SURFACE ENHANCED RAMAN SPECTROSCOPY OF HISTIDINE ON AN ELECTROCHEMICALLY PREPARED SILVER SURFACE

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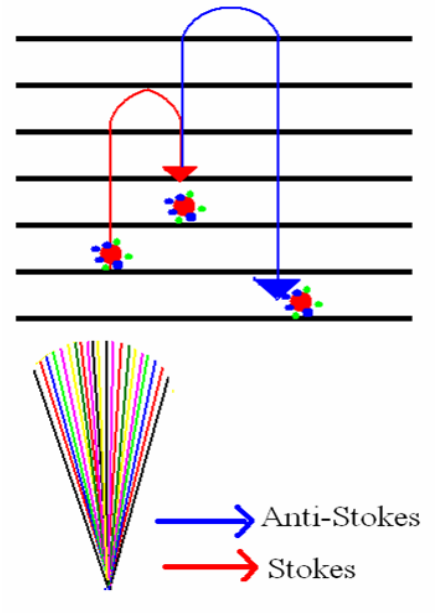
Introduction:

Spectroscopy is the study of light. Specifically, Spectroscopy is the study of how a 'species', such as an atom, molecule, or a solution, reacts to light. Some studies depend on how much light an atom absorbs. The electromagnetic radiation absorbed, emitted or scattered by the molecule is analyzed. Typically, a beam of radiation from a source such as a laser is passed through a sample, and the radiation exiting the sample is measured. There are various forms of spectroscopy. Many are familiar with the standard Infrared spectroscopy. A very common form of spectroscopy is X-ray. Some, like Raman, depend on a molecule's vibrations in reaction to the light.

Surface Enhanced Raman Spectroscopy (SERS) was discovered in 1974, when a sample of Pyridine was absorbed on an electrochemically roughened surface of silver. To enhance a surface in Raman spectroscopy, typically, a silver, gold, or copper electrode is subject to an electrochemical roughening. This is done to increase the Surface area of the metallic surface, so that more solution may be absorbed, as to allow for more scattering to be measured. The surface is also roughened because it was found that a significant enhancement of the Raman Signal occurred when the organic compound came in close contact with a roughened metallic surface. This is because there is more of a metallic surface for light reflection to occur, when the surface is roughened. SERS is typically used to measure a molecule's vibrations. Each molecule

will have its own characteristic spectrum. This means, generally, each atom or structure has its own special vibration, like a finger print. By analyzing the specific wavelength that a structure vibrates at most, or least, we can: identify unknown samples, define the structure of little known/studied atoms, and define the stability of a substance. The general assumption is that as a result of electromagnetic enhancement, the SERS of a molecule is virtually an enhanced version of that compound's normal Raman Spectrum. However, during charge transfer, some vibrations will be altered due to a molecule's interaction with the surface, resulting in some of the SER bands being shifted in respect to the normal Raman Spectrum. SER spectra of a different small peptides and proteins were obtained from a roughened surface using a Raman spectrometer. From this, the structures of these molecules were determined

Each vibration measured constitutes a peak (when measured) and certain peaks imply a specific structure. One photon in a million interacts with the moving atoms within a molecule; when the wave exits the sample, it has a different wavelength. This difference offers a clue to what kind of molecule the photon encountered and what kind of motion affected it. Lasers are specific to this type of experiment. Unlike sunlight or light from an ordinary light bulb, laser light is all one wavelength. Not only that, but the light waves are all polarized. Thus, lasers can put a lot of photons onto a very small spot. There are so many photons hitting the sample that "one-in-a-million" adds up to a signal that is strong enough to detect. In Raman spectroscopy, by varying the frequency of the radiation, a spectrum can be produced, showing the intensity of the exiting radiation for each frequency. This spectrum will show which frequencies of radiation have been absorbed by the molecule to raise it to higher vibrational energy states. These vibrations and their vibrational energy states are referred to as “Stokes”. When Light hits a sample, it is excited. Most of the time, an atom is naturally vibrational.



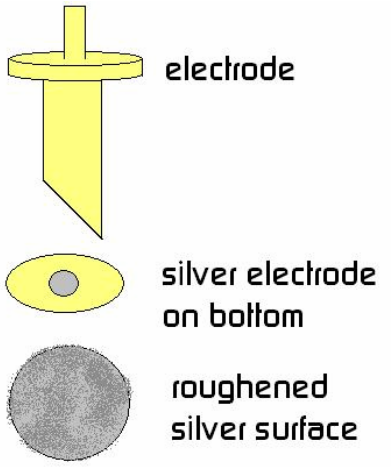
In either situation, the atom is forced to vibrate and move. It is these vibrations which are being measured. Atoms are at a certain energy level at any given time. As a laser light hits the atom, it is excited and reaches a higher level of energy, and then is brought back down. This occurs Numerous times. Raman measures these differences in Energy levels, which are the Stokes. Sometimes, if an atom is at a given energy level, it can be excited then fall below the original level. There are also mirror images of peaks. On a chart, before major peaks, there are softer peaks, in the negative wave length area of the X-axis. These are the Anti stokes. Anti-stokes spectrum are mirror spectrums of Raman Stokes, and are often not measured because even though the peaks are at the negative wavelength, (i.e., 400, -400) the peaks are much, much lower, and are almost undetectable in comparison to other waves.

Fifty percent of the basic dry human cell is composed of proteins and amino acids. The importance and the ability to analyze and identify them without having to damage the cell has led to Raman spectroscopy. The cell is not dissected or dismembered: it is easily placed in a neutral solution (to simulate blood, such as Potassium Chloride or other pH/pOH 7-pH 7.6). Mostly, SERS, and RS spectrums are not only used to identify basic proteins and amino acids, but also, to infer different orientations of such amino acid compounds and dipeptides, tripeptides, and polypeptides, such as tyrosine-glycine, or alanine-leucine. This can lead to further studies such as reactions between different combinations of peptides, amino acids, how different peptides can be put together, their reactions to different surfaces, and/or solutions.

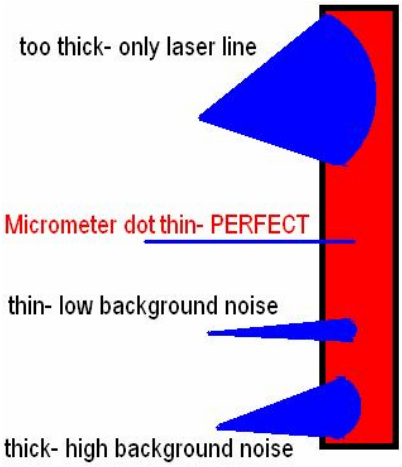
In Raman spectroscopy, by

varying the frequency of the radiation, a spectrum can be produced, showing the intensity of the exiting radiation for each frequency. This spectrum will show which frequencies of radiation have been absorbed by the molecule to raise it to higher vibrational energy states. A substance may not absorb any radiation at 300 wavelengths, but may absorb at 700 or 720. Typically, higher waves denote different structures. At 1300, a peak might show that there is a nitrogen-hydrogen bond. Commonly, at 700, hydrogen bonds absorb the radiation. Depending on how much is already known about the spectrum, one could know how far to run the spectrum. Basic substances, like sulfur and pyridine can be run to 2000 waves, and a structure can be obtained from just the peaks within that wave. For a structure of histidine, if a person only knew that there was a nitrogen bond in the structure, they would run it to at least 3200 waves. To be safe, a histidine spectrum would be run to 4000 waves. With a nitrogen laser, if we would start a spectrum at 200 with an argon laser, with nitrogen, we would start at 5682 waves. This is because before this number, the nitrogen laser would give a laser line spectrum. It would give a spectrum of nitrogen gas. After 5682, the nitrogen is exhausted and there is no other spectrum to be taken except that of the sample. It becomes confusing when using a nitrogen laser and a sample containing nitrogen. Therefore, if we were to run histidine with a nitrogen laser, there would be an excess of peaks implying nitrogen.

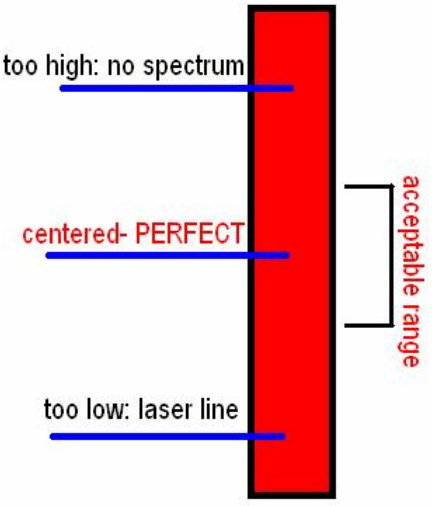
Typically, there is a universal specific method to obtaining a SER spectrum. The main focus is on the sample on the electrode. With SERS, gold, silver, or copper are the common used metals for the electrode: these occur in the form of a roughened surface, island film, or a colloid. It was found that a significant enhancement of the Raman Signal occurred when the organic compound came in close contact with a roughened metallic surface, therefore, an insulated conductor was soldered to a piece of pure silver which was the embedded into a chemically resistant resin, leaving one face exposed. This face was the working electrode.



An electrode may be reused, the surface of which must, at each experiment, be subject to an oxidation reduction reaction and be roughened with Aluminum powder. The electrode may be reused so long as none of the samples used contain nitrogen or sulfur. To obtain the SERS of wet compounds, one micro liter of sample solution was placed on the roughened surface. A glass cover slip was placed over the sample, so as not to let the sample dry out too much. Sometimes, depending on the sample, the sample may be left to evaporate, so as to leave only dried sample, or the sample can be rum as a single drop of solution. There are three main filters used in an argon laser (a krypton laser would use two; a nitrogen laser would use one). If the sample is dried, then it is placed on the laser light. If the sample is wet, then a glass cover slip is suspended from the Teflon electrode-case. Now, it is to be understood that, the light reflects off of nothing except the mirror/reflector and the electrode. Even though one main point of SERS is reflecting the light off the particles, in order to focus the laser, the metallic surface of the electrode and the placement of the mirror add more light to the focus (see end for diagram).



Therefore, focusing the laser into the slit perfectly is crucial to the spectrum. If the laser is diffuse, then there will either be a very high background, which blocks any possible peaks. If it is too high or too low, there may not even be a spectrum.

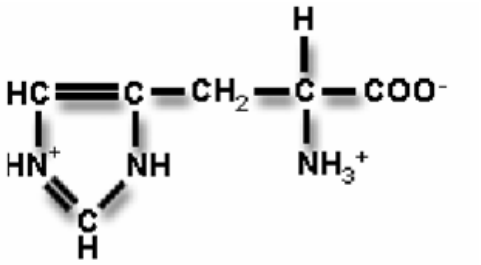


The laser must be a single dot, the diameter measurable in only micrometers, in the center of the slit. When the laser hits the slit, it goes into the spectrograph, where the changes in vibrational energies are measured.

There are two main ways to put a sample in the laser's path. One way is simply placing the surface enhanced electrode in the laser's path and taking a spectrum. Another way is what is called *In Situ*. In an *In Situ* spectrum, the electrode is placed within a cell. This cell is filled with the solution (in our experiment, it is filled with 10ml of histidine and potassium chloride) and an oxidation reduction reaction is performed on the electrode while it is in the solution. Then the solution is sealed off, and filled with nitrogen gas, to degas the solution, and to remove excess oxygen. The cell is sealed off completely, and the spectrum in run, while the sample is still in solution. The positive point of using an *In Situ* method is that the sample never dries out, and there is always enough sample to take a spectrum. The negative of using such a method is that if there is any gas in the cell, the spectrum will be of the present gas, and not of the solution; also, consider that on an electrode surface, the surface itself is a few millimeters in diameter. Our surface was 3.5 mm in diameter. If the sample is in a drop form, then there is almost no place for the substance to go: meaning, if the molecules are excited, they cannot go anywhere out of the single drop. The laser line will pick up all molecules in that single drop. If there is a large cell which holds 50 times that amount, the excited atoms could escape the path of the laser, and our spectrum might be weaker than is possible. Using an *In Situ* method is up to the scientist and the sample they choose to use.

Histidine is an amino acid. It is one of the 20 common natural amino acids nutritionally; histidine is

considered an essential Amino Acid, but mostly only in children. This amino acid side chain finds its way into considerable use as a coordinating enzyme, and also as a catalytic site in certain enzymes.



Chemical name Histidine
Chemical formula
C6H9N3O2
Molecular mass 155.16 gmol-1

In Catalytic Triads, the basic nitrogen of histidine is used to extract a proton from Serine, Threonine or Cysteine. Histidine is also used as a ‘Proton Shuttle In a histidine proton shuttle, histidine is used to quickly transport protons, it can do this by abstracting a proton with its basic nitrogen to make a positively charged intermediate and then use another molecule, example, a buffer, to extract the proton from its acidic nitrogen.

In our experiment, we tried obtaining SERS of various substances. Our main concentration was on Histidine. At first, we used an argon laser. Solutions of .001, .01, and .one molar solution were made in 25 milliliters of sodium Chloride. We diluted each amount (0.001 M=0.00389g solid; 0.01 M=0.0389g; 0.1 M=0.389g). For the sample itself, we had to prepare various electrodes. Our first attempt was at a silver electrode. The electrode had to be roughened with aluminum powder. Then sonicated, and roughened once again. After it was roughened a second time, it was sonicated and subject to an Oxidation reduction cycle. This was to make the solution cling more easily tot he roughened surface; not only was the surface roughened to hold and trap particles in the histidine solution, but the electrode became a magnet, attracting the solution. Then, we placed the electrode in the

Spectrometer, and ran a Raman program. We started each trial at 200 wavelengths, and ran it until it reached 3800 waves. We discovered that the spectrum was not what was needed: we knew, for instance, there was a nitrogen bond, which should have shown at 3200: instead, there was an excess of peaks. These excess

peaks we discovered, were photoluminescence from the beam. We inferred that the sodium was actually reflecting light as well as the histidine, which blended together. We re-ran the sample, using instead of 25 milliliters of Sodium chloride, we used Potassium chloride on the roughened silver surface. After running the sample again, we discovered that our oxidation reduction cycle had ruined our electrode.

At .001 Molar, we discovered that there might not have been enough Histidine solid to obtain a spectrum. We kept obtaining a spectrum of silver (the electrode) and even, sometimes, of Teflon (holding the electrode). We ran this spectrum numerous times, realizing that our concentration of histidine was too small. When we escalated to 0.01 molar histidine in Sodium Chloride, we ran the spectrum, but realized that there was a photoluminescence. We determined that this was because the sodium in the sodium chloride was absorbing the laser light, and that there was a mixed spectrum, of histidine, sodium, and also, laser noise. The laser was set to such a high frequency (a normal spectrum would use a frequency of 20-30: the histidine was producing an intense frequency and increasing the laser frequency to almost 67) that it was producing a noise, which resembled a frantic, scattered line that went up and down aimlessly. Therefore, we increased to 0.1 molar, and instead of using sodium chloride, we used potassium chloride. We received some photoluminescence, but there were enough peaks to constitute a spectrum. From this spectrum, we could only conclude that there were hydrogen bonds and a Nitrogen bond. There is also NH3 and COO bond present, but these were not present in our spectrum.

In conclusion, most of our Peaks are Consistent within ranges of 30-40 wavelengths. This means that we have found our own spectrum of Histidine, but, our problem is getting it more accurate. We have run the spectrum with 99% pure histidine solid, .001 M solution, and .01 M solution. Our spectrum has excess noise from the laser, which prevent us from finding all the peaks in the

spectrum. Also, we are trying different methods, such as if De-gassing the solution makes for a better spectrum, adding or taking away light, Oxidizing/Reducing the sample more than one time, etc. With these results, we discovered that we would have to run our spectrum again, with different possibilities:

1) We would have to run our spectrum to at least 8000 in order to get peaks for the compounds in Histidine with the Argon laser

2) We use the nitrogen laser from 5682-20000 waves in order to pick up all compounds and structures.

3) We could run a spectrum using a gold electrode, since a gold electrode would not only hold more substance, but it is highly metallic and would produce stronger peaks, on an argon laser.

We hope, that in the future, we can experiment not only with different electrodes, different lasers, but also, different methods. Before our summer session had ended, we were experimenting with degassing the substance. Also, comparing the spectrums in situ versus on the electrode may give different spectrums. Therefore, our only goal is to obtain a solid, repeatable spectrum with histidine by any of the various available methods, in the future.

References

1. Benevides, J. A., Overman, S. A., & Thomas, G. J. (2005, May). Raman, polarized Raman and ultraviolet resonance Raman spectroscopy of nucleic acids and their complexes. *Journal of Raman Spectroscopy, 36*(6-7), 279-299.

2. Habuchi, S., Cotlet, M., Gronheid, R., Michiels, J., Vanderleyden, J., De Schryver, F., et al. (2003). Single molecule Surface Enhanced Resonance Raman Spectroscopy of the Enhanced Green Flourescent Protein. *Journal of the American Chemical Society, 125*(28), 8446-8447.

3. Ooka, A. A., & Garrell, R. L. (2000). Surface enhanced Raman Spectroscopy of DOPA- Containing Peptides Related to Adhesive Protein of Marine Mussel, Mytilus Edulis. *Biospectroscopy, 57*, 92-102.

4. Pinzaru, C., Pavel, I., Leopold, N., & Kiefer, W. (2004, May). Identification and characterization of pharmaceuticals using Raman and surface-enhanced Raman scattering. *Journal of Raman Spectroscopy, 35*(5-6), 338-346.

5. Roth, E., Hope, A., Schweinsberg, D. P., Kiefer, W., & Fredericks, P. M. (1993). Simple Techniqe for measuring Surface Enhanced Fourier Transform Raman Spectra of Organic Compounds. *Applied Spectroscopy, 47*(11), 1794-1800.

6. Stewart, S., & Fredericks, P. M. (1999). Surface Enhanced Raman Spectroscopy of Peptides and Proteins absorbed on an Electrochemically Prepared Silver Surface. *Spectrochima Acta, Part A*(55), 1615-1640.

7. Tian, Z. Q. (2005). Surface-enhanced Raman spectroscopy: advancements and applications. *Journal of Raman Spectroscopy, 36*(6-7), 446-470.

8. Yang, H., Zhang, Z., Shen, G., & Yu, R. (2004, March). In situ Raman spectra of an NAD+-modified silver electrode at various potentials. *Journal of Raman Spectroscopy, 35*(5-6), 190-194.

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