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Application News

No. FTIR/UV-1402

Spectrophotometric Analysis

Spectroscopic Characterization of Nanoparticles for Potential Drug Discovery

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■ Introduction

Nanoscience/nanotechnology has emerged as one the fastest growing fields of science this decade.[1] Various nanoparticles, including carbon nanotubes, quantum dots, and gold nanoparticles, have been extensively studied for biomedical applications. Of particular interest is the improvement in efficacy and reduction in toxicity that has been demonstrated with these nanoparticle-based drug delivery systems.[2]

The inertness and biocompatibility of gold nanoparticles (Au-NPs) make them very promising for specific applications such as medical imaging, drug delivery, gene delivery, and molecular sensing.[3]

In each of these applications the particle size and shape of Au-NPs is critical. The distinct absorption peak from the surface plasmon absorption of the gold nanoparticles is located between 510 and 530 nm. The surface plasmon is a collective excitation of electrons at the interface between the conductor and insulator.[4] Absorption of light by the surface plasmon of Au-NPs accounts for the colorful appearance of these solutions, which in turn is a direct characteristic of their size and aggregation.

Gold nanoparticles ranging in sizes from 15 to 100 nm are readily available and can be easily synthesized. In a simple preparation scheme Gold nanoparticles can be stabilized by the citrate ion and further functionalized by a wide array of compounds. This scheme allows for the easy preparation of chemistries between the Au-NPs and other compounds of interest.

In this study, Au-NPs were prepared from the citrate reduction of AuCl3 and Au-NP size and refractive index were evaluated using UV-Vis and laser diffraction particle size instrumentation. The Au-NPs were then mixed with various organic compounds and the complexes formed further evaluated spectroscopically. The remainder of this presentation will be a report of the results of these evaluations.

■ Experimental

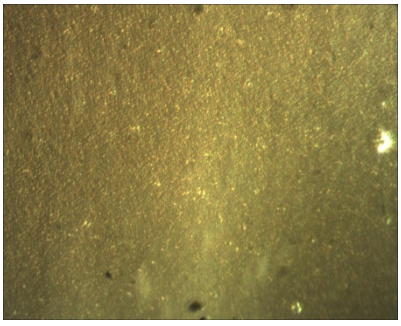
Preparation of Gold Nanoparticles

Gold nanoparticles were synthesized and stabilized by the citrate reduction of a 0.001 Molar chloroauric acid solution. Gold nanoparticles were synthesized using various molar ratios of citrate in order to investigate particle size control in the synthesis. Completion of the reduction reaction could be readily evaluated by polarized optical microscopy.

Particle size of the synthesized Au-NPs was evaluated by monitoring the Au-NP visible absorption band using a Shimadzu UV-2600 UV-Vis spectrophotometer and 2mm quartz cell.

Gold Nano particles newly synthesized demonstrating a deep crimson color

The particle size was confirmed by laser diffraction particle size analysis using a Shimadzu SALD-7101 particle size analyzer. In addition, the refractive index of the An-NPs was estimated by the Becke line method of optical microscopy and confirmed by the SALD-7101 particle size software.



Synthesized Au-NP (after agglomeration) 800X Hoffman Modulation

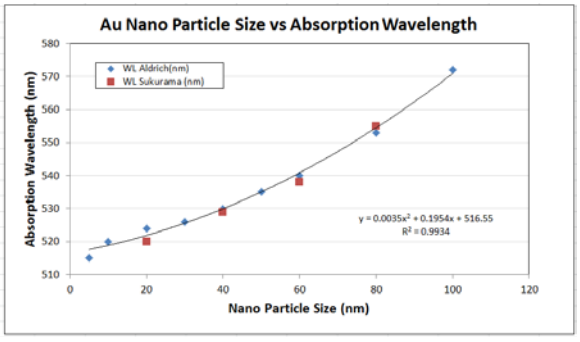
Preparation of Gold Nanoparticles – Organic Complexes
Gold nanoparticle-organic complexes were prepared by mixing the citrate-stabilized Au-NPs with various organic compounds of interest, followed by vortexing. To assure Au-NPs size uniformity freshly prepared Au-NP were used. Organic compounds used were lysozyme, casein, prednisone, protein, posphatidyl choline, and Igg.

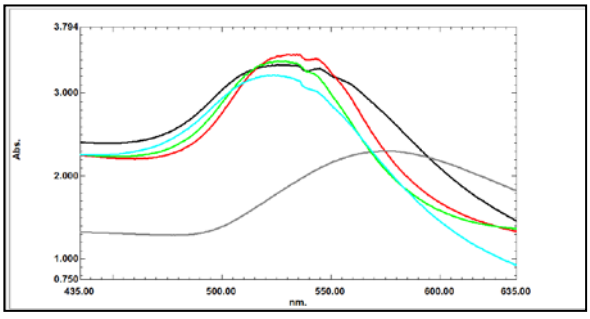
■ Spectral Analysis

UV-Vis spectra of the neat organic solutions and Au-NP complexes were acquired as transmission spectra through a quartz cuvette with 2 mm path length. As such, dilution of the complex mixtures was not necessary. FTIR transmission spectra of the organic neat and complexed Au-NP solutions were acquired by placing 30 uL of the Au-NP solution on a section of IR transparent silicon wafer. The silicon wafer was placed on the surface of a warm hot plate (approximately 130 degree Celsius) to allow evaporation. The silicon wafers were then placed in the FTIR and scans were acquired either by transmission or diffuse reflectance.

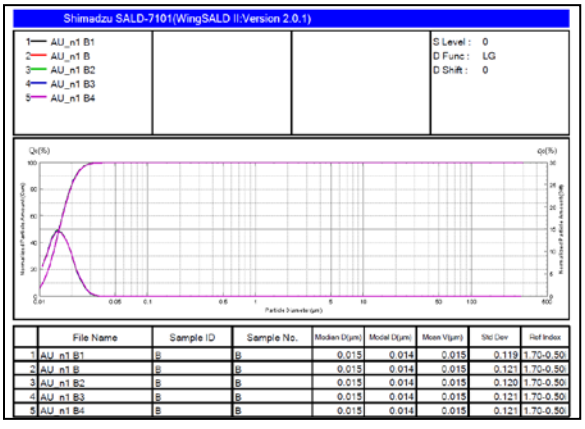
■ Results/Discussion

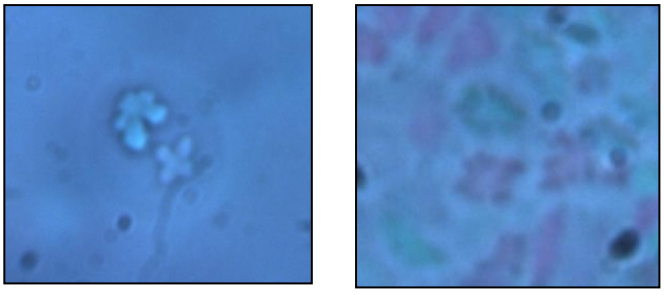
Size Analysis – Spectroscopy
Gold NP Visible absorption directly related to nanoparticle size (left figure below).[6,7] Addition of citrate to the chloroauric acid solution in molar ratios of 0.17 to 1.4 acts to stabilize various nanoparticle size. The Au-NP absorption band moves from 577 nm to 523 nm suggesting an initial Au-NP size of 100 nm and a final Au-NP size of 20 nm at the 1.4:1 citrate to gold molar ratio. All further studies were focused on the 1.4:1 citrate to gold molar ratio with a large Au-NP size of approximately 20 nm.





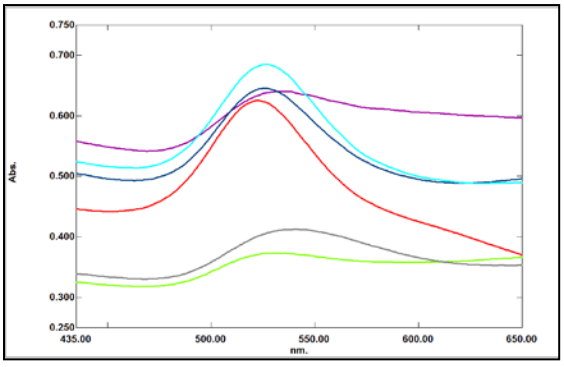
Refractive Index Analysis – Spectroscopy
Particles size measurements using a Shimadzu SALD-7101 provided a Au-NP particles size measurement of 15 nm (+/- 23) and suggested a refractive Index of 1.70. The refractive index of the agglomerated Au-NPs via the Becke line method also suggests an RI of between 1.648 and 1.709.





Becke line into the Au-NP with RI of 1.648 (left) and into RI solution with RI of 1.709 (right).

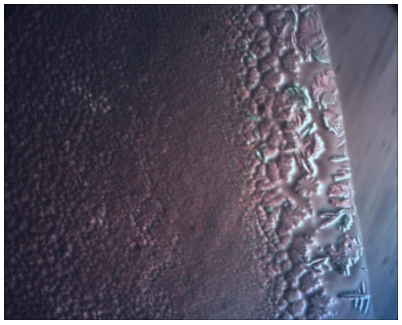
UV-Vis Analysis of Au-NP Complexes – Conjugate formation
UV-Vis spectra of the conjugated Au-NP complexes show the change in the Au-NP absorption as different complexes are formed.



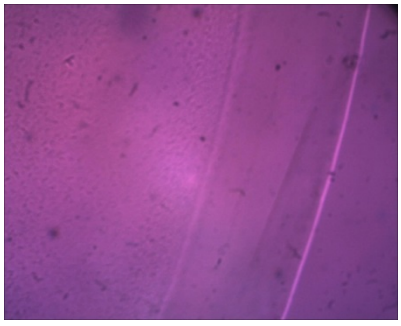
UV-Vis spectra for Au-NP conjugated with organics to form Au-NP complexes.

- Red – Au-NP
- Grey – casein
- Cyan – Igg
- Magenta – phosphatidyl choline
- Green – prednisone
- Blue – protein

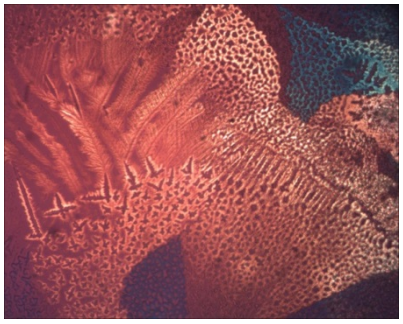
Verification of Crystal Formation
Further confirmation of conjugation was the change in crystal form of the final conjugated complex.



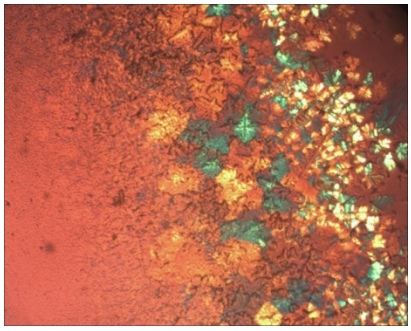
Au-NP 400X Pol-tint



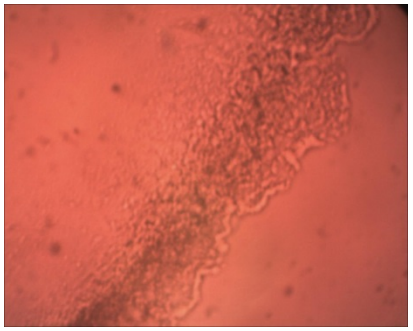
Casein 400X Pol-tint



Casein-AuNP 400X Pol

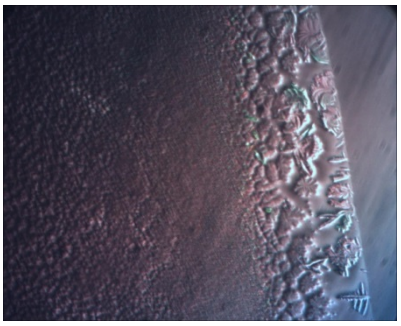


Igg 400X Pol-tint

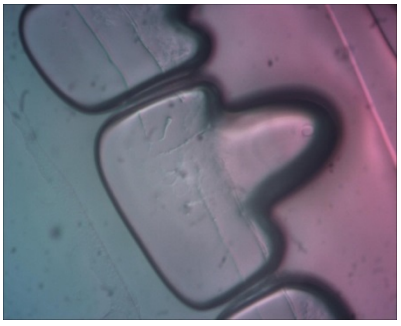


Igg-AuNP 400X Pol

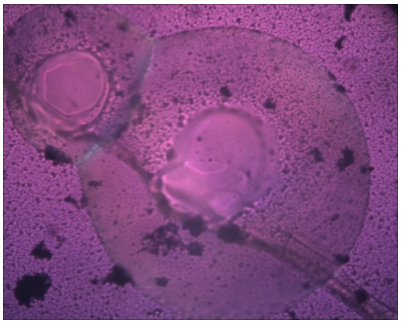
Again, confirmation of conjugation was the change in crystal form of the final conjugated complex.



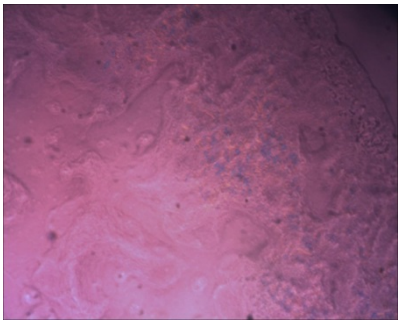
Au-NP 400X Pol-tint



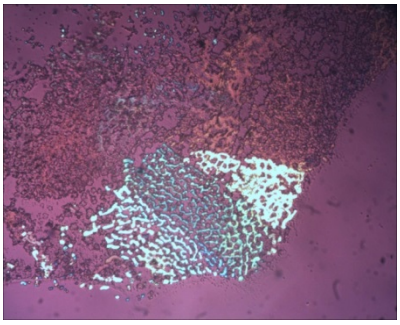
Lysozyme 400X Pol-tint



Lysozyme-AuNP 400X Pol-tint

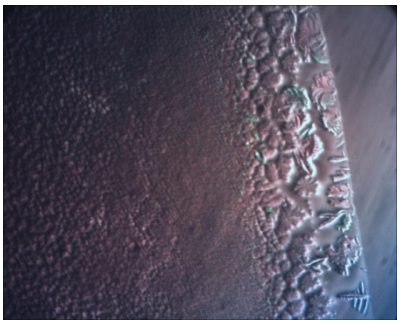


Phosphatidyl choline 400X Pol-tint

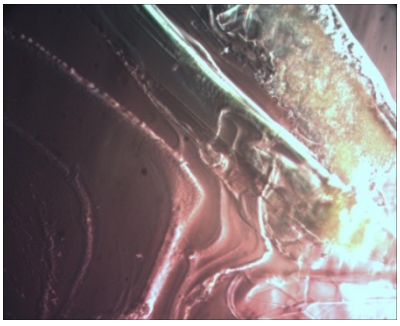


Phosphatidyl choline-AuNP 400X Pol-tint

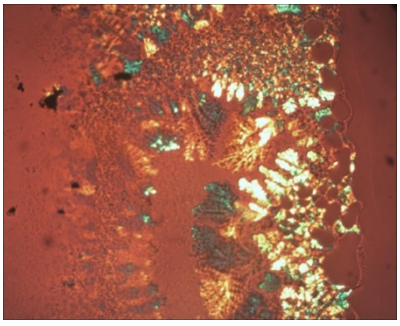
In all complex formation the significant changes in crystal form and anisotropy strongly support the formation of a Au-NP conjugate complex.



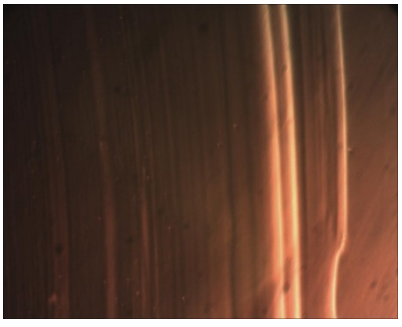
Au-NP 400X Pol-tint



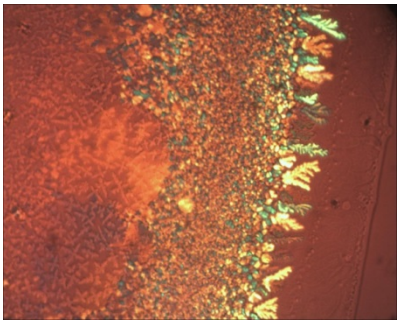
Prednisone 400X HF-PACO



Prednisone-AuNP 400X Pol-tint



Protein 400X HF-PACO

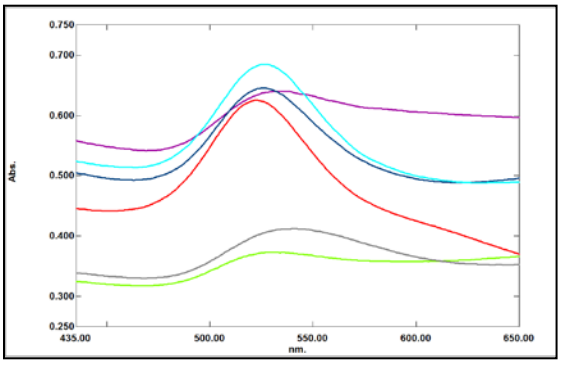


Protein 400X POL-tint

UV-Vis Analysis of Au-NP Complexes – Optimization

The microscopic crystal study suggests that the differences observed in the UV-Vis spectra for the conjugate complex formations are real and may provide a method to easily monitor and characterize complex chemistry. In this quick study, Au-NP formed conjugated complexes were not optimized for any given complex.

Optimization would consist of adjusting the Au-NP size and buffer (pH) for the desired task at hand. UV-Vis analysis is again suggested as an excellent method for monitoring and optimizing Au-NP and Au-NP conjugated complexes.

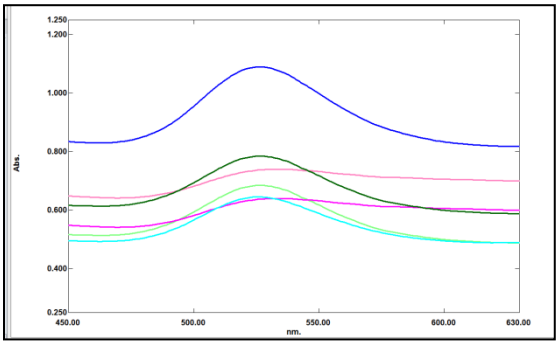


UV-Vis spectra for Au-NP conjugated with organics to form Au-NP complexes.

- Red – Au-NP
- Grey – casein
- Cyan – Igg
- Magenta – phosphatidyl choline
- Green – prednisone
- Blue – protein

UV-Vis Analysis of Au-NP Complexes – Stability

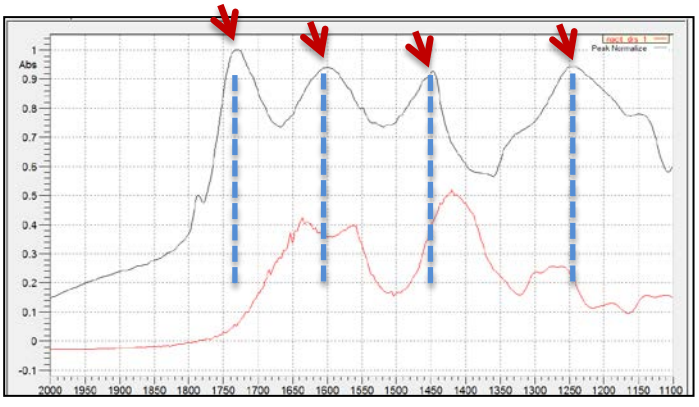
UV-Vis spectra of Au-NP complexes immediately after synthesis and several days after formation showing the stability of the conjugated complex.



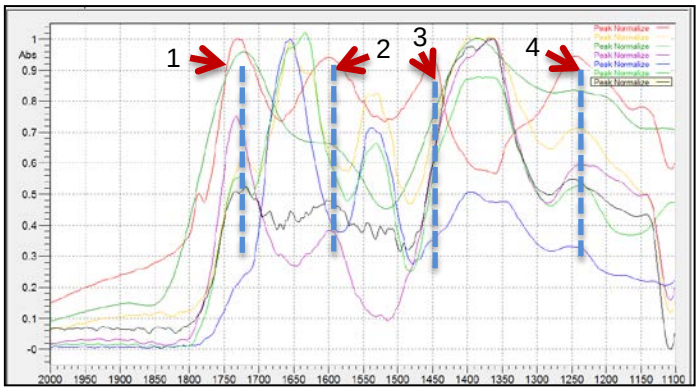
- Blue/Cyan – protein complex
- Green/Light Green – Igg complex
- Magenta/Pink – phosphatidyl choline complex

FT-IT Analysis of Au-NP Complexes

FTIR spectra of the gold nanoparticles (black) and sodium citrate (red). The Au-NP spectrum shows four distinct absorption bands at 1734, 1599, 1448, and 1245 cm^{-1}.

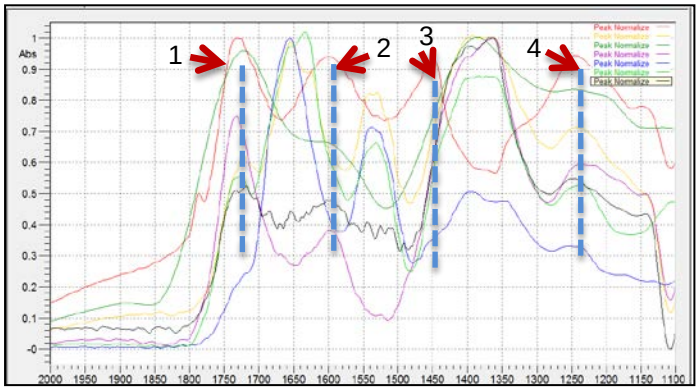


The four characteristic absorption bands of the Au-NP can be readily seen in ALL of the conjugated complexes. The Au-NP absorption bands either show as distinct absorption bands in the conjugated complex or can be observed in the shoulders of other complex characteristic absorption bands.



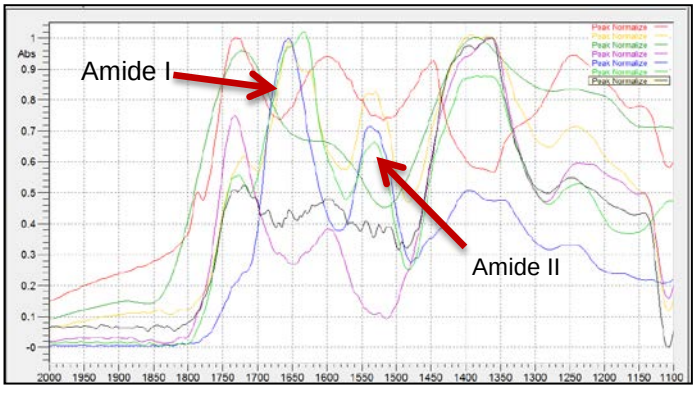
- Red – Au-NP
- Light Green - Igg
- Orange - Protein
- Dark Green – prednisone
- Magenta – phosphatidyl choline
- Black - Casein
- Blue – Lysozyme

The characteristic Au-NP absorption bands do not shift with conjugation. This suggests that the conjugate formation does not involve significant changes to the molecular bonding of the Au-NPs.



- Red – Au-NP
- Light Green - Igg
- Orange - Protein
- Dark Green – prednisone
- Magenta – phosphatidyl choline
- Black – Casein
- Blue – Lysozyme

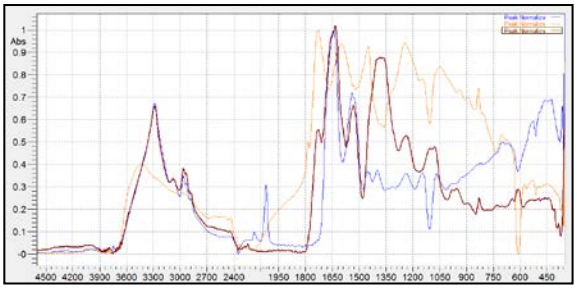
FTIR spectra of the protein, lysozyme, and Igg conjugated complexes show the characteristic Amide I and II bands at 1665 and 1534 cm^{-1}.



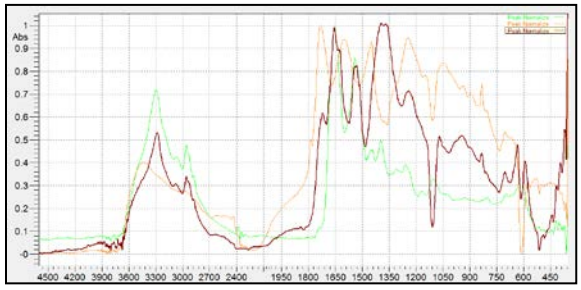
- Red – Au-NP
- Light Green - Igg
- Orange - Protein
- Dark Green – prednisone
- Magenta – phosphatidyl choline
- Black – Casein
- Blue – Lysozyme

These Amide absorption bands do not show shifts from their non-conjugated spectra suggesting that the amide moieties (C=O and N-H) do not take part in formation of the Au-NPs conjugated complexes. This also suggests that any Inter on intra-molecular H-bonding that these functionalities may be involved in are not affected by complexation with the Au-NPs.

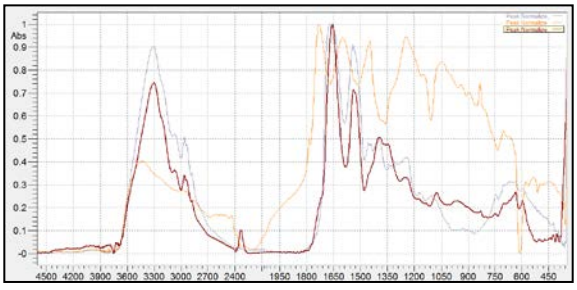
Furthermore, no significant band shape or wavenumber shift is observed in the OH absorptions of these compounds either before or after Au-NP complex formation suggesting that the OH moieties do not play a role in conjugate complex formation. H-bonding does not appear to be a part of the complex formation.



Igg and Igg-Au-NP (red)

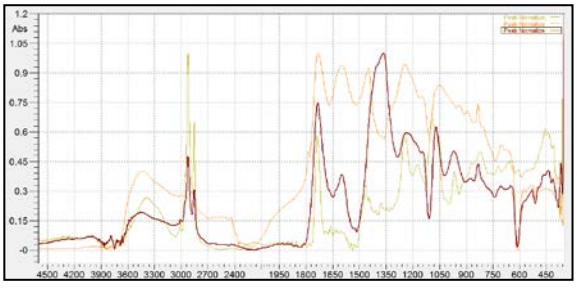


Protein and Protein-Au-Np (red)

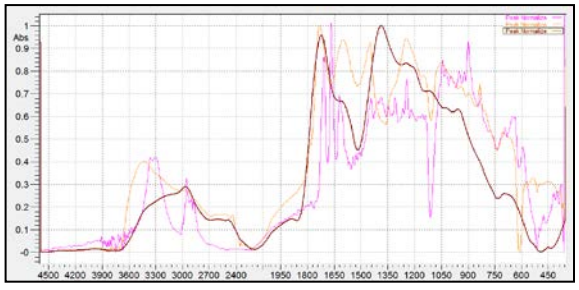


Lysozyme and Lysozyme-Au-NP (red)

The phosphatidyl choline and prednisone spectra, however, do show significant changes in the OH absorption bands before and after Au-NP complex formation suggesting that the OH functionalities and/or H-bonding associated with the OH functionalities of those molecules may either be disrupted with complex formation or may take an active part in the complex formation itself. Shifts and changes in the C=O absorption band also suggest possible H-bonding changes with complex formation.

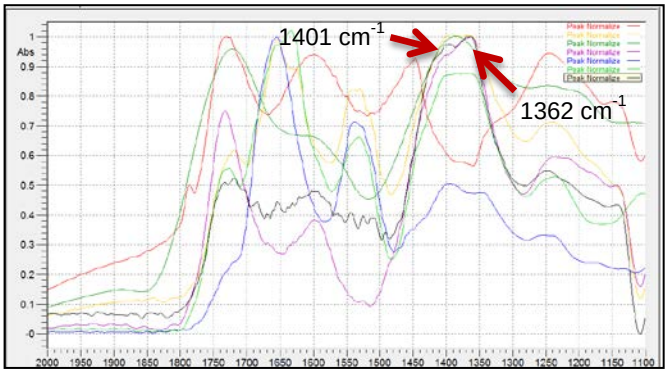


Phosphatidyl choline and PC-Au-NP (red)



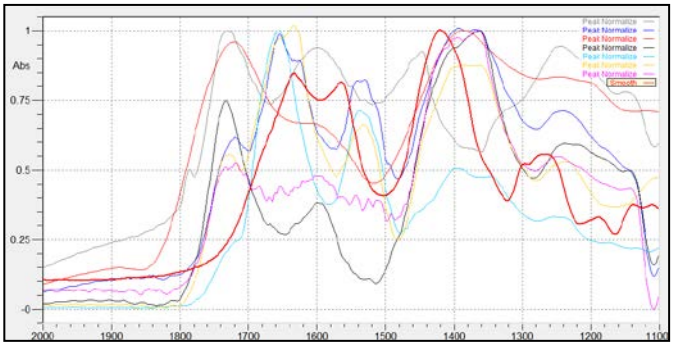
Prednisone and Pred-Au-NP (red)

FTIR spectra of all conjugated complexes show a unique absorption band between 1428 and 1314 cm^{-1} that is not found in the non-conjugated organic spectra. This absorption band is composed of two underlying peaks at 1401 cm^{-1} and 1362 cm^{-1}.



- Red – Au-NP
- Light Green - Igg
- Orange - Protein
- Dark Green – prednisone
- Magenta – phosphatidyl choline
- Black – Casein
- Blue – Lysozyme

The absence of several of the citrate absorption bands in the spectra of the complexes further suggests that the citrate was replaced in the organic complexes (citrate – red).



■ Conclusion

The data presented here have clearly shown the power and advantages of using spectroscopic techniques such as UV-Vis and FTIR to monitor and characterize gold nanoparticles and gold nanoparticle conjugated systems. UV-Visible spectra of conjugated complexes gave information on nanoparticle size and also offered a quick method to aid in the optimization of particle size and buffer pH in conjugate complex formation.

Acquired FTIR spectra readily showed the formation of the conjugate complexes and further may provide information about bonding and structural characteristics of such systems.

Modern UV-Vis spectrophotometers, such as those used in this study, offer wider spectral ranges up to 1400nm and lower stray light values, allowing for photometric ranges easily extending to 8 Abs. These modern benches provide the nanoparticle researcher opportunities to study conjugated systems without the need for dilution. In addition, modern spectrophotometers reaching into the NIR provide the possibility of monitoring conjugated phenomenon, such as a NIR-trigger mechanism for conjugate release.

Newer FTIR systems easily offer rapid scanning with high sensitivity, resolution, and signal-to-noise ratios providing for the possibilities of monitoring nanoparticle reaction kinetics.

Investigations into nanoparticles have already impacted current manufacturing industries, including electronics, photovoltaics, ophthalmics, and medicine. Further advances are on the near horizon. Modern analytical instrumentation will continue to advance to provide the best tools for the researcher's needs and be the support structure that helps bring these advances to fruition.

■ **Acknowledgements**

This work was conducted at Shimadzu Scientific Instruments in Columbia, Maryland as part of an ongoing effort to provide state of the art scientific instrumentation that readily meets the needs of current researchers.

This author acknowledges the efforts of the Shimadzu staff in support of this goal.

Acknowledgements also go to: Dr. Suja Sukuraman, Andrew Shaff, Dr. Robert Clifford.

■ **References**

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