# Determination of the optimal conditions for bovine serum albumin surface enhanced Raman scattering on silver colloids

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## Introduction

In proteomic studies, surface enhanced Raman scattering(SERS) has been used for protein identification[1,2]. SERS is a sensitive surface technique used to identify various molecules through the enhancement of inelastic scattering. Raman scattering occurs when adsorbed to a nanometer sized metal (typically Au or Ag) surface. Serum albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues[3]. It also is responsible for maintaining the pH of blood and is the most abundant protein in the circulatory system[4]. Acidified sulfate was used as an aggregating agent for SERS of bovine serum albumin(BSA) because the sulfates have a weaker binding affinity to the metal surface[5].

The purpose of this particular study is to find the optimal condition to take SERS spectra of BSA. If such conditions can be found for one particular type of protein, then one could design a systematic approach to determine label-free detection of any protein molecule.

## Materials and methods

BSA, sodium citrate, and $AgNO_3$ were used as purchased from Sigma Aldrich (Saint Louis, Missouri). Silver colloids were made with $AgNO_3$, sodium citrate (1%), and $H_2O$ following Lee & Meisel[6]. BSA (0.05 g) was dissolved in a phosphate buffer (100 mL). BSA solution (0.1 mL) was pipetted into a micro-well plate followed by $Na_2SO_4$ (0.3 M, 0.1 mL) and silver colloids (0.1 mL). The spectra were exposed for 1-10 s at a wavelength of 570 nm, 600 g/mm, 50 µm slits, and dynamic auto background subtraction. The pH of the BSA solution was adjusted with $Na_2SO_4$ and $H_2SO_4$ until the desired pH (2, 4, 6, and 10) was reached.

## Instrumentation

Home built Raman Microscope was used to obtain SERS spectra:

- •Laser- Spectra Physics, Excelsior, 532 nm, 150 mW
- •Microscope- Leica DMLM, 20x objective
- •Beam Splitter- Omega Optical 540 DRLP
- •Edge Filter- Razor Edge LWP filter, 532 nm, U-Grade, 25 mm
- •Monochromator- CVI Digikrom 240, 600 gr/mm grating, 50 µm slit width
- •Detector- Apogee thermoelectrically cooled CCD

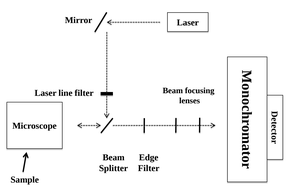


**Figure 1.** A diagram of the Raman spectrometer.

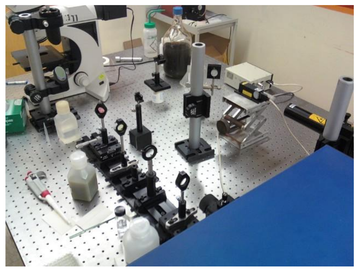

**Figure 2.** A picture of the Raman spectrometer.

## Results

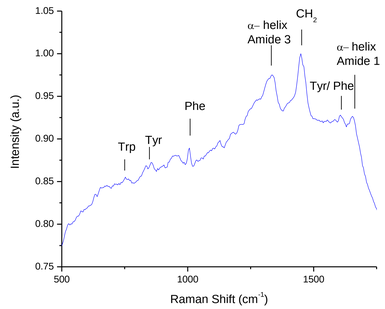


**Figure 3.** Raman spectrum of pure BSA with 10 second exposure.

**Table 1.** Tentative peak assignments from Figure 4.

| Raman Shift ($cm^{-1}$) | Assignments |
|---|---|
| 752 | tryptophan (Trp) |
| 854 | tyrosine (Tyr) |
| 1006 | phenylalanine (Phe) |
| 1334 | alpha helix- amide 3 |
| 1448 | $CH_2$ |
| 1606 | tyrosine or phenylalanine |
| 1654 | alpha helix- amide 1 |

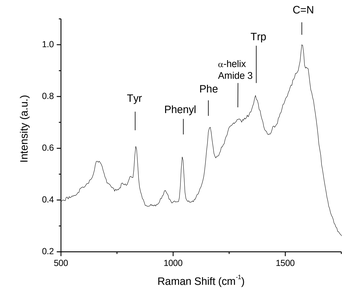


**Figure 4.** SERS spectrum of BSA (pH 4) with 2 second exposure.

**Table 2.** Tentative Raman shift assignments for the bands shown in Figure 3

| Raman Shift ($cm^{-1}$) | Assignments |
|---|---|
| 833 | tyrosine (Tyr) |
| 1040 | phenyl |
| 1165 | phenylalanine (Phe) |
| 1297 | alpha-helix amide 3 |
| 1367 | tryptophan (Trp) |
| 1574 | C=N stretch |

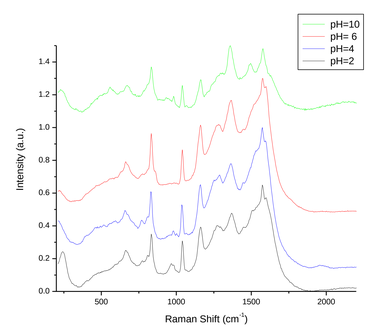


**Figure 5.** SERS spectra of BSA solution with varying pHs (In descending order, 10, 6, 4, and 2).

## Conclusions

Surface enhanced Raman scattering could be used to investigate label free detection of proteins by determining the secondary structure of the protein. The pH dependent spectra of BSA showed that when the pH of the protein was below its isoelectric point (pI), the more prominent the Raman bands. This was likely caused by the protein's increase in negative charge having a stronger attraction with the positively charged colloids. The optimal conditions of SERS for BSA were 500ug/mL and pH=4, however the optimal conditions for another protein could be very different because proteins interact differently with the colloids and have different pIs.

The observed vibrations in SERS identifies which part of the molecule is interacting with the silver colloids. The various amino acid stretches help depict the overall conformation of molecule. The amide stretches determine the secondary structure of the molecule. The most common conformations in secondary structure are alpha helix and beta sheet. In the spectra of BSA, the amide stretches show a alpha-helical structure.

SERS is an important process to explore because label free detection of molecules can form a hypothesis about the functions and interactions of the compound in its environment. Further investigation with this process could lead to the discovery of new proteins and a better understanding of protein interactions in their existing environments.

## Literature cited

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## For further information

Please contact *jreyes@linfield.edu*. More information on this and related projects can be obtained at *www.linfield.edu/chem.*

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