UV-VIS Absorption Spectroscopy

Objectives

The objective of this experiment is to gain hands-on experience with

(1) calibration of the UV-VIS instrument,

(2) use of multichannel absorption spectrophotometers, and

(3) derivative spectroscopy in chemical analysis.

Background

Absorption of light by solution is one of the oldest and still one of the more useful instrumental methods. The wavelength of light that a compound will absorb is characteristic of its chemical structure. Specific regions of the electromagnetic spectrum are absorbed by exciting specific types of molecular and atomic motion to higher energy levels. Absorption of microwave radiation is generally due to excitation of molecular rotational motion. Infrared absorption is associated with vibrational motions of molecules. Absorption of visible and ultraviolet (UV) radiation is associated with excitation of electrons, in both atoms and molecules, to higher energy states. All molecules will undergo electronic excitation following absorption of light, but for most molecules very high energy radiation (in the vacuum ultraviolet, <200 nm) is required. For molecules containing conjugated electron systems however, light in the UV-visible region is adequate (e.g., benzene absorbs in the 260 nm region). As the degree of conjugation increases, the spectrum shifts to lower energy. Thus naphthalene absorbs light up to 300 nm, and anthracene absorbs to about 400 nm. Because absorption spectra are characteristic of molecular structure, they can be used to qualitatively identify atomic and molecular species.

The amount of light, I, transmitted through a solution of an absorbing chemical in a transparent solvent can be related to its concentration by Beers Law:

$$-\log \frac{I}{I_0} = A = \varepsilon_\lambda bc \tag{1}$$

where I_0 is the incident light intensity, A is the absorbance (a defined quantity, also referred to as the optical density, or OD), b is the cell path length in cm, c is the solution concentration in moles/liter, and ε_λ is the molar absorptivity, (also referred to as the molar extinction coefficient) which has units of liter/mole/cm (i.e., A is a unitless quantity). Notice that ε_λ is a function of wavelength, and it is the quantity which represents the spectrum of the solution. When its value is stated, it must be stated for a particular wavelength (e.g. ε_{532}). The only exception to this is when its value at the peak of the spectrum is given, in which case it may be denoted as ε_{max}. Thus absorption spectroscopy can be used to quantify the amount of chemical present in an unknown solution.

Instrumentation

Currently, research grade UV-VIS absorption instruments come in two configurations. The first is called a scanning spectrophotometer because it measures the intensity of transmitted light of a narrow bandpass, and scans the wavelength in time in order to collect a spectrum. Because absorption is a ratiometric measurement, these instruments generally require the user to measure two spectra, one sample and one blank. The blank should be identical to the sample in every way except that the absorbing species of interest is not present. This can be done either consecutively with a single beam instrument followed by the ratio calculation, or simultaneously with a dual beam instrument. The dual beam method is faster, and has the added advantage that lamp drift and other slow intensity fluctuations are properly accounted for in the ratio calculation. Collecting spectra with scanning spectrophotometers is slow, but the instruments often have very high resolving power owing to the use of photomultiplier tube detectors, which can be used with very narrow slit widths.

A photodiode array is a 1 or 2 dimensional stack of individual photodiode detectors, each of which makes an independent measurement of the incident light intensity at its particular location. Typically, 2-dimensional arrays are used for electronic imaging applications, for instance the photodetector of a camcorder. One dimensional arrays are often used in spectroscopic instrumentation. If the array is placed at the focal plane of a monochromator, then the position of each photodiode will be associated with a specific bandwidth of light. In the case of the diode array spectrograph that we will use for the UV-Vis experiment (a Hewlett Packard model HP 8453) each of 1024 diodes in the linear array is associated with a 1 nm band of light spanning from 190 nm to 1000 nm. The individual diodes are separated by about 25 microns, and the physical slit width matches this spacing. Thus the maximum resolution of the instrument is 1 nm. This is not as good as the typical resolution of a scanning instrument (resolutions of about 0.2 nm are not uncommon with scanning instruments), but for many applications (especially molecular solution spectroscopy where absorption bands are typically very broad) 1 nm is adequate. The real advantage of the instrument is its speed. A single spectrum can be taken in about 0.1 seconds.

Preliminary Preparations

Before turn the instrument so that the light sources have a chance to warm up and stabilize. Next prepare or gather all of the samples which you will need to perform the experiment.

Solutions which students need to prepare are marked with *.

*1. a) Use 50 ml volumetric flasks to prepare 4 solutions containing 0.04, 0.03, 0.02, and 0.01 g/l of K_2CrO_4 in 0.05 N KOH prepared from stock solutions provided. (0.1 g/l K_2CrO_4 and 0.5 N KOH)
b) A 0.05 N KOH blank

2. a) A sample of phenol in turbid water
b) A water blank

*3. a) Solutions containing 25, 50, and 75 ppm of phenol in water from a stock solution of 250 ppm phenol in water
b) A water blank

Experiments

I. Calibration of a UV-Vis Spectrometer

A. Wavelength Calibration

Solutions of rare earth ions exhibit very narrow absorption bands, the wavelengths of which are well known. Holmium has many peaks well spaced in wavelength which provide convenient calibration points. You will calibrate the wavelength of the HP 8453 with a sample of Holmium oxide immobilized in a polymer block.

Measure the spectrum of the solution using the HP UV/Vis Photodiode Array Spectrometer. Check the apparent wavelength of the absorption peaks which should be at 287.0, 361.1, 450.8, 537.0, and 640.4 nm. Use the Peak/Spectrum function to annotate each peak, then print the entire spectrum and the wavelength report to determine the exact wavelength maxima.

B. Photometric Calibration

Basic potassium chromate is recommended as a photometric standard by the National Bureau of Standards. You have been given a solution containing 0.1 g/l of K_2CrO_4 and a solution 0.5 N in KOH, from which you prepared solutions containing 0.04, 0.03, 0.02 and 0.01 g/l K_2CrO_4 in 0.05 N KOH. Measure the spectra of these solutions on the HP 8453 and read the absorbance at 370 nm. Use FIXED WAVELENGTH under METHOD then specify the wavelength. To save time and paper, all the spectra can be overlaid then printed. *Be sure to correct the wavelength as needed according to the results in part I.A*. Check for linearity of response and also for proper slope in the response curve. The absorbance of your most concentrated solution should be 0.9914. Any relative deviation of over 1% from linearity or from the proper slope is cause for concern.

Calculate the molar absorptivity for K_2CrO_4 from the slope of a plot of absorbance versus concentration, and compare it with the accepted value of 4790 liter/mole/cm.

II. Derivative Spectroscopy and Quantitative Analysis

This HP 5483 performs derivative spectroscopy by measuring the actual spectrum and performing the derivative operations in the computer. It is capable of 1st through 4th derivatives, though only 1st and 2nd derivatives are used regularly. The advantages of derivative spectroscopy include: (1) Precise determination of the wavelength of peak maxima can be obtained from the zero crossing of the first derivative. (2) Improved spectral resolution is obtained, especially with the second derivative. Spectral features which appear as barely noticeable shoulders in the original spectrum become much more prominent. (3) Quantitative analysis can be performed in the presence of turbidity. Turbid solution generally show steadily increasing absorbance toward shorter wavelength, which can hinder the accurate measurement of absorbances due to the changing background level. One solution to this problem is to calculate the expected background and subtract it off. Another approach is to calculate the spectrum's second derivative. The HP8453 is capable of both methods. We will use the spectrum of benzene

to study the properties of derivative spectra and then measure the concentration of phenol in a turbid solution.

A. Phenol in turbid solution and phenol standard solutions (with water blanks)

Measure the spectrum of 25, 50, and 75 ppm phenol in water solutions and of the phenol in turbid solution. Measure, mark, and print the absorbance and the 1st and the 2nd derivative spectra of these. From the 2nd derivative spectra of the phenol in turbid solution, construct a calibration curve containing 0 (i.e., use blank as sample), 25, 50, and 75 ppm and **determine the phenol concentration in the turbid solution.**

UV-Vis Report Format

A formal report format will be used for this lab. While the reports that each student turns in should be typed, ALL OBSERVATIONS SHOULD BE RECORDED IN YOUR LAB NOTEBOOKS! **We will require you to provide photocopies of all lab pages including spectra and calibration plots with your lab report.** Make sure that your lab notebook contains the standard sections expected in a proper entry, including Title of Experiment, Date of Experiment, Purpose of Experiment, Brief Procedural Outline of Experiment in Checklist format. All instrument settings, readings, and pertinent information should be written down in the Notebook at the time the lab is performed. Sample calculations should also be performed in the lab Notebook including the calculations used for preparation of standards.

For this lab, the student should imagine that he or she is an analytical chemist working for a company, and is approached by a supervisor to use absorption spectroscopy to develop a method for the determination of the amount of some species. When the report is turned in, it should follow a format which would be suitable to hand to a supervisor for review of the process and results. This should be neatly typed, double spaced, brief and to the point. **The main purpose of this type of report is to present the results of the analysis to a customer. It must be complete enough for the customer to have confidence in the results, and to understand the limitations of the analysis.** The reports should be formatted using the following outline.

Title Page: This should include the name of the lab, the student’s name, date the lab was performed, the phenol unknown number and determined concentration of the unknown **with an uncertainty assignment**, and the date the paper is submitted for grading.

Introduction: This section should be brief and should explain the scope of the project (general purpose) as well as some of the methodology used in the lab. This should be no more than 200 words, but should be thorough enough to give some idea as to what the rest of the paper will cover. This lab covers a phenol unknown determination and some calibration experiments.

Experimental Section: This should give a more detailed description of the actual procedural steps taken during the lab. This should be in enough detail (but in the student’s own words) that

someone reading the paper would be able to exactly reproduce the experiment that YOU performed.

Results/Raw Data: This section should contain the actual raw data obtained in the analysis. Numbers should be compiled in to tables (labeled with "Table 1", "Table 2", etc), and tables should be neatly organized for someone who might read your paper (namely the one grading it). This section should not contain explanations or interpretations of the data.

Data Analysis: This is the main text of the report. This should contain any figures, graphs, calibration plots and explanations that might help the reader in interpreting the data presented in the Results/Raw Data section. This is where you will present your calibration plots and your error analysis. Any calculations required to complete the analysis should be described in detail in this section.

Questions: Answer the following questions in this section:

1. What effect would drift caused by a gradual increase in source intensity have on a measurement of absorbance versus time in a single beam spectrometer? How is this accounted for in the instruments used in this experiment?

2. Why are we unconcerned about the exact Holmium concentration in the wavelength calibration?

3. What effect would an error in cell dimensions (say 10.50 mm instead of 10.00 mm) have on the value of ε (assuming A and c are constant)?

4. Why use a rare earth ion for wavelength calibration?

5. What are the advantages and disadvantages of using derivative spectroscopy?

Discussion: This section will contain a discussion of the results including explanation of any unusual data, circumstances that might affect interpretation of the data, or comments on observations that you may consider important. It does not need to be very long. A paragraph or two will usually be enough unless you have significant problems performing that analysis.

It is not necessary to include every piece of information from the lab notebook in the report.