A SERS-BASED INVESTIGATION OF THE MOLECULAR INTERACTION BETWEEN NATURAL ORGANIC MATTER AND COLLOIDAL SILVER NANOPARTICLES IN POROUS MEDIA

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science

By

JESSICA LAUREN FRALEY
B.S., Wilmington College, 2012

Wright State University
2014
WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

July 25, 2014

ABSTRACT

Fraley, Jessica Lauren M.S., Department of Chemistry, Wright State University. 2014. A SERS-Based Investigation of the Molecular Interaction between Natural Organic Matter and Colloidal Silver Nanoparticles in Porous Media.

The significant presence of silver nanoparticles (AgNPs) in numerous consumer products and various applications of today’s society raises major concerns with respect to their release into the environment. Because groundwater represents an important transport pathway from contamination sources to human and environmental receptors, it is critical to understand how natural organic matter (NOM), a main component of groundwater, interacts with colloidal AgNPs and influences their stability and mobility. Thus, this study uses Raman and surface-enhanced Raman spectroscopy (SERS) to examine the possible molecular interactions between widely-used Creighton AgNPs (11 mg L\(^{-1}\)) and NOM (0-160.0 mg L\(^{-1}\) of humic and fulvic acids) in porous media (glass beads) and at various benchmark incubation times (0, 1 hr, 3 hrs, 1 day, 1 week, and 60 days). Additionally, fluorescence spectroscopy was employed to quantify the amount of NOM adsorbed onto AgNPs. It was found that NOM forms surface-complexed species with AgNPs mainly through the ionized carboxylic moieties located in the immediate vicinity of the metallic nanosurface, and the interaction becomes stronger with the increase in incubation time. The fluorescence emission spectra confirmed the Raman/SERS observations of significant fluorescence quenching upon the AgNP addition and the formation of AgNP-NOM-AgNP aggregates, which may decrease AgNP mobility during their groundwater transport.
# TABLE OF CONTENTS

1. Introduction
   1.1 Importance of Silver Nanoparticles
   1.2 Fabrication of Silver Nanoparticles
   1.3 Silver in the Environment
   1.4 Natural Organic Matter and Silver Nanoparticles

2. Hypothesis and Specific Aims
   2.1 Hypothesis
   2.2 Specific Aims
      2.2.1 Specific Aim 1
      2.2.2 Specific Aim 2

3. Materials and Methods
   3.1 Materials
   3.2 Synthesis of AgNPs
   3.3 Characterization of AgNP Samples by UV-Vis Absorption Spectroscopy
      3.3.1 UV-Vis Absorption Spectroscopy Working Principle
      3.3.2 Sample Preparation
      3.3.3 Data Collection
   3.4 Characterization of AgNP Samples by Inductively coupled Plasma Optical Emission Spectroscopy (ICP-OES)
      3.4.1 ICP-OES Working Principle
      3.4.2 Sample Preparation
      3.4.3 Data Collection
   3.5 Characterization of AgNP Samples by Raman and Surface Enhanced Raman Spectroscopy (SERS)
      3.5.1 Raman Working Principle
      3.5.2 Sers Working Principle
      3.5.3 Sample Preparation
      3.5.4 Data Collection
   3.6 Fluorescence Emission Spectroscopy
      3.6.1 Fluorescence Emission Spectroscopy Working Principle
      3.6.2 Sample preparation
      3.6.3 Data collection

4. Results & Discussion
   4.1 Characterization of Colloidal AgNPs
   4.2 Raman and SERS Measurements
   4.3 Fluorescence Spectrophotometry Measurements
5. Conclusions .................................................................................................................. 49
6. References .................................................................................................................. 51
LIST OF FIGURES

Figure 1. Bar chart showing the number of consumer products associated with different nanomaterials. .......................................................... 2

Figure 2. Pie chart illustrating the presence of nanosilver in different sectors of consumer products................................................................. 3

Figure 3. The synthesis setup for producing Creighton colloidal AgNPs. ............................................. 11

Figure 4. Potential electron transitions of π and non-bonding (n) electrons......................... 12

Figure 5. Varian Inc. Cary 50 Bio UV-Vis spectrophotometer located in Dr. Sizemore’s research laboratory at Wright State University.............................. 14

Figure 6. Varian Inc. 710-ES ICP-OES located in the Instrumental Analysis Laboratory of the Chemistry Department at Wright State University.......................... 16

Figure 7. Raman and Rayleigh scattering energy diagram. The lowest energy ground electronic state is represented by m and increasing energy states follow above it... 17

Figure 8. The Horiba Jobin Yvon Inc. LabRamHR 800 system used for the collection of Raman and SERS spectroscopy (located in Dr. Sizemore’s research laboratory at Wright State University). ...................................................... 21

Figure 9. A fluorescence emission energy diagram................................................................. 22

Figure 10. An Agilent Technologies Cary Eclipse fluorescence spectrophotometer located in Dr. Sizemore’s research laboratory at Wright State University........... 23

Figure 11. Image of a Creighton colloid (a), UV-Vis absorption spectra of the Creighton colloid used for the SRHA (b) and SRFA study (c) ........................................ 24
Figure 12. The ICP-OES calibration curve developed from the following concentrations of silver standards: 4.0, 6.0, 20.0, 40.0, 60.0, 200.0, 400.0, 600.0, and 800.0 μg L\(^{-1}\).

Figure 13. Raman and SERS spectra of control samples: a) I) glass beads in water, (II) glass beads in colloidal AgNPs, and (III) 13.4 mg L\(^{-1}\) of Creighton colloidal AgNPs, b) 160.0 mg L\(^{-1}\) of SRHA, c) 160.0 mg L\(^{-1}\) of SRHA mixed with AgNPs, and d) 160.0 mg L\(^{-1}\).

Figure 14. Raman spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) of SRHA. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. Acquisition parameters were a 10 s accumulation time, 5 cycles, and a 200 μm confocal hole. Raman spectra (I)-(IV) were manually shifted upwards for comparison purpose.

Figure 15. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRHA incubated for 1 hr with AgNPs. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition parameters were a 10 s accumulation time, 5 cycles, and a 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purpose.

Figure 16. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRHA incubated for 1 hr with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition parameters were a 10 s accumulation time, 5 cycles, and a 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.
Figure 17. Raman and SERS spectra of control samples: a) I) glass beads in water, (II) glass beads in colloidal AgNPs, and (III) 12.7 mg L\(^{-1}\) of Creighton colloidal AgNPs, b) 160.0 mg L\(^{-1}\) of SRFA, c) 160.0 mg L\(^{-1}\) of SRFA mixed with AgNPs, and d) 160.0 mg L\(^{-1}\) of SRFA mixed with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) region for spectra b)-d). Acquisition time was 10 s and confocal hole was set at a) I) 300 μm, and a) II)-III), b)-d) 200 μm. All spectra were averaged over 5 cycles to improve signal-to-noise ratio.

Figure 18. Raman spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRFA. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. Acquisition parameters were 10 s accumulation time, 5 cycles, and 200 μm confocal hole. Raman spectra (I)-(IV) were manually shifted upwards for comparison purposes.

Figure 19. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRFA incubated for 1 hr with AgNPs. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition parameters were 10 s accumulation time, 5 cycles, and 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.

Figure 20. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRFA incubated for 1 hr with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition
parameters were 10 s accumulation time, 5 cycles, and 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.

Figure 21. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

Figure 22. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

Figure 23. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA-AgNPs immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

Figure 24. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA-AgNPs in the presence of glass beads immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

Figure 25. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRFA immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

Figure 26. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRFA-AgNPs immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

Figure 27. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRFA-AgNPs in the presence of glass beads immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.
### LIST OF TABLES

**Table 1** Observed Raman and SERS vibrational modes (in cm\(^{-1}\)) for SRHA, and their tentative assignment according to literature .......................................................................................................................... 28

**Table 2** Observed Raman and SERS vibrational modes (in cm\(^{-1}\)) for SRFA, and their tentative assignment according to literature .......................................................................................................................... 35

**Table 3** Fluorescence emission intensity of the 160.0 mg L\(^{-1}\) SRHA-AgNPs-glass beads and SRFA-AgNPs-glass beads samples and the corresponding controls .......................... 48
ACKNOWLEDGEMENTS

The Raman and SERS work described in this thesis was submitted for publication together with our research collaborators, Dr. Kanel Sushil and Dr. Mark Goltz, from the Air Force Institute of Technology at Wright Patterson Air Force Base. The manuscript is currently under review by the Journal of Nanoparticle Research. Drs. Sushil and Goltz are highly acknowledged for providing us with some of the natural organic matter samples.

Manuscript title: Influence of Natural Organic Matter on Fate and Transport of Silver Nanoparticles in Saturated Porous Media: Laboratory Experiments and Modeling

Authors: Sushil R. Kanel*, Jason Flory¹, Allie Meyerhoefer², Jessica L. Fraley², Deborah Roose³, Ioana E. Sizemore², and Mark N. Goltz*¹

¹Department of Systems Engineering and Management, Air Force Institute of Technology, 2950 Hobson Way, Wright-Patterson AFB OH 45433
²Department of Chemistry, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435
³United States Environmental Protection Agency, National Risk Management Research Laboratory, Water Supply and Water Resources Division, Water Quality Management Branch, 26 W. Martin Luther King Dr., Cincinnati, Ohio 45268
1. INTRODUCTION

1.1. IMPORTANCE OF SILVER NANO PARTICLES

With the recent innovations in the field of nanoscience, nanotechnology has merged with practical consumer demands.\(^1\) Any time we gain access to new technology that allows businesses to invent or improve current products, economic growth and interest in that industry will follow. The global demand for nanomaterials in every day products has elevated the term “nano” from esoteric jargon to being ubiquitous in our society.\(^2\)

Nanomaterials consist of particles with an external dimension in the 1-100 nm-size range and an internal structure of one or more dimensions in the 1-100 nm-size range.\(^3\) The global market’s demand for nanoscience is experiencing unprecedented growth as over 4,000 patents have been issued in the U.S. alone, and the industry’s linear growth is expected to surpass 3 trillion USD by the year 2020.\(^2,4,5\)

While a diverse selection of nanomaterials are available, silver nanoparticles (AgNPs) have received pronounced attention mostly due to their unique antimicrobial and optical properties that arise from their small dimensions and high ratio of surface area to volume.\(^6\) The Project on Emerging Nanotechnologies indicated that there are currently over 380 global consumer products that contain nanosilver (Figure 1).\(^4\) Figure 1 demonstrates the vast growth of consumer products containing nanomaterials from 2006
to 2013. Among these, silver emerges as the leading major material in consumer products.

![Bar chart showing the number of consumer products associated with different nanomaterials.](image)

**Figure 1.** Bar chart showing the number of consumer products associated with different nanomaterials.\(^4\)

AgNPs are present in a variety of sectors such as cosmetics, athletic gear, target drug delivery, automotive maintenance products, and SERS-based sensing, to reference just a few (Figure 2).\(^4\) For instance, the antimicrobial properties of AgNPs are largely exploited in the production of athletic apparel (Figure 2) due to their ability to eliminate body odor and bacteria.\(^6\) Similarly to the athletic apparel in the health and fitness sector, most nanosilver-based consumer products take advantage of its antibacterial properties. The AgNP main mechanism of antibacterial action involves the attachment to and penetration of bacterial cell wall, which induces structural changes to the cell membrane and causes cell death.\(^6\)
Figure 2. Pie chart illustrating the presence of nanosilver in different sectors of consumer products.4

1.2. Fabrication of Silver Nanoparticles

The synthesis of nanosilver can occur by “top-down” methods, used largely by engineers, or “bottom-up” methods, the preferred technique of chemists.7,8 In a top-down method, starting bulk material is reduced into nanomaterials by a chemical, physical, or mechanical process such as the laser ablation of thin, bulk silver sheets.7,8 Bottom-up methods encompass physical or chemical environments for atoms or molecules to react under in order to generate nanomaterials.7,8 The Creighton method for synthesizing colloidal AgNPs is a commonly used bottom-up technique due to its low cost, minimal time constraint, and manageability.9 Furthermore, the Creighton method was employed in this study because the metal salt precursor, silver nitrate, and the reducing agent, sodium borohydride, are among the most widely used reagents for the production of AgNPs.10
Tolaymat et al. (2010) investigated 200 articles describing different bottom-up techniques for the synthesis of AgNPs.\textsuperscript{10} The review indicated that out of the eight different silver salt precursors that are heavily used in bottom-up methods, silver nitrate accounted for 83\% and was the most commonly employed.\textsuperscript{10} Additionally, out of the 14 different reducing agents observed, 23\% of the studies used sodium borohydride, making it the most commonly used as well.\textsuperscript{10}

\section*{1.3. Silver in the Environment}

Currently, it is known that silver metal has a natural abundance in the earth’s crust of 0.1 ppm, 0.2-2.0 ppb in surface waters, and 0.3 ppm in soil.\textsuperscript{11} In the U.S., the estimated annual anthropogenic release of silver metal was 77,700 kg in air, 125,000 kg in water, and 1,010,000 kg in soil in 1978.\textsuperscript{11} To my best knowledge, there is no other current public record of the estimated annual anthropogenic release of silver in the U.S. Major contributors to the anthropogenic release of silver metal into the environment are metal production, urban waste, sewage treatment plants, and the photographic industry.\textsuperscript{11} Furthermore, the U.S. Environmental Protection Agency (U.S. EPA) established nanosilver as a primary water pollutant in 1977, and imposed an oral intake limit of 5.0 \textmu g/kg/day of nanosilver based upon its ability to cause argyria.\textsuperscript{6} Argyria is a medical condition that was found to cause skin darkening in sunlight as a result over exposure and bioaccumulation of nanosilver in the body.\textsuperscript{12} Thus, U.S. EPA is currently funding research to fully understand and regulate the toxicity and environmental implications of nanosilver.\textsuperscript{6} Although silver metal is being released into the environment in considerable
amounts and as indicated above, some regulations have been imposed, marginal
information is known about the fate and transport of AgNPs. Most studies have been
focused on AgNP production and toxicity. This may be due to the fact that
environmental samples contain an immense assortment of organic materials and
impurities that prove to be a challenge to analyze. In order to fully understand if the
environmental release of AgNPs poses a health risk to humans, it is imperative to study
their fate and transport. Recent studies have provided some insight into the fate and
transport of AgNPs. One such study demonstrated a majority of nanosilver is released
from plastics and textiles into wastewater and subsequently ends up in sewage that is
spread over agricultural fields. Another study conducted in the UK revealed that around
8.8 tons of AgNPs are released into waste water systems annually, with sewage sludge
being the primary receiver of AgNPs from waste water systems. Additionally, it has
been suggested that AgNP accumulation could be detrimental to waste water treatment
plants, if their concentration reaches 0.1 mg L$^{-1}$ due to their ability to hinder the growth
of needed “good” bacteria. Groundwater, the focus of this project, is another important
potential pathway of AgNPs release because of its ability to intersect contaminated
sources (i.e., run off from manufacturing plants) and lead them to both humans and the
environment.

1.4. NATURAL ORGANIC MATTER AND SILVER NANOPARTICLES

The primary component of groundwater is natural organic matter (NOM). NOM is a
material that is produced when microorganisms decompose organic matter under either
aerobic or anaerobic conditions.\textsuperscript{16} Numerous researchers have attempted to unveil the chemical structure of the complex matrix of NOM.\textsuperscript{16} Three main components of NOM were obtained using various extraction techniques: humic acid, fulvic acid, and humin.\textsuperscript{16} Humin is an insoluble substance that remains after soil is treated with an alkali solution for extraction.\textsuperscript{16} The residual alkali solution contains humic and fulvic acids. Humic acids can be further separated by lowering the pH of the solution to 1.0.\textsuperscript{17}

There have been several opposing theories reported as to whether or not the presence of NOM stabilizes or destabilizes NPs. One such study conducted by Chen et al.\textsuperscript{2007} investigated the interaction between fullerene C\textsubscript{60} NPs and humic acid using dynamic light scattering (DLS).\textsuperscript{18} The fullerene NPs were synthesized using the method of Andrievsky et al. to produce mostly spherical NPs with electrophoretic mobility.\textsuperscript{18} The study found that fullerene NPs in the presence of humic acid and NaCl or MgCl\textsubscript{2} electrolytes showed increased stability through steric repulsion due to the adsorption of humic acid on the fullerene NPs.\textsuperscript{18} In contrast, fullerene NPs became destabilized when exposed to high concentrations of CaCl\textsubscript{2} due to humic acid macromolecules bridging to the NPs and forming large aggregates.\textsuperscript{18} Another study conducted by Cumberland et al.\textsuperscript{2009} reported that citrate-stabilized AgNPs showed size-reduction and increased steric and charge stabilization in the presence of NOM due to the formation of a surface coating on AgNPs.\textsuperscript{19} However, the mechanism of charge stabilization of AgNPs in the presence of NOM is not yet fully understood.\textsuperscript{19} The same research group also noted that the size of AgNPs increased with the increase in pH from 5 to 8 at low ionic strength.\textsuperscript{19} In
turn, this facilitated AgNP-aggregation and decreased their stability under the mentioned environmental conditions. Increasing the ionic strength from $10^{-3}$ to $10^{-2}$ M of Na$^+$ ions increased the stability of AgNPs.\textsuperscript{9} Sagee et al. (2012) found that 30 nm AgNPs synthesized in a similar manner by the reduction with sodium citrate displayed a significant increase in mobility during column experiments in the presence of NOM.\textsuperscript{20} El Badawy et al. (2010) observed that uncoated AgNPs (hydrogen-reduced) and electrostatically stabilized AgNPs (citrate-, borohydride-, and branched polyethyleneimine-stabilized) aggregated at higher ionic strength and/or acidic pH. In contrast, pH or ionic strength had no influence on the aggregation state of sterically stabilized AgNPs (PVP-capped).\textsuperscript{21} Overall, these studies further emphasize the need for understanding and defining the complex nature of the mechanisms of interaction that occur between NPs and NOM.\textsuperscript{20}

Groundwater represents an important transport pathway from contamination sources to human and environmental receptors. Thus, it is critical to understand how NOM interacts with colloidal AgNPs, and influence their stability and mobility, i.e., their fate and transport through groundwater. Because very little is known in this regard, this study will examine the possible molecular interactions between water soluble NOM (0-160 mg L$^{-1}$ of humic and fulvic acids) and the widely-used Creighton AgNPs (~13 mg L$^{-1}$ of nanosilver) in the presence of a porous media and at various benchmark incubation times (0, 1 hr, 3 hrs, 1 day, 1 week, and 60 days).\textsuperscript{15} Glass beads will be utilized as porous media to simulate the environment of groundwater, and consequently NOM and AgNPs,
traveling through soil and sediment. The selection of the NOM concentration was based on the fact that the concentration of NOM in groundwater is in general a few mg L$^{-1}$, while contaminated groundwater may have NOM amounts in the hundreds of mg L$^{-1}$ range. No other ions will be added and pH (7.0-7.8) will be maintained constant in this initial study.
2. HYPOTHESIS AND SPECIFIC AIMS

2.1. HYPOTHESIS

It is hypothesized that NOM forms surface-complexed species with the Creighton AgNPs through the ionized carboxylic moieties located in the immediate vicinity of the nanosurface.

2.2. SPECIFIC AIMS

2.2.1. SPECIFIC AIM 1

To determine the possible molecular interactions between varying concentrations of NOM (0, 1, 10, 40, 80, and 160 mg L\(^{-1}\) of humic and fulvic acids in the final mixtures) and Creighton AgNPs (~11 mg L\(^{-1}\) in the final mixtures) in the presence and absence of glass beads and for various incubation times (0, 1 hr, 3 hrs, 1 day, 1 week, and 60 days) using Raman and surface-enhanced Raman spectroscopy (SERS).

2.2.2. SPECIFIC AIM 2

To quantify the amount of NOM that attaches to AgNPs under the above mentioned conditions using fluorescence emission spectroscopy.
3. MATERIALS AND METHODS

3.1. MATERIALS

Suwannee River Humic Acid (SRHA) and Suwannee River Fulvic Acid (SRFA) were purchased from the International Humic Substances Society (International Humic Substances Society, St. Paul, MN). The elemental composition of SRHA is H₂O (20.4 % w/w), Ash (1.04 % w/w), C (52.63 % w/w), H (4.28 % w/w), O (42.04 % w/w), N (1.17 % w/w), S (0.54 % w/w), and P (0.013 % w/w). The elemental composition of SRFA is H₂O (16.9 % w/w), Ash (0.58 % w/w), C (52.34 % w/w), H (4.36 % w/w), O (42.98 % w/w), N (0.67 % w/w), S (0.46 % w/w), and P (0.004 % w/w). All solutions were prepared with ultrapure water (Millipore, 18.2 MΩ cm). Acid washed glass beads (425-600 μm) were purchased from Sigma-Aldrich (Sigma-Aldrich, Co., St. Louis, MO). The pH meter (SevenMulti™) and pH probe (InLab™ Expert Pro-ISM-IP-67) were from Mettler Toledo™. Standard buffer solutions of pH 4.01, 7.00, and 10.01 were purchased from Thermo Scientific. Glassware was cleaned before use with 10 % nitric acid (HNO₃) and rinsed with ultrapure water. All chemicals used for AgNP synthesis and sample preparation for Raman, SERS, and fluorescence emission spectroscopy measurements were high-grade reagents purchased from Fisher Scientific.
3.2. SYNTHESIS OF AgNPs

Colloidal AgNPs were synthesized using the Creighton method by the reduction of silver nitrate (1 mM of AgNO$_3$) with sodium borohydride (2 mM of NaBH$_4$) in ultra-pure water. Briefly, 50 mL of AgNO$_3$ solution was added drop-wise to 300 mL of NaBH$_4$ solution, while continuously being stirred (225 rotations per minute). Both solutions were pre-cooled to 0°C before use. The complete set up for the synthesis of Creighton colloidal AgNPs can be observed in Figure 3. The NaBH$_4$: AgNO$_3$ mM ratio of 2: 1 was found to produce a stable colloid and to effectively reduce Ag$^+$, while minimizing the excess NaBH$_4$.²

Figure 3. The synthesis setup for producing Creighton colloidal AgNPs.
3.3. CHARACTERIZATION OF AgNP SAMPLES BY UV-Vis ABSORPTION SPECTROSCOPY

3.3.1. WORKING PRINCIPLE

Ultraviolet-visible (UV-Vis) absorption spectroscopy is an effective spectroscopic technique at obtaining quantitative (*i.e.*, identifying impurities in organic samples) and qualitative (*i.e.*, determining the presence organic compounds in samples) data about various molecules and compounds.\(^{23}\) Molecules that are irradiated with UV (200 to 400 nm) and visible (400 to 800 nm) radiation are provided with enough energy to excite a molecular electron, namely \(\pi\)-electrons or non-bonding electrons, to a higher energy anti-bonding orbital, \(\pi^*\) and \(\sigma^*\) (Figure 4).\(^ {23, 24}\) The most favored of these electron transitions will be the one from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).\(^ {23}\)

![Potential electron transitions of \(\pi\) and non-bonding (\(n\)) electrons.](image)

**Figure 4.** Potential electron transitions of \(\pi\) and non-bonding (\(n\)) electrons.\(^ {24}\)

After molecules are exposed to light that have the same energy of a potential electronic transition within the molecules, a portion of that energy is absorbed when an electron is excited to a higher energy orbital.\(^ {23}\) A UV-Vis absorption spectrophotometer records this absorption of UV-Vis radiation as a function of wavelength. A UV-Vis
absorption spectrum may be generated to display transmittance, absorbance, or molar absorptivity ($\varepsilon$) versus wavelength.\textsuperscript{23}

AgNPs exhibit special optical properties that are not present in bulk silver. These optical properties emerge due to the combined oscillations of non-localized electrons in nanosilver when exposed to electromagnetic radiation.\textsuperscript{25} This collective oscillation displaces particle electrons from equilibrium causing an electrostatic force to arise between the electrons and nuclei in order to restore balance.\textsuperscript{9, 25} The restoring electrostatic force induces an oscillation of the electron cloud comparative to the nuclear structure, termed surface plasmon resonance (SPR), which can be experientially detected near 400 nm for Creighton AgNPs using a UV-Vis absorption spectrophotometer.\textsuperscript{9, 25}

3.3.2. Sample Preparation

In order to confirm the formation of Creighton AgNPs, 2.5 mL of ultrapure water and 0.25 mL of the Creighton colloid were mixed and placed in a 3 mL cuvette for SPR analysis by UV-Vis absorption spectroscopy. This consists of 1:10 volume dilution that typically leads to an absorbance value below the detection limit of the used instrument.

3.3.3. Data Collection

The SPR peak of the AgNP samples was analyzed using a UV-Cary 50 absorption spectrophotometer (Varian Inc.).
3.4. **Characterization of AgNP Samples by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)**

3.4.1. **Working Principle**

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is a common analytical technique used for trace elemental analysis (down to the ppb range) in a variety of samples. To begin, a chemically digested sample is injected into the instrument via a peristaltic pump, the sample is then sent to the nebulizer, where it becomes an aerosol that is atomized in the ICP torch and excited within the argon plasma.

The plasma, ionized gas at extremely high temperatures (6,500 to 10,000 K), serves as a source to thermally excite analyte ions to emit element-specific wavelengths. The ICP torch is made up of three quartz tubes, through which are argon gas flows, which are surrounded by a water-cooled induction coil that is powered by a radio-frequency (RF). The applied RF power induces an alternating current that fluctuates within the induction coil, which consequently triggers a fluctuating magnetic field to arise around...
the coil. The flowing argon gas is first ionized by a spark from a Tesla coil, and the ions produced interact with the oscillating magnetic fields within the induction coil. This interaction between ions and the magnetic field causes the ions to rapidly alternate directions, which generates inductively coupled or induced energy.

Once analyte ions are thermally excited by the energy provided from the plasma, the ions relax back to the ground state by emitting a photon. The photons released have element-specific wavelengths, which allows for the identification of the elements within the analyte. A spectrometer then separates and quantifies this emitted energy to correlate its intensity to the element concentration using a calibration curve.

3.4.2. Sample Preparation

In order to remove any trace metal contaminants, all glassware was stored in 10 % HNO₃ and washed with ultrapure water prior to the sample preparation. A 10,000 μg mL⁻¹ ICP-OES silver standard (Ultra Scientific) was utilized for the preparation of nine Ag⁺ standards through quantitative dilutions in a 2% HNO₃ matrix in order to avoid silver leaching. The Ag⁺ standards, which were employed for the construction of an external calibration curve, had the following concentrations: 4, 6, 20, 40, 60, 200, 400, 600, and 800 μg L⁻¹. The method blank and aliquot samples of the Creighton colloid were first subjected to a cold digestion for 15 min and then to a hot digestion (225 °C) for 25 min until around 200 μL of sample remained. The samples were chemically digested using 70 % HNO₃ and kept in a 2% HNO₃ matrix.

3.4.3. Data Collection

Measurements were carried out using a Varian Inc. 710-ES inductively coupled optical emission spectrometer (Figure 6) for the quantification of nanosilver in the
Creighton colloids. The original Creighton colloid samples were measured in triplicate with an acquisition time of 10 sec, stabilization time of 25 sec, and a sample uptake delay of 45 sec. The ICP-OES torch of this instrument is made up of three concentric quarts tubes, in the axial position, through which argon gas flows. The other acquisition parameters were the following: a wavelength of 328.068 nm for Ag, a radio frequency (RF) power of 1.20 kW, a plasma flow of 15 L min\(^{-1}\), auxiliary flow of 1.50 L min\(^{-1}\), and a nebulizer pressure of 200 kPa.

Figure 6. Varian Inc. 710-ES ICP-OES located in the Instrumental Analysis Laboratory of the Chemistry Department at Wright State University.
3.5. **CHARACTERIZATION OF AgNP SAMPLES BY RAMAN AND SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)**

3.5.1. **RAMAN WORKING PRINCIPLE**

Raman spectroscopy provides vibrational information about the different bonds within chemical compounds through the inelastic backscattering of monochromatic light. The information gathered from Raman spectroscopy is extensively utilized in the identification of sample components and obtaining structural and physical information about chemical compounds. In Raman scattering, a single beam radiation (usually in the form of a laser), that should not be absorbed, is used to excite the vibrations and rotations in a molecule. When this radiation occurs, a molecule is excited from its ground state to an unstable and short-lived virtual energy state, and the radiation beam is merely scattered into space. As the molecule relaxes, it emits a photon and returns to a different rotational or vibrational state (Figure 7), and the energy difference is measured.

Figure 7. Raman and Rayleigh scattering energy diagram. The lowest energy ground electronic state is represented by $m$ and increasing energy states follow above it.
The scattered radiation may disperse in the form of Rayleigh, Stokes, or anti-Stokes scattering (Figure 5). In Rayleigh (elastic) scattering, photons do not experience an energy change and the molecules return to the ground electronic state, emitting a photon of the same energy. Most of the photons present experience Rayleigh scattering. A minimal number (roughly one in $10^6$) experience Raman or inelastic scattering. In Raman scattering, a photon that is scattered with lower energy than the incident radiation is referred as Stokes scattering and molecules that return to a higher energy state than the one corresponding to the incident radiation experience anti-Stokes scattering.

### 3.5.2. SERS Working Principle

Surface-enhanced Raman Spectroscopy (SERS) is a division of Raman spectroscopy that retains all of the vibrational quantification capabilities of Raman spectroscopy, but has much lower detect limits (down to the single molecule level). Obtaining SERS involves getting an enhanced Raman signal from nanoscale noble metal substrates, to which molecules have adsorbed on. The two theories currently used to explain the great surface-enhancement experienced in SERS are the electromagnetic and charge transfer enhancement theories.

The electromagnetic enhancement theory suggests an interaction between the laser beam and the surface of a nanometal substrate. This interaction is believed to cause the non-localized electrons on the metals surface to go into an excited state proceeding to surface plasmon resonance and an increased incident and scattered electromagnetic (EM) fields. This enhancement is the most prominent when the plasmon frequency is in tone with the radiation and oscillating perpendicular to the surface.
The charge transfer theory shows that chemical bonds may be formed between the metal surface and the sample producing a chemisorbed species. This chemisorbed species allows for metal surface to analyte charge transfer to occur, thus causing the molecule to become more polarized. The enhancement is understood to occur due to the formation of new electronic states as a result of the metal surface to analyte bond. Hence, in the charge transfer theory, radiation is absorbed into the metal surface rather than absorbed or scattered onto surface plasmons.

### 3.5.3. Sample Preparation

In order to study the potential interaction between Creighton AgNPs and SRHA, mixtures consisting of 1, 10, 40, 80, and 160 mg L\(^{-1}\) of SRHA and 13.4 mg L\(^{-1}\) of AgNPs were prepared. For the preparation of 160 mg L\(^{-1}\) of SRHA-AgNP sample, 1.6 mL of 1,000 mg L\(^{-1}\) of SRHA stock solution (pH 3.4) was combined with 8.4 mL of 12.7 mg L\(^{-1}\) of Creighton colloid in a test tube. Serial dilutions were then performed to prepare the remaining samples (1, 10, 40, and 80 mg L\(^{-1}\)) so that each SRHA-AgNP sample had a final volume of 10 mL and the same AgNP amount. Two milliliter aliquots of each SRHA-AgNP sample were combined with 0.50 g of glass beads in a test tube. The test tubes were manually shaken for 5 min and measurements were taken immediately, at 0, 1 hr, 3 hrs, 1 day, 1 week and 60 days after preparation. This procedure was followed to study the possible interaction between another batch of Creighton AgNPs (12.7 mg L\(^{-1}\)) and SRFA at the same concentrations and for identical time periods. Samples were prepared from a 1,000 mg L\(^{-1}\) of a stock solution of SRFA at pH 3.1. The pH of the final SRHA- and SRFA-AgNPs sample mixtures were from 7.0-7.8. Control samples included AgNPs, SRHA, SRFA, glass beads, AgNPs-glass beads, AgNPs-SRHA samples without
glass beads, and AgNPs-SRFA- without glass beads, all in the same concentrations and time durations.

3.5.4. DATA COLLECTION

The Raman and SERS measurements were performed (in duplicate) using a Horiba LabRamHR 800 system (Jobin Yvon Inc., Figure 8) and a HeNe laser (wavelength of 632.8 nm and laser power at the sample of 15 mW). Two milliliter sample aliquots were placed in a quartz cuvette, onto the Raman microscope stage. A high resolution confocal Raman microscope (high stability BX41) and an Olympus objective (50x) were used to direct the laser beam onto the sample. Spectra were collected using an open electrode thermo-electric cooled charge-coupled device (CCD) detector (1024x526 pixels), a holographic grating of 600 grooves mm$^{-1}$, a confocal hole of 200 or 300 μm, and the LabSpec v.5 software. Under these experimental conditions, the spectral resolution was about 1 cm$^{-1}$. All spectral data were the processed in Origin 8 software.
Figure 8. The Horiba Jobin Yvon Inc. LabRamHR 800 system used for the collection of Raman and SERS spectroscopy (located in Dr. Sizemore’s research laboratory at Wright State University).

3.6. **Fluorescence Emission Spectroscopy**

3.6.1. **Working Principle**

Fluorescence can be measured when a sample is excited from its ground state to a higher vibrational state with an excitation wavelength, and the resulting short-lived emission (fluorescence) is evaluated at a longer emission wavelength. When sample molecules decay from their excited state and a photon of the same energy as the excitation photon may not be released, molecules may de-excite through a radiationless transition (Figure 9).
In this radiationless process, there is a decrease in energy but no light is emitted, which allows the molecule to release a photon of proportional energy to the difference between the two energy levels. This emission of light at a longer wavelength (lower energy) than the absorbed one is called fluorescence.

### 3.6.2. Sample Preparation

To quantify the amount of AgNPs that is attached to SRHA at 1, 10, 40, 80, and 160 mg L\(^{-1}\), 2 mL of SRHA-AgNPs was combined in test tubes with approximately 0.50 g of glass beads. The test tubes were manually shaken for 5 min and measured in a 1 mL fluorescence quartz cuvette immediately, at 0, 1 hr, 3 hrs, 1 day, 1 week, 60 days after preparation. The above protocol was followed in order to quantify the amount of AgNPs attached to SRFA at the same concentrations and for identical time periods. Control samples included AgNPs, SRHA, SRFA, glass beads, AgNPs-glass beads, SRHA-AgNPs-samples without glass beads, and SRFA-AgNPs-samples without glass beads, all in the same concentrations and time durations.

---

**Figure 9.** A fluorescence emission energy diagram.\(^{34}\)
3.6.3. **DATA COLLECTION**

The fluorescence emission spectrophotometry measurements were performed (in duplicate) using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Figure 10). The emission spectra of the SRHA samples were examined in the 387-600 nm spectral range using an excitation wavelength of 362 nm, while the emission spectra of the SRFA samples were collected in the 337-600 nm spectral range using an excitation wavelength of 338 nm. The PMT detector voltage was set at 680 V, and a scan rate of 600 nm min\(^{-1}\) was selected for all measurements.

![An Agilent Technologies Cary Eclipse fluorescence spectrophotometer located in Dr. Sizemore’s research laboratory at Wright State University.](image)

**Figure 10.** An Agilent Technologies Cary Eclipse fluorescence spectrophotometer located in Dr. Sizemore’s research laboratory at Wright State University.
4. RESULTS & DISCUSSION

4.1. CHARACTERIZATION OF COLLOIDAL AgNPs

Two lots of Creighton colloidal AgNPs were synthesized for SRHA and SRFA studies. Both lots of Creighton colloid expressed the expected golden yellow color (Figure 11a) along with a sharp symmetric surface plasmon resonance peak (SPR) at about 390-400 nm (Figure 11b and 11c). The uniform SPR peak is indicative of the formation of round AgNPs mostly in the 1-100 nm size range. Previous studies showed that these AgNPs are negatively charged and have an average diameter of about 11 nm.

The quantity of silver within the Creighton colloid used for the NOM studies was determined by ICP-OES. The emission intensity (c s⁻¹) was plotted versus the concentration of the nine silver standards (μg L⁻¹) to create an external calibration curve (Figure 12). The final concentration of silver in the Creighton colloid employed in the
SRHA and SRFA studies was interpolated from the calibration curve: $13.4 \text{ mg L}^{-1}$ and $12.7 \text{ mg L}^{-1}$, respectively.

![Graph showing the ICP-OES calibration curve](image)

**Figure 12.** The ICP-OES calibration curve developed from the following concentrations of silver standards: 4.0, 6.0, 20.0, 40.0, 60.0, 200.0, 400.0, 600.0, and 800.0 μg L$^{-1}$.

### 4.2. **Raman and SERS Measurements**

To better understand the influence of NOM on the fate and transport of AgNPs through groundwater, it is imperative to determine if any molecular interactions occur between these components.$^{15}$ SERS is a powerful sensing technique, which has the
molecular fingerprinting capabilities of Raman spectroscopy in water, but much higher sensitivity due to the presence of Raman-active molecules (e.g., NOM) to a nano-roughened, noble-metal substrate (e.g., AgNPs). Thus, in this study, Raman and SERS measurements were performed to study the possible molecular interactions between NOM and AgNPs. Samples included controls (AgNPs, glass beads, SRHA, SRFA, SRHA-AgNPs, SRFA-AgNPs, AgNPs-glass beads, SRHA- and SRHA-glass beads alone) and column samples (SRHA-AgNPs and SRFA-AgNPs in the presence of glass beads). All measurements were performed in duplicates.

The Raman spectrum of AgNPs was dominated by three bands resulting from the bending (1638 cm⁻¹) and symmetric and asymmetric stretching (3263-3295 cm⁻¹) motions of water (Table 1 and Figure 1a (I))⁹,¹⁵,³⁵ The same modes were observed for the wet glass beads in the presence or absence of AgNPs (Figures 1a (II) and (III)). The intensities of the water bands were higher for the glass beads immersed in water because of the larger confocal hole employed in this measurement (Figure 1a (I)).¹⁵

The Raman spectra of the SRHA control samples at 1.0 10.0, 40.0, 80.0, and 160.0 mg L⁻¹ revealed characteristic vibrational modes at 337-340, 400-409, 472-477, 559-562, 647-660, 729-736, 823-827, 1078-1083, 1349-1362, 1466-1474, 1584-1629, and 3199-3432 cm⁻¹ (Figures 1b and Figure 14).¹⁵ A tentative assignment of these Raman bands was provided in Table 1 according to the literature.¹⁵
Figure 13. Raman and SERS spectra of control samples: a) I) glass beads in SRHA (II) glass beads in water, (III) glass beads in colloidal AgNPs, and (IV) 13.4 mg L\(^{-1}\) of Creighton colloidal AgNPs, b) 160.0 mg L\(^{-1}\) of SRHA, c) 160.0 mg L\(^{-1}\) of SRHA mixed with AgNPs, and d) 160.0 mg L\(^{-1}\) of SRHA mixed with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) region for spectra b)-d). Acquisition time was 10 s and confocal hole was set at II) 300 μm, and a) I), III), and IV), b)-d) 200 μm. All spectra were averaged over 5 cycles to improve signal-to-noise ratio.\(^{15}\)
<table>
<thead>
<tr>
<th>Controls:</th>
<th>Sample:</th>
<th>Tentative Assignment:</th>
<th>Lit. Peaks:</th>
</tr>
</thead>
</table>
| - AgNPs (13.4 mg L\(^{-1}\))  
- Glass beads (0.5 g) in H\(_2\)O or AgNPs | SRHA (160.0 mg L\(^{-1}\))  
AgNPs-SRHA (11.3 mg L\(^{-1}\)) : (1.0-160.0 mg L\(^{-1}\)) | AgNPs-SRHA with glass beads (11.3 mg L\(^{-1}\)) : (1.0-160.0 mg L\(^{-1}\)) |           |
|         | 227-229 | 227-229 | Ag-O stretching mode | 2-240\(^{38}\) |
| 337-340 | 337-340 | 337-340 | Skeletal vibration of polycyclic aromatic compounds in NOM\(^{38}\) | 338\(^{38}\) |
| 400-409 | 402-411 | 406-412 | In plane deformation of stretching of -COO- group in NOM\(^{36}\) | 400-700\(^{36}\) |
| 472-477 | 476-477 | 474-480 | In plane deformation of stretching of -COO- group in NOM\(^{36}\) | 400-700\(^{36}\) |
| 559-562 | 552-560 | 555-563 | In plane deformation of stretching of -COO- group in NOM\(^{36}\) | 400-700\(^{36}\) |
| 647-660 | 648-656 | 648-657 | In plane deformation of stretching of -COO- group in NOM\(^{36}\) | 400-700\(^{36}\) |
| 729-736 | 729-733 | 725-735 | In plane stretching of aromatic compounds in NOM\(^{36}\) | 738\(^{38}\) |
| 823-827 | 826-830 | 826-833 | In plane stretching of aromatic compounds in NOM\(^{38}\) | 840\(^{38}\) |
| 1078-1083 | 1082-1101 | 1081-1091 | C-C, C-O, C-N stretching and/or N-H C-H rocking a wagging of NOM\(^{36}\) | 1000-1300\(^{36}\) |
| 1349-1362 | 1345-1352 | 1342-1356 | Symmetric stretching of -COO- group in NOM\(^{36,37}\) | 1314\(^{36}\) \(1330\(^{37}\) |
| 1466-1474 | 1462-1466 | 1463-1477 | Combination bands of the intense polycyclic aromatic groups in NOM\(^{18,36}\) | 1460\(^{36}\) \(1464\(^{18}\) |
| 1584-1629 | 1582-1625 | 1590-1638 | Asymmetric stretching of -COO- group in NOM\(^{36,37}\) | 1618\(^{36}\) \(1580\(^{36}\) |
| 1638-1640 | | | Bending of water | 1639\(^{9}\) |
| 3254-3385 | | | Symmetric and asymmetric stretching of water | 3241-3394\(^{9}\) |
| 3199-3432 | 3189-3420 | 3214-3411 | N-H and C-H stretching of NOM\(^{36}\) | 3208-3415\(^{36}\) |
Blue or red shifts were detected for most bands in these SERS spectra (Figure 10c and 10d) with respect to the ordinary Raman spectrum of SRHA at all concentrations (Figure 10b). The most dramatic changes were observed for the symmetric and asymmetric stretching of the –COO\(^{-}\) groups of SRHA located at 1353 cm\(^{-1}\) and 1584 cm\(^{-1}\) in the Raman spectrum (Figures 10b, 160 mg L\(^{-1}\) of SRHA).\(^{36,35,37}\) These broad peaks were shifted by 6-11 cm\(^{-1}\) and 7-11 cm\(^{-1}\), respectively, in the SERS spectra (Figure 10d), suggesting a possible interaction of SRHA with AgNPs through the ionized carboxylic moieties that are located in the immediate vicinity of the metallic surface. Additionally, the integrated area of the 1353 cm\(^{-1}\) peak decreased by ~ 17% in the presence of AgNPs, while the full width at half maximum (FWHM) of the 1584 cm\(^{-1}\) band increased by ~ 30 cm\(^{-1}\) (Figure 10b and 210). Previous SERS reports on humic acids from Oak Forest suggested the AgNP-attachment of ionized carboxylic groups in a unidentate complex form.\(^{36}\) This coordination was further substantiated by the downshift and upshift of the symmetric and asymmetric stretching modes of the –COO\(^{-}\) groups when compared to the free carboxylates. Similar red- and blue-shifts were observed in this study for the two –COO\(^{-}\) stretching vibrations along with significant changes in FWHM or integrated area.\(^{15}\)

Furthermore, a recent NMR study on similar Suwannee River fractions of NOM demonstrated through the relaxation times of methylene protons that carboxyl or hydroxyl groups of SRFA interacted with the surface of citrate-capped AgNPs.\(^{40}\)

The SERS peaks of the SRHA-AgNPs controls and the SRHA-AgNPs samples in the presence of glass beads (Figures 13c, 13d, 14-16) were found to have similar profiles. No peaks appeared or disappeared. Nevertheless, small shifts to smaller or higher wavenumbers were noticed in between these SERS spectra suggesting that the presence
of glass beads may slightly influence the SRHA orientation to the AgNP surface with respect to the polarization of laser light (in particular the –COO⁻ bands). However, no covalent, chemical interaction was detected between the glass beads and the SRHA or AgNPs after one week of incubation.¹⁵

![Raman spectra](image)

**Figure 14.** Raman spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L⁻¹ (V) of SRHA. Insets illustrate the corresponding 2000-4000 cm⁻¹ spectral region. Acquisition parameters were a 10 s accumulation time, 5 cycles, and a 200 μm confocal hole. Raman spectra (I)-(IV) were manually shifted upwards for comparison purposes.¹⁵
Figure 15. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRHA incubated for 1 hr with AgNPs. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition parameters were a 10 s accumulation time, 5 cycles, and a 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.\(^{15}\)
Figure 16. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRHA incubated for 1 hr with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition parameters were a 10 s accumulation time, 5 cycles, and a 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.

In the study of SRFA, Raman and SERS measurements were again performed on all controls. Similarly to the SRHA controls, the Raman spectrum of AgNPs, AgNPs-glass beads, and glass beads were dominated by the bending (1635 cm\(^{-1}\)) and symmetric and asymmetric stretching (3254-3398 cm\(^{-1}\)) modes of water (Table 2 and Figure 17).\(^9,15,35\)

The Raman spectra of the SRFA control samples at 1.0, 10.0, 40.0, 80.0 and 160.0 mg L\(^{-1}\) revealed characteristic vibrational modes at 338-340, 400-405, 469-475, 554-559, 653-656, 731-734, 824-828, 1082-1087, 1345-1350, and 3221-3398 cm\(^{-1}\) (Figure 17b and Table 2). The tentative assignment for the characteristic vibrational modes observed in
the Raman spectra us given in Table 2. The SERS spectra of SRFA-AgNPs and SRFA-AgNPs in the presence of glass beads (Figure 13c and 13d) displayed small blue or reds shifts in most of the bands observed. The most notable changes between the Raman (Figure 17b) and SERS (Figure 17d) spectra were again in the symmetric and asymmetric –COO⁻ vibrational modes. These –COO⁻ peaks of SRFA were observed at 1346 cm⁻¹ and 1582 cm⁻¹, and experienced spectral shifts of 8-10 cm⁻¹ and 9-20 cm⁻¹ respectively, in the SERS spectra with respect to the ordinary Raman spectra (Figure 17b and Figure 147).36 These spectral changes further suggests a possible interaction between SRFA and AgNPs through the ionized carboxylic moieties located at the metallic nanosurface.15

The SRFA-AgNPs control had similar spectral profiles as the SRFA-AgNPs sample in the presence of glass beads (Figure 17c, 17d and Table 2). While no peaks appeared or disappeared, small peak shifts were detected between the SRFA-AgNPs control and the SRFA-AgNPs sample in the presence of glass beads, which may be attributed to slight reorientation of SRFA with respect to the polarization of the laser light due to the presence of glass beads. Nevertheless, there appears no evidence of covalent chemical interaction between the glass beads and the NOM or AgNPs after one week of incubation.15,35-39
Figure 17. Raman and SERS spectra of control samples: a) I) glass beads in SRFA (II) glass beads in water, (III) glass beads in colloidal AgNPs, and (IV) 12.7 mg L\(^{-1}\) of Creighton colloidal AgNPs, b) 160.0 mg L\(^{-1}\) of SRFA, c) 160.0 mg L\(^{-1}\) of SRFA mixed with AgNPs, and d) 160.0 mg L\(^{-1}\) of SRFA mixed with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) region for spectra b)-d). Acquisition time was 10 s and confocal hole was set at a) II) 300 μm, and a) I), III), and IV, b)-d) 200 μm. All spectra were averaged over 5 cycles to improve signal-to-noise ratio.
Table 2. Observed Raman and SERS vibrational modes (in cm\(^{-1}\)) for SRFA, and their tentative assignment according to literature.\textsuperscript{9,32-41}

<table>
<thead>
<tr>
<th>Controls:</th>
<th>Sample:</th>
<th>Tentative Assignment:</th>
<th>Lit. Peaks:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- AgNPs (12.7 mg L(^{-1})) - Glass beads (0.5 g) in H(_2)O or AgNPs</td>
<td>SRFA (160.0 mg L(^{-1}))</td>
<td>AgNPs-SRFA (10.7 mg L(^{-1})) : (1.0-160.0 mg L(^{-1}))</td>
<td>AgNPs-SRFA with glass beads (10.7 mg L(^{-1})) : (1.0-160.0 mg L(^{-1}))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

35
Figure 18. Raman spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L$^{-1}$(V) of SRFA. Insets illustrate the corresponding 2000-4000 cm$^{-1}$ spectral region. Acquisition parameters were 10 s accumulation time, 5 cycles, and 200 μm confocal hole. Raman spectra (I)-(IV) were manually shifted upwards for comparison purposes.
Figure 19. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L$^{-1}$ (V) of SRFA incubated for 1 hr with AgNPs. Insets illustrate the corresponding 2000-4000 cm$^{-1}$ spectral region. The acquisition parameters were 10 s accumulation time, 5 cycles, and 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.
Figure 20. SERS spectra of 160.0 (I), 80.0 (II), 40.0 (III), 10.0 (IV), and 1.0 mg L\(^{-1}\) (V) of SRFA incubated for 1 hr with AgNPs in the presence of glass beads. Insets illustrate the corresponding 2000-4000 cm\(^{-1}\) spectral region. The acquisition parameters were 10 s accumulation time, 5 cycles, and 300 μm confocal hole. SERS spectra (I)-(IV) were manually shifted upwards for comparison purposes.

The presence of an Ag-O stretching mode was observed at about 227 cm\(^{-1}\) for both SRHA-AgNPs and SRFA-AgNPs samples in the presence of glass beads (Figure 21). The presence of this vibrational mode provides additional evidence of a molecular interaction between the -COO\(^{-}\) moieties of NOM and AgNPs.\(^{39}\) This peaks was not detected in the ordinary Raman spectra of SRHA and SRFA.

The large, cathedral-like background is the result of NOM fluorescence (Figure 13b-d and Figure 17b-d), which was also observed for other types of NOM or fluorescent dye molecules.\(^{9,15,35,36}\) Humic substances are known to exhibit a broad absorption in the visible spectral range.\(^{36}\) This fluorescence background exhibits considerable quenching in
SERS spectra due to the adsorption of NOM to the AgNP surface.\textsuperscript{9,35} For example, a comparison of the ordinary Raman spectra of 160.0 mg L\textsuperscript{-1} of aqueous SRHA (Figure 13b) with its SERS spectra after 1 hr exposure to 11.3 mg L\textsuperscript{-1} of AgNPs (Figure 13d) indicates a 5.4-10.6 % decrease in intensity for the broad background. This aspect further confirms the observed interaction between AgNPs and SRHA. After 60 days of incubation with AgNPs, the SERS spectra exhibited 71.7-78.9 % decrease in fluorescence suggesting that a significant amount of SRHA was adsorbed onto the noble metal substrate. A similar decrease in the fluorescence background was observed for the SRFA samples. After 1 hr incubation of 160.0 mg L\textsuperscript{-1} of SRFA with 10.7 mg L\textsuperscript{-1} of AgNPs, SERS spectrum experienced a 15.3-17.9% decrease in fluorescence compared the ordinary Raman spectrum of SRFA. After 60 days of incubation with AgNPs at the same SRFA concentrations, the fluorescence was quenched by 31.5-42.2 %. This suggests that SRFA may attach faster to AgNPs than SRHA at the beginning of the incubation period (1 hr), but experience a slower surface-complexation as time passes (60 days). In summary, the longer the time elapsed as NOM and AgNPs incubate, the stronger their interaction and possible aggregation in AgNP-NOM-AgNP assemblies. However, it is likely that not all NOM molecules will be adsorbed to the AgNP surface at such large concentrations (e.g., 160.0 mg L\textsuperscript{-1} of NOM to 11.3 mg L\textsuperscript{-1} of AgNPs).\textsuperscript{32}
Figure 21. SERS spectra of 160.0 mg L\(^{-1}\) of SRHA-AgNPS (I) and SRFA-AgNPs (II) incubated for 1 hr in the presence of glass beads. The acquisition parameters were 10 s accumulation time, 5 cycles, and 300 \(\mu\)m confocal hole. SERS spectra (I)-(II) were manually shifted upwards for comparison purposes.

Transmission electron microscopy (TEM) measurements and ICP-OES are currently in preparation by our laboratory in order to gain a better mechanistic understanding of the observed trends in the AgNP-adsorption behavior of SRHA and SRFA.

4.3. Fluorescence Spectrophotometry Measurements

Fluorescence emission spectrophotometry was employed to quantify the amount of NOM adsorbed onto AgNPs through the fluorescence quenching of NOM.\(^{37}\) More exactly, the fluorescence quenching was estimated from the decrease in fluorescence...
emission intensity after the complexation of NOM to the metal surface.\textsuperscript{37} Fluorescence measurements were performed on SRHA-AgNPs and SRFA-AgNPs samples in the presence of glass beads as well as on all controls (AgNPs, glass beads, SRHA, SRFA, SRHA-AgNPs, SRFA-AgNPs, AgNPs-glass beads, SRHA-glass beads alone, and SRFA-glass beads alone) (Table 3). The measurements were carried out immediately after the sample preparation, at 1 hr, 3 hrs, 1 day, and 1 week. The 60 days measurements are currently in progress. The fluorescence emission peaks for humic substances are between 400-460 nm; these peaks were observed for both SRHA (Figure 21-23) and SRFA (Figure 24-26).\textsuperscript{37} No fluorescence emission was observed for the original Creighton AgNPs and minimal fluorescence was detected for glass beads and AgNPs-glass beads controls (Table 3).

When 160.0 mg L\textsuperscript{-1} of SRHA or SRFA were incubated with AgNPs in the presence of glass beads, a 36.9-37.4\% and 38.9-39.3\%, respectively, decrease in fluorescence emission intensity was observed at 1 hr with respect to the SRHA and SRFA controls in the presence of glass beads. This decrease in fluorescence emission intensity suggests there is a metal complex being formed between NOM and AgNPs, which further supports the previous Raman/SERS findings of a potential molecular interaction between NOM and AgNPs. It should be noted that SRHA and SRFA exhibit the same adsorption trend as revealed by Raman and SERS at 1 hr incubation, namely SRFA has a slightly higher affinity for the Creighton AgNPs than SRHA. However, the percent quenching in NOM fluorescence appears to be up to seven-fold larger than the ones obtained by Raman and SERS. We believe that the fluorescence emission technique is more accurate in the quantification of the amount of NOM adsorbed onto AgNPs than Raman and SERS.
due to the rough estimations of the large fluorescence background in Raman. Table 3 and Figures 21-23 indicate that the fluorescence of SRFA-AgNPs sample in the presence of glass beads diminishes with the increase in incubation time (over 20% from the preparation time to 1 week of incubation). However, the change in fluorescence emission appears insignificant for the SRHA-AgNPs-glass beads sample at 1 week when compared to the time of preparation. Future fluorescence emission measurements at 60 days incubation are expected to clarify this unexpected result and to confirm the Raman and SERS data.

The fluorescence emission data (Table 3) also suggest that the glass beads may sterically hinder the complexation of both SRHA and SRFA to the metallic surface. A reduction of over 10% and 20% was noticed upon addition of the glass beads to the SRHA- and SRFA-AgNPs samples, respectively, for all incubation times. This is good agreement with the Raman and SERS data, which indicated a reorientation of the chemisorbed NOM molecules with respect to the laser polarization due to presence of the glass beads.
Figure 22. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.
Figure 23. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA-AgNPs immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.
Figure 24. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRHA-AgNPs in the presence of glass beads immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.
Figure 25. The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRFA immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.
**Figure 26.** The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRFA-AgNPs immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.
**Figure 27.** The fluorescence emission spectra of 160.0 mg L$^{-1}$ of SRFA-AgNPs in the presence of glass beads immediately after preparation, at 1 hr, 3 hrs, 1 day, and 1 week.

**Table 3.** Fluorescence emission intensity of the 160.0 mg L$^{-1}$ SRHF-AgNPs-glass beads and SRFA-AgNPs-glass beads samples and the corresponding controls.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluorescence emission intensity (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>AgNPs</td>
<td>0</td>
</tr>
<tr>
<td>Glass beads-Water</td>
<td>3-4</td>
</tr>
<tr>
<td>AgNPs-glass beads</td>
<td>3</td>
</tr>
<tr>
<td>SRHA</td>
<td>524-532</td>
</tr>
<tr>
<td>SRHA-AgNPs</td>
<td>408-412</td>
</tr>
<tr>
<td>SRHA-AgNPs-glass beads</td>
<td>317-322</td>
</tr>
<tr>
<td>SRFA</td>
<td>510-515</td>
</tr>
<tr>
<td>SRFA-AgNPs</td>
<td>424-427</td>
</tr>
<tr>
<td>SRFA-AgNPs-glass beads</td>
<td>336-345</td>
</tr>
</tbody>
</table>
5. CONCLUSIONS

The results obtained in this study demonstrate that Raman spectroscopy, SERS, and fluorescence emission spectroscopy are effective analytical techniques for the qualitative and quantitative examination of the potential molecular interactions between AgNPs (~11 mg L$^{-1}$) and NOM (1.0 10.0, 40.0, 80.0, and 160.0 mg L$^{-1}$ of humic and fulvic acids). The changes in the spectral features of the symmetric and asymmetric stretching modes of the $\text{–COO}^-$ moieties of NOM, when compared to the free carboxylates, are suggestive of their attachment to the AgNP surface. This leads to the formation of AgNP-NOM-AgNP aggregates, which may decrease the mobility of AgNPs through groundwater. The fluorescence emission spectra of NOM incubated with AgNPs at various benchmark times (0-60 days) revealed fluorescence quenching and confirmed the attachment of NOM to the metallic nanosurface. The interaction was found to become stronger with the increase in incubation time and the amount of AgNPs.

Additional studies should be conducted on the mechanism of molecular interaction between AgNPs and NOM in order to fully grasp the transport and fate of AgNPs through the environment. Future projects in our research group will examine how the ionic strength and pH affect the molecular interactions between NOM and AgNPs. Prospective techniques that could be employed to further study the complex nature of environmental samples are electrospray atomizer coupled to a scanning mobility particle sizer (ES-SMPS) for determining the behavior of NPs in aqueous suspensions and flow...
field-flow fractionation (FIFFF) for isolating the different components of environmental samples containing nanosilver. These extended studies would hopefully define a) under what environmental conditions the proposed mechanisms of interaction between NOM and AgNPs occur, b) the change in the properties of nanosilver into the environment, and c) the amount of nanosilver being released into the environment.
REFERENCES


2. International Center for Technology Assessment.


4. The Project on Emerging Nanotechnologies

5. Roco, M. C., The long view of nanotechnology development:


http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/uvvisab1.htm (accessed June, 2014)


