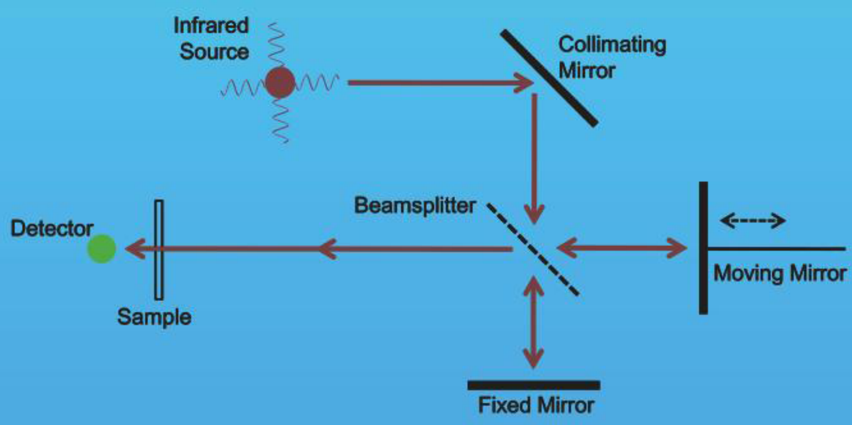
Second Edition

Fundamentals of FOURIER TRANSFORM INFRARED SPECTROSCOPY



Brian C. Smith

CRC Press
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To my loving and lovely wife, Marian.

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Preface

Why write a second edition of *Fundamentals of FTIR*? Two reasons. First, much has changed since the first edition was published in 1996, particularly the development of diamond ATRs. Second, I have taught FTIR to thousands of people since 1992, and I now do a better job of explaining the important concepts of FTIR than I did then, which is reflected in this book. Those familiar with the first edition of *Fundamentals of FTIR* will recognize very little here; the book has been entirely rewritten and there are dozens of new figures. Topics have been added, expanded, and dropped as appropriate.

So, who do I think you are? This book is aimed at those new to FTIR, particularly non-chemists who need to use FTIR equipment and who seem to be the majority of new users these days. However, journeyman and expert spectroscopists should find this volume a useful reference for its concise and comprehensible explanation of FTIR topics. A nodding familiarity with freshman chemistry and physics will help while reading this volume, but is by no means a necessity. Chemists and non-chemists have told me they benefited from reading the first edition, and I expect the same will be true of this edition.

This book contains six chapters. Chapter 1 is an introduction to the field of infrared spectroscopy, including the strengths and weaknesses of FTIR as a chemical analysis tool. The second chapter, "How an FTIR Works," is all about the instrument. Topics include how an interferometer generates a spectrum, optimizing spectral quality, and tests to monitor instrument health. Chapter 3 is a discussion of how to properly use spectral processing to increase the information content of a spectrum without damaging the data. My approach to this topic is as unique now as it was in the first edition. Chapter 4 is dedicated to sample preparation. Preparing samples is half the battle in getting a good spectrum—Chapter 4 will teach you to win that battle. The chapter is significantly expanded compared to the first edition, including a lengthening of the ATR section. Chapter 5 is dedicated to single analyte quantitative analyses. Entire books have been written on this topic (including one by me), but the goal here is to introduce you to enough of the theory and practice of this field to allow you to generate your own quality calibrations. Chapter 6 is an overview of infrared microscopy. I estimate that one third of the FTIRs in use have infrared microscope attachments, hence the need to cover this topic in an introductory book.

I hope you find this book a readable and enjoyable introduction to an important field of chemical analysis. I take full responsibility for the contents of the book including all errors, and greatly appreciate your comments whether positive or negative. You can find me by doing an Internet search on "Spectros Associates."

Happy reading.

Brian C. Smith, Ph.D.

Acknowledgments

No book is an island; authors need the assistance of numerous people to turn a book idea into reality, all of whom deserve credit. I would first like to thank the folks at CRC Press. I have been one of their authors for 17 (!) years, and I still enjoy my relationship with them. I would particularly like to thank my publisher, Fiona Macdonald, and my editor, Barbara Glunn. Barb has been particularly patient and gracious with me through many delays and frustrations. I am grateful that my Ph.D. advisor from Dartmouth College, Prof. John Winn, is still part of my life, and I would like to thank him for reviewing parts of this book. Much of the spectral data in this book was processed and plotted using the GRAMS/AI™ software package from ThermoFisher Scientific. I would like to thank them for supplying me with the software. Some of the spectra were measured using an ALPHA FTIR from Bruker Optics. I would like to thank Haydar Kustu from Bruker Optics for making the ALPHA available to me. Ken Kempfert of PIKE Technologies supplied many of the pictures of sampling accessories. Thanks Ken! I also thank my "partner in crime" here at Spectros Associates, Peg Veal, for her many years of dedicated service.

I have made my living as an FTIR trainer and consultant since 1992. In that time thousands of people have patronized my business, and I would like to thank each and every one of them, including you, for allowing me to have such a fun and interesting career.

1 Introduction to Infrared Spectroscopy

The purpose of this book is to introduce the reader to the fundamental concepts of Fourier Transform Infrared (FTIR) spectroscopy. The discussion assumes no previous background in FTIR, but a familiarity with the basic concepts of chemistry and physics will be helpful in understanding this text. This book teaches the basics of FTIR to those new to the field, and will serve as an excellent reference guide for experienced users. All terms shown in *italics* will be defined in the glossary at the end of the book.

I. TERMS AND DEFINITIONS

Half the battle in learning any new field is understanding the jargon. To aid you in learning about FTIR, a number of the terms used in the field of infrared spectroscopy are defined below.

Spectroscopy – the study of the interaction of light with matter.
Infrared Spectroscopy – the study of the interaction of infrared light with matter.
Mid-Infrared – light from 4000 to 400 *wavenumbers* (cm^{-1}).
Spectrum – a plot of measured light intensity versus some property of light such as *wavelength* or *wavenumber.*
Spectrometer – an instrument that measures a spectrum.
Infrared Spectrometer – an instrument that measures an infrared spectrum.
FTIR – Fourier Transform Infrared, a specific type of infrared spectrometer.

Analysis of infrared spectra can tell you what molecules are present in a sample and at what concentrations; this is why infrared spectroscopy is useful. There are several types of infrared spectrometers in the world, but the most widely used ones are FTIRs, which is the focus here. This book will teach you how FTIRs work, how to use them to obtain the best spectra, how to use FTIR software to assist in data analysis, how to properly prepare samples for FTIR analysis, how to quantify concentrations in samples using FTIR spectra, and infrared microscopy. In essence, we will be studying everything involved in obtaining a good infrared spectrum. For information on how to interpret an infrared spectrum to determine the structures of molecules present in a sample please consult my book on infrared spectral interpretation [1].

II. THE PROPERTIES OF LIGHT

The proper term used to describe light is *electromagnetic radiation*. Light is composed of electric and magnetic waves called the *electric vector* and the magnetic vector. These two waves undulate in planes mutually perpendicular to each other, and move through space in a third direction perpendicular to the planes of undulation. It is the interaction of the electric vector with matter that leads to the absorbance of light. The amplitude of the electric vector changes over time and has the form of a sine wave as shown in Figure 1.1. The + and – signs in the figure indicate that the polarity of the electric vector alternates over time.

Since the motion of waves is repetitive, they go through *cycles*. For a wave a *cycle* begins at zero amplitude and ends when the wave has crossed zero amplitude a third time as illustrated in Figure 1.1. The distance forward traveled by a wave during a cycle is called its *wavelength*. The units of the wavelength are distance per cycle, although typically just the distance units are noted. Different types of light waves have different wavelengths. For example, the mid-infrared radiation typically used to measure infrared spectra has wavelengths of about 10 microns, which is a little smaller than the diameter of a human hair. Scientists use the Greek letter lambda (λ) to denote wavelength. The arrows in Figure 1.1 show the wavelength of the light wave. In the older scientific literature you will sometimes see infrared spectra plotted with wavelength on the x-axis.

Another important property of a light wave is its *wavenumber*, which is denoted by the letter W. The wavenumber measures the number of cycles a wave undergoes per unit length. Wavenumbers are measured in units of cycles per centimeter, which are frequently abbreviated as cm^{-1} and can be pronounced as "inverse centimeters," "reciprocal centimeters," or "wavenumber." If a spectrum has a peak at 3000 cm^{-1} it means the sample absorbed infrared light that underwent 3000 cycles per centimeter. Most infrared spectra are plotted from 4000 to 400 cm^{-1} on the x-axis, as seen in Figure 1.2.

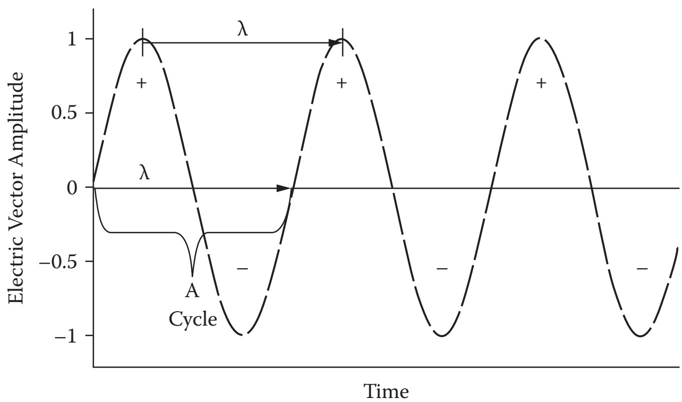

FIGURE 1.1 An example of the electric vector of a light wave. The + and – signs indicate the alternating polarity of the electric vector. The arrows show the wavelength (λ) of the wave. Note where the wave's cycle begins and ends.

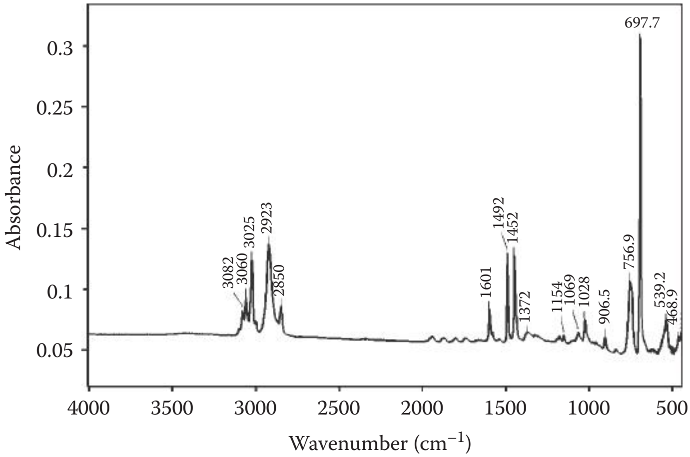

FIGURE 1.2 The infrared spectrum of polystyrene. Note that the x-axis is plotted in wavenumber and that the y-axis is in absorbance.

Since the wavelength has units of distance/cycle and wavenumbers have units of cycles/distance, the two quantities are reciprocals of each other as such:

$$W = 1/\lambda \tag{1.1}$$

Where

W = Wavenumber
λ = Wavelength

If λ is measured in centimeters, then W is calculated in cm^{-1}. One of the interesting properties of W is that it is proportional to the energy of a light wave as follows:

$$E = hcW \tag{1.2}$$

Where

E = Light energy in Joules
c = The Velocity of light (~3×10^{10} cm/second)
h = Planck's constant (6.63×10^{-34} Joule-second)
W = Wavenumber

Since energy is proportional to W, high wavenumber light has more energy than low wavenumber light. Thus, the x-axis of Figure 1.2 is an energy axis with higher energy to the left and lower energy to the right.

Another important property of light waves is their *frequency*, which is a measure of the number of cycles a wave undergoes per unit time. Frequency is typically measured in cycles/second or Hertz (Hz) and the units are frequently written as sec^{-1}. Mid-infrared frequencies are on the order of 10^{14} Hz or ~10 terahertz. Scientists represent frequency with the Greek letter nu (ν).

The different properties of light waves are related to each other by the following equation:

$$c = \nu\lambda \tag{1.3}$$

Where

c = The Velocity of light (~3×10^{10} cm/second)
ν = Frequency in Hertz (sec^{-1})
λ = Wavelength in cm

A close look at Equation 1.3 shows that the units make sense. When wavelength is measured in centimeters and is multiplied by frequency measured in sec^{-1}, we obtain units of cm/sec, which are the units of velocity. Equation 1.3 shows that the product of frequency times wavelength for a light wave equals a constant, the speed of light. Thus for any light wave you can calculate the frequency if you know the wavelength, and you can calculate the wavelength if you know the frequency. From Equation 1.1 we know that $W = 1/\lambda$, which can be substituted into Equation 1.3 and rearranged to obtain the following:

$$c = \nu/W \tag{1.4}$$

Where

c = The Velocity of light (~3×10^{10} cm/second)
ν = Frequency in Hertz (sec^{-1})
W = Wavenumber in cm^{-1}

Equation 1.4 allows the calculation of the wavenumber of a light wave if frequency is known, or frequency if wavenumber is known.

There are many types of electromagnetic radiation in the universe besides the mid-infrared, the collection of which is called the *electromagnetic spectrum*. A diagram of part of the electromagnetic spectrum is seen in Figure 1.3.

The mid-infrared has been intentionally placed at the center of Figure 1.3. To the right of the mid-infrared between 400 and 4 cm^{-1} is the *far infrared*. When molecules absorb far infrared light they vibrate. Molecules with heavy atoms in them, including many inorganics, absorb in this region. Some FTIRs work in the far infrared. Lower in energy than the far infrared are *microwaves*. When molecules absorb microwaves they rotate faster. Microwave spectra of rotating gas phase molecules have been measured, and this type of spectroscopy can be used to identify and quantitate gases in samples. A microwave oven gives off radiation tuned to an absorbance of liquid water. The liquid water molecules in food absorb this energy and rotate rapidly. Collisions with neighboring food molecules transfer energy to them raising their temperature and making your dinner warm.

Higher in energy than the mid-infrared, from 14,000 to 4000 cm^{-1}, is the *near infrared*. Molecules vibrate when they absorb near infrared radiation, but the spectral features are fewer, broader, and more difficult to interpret than in the mid-infrared. Because of certain instrumental advantages, near infrared radiation is frequently

>14,000 cm^{-1} Visible & UV	14,000 to 4000 cm^{-1} Near IR	4000 to 400 cm^{-1} Mid-Infrared	400 to 4 cm^{-1} Far Infrared	< 4 cm^{-1} Microwaves
Electronic Transitions	Molecular Vibrations	Molecular Vibrations	Molecular Vibrations	Molecular Rotations

Higher Wavenumber	**Lower Wavenumber**
Higher Frequency	**Lower Frequency**
Higher Energy	**Lower Energy**
Shorter Wavelength	**Longer Wavelength**

FIGURE 1.3 A part of the electromagnetic spectrum.

used to measure sample properties in difficult environments such as in the middle of a chemical reactor or of liquid flowing through a pipe. Some FTIRs work in the near infrared. Higher in energy than the near infrared are visible light and ultraviolet (UV) radiation. These higher energy light waves fall above 14,000 cm^{-1}. When a molecule absorbs visible or ultraviolet light the electrons in the molecule transition from a lower electronic energy level to a higher one. Many molecules have measurable UV and visible spectra, and these spectra can be used to identify and quantitate molecules in samples. FTIRs can be equipped to work in the visible and UV. Figure 1.3 shows that as you move from right to left across the electromagnetic spectrum there is an increase in energy, wavenumber, and frequency, but a decrease in wavelength. Similarly, moving from left to right across Figure 1.3 there is a decrease in energy, frequency, and wavenumber and an increase in wavelength.

III. WHAT IS AN INFRARED SPECTRUM?

A plot of measured infrared light intensity versus a property of light is called an *infrared spectrum*. An example of an infrared spectrum is shown in Figure 1.2. By convention the x-axis of an infrared spectrum is plotted with high wavenumber to the left and low wavenumber to the right. Plots of your FTIR spectra should always follow this convention. Note in Figure 1.2 that 4000 cm^{-1} is to the left and 500 cm^{-1} is to the right, and that the spectrum is plotted in Absorbance units, which measure the amount of light absorbed by a sample. As you can see in the figure the peaks point up and their tops denote wavenumbers at which significant amounts of light were absorbed by the sample. The absorbance spectrum of a sample is calculated from the following equation:

$$A = \log(I_0/I) \tag{1.5}$$

Where

$A =$ Absorbance
$I_0 =$ Intensity in the background spectrum
$I =$ Intensity in the sample spectrum

Absorbance is also related to the concentration of molecules in a sample via an equation called *Beer's Law*:

$$A = \varepsilon lc \quad (1.6)$$

Where

$A =$ Absorbance
$\varepsilon =$ Absorptivity
$l =$ Pathlength
$c =$ Concentration

The height or area of a peak in an absorbance spectrum is proportional to concentration, which is why Beer's Law can be used to determine the concentrations of molecules in samples. This topic will be covered in Chapter 5.

The y-axis of an infrared spectrum can also be plotted in units called percent transmittance (%T), which measures the percentage of light transmitted by a sample. %T spectra are calculated as follows:

$$\%T = 100 \times (I/I_0) \quad (1.7)$$

Where

$\%T =$ Percent Transmittance
$I_0 =$ Intensity in the background spectrum
$I =$ Intensity in the sample spectrum

Note that in the %T spectrum seen in Figure 1.4 the peaks point down. The peak bottoms represent wavenumbers where the sample transmitted measurably less than 100% of the incident infrared light.

Absorbance and %T are mathematically related to each other, and it is easy using FTIR software to convert from one to the other. When this conversion is made only the y-axis changes; the peak positions are not affected. In the scientific literature you will see spectra plotted in both absorbance and %T. The spectra in this book will be plotted both ways to give you practice looking at both types of spectra.

Since there are two different y-axis units used in infrared spectroscopy, you may be wondering which unit you should use. Since absorbance is linearly proportional to concentration (Equation 1.6), absorbance units must be used for quantitative analysis. Your spectra must also be in absorbance if you are going to perform a spectral subtraction or library search [for reasons outlined in Chapter 3]. Absorbance spectra are perfectly well suited to qualitative analysis as well. The size of the peaks in %T spectra are not linearly proportional to concentration, so these spectra should not be used for quantitative analysis, spectral subtraction, or library searching.

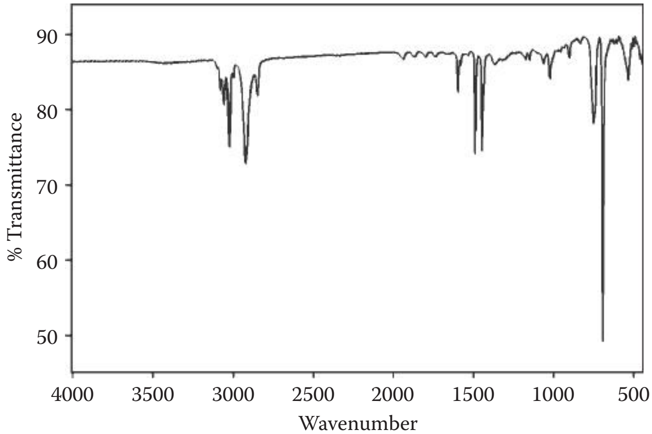

FIGURE 1.4 The infrared spectrum of polystyrene plotted in percent transmittance (%T).

However, %T spectra are perfectly fine for qualitative analysis. Thus, if you are identifying unknowns or performing spectral comparisons, either absorbance or %T spectra can be used, and the choice is often a matter of personal preference, your supervisor's preference, or as stated in the standard procedure you are following.

When preparing samples for infrared analysis it may be tempting to cram as much material as possible into the infrared beam to try and maximize the amount of light absorbed. This is actually a bad idea because FTIRs are very sensitive; it typically takes only milligrams of material to obtain a good spectrum. In fact, in infrared spectra there is such a thing as the peaks being too big as seen in Figure 1.5.

Recall from Equation 1.6 that absorbance depends upon pathlength times concentration. The top spectrum in Figure 1.5 was measured with an appropriate pathlength and concentration and these are normal-looking infrared peaks. The bottom

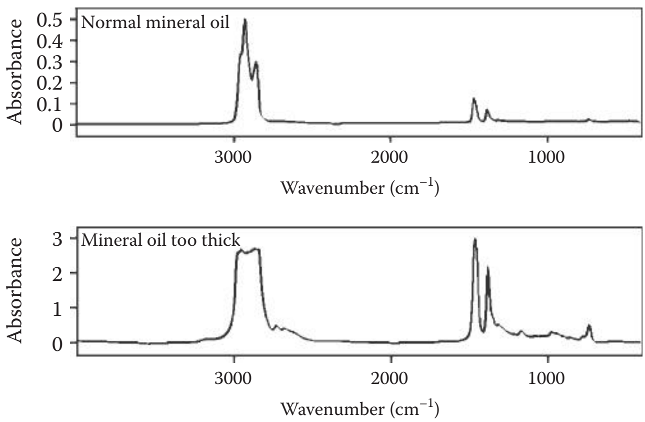

FIGURE 1.5 An example of what happens when a sample is too thick or too concentrated, using mineral oil as an example.

spectrum in Figure 1.5 was intentionally measured with a sample that was too thick. You can see that at around 3000 cm^{-1} the peak has a "chopped-off" or box-like appearance. This is because the sample is literally absorbing all the light in this wavenumber region. Since the sample spectrum intensity (I) in Equation 1.5 appears in the denominator, as this quantity approaches zero, the value of A approaches infinity. Rather than trying to measure infinitely large absorbances, the instrument truncates the intensity measurement at some value, giving the chopped-off peak appearance seen in Figure 1.5. These peaks are a problem because their shapes are distorted; one could easily conclude that the two spectra in Figure 1.5 are of different materials when in fact they are both of mineral oil. In general, when you measure spectra your peaks should be less than 2 absorbance units or greater than 10%T to avoid the truncated peaks seen in Figure 1.5. Any time you measure a spectrum, check the peak sizes and shapes to make sure they are on scale and of the correct shape. With proper sample preparation you can reduce the pathlength or concentration of a sample to bring its peaks on scale.

IV. WHAT ARE INFRARED SPECTRA USED FOR?

We measure infrared spectra to answer questions about samples. One question we commonly try to answer is, "What molecules are present in this sample?", otherwise called unknown analysis. The peak positions in an infrared spectrum correlate with molecular structure, which is part of why infrared spectroscopy is useful. Over the last 100-plus years a great number of infrared spectra have been measured, and the peak positions of known molecules derived from these spectra can be used to identify the molecules in an unknown sample [1].

When performing a spectral comparison, or what is sometimes called identity testing, we are asking the question of the spectrum, "Are these two samples the same?" This question is answered by comparing the unknown spectrum to a reference spectrum and noting how well the peak positions, heights, and widths in the two spectra match. Comparing spectra to each is easier than interpreting an unknown spectrum, but it still needs to be done properly [1].

A third question an infrared spectrum can answer is, "What are the concentrations of molecules in this sample?" To do this one must measure the spectra of samples of known concentration, and then use Beer's Law (Equation 1.6) to prepare a calibration line relating absorbance to concentration. Once the calibration has been generated and validated it can be used to determine the concentration of molecules in unknown samples. For more on quantitative analysis see Chapter 5 of this volumes or my book on quantitative spectroscopy [2].

V. THE ADVANTAGES AND DISADVANTAGES OF INFRARED SPECTROSCOPY

The advantages and disadvantages of infrared spectroscopy as a chemical analysis technique are listed in Table 1.1. These attributes are for the technique generally, regardless of the type or brand of infrared spectrometer being used to measure a spectrum.

TABLE 1.1
The Advantages and Disadvantages of Infrared Spectroscopy

Advantages	Disadvantages
Almost universal	Can't detect some molecules
Spectra are information rich	Mixtures
Relatively fast and easy	Water
Relatively inexpensive	
Sensitivity	

The first advantage of infrared spectroscopy is that it is almost universal. Many molecules have strong absorbances in the mid-infrared, which is part of why we measure spectra in this region. Many types of samples including solids, liquids, gases, semi-solids, powders, polymers, organics, inorganics, biological materials, pure substances, and mixtures can have their infrared spectra measured. The second advantage of infrared spectroscopy is that infrared spectra are information rich. The peak positions give the structures of the molecules in a sample, the peak intensities give the concentrations of molecules in a sample, and the peak widths are sensitive to the chemical matrix of the sample including pH and hydrogen bonding [1].

The third advantage of infrared spectroscopy is that measuring infrared spectra is relatively fast and easy. Of course, the nature of the sample and the sampling technique chosen will affect the speed and ease of analysis, and some samples will be more difficult than others. However, many samples can have their infrared spectra measured in five minutes or less. For certain samples and sampling techniques (such as ATR, see Chapter 4) quality spectra can be obtained in a matter of seconds. This compares favorably to gas and liquid chromatography where it can take 15 to 45 minutes to analyze one sample. The fourth advantage of infrared spectroscopy is that it is relatively inexpensive. A quality infrared spectrometer can be had today (2010) for ~$15K. This may sound like a lot of money, but in the world of lab instruments it is not an unusually large expense. Instruments such as Nuclear Magnetic Resonance spectrometers (NMR), gas chromatography-mass spectrometers (GC-MS), and liquid chromatography-mass spectrometers (LC-MS) routinely cost more than $100K, several times more expensiv4e than most FTIRs.

The fifth advantage of infrared spectroscopy is its sensitivity, which is a measure of the minimum amount of material that gives a usable spectrum. For the average FTIR, milligram (10^{-3} gram) samples are ideal, and micrograms (10^{-6} gram) of material can be detected routinely. If a gas chromatograph is hooked up to an FTIR, nanograms (10^{-9} gram) of material can be routinely detected. Finally, if money is no object, picograms (10^{-12} gram) of some materials can be detected by freezing the eluant from a gas chromatograph to an infrared transparent window and then taking its spectrum [3]. The ability to detect picograms of material puts infrared spectroscopy in the same sensitivity ballpark as mass spectrometry, which is an underappreciated fact among analytical chemists.

The disadvantages of infrared spectroscopy are also listed in Table 1.1. Again, these are independent of the type or brand of infrared spectrometer being used. The first issue with infrared spectroscopy is that there are several materials that do not have measurable mid-infrared spectra. Since the absorbance of infrared light by molecules excites vibrations, a chemical species without vibrations will not have an infrared spectrum. Individual atoms, such as the noble gases helium and argon, are not chemically bonded to anything, have no vibrations, and thus do not have an infrared spectrum. Similarly, monatomic ions, a single atom with a charge, do not have an infrared spectrum because they are not chemically bonded to anything and do not possess vibrations. Now, monatomic ions may affect the spectrum of solvent molecules around them, for example, the presence of enough Pb^{+2} may change the spectrum of liquid water, but the monatomic ions cannot be detected directly by infrared spectroscopy. Since infrared spectroscopy has trouble detecting atoms and monatomic ions, atomic spectroscopy techniques should be used for these analyses instead.

Another group of materials that does not possess a mid-infrared spectrum are homonuclear diatomic molecules. These are molecules that contain only two atoms and the two atoms are identical. Examples include oxygen gas (O_2) and nitrogen gas (N_2). These molecules possess one vibration, a symmetric stretch. The symmetric stretch of a symmetric molecule has a peak intensity of zero (for an explanation see [1]), so oxygen and nitrogen molecules have no peaks in the mid-infrared. This is probably a good thing. These two gases make up greater than 99% of the earth's atmosphere, and if they absorbed in the mid-infrared it would strongly interfere with the spectra of samples.

The second disadvantage of infrared spectroscopy is mixtures. The problem is that the more complex the composition of a sample, the more complex its spectrum becomes, and the more difficult it is to determine what peaks are from what molecules. This difficulty is the biggest practical disadvantage infrared spectroscopy faces. Fortunately, there are ways around the mixture problem. First, anything you can do to purify a mixture will make its composition and hence its spectrum simpler. Any of the purification techniques that chemists routinely use can be applied to infrared samples. For example, liquid mixtures can be distilled, solid mixtures can be recrystallized, and solid/liquid mixtures can be filtered. Physically separating the parts of a sample can be a way of purifying it. For example, if a powdered mixture has black and white crystals in it, a pair of forceps can be used to separate the crystals into black and white piles. The spectra of these now purified crystals can be measured separately. Extractions, which utilize solubility differences between materials to purify them, can be used to purify infrared samples. Lastly, chromatographs are excellent at separating mixtures into their components. A low-tech way of "interfacing" an FTIR to a liquid chromatograph (LC) is to collect LC peaks in different containers, evaporate off the solvent, and analyze each peak residue by FTIR.

Another way of dealing with mixtures is to use the power of computation. *Spectral subtraction* software routines allow the spectra of pure substances to be subtracted from the spectra of mixtures to simplify their spectra. The spectrum of liquid water has broad, intense peaks as seen in Figure 1.6 that can mask the spectrum of any solute present, such as soap.

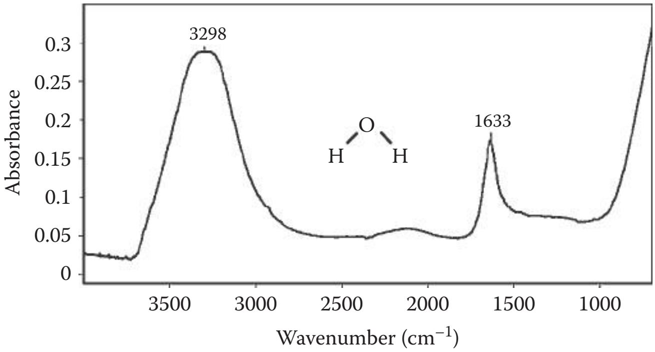

FIGURE 1.6 The infrared spectrum of liquid water. Note the broad peaks around 3500 and 1600 cm^{-1}.

By subtracting the spectrum of pure water from the spectrum of a mixture of soap and water, the water peaks can be removed making the soap peaks much easier to see. Spectral subtraction is discussed in detail in Chapter 3.

One last way of dealing with mixtures is *library searching*. This technique uses a computer to automate and speed the spectral comparison process. Thousands of spectra can be quickly compared to a mixture spectrum, which will hopefully yield one or more close matches. These matches can then be used to help identify the components in a mixture. Some software packages allow the library spectrum to be subtracted from the unknown spectrum and then the subtraction result can be used in a subsequent search in a technique called *subtract and search again*. Library searching and subtract and search again are discussed in detail in Chapter 3.

The final disadvantage of infrared spectroscopy is water. Liquid water is a problem because its broad and intense peaks as seen in Figure 1.6 can mask the spectra of solutes dissolved in water. Even using spectral subtraction, my experience is that a solute in liquid water needs to be present in a concentration greater than 0.1% to be seen. A way of dealing with this issue is to extract the molecule of interest (the *analyte*) out of the water, or to evaporate off the water and analyze the residue. However, neither of these approaches is trivial and there are no guarantees they will work. Ultimately, infrared spectroscopy is not well suited for the analysis of trace amounts of solutes in liquid water. Other techniques such as gas chromatography-mass spectrometry (GC-MS) are better suited.

Another issue with liquid water is that it dissolves some of the materials used in IR sample preparation. Materials such as KBr and NaCl are transparent in the mid-infrared and are used to make windows and cells to hold infrared samples. These materials are highly water soluble, and any liquid water present in a sample will damage these cells or windows. There are infrared transparent materials that are not water soluble that can be used (see Chapter 4), but they tend to be more expensive than KBr and NaCl.

Like liquid water, water vapor can interfere with infrared analyses as well. The spectrum of the atmosphere contains peaks from water vapor as shown in Figure 1.7.

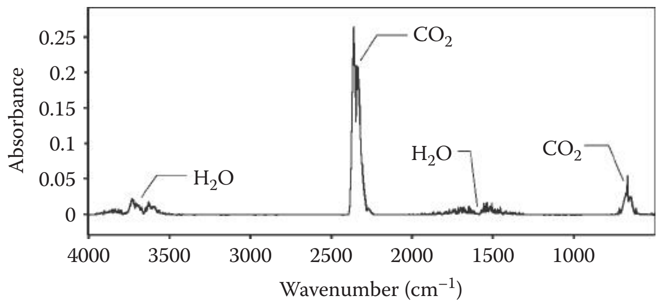

FIGURE 1.7 The infrared spectrum of the atmosphere. Note the water vapor peaks around 3700 and 1600 cm^{-1}.

Water vapor has a series of sharp features around 3700 and 1600 cm^{-1} that can interfere with spectra measured on an FTIR. Purging an FTIR with dry nitrogen or sealing and desiccating it are ways of minimizing the concentration of water vapor inside the instrument. One can also try subtracting the spectrum of water vapor from a sample spectrum to eliminate water interference. However, none of these techniques is perfect; the ultimate way of dealing with water vapor peaks it to learn to recognize and ignore them.

VI. THE ADVANTAGES AND DISADVANTAGES OF FTIR

Recall from above that an FTIR is a specific type of infrared spectrometer. Thus, FTIRs have advantages and disadvantages compared to other types of infrared instruments above and beyond the advantages and disadvantages of infrared spectroscopy generally, which was discussed above.

A. The Advantages of FTIRs

To be able to compare different types of spectrometers to each other we need a measure of spectral quality. One common measure is called the *signal-to-noise ratio* of a peak, or SNR for short. SNR is defined by Equation 1.8.

$$\text{SNR} = \text{Signal/Noise} \tag{1.8}$$

Where

SNR = Signal-to-Noise Ratio

An example of how to measure an SNR is shown in Figure 1.8.

The signal-to-noise ratio is a measure of the quality of a peak. The signal is determined by measuring the size of a peak, which can easily be done using the cursor in the software program used to display the spectrum. In Figure 1.8 the size of the peak is 0.0215 absorbance units. *Noise* is error, in this case in the y-axis of a spectrum.

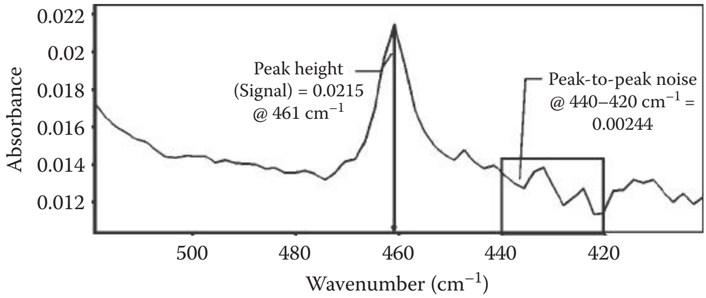

FIGURE 1.8 How to measure signal and noise to calculate a signal-to-noise ratio (SNR). In this example the SNR is (0.0215)/(0.00244) ≅ 9.

It appears jagged, is present in every spectrum, and can be seen by expanding the y-axis display limits of the baseline of any spectrum. Perhaps the simplest way to measure the magnitude of noise is called the *peak-to-peak noise* (PPN). In this measurement a peak-free region of baseline spectrum is selected, and the lowest noise point is subtracted from the highest noise point. In Figure 1.8 the PPN between 440 and 420 cm^{-1} is 0.00244 absorbance units. The SNR for this spectrum then is (0.0215/0.00244) or about 9. In this author's view a spectral feature is real if it has an SNR ≥ 3. A feature with an SNR less than 3 is suspect because it may be a noise spike or artifact. An example of a spectrum with a good SNR is shown in Figure 1.9.

The PPN of the spectrum in Figure 1.9 is 0.001 absorbance units. The portions of this spectrum that fall above the dashed line at 0.1 absorbance units have an SNR of 100 or better. Also note in Figure 1.9 that the spectrum has a flat baseline, little noise, the peaks are well resolved, there are no artifacts such as water vapor or CO_2 peaks, and the overall SNR of the spectrum is good. This is what a good spectrum looks like. In contrast to Figure 1.9, the spectrum in Figure 1.10 is of low quality.

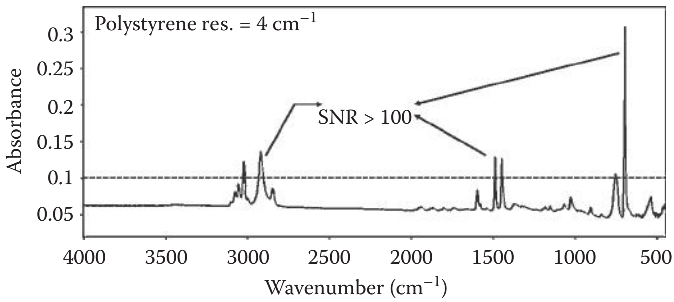

FIGURE 1.9 An example of a spectrum with a good SNR. The PPN is 0.001, so the portions of the spectrum above 0.1, as denoted by the dashed line, have a signal-to-noise ratio of greater than 100.

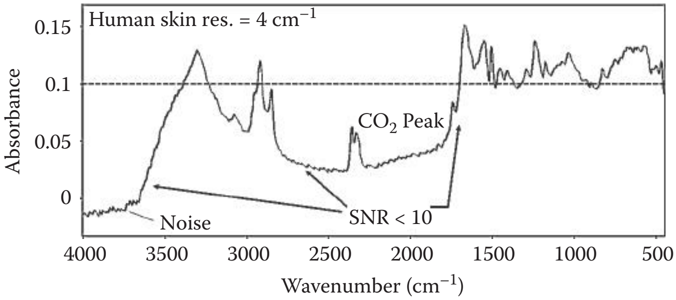

FIGURE 1.10 An example of a spectrum with a bad SNR. The PPN is 0.01 so the portions of the spectrum below 0.1, as denoted by the dashed line, have a signal-to-noise ratio of less than 10.

The spectrum in this figure (an ATR spectrum of the author's thumb) has a PPN of 0.01, so the regions of this spectrum with an absorbance of less than 0.1, as denoted by the dashed line, have an SNR of less than 10. Note there is noise clearly visible in this spectrum as "fuzz" in the baseline. Also, the spectrum has a sloping baseline and a CO_2 artifact peak near 2350 cm^{-1}. This is an example of a poor spectrum, and changes in sampling or scanning conditions should be pursued to try and improve its quality.

One of the major advantages FTIRs enjoy over other infrared spectrometers is their ability to measure spectra with high signal-to-noise ratios. SNRs of 100 or better are routinely measured by FTIRs, as seen in Figure 1.9. Why do FTIRs measure spectra with high SNRs? One reason is the *throughput* advantage of FTIR. The amount of signal in a spectrum depends upon the amount of light hitting the detector; the more light the better. Throughput is a measure of the amount of light from the source that makes it to the detector. The infrared beam in non-FTIRs spectrometers may pass through slits, prisms, and gratings that decrease the intensity of the beam and thus reduce throughput. In an FTIR, as we will see in Chapter 2, the infrared beam does not pass through a prism, grating, or slit so a high-intensity infrared beam impinges on the detector increasing the signal level.

A second advantage FTIRs enjoy compared to other infrared spectrometers is the *multiplex advantage*. It has been shown [4] that the SNR of a spectral region is proportional to the square root of the amount of time (t) spent observing the intensity of light in that region. This gives Equation 1.9.

$$\mathrm{SNR} \propto t^{1/2} \tag{1.9}$$

Where

SNR = Signal-to-Noise Ratio
t = Observation time

Observation time in FTIR is determined by the number of scans added together, and the more scans that are taken of a sample the more time the intensity can be observed. So, there is a relationship between the number of scans used to measure a spectrum (N) and observation time. Since N is proportional to time we can rewrite Equation 1.9 as follows:

$$SNR \propto N^{1/2} \tag{1.10}$$

Where

SNR = Signal-to-Noise Ratio
N = Number of scans added together to comprise a spectrum

Equation 1.10 is the practical expression of the multiplex advantage, and it tells us that adding more scans together is a way of improving the SNR of a spectrum measured with an FTIR. For example, a spectrum measured using 100 scans, the square root of which is 10, will have a ten times better SNR than a spectrum measured with only one scan. Since for many instruments measuring 100 scans takes about 2 minutes, it is worth waiting this short period of time to achieve a tenfold improvement in spectral quality. Thus, increasing the number of scans used to measure a spectrum is a convenient and straightforward way of improving spectral quality.

A third advantage FTIRs enjoy over other infrared spectrometers is wavenumber precision (remember that precision is a measure of reproducibility and is not the same thing as accuracy [2]). It is vital that the wavenumbers, and hence peak positions, in an infrared spectrum be measured reproducibly. FTIRs contain a laser that acts as an internal wavenumber standard. This allows the wavenumbers in a measured FTIR spectrum to be determined with a precision of $\pm 0.01\ cm^{-1}$. The reasons for this will be discussed in detail in Chapter 2.

Because of its advantages, an FTIR is capable of measuring spectra with an SNR 10 to 100 times better than other types of infrared spectrometers, all things being equal [4]. This is why FTIRs are the predominant type of infrared spectrometer in use in the world today. What are the practical advantages of a high SNR? There are many. First, a higher SNR increases the sensitivity of the instrument. The smaller the noise the easier it is to see small peaks, thus increasing the minimum amount of sample that can be detected. Another advantage of a high SNR? is quantitative accuracy. Recall from Equation 1.6 that the height and area of the peaks in absorbance spectra are proportional to concentration. The lower the noise level in a spectrum the more accurately peak heights and areas can be measured, leading to more accurate concentration determinations. But perhaps the biggest advantage of a high SNR is an increase in applications. Samples that were previously considered intractable using other types of spectrometers now yield usable spectra thanks to the SNR advantages of FTIR. For example, sample preparation techniques such as Attenuated Total Reflectance (ATR, see Chapter 4) are now making analysis of many samples fast and easy. Objects 8 microns in diameter and larger can now have their spectra measured thanks to the development of infrared microscopes (Chapter 6). In a potentially lifesaving development, the spectra of healthy and cancerous human cells have been measured [5]. These are just some of the numerous applications that have been made possible by the SNR advantages enjoyed by FTIRs.

TABLE 1.2
The Advantages and Disadvantages of FTIR Spectrometers

Advantages	Disadvantages
Throughput advantage	Artifacts
Multiplex advantage	
Better SNR	
Precise wavenumber measurement	

B. The Disadvantage of FTIR

Despite the virtues of FTIRs, they are not perfect instruments. FTIRs suffer from the disadvantage of *artifacts*. These are features present in the spectrum of a sample that are not from the sample. Common examples of artifacts in FTIR spectra include the water vapor and carbon dioxide peaks seen in Figure 1.7. Recall from Equations 1.5 and 1.7 that both Absorbance and % Transmittance spectra are calculated by ratioing the *background spectrum* and the sample spectrum. If the contribution of water vapor and CO_2 are the same when the background and sample spectra are measured, their contributions will ratio out, giving an artifact-free sample spectrum like the one seen in Figure 1.9. However, when using an FTIR the background and sample spectra must be measured at different points in time. If anything changes inside the instrument between when the two spectra are measured, such as a change in H_2O or carbon dioxide concentration, peaks from these gases will contaminate the sample spectrum, such as the CO_2 peak seen in Figure 1.10. When using an FTIR one must be careful not to interpret atmospheric gas peaks as being from the sample. Thus, you should familiarize yourself with the spectrum of the atmosphere as seen in Figure 1.7.

Although the artifact problem is annoying, the many advantages of FTIR make it the spectrometer of choice for analyzing most samples. Table 1.2 summarizes the advantages and disadvantages of Fourier Transform Infrared spectrometers.

VII. FTIR: THE REST OF THE STORY

Infrared spectroscopy is an excellent chemical analysis technique. However, both infrared spectroscopy and FTIR spectrometers have disadvantages and limitations as discussed above. This means infrared spectroscopy will solve many but not all chemical analysis problems. When dealing with an unknown you may be tempted to identify a sample using just its infrared spectrum. This can be dangerous because spectra can contain incomplete information or be misleading. It is always good analytical practice to gather as much information as possible about unknown samples. Note the sample's color, texture, and physical state (solid? liquid? gas? polymer?). Interrogate the person handing you the sample about where the sample came from, what chemical species might be present, and what they are trying to learn from the sample. Perform physical tests on the sample such as determining its melting point, boiling point, and tensile strength. Finally, make use of all the instruments in your lab when analyzing unknowns. Other types of spectroscopy that provide chemical information include Raman Scattering, Nuclear Magnetic Resonance (NMR), Mass Spectrometry, and UV-Visible spectroscopy. FTIRs are good for analyzing unknowns, but you will have more success if you use them in conjunction with other instrumental techniques and gather as much information as possible about your unknown samples.

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2 How an FTIR Works

The purpose of this chapter is to familiarize you with the inner workings of an FTIR and how to use this knowledge to optimize the quality of the spectra you measure. The chapter concludes with a discussion of tests you can run to insure your FTIR is working properly.

I. INTERFEROMETERS AND INTERFEROGRAMS

At the heart of every FTIR is an optical device called an *interferometer*. A diagram of an interferometer is shown in Figure 2.1.

Can interferometer or "interference meter," measures the interference pattern between two light beams. The light from an infrared source is shown entering the interferometer from the left in Figure 2.1. The interferometer splits the single light beam into two light beams. The interferometer then causes the two light beams to travel different paths, which are denoted D_1 and D_2 in Figure 2.1. After the two light beams have traveled their different paths they are recombined into one beam, and then the light beam leaves the interferometer in Figure 2.1.

Note in Figure 2.1 that the two light beams in this interferometer travel different distances; path D_1 is 4 cm long and path D_2 is 10 cm long. The *optical path difference* of an interferometer, denoted by the Greek letter small delta (δ), is the difference in distance traveled by the two light beams. For the interferometer in Figure 2.1 the optical path difference is (10 − 4 cm) = 6 cm. If the distances the two light beams travel in the interferometer happen to be identical, then $\delta = 0$ and the condition is called *zero path difference*, or ZPD for short.

There are a number of interferometer designs used by FTIR manufacturers. The oldest and perhaps the most common type of interferometer in use today is the Michelson interferometer. It is named after Albert Abraham Michelson (1852–1931) who first built his interferometer in the 1880s [1] and went on to win a Nobel Prize in Physics for the discoveries he made with it. The optical design of a Michelson interferometer is shown in Figure 2.2. Even if your FTIR does not have a Michelson interferometer in it, the following discussion will be relevant because the basics of interferometer operation are similar for all interferometer types.

The Michelson interferometer consists of four arms. The top arm in Figure 2.2 contains the infrared source and a *collimating mirror* to collect the light from the source and make its rays parallel. The bottom arm of the Michelson interferometer contains a fixed mirror, i.e., a mirror that is in a fixed position and does not move. This is in contrast to the right arm of the interferometer, which contains a moving mirror which is capable of moving left and right. The left arm of the interferometer contains the sample and detector. At the heart of the interferometer is an optical

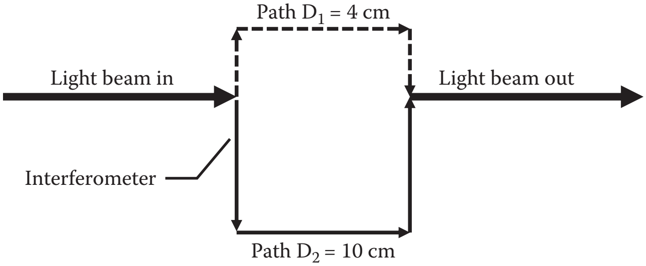

FIGURE 2.1 A simplified diagram of an interferometer.

device called a *beamsplitter*. A beamsplitter is designed to transmit some of the light incident upon it and reflect some of the light incident upon it. In Figure 2.2 the light transmitted by the beamsplitter travels toward the fixed mirror, and the light reflected by the beamsplitter travels toward the moving mirror. Once the light beams reflect from these mirrors they travel back to the beamsplitter, where they are recombined into a single light beam that leaves the interferometer, interacts with the sample, and strikes the detector.

How do the two light beams recombine into one at the beamsplitter? It is a property of waves that when superposed they will *interfere* with each other, which means their amplitudes add together to form a single wave. In a Michelson interferometer the light beams reflected from the fixed and moving mirrors interfere as follows:

$$A_f = A_1 + A_2 \tag{2.1}$$

Where

$A_f =$ Final amplitude
$A_1 =$ The amplitude of the fixed mirror beam
$A_2 =$ The amplitude of the moving mirror beam

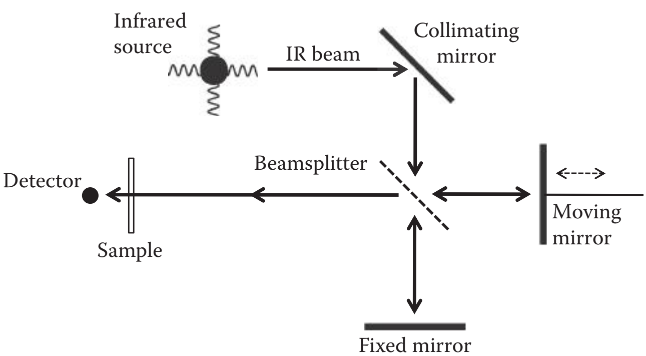

FIGURE 2.2 The optical diagram of a Michelson interferometer.

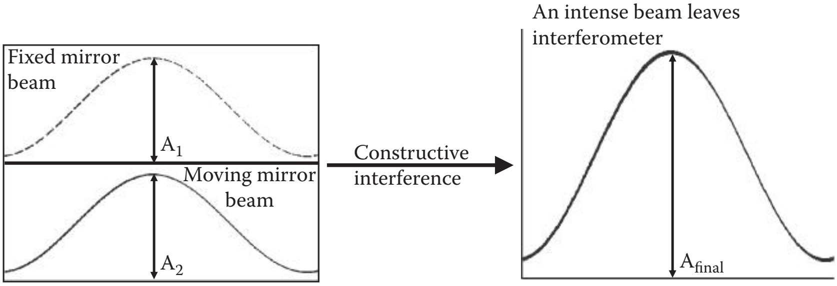

FIGURE 2.3 An example of two light beams undergoing constructive interference.

If A_f is greater than A_1 or A_2 the two beams are said to undergo *constructive interference*. If A_f is less than A_1 or A_2 *destructive interference* is said to take place. The square of the amplitude of a light beam is proportional to its intensity. For example, a candle is dim because it gives off visible light of low amplitude, and the sun is intense because it gives off visible light of high amplitude. When the two light beams in an interferometer interfere with each other, the interferometer measures their interference pattern, hence the meaning of the word interferometer.

Our goal is to understand how light interference and an interferometer produce a spectrum. To reach that goal it is necessary to make two simplifying assumptions. First, we will assume that the infrared source in Figure 2.2 gives off only one wavelength of light, λ. In reality, the infrared source in an FTIR gives off light composed of many wavelengths. Second, we will assume for the moment that the interferometer is at zero path difference, as shown in Figure 2.2. What will it look like when the two light beams of wavelength λ interfere when they reach the beamsplitter? This is illustrated in Figure 2.3.

The light beam reflected from the fixed mirror and the light beam reflected from the moving mirror travel the same distance at the same speed (remember that the speed of light is a constant). Thus, when the two beams recombine at the beamsplitter their crests overlap, as seen in Figure 2.3. These two light beams are said to be *in phase* with each other, and they constructively interfere, giving a final beam more intense than either of the two beams by themselves as seen in figure 2.3. These two light beams will be in phase with each other when their optical path difference is a multiple of their wavelength, as follows:

$$\delta = n\lambda \tag{2.2}$$

Where

$\delta =$ Optical path difference
$\lambda =$ Wavelength
$n =$ 0, 1, 2, … (any integer)

The n = 0 value corresponds to zero path difference and gives the picture seen in Figure 2.3. The n = 1 value corresponds to the light beams being exactly one

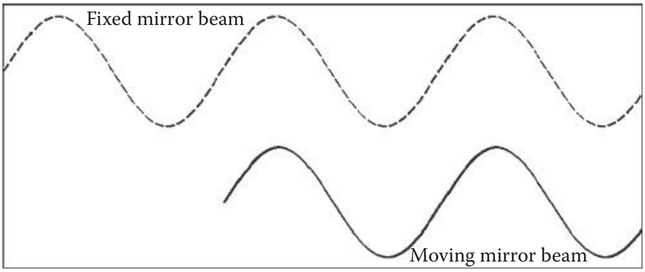

FIGURE 2.4 Two light beams constructively interfering when they are exactly 1 cycle out of phase with each other.

cycle out of phase as shown in Figure 2.4. The n = 2 value corresponds to the beams being two cycles out of phase, and so on. As long as the two beams are a whole number of cycles out of phase with each other their crests and troughs will overlap and constructive interference will take place. For example, if $\lambda = 10\mu$ then constructive interference will take place at optical path differences of 0μ, 10μ, and 20μ, etc.

What happens if we translate the moving mirror and introduce a non-zero optical path difference between the two light beams? The distance the mirror moves in an interferometer is called the *mirror displacement*, and it is denoted with the Greek letter capital delta (Δ). A mirror translation of Δ gives an optical path difference of 2Δ because the light traverses the displaced distance twice on the way to and from the moving mirror. For example, in a Michelson interferometer a mirror displacement of 1 cm gives an optical path difference of 2 cm as quantified in Equation 2.3 and shown in Figure 2.5.

$$\delta = 2\Delta \tag{2.3}$$

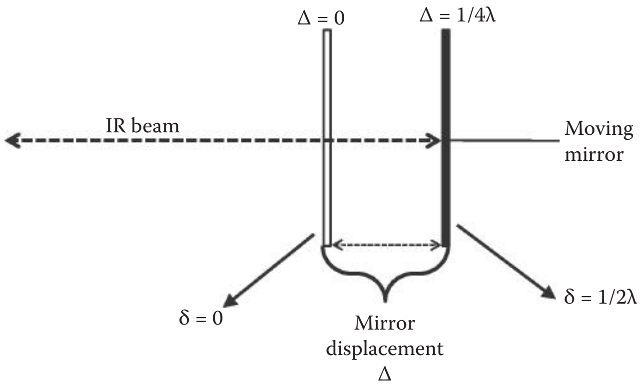

FIGURE 2.5 An illustration of how moving the mirror in a Michelson interferometer produces a non-zero optical path difference. Note that optical path difference is twice mirror displacement because the light beam traverses the extra distance twice.

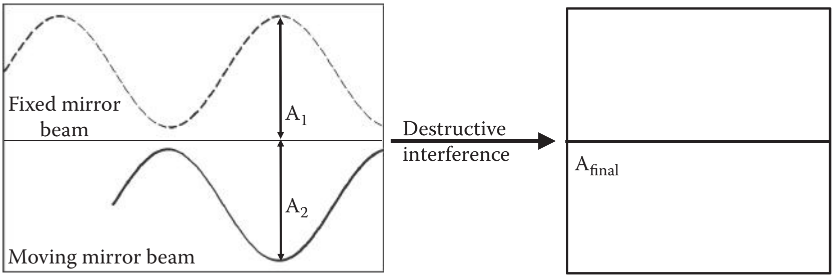

FIGURE 2.6 An example of two light beams that are 1/2 cycle out of phase with each other and undergo destructive interference.

Where

$\delta =$ Optical path difference
$\Delta =$ Mirror displacement

If $\delta = 1/2\lambda$, the interference pattern illustrated in Figure 2.6 is obtained.

Since we have done nothing to the path of the fixed mirror light beam it is unchanged and looks the same in Figure 2.6 as it does in Figure 2.3. However, the moving mirror light beam has traveled a distance equivalent to half a wavelength farther and is now 1/2 cycle out of phase with the fixed mirror beam; the crest of one beam now overlaps with the trough of another. When two out-of-phase beams interfere with each other, their amplitudes will cancel and the final amplitude will be less than the amplitude of either beam by itself, which is an example of destructive interference. By moving the mirror a fraction of a wavelength in distance, there has been a large change in the intensity of the beam leaving the interferometer.

The constructive interference illustrated in Figure 2.3 occurs whenever the two beams are a whole number of cycles out of phase. Conversely, the destructive interference illustrated in Figure 2.6 occurs when the two beams are some number of cycles plus a half out of phase with each other. Thus, we can write for destructive interference that

$$\delta = (n + 1/2)\lambda \tag{2.4}$$

Where

$\delta =$ Optical path difference
$\lambda =$ Wavelength
$n =$ 0, 1, 2, … (any integer)

If $\lambda = 10\mu$ then destructive interference will take place at optical path differences of 5, 15, and 25μ, etc.

We have seen how moving the mirror in a Michelson interferometer a small distance affects the intensity of the beam exiting the interferometer. Now, what would happen to the intensity of this beam if we moved the mirror a greater distance away from the beamsplitter? We know at zero path difference the intensity is large because the fixed mirror and moving mirror beams are in phase and constructive interference takes place. As we gradually move the mirror away from the beamsplitter, these beams grow out of phase with each other and the resultant beam becomes dimmer due to destructive interference. At $\delta = 1/2\lambda$ the resultant beam intensity passes through a minimum as dictated by Equation 2.4. As the mirror continues to move, the beams become more in phase and the resultant beam gains in intensity until it passes through a maximum at $\delta = \lambda$ as dictated by Equation 2.2. As the mirror continues to move, the resultant beam intensity grows dimmer and brighter as it passes through maxima and minima as determined by Equations 2.2 and 2.4. If the measured light intensity versus optical path difference is plotted, the graph in Figure 2.7 is obtained. A plot of light intensity versus optical path difference is called an *interferogram*, which means "interference writing."

An interferogram is the fundamental measurement obtained by an FTIR. Note that the shape of the interferogram in Figure 2.7 is a cosine wave, and although it looks like a light wave the interferogram is the electrical signal coming out of the detector. This is why the y-axis units in Figure 2.7 are in voltage. Remember, we have made the simplifying assumption here that there is only one wavelength of light passing through the interferometer. Thus Figure 2.7 represents the interferogram of

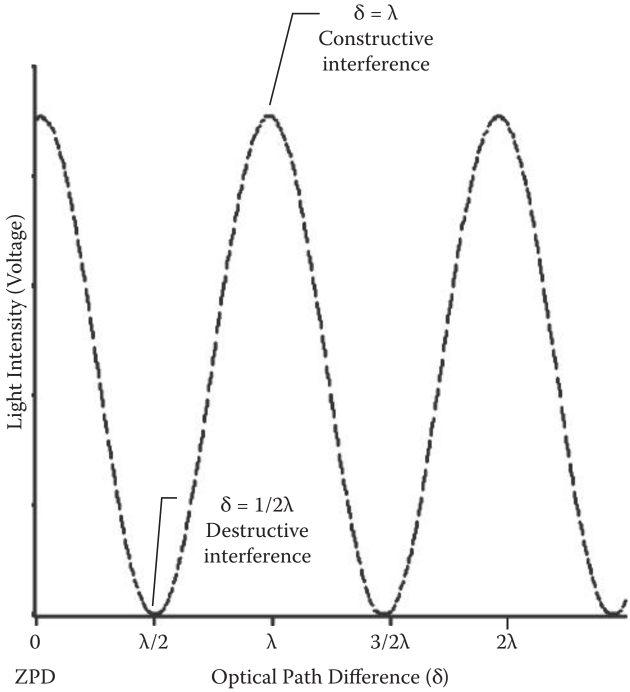

FIGURE 2.7 A plot of light intensity (or detector signal) versus optical path difference for a mirror moving away from the beamsplitter in a Michelson interferometer. Such a plot is called an *interferogram*. This is the interferogram for a single wavelength of light passing through the interferometer.

a single wavelength of light. A laser gives off individual wavelengths of light, and so Figure 2.7 represents the interferogram of a laser line.

To measure an interferogram using a Michelson interferometer the mirror is moved back and forth once. This is called a *scan*. The interferograms measured while scanning are Fourier transformed to yield a spectrum, hence the term *Fourier Transform Infrared* (FTIR) spectroscopy.

Remember that in real life all the wavelengths of light given off by the source pass together through the interferometer. To come slightly closer to that picture, let's now assume that light of wavelengths λ and 3λ pass through the interferometer together. What does the interferogram of 3λ light look like? Recall that Equations 2.2 and 2.4 gave the locations of the maxima and minima of the interferogram seen in Figure 2.7. If we substitute 3λ for λ in these equations we find that the maxima in the interferogram for 3λ light fall at

$$\delta = 3n\lambda \tag{2.5}$$

and that the minima in the interferogram for 3λ light are given by

$$\delta = 3(n + 1/2)\lambda$$

Where

$\delta =$ Optical path difference
$n =$ 0, 1, 2, …
$\lambda =$ Wavelength

A plot of the interferogram of 3λ light is seen in Figure 2.8.

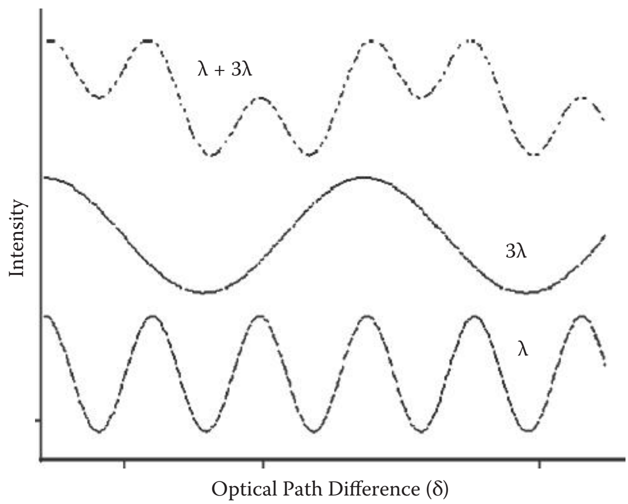

FIGURE 2.8 The interferograms of λ light, 3λ light, and their sum.

The wavelengths λ and 3λ have different equations governing where the maxima and minima in their interferograms fall; hence their interferograms are different. We learned in Chapter 1 that one property of light waves is their frequency, which is measured in cycles/second or Hertz. An infrared detector measures an interferogram as an electrical signal, and these signals have the shape of a cosine wave as seen in Figure 2.8. These electrical signals have frequencies that can also be measured in units of Hertz. The reason the interferograms of λ and 3λ light seen in Figure 2.8 are different is because they have *different frequencies*. There is a relationship between the frequency of a light wave and the frequency of its corresponding interferogram as follows [2]:

$$F = (2\nu v)/c \tag{2.6}$$

Where

- $F =$ *Fourier Frequency* of the interferogram in Hertz
- $\nu =$ Frequency of the light wave in Hertz
- $v =$ Moving mirror velocity (assumed constant) in cm/sec
- $c =$ The speed of light in cm/sec

The quantity F is sometimes called the *Fourier frequency* of an interferogram. Using Equation 1.4 we can rewrite Equation 2.6 as

$$F = 2vW \tag{2.7}$$

Where

- $F =$ Frequency of the interferogram in Hertz
- $v =$ Moving mirror velocity (assumed constant) in cm/sec
- $W =$ Wavenumber in cm^{-1}

and rearranging obtain

$$W = F/2v$$

Equation 2.6 may seem surprising at first, but its units do make sense. If the moving mirror velocity is measured in cm/sec, the velocity of light is measured in cm/sec, and the frequency of the light is measured in Hertz, the units of the two velocities cancel, giving the correct units of Hertz. The beauty of Equation 2.6 is that it gives a direct relationship between the frequency of a light wave and the Fourier frequency of its corresponding interferogram. Additionally, Equation 2.7 shows that for each different wavenumber of light a different frequency interferogram exists. Thus the different wavenumbers of light do not have to be separated in time or space to be distinguished.

An infrared spectrum contains two pieces of information, light intensity and wavenumber. Since the spectrum is calculated from the interferogram, the intensity and wavenumber information are present in the interferogram. The Fourier frequencies present in an interferogram tell us what wavenumbers of light are present in a given light beam thanks to Equation 2.7. The amplitude of a Fourier frequency is proportional to the amount of light hitting the detector at that wavenumber. Imagine if the interferogram for wavelength λ seen in the bottom of Figure 2.8 were measured with no sample present. If we put a sample in the infrared beam that happens to absorb at wavelength λ, the amplitude of this interferogram will go down. This reduction in intensity, upon Fourier transformation, gives rise to a peak in the absorption spectrum of the sample.

In the real world all the wavenumbers of light given off by the source pass through the interferometer together. For each of these wavenumbers there exists an interferogram with a unique Fourier frequency. What the detector actually measures is the sum of these individual interferograms. For λ and 3λ light passing together through an interferometer, the measured interferogram is the sum of their interferograms as seen on the top of Figure 2.9. For a broadband infrared source that gives off light many wavenumbers the resultant interferogram is shown in Figure 2.9.

There is a large burst of infrared intensity at zero path difference, called the *centerburst*, because all wavelengths of light are in-phase and constructively interfere at ZPD. The size of the centerburst is proportional to the total amount of infrared light striking the detector. Note in Figure 2.9 that as the optical path difference changes the infrared intensity falls off quickly on both sides of the interferogram. The low intensity regions of an interferogram are called its *wings*. The wings have low intensity because as optical path difference increases the various Fourier frequencies fall increasingly out of phase with each other and destructively interfere [3].

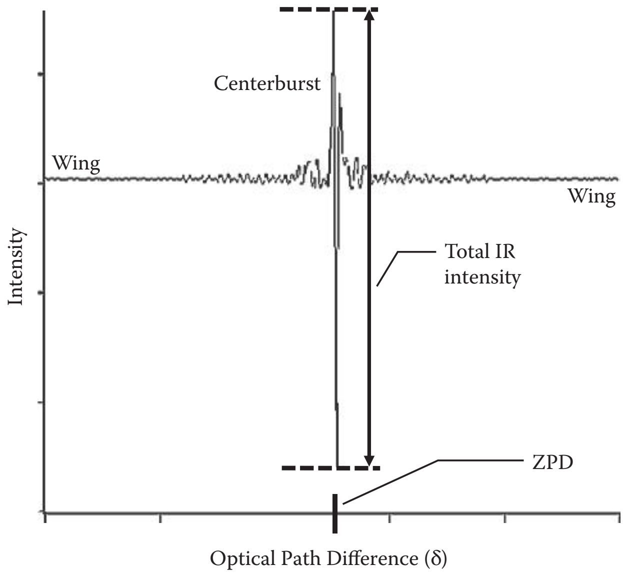

FIGURE 2.9 A real-world interferogram obtained when many wavenumbers of light pass through the interferometer together.

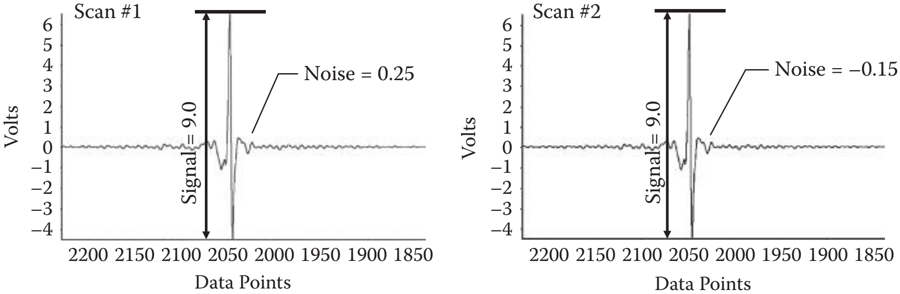

FIGURE 2.10 Left: An interferogram measured with a signal of 9.0 and a noise level of 0.25. Right: An interferogram measured a moment later with a signal of 9.01 and a noise level of −0.15. Note that the sign of the noise has changed while the sign of the signal has not.

A. How Many Scans Should Be Used?

The number of scans used to measure a spectrum is easily adjusted by simply typing the desired number in the correct field of the FTIR software program being used to run the instrument. If 32 scans are chosen, the scanning mirror will be moved back and forth 32 times, 32 separate interferograms will be measured, and then averaged together in a process called *signal averaging*. Typically, the averaged interferogram is sent to the computer where the FTIR software program performs a Fourier transform on it to obtain a spectrum.

Equation 1.10 states that the signal-to-noise ratio in a spectrum is proportional to the square root of the number of scans added together. We are now in position to understand why this relationship is true. Imagine that for scan no. 1 of a sample the signal in the interferogram has a value of 9.0 units and the noise a value of 0.25 units, as shown in Figure 2.10.

Now, the sign of signal is always considered to be positive, but the sign of noise is random and fluctuates between positive and negative. When scan no. 2 is measured, the signal still has a value of 9.0, but the noise now has a value of −0.15, as illustrated in Figure 2.10. When the two scans are averaged the noise levels are averaged as well, giving a final noise level of $[(0.25) + (-0.15)]/2 = 0.05$. The noise cancels itself because its sign fluctuates. The signal does not cancel because its sign is constant. As more scans are added together the noise cancels itself to a greater extent according to Equation 1.10.

Figure 2.11 illustrates that the reduction in noise level with number of scans is real.

Note in Figure 2.11 how the noise level in the bottom spectrum for 100 scans is less than the noise level in the top spectrum for 1 scan. Both spectra were plotted with the same y-axis scale, so comparison is legitimate. Table 2.1 illustrates the expected improvement in SNR for different numbers of scans compared to 1 scan. Note that the right hand column in Table 2.1 is the square root of the left hand column. Figure 2.12 graphically illustrates how using experimental data the relationship between SNR and $N^{1/2}$ is linear as expected.

The improvement of SNR with the number of measurements added together is not isolated to FTIR. All measurements of any quantity have a signal, a noise level, and

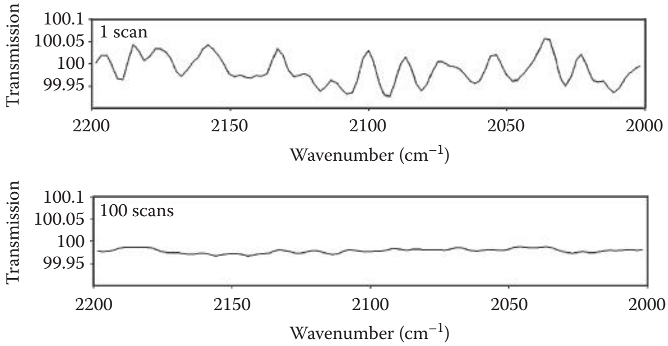

FIGURE 2.11 Top: The noise level in an infrared spectrum between 2200 and 2000 cm^{-1} after 1 scan. Bottom: The noise level in an infrared spectrum between 2200 and 2000 cm^{-1} after 100 scans. The y-axis scales in the two spectra are identical. Note the reduction in noise for 100 scans versus 1 scan.

a signal-to-noise ratio (SNR). Equation 1.10 applies to measurements of any quantity and N is the number of measurements added together. Scientists frequently make multiple measurements of the same quantity, add the results together, and average them because the average will have a better SNR than any individual measurement. So, an FTIR spectrum where more than 1 scan is used is the average spectrum for the period of time spent scanning.

The number of scans is perhaps the most important user-adjustable scanning parameter in FTIR, which begs the question, "How many scans should I use?" Ultimately, it depends on your sample. For routine samples that are analyzed in the FTIR sample compartment using sample preparation techniques such as ATR, KBr pellets, and capillary thin films (more on these in Chapter 4), anywhere between 10 and 100 scans will typically yield a spectrum with a good SNR. For example, if 16 scans give a poor spectrum, by all means more scans should be tried. On the other

TABLE 2.1
The Noise Reduction Expected for a Given Number of Scans Compared to the Noise Level in a Spectrum Measured with One Scan

No. of Scans	Noise Reduction
1	—
4	2
16	4
64	8
100	10
256	16
1000	31.6

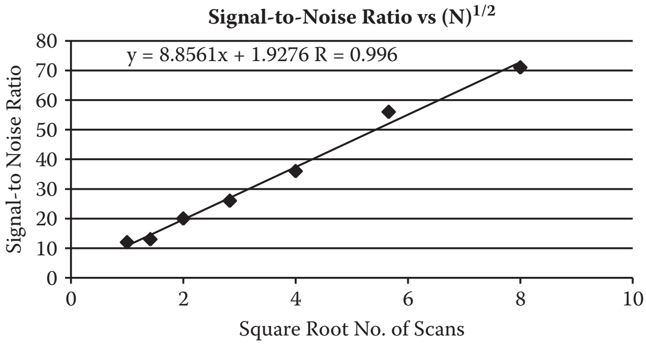

FIGURE 2.12 A plot of the signal-to-noise ratio for a spectrum versus the square root of the number of scans used to measure that spectrum. Note that as the square root of the number of scans increases the signal-to-noise ratio increases.

hand if 16 scans gives a good SNR (say ≥100) then there is probably no need to do more scans. Difficult samples may have a poor SNR because they are too small, too thin, too dilute, or may scatter the infrared beam, preventing much of the light from reaching the detector. For these samples, 100 to 1000 scans can be tried. Note that in general using more than 1000 scans for any sample is not recommended. On most FTIRs 1000 or more scans can take 20 or more minutes, which is a significant investment in time. Despite Equation 1.10, for every FTIR there is some number of scans beyond which there is no further improvement in noise level. This has to do with limitations of the spectrometer electronics explained in ref. [2]. It is the author's experience that for FTIRs, after 1000 scans, there is no further improvement in noise level, so it does not make sense to scan longer if no spectral improvement is obtained.

Since increasing the number of scans can improve spectral quality, in general the user of the instrument should be allowed to adjust this parameter to optimize the spectrum of each sample. Thus, when writing a standard operating procedure it would be best to recommend a range for the number of scans or include a suggested value, but leave the instrument operator free to alter the number of scans as needed. You may have noticed that many FTIR operators routinely use a number of scans that is a multiple of 2, such as 16 or 32 scans. There is no magic in these numbers. In most cases there is no reason why 15 or 33 scans could not be used instead. It's just that these multiples of 2 are small, round numbers that are easy to remember.

II. HOW AN INTERFEROGRAM BECOMES A SPECTRUM

As about mentioned, an interferogram is Fourier transformed to yield a spectrum. However, we have said nothing about how the Fourier transform (FT) works. A detailed discussion of the mathematics of the FT and its use in spectroscopy is available in ref. [4]. Here we will use words and pictures to give the reader a feeling for the qualitative aspects of how a Fourier transform works.

The FT in FTIR stands for *Fourier Transform*, which is named in honor of the French mathematician Joseph Fourier (1768–1830). Fourier's theorem states that many mathematical functions (which are simply x, y plots) can be expressed as a superposition of sine and cosine waves. The mathematical calculation known as the Fourier transform can be used to calculate the superposition of sine and cosine waves for a given function and the function that corresponds to a superposition given of sine and cosine waves. Recall from above that an interferogram is a superposition of cosine waves. Hence when we Fourier transform an interferogram a mathematical function is obtained that corresponds to the interferogram. This function is the spectrum of the infrared beam that hits the detector. By passing the light through the interferometer the spectrum of the infrared beam is optically transformed into an interference pattern (the interferogram). The infrared detector measures this interference pattern, and the Fourier transform turns it back into a spectrum. The net effect of placing a sample into the infrared beam is to simply alter the interference pattern.

To understand the relationship between the spectrum of a light beam and its interferogram it is best to start by examining the spectrum of a simple light source such as a laser. An idealized laser has high intensity at a specific wavenumber and little or none at other wavenumbers so its spectrum is a line as illustrated in the right-hand side of Figure 2.13.

From above we know that the interferogram of a specific wavenumber of light is a cosine wave. A laser line comes dose to this ideal and its interferogram is a cosine wave, as illustrated in the left-hand side of Figure 2.13. Thus, the Fourier transform of a line is a cosine wave, and the Fourier transform of a cosine wave is a line. There really is a relationship between the wiggles in an interferogram and the peaks in an infrared spectrum.

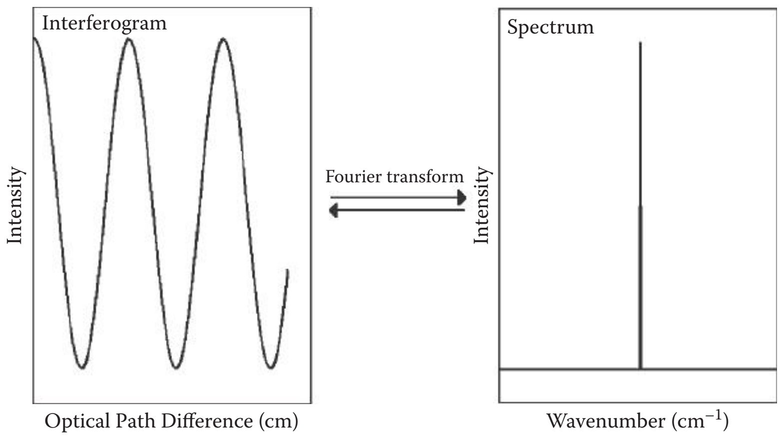

FIGURE 2.13 An illustration of the relationship between the spectrum of an idealized laser line and its interferogram. The Fourier transform of a line is a cosine wave, and the Fourier transform of a cosine wave is a line. Note that the x-axis units of the interferogram are transformed from cm to cm^{-1} when the spectrum is calculated.

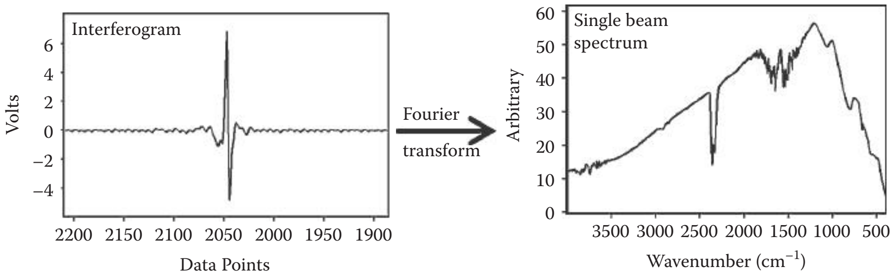

FIGURE 2.14 When an interferogram is Fourier transformed it produces a single beam spectrum.

A better understanding of the relationship between interferograms and spectra can be obtained by considering their units. When a Fourier transform is applied to a function one of the things it does is invert the x-axis units of that function. Recall that an interferogram is a plot of infrared intensity versus optical path difference, which can be measured in centimeters as shown in Figure 2.13. When an interferogram is Fourier transformed we obtain a function that is a plot of infrared intensity versus cm^{-1}. The units of wavenumber are cm^{-1}, and a plot of infrared intensity versus wavenumber is a spectrum as shown in Figure 2.13. Again, there is a very real relationship between the information in the interferogram and the information in a spectrum.

There are several steps between the measurement of the interferogram and the final absorbance or transmittance spectrum we normally interpret. The Fourier transform of any interferogram yields a *single beam spectrum* as illustrated in Figure 2.14. A single beam spectrum is a plot of arbitrary infrared intensity versus wavenumber. The term "single beam" is from the fact that there is only one infrared beam used in an FTIR; some other types of infrared spectrometer use two beams. If interferograms are measured with no sample present, are added together, and then Fourier transformed, a single beam spectrum called a *background spectrum* is obtained. An example of a background spectrum is shown in Figure 2.15.

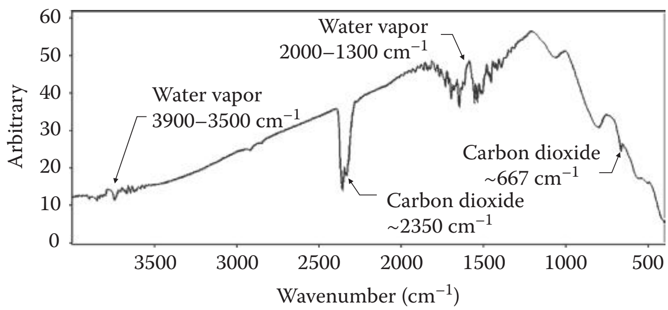

FIGURE 2.15 An example of a background spectrum, a single beam spectrum measured with no sample present.

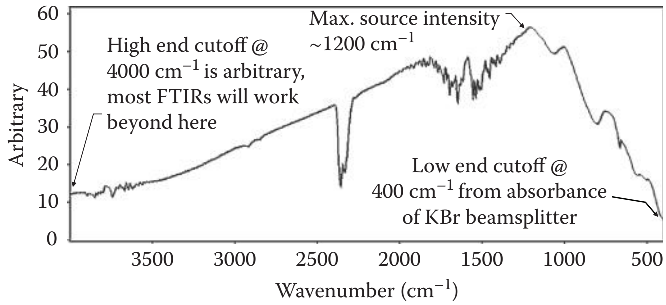

FIGURE 2.16 A typical background spectrum with the contributions of the instrument response function noted.

The background spectrum includes spectral contributions from the instrument and the environment. Much of the environmental contribution is due to infrared absorbing atmospheric gases such as water vapor and carbon dioxide, whose peaks are indicated in Figure 2.15. The contribution of the instrument to the spectrum is called the *instrument response function*. Portions of the instrument response function are noted in the background spectrum shown in Figure 2.16. All of the optical elements in the FTIR, including the source, beamsplitter, mirrors, and detector, contribute to the instrument response function. The overall shape of the background spectrum is determined by the spectrum of the source, which in turn is determined by its temperature. The low wavenumber cutoff at 400 cm^{-1} is due to absorbance by the KBr windows in the beamsplitter. Most infrared spectra are measured with a high wavenumber cutoff of 4000 cm^{-1}. Many FTIRs work at wavenumbers higher than this, but there are few strong fundamental absorbances of interest above this wavenumber, which is why most spectra are not plotted above 4000 cm^{-1}.

Typically, once the measurement of the background spectrum is complete, the sample is placed in the infrared beam. Interferograms are measured with the sample present, are added together, and then Fourier transformed to obtain the *sample single beam spectrum*, an example of which is shown in Figure 2.17. A sample single beam spectrum contains contributions from the environment, instrument, and sample. Note

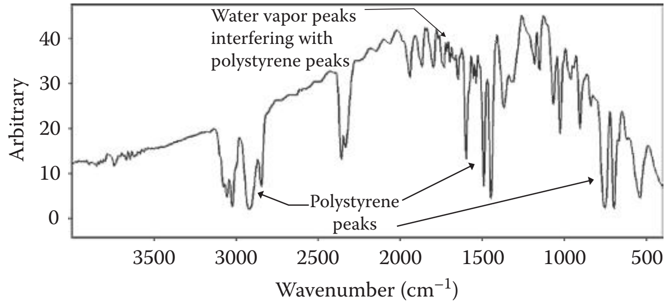

FIGURE 2.17 The sample single beam spectrum of polystyrene.

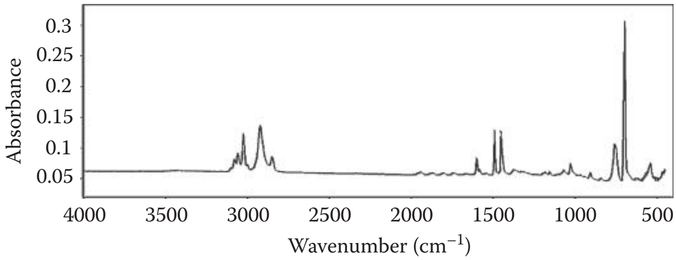

FIGURE 2.18 The absorbance spectrum of polystyrene. Note the lack of artifacts and an instrument response function.

in Figure 2.17 the instrument response function is present, water vapor and carbon dioxide peaks are present, and there are also sample peaks from polystyrene. A sample single beam spectrum is measured every time a sample is analyzed using an FTIR, even though it might not be displayed. In theory, this spectrum could be interpreted because it contains sample information. However, it is a low-quality spectrum because of the curvature in the baseline and the interference of water vapor with the spectrum of polystyrene around 1600 cm^{-1}.

Ideally, we want to obtain a spectrum that contains sample information only as we are generally not interested in the spectrum of the instrument or the environment. But how do we do this? Recall from Equations 1.5 and 1.7 that calculating an absorbance spectrum or %T spectrum involves ratioing the background spectrum and the sample single beam spectrum. If the contribution of the instrument and the environment is the same to the sample single beam and background spectra, these will cancel when the ratio is calculated, leaving just the sample spectrum behind. For the instrument contribution to be held the same the scanning parameters must be identical, including the number of scans and the resolution. The only difference between the measurement of the background and sample spectra should be the presence of the sample for the latter.

An example of an absorbance spectrum with the instrument and environment contributions ratioed out can be seen in Figure 2.18, which is the absorbance spectrum of polystyrene. The spectrum in Figure 2.18 was calculated using the background spectrum shown in Figure 2.16 and the sample single beam spectrum shown in Figure 2.17. Note in Figure 2.18 that the curved baseline from the instrument response function and the water vapor and carbon dioxide peaks are gone. This means the instrument and environment contributions were the same for the sample and background spectra, and were ratioed out. The result is a spectrum with a flat baseline, a good signal-to-noise ratio, and a wealth of sample information. If you ever need an example of what a good infrared spectrum looks like, keep Figure 2.18 in mind.

A. Scanning Advice

As many FTIR users are well aware, water vapor and carbon dioxide peaks can appear in FTIR sample spectra and interfere with sample peaks. This is because when using an FTIR the background and sample spectra must be measured at different points in time. If the concentration of water vapor and CO_2 changes inside the instrument between these measurements, their peaks will not ratio out and will appear as artifacts in the sample spectrum. For the sample spectrum to be free of water vapor and CO_2, their concentrations must be identical when the background and sample spectra are measured.

How is this insured? Many instruments can be purged with gases such as dry nitrogen. The flow of purge gas through the instrument reduces the concentration of water vapor and carbon dioxide. Additionally, the subsequent reduction in humidity helps to preserve sensitive optical parts such as the KBr windows used in beamsplitters. Many instruments currently manufactured have sealed and desiccated interferometers. In these instruments the interferometer has a cover over it and a seal isolating it from the atmosphere. A desiccant pack is contained under the cover that removes water vapor and CO_2 from the atmosphere around the interferometer. This again helps reduce the concentration of water vapor and CO_2 and helps to protect the beamsplitter.

Despite all this, if water vapor and CO_2 peaks are still a problem with your FTIR there are two more things to try. One is simply measuring background spectra as close in time as possible to sample spectra. The longer the time between background and sample spectra the higher the probability something will change in the atmosphere or the environment, and the higher the probability that artifacts will occur. Another thing to try is to be reproducible opening and closing the sample compartment. To do this, open the sample compartment for a few moments and then close it. Wait a period of time, perhaps one minute, and then run the background spectrum. Place the sample in the sample compartment, close it, and then wait the same amount of time as before, and then measure the sample spectrum. By reproducibly opening and closing the sample compartment you will hopefully reproduce the concentrations of water vapor and CO_2 well enough so that their contributions will ratio out. This routine need not be followed for every sample, but it should certainly be tried if water vapor and CO_2 peaks are a problem for you.

Occasionally the water vapor and CO_2 can be seen pointing the "wrong way" in a spectrum, as illustrated in Figure 2.19.

Note that the y-axis scale says absorbance units but the peaks point down. This "negative absorbance" is observed when atmospheric gas concentrations *decrease*

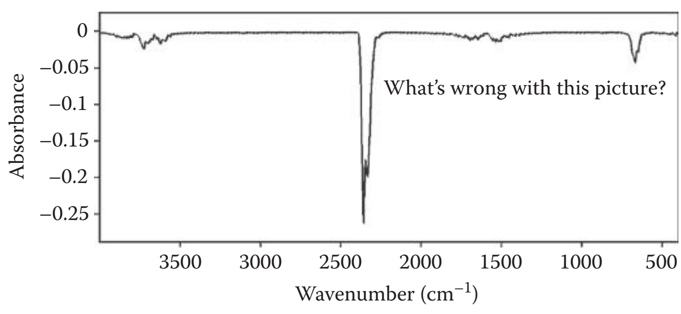

FIGURE 2.19 Atmospheric water vapor and carbon dioxide peaks showing "negative absorbance" caused by a reduction in their concentrations between when the background and sample spectra were measured.

between the measurement of the background and sample spectra. This is expected since these features would be pointing up if the atmospheric gas concentrations increased between the measurement of the background and sample spectra. To solve this problem, try measuring fresh background and sample spectra, minimizing the time between the two.

III. INSTRUMENTAL RESOLUTION

The *resolution* of a spectrometer is a measure of how well an instrument distinguishes peaks that are close together. For example, if a sample has peaks at 2999 and 3000 cm^{-1} and the two peaks are well separated in the sample's spectrum, we can say the instrumental resolution used to measure the spectrum was 1 cm^{-1}. This is considered "high resolution." On the other hand, if the instrumental resolution were changed so only peaks 32 cm^{-1} apart could be distinguished we would say the instrumental resolution used to measure the spectrum was 32 cm^{-1}. This is considered "low resolution." The confusing part of this is that high resolution is denoted by small numbers and low resolution is denoted by large numbers.

The effect of instrumental resolution on spectral appearance is dramatically illustrated in Figure 2.20. The top spectrum in Figure 2.20 is of water vapor measured at 0.5 cm^{-1} resolution. Note the series of sharp, well-resolved peaks. The bottom spectrum is also of water vapor but was measured at 32 cm^{-1} resolution. Note that there is a big, fat blob instead of a series of well-resolved peaks. The bottom spectrum was not measured with sufficient resolution to distinguish all the peaks in the water vapor spectrum, so the individual peaks merge together to form the broad feature seen. The top spectrum was measured with sufficient resolution to distinguish the peaks present in the spectrum of water vapor. These two spectra are of the same material but have radically different appearances because of the difference in instrumental resolution used. Also, note that the number of peaks in the high-resolution spectrum is greater than in the low-resolution spectrum. Since the peaks in a sample's spectrum are information, a spectrum with more peaks has more information in it than a spectrum with fewer peaks. Thus, in general, the higher the resolution the higher the information content of a spectrum.

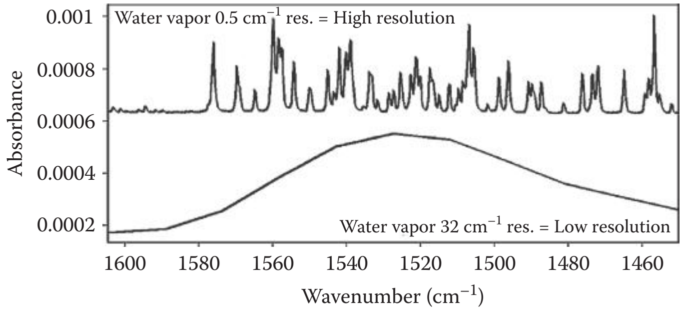

FIGURE 2.20 Top: The spectrum of water vapor measured at 0.5 cm^{-1} resolution. Bottom: The spectrum of water vapor measured at 32 cm^{-1} resolution.

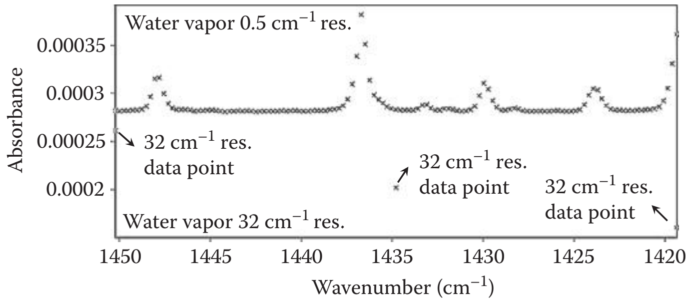

FIGURE 2.21 Top: Part of the infrared spectrum of water vapor measured at 0.5 cm^{-1} resolution. Each x represents an individual data point in the spectrum. There are over 100 data points in this spectrum. Bottom: Part of the infrared spectrum of water vapor measured at 32 cm^{-1} resolution. The three data points in this region have been labeled for clarity.

A. What Determines Resolution in an FTIR Scan?

During an FTIR scan, as the moving mirror is translated the intensity of the interferogram is measured by the infrared detector at discrete optical path difference intervals. In many instruments this is approximately every 0.6 microns. This gives an interferogram that consists of discrete data points, and the spectra calculated from them consist of discrete data points as well, as shown in Figure 2.21.

The top of Figure 2.21 shows part of the spectrum of water vapor measured at 0.5 cm^{-1} resolution. Each x represents an individual data point, and there are over 100 data points shown. The peaks present are well resolved because the data points are close enough together to capture the width of the peaks. The bottom of Figure 2.21 shows part of the spectrum of water vapor measured at 32 cm^{-1} resolution. The three data points present are labeled for clarity, and there are no peaks seen because they fall between the measured data points.

Since interferogram data points are obtained at evenly spaced intervals, high-resolution scans require more data points and hence greater optical path differences than low-resolution scans. Thus there is a relationship between resolution and optical path difference as follows:

$$\text{Resolution} \propto 1/\delta \tag{2.8}$$

Where

$\delta =$ Maximum Optical path difference for a scan

Equation 2.8 takes the form of a reciprocal because high resolution is denoted by small numbers and low resolution by large numbers.

When one types the desired instrumental resolution into the FTIR software, the instrument determines the maximum optical path difference to use in the measurement by rearranging Equation 2.8 as follows:

$$\delta \propto 1/\text{Resolution} \tag{2.9}$$

TABLE 2.2
The Relationship between Instrumental Resolution, Optical Path Difference, and Mirror Translation for a Michelson Interferometer

Resolution in cm^{-1}	δ in cm	Δ in cm
8	0.125	0.0625
4	0.25	0.125
2	0.5	0.25
1	1.0	0.5
0.5	2.0	1.0

According to Equation 2.9 a 4-cm^{-1} resolution spectrum requires an optical path difference of 1/4 cm, an 8-cm^{-1} resolution spectrum requires an optical path difference of 1/8 of a cm, and so on. Equation 2.3 shows that for a Michelson interferometer optical path difference is twice mirror translation. For example, if the maximum mirror translation during a scan is 1/8 cm the optical path difference is giving 1/4 cm and hence a 4-cm^{-1} resolution spectrum. Table 2.2 shows the relationship between instrumental resolution, optical path difference, and mirror translation for a number of common resolution settings.

Note in Table 2.2 that the middle column is the inverse of the left-hand column, and that the right-hand column is half the middle column. Using Equations 2.9 and 2.3 you can calculate the optical path difference and mirror translation needed to measure a spectrum at any resolution. Also note in Table 2.2 that for instrumental resolution settings typically used in FTIR, the amount of mirror translation is not large, just fractions of a centimeter. This means interferometers do not need to be large to achieve reasonable resolutions.

In theory, one can easily measure high-resolution spectra with an FTIR by moving the mirror further. Since high-resolution scans contain more information than low-resolution scans, this would argue in favor of measuring the spectra of all samples at the highest resolution possible. Unfortunately, this is not the case. High-resolution scans are proportionally noisier than low-resolution scans, as given by Equation 2.10.

$$\text{SNR} \propto \text{Resolution} \tag{2.10}$$

Where

SNR = Signal-to-noise ratio

Recall that high resolution is denoted by small numbers, so according to Equation 2.10 high-resolution spectra are inherently noisy. Conversely, low resolution is denoted by large numbers, so these spectra generally have good SNRs. Equation 2.10 is a consequence of the shape of interferograms like the one shown in Figure 2.9. A low-resolution interferogram with relatively few data points is measured around the centerburst. The SNR near the centerburst is large because there is a lot of infrared light hitting the detector there. This is why low-resolution spectra have good SNRs. On the other hand, a high-resolution interferogram with many data points measures the centerburst and the wings of the interferogram. The wings are light starved so the SNR there is poor; hence high-resolution spectra are noisy.

B. What Resolution Should Be Used?

The trade-off between resolution and noise level leaves us choosing between the lesser of two evils—less information and low noise or much information and high noise. This raises the question, "What instrumental resolution should I use?" The physical state of your sample will answer this question. Because in solids and liquids their molecules are packed closely together, their infrared bands are 10 cm^{-1} wide or more. Thus, it takes a resolution setting somewhat higher than 10 cm^{-1} to resolve most of the peaks in the spectra of many solids and liquids. As a result, resolutions of 8 cm^{-1} and 4 cm^{-1} are generally used to measure the spectra of solids and liquids. The effect of measuring the spectrum of a liquid at too high a resolution is shown in Figure 2.22.

The top spectrum in Figure 2.22 is of liquid water measured at 8 cm^{-1} resolution. Note that there is one peak over 100 cm^{-1} wide. The bottom spectrum in Figure 2.22 is of liquid water measured at 1 cm^{-1} resolution. The two spectra both show only one broad peak since this is the only feature present in this region of water's spectrum. This means the two spectra have the same information content. Note, however, how much noisier the 1 cm^{-1} spectrum is, a direct result of Equation 2.10. The 1-cm^{-1} resolution spectrum has no more information but a lot more noise than the 8-cm^{-1} resolution spectrum. In this case it makes no sense to use the higher resolution setting, and in general for solids and liquids it does not make sense to use high resolution.

The spectra of gases are inherently different than those of liquids and solids and typically require a higher resolution setting. As can be seen in the top spectrum of Figure 2.20, the spectrum of water vapor consists of a series of sharp lines, due in

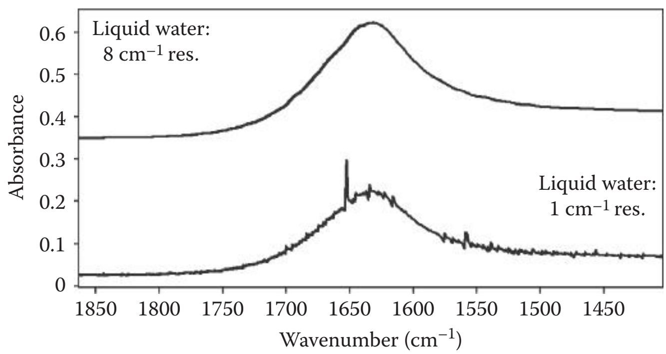

FIGURE 2.22 Top: The infrared spectrum of liquid water measured at 8 cm^{-1} resolution. Bottom: The infrared spectrum of liquid water measured at 1 cm^{-1} resolution.

TABLE 2.3
Typical Instrumental Resolution Settings for Different Types of Samples

Sample	Suggested Resolution
Solids/liquids	8 or 4 cm^{-1}
Gases	2 cm^{-1} or higher

part to the fact that water vapor molecules are well separated compared to liquid water molecules. In general then, measuring gas and vapor phase spectra at higher resolution makes sense because more usable information may result, which is dramatically illustrated by the two spectra in Figure 2.20. This is why gas phase spectra are typically measured at a 2 cm^{-1} resolution or higher. Bear in mind, however, that high-resolution spectra will be noisier than low-resolution spectra, all things being equal. Table 2.3 summarizes the typical resolution settings for different types of samples.

IV. FTIR TRADING RULES

It is the goal of every FTIR analysis to maximize spectral quality while minimizing analysis time. This makes the best use of you and your instrument, and makes you more valuable to your employer. We are now in a position to summarize the relationships between scanning parameters, spectral quality, and analysis time that will enable you to achieve these goals. These relationships are called "FTIR Trading Rules" [2] because they describe the trade-offs between different analysis parameters and spectral quality. The parameters we will be discussing are

SNR = Signal-to-noise ratio
Res. = Instrumental resolution
T = Analysis time
N = Number of scans

We are already familiar with some of the trading rules. The first is the relationship between SNR and the number of scans, originally stated as Equation 1.10

$$\mathrm{SNR} \propto N^{1/2} \tag{2.11}$$

Equation 2.11 tells us that the signal-to-noise ratio, which is a measure of spectral quality, increases as the square root of the number of scans added together. However, recall that there are limits to this idea as discussed earlier in this chapter.

The second trading rule is also familiar and was originally presented as Equation 2.10:

$$\mathrm{SNR} \propto \mathrm{Res.} \tag{2.12}$$

This equation reminds us that high-resolution spectra are inherently noisier than low-resolution spectra, and the increase in information obtained at high resolution comes at the cost of increased noise. Thus, one should use an instrumental resolution setting appropriate to the sample as seen in Table 2.3.

The third trading rule has not been mentioned before but makes sense. It states the relationship between analysis time and the number of scans as follows:

$$T \propto N \tag{2.13}$$

Equation 2.13 shows that more scans take more time than fewer scans. For example, it takes more time to move the scanning mirror in an interferometer back and forth 100 times than it does 10 times. If after 32 scans you get a good-looking spectrum that is sufficient for your needs, there is no point in scanning for several more minutes to get a small improvement in SNR; this is equivalent to gilding a lily. On the other hand, if after 32 scans the SNR is lousy, then by all means more scans should be used.

The fourth trading rule describes the relationship between resolution and analysis time as follows:

$$T \propto 1/\text{Res.} \tag{2.14}$$

Recall that each high-resolution scan involves a larger optical path difference than each low-resolution scan. Literally, it takes more time to move the mirror a long distance than a short distance. As a result, high-resolution scans take more time than low-resolution scans, as summarized in Equation 2.14.

Equations 2.12 and 2.14 show that working at high resolution creates two problems. First, high-resolution scans are inherently noisier than low-resolution scans. Second, high-resolution scans take more time than low-resolution scans. Again, in general high-resolution scans should only be used on appropriate samples, such as gases.

V. FTIR HARDWARE

So far we have discussed how the parts of an FTIR work together to measure an interferogram and ultimately obtain a spectrum. It is now time to look at how each of these pieces works by themselves.

A. Interferometers

The principles of operation of the Michelson interferometer were discussed above. What we have not discussed is how the mirror is moved during a scan. It is vital that the mirror moves freely and at constant velocity. Recall from Equation 2.7 that the Fourier frequency of the interferogram for a given wavenumber of light depends upon the speed of the moving mirror. If the moving mirror's velocity changes during a scan, incorrect Fourier frequencies would be measured, leading to incorrect wavenumbers being calculated from them. Since the x-axis of an interferogram is optical

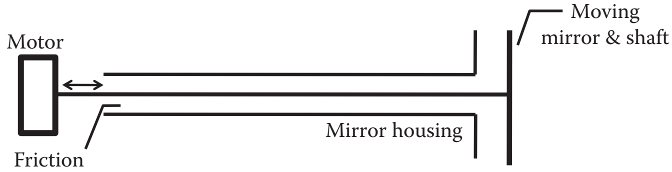

FIGURE 2.23 An illustration of how the moving mirror of a Michelson interferometer can be translated back and forth by use of a shaft attached to a motor. Note that the mirror housing is stationary, and that friction occurs between it and the shaft.

path difference, it is important that the moving mirror position be known accurately. If the mirror is moving in fits and starts it will be difficult to accurately know the optical path difference at any given point during a scan. One cause of inconsistent mirror movement is friction. In many FTIRs the moving mirror in the Michelson interferometer is attached to a shaft, which is attached to a motor, which is supported by a stationary housing, as seen in Figure 2.23.

Friction will occur between the moving mirror shaft and the housing as the mirror translates back and forth; thus there is a need for lubrication between the two. Over the years manufacturers have used different methods to insure the smooth translation of the moving mirror. Many first-generation FTIRs used a cushion of air to elevate and support the moving mirror shaft. This type of design was called an *air bearing*. The cushion of air made for practically frictionless travel. Additionally, the lack of friction means these interferometers rarely wear out. However, these designs require a constant flow of air through them, which can be impractical in some settings, and they are expensive to make.

To get around these problems FTIR manufacturers have invented what are called *mechanical bearing* interferometers. These use a mechanical means to lubricate the travel of the moving mirror. For example, some manufacturers use ball bearings while others use a lubricant such as graphite or Teflon. In general, mechanical bearings are more rugged and cheaper to make than air bearings, which is why the vast majority of interferometers in use today contain mechanical bearings. One potential drawback to mechanical bearings is that since the friction inside is not zero, they may wear out.

B. Infrared Sources

The source in any spectrometer provides the light with which to measure a spectrum. Recall that infrared radiation and heat are the same thing. We then need a source that is hot since hot things give off more infrared light than cool things. The source used in many FTIRs these days is called an *air-cooled source*, a diagram of which is shown in Figure 2.24. The term "air cooled" indicates that the source temperature is regulated by emitting heat to the atmosphere.

An air-cooled source consists of a coil or ribbon of nichrome wire. Nichrome is an alloy of nickel and chromium and has high resistance to electrical flow. As a result it gets hot and glows in the mid-infrared when electricity is passed through it.

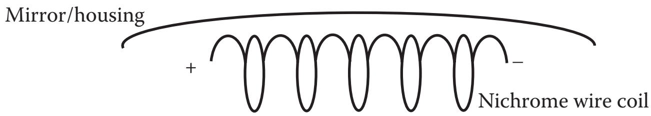

FIGURE 2.24 A diagram of an air-cooled infrared source.

Another advantage of nichrome is that it resists oxidation, which is important since this hot wire is exposed to the atmosphere. A common item that also contains an air-cooled infrared source is a toaster. The nichrome wire may in an FTIR's source be in thermal contact with a piece of ceramic, which allows it to operate at higher temperatures. Air-cooled sources can attain temperatures of 1200°K to 1400°K. In most FTIRs the source is backed by a mirror, as shown in Figure 2.24, or surrounded by a housing to collect as much infrared light as possible and send it toward the interferometer.

Like the filament in any light source, air-cooled infrared sources will eventually fail. This makes the source one of the "consumables" in an FTIR. The author's experience is that most air-cooled infrared sources last for three or more years. For many FTIRs, replacing the source is something users can perform themselves. Air-cooled sources are not expensive, and it is a good idea to keep a spare one handy so when the day comes when your source dies you can replace it quickly. Alternatively, if you have a service contract on your instrument, the source is something the manufacturer should replace on a routine basis.

The advantages of air-cooled sources are that they are rugged, simple, and cheap. The only potential drawback to air-cooled sources is that in a few very demanding applications they are not intense enough. In these cases water-cooled sources are used. However, for the vast majority of samples and applications, air-cooled sources provide enough intensity to give spectra of high SNR.

C. Beamsplitters

An examination of Figure 2.2 shows that the beamsplitter is at the heart of the interferometer. Recall that it is the job of the beamsplitter to take an infrared beam, split it in two, and then recombine these beams into one, but we have said nothing about how the beamsplitter accomplishes this task. Figure 2.25 illustrates how a beamsplitter works.

The beamsplitter in most FTIRs consists of a thin film of germanium sandwiched between two infrared transparent windows. The germanium is of the right thickness to transmit some infrared radiation and reflect some infrared radiation. It thus works like a two-way mirror that contains a thin layer of silver that is thick enough to reflect some light but thin enough to transmit some light. A simple pane of glass can also behave like a beamsplitter. If observed from the right angle you can see through a pane of glass, which means it is transmitting visible light, but you can also see your reflection, which means it is also reflecting visible light.

The purpose of the infrared transparent windows in a beamsplitter is to support and protect the germanium film. Many FTIRs contain beamsplitters with KBr

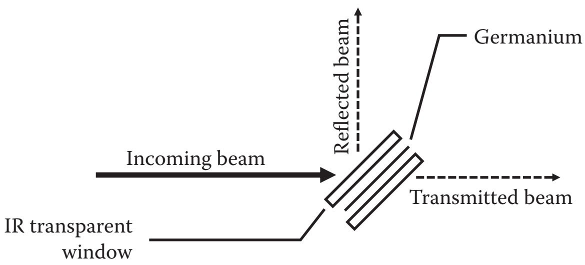

FIGURE 2.25 An illustration of how the beamsplitter in an FTIR works.

windows. KBr is useful because it is transparent across a broad spectral range, from 400 cm^{-1} out into the near infrared, and it is easily machined. The reason most FTIRs don't measure spectra below 400 cm^{-1} is because the KBr windows in the beamsplitter start to absorb strongly at that point. A disadvantage of KBr is that it is *hygroscopic*, which means that it absorbs water from the atmosphere. Over time a layer of adsorbed water will build up on the surface of the KBr windows, which will absorb infrared light and cut down on the amount of light reaching the detector. For this reason the humidity should be kept low inside an FTIR. This can be accomplished via a dry purge gas or by sealing and desiccating the interferometer as discussed earlier in this chapter.

In some applications, such as in the field or in humid environments, KBr is simply too moisture sensitive to be used as a beamsplitter window. An alternative is zinc selenide (ZnSe). This material is infrared transparent and is not hygroscopic, so it is frequently used in FTIRs that are used outdoors, on factory floors, outside the lab, etc. For all its utility there are two problems with ZnSe beamsplitters. First, ZnSe absorbs strongly starting around 700 cm^{-1}, and any spectral features below this will be lost. Second, a ZnSe beamsplitter adds several thousand dollars to the cost of an instrument. Another beamsplitter window material in use is cesium iodide (CsI). This material is transparent down to 200 cm^{-1} in the far infrared and can be used if your samples have important features between 400 and 200 cm^{-1}. However, CsI is very hygroscopic, and windows made from it have to be replaced frequently due to excessive absorption of moisture.

D. Infrared Detectors

The job of any infrared detector is to turn light intensity into an electrical signal, typically a voltage. Ideally, changes in light intensity will ultimately lead to proportionate changes in electrical signal coming out of the detector. In this task an infrared detector acts as a transducer, turning one form of energy (light) into another form of energy (electrical impulses). Since infrared radiation is the same thing as heat, an infrared detector may respond to changes in temperature. Thus, materials whose properties change with temperature are candidates to act as infrared detectors.

At the heart of any infrared detector is a small piece of material called the *detector element*, whose job is to turn changes in infrared intensity into an electrical signal.

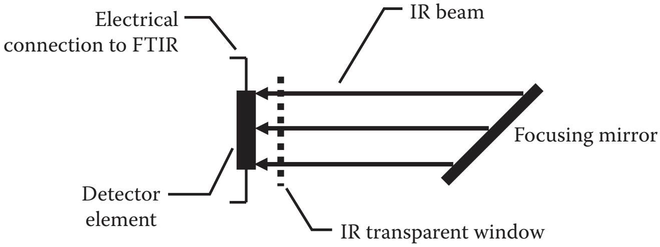

FIGURE 2.26 An illustration of how the final mirror in an FTIR focuses the infrared beam onto the detector element, how the element is protected by an infrared transparent window, and how the detector is connected to the instrument electronics.

The detector element determines many of the important properties of a detector including its usable wavenumber range, its speed of response, and its noise level. The last mirror in an FTIR brings the infrared beam to a focus on the detector element as illustrated in Figure 2.26. Ideally, the infrared beam should exactly fill the detector element. Since for infrared detectors the area of the detector element is proportional to noise level, detector elements are generally small, typically less than 1-mm square.

Detector elements need to be protected from the environment to work well. To this end an infrared transparent window, frequently made of the same material as the beamsplitter window, is placed just in front of the detector as is illustrated in Figure 2.26. Commonly used window materials include KBr and ZnSe. There are wires attached to the detector element that connect it to the instrument's electronics. The signal from the detector element passes through these wires and can be amplified, digitized, and then sent to a computer for Fourier transformation.

A common type of detector element is called deuterated triglycine sulfate, or DTGS for short. The unique property of DTGS is that its electrical polarization (the distribution of charges) varies with temperature, causing a current flow when its temperature changes. This electrical impulse is measured by electrical contacts placed on the surface of the DTGS element. When equipped with a KBr window, DTGS detectors are sensitive from 400 cm^{-1} to beyond 4000 cm^{-1} and thus do a good job of covering the mid-infrared. They are also relatively inexpensive, rugged, and do not require any special treatment.

However, there are two significant disadvantages to DTGS detectors. First, DTGS is a "slow" detector. Different detector element materials respond to changes in infrared intensity at different speeds. This is ultimately what determines the speed at which the moving mirror can be translated in an interferometer. DTGS is relatively slow to respond to changes in infrared intensity compared to other detector materials. A second major disadvantage of DTGS is its noise level. Compared to other types of infrared detectors, DTGS detectors are relatively noisy. DTGS detectors are fine for routine analysis, such as for many of the samples analyzed in an FTIR's sample compartment. However, for demanding applications such as infrared microscopy, DTGS detectors give spectra that are too noisy to be useful.

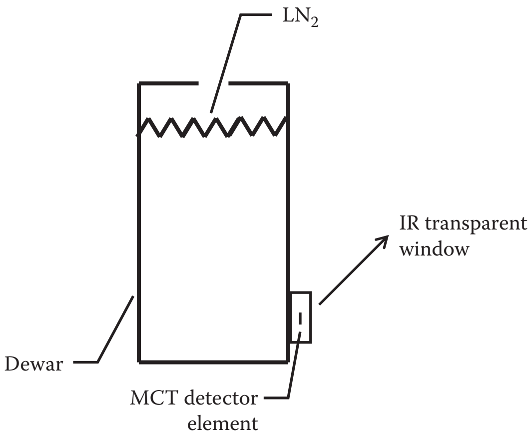

FIGURE 2.27 How an MCT detector is cooled using liquid nitrogen.

As a result of the drawbacks of DTGS detectors, other types of detector element materials have been developed such as mercury cadmium telluride (MCT). This material is an alloy of the elements mercury (Hg), cadmium (Cd), and tellurium (Te). MCT is a photoconductor; when it absorbs infrared light, electrons are knocked loose creating an electrical current. The more intense the infrared light, the larger the current. MCT detector elements are frequently 250 microns square. The beauty of MCT detectors is that they are 4 times faster and 10 times less noisy than DTGS detectors. Given this fact, why don't all FTIRs have MCT detectors in them? Because MCT detectors are thousands of dollars more expensive than DTGS detectors. Thus, MCT detectors are only used in demanding applications where a DTGS detector will not work and the extra expense is justified. The classic application of MCT detectors is in infrared microscopy, which will be discussed in Chapter 6.

In addition to their expense, MCT detectors suffer from other practical limitations. First, their spectral range is not as good as that of DTGS detectors; many cut off around 700 cm^{-1} obscuring part of the mid-infrared. This can be a problem if your sample has peaks in this wavenumber range. A second disadvantage of MCT detectors is that they must be cooled with liquid nitrogen (LN_2) before use to reduce noise. This is accomplished by placing the MCT detector element in contact with a reservoir of LN_2, as shown in Figure 2.27.

The LN_2 is poured into a container called a dewar, which consists of two containers nested within each other separated by a vacuum. The liquid nitrogen is poured into a hole in the top of the dewar, and it may take several minutes for some of the LN_2 to boil off as the dewar is chilled to temperature. It may take upwards of a liter of LN_2 to accomplish this task. Once the dewar is cooled and filled, it will typically stay chilled for 8 hours, so the detector usually only needs to be filled once per work day. In theory, MCT detectors can be used at room temperature, but they will give very noisy spectra; they work best when cooled to LN_2 temperatures. The problem with having to cool MCT detectors is the LN_2 has to be purchased, transported, and poured into the detector. The detector literally needs care and feeding, adding to the expense and complexity of its use. DTGS detectors do not suffer from this problem.

E. The Laser

Virtually every FTIR contains a laser whose light follows the infrared beam through the interferometer. This laser light is NOT the infrared source. Recall that a laser gives off light in a wavenumber. To measure a spectrum, many wavenumbers of light are required, which is why lasers cannot be used as FTIR sources. Instead, they measure the optical path difference of the interferometer. Most FTIRs use a helium-neon (He-Ne) laser for this purpose. He-Ne lasers are also used in grocery store scanners and give off red visible light at 15,798 cm^{-1}. After the laser light follows the infrared beam through the interferometer it is picked off by a laser light detector. Some of the laser beam may make it past the detector to the sample compartment, where its appearance can be used to align accessories or give one an idea of where the infrared beam is located since the human eye cannot see in the mid-infrared.

So far we have said little about how an FTIR measures the optical path difference of the interferometer. Here's how: Since the laser hits its detector after passing through the interferometer, the interferogram of the laser is measured as shown in Figure 2.28.

Recall that the interferogram of a laser line is a cosine wave, hence the appearance of the interferogram in Figure 2.28. We also know from Equation 2.2 that for the interferogram of an individual wavelength of light the maxima fall at $\delta = n\lambda$. For the laser interferogram we know the wavelength of light, which for a He-Ne laser is 0.632 microns. If we somehow knew n for a given maximum in the interferogram, we could simply multiply n times the known wavelength of the laser to determine

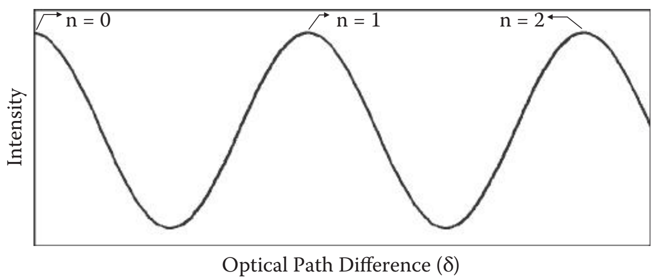

FIGURE 2.28 An illustration of what the interferogram measured by the laser detector on an FTIR might look like. Note that each maximum in the interferogram corresponds to a different value of the integers n = 0, 1, 2, …

the optical path difference of the interferometer. We can determine n by counting the maxima in the laser interferogram as the moving mirror is being scanned. As each n is determined it is multiplied by the known laser wavelength to give the optical path difference of the interferometer at that point in time. This triggers the instrument to sample the intensity of the signal coming out of the infrared detector. In this way discrete values of infrared intensity are obtained. Ultimately, then, an FTIR measures two interferograms during a scan. The laser interferogram provides the optical path difference data. The infrared detector provides the infrared intensity data, which is then plotted against the optical path difference to obtain the infrared interferogram.

Since the laser measures the optical path difference of the interferometer, an FTIR cannot measure anything without a functional laser. Like other light sources, lasers will wear out, typically lasting for 3–5 years. Laser power supplies also wear out after several years and are frequently replaced at the same time as the laser. The infrared source, laser, and its power supply are the most commonly replaced components on an FTIR. Some FTIRs are designed so that the user can replace the laser. In this case it makes sense to keep a spare laser and spare laser power supply in your lab. Alternatively, if you have a service contract on your instrument, the laser and its power supply are among the things that will be tested and replaced on a regular basis. If you cannot replace the laser, and you do not have a service contract, you may have to pay for a repair person to visit your lab for the repairs, or you may even have to ship the instrument back to the manufacturer to get it fixed.

To obtain a spectrum with a precise wavenumber scale, an interferogram with precise optical path differences must be measured. It is the job of the laser in an FTIR to ensure that optical path differences are measured precisely, and in so doing precise wavenumbers are obtained. In this sense then, the laser acts as an internal wavenumber standard for the FTIR. It is important to emphasize that accuracy and precision are different things. Precision is a measure of reproducibility and measures only random error. Accuracy is a measure of how far away a measurement is from its true value and measures random and systematic error. Thanks to the laser, the precision of the wavenumber measurements in an FTIR is very good, generally on the order of ± 0.01 cm^{-1}. The accuracy of the wavenumber measurements in an FTIR is ± half the instrumental resolution used to measure the spectrum. Thus if a spectrum is measured at 4 cm^{-1} resolution the wavenumbers will have an accuracy of ±2 wavenumbers.

In rare instances the laser may fail to do its job, and incorrect wavenumbers will be measured. There is then a need for a calibration check on the wavenumbers to ensure that the laser is functioning properly. This is often done by measuring the infrared spectrum of a standard material such as polystyrene. In fact, when you buy an FTIR you are frequently provided with a polystyrene film mounted in a cardboard frame for this purpose. In many labs the position of the polystyrene peak at 1601 cm^{-1} is used as a standard peak position. This is seen in the spectrum of polystyrene featured in Figure 2.29.

To be clear: the laser on the FTIR maintains the precision of wavenumber measurements, while the polystyrene test measures their accuracy. The author

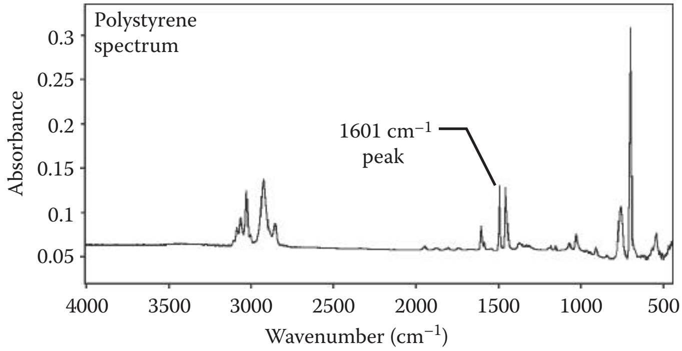

FIGURE 2.29 The infrared spectrum of polystyrene. Note the position of the peak at 1601 cm^{-1}, which is used as a wavenumber standard.

recommends measuring the spectrum of polystyrene on a regular basis to ensure that the wavenumber scales of your spectra are always in calibration. For those interested in running a more stringent test, a standard polystyrene film, along with instructions on how to measure its spectrum and use it as a standard, is available from the National Institutes of Standards and Technology, a branch of the US government. It is standard reference material 1921b [5].

VI. TESTING INSTRUMENT QUALITY AND TROUBLESHOOTING

Like all pieces of instrumentation, the performance of an FTIR should be monitored on a regular basis. This is done for two reasons. First, you need to know if the instrument is working properly before performing a given analysis. Second, keeping track of the instrument's performance over time will flag potential problems, such as a part about to fail, and allow corrective action to be taken before a catastrophic failure occurs. The tests described here are quick and easy procedures that the author uses on a regular basis to monitor the health of his instrument. A more thorough and detailed set of tests for FTIRs has been developed by the American Society for Testing and Materials (ASTM) [6].

Every spectrum measured on your FTIR should be examined for quality; a poor sample spectrum can indicate problems with the FTIR. Figure 2.18 shows an example of a quality FTIR spectrum for your reference. For starters, all the peaks in an infrared spectrum need to be on scale. For Absorbance spectra the peaks should have a value of 2 or less, and for %Transmittance spectra all peaks should be above 10%. Recall that the minimum SNR for a peak to be real is 3 and that SNRs of >100 are routinely obtained with FTIRs. A quick look at the "fuzziness" in the baseline of a spectrum can give you a qualitative feel for the amount of noise in a given spectrum. Sample spectra should also have a flat baseline and be free of artifacts such as water vapor or CO_2 peaks. If your sample spectrum suffers from any of these problems it could mean there is a problem with your instrument that should be fixed before any other spectra are measured.

An excellent way of monitoring the health of an FTIR is to examine its interferogram. Every FTIR software program has a command that will allow you to see interferograms being measured in real time. The first thing to note about the interferogram is its shape; it should have a centerburst and wings like the interferogram shown in Figure 2.9. If you do not see a centerburst, there is no infrared signal being measured by the detector. This could mean your source has died and needs to be replaced. Alternatively, there may be something blocking the infrared beam such as an incorrectly installed sampling accessory or a paper towel carelessly left in the sample compartment (the author speaks from experience on both counts). Your FTIR software will display a number that tells you the size of the centerburst. The unit in which this number is measured varies among manufacturers. Its value should be monitored while aligning instruments or accessories. If you see the size of the centerburst decrease over time it may mean your instrument needs aligning, your source is dying and may need replacing or the beam splitter is fagging up.

From scan to scan the size of the centerburst will change a small amount because of noise. However, wild fluctuations in the centerburst size there is instability either in your sample or your instrument. For example, if the concentration of the sample is changing rapidly, perhaps due to evaporation or chemical reaction, this will cause the centerburst height to vary. Alternatively, the source may be sputtering on and off, indicating that it is about to die. If there is a vibration shaking the instrument, this will cause the infrared beam to go in and out of focus at the detector, causing rapid changes in measured infrared intensity. To solve these problems, the sample should be examined, a new source should be installed, or the source of vibration should be eliminated.

A second number displayed while viewing interferograms is the position of the centerburst on the x-axis. The units in use here vary among manufacturers, but what is important is that this number be steady from scan to scan. If the centerburst position jumps around from scan to scan it may mean the laser is dying or is dead. In this case the instrument has difficulty determining optical path difference during a scan, causing the measured position of the centerburst to be incorrect. Additionally, if the mechanical bearing in your interferometer has worn out, the mirror may be moving in fits and starts, making it difficult to find ZPD. If you see your interferogram position jumping around, turn the instrument off and call your manufacturer.

An important test that measures the health of an FTIR is called the *100% line*. A 100% line is calculated by first measuring a background spectrum and then measuring a second background spectrum. The two spectra are then ratioed to obtain a percent transmittance spectrum. You may have to call the second background spectrum a "sample" spectrum to fool your FTIR software into measuring the two backgrounds and ratioing them. There should be no sample or sampling accessory present when a 100% line is measured; this measurement should be of the instrument by itself. An example of a 100% line is shown in Figure 2.30.

In theory, the two background spectra used to calculate the 100% line should be identical because they were measured on the same instrument under the same

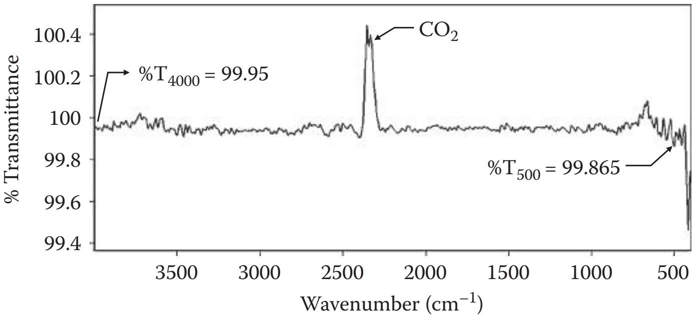

FIGURE 2.30 An FTIR 100% line. Sixteen scans and 8 cm^{-1} resolution were used. The data used to calculate the slope of the line are indicated.

conditions with an empty sample compartment. When these two spectra are ratioed, a flat line at 100% transmittance should be obtained. In reality, a mostly flat line is obtained that contains noise and perhaps artifacts, as seen in Figure 2.30.

The slope of a 100% line is a measure of instrument stability; the more unstable the FTIR the greater the slope or curvature will be. The slope can be measured by taking the %T at 4000 cm^{-1} and subtracting from it the %T at 500 cm^{-1} (in theory 400 cm^{-1} should be used, but the region below 500 cm^{-1} is frequently very noisy due to KBr window absorbance, making it difficult to determine the exact location of the baseline). For the 100% line shown in Figure 2.30, the relevant numbers for the slope calculation are (99.95% – 99.865%) = 0.085%. For the instrument on which this 100% line was measured, this is a good result. The numbers obtained will depend upon your instrument; consult with your manufacturer on what a good slope number is for your make and model of FTIR. Severe slope or curvature in the baseline indicates problems with your FTIR that should be resolved before any sample spectra are measured.

Another important measurement to make on the 100% line is the peak-to-peak noise (see Chapter 1). The region between 2200 and 2000 cm^{-1} is frequently used for this measurement because it is generally free of water vapor and CO_2 interferences and is usually the least noisy part of a spectrum. The simplest way to measure the peak-to-peak noise of a 100% line is to use your FTIR software to expand the spectrum so the x-axis display limits are at 2200 and 2000 cm^{-1}, and then scale the y-axis display limits so the highest and lowest points in this region are visible. Then the highest and lowest points in this region are subtracted from each other to obtain the peak-to-peak noise, as illustrated in Figure 2.31.

For this particular 100% line the peak-to-peak noise between 2200 and 2000 cm^{-1} is (99.961% − 99.928%) = 0.033%. This is an excellent result for this particular spectrometer. The results you obtain will depend upon your FTIR and the number of scans and resolution used to measure the 100% line. Your FTIR software may use a different unit of noise measurement than used here, such as RMS (Root Mean Square) noise. Consult with your manufacturer about what noise levels are typical for your FTIR.

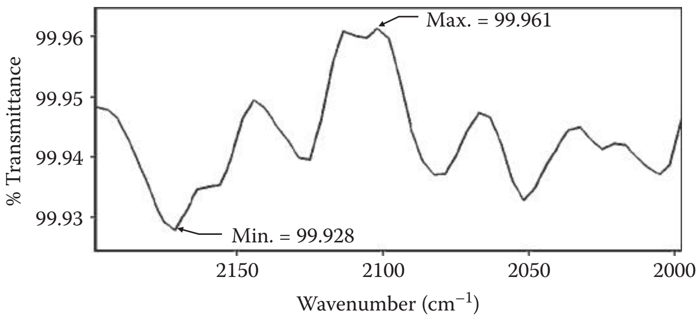

FIGURE 2.31 The 2200–2000 cm^{-1} region of the 100% line shown in Figure 2.30. The maximum and minimum y-axis values indicated were used to calculate the peak-to-peak noise in this region.

To ensure the long-term health of your FTIR, and to make sure the data you measure from day to day are correct and of high quality, you must run the tests discussed here on a regular basis. As mentioned, every sample spectrum measured should be examined for quality. Looking at the interferogram should be done at intervals during the day since it is quick and tells a lot about current instrument status. The spectrum of polystyrene should be measured daily to check the calibration of the wavenumber scale. The 100% line test should be run before any samples are analyzed on a given day. If the instrument will be in use all day, the author recommends running a 100% line in the morning and another after lunch.

Your FTIR software package may have a “validation” or “calibration” function that may automatically run some or all of the tests noted here. This is fine because it saves you the trouble of running the tests yourself. However, you should familiarize yourself with what tests are run, where the results are stored, and what the numbers mean. You must look at this data on a regular basis to check for any disturbing trends. Just because the computer runs the tests for you does not mean you can sit back and ignore the data.

The results of all these tests should be recorded in some fashion. In many labs, test results are recorded in an instrument logbook. In addition to the results obtained on a given day, tracking the results over time is useful because you can spot trends and fix problems before they happen. Instrument repairs and the installation of new FTIR software should also be noted in the instrument’s logbook. The author, records every spectrum he measures on his FTIR in a logbook. The spectrum’s file name, number of scans, instrumental resolution, and sampling technique are noted along with the time, date, and sample information. This way, if there is ever any question about the data, there is a record of how and when the spectrum was measured. Running these tests so frequently may seem like an annoyance. However, each of these tests is easy to run and the full battery of them takes only a few minutes. It is worth investing a little effort every day to make sure your FTIR is healthy and is measuring the best possible data.

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3 Proper Use of Spectral Processing

Modern FTIR software programs contain powerful algorithms for processing spectra. If used properly, these algorithms can enhance the information content of a spectrum, speed mixture analysis, and make spectral interpretation easier. However, if used improperly these algorithms can alter and destroy spectroscopic data and can lead to erroneous conclusions. Hence the need for this chapter. The goal here is to explain how these processing algorithms work and how to use them properly to maximize the information obtained without sacrificing spectral quality.

This chapter begins with a discussion of *spectral subtraction*, which is a way of simplifying mixture spectra to make them easier to interpret. Next, *baseline correction* is discussed, which is a method of removing baseline slope and curvature from a spectrum. Third, the use of spectral *smoothing* to reduce the noise level in a spectrum is covered. Fourth, spectral *derivatives* and their use in determining peak positions and analyzing overlapped peaks is presented. Fifth, a method of mathematically enhancing the resolution of a spectrum called *deconvolution* is examined. Last, *library searching*, which makes spectral interpretation easier and aids in mixture analysis.

I. THE RULES OF SPECTRAL PROCESSING

To avoid potential problems in the use of spectral processing algorithms, the author recommends you follow these rules.

1. Always closely examine the original spectrum before deciding on whether to process it or not. If you proceed, always have a good reason for doing so. Never process spectra automatically.
2. Have a clear idea of how the processing algorithm you propose to use works, the problems it can cause, and how to avoid them.
3. Always retain the original data so if something goes wrong, you have the original to fall back on.
4. Always record how, when, why, and by whom the processing was performed.

Rule 1 insists that you look at a spectrum first before deciding whether to process it; each spectrum is unique, and this decision needs to be made on a case-by-case basis. You should never automatically process all the spectra you measure. Spectral processing can involve data compromise, and you should only process spectra if there is a real need. Processing spectra just to make them look better is scientifically illegitimate. It's not worth the risk of trashing your spectrum just to make a prettier picture. If you spectrum is of poor quality, study Chapters 2 and 4 for ideas on how to change scanning conditions or sample preparation to optimize spectral quality. If you still have problems, then spectral processing can be legitimitely applied.

Rule 2 refers to having a clear understanding of how spectral processing algorithms work, how to use them properly, and the problems that can be encountered in their use. This understanding can be gained by reading this chapter, reading the documentation that came with your FTIR software package, and practice. For example, to properly interpret a spectral subtraction result properly you need to learn to recognize and ignore the artifacts that the process can introduce into a spectrum.

Rule 3 says to always retain the original spectrum, which can be saved as a hard copy, stored on your computer's hard disk, or archived at some other location. Since spectral processing may involve data compromise, you need to save the original spectrum in case something goes wrong. You should process electronic copies of the original data, and you should never overwrite original data with processed data.

Rule 4 tells you to show your work. The information to record includes who did the processing, when it was performed, what software brand and version number was used, what algorithm and parameters were used, and why the manipulation was performed. This data can be written on a hard copy of the spectrum, recorded in a lab notebook, or saved in electronic form. Some software packages record some or all of this information for you automatically. What matters is that this information be recorded because processing a spectrum can alter its appearance, and anyone who looks at a spectrum needs to know what was done so they can take it into account when interpreting the spectrum. Also, publishing a processed spectrum without stating how and why it was processed is a form of scientific fraud—a behavior best avoided if you value your career.

The purpose of these rules is not to scare you away from using spectral processing, but to encourage you to use these algorithms properly so you can enjoy their benefits.

II. SPECTRAL SUBTRACTION

Spectral subtraction is a spectral processing technique used to simplify mixture spectra by removing unwanted contributions to a spectrum such as water vapor, CO_2, solvents, impurities, interferents, and known components. Ideally, by removing these unwanted contributions, peaks of interest will be more easily readily be observed. A good example of the power of spectral subtraction is shown in Figure 3.1.

The *sample spectrum* in the bottom of Figure 3.1 is of a solution of the amino acid glutamine dissolved in water. Sample spectra used in subtraction are typically mixtures containing some contribution that we wish to remove. Note that the broad, intense peaks of water in the sample spectrum make the glutamine peaks difficult to see. The *reference spectrum* in the middle of Figure 3.1 is of pure liquid water. Reference spectra are typically of a pure substance that is also present in the sample.

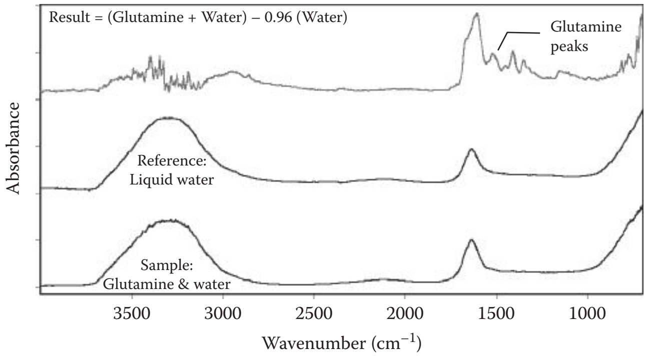


FIGURE 3.1 An example of a spectral subtraction. The sample spectrum displayed at the bottom is of the amino acid glutamine dissolved in water. The reference spectrum shown in the middle is of pure liquid water. The top spectrum is of the subtraction result.

When the water spectrum was subtracted from the glutamine/water spectrum, the water peaks were removed and the result at the top of Figure 3.1 was obtained. The beauty of this result is that the glutamine peaks are now much easier to identify.

For subtraction to work properly the two spectra must be plotted with y-axis units that are linearly proportional to concentration, such as absorbance. Single beam spectra or spectra plotted in percent transmittance have peak sizes that are not linear with concentration and should not be used in spectral subtraction. The mathematical algorithm behind spectral subtraction is relatively simple and is given by Equation 3.1:

$$\text{(Sample Absorbance)} - \text{(Reference Absorbance)} = \text{Result Absorbance} \quad (3.1)$$

This equation states that the absorbance at a given wavenumber in the result is equal to the difference in absorbance between the sample and reference spectrum at that wavenumber. For example, if the absorbance of the sample spectrum at 3000 cm^{-1} is 0.5, and the absorbance of the reference spectrum at 3000 cm^{-1} is 0.2, the result absorbance will be 0.3. A subtraction result then is a plot of the difference in absorbance between the sample and reference spectra plotted versus wavenumber.

Ideally, a subtraction result will contain no features from the reference material, i.e., the reference features will have zero absorbance and be part of the spectrum's baseline. For this to happen, the reference features present in the spectra being subtracted must be perfect overlays, i.e., have the same width, height, shape, and x-axis position. This situation is illustrated in Figure 3.2.

The left-hand spectrum in Figure 3.2 shows two peaks that are excellent overlays. Since almost all the absorbances in these two peaks are the same, when they are subtracted they will give a flat line at zero absorbance. In the right-hand spectrum in Figure 3.2, the two peaks to be subtracted are of different sizes. This could be due to differences in pathlength or concentration between the sample and reference. When these two peaks are subtracted the result will have a feature in this region

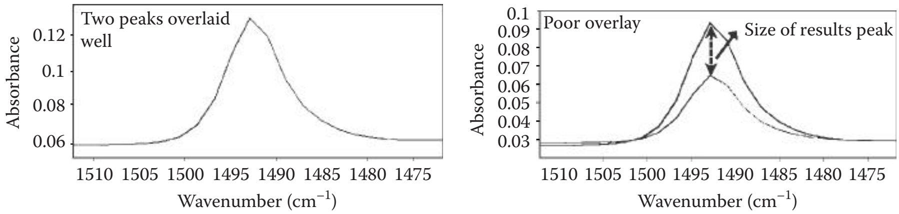


FIGURE 3.2 Left: Two well-overlaid peaks. When subtracted they will give a flat baseline in the result. Right: Two peaks of different sizes that when subtracted will give a peak of the size noted in the result spectrum.

equal in size to the difference in absorbance between the two peaks. This defeats the purpose of the subtraction, which is to get rid of unwanted features. This presents a problem, unless by chance the peaks in the sample and reference are the same size. They will not subtract cleanly and the subtraction result will have unwanted peaks in it. One could get around this problem by measuring many different reference spectra at many different concentrations and pathlengths to obtain a spectrum that hopefully matches the absorbances in the sample spectrum. However, this is a long and tedious process.

Fortunately, there is an easier away around this problem. Most FTIR software programs allow you to multiply the absorbances in the reference spectrum by a *subtraction factor* (sometimes called a "scaling factor"), which is used to scale the reference spectrum absorbances to match the sample spectrum absorbances so that unwanted peaks subtract cleanly. The subtraction algorithm then becomes

$$\text{(Sample Absorbance)} - \text{(Subtraction Factor)(Reference Absorbance)} \\ = \text{Result Absorbance} \quad (3.2)$$

For example, if the sample absorbance at a given wavenumber is 1.0 and the reference absorbance at the same wavenumber is 0.5, multiplying the reference spectrum times a subtraction factor of 2.0 will make the two absorbances equal, which will give an absorbance of zero when subtracted. In most FTIR software packages, dragging a scroll bar or clicking a button easily changes the subtraction factor. Normally, the result is displayed quickly so the effect of changing the subtraction factor on the result can be easily seen. The subtraction result shown in the top of Figure 3.1 was obtained with a subtraction factor of 0.96.

The subtraction factor is a user adjustable parameter, and the skill with which you set the subtraction factor will determine the quality of the subtraction result. How then do you set the subtraction factor properly? The subtraction factor that works best will vary with the nature of each sample and reference spectrum. That being said, there is a process to follow that will assist you in obtaining a good subtraction factor. First, before performing a spectral subtraction, compare the sample and reference spectra and look for peaks present in both spectra. These reference peaks are from molecules present in both the sample and the reference, and in general will be the peaks you will want to remove. For example, a comparison of the sample and reference spectra in Figure 3.1 shows that both contain liquid water reference peaks at 3400 and 1600 cm^{-1}. Unfortunately, experience has shown that large peaks, ones whose absorbance is greater than 0.8, tend to not subtract cleanly [1]. This is because large peaks sometimes have a nonlinear absorbance/concentration relationship. That is, they do not follow Beer's Law as stated in Equation 1.6. However, this limit is empirical and has exceptions. When comparing sample and reference spectra, we look for medium- to small-sized reference peaks because these are the ones most likely to subtract cleanly.

Next, the subtraction factor is adjusted while keeping an eye on the reference peaks. Ideally, all of the peaks you want to get rid of will become even with the baseline at the same value of the subtraction factor. The reality is more complicated. It may happen that different peaks will zero out at different subtraction factors. In general, if you are interested in a specific wavenumber region in the result, reference peaks in that wavenumber region should be used to set the subtraction factor. If you are interested in the subtraction result as a whole, you may want to use a subtraction factor that minimizes the size of a number of reference peaks. Ultimately, choosing the best subtraction factor and reference peaks is a judgment call, and the experience of the user does come into play. Subtraction seems to work best with subtraction factors close to 1.0. Thus, if you have control over the measurement of the reference spectrum, experiment with pathlength and concentration to try to get the reference absorbances to closely match the sample absorbances. In the subtraction shown in Figure 3.1 the water peak at ~1630 cm^{-1} was used as the reference peak. Its absorbance was zeroed by adjusting the subtraction factor to 0.96. In theory, the peak near 3400 cm^{-1} could have been used, but it was too intense to subtract cleanly.

We can now summarize the subtraction process. First, before doing a subtraction, the sample and reference spectra should be compared to determine what reference peaks are going to be used to set the subtraction factor. Second, the subtraction factor is adjusted to try to minimize the size of the reference peaks. Lastly, the reference spectrum is compared to the result to see if the peaks of interest have disappeared. More simply, find the peaks to get rid of, try to get rid of them, and see if you got rid of them.

Optimizing the subtraction factor properly is a balancing act, and the problems encountered when that balance is not achieved are shown in Figure 3.3. The subtraction results shown in Figure 3.3 were calculated using a sample that was a mixture of polystyrene and another polymer, while the reference spectrum was of pure polystyrene. In the top spectrum the subtraction factor of 0.5 was too small and the reference peaks point up. In the middle spectrum the subtraction factor of 1.8 was just right since the reference band absorbances are minimized. In the bottom spectrum the subtraction factor of 2.2 was too large and the reference peaks point down. As in this example, the best subtraction factor is often in the middle. There are automated computer programs that attempt to calculate the optimal subtraction factor. However, it is the author's experience that a trained human being can do a better job of setting the subtraction factor than these programs.

Spectral subtraction works even when there are overlapped peaks, as illustrated in Figure 3.4. The bottom spectrum in Figure 3.4 shows two polystyrene bands overlapped with two unknown bands. The top spectrum shows that upon subtraction the

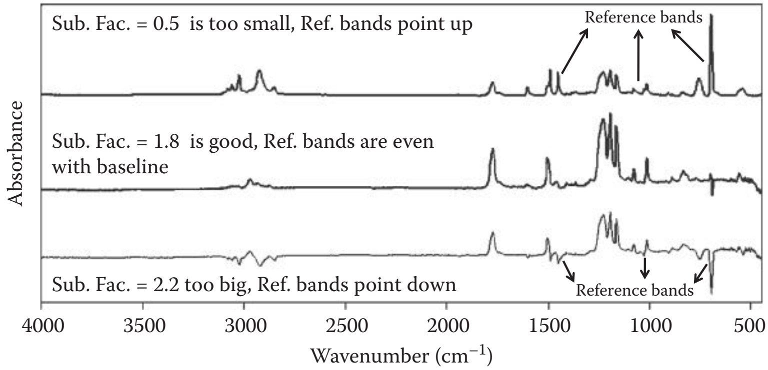


FIGURE 3.3 The effect of varying subtraction factors on the subtraction result. Top: A subtraction factor of 0.5 is too small and the reference bands in the result point up. Middle: A subtraction factor of 1.8 is just right since it minimizes the size of the reference peaks. Bottom: A subtraction factor of 2.2 is too large since the reference peaks point down.

polystyrene bands have gone away, but the unknown peaks are still visible. This shows that even when there is peak overlap, subtraction can make unknown peaks easier to see.

A. Subtraction Artifacts

A problem with subtraction results is they are generally noisier than the sample and reference spectra from which they are calculated. Thus the SNR of spectra to be subtracted must be high to begin with to obtain usable subtraction results. Another problem encountered with subtraction results are artifacts, i.e., unwanted spectral features. An unsubtracted reference band, a type of subtraction artifact, is shown in Figure 3.5.

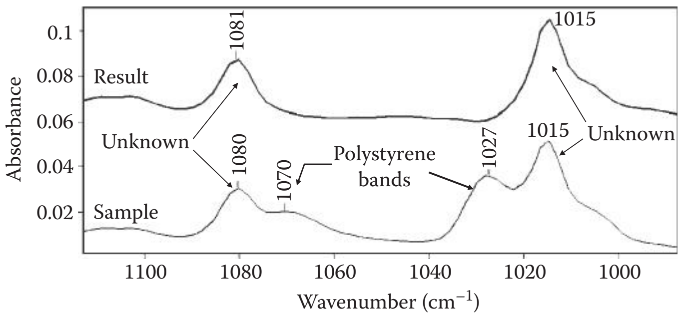


FIGURE 3.4 Bottom: A sample spectrum of a mixture two polymers. Note that there are polystyrene and unknown bands overlapped with each other. Top: A subtraction result. The polystyrene peaks have been removed and the unknown peaks are now more clearly visible.

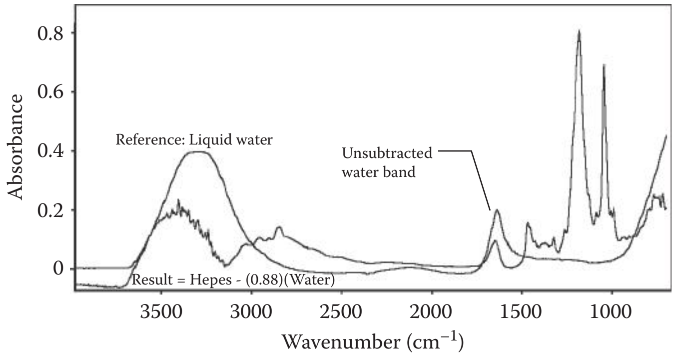


FIGURE 3.5 A comparison of a reference and a result spectrum reveals an unsubtracted reference band due to liquid water.

Recall that large peaks frequently do not subtract completely because they may have a nonlinear absorbance/concentration relationship. These peaks are present in subtraction results and are a form of subtraction artifact. Comparison of the reference spectrum and result can help spot these artifacts; any features common to both are unsubtracted reference peaks. This type of comparison is illustrated in Figure 3.5 where an unsubtracted reference peak from liquid water was spotted by comparing the reference to the result. There is nothing we can do to force a particular peak to follow Beer's Law. Hence there is nothing that can be done about unsubtracted reference bands except to learn to recognize them and ignore them.

A second type of subtraction artifact are derivative-shaped peaks, as shown in Figure 3.6. These features like the calculus derivative of an absorbance band. Figure 3.6 shows a comparison of a sample and a reference spectrum, both of which contain liquid water. In theory these two spectra could be subtracted from each other, resulting in the removal of the water peak. However, note that the water peaks in Figure 3.6 are at slightly different wavenumbers. This wavenumber shift is a result

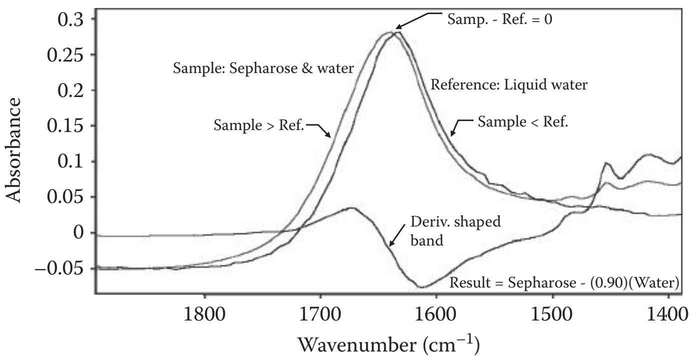


FIGURE 3.6 An illustration of how subtracting two peaks that are not at the same wavenumber can lead to derivative-shaped bands in the subtraction result.

of chemical interactions between the water molecules and solute molecules in the sample. These interactions are strong enough to change the spectrum of the water and cause its peak position to shift. To the left of Figure 3.6 the sample absorbances are greater than the reference absorbances, so the result is positive as seen in the result spectrum at the bottom of Figure 3.6. At the point where the two spectra cross they have the same absorbance, and the subtraction result equals zero as indicated in the figure. To the right of Figure 3.6 the sample absorbances are less than the reference absorbances, so the subtraction result is negative. The result spectrum then contains a feature that has positive and negative lobes. Since we cannot prevent molecules from interacting with each other, we can not prevent the type of peak shifts seen in Figure 3.6. Thus, like unsubtracted reference bands, there is nothing to be done about derivative-shaped bands in spectral subtraction results except to learn to recognize them and ignore them.

Despite the problems listed here, spectral subtraction, if used properly, is a legitimate way of simplifying mixture spectra, and making them easier to interpret.

III. BASELINE CORRECTION

Ideally, a measured infrared spectrum should have a flat baseline that falls at zero absorbance or 100% transmittance [2]. In reality, there will be times when you measure spectra that do not have ideal baselines. These can fall into three categories. First, the entire baseline of a spectrum can be offset from zero, as illustrated in Figure 3.7.

This type of offset is equivalent to adding a constant value to all the absorbances in a spectrum. Offset is caused by any problem where the effect on the spectrum's y-axis values is about the same at all wavenumbers. The KBr pellet spectrum of aspirin shown in Figure 3.7 has an offset of about 0.2 absorbance units. The pellet was too thick and reflected and absorbed significant amounts of the infrared beam at all wavenumbers. This problem can be corrected by making a thinner KBr pellet.

The second type of baseline problem is slope as illustrated in Figure 3.8 which shows the spectrum of a KBr pellet where the sample and KBr were not ground

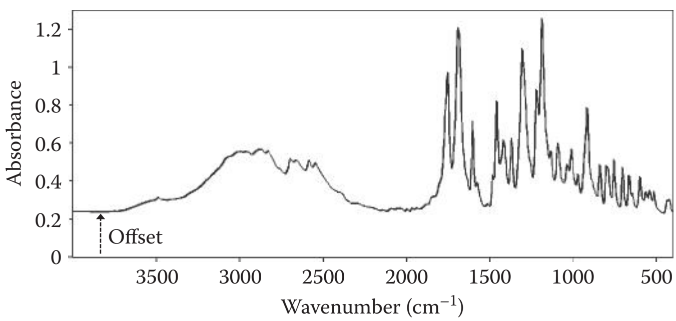


FIGURE 3.7 An example of an infrared spectrum whose baseline is offset by ~0.2 absorbance units. This is the spectrum of an aspirin tablet obtained with the KBr pellet sampling method. The baseline offset is caused by the pellet being too thick.

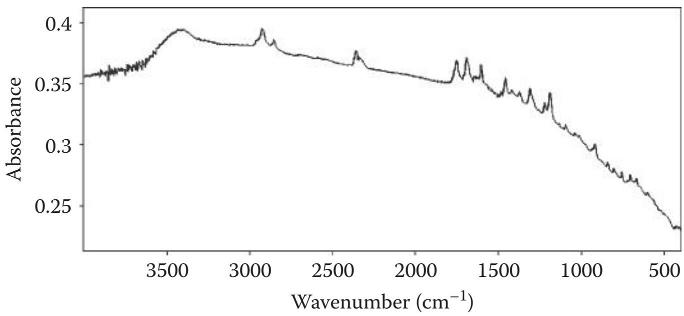


FIGURE 3.8 An example of a spectrum with a sloped baseline. This is the infrared spectrum of a KBr pellet where the KBr and sample were not ground enough. The resultant scattering of the light beam increases with wavenumber, causing the baseline distortion.

sufficiently. The resultant large particles in the sample scattered the infrared beam [3], whose intensity increases with wavenumber giving the sloped spectrum shown. This problem can be fixed by simply remaking the pellet with sufficiently ground sample and KBr. Another problem that causes sloped baselines is instrument drift, which can be caused by variation in temperature inside the instrument and voltage fluctuations in the electrical line powering the spectrometer. This problem can sometimes be corrected by running a fresh background.

The third type of baseline problem is curvature. The spectrum in Figure 3.8 shows some curvature, but a more severe example is seen in Figure 3.9. In this particular case, an issue with the instrument's detector caused the curvature. Other things that can cause curved baselines include problems with the FTIR's laser or interferometer.

Note that these three types of baseline problems (offset, slope, and curvature) have known causes, which means *they have known solutions*. You should do everything in your power to cure the experimental causes of baseline problems before applying any correction algorithms. However, sometimes, despite our best efforts, baseline problems cannot be fixed experimentally. For example, if there are particles embedded

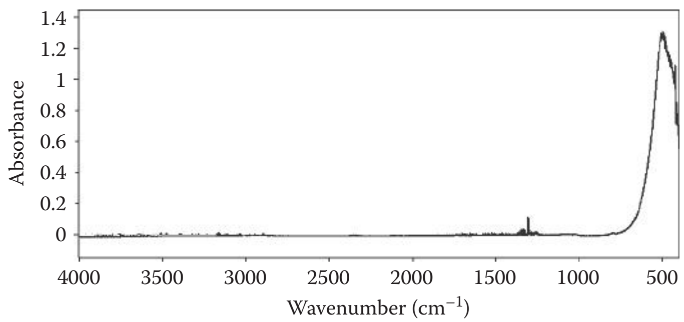


FIGURE 3.9 An example of a spectrum with a severely curved baseline.

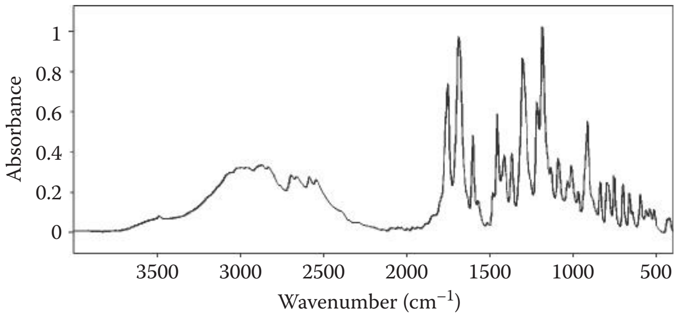


FIGURE 3.10 The KBr pellet spectrum from Figure 3.7. Its offset has been removed by subtracting the minimum absorbance from the entire spectrum.

in a polymer sample that are scattering light and causing a sloped baseline, it may be impossible to tease the particles out to prevent the scattering. In these cases the spectral processing technique called *baseline correction* can be used. The type of baseline correction to use depends upon the baseline problem you are encountering. Since with offset the entire spectrum is raised or lowered by a constant along the y-axis, correcting it involves adding or subtracting an appropriate constant from the spectrum to get the baseline down to zero. Taking the minimum absorbance in the spectrum and subtracting it from all the other absorbances in the spectrum can correct offset, as follows:

$$A_c = (A_i - A_{min}) \tag{3.3}$$

Where

A_c = Absorbance in baseline corrected spectrum
A_i = Absorbance at any wavenumber i in the uncorrected spectrum
A_{min} = Minimum absorbance in uncorrected spectrum

The offset of the spectrum shown in Figure 3.7 was corrected by subtracting the minimum absorbance (= 0.23) from the entire spectrum to obtain the offset corrected spectrum shown in Figure 3.10.

To correct slope or curvature in a baseline, a function that closely parallels the spectrum's baseline is drawn. This baseline is then subtracted from the spectrum. Ideally this removes the baseline problem without altering the spectral data of interest. The author has found that programs that allow you to use a series of line segments to parallel a sloped or curved baseline work well in many instances. Users of these programs must choose the number and length of these line segments, trying to mimic the baseline of the spectrum as closely as possible. This is illustrated in Figure 3.11, which shows how eight line segments can be used to approximate the slope of the spectrum originally seen in Figure 3.8.

There is an art to drawing a quality parallel function. Note in Figure 3.11 that absorbance minima and places where the slope noticeably changes are good places

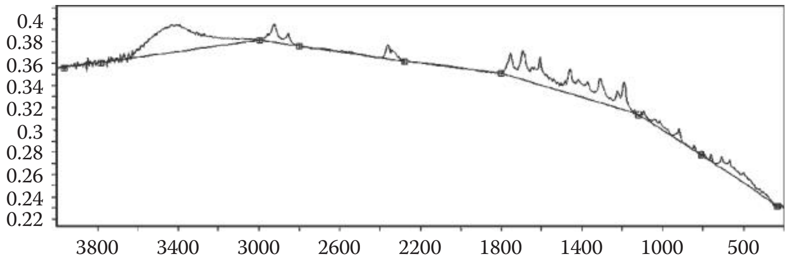


FIGURE 3.11 The sloped spectrum from Figure 3.8 with a function drawn parallel to its baseline consisting of 8 line segments.

to place line segment endpoints. The result of using the eight line segments shown in Figure 3.11 for baseline correction is seen in Figure 3.12.

Note in Figure 3.12 that the slope of the baseline is now essentially gone, and the baseline is at zero absorbance units. In general, you should use the minimum number of line segments that do a good job of paralleling the baseline. If there is curvature in a baseline, a series of short line segments can sometimes be used as a parallel function. Additionally, some software packages allow you to draw non-linear functions to parallel a curved baseline.

Problems with baseline correction arise when the function drawn does not parallel the spectrum's baseline well. This is illustrated in Figure 3.13. The bottom spectrum is identical to the spectrum with a curved baseline shown in Figure 3.9. You can also see a straight line drawn across the spectrum that will be used as a "parallel" function. The result of using this nonparallel function in a baseline correction is shown at the top of Figure 3.13; rather than reducing baseline slope, the result's slope is worse than the original. Improperly drawn parallel functions can also introduce spurious peaks or hide real peaks. This is why it is imperative that the parallel function in baseline correction be drawn properly

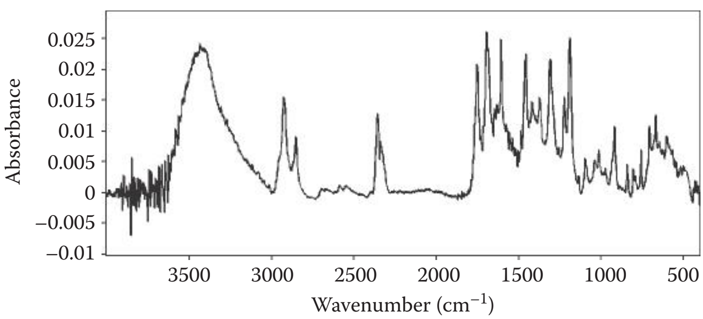


FIGURE 3.12 The spectrum from Figure 3.8 after being baseline corrected using the line segments shown in Figure 3.11. Note that the baseline is now essentially straight, and that the baseline is at zero absorbance units.

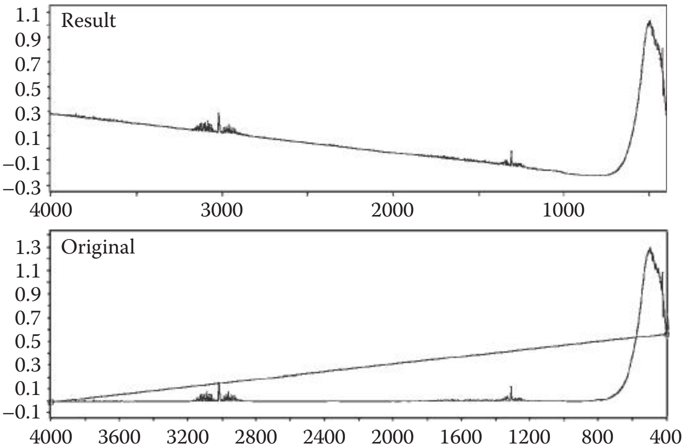


FIGURE 3.13 Bottom: A spectrum in need of baseline correction, and a very poorly drawn parallel function. Top: The result of using such a parallel function.

IV. SMOOTHING

If a spectrum has a poor SNR, all experimental methods for improving it, including increasing the number of scans and choosing an appropriate sampling method, should be tried first. However, if this does not succeed you can consider a spectral processing technique called *smoothing*, which is used to reduce the noise level in a spectrum and hence increase its signal-to-noise ratio [2,4–6]. An example of the effect of smoothing on a spectrum is shown in Figure 3.14.

The bottom spectrum in Figure 3.14 is a noisy spectrum of the molecule benzonitrile. The top spectrum is the result obtained after smoothing the spectrum. Note that in the top spectrum there is much less noise and the peaks are much easier to see than in the bottom spectrum. For example, the peak at 1599 cm^{-1} is much easier to

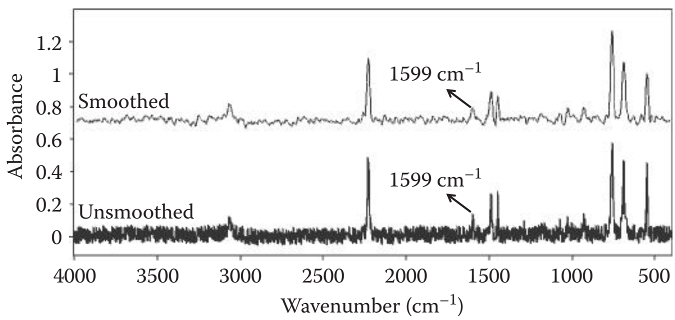


FIGURE 3.14 Bottom: A noisy spectrum of benzonitrile. Top: The same spectrum after smoothing (17-point Savitsky–Golay smooth, polynomial order 2). Note that the peak at 1599 cm^{-1} is much easier to see after the smooth.

see in the smoothed spectrum than in the unsmoothed spectrum. When interpreting peaks in a noisy spectrum, remember that, in general, noise features are narrow and sample peaks, particularly for solids and liquids, are wide.

Many FTIR software packages contain smoothing functions, and there are typically several smoothing algorithms from which to choose. The simplest one is called boxcar smoothing, which is not often used but is the easiest to explain. For boxcar smoothing, imagine taking several data points in a spectrum and drawing a box around them. This box is known as the smoothing window. Assume for a moment the smoothing window contains 9 data points. Now, take the y-axis values of these 9 data points, add them together, and then divide by 9 to calculate their average. Then, assign the average y-axis value to the middle x-axis point in the smoothing window. Next, move the box over one data point, calculate the average for this set of 9 data points, assign a new average to the middle x-axis data point, and so on. The smoothing window is dragged across the entire spectrum to obtain a running average of the y-axis values. The smoothing result then is an average y-axis value plotted versus wavenumber. The noise in the smoothed spectrum averages out, as given by Equation 2.11 where N is the number of points in the smoothing window. The amount of smoothing is proportional to the number of points included in the smoothing window. Smoothing windows must always have an odd number of data points or else there is not a middle data point in the window. Smoothed spectra are truncated at their ends because these data points are never the center point in a smoothing window.

Smoothing algorithms differ in how the average in the smoothing window is calculated. For instance, averages that apply more or less weight to midpoints and end points in the smoothing window can be used. Some of the more common smoothing algorithms are boxcar, triangle, fast Fourier transform, and Savitsky–Golay. When describing how a smooth was performed, at a minimum you must report the smoothing algorithm used and the number of data points in the smoothing window. Thus, terms such as "7-point boxcar" and "9-point Savitsky–Golay" are proper ways of denoting how a spectrum was smoothed.

The Savitsky–Golay method [7] is probably the best-known and most widely used smoothing algorithm. It works by least-squares fitting a polynomial function to the set of data points in the smoothing window. The y-axis value assigned to the center x-axis point is the value of the polynomial function in the center of the smoothing window [8]. When using the Savitsky–Golay algorithm, in addition to setting the number of points in the smoothing window, the order of the equation used in the least-squares fit must be specified as well. For example, the highest order term in a polynomial of order 2 is x^2. The higher the degree of the polynomial that is fit to the points in the smoothing window, the less smoothing is achieved, as shown in Figure 3.15.

The bottom spectrum in Figure 3.15 is an unsmoothed and noisy spectrum of benzonitrile (as also shown in the bottom of Figure 3.14). Each of the other spectra in Figure 3.15 were smoothed using the Savitsky–Golay algorithm with polynomial orders of 10, 6, and 2, respectively. Note that as the polynomial order goes down, the amount of smoothing goes up.

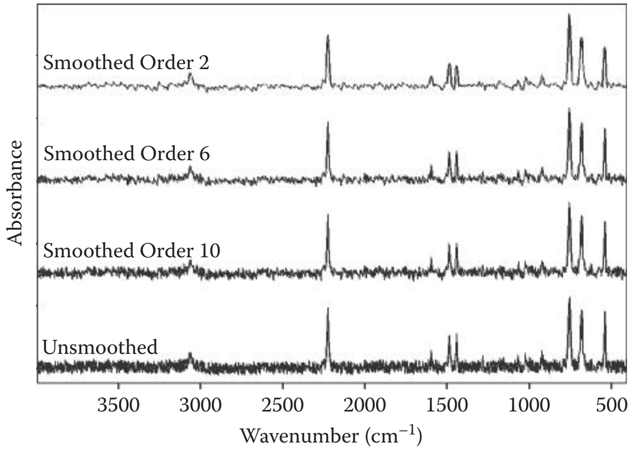


FIGURE 3.15 An example of how changing the order of the polynomial in Savitsky–Golay smoothing changes the smoothing results. The bottom spectrum is of benzonitrile and is unsmoothed. The other three spectra were smoothed using the Savitsky–Golay algorithm, 15 points, with polynomial orders of 10, 6, and 2, respectively. Note how the amount of smoothing goes up as the polynomial order goes down.

A drawback of smoothing is that it degrades a spectrum by causing the peaks to broaden. If a spectrum is oversmoothed, peak shapes can be distorted, and peaks can even merge together, as shown in Figure 3.16.

Note how in the top spectrum in Figure 3.16 the peaks are considerably broadened compared to the bottom spectrum. Also, the broadening is significant enough that the pairs of peaks around 1450 and 725 cm^{-1} have grown together, destroying important spectral information. You can hardly tell that the two spectra in Figure 3.16 are of the same molecule!

To prevent oversmoothing from affecting your data, start with a small number of data points in the smoothing window. Increase this number in small increments, and

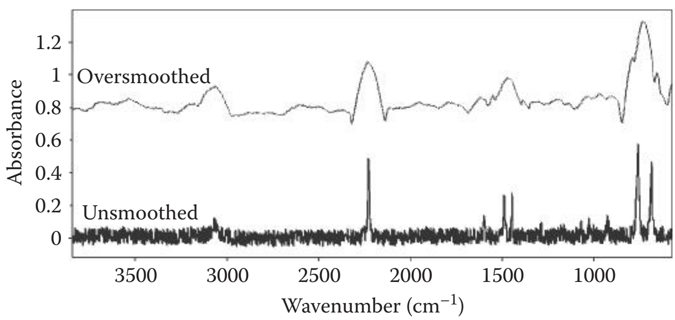


FIGURE 3.16 Bottom: A noisy spectrum of benzonitrile. Top: The same spectrum oversmoothed. Note how broadened the peaks are and that some peaks have merged together (99-point Savitsky–Golay smooth, 2nd order).

keep a close eye on the peaks in the smoothed spectrum. If the peaks start getting noticeably broadened, or if peaks start merging together, you need to reduce the size of the smoothing window.

V. SPECTRAL DERIVATIVES

Calculus tells us that the slope of any mathematical function can be determined by calculating its first *derivative* [9]. Since an infrared spectrum is a mathematical function, derivatives of infrared spectra can be calculated, as illustrated in Figure 3.17.

The two sides of an absorbance band have different slopes; one is positive and the other is negative, which is why the derivative in Figure 3.17 has positive and negative lobes. At the top of an absorbance peak the spectrum is horizontal and the slope of a horizontal line is zero, as illustrated by the intersecting dotted lines in Figure 3.17. First derivative algorithms can be used to pick peaks by calculating a spectrum's derivative and finding where it equals zero.

One thing that affects the look of derivative features is whether the spectrum being differentiated is plotted in absorbance or transmittance. Figure 3.17 shows an absorbance band and its first derivative. Figure 3.18 shows the same peak plotted in percent transmittance and its first derivative.

Note in Figure 3.17 that the first derivative of an absorbance peak has a negative and then a positive lobe when viewing it from left to right along the x-axis, whereas in Figure 3.18 the first derivative of a percent transmittance peak has a positive lobe and then a negative lobe. Another thing that affects the look of a derivative is whether the spectrum being processed has high wavenumbers plotted to the left or the right. The point is that both the y-axis units and x-axis orientation affect what a derivative looks like, and when comparing derivatives to each other you have to make sure these things are held constant to get a true comparison.

Note that the baselines of the first derivatives shown in Figures 3.17 and 3.18 fall at zero. Recall that adding or subtracting a constant from a spectrum's y-axis values causes baseline offset. Calculus tells us that the first derivative of a constant is zero [9]. Thus, first derivatives do not contain offset and their baselines fall at zero. They

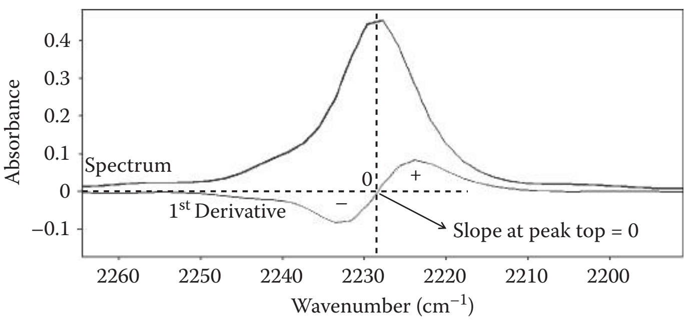


FIGURE 3.17 Top: Part of the spectrum of benzonitrile plotted in absorbance. Bottom: The first derivative of this spectrum (Savitsky–Golay algorithm, 2nd order, 5 points). The intersecting dotted lines indicate that the top of the absorbance band has a slope of zero.

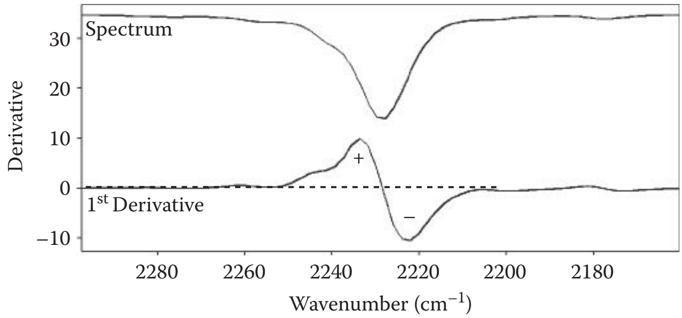


FIGURE 3.18 Top: A benzonitrile peak plotted in percent transmittance. Bottom: Its first derivative (Savitsky–Golay algorithm, 2nd order, 5 points).

can then be used in library searching to give better search results, and in quantitative analysis to give better calibrations. There are a number of different algorithms used to calculate spectral derivatives, but a detailed discussion of them is beyond the scope of this book [2,4,6]. Perhaps the most commonly used derivative technique is the Savitsky–Golay algorithm [7].

Some derivative algorithms can be used to calculate derivatives of different orders. For example, the derivative of a first derivative is a second derivative. The second derivative of a function measures its concavity, which is a measure of the direction of the curvature of a function. You can visualize the concavity of a spectrum by thinking about pouring water on it. The places where the water collects are concave up like a bowl. The places from which the water drains are concave down like a hillside. Concavity can also be thought of as a measure of the change in slope of a function. The second derivative of an absorbance feature is shown in Figure 3.19.

Note that the second derivative has three lobes in it. As we proceed from left to right along the x-axis, the value of the second derivative goes from positive to negative to positive because the concavity of the spectrum follows the same pattern. Note that the vertical dashed line in Figure 3.19 passes through the maximum of the

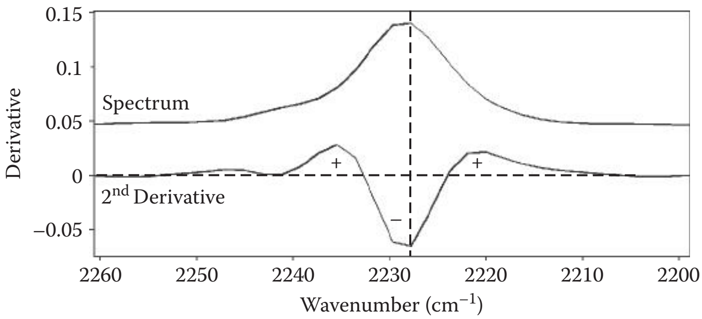


FIGURE 3.19 Top: A benzonitrile peak plotted in absorbance. Bottom: Its second derivative (Savitsky–Golay algorithm, 2nd order, 5 points).

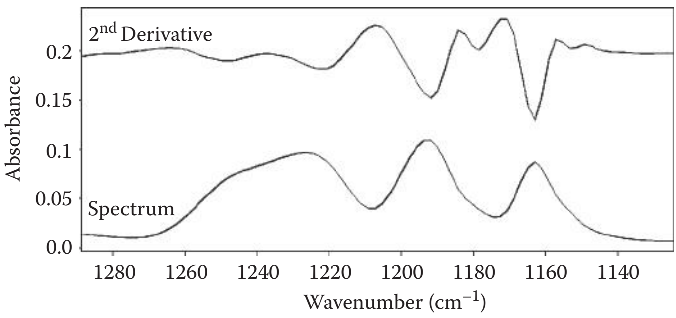


FIGURE 3.20 Bottom: Part of the absorbance spectrum of a mixture of polystyrene and a polycarbonate. Top: The second derivative of this spectrum (Savitsky–Golay algorithm, 2nd order, 5 points). Note how the negative lobes of the 2nd derivative point at the peaks in the spectrum.

absorbance peak and through the minimum in its second derivative; the minimum in a second derivative corresponds to the peak maximum in the peak from which it was calculated. This is why second derivatives are used in peak picking, peak identification, and library searching. One of the beauties of second derivatives is that they can be useful in pulling apart overlapped features as well, as illustrated in Figure 3.20.

The spectrum at the bottom of Figure 3.20 is of a mixture of polystyrene and a polycarbonate. In Figure 3.20 there are six negative lobes in the second derivative in this wavenumber region, indicating that there are six peaks in this region of the spectrum located at the same wavenumbers. This happens because of how the presence of shoulders and overlapped peaks affects the change in slope of the spectrum. Thus second derivatives can be used to pull apart overlapped features, determine how many peaks have grown together to make up a feature, and where those peaks are located.

The second derivative measures the change in slope of a function, and since constants have no change in slope their second derivative is zero. Similarly, a straight line has a constant slope, thus no change in slope, hence a second derivative of zero. This means that second derivatives contain no slope or offset, as illustrated in Figure 3.21.

The areas of the features in derivative spectra are proportional to the area of the peaks in the absorbance spectrum from which they were calculated. Hence, the size of features in derivative spectra calculated from absorbance peaks is proportional to concentration. Thus derivatives can be used to obtain quantitative calibrations. First derivatives are used on data to remove offset from standard spectra, and second derivatives are used to remove offset and slope [10].

A problem with derivative spectra is that they contain more noise than the spectra from which they are calculated. This means in general a spectrum needs to have a good SNR to give a usable derivative. The Savitsky–Golay derivative algorithm [7], among others, allows some smoothing to be applied while the derivative is calculated. This reduces the noise level in the derivative, sometimes making certain peaks easier to see. However, there is also a concomitant broadening of peaks as a result of the smoothing.

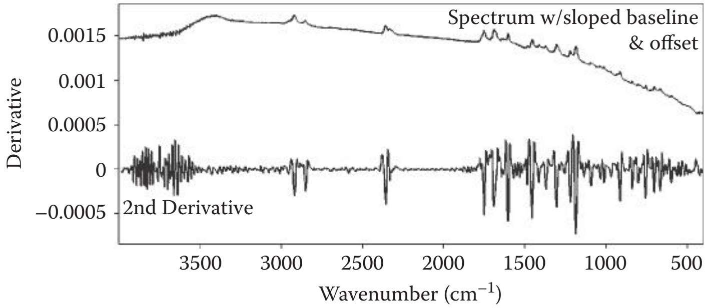


FIGURE 3.21 Top: An absorbance spectrum with slope and offset in it. Bottom: The 2nd derivative of this spectrum. Note that the 2nd derivative has no slope or offset (Savitsky–Golay algorithm, 2nd order, 13 points).

VI. DECONVOLUTION

In infrared spectra, two or more peaks can by chance fall on top of each other. A spectral processing technique called *deconvolution* (sometimes called Fourier self-deconvolution or FSD) can be used to mathematically enhance the resolution of a spectrum, allowing us to pull apart overlapped peaks to more clearly see what is going on in a crowded spectrum [2,4,11]. A group of overlapped peaks potentially in need of deconvolution is shown in the bottom of Figure 3.22.

The top spectrum in Figure 3.22 is a successful deconvolution of the spectrum found in the bottom of the figure. Deconvolution has sharpened the peaks, making features previously hidden by overlap easier to see. There are now six peaks in this region, but there were only four in the original spectrum. The deconvolved spectrum contains more peaks and hence more information than the original spectrum. So, when used properly, deconvolution enhances the information content of a spectrum. The deconvolution process is illustrated in Figure 3.23.

A section of a spectrum with overlapped peaks is shown in the upper-left-hand corner of the figure (this is the same spectrum shown in the bottom of Figure 3.22).

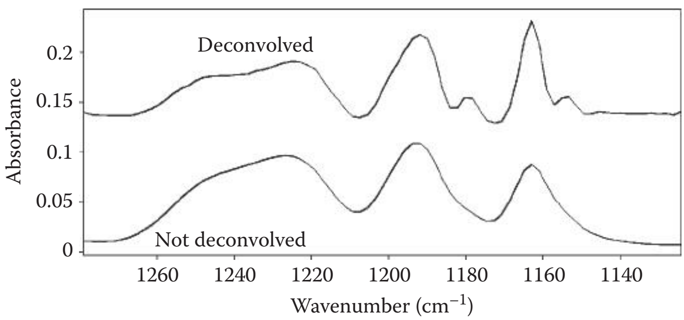


FIGURE 3.22 Bottom: A spectral region with overlapped peaks. Top: After deconvolution, the number and position of the peaks in this region are easier to see.

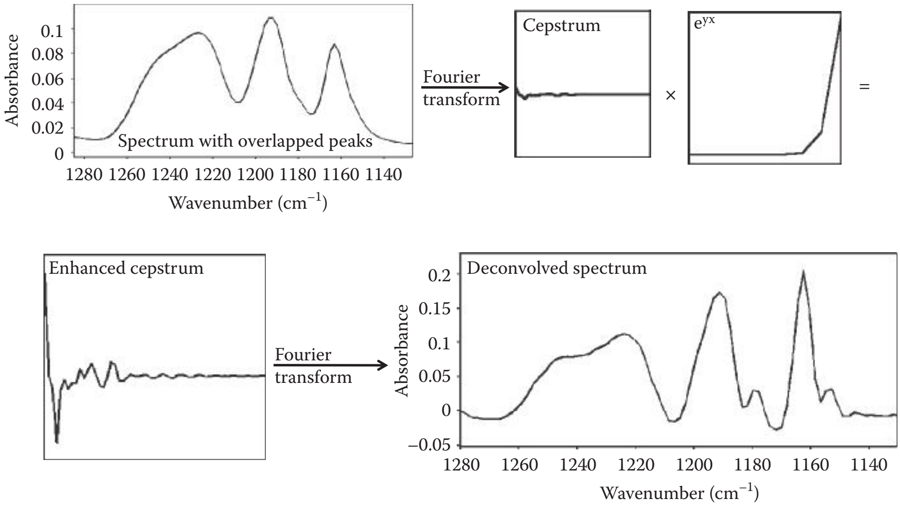


FIGURE 3.23 A schematic diagram of the deconvolution process.

This spectral section is Fourier transformed to give a mathematical function called a *cepstrum* [12] as shown in the top of Figure 3.23. A cepstrum is similar to but not identical to an interferogram. To perform a deconvolution we take advantage of one of the properties of cepstra illustrated in Figure 3.24.

The cepstrum in the top of Figure 3.24 is of a broad infrared band, and note that its intensity decays rapidly going from left to right. The cepstrum in the bottom of

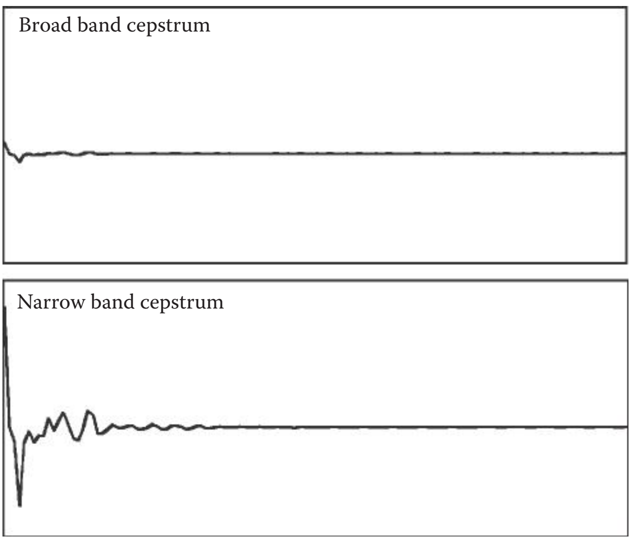


FIGURE 3.24 The cepstra of a narrow and a broad infrared band. Note that the features in the narrow band cepstrum are larger than the features in the broad band cepstrum.

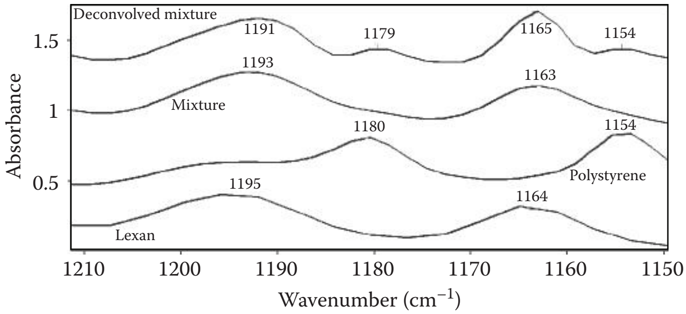


FIGURE 3.25 Bottom: The spectrum of the polycarbonate Lexan® from 1210 to 1150 cm^{-1}. Next to bottom: The spectrum of polystyrene. Next to top: A spectrum of a Lexan and polystyrene mixture. Top: A deconvolved spectrum of this mixture.

Figure 3.24 is of a narrow infrared band, and note how its intensity decays slowly. The idea behind deconvolution is to make the cepstrum of a broad band look like the cepstrum of a narrow band. This is accomplished by multiplying the broad band cepstrum by an exponential function, $e^{\gamma x}$, where X is optical retardation and γ is related to the amount of resolution enhancement. This makes the broad band cepstrum look like a narrow band cepstrum, as shown in the bottom Figure 3.23. This enhanced cepstrum is Fourier transformed to produce a spectral region with narrower peaks than the original, as is also illustrated in Figure 3.23.

Thanks to the resolution enhancement powers of deconvolution, the deconvolved spectrum in Figure 3.23 has more peaks in it than the original spectrum. Are these new features real? This question is answered in Figure 3.25.

The bottom spectrum in Figure 3.25 is of the polycarbonate Lexan®. Just above this is the spectrum of polystyrene. Note that Lexan has peaks at 1195 and 1164 cm^{-1} while polystyrene has peaks at 1180 and 1154 cm^{-1}. A spectrum of a mixture of these two polymers is shown next to the top in Figure 3.25. One might think that this spectrum would contain four peaks, two from each of the components in the mixture. However, only the Lexan peaks at 1195 and 1164 cm^{-1} are seen because they are large enough to mask the polystyrene peaks in this region. The top spectrum in Figure 3.25 is a deconvolved spectrum of the Lexan/polystyrene mixture. There are now four peaks visible at 1191, 1179, 1163, and 1154 cm^{-1}, and these peaks line up well with those in the spectra of Lexan and polystyrene. We can see the polystyrene peaks in the deconvolved spectrum even though they were masked in the original spectrum. This illustrates how deconvolution can find real infrared peaks that are not normally visible due to spectral overlap, and that deconvolution can be used to successfully analyze the spectra of mixtures.

A. Guidance, Precautions, and Limitations

For all its utility in pulling apart overlapped peaks, if deconvolution is not performed properly it can distort and destroy spectral data. This discussion will give you guidance on how to use deconvolution properly, and familiarize you with its limitations. Recall from Chapter 2 that all FTIR spectra are measured at a given instrumental

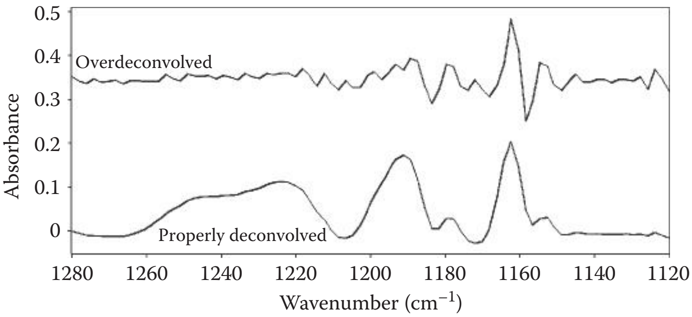


FIGURE 3.26 Bottom: A properly deconvolved spectrum. Top: An overdeconvolved version of the same spectrum.

resolution, and that the instrumental resolution also determines the data point spacing of a spectrum. For example, a spectrum measured at 8 cm^{-1} resolution will nominally have a data point every 8 wavenumbers. When deconvolving this spectrum you cannot mathematically enhance its resolution beyond 8 cm^{-1} because a peak cannot be narrower than its data point spacing. In essence, deconvolution is applied to peaks broader than the instrumental resolution used to measure the spectrum, and peaks cannot be made narrower than this resolution.

It is not obvious after looking at a spectrum how much resolution enhancement is appropriate. Deconvolution software allows you to adjust the resolution enhancement factor of a spectrum an arbitrary amount, which can result in hundreds or thousands of new peaks appearing. This phenomenon is called overdeconvolution, and it is illustrated in Figure 3.26.

The bottom spectrum in Figure 3.26 is the result of a proper deconvolution. The top spectrum is overdeconvolved, and it is not obvious what peaks are real. Because of the nature of the mathematics of the Fourier transform, baseline undulations are introduced into deconvolved spectra. If these undulations are large enough they can be mistaken for sample peaks, which is partially why the spectrum in the top of Figure 3.26 has so many features. Thus, while adjusting the resolution enhancement factor keep your eye on the baseline and never use a value that gives visible baseline undulations. Another problem with the overdeconvolved spectrum in Figure 3.26 is noise. When the sample cepstrum is multiplied by an exponential function during the deconvolution process, the intensity of the cepstrum is enhanced—and so is its noise. As a result, deconvolved spectra are noisier than normal spectra. This is partially why the overdeconvolved spectrum in the top of Figure 3.26 bears little resemblance to the properly deconvolved spectrum in the bottom of Figure 3.26. When deconvolving, limit yourself to resolution enhancement factors that introduce little or no noise to the result. In general, you will get better results by deconvolving spectra that have a high SNR to begin with.

The top spectrum in Figure 3.26 is ample proof that overdeconvolution should be avoided. An excellent way to prevent this is to examine the second derivative of a spectrum *before* deconvolving it. As seen in Figure 3.19 the negative lobe in a second derivative occurs at the same wavenumber as the top of the absorbance peak from which it was calculated. Second derivatives are also used to help locate overlapped peaks as

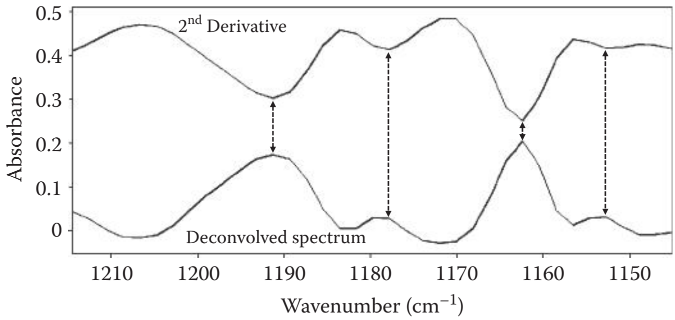


FIGURE 3.27 Bottom: The deconvolved spectrum of the Lexan/polystyrene mixture. Top: The 2nd derivative of the original mixture spectrum. Note how the negative lobes in the 2nd derivative point at the four peaks in the deconvolved spectrum.

illustrated in Figure 3.20. In general, the downward-pointing features in a second derivative will give the number and position of the peaks for a group of overlapped bands. One should only deconvolve a spectrum until one sees the features that the second derivative tells you are real. How to go about this is illustrated in Figure 3.27.

The bottom spectrum in Figure 3.27 is the deconvolved spectrum of the Lexan/polystyrene mixture shown in Figure 3.25. The second derivative of the Lexan/polystyrene mixture spectrum is shown in the top of Figure 3.27. Note how there are four features in the second derivative pointing down at the four peaks in the deconvolved mixture spectrum. When the peaks seen in the second derivative appeared in the deconvolution result, the process was stopped. This is an indication of the power of second derivatives in helping pull apart overlapped peaks and as a guide in deconvolution.

Another feature of the deconvolution process is that in a group of overlapped bands, the narrower bands appear first as resolution is enhanced. For example, water vapor lines are very narrow, and if the band being deconvolved overlaps with these lines, they will appear in the deconvolved spectrum before all other features. This can be misleading since one may mistake the plethora of sharp bands from water vapor as being from your sample. When interpreting the results of deconvolutions performed on regions where atmospheric gases absorb, make sure the features you see are not due to carbon dioxide or water vapor. This can be accomplished by comparing the deconvolved spectrum with a spectrum of the atmosphere.

In summary, it has been shown that deconvolution can be used to pull apart overlapped features and help in the interpretation of mixture spectra. However, remember the limitations of this technique and the precautions that must be followed to use it properly. On the whole, deconvolution is a useful spectral manipulation technique when used with proper precautions.

VII. SPECTRAL LIBRARY SEARCHING

Visually comparing unknown and reference spectra to each other to aid in interpretation is part of infrared spectroscopy. In the days before personal computers, collections of reference spectra called *spectral libraries* were plotted on paper and kept in three-ring binders. These collections were searched by eye, with sometimes hundreds or thousands of spectra needing to be viewed to find a match. Needless to say, this was tedious. Today, spectral libraries in digital form can be purchased or constructed by FTIR users. Computers are used to mathematically compare an unknown spectrum to these libraries in a matter of seconds. This technique of computerized spectral comparison is called *spectral library searching* or just *library searching* for short. Library searching is as an interpretation aid and is a powerful tool for the analysis of mixtures.

The first things needed to perform a library search are the libraries themselves. These libraries may be stored in your hard disk, CD, your company's network, or the Internet. There are several commercial sources of infrared spectral libraries. Some FTIR library vendors sell large collections of spectra that can be searched by commercial FTIR software packages. These and other companies also sell smaller databases of spectra organized by sample type, application, functional group, or sampling technique. For example, there are polymer, inorganic, gas phase, pharmaceutical, forensic, ATR, and ester libraries available. Perhaps the largest vendor of infrared commercial libraries is the Informatics division of the Bio-Rad Corporation, sometimes known as the Sadtler Division of Bio-Rad (Philadelphia, PA). The Sadtler Research Co. was one of the first to start compiling and cataloging paper plots of spectra in the middle part of the 20th century. With the advent of personal computers, Sadtler digitized their data, and they now have over 220,000 spectra available, which they claim is the largest collection in the world. The entire database is available for searching by subscription, or smaller libraries for specific purposes can be leased or purchased.

Another important supplier of infrared spectral libraries is Aldrich Chemical (Milwaukee, WI). They have over 70,000 spectra available, including libraries organized by application and sample type. An interesting thing Aldrich has done with their database is make it available over the internet at www.ftirsearch.com. For a small fee, anyone can search the Aldrich database, compare library and unknown spectra, and download and plot the results. In addition to Sadtler and Aldrich, there are other vendors of FTIR libraries generally selling smaller, more specialized collections of spectra, including S.T. Japan-USA and Fiveash Data Management. The Web sites of FTIR library vendors can be found via Internet search.

In addition to purchasing FTIR libraries, an important source of infrared spectral libraries is you. Most FTIR software packages will allow you to build your own libraries. This is a good idea because only you have access to the samples that are typical of your work. Every time you obtain a good identification of a sample you should put its spectrum in a library. The next time you analyze a similar sample the library search will quickly help you identify the unknown, saving time and money. You can also build multiple libraries of your own, organizing them by type of sample. However, you do have to be careful when building your own libraries because any spectrum you place in a library becomes a de facto reference. If your library spectra are noisy, have baseline issues, or are incorrectly identified, they will degrade the quality of your library searches and possibly lead to misidentifications. Only high-quality spectra whose identification you are certain of should be placed in a user-constructed infrared spectral library. Additionally, all the spectra placed in a given library should be measured at the same instrumental resolution and using the same wavenumber range. The author has found that for many labs a combination of relevant commercial libraries and user-built libraries works well.

A. The Search Process

Most commercial FTIR software packages come equipped with a library searching capability, or it can be purchased for an additional fee. When a spectral library search is performed, the unknown spectrum is compared to each spectrum in the libraries selected. Thus, if there are 1000 spectra in a library, 1000 comparisons are performed. As a result of each comparison a number called the *hit quality index* (HQI) is calculated, which is a numerical measure of the similarity between two spectra. In an ideal world the library search will turn up a spectrum similar to the unknown spectrum. It is then assumed the unknown sample is chemically similar to the library sample. This way known spectra can be used to identify unknown spectra. Library searching is so useful in identifying unknowns and interpreting mixture spectra that I encourage *all* FTIR users to have access to library searching capabilities.

There are different types of library searches possible that are, in part, a function of the software package you are using. A *text search* uses keywords to find spectra in a library and is very useful if you know the name of the spectrum you are seeking. For example, if you need to look up a reference spectrum of acetone, you could simply type this word into a dialog box and the software will show you any spectra of acetone that may be contained in a library. Some libraries also contain physical property information such as melting points. So, if you had a sample with a melting point of 140°C you could look up the spectra of all materials with that melting point and compare your unknown spectrum to them.

Another type of library searching is called peak searching. In this case the peak positions in your unknown spectrum are determined by an algorithm and are compared to the peak positions in the library spectra determined by the same algorithm. This type of search was very popular in the past because it was fast and used very little computer memory. However, by only looking at the peaks in a spectrum, thousands of data points are ignored, reducing the accuracy of search results. With the advent of fast and powerful personal computers with large memories, there is no longer a need to perform peak searches to save computer time. However, peak searching can be useful if a spectrum is of very low quality. In this case by just including the peak positions in a search and leaving out all the bad data points, search quality may be enhanced. The type of library search most typically performed by FTIR search software packages is called a *full spectrum search*. In this type of search all the data points in a spectrum are used. Since more data are normally better, this type of search typically provides a more accurate comparison than peak searching.

Before a library search can be performed, a number of pieces of information must be entered into the software. Your unknown spectrum should be in absorbance since it will be compared to library spectra also measured in absorbance. Some software packages may be smart enough to convert a transmittance spectrum to absorbance before spectral comparisons are performed, but it can not be guaranteed. The actual libraries to use in a search must also be chosen. It is tempting to search every unknown spectrum against every spectral library you own. However, this can waste search time and dilute the quality of your results. It is best to take a few moments to think about what libraries might contain spectra that are potentially well matched to your unknown. For example, if your sample is a polymer it makes sense to search its spectrum against a polymer library, but not against a vapor phase library, as no polymers, to the best of the author's knowledge, exist in the vapor phase at room temperature.

Another thing to consider when setting up a library search are the parts of the spectrum to include in the search. By default a full spectrum search uses the entire spectrum. However, some FTIR search software packages allow you to include or exclude regions of a spectrum from a search. This is a good idea if there are spectral regions that contain large water vapor and CO_2 peaks, lots of noise, artifacts, or baseline problems. For example, if a mixture spectrum contains a known component, its peaks can be eliminated from the search to more effectively identify the unknown components in the sample.

In many FTIR search software packages the unknown spectrum is processed before the spectral comparisons are performed; this is why the topic of library searching is being included in a chapter on spectral processing. The purpose of spectral processing prior to library searching is to remove information from a spectrum that does not relate to the chemical composition of a sample. For example, baseline slope and curvature in a spectrum are frequently caused by problems with sample preparation or with the FTIR itself. These problems can mask important chemical information, and their variability across spectra can hurt the quality of search results. It is best, then, to remove baseline problems from spectra before comparisons are performed. Baseline correction algorithms, similar to the ones discussed earlier in this chapter, are applied automatically to unknown spectra and library spectra before comparison in an attempt to remove the impact of baseline problems on search results.

Another spectral processing step performed prior to library searching is *normalization*. In this step all the absorbances in a spectrum are divided by the largest absorbance in present, as shown in Equation 3.4.

$$A_n = A_i/A_{max} \tag{3.4}$$

Where

A_n = Normalized absorbance value
A_i = Un-normalized absorbance value at given wavenumber i
A_{max} = Maximum absorbance in a spectrum

Applying Equation 3.4 to a spectrum gives a new spectrum with a y-axis scale of zero to one. An example of a normalized spectrum is shown in Figure 3.28.

In this particular example, the biggest peak in the original spectrum had an absorbance of 0.308. When all the other absorbances in the spectrum are divided by this number, the biggest peak takes on a value of 1.0 (0.308/0.308 = 1.0). The absorbances at all other wavenumbers are less than 0.308, so their normalized values are less than one. Normalization is performed for two reasons. First, it is best to compare spectra

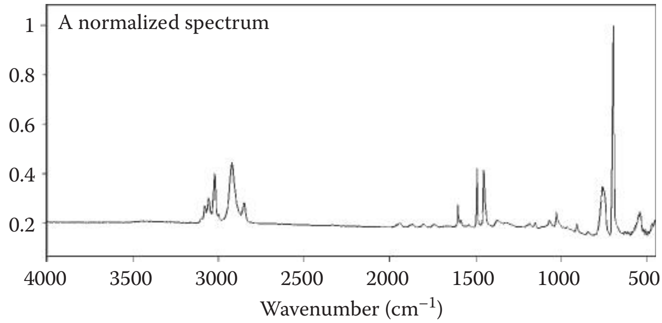


FIGURE 3.28 An example of a normalized infrared spectrum, a spectral processing step frequently used before library searching. Note the zero to one y-axis scale.

with a common intensity scale, and normalization provides this since library spectra and unknown spectra are typically normalized before spectral comparisons are performed. Second, spectral normalization removes the effect of varying pathlengths on the data. Recall from Equation 1.6 that absorbance is proportional to pathlength, which is a physical property of a sample that tells us nothing about chemical composition. Its variability across samples can cause peak intensities to vary and throw off the quality of matches. Thus, this information is best removed from spectra that are being compared. In general, for any two absorbances in a given spectrum the pathlengths are the same. When these two absorbances are divided as is done in normalization, the pathlengths in the numerator and denominator are the same and will cancel. This is why normalized spectra are pathlength independent and hence rendered more appropriate for spectral library searching. Most FTIR software search packages automatically baseline correct and normalize a sample spectrum before library searching. Most spectral libraries contain spectra that have already been baseline corrected and normalized.

Once all the needed information has been entered into the software and the spectral pre-processing is completed, the actual spectral comparisons begin. The mathematical comparison between two spectra is performed using a *search algorithm*. There are a number of search algorithms available depending upon the FTIR search software you use. A detailed explanation of the mathematics behind most search algorithms is beyond the scope of this book. The help functions in some search software packages provide a good introduction to the mathematics of search algorithms [13]. Search algorithms differ in how much they emphasize peak position versus intensity. Two search algorithms that are commonly used are called Correlation and Euclidean Distance. These two algorithms weigh peak intensity and positions equally. Any search algorithm with the word “derivative” in it compares the first or second derivative of spectra to each other. Any search algorithm with the word “derivative” in it will emphasize peak position over intensity. As discussed earlier, derivative spectra are free of baseline slope and offset, and derivative search algorithms can work well with data with baseline problems.

A search algorithm whose mathematics is understandable, called the “absolute value” algorithm, is based on spectral subtraction. This algorithm works by subtracting

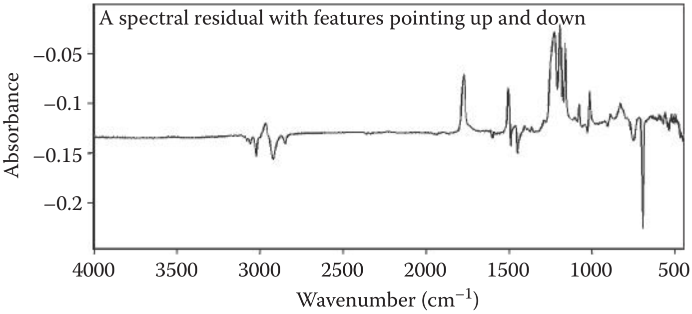


FIGURE 3.29 A spectral residual calculated by subtracting two spectra from each other. Note the spectral features pointing up and down.

the unknown spectrum from a library spectrum to produce a spectral subtraction result called a *residual*. An illustration of a residual is shown in Figure 3.29.

Note that there are features in Figure 3.29 pointing up and down. This is because at some wavenumbers the unknown spectrum is less intense than the library spectrum, and at other wavenumbers the unknown is more intense than the library spectrum. The sizes of the features in a residual are a measure of spectral similarity. The bigger the difference between two spectra, the greater the size of the features in their residual; the smaller the difference, the smaller the features in their residual. To quantify the features in a residual, the absolute values of the absorbances in the residual are calculated, giving all positive values (hence the name of the algorithm). These are then added together to give a single number, which is divided by the total number of data points used in the comparison. The formula for this calculation is as follows [13]:

$$\mathrm{HQI} = (\Sigma_i^n |\mathrm{Lib}_i - \mathrm{Unk}_i|)/n \tag{3.5}$$

Where

HQI = Hit Quality Index
Lib_i = Absorbances in library spectrum
Unk_i = Absorbances in unknown spectrum
n = Number of data points in the spectrum
i = Index of data points

Equation 3.5, calculates the average absolute absorbance in the residual, from which the HQI is determined. The absolute value algorithm and any algorithm with the word “absolute” in its name emphasize peak intensities over peak positions.

B. Interpreting Library Search Results

When the search algorithm compares the unknown spectrum to each library spectrum, a large number of hit quality indices will be calculated. Sorting through these numbers could be a difficult task, but fortunately FTIR search software programs

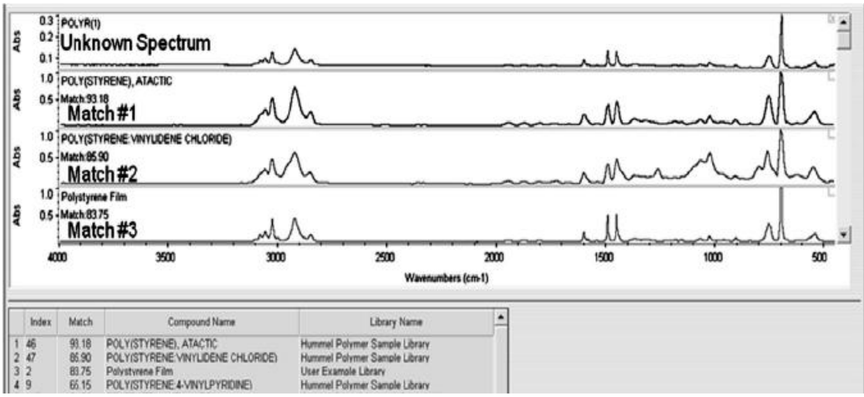


FIGURE 3.30 A library search report. The Correlation search algorithm was used, and the HQI for a perfect match is 100.

use a *search report* to organize and display the best library matches for you. An example of a library search report is shown in Figure 3.30.

Typically, an FTIR search report will include a table listing the best matches along with their HQIs and the library where they were found. Additionally, the spectra of several of the best matches will be displayed. It is vital that the information in a search report be interpreted properly. The author has seen FTIR users perform a search, look at the best match in the search report, and declare "that's the answer" without looking at the HQI or the spectra themselves. This is a recipe for disaster.

We need to be very clear about the meaning of the HQI. Some FTIR search software packages use an HQI indexing system that goes from 0 to 100. It is then tempting to interpret an HQI of 95 as meaning there is a 95% probability that you have found the right answer, or that the two spectra are 95% the same. Both of these conclusions are wrong. The HQI is a number whose value depends upon the parameters used in a search. For example, the HQI from the comparison of two specific spectra will depend upon the search algorithm, spectral pre-processing steps, and wavenumber regions used in the search. The HQI is not an absolute measure of anything; it is a relative measure of spectral similarity for a given search and should never be interpreted outside that context. Noise and artifacts can make spectra look more different or more similar than they are. Thus it is vital that for every search report you view, ALWAYS VISUALLY COMPARE the spectrum of your unknown to the library spectra. If your visual comparison of two spectra tells you one thing and the HQI tells you another, trust your visual comparison. The purpose of the HQI is to order the matches for you for a given search. It is your job as the FTIR user to decide for yourself whether something is a good match or not.

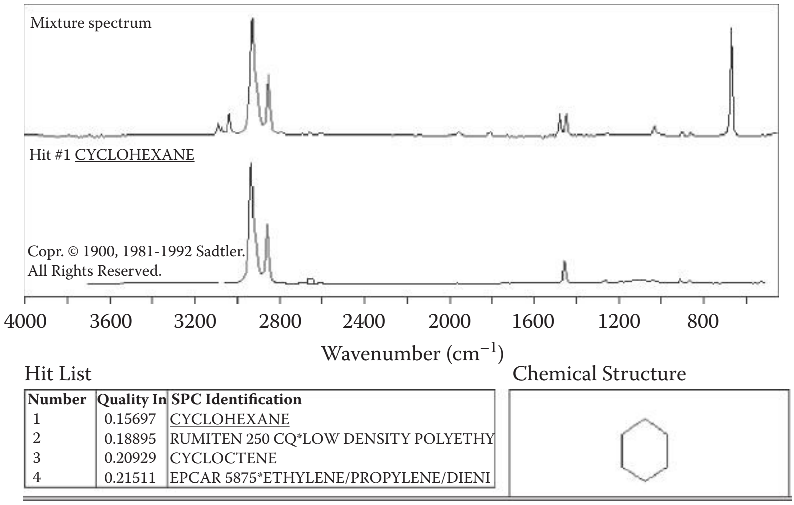
What then does the HQI mean? Unfortunately, different software vendors use different HQI indexing systems. Values of 0, 1, 10, 100, and 1000 have all been used to denote a perfect match, and in some software packages different search algorithms use different numbering systems! So, you need to read the manual for your FTIR software package to determine what the HQI values really mean. That being said, if your search software HQI indexing system has a power of 10 as a perfect match (1, 10, 100, 1000) it is possible to ascertain the overall quality of a match from the HQI. A hit greater than 0.8, 8, 80, 800, (depending on indexing system) is good and means the two spectra being compared are very similar. It may be possible to get a complete identification with a match of this quality. For medium quality hits (0.5 to 0.8, 5 to 8, 50 to 80, or 500 to 800, depending on indexing system) this means the spectra are somewhat similar. A complete identification is probably not possible, but the presence of certain functional groups may be inferred from the match. For poor hits (less than 0.5, 5, 50, or 500, depending on indexing system) the two spectra are probably quite different, and there is normally very little useful information available from such a low-quality match.

Another thing to look at in a search report is the change in HQI for consecutive hits. Any change of greater than 10% from one hit to another is considered significant. For example, if two consecutive hits had values of 95 and 92 (assuming 100 = perfect match) they are ~3% different and for most purposes should be considered equally likely candidates to be the best match. On the other hand, if two consecutive hits had values of 95 and 80, this is ~16% change. When there is a greater than 10% change in HQI between consecutive hits, it should be considered significant. An imaginary line drawn at the first change of HQI of >10% in a search report can then be used to divide hits into different quality classes. The hits above this line are much more likely to provide a good match than the hits below this line.

What should you do about poor search results? You could panic, but that is never an appropriate response for a scientist. The answer is to experiment with the search parameters. Try changing search algorithms, adjusting the spectral regions included in the search, or searching different libraries. The probability of finding a library spectrum similar to your unknown increases with the number of spectra you search against. Ultimately, though, you need to realize that poor library search results are not necessarily your fault. If by chance there are no spectra similar to your unknown in your libraries, you will get poor HQIs regardless of what you do. The best way around this problem is to have access to a large number of library spectra.

VIII. ANALYSIS OF MIXTURES: SUBTRACT AND SEARCH AGAIN

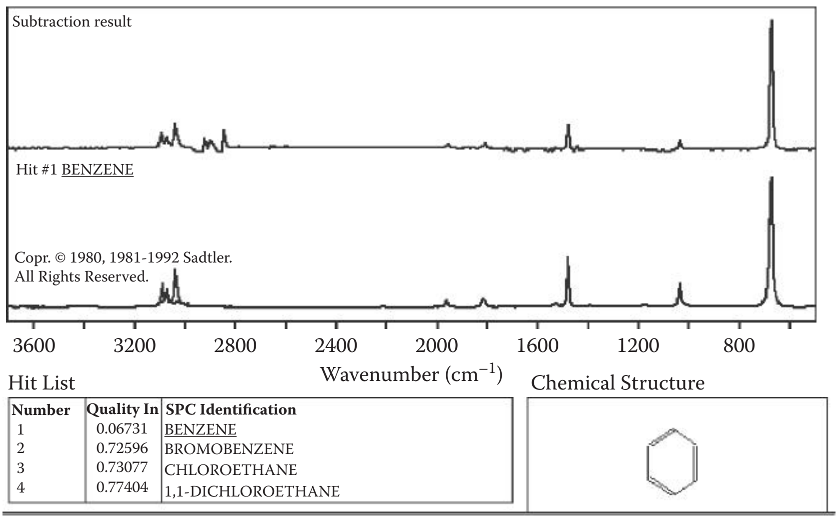
A powerful way of using a library search to analyze mixtures is to combine it with spectral subtraction in a technique called *subtract and search again*, which works as follows. First, a library search using a mixture spectrum as the unknown is performed. Ideally, the search allows you to identify one of the components in the mixture. This can be done if, for example, all the peaks in the library spectrum are also present in the mixture spectrum. If your FTIR search software package allows, use the library spectrum of the identified mixture component as the reference spectrum in a spectral subtraction. Once the subtraction result is obtained, search it against your libraries ("search again"). This will hopefully identify a second component in the mixture. The subtract and search again process is illustrated in Figures 3.31 and 3.32.



Number	Quality In	SPC Identification
1	0.15697	CYCLOHEXANE
2	0.18895	RUMITEN 250 CQ*LOW DENSITY POLYETHY
3	0.20929	CYCLOCTENE
4	0.21511	EPCAR 5875*ETHYLENE/PROPYLENE/DIENI

FIGURE 3.31 The library search result for a mixture of cyclohexane and benzene. Note that cyclohexane is the best match (correlation algorithm, 0.0 is a perfect match).

Figure 3.31 shows a spectrum of a mixture of cyclohexane and benzene. A library search of this spectrum produced cyclohexane as the best match. Note that all of the cyclohexane peaks are present in the mixture spectrum, indicating that this molecule is present in the sample. The cyclohexane library spectrum was used as the reference in a spectral subtraction. The result of this subtraction is shown in Figure 3.32 along with its search results.



Number	Quality In	SPC Identification
1	0.06731	BENZENE
2	0.72596	BROMOBENZENE
3	0.73077	CHLOROETHANE
4	0.77404	1,1-DICHLOROETHANE

FIGURE 3.32 The two spectra shown in Figure 3.31 were subtracted from each other, and the result was library searched to produce the search report seen here. Note that benzene is the best match (correlation algorithm, 0.0 is a perfect match).

Note that the best match in Figure 3.32 is benzene, and that all the benzene peaks are present in the sample spectrum. Thus, the subtract and search again method identified the two components in this mixture. This is a simple but real example of the power of this technique to analyze the spectra of mixtures.

Unfortunately, there are some limitations to the subtract and search again method. In practice, it is usually only possible to identify the two or three most concentrated components in a given mixture. The problem is that every time a subtraction is performed, noise and artifacts are added to the result. After performing multiple subtractions using the same sample spectrum, the result will be so noisy and full of artifacts that the quality of the subsequent library search is poor as well, limiting the ability to identify more components. Despite this limitation, the subtract and search again technique is a useful way of dealing with the problem of mixture spectra.

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4 Preparing Samples Properly

Properly preparing samples for FTIR analysis is half the battle in obtaining a good spectrum—this chapter will help you *win that battle* by emphasizing what techniques work best on what samples. You will learn to not waste time trying techniques that won't work. There are two broad families of infrared sampling techniques—transmission and reflectance—so the sections of this chapter are organized by sampling method and sample type. For example, there will be sections on the transmission sampling of liquids and reflectance sampling of solids. The chapter also contains convenient tables summarizing the advantages and disadvantages of each sample preparation technique.

I. TRANSMISSION SAMPLING OVERVIEW

In *transmission sampling* the infrared beam passes through a thin film of sample and then impinges on the detector as shown in Figure 4.1.

The letter L in the figure denotes the pathlength of the sample, which is the thickness of sample encountered by the infrared beam. Since the beam passes through the entire sample, bulk contributions are emphasized and surface contributions are minimized, as shown in Figure 4.1. Sample thicknesses of 1 to 20 microns are typical. Transmission sampling should not be confused with *transmittance*, which is a y-axis unit used to plot spectra, which was discussed in Chapter 1.

An important advantage of transmission sampling techniques is that they are practically universal; there are few sample types that will not yield usable transmission spectra. An additional advantage of transmission sampling is that the tools needed to prepare the samples are relatively inexpensive—in general, transmission sampling tools cost less than reflectance sampling tools. A third important advantage of transmission sampling techniques is that they often give spectra with a good signal-to-noise ratio, flat baseline, and clearly resolved sample features. Finally, many of these techniques have been in use for over 50 years, and there is a history and tradition behind them; people trust the results they obtain with transmission sampling. The advantages of transmission sampling are summarized in Table 4.1.

Given the litany of transmission sampling advantages, why would you bother preparing your samples any other way? Because transmission techniques suffer from what is called an *opacity problem*. Recall from Equation 1.6 that absorbance is proportional to the product of pathlength times concentration. If the product of pathlength times concentration is too small, the sample will not absorb enough light, and no spectrum will be detected. If the product of pathlength times concentration is too large, the sample will absorb all the light and the spectrum will contain

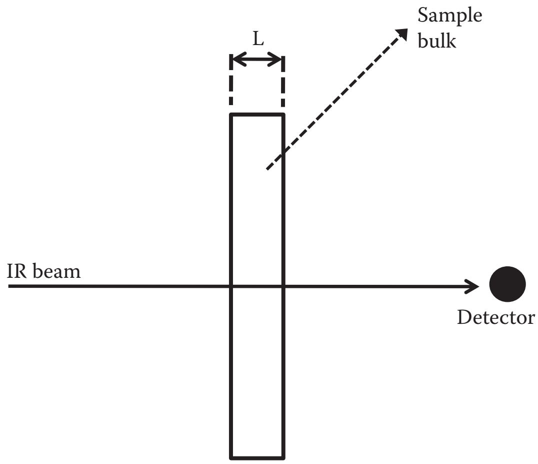

FIGURE 4.1 An illustration of transmission sampling, where the infrared beam passes through a thin film of sample before impinging on the detector. The letter L denotes the pathlength, which is the thickness of sample encountered by the infrared beam.

distorted peaks as seen and discussed in Chapter 1. The solution to these problems is to prepare the sample so its pathlength/concentration product produces peaks that are less than 2 absorbance units of greater than 10% T. The opacity problem exists because most materials absorb strongly in the mid-infrared, which is why only thin film samples from 1 to 20 microns thick can generally be analyzed. For many samples, particularly solids, getting them into thin film form is difficult. Grinding, squishing, diluting, and other types of tedious, manual sample preparation may be necessary. Typically, the only way to know if you have a sample with a usable pathlength/concentration product is to actually prepare the sample and measure its spectrum. If the product is too big or too small, you will have to start over and re-prepare the sample. Many tries and many minutes or hours of sample preparation ever may be necessary to get the sample right. If you have spent an entire afternoon trying to make a KBr pellet you know what I am talking about. In this day and age where all labs are expected to do more with less, spending minutes or hours preparing a single sample for FTIR analysis is not justifiable, particularly since there are reflectance techniques available that are frequently faster and easier to use.

TABLE 4.1
The Advantages and Disadvantages of Transmission Sampling

Advantages	Disadvantages
Works on many sample types	Opacity problem
Inexpensive tools	Trial and error may be involved
Quality spectra	May be time-consuming
History/tradition	May be destructive of sample

Another disadvantage of transmission techniques is that they can be destructive. Transmission techniques involve grinding, compressing, or dissolving the sample, all of which make it impossible to recover the sample intact. If there is not much sample, the sample is valuable, or if it needs to be preserved for some other purpose, this is a problem.

A. Windows, Cells, and Materials for Transmission Analysis

For transmission analyses the sample is frequently put into an infrared transparent cell, placed between infrared transparent windows, or mixed with infrared transparent powder. It is up to the FTIR user to choose the right infrared transparent material for a given sample. The ideal infrared transparent material would be usable over a large wavenumber range, not react with or dissolve in the sample, and be rugged, tough, and inexpensive. No one material meets all these criteria perfectly. For example, some materials absorb strongly above 400 cm^{-1}, some materials are water soluble, and others are quite expensive. This is why a number of different infrared transparent materials are used in transmission analysis. A listing of the more common materials and their relevant properties is given in Table 4.2.

The "Low cm^{-1} Cutoff" column in Table 4.2 indicates the lowest wavenumber to which a material is infrared transparent enough to allow usable spectra to be measured. For example, KBr begins to absorb strongly near 400 cm^{-1}, so spectra should not be measured beyond that point when working with it. The cost data in Table 4.2 is inexact because the prices of things vary over time. The number of dollar signs is meant to indicate the relative cost of these different infrared transparent materials.

Potassium bromide (KBr) is the most commonly used infrared transparent material. It is transparent over a broad spectral range, from 400 cm^{-1} up through the visible. KBr windows are commonly used in the beamsplitters found in FTIRs, and their low wavenumber cutoff normally determines the low wavenumber cutoff of the FTIR as well. KBr is also relatively cheap and is easy to machine into windows and cells. A major drawback is that it is highly polar, which leads to two problems. First, KBr is *hygroscopic*, which means it absorbs water from the atmosphere. Over time a thick layer of water can build up on the surface of KBr, masking sample absorbances. Therefore, KBr in all forms—windows, cells, and powder—should

TABLE 4.2
Infrared Transparent Materials Commonly Used in Transmission Analysis

Material	Low cm^{-1} Cutoff	Water Soluble?	Cost
KBr	400	Yes	$
CsI	200	Yes	$
AgCl	400	No	$$
AgBr	300	No	$$
ZnSe	700	No	$$$
Ge	600	No	$$$

be kept dry and away from atmospheric moisture. This is achieved by keeping the materials in a desiccator or in an oven at a temperature above 100°C to drive off absorbed water.

The second problem with KBr is that it is water soluble, so any sample that contains liquid water cannot be analyzed using this material. Cesium iodide (CsI) cuts off at 200 cm^{-1} and has a broader spectral range than KBr. However, it is more hygroscopic and expensive than KBr. It is typically used only when the sample has an important infrared feature between 400 and 200 cm^{-1}. The final four materials listed in Table 4.2 share the advantage that they are not water soluble, are not hydroscopic, and can be used with samples that contain liquid water. Their major disadvantage is that they are expensive.

II. TRANSMISSION ANALYSIS OF SOLIDS AND POWDERS

A. KBr Pellets

Potassium bromide (KBr) pellets are used to obtain the infrared spectra of powders and anything that can be ground into a powder. KBr is used because it is inert and transparent above 400 cm^{-1}. In this technique, KBr and sample particles are mixed together. The KBr acts as an infrared transparent diluent, helping reduce the product of pathlength times concentration to keep the sample peaks on scale. Pressure is applied to the KBr/sample mixture, which flows into a glass, embedding sample particles in it. The resulting pellet holds sample particles in the infrared beam, allowing them to be analyzed in transmission.

Here are the steps for preparing successful KBr pellets. First, the sample and the KBr must be ground to reduce the particle size to less than 2 microns in diameter. Particles bigger than this scatter the infrared beam, whose intensity increases with wavenumber and causes a sloping baseline, as shown in Figure 4.2.

Manual grinding is performed with an agate mortar and pestle, but mechanical grinding can also be used (see below). For manual grinding, a gram or so of KBr should be placed in the mortar and ground for at least 1 minute. While grinding you will notice that the size of the crystallites will decrease and the KBr will become pasty, stick to the sides of the mortar, and take on a dull appearance. This is due to a reduction in scattered light intensity, indicating you have reached the correct particle size. The KBr and the sample should be ground in separate mortars. If ground together the heat and pressure of grinding may cause the KBr and sample to react with each other, altering the chemical composition of the sample.

Once the grinding is accomplished the sample is diluted with the KBr; 1% to 10% sample should be sufficient. Weighing out the amounts gives more reproducible pellets. It is important that the sample be well dispersed in the KBr; the sample needs to be homogeneous to ensure that a typical spectrum is measured. An easy way to mix the sample and KBr together is to place them in a mortar and stir them together with a spatula for one minute. Next, the sample/KBr mixture is placed in a press and put under 5 to 10 thousand PSI of pressure to create the pellet. There are a number of different presses on the market used to make KBr pellets. A "bolt press" consists

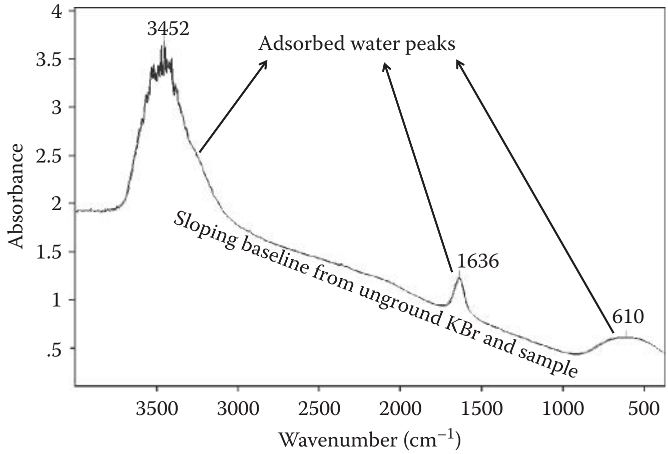

FIGURE 4.2 The spectrum of a poorly prepared KBr pellet. The sloping baseline is from scattering caused by sample and KBr particles being too large. The peaks around 3540, 1630, and 610 cm^{-1} are due to water adsorbed on the KBr.

of a stainless steel barrel, two stainless steel bolts, and a device that slides into the sample compartment to hold the barrel, as shown in Figure 4.3.

Bolt presses provide a simple and inexpensive way to make KBr pellets. To use this device, one bolt is screwed about halfway into the barrel. The barrel is held upright, and enough KBr/sample mixture is added to cover the face of the bolt. The second

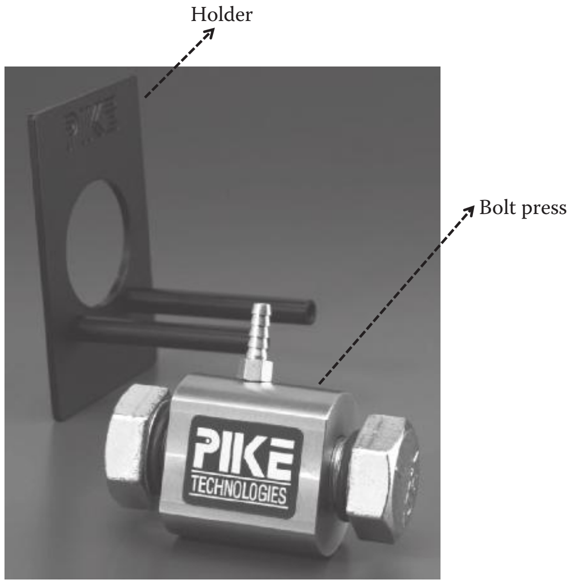

FIGURE 4.3 A photo of a “bolt press” KBr pellet maker and its holder. (Photo courtesy of PIKE Technologies.)

bolt is screwed into the barrel while still holding the device upright. Next, the bolts are tightened using wrenches to apply the pressure needed to make the pellet. After a minute or so, the barrel is held horizontally and the bolts are gently unscrewed. Hopefully, a thin, semitransparent KBr disk will be visible inside the barrel. The barrel also acts as the pellet holder and is placed on the holder seen in Figure 4.3, which is then installed in the FTIR's sample compartment. The infrared beam then passes into the barrel, through the KBr glass and embedded sample particles, out of the barrel, and is focused onto the detector.

You might think that a pellet made from pure ground KBr should be used for the background spectrum. Remember from Chapter 2 that the only difference between the measurement of the background and sample spectra should be the absence or presence of the sample, and that for contributions to ratio out they need to be identical in both measurements. It is difficult to make two KBr pellets with the same optical properties, such as thickness and shape. Thus an analyte-free pellet will not provide a good background because its contributions will not ratio out. However, the optical properties of the pellet holder are reproducible, which is why it should be used to run the background spectrum for KBr pellets.

A way to judge pellet quality is to hold it up to the light and look at it. Ideally, you will see a homogeneous, translucent pellet, which means you should be able to see light through it but will probably not see images. If the pellet is opaque it is too thick, and too much material was used in making the pellet. Opaque pellets will give poor spectra because very little light will pass through them, giving totally absorbing peaks (as seen in Figure 1.5) and offset baselines. The solution is to make a new pellet using less material. If the pellet contains white spots it indicates that there are large particles present in the pellet because the sample was not ground sufficiently, or that the sample was not well dispersed in the KBr matrix. The solution is to make a new pellet by grinding the sample longer and mixing the sample and KBr more thoroughly. If upon opening the press there is no pellet but just little piles of powder, it means not enough KBr/sample mixture or pressure was used. The pellet should be remade using more material and/or pressure. Finally, pellets are thin and brittle, can fall out of pellet holders, can crack, or can disintegrate, so they should be handled with care. A final problem is that KBr is hygroscopic. Water adsorbs on it, which can contaminate sample spectra, as shown in Figure 4.2. Upon visual examination a "wet" KBr pellet will have cloudy regions. To keep KBr dry it should be kept in an oven at a temperature >100°C to drive off the adsorbed water. Alternatively, the KBr can be kept in a desiccator, although it is the author's experience that an oven works better. Despite all these problems, KBr pellets produce quality spectra of solid and powdered samples. The KBr pellet spectrum of an aspirin tablet is shown in Figure 4.4. Note this spectrum has a low noise level, flat baseline, well-resolved peaks that are on scale, and a small amount of offset.

1. Mechanical Grinding

An agate mortar and pestle is capable of grinding the KBr and sample sufficiently to obtain a quality pellet. However, it requires several minutes of manual labor and because different people grind differently, the particle sizes and shapes obtained are not necessarily reproducible, which can lead to varying results. A way around this

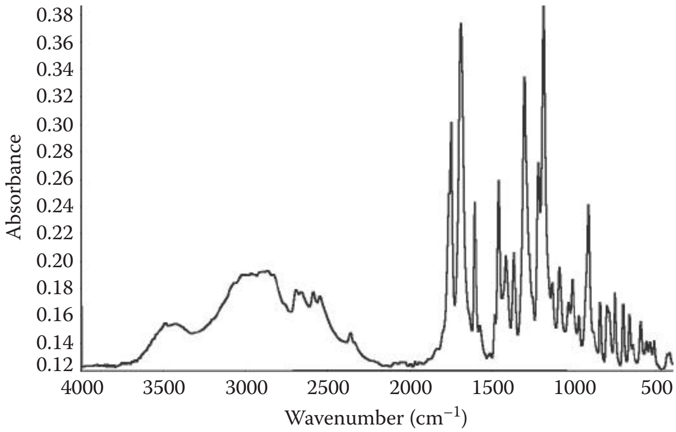

FIGURE 4.4 The KBr pellet spectrum of an aspirin tablet.

problem is to use a mechanical device to grind the sample and the KBr such as the Wig-L-Bug™ shown in Figure 4.5.

To obtain a ground powder with a Wig-L-Bug, the KBr or sample is placed in a stainless steel capsule along with a ball bearing. These are shown in the bottom of Figure 4.5. The capsule is positioned in a c-clamp that is attached to an electric motor. When the motor is turned on it violently shakes the capsule back and forth. The ball bearing flies around, smashing to bits whatever is inside the capsule. A minute of shaking is sufficient to pulverize many samples.

In the author's experience the Wig-L-Bug is faster and easier than manual grinding. Also, as long as the amount of time spent grinding is held constant, the particle sizes and shapes will be more reproducible, leading to consistently better pellets. To avoid a possible chemical reaction between the sample and KBr, they should be ground in separate capsules. To mix the KBr and sample together the two materials can be placed together in the same capsule but without a ball bearing. Then the capsule is shaken and the materials will mix well, but no further grinding will occur in the absence of the ball bearing. Another advantage of the Wig-L-Bug is that the stainless steel capsules are convenient vessels for weighing out the KBr and sample. Many laboratory supply companies and FTIR accessory manufacturers sell Wig-L-Bugs, and they cost several hundred dollars. If you make KBr pellets occasionally a Wig-L-Bug might not be worth the expense, and an agate mortar and pestle will work well. However, if you make KBr pellets on a regular basis, investing in a Wig-L-Bug makes sense because it will make the pellet fabrication process faster and easier.

2. Advantages and Disadvantages of KBr Pellets

One of the beauties of KBr pellets is that practically any powdered solid can be analyzed. If a solid is not a powder, but if it can be broken into pieces and ground into a powder, its spectrum can be obtained as well. If only small amounts of sample are available, "micro" KBr pellet presses can be used to make pellets as small as several millimeters in diameter. KBr pellets can also be used for quantitative work.

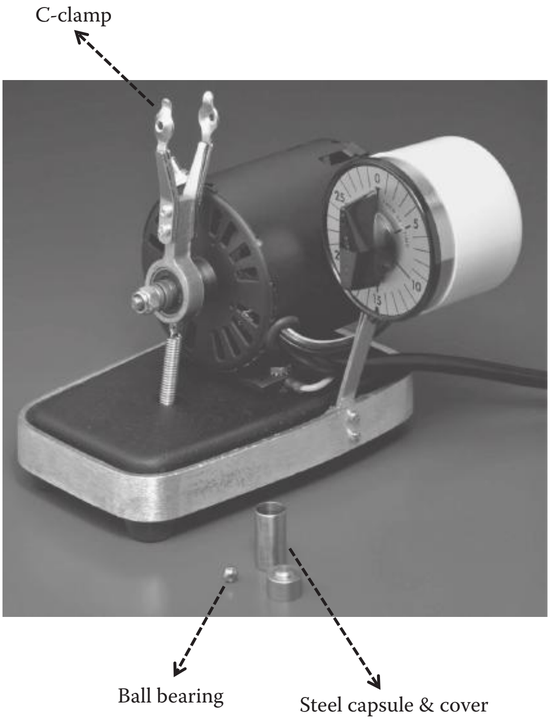

FIGURE 4.5 A Wig-L-Bug™, which can be used to mechanically grind KBr and samples to make KBr pellets. (Photo courtesy of PIKE Technologies.)

However, this requires measuring out precise amounts of KBr and sample to make the pellet. Also, since the pathlength of the pellet is irreproducible, band area ratios must be used to eliminate pathlength as a variable.

A problem with KBr pellets is that since KBr is hygroscopic, it readily adsorbs water, which can degrade moisture-sensitive samples in a pellet. Another problem with KBr pellets is that many things can go wrong with the technique, wasting precious analysis time. It will take 5 or more minutes to analyze a cooperative sample in a skilled pair of hands. An uncooperative sample, regardless of the user's skill, can take hours to analyze. Investing this much time and effort in analyzing one sample is a bad idea in today's highly efficient lab environment. Fortunately, there are faster ways to analyze solids and powders as discussed below. This means over time that KBr pellets will probably become less popular. The overall advantages and disadvantages of the KBr pellet method are listed in Table 4.3.

B. Mulls

The *mull* technique is a second way of taking the spectra of solids in transmission. It works on powders or anything that can be ground into a powder, and competes directly with the KBr pellet method. Mulls are made by first grinding the sample to reduce particle size. This is to avoid light scattering and sloped

TABLE 4.3
The Advantages and Disadvantages of KBr Pellets

Advantages	Disadvantages
Works on many types of solids	Opacity problem
Inexpensive tools	Trial and error may be involved
Quality spectra	Time-consuming
History/tradition	Moisture-sensitive samples
Can do quantitation	Hygroscopic KBr can give moisture bands
	KBr must be stored properly
	Baseline slope with improper grinding
	KBr can react with samples

baselines. Next, a drop or two of oil, called the *mulling agent*, is added to the powdered sample, and the two are stirred together to form a slurry. The most common mulling agent is mineral oil, which is a mixture of long-chain alkanes. A brand of mineral oil called "Nujol™" is commonly used to make mulls; hence this technique is frequently called the "Nujol mull" method. The oil and sample should be thoroughly mixed together by stirring with a spatula until a pasty consistency is obtained. A small amount of the slurry is then placed on the surface of an infrared transparent window (typically KBr), a second window is placed on top, and the two windows are pressed together to form a "sandwich," as shown in Figure 4.6.

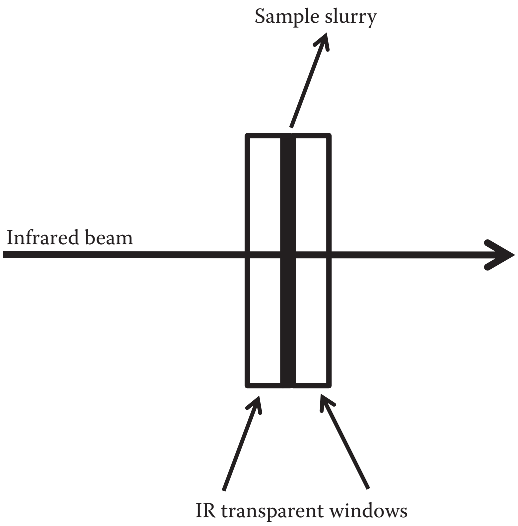

FIGURE 4.6 A mull, consisting of a slurry of oil and sample compressed between two infrared transparent windows.

FIGURE 4.7 IR window holders used for transmission analysis. These holders can be used with mull, polymer film, and capillary thin film samples. (Photo courtesy of PIKE Technologies.)

The capillary action of the mulling agent will hold the windows together. However, it is usually best to use a mechanical means of placing gentle pressure on the windows to thin out the slurry and to position the windows reproducibly, such as the window holders shown in Figure 4.7.

The sample and holder are placed in the infrared beam and the spectrum is measured. The background spectrum should be run on the same KBr windows and holder that are used for the mull, but with nothing between the windows. An example of an infrared spectrum obtained using a mineral oil mull is shown in Figure 4.8. Note that the spectrum in Figure 4.8 has a good SNR, has a low offset, is well resolved on scale peaks, and a flat baseline.

The advantage of mulls over KBr pellets is that mulls are easier and faster to make since there is no time-consuming pellet pressing involved. Mulls are also well suited to samples that will degrade upon exposure to atmospheric moisture. The mulling oil coats and protects sample particles from the environment, and since the oil is hydrophobic water is effectively repelled.

1. The Split Mull Method

A major disadvantage of the mull technique is that the mulling agents have infrared peaks that can mask sample features. This is illustrated by the spectrum of mineral oil shown in Figure 4.9. Note that mineral oil has features around 3000, 1400, and 720 cm^{-1}, all of which can interfere with the spectrum of your sample. In theory, one could subtract the spectrum of the mineral oil from the sample spectrum. However, the bands from the oil are usually too intense for clean subtractions to take place for reasons cited in Chapter 3.

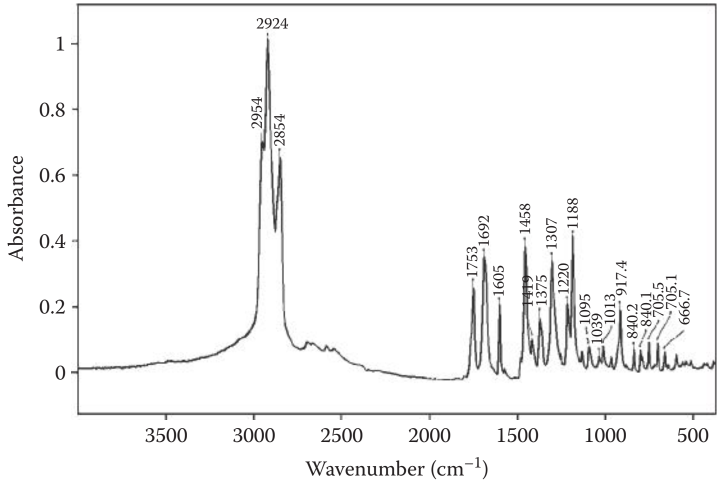

FIGURE 4.8 The infrared spectrum of an aspirin tablet obtained using a mineral oil mull.

Fortunately, a way around the problem of mineral oil's infrared bands is a technique called the *split mull method*. The sample is first prepared as a mull in mineral oil and its spectrum is measured. Then a second mull of the sample is made using a mulling agent called Fluorolube®, which is a mixture of long-chain fluorocarbons made by replacing all the C-H bonds in long-chain alkanes with C-F bonds. The infrared spectrum of Fluorolube is shown in Figure 4.10.

Note in Figure 4.10 that Fluorolube has no infrared features above 1350 cm^{-1}, and note from Figure 4.9 that the mineral oil is transparent below 1350 cm^{-1} (except for the small peak near 720 cm^{-1}). In the split mull technique the spectrum of the same sample as measured using these two mulling agents, and then the 4000 to

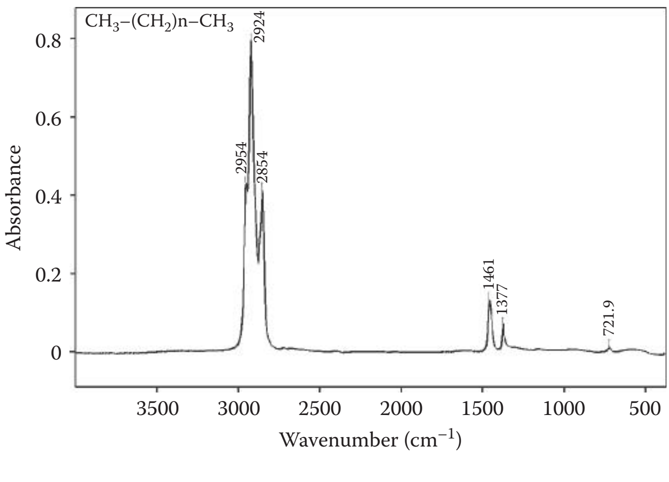

FIGURE 4.9 The infrared spectrum of mineral oil, which is used as a mulling agent. Note its bands around 3000, 1400, and 720 cm^{-1}, which can interfere with the spectra of samples.

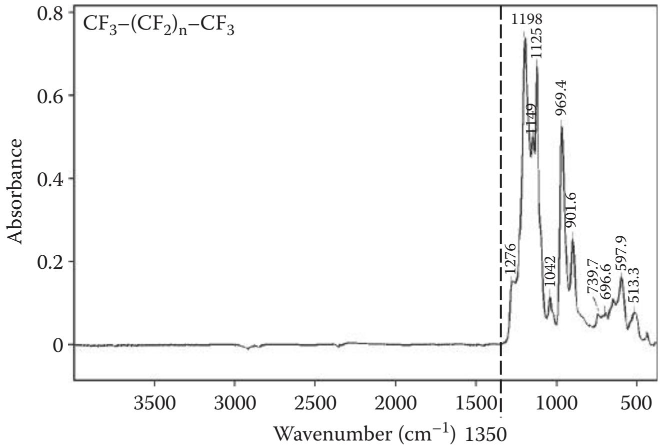

FIGURE 4.10 The infrared spectrum of Fluorolube, a mulling agent. Note the bands below 1350 cm^{-1} and the lack of bands above 1350 cm^{-1}.

1350 cm^{-1} region of the Fluorolube mull spectrum to the 1350 and 400 cm^{-1} section of the mineral oil spectrum are spliced together. This can be done electronically using a computer, or the old-fashioned way of cutting and pasting paper copies of the spectra. Figure 4.11 shows a spectrum that resulted from electronically splicing the relevant sections of the spectra of mineral oil and Fluorolube. Note the lack of features except for the small mineral oil feature at 720 cm^{-1}.

A problem with the split mull technique is that by the time the mulls are prepared and the spectra are measured and spliced together, it may be faster and easier to obtain the sample spectrum using a KBr pellet.

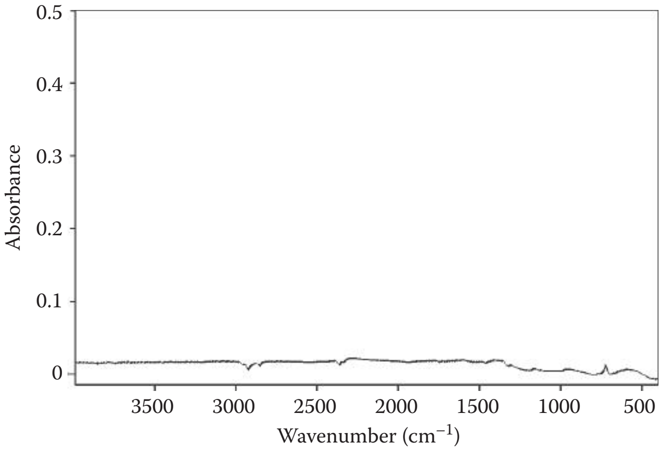

FIGURE 4.11 The spectrum of mineral oil from 4000 to 1350 cm^{-1} electronically spliced to the spectrum of Fluorolube from 1350 to 400 cm^{-1}. Note the lack of features except for the small mineral oil peak at 720 cm^{-1}.

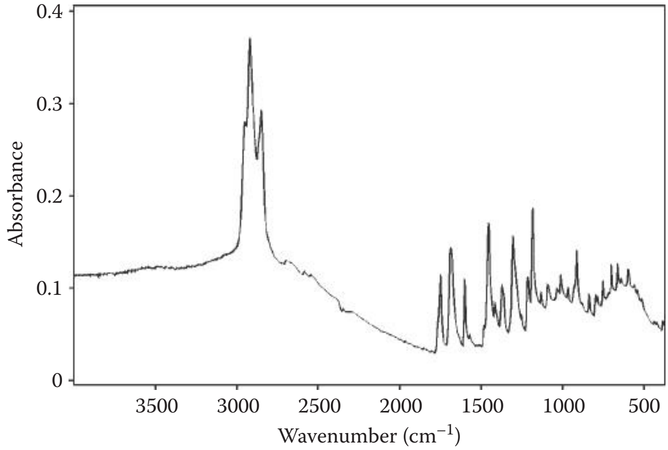

FIGURE 4.12 A spectrum of an aspirin tablet obtained with a mineral oil mull. Note the sloped baseline at high wavenumber caused by light scattering, which is a result of the sample particles being too big from insufficient grinding.

Another problem that can be encountered with mulls is that if the sample is not ground enough the large particles will scatter the infrared beam, giving a sloped baseline. This problem is illustrated in Figure 4.12, which shows the mineral oil mull spectrum of an aspirin tablet that was not ground enough.

Like other transmission techniques, mulls can be time-consuming. The only way to know if the mull was made properly is to measure its spectrum. If the spectrum is bad, more time must be spent preparing a new sample. In a pair of skilled hands each sample will take several minutes via this technique. Using the split mull method at a minimum doubles sample preparation time. Mulls are typically used for qualitative work, but not for quantitative work since concentrations and pathlengths are difficult to reproduce with this method. The advantages and disadvantages of mulls are summarized in Table 4.4.

TABLE 4.4
The Advantages and Disadvantages of Mulls

Advantages	Disadvantages
Works on many types of solids	Opacity problem
Inexpensive tools	Trial and error may be involved
Quality spectra	Time-consuming
History/tradition	No quantitation
Good for moisture-sensitive samples	Baseline problems with improper grinding
Somewhat faster and easier than KBr pellets	Oil bands interfere with sample spectrum
Split mulls avoid oil band problem	

III. TRANSMISSION ANALYSIS OF POLYMERS

Polymers, like powders, are solids at room temperature and in theory can be analyzed using KBr pellets or mulls. For hard polymers that can be ground into a powder, this works. However, polymers that are soft, rubbery, or too tough cannot readily be ground into powders and so are not easy to analyze. Fortunately, there exist techniques for turning polymers into thin films, which allows them to be analyzed in transmission as described below.

A. The Cast Film Method

Casting a film can refer to picking people to be in a movie, but here it refers to a method of preparing polymers for infrared transmission analysis. In this technique, the polymer is first dissolved in a volatile solvent, which is required because the solvent is evaporated later. Examples of solvents that work well include acetone, methyl ethyl ketone, chloroform, methylene chloride, tetrahydrofuran, and toluene. The polymer can be in any form, be it a sheet, formed part, chunk, or pellet. As long as the polymer will dissolve in a volatile solvent, in theory its spectrum can be measured. Thermoset polymers, which consist of three-dimensional cross-linked polymer networks, do not dissolve in solvents and hence cannot be analyzed using this technique. The amount of polymer and solvent used can be "eyeballed," but more consistent results are obtained if the amount of polymer and solvent are measured.

Once the polymer is dissolved, drops of the solution are placed onto the surface of an infrared transparent window, and the solvent is allowed to evaporate. KBr windows are frequently used for this purpose, but other infrared transparent windows as described in Table 4.1 can be used as well. As the solvent evaporates it will leave behind a thin film of polymer. This is called "casting a film," hence the name of the technique. To build up a sufficient film thickness, it may be necessary to apply several drops of solution to the window, allow it to evaporate, and then apply more drops in a step-wise fashion. This is illustrated in Figure 4.13.

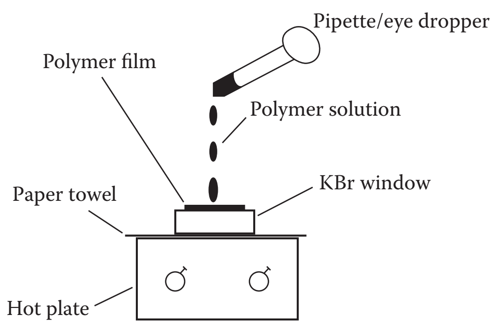

FIGURE 4.13 The process of building up a cast polymer film by placing drops of solution on an IR transparent window. The hot plate is used to speed the evaporation of the solvent.

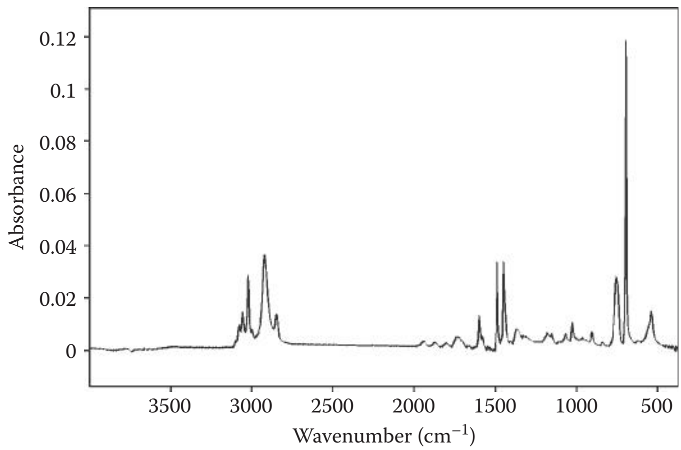

FIGURE 4.14 The infrared spectrum of a polystyrene film cast from a methyl ethyl ketone solution. A KBr window was used.

Once the solvent has evaporated, the infrared transparent window and cast film are placed in a holder in the sample compartment of an FTIR, and the spectrum is measured. Holders such as the ones shown in Figure 4.7 can be used for this purpose. Ideally, the background spectrum should be run on the same window holding the cast film. This technique could work on powders because they can be dissolved and evaporated on a window. However, once the window is held vertically to place it into the IR beam, the powder will fall off the window. This technique works with polymers because of their ability to form films and adhere to flat surfaces. A spectrum of polystyrene cast from methyl ethyl ketone solution onto a KBr window is shown in Figure 4.14. Note that the SNR is good, the baseline is flat, the sample peaks are well resolved and on scale, and there is no offset.

Ideally, all of the solvent should be evaporated from the film before its spectrum is measured to prevent the solvent from contaminating the sample spectrum. You can wait for the solvent to evaporate on its own, which can be time-consuming, depending upon the volatility of the solvent chosen. Alternatively, there are a number of ways of speeding the process of evaporation, such as using a hot plate as illustrated in Figure 4.13. The infrared transparent window is placed on a paper towel and *gentle* heat is applied. If the window gets too hot it may crack from thermal stress, and if the paper towel gets too hot it may catch fire (in both cases the author speaks from experience). Another way of speeding solvent evaporation is to use a heat gun. Some labs have these devices, but a simple and low-cost alternative is simply to use a blow dryer. However, be careful to only apply a gentle flow of hot air to the polymer solution. Otherwise, you will create ripples in the resulting film or, worse yet, blow the solution completely off the window. A final way to speed evaporation of the solvent is to place the polymer solution/window under a heat lamp similar to the ones used in restaurants to keep food warm.

TABLE 4.5
The Advantages and Disadvantages of Cast Polymer Films

Advantages	Disadvantages
Good SNR	Must evaporate solvent
Inexpensive	Does not work on all polymers
Amenable to multi-tasking	Time-consuming
	Alters polymer morphology
	Difficult Quantitation

One advantage of the cast film method is that it is inexpensive. All you need is a little polymer, a little solvent, a test tube, an eyedropper, and an IR transparent window. Also, like other transmission techniques, it is capable of producing quality spectra as shown in Figure 4.14. However, there are a number of problems with this technique (does this make me a film critic?). The first is the solvent itself. If the solvent does not evaporate completely it will contaminate the sample spectrum. Not all polymers, such as thermosets, will dissolve in volatile solvents, thus limiting applications. Another disadvantage of the cast film method is that it is time-consuming; it can take several minutes to make the polymer solution and evaporate it onto an infrared transparent window. The only way to know if a cast film is of the right thickness is to measure its spectrum. If the film is too thin, more drops of polymer solution can be applied and allowed to evaporate to build up a thicker film. If the cast film is too thick, it must be dissolved off the window and a new film must be made. These trial and error attempts to make a film of the right thickness can waste minutes or even hours of your time. That being said, while waiting for the solvent to evaporate you can multitask because the sample does not need babysitting. Another disadvantage of the cast film method is that it alters *polymer morphology*, which describes how polymer chains pack together. This information is lost when the polymer is dissolved in a solvent. Finally, quantitative analysis is difficult with cast films because there is poor control over sample thickness. Table 4.5 lists the advantages and disadvantages of cast films for analyzing polymers.

B. The Heat and Pressure Method

A second way of taking the spectra of polymeric samples in transmission is the *heat and pressure* method. This method works by compressing a polymer and heating it to above its glass transition temperature, where it will soften and flow to form a thin film. A diagram of how the technique works is shown in Figure 4.15.

The sample is placed between the platens of a hydraulic press. The sample can be a polymer pellet, chunk, piece, or film. In theory, as long as the sample fits into the press it can be turned into a thin film. Using the press, pressures of 10,000 pounds per square inch (PSI) or more are applied. The platens need to be of a special type that can be heated. Temperatures upwards of 300°C may be needed, depending upon the material, to get the sample to soften and flow. A hydraulic press that can be used to make heat and pressure films is shown in Figure 4.16.

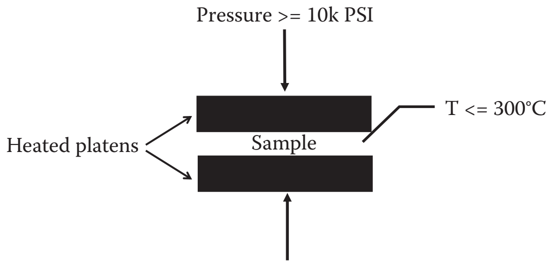

FIGURE 4.15 A diagram of how heat and pressure are applied to a polymeric sample to turn it into a thin film.

The sample may need to be heated and pressed for 5 or more minutes to form a film. Once the pressure is released and the polymer film cools, it may stick to the platens. To avoid this problem, the sample can be placed between sheets of Teflon™ or Teflon-coated aluminum foil before being placed in the press. Sample clamps such as those shown in Figure 4.7 can be used to hold polymer films in the infrared beam. The background spectrum would be run on the empty film holder. An example of a polymer whose spectrum was obtained using the heat and pressure method is shown in Figure 4.17.

Some labs use heat and pressure to turn awkwardly shaped parts into thin films, and then take their sample spectra via ATR (see later in this chapter).

The thickness of heat and pressure films depends upon a number of variables, and the only way to know if a film is of the proper thickness is to measure its spectrum. If

FIGURE 4.16 An example of a hydraulic press used in the heat and pressure method of analyzing polymers in transmission. (Photo courtesy of PIKE Technologies.)

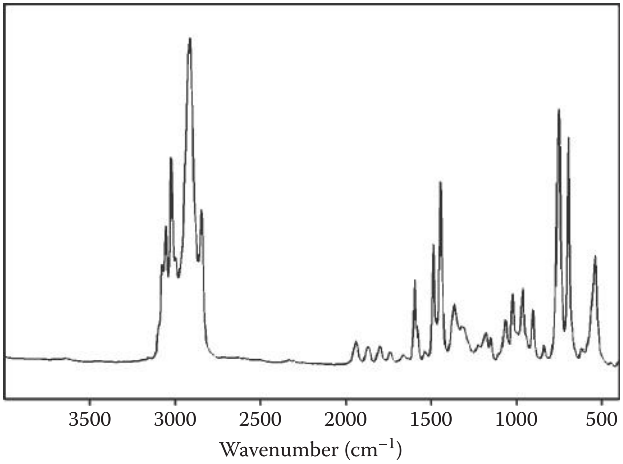

FIGURE 4.17 The infrared spectrum of a polystyrene-butadiene co-polymer obtained using the heat and pressure method.

the sample is too thin or too thick it will have to be remade. Many minutes or hours may be spent experimenting with variables to obtain a polymer film of the right thickness. Even if a film of the proper thickness is formed on the first try, it takes 5 or more minutes for a film to form. Another problem with this technique is that since the sample pathlength is not controlled, it will be difficult to perform quantitative analyses.

The solution to these problems is the constant thickness film maker. As its name implies, this device is used to make heat and pressure films of known thickness. A photo of the device is shown in Figure 4.18.

To use the constant thickness film maker, a spacer ring of a given thickness is placed in the bottom half of the device along with the sample. The other half of the device is put in place on top of the sample. The entire unit is then placed between the heated platens of a hydraulic press. Once the correct temperature is reached, the sample will soften and flow and fill the space between the top and the bottom of the unit. The film thus formed will be of the same thickness as the spacer ring. Spacer rings of different thicknesses are available to give films of different pathlengths. A small hole in the side of the device allows excess polymer to flow out. Once the device is removed from the press and the polymer film is allowed to cool, its spectrum is measured like any other heat and pressure film, as described above.

The beauty of the constant thickness film maker is that it saves sample preparation time because there will be less experimenting required to obtain a film of the desired thickness. Additionally, since the pathlength is controlled, this technique can be used for quantitative analysis. For example, thin films can be made to quantify the presence of species present in high concentration such as co-monomers. Alternatively, thick films can be made to quantify chemical species present in low concentration such as polymer additives. In these cases the polymer peaks may go off scale, but as long as the additive peaks are on scale, and their peak size correlates to

FIGURE 4.18 A constant thickness film maker, used with the heat and pressure method to make polymer films of reproducible thickness. (Photo courtesy Specac Inc.)

concentration, they can be used for quantitation. A final advantage of the heat and pressure method is that, like other transmission sampling techniques, it gives spectra with a good SNR.

Another issue with the heat and pressure method is that it alters polymer morphology. This information is lost once the polymer softens and flows. A final problem with the heat and pressure method is that it does not work on all polymers. Polymers with very high glass transition temperatures or melting points, such as PTFE, are difficult to analyze. Additionally, cross-linked or thermoset polymers cannot be analyzed by this method because they will not soften and flow.

Despite its issues, the heat and pressure method has some advantages compared to the cast film method of analyzing polymers. Although there is trial and error in finding the parameters that give the correct thickness for a given polymer, once these parameters are known they can be applied to the same material over and over again with little further experimentation. In these cases one can go off and do other things while the polymer film is forming. There is no solvent needed for this method, saving on exposure hazards and waste disposal costs. Also, the heat and pressure method works on more types of polymers than the cast film technique, since there is no requirement for the sample to dissolve in a volatile solvent. Lastly, the development of the constant thickness film maker has solved many of the problems with this technique. The trial and error is removed from obtaining films of the right thickness, and quantitative analysis is now possible. One issue that is unavoidable with this technique is the cost

TABLE 4.6
The Advantages and Disadvantages of the Heat and Pressure Method

Advantages	Disadvantages
Good SNR	$$$$ for heated hydraulic press
No solvent required	Does not work on all polymers
Works on many polymers in many forms	Can be time-consuming
Amenable to multi-tasking	Alters polymer morphology
Quantitation possible	
Constant thickness film maker	

of the equipment. The special hydraulic presses with heated platens and the constant thickness film maker each cost thousands of dollars. Table 4.6 summarizes the advantages and disadvantages of the heat and pressure method.

IV. TRANSMISSION ANALYSIS OF LIQUIDS

In general, transmission sampling of liquids is faster and easier than that of solids because it is easier to turn liquids into a thin film. There are two widely used methods for obtaining the transmission spectra of liquids: the capillary thin film method and sealed cells.

A. Capillary Thin Films

The beauty of the capillary thin film method is that it takes longer to say its name than to prepare the sample. To make a capillary thin film, place a drop of sample between two infrared transparent windows. Any of the window materials listed in Table 4.1 can be used, with KBr being the most common. The capillary action of the liquid holds the windows together and thins out the sample, hence the name of the technique. It helps to rotate one window against the other to thin and spread out the liquid film. The film is then placed in the infrared beam. Window holders such as those shown in Figure 4.7 may be used for the capillary thin film method. Alternatively, a simple sample holder like the one shown in Figure 4.19 can also be used.

Note how the holder slides into the mount in the FTIR sample compartment, and how the windows rest on the two posts protruding from the metal plate. This type of device can also be used to support KBr pellet holders, mulls, and cast films. The background spectrum is run on the same two windows that will be used to prepare the sample.

A quality spectrum obtained using the capillary thin film method and the holder shown in Figure 4.19 is shown in Figure 4.20. Note how the spectrum in Figure 4.20 has a flat baseline, low noise, well-resolved peaks that are on scale, and negligible offset. Cleanup with the capillary thin film method involves rinsing the windows with a solvent that will dissolve the sample but not the windows. For example, acetone, ethanol, methylene chloride, and tetrahydrofuran have been used.

In addition to being useful with liquids, the capillary thin film technique can be used with soft solids or viscous liquids using the elegantly named "smear" technique.

FIGURE 4.19 A sample holder for transmission analysis shown installed in the sample slide mount of an FTIR. The protruding metal arms can support the KBr windows, KBr pellets, mulls, cast films, and capillary thin films.

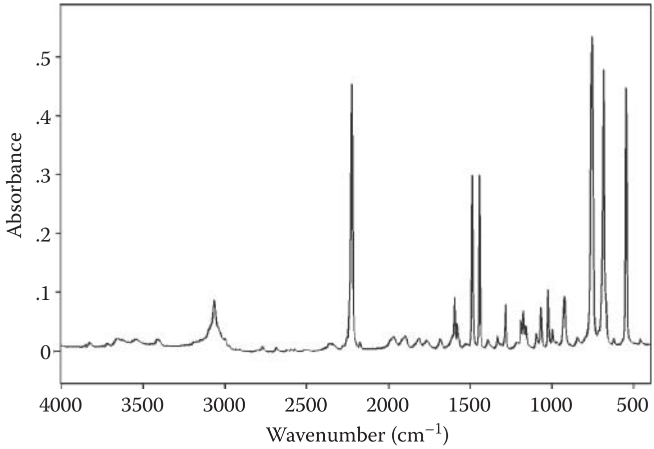

FIGURE 4.20 The infrared spectrum of benzonitrile obtained using the capillary thin film method.

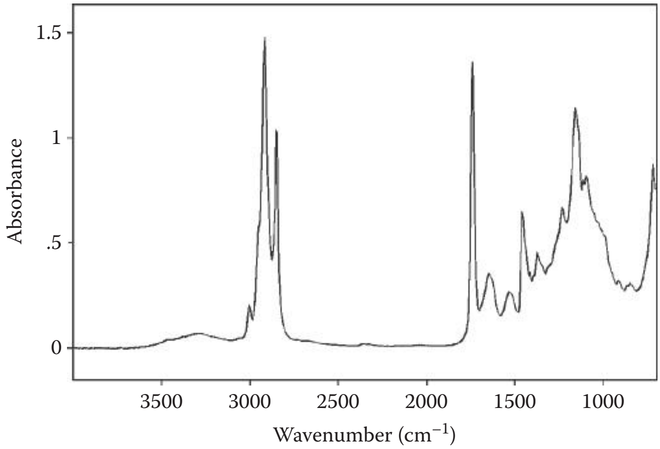

FIGURE 4.21 The capillary thin film spectrum of a smear of chunky peanut butter.

Semi-solids with the consistency of soap or peanut butter, or liquids with the consistency of maple syrup or tar, can be smeared onto a window, a second window placed on top, and then the windows can be rotated in opposite directions to smooth and flatten the sample. The resultant sample sandwich can be placed into the infrared beam as shown in Figure 4.19. The infrared spectrum of a smear of chunky peanut butter is shown in Figure 4.21.

A frequent problem encountered with the capillary thin film method is using too much sample. This technique is very sensitive; it takes less than one drop of liquid or a spatula tip's worth of soft solid to obtain a good spectrum. If your sample is too thick you can reduce its pathlength by taking the windows apart, wiping one of them on a paper towel, and putting them together again. The windows used with this technique are frequently 1 inch in diameter, but the infrared beam in most FTIRs is 1 cm in diameter at the focal point in the sample compartment. Thus, it is not necessary to cover the entire window, just the center of it so the sample fills the IR beam.

The capillary thin film method offers many advantages. The ease of forming a liquid into a thin film means there is less trial and error than with preparing solids for analysis. The technique is also versatile since via the smear technique it can be used to obtain spectra of soft solids and viscous liquids. The quality of the spectra included in this section shows that this technique is capable of delivering spectra with flat baselines and good SNRs.

The capillary thin film method works well with organic liquids, but in general is not used with aqueous solutions because many infrared transparent windows such as KBr and NaCl are water soluble; any sample containing liquid water will damage them. An alternative is to use infrared transparent windows that are water insoluble such as zinc selenide (ZnSe) or silver chloride (AgCl), but they are expensive. Additionally, when using the capillary thin film method liquid water features will be very broad and strong and will be difficult to remove by spectral subtraction (the spectrum of liquid water is seen in Figure 1.6). For these reasons the capillary thin film method is in general not compatible with aqueous samples.

TABLE 4.7
The Advantages and Disadvantages of the Capillary Thin Film Method

Advantages	Disadvantages
Fast and easy	Sample evaporation
Good SNR	Incompatible with aqueous solutions
Works on liquids and soft solids	Difficult quantitation

There are other limitations of the capillary thin film method as well. The liquid is not sealed between the windows, and if the sample is volatile it will evaporate while you are trying to take its spectrum. Materials like acetone and ether will disappear before you get them into the sample compartment. Additionally, if the volatile sample is smelly or toxic you will be contaminating yourself, your lab, and your instrument while taking the spectrum (the author speaks from experience here). Lastly, it is difficult to use the capillary thin film method for quantitation because of the lack of control over pathlength. On the whole, this technique is a fast and easy way of obtaining qualitative transmission spectra of many organic liquids. The advantages and disadvantages of the capillary thin film method are summarized in Table 4.7.

B. Sealed Liquid Cells

Sealed liquid cells have a gasket that seals the liquid in the cell, thus preventing evaporation and counteracting the evaporation problem suffered by the capillary thin film method. It also means that volatile, smelly, and toxic liquids can be analyzed because they will not escape and cause harm. A schematic diagram of how a sealed liquid cell works is shown in Figure 4.22.

A sealed liquid cell consists of two infrared transparent windows, which are typically made from KBr or one of the other infrared transparent materials listed in Table 4.1. Two small holes are drilled in one of the windows, as can be seen in Figure 4.23.

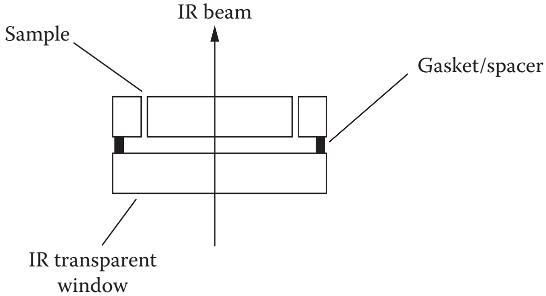

FIGURE 4.22 A schematic diagram of a sealed liquid cell. The gasket/spacer seals the liquid between the infrared transparent windows and holds the windows a fixed distance apart, giving a known pathlength.

FIGURE 4.23 A picture of a sealed liquid cell. Note the window with the holes drilled in it, the Teflon gasket, and the syringe with a Luer lock fitting. (Photo courtesy of PIKE Technologies.)

Cells with thicknesses from 1 to 500 microns have been used. Gaskets can be made of Teflon, or a lead/mercury amalgam. A Teflon gasket can be seen in Figure 4.23. Since the sample holes are small, the cell is usually filled using a syringe. The syringe in Figure 4.23 has a Luer lock tip on it, and many sealed liquid cells have Luer lock fittings on them like the one shown in Figure 4.24. Luer lock fittings provide a means of transferring a liquid from one vessel to another without leaks or exposure to the environment.

An easy way to fill a sealed liquid cell is to use two syringes. The syringe containing the sample is attached to one of the Luer lock fittings, and an empty syringe with its plunger all the way down is attached to the second fitting on the cell. As the plunger on the sample syringe is pressed down the plunger on the empty syringe is pulled up. The first pushes the liquid into the cell; the latter creates a small vacuum and pulls the liquid into the cell. Once the cell is filled, hold it up to the light to make sure there are no air bubbles present. If there are, refill the cell to eliminate the bubbles so you take the spectrum of your sample and not of air.

A sealed liquid cell is held in a metal frame like the one seen in Figure 4.24. The frame slides into the slide mount in the sample compartment of the FTIR. Plugs like the ones seen in Figure 4.24, frequently made from Teflon, keep the sample from evaporating through the holes in the infrared transparent window. The background spectrum is run of the same clean, empty liquid cell as used in the analysis of the sample. The spectrum of methyl ethyl ketone obtained using a sealed liquid cell is shown in Figure 4.25. Note the flat baseline and high signal-to-noise ratio of the spectrum.

FIGURE 4.24 A sealed liquid cell holder equipped with Luer lock fittings and Teflon stoppers. (Photo courtesy of PIKE Technologies.)

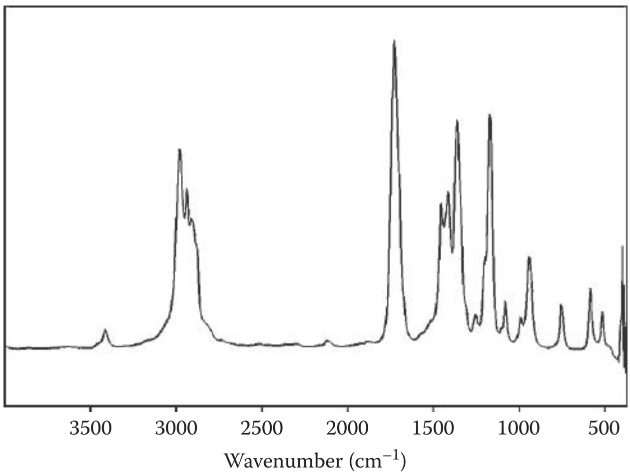

FIGURE 4.25 The infrared spectrum of methyl ethyl ketone (MEK) obtained using a sealed liquid cell.

FIGURE 4.26 A variable pathlength sealed liquid cell. (Photo courtesy International Crystal Labs.)

The fixed pathlength of sealed liquid cells makes them very useful for quantitative analysis because one of the variables in Beer's Law (Equation 1.6) is controlled. Sealed liquid cells come in different pathlengths, allowing the cell to be customized to the application. Short pathlength cells can be used for samples where the analyte concentration is high; long pathlength cells can be used when the analyte concentration is low. For this reason, many labs are equipped with multiple sealed liquid cells of different pathlengths. However, during method development one does not necessarily know what the optimum pathlength for a given analysis will be, and determining this may involve buying and trying many different cells to find the right one. A way around this problem is to use a variable pathlength sealed liquid cell like the one shown in Figure 4.26. This device contains a dial that turns to change the pathlength of the cell, and a scale on the dial tells you the pathlength.

Many of the infrared transparent windows used in sealed liquid cells, such as KBr and NaCl, are water soluble. Thus, samples containing any liquid water should be avoided when working with these windows. To avoid this problem, cells made with water insoluble windows such as ZnSe and AgCl are available, but at added expense. The real issue here is that the spectrum of any sample where water is the solvent is going to have strong, broad water absorbances like those seen in Figure 1.6. There will be a need then to subtract the water spectrum so the solute can be clearly seen. The water peaks in the spectra of aqueous solutions obtained using sealed liquid cells are frequently too intense to subtract well. In theory, reducing the pathlength

TABLE 4.8
The Advantages and Disadvantages of Sealed Liquid Cells

Advantages	Disadvantages
No evaporation	Tedious filling and cleaning
Good SNR	Cross contamination possible
Easy quantitation	Can be - (retain allignment) Incompatible with aqueous solutions

will reduce the water absorbance, but it reduces the analyte absorbance as well, making its spectrum more difficult to see.

In addition to running the spectra of liquids, the spectra of solids can be obtained using sealed liquid cells. The solid is dissolved in an appropriate solvent, the solution is placed in the cell, and its spectrum is measured. Much of the early work in infrared spectroscopy was done on such samples. The advantage of this approach is that it gives very nice-looking spectra. The choice of solvent here is critical as it needs to dissolve the sample, not react with it, and ideally be infrared transparent. However, there are no known solvents free of infrared features from 4000 to 400 cm^{-1}. Solvents with few peaks that have been used to dissolve samples include carbon tetrachloride and carbon disulfide. These two solvents mask different parts of the infrared spectrum, and by obtaining the spectra of a sample in both solvents and splicing them, a spectrum mostly free of solvent bands can be obtained. Listings of infrared transparent solvents and their useful wavenumber ranges are found in the literature [1].

The major disadvantage of sealed liquid cells is that they are difficult to fill and clean. The sample is extremely small, making it difficult to get the sample into the cell and more difficult to get the sample out of the cell [2]. It is frequently necessary to flush many volumes of solvent through the cell to completely cleanse it of sample. Failure to do so means one sample can contaminate another, known as cross contamination. Additionally, if sample spills onto the outside of the cell windows it can contaminate other samples' spectra. The best way to discover a dirty cell is to take a background spectrum of an empty sample compartment and a "sample" spectrum of the empty sealed liquid cell. A clean cell will have no absorbances. If there are any sample features present, the cell needs to be thoroughly cleansed inside and out before use. Because of these issues it takes several minutes or more per sample to fill the cell, take the spectrum, and clean it. For many organic liquids the capillary thin film method discussed above is easier and faster than using sealed liquid cells. A summary of the advantages and disadvantages of sealed liquid cells is shown in Table 4.8.

V. TRANSMISSION ANALYSIS OF GASES AND VAPORS

A gas is any material whose boiling point is at or below ambient temperature, resulting in all the molecules of the material being in the gas phase at that temperature. Well-known examples of gases include oxygen, nitrogen, and carbon dioxide. A vapor is composed of molecules of a material in the gas phase that exists in equilibrium

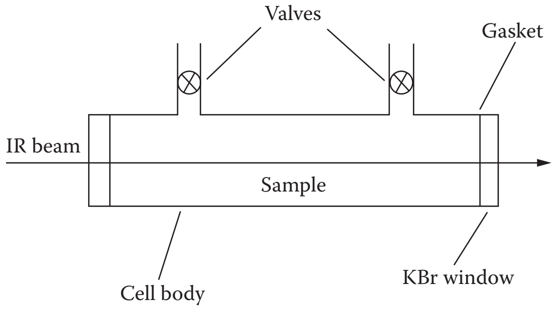

FIGURE 4.27 A diagram of a 10-cm gas cell.

with its liquid form at ambient temperature. For example, we all know from experience that water exists as a liquid and a vapor at room temperature. It is not appropriate to say water *gas* unless the sample being discussed is in an environment where the ambient temperature is greater than 100°C. Other examples of materials that exist in both the liquid and vapor state at room temperature include the well-known organic solvents acetone, ether, and toluene. The spectra of both gases and vapors can be measured using infrared spectroscopy. Throughout the rest of this discussion the word "gas" will refer to both gases and vapors. This section will focus on what is called "extractive" gas monitoring, where the gas is contained in a cell to enable its spectrum to be measured. "Open path" FTIR measurements of the atmosphere, where the infrared beam passes from the spectrometer and through the environment and onto the detector, are also possible [3].

Gases are much more dilute than liquids and solids and hence require longer pathlengths to obtain usable spectra. For solids and liquids, pathlengths on the order of microns are usually sufficient. For gases, pathlengths on the order of meters or even kilometers may be necessary to see the spectrum of the analyte. For high concentrations of gases (greater than 1%) samples are frequently analyzed in transmission using what is called a "10-cm cell," a diagram of which is shown in Figure 4.27.

As their name suggests, these cells have a pathlength of 10 cm and typically have a glass or metal body. KBr or other infrared transparent windows at either end of the cell seal the gas inside while allowing the infrared beam to pass through. The windows may be permanently adhered to the cell body, or may be sealed using a retaining ring and a gasket. The latter allows the cell to be taken apart for cleaning or for the introduction of samples. The valves on the cell allow gases in and out. A picture of a 10-cm gas cell is shown in Figure 4.28.

This particular cell has a glass body, KBr windows sealed with a gasket and retaining rings, and ground glass stopcocks for valves. Ten-centimeter gas cells are small enough to fit into most FTIR sample compartments and sit on holders that install into the sample compartment slide mount like the ones shown in Figure 4.29.

Gases already contained in a vessel, such as a gas cylinder or lecture bottle, are introduced into gas cells using a vacuum manifold, a diagram of which is shown in Figure 4.30.

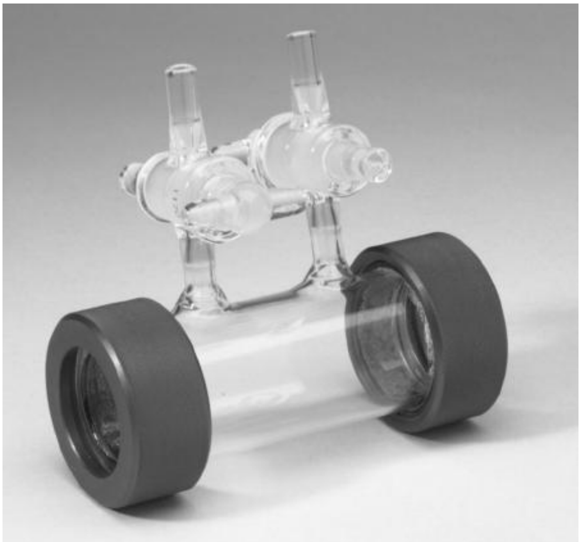

FIGURE 4.28 A 10-cm gas cell with a glass body, KBr windows sealed with a gasket and retaining rings, and ground glass stopcocks for valves. (Photo courtesy PIKE Technologies.)

A typical gas-handling manifold consists of a vacuum pump, valves, appropriate plumbing, and a pressure gauge. The gas or vapor sample is contained in a lecture bottle, glass bulb, gas cylinder, or other vessel and is connected to the manifold via plumbing. To introduce a sample into the gas cell, the manifold is evacuated and then the valve leading to the cell is opened to allow it to be evacuated as well. The valve to the vacuum pump is then closed, and the valve on the vessel holding the gas is opened. Gas will flow out of the vessel and fill the vacuum manifold and the gas cell. The pressure gauge can be used to monitor how much gas fills the cell. Once the desired pressure is reached, the valves to the cell and gas vessel are closed, and the valve to the vacuum pump is opened to pump away excess gas in the manifold. The filled cell can now be placed in the sample compartment of an FTIR.

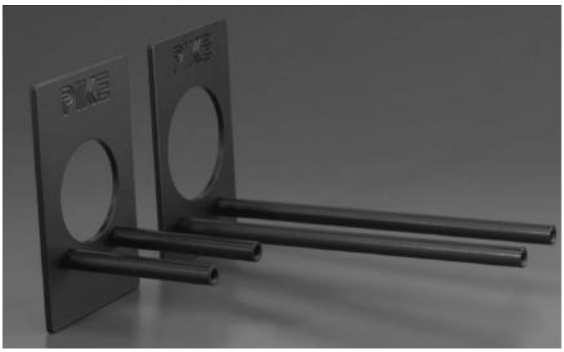

FIGURE 4.29 Two holders for FTIR gas cells. (Photo courtesy PIKE Technologies.)

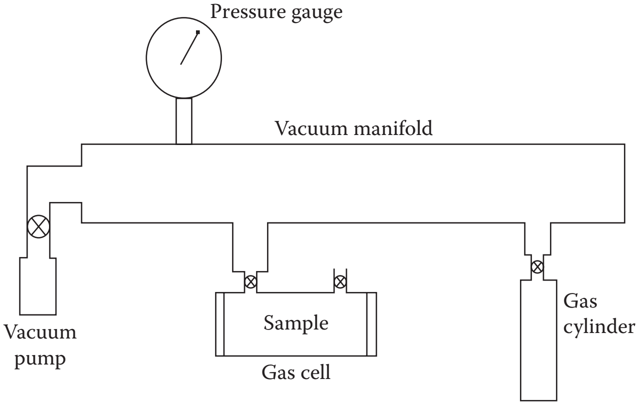

FIGURE 4.30 A gas-handling manifold that can be used to fill and evacuate FTIR gas cells.

The background spectrum can be run on an evacuated cell or a cell filled with an infrared transparent gas such as nitrogen.

In addition to measuring the spectra of gases contained in a vessel, the spectra of gases in the atmosphere can be obtained with an FTIR gas cell using what's called "grab sampling." In this technique, a previously evacuated gas cell is carried to some location where one desires to measure the composition of the atmosphere such as a smoke stack, car tailpipe, or workplace. The valve on the gas cell is cracked open, and the pressure difference will cause air to be pulled into the cell. Once the sample is safely inside, the valve on the cell is closed. The gas cell is then brought to the FTIR and the sample spectrum is measured. In situations where monitoring the composition of a gas in real time is desired, flow-through analysis can be used. In this case the gas is pumped through the gas cell and spectra are collected over time. This can be used, for example, to monitor a chemical reaction.

In many gas phase applications there is a need to monitor gases at the parts per million or even parts per billion levels. Certain pollutants represent a health hazard at these low levels, so their concentrations in the environment need to be measured. To detect gases at these levels, pathlengths of meters are necessary. Of course, using a gas cell that is, say, 10 meters long is impractical for it will take a lot of gas to fill the volume, not to mention that it would be difficult to find a room big enough to hold it! By putting mirrors at either end of a gas cell the light can be bounced back and forth a number of times, allowing a large pathlength to be obtained from a cell of short length and small volume. Such a cell is called a "White Cell" [4], which is named after its discoverer. A diagram of a White cell is shown in Figure 4.31.

A White cell consists of a tube, typically made from glass or metal, with mirrors at each end. As seen in Figure 4.31, one end of the cell contains a spherical mirror called the field mirror, and the other end of the cell contains a pair of spherical mirrors called the objective mirrors. The focal lengths of these mirrors are identical and equal the length of the tube. For example, a 1-meter-long White cell requires mirrors with a 1-meter focal length. Once the infrared beam enters the cell it is brought to a focus in the focal plane of the field mirror. The beam expands to fill one of the

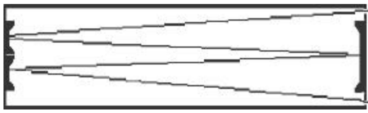

FIGURE 4.31 A diagram of a White cell, whose design gives gas cells of high pathlength and small volume.

objective mirrors at the other end of the cell. The beam is then focused to a point on the surface of the field mirror, is reflected, and fills the second objective mirror. Finally, the beam is brought to a focus in the focal plane of the field mirror, and then leaves the cell. At a minimum, then, the infrared beam traverses the length of the cell four times before exiting. Thus, the minimum pathlength for any White cell is four times its length. For example, a 1-meter-long White cell has a minimum pathlength of 4 meters. In some White cells the position of the objective mirrors can be adjusted to increase the number of passes of the beam through the cell by multiples of four. Thus, 4 passes, 8 passes, 12 passes, etc., are possible. This allows long pathlengths to be obtained with a relatively short and compact cell. For example, a cell 0.5 meters long set for 16 passes can give a pathlength of 8 meters, which is sufficient to detect many gases in the parts per million range. A picture of a pair of short length, low volume, multi-pass, and high pathlength White cells with glass bodies is shown in Figure 4.32.

The cells seen in Figure 4.32 mount onto the base plate of the sample compartment in most FTIRs, with the cell sticking up out of the sample compartment. Cells in this configuration with pathlengths up to 16 meters or more are commercially available. White cells up to 1 kilometer in pathlength have been built and used [5]. White cells can be heated or cooled as needed for an analyte, and can be constructed of inert materials for analysis of corrosive gases.

Gas phase spectra look completely different than solid and liquid phase spectra. Gas phase molecules are separated by large distances (at least on a molecular scale) and not only vibrate but rotate as well. The molecules in liquid and solid phase samples are crammed too close together to rotate freely. Both the vibrational and rotational energies in molecules are quantized. As a result, molecules contain what are called *rovibrational* energy levels. When a gas phase molecule absorbs mid-infrared light it is promoted from a specific rotational energy level in the ground vibrational state to a specific rotational energy level in the excited vibrational state. The practical import of this is that there are features in the spectra of gases than in the spectra of solids and liquids. This can be seen in the gas phase spectrum of carbon monoxide ($C\equiv O$) shown in Figure 4.33.

The series of peaks in this spectrum comprise what is called a single rovibrational band. Notice that the peaks in Figure 4.33 are relatively narrow. The peak width in infrared spectra is determined, among other things, by the strength of the intermolecular interactions between neighboring molecules [6]. In the gas phase these interactions are weak because the molecules are far apart, leading to narrow bands. If you take $C\equiv O$, condense it into the liquid phase, and measure its infrared spectrum, the series of sharp lines seen in Figure 4.33 would coalesce into one broad peak

FIGURE 4.32 A pair of short length, low volume, multi-pass, high pathlength White cells with glass bodies. (Photo courtesy PIKE Technologies.)

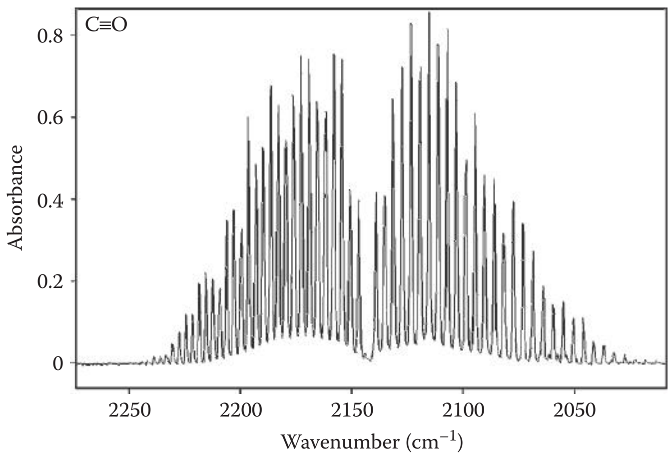

FIGURE 4.33 The infrared spectrum of 55 Torr of carbon monoxide obtained with a 10-cm gas cell. Note the large number and narrowness of the peaks.

centered near 2142 cm^{-1}. So, despite their differences, there is a relationship between gas phase and condensed phase spectra.

In addition to taking the spectra of gases and vapors, infrared gas cells can be used to obtain the spectra of vapors given off by solids and liquids. If the sample of interest is contained in a sealed vessel, the vacuum manifold illustrated in Figure 4.30 can be used to pull vapor off the sample and into the gas cell. Alternatively, if one possesses a gas cell with removable windows like the one seen in Figure 4.28, the solid or liquid can be placed in the cell, the cell sealed, and the infrared beam passed through the vapor above the sample. Since the solid or liquid is sitting at the bottom of the cell it does not interfere with the infrared beam. The author has used this technique to take the spectra of noxious smelling solids.

VI. REFLECTANCE ANALYSIS

A. Different Types of Reflectance

Reflectance sampling is the second major family of infrared sample preparation methods. In reflection techniques the light is reflected from the surface of the sample, as shown in Figure 4.34.

It is convenient when talking about reflectance spectroscopy to define a line drawn perpendicular to the reflecting surface called the *surface normal*, which is shown in Figure 4.34. When an infrared beam approaches a sample, the angle it makes with the surface normal is called the *angle of incidence* and is denoted θ_i. The angle the reflected beam makes with the surface normal after leaving the sample is called the *angle of reflectance* and is denoted θ_r. There are several different types of reflection that can take place depending upon the nature of the reflecting surface. *Specular Reflectance* occurs when $\theta_i = \theta_r$ and is created by smooth surfaces such as mirrors and shiny pieces of metal. Figure 4.34 shows an example of specular reflectance.

When an infrared beam encounters a rough surface, *diffuse reflectance* takes place, as illustrated in Figure 4.35. In diffuse reflectance the angle of incidence is

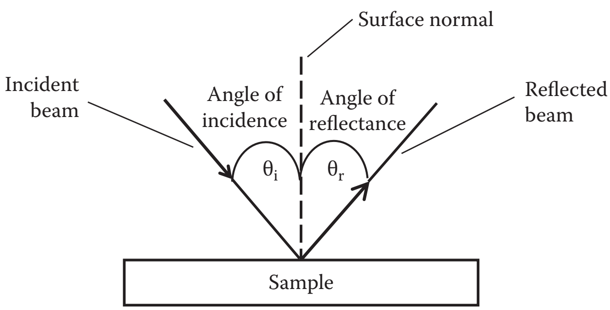

FIGURE 4.34 An illustration of an infrared beam reflecting from the surface of a sample. The dotted line perpendicular to the surface of the sample is called the *surface normal*. The angle the incoming beam makes with the surface normal is called the angle of incidence θ_i. The angle the outgoing beam makes with the surface normal is called the angle of reflectance θ_r.

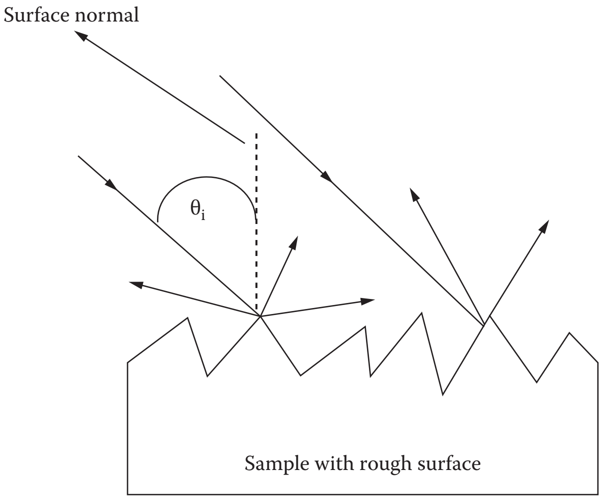

FIGURE 4.35 An example of diffuse reflectance from a rough surface. The angle of incidence is fixed, but the angle of reflectance has many values.

fixed, but the angle of reflectance has many values. Diffuse reflectance is the net result of a number of different phenomena including reflection, refraction, diffraction, scattering, absorbance, and transmittance. This is why the reflected light leaves the sample at so many different angles. Diffuse reflectance is used to take infrared spectra of samples, as will be discussed below.

Another type of reflectance is *total internal reflectance*, which is illustrated in Figure 4.36. In this figure the infrared beam is traveling through a crystal with high refractive index (n_c), which is greater than that of the refractive index of the sample above it (n_s). When the light beam reaches the boundary of the two media, and if the angle of incidence θ_i is large enough, *all* of the light will reflect from the internal surface of the crystal, hence the term total internal reflectance. This phenomenon is how optical fibers work. The light beam reflects off the internal surfaces of the fiber many times, which enables the beam to traverse the fiber from one end to the other.

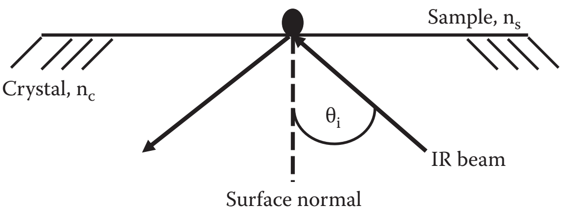

FIGURE 4.36 An example of total internal reflectance. The refractive index of the crystal through which the beam is passing, n_c, is greater than the refractive index of the sample above it, n_s.

B. Advantages and Disadvantages of Reflectance Sampling

In reflectance sampling it takes extra mirrors to focus the light onto the sample and to collect the reflected light. These extra mirrors are contained in reflectance accessories that either mount on the base plate in the FTIR sample compartment or slide into the sample slide mount. These accessories must be custom built for the make and model of FTIR in which they are going to be used. A problem with reflectance accessories is that they are thousands of dollars more expensive than transmission accessories. There are several companies that make customized FTIR reflectance accessories, and they can be found via an Internet search.

Another problem with reflectance techniques is that it is not always possible to collect all the light once it has reflected from the surface of a sample, which is particularly true for diffuse reflectance. As a result, reflectance spectra can be noisy. Another problem with reflectance techniques is unknown pathlength. In transmission, the pathlength is the thickness of sample traversed by the infrared beam. In reflectance the depth the beam penetrates into the surface depends upon the angle of incidence, surface roughness, sample geometry, and the sample's absorptivity, among other things. Thus, we do not always have a precise idea of the pathlength in a reflectance sampling experiment. This can make quantitation difficult because pathlength is one of the variables in Beer's Law (see Equation 1.6) that needs to be controlled to achieve accurate quantitative results.

In transmission, the spectrum is of the bulk of the sample with little surface contribution. In reflectance, the light bounces off the surface of the sample so the surface contributes strongly to the overall spectrum. This surface sensitivity of reflectance techniques is good if you are interested in knowing the composition of the surface of a sample. However, if you are interested in the spectrum of the bulk of a sample, and the surface and the bulk have different chemical compositions, the reflectance spectrum of the sample will not give a complete picture of the composition of the sample.

After reading through the litany of disadvantages for reflectance sampling techniques, you may wonder, why bother using them? Recall that transmission techniques suffer from an opacity problem, and getting around this can entail tedious, time-consuming, manual sample preparation. Reflectance techniques do not suffer from an opacity problem. There is no need to present the sample to the infrared beam in thin film form, so the pressing, grinding, dissolving, and diluting that are typical of transmission analyses evaporate. In general, samples for reflectance analysis are faster and easier to prepare than samples for transmission analysis. Given the great pressure that labs are under these days to run more efficiently, the opportunity to save time and money by using reflectance techniques makes sense. One of the disadvantages noted above of reflectance accessories is that they cost thousands of dollars. However, it has been the author's experience that in many labs, the time and money saved in sample preparation quickly pays for the cost of reflectance accessories. A final advantage of reflectance techniques is that they can be nondestructive. Certain transmission techniques, such as KBr pellets, make it difficult to recover the sample. Some reflectance techniques allow the sample to be analyzed as is, preserving it. This can be useful if the sample is

TABLE 4.9
The Advantages and Disadvantages of Reflectance Sampling Techniques

Advantages	Disadvantages
No opacity problem	Expensive tools
Fast and easy sample preparation	Spectra sometimes noisy
Can be nondestructive	Surface sensitivity
	Sometimes unknown pathlength

valuable, if there is not much of it, or if it needs to be preserved for other analyses. The advantages and disadvantages of reflectance sampling are summarized in Table 4.9.

VII. SPECULAR REFLECTANCE

Specular reflectance is used in infrared spectroscopy to take spectra of samples. Since specular reflectance takes place from smooth surfaces, this technique works well on shiny, smooth metal surfaces. If there is a coating on a metal surface, a technique called *reflectance-absorbance* can be used to obtain the spectrum of the coating, as illustrated in Figure 4.37.

In reflectance-absorbance, the infrared beam passes through the coating, reflects from the metal substrate, passes through the coating a second time, then is captured and focused onto the FTIR's detector. A specular reflectance accessory that can measure reflectance-absorbance spectra is shown in Figure 4.38.

This particular specular reflectance accessory slides into the sample slide mount in the FTIR's sample compartment. Its optical diagram is seen on the right in Figure 4.38. The sample is placed on top of the device, covering the hole. The infrared beam reflects from a flat mirror, from the sample, off a second flat mirror, and is then focused onto the FTIR's detector. The reflectance-absorbance spectrum of the paint on the outside of a full soda can taken with this device is shown in Figure 4.39.

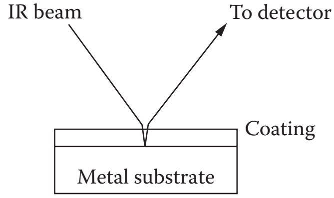

FIGURE 4.37 An example of reflectance-absorbance, where a light beam passes through a sample, reflects from a substrate, passes through the sample a second time, and then is focused onto the infrared detector.

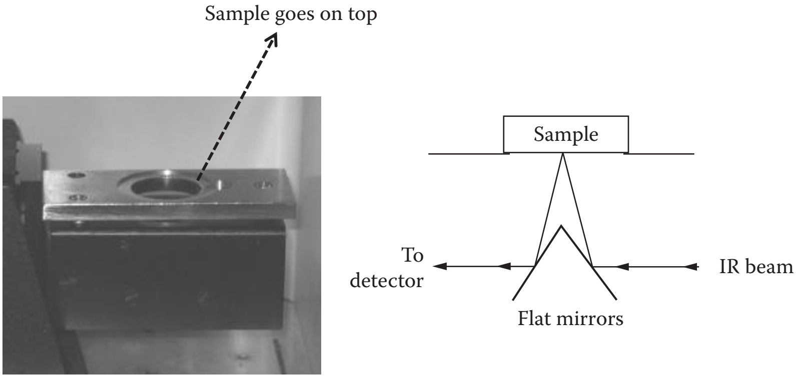

FIGURE 4.38 Left: A picture of a specular reflectance accessory. Right: Its optical diagram.

This spectrum was obtained by holding the soda can on top of the specular reflectance accessory while the FTIR was scanning. The infrared beam passed through the paint, reflected off the aluminum can beneath, passed through the paint a second time, and then onto the detector (the soda can was then removed and the soda was consumed). This technique involves no sample preparation so it is fast and easy, and it is nondestructive. In theory, specular reflectance spectra can be collected of any sample that is placed on top of the accessory. This can include large, bulky objects such as plastic bottles, in addition to shiny metal surfaces and things coated on metal. In reality, there are not many surfaces smooth enough to exhibit significant amounts of specular reflectance, which limits the applications of this technique. A summary of the advantages and disadvantages of specular reflectance sampling is provided in Table 4.10.

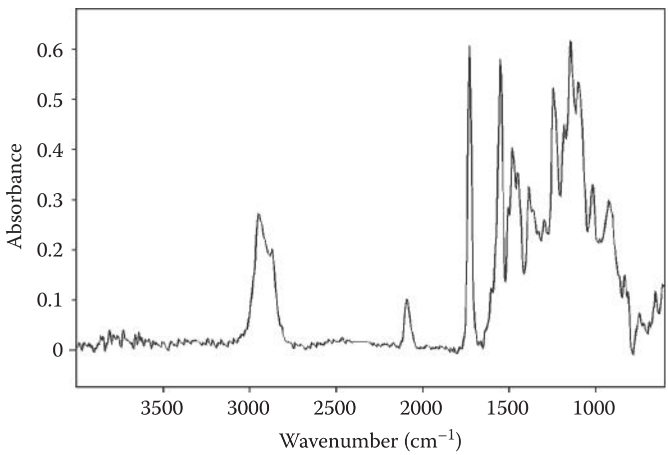

FIGURE 4.39 The reflection-absorbance spectrum of the paint on the outside of a full soda can.

TABLE 4.10
The Advantages and Disadvantages of Specular Reflectance

Advantages	Disadvantages
Fast and easy	Limited applications
Nondestructive	
Surface sensitive	

VIII. DIFFUSE REFLECTANCE (DRIFTS)

When an FTIR is used to collect diffuse reflectance spectra of samples the technique is called Diffuse Reflectance Infrared Fourier Transform Spectroscopy, or DRIFTS for short [7]. DRIFTS is used to analyze powders and things that can be ground into a powder, so it competes directly with the KBr pellet method discussed previously. In both the DRIFTS and KBr pellet methods, the sample and KBr powder are ground to reduce particle size and avoid light scattering. These materials should be ground separately to avoid possible chemical reaction. Grinding can be accomplished using a mechanical grinder, as shown in Figure 4.5, or the agate mortar and pestle shown in Figure 4.40.

The measured intensity of the reflected light in a DRIFTS experiment depends upon the size and shape of the particles and how they are packed together. Mechanical grinding gives more reproducible particle sizes and shapes than manual grinding. Once ground, the sample and KBr are mixed together thoroughly to dilute the sample, which prevents too much light from being absorbed. DRIFTS is not as sensitive as the KBr pellet method and needs 5% to 15% sample should be used. At this point in making a pellet, the KBr/sample mixture would have to be compressed. With DRIFTS, compressing the sample is not necessary. Instead, the sample/KBr powder is placed in a small metal cup like the one shown in Figure 4.40.

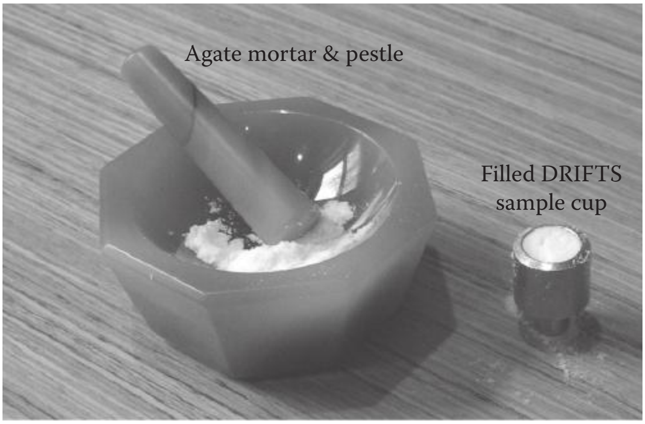

FIGURE 4.40 Left: An agate mortar and pestle used to grind powders for infrared analysis. Right: A filled DRIFTS sample cup.

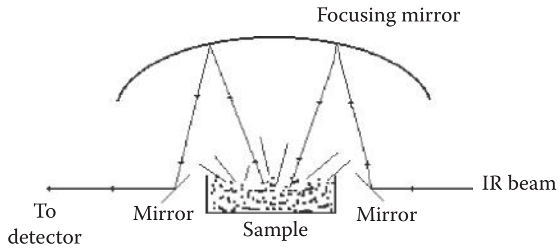

FIGURE 4.41 An optical diagram of a diffuse reflectance (DRIFTS) accessory.

Filling DRIFTS sample cups properly is important, since filling the cups in different ways leads to different packing densities and hence different band heights. To achieve a uniform packing density, fill the sample cup to overflowing using a spatula and then drag its edge across the top of the cup to remove excess powder. When you are done, the surface of the powder should be level, and the sample cup should be full up to the brim. This was how the sample cup in Figure 4.40 was filled. *Do not* tap the sample cup against anything to make the particles settle; it causes the big particles to rise to the top, possibly altering the intensity of reflected radiation. Once the sample cup is properly filled it is placed at the focal point of a diffuse reflectance accessory like the one shown in Figure 4.41.

DRIFTS accessories are typically either mounted on the base plate in the FTIR sample compartment or slide into the sample slide mount. In most DRIFTS accessories, one or more flat mirrors direct the infrared beam to a large focusing mirror mounted above the sample. The focusing mirror is typically either spherical or ellipsoidal. The focused infrared beam then impinges on the surface of the powdered sample. Since the powdered surface is rough, the reflected beams travel in many directions, and the challenge is collecting enough of that light to obtain a usable spectrum. In Figure 4.41 the diffusely reflected light is collected by part of the focusing mirror, reflects off of flat mirrors, and is then focused onto the infrared detector. A DRIFTS spectrum of an aspirin tablet is shown in Figure 4.42.

When enough material is available, grinding and diluting DRIFTS samples in KBr gives the best results. However, the drawback of this method is that it is destructive. Also, if you don't have much sample, or if the sample is valuable, you may not want to destroy it to take its spectrum. In these cases a small amount of sample can be placed in the bottom of a DRIFTS sample cup. The focused infrared beam is reflected directly off the sample, is collected, and is focused onto the detector like in any other DRIFTS experiment. The background spectrum is taken of the empty sample cup is taken. This technique is sometimes called "micro-DRIFTS" because it works on small amounts of sample. A pair of DRIFTS sample cups specially made to hold small amounts of sample are shown in Figure 4.43.

A discussion of the y-axis units used to plot DRIFTS spectra is needed. When percent transmittance or absorbance units are used to plot a spectrum, it is assumed that transmission sampling was used, as illustrated in Figure 4.1. DRIFTS is not a transmission technique but a reflectance technique, and the proper y-axis units for DRIFTS spectra should be reflectance or some unit related to it. It is technically

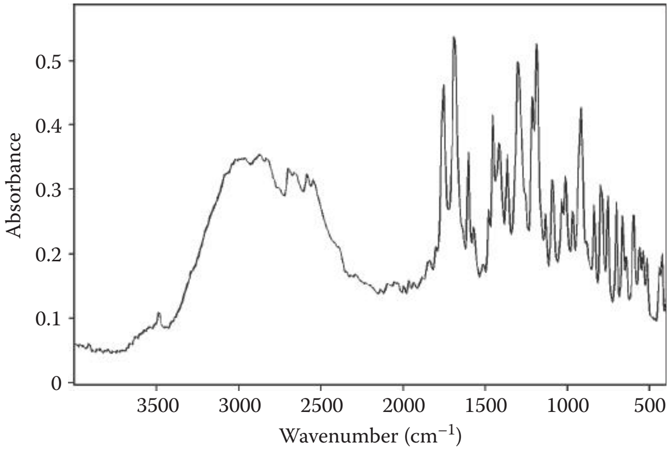

FIGURE 4.42 The DRIFTS spectrum of a ground aspirin tablet diluted in KBr.

incorrect to plot DRIFTS spectra in absorbance or transmittance. The correct units to use are called *Kubelka–Munk units* [8], named after two gentlemen who studied diffuse reflectance. Many FTIR software packages have routines that will convert absorbance or percent transmittance spectra to Kubelka–Munk units. Figure 4.44 shows a comparison of the DRIFTS spectrum of an aspirin tablet plotted in absorbance and Kubelka–Munk units.

As you can see, the peaks in the Kubelka–Munk spectrum are smaller and have different relative intensities than those in the absorbance spectrum. To be clear, the only difference between these two spectra is how the y-axis units are plotted; peak positions are the same. The equation derived by Kubelka and Munk relates the intensity of diffusely reflected light to concentration [8]. If you are going to perform quantitative DRIFTS you must measure the spectra in Kubelka–Munk units to obtain a calibration line, similar to how absorbance spectra must be used for quantitation in transmission experiments (see Chapter 5). However, if one is doing qualitative analysis, DRIFTS spectra plotted in absorbance or Kubelka–Munk units may be used. Since the DRIFTS experiment does not produce a true absorbance spectrum, it is best to call the y-axis units of a DRIFTS spectrum plotted in absorbance "diffuse absorbance." All the DRIFTS spectra in this chapter are plotted in diffuse absorbance.

FIGURE 4.43 A picture of two different micro-DRIFTS sample cups.

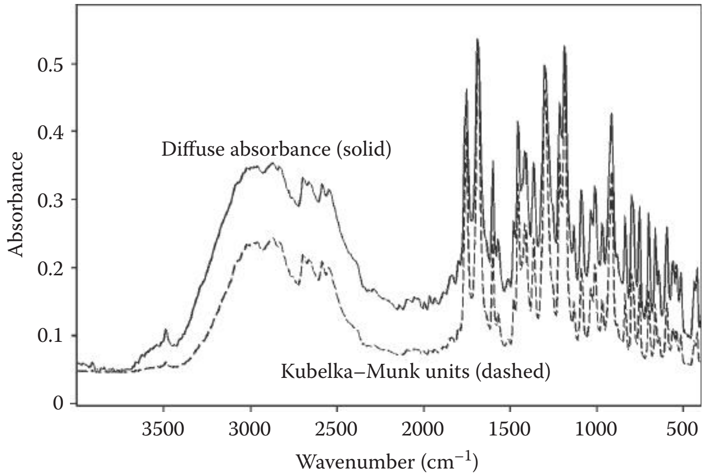

FIGURE 4.44 Top (solid): DRIFTS spectrum of an aspirin tablet plotted in "Diffuse Absorbance." Bottom (dashed): DRIFTS spectrum of an aspirin tablet plotted in Kubelka–Munk units.

A. Abrasive Sampling

In addition to obtaining the spectra of powdered samples, DRIFTS accessories can be used to take the spectra of large intractable solids using a technique known as *abrasive sampling*. In this technique, abrasive paper is used to collect particles from the sample of interest; silicon carbide is commonly used. A convenient way of obtaining abrasive sampling spectra is to purchase (from an FTIR accessory company) pieces of precut abrasive paper with an adhesive on the back, one of which is seen on the left in Figure 4.45.

To obtain a sample, the adhesive backing must first be removed and the disk adhered to a metal post like the one seen in the middle of Figure 4.45, which gives the arrangement seen on the right in Figure 4.45. Then the abrasive paper is rubbed against the sample to collect particles. The sample is then put at the focal point of a DRIFTS accessory, and the light is reflected off the sample particles and is collected

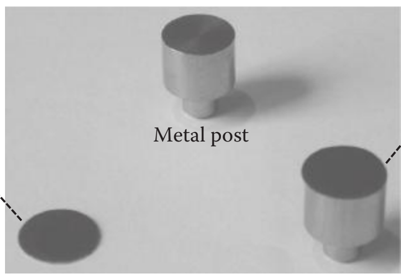

FIGURE 4.45 Left: A silicon carbide disk with an adhesive back. Center: A metal post that fits into a DRIFTS accessory. Right: A silicon carbide disk adhered to the metal post.

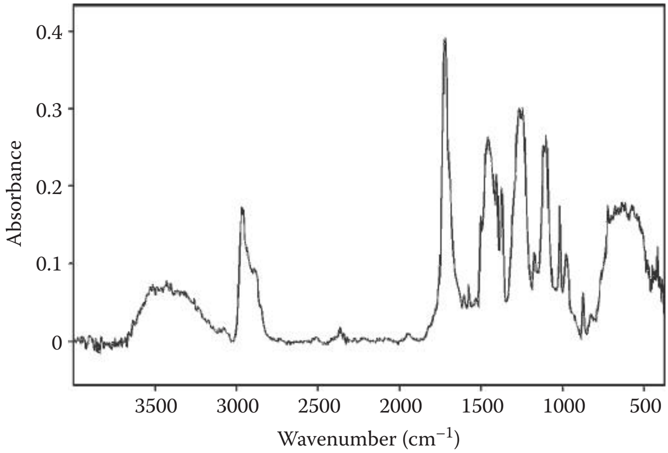

FIGURE 4.46 The abrasive sampling spectrum of white paint from a light fixture.

as in any other DRIFTS experiment. The background spectrum is obtained on a piece of clean abrasive paper. You can cheaply make your own SiC paper discs by buying silicon carbide paper and using a cork borer to cut out disks of the right size to fit into your DRIFTS sample cup. An abrasive sampling spectrum of the white paint from a light fixture is shown in Figure 4.46.

The beauty of the abrasive sampling technique is that it is fast, easy, and in theory can work on many solid samples. The drawback to the technique is that the spectra are frequently noisy, as can be seen in Figure 4.46. The problem is the infrared beam is scattered and reflected in many directions by the sample particles, reducing the amount of signal measured and causing unwanted noise. Frequently, more scans are needed to obtain usable spectra via abrasive sampling spectra than for other sample preparation techniques.

An advantage of DRIFTS is that it is faster and easier than the KBr pellet method because the sample does not need to be compressed. The technique can be nondestructive if desired by using the micro-DRIFTS technique, and abrasive sampling allows spectra of large, intractable objects to be obtained easily. The major drawback of DRIFTS is that since light is lost when it reflects from a rough surface, DRIFTS spectra can sometimes be noisy. Finally, the accessories cost a few thousand dollars, so the upfront investment in a DRIFTS accessory is greater than for other sampling techniques. The advantages and disadvantages of DRIFTS are summarized in Table 4.11.

TABLE 4.11
The Advantages and Disadvantages of DRIFTS

Advantages	Disadvantages
Comparatively easy sample preparation	Spectra may be noisy
Nondestructive	Accessory expense
Abrasive sampling	

IX. ATTENUATED TOTAL REFLECTANCE (ATR)

Attenuated total reflectance (ATR) is based upon internal reflectance, which is shown in detail in Figure 4.47. The figure shows a beam of light traveling through a crystal of high refractive index n_c, which encounters a boundary with a sample with lower refractive index n_s. The dotted line in Figure 4.47 is the surface normal, and the angle the incoming beam makes with the surface normal is the angle of incidence, θ_i. What happens to the infrared beam when it reaches the boundary of the two media depends upon θ_i. If the angle of incidence is small, some of the beam will reflect off the internal surface of the crystal, and some of the beam will refract out of the crystal into the sample giving the refracted beam shown in Figure 4.47. The *angle of refraction*, denoted by θ_R, is the angle the refracted beam makes with the surface normal. The laws of refraction tell us [9] that as θ_i increases θ_R increases. At some angle of incidence the angle of refraction will become 90° and the infrared beam will no longer leave the crystal, but will remain within it. In this case all the light reflects off the internal surface of the crystal, hence the term *total internal reflectance*. The minimum angle of incidence at which total internal reflectance occurs in a material is called its *critical angle*, θ_c. Total internal reflection will take place at θ_c and all angles of incidence greater than θ_c. The critical angle depends upon the refractive indices of the crystal and sample as follows:

$$\theta_c = \sin^{-1}(n_s/n_c) \tag{4.1}$$

Where

θ_c = Critical angle
n_s = Refractive index of sample
n_c = Refractive index of crystal

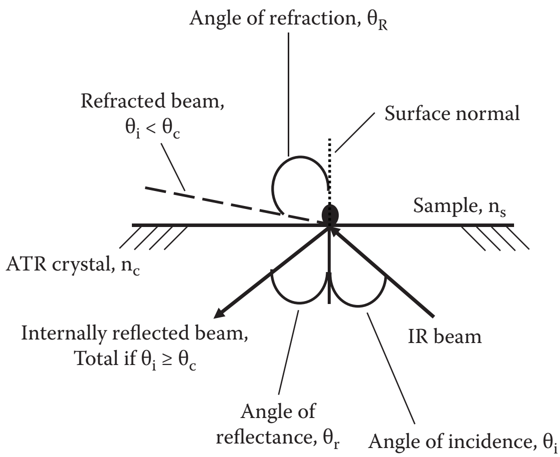

FIGURE 4.47 The optical processes that take place when an infrared beam in a crystal of high refractive index, n_c, encounters a sample of lower refractive index n_s. θ_i is the angle of incidence, θ_R is the angle of refraction, θ_r is the angle of reflectance, and θ_c is the critical angle above which total internal reflection takes place. Total internal reflectances to take place when $n_s < n_c$ and $\theta_i > \theta_c$.

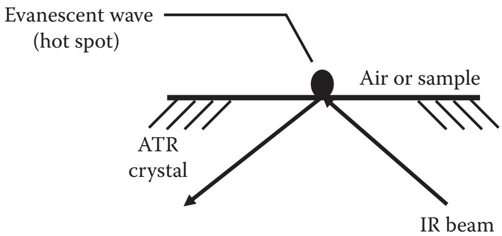

FIGURE 4.48 An illustration of the evanescent wave, or hot spot, that forms when an infrared beam undergoes total internal reflectance.

If the sample is a typical organic material with n = 1.5 and the crystal is diamond with n = 2.42, the critical angle is 38.3°. Note that Equation 4.1 only has physical meaning if $n_s < n_c$. By definition the sin of an angle cannot be greater than 1. If $n_s > n_c$ in Equation 4.1 you will be trying to find the angle whose sin is greater than one, which is impossible. Practically, this means total internal reflectance ceases when $n_s > n_c$. Total internal reflection is similar to specular reflectance in that $\theta_i = \theta_r$.

It is not yet obvious how to make use of total internal reflectance to take spectra of samples since the infrared beam appears to never leave the crystal. In reality, at the point of internal reflectance the incoming and outgoing infrared beams occupy the same volume. Under the right conditions (including $\theta_i \geq \theta_c$) these two beams undergo constructive interference. This means the infrared amplitude at the point of internal reflectance will be greater than the amplitude on either side. Since we are already at the surface of the crystal, the enhanced amplitude has nowhere to go but up, which results in infrared light sticking up into the space above the crystal surface, which is called an *evanescent wave* as illustrated in Figure 4.48. Evanescent waves are also sometimes called "hot spots."

The evanescent wave sticks up above the surface of the crystal by less than a micron to upwards of 10 microns depending upon the experiment. To take the spectrum of a sample it is brought into contact with the hot spot, some of the beam is absorbed by the sample, and the beam is then focused on the detector. The background spectrum is obtained of the clean, dry crystal. Since the sample absorbances attenuate the intensity of the totally reflected infrared beam the term *attenuated total reflectance* (ATR) is used. An example of how an ATR crystal can be used to take the spectrum of a sample is shown in Figure 4.49.

In theory any sample that can be brought into contact with a hot spot will have its spectrum measured. For solids, polymers, and powders a clamp should be used to apply pressure to the sample to obtain good sample/hot spot contact.

In the past ATR crystals were shaped into long, thin rods or parallelograms and were made of high-refractive-index materials that were transparent in the mid-infrared such as zinc selenide (ZnSe), silicon, and germanium. These crystals frequently had multiple hot spots on their surface, as illustrated in Figure 4.49. The total pathlength for this type of ATR experiment is simply equal to the depth of penetration of the beam into the sample for each hot spot times the number of hot spots covered by the sample. For example, in Figure 4.49 if the beam penetrated 1 micron at each hot

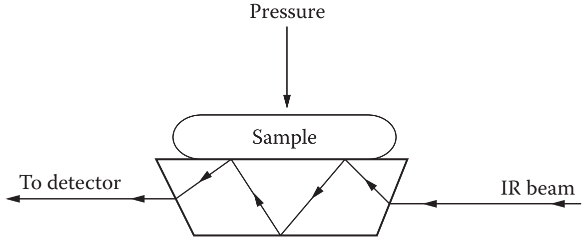

FIGURE 4.49 An example of an ATR crystal with two hot spots on its top surface. Pressure is applied to some solid samples using a clamp to facilitate sample/hot spot contact.

spot, and the sample covered both hot spots, the total pathlength would be 2 microns. The advantage of long, thin ATR crystals is that multiple hot spots boost pathlength, increasing the sensitivity of the experiment. With the more recent introduction of diamond as an ATR crystal material, the size of crystals has shrunk dramatically because of diamond's high cost. Most diamond ATRs contain only one hot spot and are sometimes called "single bounce" accessories because the infrared beam only reflects once off the crystal surface.

A. Depth of Penetration

An important thing to know about any ATR experiment is how far the infrared beam penetrates into the sample. The intensity of the evanescent wave decreases exponentially with distance above the surface of the ATR crystal. The *depth of penetration* (DP) is the depth at which the evanescent wave intensity decreases to 36.8% (= 1/e where e is the base of natural logarithms) of its initial value. The depth of penetration for an ATR experiment is given by Equation 4.2:

$$DP = 1/[2\pi W n_c(\sin^2\theta - n^2_{sc})^{1/2}] \tag{4.2}$$

Where

DP = Depth of penetration
W = Wavenumber
n_c = Refractive index of ATR crystal
θ = Angle of incidence
n_{sc} = $n_{sample}/n_{crystal}$

Note that all of the parameters in Equation 4.2 are in the denominator, so if any of them go up, DP goes down. Since wavenumber units of cm^{-1} are used in Equation 4.2, the DP calculated will be in centimeters; multiply this number by 10^4 to obtain DP in microns. At 1000 cm^{-1} a diamond ATR crystal with n_c = 2.42, angle of incidence of 45°, and sample refractive index of 1.4 (typical of many organic materials) gives a DP of 1.6 microns. Each of the parameters in Equation 4.2 has important things to teach us about the ATR technique and its applications.

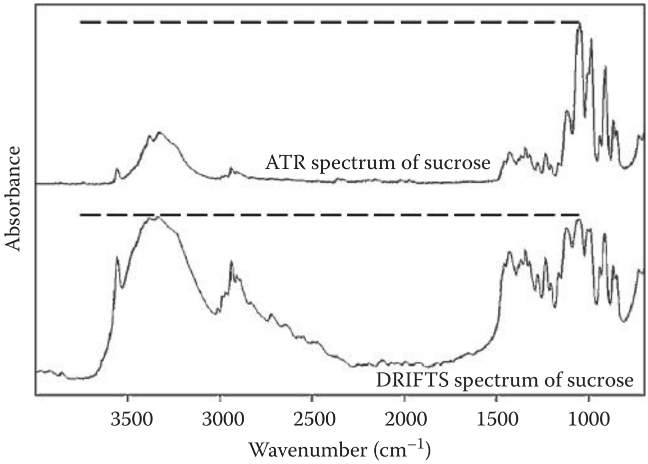

FIGURE 4.50 Bottom: The DRIFTS spectrum of sucrose (table sugar). The dashed line shows that the peaks at high and low wavenumber are about the same intensity. Top: The ATR spectrum of sucrose. The dashed line shows that the peaks at high wavenumber are less intense than the peaks at low wavenumber.

The W in Equation 4.2 is wavenumber, and since it is in the denominator, as W goes up, DP goes down. Thus, 1000 cm^{-1} light penetrates three times farther into a sample than 3000 cm^{-1} light. For a spectrum plotted with low wavenumber to the right, DP goes down as you read the spectrum from right to left, so peak height goes down as well. For the sampling techniques discussed earlier in this chapter pathlength did not depend on W, so the peaks do not necessarily get smaller at high W. The net effect is that the relative intensities in ATR spectra and non-ATR spectra of the same sample are different, as illustrated in Figure 4.50.

The bottom spectrum in Figure 4.50 is the DRIFTS spectrum of sucrose: the depth the infrared beam penetrated into the sample did not depend upon wavenumber, and the dashed line shows that the peak heights at high and low W are about the same. The top spectrum in Figure 4.50 shows the ATR spectrum of sucrose, and the dashed line shows that the peaks near 1000 cm^{-1} are much bigger than the peaks around 3300 cm^{-1}. Note that the peak positions in the two spectra are similar and the relative intensities of the peaks are different.

The dependence of ATR intensities on wavenumber has important implications for how we can use and interpret ATR spectra. It is difficult to compare ATR spectra to spectra measured using other sampling techniques because the spectra of the same sample will look different, as illustrated in Figure 4.50. This is why it is best to only compare ATR spectra to other ATR spectra. If you must compare ATR and non-ATR spectra to each other, be aware that the relative intensities will be different. Similarly, it is best to only search ATR spectra against libraries containing ATR spectra. Searching ATR spectra against non-ATR libraries will produce poor search results because the relative intensities in the sample and library spectra will be different, based not on chemical differences but sample preparation. One way around

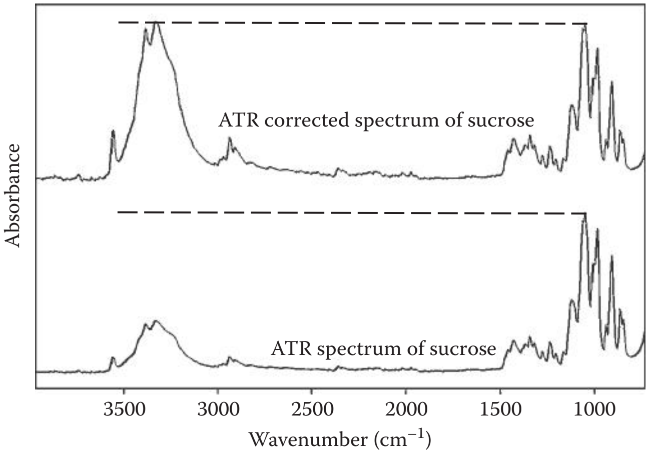

FIGURE 4.51 Bottom: The ATR spectrum of sucrose. Top: The ATR corrected spectrum of sucrose. The dashed lines show that in the uncorrected spectrum the peaks at high and low wavenumber are different in size, and that in the corrected spectrum they are similar.

this problem is to purchase libraries of ATR spectra (see Chapter 3) or to build your own ATR libraries.

If you must search ATR spectra against non-ATR libraries there is help. Some FTIR software packages contain an "ATR Correction" function. This function adjusts the relative intensities in an ATR spectrum so it looks more like a spectrum measured using a transmission experiment; the net effect is to make the peaks at higher wavenumber bigger than they were in the original ATR spectrum. A comparison of the ATR spectrum and ATR corrected spectrum of sucrose is seen in Figure 4.51.

The bottom spectrum in this figure is sucrose measured via ATR; note that the peaks at high and low wavenumber are different sizes. The top spectrum in Figure 4.51 is the ATR corrected spectrum of sucrose. Note how the peaks at high and low wavenumber are the same size, and that this spectrum looks more like the DRIFTS spectrum of sucrose seen in Figure 4.51 than its the ATR spectrum. Once the ATR correction function has been applied the quality of matches obtained with non-ATR libraries should improve. However, the term "ATR correction" is a misnomer. There is nothing wrong with ATR spectra that do not need correcting; these functions try to get around the depth of penetration's dependence on wavenumber. Some labs mistakenly apply the ATR correction function to all their ATR spectra, including ones that are not going to be searched against or compared to non-ATR spectra. This is a bad idea because the ATR correction alters your original experimental data. A spectrum should only be processed for a reason. Needing to search an ATR spectrum against a non-ATR library is a legitimate reason to ATR correct a spectrum. Altering an ATR spectrum in the mistaken belief that it needs "correcting" is not a legitimate reason to use this function.

The second parameter that appears in the denominator of Equation 4.2 is the refractive index of the ATR crystal, n_c, which means as n_c goes up, the depth of penetration

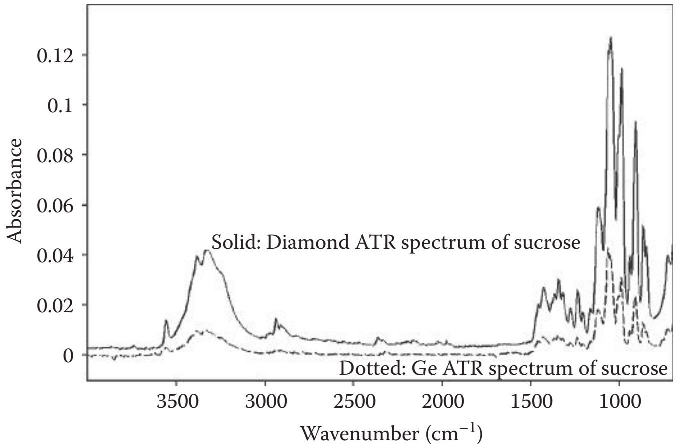

FIGURE 4.52 Bottom: The ATR spectrum of sucrose obtained using a germanium (Ge) crystal. Top: The ATR spectrum of sucrose obtained using a diamond crystal. Note that the peaks in the diamond crystal spectrum are bigger because of greater depth of penetration.

goes down. Thus for ATR crystals of different refractive indices the infrared beam will penetrate to different depths. ATR accessories are available that make it easy to switch between crystals with different values of n_c. This allows spectra of a sample to be measured at different depths nondestructively—an ability know as *depth profiling*. Figure 4.52 shows a comparison of ATR spectra of the same sample obtained with diamond and Ge crystals.

Note in Figure 4.52 that all the peaks in the diamond ATR spectrum are more intense than the peaks in the Ge ATR spectrum. This is because the depth of penetration was greater using the diamond than using the Ge crystal. The ability to obtain depth profiling spectra can be very helpful if you are interested in knowing how the composition of a sample changes with depth, such as for polymer laminates. In theory, one could take the spectrum of the top two layers of a laminate with a diamond ATR crystal with a relatively large DP, obtain the spectrum of the top layer by itself using a germanium (Ge) ATR crystal with a shallower depth of penetration, then subtract the Ge spectrum from the diamond spectrum to obtain the spectrum of the second layer to the laminate.

The refractive indices and other relevant properties of some common ATR crystals are shown in Table 4.12. It is important to choose the correct ATR crystal for a given application. In addition to the crystal's refractive index, properties such as its useful wavenumber range, pH sensitivity, durability, and toughness matter as well. KRS-5 is a mixture of thallium bromide and thallium iodide and was one of the first materials used as an ATR crystal. Its one advantage is that it works over a wide wavenumber region, including the entire mid-infrared. However, it is soft and easily scratched so it cannot be used with powders and many other solids, and it is highly toxic and should never be handled with bare hands.

Zinc selenide (ZnSe) was for many years the most popular ATR crystal material. It is tougher and harder than KRS-5, and is transparent throughout most of the

TABLE 4.12
The Properties of Common ATR Crystals

Crystal Material	Refractive Index	Wavenumber Range(s) (cm^{-1})	Color	pH Range	Comments
KRS-5	2.37	20,000–250	Red	5–8	Soft, highly toxic, rarely used today
ZnSe	2.42	15,000–600	Yellow	5–9	Once very common, brittle, attacked by strong acids and bases
Si	3.42	8900–660	Grey	1–12	
Ge	4.0	5500–600	Grey	1–14	Shallow DP, durable
Diamond	2.42	30,000–2200, 2000–400	Clear	1–14	Tough, durable, absorbs in mid-infrared, $$$

mid-infrared. However, hard powders and solids will scratch it, it will snap if too much pressure is applied, and it is attacked by strong acids and bases. This material works well with liquids, polymers, and soft solids but has difficulty with powders. Silicon and germanium are useful ATR crystals because they are hard, durable, and not that sensitive to pH. Germanium is particularly useful because its high refractive index of 4.0 gives it the shallowest depth of penetration of any of the ATR crystals listed. This is important in the analysis of filled polymers, which are frequently rubbery materials such as o-rings and gaskets filled with carbon black, silica, or limestone. These fillers present a problem because they absorb strongly, masking the spectrum of the resin, and their particles scatter the infrared beam, giving offset and sloped baselines. This is illustrated in the top spectrum of Figure 4.53, which is the diamond ATR spectrum of a carbon black-filled o-ring.

The baseline offset of over 0.8 absorbance units in this spectrum is real. Also, note the sloped baseline and the distorted peak shapes. These spectral problems are caused by the absorbance, scattering, and reflectance of the carbon black filler. It would be difficult to identify the resin used to make this o-ring from such a low-quality spectrum. A library search of this spectrum produced a best match of fructose, which is a far cry from the structure of the resin actually in the o-ring. The bottom spectrum in Figure 4.53 is the spectrum of the same o-ring measured using a Ge crystal. The lower pathlength means the infrared beam sees less carbon black filler, so this spectrum, although not perfect, has much less baseline offset and slope than the diamond crystal spectrum. Also, the peak shapes in the Ge crystal spectrum are not distorted, making it easier to identify the resin used to make the o-ring. A library search of this spectrum produced a top match of acrylonitrile/butadiene rubber, which is what the o-ring is made from. This example shows why Ge ATR is the method of choice for analyzing filled polymers.

The use of diamond as an ATR crystal began in the mid-1990s and has increased exponentially since then. Diamond is in some ways the perfect ATR crystal material. Its refractive index is almost identical to that of ZnSe, which means the two crystals produce similar-looking spectra, which is very useful when performing spectral

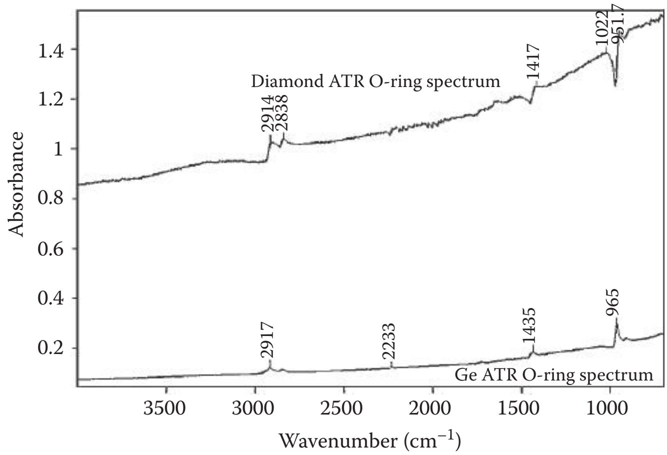

FIGURE 4.53 Top: The diamond ATR spectrum of a carbon black-filled o-ring. Note the baseline slope, offset, and distorted peak shapes. Bottom: Ge ATR spectrum of the same o-ring. The baseline slope and offset are greatly reduced, and the peak shapes appear normal.

comparisons or library searching. Diamond is chemically inert; it will not react with most samples, and it is impervious to strong acids and bases. Diamond is the hardest substance known to man, so samples will not scratch it. Finally, diamond is tough. A lot of pressure can be applied to it to flatten any manner of samples and the crystal will not break. There are very few materials whose spectra cannot be obtained using a diamond ATR, making it the most versatile ATR crystal material. This crystal has become so popular that some in the literature have started calling the technique DATR, which stands for Diamond Attenuated Total Reflectance.

Despite their popularity, diamond ATR crystals do have some disadvantages you need to understand. First, diamond ATRs cost several thousand dollars more than other ATR accessories. A close inspection of Table 4.12 shows another disadvantage of diamond: it absorbs strongly in the mid-infrared between 2200 and 2000 cm^{-1}. In theory, these bands should ratio out when the sample single beam and background spectra are ratioed. In reality, the diamond bands in this region are almost totally absorbing so that sample absorbances in this region are difficult to see. For example, C≡C and C≡N bonds absorb between 2200 and 2000 cm^{-1} [6], but these peaks will not appear in DATR spectra. If analyzing for functional groups that absorb in this region it is useful to use a crystal such as germanium in addition to diamond. Despite these disadvantages, diamond is still an excellent ATR crystal material.

The third parameter in the denominator of Equation 4.2 is $\sin^2\theta$ where θ is the angle of incidence, as shown in Figure 4.47. Since $\sin\theta$ is in the denominator, as in θ goes up, depth of penetration goes down. This means if we had a way of adjusting θ we'd have another means of depth profiling. There are ATR accessories you can buy in which the positions of the mirrors in the transfer optics can be easily adjusted. By changing the angle at which the light beam approaches the ATR crystal, the angle of incidence of the beam at the top surface of the crystal can be changed. Figure 4.54

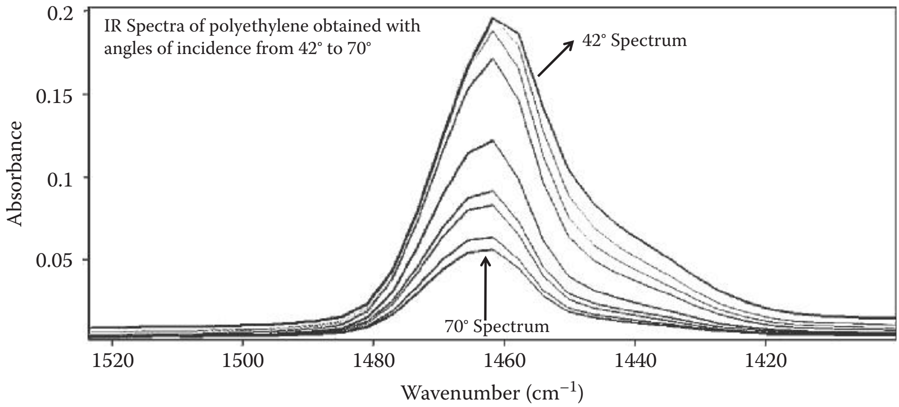

FIGURE 4.54 A peak in the DATR spectrum of polyethylene measured with angles of incidence from 42° to 70°. The peaks are of different sizes because the depth of penetration changed with angle of incidence.

shows a peak in the spectrum of polyethylene taken at many angles of incidence using such an ATR accessory.

The top spectrum in Figure 4.54 was measured at 42°, the bottom spectrum at 70°, and the middle spectra were measured at angles of incidence in between. Since the size of an absorbance peak depends upon pathlength, the size of the peak in Figure 4.54 varies with angle of incidence. Changing the angle of incidence gives the experimenter more control over DP than changing the refractive index of the ATR crystal. Mother Nature determines the latter quantity, and we have no control over it. The former quantity can be easily controlled using the appropriate ATR accessory. This ability to fine-tune the depth of penetration is very useful for samples where composition changes with depth are of interest, such as for polymer laminates.

The final parameter in the denominator of Equation 4.2 is the ratio of the refractive index of the sample, n_s, to the refractive index of the crystal, n_c. A close look at Equation 4.2 shows that as n_s goes up, DP goes up as well. Because of this dependence, DP will change from sample to sample. However, for many organic and biological substances the refractive index falls in a narrow range between 1.4 and 1.5. Since this parameter is essentially constant across this broad class of materials, the DP is about the same for them as well. It is sometimes safe to assume, to a first approximation, that the DP is sample independent for many organic and biological materials. This assumption is *not* true for inorganics, where n_s varies significantly from substance to substance. Since the DP is essentially constant for some materials, and since Beer's Law (Equation 1.6) depends upon pathlength, ATR is well suited for quantitative analysis since DP may be controlled. An example calibration obtained using ATR is shown in Chapter 5.

Figure 4.49 shows that for some solids pressure must be applied to obtain ATR spectra, and as pressure goes up, DP goes up. In theory, then, pressure should appear in the numerator of Equation 4.2. It does not for two reasons. First, since pressure is not applied to all samples, and an equation that applies to all samples is desired, pressure is not included. Second, the relationship between pressure and DP for a given sample can be complex and is not necessarily linear. For example, doubling the pressure does not necessarily double the DP. In the absence of the ability to control the pressure applied to solid samples, the DP will vary, giving irreproducible spectra and making quantitation difficult. Fortunately, there is a solution to this problem. You can buy ATR clamps with pressure gauges on them that allow reproducible amounts of pressure to be applied to solids, making it possible to control the DP for these materials, obtain reproducible spectra, and make quantitation possible.

X. APPLICATIONS OF ATR

A. Liquids

Taking the ATR spectra of liquids is simply a matter of bringing the liquid into contact with the hot spot on the surface of the ATR crystal. Liquids will normally make good contact with the hot spot, so there is no need to clamp them. Depending upon the ATR accessory, a trough or well may be used to hold the liquid in place atop the ATR crystal. Otherwise, a drop of liquid is placed on the crystal. ATR crystals will not dissolve in or react with most organics, so this technique is well suited to organic liquids. Similarly, because ATR crystals will not dissolve in water, this technique can be used to analyze aqueous solutions as well, which is illustrated in Figure 4.55.

The bottom spectrum in Figure 4.55 is the DATR spectrum of the amino acid glutamine dissolved in water. The water peaks are dominant and the glutamine peaks

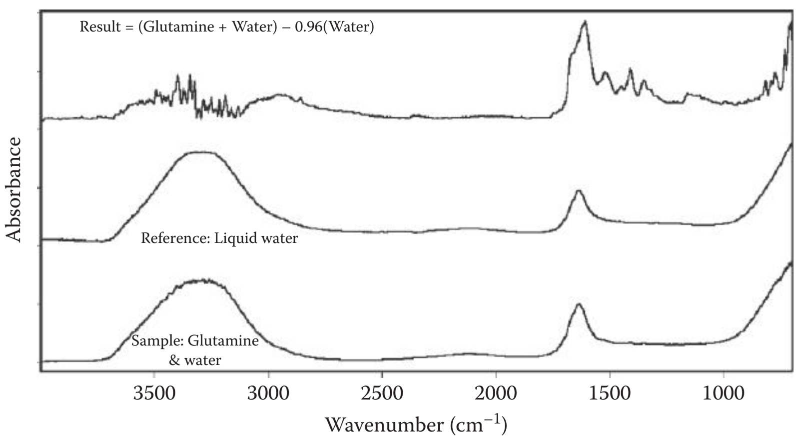

FIGURE 4.55 Bottom: The ATR spectrum of the amino acid glutamine dissolved in water. Note that the glutamine peaks are difficult to see. Middle: A reference spectrum of pure liquid water obtained by ATR. Top: The result of subtracting the pure water spectrum from the glutamine dissolved in water spectrum. Note how clearly the glutamine peaks now appear.

are barely visible. The middle spectrum in Figure 4.55 is the DATR spectrum of pure water. The top spectrum is the result obtained by subtracting the pure water spectrum from the water + glutamine spectrum. Note how the water contribution is almost completely removed, and there are now a number of glutamine peaks clearly visible. ATR is well suited to the analysis of aqueous solutions because the small depths of penetration keep the water absorbances small enough so they can subtract cleanly.

Something to be careful about when studying liquids is that some crystals, such as ZnSe, will react with or dissolve in strong acids or bases. The usable pH ranges for a number of ATR crystals are listed in Table 4.12. Another issue with ATR is that volatile liquids, such as acetone, may evaporate before their spectrum is measured. Many ATR accessories are equipped with "volatiles covers" that are placed over the liquid in an attempt to prevent evaporation. The author has had mixed results with these, and in his experience, highly volatile liquids may be best sampled using the sealed liquid cell method discussed earlier.

B. Semi-Solids

Semi-solids include soft things like peanut butter and toothpaste, and viscous liquids like maple syrup or motor oil. These materials are easy to analyze by ATR; simply smear them on the ATR crystal, measure the spectrum, and then clean the crystal with an appropriate solvent. As always, the background spectrum is run on a clean, dry ATR crystal. Since semi-solids spread out nicely and make good contact with the ATR crystal, they generally do not need to be clamped. The spectrum of a well-known semi-solid, butter, is shown in Figure 4.56.

The butter spectrum was obtained by smearing it on the ATR crystal with a butter knife and then cleaning it off with a paper towel and glass cleaner. As you can see, ATR is a fast and easy method for obtaining infrared spectra of semi-solids. That being said, the "smear" method of analyzing semi-solids in transmission discussed earlier in this chapter is fast and easy as well. The ultimate choice of which technique to use may be guided by which is more convenient for a given sample.

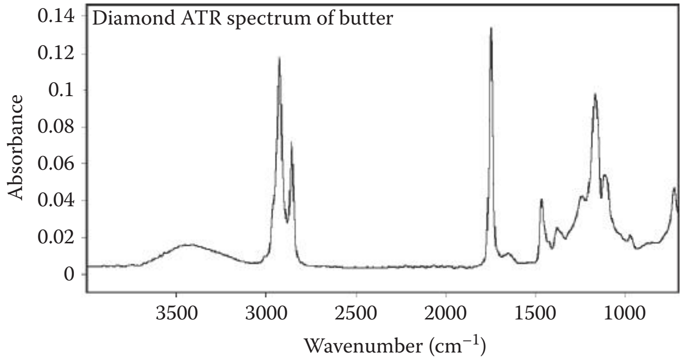

FIGURE 4.56 The diamond ATR spectrum of butter, an example of a semi-solid.

C. Polymers

The transmission techniques for polymers discussed earlier in this chapter involved either dissolving the polymer in a solvent and casting a film, or heating and pressing the polymer into thin film form. Both of these methods can be time-consuming and involve trial and error to get a film of the right thickness. Additionally, both of these methods involve altering the morphology of the polymer, and important information such as percent crystallinity is lost. ATR suffers from none of these problems; it provides a fast and easy method of obtaining polymer spectra. Since polymers are solids they will generally need to be clamped to the ATR crystal to promote good sample/hot spot contact. Taking a spectrum of a polymer, then, is simply a matter of running a background spectrum on a clean, dry ATR crystal, clamping the sample to the crystal, taking the spectrum, and then cleaning the crystal. In theory, the spectrum of any polymer sample can be obtained if it can be clamped to the ATR crystal, including polymer films, pieces, pellets, and parts. The ATR spectrum of a polymer film made of polyethylene terephthalate (otherwise known as a soda bottle) is shown in Figure 4.57.

The entire measurement process for the spectrum in Figure 4.57, including scanning, took less than one minute. Note the high SNR in the spectrum, and how the peaks at low wavenumber are significantly smaller than the peaks at high wavenumber—a result of the depth of penetration's dependence on wavenumber as discussed above.

Pellets of polymeric material are important in commerce. Many of the plastic pieces and parts that populate our everyday lives are formed by a technique called injection molding, where polymer pellets are fed into a machine, melted, and injected into a mold. The polymer conforms to the shape of the mold, cools, the molded part is removed, and the process is repeated. Being able to analyze polymer pellets before they are made into a product is important for quality control purposes. In the past, polymer pellets were hard to analyze by the transmission methods discussed

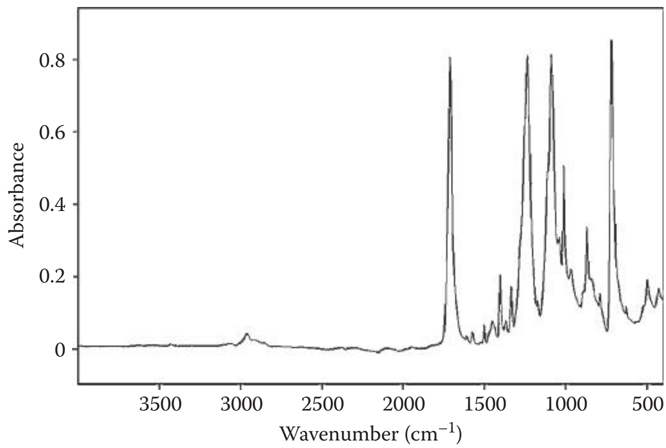

FIGURE 4.57 The diamond ATR spectrum of a polymer film of polyethylene terephthalate, otherwise known as a soda bottle.

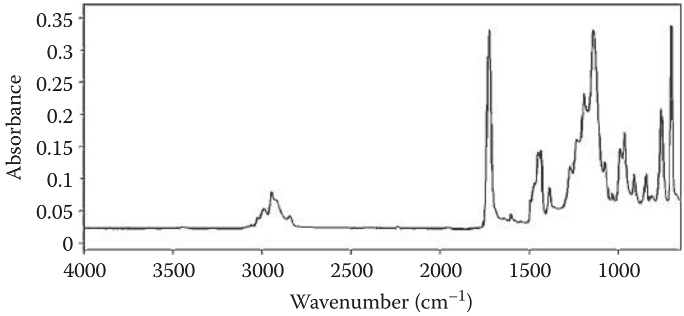

FIGURE 4.58 The diamond ATR spectrum of a pellet of styrene/methyl methacrylate co-polymer.

above. Analyzing a polymer pellet by ATR is simply a matter of clamping the pellet to the ATR crystal and taking its spectrum. The diamond ATR spectrum of a polymer pellet composed of a styrene/methyl methacrylate co-polymer is shown in Figure 4.58.

D. Powders

Powders are difficult to analyze via ATR because they are hard and can scratch soft ATR crystals such as KRS-5 and ZnSe. Additionally, they are composed of irregularly shaped crystallites that do not easily flatten and do not make good contact with the ATR crystal, as illustrated in Figure 4.59.

Typically, high pressure is needed to flatten powders so they make good contact with the ATR crystal surface. This much pressure can bend or break softer ATR crystals. In the mid-1990s a valuable development in ATR occurred: the introduction of diamond ATR (DATR) crystals. Diamond is an almost perfect ATR crystal material. It is the hardest substance known to man so it will not scratch easily, is very tough, and endures a lot of pressure without breaking. This means almost any solid or powdered sample can be examined by DATR. Since diamond is expensive, almost all DATR accessories contain single-bounce crystals. An optical diagram of a commercially available DATR accessory is shown in Figure 4.60.

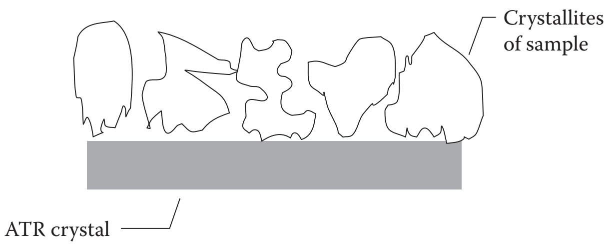

FIGURE 4.59 A diagram of irregularly shaped crystallites of a powder sitting on an ATR crystal. The crystallites do not flatten easily, hence high pressure must be applied so they make good contact with the crystal surface.

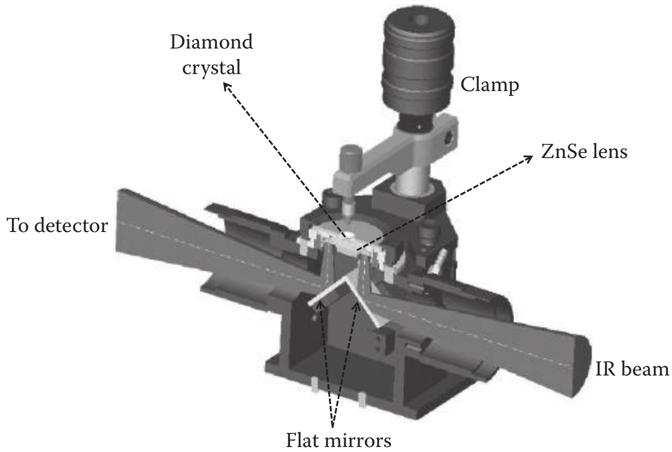

FIGURE 4.60 The optical diagram of a commercially available single-bounce diamond ATR accessory. (Diagram courtesy of PIKE Technologies.)

In this accessory the infrared beam reflects from a flat mirror and is focused by a ZnSe lens onto the diamond crystal. The sample interacts with the hot spot formed on the surface of the diamond. The beam then passes through the lens again, reflects off another flat mirror, and is focused onto the detector.

The importance of applying high pressure in analyzing powders is shown in Figure 4.61. The bottom spectrum in Figure 4.61 is the DATR spectrum of a ground rock sample measured at low pressure. Note how small the peaks are. The top spectrum in Figure 4.61 is of the same sample measured at high pressure. The peaks in this spectrum are several times larger because of the increased DP at high pressure.

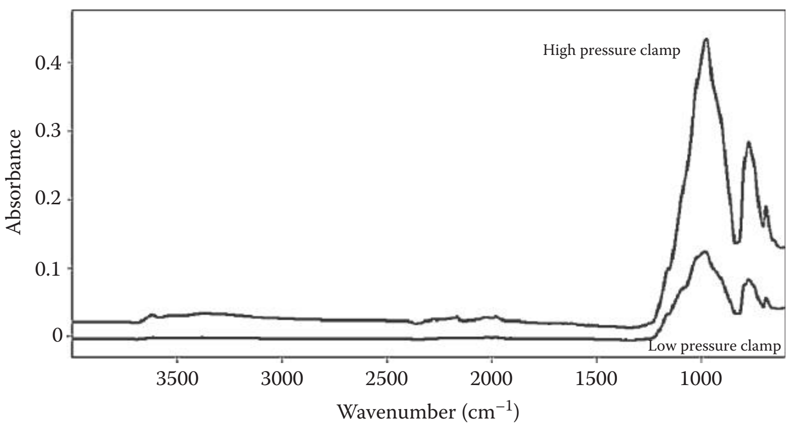

FIGURE 4.61 Bottom: The spectrum of a ground rock sample obtained by diamond ATR at low pressure. Top: The spectrum of the same ground rock sample taken at high pressure.

Figure 4.61 shows that things as hard as rocks can have their spectra measured using DATR. This means that any solid or powder softer than rocks, including most organics, can be analyzed by DATR. However, the author has found that grinding powdered samples for 1 minute in an agate mortar and pestle noticeably improves the quality of powder ATR spectra. Pressure is force per area as defined in Equation 4.3:

$$P = F/A \tag{4.3}$$

Where

P = Pressure
F = Force
A = Area

As a particle's size goes down its area decreases, and grinding reduces particle size. For a constant force the amount of pressure on a small particle is greater than for a large particle. Thus, smaller particles are easier to flatten, will make better contact with an ATR crystal, and will give better spectra, as shown in Figure 4.62.

The bottom two spectra in Figure 4.62 are of an intact piece of brick and of brick dust obtained by hitting the brick with a hammer (rarely is FTIR sample preparation so much fun). The top spectrum in Figure 4.62 was obtained from brick dust that was ground for 1 minute in an agate mortar and pestle. Note how the ground brick spectrum has peaks several times bigger than those of the other samples. One of the highly touted advantages of ATR is that there is no sample preparation, but for the top spectrum in Figure 4.62 there was one minute of sample preparation, contradicting this idea. However, the 1 minute invested here in sample preparation produced a significantly better spectrum, so the time invested was worthwhile. This is why the author recommends all powders be ground before ATR analysis.

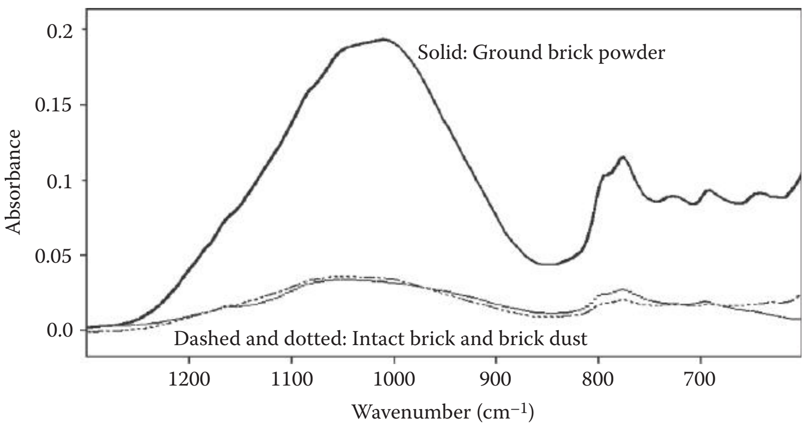

FIGURE 4.62 Bottom: Spectra of a brick piece and unground brick dust. Top: Spectrum of brick dust ground for 1 minute in an agate mortar and pestle.

XI. ATR: ADVANTAGES AND DISADVANTAGES

ATR enjoys a number of advantages compared to the other sampling techniques discussed in this chapter. It is a reflectance technique and does not suffer from the opacity problem that plagues transmission methods. As a result there is little grinding, squishing, pressing, and diluting needed to prepare a sample for ATR analysis. For many samples there is little sample preparation—the ATR crystal is cleaned, the sample is placed on the crystal, it is clamped if needed, the spectrum is measured, the sample is removed, and the crystal is cleaned. This means ATR is a fast and easy technique. Another advantage of ATR is the enormous variety of samples it works on including liquids, semi-solids, polymers, powders, and solids. The only sample type ATR cannot analyze is gases; it is hard to clamp a gas to an ATR crystal. Also, ATR can be nondestructive. Under most circumstances the sample is not altered, damaged, or chemically changed by the analysis. This is important if the sample is valuable, if you do not have much of it, or if you need to preserve it for further analysis. Another advantage of ATR is that it is useful for quantitation. Recall from Equation 1.6 that absorbance depends upon pathlength. In an ATR experiment the depth of penetration (DP) is a measure of pathlength, and is given by Equation 4.2. The only sample-related variable present in the equation is the refractive index of the sample, n_s. As long as n_s for the standards and samples falls within a narrow range, DP will be effectively controlled, making quantitation easier.

For all its advantages ATR has important disadvantages. The crystal must be kept clean and scratch-free. Scratches are a problem because they interfere with sample/hot spot contact. Be careful of paper towels—some of them contain wood fibers that can scratch softer ATR crystals such as ZnSe. These crystals can also be scratched by some samples, which is generally not a problem with diamond crystals. Since the evanescent wave passes above the crystal, any dirt or crud on the surface of the crystal will contaminate the spectrum of the sample. Lastly, don’t drop an ATR crystal on the floor—it may break, and they are not cheap to replace.

Another issue with ATR is its wavenumber range. Many ATR crystals absorb in the mid-infrared, which is why there is a “wavenumber range” column in Table 4.12. Specifically, diamond absorbs between 2200 and 2000 cm^{-1} and ZnSe absorbs below 700 cm^{-1}, masking sample features in these regions. Some diamond ATRs use ZnSe lenses to focus the infrared beam; for these accessories sample peaks between 2200 to 2000 cm^{-1} and below 700 cm^{-1} will be hidden. It is a good idea to have two crystals with complimentary wavenumber ranges to cover the entire mid-infrared. For example, diamond and Ge together are transparent from 4000 cm^{-1} down to 600 cm^{-1}. Before measuring any samples you should know what type of ATR crystal you have and what wavenumber regions it masks so you can take that into account when interpreting your sample spectra.

Perhaps the most significant disadvantage of ATR is its lack of sensitivity, and the cause of the issue is pathlength. The DP in ATR given by Equation 4.2 is rarely greater than 10 microns and can be less than 1 micron. At these shallow DPs the size of the absorbances measured are relatively small, leading to a loss of sensitivity. In general, ATR can only detect molecules present in concentrations greater than 0.1%.

TABLE 4.13
The Advantages and Disadvantages of the ATR Sample Preparation Method

Advantages	Disadvantages
Fast and easy	Lack of sensitivity
Works on many different types of samples	$$$
Nondestructive	Crystal care
Straightforward quantitation	Limited cm^{-1} range

Lastly, ATR is probably the most expensive FTIR sampling technique available. Very basic accessories cost several thousand dollars, and diamond ATRs cost even more. That being said, the time saved in sample preparation often pays for the ATR accessory. Table 4.13 lists the advantages and disadvantages of the ATR sample preparation technique.

XII. FTIR SAMPLE PREPARATION: OVERVIEW AND RECOMMENDATIONS

As you can see from reading this chapter, there are many different types of infrared sampling techniques. The goal of this chapter has been to cut through the potential confusion that surrounds sample preparation by emphasizing the advantages and disadvantages of each technique, and to clearly state what techniques work best on what samples. It is now time to summarize what we have learned, and for me to share with you my basic strategy of FTIR sample preparation.

Many of the transmission techniques discussed earlier in this chapter were developed over 50-plus years ago and involve a certain amount of skill and sometimes significant amounts of manual sample preparation. Back then labor was cheaper, and it was okay for it to take many minutes or hours for one sample to be analyzed. However, in the 21st century, labor costs are significantly higher, and all labs are under pressure to do more with less to increase profitability. Therefore, the main criterion the author uses to evaluate sample preparation techniques is speed and ease of use. For many samples, reflection techniques are faster and easier than transmission techniques. My approach, then, is that for a given sample I will try reflection techniques first. If the reflection technique works well, it will do so quickly and I can move on to the next sample. If the reflection technique does not work well, I find out quickly so not much time is wasted. Then, I will try one of the transmission techniques appropriate for the particular sample.

The introduction in the mid-1990s of diamond ATR accessories has revolutionized FTIR sample preparation. The toughness and durability of diamond now means that materials that would have formerly scratched or pressures that would have broken ATR crystals are now tolerated, and all manner of liquids, semi-solids, solids, and powders can be analyzed quickly and easily using this device. For these reasons diamond ATR is the premier type of sample preparation in use today. It does not work well on all

TABLE 4.14
Summary of FTIR Sample Preparation Techniques

Sample Type	Techniques That Work	Technique Recommended
Solids	Specular reflectance, KBr pellets, mulls, DRIFTS, abrasive sampling, ATR	ATR
Powders	KBr pellets, mulls, DRIFTS, abrasive sampling, ATR	ATR
Polymers	Cast films, heat and pressure, ATR	ATR
Semi-solids	Capillary thin films, ATR	ATR
Liquids	Capillary thin films, sealed liquid cells, ATR	ATR
Gases	Transmission gas cell	Transmission gas cell

samples, but it is the closest thing FTIR has to a universal sample preparation technique. Diamond ATR is my technique of choice for many samples, and I strongly recommend that all labs that have an FTIR obtain a diamond ATR accessory.

Table 4.14 summarizes different types of samples, the sampling techniques that work on them, and the author's opinion of which techniques are recommended for a given sample type. If the recommended technique does not work well for some reason, the other techniques listed for that sample type can be tried.

FTIR sample preparation equipment can be purchased from FTIR companies, but there are also a number of small, independent companies that supply FTIR sampling accessories. An Internet search of the names of any of the techniques mentioned in this chapter should turn up their Web sites. Alternatively, the author's Web site at www.spectros1.com maintains links to many of them.

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5 Quantitative Infrared Spectroscopy

An important application of FTIR is *quantitative spectroscopy*, where infrared spectra are used to determine the concentrations of chemical species in samples. Entire books have been written on this subject [1], and a lengthy description of quantitative spectroscopy is beyond the scope of this volume. My goal here is to introduce you to the fundamentals of quantitative spectroscopy by focusing on the determination of one chemical species at a time. Quantitating multiple chemical species at a time via infrared spectroscopy is doable given proper technique, equipment, software, and skill and is discussed elsewhere [1].

This chapter begins with an introduction to the terms and definitions of quantitative analysis. Then Beer's Law, the equation that relates peak size to concentration, is introduced. An illustration of how to calibrate and predict concentrations using Beer's Law is presented. The chapter wraps up with practical advice on measuring absorbances and how to avoid experimental problems.

I. TERMS AND DEFINITIONS

Like many fields, quantitative spectroscopy has its own jargon that must be learned before you can understand the topic. The following terms will be used throughout this chapter, and you should familiarize yourself with them.

Analyte – The chemical species whose concentration is being quantitated.
Analyst – The person performing the analysis.
Standards – Samples containing a known concentration of the analyte.
Calibration – A mathematical model that relates two or more sets of data to each other. Our interest will be in relating the size of the absorbance bands in an infrared spectrum to concentration.
Unknown – The sample whose analyte concentration is sought.
Prediction – The process of applying a calibration to an unknown spectrum to obtain the analyte concentration of an unknown sample.

Although it seems like a semantic nicety, it is imperative that you use the term *prediction* in quantitative spectroscopy to describe the process of finding the concentration of analyte in an unknown sample. The fundamental assumption of quantitative spectroscopy is that the calibration applies equally well to the standards and the unknowns. Despite careful experimental technique and the skills of the analyst, this assumption is not always true, and you may not necessarily know it. As a result the numbers obtained from quantitative spectroscopy inherently contain errors. Use of the terms "calculate" and "determine" give the numbers an aura of certainty they do not possess. The terms "predict" and prediction" are a better description of the truth behind these numbers.

II. BEER'S LAW

Beer's Law relates concentration, a sample property, to absorbance, a spectral property, and forms the basis for many quantitative spectroscopic analyses. It assumes that the spectrum is being measured using a transmission sampling method as illustrated in Figure 5.1.

In Figure 5.1 the infrared beam impinges on a thin film of sample of pathlength L. The sample absorbs some of the infrared beam, and the light that passes through the sample is focused onto a detector to obtain the sample's spectrum. Technically, reflectance spectra should not be used in Beer's Law since it is assumed the experiment is done in transmission. For DRIFTS experiments (see Chapter 4) Kubelka–Munk units and the Kubelka–Munk equation should be used for quantitative analysis [2]. In ATR (Chapter 4) the spectra are measured in absorbance and Beer's Law is used for quantitation, even though technically this is incorrect since ATR is a reflectance technique. The fact that many quality calibrations have been obtained using ATR spectra in absorbance [1] indicates that this violation of Beer's Law is generally not a problem.

Beer's Law applies to the absorbance process, whose energetics are illustrated in Figure 5.2. Before interacting with a light beam a molecule is in low energy state E_l. When molecules absorb energy from a light beam they are promoted to an upper energy level E_u. The amount of energy lost by the light beam equals the amount of energy gained by the molecule since the law of conservation of energy must be followed. The FTIR detects the decrease in light intensity, giving a peak in the sample's absorbance

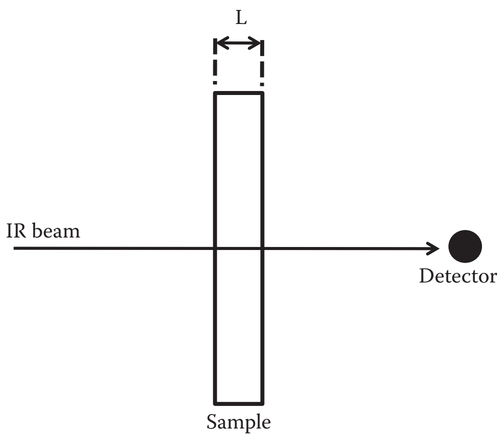

FIGURE 5.1 An illustration of transmission sampling where the infrared beam passes through the sample before impinging on the detector. The letter L denotes the pathlength, the thickness of sample through which the IR beam passes. Beer's Law is derived assuming this experimental setup.

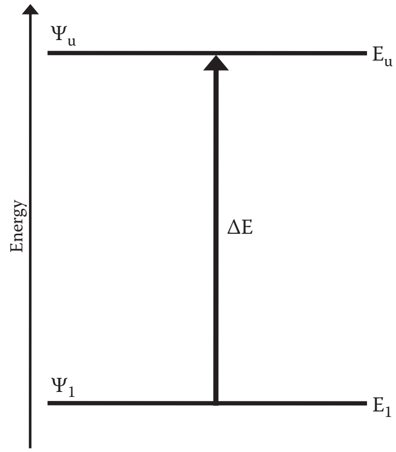

FIGURE 5.2 An illustration of the energetics of the absorbance process. When molecules absorb light they are promoted from a lower energy level E_l to a higher energy level E_u with a corresponding decrease in energy of the light beam.

spectrum. Beer's Law is the equation that relates the amount of light absorbed by a sample to the concentration of absorbing species in the sample, as follows:

$$A = \varepsilon l c \tag{5.1}$$

Where

A = Absorbance
ε = Absorptivity
l = Pathlength
c = Concentration

The absorbance, A, is measured as the peak height or peak area in a spectrum. Percent transmittance spectra (%T) should *not* be used for quantitative analysis since the relationship between %T and concentration is complex and nonlinear. The concentration, c, in Beer's Law is the concentration of analyte in the sample. The analyte must be an absorbing species; that is, it must have at least one peak in the infrared spectrum to be analyzable. The concentration units used in Beer's Law can be whatever is convenient for the analyst such as moles/liter, parts per million (ppm), weight percent, etc. The predicted concentrations in the unknown sample will be in the same units as the standards used to obtain the calibration. The pathlength in Equation 5.1 is the thickness of sample seen by the infrared beam as seen in Figure 5.1. For solids and liquids analyzed in the mid-infrared, pathlengths on the order of microns are typical.

The absorptivity in Equation 5.1 is the proportionality constant between concentration and absorbance. For a given molecule and a given wavenumber of light, the absorptivity is a fundamental physical constant of a molecule, as invariant as its boiling point or molecular weight. For example, the absorptivity of water at

1600 cm^{-1} is one of its physical constants. However, the absorptivity of water is different at different wavenumbers. as follows:

$$\varepsilon_{water}^{1600} \neq \varepsilon_{water}^{1700}$$

which states that the absorptivities of water at 1700 and 1600 cm^{-1} are different. Also, in general, different molecules will have different absorptivities at the same wavenumber, thus

$$\varepsilon_{water}^{1700} \neq \varepsilon_{acetone}^{1700}$$

which states that the absorptivity of water and acetone are different at 1700 cm^{-1}. The absorptivity can be thought of as an "inherent absorbance"; it measures the amount of light a pure substance will absorb under controlled conditions such as pathlength, wavenumber, and temperature. So, strongly absorbing molecules have high absorptivities, and weakly absorbing molecules have low absorptivities.

The absorbance is a unitless quantity, so the units on the right-hand side of Equation 5.1 must cancel. Since the l is in units of length and c is in units of concentration, the absorptivity must be in units of $(\text{length} \times \text{concentration})^{-1}$. This leads to the absorptivity being expressed in units such as liter/mole-cm, which might seem strange but are correct. Peak heights and areas in absorbance spectra are measured in "absorbance units."

III. CALIBRATION AND PREDICTION WITH BEER'S LAW

A. Calibration

To obtain a calibration using Beer's Law, notice that its form is analogous to the formula for a straight line, which is

$$Y = mx + b \tag{5.2}$$

Where

Y = Y-axis value
m = Slope of line
X = X-axis value
b = Y-intercept of the line

Comparison of Equations 5.1 and 5.2 shows that if absorbance is plotted on the y-axis and concentration is plotted on the x-axis, a straight line should be obtained whose slope is given by

$$m = \varepsilon l \tag{5.3}$$

To obtain the data needed to plot a *calibration line* the absorbances of a series of standard samples are measured either as a peak height at a specific wavenumber, or as an integrated peak area. The concentrations of the standards must be obtained by some method other than FTIR. Frequently, standards are made with measured

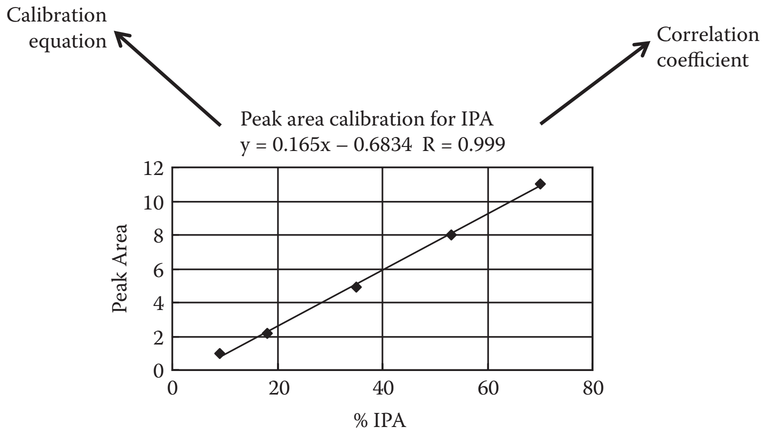

FIGURE 5.3 A calibration line for the analysis of isopropanol (IPA) in water. The y-axis is the area of the isopropanol peak centered at 2973 cm^{-1}. The x-axis is volume percent isopropanol in water.

masses or volumes of analytes. Alternatively, a method such as titration or chromatography can be used to determine the concentration of analyte in standards. The measured absorbances and the known concentrations are then plotted against each other. An example of a calibration line obtained using Beer's Law is shown in Figure 5.3, which is a plot of peak area versus the volume percent of isopropanol (IPA, also known as rubbing alcohol) dissolved in water.

Plots like Figure 5.3 are called Beer's Law plots. The measured absorbances in Figure 5.3 are the area of the IPA peak centered at 2973 cm^{-1} (the methyl group asymmetric C-H stretch, integration limits 3008 to 2947 cm^{-1}). The data used in making this plot are shown in Table 5.1. An overlay of the peaks analyzed is shown in Figure 5.4.

Note that the calibration plot in Figure 5.3 is a straight line. If a plot of absorbance versus concentration is linear, the chemical system is said to follow Beer's

TABLE 5.1
Volume Percent and Peak Area for Isopropanol Calibration Line Shown in Figure 5.3

%IPA	Peak Area
9	1
18	2.2
35	4.9
53	8
70	11

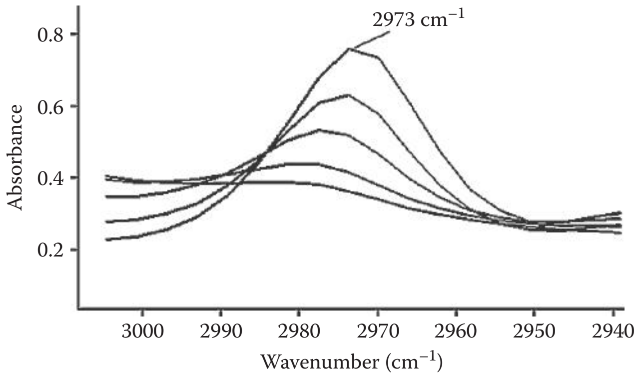

FIGURE 5.4 The CH_3 asymmetric stretching peak for five standard samples of IPA dissolved in water. The areas of these peaks are listed in Table 5.1 and were used to obtain the calibration line shown in Figure 5.3.

Law. For single-component analysis, the calibration line is the mathematical model relating the absorbance of the samples to the concentration of analyte. Any number of computer programs can be used to plot data, and many of them will calculate the straight-line equation and other metrics for you. The plot in Figure 5.3 was made with Microsoft Excel® and the calibration line has a slope of 0.165 and its Y intercept is −0.6834. This means that the product of the absorptivity and pathlength for this chemical system is 0.165. Although Beer's Law predicts that absorbance versus concentration plots have a Y intercept of zero and should pass through the origin, clearly that is not the case here. This happens for two reasons. First, it is possible that the sample may have a non-zero absorbance at the wavenumber of interest even if the analyte concentration is zero. This means chemical species other than the analyte are contributing to its measured peak area. Since absorbance is positive, this will cause a positive deviation of the Y intercept from zero. The second cause of non-zero Y intercepts is noise. Since the sign of random noise is random, this can result in a positive or negative deviation from zero for the Y intercept.

Figure 5.3 contains the statement "R = 0.999" where R is the *correlation coefficient*, which is a measure of model quality, and in this case is a measure of linearity. If all the data points were perfectly centered on the line we would have a perfect correlation between absorbance and concentration, and R would equal 1.0. Of course, this does not happen because of noise. On the other hand, if there were absolutely no correlation between absorbance and concentration, R would equal zero. The closer R is to 1.0 the better the calibration. The correlation coefficient for the calibration line in Figure 5.3 is 0.999, which is very good.

B. Prediction

The point of obtaining a calibration is to be able to predict the concentration of the analyte in unknown samples. We first need to derive an equation to enable us to do this. We can write Beer's Law as follows for an unknown:

$$A_{unk} = \varepsilon l C_{unk} \tag{5.4}$$

Where

A_{unk} = Absorbance of the unknown sample
εl = The product of the absorptivity and pathlength
C_{unk} = The analyte concentration in the unknown sample

Ultimately, it is the concentration of analyte in the unknown sample, C_{unk}, in which we are interested. Rearranging Equation 5.4 gives

$$C_{unk} = A_{unk}/\varepsilon l$$

From Equation 5.3 we know $m = \varepsilon l$, so

$$C_{unk} = A_{unk}/m \tag{5.5}$$

According to Equation 5.5, predicting the concentration of an analyte in an unknown sample is a matter of dividing the measured absorbance of the unknown sample by the slope of the calibration line. Note that to use Equation 5.5 you do not need to know the absorptivity and pathlength explicitly, just their product.

In deriving Equation 5.5 we have assumed that εl is the same for the standards and the unknowns. This is another way of stating the fundamental assumption of quantitative spectroscopy, that the calibration applies equally well to standards and unknowns. This assumption is unavoidable since by definition εl is not knowable for the unknown samples. Making this assumption means you must control as many experimental variables as possible when performing quantitative spectroscopic analysis to ensure εl really is constant for both the samples and unknowns. If for any reason you suspect this is not the case, the calibration should not be applied to unknown samples.

C. An Experimental Protocol for Single Component Analyses

The following is an experimental protocol that, if followed, will help in the attainment of accurate single analyte calibrations.

1. Analyzing for a Single Analyte

1. Prepare the standards. Be as accurate as possible since any concentration error in the standards is carried through as error in the predicted concentrations of the unknown samples.
2. Take two aliquots of each sample and obtain separate spectra of them. In theory, these two spectra should be identical because they were obtained with the same sample, instrument, sampling accessory, and experimental conditions.
3. Subtract these two spectra using a subtraction factor of one, obtaining a subtraction result called a *residual*. Look closely at the residual. Two spectra of the same sample should be identical, and should give a residual

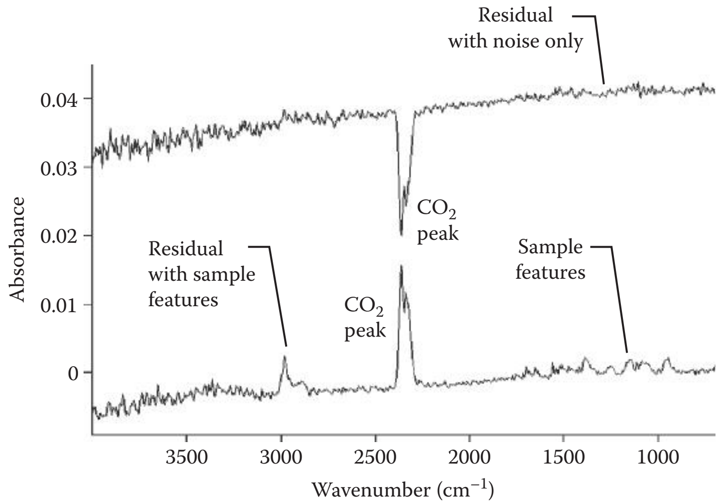

FIGURE 5.5 Spectral residuals obtained by subtracting two spectra of the same sample. Top: No sample features are seen, just noise and a CO_2 peak. Bottom: Sample spectral features are present.

that is a reasonably flat line containing only noise. Such a residual is seen in the top of Figure 5.5, whose only feature is a CO_2 peak at 2350 cm^{-1} since the analyte absorbance between the two spectra was constant and subtracted out. The bottom residual in Figure 5.5 shows analyte peaks pointing up between the two measurements, meaning something caused the absorbances to change. Since these are spectra of the same sample, the absorbance variability may be caused by the instrument or sample. At a minimum, the spectra of the standard should be remeasured and a new residual calculated.

4. Examine the spectra and choose a band whose height or area changes with concentration.
5. Measure the appropriate absorbance and plot the calibration line (see above). Examine the plot to insure linearity, and then calculate its slope.
6. Obtain the spectrum of the unknown sample and predict its concentration using Equation 5.5.

IV. MEASURING ABSORBANCES PROPERLY

A. Peak Areas versus Peak Heights

Correctly measuring the absorbance of a spectroscopic feature is more difficult than might initially be thought. First, you must ensure that the spectral feature being measured is due to the analyte, and that there are no interfering absorbances from other components. This is best accomplished by examining spectra of the known components in the sample of interest. Second, it may appear that reading the absolute

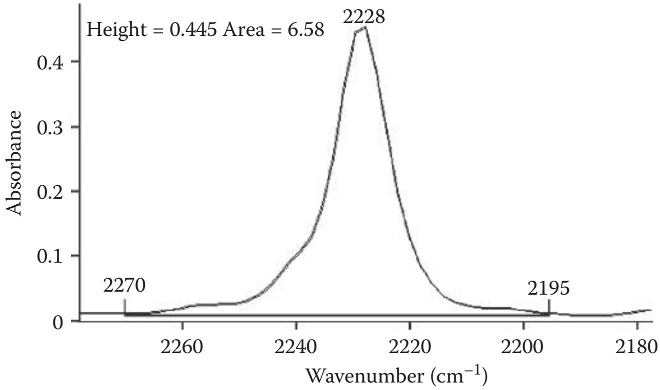

FIGURE 5.6 An example of a spectroscopic absorbance band with a correctly drawn baseline and edges. By measuring the peak height or area with respect to this baseline, offset is removed from the measured absorbances (this peak is the −C≡N stretch of pure benzonitrile).

peak height directly off a spectrum will give an accurate absorbance. However, the baselines of spectra can drift up and down on the y-axis due to instrumental and sample problems, introducing an unwanted offset to the absorbance data. To avoid this problem, it is common to draw a baseline connecting the beginning and ending points of a peak. This baseline gives a new "zero" absorbance point, and any heights or areas measured with respect to this baseline will be free of baseline offset. Figure 5.6 shows an example of an infrared peak with a correctly drawn baseline.

Note the vertical lines in Figure 5.6 at 2270 and 2195 cm^{-1}, which are the beginning and ending baseline points. It is critical that these end points be chosen so they include the entire analyte peak but no spectral features due to other molecules. The peak height is measured from the baseline to the top of the peak. The peak area is measured by integrating between the baseline's end points.

Many of us instinctively want to measure peak heights at the peak top. Why are things done this way? The peak shown in Figure 5.6 consists of 40 absorbances measured at different wavenumbers. In theory, the absorbance at any of these wavenumbers could be used to create a calibration line since all of them respond to changes in analyte concentration. However, we use the data point at the top of the peak for two reasons. First, assuming the noise level across the peak is constant, the maximum absorbance will have the best signal-to-noise ratio of all the data points in the peak. Second, the slope of the spectrum at the top of a peak is close to or equal to zero, so a small error in measured wavenumber will produce a smaller absorbance error than on the steep sides of the peak.

There is an ongoing debate in the spectroscopic world as to whether peak areas or peak heights provide better calibrations. When a peak height is measured, only one data point that is sensitive to analyte concentration is used. This is literally putting all your eggs in one basket. If anything causes the accuracy of this one data point to be compromised (an interferent in the unknown, for example), the entire calibration is thrown off. Peak areas are measured by integrating many absorbances together. For the band shown in Figure 5.6, the peak area was calculated by adding 40 different absorbances together. If one of these absorbances is inaccurate, it is averaged over the other 39 data points that are accurate. By using more data points, the calibration is less sensitive to error in any one specific data point. Additionally, by adding the absorbances together the signal-to-noise ratio for a peak area is greater than for a peak height. Thus, peak areas should produce more accurate calibrations because they are not as dependent on a single data point, and the raw data has a better signal-to-noise ratio. However, there are examples of chemical systems where peak height calibrations work better than peak area calibrations. Therefore, the author recommends that when establishing a calibration on a new chemical system peak areas be tried first. If you have difficulty getting a good calibration with peak areas, then try peak heights. There is no guarantee this will fix your calibration, but it might.

B. Dealing with Overlapped Peaks

In an ideal world we would measure the absorbance of an analyte band that is baseline resolved and free from interference, an example of which is shown in Figure 5.6. However, in the real world there are samples where analyte bands are overlapped with those from other molecules, such as the IPA peak in Figure 5.7, which is a shoulder superimposed upon a much broader and stronger water band. How would we accurately measure the peaks height or area?

In general, you want to draw the baseline for shoulder peaks between minima to the left and right of the peak, and include as much of the peak and exclude as much of the interferent as possible. This can be seen from the baseline drawn in Figure 5.7. The 2973 cm^{-1} peak in the figure was used to generate the calibration shown in Figure 5.3. This calibration is of high quality, indicating that the baseline end points were chosen correctly.

Another time when it can be difficult to measure absorbances is when a group of peaks overlap and are not baseline resolved, as illustrated in Figure 5.8.

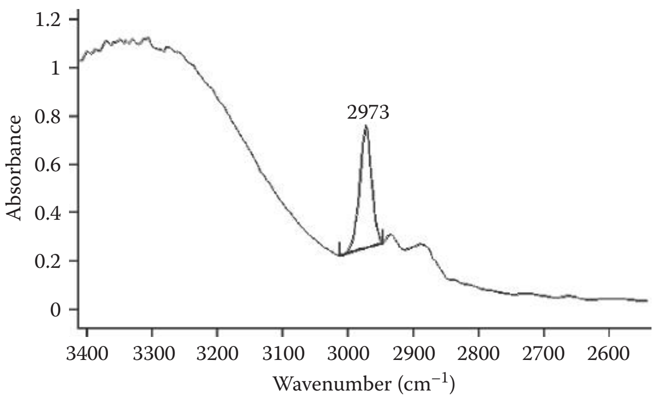

FIGURE 5.7 An example of a shoulder peak and how to draw a baseline to accurately measure its peak height or area.

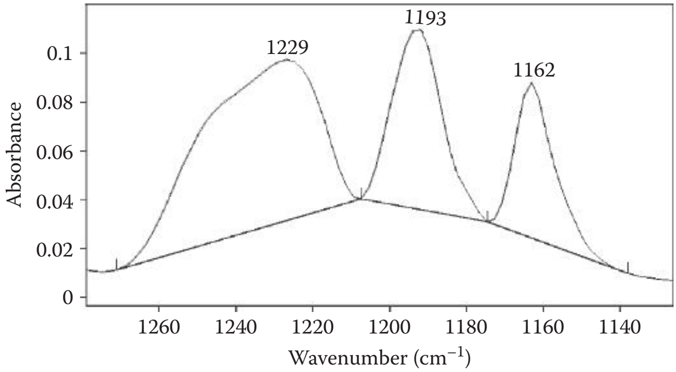

FIGURE 5.8 A series of overlapped peaks that share a common baseline and how to draw a baseline to measure their absorbances.

Note how in Figure 5.8 the minima between the overlapped peaks are used as baseline end points. Used properly, this approach isolates the absorbances of the analytes from those of interferents.

V. AVOIDING EXPERIMENTAL ERRORS

Following is a list of common experimental problems that can ruin quantitative analyses. Avoiding these problems is key to insuring the accuracy of your calibrations.

1. Use standards with concentrations that bracket the expected concentration range of components in your unknowns. For example, if the concentration of the analyte in your standards runs from 10% to 70%, only unknowns whose concentrations fall in this range can be analyzed. Your mathematical model cannot be applied to concentrations greater than 70% or less than 10% because you have no information on the behavior of the model in those concentration ranges. It is always tempting to extrapolate a model into a concentration range where there is no data; it is also always wrong.
2. Use the actual components to be measured when preparing standards. For example, imagine you need to develop a calibration to measure the amount of acetone dissolved in water. The unknown samples will come from a factory floor and are made using 95% pure technical-grade acetone. You may have 99% pure reagent-grade acetone in your lab, and since we are taught that "purer is better" you may be tempted to make up the standard samples using the reagent-grade acetone. You may even get a perfectly good calibration with the reagent-grade material. The problem is that it will be the wrong calibration! The reagent-grade calibration would systematically overpredict the concentration of acetone in unknowns made with technical-grade material by 4%. The technical-grade material has 4% more impurities than the reagent-grade material, so the acetone in it is 4%

more dilute than in the reagent-grade material. Also, remember that the fundamental assumption of quantitative spectroscopy is that the calibration describes the samples and unknowns equally well. The reagent-grade and technical-grade acetone are chemically distinct, so a calibration for one should not be used for the other.

3. Run standards in random order to insure that sample order has no effect on the results.
4. Obtain two spectra of each standard, and subtract them using a subtraction factor of 1.0 to see if a flat residual is obtained. If not, it means that a variable in the sample or the experiment has caused spectra of the same sample to be different. The source of variability must be found and eliminated before continuing.
5. Obtain standards with accurate concentrations. The accuracy of a predicted concentration is no better than the accuracy to which we know the concentration in the standards.
6. The minimum number of standards to run is $2n + 2$, where n is the number of analytes. Thus, for one analyte the minimum number of standards to run is $[(2 \times 1) + 2]$ or 4. However, running more standards typically will give a better calibration.
7. Cleanliness is crucial! Thoroughly clean everything your sample comes in contact with before performing an analysis. Occasionally obtain the spectra of "clean" sample cells and accessories to check for contamination.
8. Try to use absorbance bands that are less than 0.8 absorbance units in calibrations. Beer's Law is frequently not obeyed by bands whose absorbances are greater than this, which will manifest itself as a curvature in the calibration line.
9. Make sure your sample components do not react with each other. This can be checked by obtaining spectra of the sample over time and looking for changes. Also, be aware of the materials the sample cell is made of and how they might react with any components in the sample. For example, KBr is a commonly used cell and window material in infrared spectroscopy, but it is water soluble. Prior knowledge of the properties of cell and window materials can prevent this type of mistake.
10. The physical condition of the standards and the unknowns, such as temperature and pressure, must be held constant when their spectra are acquired. Daily fluctuations in temperature and pressure are difficult to control because of the weather. However, for example, do not intentionally run your standards at room temperature and then measure an unknown spectrum at 150°C.
11. Use the same instrumental parameters for the standards and unknowns including the number of scans, resolution, and spectral range. You don't want changes in the scanning conditions to change your results. Many instrument software packages allow you to save a set of instrumental parameters to the computer's hard disk. By using the same parameter set for standards and unknowns, you ensure that their spectra are measured under identical conditions.

12. Do as little spectral processing as possible. Techniques such as baseline correction, subtraction, and smoothing alter the absorbances in a spectrum and are difficult to reproduce from one spectrum to another. If you must process your data before quantitation, all standard and unknown spectra should be processed in exactly the same fashion.
13. Be consistent in the use of cells, windows, crystals, and sampling accessories for your standards and unknowns. For example, KBr and NaCl are both commonly used window materials in infrared spectroscopy, but have different optical properties. If a calibration is developed with a sample cell containing KBr windows, and unknown spectra are obtained with a cell with NaCl windows, the unmodeled spectral difference may be enough to introduce error into the predicted concentrations.
14. Once a calibration is obtained and is working properly, occasionally check it by running a new standard and comparing its known and calculated concentrations. If the concentrations are similar, the calibration is still good. If the concentrations do not agree, then changes in the sample, sampling accessory, and instrument over time may have contributed to drift in quantitative results. You must track down the source of variability, eliminate it, and then run a new set of standards and plot a new calibration line. Daily or weekly calibration checks can ensure accurate results and contribute to the peace of mind of the analyst.
15. Different sampling techniques present their own quantitative analysis challenges. These are discussed in Chapter 4.

Despite the problems listed here, quantitative FTIR analyses are commonplace. The point to remember is that if you develop the method properly using the information contained here, you will have fewer problems later when the method is actually being used.

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6 Infrared Microscopy

I. HYPHENATED INFRARED TECHNIQUES

Hyphenated techniques are obtained when two different types of instruments are mated together. The names of these techniques are constructed from the names of the instruments interfaced and be then adding a hyphen. For example, the technique that interfaces a gas chromatograph to a mass spectrometer is called gas chromatography-mass spectrometry, or GC-MS for short. The purpose of hyphenated techniques is to allow multiple pieces of information to be obtained on the same sample by having two or more instruments working together. In GC-MS the gas chromatograph purifies the sample into its components and can quantify those components, and the MS identifies each component. This provides a powerful technique for purifying, identifying, and quantifying mixtures. Hyphenated infrared techniques make use of the ability of FTIR to provide chemical information. Frequently, FTIRs are interfaced to instruments that provide physical information to give hyphenated techniques that provide physical and chemical information on the same sample.

Although its name does not contain a hyphen, an infrared microscope is considered a hyphenated technique because it involves interfacing a microscope to an FTIR. The microscope provides physical information on samples including size, shape, color, and morphology. The sample can then be illuminated with infrared light and its chemical composition determined [1,2]. Other examples of instruments that have been interfaced to FTIRs to give hyphenated infrared techniques include thermogravimetric analyzers, gas chromatographs, and liquid chromatographs [3–7].

Many hyphenated infrared techniques have structural elements in common. In general, the infrared beam needs to be removed from the FTIR to interface with another instrument. This is accomplished by a “pickoff mirror” placed in the infrared beam after it has left the interferometer, as shown in Figure 6.1.

On some FTIRs the pickoff mirror is moved into and out of position via a motor under software control. On older instruments the mirror may need to be placed and removed by hand. Once the IR beam has reflected off the pickoff mirror, it leaves the FTIR via a hole in the side of the instrument’s cover which is frequently covered with an infrared transparent window. Once the beam leaves the FTIR it enters an interface box that contains transfer optics and other devices, allowing the FTIR and the second instrument to work together.

II. INFRARED MICROSCOPY INSTRUMENTATION

Infrared microscopes are used to take infrared spectra of small samples and are typically quality visible-light microscopes that have been re-engineered to work with mid-infrared radiation. In many cases the infrared microscope mounts on the side of

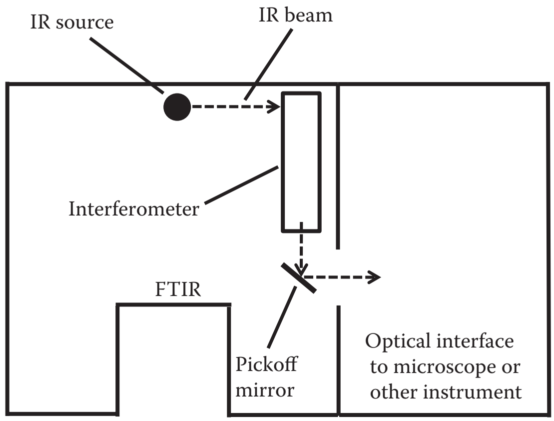

FIGURE 6.1 The IR beam path and interface used to create hyphenated infrared techniques.

the FTIR, as shown in Figure 6.1, or may mount directly in the FTIR's sample compartment. A schematic diagram of an infrared microscope is shown in Figure 6.2.

The visible light source for microscopes is a light bulb; the infrared light comes from the FTIR. Both beams may enter the bottom of the microscope, as illustrated in Figure 6.2. A movable input mirror determines whether infrared or visible light irradiates the sample.

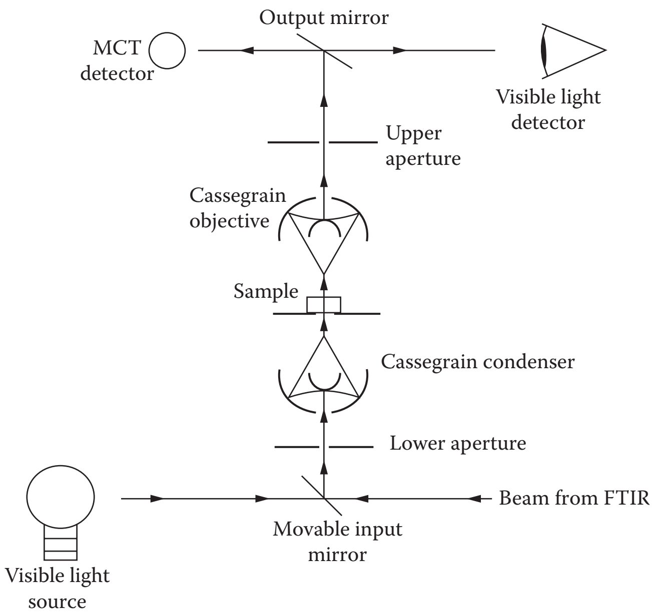

FIGURE 6.2 An optical diagram of an infrared microscope.

In some microscope designs the light beam passes through a lower aperture, which is used to define the size and shape of the beam before it impinges on the sample. The next optic the light beam encounters is the *condenser*, which focuses the light beam onto the sample. In some infrared microscopes the condenser consists of an arrangement of mirrors called a Cassegrain, which has a small convex mirror suspended above a larger concave mirror, as illustrated in Figure 6.2. Note that in this Cassegrain the concave mirror faces up. A hole in the concave mirror allows the infrared light through, it reflects off the convex mirror, and then the concave mirror focuses the light onto the sample. The sample is placed on a translation stage so it can be moved up and down, left and right, and back and forth for focusing and viewing. Figure 6.2 shows the sample being examined in transmission mode, where light passes through the sample before being focused on the detector.

The optic in a microscope that collects the light after it has interacted with the sample is called the *objective*. In many infrared microscopes it is another Cassegrain identical to the condenser, except that it has been flipped over and faces down. The concave mirror in the objective gathers the light that has interacted with the sample, focuses it on the convex mirror, and then sends the beam upward through the hole in the concave mirror. In visible-light microscopes, glass lenses are frequently used as the condenser and objective. These cannot be used in an infrared microscope because glass is a strong infrared absorber. To avoid this problem, all the optics in an infrared microscope consist of mirrors, which is properly called “all reflective optics.”

Most infrared microscopes contain an upper aperture, which determines the size and shape of the infrared beam and is used to block stray light, which is a problem because the sample is frequently smaller than the infrared beam, as illustrated in Figure 6.3.

In some infrared microscopes the infrared beam is 250 microns in diameter at the focal point where the sample is placed, and many samples are smaller than this. As a result, some of the infrared beam does not interact with the sample. This stray light causes problems if it encounters things such as fingerprints or dust whose spectrum can contaminate the sample spectrum. Also, stray light contains no sample information, and if it is allowed to hit the detector valuable detector capacity is wasted, essentially becoming a source of noise. It is the job of the upper

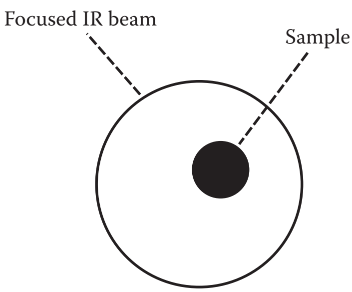

FIGURE 6.3 An illustration of how the sample can be smaller than the IR beam in an infrared microscope.

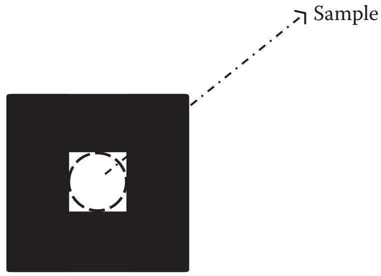

FIGURE 6.4 An example of what an infrared microscope sample might look like after the image of upper aperture's knife edges has been adjusted to make contact with the image of the sample.

aperture in an infrared microscope to block stray light. This is done by first focusing on the sample using visible light, and then bringing the image of the aperture edges into contact with the image of the sample. In many microscopes the aperture consists of four black knife edges. When looking through the microscope it looks like the aperture edges are actually touching the sample, an example of which is shown in Figure 6.4.

In reality it's the images that are touching—the sample and aperture are physically separated by several inches. The aperture's edges block the stray light so only infrared radiation that has interacted with the sample reaches the detector. Next, the light beam is brought to a focus onto a detector. For visible light the detectors are your eyeballs, and there is usually a binocular eyepiece to accommodate them. Infrared microscope spectra are inherently noisy due to reflectance losses at the many mirrors in the microscope, the aperture(s) blocking parts of the beam, and the small size of the samples. This means that the use of a high-sensitivity, low-noise, and liquid-nitrogen-cooled MCT detector is essential in infrared microscopy. The principles of operation of these detectors were discussed in Chapter 2.

In infrared microscopy the sample and background spectra must be run using the same aperture setting. Since the sample size determines the aperture setting, the sample must be brought into the field of view, the focus adjusted, and the apertures set. Then the sample must be taken out of the field of view for the background to be run, and then brought back to the same position to measure the sample spectrum. This is not necessarily easy to accomplish. Another problem with this procedure is that microscopic samples can disappear due to vibrations or wind puffs while the background is being measured. Therefore, it may be best, once you have the sample in focus and the aperture(s) adjusted, to measure the sample single beam spectrum while the sample is literally in your crosshairs. The background can then be measured and the two spectra ratioed later to obtain an absorbance or percent transmittance spectrum.

In addition to being used in transmission as illustrated in Figure 6.2, infrared microscopes can use reflectance to examine samples. In this case the infrared radiation comes down from above and is focused on the sample it using the upper

Cassegrain. The light reflects from the sample, is collected by the upper Cassegrain, and is then focused on the detector. The upper Cassegrain acts as both condenser and objective, and the lower half of the microscope is not involved in this measurement. We have now seen that an infrared microscope can be used in four different ways: visible light transmission, visible light reflectance, infrared transmission, and infrared reflectance. The mode that will provide the best viewing and spectra will depend upon the sample.

III. SAMPLE PREPARATION

The principles behind preparing microscopic samples for infrared analysis are similar to those for preparing macroscopic samples, as discussed in Chapter 4. Since an infrared microscope can be used in transmittance and reflectance, both types of sample preparation come into play. Unfortunately, the preparation of microscopic samples for infrared analysis may be the hardest part of using an infrared microscope. Frequently, samples are too small to be seen by the naked eye, so the preparation of samples for infrared microscopy requires the use of a low-power stereo microscope, as shown in Figure 6.5.

The user looks at a magnified image of the sample through the binocular eyepiece. Preparative microscopes typically have a magnification of 5× to 25×. The sample is then placed on the working surface of the microscope and may be illuminated by a lamp for better viewing. The focusing knob is adjusted to bring the sample into view. Special tools such as probes, scalpels, and tweezers are used to cut the sample, move it around, and mount it. It takes skill, patience, and practice

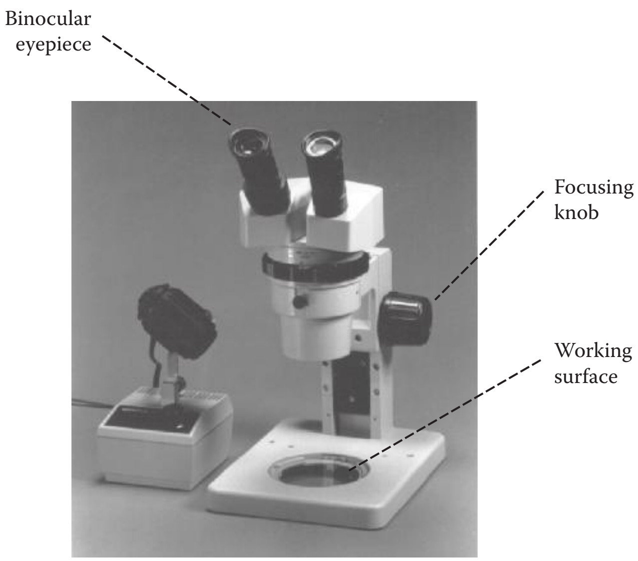

FIGURE 6.5 A photo of a preparative microscope: a low-power visible-light microscope used to prepare microscopic samples for infrared analysis.

to manipulate small samples while looking through a microscope. The challenge is that in a magnified view of a sample, a small amount of movement can cause it to leave your field of view. Steady hands are a necessity, and one way to steady them is to plant your little fingers on either side of the working surface, and then manipulate the sampling tools with your other fingers.

Microscope transmission samples suffer from the same opacity problem as macroscopic samples. The solution to this problem is to prepare samples that are 1 to 20 microns thick. Fortunately, many microscopic samples can be turned into thin films by applying pressure and flattening them. This works because it does not take a lot of force to put a lot of pressure on a sample of small area, as shown in Equation 4.3. There are many ways to apply pressure to a microscopic sample to flatten it. In the author's opinion the best tool for this purpose is a "roller knife," as shown in Figure 6.6, so called because it has a roller at one end and a scalpel at the other.

Roller knives are available from companies that make infrared microscopes. To use one, the sample is typically placed on the working surface of a preparative microscope like the one shown in Figure 6.5. The roller is pressed and rolled over the sample several times while applying pressure, which serves to spread the sample

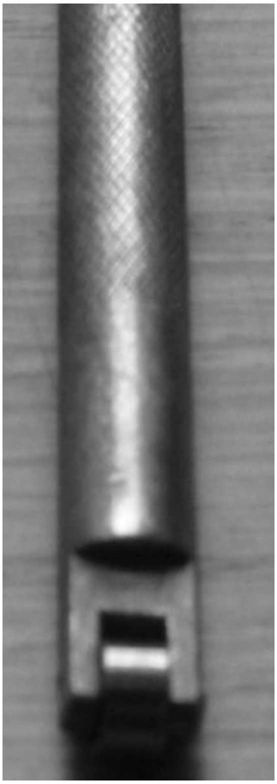

FIGURE 6.6 A picture of a roller used to flatten microscopic samples for infrared analysis.

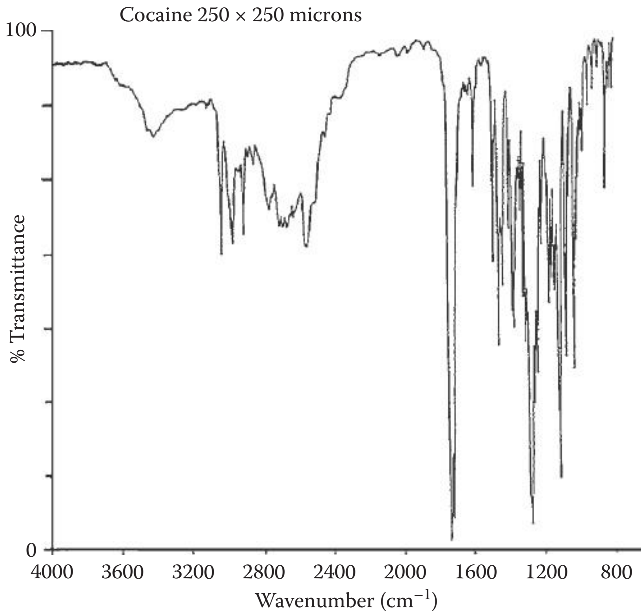

FIGURE 6.7 The infrared spectrum of a cocaine hydrochloride crystal obtained by flattening it with a roller, mounting it on an infrared transparent window, and measuring its spectrum using infrared transmission mode.

out and thin it. Many samples can be flattened in this manner including polymers, particles, and fibers. The flattened sample is then picked up with tweezers or a probe while still being viewed through the preparative microscope, and then placed on an infrared transparent window (typically KBr). The sample and window are then placed at the focal point of the infrared microscope. Using visible light the sample is found and brought into focus. The aperture(s) of the microscope are then adjusted so their image touches that of the sample, the microscope is switched to infrared transmission mode, and the sample spectrum is measured. The background spectrum is obtained on a clean portion of the infrared transparent window using the same aperture as for the sample. The infrared spectrum of a cocaine hydrochloride crystal flattened with a roller knife, mounted on an infrared transparent window, and obtained in transmission mode is shown in Figure 6.7.

Since microscopic samples are much smaller than the infrared transparent window upon which they sit, they are sometimes difficult to locate. A probe or tweezers can be used to place an identifying scratch on the window near the sample. Once the window is placed in the microscope the scratch can easily be found, and moving the field of view along the scratch will lead to the sample. Another problem with microscopic samples is that they can be easily blown away by a sneeze or a puff of wind, such as from a door opening, a ventilation system turning on, or by someone walking past. A way around this problem is to flatten the sample into the surface of the infrared transparent window, thereby embedding it. This works because KBr

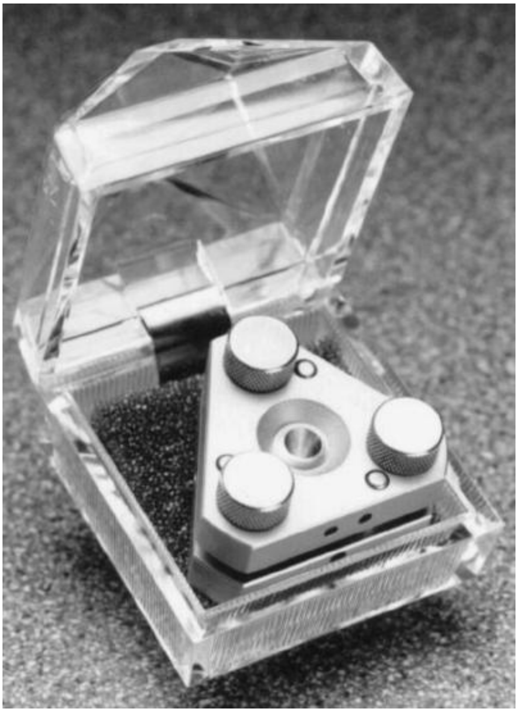

FIGURE 6.8 A photo of a diamond anvil cell used to flatten samples for transmission analysis. (Photo courtesy of High Pressure Diamond Optics.)

is soft. Embedding the sample makes it more secure and less prone to blowing away, and a number of samples can be embedded in the same window. However, if there are multiple samples embedded in a window one must be careful to take the spectrum of the correct one. Samples embedded in windows can be removed by rubbing the KBr window briefly on a small piece of wet paper towel and then quickly drying it. This removes the first few atomic layers of the window along with any samples that are embedded.

Hard samples can be difficult to flatten with a roller knife, and rubbery samples such as elastomers will spring back to their original size and shape after pressure is removed. For these samples a device called a diamond anvil cell can be used to flatten them, a picture of which is shown in Figure 6.8. A diamond anvil cell consists of two metal halves that mate together, with each half containing a small gem-quality diamond. The two halves are separated and placed on the working surface of a preparative microscope like the one shown in Figure 6.5. The sample is placed on one of the diamond windows, then the two halves of the cell are brought together, sandwiching the sample in between. For softer samples finger pressure may be sufficient to flatten them; for harder samples there are screws on the device, as shown in Figure 6.8, that can be tightened. The cell is roughly 1 inch in diameter and three-quarters of an inch thick so it will fit on the sample stage of most microscopes.

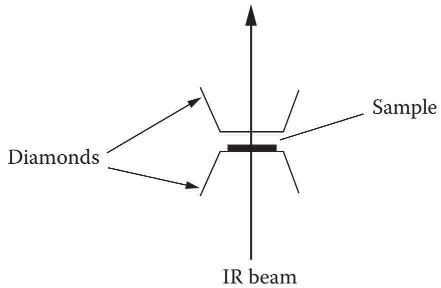

FIGURE 6.9 The path of the infrared beam through a sample mounted on a diamond anvil cell.

There are holes in the top and bottom of the cell to let the infrared light through, as shown in Figure 6.8. The beam path for the diamond windows and the sample is illustrated in Figure 6.9.

For samples that will not pop back into shape it is sometimes convenient to remove the top window and analyze the flattened sample with it resting on the bottom window. This makes the device smaller, allowing more room for focusing and moving the sample around. It also increases infrared throughput by reducing the pathlength of diamond through which the infrared beam must pass. The background spectrum should be obtained on a clean part of the diamond window using the same aperture as the sample. This can be tricky, because if the entire window is covered with sample it will be necessary to measure the sample spectrum, remove the diamond cell to clean it, place it back in the microscope, and then measure the background spectrum, all using the same aperture setting.

A beauty of the diamond anvil cell is it is hard enough to flatten almost anything including polymers, powders, particles, fibers, paint chips, and elastomers. The cell turns an infrared microscope into an almost universal sampling accessory for solids; in some labs they analyze all their macroscopic and microscopic solid samples this way. In labs without an IR microscope the diamond anvil cell can be used to analyze solids by placing it in a beam condenser mounted in the FTIR sample compartment.

The utility of the diamond anvil cell is great, but it comes with a price: the device costs several thousand dollars. This is no surprise since diamonds are expensive. Add to this the cost of an infrared microscope, which is typically tens of thousands of dollars, and you end up with a hefty price tag. Another disadvantage of the diamond anvil cell is that diamond absorbs in the mid-infrared between 2200 and 2200 cm^{-1}. It will typically mask the peaks of samples that absorb in this region such as materials containing C≡C and C≡N bonds. If your sample has important peaks in this wavenumber range, it may be necessary to analyze the sample using some other sample preparation technique.

Infrared spectra of microscopic samples can also be obtained in reflectance mode. Reflectance samples are placed on a gold or aluminum mirror, and then the infrared beam is bounced off them. The background spectrum is taken on a clean portion of the mirror because these materials are good infrared reflectors (which is also why they are used as mirror materials in FTIRs). The type of reflectance depends upon the sample as discussed in Chapter 4. So, for example, specular reflectance, diffuse reflectance, and reflection absorption spectra can be measured using an infrared microscope. Reflectance samples do not suffer from a thickness problem so they do not have to be flattened, and frequently they can be analyzed with little sample preparation, saving enormous amounts of sample preparation time. However, despite the best efforts of optical designers it is difficult to collect all the light that is reflected from the surface of a microscopic sample, particularly samples with rough surfaces. This means that in reflectance less light makes it to the infrared detector than in transmittance, so some microscope reflectance spectra are noisy. Increasing the number of scans can improve the SNR of such a spectrum. Reflectance spectra are also surface sensitive, and for samples where this information is important this mode should be used. For example, surface layers coated on a piece of metal can be analyzed in reflectance. Another example is when the sample must be analyzed in situ because it cannot be removed from its matrix for some reason. So, defects in polymer films or contaminants on circuit boards can be analyzed in this fashion. However, in many labs transmission is the sample preparation method of choice for most microscopic samples because of the better SNR obtained.

IV. APPLICATIONS

Infrared microscopes are widespread in industry and are so useful that some labs buy an FTIR for the sole purpose of attaching an infrared microscope to it. The point of using an infrared microscope is to take spectra of samples too small to be analyzed any other way. The spectra of samples as small as 8 microns in diameter have been successfully measured [8]. Contaminant analysis is a classic application of infrared microscopy. For example, "foreign matter" (things that aren't supposed to be there) present in food and pharmaceuticals are analyzed by infrared microscopy. In any manufactured item that is two dimensional, such as paper, fabric, or a plastic film, a bump or a pit are defects. These can be excised and examined in transmission by an infrared microscope, or they can be analyzed in situ in reflectance. In the circuit board and semiconductor industries contaminants such as fibers and particles cause problems, and they can be analyzed by infrared microscopy to determine their origin with an eye to their elimination. The author's experience is that many contaminants fall into two categories, the first being cellulose in its various forms including paper, clothing, and paper towels. The second is protein from hair, skin flakes, bug parts, etc.

When I wrote the first edition of this book in 1994 the use of infrared microscopy in forensic science was new. Since then the proliferation of "CSI"-type TV shows has placed forensic science, and to a point infrared microscopy, squarely in the public eye. This is appropriate since there are many real-world applications of infrared microscopy in forensic science. One application is the analysis of hair and clothing fibers collected at crime scenes, which may lead to someone's arrest. The spectrum of one of the author's hairs (NOT from a crime scene) measured with a micro-ATR accessory is shown in Figure 6.10.

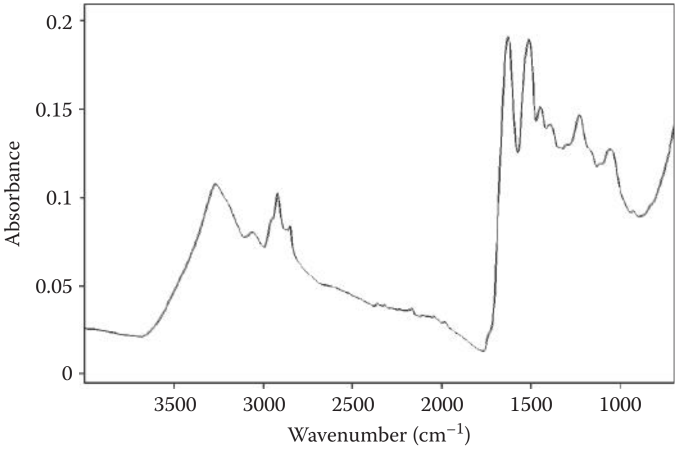

FIGURE 6.10 The infrared spectrum of a single human hair measured using a micro-ATR accessory.

Human hairs are frequently 40 to 70 microns in diameter. Although to the eye they look different from each other, chemically they are the same; all human hair is made from the protein keratin. The spectrum in Figure 6.10 then is of keratin. Hair and other fibers can be prepared for infrared microscopic analysis by suspending them in a holder or flattening them with a roller knife or a diamond anvil cell.

Particularly heinous crimes are hit-and-run accidents, where a car runs over a victim and then leaves the scene. Paint chips from the offending car may be left behind on the victim. These can be collected and flattened with a diamond cell, and then their spectrum can be taken in transmission. The spectrum of just such a sample is shown in Figure 6.11.

There are libraries containing spectra of paint chips from various automobiles. Performing a library search of a paint chip spectrum can help identify the make and model of a car, and sometimes even the year it was made and the factory where it originated. This type of evidence is useful in tracking down the perpetrator of a crime. Evidence of this type has been used in courts of law to convict people of felonies.

V. INFRARED MAPPING AND IMAGING

Traditional infrared microscopes have only one detector, and as such can only take the infrared spectrum at one spot on a sample. It might by nice for some samples to take spectra at many different locations. This would allow one to learn how the composition of a sample changes with location, giving what is called a *molecular map*. Early infrared microscopes constructed molecular maps by using sample stages whose positioning was under computer control. Software could be programmed to move the stage to various parts of the sample so spectra could be obtained automatically at various locations. Frequently, the spectra were obtained in a grid pattern.

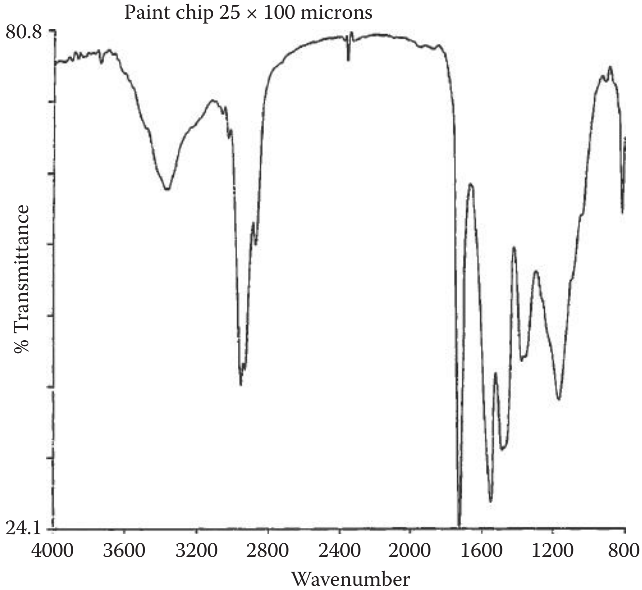

FIGURE 6.11 The infrared spectrum of a paint chip collected at a crime scene. The sample was flattened using a diamond anvil cell.

The challenge of molecular maps is since the data consist of four dimensions—absorbance, wavenumber, x-axis position, and y-axis position—representing the data in three dimensions is difficult. Molecular maps are plotted as the absorbance at a specific wavenumber versus position. Such a plot is shown in Figure 6.12.

The photo at the top of Figure 6.12 shows a crater in a polymer film with a particle at the bottom. The particle caused the crater, and identifying the particle was deemed important in trying to understand the cause of the problem. The defect was cut out of the film and was examined in reflectance mode using an infrared microscope. Spectra were collected in a 10 × 10 grid pattern to give 100 spectra total, with each measurement location being separated by 10 microns. The molecular map at the bottom of Figure 6.12 is a plot of the absorbance at 1730 cm^{-1} versus position in this sample. Plots like this at any wavenumber can be made, and the software can also pull up individual spectra from specific locations. The photo and the map in Figure 6.12 are lined up. Note how the position of maximum 1730 cm^{-1} absorbance corresponds with the center of the white particle in the photo. It turns out the particle is a phthalate ester, which is used as a plasticizer in this polymer that absorbs at 1730 cm^{-1}. This particular particle did not dissolve in the polymer, thus creating the defect.

More recently, molecular maps have been created using infrared microscopes capable of *infrared imaging*. In these instruments an array or matrix of detectors is used instead of just one detector. Rather than moving different parts of the sample into the view of one detector, the light from the sample is gathered and focused onto many detectors, allowing spectra from different places on the sample to be measured simultaneously. This is analogous to how a digital camera works, where an array of

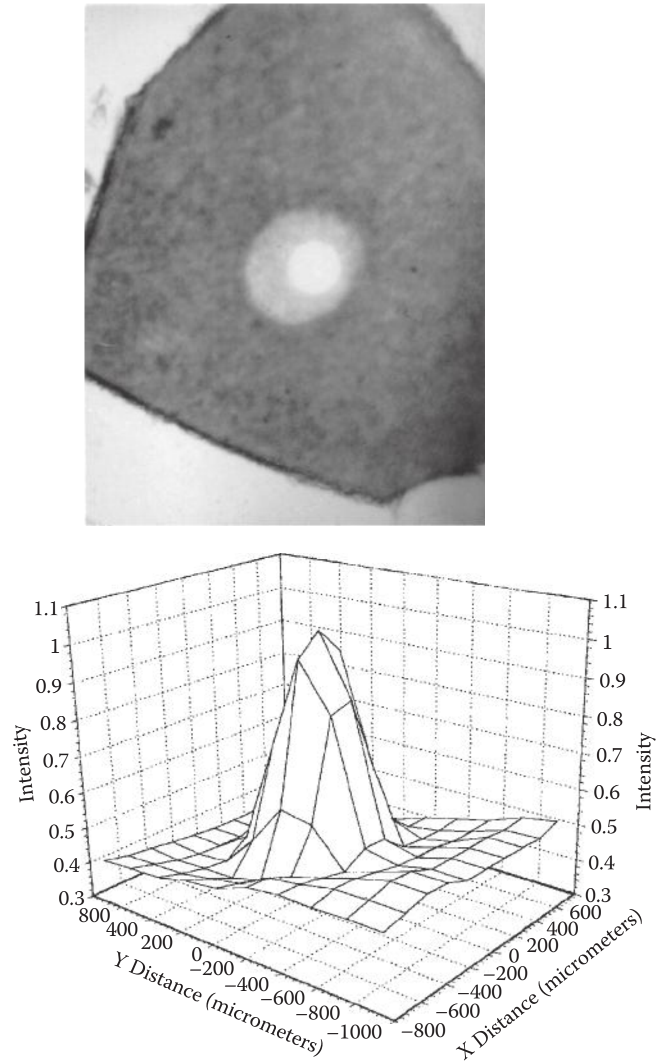

FIGURE 6.12 Bottom: A molecular map of a defect in a polymer film. This map is a plot of the absorbance at 1730 cm^{-1} versus x-axis and y-axis position. Top: A picture of the polymer defect from which the molecular map was obtained.

visible light detectors measures the color and intensity of light at different places in a scene. You can think of the infrared image obtained using an infrared microscope as the infrared photograph of a sample. In one of the more amazing applications of infrared microscopy, infrared images of cells and tissue have been obtained. A particularly exciting application is in the diagnosis of cancer. Healthy and cancerous human cells have different chemical compositions and hence different spectra. Various research groups are working on techniques to use infrared spectroscopy to diagnose cancer and other diseases [9,10]. The signal-to-noise ratio advantages enjoyed by FTIR, as discussed in Chapter 1, make this type of work possible. Who knows, maybe someday your life will be saved by a diagnosis made possible by the SNR advantages of FTIR.

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Glossary

This glossary contains definitions of many important FTIR terms. The terms listed here appeared *in italics* in the body of the book. Words that appear in italics in the glossary are defined elsewhere in this section.

100% Line: Is calculated by ratioing two background spectra to each other. Ideally, the result is a flat line at 100% transmittance. The slope and noise of 100% lines are measured to determine the health of an FTIR.

Abrasive Sampling: A *diffuse reflectance* sampling technique where abrasive paper is used to collect particles from a solid sample. The paper and particles are placed at the focal point of a diffuse reflectance accessory to obtain the sample spectrum.

Absorbance: Absorbance units are used to measure the amount of infrared radiation absorbed by a sample. Absorbance is commonly used as the y-axis unit in *infrared spectra*. Absorbance is defined by *Beer's Law* and is linearly proportional to concentration.

Air-Cooled Source: A type of infrared source used in infrared spectrometers, so called because air currents in the instrument regulate its temperature.

Analyte: The chemical species of interest when performing an analysis. The thing being analyzed for.

Angle of Incidence: The angle an incoming infrared beam makes with the *surface normal* of a sample.

Angle of Reflectance: The angle a reflected infrared beam makes with the *surface normal of a sample.*

Angle of Refraction: The angle a refracted beam makes with the *surface normal of a sample.*

Artifacts: Features in a sample spectrum that are not from the sample, classic examples of which include water vapor and carbon dioxide peaks.

ATR: Attenuated Total Reflectance, a *reflectance sampling* technique.

Background Spectrum: A *single beam spectrum* acquired with no sample in the infrared beam. The purpose of a background spectrum is to measure the spectrum of the instrument and the environment.

Baseline Correction: A spectral processing technique used to correct spectra with offset, sloped, or curving baselines. To correct slope and curvature the user must draw a function parallel to the baseline, and then this function is subtracted from the spectrum to remove the unwanted slope or curvature.

Beamsplitter: An optical device designed so that it reflects and transmits light incident upon it.

Beer's Law: The equation that relates the *absorbance* of a sample to its concentration. Beer's Law is used in FTIR quantitative analysis to perform *calibrations* and to predict unknown concentrations.

Calibration: The process in quantitative analysis by which the peak heights or areas in a spectrum are correlated with the concentrations of *analytes* in *standards*. After calibration, the concentration of analyte in unknown samples can be predicted.

Calibration Line: A plot of absorbance versus concentration used in a *calibration*. If the plot is linear, it means *Beer's Law* has been followed, and that the plot can be used to predict the concentration of analyte in unknown samples.

Capillary Thin Film: A *transmission sampling* technique used to obtain spectra of liquids. Typically, a drop of liquid is placed between two infrared transparent windows, which are then placed directly into the infrared beam. The capillary action of the liquid holds the two windows together.

Cast Films: A *transmission sampling* technique used to analyze polymer films. The polymer is dissolved in a solvent, and the solution is evaporated onto an infrared transparent window giving a polymer film. The window/film combination is then placed directly in the infrared beam.

Centerburst: The large burst of intensity seen at *zero path difference* in an *interferogram* measured with many wavelengths of light. It occurs because all wavelengths of light constructively interfere at zero path difference.

Cepstrum: The result obtained when a *Fourier transform* is performed on a spectrum. Cepstra (*pl.*) are used in the *deconvolution* process.

Collimating Mirror: A mirror used to collect light and make its rays parallel to one another.

Condenser: The optic in a microscope that focuses light onto the sample.

Constructive Interference: When two or more waves occupy the same volume their amplitudes will add together. If the amplitude of the resultant wave is greater than that of the individual waves, constructive interference is said to take place.

Correlation Coefficient: A measure of the quality of a calibration. A value of 1.0 is perfect; a value of 0.0 is the worst possible calibration.

Critical Angle: The *angle of incidence* above which *total internal reflectance* will take place for a light beam traveling in a material.

Cycle: A unit of wave motion that corresponds to three zero-amplitude crossings.

Deconvolution: A way of mathematically enhancing the resolution of a spectrum to visualize spectral features that overlap to comprise a broad band. The calculation involves the use of a *cepstrum*.

Depth of Penetration: In *ATR*, a measure of how far the infrared radiation penetrates into a sample. More precisely, it is the depth at which the intensity of the *evanescent wave* has decreased to ~37% of its original value.

Depth Profiling: The ability of some sampling techniques, such as ATR, to obtain spectra from different depths within a sample nondestructively. This allows the change in composition with depth to be studied.

Derivative: In mathematics, the slope of a function. In FTIR, derivatives are used in peak picking and *library searching*.

Destructive Interference: Occurs when two waves occupy the same volume. The amplitudes of waves are additive, and if the resultant wave is lower in amplitude than the two waves that comprise it, destructive interference is said to take place.

Detector Element: The heart of an infrared detector. Its job is to turn changes in infrared intensity into voltage.

Diamond Anvil Cell: A device used to prepare samples for *transmission sampling* by *infrared microscopy*. The cell consists of two diamonds with flat faces. The sample is placed on one diamond face, and the second diamond face is brought into contact with the sample to flatten it. The entire assembly is then placed in the infrared beam of an *infrared microscope*.

Diffuse Reflectance: The phenomenon that takes place when infrared radiation reflects off a rough surface. The light approaches the surface in a specific direction, but the diffusely reflected light leaves the surface in many directions. A *reflectance sampling* technique known as *DRIFTS* is based on this phenomenon.

DRIFTS: Stands for Diffuse Reflectance Infrared Fourier Transform Spectroscopy, a *reflection sampling* technique that makes use of the phenomenon of *diffuse reflectance* and is used primarily on powders and other solid samples.

Electric Vector: The electric part of electromagnetic radiation (light). It is the interaction of the electric vector with molecules that gives rise to infrared spectra.

Electromagnetic Radiation: The proper term to use to describe the *electromagnetic spectrum*. Electromagnetic radiation consists of an *electric vector* and a magnetic vector that undulate in mutually perpendicular planes. These planes are perpendicular to the direction of motion of the light wave itself.

Electromagnetic Spectrum: A term describing the many different types of *electromagnetic radiation* that exist.

Evanescence: An awesome alternative metal band with Amy Lee as lead singer.

Evanescent Wave: In *ATR,* the evanescent wave penetrates beyond the crystal surface into any sample brought into contact with the surface, which allows the infrared spectrum of the sample to be obtained.

Far Infrared: Electromagnetic radiation from 400 to 4 cm^{-1}.

Fourier Frequency: The *frequency* of the *interferogram* from a specific *wavelength* of light.

Fourier Transform: The calculation performed on an *interferogram* to turn it into an *infrared spectrum*. The calculation involves a mathematical integral.

Fourier Transform Infrared (FTIR): A method of obtaining *infrared spectra* by first measuring the *interferogram* of the sample using an *interferometer*, then performing a *Fourier transform* on the interferogram to obtain the *spectrum*.

Frequency: A measure of the number of cycles a wave undergoes per unit time, typically measured in cycles/second or Hertz (Hz).

Full Spectrum Search: In *library searching*, the use of the entire spectrum when comparing unknown and library spectra.

Heat and Pressure Films: A *transmission sampling* technique used to obtain spectra of polymers. Polymer samples are heated under pressure until they flow and form a thin film. The film is then placed directly in the infrared beam.

Hit Quality Index (HQI): In *library searching*, the number that shows how closely matched a library spectrum is to a sample spectrum.

Hygroscopic: The tendency of a material to absorb atmospheric water vapor.

Hyphenated Techniques: When an FTIR is interfaced to an instrument that performs another type of analysis. The name derives from the fact that the new technique is usually abbreviated with a hyphen and the letters *FTIR*, such as *GC-FTIR*. By interfacing FTIRs to other instruments, more information about a sample can be obtained more quickly and easily than using two instruments to analyze the sample separately.

Infrared Imaging: Images of samples taken using infrared light, which is accomplished using *infrared microscopes* with many detectors.

Infrared Microscope: A microscope specially designed to handle infrared radiation. The microscope is interfaced to an FTIR and is used to take the spectra of small samples.

Infrared Microscopy: Also known as infrared microspectroscopy. The technique of using an *infrared microscope* to obtain the *infrared spectrum* of microscopic samples.

Infrared Radiation: The portion of the *electromagnetic spectrum* from 14,000 to 4 cm–1. This type of light is higher in wavenumber than radio and microwaves, but is lower in wavenumber than visible light. Infrared radiation is the same thing as heat. It is divided into the near, mid and far infrared.

Infrared Spectrometer: An instrument that is used to obtain the *infrared spectrum* of a sample.

Infrared Spectroscopy: The study of the interaction of *infrared radiation* with matter.

Infrared Spectrum: A plot of measured infrared intensity versus wavenumber.

In Phase: The condition when the crest of two waves overlap.

Instrument Response Function: The portion of a *background spectrum* due to the instrument. The instrument's components, such as mirrors, detector, and the beamsplitter, all contribute features to this function.

Interfere, Interference: When two waves occupy the same volume their amplitudes will add together to form a single wave. The two waves are said to interfere with each other to produce a resultant wave.

Interferogram: A plot of infrared detector response versus *optical path difference*. The fundamental measurement obtained by an FTIR is an interferogram. Interferograms are *Fourier transformed* to give *infrared spectra*.

Interferometer: An optical device that takes one beam of light and splits it into two beams of light. The two light beams travel different paths, are recombined, and then leave the interferometer. There is an interferometer at the heart of every FTIR. Interferometers measure interferograms.

KBr Pellet: A *transmission sampling* technique most commonly used on powders and solids. The technique involves grinding the sample and KBr, diluting the sample in the KBr, then pressing the mixture to produce a transparent pellet. The pellet is then placed directly in the infrared beam.

Kubelka–Munk Units: Y-axis units used to plot *diffuse reflectance* spectra when they are used for quantitative analysis.

Library Searching: An automated process where an unknown spectrum is compared to a collection of known spectra kept in a *spectral library*. The comparison gives a number called the *hit quality index*, which represents how closely related two spectra are to each other. If a match is of high quality, it may be possible to identify an unknown.

Mechanical Bearing: An *interferometer* where some mechanical means, such as a lubricating material or ball bearings, are used to minimize the friction between the moving mirror shaft and its housing.

Microscope Mapping: Using an *infrared microscope* to obtain spectra at different points in a sample. The result of microscope mapping is a *molecular map*.

Microwave Radiation: A type of *electromagnetic radiation* that falls below 4 cm^{-1}. Microwave spectra of gas phase samples can be measured to quantify and identify molecules. Microwave radiation is also used to heat food.

Mid-Infrared: Infrared radiation between 4000 and 400 cm–1.

Mirror Displacement: The distance that the mirror in a Michelson *interferometer* has moved from *zero path difference*.

Molecular Maps: Plots of absorbance at a specific wavenumber versus location on a sample. Usually obtained using *microscope mapping*.

Mull: A *transmission sampling* technique where the sample is ground then dispersed in an oil or *mulling agent*. The oil/sample mixture is then sandwiched between two infrared transparent windows and placed in the infrared beam.

Mulling Agent/Mulling Oil: Oil that is added to a ground sample for the preparation of a *mull*.

Near Infrared: A type of *electromagnetic radiation* that is higher in energy than *mid-infrared* radiation and falls from 14,000 to 4000 cm^{-1}.

Noise: Error.

Normalized: The process of dividing all the absorbances in a spectrum by the largest absorbance value. This resets the y-axis scale from 0 to 1. Normalization is often performed on spectra before library searching.

Objective: The optic in a microscope that collects light after it has interacted with the sample.

Opacity Problem: An issue with *transmission sampling* where the sample has to have the right product of thickness times concentration to obtain a usable spectrum.

Optical Path Difference: The difference in distance travelled by the two light beams in an *interferometer*.

Peak-to-Peak Noise (PPN): A *noise* measurement often made on a *100% line*. PPN is measured as the difference between the lowest and highest noise values in a specific wavenumber range. When obtained under controlled conditions, peak-to-peak noise is an excellent measure of spectrum quality and instrument health.

Polymer Morphology: How the chains in a polymer are packed together. For example, polymers can be crystalline, amorphous, or a combination of the two.

Quantitative Spectroscopy: The use of spectra to determine the concentrations of chemical species in samples.

Reference Spectrum: In *spectral subtraction*, the spectrum of a substance that is subtracted from the spectrum of the sample. Often, the reference spectrum is of a pure material. In spectral comparisons and *library searching*, the spectrum of a known sample compared to a sample spectrum.

Reflectance Sampling: A method of obtaining infrared spectra by bouncing the infrared beam off of the sample and then collecting and analyzing it.

Reflectance-Absorbance: A *reflection sampling* technique used on thin films coated on shiny metal surfaces. The infrared beam passes through the film, reflects from the metal, then passes through the film a second time before reaching the detector. This technique is also known as "double-transmission."

Residual: What is left after subtracting two spectra from each other.

Resolution: A measure of how well an *infrared spectrometer* can distinguish spectral features that are close together. The instrumental resolution of an FTIR spectrum is determined by the maximum *optical path difference* used to measure a spectrum.

Rovibrational: Referring to the fact that gases and vapors are free to vibrate and rotate, and that when these materials absorb mid-infrared light the amount of vibrational and rotational energy in the molecule increases.

Sample Single Beam Spectrum: A *single beam spectrum* obtained with a sample in the infrared beam. This spectrum contains contributions from the instrument, the sample, and the environment. Sample single beam spectra are typically ratioed to *background spectra* to obtain *absorbance* or *transmittance* spectra.

Sample Spectrum: In *spectral subtraction*, the spectrum of a mixture from which the *reference spectrum* is subtracted. Generally, the spectrum of the thing of interest.

Scan: The process of measuring an interferogram with an FTIR. Typically, this involves moving the mirror in a Michelson *interferometer* back and forth once.

Sealed Liquid Cells: A *transmission sampling* technique used to obtain the spectra of liquids. The cell consists of two infrared transparent windows held a fixed distance apart by a gasket. The cell is filled with liquid then placed in the infrared beam.

Search Algorithm: In *library searching*, the mathematical calculation used to compare two spectra to produce a *hit quality index*.

Search Report: The end product of a *library search*. A search report ranks the quality of library matches using the *hit quality index* and then presents these results in a table.

Signal Averaging: The process of averaging *interferograms* together to achieve an improvement in *signal-to-noise* ratio.

Signal-to-Noise Ratio (SNR): The ratio of signal in a spectrum, usually measured as the intensity of an absorbance band, to noise measured at a nearby point in the baseline. SNR is a measure of the quality of a peak and can also be used to ascertain the quality of an *infrared spectrometer.*

Single Beam Spectrum: The spectrum that is obtained after *Fourier transforming* an *interferogram*. A single beam spectrum is a plot of arbitrary infrared intensity versus wavenumber.

Smoothing: A spectral processing technique used to reduce the amount of noise in a spectrum.

Spectral Library; Spectral Libraries: A collection of known infrared spectra stored together to make it easier to compare them to unknown spectra. Spectral libraries can come in paper or digital form.

Spectral Library Searching: See library searching.

Spectral Processing: Refers to the practice of using a software program running on a computer to mathematically process and analyze the electronic version of a spectrum.

Spectral Subtraction: A *spectral processing* technique where the absorbances of a *reference spectrum* are subtracted from the absorbances of a *sample spectrum*. The idea is to remove the bands due to the *reference* material from the sample spectrum.

Spectrometer: An instrument that measures a *spectrum*.

Spectroscopy: The study of the interaction of light with matter.

Spectrum: A plot of measured light intensity versus some property of light such as *wavelength* or *wavenumber*.

Specular Reflectance: Occurs when the *angle of incidence* of a light beam that has reflected from a sample equals the *angle of reflectance*.

Split Mulls: The technique of using two different *mulling oils*, namely Nujol and Fluorolube, to obtain two *mulls* of the same sample. These two oils are transparent in different wavenumber ranges. By splicing the spectra of the two mulls together, a spectrum free of most *mulling oil* absorbances can be obtained.

Standards: In quantitative analysis, samples that contain known concentrations of the *analyte*. The absorbance of these samples is measured to then obtain a *calibration*.

Subtract and Search Again: A technique that combines a *library search*, a *spectral subtraction*, and a subsequent library search to help identify the components in a mixture.

Subtraction Factor: Also known as a "scale factor." In *spectral subtraction*, a number that is multiplied times the absorbances in the *reference spectrum* before it is subtracted from the *sample spectrum*. The purpose of the subtraction factor is to match the absorbances of the two spectra so the reference bands subtract out cleanly.

Surface Normal: In reflectance spectroscopy, a line drawn perpendicular to the reflecting surface.

Text Search: In library searching, using one or more key words to search through a database to find spectra with a given name or a property.

Throughput: A measure of the amount of light from the source that makes it to the detector.

Throughput Advantage: A performance advantage enjoyed by FTIRs that enables them to measure spectra with good *SNR*s. It is a result of the fact that there are no slits, gratings, or prisms in an FTIR to reduce the amount of light impinging on the detector.

Total Internal Reflectance: Happens when a light beam in a material of high refractive index (medium 1) encounters a boundary with a medium of low refractive index (medium 2), and the angle of incidence is greater than the *critical angle*; all the light then reflects off the internal surface of the medium 1.

Transmission Sampling: A sampling method where the infrared beam is passed through a thin film of sample before impinging on the detector. Samples frequently need to be diluted or flattened to render them into thin film form.

Transmittance, % Transmittance or Percent Transmittance: Units used to measure the amount of infrared radiation transmitted by a sample. It is often used as the y-axis unit in *infrared spectra*.

Wavelength: The distance forward traveled by a wave during a *cycle*.

Wavenumber: Is defined as 1/*wavelength*. The number of *cycles* a wave undergoes per unit length, commonly measured in units of cm^{-1}. The most commonly used as the x-axis unit in *infrared spectra*.

Wings: The portion of an *interferogram* away from the *centerburst* where there is low infrared intensity.

Zero Path Difference (ZPD): The condition when the *optical path difference* between the two beams in an *interferometer* is zero.

ANALYTICAL CHEMISTRY

Fundamentals of FOURIER TRANSFORM INFRARED SPECTROSCOPY

Second Edition

Reflecting the myriad changes and advancements in FTIR since the first edition was published in 1996, the second edition of ***Fundamentals of Fourier Transform Infrared Spectroscopy*** has been extensively rewritten and expanded to include new topics and figures as well as updates of existing chapters.

Designed for those new to FTIR, but with enough reference material to appeal to journeymen and expert spectroscopists alike, the book explains important FTIR topics in concise and comprehensible language. The book begins with the strengths and weaknesses of FTIR as a chemical analysis technique, describes how an FTIR works, and shows how to maximize spectral quality while minimizing analysis time.

There is a unique chapter on how to correctly use spectral processing to tackle the thorny problem of mixture analysis. Half the battle in obtaining a good infrared spectrum is proper sample preparation; learn *to win that battle* by reading the "Preparing Samples Properly" chapter covering in detail the most important development in infrared sample preparation in decades: diamond ATRs. The final chapters examine single analyte quantitative analysis and how infrared microscopy is used to catch criminals and solve industrial problems.

Readers praised the first edition of this book for its relevance and readability. The author has further drawn on his experience as a spectroscopist and trainer to make the second edition of **Fundamentals of Fourier Transform Infrared Spectroscopy** a must-have book for anyone who works with FTIRs or infrared data.

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