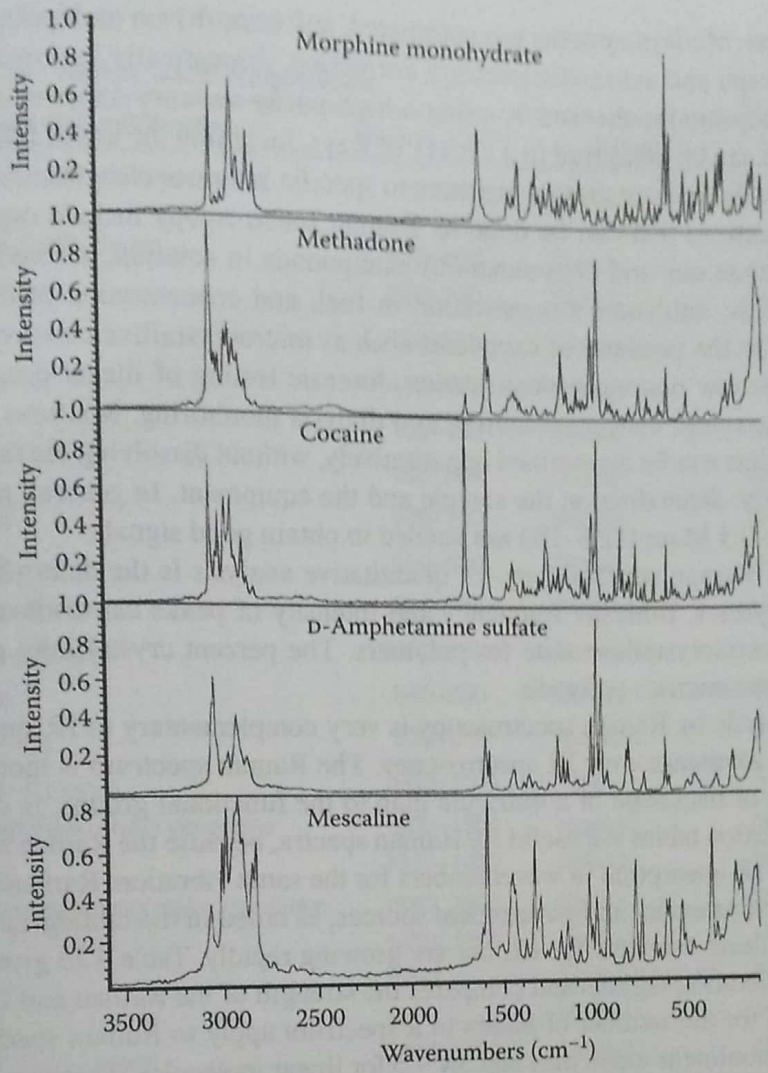


Figure 4.70 Raman spectra of common drugs of abuse, showing how sharp the Raman spectral lines are. (© Thermo Fisher Scientific (www.thermofisher.com). Used with permission.)

K, J, and ν are all constants for a given sample measurement. The frequency and intensity of the desired Raman lines are measured, and the intensity of an unknown compared to the calibration curve. It is common to use an internal standard for Raman analysis, because of the dependence of the signal on the laser power (in the K term). Without an internal standard, the laser power, sample alignment, and other experimental parameters must be carefully controlled. If an internal standard is used, the intensity of the internal standard peak is also measured, and the ratio of intensities plotted versus concentration. Upon division, this reduces Equation 4.14 to

$$\frac{I_{\text{unk}}}{I_{\text{std}}} = K'c \tag{4.15}$$

The use of the internal standard minimizes the effect of changes in instrumental parameters and can result in better accuracy and precision. Newer Raman systems such as the Thermo Scientific DXR series incorporate a laser power regulator that delivers reproducible laser power to the sample and compensates for laser aging and variability, minimizing the need for internal standards.

Two other major factors had impacted Raman spectroscopy for both qualitative and quantitative work. Older systems had problems with reproducibility due to wavelength dependence of the silicon CCD detector response and x-axis nonreproducibility, a result of inadequate spectrograph

and laser calibration. Modern systems use automated, software-driven multipoint calibration of the laser and spectrograph and automatic intensity correction, dramatically improving reproducibility. Wavelength accuracy can be checked by using a high-purity mercury vapor or argon light source. Laser power output can be measured in a variety of ways, including the use of NIST Raman relative intensity standards, fluorescent glasses sensitive to specific laser wavelengths.

Quantitative analyses that can be done by Raman spectroscopy include organic pollutants in water, inorganic oxyanions and organometallic compounds in solution, aromatic/aliphatic hydrocarbon ratios in fuels, antifreeze concentration in fuel, and concentration of the active pharmaceutical ingredient in the presence of excipients such as microcrystalline cellulose. Other common applications include raw materials identification, forensic testing of illegal drugs, explosives and other hazardous materials, cancer screening, and dialysis monitoring. Mixtures of compounds in pharmaceutical tablets can be determined quantitatively, without dissolving the tablets. Raman sensitivity varies greatly, depending on the sample and the equipment. In general, analyte concentrations of at least 0.1–0.5 M (or 0.1%–1%) are needed to obtain good signals.

Another use of Raman spectroscopy for quantitative analysis is the determination of percent crystallinity in polymers. Both the frequency and intensity of peaks can shift on going from the amorphous to the semicrystalline state for polymers. The percent crystallinity can be calculated with the help of chemometrics software.

Qualitative analysis by Raman spectroscopy is very complementary to IR spectroscopy and in some cases has an advantage over IR spectroscopy. The Raman spectrum is more sensitive to the organic framework or backbone of a molecule than to the functional groups, in contrast to the IR spectrum. IR correlation tables are useful for Raman spectra, because the Raman shift in wavenumbers is equal to the IR absorption in wavenumbers for the same vibration. Raman spectral libraries are available from commercial and government sources, as noted in the bibliography. These are not yet as extensive as those available for IR, but are growing rapidly. Table 4.19 gives common functional groups and vibration regions and compares the strength of the Raman and IR bands.

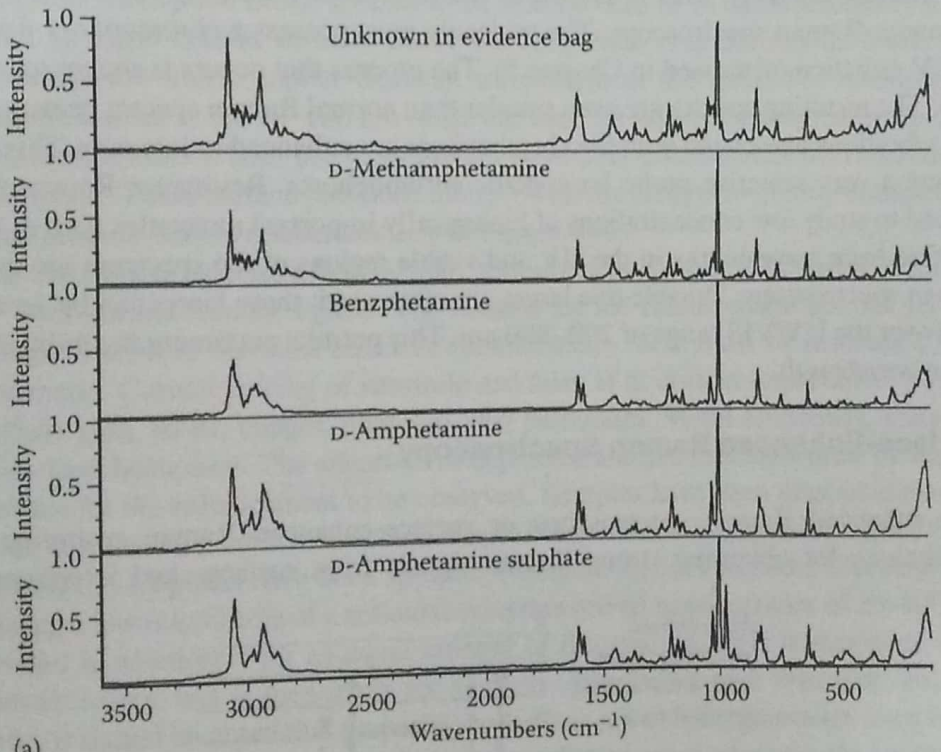
The same rules for the number of bands in a spectrum apply to Raman spectra as well as IR spectra: $3N - 6$ for nonlinear molecules and $3N - 5$ for linear molecules. There may be fewer bands than theoretically predicted due to degeneracy and nonactive modes. Raman spectra do not usually show overtone or combination bands; they are much weaker than in IR. A "rule of thumb" that is often true is that a band that is strong in IR is weak in Raman and *vice versa*. A molecule with a center of symmetry, such as CO_2, obeys another rule: if a band is present in the IR spectrum, it will not be present in the Raman spectrum. The reverse is also true. The detailed explanation for this is outside the scope of this text, but the rule "explains" why the symmetric stretch in carbon dioxide is seen in the Raman spectrum, but not in the IR spectrum, while the asymmetric stretch appears in the IR spectrum but not in the Raman spectrum.

Since the physical properties of the sample do not greatly impact the Raman spectrum, it is not able to differentiate between materials that differ only in physical form, for example, powder versus tablet. This does have an advantage in that a Raman spectral library can be built using only a single reference for each substance. Raman is an excellent tool for substances that differ chemically, even only a little. Raman can differentiate polymorphs, isomers, anhydrates from hydrates, monohydrates from polyhydrates, L and D forms, and other chemically similar forms. A good example is the ease of distinguishing between pseudoephedrine and ephedrine, diastereomers that differ only in the position of a hydroxyl group, or the ease of distinguishing between methamphetamine (*N*-methyl-1-phenylpropan-2-amine, a drug widely abused) and phentermine (a diet pill, 2-methyl-1-phenylpropan-2-amine). The two compounds have the same MW and, as we'll see in Chapter 10, are very difficult to distinguish based on their mass spectra. The Raman spectra (Figure 4.71a) are clearly different; even without any knowledge of Raman spectroscopy, one can see that these are not the same spectra. The sharpness of the Raman bands is the key to using this technique for identification.

Structural identification of an unknown is usually done with both IR and Raman spectra or by matching Raman spectra to spectral libraries of known compounds. The subtraction of known spectra from the spectrum of an unknown mixture to identify the components of the mixture works better for Raman spectra than for IR spectra, because there are fewer Raman peaks, the peaks are sharp, and their position and shape are not affected by hydrogen bonding. For example, it is possible to identify the components of a commercial pain relief tablet by spectral subtraction from the Raman spectrum of the intact tablet, as shown in Figure 4.71b.

The sharpness of Raman spectra also makes library searching very reliable, as seen in Figure 4.72a and b. The spectrum of an unknown white powder collected through an evidence bag, as in Figure 4.69, is compared to spectra of a variety of drugs. The match to methamphetamine is significantly better than the other possibilities (83 vs. 62–65 for the other compounds).

Raman spectroscopy is particularly useful for studying inorganic and organometallic species. Most inorganic, oxyanionic, and organometallic species have vibrations in the far-IR region of the spectrum, which is not easily accessible with commercial IR equipment. These metal–ligand and metal–oxygen bonds are Raman active and are easily studied in aqueous solutions and in solids.



(a)

Index	Match	Compound Name	Library
90	82.62	Methamphetamine	FT-Raman Forensic Library
18	65.39	Benzphetamine	FT-Raman Forensic Library
15	64.02	D-amphetamine	FT-Raman Forensic Library
14	63.29	D-amphetamine sulfate	FT-Raman Forensic Library
10	62.21	Aspartame	FT-Raman Forensic Library
102	61.89	α-Methyl fentanyl	FT-Raman Forensic Library

(b)

Figure 4.72 (a) Raman spectra of an unknown material (top) and four related drug compounds. (b) The library search match numbers (second column) show that the unknown is very probably methamphetamine. Note that the entire analysis was performed in 30 s. (© Thermo Fisher Scientific (www.thermofisher.com). Used with permission.)

Raman spectroscopy is used in geology and gemology for identification and analysis and in art restoration and identification and verification of cultural objects.

As noted earlier, fused silica optical fiber is used for remote NIR measurements. The same type of fiber-optic probe can be used for Raman spectroscopy and enables remote measurement of samples and online process measurements. *In situ* reaction monitoring by Raman spectroscopy has been used to study catalytic hydrogenation, emulsion polymerization, and reaction mechanisms. It is a powerful tool for real-time reaction monitoring, allowing feedback for reaction control. Remote sensing of molecules in the atmosphere can be performed by Raman scattering measurements using pulsed lasers.

4.8.4 Resonance Raman Effect

When monochromatic light of a frequency that cannot be absorbed by the sample is used, the resulting Raman spectrum is the **normal** Raman spectrum. Normal Raman spectroscopy, as has been noted, is an inefficient process resulting in low sensitivity and it suffers from interfering fluorescence in many samples. If a laser excitation wavelength is used that falls within an excited electronic state of the molecule, the intensity of some Raman lines increases by as much as 10^3–10^6 over the normal Raman intensity. This is known as the *resonance Raman effect*, and the technique is called resonance Raman spectroscopy. The molecule must possess a *chromophore* that can absorb visible or UV radiation (discussed in Chapter 5). The process that occurs is shown schematically in Figure 4.73. The resulting spectra are even simpler than normal Raman spectra because only totally symmetric vibrations associated with the chromophore are enhanced in intensity. This makes resonance Raman a very selective probe for specific chromophores. Resonance Raman spectroscopy has been used to study low concentrations of biologically important molecules such as hemoglobin.

Lasers that have wavelengths in the UV and visible regions of the spectrum are used for resonance Raman spectroscopy. Tunable dye lasers are often used; these lasers can be set to a selected wavelength over the UV/VIS range of 200–800 nm. This permits maximum flexibility in the choice of excitation wavelength.

4.8.5 Surface-Enhanced Raman Spectroscopy

Surface-enhanced Raman spectroscopy or surface-enhanced Raman scattering (SERS) is another technique for obtaining strong Raman signals from surfaces and interfaces, including

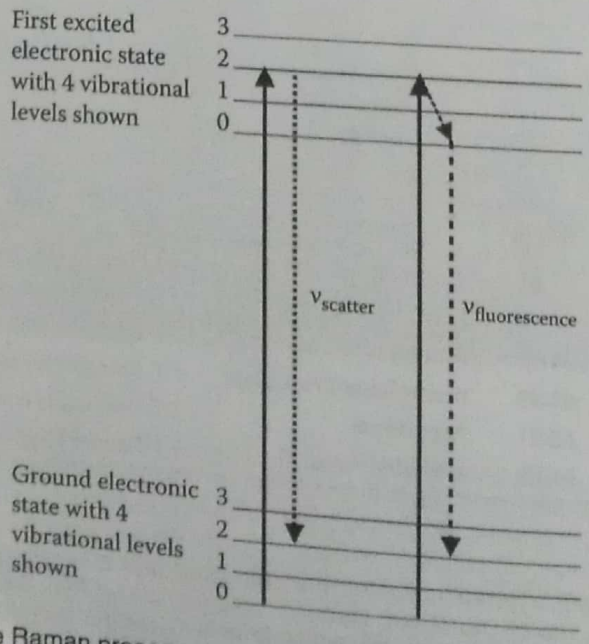


Figure 4.73 The resonance Raman process.

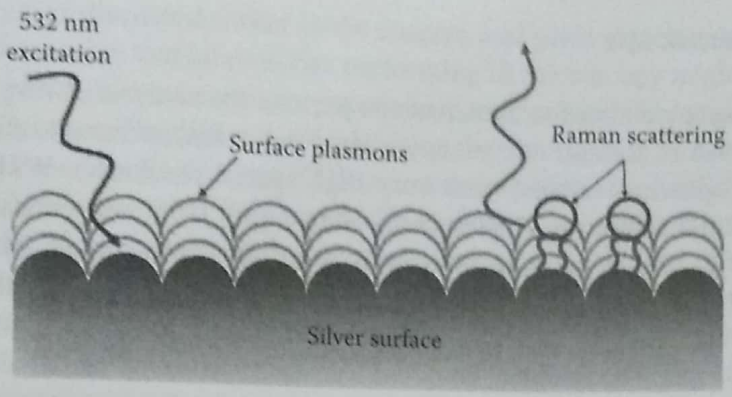


Figure 4.74 The SERS process using 532 nm excitation of a silver substrate with adsorbed species (the circles). Surface plasmons interact with the adsorbed species to enhance the Raman emission, shown on the right side of the diagram. (© Thermo Fisher Scientific (www.thermofisher.com). Used with permission.)

species adsorbed onto surfaces. Fleischmann and coworkers developed SERS in 1974. The SERS technique requires adsorption of the species to be studied onto or in close proximity to a prepared "rough" metal or metal colloid surface, where the roughness is at the atomic level. The surface roughness features are much smaller than the wavelength of the laser. For example, roughness features or particle sizes in the 20–100 nm range are used for laser wavelengths in the 532–780 nm range. The Raman excitation laser produces *surface plasmons* (coherent electron oscillations) on the surface of the metal. These surface plasmons interact with the analyte to greatly enhance the Raman emission. The process is shown schematically in Figure 4.74.

Inorganic and organic species adsorbed onto such surfaces show enhancement of Raman signals by up to 10^6 over normal Raman signals. The reasons for the enhancement are not yet well understood, although to achieve the most effective enhancement, there must be resonance between the laser and the metal. Correct pairing of substrate and laser is of critical importance. Metals used as surfaces include gold, silver, copper, platinum, and palladium. Metal electrodes, metal films, and metal colloids have been used. The adsorbed or deposited analyte molecule must be less than 50 Å from the surface for the enhancement to be observed. Samples have been deposited electrolytically onto electrode surfaces or mixed with colloidal metal suspensions. Samples separated on thin layer chromatography (TLC) plates have been sprayed with metal colloid solution. One simple approach involves mixing a few microliters of a colloidal substrate (metal nanoparticles of 20–100 nm dimensions suspended in solution) with an equal amount of the analyte. This is deposited onto a glass slide, is allowed to dry, and is then ready for analysis (Deschaines and Wieboldt, 2010 and references therein). It should be noted that preparing colloids is not simple; glassware must be extremely clean and ultrapure water must be used. Reaction conditions must be carefully controlled so that particles of the correct size and shape form.

Preparing metal surfaces requires expertise and can be expensive. Commercial sources for SERS substrates, colloids, and sample holders are available from companies such as Thermo Fisher Scientific, Renishaw, and Real-Time Analyzers (www.rta.biz), which focuses on high-throughput needs with SERS 96 well plates.

The enhancement leads to the ability to detect extremely small amounts of material, making SERS an effective tool for a variety of problems, including corrosion studies, detection of chemical warfare agents, bacteria on food, trace evidence in forensic science, blood glucose, research into infectious diseases, and many more. Detection limits for SERS are in the nanogram range. SERS can be used to study the way in which an analyte interacts with or binds to a surface. SERS therefore can be used to obtain information on very dilute solutions and small amounts of material that cannot be obtained by regular Raman spectroscopy. Current research in SERS includes practical biomedical applications such as detecting disease states based on deoxyribonucleic acid (DNA) signatures.

4.8.6 Raman Microscopy

Raman spectroscopy coupled to a microscope permits the analysis of very small samples nondestructively. The use of Raman microscopy allows the characterization of specific domains or inclusions in heterogeneous samples with very high spatial resolution. With dispersive Raman microscopy, the spatial resolution is often better than 1 μm. FT-Raman microscopy is limited to spot sizes of about 2–10 μm, but with no interference from fluorescence. The use of a confocal microscope (Figure 4.75) allows only the light at the sample focus to pass into the detector; all other light is blocked. This permits nondestructive depth profiling of samples without the need for cross sectioning of the sample. For example, confocal Raman microscopy can identify the polymers in complex layered structures, such as multilayer films used for food packaging. Characterization of heterogeneous materials includes inclusions in minerals, the pigments, and other components in cosmetics and the study of pigments, resins, and dyes in art and archeological objects. Using Raman microscopy, it is possible to identify if a red pigment in a painting is expensive cinnabar (HgS), cheaper hematite (Fe_2O_3), a mixture of the two, or an organic dye. The article by Edwards provides a detailed table of Raman bands from common minerals used in art and an overview of the use of Raman microscopy for the study of art objects. Raman microscopy is particularly useful for art and archeological objects, because there is no sample preparation required and the natural water present in paintings, manuscripts, and ancient textiles does not interfere, as it does in IR spectroscopy.

Since spatial resolution is diffraction limited, short wavelength lasers are optimal for analyzing small sample features. In order to achieve micron-level spatial resolution, the alignment of the Raman microscope is critical. The visual light path, the excitation laser beam path, and the Raman scatter beam path from the sample to the detector must all be targeted precisely on the same spot.

Raman microscopes are available from a number of instrument companies including Bruker, HORIBA Scientific, Jasco, Thermo Fisher Scientific, WITec, and Renishaw plc, among others.

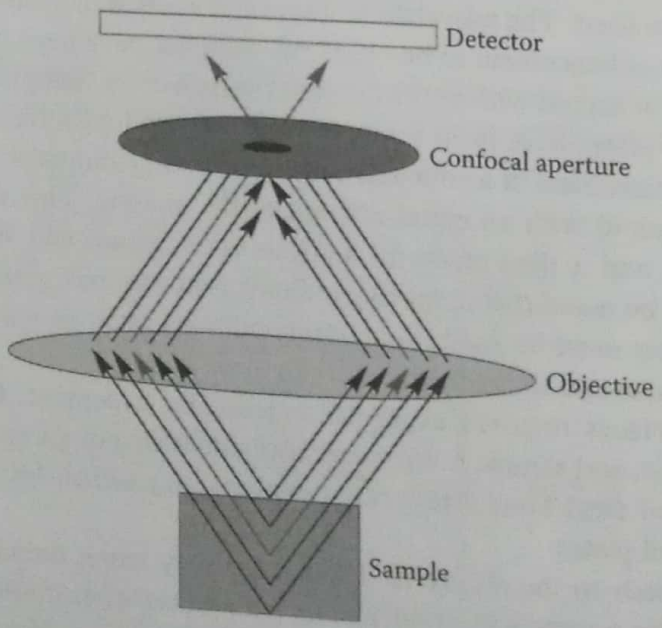


Figure 4.75 A simplified illustration of confocal microscopy. A spectrum is collected only from the area of the sample denoted by the middle arrow; only that light exits the confocal aperture. Information from all other sample areas is blocked. Raising or lowering the sample will allow the detector to collect Raman scatter from levels in the sample lower or higher than the area in the middle. (Reprinted from Weesner, F. and Longmire, M., *Spectroscopy*, 16(2), 68, 2001. With permission from Advanstar Communications, Inc.)

FTIR microscopy was discussed earlier in the chapter, and given the complementary nature of IR and Raman, it is reasonable that laboratories performing IR microscopy might well need Raman microscopy and *vice versa*. Two microscope systems were required and the sample had to be moved from one system to the other. The difficulty of relocating the exact spot to be sampled can be imagined. A combination dispersive Raman and FTIR microscopy system, the LabRAM-IR2 (HORIBA Scientific, Edison, NJ), allows both Raman and IR spectra to be collected at exactly the same location on the sample. The resolution depends on the wavelength observed, because resolution is limited by diffraction, but is <1 μm for the Raman spectrum and 10–20 μm for the IR spectrum. Examples of the type of data that can be obtained with this combination microscopy system are shown in Figures 4.76 and 4.77.

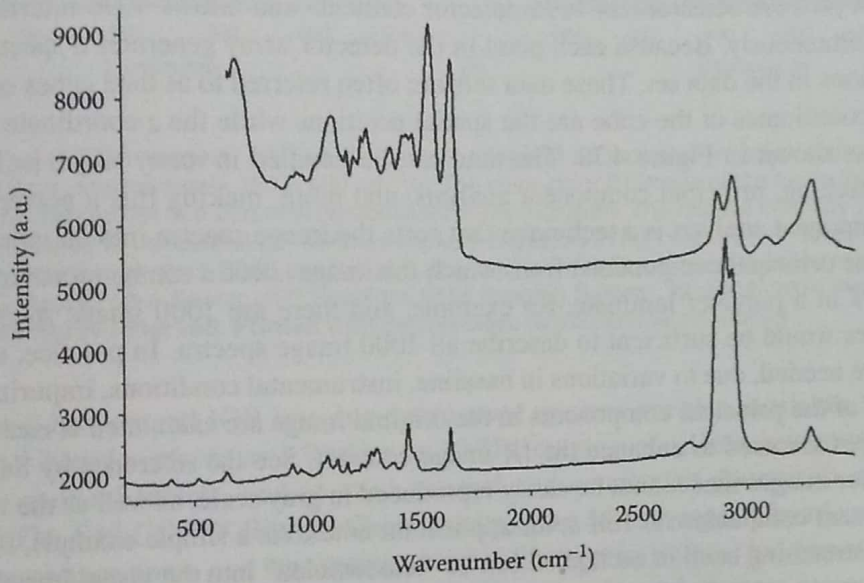


Figure 4.76 Raman and FTIR spectra of a 10 μm fiber of a nylon 6-PEG block copolymer. Spectra were collected at exactly the same spot on the fiber. (Courtesy of HORIBA Scientific, www.horiba.com.)

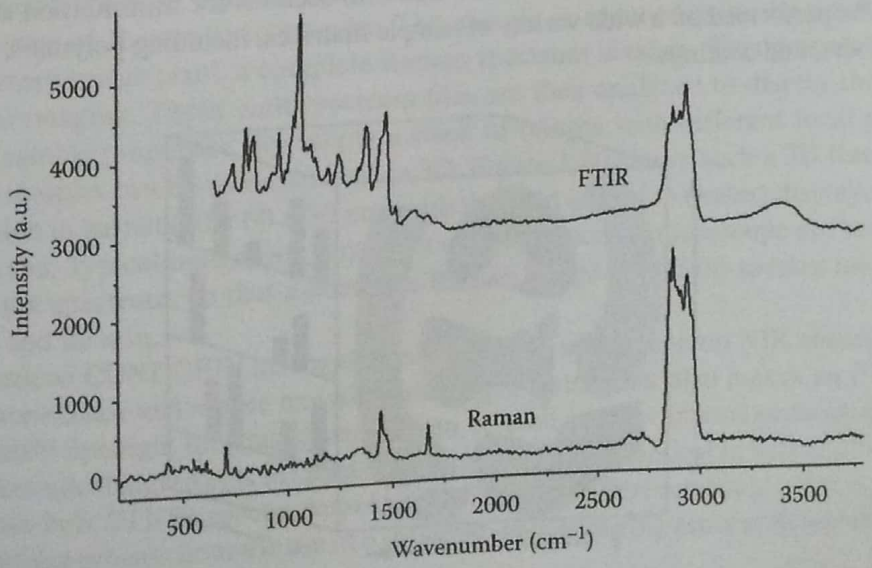


Figure 4.77 Raman and FTIR spectra of a gallstone. The spectra were recorded with the LabRAM-IR microscope using a 532 nm laser. The FTIR spectrum is more sensitive to the OH bands, while the Raman spectrum starts below 600 cm^{-1} and shows details of the cholesteric species and the C=C bands. (Courtesy of HORIBA Scientific, www.horiba.com.)