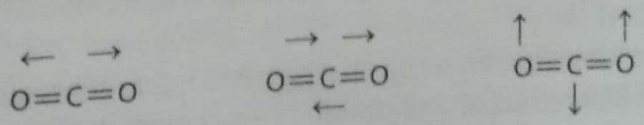
4.8 RAMAN SPECTROSCOPY

Raman spectroscopy is a powerful, noninvasive technique for studying molecular vibrations by light scattering. Raman spectroscopy complements IR absorption spectroscopy because some vibrations, as we have seen, do not result in absorptions in the IR region. A vibration is only seen in the IR spectrum if there is a change in the dipole moment during the vibration. For a vibration to be seen in the Raman spectrum, a change in polarizability is necessary. That is, a distortion of the electron cloud around the vibrating atoms is required. Distortion becomes easier as a bond lengthens and harder as a bond shortens, so the polarizability changes as the bound atoms vibrate. As we learned earlier in the chapter, homonuclear diatomic molecules such as Cl_2 do not absorb IR radiation, because they have no dipole moment. The Cl–Cl stretching vibration is said to be *IR inactive*. Homonuclear diatomic molecules do change polarizability when the bond stretches, so the Cl–Cl stretch is seen in Raman spectroscopy. The Cl–Cl stretching vibration is said to be *Raman active*. Some molecular vibrations are active in IR and not in Raman, and *vice versa*; many modes in most molecules are active in both IR and Raman. Looking at CO_2 again, shown in the following diagrams, the mode on the left is the IR-inactive symmetric stretch, while the other two asymmetric vibrations are both IR active. The symmetric stretch is Raman active:



In general, symmetric vibrations give rise to intense Raman lines; nonsymmetric ones are usually weak and sometimes unobserved.

Raman spectroscopy offers some major advantages in comparison to other analytical techniques. Because it is a light-scattering technique, there are few concerns with sample thickness and little interference from ambient atmosphere. Therefore, there is no need for high-vacuum systems or instrument purge gas. Glass, water, and plastic packaging have weak Raman spectra, allowing samples to be measured directly inside a bottle or package, thereby minimizing sample contamination. Aqueous samples are readily analyzed. No two compounds give exactly the same Raman spectra and the intensity of the scattered light is proportional to the amount of material present. Raman spectroscopy is therefore a qualitative and quantitative technique.

4.8.1 Principles of Raman Scattering

When radiation from a source is passed through a sample, some of the radiation is scattered by the molecules present. For simplicity, it is best to use radiation of only one frequency and the sample should not absorb that frequency. The beam of radiation is merely dispersed in space. Three types of scattering occur. They are called *Rayleigh scattering*, *Stokes scattering*, and *anti-Stokes scattering*. Most of the scattered radiation has the same frequency as the source radiation. This is Rayleigh scattering, named after Lord Rayleigh, who spent many years studying light scattering. Rayleigh scattering occurs as a result of elastic collisions between the photons and the molecules in the sample; no energy is lost on collision. However, if the scattered radiation is studied closely, it can be observed that slight interaction of the incident beam with the molecules occurs. Some of the photons are scattered with less energy after their interaction with molecules and some photons are scattered with more energy. These spectral lines are called *Raman lines*, after Sir C.V. Raman, who first observed them in 1928. Only about 1 photon in a million will scatter with a shift in wavelength. The Raman–Stokes lines are from those photons scattered with less energy than the incident radiation; the Raman–anti-Stokes lines are from the photons scattered with more energy. The slight shifts in energy and therefore slight shifts in the frequencies of these scattered photons are caused by inelastic collisions with molecules. The differences in the energies of the scattered photons from the incident photons have been found to correspond to vibrational transitions. Therefore, the molecules can be considered to have been excited to higher vibrational states, as in IR spectroscopy, but by a very different mechanism. Figure 4.62 shows a schematic diagram of the Rayleigh and Raman scattering processes and of the IR absorption process.

The energy of the source photons is given by the familiar expression $E = h\nu$. If a photon collides with a molecule, the molecule increases in energy by the amount $h\nu$. This process is not quantized, unlike absorption of a photon. The molecule can be thought of as existing in an imaginary state, called a virtual state, with an energy between the ground state and the first excited electronic state. The energies of two of these virtual states are shown as dotted lines in Figure 4.62. The two leftmost arrows depict increases in energy through collision for a molecule in the ground state and a molecule in the first excited vibrational state, respectively. The arrows are of the same length, indicating that the interacting photons have the same energy. If the molecule releases the absorbed energy, the scattered photons have the same energy as the source photons. These are the Rayleigh scattered photons, shown by the two middle arrows. The molecules have returned to the same states they started from, one to the ground vibrational state and the other to the first excited vibrational state. The arrows are the same length; therefore, the scattered photons are of the same energy.

If the molecule begins to vibrate with more energy after interaction with the photon, that energy must come from the photon. Therefore, the scattered photon must decrease in energy by the amount equal to the vibrational energy gained by the molecule. That process is shown by the second arrow from the right. Instead of returning to the ground vibrational state, the molecule is now in the first excited vibrational state. The energy of the scattered photon is $E - \Delta E$, where ΔE is the difference

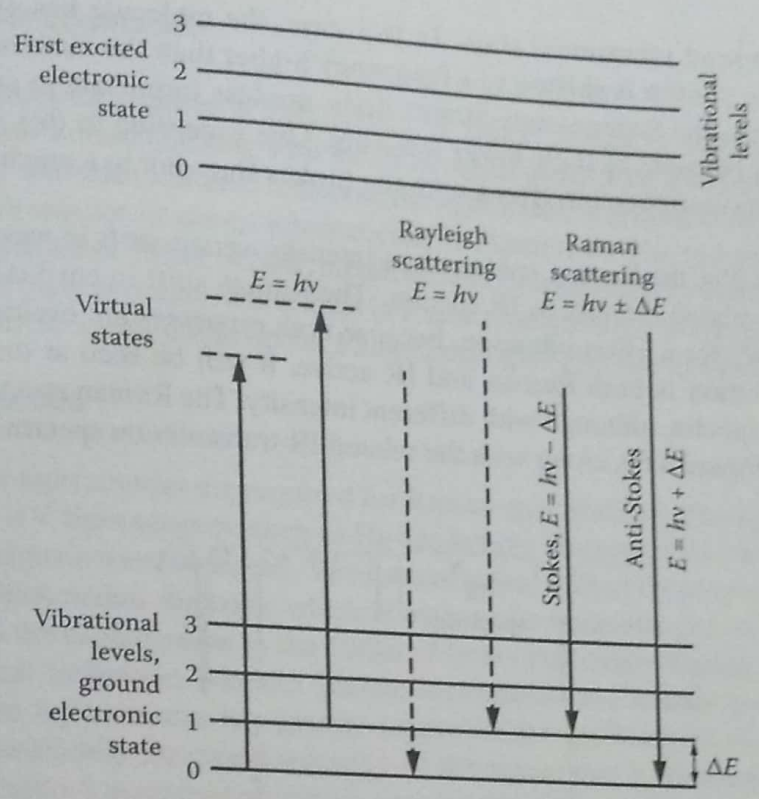


Figure 4.62 The process of Rayleigh and Raman scattering. Two virtual states are shown, one of higher energy. Rayleigh and Raman scattering are shown from each state. Normal IR absorption is shown by the small arrow on the far right marked ΔE, indicating a transition from the ground-state vibrational level to the first excited vibrational level within the ground electronic state.

in energy between the ground and first excited vibrational states. This is Raman scattering, and the lower energy scattered photon gives rise to one of the Stokes lines. Note that ΔE is equal to the frequency of an IR vibration; if this vibration were IR active, there would be a peak in the IR spectrum at a frequency equal to ΔE. In general, the Raman–Stokes lines have energies equal to $E - \Delta E$, where ΔE represents the various possible vibrational energy changes of the molecule. This relationship can be expressed as

$$E - \Delta E = h(\nu - \nu_1) \tag{4.13}$$

where

ν is the frequency of the incident photon
ν_1 is the *shift* in frequency due to an energy change ΔE

Several excited vibrational levels may be reached, resulting in several lines of energy $h(\nu - \nu_1)$, $h(\nu - \nu_2)$, $h(\nu - \nu_3)$, and so on. These lines are all shifted in frequency from the Rayleigh frequency. The Stokes lines, named after Sir George Gabriel Stokes, who observed a similar phenomenon in fluorescence, are shifted to lower frequencies than the Rayleigh frequency. The Raman shifts are completely independent of the wavelength of the excitation source. Sources with UV, visible, and NIR wavelengths are used, and the same Raman spectrum is normally obtained for a given molecule. There are exceptions due to instrumental variations and also if a resonance or near-resonance condition applies at certain wavelengths (Section 4.8.4).

Less commonly, the molecule *decreases* in vibrational energy after interacting with a photon. This might occur if the molecule is in an excited vibrational state to begin with

and relaxes to the ground vibrational state. In this case, the molecule has given energy to the scattered photon. The photon is shifted to a frequency higher than the incident radiation. These higher-frequency lines, the Raman–anti-Stokes lines, are less important to analytical chemists than the Stokes lines because of their lower intensity. One exception to this is for samples that fluoresce strongly. Fluorescence interferes with the Stokes lines but to a much lesser extent with the anti-Stokes lines.

It is convenient to plot the Raman spectrum as intensity versus shift in wavenumbers in cm^{-1}, because these can be related directly to IR spectra. The Raman shift in cm^{-1} is identical to the IR absorption peak in cm^{-1} for a given vibration, because both processes are exciting the same vibration. That is, if a vibration is both Raman and IR active, it will be seen at the same position in wavenumbers in both spectra, although with different intensity. The Raman spectra for benzene and ethanol are shown in Figure 4.63, along with the related IR transmission spectra.

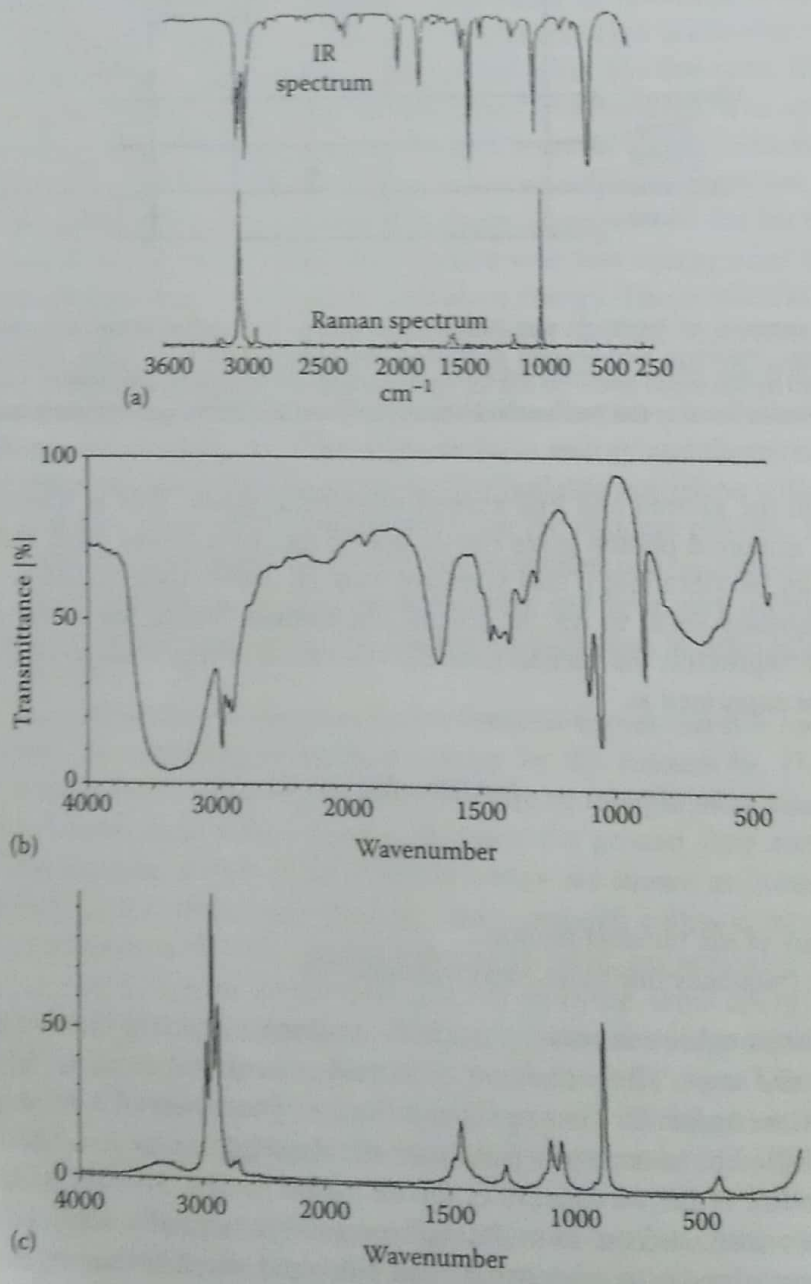


Figure 4.63 (a) The IR spectrum and Raman spectrum of benzene. (b) The IR spectrum of ethanol. (c) The Raman spectrum of ethanol. (b) and (c) are not plotted on the same scale. (The ethanol spectra are courtesy of http://www.aist.go.jp/RIODB/SDBS.)

4.8.2 Raman Instrumentation

A Raman spectrometer requires a light source, a sample holder or cell, a wavelength selector (or interferometer), and a detector, along with the usual signal processing and display equipment. Since Raman spectroscopy measures scattered radiation, the light source and sample cell are usually placed at 90° to the wavelength selector, as shown schematically in Figure 4.64. The radiation being measured in Raman spectroscopy is either visible or NIR; therefore, spectrometer optics, windows, sample cells, and so on can be made of glass or quartz. It is critical in Raman spectroscopy to completely exclude fluorescent room lights from the spectrometer optics. Fluorescent lights give rise to numerous spurious signals.

4.8.2.1 *Light Sources*

Monochromatic light sources are required for Raman spectroscopy. The light sources used originally were simple UV light sources, such as Hg arc lamps; however, these were weak sources and only weak Raman signals were observed. The Raman signal is directly proportional to the power of the light source, which makes the laser, which is both monochromatic and very intense, a desirable light source. It was the development in the 1960s of lasers that made Raman spectroscopy a viable and useful analytical technique. Modern Raman instruments use a laser as the light source. The use of these intense light sources has greatly expanded the applications of Raman spectroscopy, because of the dramatically increased intensity of the signal and a simultaneous improvement of the signal-to-noise ratio. Lasers and excitation wavelengths commonly used for Raman instruments include visible wavelength helium/neon lasers and ion lasers such as the argon ion laser (488 nm) and the krypton ion laser (532 nm). The intensity of Raman scattering is proportional to the fourth power of the excitation frequency or to $1/\lambda^4$, so the shorter wavelength blue and green ion lasers have an advantage over the red helium/neon laser line at 633 nm. The disadvantage of the shorter wavelength lasers is that they can cause the sample to decompose on irradiation (photodecomposition) or fluoresce, an interference discussed subsequently. NIR lasers, such as neodymium/yttrium aluminum garnet (Nd/YAG) with an excitation line at 1064 nm, and the 785 nm NIR diode laser are used to advantage with some samples, such as biological tissue, because they do not cause fluorescence or photodecomposition. However, longer integration time or a higher-powered laser may be needed to compensate for the decrease in scattering efficiency of NIR lasers. Because of the wavelength-dependent nature of fluorescence excitation, a Raman spectrometer that integrates multiple laser sources and makes changing lasers easy should be considered if a diverse sample load is expected.

4.8.2.2 *Dispersive Spectrometer Systems*

Traditional Raman spectrometers used a monochromator with two or even three gratings to eliminate the intense Rayleigh scattering. The optical layout is similar to that for the UV/VIS single grating monochromators discussed in Chapters 2 and 5. Holographic interference filters, called

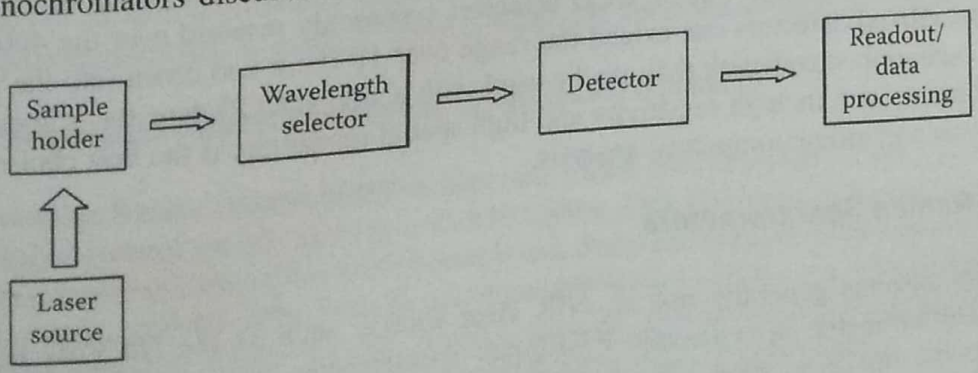


Figure 4.64 Idealized layout of a Raman spectrometer.

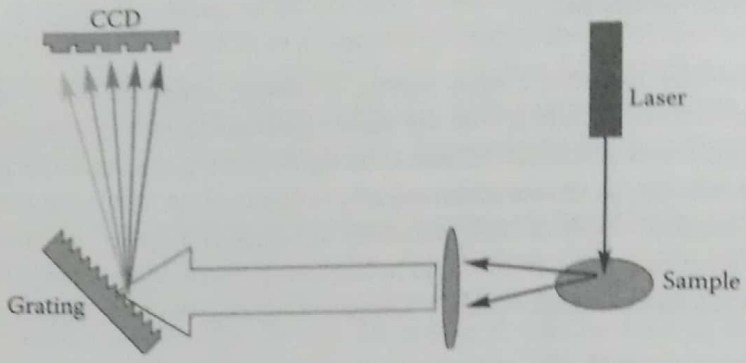


Figure 4.65 Schematic of a dispersive Raman spectrometer. (Reprinted from Weesner, F. and Longmire, M., *Spectroscopy*, 16(2), 68, 2001. With permission from Advanstar Communications, Inc.)

super notch filters, have been developed that dramatically reduce the amount of Rayleigh scattering reaching the detector. These filters can eliminate the need for a multiple grating instrument unless spectra must be collected within 150 cm^{-1} of the source frequency. Dispersive systems generally use a visible laser as the source. The low-end cutoff of the Raman spectrum is determined by the ability of the filters to exclude Rayleigh scattering. Since inorganic compounds have Raman bands below 100 cm^{-1}, modern instruments should provide a 50 cm^{-1} low-end cutoff.

The traditional detector for these systems was a photomultiplier tube. Multichannel instruments with PDA, charge injection device (CID), or charge-coupled device (CCD) detectors are commonly used today. All three detectors require cryogenic cooling. Room-temperature InGaAs detectors are also available. The PDA has the advantage of having the fastest response but requires more complicated optics than the other detectors. The CID has the advantage over both the PDA and CCD of not "blooming." Blooming means having charge spill over onto adjacent pixels in the array, which would be read in error as a signal at a frequency where no signal exists. CCDs are the slowest of the three array detectors because they have to be read out by transferring the stored charge row by row, but they are also the least expensive of the detector arrays. Sensitivity is improved in newer CCD designs as well. A dispersive Raman spectrometer with a CCD detector is shown schematically in Figure 4.65.

As described in Chapter 2, spectral resolution determines the amount of detail that can be seen in the spectrum. If the resolution is too low, it will be impossible to distinguish between spectra of closely related compounds; if the resolution is too high, noise increases without any increase in useful information. Spectral resolution is determined by the diffraction grating and by the optical design of the spectrometer. With a fixed detector size, there is a resolution beyond which not all of the Raman wavelengths fall on the detector in one exposure. Ideally, gratings should be matched specifically to each laser used. A dispersive Raman echelle spectrometer from PerkinElmer Instruments covers the spectral range 3500–230 cm^{-1} with a resolution better than 4 cm^{-1}.

CCDs used in modern instruments are generally Si-based 2D arrays of light-sensitive elements, called pixels. Each pixel, typically <30 μm, acts as an individual detector. Each dispersed wavelength is detected by a different pixel. CCD detectors commonly respond over the 400–1100 nm range, but specialized detectors can extend the range over 1100 nm and down into the UV range. The longest excitation wavelength that can be used with a silicon CCD detector is about 785 nm. Dispersive Raman, with its high sensitivity and high spatial resolution, is the best choice for small particle analysis and minor component analysis.

4.8.2.3 FT-Raman Spectrometers

FT-Raman systems generally use an NIR laser source, such as the Nd/YAG laser, and a Michelson interferometer. A schematic FT-Raman spectrometer is shown in Figure 4.66. The NIR laser source line is at 1064 nm, so the Raman–Stokes lines occur at longer wavelengths.

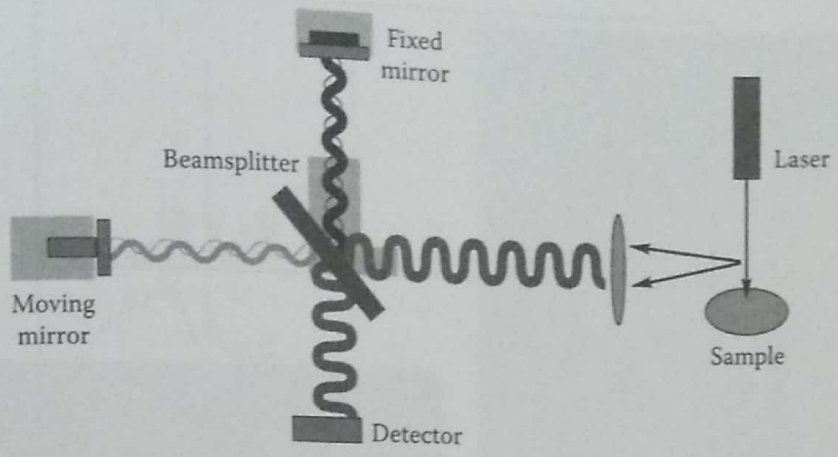


Figure 4.66 Schematic of an FT-Raman spectrometer. (Reprinted from Weesner, F. and Longmire, M., *Spectroscopy*, 16(2), 68, 2001. With permission from Advanstar Communications, Inc.)

This is beyond the detection range of the materials used in array detectors. The detector for an NIR-laser-based FT-Raman system is a liquid nitrogen-cooled photoconductive detector such as Ge or InGaAs. InGaAs detectors that do not require cooling are also available.

FT-Raman has many of the advantages of FTIR. There is high light throughput, simultaneous measurement of all wavelengths (the multiplex advantage), increased signal-to-noise ratio by signal averaging, and high precision in wavelength due to the internal interferometer calibration provided by the built-in He–Ne laser. A major advantage is in the use of the NIR laser excitation source, which dramatically reduces fluorescence in samples. Fluorescence occurs when the virtual states populated by excitation overlap excited electronic states in the molecule. Then, the molecule can undergo a radiationless transition to the lowest ground state of the excited electronic state before emitting a fluorescence photon on relaxation to the ground state. The fluorescence photon is of lower energy than the exciting radiation, and so fluorescence occurs at longer wavelengths, interfering with the Stokes scattering lines. The NIR laser is of low energy and does not populate virtual states that overlap the excited electronic states, as higher energy visible lasers can. As an example, the Raman spectrum of cocaine is shown in Figure 4.67. The spectrum in Figure 4.67a was collected with an FT-Raman spectrometer using an NIR laser, while that in Figure 4.67b was collected with a dispersive Raman system and a visible laser. Figure 4.67b shows a large fluorescence band that obscures most of the Raman spectrum below 2000 cm^{-1}. With appropriate mathematical "smoothing" algorithms and multipoint baseline correction, it is possible to extract a useable Raman spectrum from samples that exhibit strong fluorescence, as shown in Figure 4.68. One consideration in FT-Raman is that the laser line at 1064 nm is very close to a water absorption band. While this does not prevent aqueous solutions from being studied by FT-Raman, aqueous solutions cannot be studied as easily as they can with dispersive Raman. FT-Raman is the better choice for samples that fluoresce or contain impurities that fluoresce. FT-Raman is widely used in the analysis of illicit drugs and pharmaceuticals, as many of these compounds fluoresce strongly at visible wavelengths.

4.8.2.4 *Fiber-Optic-Based Modular and Handheld Systems*

Low-resolution Raman systems based on fiber optics have been developed to provide miniaturized, low-cost, rugged systems for lab, process, and field testing. This development has occurred in the last 10 years, due to improved, low-cost semiconductor lasers, cheap and highly sensitive solid-state detectors, and cheaper, faster computer chips. Portable and handheld Raman systems are now available from several companies, including Rigaku Raman Technologies, Inc. (www.rigakuraman.com), BaySpec, Inc. (www.bayspec.com), Thermo Fisher Scientific (www.thermo.com), Enwave Optronics, Inc. (www.enwaveopt.com), B&W Tek, Inc. (www.bwtek.com), and Ocean Optics (www.oceanoptics.com) for screening

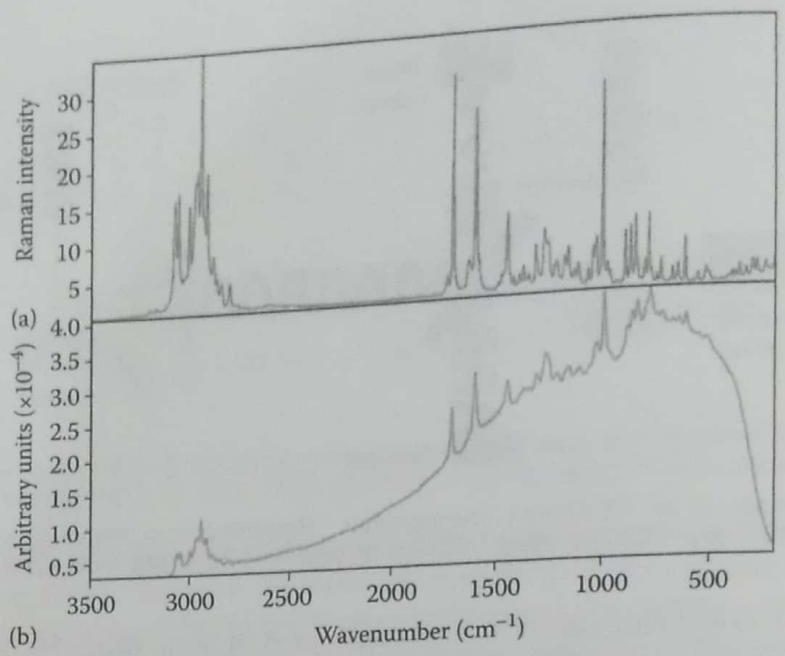


Figure 4.67 Analyses of crack cocaine using (a) FT-Raman and (b) 785 nm dispersive Raman. Note the lack of fluorescence in the FT-Raman spectrum (flat baseline vs. the big "hump" due to fluorescence in the lower spectrum) and the rich spectral information in the upper spectrum. This information is partially obscured by the fluorescence band in the dispersive spectrum. (Reprinted from Weesner, F. and Longmire, M., *Spectroscopy*, 16(2), 68, 2001. With permission from Advanstar Communications, Inc.)

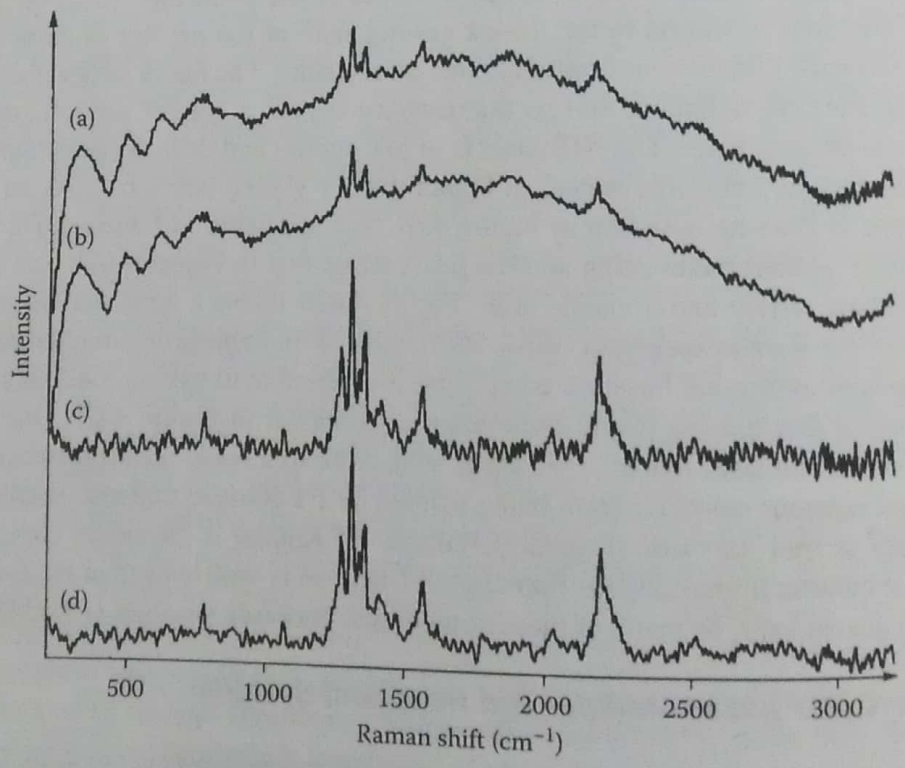
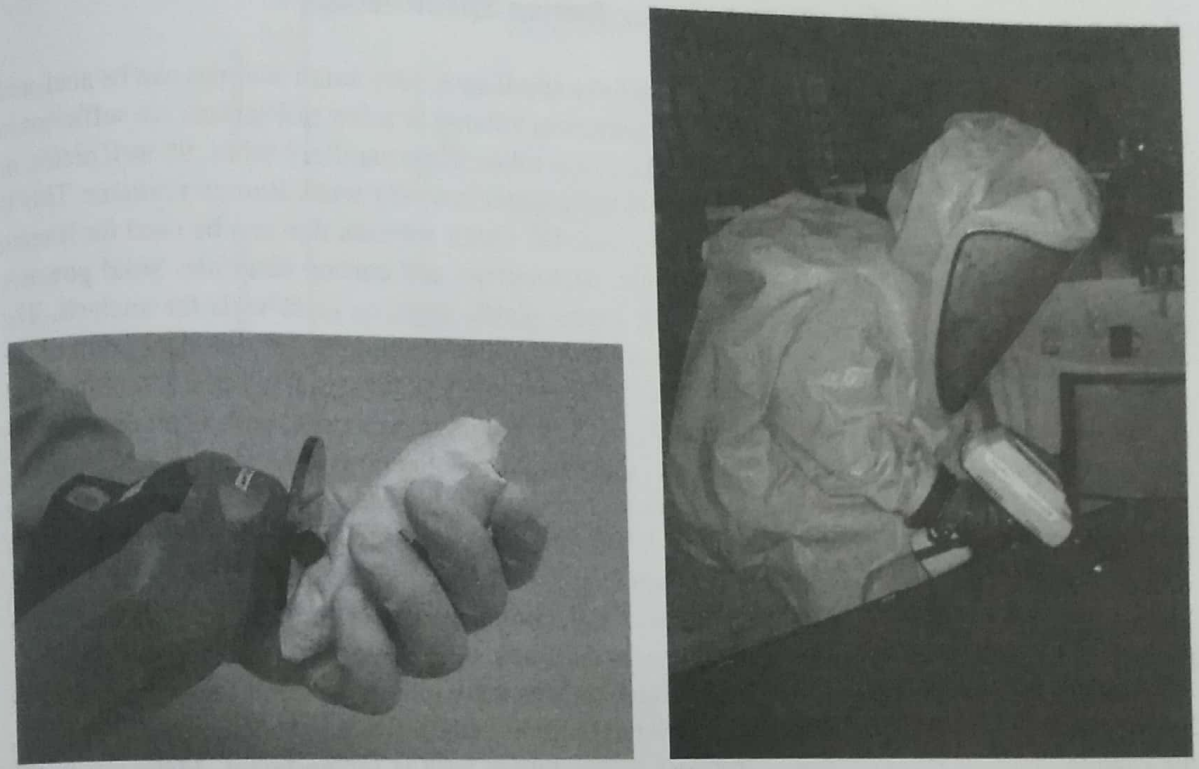


Figure 4.68 It is possible to extract data from a Raman spectrum that exhibits fluorescence interference by the application of mathematical data treatments. The data treatments were applied sequentially to give the final spectrum shown in (d). (a) is the raw spectrum of a weak scatterer with a fluorescence background, (b) is the spectrum after Savitzky–Golay smoothing, (c) after a multipoint baseline correction performed by the analyst, and (d) after a Fourier smoothing. (Reprinted from Kawai, N. and Janni, J.A., *Spectroscopy*, 15(10), 32, 2000. With permission from Advanstar Communications, Inc.)



(a) (b)

Figure 4.69 (a) Handheld TruScan® Raman instrument collecting a spectrum from a powder sample directly through the plastic bag. (© Thermo Fisher Scientific (www.thermofisher.com). Used with permission.) (b) Hazardous material identification by a first responder using the handheld FirstGuard™ 1064 nm advanced Raman system. (Used with permission of Rigaku Raman Technologies, Inc., www.rigakuraman.com.)

of incoming raw materials, hazardous materials, forensic applications, in-line pharmaceutical processing, gem and semiprecious stone quality, authentication and anticounterfeiting, and QC (Figure 4.69). These field and portable instruments weigh between 2 and 5 lb, can be configured with one or more lasers and different detectors. The field portable EZRaman-I series instruments from Enwave Optronics, Inc., for example, are lithium-battery-powered, compact instruments with built-in laptop; one of three lasers (or configured with two different lasers); a high-sensitivity CCD spectrograph, with the CCD thermoelectrically cooled to –50°C; spectral ranges from 100 to 3300 cm^{-1}; and average resolution of 6–7 cm^{-1}.

Handheld and portable systems may have less resolution and spectral range than laboratory systems but can be customized for specific applications with customized spectral libraries. Specialized applications for these instruments include measuring the "freshness" of fish by monitoring the concentration of dimethylamine, which is directly related to age and temperature of processed frozen fish (Herrero et al., 2004). Fat concentration, composition, and saturation index allows a handheld Raman instrument to determine in less than 1 min if edible oils such as olive oil have been adulterated or if cheaper oil is being sold as more expensive olive oil.

Another critical food safety application is the determination of melamine in human and pet food. Melamine has been found in milk and pet food, deliberately added to watered-down or inferior products to boost the apparent protein concentration. The standard wet chemical method for "protein" in food is the Kjeldahl method, which actually measures nitrogen and is an indirect measure of protein. Melamine responds to the Kjeldahl method just like protein, but is not a protein and is toxic; it combines with uric acid to form kidney stones. The USDA has set a maximum allowable limit of 2.5 ppm melamine for adults. Using their portable Nunavut™ Raman System, BaySpec, Inc., scientists demonstrated the measurement of melamine at levels down to 3 ppm in the field; the analysis takes less than 10 s, is accurate, is repeatable, and is nondestructive.

4.8.2.5 Samples and Sample Holders for Raman Spectroscopy

Because the laser light source can be focused to a small spot, very small samples can be analyzed by Raman spectroscopy. Samples of a few microliters in volume or a few milligrams are sufficient in most cases. Liquid samples can be held in beakers, test tubes, glass capillary tubes, 96 well plates, or NMR tubes. Aqueous solutions can be analyzed since water is a very weak Raman scatterer. This is a significant advantage for Raman spectroscopy over IR. Other solvents that can be used for Raman studies include chloroform, carbon tetrachloride, acetonitrile, and carbon disulfide. Solid powders can be packed into glass capillary tubes, NMR tubes, plastic bags, or glass vials for analysis. The spectra are obtained through the glass. Solid samples can also be mounted at the focal point of the laser beam and their spectra obtained "as is" or pressed into pellets. Gas samples do not scatter radiation efficiently, but can be analyzed by being placed into a multipath gas cell, with reflecting mirrors at each end. The body of the gas cell must be of glass to allow collection of the scattered light at 90°.

The sample must be placed at the focal point of an intense laser beam, and some samples may be subject to thermal decomposition or photodecomposition. Accessories that spin the sample tube or cup are available, to distribute the laser beam over the sample and reduce heating of the sample. Spinning or rotating the sample minimizes thermal decomposition, but does not stop photodecomposition. Sample spinning is required for *resonance Raman spectroscopy*, discussed later.

Raman spectroscopy does not suffer interference from atmospheric water vapor or carbon dioxide, as does IR. Gases do not scatter well, so even though Raman-active bands occur for these gases, the contribution to the Raman signal from air in the optical path is insignificant. Materials in the optical path outside of the laser focus also have negligible scattering.

Sample cells have been designed for low- and high-temperature operation, well-plate accessories allow high-throughput analyses, and remote probes and user-friendly video stages are available for many systems.

4.8.3 Applications of Raman Spectroscopy

Quantitative and qualitative analyses of inorganic and organic compounds can be performed by Raman spectroscopy. Raman spectroscopy is used for bulk material characterization, online process analysis, remote sensing, microscopic analysis, and chemical imaging of inorganic, organic, and organometallic compounds, polymers, biological systems, art objects, carbon nanomaterials, and much more. Forensic science applications include identification of illicit drugs, explosives, and trace evidence like hair, fibers, and inks. Raman spectra have fewer lines and much sharper lines than the corresponding IR spectra, as seen in Figure 4.70. This makes quantitative analysis, especially of mixtures, much simpler by Raman spectroscopy than by IR spectroscopy.

Quantitative analysis had not been as common as in IR spectroscopy until recently, due to the high cost of Raman instruments. With prices for Raman systems dropping below $40,000, and even as low as $10,000, the use of Raman spectroscopy for quantitative analysis is increasing. Quantitative analysis requires measurement of the intensity of the Raman peaks and the use of a calibration curve to establish the concentration–intensity relationship. The intensity of a Raman peak is directly proportional to the concentration:

$$I = KJ\nu^4 c \tag{4.14}$$

where

I is the intensity of Raman signal at frequency ν
K is the proportionality constant including instrument parameters such as laser power
J is the scattering constant for the given Raman peak
ν is the scattering frequency of the Raman peak
c is the concentration of analyte