8
chapter

Infrared and Raman Spectroscopy

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8.1 INTRODUCTION

Infrared (IR) **spectroscopy** refers to measurement of the absorption of different frequencies of IR radiation by foods or other solids, liquids, or gases. IR spectroscopy began in 1800 with an experiment by Herschel [1]. When he used a prism to create a spectrum from white light and placed a thermometer at a point just beyond the red region of the spectrum, he noted an increase in temperature. This was the first observation of the effects of IR radiation. By the 1940s, IR spectroscopy had become an important tool used by chemists to identify functional groups in organic compounds. In the 1970s, commercial near-IR (NIR) reflectance instruments were introduced that provided rapid quantitative determinations of moisture, protein, and fat in cereal grains and other foods. Today, IR spectroscopy is used widely in the food industry for both qualitative and quantitative analyses of ingredients and finished foods.

In this chapter, the techniques of mid- and near-IR and Raman spectroscopy are described, including the principles by which molecules absorb IR radiation, the components and configuration of commercial IR spectrometers, sampling methods for IR spectroscopy, and qualitative and quantitative applications of these techniques to food analysis. Infrared and Raman microspectroscopy will not be covered in this chapter, but rather are covered in Chap. 32, Sects. 32.3.2 and 32.3.3.

8.2 PRINCIPLES OF IR SPECTROSCOPY

8.2.1 The IR Region of the Electromagnetic Spectrum

Infrared radiation is electromagnetic energy with **wavelengths** (λ) longer than visible light but shorter than microwaves. Generally, wavelengths from 0.8 to 100 micrometers (μm) can be used for IR spectroscopy and are divided into the **near-IR** (0.8–2.5 μm; 12,500–4000 cm^{-1}), the **mid-IR** (2.5–15.4 μm; 4000–650 cm^{-1}), and the **far-IR** (15.4–100 μm; 650–100 cm^{-1}) regions. One μm is equal to 1×10^{-6} m. The near- and mid-IR regions of the spectrum are most useful for quantitative and qualitative analysis of foods.

IR radiation also can be measured in terms of its **frequency**, which is useful because frequency is directly related to the energy of the radiation by the following relationship:

$$E = h\nu \tag{8.1}$$

where:

E = energy of the system
h = Planck's constant
ν = frequency in hertz

Frequencies are commonly expressed as **wave numbers** ($\bar{v}$, in reciprocal centimeters, cm^{-1}). Wave numbers are calculated as follows:

$$\bar{v} = 1/(\lambda \text{ in cm}) = 10^4/(\lambda \text{ in } \mu\text{m}) \tag{8.2}$$

8.2.2 Molecular Vibrations

A molecule can absorb IR radiation if it vibrates in such a way that its charge distribution, and therefore its electric dipole moment, changes during the vibration. Although there are many possible vibrations in a polyatomic molecule, the most important vibrations that produce a change in dipole moment are stretching (symmetric and asymmetric) and bending (scissoring, rocking, twisting, wagging) motions. Examples of these vibrations for the water molecule are shown in Fig. 8.1. Note that the stretching motions vibrate at higher frequencies than the scissoring motion. Also, asymmetric stretches are more likely to result in a change in dipole moment, with corresponding absorption of IR radiation, than are symmetric stretches.

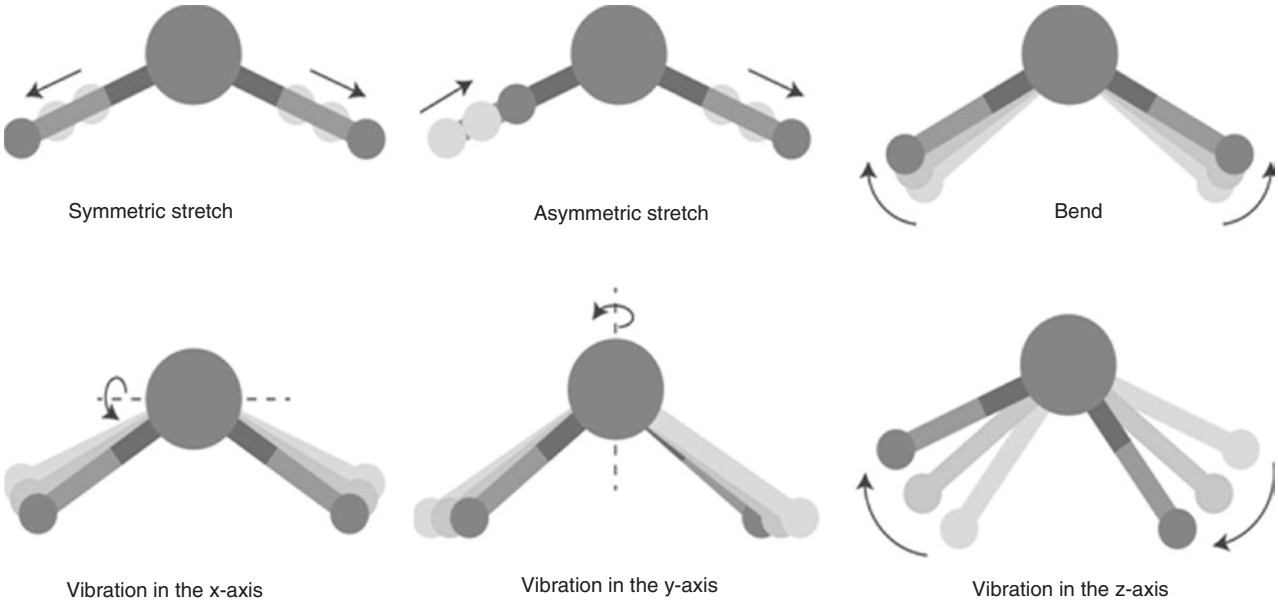
8.2.3 Factors Affecting the Frequency of Vibration

The basic requirement for absorption of infrared radiation is that there must be a net change in dipole moment during the vibration of the molecule or functional group. A molecular vibration can be thought of as a **harmonic oscillator** (Fig. 8.2a), with the energy level for any molecular vibration given by the following equation:

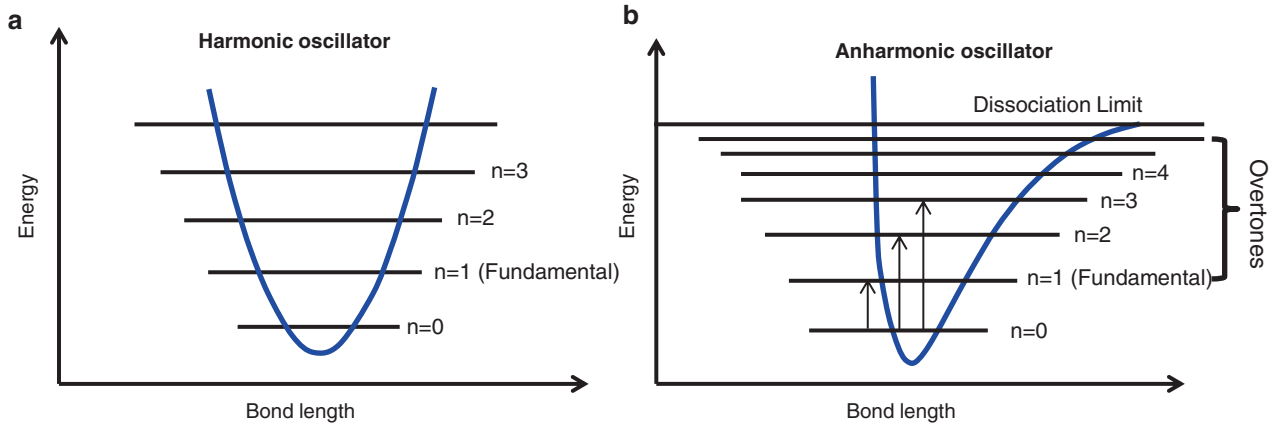
$$E = \left(v + \tfrac{1}{2}\right)\left(\tfrac{h}{2\pi}\right)\sqrt{k/\frac{m_1 m_2}{m_1 + m_2}} \tag{8.3}$$

where:

v = vibrational quantum number (positive integer values, including zero, only)
h = Planck's constant
k = force constant of the bond
m_1 and m_2 = masses of the individual atoms involved in the vibration



8.1 figure Vibrational modes of the water molecule (Adapted from the SKC chemistry [67])



8.2 figure Diagram of the differences in potential energy curves between the (**a**) harmonic and (**b**) anharmonic oscillator model

Note that the vibrational energy, and therefore the frequency of vibration, is directly proportional to the strength of the bond and inversely proportional to the mass of the molecular system. Thus, different chemical functional groups will vibrate at different frequencies. A vibrating molecular functional group can absorb radiant energy to move from the lowest ($v=0$) vibrational state to the first excited ($v=1$) state, and the frequency of radiation that will make this occur is identical to the initial frequency of vibration of the bond. This frequency is referred to as the **fundamental absorption**. The harmonic oscillator provides a good fit to explain bond stretching vibrations for fundamental vibrations. However, molecules also can absorb radiation to move to a higher ($v=2$ or 3) excited state, such that the frequency of the radiation absorbed is two or three times that of the fundamental frequency. These absorptions are referred to as **overtones**, and the intensities of these absorptions are much lower than the fundamental since these transitions are less favored. The **anharmonic oscillator model** (Fig. 8.2b) accounts for repulsion and attraction of the electron cloud and accommodates bond dissociation at higher energy levels. Overall, the fundamental vibrations are unaffected by the anharmonicity terms, but overtone transitions are influenced by anharmonicity, which

must be taken into account when assessing the frequency of these higher frequency vibrations. Combination bands also can occur if two or more different vibrations interact to give bands that are sums of their fundamental frequencies. The model of the harmonic oscillator and its modification to account for anharmonicity allows explanation of the origin of many of the characteristic frequencies that can be assigned to particular combinations of atoms within a molecule [2]. The overall result is that each functional group within the molecule absorbs IR radiation in distinct wavelength bands rather than as a continuum.

8.3 MID-IR SPECTROSCOPY

Mid-IR spectroscopy measures a sample's ability to absorb light in the 2.5–15 μm (4000–650 cm^{-1}) region. Fundamental absorptions are primarily observed in this spectral region. Mid-IR spectroscopy is very useful in the study of organic compounds because the absorption bands are related to the vibrational modes of specific functional groups. The positioning of the band and its intensity are correlated with the energy of the bond, its environment, and its concentration in the matrix, making mid-IR spectroscopy ideal for both qualitative and quantitative applications.

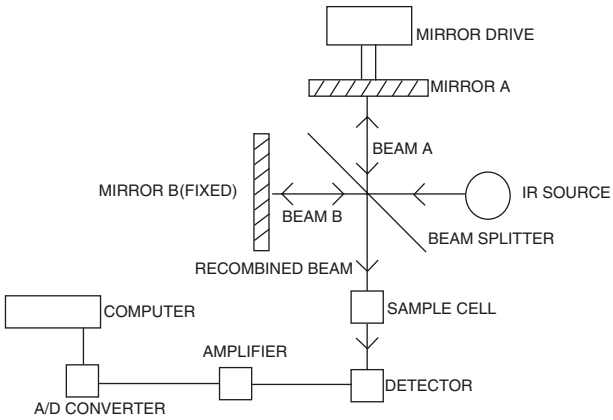
8.3.1 Instrumentation

8.3.1.1 *Overview*

There are two types of spectrometers available for mid-IR analysis, dispersive and Fourier transform (FT) instruments. Dispersive systems have been available since the 1940s using prisms or gratings as dispersive elements. These systems contain components similar to ultraviolet–visible (UV–Vis) spectrometers, including a radiation source, a monochromator, a sample holder, and a detector connected to an amplifier system to record the spectra. In these systems, a filter, grating, or a prism is used to separate the IR radiation into its individual wavelengths. A major advance in the field of mid-IR spectroscopy was the development of Fourier transform infrared spectrometers (FTIR), which have mostly replaced the dispersive instruments due to dramatically improved quality of spectra and decreased time required to obtain data.

8.3.1.2 *Fourier Transform Instruments*

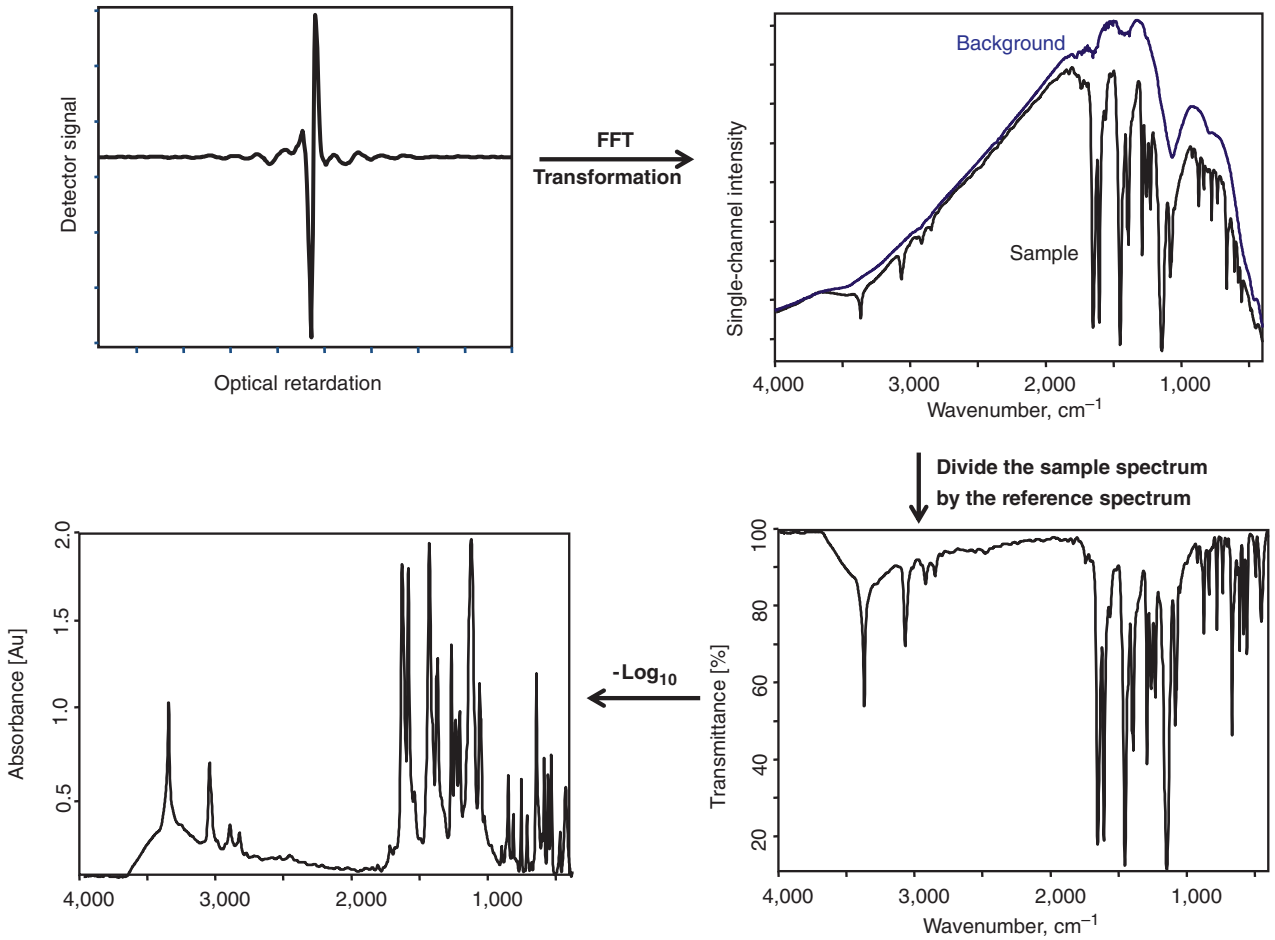
Compared to mid-IR dispersive instruments, FTIR spectrometers in food analysis allow for greater speed, higher sensitivity, superior wavelength resolution, and wavelength accuracy (details for advantages are in references [3, 4]). In **Fourier transform** (FT) instruments, the radiation is not dispersed, but rather all wavelengths arrive at the detector simultaneously,



8.3 figure Block diagram of an interferometer and associated electronics typically used in an FTIR instrument

and a mathematical treatment is used to convert the results into a typical IR spectrum. Instead of a monochromator, the instrument uses an **interferometer**. A Michelson interferometer is the most commonly used design, and its mechanism is simple (Fig. 8.3). The infrared radiation from the source is split into two beams by a beam splitter, and each half of the beam goes to a mirror (either a fixed or moving mirror). The beams are reflected back and recombined at the beam splitter, resulting in interference that is directed to the sample (or reference) and then the detector. Motion of the moving mirror results in the change of optical path length between the two split beams so that constructive, destructive, and intermediate interference states occur (with destructive interference being dominant). The resulting output is referred to as an **interferogram**, which is the intensity measured by the detector as a function of the position of the moving mirror. When a sample interacts with the recombined beam ahead of the detector, molecules absorb at their characteristic frequencies, and thus the radiation reaching the detector is modified (Fig. 8.4). Once the data are collected, a mathematical transformation called a "Fourier transform" converts the interferogram from time domain (intensity versus time) to an IR spectrum in the frequency domain (intensity versus frequency). A computer allows the mathematical transformation to be completed rapidly.

The common **radiation sources** for mid-IR spectrometers are inert solids heated electrically to 1000–1800 °C. Three popular types of sources are the Nernst glower (constructed of rare-earth oxides), Globar (constructed of silicon carbide), and a Nichrome coil wrapped around a ceramic core that glows when an electrical current is passed through it. They all produce continuous radiation, but with different radiation energy profiles.



8.4 figure Illustration on the process to convert an interferogram into an infrared spectrum by using the Fourier transform algorithm

Detectors include **thermocouples** for which output voltage varies with temperature changes caused by varying levels of radiation striking the detector. In a **Golay detector**, the radiation strikes a sealed tube of xenon gas warming the gas and causing pressure changes within the tube. However, most modern instruments use either **pyroelectric detectors**, such as deuterated triglycine sulfate (DTGS) crystals, or solid-state **semiconductor detectors**. Variation in the amount of radiation striking a DTGS detector causes the temperature of the detector to change, which results in a change in the dielectric constant of the DTGS element. The resulting change in capacitance is measured. Semiconductor detectors, such as those made from a mercury–cadmium–telluride (MCT) alloy, have conductivities that vary according to the amount of radiation striking the detector surface. MCT detectors respond faster to smaller changes in radiation intensity than other detectors; however, they typically require cryogenic cooling. DTGS and MCT detectors are the most commonly used detectors in Fourier transform instruments.

8.3.2 Sample Handling Techniques

Transmission mode is based on the IR beam passing through a sample that is placed in between two IR transparent windows. Liquids are often measured by **transmission IR spectroscopy**. Because absorptivity coefficients in the mid-IR are high, cells with path lengths of only 0.01–1.0 mm are commonly used. Quartz and glass absorb in the mid-IR region, so cell windows are made of non-absorbing materials such as halide or sulfide salts. Halide salts are soluble in water, and care must be taken when selecting cells for use with aqueous samples. Cells also are available with windows made from more durable and less soluble materials, such as zinc selenide, but are more expensive than those with halide

salt windows. Liquid cells must be free of air bubbles and extra care needs to be taken when cleaning between samples.

Transmission spectra of solids can be obtained by finely grinding a small amount of the sample with potassium bromide (KBr), pressing the mixture into a pellet under high pressure and inserting the pellet into the IR beam. Limitations of this technique include difficulty of handling and storing the hygroscopic KBr and the complexity and time required to make a good KBr pellet. An alternative technique is to disperse a finely divided solid in Nujol mineral oil to form a mull.

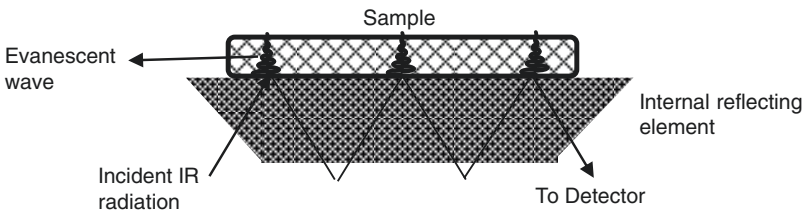
Transmission spectra can be obtained from gas samples using a sealed 2–10 cm glass cell with IR transparent windows. For trace analysis, multiple-pass cells are available that reflect the IR beam back and forth through the cell many times to obtain path lengths as long as several meters. FTIR instruments also can be interfaced to a gas chromatograph, to obtain spectra of compounds eluting from the chromatography column.

Attenuated total reflectance (ATR) is a widely applied sampling technique in infrared spectroscopy because it requires little or no sample preparation, eliminates variation in cell path lengths, and provides consistent spectra collection. ATR allows for obtaining spectra from solid samples that are too thick for transmission measurements, e.g., pastes such as peanut butter and viscous liquids. ATR works based on the attenuation effect of infrared light (Fig. 8.5) when it is directed at an interface between an internal reflection element (crystal) with high refractive index properties (i.e., zinc selenide (ZnSe), thallium iodide–thallium bromide (KRS-5), germanium (Ge), silicon (Si), and diamond) and a low refractive index material (food sample) on its surface. Upon the interaction with the reflecting surface, radiation called an "evanescent wave" is formed, exits the high refractive index material, and slightly penetrates into the sample. The sample material selectively absorbs, the intensity of the reflected radiation is decreased at wavelengths for which the sample absorbs radiation, and the final attenuated radiation exiting the crystal is measured as being unique for the sample analyzed.

Radiation is not transmitted through the sample; therefore, there is no need for the sample to be thin enough to allow the transmission of the incident light. Since the penetration depth of the radiation is limited to a few micrometers (μm), the same spectrum is obtained regardless of the amount of the sample placed on the surface, and there is no need to dilute the samples.

The physical state of the sample is an important factor because it must be in intimate contact with the ATR crystal to obtain a good ATR spectrum. Liquids and pastes usually exhibit better ATR spectra than solid samples. A pressure clamping system is used

a



Triple reflection ATR crystal

b

Guidelines for selection of ATR crystals

Material	Spectral range (cm^{-1})	Hardness (Kg/mm)	pH range	Refractive Index	Depth of penetration at 45°
Diamond	50,000–2,500	9,000	1–14	2.4	1.66
Germanium	5,000–550	780	1–14	4.0	0.65
Silicon	8,333–33	1,150	1–12	3.4	0.81
KRS-5	17,900–250	40	5–8	2.4	0.85
ZnSe	20,000–500	130	5–9	2.4	1.66
AMTIR	11,000–725	170	1–9	2.5	1.46

8.5 figure

(**a**) Illustration of the reflection phenomena in a triple reflection attenuated total reflection accessory and formation of the evanescence wave into the sample. (**b**) Characteristics of common ATR crystals

with solid samples to deform the sample, increasing the extent of contact between the ATR crystal and the sample.

8.3.3 Applications of Mid-IR Spectroscopy

8.3.3.1 *Absorption Bands of Organic Functional Groups*

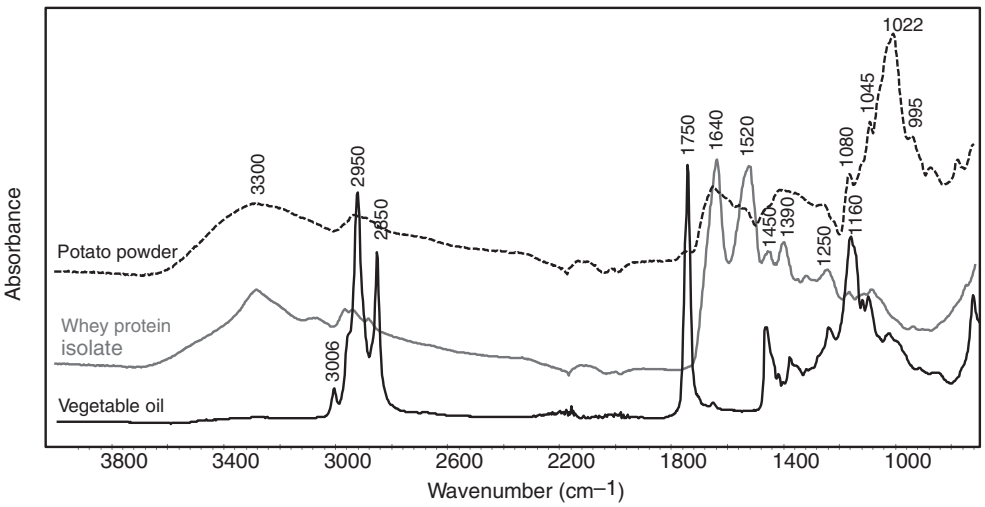
Infrared spectroscopy monitors the interaction of functional groups in chemical molecules with infrared light resulting in predictable vibrations that provide a "fingerprint" characteristic of chemical substances present in the sample. Spectra in the mid-IR region have well-resolved bands that can be assigned to functional groups of the components of foods. The positioning of the band facilitates structural characterization, and its intensity correlates with its concentration in the matrix, allowing for both qualitative and quantitative applications.

Spectra are commonly presented in wave numbers plotted on the *x*-axis and either percent transmittance or absorbance plotted on the *y*-axis. The mid-IR spectra of selected foods are shown in Fig. 8.6 displaying the major absorption bands that can be associated with functional groups (Table 8.1) in fat-, protein-, and carbohydrate-rich commodities.

The unique spectral profile can be used to identify specific functional groups present in an unknown substance. Comparing the mid-IR spectrum to a set of standard spectra and determining the closest match can accomplish identification of chemical compounds. Spectral libraries are available from several sources, but probably the largest collection of standards is the Sadtler Standard Spectra (Sadtler Division of Bio-Rad Inc., Philadelphia, PA) that contains over 225,000 infrared spectra. Algorithms are used to compare the unknown spectrum to each spectrum in the reference database, and the hit quality index (HQI) is determined, indicating the similarity between spectra. Several HQI values can be generated for the unknown compounds, and the software will sort and display the best matches in a search report. Noise and spectral artifacts can impact the HQI and lead to mistakes in identification; thus, it is imperative to perform visual comparisons to confirm a good match. Spectral searches will most commonly be done on purified substances, rather than foods or commodities.

8.3.3.2 *Applications*

Mid-IR spectroscopic measurements obey **Beer's law**, although deviations may be greater than in UV–Vis spectroscopy due to the low intensities of IR sources, the low sensitivities of IR detectors, and the relative narrowness of mid-IR absorption bands. One of the first and most extensive uses of this technique is the **infrared milk analyzer**, which has the ability to analyze hundreds of samples per hour. The fat, protein, and lactose contents of milk can be determined simultaneously with one of these instruments. The ester carbonyl groups of lipid absorb at 5.73 μm (1742 cm^{-1}), the amide groups of protein absorb at 6.47 μm (1545 cm^{-1}), and the hydroxyl groups of lactose absorb at 9.61 μm (1045 cm^{-1}). These automated instruments homogenize the milk fat globules to minimize light scattering by the sample and then pump the milk into a flow-through cell through which the IR beam is passed. The instrument is calibrated using samples of known concentration to establish the slope and intercept of a Beer's law plot. Official methods have been adopted



8.6 figure

Mid-IR spectra of corn oil, whey protein isolate, and potato flour measured by triple-reflection ATR unit. Frequency in wave numbers is plotted on the *x*-axis, with intensity on the *y*-axis

8.1 table

Characteristic mid-IR and near-IR absorption frequencies of major food components

Food components	*Infrared region*	*Frequency (cm^{-1})*	*Assignment*
Fats	Mid-IR	3006	*cis*-Olefinic groups
		3000–2800	C–H asym. and sym. str. of long-chain fatty acids
		1740	C=O str. of fatty acid esters
		1711	C=O str. of fatty acids
		1475–1435	Asym. bending defor. $-CH_3$ in branched alkanes
		1465	=C–H *cis*-bending
		1350–1395	C–H symmetric bending in $-CH_3$
		1350–1150	C–H bending
		1240 and 1163	–C–O and $-CH_2-$ str. bending
		1150–1000	–C=O str.
		966	Isolated out of plane *trans*-CUC bending
		914	*cis* –C=C–H bending out of plane
	Near-IR	8700–8100 (1150–1235 nm)	C–H str., second overtone
		8563 (1168 nm)	cis Double bond
		7209 (1387 nm)	Combination C–H str. and C–H bending
		5807 (1722 nm), 5681 (1760 nm)	C–H str., first overtone
		4705 (2125 nm)	C–H vibrations in isolated C=C bonds
		4336 (2306 nm), 4269 (2342 nm)	Combination bands of C–H and C–O str.
Water	Mid-IR	3500	O–H str.
		1650	O–H bending
	Near-IR	6900 (1450 nm)	First overtones str. free OH
		5150 (1940 nm)	OH combination bands
Proteins	Mid-IR	3300	Amide A
		1640	Amide I
		1540	Amide II
		1330–1230	Amide III
	Near-IR	5000–4550	Amides
		4855 (2016 nm)	Combination of amide A and amide II
		4580 (2180 nm)	Combination of amide A and amide III
Carbohydrates	Mid-IR	1745	Ester, pectin
		1630–1605	Carboxylate, pectin
		Shifts at 1617 and 1420	Metal coordination by the pectate chains
		1460–1340	C–C–H and C–O–H deformations
		1250–950	Endo- and/or exocyclic C–C and C–O bonds
		1150	Characteristic of pyranose ring
		1110	CO ring, C–4–O, C–6–O
		1080	Typical bending of C–1–H
		1060	C–1–OH (fructose residue)
		1030	Typical C–4–OH vibration
		1050–1020	Starch retrogradation, increased crystallinity
		995	Glycosidic linkage
		950–750	α and β anomeric region of saccharide
	Near-IR	69405 (1440 nm)	OH str., first overtone, crystalline structure
		5924 (1688 nm)	C–H stretch first overtone
		5882 (1700 nm)	C–H str. methyl groups from cellulose or lignin
		4662 (2100 nm)	Combination C–O str./OH bend – carbohydrates
		4280 (2336 nm)	Cellulose and starch
		4386 (2280 nm)–4292 (2330 nm)	Combination C–H str. and CH_2 deformation
Aromatics	Mid-IR	3100–3000	Aromatic –CH stretch
		1600	–C=C– stretch
Alcohols	Mid-IR	3600–3200	–OH stretch
		1500–1300	–OH bend
		1220–1000	C–O stretch
	Near-IR	6850 (1460 nm)–6240 (1600 nm)	First overtone –OH
		6800 (1470 nm) and 7100 (1280 nm)	Typical first overtone bands of bonded OH–H bonding
		4550 (1800 nm)–5550 (2200 nm)	Combination OH str. and OH bending
Ethers	Mid-IR	1220–1000	C–O asymmetric stretch

for the IR milk analyzers, and specific procedures for operation of these instruments are given [5, 6].

There are many additional applications of mid-IR spectroscopy to food analysis. Examples of applications of mid-IR spectroscopy to foods are found in review articles [7–9]. Due to the complex nature of infrared spectra, multivariate statistical analysis techniques (chemometrics) must often be used to extract information from the infrared spectra, allowing for classification and quantitative analysis of multiple components in foods. Instrument calibration using chemometric techniques is discussed in more detail in Sect. 8.5.3.

8.4 NEAR-IR SPECTROSCOPY

Measurements in the **near-IR** (NIR) spectral region (0.7–2.5 μm, equal to 700–2500 nm) are more widely used for quantitative analysis of foods than are mid-IR measurements. Several commercial instruments are available for compositional analysis of foods using near-IR spectroscopy. A major advantage of near-IR spectroscopy is its ability to measure directly the composition of solid food products by the use of diffuse reflection techniques.

8.4.1 Principles

8.4.1.1 *Principles of Diffuse Reflection Measurements*

When radiation strikes a solid or granular material, part of the radiation is reflected from the sample surface. This mirrorlike reflection is called **specular reflection** and gives little useful information about the sample. Most of the specularly reflected radiation is directed back toward the energy source. Another portion of the radiation will penetrate through the surface of the sample and be reflected off several sample particles before it exits the sample. This is referred to as **diffuse reflection**, and this diffusely reflected radiation emerges from the surface at random angles through 180°. Each time the radiation interacts with a sample particle, the chemical constituents in the sample can absorb a portion of the radiation. Therefore, the diffusely reflected radiation contains information about the chemical composition of the sample, as indicated by the amount of energy absorbed at specific wavelengths.

The amount of radiation penetrating and leaving the sample surface is affected by the size and shape of the sample particles. Compensation for this effect may be achieved by grinding solid or granular materials with a sample preparation mill to a fine, uniform particle size, or by applying mathematical corrections when the instrument is calibrated [10].

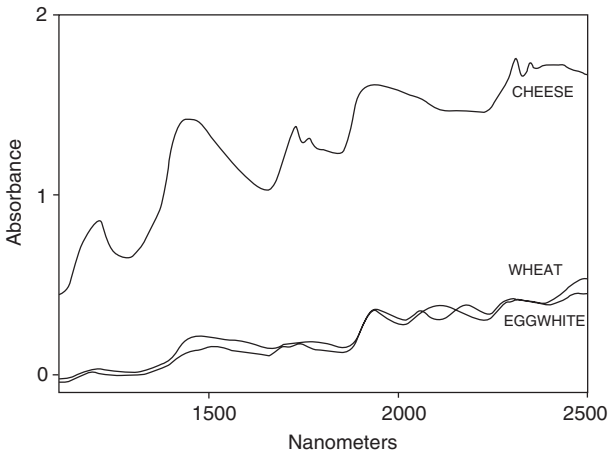
8.4.1.2 *Absorption Bands in the Near-IR Region*

The absorption bands observed in the near-IR region are primarily overtones and combinations. Therefore, the absorptions tend to be weak in intensity. However, this is actually an advantage, since absorption bands that have sufficient intensity to be observed in the near-IR region arise primarily from functional groups that have a hydrogen atom attached to a carbon, nitrogen, or oxygen, which are common groups in the major constituents of food such as water, proteins, lipids, and carbohydrates. Table 8.1 lists the absorption bands associated with a number of important food constituents.

The absorption bands in the near-IR region tend to be broad and frequently overlap, yielding spectra that are quite complex. However, these broad bands are especially useful for quantitative analysis. Typical near-IR spectra of wheat, dried egg white, and cheese are shown in Fig. 8.7. Note that strong absorption bands associated with the -OH groups of water which are the dominant features in the spectrum of cheese, containing 30–40% moisture, and they are still prominent even in the lower moisture wheat and egg white samples. Bands arising from protein (2060 and 2180 nm) in the wheat sample are partially obscured by a strong starch absorption band and centered at 2100 nm. Relatively sharp absorption bands arising from -CH groups in lipid can be observed at 2310, 2350 nm, and 1730 nm and are distinctly observable in the cheese spectrum.

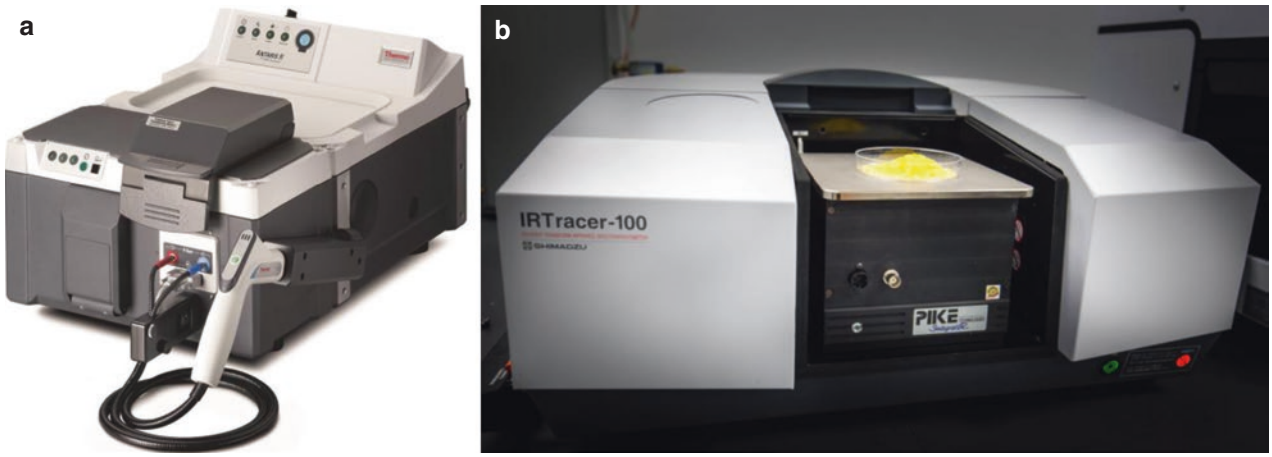
8.4.2 Instrumentation

Two commercial near-IR spectrometers are shown in Fig. 8.8. The radiation source in most near-IR instruments is a tungsten–halogen lamp with a quartz enve-



8.7 figure

Near-IR spectra of cheese, wheat, and dried egg white plotted as log (1/*R*) vs. wavelength in nm



8.8 figure

Modern commercial near-IR instrument. (**a**) Thermo Scientific Antaris II. (**b**) Shimadzu IRTracer-100 equipped with an NIR integrating sphere accessory from PIKE Technologies (Photographs courtesy of Thermo Fisher Scientific and Shimadzu Scientific Instruments)

lope, similar to a projector lamp. These lamps emit significant amounts of radiation in both the visible and near-IR spectral regions. Semiconductor detectors are most commonly used in near-IR instruments, with silicon detectors used in the 700–1100 nm range and lead sulfide used in the 1100–2500 nm region. In situations for which a rapid response to changing light intensity is needed, such as in online monitoring, indium–gallium–arsenide (InGaAs) detectors can be used. Many InGaAs detectors are limited to a maximum wavelength of 1700 nm, although commercial InGaAs detectors with a range extended to longer wavelengths are now available. Most commercial near-IR instruments use monochromators, rather than interferometers, although some commercial instruments are now using FT technology. Monochromator-based instruments may be of the scanning type, in which a grating is used to disperse the radiation by wavelength, and the grating is rotated to impinge a single wavelength (or more appropriately, a narrow band of wavelengths) onto a sample at any given time. Using this arrangement, it takes several seconds to collect a spectrum from a sample over the entire near-IR region. Some rapid scanning instruments impinge light over the entire near-IR region onto the sample. The reflected or transmitted light then is directed onto a fixed grating that disperses the light by wavelength and also focuses it onto a multichannel array detector that measures all wavelengths at once. These instruments can obtain a spectrum from a sample in less than 1 s.

Instruments dedicated to specific applications can use optical interference filters to select 6–20 discrete wavelengths that can be impinged on the sample. The filters are selected to obtain wavelengths that are known to be absorbed by the sample constituents. The instrument inserts filters one at a time into the light beam to direct individual wavelengths of radiation onto the sample.

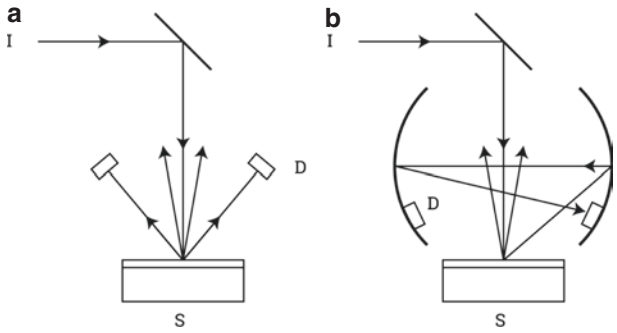
Either **reflection** or **transmission** measurements may be made in near-IR spectroscopy, depending on the type of sample. In the reflection mode, used primarily for solid or granular samples, it is desirable to measure only the diffusely reflected radiation that contains information about the sample. In many instruments, this is accomplished by positioning the detectors at a 45° angle with respect to the incoming IR beam, so that the specularly reflected radiation is not measured (Fig. 8.9a). Other instruments use an integrating sphere, which is a gold-coated metallic sphere with the detectors mounted inside (Fig. 8.9b). The sphere collects the diffusely reflected radiation coming at various angles from the sample and focuses it onto the detectors. The specular component escapes from the sphere through the same port by which the incident beam enters and strikes the sample.

Samples often are prepared by packing the food tightly into a cell against a quartz window, thereby providing a smooth, uniform surface from which reflection can occur. Quartz does not absorb in the near-IR region. At each wavelength, the intensity of light reflecting from the sample is compared to the intensity reflected from a non-absorbing reference, such as a ceramic or fluorocarbon material. Reflectance (R) is calculated by the following formula:

$$R = I / I_0 \tag{8.4}$$

where:

I = intensity of radiation reflected from the sample at a given wavelength
I_0 = intensity of radiation reflected from the reference at the same wavelength



8.9 figure

Typical instrument geometries for measuring diffuse reflectance from solid food samples. Radiation from the monochromator (*I*) is directed by a mirror onto the sample (*S*). Diffusely reflected radiation is measured directly by detectors (*D*) placed at a 45° angle to the incident beam (**a**) or is collected by an integrating sphere and focused onto the detectors (**b**). In both cases, the specularly reflected radiation is not measured

Reflectance data are expressed most commonly as log (1/*R*), an expression analogous to absorbance in transmission spectroscopy. Reflectance measurements also are expressed sometimes as differences, or derivatives, of the reflectance values obtained from adjacent wavelengths:

$$\left(\log R_{\lambda 2} - \log R_{\lambda 1}\right) \tag{8.5}$$

or

$$\left(2\log R_{\lambda 2} - \log R_{\lambda 1} - \log R_{\lambda 3}\right) \tag{8.6}$$

These derivative values are measures of the changes in slope of the spectrum, where λ_1, λ_2, and λ_3 are adjacent wavelengths typically separated by 5–20 nm, with the higher numbers representing longer wavelengths.

Transmission measurements also can be made in the near-IR region, and this is usually the method of choice for liquid samples. A liquid is placed in a quartz cuvette and the absorbance measured at the wavelengths of interest. Transmission measurements also can be taken from solid samples, but generally only in the 700–1100 nm range. In this wavelength region, the absorption bands are higher overtones that are very weak, allowing the radiation to penetrate through several millimeters of a solid sample. The use of transmission measurements can minimize the degree of sample preparation needed. Since the IR beam passes through the entire sample, the need for a smooth, homogeneous sample surface is reduced.

Near-IR energy can be transmitted through a fiber optic cable some distance from the monochromator or interferometer, allowing reflection or transmission measurements to be made remotely from the instrument. This is very useful to take measurements in a processing plant environment. Commercial probes are available that can be inserted directly into bulk granular materials, or inserted into a pipe carrying a liquid.

As with mid-IR, near-IR imaging instruments are now commercially available. These instruments use an array detector so that a digital image of a food sample can be obtained at various wavelengths or a spectrum can be obtained from a single pixel in a digital image. This technique is often referred to as hyperspectral imaging. It holds much potential for evaluating sample heterogeneity, or identifying small features or contaminants on an intact food sample.

8.4.3 Quantitative Methods Using Infrared Spectroscopy

Infrared instruments can be calibrated to measure various constituents in foods and agricultural commodities. Because of the overlapping nature of the near-IR absorption bands, it is usually necessary to take measurements at two or more wavelengths to quantify a food component reliably. Multivariate statistical techniques (**chemometrics**) are used to relate the spectral data collected at multiple wavelengths to the concentration of the component of interest in the food [11–13]. The simplest statistical technique used is **multiple linear regression** (MLR), which applies an equation of the following form to predict the amount of a constituent present in the food from the spectral measurements:

$$\%\ \text{constituent} = z + a\log\left(1/R_{\lambda 1}\right) + b\log\left(1/R_{\lambda 2}\right) + c\log\left(1/R_{\lambda 3}\right) + \ldots \tag{8.7}$$

where each term represents the spectral measurement at a different wavelength multiplied by a corresponding coefficient. Each coefficient and the intercept (*z*) are determined by multivariate regression analysis.

Absorbance or derivatized reflectance data also can be used in lieu of the log (1/*R*) format. The use of derivatized reflectance data has been found to provide improved results in some instances, particularly with samples that may not have uniform particle sizes. Other mathematical techniques also are available that can be applied to the reflectance data to correct for the effects of nonuniform particle size [10].

To **calibrate** an infrared instrument for quantitative measurement, a set of samples is obtained that represents the product to be measured and contains the component of interest over the expected range of interest. The samples are then analyzed using the conventional method of analysis (e.g., for protein analysis, use Kjeldahl or Dumas methods; Chap. 18, Sects. 18.2.1 and 18.2.2), and the infrared spectrum of each sample is collected. A computer-assisted MLR analysis is then

performed to determine the combinations of wavelengths that best predict the concentration of the component of interest and the coefficients associated with each wavelength, as shown in Eq. (8.7). In chemometric techniques such as MLR, the wavelengths are chosen based on statistical correlation with the component being measured. However, the results should always be inspected to make sure that the wavelengths selected make sense from a spectroscopic perspective. Each calibration also should be tested using a second set of independent samples. Then, if the calibration yields satisfactory results, it can be used for routine analysis.

When using MLR, it may sometimes be difficult to include enough wavelengths to adequately define the relationship between the spectral and composition data. Adding too many wavelengths may "overfit" the calibration so that it does not apply well to samples that were not part of the original set. This can occur because the responses of individual wavelengths are highly intercorrelated. To overcome this problem and to obtain more robust predictions, multivariate calibration methods such as **partial least squares** (PLS) regression and **principal component regression** (PCR) can be used. PLS and PCR are often referred to as "data compression" techniques, since they take the spectral variation from the entire wavelength range and express most of that variation with a smaller number of variables that are not correlated. These variables then are used in to develop a regression equation. PLS and PCR often provide improved results compared to MLR because they can use information from the entire spectrum with less risk of "overfitting" the results. For this reason, these two techniques are now the most widely used methods for calibrating mid-IR and near-IR instruments for quantitative analysis. Readers interested in a more detailed explanation of these techniques should consult the references [11–16].

8.4.4 Qualitative Analysis by Infrared Spectroscopy

Infrared spectroscopy can be used to classify a sample into one of two or more groups, rather than to provide quantitative measurements. Classification techniques, such as **principal component analysis** (PCA), soft independent modeling of class analogy (SIMCA), or discriminant analysis, can be used to compare the infrared spectrum of an unknown sample to the spectra of samples from different groups. The unknown sample then is classified into the group to which its spectrum is most similar. While this technique has historically been used in the chemical and pharmaceutical industries for raw material identification, it is becoming more widely used for food applications, including the classification of wheat as hard red spring or hard red winter [17], the identification of orange juice samples from different sources [18, 19], authentication of the source of olive oils [20–22], and discrimination of beef [23–25]. Readers interested in a more detailed explanation of these classification techniques should consult the references [11, 26].

8.4.5 Applications of Near-IR Spectroscopy to Food Analysis

Theory and applications of near-IR spectroscopy to food analysis have been discussed in several publications [27–30]. The technique is widely used throughout the grain, cereal products, and oilseed processing industries. Near-IR techniques using measurements from ground or whole grain samples have been adopted as approved methods of analysis by AACC International [31] for measuring protein in barley, oats, rye, triticale, wheat, and wheat flour, as well as moisture, protein, and oil in soybeans. These approved methods describe the instruments available for making these measurements, including a list of current manufacturers with contact information in Method 39–30, as well as the proper techniques for preparing samples and calibrating the instruments. Near-IR instruments now are used by the official grain inspection agencies in both the US and Canada for measuring protein, moisture, and oil in cereals and oilseeds.

Food components such as protein and dietary fiber can be determined successfully in a number of cereal-based foods using near-IR spectroscopy [32–34]. Modern instruments and calibration techniques allow a wide variety of products, such as cookies, granola bars, and ready-to-eat breakfast foods, to be analyzed using the same calibration.

Near-IR spectroscopy also can be used for numerous other commodities and food products. The technique has been used successfully to evaluate composition and quality of red meats and processed meat products [35–37], poultry [38], and fish [39]. Near-IR spectroscopy is useful also for analyzing a number of dairy products and nondairy spreads, including measuring moisture in butter and margarine [40]; moisture, fat, and protein in cheese [41, 42]; and lactose, protein, and moisture in milk and whey powders [43]. Near-IR techniques also have shown promise for measuring total sugars and soluble solids in fruits, vegetables, and juices [44–46], are being used commercially for monitoring the sugar content in corn sweeteners [47], and can be used to quantitate sucrose and lactose in chocolate [48].

Near-IR spectroscopy also is showing potential for measuring specific chemical constituents in a food that affect its end-use quality, for monitoring changes that occur during processing or storage, and for directly predicting processing characteristics of a commodity that are related to its chemical composition. Examples include determining the amylose content in

rice starch, an important determinant of rice quality [49, 50], monitoring peroxide value in vegetable oils [51], monitoring degradation of frying oils [52], and predicting corn-processing quality [53, 54].

These are only a few examples of current applications. If a substance absorbs in the near-IR region and is present at a level of a few tenths of a percent or greater, it has potential for being measured by this technique. The primary advantage of near-IR spectroscopy is that once the instrument has been calibrated, several constituents in a sample can be measured rapidly (from 30 s to 2 min) and simultaneously. To measure multiple constituents, a calibration equation for each constituent is stored into the memory of the instrument. Measurements are taken at all wavelengths needed by the calibrations, and each equation then is solved to predict the constituents of interest. No sample weighing is required, and no hazardous reagents are used or chemical waste generated. It also is adaptable for **online measurement systems** [55]. Disadvantages include the high initial cost of the instrumentation, which may require a large sample load to justify the expenditure, and the fact that specific calibrations may need to be developed for each product measured. Also, the results produced by the instrument can be no better than the data used to calibrate it, which makes careful analysis of the calibration samples of highest importance.

8.5 RAMAN SPECTROSCOPY

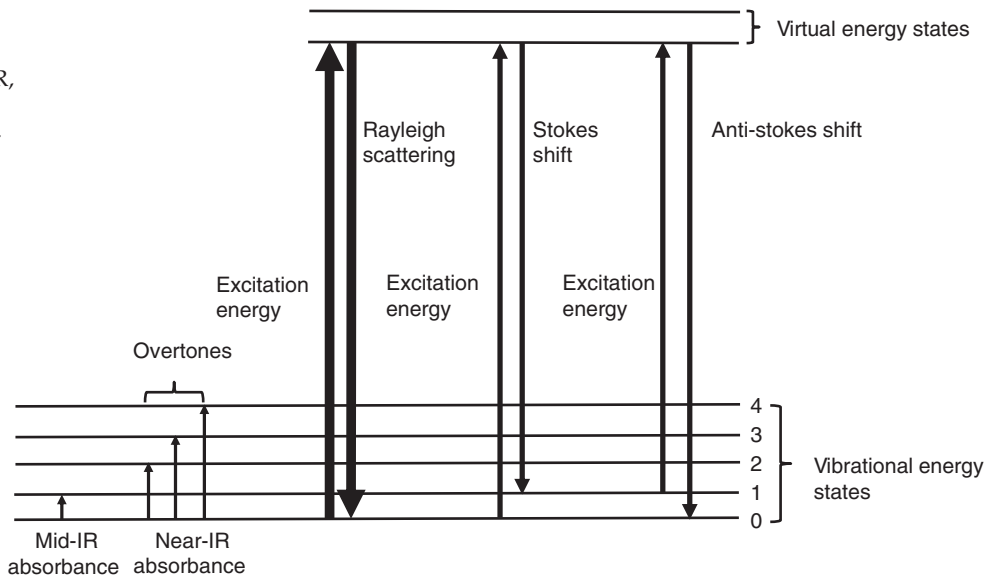
8.5.1 Principles

Raman spectroscopy is a vibrational spectroscopic technique that is complementary to IR measurements [56]. When a photon of light collides with a molecule, the collision can result in the photon being scattered. Molecules in the sample can be excited and reach an unstable virtual energy state when they interact with the incident light as shown in Fig. 8.10. However, this transition to a high-energy state in the molecule is a short-lived process, and most of the molecules relax back to their initial low energy level resulting in the scattered photon having the same energy as the incident light. This is called elastic scattering or **Rayleigh scattering**. However, a few molecules relax to a higher vibrational state with a change in the vibrational and rotational energy of the molecule causing a shift in the wavelength of the scattered radiation. This is called as inelastic scattering or **Raman scattering**. For Raman scattering to occur, a molecule must undergo a change in polarizability of the electron cloud of the molecule, but does not need to undergo a change in dipole moment. Thus, Raman can observe symmetrical vibrations that cannot be detected by IR spectroscopy. Raman is complementary to IR spectroscopy in that some vibrations are only Raman active, some are only IR active, and some are both.

During Raman scattering, scattered photons (approximately 1 in 10^7 photons) will shift to a longer wavelength (lower frequency), and this shift in frequency is called a **Stokes lines** or Stokes shift. If the final energetic state is lower than the initial state, scattered photons will shift to a shorter wavelength (higher frequency), and this is called an **anti-Stokes lines** or anti-Stokes shift [57]. Intensities of the Stokes lines are higher than those of the anti-Stokes lines, and therefore the Stokes lines are usually measured as the Raman spectrum [58].

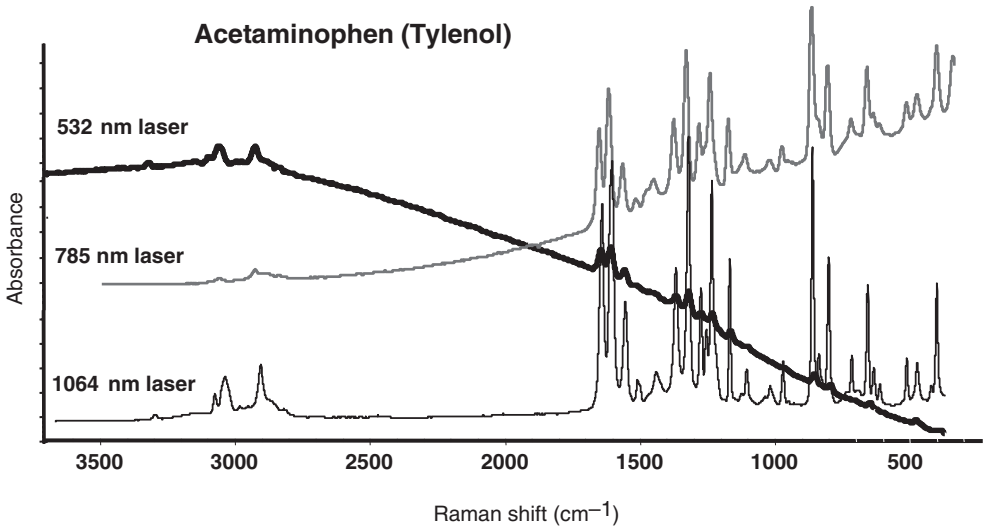
A typical Raman spectrum includes scattering intensity (photons per second) on the y-axis versus

8.10 figure



Energy-level diagram of mid-IR, near-IR, Rayleigh, and Raman scattering (Adapted from Boyaci et al. [57])

8.11 figure



Raman spectrum of acetaminophen (Tylenol) generated using different laser sources

either increasing wavelength (nm) or Raman shift (cm^{-1}) on the x-axis. Each band in a Raman spectrum corresponds to a vibration of a chemical bond and/or functional group in the molecule as illustrated in a representative Raman spectrum of a pharmaceutical in Fig. 8.11. Similar to IR, a fingerprint of a molecule can be acquired, and by employing chemometric tools previously described in Sect. 8.5.3, both qualitative and quantitative analyses can be generated since the intensity of the Raman bands is proportional to the concentration of the analyte [57].

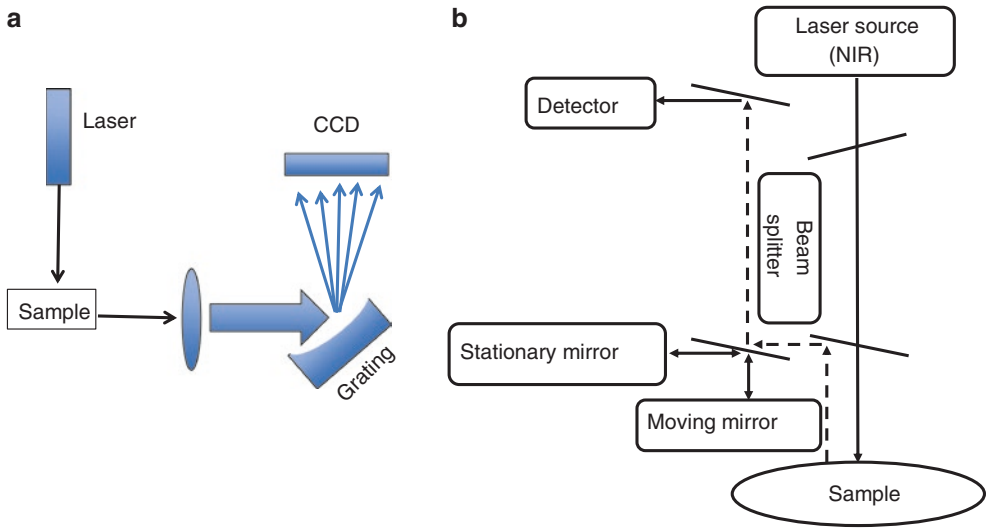
Raman spectroscopy is positioning as an attractive technique because: (1) it requires little or no sample preparation; (2) water and alcohols are weak Raman scatterers, allowing measurement of aqueous samples without any special accessories or sample preparation; and (3) it allows measurements through common transparent containers such as glass, quartz, and plastic eliminating the need to open containers to analyze the contents. For food applications of Raman spectroscopy, the challenge of sample fluorescence may be a limiting factor. In general, the intensity of the Raman scatter is proportional to $1/\lambda^4$, so shorter excitation wavelengths deliver a much stronger Raman signal. The caveat is that when using short excitation wavelengths, **fluorescence** is more likely to occur under these conditions.

8.5.2 Instrumentation

Raman spectrometers are based on **dispersive** and **Fourier transform** technologies (Fig. 8.12). Each technique has its unique advantages, and each is ideally suited to specific types of analysis.

A Raman dispersive system is composed of a laser source, sample, dispersing element (diffraction grating), detector, and a computer. During a typical Raman spectrum collection process, a laser source gives a coherent beam of monochromatic light that is focused on the sample. The scattered light is passed through a notch filter that rejects the Rayleigh-scattered light, resulting in an important gain in sensitivity. Raman scattered photons enter the monochromator, where they are separated by wavelength and are collected by a detector that records the intensity of the Raman signal at each wavelength. Lasers are typically used as the radiation source, as the strength of the Raman signal is proportional to the intensity of the incident light. The use of the lasers as a source of radiation to generate the Raman scattering has been a crucial development in Raman instrumentation [59]. Typical wavelengths are 785, 633, 532, and 473 nm. Raman detectors are frequently photodiode arrays (PDA) or charge-coupled device (CCD) cameras. CCD detectors are extremely sensitive to light and contain thousands of picture elements (pixels) that acquire the whole spectrum at once in less than a second. CCD detectors allow the use of very low laser power, to prevent thermal or photochemical destruction of the sample.

FT-Raman spectrometers commonly use a near-IR laser, usually at 1030 nm or 1064 nm. Using lasers with excitation wavelengths in the near-IR region almost completely eliminates fluorescence; however, the Raman scattering intensity is weak. FT-Raman uses sensitive, single-element near-IR detectors such as **InGaAs** or liquid nitrogen-cooled germanium (**Ge**) detectors. An **interferometer** converts the Raman signal into an interferogram, permitting the detector to collect the entire spectrum simultaneously. Application of the Fourier transform algorithm to the **interferogram** converts the results into a conventional Raman spectrum. Besides removal of the fluorescence interference, FT-Raman spectroscopy provides excellent wave number accu-



8.12 figure Basic illustration of the (**a**) dispersive Raman and (**b**) FT-Raman instrumentation (Adapted from Das and Agrawal [68])

racy as a result of the internal interferometer calibration by a built-in helium–neon laser.

8.5.3 Surface-Enhanced Raman Scattering (SERS)

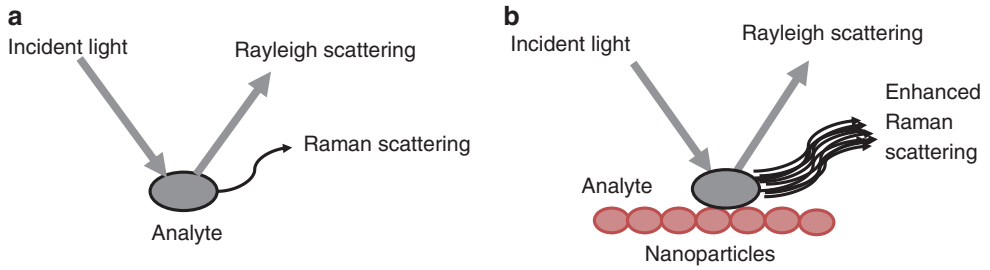
As mentioned earlier, one of the drawbacks of traditional Raman spectroscopy is the very low level of Raman signals. One of the most popular techniques used to overcome this problem is surface-enhanced Raman scattering (SERS). Nanostructures on a metallic surface (typically gold or silver) can tremendously enhance the Raman signal of sample molecules on the order of 10^4–10^{11}, allowing detection in the ppb or single-molecule level [60, 61]. Simple mechanisms of traditional Raman and SERS are compared in Fig. 8.13. Variations in the magnitude of the signal enhancement depend on the morphology of the particle (roughened surfaces can provide much more enhancements compared to flat (smooth) metal surfaces) [62].

The SERS phenomena result from an enhanced electromagnetic field produced at the surface of the metal and a chemical enhancement due to a charge–transfer interaction between the metal and adsorbed molecules. When the wavelength of the incident light is close to a surface plasmon resonance (collective excitation of conductive electrons in small metallic structures), molecules adsorbed or in close proximity to the surface experience an exceptionally large electromagnetic field. In addition, the electronic transitions of many charge–transfer complexes between the metal surface and the molecule result in resonance.

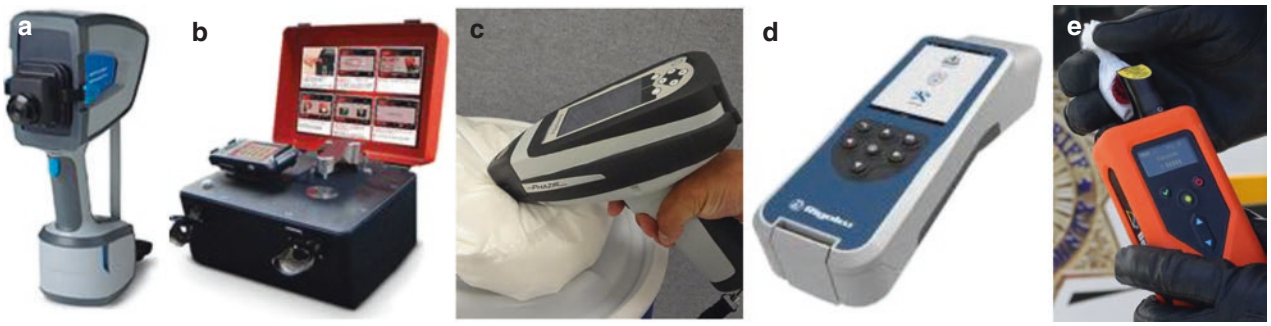
The enormous signal enhancement achieved by SERS has positioned it as a very promising analytical tool for the biochemical, biomedical, and pharmaceutical fields. Food applications have been directed to food safety for the detection of food-borne pathogens as an alternative to current microorganism diagnostic tools, providing the possibility of developing portable pathogen sensors for on-site food inspection [60]. Other applications of SERS include the detection of food contaminants (pesticides and antibiotic residues) and adulteration (melamine, illegal food dyes, and mycotoxins) [60, 63].

8.6 HANDHELD AND PORTABLE TECHNOLOGY

Vibrational spectroscopy techniques are extremely well suited to be used as portable or handheld instruments. Their simplicity, speed, selectivity, and ability to operate without sample preparation make them ideal to be used outside the laboratory for process monitoring in challenging environments. A single spectrometer can be used to verify the identity of bulk materials, check for contamination, control processes, and confirm final product specifications. Field-based instruments have to tolerate harsh conditions, maintain reliability and accuracy, be easy to operate, battery powered, lightweight with an ergonomic design and intuitive user interface. Sample accessories must be robust, with limited or no sample preparation required and capable of fast analysis (Fig. 8.14).



8.13 figure Comparison of (**a**) traditional Raman and (**b**) SERS mechanism (Adapted from Zheng and He [63])



8.14 figure Portable/handheld vibrational spectrometers commercially available. (**a**) Agilent 4300 handheld FTIR, (**b**) Agilent 4500 Portable FTIR, (**c**) Thermo microPhazir Handheld Near-IR, (**d**) Progeny™ 1064 nm handheld Raman analyzer by Rigaku Analytical Devices, and (**e**) DeltaNu ReporteR handheld Raman spectrometer

The advantage of portable mid-IR spectrometers (compared to near-IR) is the higher fundamental signal, allowing detection of low analyte levels and its unique fingerprinting capabilities. However, if a sample contains water, its strong mid-IR absorption may swamp useful information. Near-IR allows analysis without any sample preparation. The most commonly used mode of sampling for solids is diffuse reflectance, while transflectance (combined transmission and reflectance) and transmission are suitable configurations for liquid analysis. Although the near-IR signal is 10–1000 times lower than mid-IR bands, the lower absorptivities permit the near-IR beam to penetrate deeper into the sample, resulting in more representative analysis. Advantages of portable Raman instruments for field deployment include little or no sample preparation, noncontact and nondestructive capabilities, and the relatively weak Raman response of water to allow measuring aqueous solutions. Near-IR and Raman analysis permit measurements through glass and plastic films.

Portable and handheld vibrational spectrometers are attractive fingerprinting techniques for various applications including food, pharmaceuticals, petrochemicals, and law enforcement [64]. Ellis et al. [65] summarized the applicability of various commercially available spectroscopy-based approaches for rapid on-site food fraud analysis. In addition, dos Santos et al. [66] reviewed the application of portable near-IR spectrometers in the agro-food industry.

8.7 SUMMARY

IR spectroscopy measures the absorption of radiation in the near-IR ($\lambda = 0.8$–2.5 μm) or mid-IR ($\lambda = 2.5$–15 μm) regions by molecules in food or other substances. IR radiation is absorbed as molecules change their vibrational energy levels. A summary of the most important characteristics of spectroscopy techniques is presented in Table 8.2. Mid-IR spectroscopy is especially useful for qualitative analysis, such as identifying specific functional groups present in a substance. Different functional groups absorb different frequencies of radiation, allowing the groups to be identified from the spectrum of a sample. Quantitative analysis also can be achieved by mid-IR spectroscopy, with milk analysis being a major application. Near-IR spectroscopy is used most extensively for quantitative applications, using either transmission or diffuse reflection measurements that can be taken directly from solid foods. By using multivariate statistical techniques, infrared instruments can be calibrated to measure the amounts

8.2 table Comparison of spectroscopy methods common in food analysis

Type of spectroscopy	*Principles*	*Order of components*	*Application*
Ultraviolet–visible (UV–Vis)	Based on absorption by molecules of radiant energy of specific wavelength from tungsten or UV lamp. Measure difference between amount of energy emitted by lamp and that reaching the detector. Absorption is proportional to concentration of molecule	UV or visible lamp, monochromator, sample, detector, readout	Quantitate molecules (by themselves or as the result of a chemical reaction) that will absorb radiant energy in the UV or vis region
Fluorescence	Based on emission, by molecules, of radiant energy of specific wavelength. Molecule is activated from its ground state to an excited electronic energy level by the absorption of radiation from a UV or vis lamp. Measure radiant emitted by the molecule as it relaxes from an excited electronic energy level to its corresponding ground state. Emitted energy is proportional to concentration of molecule	UV or visible lamp, monochromator, sample, monochromator, detector, readout	Quantitate molecules (by themselves or as the result of a chemical reaction) that will emit radiant energy in the UV or vis region
Mid-IR (MIR)	Based on absorption, by molecules, of radiant energy of specific wavelength (2.5–15 μm; 4000–670 cm^{-1}) from a mid-IR source. Molecules in the ground state absorb mid-IR radiation and make a transition to the first excitation state of vibration (fundamental vibration). When the frequency of the radiation matches the vibrational frequency of the molecule or functional group, then radiation causes vibrational and rotational changes and a net change in the dipole moment of the molecule. Measured by difference between amount of energy emitter by the mid-IR source and that reaching the detector. Absorption is proportional to concentration of the analyte	For dispersive systems: mid-IR source, grating, slit, sample, detector, readout For FT systems: mid-IR source, interferometer (He:Ne alignment laser, beam splitter, movable mirror, fixed mirror), sample, detector, readout	Qualitative and quantitative analysis of sample(s)
Near-IR (NIR)	Based on absorption, by molecules, of radiant energy of specific wavelength (0.8–2.5 μm; 12,500–4000 cm^{-1}) from a NIR source. Molecules absorb near-IR radiation and make transitions to higher excited states (v=2, 3 or higher) resulting in overtones and combination vibrations of C–H, O–H, and N–H groups. A net change in the dipole moment and large mechanical anharmonicity of the vibrating atoms are required. Measured by the difference between amount of energy emitted by the near-IR source and that reaching the detector. Absorption is proportional to concentration of molecule	For dispersive systems: near-IR source, grating, slit, sample, detector, readout For FT systems: near-IR source, interferometer (He:Ne alignment laser, beam splitter, movable mirror, fixed mirror), sample, detector, readout	Qualitative and quantitative analysis of sample(s)
Raman	Based on inelastic scattering process of monochromatic laser beam through its interaction with vibrating molecule resulting in a change in polarizability of the electron cloud of the molecule. Scattered photon is shifted to higher (anti-Stokes shift) or lower (Stokes shift) frequencies than the incident photon, giving information about the vibrational modes in the system. Measure Raman scattered photons by a detector. Band intensities are proportional to concentration of molecule	For dispersive systems: UV or visible laser, sample, dispersing element (diffracting grating), detector, readout For FT systems: near-IR laser, interferometer (He:Ne alignment laser, beam splitter, movable mirror, fixed mirror), sample, detector, readout	Qualitative and quantitative analysis of sample(s)

(continued)

8.2 table (Continued)

Type of spectroscopy	*Principles*	*Order of components*	*Application*
Atomic absorption (flame)	Based on absorption, by atoms, of energy of specific wavelength from hollow cathode lamp (HCL). Flame converts molecules to atoms. Energy from HCL activates atoms from ground state to excited state. Absorption is proportional to concentration of atoms	HCL, sample inserted in flame, monochromator, detector, readout	Quantitate minerals
Atomic emission (plasma)	Based on emission, by atoms, of energy of specific wavelength. Plasma converts molecules to atoms. Plasma activates atoms from ground state to excited state. Emission is proportional to concentration of atoms	Sample inserted in plasma, monochromator (or echelle optics), detector, readout	Quantitate minerals

of various constituents in a food sample based on the amount of IR radiation absorbed at specific wavelengths. Mid-IR, near-IR, and Raman spectroscopy requires much less time to perform quantitative analysis than do many conventional wet chemical or chromatographic techniques.

8.8 STUDY QUESTIONS

1. Describe the factors that affect the frequency of vibration of a molecular functional group and thus the frequencies of radiation that it absorbs. Also, explain how the fundamental absorption and overtone absorptions of a molecule are related.
2. Describe the essential components of an FT mid-IR spectrometer and their function, and compare the operation of the FT instrument to a dispersive instrument. What advantages do FT instruments have over dispersive IR spectrophotometers?
3. Describe the similarities and differences between mid-IR spectroscopy and Raman spectroscopy.
4. Of the three antioxidants, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate, which would you expect to have a strong IR absorption band in the 1700–1750 cm^{-1} spectral region? Look up these compounds in a reference book if you are uncertain of their structure.
5. Describe the two ways in which radiation is reflected from a solid or granular material. Which type of reflected radiation is useful for making quantitative measurements on solid samples by near-IR spectroscopy? How are near-IR reflectance instruments designed to select for the desired component of reflected radiation?
6. Describe the steps involved in calibrating a near-IR reflectance instrument to measure the protein content of wheat flour. Why is it usually necessary to make measurements at more than one wavelength?

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