UV RECORDING SPECTROPHOTOMETRY

Basis of the Experiment

The UV spectrum of benzene is used to explore the variables and capabilities of scanning UV-Vis spectrophotometry. Solvent and substituent effects are examined. Extinction coefficients are calculated from Beer's law, and the effect of instrumental parameters on bandwidths and the extinction coefficient is studied.

Apparatus

Shimadzu 2401PC recording spectrophotometer, two 1 cm Quartz cells.

Reagents & Solutions

Reagent grade benzene, spectra-grade hexane, spectra-grade methanol, toluene, phenol, 1.0 M benzene in spectra-grade hexane solution

BENZENE IS A KNOWN CARCINOGEN. BE CAREFUL, USE IT IN THE HOOD. Since many chemicals absorb in the UV, this experiment is sensitive to contaminants in the solvent, on the cuvettes, on your glassware, etc. Remember, as always, **never** pipet out of the reagent bottle, **never** return a chemical to a reagent bottle, and dispose of waste reagents in a properly labeled container. Sign the waste manifest sheet. Wear your safety glasses.

Do not dry any of your glassware with acetone. Dilute the stock 1 M benzene in hexane solution with spectra-grade hexane to prepare a 1.0×10^{-2} M benzene solution. Use this solution to prepare 25 ml of each of the following concentrations: 3.00, 2.50, 2.00, 1.50, 1.00, and 0.500×10^{-3} M benzene in spectra-grade hexane. Also prepare a 2.00×10^{-3} M benzene in spectro-grade methanol, 2.00×10^{-3} M toluene in hexane and a 2.00×10^{-4} M phenol in hexane solutions from the stock solutions available in the lab.

Waste: **organic**

Procedure

1. Background information on UV - VIS molecular spectroscopy theory, instrumentation, and techniques may be found in Skoog and Leary - Chapter 8, or any good instrumental text. The Shimadzu 2401PC is a double beam computer controlled scanning spectrophotometer. A condensed set of operating instructions is located near the instrument. Read it carefully and check with your instructor before you begin. The first part of the experiment deals with the UV range from 300 to 220 nm so you will use the D_2 (UV) source. On this instrument the correct source is chosen automatically but on some instruments you may need to manually select the source.

2. Clean the cells; rinse with hexane and let dry. Handle cells by the fogged sides, not the clear sides. Also, always use the same cell for the reference and replace the sample cell in the same orientation after removing it to change solutions.

3. Turn on the spectrometer (switch on the front of the instrument), computer, and monitor.

4. On the Windows Desktop, double click (with the left button of the mouse) on the "Shimadzu UV-Vis" icon. Click on the connect icon on the bottom menu of the initial screen. The system will go through a series of instrument checks. Wait until this is completed (this only occurs when the instrument is turned on). All bullets should be green after the self-test has completed successfully. If one is red, please see Tom Allston.

5. When self-test finishes you will need to select the proper mode for the instrument. There are three icons on the top toolbar toward the middle. They start just to right and below the Tools drop down command. The three icons will set the instrument to Kinetics mode (not used in SCHA311), Photometric (not used in SCHA311), and Spectrum (for the UV and Fluorescence labs). Select the spectrum mode.

6. Click the large green M icon (located toward the right). This takes you to your method and in the dialog box there are four tabs that will allow you to set up the experimental parameters. The following table summarizes the parameters to be set under each tab. When all the parameters have been entered, close this box.

Spectrometer Parameters Available Under The Large Green M.		
Tab	**Parameter**	**Values**
Measurement	Wavelength Range	300 to 220 nm
	Scan Rate	Medium
	Sampling Interval	Auto
	Scan Mode	Single
	Report File Name	AutoPrint Report 1
Sample Preparation	Enter your sample's characteristics	
Instrument Parameters	Measurement Mode	Absorbance
	Slit Width	0.1 nm Not the true slit width, but the spectral bandwidth.
Attachments	None	

7. With empty cuvettes in the instrument. Click on "Autozero" to zero the instrument at the starting wavelength. Click on "Baseline" to zero the baseline over the spectrum region of interest. The wavelength window in the lower left corner will display the scanning wavelength. Wait until the wavelength returns to the original value.

8. Remove the sample cell and place a drop of benzene in the quartz cell and then recap it. Place this cell in the sample compartment an empty cell in the reference compartment. Record the vapor phase spectrum from 300 to 220 nm by clicking on "Start". *You will note the appearance of a broad envelope of several narrow peaks. What is the cause of the multiple maxima?*

9. When the scan is completed, the File Name Dialog box will appear. In the box adjacent to "Enter File Name", accept the default name or type in your own file name. A comment may, and should be in good laboratory practice, be typed in the "Enter Comment" box (press tab to move to this box).

10. Select "OK" to save the data. If "Cancel" is selected, the data will be erased.

11. Right click in the plot window and select customize to adjust the display options.

12. Run the "Peak Pick " icon in the top menu bar. This will open a window with the results of the peak pick. (You might need to copy this data to the clipboard so that it may be included with the printout.) To print your spectrum, click on "File", then "Print". You will repeat steps 6 to 11 for all samples in your experiment.

13. Rinse the cell well with hexane to remove the benzene and place both cell filled with hexane in both beams. Depress the AUTO ZERO.

14. Run spectra of the benzene in hexane solutions at slit = 0.2 nm and for your highest concentration solution again at 5 nm. (Note: the remaining spectra for this experiment involve filling the cuvette with the solution, not just adding a drop of solution, as was done above). Change the ordinate maximum if necessary. Use hexane as the reference. Carry out a peak pick to determine the absorbance of the various peaks. Construct a Beer's law plot of the data and calculate ε_{max} for several bands. Compare your results with accepted literature values with respect to peak location and molar absorptivity. *What type of transition causes this absorption? What is the reason for the multiple maxima? Also discuss the difference between the spectra at the two different slit widths.*

15. Run spectra of the toluene and phenol solutions using the appropriate reference solvents. Change the ordinate max and dilute the solutions if necessary. Use 0.2 nm slit.

16. Run a scan of the benzene in methanol solution using methanol as the reference. Use 0.2 nm slit.

17. Determine the wavelength calibration by using a holmium oxide glass filter or a lamp that emits line spectra. For Holmium oxide collect the spectrum from 700 nm to 320 nm. Use 0.5 nm slit. Next observe the effect on slit with on the peak at about 361 nm by scanning from 385 nm to 345 nm at all the available slit widths. Do the scan five times at 0.1 nm slit and calculate the wavelength and absorbance precision and accuracy.

18. To turn off the spectrometer, click on “File”, and then “Exit”. Shut down Windows 98, then turn off the computer, monitor, and spectrometer. Remove your sample.

Report

Figures.

1. Prepare Beer's law plots (absorbance *vs.* concentration) for the four most prominent peaks from the benzene solutions in hexane for the slit width of 0.2 nm.

Calculations.

1. Determine the molar absorptivity for the four peaks you obtained with benzene in hexane using 0.2 nm slit width. *Why do the four peaks have different molar absorptivities?* Compare your results with accepted literature values with respect to peak location (λ) and molar absorptivity (ε).

Questions for your write-up (Discussion).

1. The questions for this write-up are interspersed in italics in the procedure section of the handout.
2. Discuss any differences you observe when you took spectra of compounds closely related to benzene. Include these structures in your report. Also discuss the solvent effect you observed.

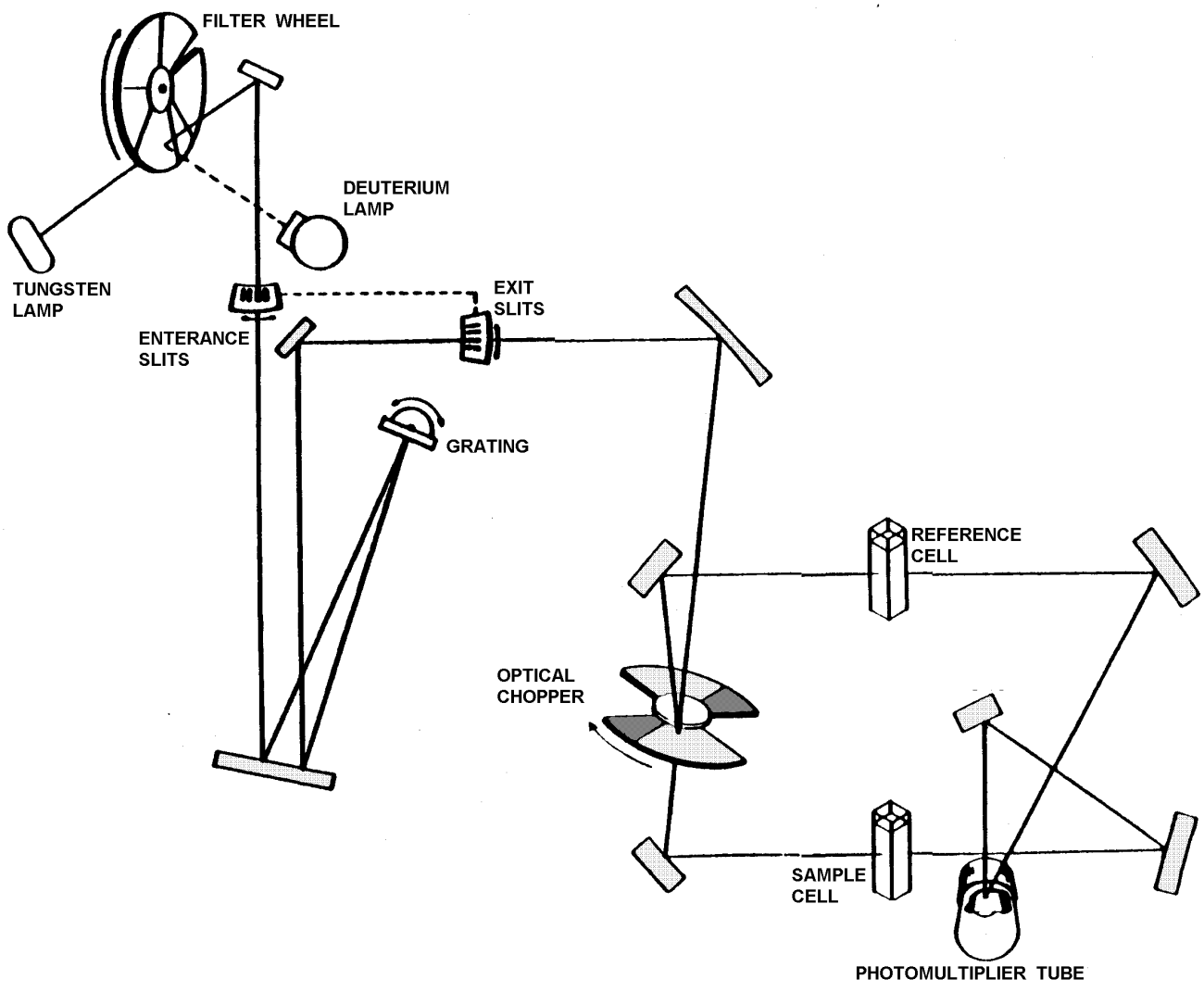

Figure 1. Light Path in a Typical Recording UV-Vis Spectrophotometer.