Educational experiment package
Volume 1

Molecular spectroscopy

Overview

Thermo Fisher Scientific is proud to offer a variety of educational experiments for use with Fourier transform infrared (FTIR) spectrometers. These educational experiments present the theory and operation of an FTIR spectrometer, and then apply that learning to a series of significant applications. By combining this tool with lecture and lab reporting, students should gain significant experience with FTIR as a laboratory tool.

Contents

Experiment 1
Theory of FTIR

Objective
To understand the steps of data collection and processing used in an FTIR.

Materials Required

- Nicolet FTIR spectrometer

- Polystyrene card

Introduction
A Fourier transform infrared spectrometer is a powerful tool used in quality control labs, analytical laboratories and forensics settings. This experiment will examine the basics of how an FTIR works and some of the data manipulations which take place to produce the sample spectrum. The complex Fourier transform mathematics is not presented, as the primary aim is to develop an understanding of "what is under the hood."

Discussion
Infrared (IR) spectrometry provides keen insights into molecular structures, and is thus a core part of many analytical laboratories. The earliest IR spectrometers were based on dispersive technology, which uses a diffraction grating to spread out the IR light into different wavelengths, like a rainbow does for visible light. A narrow range of this dispersed light passes through a slit and then impinges on a detector. The amount of light transmitted through the sample is measured, resulting in an infrared spectrum. This measurement process is very slow and not very sensitive since both the energy and the spectral range reaching the detector is limited by the slit. Dispersive instruments also need to be calibrated with an external reference source to verify the wavelength (x-axis) scale.

Interferometer based instruments overcoming these issues were developed in the 1890's by A. A. Michelson. The interferometer replaces the diffraction grating and slit, allowing all of the energy and all of the wavelengths of the IR light to pass through the spectrometer simultaneously. The signal from the detector emerges in a time-domain format, which is converted to the more familiar (wavenumber) spectrum using the Fourier transform mathematical computation. The first FTIR spectrum obtained using an interferometer was acquired in 1949 by Peter Fellgett.

The main elements of the interferometer are an infrared source, a beamsplitter, two mirrors, and a detector. The infrared energy travels from the source to the beamsplitter. The most common beamsplitter is made primarily of KBr coated so that it reflects half of the light and transmits the other half. The optics are then arranged to direct these two beams to the two mirrors.
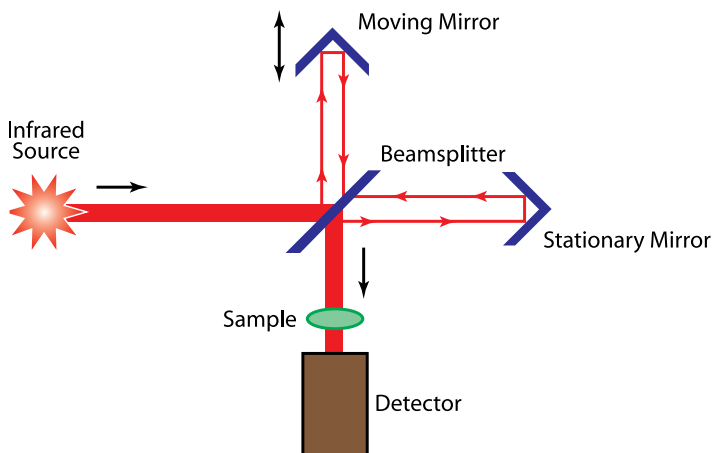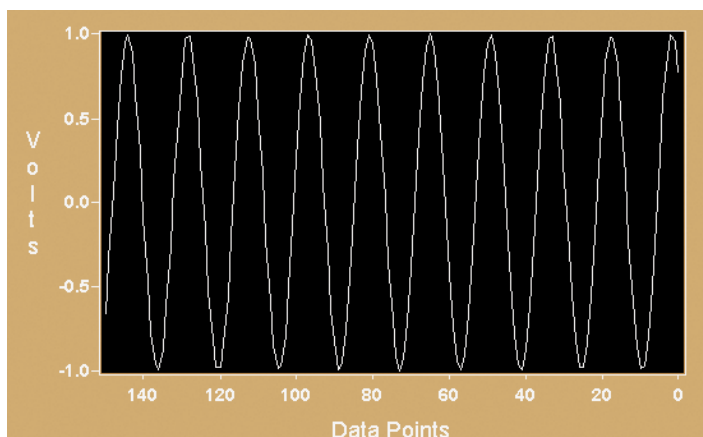

FTIR Interferometer Diagram

One of the two mirrors is stationary, so that the distance traveled by one of the half-beams is fixed and known. The other mirror moves back and forth at a constant velocity, which is controlled by monitoring the signal from a laser with a known wavelength. Thus, the distance traveled by the second half beam mirror is constantly changing. The two half beams recombine at the beamsplitter. The difference in path lengths for the two beams results in interference when the two recombine, as discussed below. The recombined beam travels through the sample compartment and then strikes a detector. The signal recorded at the detector due to the interference of the two beams is called an interferogram.
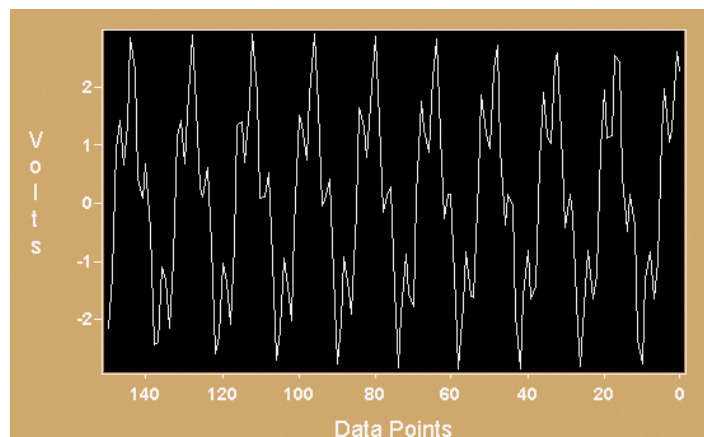
The adjacent graphs show the patterns resulting from the interference of one, two and multiple wavelengths beams. The pattern seen in the last figure occurs when a large number of wavelengths are interfering. This is the classic interferogram seen on most FTIR spectrometers.

An interferogram represents the energy striking the detector in the time domain, converted in these figures by an analog-to-digital converter. Every time the moving mirror completes one sweep, one interferogram is collected. Although the desired absorption information is present in the time domain signal, interpreting interferograms is extremely difficult. Instead, the data is converted from the time domain interferogram to the frequency domain spectrum using a Fourier transform (FT). The advent of high speed computers able to rapidly perform the FT in real time was the seminal moment in making FTIR a practical tool. After the FT is performed, the result is a single beam
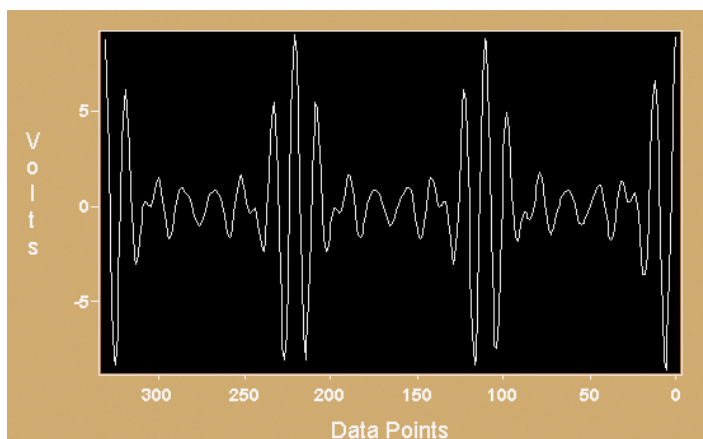
spectrum, showing the response of the instrument and the sample at the same time. In order to obtain the spectrum of the sample alone, a background is collected with no sample present. This background spectrum contains information about the wavelength-dependent energy response of the detector as well as background interferences, like water vapor and carbon dioxide.
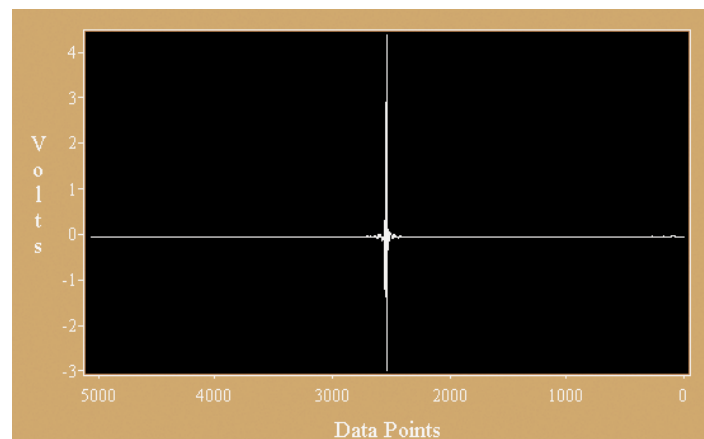

One Wavelength Interference Pattern

Two Wavelength Interference Pattern

The two single beam spectra – with and without sample present – are collected first. Both single beams are processed using the same steps (FT with all the parameters the same). The next step in obtaining the final spectrum of the sample is typically done automatically, so the analyst may never see the single beam spectrum from the sample.

Multiple Wavelength Interference Pattern Infrared

Infrared Interferogram

The background signals are in both the background and sample single beams, while the sample is only present in the latter. Forming the ratio of the two removes the background signals. This spectrum multiplied by 100 is called a %Transmittance (%T) spectrum, as it represents the percentage of light that passes through unaffected by the sample. This ratio is the same as taking each data point in the spectrum for the two spectra and performing the following function:
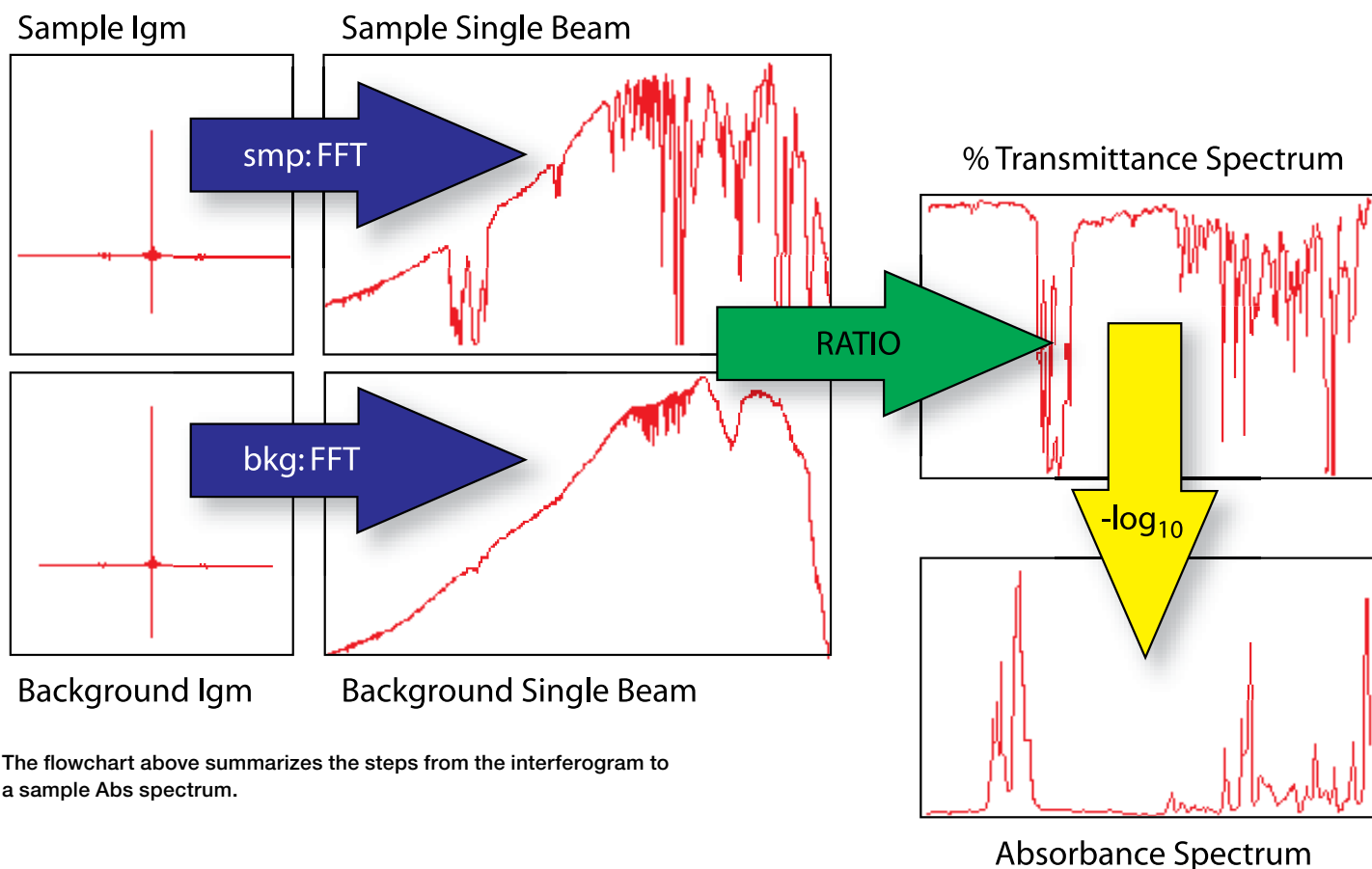
$$\%T = \frac{\text{Energy of the Sample Single Beam}}{\text{Energy of the Background Single Beam}}$$

This ratio process is NOT a "subtraction", although the two terms have been frequently used incorrectly to mean the same thing. The ratio process ends with a graph of the percent of energy transmitted through the sample to the detector versus the wavenumbers. Basically, %T spectra show the peaks used to identify compounds. The %T peaks

point downward, showing the percentage of energy which makes it through the sample to the detector at each infrared energy. As energy is directly proportional to wavenumber (cm^{-1}), rather than wavelength (microns), the wavenumber plot has been the preferred method for presentation of IR spectra.

The intensities of the peaks in a %T spectrum are not linear with respect to concentration. Infrared is being used more frequently for quantitative analyses, so a linear relationship between concentration and intensity is desirable. Linearity is accomplished by taking the negative of the base ten logarithm ($-\log10$) of the %T data points. This results in the Absorbance (Abs) spectrum where the intensities can be used directly in a Beer-Lambert's law calculation for quantitation. The peaks in an absorbance spectrum point upward, indicating the amount of energy which has been absorbed by the sample itself. The information content of the two displays is the same, and most reference materials use %T for historical reasons.

Sample Igm   **Sample Single Beam**

smp:FFT

% Transmittance Spectrum

RATIO

bkg:FFT

$-\log_{10}$

Background Igm   **Background Single Beam**

Absorbance Spectrum

The flowchart above summarizes the steps from the interferogram to a sample Abs spectrum.

Experimental

Collect the infrared signal at the detector without a sample present. This should be in the form of an interferogram. Note the x- and y-axis labels.

x-axis = _____

y-axis = _____

Check that the spectrometer has no samples in the sample compartment; then collect a background single beam spectrum. Print this out to include with your laboratory report. Note the x- and y-axis labels.

x-axis = _____

y-axis = _____

Place the polystyrene card or other sample provided into the sample compartment and collect and print the sample single beam spectrum. Note the differences in the two. Now, ratio the two single beams to produce the %T and absorbance spectrum of the sample. Print out the sample spectrum to include with your laboratory report in both %T and Abs format. Note the x- and y-axis labels.

%T: x-axis = _____

%T: y-axis = _____

Abs: x-axis = _____

Abs: y-axis = _____

Questions

1. What signal format does the detector on the infrared spectrometer actually see? Read a bit about analog-to-digital converters, and add a short description to your report.

    _____

    _____

    _____

2. List the steps in collecting and processing an FTIR spectrum. What is the function of taking the ratio of the two single beams?

    _____

    _____

    _____

Look at your %T and Abs spectrum of the samples. Note any differences in peak shapes or relative intensities you observe. Does "turning over" one of the two make it look like the other one?

_____

_____

_____

_____

Experiment 2
Functional group identification

Objective
To correlate known organic functional groups to their infrared spectra, and use that to interpret IR spectra.

Materials Required

- Nicolet FTIR spectrometer

- Unknown sample

Introduction
IR spectrum can be correlated with known information to identify the compound's functional groups. Second, comparison of the compound's full or partial spectrum to others in the literature or in digital databases can narrow the list of possible candidates to a few or even one compound. Identification of materials is the single most common use of FTIR spectrometry.

This experiment introduces the idea of correlation tables and decision trees to assign IR peaks to common functional groups. In addition, you will perform a search of the SUNY Stony Brook Educational Infrared Database to verify the unknown.

NOTE: The SUNY Stony Brook Educational Infrared Database was provided by Marjorie Kandel and James Marecek, Department of Chemistry, SUNY Stony Brook, Stony Brook, NY, USA. This library is available for purchase upon request from Thermo Fisher Scientific. Please contact your local representative for details.

Discussion
There are many factors to consider in interpreting the infrared spectrum of an unknown. The basic steps presented here lead to a reliable interpretation. However, the wavenumber positions in the interpretation tables are only approximate, so care must be used. Keep in mind that electronegative atoms and groups close in proximity to the bonds can raise the expected wavenumber location of the bands, while conjugation and other influences (like hydrogen bonding) may lower the wavenumber. Also, every possible vibrational mode may not manifest as a peak in the spectrum. Some vibrations in symmetric molecules may be infrared inactive (the C-C stretch in benzene, for instance), so absorption peaks may not be observed or may be very weak.

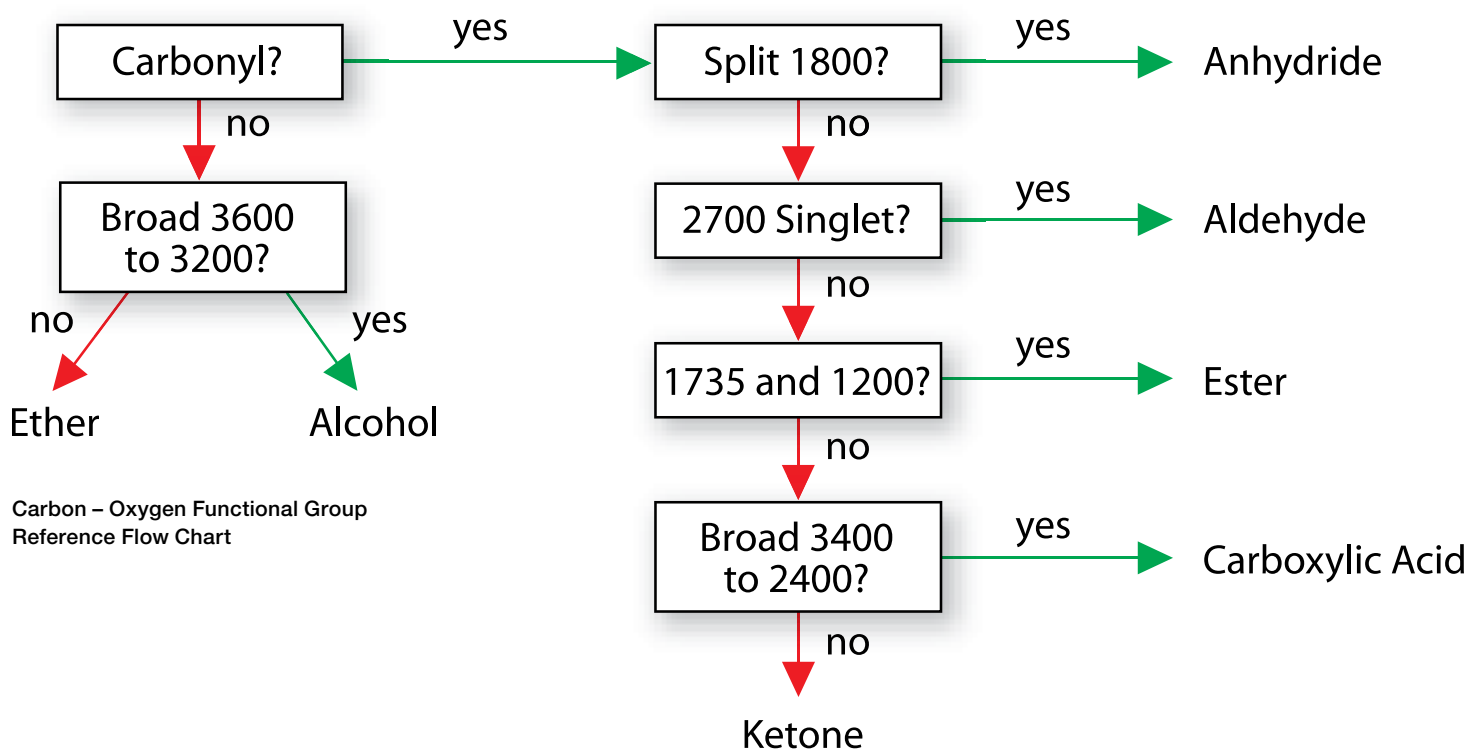Peaks at frequencies above 1500 cm^{-1} can usually be assigned to particular functional groups. Peaks below 1500 cm^{-1} are harder to specifically assign. It is generally not practical to deduce the exact carbon skeleton structure of an unknown from the infrared spectrum alone. Rather, one can determine which groups are definitely present, which may be present, and which are definitely not present. Experience is by far the best teacher. Information derived from other methods such as Nuclear Magnetic Resonance (NMR), or mass spectrometry (MS) can enhance interpretation.

Basic Steps to Interpret an Infrared Spectrum
1. **C-H Stretches 3050-2800 cm^{-1}:** Absorptions due to the stretching vibration of C-H bonds are seen in this region of the spectrum. The sp3 C-H stretches on aliphatic carbons always appear below 3000 cm^{-1}. Unsaturated sp2 C-H stretches always appear slightly above 3000 cm^{-1}. Unsaturated sp C-H stretches much higher, at around 3300 cm^{-1}. A distinctive variant of C-H stretching is found in aromatic hydrocarbons. These peaks are particularly sharp due to the constraints of molecular movement within the ring compound.
2. **C=C Stretches 1650-1450 cm^{-1}:** Linear alkene C=C stretching is usually seen near 1650 cm^{-1}. Aromatic C=C stretching gives rise to several sharp peaks between 1600 and 1450 cm^{-1}.
3. **C-H Bends:** Other peaks can be used along with the C-H and C=C stretching modes to determine other probable structural features in the compound. For example, many aromatic compounds have a strong C-H bending mode at around 700 cm^{-1}. Branched groups may have multiple peaks, like the t-butyl doublet at 1385 and 1365 cm^{-1}.
4. **C=O Stretch 1850-1650 cm^{-1}:** The carbonyl bond gives rise to a very prominent absorption peak, usually the most intense in the spectrum. The carbonyl peak is observed in the region centered around 1720 cm^{-1}. The particular type of carbonyl compound (ketone, carboxylic acid, ester, etc.) may be determined by analyzing other regions of the spectrum. The flow chart on the following page illustrates the steps that can be followed to further identify the carbonyl. Although this chart is useful for simple molecules, it may not apply to multi-functional or atypical compounds, like some inorganics.
5. **O-H Stretch 3600-3200 cm^{-1}:** The alcohol O-H stretching mode is particularly characteristic, with a broad, intense peak between 3600 and 3200 cm^{-1}. The carboxylic acid O-H stretch is also broad and strong, but appears at a lower frequency (3400-2400 cm^{-1}) due to the proximity of the C=O group. If the sample contains water, the water O-H absorption (in the same region as an alcohol) will be observed along with a broad peak of moderate intensity at approximately 1600 cm^{-1}. Note that carbonyl compounds, due to their very strong absorption at around 1720 cm^{-1}, exhibit weak overtone peaks in the O-H region. These peaks can mislead the inexperienced spectroscopist.

6. **Characteristic Nitrogen Bond Stretches:** The N-H stretch is seen in the same region as the alcohol O-H (around 3300 cm^{-1}) but is not as broad or intense. The NH2 group generates two peaks in this region. Two low frequency carbonyl peaks in the 1650 cm^{-1} region, in addition to the N-H stretching absorption, point to an amide. The nitrile stretch occurs at around 2250 cm^{-1}, while the nitro group shows strong asymmetric and symmetric stretching at 1570 and 1380 cm^{-1}, respectively.

Carbonyl? — no → **Broad 3600 to 3200?** — no → Ether; yes → Alcohol

Carbonyl? — yes → **Split 1800?** — yes → Anhydride; no → **2700 Singlet?** — yes → Aldehyde; no → **1735 and 1200?** — yes → Ester; no → **Broad 3400 to 2400?** — yes → Carboxylic Acid; no → Ketone

Carbon – Oxygen Functional Group Reference Flow Chart

Once you have interpreted a spectrum and determined a partial structure, you can conduct a search to identify the specific compound. This can be done using printed sources such as The Aldrich Library of FTIR Spectra or a digital spectral database. Entire books have been written about interpretation of infrared spectra. Most organic textbooks contain charts with this information, to help extend the interpretation. The OMNIC™ spectroscopic software contains tools automating these charts, also.

Experimental

Collect the FTIR sample spectrum of the unknown compound provided to you by your instructor. Visually check the quality of the spectrum collected. Look for peaks that are flat and showing very low %T values, called saturated, which can hinder interpretation of the spectrum. In addition, check for water vapor and carbon dioxide peaks in your spectrum. If the spectral quality is low for any of these reasons, collect a new background reference spectrum or recollect your sample spectrum with thinner samples (to avoid saturation) to get the best results. Print and save the spectrum. Use whatever information you have access to – the tables above, peak positions identified using the software, the Correlation Chart feature in the software or your textbook, and other outside resources to identify your sample's functional groups.

My sample contains these functional groups:

_____

_____

_____

_____

_____

Search the SUNY Stony Brook Educational Infrared Database to obtain the best possible match candidates for your sample. Visually compare similarities and differences between the best library match(es) and the sample. Remember, database searches always provide the highest matches to the sample available within the database, but these matches are not necessarily the correct identification of the sample.

Indicate the top three library match names and their hit index below:

Name: _____ Hit Index: _____

Name: _____ Hit Index: _____

Name: _____ Hit Index: _____

Generally, a correlation coefficient of 0.95 or better indicates an acceptable match. If more than one spectrum has achieved this, determine which is the closest match by visually comparing each to the sample spectrum. If no matches seem to provide the same spectrum as your sample, look at the matches for closely related spectral features. These can indicate functional groups present in the sample.

The best match for my sample is:

_____

Use the information you have collected on functional groups and library matches to identify your unknown sample.

My sample identity is:

_____
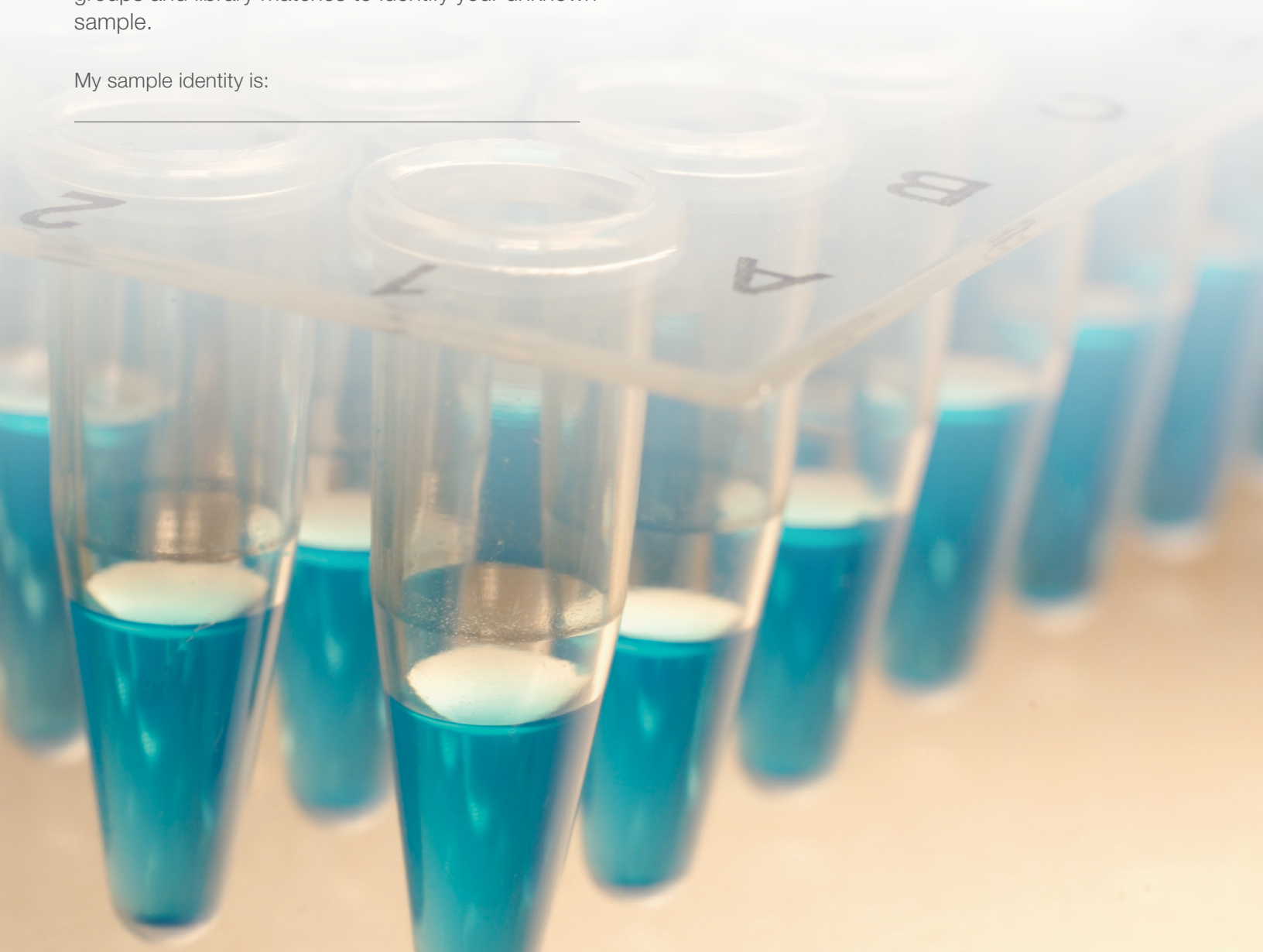
Experiment 3
Quantitative analysis using an FTIR spectrometer

Objective
To calibrate the response of an FTIR spectrometer and then predict the concentration of a component in a mixture using that calibration.

Materials Required

- FTIR spectrometer, graphing calculator or computer spreadsheet package, hexane and cyclohexanone

- Sampling cell (one or the other):

  – *Liquid cell with 0.015 mm pathlength*

  – *Attenuated Total Reflectance (ATR) accessory*

Introduction
The infrared spectrum of a sample is traditionally used as a tool to identify and characterize compounds based on the peaks present (as in the previous experiment of this set). However, the infrared spectrum can also be used to make quantitative measurements. The concentration of a component in a mixture is related to the intensity of the absorption band(s) which are characteristic of that component. When a component peak is well separated from other spectral features (free from interferences), the relationship between the absorbance of the peak to the component's concentration is fairly linear. This relationship is predicted by the Beer-Lambert law:

Absorbance = A = εlc
ε = absorptivity of the component
l = pathlength of the infrared beam through the sample
c = concentration of the component

Discussion
When calculating a quantitative calibration curve from actual FTIR data, standards are prepared to cover a range of concentrations for the mixture. The spectrum of each standard needs to be acquired to set up the calibration. A best fit line is generated with the equation following the form:

Absorbance = y = mx + b
m = slope of the best fit line
x = concentration of the component in the standard
b = intercept of the best fit line

Ideally, b should be zero (no absorbance when the component is absent), but in most cases there is a small, non-zero, contribution. The slope m combines ε and l from the Beer-Lambert Law above – you do not need to know either explicitly, though you can calculate ε knowing l.

The absorbance value is best measured as the peak area for the peak being used, although in some cases the net absorbance (peak height) is also acceptable. The basic linear relationship holds well for mixtures where the absorbance peak used for the quantitation is isolated and due only to the component of interest. More complicated mixtures with overlapping peaks of multiple components can also be quantified, although more powerful algorithms like partial least squares (PLS) are necessary.

Experimental
You will prepare a set of two-component liquid mixtures. The standards are formulated by combining cyclohexanone and hexane. The peak area of the carbonyl band of the ketone is measured for each standard, and tabulated versus known concentration. The best fit line of the concentration versus peak area graph is calculated, along with the correlation coefficient for the line. Two unknowns are then measured and the concentration of cyclohexanone in each is calculated from the best fit line.

Set up a calibration set of five standards from approximately 15 to 35 volume percent cyclohexanone. You will need about 20 ml of hexane and less than 10 ml of cyclohexanone – be sure to cover the whole range. Mix all standards thoroughly. Prepare a table similar to the one following for your standards, using the actual volume percent values based on your preparation tools.

% Cyclohexanone (Vol/Vol)	Hexane (ml)	Cyclohexanone (ml)
15	4.25	0.75
20	4.00	1.00
25	3.75	1.25
30	3.50	1.50
35	3.25	1.75

Start by collecting a single beam background spectrum (no sample present). Next, collect a spectrum in absorbance format for each standard you made. Flush the cell, or ATR crystal, with each new standard about three times before collecting the spectrum, to ensure there is no dilution problems with subsequent standards. It is generally best practice to NOT run standards in order – run them randomly.

Otherwise, biases and cumulative dilution errors can arise. It also does not matter if the peaks around 3000 cm^{-1} are saturated, as the analysis is focusing on the band near 1750 cm^{-1}. Save all the spectra you collect, and title them with their cyclohexanone concentration. The mixture spectra will look approximately like the spectrum below.
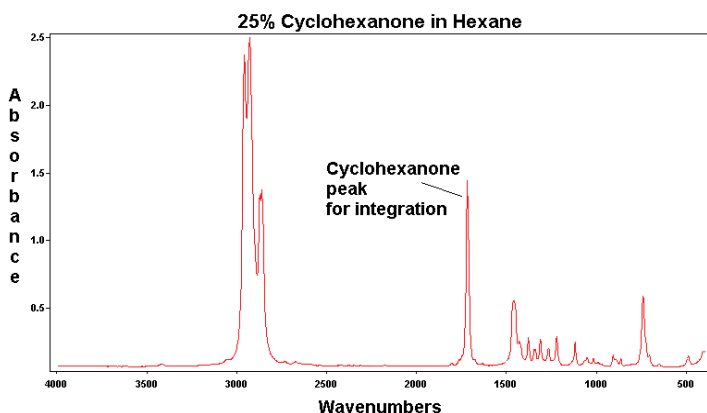
Measure and record the area and heights of the carbonyl peak in each sample. These measurements will be used as the y-values in your linear concentration calibration curve. Be sure to correct for baseline drifts – starting and ending baseline points for the area of integration should be selected at approximately 1680 cm^{-1} to 1760 cm^{-1}. Choose the same baseline points for every standard to obtain the best results.

Using the peak areas as your y-values and the percent by volume cyclohexanone concentrations as your x-values, calculate the best fit line of the data using your graphing calculator or spreadsheet package. Your data will look something like the % Cyclohexanone figure below.

Evaluate the fit of the generated equation. A good calibration for a linear system like cyclohexanone in hexane should have a correlation coefficient of 0.95 or higher. If this is not the case, the standards may need to be remeasured, additional standards may be added, or duplicates of the standards may be measured and added to the best fit calculation.

When you have achieved a satisfactory correlation coefficient, measure the spectra of the two unknowns which will be provided to you. Integrate the peak area of the carbonyl peak as above and record the Abs values. Calculate the percent cyclohexanone in each unknown.

Your final results should include the table of values used for the best fit line calculation, the % Cyclohexanone graph, and the unknown measured quantitation results.

Prepare a similar calibration using the peak heights, with baseline correct. How does this calibration compare to the previous one? Which might be better, and why?
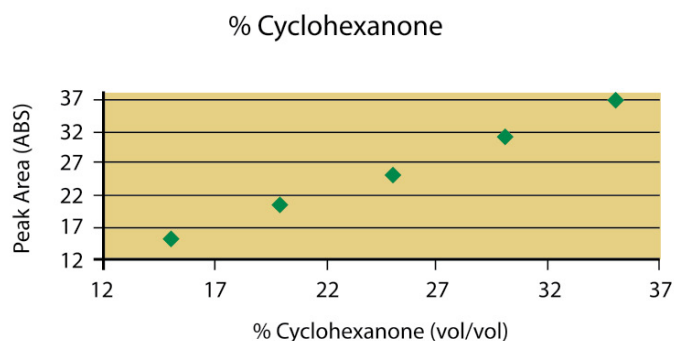
% CYCLO	ABS
15	15.06
20	20.56
25	25.34
30	31.15
35	37.07
CorrCoeff	0.99929
Slope	1.0922
Intercept	-1.469

Write the equation for the calibration equation as:
Abs = (Slope)(% Cyclo) + Intercept

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