

Raman Scattering Spectroscopy

Raman scattering, and the related technique of resonance Raman scattering, are methods that examine the vibrational states of molecules. Raman spectroscopy therefore involves transition energies similar to those in infrared spectroscopy. However, because the selection rules for Raman scattering and infrared absorbance differ, and because the underlying mechanism by which the electromagnetic radiation interacts with the molecule differs for the two processes, the techniques yield somewhat different results.

Infrared spectroscopy, like ultraviolet-visible spectroscopy, is a process that involves absorption of light by the molecules of the sample. The intensity of infrared absorbance bands is therefore dependent on the extinction coefficient and concentration of the absorbing molecules at the relevant wavelength. This makes properly obtained infrared spectra readily comparable between samples. However, “properly obtained” infrared spectra are difficult to obtain for proteins, due to the difficulty in maintaining infrared cuvettes (sodium chloride tends to dissolve in the aqueous buffers required to maintain most proteins in their native conformation), and due to the intense infrared absorption of water. In addition, infrared bands tend to overlap, which complicates the interpretation of the infrared spectrum.

In contrast, water has a weak Raman spectrum. In addition, as is described below, Raman is a function of the polarizability of the chemical group, and therefore groups containing π -bonds or other sources of relatively loosely bound electrons dominate the Raman spectrum. Although the weak Raman bands of π -bonded atoms may become important due to the presence of these bonds in large numbers, in general the Raman spectrum is more easily interpreted than the infrared spectrum.

Raman signals are due to inelastic scattering of the incident electromagnetic radiation. The time-varying electric field of the radiation is:

$$\mathbf{E}(t) = \mathbf{E}_0 \cos 2\pi \nu t$$

This electric field induces a dipole in the molecule:

$$\mathbf{p}(t) = \mathbf{a}(\nu) \cdot \mathbf{E}_0 \cos 2\pi \nu t$$

where $\mathbf{a}(\nu)$ is the polarizability of the molecule. Because molecules vibrate, the polarizability term will vary with time:

$$\mathbf{a}(\nu) = \mathbf{a}_0(\nu) + \mathbf{a}'(\nu) \cos 2\pi \nu' t$$

where $\mathbf{a}_0(\nu)$ is the polarizability of the equilibrium conformation of the molecule, $\mathbf{a}'(\nu)$ is the polarizability change with molecular vibration, and ν' is the vibrational frequency of the molecule. (Note that the equation refers to two different frequencies, the frequency of the electromagnetic radiation, ν , and the molecular frequency, ν' .)

Combining the two equations above gives:

$$\mu(t) = [\mu_0(\nu) + \mu(\nu)] \cos 2 \nu t \cdot \mathbf{E}_0 \cos \omega t$$

Since $\cos A \cos B = 0.5[\cos(A+B) + \cos(A - B)]$:

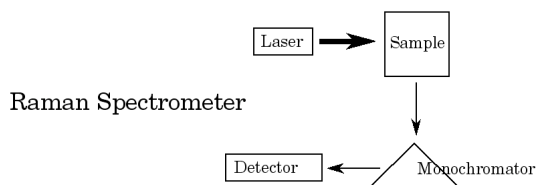
$$\mu(t) = \mu_0(\nu) \cdot \mathbf{E}_0 \cos 2\nu t + 0.5\mu(\nu) \cdot \mathbf{E}_0 [\cos 2 \nu(\nu + \omega)t + \cos 2 \nu(\nu - \omega)t] = \mu(t) + \mu(\omega)$$

The induced dipole therefore varies in two ways; the strength of the induced dipole varies with the electric field, and the strength of the induced dipole varies with molecular vibration. Oscillating dipoles emit radiation at their frequency of oscillation, with an intensity proportional to $(\ddot{\mu})^2$. Thus, a vibrating molecule exposed to radiation at ν will emit at $\nu + \nu'$ (the Stokes band) and at $\nu - \nu'$ (the anti-Stokes band). (The molecule will also emit at ν , which is the source of Rayleigh elastic scattering and of the index of refraction.)

Raman signals require a change in polarizability with motion, while infrared absorbance requires a change in the vibrational energy level of a permanent dipole. The intensity of Raman and infrared signals therefore tends to differ, because the underlying mechanism is different. As an example, water, which has a strong infrared absorbance, has a weak Raman spectrum. The low level of interference from water is a major reason for the popularity of Raman for the study of biological molecules.

Raman spectroscopy is associated with some problems. The frequency shift $\nu + \nu'$ or $\nu - \nu'$ tends to be fairly small, and may be lost in the light scattering from the sample unless a very good monochromator is used. In most cases, laser light sources are used, because these can generate intense monochromatic light. A second problem is that the Raman signal tends to be fairly weak (1 part in $\sim 10^5$ of the incident light). A third problem is that fluorescent signals (from the molecule of interest or from contaminants), which may have much higher intensity, will also be observed using standard instrumentation. This last problem can usually be solved by using long wavelength incident beams (such as the 514.5 nm wavelength of an argon-ion laser); this works because the **Raman frequency shift is independent of wavelength**. (The relatively long wavelength also reduces the effect of Rayleigh scattering, which is proportional to λ^{-4} , and is therefore much stronger at shorter wavelengths.)

In Raman spectrometers, as in fluorescence spectrometers, the instrument is usually designed to detect light emitted in a direction perpendicular to the incident light beam. This reduces interference from vastly more intense incident light. In the drawing at right, a laser is used as a light source. The laser is, in effect, a monochromator, and therefore no monochromator is necessary before the light interacts with the sample.



In standard Raman spectroscopy, the laser usually has a fixed wavelength (such as the 514.5 nm of the argon ion-laser mentioned above). In resonance Raman

spectroscopy, the incident wavelength is chosen to be close to the absorption maximum of a chromophore. This requires variable wavelengths, because all molecules do not exhibit the same absorbance spectrum. In most instruments, this wavelength variation is achieved by the use of tunable lasers.

In resonance Raman spectroscopy, the molecular interaction of the light by the absorbing chromophore has a much higher probability than other groups. As a result, the Raman bands of the chromophore will be much stronger than those of other groups, and can be examined preferentially.

Raman spectroscopy can be used to assess secondary structure, because different secondary structural elements have characteristic Raman bands. In addition, as with most spectroscopic techniques, Raman is sensitive to changes in the bonding patterns of a molecule. Thus, Raman can be used to study hydrogen-deuterium exchange (which is proportional to exposure of the ionizable protons) in molecules too large to be amenable to NMR analysis. Raman can also be used to monitor conformational changes. Finally, Raman and resonance Raman can be very useful for studying changes in prosthetic groups.

A relatively new variant on Raman scattering is Raman optical activity, which measures the differential scattering of left and right circularly polarized light. Because the difference in scattering for the polarized light is small, and because the polarizer decreases the incident light intensity, the technique tends to require long measurement times in order to obtain reasonable signal to noise ratios. In addition, due to the fact that the optical activity is most apparent in backscattered light, the instrument must have a considerably different design from a normal Raman spectrometer. In the drawing below, the mirror has a hole that permits the incident light to reach the sample. Light backscattered to angles close to 180° is reflected by the mirror, and reaches the monochromator. The separated light is then directed to a CCD detector, allowing light of multiple wavelengths to be measured simultaneously. The use of a CCD allows the integration of the scattered light signal over the prolonged periods necessary for useful results.

