: Histogram of size of nanoparticles, generated by NanoSight Microscopy

NanoSight microscopy was used to study several silver nanoparticles. For this report, only the 100nm silver nanocubes will be discussed. As can be seen from the UV-visible spectra, P4, Pep1 and Pep3 all look reasonable, with some changes in the absorbance. Interestingly, the uncharged Pep3 looked very good for stability (Figure 11).
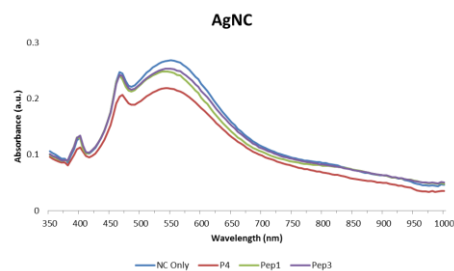

Figure 11: Spectra of Silver Nanocubes with 3 selected ligands – P4, Pep1, Pep3

From the NanoSight analysis, a graph of sizes of stock uncapped nanocubes was generated. The mode size of uncapped cubes is 133 nm. This means that the silver cubes, taking into consideration the charged shell of salt around the particle (electric double layer), behaves in movement as a 133 nm particle. Some larger sizes are observed, possibly due to some slight aggregation or reversible interactions of the stock nanocubes, which is considered normal. On the other hand, the mode for the CALNN-capped particles is 146 nm, and 122 nm for the CALNN-ol capped nanocubes (Supplementary Information Figure S7). From this, it can be inferred that the stability and single particle suspension for CALNN-ol capped nanoparticles is greater. Furthermore, the lower mode (and mean of the main population) for CALNN-ol suggests that the electric double layer observed in the uncapped nanocubes has disappeared. This is as expected for the uncharged surface provided by CALNN-ol, hence showing that CALNN-ol has successfully capped the nanocubes.

IV. Conclusion

While silver nanoparticles are more useful than gold nanoparticles, it can be concluded that they are also more difficult to stabilize. In order to expound on the uses of silver nanoparticles, more research into different methods of nanoparticle capping will be needed in order to come up with a simple, yet effective method of stabilizing nanoparticles by capping ligands. From the experiments, we can conclude that P4, Pep1 (CALNN) and Pep3 (CALNN-ol) are viable peptide ligands for the capping of silver nanoparticles. However, they do need further refinement to make them more resistant, especially towards salt-induced aggregation. Resistance against salt-mediated aggregation is especially important for these nanoparticles as nanoparticles are emerging as potential effective biosensors (Huang. L, Porter. A. L, Guo. Y, n.d.); as biosensors, the nanoparticles would likely be exposed to environments with high salt content.

There are many improvements possible in order to successfully stabilize these gold and silver nanoparticles.

Firstly, more mixes of peptides could have been tested in different ratios in order to maximize the stabilization of the nanoparticle (Free. P, 2012). This is because the capping ligands bind at different rates to the nanoparticle; as a result, one ligand might preferentially cap the nanoparticles. By testing out different mixes with different ratios of peptides, it is hoped that the monolayer protecting the nanoparticle will be optimized to prevent aggregation through both the electrostatic repulsion and steric hindrance, hence increasing its effectiveness.

Secondly, the P4 ligand could be changed to use longer chain ethylene glycols, allowing for even greater steric hindrance and hence, greater stability. This might provide additional stabilization against high-salt environments.

Thirdly, different techniques (such as NanoSight microscopy) could be used in greater depth in order to more accurately determine whether capping was successful. This would greatly improve the accuracy of the results, hence laying the foundation for future research into stabilizing silver nanoparticles.

In conclusion, while several ligands showed potential in stabilizing silver nanoparticles, there is still much work to be done in order to fully stabilize silver nanoparticles for future usage in many different applications, especially since different sized nanoparticles are shown to react differently to the capping ligands.

V. Acknowledgment

VI. References

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Solutions for coping with haze:

- Better early warning systems
  - By incorporating meteorological predictions, a predictive model of the Pollutant Standards Index (PSI) can be made, whereby the estimated PSI for different areas in Singapore can be made known to the public.
  - This would increase public preparedness in the event of a sudden bout of haze attack, as the public would be informed of the impending haze
- Guidelines on how to respond, for the public
  - There should be simple guidelines on how to respond to haze once it sets in
  - The guidelines should be tweaked real-time in accordance to the severity of the haze situation
- Stockpile of face masks
  - The relevant authorities should keep an emergency stock of face masks to be deployed anytime
  - The supply of masks would be guaranteed, preventing price markups which would adversely affect the lower-income families
- Emergency Response Teams
  - Similar to how some cities have Ebola Crisis Teams, which are on standby to deal with any threats related with Ebola
  - Such haze response teams can distribute face masks to both stores and the lower income families to ensure that every person will be able to protect themselves from the haze
  - The teams can also give out information leaflets on what to do in the event of a haze, ensuring that everybody is prepared.
- Curtain of water between Singapore and Indonesia
  - Airships carrying tanks filled with water can suck up seawater and spray it out as a fine mist
  - With numerous airships, the mist can combine to form a curtain of water, whereby the dust particles will be forced downwards out of the air
  - This natural purification has low cost apart from the maintenance cost of the fleet of airships