QUANTITATIVE INFRARED SPECTROSCOPY

Objective:

The objectives of this experiment are:

(1) to learn proper sample handling procedures for acquiring infrared spectra.

(2) to determine the percentage composition of a liquid sample mixture by the application of Beer's law.

Text Reference:


Introduction:

Infrared spectroscopy is most often used for qualitative identification. An unknown material can be determined by comparing the infrared spectrum acquired on this sample to the spectra of known compounds. For a conclusive identification, all features of both spectra that are more intense than instrumental noise must match. Alternatively, IR spectral lines may be interpreted to provide clues to the structure of an unknown. This approach is rarely conclusive, but must be supplemented with other types of evidence.

The infrared spectrum is rich in structural information. The spectral lines are produced by the absorption of incident radiation by the vibrational modes of functional groups in the molecule. The absorptions adhere to Beer's law. Thus, analysis of the infrared spectral band intensities as a function of solute concentration provides a straightforward means for determining the concentration mixture components.

IR cells used for analytical purposes are generally fabricated from NaCl and accordingly require very special treatment to avoid damage. Cells must be kept in a desiccator except when actually in use and when in use must be protected from the operator's hands. The most effective protection is to wear gloves when handling the salt windows. Samples introduced into cells must be rigorously dried. Solvents may be stored over drying agents; special care must be taken to avoid disturbing this material, otherwise solutions will be contaminated by fine particles. Bottles should be opened only while samples are being withdrawn. Syringes and other glassware must be dried before use.

Fixed cells are cleaned after use with chloroform or carbon tetrachloride. They must be dried by drawing dry air or nitrogen through to evaporate the solvent. Note that if
moisture is present, the evaporation of the cleaning solvent may cause the temperature inside of the cell to drop below the dew point. If this occurs, water will condense on the inside of the cell and damage the salt window.

**Experimental:**

1. Ask the instructor to show you the procedure for initiating the FTIR and collecting a spectrum. FTIR spectroscopy is a single beam technique. The acquired spectral information must be stored in separate memory locations and manipulated (added, subtracted, ratioed, etc.) post-run. An IR spectrum of the gases in the sample compartment (air!) must be initially stored in the "BACKGROUND" memory location. Acquire a "BACKGROUND" by selecting 'Collect Background' under the Collect menu and add to Window 1. Plot the background spectrum by selecting 'Print' under the File menu and responding 'OK'. The computer will subsequently subtract the absorption lines from any data in "SAMPLE" or "REFERENCE" memory location before displaying it on the monitor.

   Note that the instrument may be set to display the spectral data in % T. If this is the case, select 'Absorbance' under the Process menu.

2. After cleaning and drying the fixed cell(s), record the spectrophotometer response from 4000-600 cm\(^{-1}\) with only dry air in the cell. If the cell is reasonably transparent in the IR spectral region, an interference pattern will be seen. The true path length can be calculated from this pattern. Expand the spectrum until you can easily count the number of peaks present and then plot it out. The spectrum can be expanded by using the 'spectrum selection tool' \([\text{ô}]\) to draw a box around the area and then clicking inside the box and then selecting 'Full Scale' under the View menu. The limits will be displayed on the reduced spectra at the bottom of the window. To label the peaks select 'Find Peaks' under the Analyze menu. Print this spectra using the same procedure as indicated above.

3. Check the wavelength calibration by inserting the polystyrene calibrating film into the sample beam and record the spectrum from 4000-600 cm\(^{-1}\). A sharp, intense peak should be at 2924 cm\(^{-1}\). Expand the scale around the most intense peak. Use the 'annotation tool' \([\text{T}]\) to find the absorbance and wavenumber of the most intense peak and record this information.

4. Fill the fixed liquid cell with solution 1 and record the spectrum from 4000-600 cm\(^{-1}\). Take special care when handling the syringes. They are delicate and expensive laboratory items. Obtain a plot of the spectrum with the peaks labelled using the procedure detailed in part 2. Conduct a library search to determine the identity of the solution. Under the Analyze menu, choose 'Search Setup'. Add the libraries you wish to use and press 'OK'. Then choose 'Search'. Obtain a printout of the search window and an overlayed spectra of your solution and the best match obtained. Repeat this completely for solutions 2 and 3.
5. Thoroughly rinse the cell and then fill it with solution 2. Record the spectrum, obtain a hardcopy with the peaks labelled on the plot and conduct a library search.

6. Thoroughly rinse the cell with solution 3. Fill the fixed cell with solution 3 and record the spectrum. Obtain a hardcopy of the spectrum with the peaks labelled on the plot and conduct a library search.

7. Inspect each of the spectra obtained in parts 4 through 6. Select the peak or peaks which would be most useful in the quantitative analysis of a mixture containing solutions 1, 2 and 3.

8. Obtain a liquid unknown mixture from your instructor.

9. Prepare accurately the following ternary standards (by % volume):

<table>
<thead>
<tr>
<th>solution</th>
<th>% #1</th>
<th>% #2</th>
<th>% #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>D</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>70</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

Prepare these standard solutions using the three burets and five vials. Note: you need not prepare standard A which is exactly 15 % solution 1, 50 % solution 2 and 35 % solution 3. Rather, prepare a standard which is about the concentration but know precisely what volume of each liquid you have delivered to the flask.

10. Record the spectrum and obtain a hardcopy of the spectrum for each of the standard solutions prepared in part 9. Label the peaks and use the 'annotation tool' as necessary. You may save your data to disk to avoid repeating experiments. Obtain an estimate of the uncertainty in the absorbance readings by recording the spectrum of one the standard mixtures in triplicate. Use the cursor to find the absorbances of each of the key peaks you have selected for quantitation. You may choose to plot the section of your spectrum containing the key peaks. For ease of analysis, once you have identified the "section", keep the x-axis and y-axis limits constant.

11. Record the spectrum of your liquid unknown mixture in triplicate.

12. Open the cell door, remove the cell and record a background spectrum with the door open. Compare this spectrum to that obtained in part 1. Obtain a printout of this spectrum as well.
Reporting the Results:

The report should consist of the assembled printouts (appropriately labelled). In addition to an assessment of the precision in the measurements and an analysis of sources of error in this experiment, the discussion section should contain answers to the following questions:

1. Identify the major peaks in the background spectrum. What differences did you find between the first background spectrum and the one acquired with the door open? What causes the differences (if any) that you observed? Explain the function(s) of the gas scrubbers mounted on the back wall.

2. Calculate the thickness of the fixed cell. Note that in addition to being a method for measurement of cell window spacing, the observation of fringes provides a check of smoothness and parallel orientation of the cell windows. The windows must be in reasonably good shape to give fringes. The equation for an interference pattern is given by:

\[ 2t = \frac{m}{n} (\nu_1 - \nu_2) \]

where 
- \( t \) = path length 
- \( m \) = # of peaks in the wavenumber interval 
- \( n \) = the refractive index of the sample 
- \( \nu \) = wavenumber

3. Identify each solution and compare the sample with the library spectra. Discuss the validity of your identification based on similarities in the spectra.

4. Describe the criterion you used in selecting the particular peaks you used for quantitation. Also identify the major functional groups of your standards. Neatly draw a straight line across the bottom of the peak(s) whose absorbance is to be measured in the quantitative analysis of your unknown liquid mixture. The corrected absorbance can be measured by the difference in the absorbances of the baseline from that of the peak at the same wavelength (if all spectra have the same y-axis). Tabulate the absorbance data.

5. Plot the corrected absorbance as a function of percent volume for each of the components of your liquid unknown mixture. Using this plot, determine the percent composition (and uncertainty) of your mixture.

6. The discussion section of your report should contain comments on whether the plots drawn in #3 above show conformity to Beer's law. Explain any nonconformity. The discussion section should also include reasons for your selection of wavelengths. What other peaks could have been suitable for the analysis of the mixture?
Grade Breakdown:

- **Introduction:** 20 points
  A discussion or the theory of IR, how IR spectrometers work, the meaning of “fourier transform” and the quantitative use of IR. Please include diagrams.

- **Procedure:** 5 points
  Deviations from the manual.

- **Tables & Plots:** 15 points
  Neatly and correctly labeled plots of absorbance vs. % volume of each of the three standards.

- **Spectra Interpretation:** 10 points
  Includes correct labeling of the major functional groups in each of the three standards, and a reasonable choice of which peak to use in calibration (peak must be on scale and must increase linearly with increasing concentration of the analyte to be useful in describing that analyte).

- **Quantitative Accuracy:** 20 points
  Includes your correct identification of the three standard compounds and a reported assessment of the unknown composition that is within 10% of the actual value.

- **Discussion:** 30 points
  Should include a discussion of the questions in the “Reporting the results” section. The questions should not be answered by number, but rather woven into the overall discussion.