

# Investigation of the link between media acidity and pI in obtaining optimum surface-enhanced Raman scattering for proteins

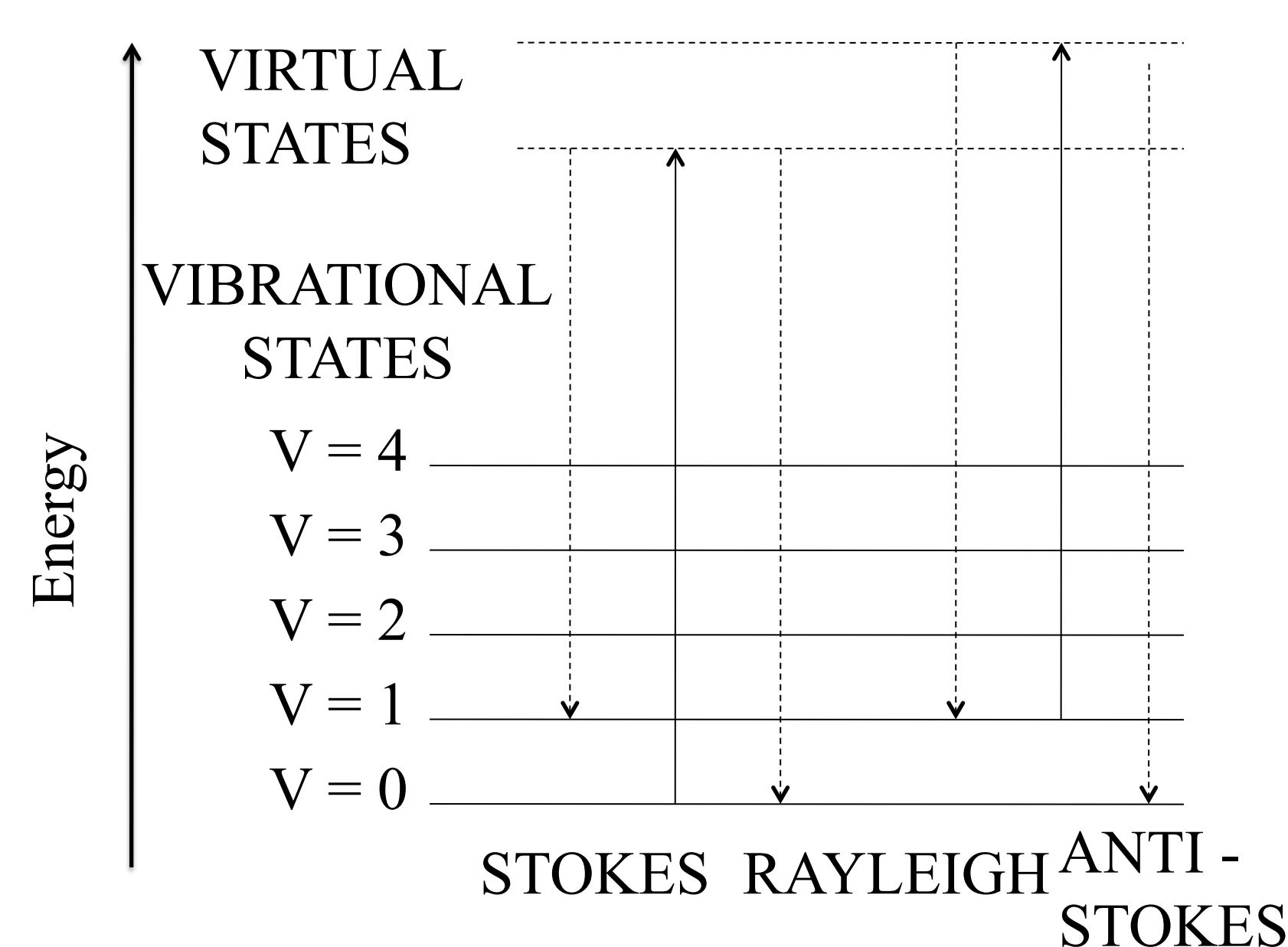
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## Introduction

- Surface enhanced Raman spectroscopy is a highly sensitive surface technique used for the identification of different molecules through the enhancement of inelastic (Raman) scattering.
- Surface enhanced Raman scattering (SERS) occurs through the adsorption of the molecule onto a roughened metal surface and is a valuable tool in protein detection.
- Halides used in colloid aggregation can form a strongly bonded surface layer that repels the adsorption of proteins, which strongly inhibits SERS from occurring.
- A technique has been found using acidified sulfate as the aggregating agent that allows SERS of proteins to take place due to sulfates weaker binding affinity to the metal surface.
- We have conducted further investigation into the level of acidity needed to obtain optimum SERS spectra. A link between the pH of the media and the pI of the individual proteins was investigated.

## Background



Inelastic (Raman) scattering occurs when an excited molecule relaxes to a different vibrational level than its original state. The energy difference between the incident and scattered light appears as a frequency shift. If the final vibrational state of the molecule is lower, a shift to the blue of the excitation frequency occurs, yielding Raman bands known as anti-Stokes lines. A shift to the red of the excitation frequency, caused by a higher final vibrational state, yields Raman bands known as Stokes lines. Rayleigh scattering occurs when the excited molecule relaxes to the original vibrational state, re-emitting a photon at the same frequency as the incident light.

## Materials and methods

- Proteins:** Lysozyme, Bovine Serum Albumin (BSA), Catalase, and Hemoglobin. Purchased from Sigma Aldrich and used as received.
- Colloids:**  $\text{NaBH}_4$  and  $\text{AgNO}_3$  were acquired from Sigma Aldrich. All glassware cleaned with Aqua Regia. Ag colloids were made by borohydride reduction according to the method published by Lee & Meisel<sup>1</sup>.
- Solutions of the four proteins were prepared (500  $\mu\text{g}/\text{mL}$ ) and pipetted (0.01 mL) into a plastic micro-well plate.
- The solutions were then diluted 30:1 with equal amounts of the silver colloids and  $\text{NaSO}_4$  (0.1 M) aggregating agent.
- The aggregating agent's pH was adjusted to values of 2, 4, 6, 9, and 11, with sulfuric acid and sodium hydroxide. SERS spectra were taken of each of the protein solutions at those various values.
- A 20 sec integration time, excitation wavelength of 532 nm, 600 gr/mm grating, and 50  $\mu\text{m}$  slit width were used to when obtaining the spectra.
- Spectra were collected over a range of 416 to 2206  $\text{cm}^{-1}$ .

## Instrumentation

Home built Raman microscope was used to obtain SERS spectra:

- Laser- SpectraPhysics, 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  $\mu\text{m}$  slit width
- Detector- Apogee thermoelectrically cooled CCD

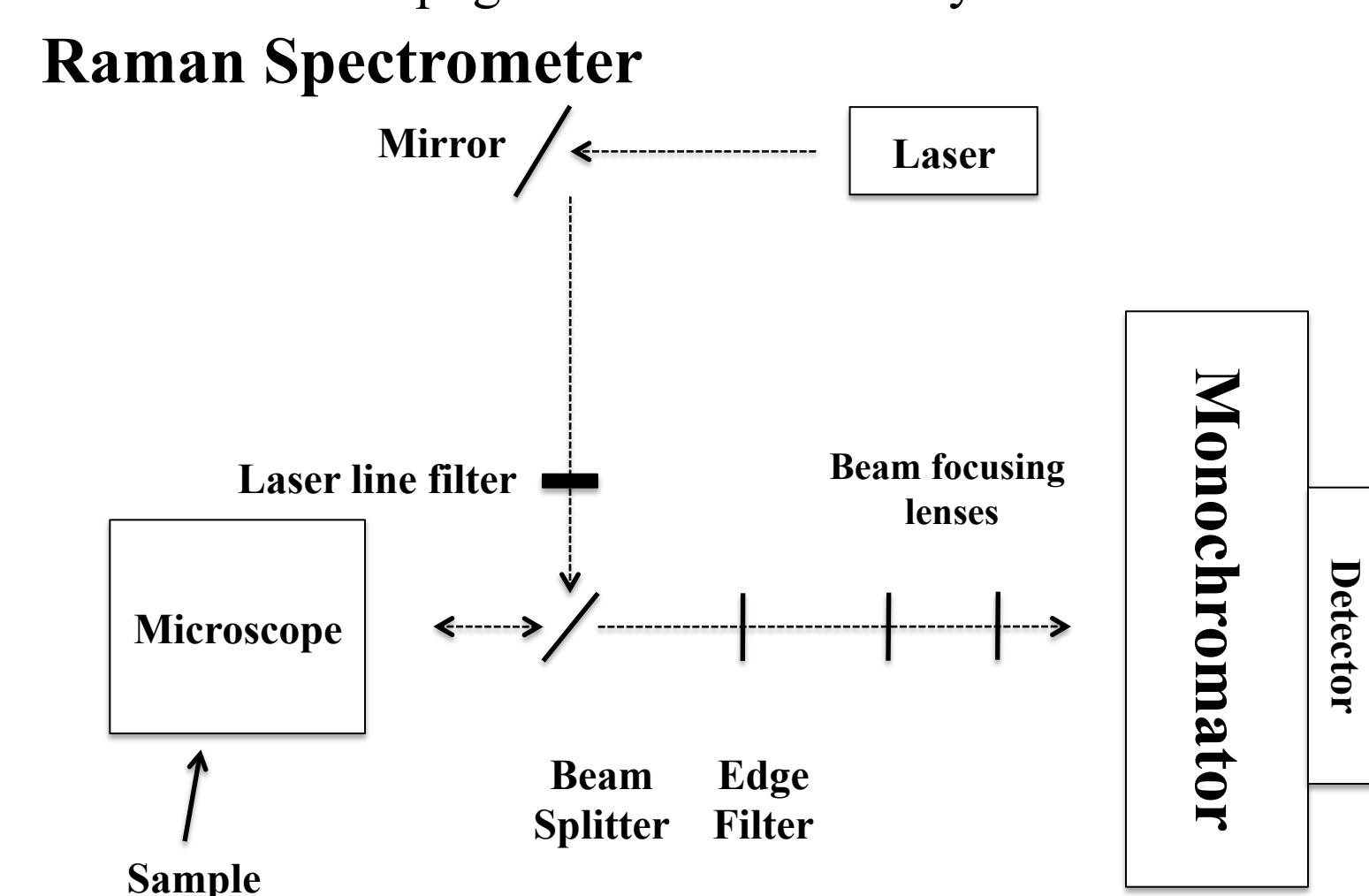


Figure 1: Block diagram of the Raman spectrometer.



Figure 2: Real life picture of Raman spectrometer.

## Results

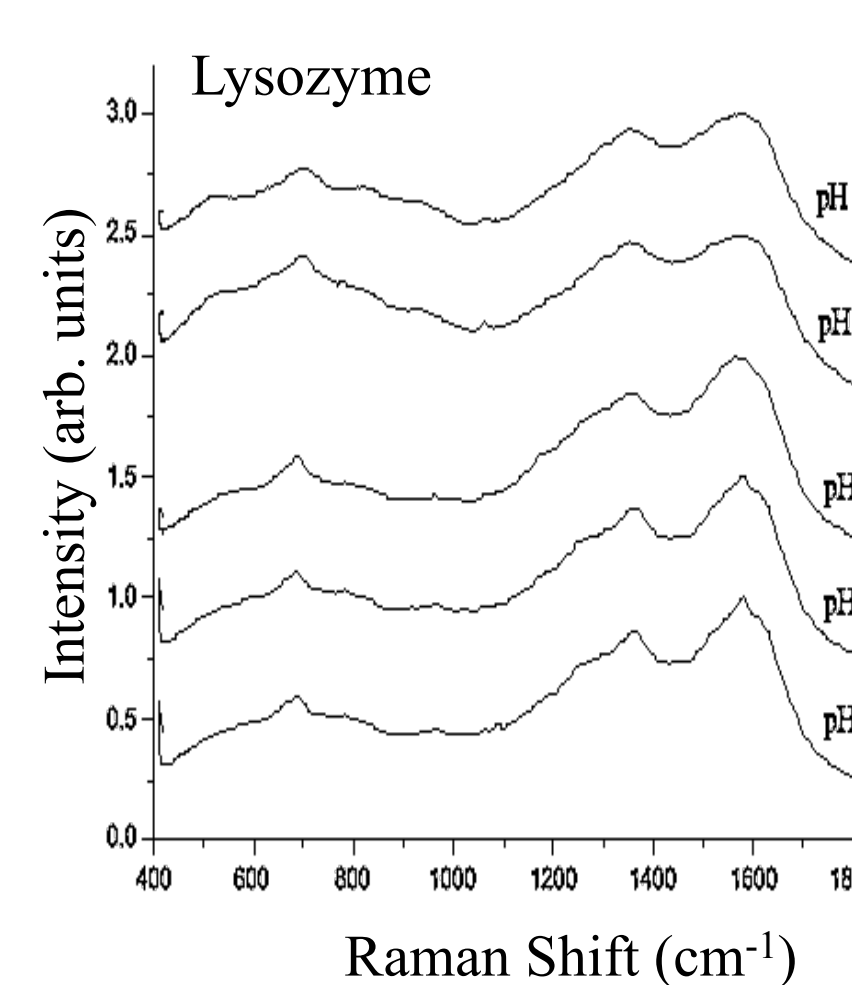


Figure 3: pH-dependent SERS spectra of lysozyme.

Tentative Peak Assignments	Raman Shift ( $\text{cm}^{-1}$ )
Phe	685.05
Trp	1085.89
Trp and/or CH	1369.9
Tyr	1579.82

Table 1: Tentative peak assignments for lysozyme Raman bands.

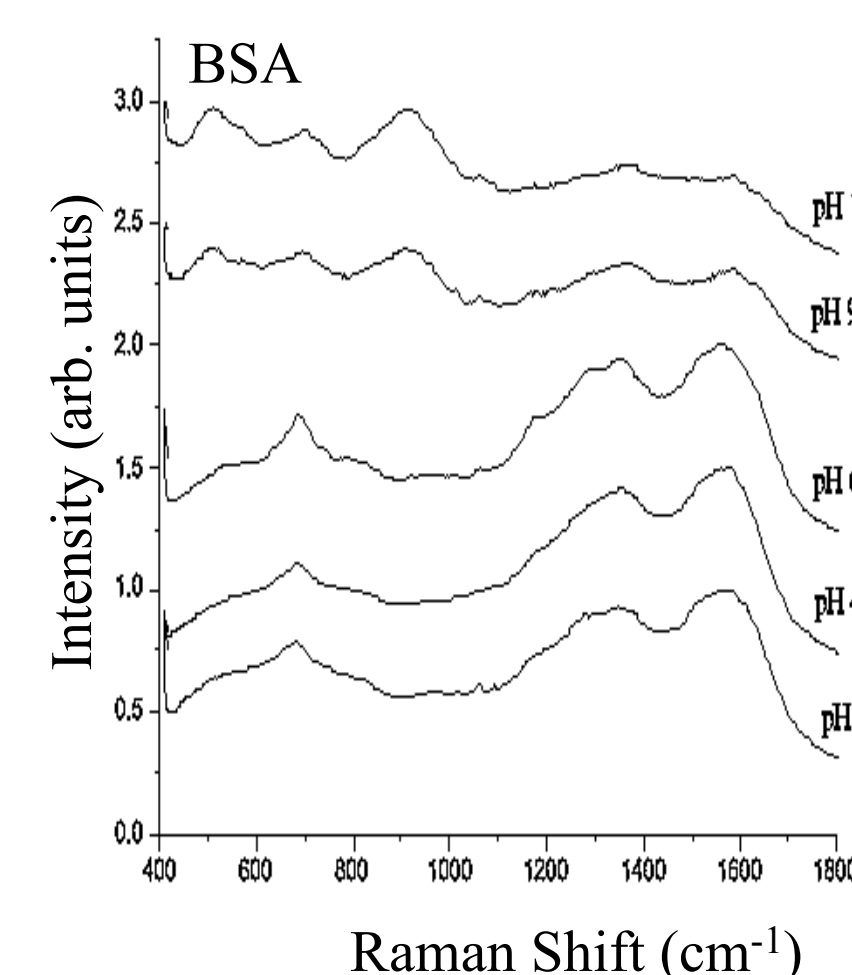


Figure 4: pH-dependent SERS spectra of BSA.

Tentative Peak Assignments	Raman Shift ( $\text{cm}^{-1}$ )
Phe	685.05
Trp	783.56
Tyr	1180.01
Amide III	1279.98
Trp	1346.03
Tyr	1563.64

Table 2: Tentative peak assignments for BSA Raman bands.

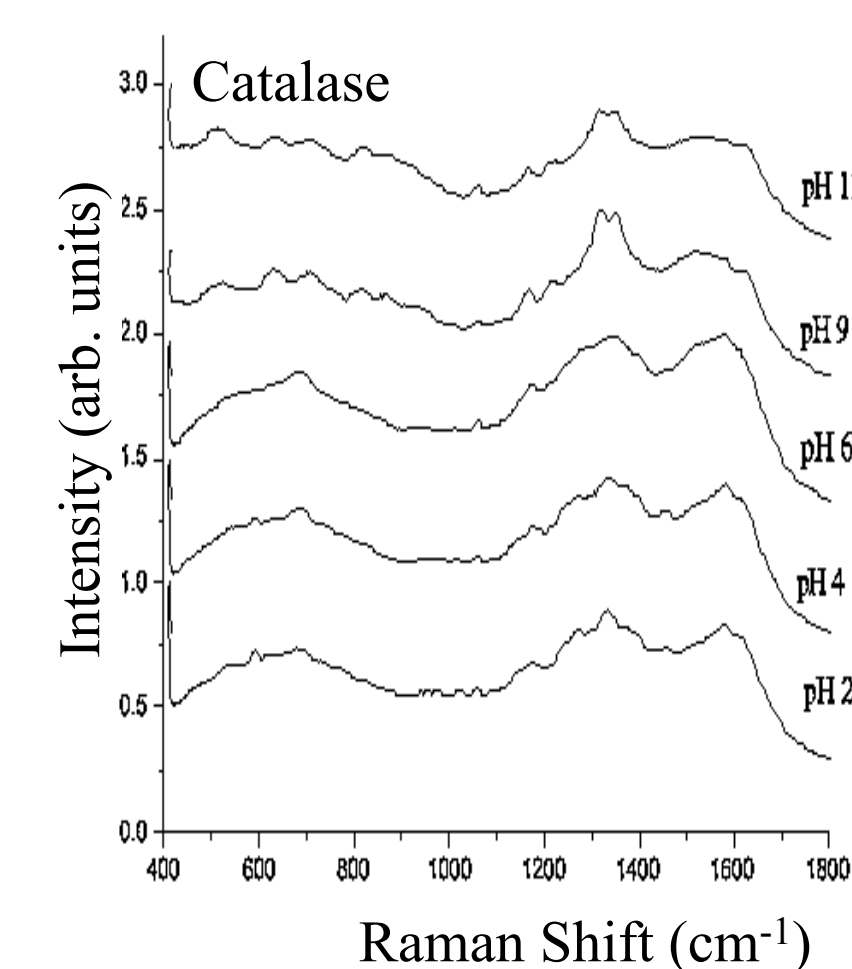


Figure 5: pH-dependent SERS spectra of catalase.

Tentative Peak Assignments	Raman Shift ( $\text{cm}^{-1}$ )
Phe	680.52
Amide III	1271.11
Tyr	1579.82
Trp	1608.06

Table 3: Tentative peak assignments for catalase Raman bands.

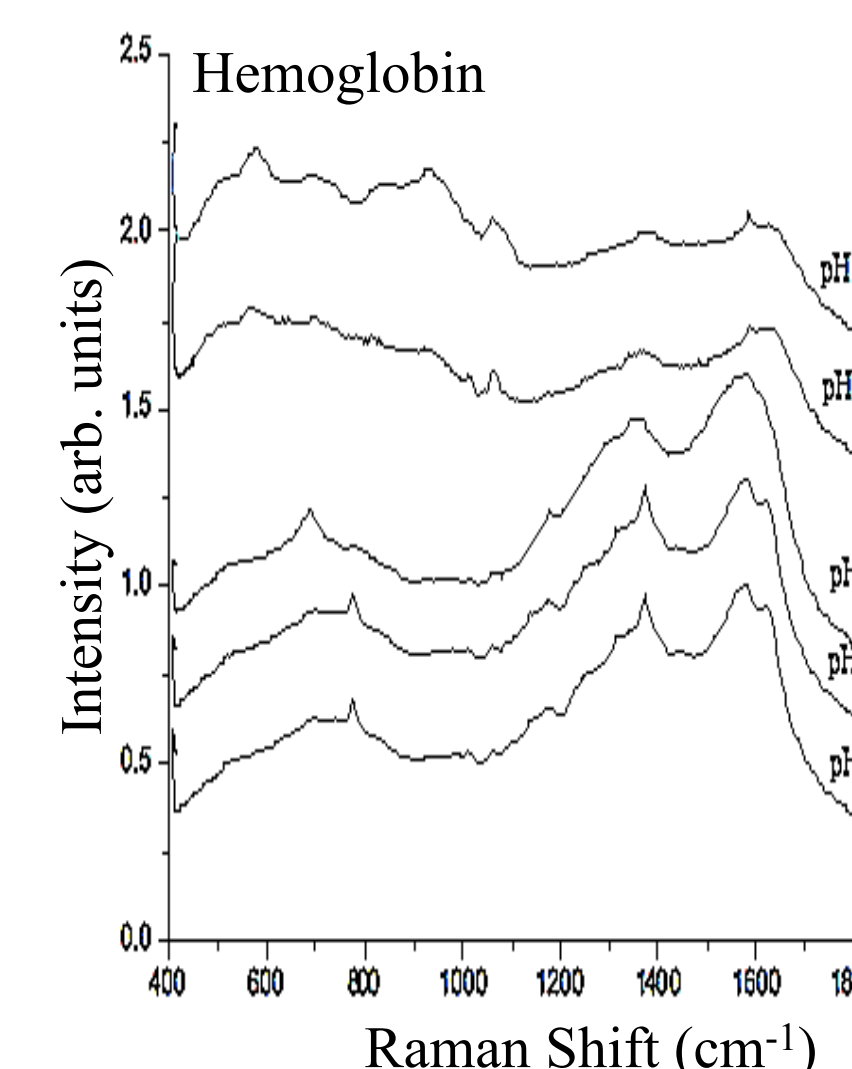


Figure 6: pH-dependent SERS spectra of hemoglobin.

Tentative Peak Assignments	Raman Shift ( $\text{cm}^{-1}$ )
Porphyrin Stretching	774.84
CH Bending	1176
Heme Group	1373.89
	1575.77
	1616.11

Table 4: Tentative peak assignments for hemoglobin Raman bands.

## Conclusions

- The pH-dependent SERS spectra of the proteins seem to exhibit a trend, which shows no distinctive Raman bands when the pH of the media is set above the pI of the protein. This is likely caused by repulsion between the metal surface and negatively charged protein that is produced when the media of the pH is set above the proteins pI.
- When the pH was set below the pI of the protein, the Raman bands became apparent, with enhancement occurring as the pH was lowered. The net positive charge placed on the protein attracts to the roughened metal surface through electrostatic interactions and ionic attractions to the negatively charge sulfate anions, allowing enhancement of the Raman scattering to occur.
- An apparent link between the pI of the protein and pH of the media needed to give optimum enhancement has seemingly been found. This link may increase the ability for future SERS of proteins to be made as long as knowledge of the particular protein's pI exists.

## Literature cited

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- Lin-Vien, D.; Colthup, N.; Fateley, W.; Grasselli, J. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*; Academic Press: San Diego, **1991**; pp 2-3.
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## For further information

Please contact [dsorber@linfield.edu](mailto:dsorber@linfield.edu). More information on this and related projects can be obtained at [www.linfield.edu/chem](http://www.linfield.edu/chem).