CHL311 Instrumental Analysis Laboratory

Qualitative UV-VIS Spectrophotometry Laboratory

Introduction

UV-VIS absorption spectrophotometry can be applied both quantitatively (such as Beer's Law analysis) and qualitatively (compound identification and purity). This lab will explore the use of the UV-VIS spectrophotometer to analyze various UV-VIS absorption organic and inorganic species. Your goal should be to gain intuition into what types of chemical systems benefit most from UV-VIS spectrophotometry and those that do not. You will also gain experience into what the various scan parameters do and why and when you should change them.

Wavelength Accuracy

This procedure does NOT need to be performed every time you use the spectrophotometer. However, it is important to realize that just because the readout from the instrument indicated that 500.00-nm light is being measured, this does not absolutely guarantee that this is the case. If wavelength accuracy is of primary concern, then you should verify the spectrophotometer's calibration by measurement of the absorption lines of holmium glass or emission lines of the deuterium (D₂) lamp. The following procedure will allow you to check the wavelength accuracy of the spectrophotometer.

Holmium Glass Method. This method allows you to verify the wavelength calibration at three different wavelengths, 460.0 nm, 360.9 nm and 279.4 nm. The performance is considered satisfactory if the wavelength errors of the holmium glass absorption lines are within +/1 0.3 nm

a) With the Holmium Glass Filter in the sample compartment, measure the 460.0 nm absorption from between 455.0 to 465.0 nm at a 0.5 nm bandwidth, 10 nm/min scan speed, and a data interval of 0.1 nm.

b) Determine the wavelength of maximum absorbance using the Peak Pick Table.

Effects of Bandwidth

1. Place one drop of benzene in the bottom of a clean dry cell and seal the cell. Do not get benzene on the walls of the cell. Put cell in the sample compartment; no reference is necessary.

2. Obtain absorbance spectra of the benzene vapor from 220 to 280 nm (30 nm/min, 0.1 nm data interval) at the following bandwidth settings: (Plot on same graph to see differences/similarities).

a) 4 nm
b) 2 nm
c) 1.0 nm
d) 0.5 nm
e) 0.2 nm
f) 0.2 nm

Cuvettes and Solvents

1. Obtain spectrum of empty quartz cuvette across the maximum wavelength range of the instrument.

2. Obtain spectrum of empty glass cuvette across the maximum wavelength range of the instrument.

3. Using the quartz cuvette, obtain spectra of the following solvents:

a) waterb) methanolc) hexane

Electronic Transitions of Organic Molecules

1. Obtain UV-VIS spectra of the following compounds using the solvent as the reference. You must prepare the sample in order that the absorbance is "on scale" (less than about 1.5). You should choose the appropriate wavelength range (which will necessitate a quick survey run on the samples before the final spectra is obtained) in order to get all of the important spectral features. Use a bandwidth of 1.0 nm unless otherwise noted. Also record the peak positions.

benzene in cyclohexane (at 0.5 nm and 1 nm bandwidth) naphthalene in cyclohexane anthracene in cyclohexane

2. Obtain a UV-VIS Spectrum of benzene in ethanol (at 0.5 nm bandwidth) Superimpose the gas phase, the cyclohexane and ethanol spectra of benzene at the same bandwidth.

Note: Many of these compounds are toxic and/or carcinogens. Do not leave bottles open or samples sitting around. Prepare solutions in the hood. Clean up spills. Dispose of organic wastes in proper containers.

Include in the Report:

1. Sketch the basic components (block diagram) of a UV-VIS scanning spectrometer.

2. For what types of molecules is UV-VIS spectroscopy most useful? For which is it not? As a consequence, what are the best solvents for UV-VIS spectroscopy?

3. What is meant by bandwidth? As you narrow the bandwidth of the UV-VIS spectrometer, what might you expect to happen to the spectrum?

4. Labeled copies of all the spectra and peak data.

5. Discuss in what situations would an accurate calibration of the UV-VIS be most important?

6. Discuss how changing the bandwidth changes the spectrum of the benzene vapor.

7. Compare the different optical material used to make cuvettes. What wavelength ranges is each material useful for?

8. Compare the spectra of the different solvents. State which of these solvents could be used in UV-VIS absorption spectroscopy.

9. Discuss the difference and similarities between the following spectra.

a) benzene in the vapor phase vs. benzene in cyclohexane (at 0.5 nm bandwidth)

b) benzene vs. naphthalene vs. anthracene (all in cyclohexane)

Do you find any correlation between molecular structure and the spectra?

10. Do you notice any differences between the gas phase and the solution spectra (cyclohexane and ethanol) of benzene. What correlation can be made?

11. How would you quantitatively describe the concentration of the organic samples you prepared? Suggest a procedure for preparing these concentrations quantitatively.