Raman Spectroscopy

Introduction:

Raman spectroscopy, a molecular spectroscopy which is observed as inelastically scattered light, allows for the interrogation and identification of vibrational (phonon) states of molecules. As a result, Raman spectroscopy provides an invaluable analytical tool for molecular finger printing as well as monitoring changes in molecular bond structure (e.g. state changes and stresses & strains).

In comparison to other vibrational spectroscopy methods, such as FT-IR and NIR, Raman has several major advantages. These advantages stem from the fact that the Raman effect manifests itself in the light scattered off of a sample as opposed to the light absorbed by a sample. As a result, Raman spectroscopy requires little to no sample preparation and is insensitive to aqueous absorption bands. This property of Raman facilitates the measurement of solids, liquids, and gases not only directly, but also through transparent containers such as glass, quartz, and plastic.

Similar to FT-IR, Raman spectroscopy is highly selective, which allows it to identify and differentiate molecules and chemical species that are very similar. Figure R-1 shows an example of five similar molecules – Acetone, Ethanol, Dimethyl Sulfoxide, Ethyl Acetate, and Tolune. Although each chemical has a similar molecular structure, their Raman spectra are clearly differentiable, even to the untrained eye. Using Raman spectral libraries, it is easy to see how easily Raman spectra can be used for material identification and verification.
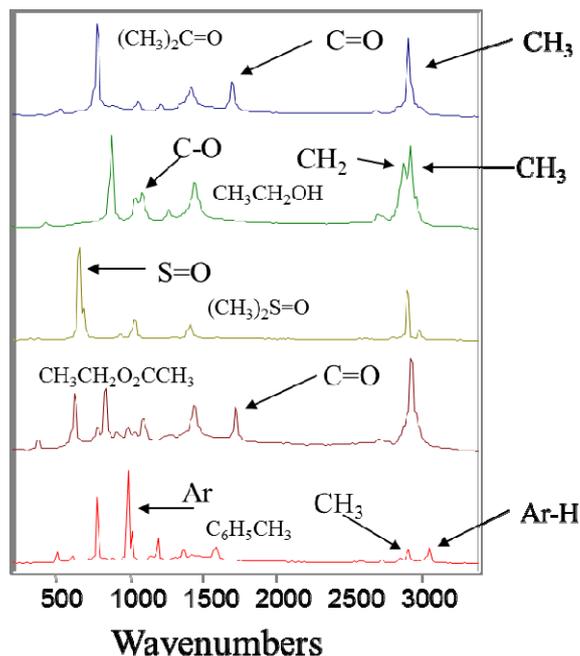

Figure R-1 Example Raman Spectra of Various Molecules

Theory of Raman Scattering:

When considering Raman scattering, we can think about the physics in one of two ways: the classical wave interpretation or the quantum particle interpretation. In the classical wave interpretation, light is considered as electromagnetic radiation, which contains an oscillating electric field that interacts with a molecule through its polarizability. Polarizability is determined by the electron cloud's ability to interact with an electric field. For example, soft molecules such as benzene tend to be strong Raman scatterers while harder molecules like water tend to be fairly weak Raman scatterers.
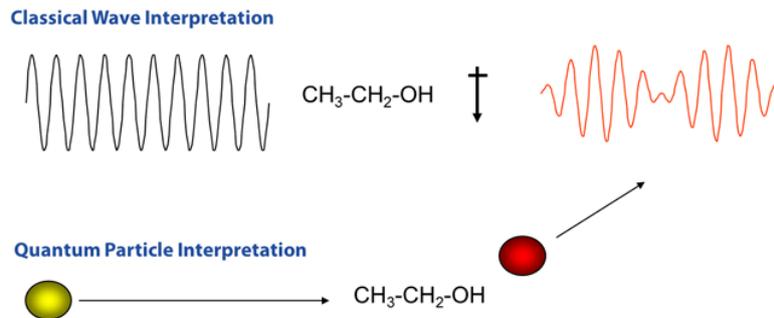

Figure R-2 Comparison of Raman Scattering Interpretations

When considering the quantum particle interpretation, light is thought of as a photon which strikes the molecule and then inelasticaly scatters. In this interpretation the number of scattered photons is proportional to the size of the bond. For example, molecules with large Pi bonds such as benzene tend to scatter lots of photons, while water with small single bonds tends to be a very weak Raman scatterer. Figure R-2 shows a visual comparison of the two methods.

When deriving the Raman effect, it is generally easiest to start with the classical interpretation by considering a simple diatomic molecule as a mass on a spring (as shown in figure R-3) where m represents the atomic mass, x represents the displacement, and K represents the bond strength.
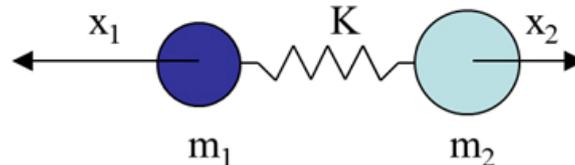

Figure R-3 Diatomic Molecule as a Mass on a Spring

When using this approximation, the displacement of the molecule can be expressed by using Hooke's law as,

$$\frac{m_1 m_2}{m_1 + m_2}\left(\frac{d^2 x_1}{dt^2} + \frac{d^2 x_2}{dt^2}\right) = -K(x_1 + x_2)\,.$$

Equation R-1

By replacing the reduced mass ($m1m2/[m1+m2]$) with μ and the total displacement ($x1+x2$) with q, the equation can be simplified to,

$$\mu \frac{d^2 q}{dt^2} = -Kq,$$

Equation R-2

By solving this equation for q we get,

$$q = q_o \cos(2\pi \nu_m t),$$

Equation R-3

where ν_m is the molecular vibration and is defined as,

$$v_m = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}}.$$

<div align="center">Equation R-4</div>

From equations R-3 and R-4, it is apparent that the molecule vibrates in a cosine pattern with a frequency proportional to the bond strength and inversely proportional to the reduced mass. From this we can see that each molecule will have its own unique vibrational signatures which are determined not only by the atoms in the molecule, but also the characteristics of the individual bonds. Through the Raman effect, these vibrational frequencies can be measured due to the fact that the polorizability of a molecule, α, is a function of displacement, q. When incident light interacts with a molecule, it induces a dipole moment, P, equal to that of the product of the polorizability of the molecule and the electric field of the incident light source. This can be expressed as,

$$P = \alpha E_o \cos(2\pi v_o t),$$

<div align="center">Equation R-5</div>

where E_o is the intensity and v_o is the frequency of the electric field. Using the small amplitude approximation, the polorizability can be described as a linear function of displacement,

$$\alpha = \alpha_o + q\left(\frac{\partial\alpha}{\partial t}\right)_{q=0} + \cdots,$$

<div align="center">Equation R-6</div>

which when combined with equations R-3 and R-5 results in,

$$P = \alpha_o E_o \cos(2\pi v_o t) + q_o \cos(2\pi v_m t)\, E_o \cos(2\pi v_o t)\left(\frac{\partial\alpha}{\partial t}\right)_{q=0}.$$

<div align="center">Equation R-7</div>

In Equation R-7 we see that there are two resultant effects from the interaction of the molecule and the incident light. The first term is called Rayleigh scattering, which is the dominate effect and results in no change in the frequency of the incident light. The second term is the Raman scattered component and when expanded to,

$$q_o E_o \left(\frac{\partial\alpha}{\partial t}\right)_{q=0} [\cos(2\pi\{v_o - v_m\}t) + \cos(2\pi\{v_o + v_m\}t)],$$

<div align="center">Equation R-8</div>

can be shown to shift the frequency of the incident light by plus or minus the frequency of the molecular vibration. The increase in frequency is known as an Anti-Stokes shift and the decrease in frequency is known as a Stokes shift. By measuring the change in frequency from the incident light (typically only the Stokes shift is used for this measurement) the Raman effect now gives spectroscopists a means of directly measuring the vibrational frequency of a molecular bond.

Now that we have derived the Raman effect using the classical wave interpretation, we can now use the quantum particle interpretation to better visualize the process and determine additional information. As discussed earlier in the quantum interpretation, the Raman effect is described as inelastic scattering of a photon off of an molecular bond. From the Jablonski diagram shown in figure R-4, we can see that this results from the incident photon exciting the molecule into a virtual energy state.
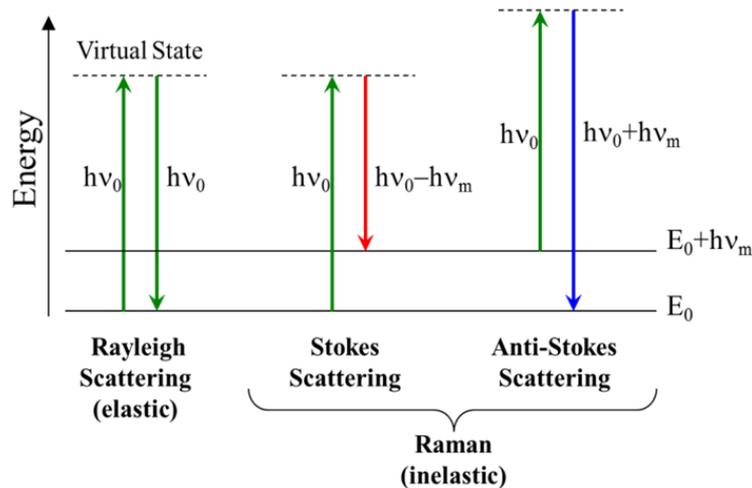
Figure R-4 Jablonski Diagram Representing Quantum Energy Transitions for Rayleigh and Raman Scattering

When this occurs, there are three different potential outcomes. First, the molecule can relax back down to the ground state and emit a photon of equal energy to that of the incident photon; this is an elastic process and is again referred to as Rayleigh scattering. Second, the molecule can relax to a real phonon state and emit a photon with less energy than the incident photon; this is called Stokes shifted Raman scattering. The third potential outcome is that the molecule is already in an excited phonon state, is excited to a higher virtual state, and then relaxes back down to the ground state emitting a photon with more energy than the incident photon; this is called Anti-Stokes Raman scattering. Due to the fact that most molecules will be found in the ground state at room temperature, there is a much lower probability that a photon will be Anti-Stokes scattered. As a result, most Raman measurements are performed considering only the Stokes shifted light.

By further investigating the quantum interpretation of the Raman effect, it can be shown that the power of the scattered light, P_s, is equal to the product of the intensity of the incident photons, I_o, and a value known as the Raman cross-section, σ_R. It can be shown that,

$$\sigma_R \propto \frac{1}{\lambda^4},$$

Equation R-9

where λ equals the wavelength of the incident photon. Therefore,

$$P_s \propto \frac{I_o}{\lambda^4}.$$

Equation R-10

From equation R-10 it is clear that there is a linear relationship between the power of the scattered light and the intensity of the incident light as well as a relationship between the power of the scattered light and the inverse of the wavelength to the fourth power. Therefore, it would appear that it is always desirable to use a short excitation wavelength and a high power excitation source based on these relationships. However, as we will see in the next section, this is not always the case.

Components of a Raman Spectrometer:

There are three primary components to any Raman spectrometer: an excitation source, a sampling apparatus, and a detector. While these three components have come in varying forms over the years, modern Raman instrumentation has developed around using a laser as an excitation source, a spectrometer for the detector, and either a microscope or a fiber optic probe for the sampling apparatus.

Since Raman spectroscopy is predicated on the ability to measure a shift in wavelength (or frequency) it is imperative that a monochromatic excitation source be employed. While a laser is typically the best excitation source, not all lasers are suitable for Raman spectroscopy, so it is imperative that the laser frequency is extremely stable and does not mode hop, since this will cause errors in the Raman shift. It is also essential to utilize a clean, narrow bandwidth laser because the quality of the Raman peaks are directly affected by the sharpness and stability of the excitation light source.

The final consideration when deciding which laser to use for a Raman spectrometer is the wavelength. From the previous section, it is clear that the shorter the wavelength the more powerful the Raman signal. However, as was already stated, this is not the only consideration especially when dealing with organic molecules. Most organic molecules will tend to fluoresce when excited by high energy (short wavelength) photons. Although fluorescence is typically considered to be a low light level process, it can still overwhelm the signal in the Raman spectrum as shown in Figure R-5. This is because the Raman effect is comprised of a very small fraction (about 1 in 10^7) of the incident photons. As a result, visible lasers are typically only used for inorganic materials such as carbon nanotubes.
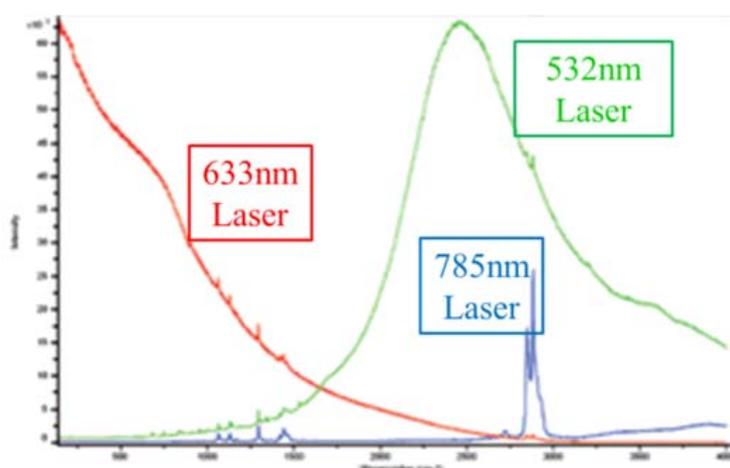

Figure R-5 Comparison of Raman Spectrum at Varying Excitation Wavelengths Demonstrating Fluorescence Interference

For organic molecules it is important to shift the laser wavelength into the near infrared to minimize fluorescence while not exceeding CCD spectral detection limits. Due to their availability and the fact that they allow for the maximum fluorescence reduction without the sacrifice of spectral range or resolution, 785nm diode lasers have become the industry standard. For increased sensitivity with inorganic molecules, a 532nm laser is the best choice because fluorescence is no longer an issue.

As previously discussed, Raman scattering is very weak and therefore tends to require long integration times in order to collect enough photons to measure a discernible signal. This makes the use of a TE cooled spectrometer a requirement in order to reduce the dark noise. For very low concentrations or weak Raman scatters, it may be necessary to use a back-thinned CCD to further increase the sensitivity of the spectrometer. By etching the detector to only a few microns thick the probability of an electron being reabsorbed as it travels through the detector based on Beer's law is greatly reduced. This increases the sensitivity of the detector from a maximum quantum efficiency of 35% to greater than 90%.

Due to the highly selective nature of Raman spectra, they may contain peaks which are fairly close together. Depending on the application, it may be necessary to resolve these closely spaced peaks, which requires the use of a high resolution spectrometer. Typically standard spectrometer configurations are for 532nm and 785nm laser excitation wavelengths, with custom excitation wavelengths also available. These spectrometers can be offer a variety of spectrometer configurations specially designed for wide spectral range and high resolution. Typical spectral ranges are available from as low as 65cm^{-1} (filter dependent) to as high as 4000cm^{-1}, with a spectral resolution as fine as 3.0cm^{-1}.

When measuring the sample, there is no more effective method of directing the laser light to the sample, collecting the Raman scatter, and directing it to a spectrometer than a fiber optic probe.

A Raman probe must be capable of directing and focusing the monochromatic excitation source (typically a laser) to the sample, collecting the scattered light and then directing it to the spectrometer. Figure R-6 shows a typical design for a Raman probe.
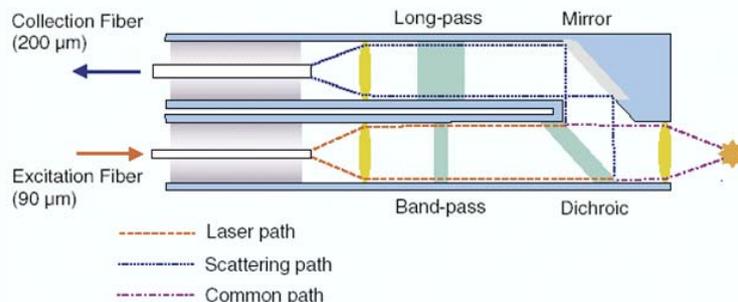

Figure R-6 Typical Design of a Raman Probe

Since a pure signal is extremely important to Raman spectroscopy, a narrow band-pass filter is placed in the optical path of the excitation source before it reaches the sample. It is also important to note that since the Raman effect is extremely weak, the signal must be collected at a 0° angle normal to the sample. This causes interference from Rayleigh scattering and therefore it is essential to filter the collected signal through the use of a long pass filter before it is directed to the spectrometer.

The flexibility afforded by fiber optics not only allows for the probe to be taken to a solid sample, but also allows it to be immersed in liquids or slurries in both laboratory and process environments (for real time kinetic measurements). It can also be connected to microscopes, cuvette holders, as well as a plethora of sampling accessories.

Applications:

Bioscience and Medical Diagnosis:

- Detection of subtle changes within biomolecules, such as drug interactions, tissue healing, cosmetics, and disease diagnosis
- ntercellular SERS localization and interaction, identification of drug binding to cells for Drug-DNA and cellular interaction analysis
- Investigation of microorganisms in single cells; yeast cell classifications, single bacterium
- Molecular level cancer detection (cervical, lung, etc.)
- Cardiovascular disease diagnosis (atherosclerosis)

Pharmaceutical Industry:

- Analysis of tablets, liquids, and gel caps
- High throughput screening techniques
- Process Analytical Technology (PAT) on-line, at-line monitoring and control: real-time monitoring of drying, coating, and blending
- Identification and analysis of API, additives, and excipients
- Drug identification control: purity and quality
- Raw material inspection: 100% incoming material identification and verification

Raman Microscopy:

- Pharmaceutical drug analysis: micro-Raman and localized molecular species analysis in complex drug mixtures, such as beta-carotene in multivitamins
- Material science thin film analysis, such as diamond film quality characterization
- Trace forensic evidence analysis, including fibers, fabrics, pigments, inks, etc.

Polymers and Chemical Processes:

- Quality Control: incoming/outgoing
- Identification of contaminants during manufacturing
- Real time monitoring of polymerization
- Predicting the morphological properties of polymers
- Multivariate analysis/chemometrics to predict physical properties: glass transition temperature, crystallization temperature, etc.
- Chemical composition analysis
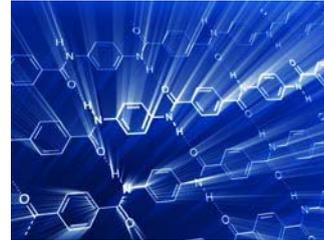
Environmental Science:

- Water pollution detection using SERS technology
- Identification of contaminants in water
- Petrochemical analysis
- Identification and analysis of sediments in water

Forensic Analysis:

- Nondestructive drug and narcotic drug identification
- Explosives: exact chemical compositions of materials, PETN, RDX and binding agents within explosive materials
- Identification and analysis of toxic solvents and bio-warfare agents
- Forensic evidence analysis and tracing, including fibers, fabrics, pigments, inks, etc., by Raman microscopy

Gemology:

- Non-invasive gemstone identification and examination
- Identification of unknown gemstone by unique Raman signal
- Identification of isomorph or subspecies of gemstone
- Analysis of gemstone origin through Raman microscopy
- Anti-counterfeiting, such as identification of diamond from zircon

Geology and Mineralogy:

- Identification of geological materials
- Examination of inclusions in minerals
- Analysis of cement clinker by Raman microscopy
- Ancient fossil analysis

Food & Agriculture Industry:

- Measurement of unsaturated fatty acid in food oils
- Detection of bacteria and/or contaminants in food products
- Identification of additive drugs: nutraceuticals in fruit drinks
- Analysis of components in grain kernel

Semiconductor & Solar Industry:

- Characterization of silicon crystallinity: monitoring of the Raman band shift as silicon crystallinity changes from amorphous to a polycrystalline structure
- Analysis of micron sized particles in situ to provide information on potential contamination
- Mechanical stress monitoring for semiconductor process