RAMAN SCATTERING

1. Introduction

Raman scattering is an inelastic scattering process in which energy is transferred to or from the molecule so that the scattered light is different in frequency from the exciting light. The energy difference is caused either by the molecule beginning or stopping to vibrate. By subtracting the frequency of the scattered radiation from the frequency of the exciting radiation, a vibrational spectrum is obtained. The spectrum is a plot of the frequency of each vibration along the x axis and of the relative Raman scattering efficiency of each vibration along the y axis. Only a small proportion of scattered photons are scattered by the Raman process, but the frequency shift makes detection relatively simple with modern equipment. Microscopes or fiber optics can be used to aid sampling. Raman scattering provides a form of vibrational spectroscopy that is complementary to infrared (ir) and the combination with ir spectroscopy can often lead to a more effective assignment of the structure of the molecule on the basis of vibrational information. In addition, the molecularly specific information that can be obtained from the spectra with minimal sample handling have made it a valuable tool in many fields including the assessment of diamond films and silicon surfaces, biodetection, and nondestructive analysis for works of art. There are many books on Raman spectroscopy (1–3) and reviews of specialist areas. In this article, a brief overview of the main features of the effect and an indication of the potential for obtaining useful information is described.

Although the effect had been predicted earlier, the first published account of Raman scattering was by C. V. Raman and K. S. Krishnan (4) in 1928. Initially, the effect was called feeble fluorescence although fluorescence involves reradiation of absorbed light and is not a scattering process in which no absorption takes place. In 1930, Raman was awarded the Nobel Prize in chemistry and this scattering effect given his name. Since then, Raman spectroscopy has been applied to many areas of industrial, medical, and academic interest.

Raman used sunlight as the excitation source in his early experiments. A series of filters were placed before and after the sample so that light that was frequency shifted by the sample could be observed by the eye. One problem with the observation of Raman scattering is that only a few photons are inelastically scattered by the Raman process, and consequently powerful light sources and sensitive detectors are required. One major reason for the recent rapid expansion of Raman spectroscopy is that readily available lasers are powerful and effective monochromatic sources and modern detectors are very sensitive in making the collection of Raman scattering much easier. Time scales in older experiments used to vary from many minutes to several hours. In a modern laboratory, Raman scattering from a strong scatterer can be detected with a simple system in a second or less, and with pulsed lasers much shorter time scales are used.

2. Basic Theory and Method

The scattering process is initiated by irradiating a sample using essentially monochromatic radiation. This is called excitation of the sample and, in a modern system, it is usually effected using a laser. The sample can be a liquid, solid, or gas. Most scattered light arises from Raleigh scattering in which the frequency of the light is not appreciably shifted from the excitation frequency, but a much weaker component, Raman scattering, accompanies it. Approximately 1 in 10^6 to 1 in 10^8 photons of the scattered photons are scattered by the Raman process. However, the appreciable shift in frequency that occurs with Raman scattering makes it possible to discriminate between the two processes by using a small monochromator. Figure 1 shows a typical spectrum and illustrates the fact that there are two different Raman scattering processes. Most molecules at room temperature are in the ground vibrational state and the Raman process involves transfer of the energy from the excitation beam to the molecule making the molecule begin to vibrate. Consequently, most Raman scattered photons have a frequency lower than that of the excitation source. This process is known as Stokes Raman scattering. However, before excitation a few molecules may be present in an excited vibrational state. Where that is the case, the Raman scattering process involves transfer of energy from the molecule to the scattered light leading to the scattering of higher energy photons. This process is called anti-Stokes scattering. By comparison with Stokes scattering the intensity is low, since only a few molecules will be present in excited states at least at room temperature. In addition, the population of an excited state will depend on its energy above the ground state with higher energy states less populated. As a result, anti-Stokes intensities drop off further compared to the corresponding Stokes intensity as the vibrational frequency increases. Since Stokes spectra are more intense than anti-Stokes spectra, the most common practice is to record only the Stokes spectrum from a sample.

During the Raman process, the beam of light interacts with the electron cloud round the molecule causing it to distort. The ability of the molecule to do this is dependent on the polarizability of the electron cloud. Thus, strong Raman scatterers have large polarizabilities. In addition, the selection rules for Raman scattering are such that symmetric vibrations cause the greatest scattering and are the most intense. By contrast, in ir absorption, a dipole moment change is required to give strong absorption and asymmetric vibrations are the most intense.

The spectra are usually recorded as a frequency shift with one shift for every effective vibration. The shift is the difference between the frequency of the exciting laser and the scattered light. The shifts are plotted along the x axis with the intensity of the scattering for each plotted on the y axis. The intensity of the scattering from any particular vibration is given by the expression $I = kl\alpha^2\nu^4$. Where, α, the polarizability, is the molecular property involved in the process and the relationship between α and the electronic, vibrational, and rotational states of a molecule is well understood through scattering theory (1,3,5). k is a constant and both l and v are properties that are in the control of the operator. The parameter l is the laser power. Higher power should give more intense scattering, but there are clear limits.

In particular, too high a laser power can cause sample degradation. The parameter υ is the frequency of the incident radiation. This is the frequency of the laser used to excite the sample and again the operator has control over the laser used, and therefore control over the frequency. Obviously, the higher the laser frequency, the more intense the scattering that would be expected. This would suggest that ultraviolet (uv) lasers would be ideal for Raman scattering. However, they can lead to sample damage and can be more expensive to buy and to run. In addition, the beam is invisible, increasing the danger of eye damage through inadvertent exposure to the beam. Thus, although there are obvious advantages in uv excitation, visible lasers are most commonly used. A small visible laser, eg, a solid-state 532-nm laser is very effective. Spectra can be taken with a laser pen, but it is usually advisable to ensure that the frequency and output power is stable with time. With visible excitation, some compounds fluoresce. Fluorescence usually occurs at lower frequencies than that of the excitation frequency and, although fluorescence can be shifted further from the exciting line than Raman scattering, there are many cases in which fluorescence interferes significantly with the collection of Raman scattering. To obtain Raman scattering from the widest range of compounds, near-ir lasers can be used. The inefficient scattering caused by the low value of ν can to some extent be offset by the higher laser powers that can be used since absorption of the radiation by the sample is usually less efficient.

3. Strengths and Weaknesses

Raman scattering is effective on fewer samples than ir absorption because of the wide variation in scattering efficiencies and because of interference from fluorescence. However, it has major advantages for specific analyses in that there is very little sample preparation. Strong scatterers give very clear spectra that can often be easily recognized and interpreted. Water is a good solvent to use, and flexible sampling using microscopes or fiber optics is easily achieved. In addition, some specialist methods have specific advantages for certain problems, where special requirements, eg, ultrahigh sensitivity, are needed. Thus, with the arrival or simpler more effective Raman spectrometers, the field is continuing to expand.

3.1. Advantages. The most significant practical advantage of Raman scattering is the ease of sample handling and preparation. However, the advantages of Raman scattering as an analysis technique should be assessed with respect to the considered problem. The main advantages are

1. No need to separate the analyte from the matrix: The spectrum depends on the molecular structure, so species can be readily identified *in situ* without separation from other molecules.
2. Minimal requirement for sample preparation: Standard samples, eg, powders and solutions, as well as complete articles, eg, pills, plastic mouldings, or pieces of fruit, can be placed in the spectrometer and a spectrum obtained without any sample preparation.

3. Variation of scattering from different molecules: Some molecules give strong, while others give weak scattering. Water in particular is a weak scatterer making it possible to obtain a vibrational spectrum from a species in aqueous solution or buffer. This is a big advantage in bioanalysis and one key advantage over ir in this field.
4. Remote detection: With only the laser beam contacting the sample, the use of the method in process analysis or to analyze potentially hazardous and toxic samples is rather straightforward.
5. Ease of use in combination with other techniques: Raman scattering is used in combination with microscopes and fiber optics to examine small samples and surfaces, for remote detection in hazardous environments, or simply for convenience.

3.2. Disadvantages. The major disadvantages are

1. Sensitivity: It is not very sensitive with dispersed analytes. However, sensitivity can be improved using a microscope allowing a spectrum to be readily recorded from extremely small crystals. In this way, <1 ng can be identified. However, for molecules in solution, detection limits are $\sim 10^{-1}$–$10^{-2} M$.
2. Interference of fluorescence signal: Excitation of a sample with a visible laser can produce a fluorescence signal that can obscure the Raman spectrum. The ratio of fluorescence to scattering depends on the molecule and, although it can be changed by adding quenchers, it is usually best to change the excitation frequency to avoid absorption of radiation by the sample. However, fluorescence need not arise from the actual analyte. The high powers used to obtain Raman scattering often mean that impurities can fluorescence sufficiently to interfere. Where impurities do cause the fluorescence, the laser beam is being preferentially absorbed by these molecules, and it is common practice to leave the sample under the beam to allow the impurities to photofade and degrade. Later, the Raman spectrum of the bulk sample can often be obtained.
3. Scattering efficiency: The wide range of scattering efficiencies means that some molecules are difficult to detect. For some selective analysis, this can be a huge advantage since a strong scatterer can be easily detected in a matrix of weaker scatterers, but Raman scattering is less effective in determining weak scatterers.

4. Instrumentation

Modern instrumentation for basic Raman scattering is much simpler and more reliable than it was 5–10 years ago. As a result, Raman scattering is becoming much more widely used. A simple modern Raman spectrometer consists of a laser, a lens to focus the scattered light, a notch or edge filter to remove Raleigh scattering and reflected light, a monochrometer and a CCD detector. A diagram of a Raman spectrometer is shown in Figure 2.

This type of equipment, especially for visible excitation, is widely available and very effective. One drawback of this kind of system is that the CCD detectors used do not work below a frequency of $\sim 10{,}000\,cm^{-1}$. Allowing for the Raman shift, this means that the lowest energy lasers that are effective with this type of system are those with wavelengths of $\sim 790-850\,nm$. For systems based on 1064-nm lasers (a popular ir laser choice), a different detection method, eg, an interferometer with an InGaS array, is required. This type of equipment is very similar to that used in Fourier transform ir spectroscopy.

The simplicity of the equipment has led to the coupling of Raman spectrometers to other optical devices, eg, microscopes with mapping stages or to fiber-optic heads for remote detection. Combined Raman spectrometers with other devices, eg, electron microscopes, chromatography columns, or AFM systems, have been constructed and are proving effective. Further, small handheld portable systems are available, enabling Raman spectra to be used in new areas of science outside the laboratory.

5. Specialized Techniques

There are a number of more selective ways of using Raman scattering that can be very effective for specific problems. Three that are used more commonly are reviewed below.

5.1. Resonance Raman Scattering. If an analyte has a suitable chromophore, a laser frequency can be chosen to approximately match the frequency of the absorption band. This can lead to absorption of light and subsequent photodegradation or efficient fluorescence. However, some photons are scattered and the process, called resonance Raman scattering, is much more efficient than ordinary Raman scattering (5). Enhancement of the Raman scattering by 10^3-10^4 is quite common and enhancements up to $\sim 10^6$ have been observed for some molecules. The relative efficiency of scattering and absorbance for any one molecule is a molecular property that is difficult to manipulate, but for a good scatterer, eg, as the heme group, resonance enhancement aids selective identification of the chromophore *in situ*. Figure 3 shows the spectrum obtained from a P450 enzyme. The most intense band arises from a vibration assigned as ν_4 for which the main displacement is a symmetric stretch of the nitrogen atoms coordinated to the central iron ion. It is an oxidation state marker for the iron in the heme system. All the other bands in the spectra are also from the heme group. They include bands that are spin state markers and bands that identify alterations in the orientation of the vinyl groups at the edge of the heme group to the protein. The P450 is a large protein with many atoms in it, but because of the selective enhancement, it is the heme spectra that dominate. This spectrum can be obtained in solution and is a good example of a case where Raman scattering can be used very selectively to probe the active site of the enzyme. In addition, resonance Raman scattering, because of the additional enhancement, can be used to obtain Raman scattering from much more dilute solutions with good spectra being recorded from solutions $\sim 10^{-4}M$ and in some cases from solutions down to 10^{-5} or $10^{-6}M$.

5.2. Surfaced Enhanced Raman Scattering. Surface enhanced Raman scattering (SERS) was discovered experimentally during studies of the adsorption of pyridine on a silver electrode (6). There is still debate over the exact mechanism of the effect, but it is believed to involve an interaction between the adsorbed molecule and surface plasmons (collective oscillations of loosely bound electrons on the surface caused by the incident light). Energy transfer through a chemical bond between the analyte and the surface may also contribute. The surface must be rough to give effective scattering. Enhancement factors for this process are calculated as $\sim 10^6$. This makes SERS a very sensitive detection technique that can be used to probe surface chemistry. Although some other metals and materials can be effective, the most commonly used metals to make effective substrates are silver and gold. A further development of this technique is to combine it with resonance Raman scattering by using a molecule containing a chromophore to obtain the surface enhanced scattering. This combined technique—surface enhanced resonance Raman scattering (SERRS)—can have an enhancement factor over Raman scattering of 10^{14} or 10^{15}. SERRS at the single molecule level has been demonstrated.

5.3. Coherent Anti-Stokes Raman Scattering. Many more advanced techniques are known. Mostly they involve complex laser systems and are limited to laboratories with specialist equipment. Some use two photon processes in which the two photons interact with one molecule to cause the scattering. One of these techniques is coherent anti-Stokes Raman scattering (CARS). In this process, two photons excite the molecule simultaneously and the scattering results from the combined effects of the two photons. This technique has been simplified recently by developing it for use with a microscope. The big advantage is that anti-Stokes Raman scattered photons are at higher energy than those from the excitation laser. As a result, fluorescence is much less of a problem. However, the technique does create a background of its own, is more complex, and is not yet widely used. There are many more techniques that can be used with pulsed lasers to obtain time dependent signals, but these lie outwith the scope of this article.

Other techniques that are becoming of more general interest include Raman optical activity (ROA) and uv Raman scattering. Both techniques can be used to probe selective features of protein structure and a brief introduction can be found in Ref. 3.

6. Uses

Raman scattering has become important in a number of fields. It is regularly used to analyze silicon surfaces and surfaces that are coated with carbon and particularly diamond, since diamond gives extremely strong Raman signals. It is also possible to discriminate crystalline from amorphous carbon because the amorphous carbon peak is much broader.

The simplicity of Raman scattering has led to its use in process analysis. One well-known example of this is in the monitoring of the manufacture of the pigment rutile. Rutile is a particular form of titanium dioxide that produces a white pigment and it is produced in bulk in various parts of the world. However,

another form, anatase, also forms, and the monitoring of the ratio of rutile to anatase is an important process analysis parameter. Raman scattering can discriminate rutile from anatase quickly and without sample preparation (Fig. 4) giving feedback on the ratio of the two in a very short time scale. Robust fiber-optic coupling systems with lenses that allow relatively long distances between the sample and the collection head have been developed and the use of fiber optics enables the spectrometer to be sited in a convenient place away from the potentially harmful conditions of the plant environment.

Raman scattering in the form of SERRS has the potential to be used in biological analysis in a manner similar to fluorescence. Fluorescence detection relies on the use of a fluorescent label attached to a DNA sequence. The sequence recognizes a complimentary strand and combines with it. This causes molecular recognition. The fluorescence probe is used as a sensitive and selective detection tag. Figure 5 shows the spectrum a SERRS label attached to a deoxyribonucleic acid (DNA) oligonucleotide at concentrations down to 0.8 f *M*. The spectra are entirely from the SERRS active dye chromophore. No scattering from the DNA itself is obtained since the concentration is so low. This degree of sensitivity makes SERRS competitive with fluorescence. The advantage of SERRS is that the pattern of sharp peaks provides very good *in situ* molecular identification of a particular label or of a number of labels in a mixture.

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W. Ewen Smith
University of Strathclyde

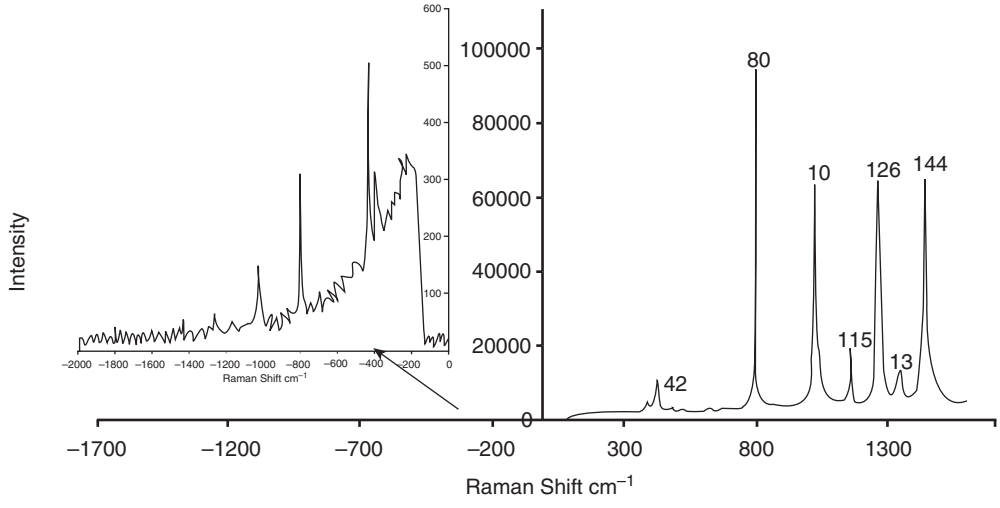

Fig. 1. Raman scattering from cyclohexane showing both Stokes and anti-Stokes spectra. Note that the anti-Stokes spectrum is much weaker because it arises from molecules in an excited vibrational state.

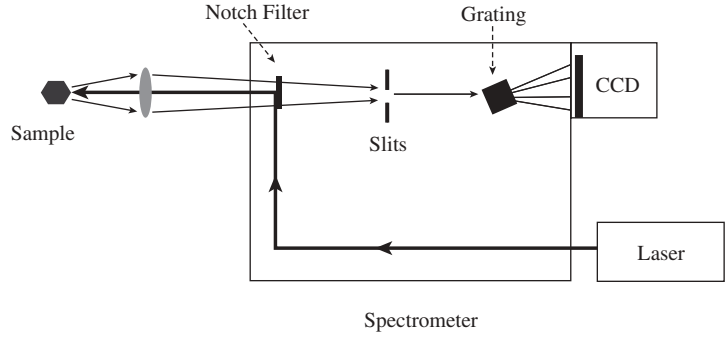

Fig. 2. Diagram of a Raman spectrometer showing a laser beam exciting a sample and the scattering being collected back along the same axis(180° scattering). Another common arrangement is to collect the scattering at 90° to the excitation direction.

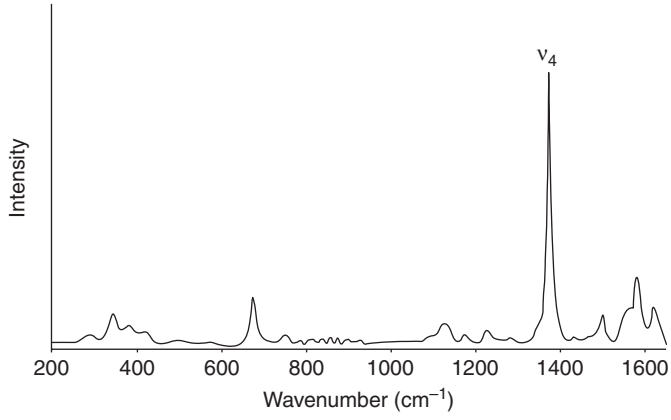

Fig. 3. Resonance Raman scattering from a $10^{-4}M$ buffered solution of P450 BM3-a heme-containing enzyme.

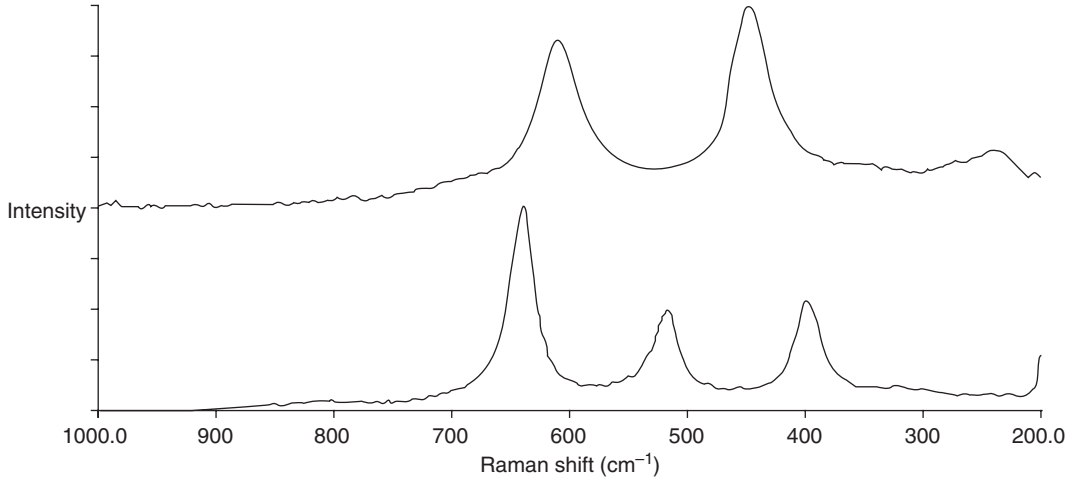

Fig. 4. Raman scattering from titanium dioxide in the form of anatase (foot) and rutile (top) indicating the ease with which each form can be discriminated. Note that the spectrum is presented as it was originally with the shift running in the other direction. Both directions are used at present.

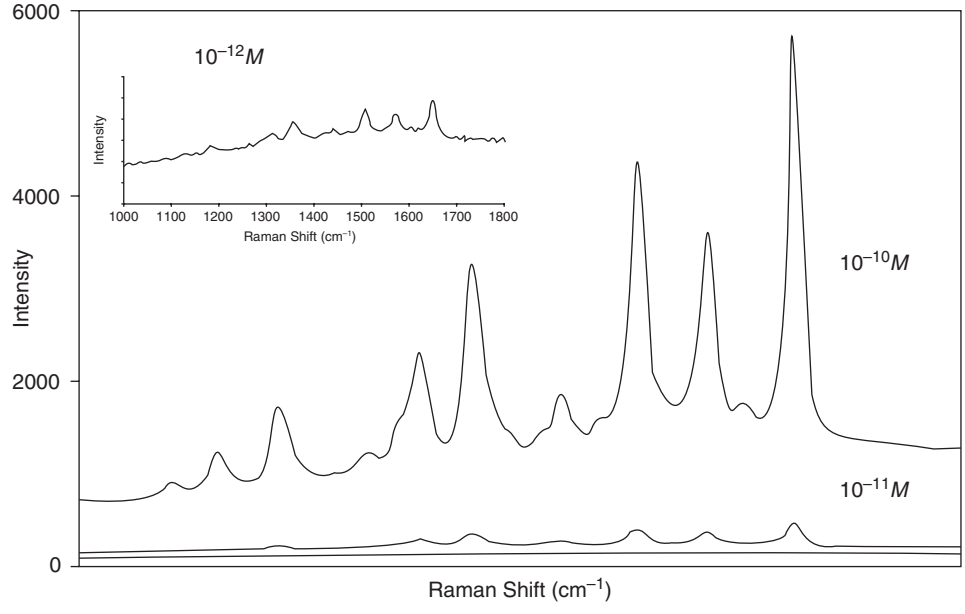

Fig. 5. The SERRS from an oligonucleotide labeled with the dye Rhodamine 6G to provide a SERRS label. All peaks can be assigned to the dye. The inset shows a spectrum from a 0.8-fM solution.