

## Molecular Spectroscopy

### Before lab:

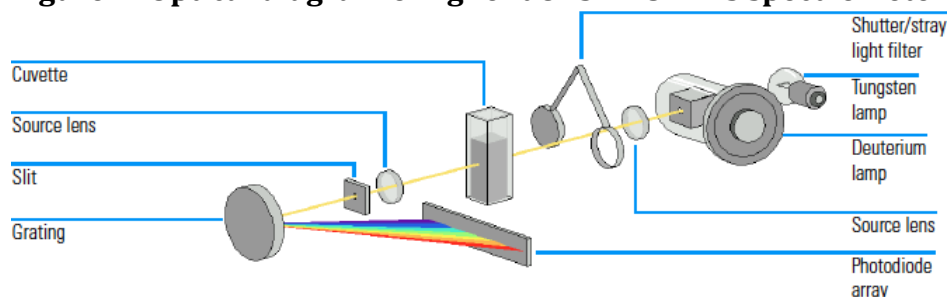
1. Diagram a conventional UV-Vis, Diagram a photodiode array. Explain what function each piece of the spectrophotometer. Compare and contrast the two instruments in terms of resolution, throughput, and speed of analysis.
2. Draw a Jablonski diagram of the transitions pictured in the benzene absorption and fluorescence spectrum.

### Introduction:

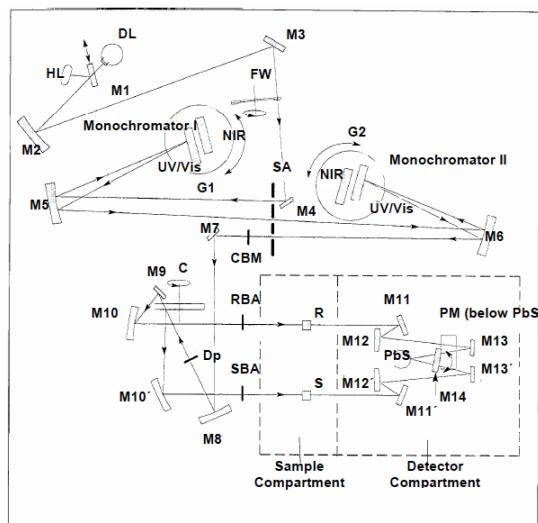
The purpose of this experiment is to compare the molecular spectroscopy instruments available in the department by examining the UV-Vis absorption and fluorescence of benzene in hexane.

Below are the instrument diagrams for several instruments. Use these to help compare and contrasting data from these instruments.

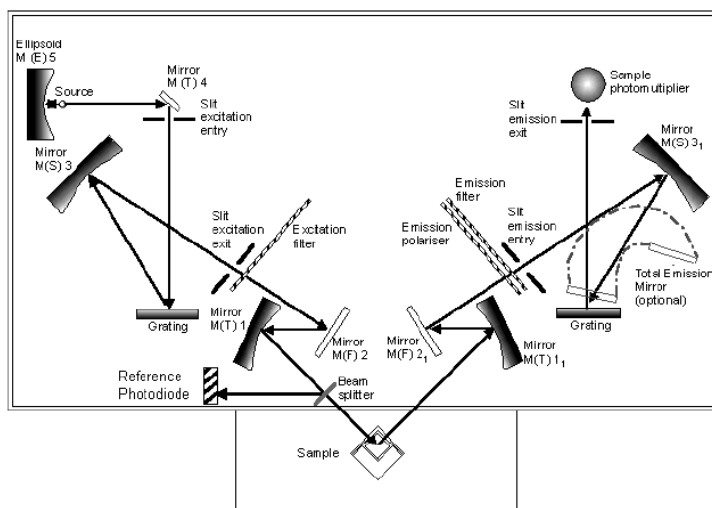
**Figure 1: Optical diagram of Agilent 8452A UV-Vis spectrometer**



**Figure 2: Optical Diagram of Perkin-Elmer Lambda 900 UV-Vis spectrometer**

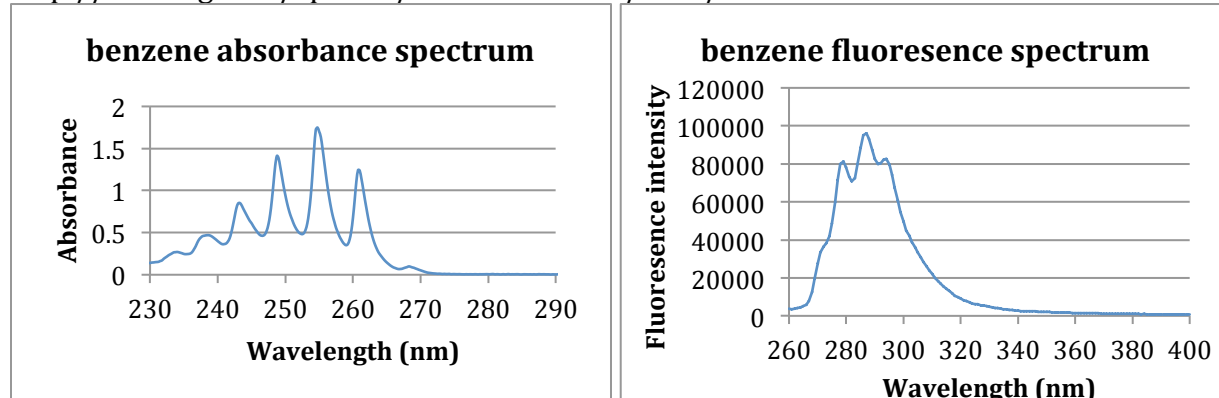


**Figure 3: Optical Diagram of Perkin-Elmer LS50B Luminescence spectrometer**



**Figure 4: Absorption and Emission Spectra of Benzene. Spectra from:**

<http://omlc.ogi.edu/spectra/PhotochemCAD/html/042.html>

**Procedure:**

In this lab, you will be collecting data on benzene in hexane on three different spectrophotometers. **Please review the MSDS for these materials available on the website- they are toxic.**

**Hewlett-Packard 8452A UV-Vis spectrometer (REIC 245)**

1. Collect absorbance spectra from 200-500nm on the bench top UV-Vis for blank (hexane) and sample (benzene in hexane).

Save the spectra

2. Click on the spectrum you want to save (diamonds will appear on spectra selected). File → Export selected spectrum → csv format → name file.csv → save. Wait until the "Exporting data..." message on the bottom of the window goes away.
3. Open files to confirm data saved. Then email them to yourselves.

**Lamda 900 UV-Vis spectrometer (REIC 139)**

1. Turn on instrument. Power switch located on top back, right corner.
2. Turn on and log into computer. Account: uvuser; password: uvuser
3. Open Lamda 900
4. In WP tab click on CHEM212  
Open method CHEM212; scans 200-400 nm; slit 0.5
5. Check method parms.
6. Click start
7. Instrument will prompt you for samples
8. Export data AFTER EACH SAMPLE! Data handling → list. Then: file → copy to clipboard
9. Paste data into excel file that will be posted to the website.

**Repeat scans for 2.5 um slits.**

10. Go to instrument tab, change slit width
11. Click setup (you may be able to hear the motor changing the slits in the instrument)
12. Scan and save.

**Perkin-Elmer LS50B Fluorimeter (REIC 139)**

- open FL WinLab from desktop or start menu
- application → scan
- Begin with an emission scan:  

Start: 260nm	End: 500nm	Excitation: 256 nm
Ex slit: 2.5nm	Em slit: 2.5nm	Scan speed: 500 nm/min

 Filename: short!
- click on traffic light to start scan
  
- Now do a slow emission scan:  

Start: 260nm	End: 500nm	Excitation: 256 nm
Ex slit: 2.5nm	Em slit: 2.5nm	Scan speed: 20 nm/min

 Filename: short!
  
- Next do an excitation scan:  

Start: 200nm	End: 260nm	Emission: 285 nm
Ex slit: 2.5nm	Em slit: 2.5nm	Scan speed: 20 nm/min

 Filename: short!
  
- Save spectra in FL WinLab window: file → open, select your file.
- You data opens in a "graph 1" window. File → save as → select your file as a .txt in ASCII format
- Data is at c:\ FL WinLab\ Data\2013\
- Paste data into excel file that will be posted to the website.

**Calculations and Discussion Questions:**

Absorption spectra, needed for the lab write-up, will be available on the webpage if needed.

Figures and food for results (observations) and discussion (interpretations)

- Overlay benzene absorption spectra from two UV-Vis instruments (three spectra).  
 What differences can you **observe** between the spectra?  
 Interpret these differences within the context of the optical components of the instruments. Throughput, bandwidth of light interacting with the sample, etc.
- Plot fluorescence excitation and emission scans  
 What observations can you make that might explain the shapes of these curves?  
 Compare the spectra with the spectrum from the introduction. How can you interpret these differences (again, consider the optical components of the instrument).
- Overlay the 500 nm/min and 20 nm/min  
 Estimate the improvement in the S/N from the 500 nm/min relative to the 20 nm/min scan  
 What is traded for improving the S/N?

**Lab write-up: Due Monday**

See example on course website.

1. **Abstract:** Background, purpose, method, results, what it means! A 250-word trash can!
2. **Introduction:** Compare and contrast molecular spectroscopy instruments you are using.
3. **Results:** Graph your results, and briefly discuss.
4. **Discussion:** respond to the questions above in paragraph form (not Q1: answer, answer). Devote 1-2 paragraphs to discuss each question.
5. **Conclusion:** Write a short paragraph concluding what was learned from the experiment.
6. **Group dynamics:** let me know how you feel your group worked together.
7. **Appendix 1- Calculations:** You can send me your spreadsheet.