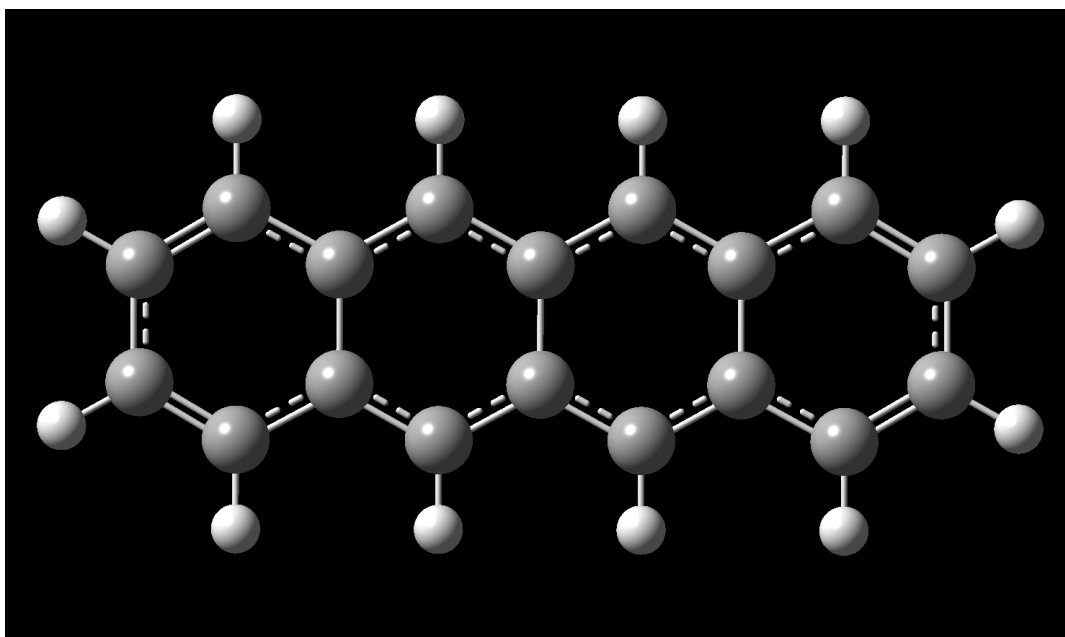# Chemistry 357

Spring 2021

LAB D

Electronic Absorption and Fluorescence of Polycyclic Aromatics in Solution

TA: Alec Beaton

Pre-Lab Activities

1. Read this entire document and review the Electronic Absorption and Emission Spectroscopy Overview document posted on Blackboard under "Lab Procedures"

2. Review and practice the exercises contained in the Computational Chemistry Lab Training Handout posted on the CHE357 Blackboard site under "Lab Procedures".

3. Watch the "Fluorescence Spectroscopy Tutorial - Basics of Fluorescence" video: https://youtu.be/5Q0n27pnQQU

4. Watch the "Molecular Probes Tutorial Series— Anatomy of Fluorescence Spectra" video: https://youtu.be/oVxpaUfTuXI

5. Watch the "Quantum Chemistry 3.8 - UV-Vis Spectra of Polyenes" video: https://youtu.be/WRYSVj-FD48

Main Learning Objectives

➤ Demonstration of the particle-in-a-box quantum model

➤ Investigate the fate of absorbed photons – emission spectra

➤ Interpret combined electronic and vibrational transitions (vibronic transitions)

➤ Use ab initio simulations to predict complex vibronic spectra of a molecule

Graded Material

Your grade in each lab is based on your completion of the Post-Lab work and questions that are detailed at the end of each lab procedure.

Text-based questions need to be answered in a narrative style using complete sentences and full explanations. You will be evaluated on accuracy, clarity, and completeness. Keep in mind that adding more text does not necessarily mean you are providing a better answer.

Number-based questions need to be answered numerically with appropriate units but must also include a clear statement or justification as to how the numbers were determined.

Graphs or plots submitted for grading must be easy to read, have properly labeled axes with units, an overall title, and a legend defining multiple traces. All graphs/plots must include a brief descriptive caption of the represented information.

Tables must be logically organized with column and row labels as appropriate. Units must be specified. All tables must include a brief descriptive caption of the represented information.

Images or figures must clearly and concisely convey information to the reader. Simple figures with clear labeling are most effective. All figures must include a brief descriptive caption of the represented information.

You need to demonstrate in your completed lab reports that you understand the answers you are providing to the questions being posed.

ATTENTION: The written lab procedures serve as guides for lab activities and associated lab reports, but you must interact with the Teaching Assistants to get the most out of each lab. There will always be aspects of lab work that are best conveyed through examples and techniques presented to you by your instructors on a one-on-one basis. Please engage with them early in your work to avoid potential frustration!

ABSTRACT

In this laboratory, you will be measuring the electronic absorption and emission (fluorescence) spectra of anthracene and tetracene in the visible/ultraviolet region of the electromagnetic spectrum. These experimental results will then be interpreted using advanced ab initio methods for simulating the ground and excited state properties of molecules.

INTRODUCTION

Electronic absorption spectroscopy is the oldest type of light-matter interaction to be recognized and is utilized in numerous scientific fields but is also encountered in everyday life. The human perception of visible colors (red, green, etc.) is a natural example of electronic spectroscopy in which our eyes detect the colors that are *not* being absorbed (are being reflected) by an object after it is illuminated with white light (containing all colors).

Electronic spectroscopy involves the excitation of an electron in the ground (stable) state configuration to an excited (metastable) state configuration. This is represented in **Figure 1** as the blue vertical arrows that connect the ground state (E = 1) to the higher energy excited state (E = 2). A pure electronic transition would represent a transition from E=1, v=0 to E=2, v=0. The other blue arrows, showing photon absorptions ending in E=2, but with v=1 and v=2, represent **vibronic** transitions. Vibronic transitions involve simultaneous electronic and vibrational transitions and the term is equally applicable to emission spectra too.

Electronic emission spectroscopy is a process that is subsequent to electronic absorption. After a photon is absorbed and an electron is promoted from a ground state orbital to an excited state orbital, the molecule is in an unstable electronic configuration (sometimes referred to as metastable) that exists for only a few nanoseconds before the excited molecule de-excites by releasing a new photon and returning to the stable ground state configuration. This process is represented as red arrows in **Figure 1**. If the entire absorption and emission process involves only singlet electronic states (those with spin-paired electrons and the most common), then the emission is termed **fluorescence**. If a triplet electronic state (those with spin-unpaired electrons, relatively rare) is involved at any stage, then the emission is termed **phosphorescence,** and the release of photons occurs much more slowly (glow-in-the-dark materials and toys rely on this phenomenon).

The pattern of spectral peaks observed in vibronic spectra is determined by the **Franck-Condon principle** which links the intensity of the vibronic transitions to the degree of overlap of the vibrational wave functions in the involved electronic states. This wave function overlap is

directly related to the differences in the potential energy surfaces of the molecule in the two electronic states. Therefore, it provides useful insights into the excited state structures and reactivities of molecules. A good description of the Franck-Condon principle can be found here: https://en.wikipedia.org/wiki/Franck-Condon_principle.

Being familiar with this principle is important since you will be using the Gaussian software package to perform a Franck-Condon spectral analysis for anthracene and then comparing the results with your own measurements. Ab initio molecular modeling can be applied to calculate the structures and vibrational motions of a molecule in both the ground and electronically excited states. These models are not perfect (as you will see), but they do a remarkably fine job at producing very good approximations to the experimental measurements.
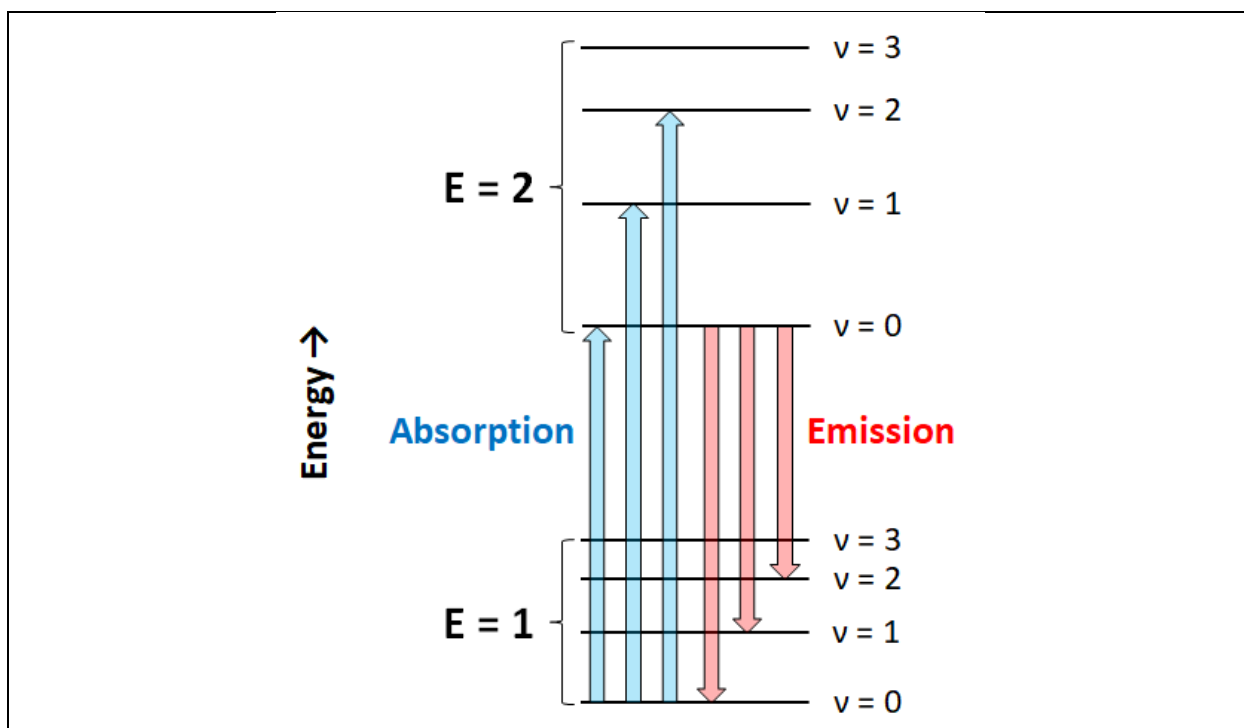
Figure 1. A schematic diagram (similar to a Jablonski diagram) showing two electronic energy levels (E = 1 and E = 2) and the vibrational energy levels (v = 0, v = 1, etc.) that are built on top of each electronic level. The blue arrows represent photon absorption, and the red arrows represent photon emission. Note that each transition connects specific electronic and vibrational levels. Also note that the energy level separations are only representative and not drawn to scale (electronic levels are *much* further apart than vibrational levels).

In addition to using ab initio simulation software, you will use the one-dimensional (1D) particle-in-a-box (PIB) quantum model to interpret some of your experimental results. The form of this equation, which gives the energies associated with each of the quantized energy levels, is as follows:

$$E_n = \frac{n^2 h^2}{8ma^2}$$

where E_n is the energy of the nth energy level, h is Planck's constant, m is the mass of the particle, and a is the length of the 1D box.

You will be using the PIB equation to interpret the results of the absorption spectra for anthracene and tetracene in solution with hexane. Anthracene and tetracene are polycyclic aromatic hydrocarbons (PAHs) which are known environmental pollutants. Their extensive system of π-bonds leads to interesting chemistry both in their ground and electronically excited states.

PROCEDURE

The procedure that follows is approximate and you will need to consult with the Teaching Assistant for any specific instructions regarding sample preparation and use.

Part I. Prediction of Spectral Trends using the Particle-in-a-Box Quantum Model

1. To use the PIB quantum model, the length (a) of the box (or the molecule) needs to be known. We can estimate molecule length by using Gaussian simulations.

2. Use GaussView to build an anthracene molecule (found in "Builder: Ring Fragment" and is in the first row of choices). Use "Tools: Point Group" (with loose tolerance) to make sure the molecule is in the high-symmetry D2h point group.

3. Run a geometry **optimization** of the structure of anthracene. For use with the PIB model, high-quality simulations of molecule structures are not necessary. When configuring the Gaussian Calculation Setup, under "Method", the default "Ground State" is right, but change the adjacent settings to "Mechanics" with "UFF". Mechanics and UFF (Universal Force Field) is a very fast (seconds) method of using classical physics (not quantum mechanics) to approximate molecular structures. It works reasonably well for simple systems, such as those you are studying in this lab.

4. Use the optimized molecule structure to estimate the length of the molecule by using the Inquire tool to select two carbon atoms from opposite ends of the molecule.

5. Repeat the Steps 2-4 for tetracene (also a D2h molecule).

6. Use the PIB equation to calculate the first two PIB energy levels (n =1, n =2) for both anthracene and tetracene. Then calculate the difference in energy (transition energy)

between the two lowest states for each molecule. Convert the transition energies to wavelengths so it is easily comparable to electronic spectroscopy measurements.

Part IIA. Collecting Absorbance Spectrum of Anthracene

1. Insert sample into Cary 60 UV-Vis spectrometer

2. Go to Setup…

3. Specify Start 550.0 nm and Stop 300.0 nm

4. Select "Slowest" under Scan Controls

5. Select OK to exit the Setup menu

6. Press the "Start" button at the top of the screen, and enter a name for your file at the prompt and specify its save location (to make your life easier, highlight and Ctrl+C to be able to easily use this same file name in later steps)

7. A prompt will appear, asking you to provide a sample name – Ctrl+V to paste the name from the previous step, then click OK (approx. 5 min)

8. Go to File… then Save Data as… Batch (you may also use Ctrl+V here, to keep file names consistent)

9. Move file over to a flash drive

Part IIB. Collecting Fluorescence Spectrum of Anthracene

1. Insert sample into Cary Eclipse Fluorescence Spectrophotometer

2. Go to Setup…

3. Specify Excitation slit width of 5 nm, and Emission slit width of 5 nm

4. Go to Prescan…

5. Select all three options, and specify Solvent as 'Other'

6. Select OK to begin Prescan

7. Once Prescan is finished, go to Setup…

8. Select 'Emission' in Scan setup

9. Set the Excitation wavelength to 355.0 nm (or the wavelength of your most intense absorbance peak in Part I)

10. Specify Start 360.0 nm and Stop 550.0 nm

11. Select "Slowest" under Scan Controls

12. Select OK to exit the Setup menu

13. Press the "Start" button ta the top of the screen, and enter a name for your file at the prompt (as in Part IA, highlight + Ctrl+C to use this file name again) (approx. 6.5 min)

14. Click on the plot containing your fluorescence spectrum, then go to File… then Save as… and be sure to specify File Type as **GRAMS**

15. Next you will run a lower resolution spectrum – go to Setup, and select "Survey" under Scan Controls

16. Repeat steps 13 and 14, being sure to save the low resolution spectrum under a different name

17. Move file over to a flash drive

POST-LAB

1. Create two spectral figures. The first should contain the anthracene absorption and emission spectra plots. The second should contain the tetracene absorption and emission spectra plots (absorption and emission data for tetracene will be provided to you by the TA). Using SpectraGryph:

   a. Scale the intensity of the fluorescence spectrum to be on scale with the absorbance spectrum – the tallest peak in each spectrum should have about the same intensity.

   b. Baseline correct the spectra.

   c. Use wavelength (nm) for your x-axis coordinate and be sure to include a legend with labels. (10 points)

2. For both anthracene and tetracene, construct a table containing the wavelengths of the peak maxima in the experimental absorption spectrum. Do the same for the emission spectrum. Why is the emission observed at longer wavelengths? (10 points)

3. In the same table, list the peak maxima wavelengths in wavenumber (cm^{-1}). In an additional table column, show the delta (difference) in wavenumbers between successive peaks. (10 points)

4. What are the average vibrational frequencies of the active modes of anthracene and tetracene in their ground electronic states? (5 points)

5. What are the average vibrational frequencies of the active modes of anthracene and tetracene in their excited electronic states? (5 points)

6. There is a similar trend in the vibrational frequency change for both molecules when they go from the ground state to the excited state. Offer an explanation as to why the excited state vibrations change the way they do. (5 points)

7. Consider the wavelength of the first peak (longest wavelength) in the absorption spectra of anthracene and tetracene. Did the PIB model correctly predict which molecule would absorb at shorter and which at longer wavelengths? Did the PIB model work well for predicting the numerical values of the absorption maxima of anthracene and tetracene? Does the model improve if you consider other transitions between other levels (n=3, n=4, n=5, etc.)? What is an obvious problem with applying the *1-dimensional* PIB model to real molecules like these? (10 points)

8. For anthracene only, construct a *simple* energy level diagram (like Figure 1) representing the electronic and vibrational <u>energy levels</u> and the <u>transitions</u> that occur between them that result in your observed experimental spectra. You will not be able to unambiguously identify every level and transition but do your best to accurately represent your observations. Be sure to label the energy levels and the absorption and emission arrows in a logical fashion so your diagram is understandable. (15 points)

9. In order to simulate the vibrational structure that is sometimes observed during electronic transitions (referred to as vibronic spectra), six calculations must be performed in Gaussian (though some can be combined into single jobs) to do a full Franck-Condon spectral analysis. Due to the length of the calculations, <u>you will be performing these calculations only on anthracene.</u>

    a. These Gaussian calculations will take several hours (each job is 1.5 to 8 hours in length) to complete using the new 12-core virtual machine (VM) workstations that ITS has developed for our use. Be sure to set your number of processors to 12 in GaussView when using the VMs for maximum efficiency. <u>Instructions for accessing the VMs will be posted on Blackboard, but please discuss this with your TA.</u>

    b. Consider the ground state molecule first. Run a combined geometry optimization and frequency analysis of the molecule using the B3LYP DFT functional and the def2TZVP basis set. The def2TZVP basis set represents a very large number of atomic orbital functions (a high accuracy basis set) and is not available through the drop-down menu in GaussView. You will need to choose "custom" and then manually type "def2TZVP" in the "Additional Keywords" text entry section. Also, make sure the checkpoint (.chk) file is being saved.

    c. Using the optimized molecule geometry, run an "energy" calculation to map out the electronically excited energy levels. Switch "ground state" to "TDA" under "Methods" in GaussView. Keep all other settings the same.

    d. Open the TDA job output in GaussView and you will see that "UV/VIS" is available under "Results". This will show you UV/VIS absorption spectrum. This gives you a preview of the PURE electronic absorption spectrum without vibrational structure. In complicated molecules, it can also give you information about which are the most important electronic states that need to be considered in the following steps.

e. Now consider the electronically excited molecule. Run a combined geometry optimization and frequency analysis of the molecule using B3LYP/def2TZVP, but make sure you have "TDA" chosen under "Methods". Also type "freq=savenormalmodes" into the "Additional keywords" box in GaussView to ensure that the checkpoint file contains extra data for later use. This will calculate the structure and vibrations of the molecule in the *excited* state and unsurprisingly these properties are very sensitive to the electronic state.

f. The setup for the full calculation of the vibronic absorption and emission spectra takes into account the vibrational frequencies of both the ground and excited electronic states. The final job must read in the checkpoint files generated for the earlier steps. GaussView is not set up to run these types of jobs, so you have to do this by hand using a text editor.

g. For calculating a vibronic absorption spectrum, your Gaussian input file will look something like this, and the blank lines are required. The yellow highlights are lab notes and not part of the file. All Gaussian input file must end with a blank line.

```
%chk=C:\RUN\ground.chk *change to your exact directory path and file name*
%mem=12GB *change to your RAM size*
%nprocshared=12 *change to your processor core count*
#FREQ(ReadFC,FranckCondon,READFCHT) NoSymm GEOM=Checkpoint

Franck Condon calculation for anthracene B3LYP/def2tvp

0 1

SpecHwHm=200 SpecRes=20 ABSORPTION

C:\RUN\excited.chk *change to your exact directory path and file name*
```

Note: For an emission (fluorescence) simulation, just change "ABSORPTION" to "EMISSION" and run the job again (do not accidently overwrite any important files!).

h. Once the Frank Condon job has run, then you can open the output in GaussView and see the predicted spectrum under "Results: Vibronic…". You will see several plots in the spectral view. The one to pay attention to is the black trace called "Final Spectrum", which includes all contributors to the spectrum. If your right-click on the spectrum window, you can "Save data" and then export the "Final Spectrum" as a text file for plotting in Excel or other software.

i. The simulated absorption and emission spectra can have very different calculated intensities, so in order to make them easily comparable, you need to normalize the y-axis values of each to 1.0. This can be done in Excel by dividing all the cells in the column that contain the intensities by the largest value in that column, then writing these new vales to a second column. You can use a function that looks like this

which is operating on Column A: "=A1/MAX(A:A)" You then need to propagate the function down the entire column by either clicking and dragging or double clicking on the bottom right hand corner of the cell with the function.

10. Using the results from Step #9, create a clear and well-labelled figure showing the ab initio absorption and fluorescence spectra of anthracene plotted together. (20 points)

11. Inspecting the peak positions in your ab initio absorption and emission spectra, what is the average vibrational frequency in the ground and excited electronic states? Do the results compare favorably with your experiments? (5 points)

12. Think about the ab initio theory you have applied here, and the physical state of the sample being studied experimentally. What major experimental factor is missing from the ab initio model and why might it be important? (5 points)

Lab D: Spectragryph Instructions

1. Open Spectragryph
2. Go to File… Open/Import… and select both the absorption and emission data to load
   a. You may see that the spectra are relatively off scale to one another. This is normal and the peaks just need to be normalized, which we will do in the following steps
3. Go to Process, then click Normalize Peaks
   a. This will normalize the max intensity of the peaks
4. Go to Plots/Views… and select Custom Boundaries.
   a. You may notice that nothing new has happened. That is ok. You have entered the 'Custom boundaries' mode, and so now when you change the boundaries of the x-axis or the y-axis, those changes will be reflected in the spectrum.
5. Change the x-axis range by double-clicking on a *tick mark* (not on a number itself) – this will open a dialog box 'Set Custom Axis Boundaries', into which you can enter the start and stop range
   a. Your x-axis should range from roughly 300 nm to 600 nm (your anthracene data should have been acquired within this range, but the tetracene data will require you to enter in these values in to remove excess space/background signal). Click 'Apply' to see the changes applied.
   b. After adjusting your axis boundaries, you should always repeat step 3 (normalize peaks), so that your peaks are normalized within the range that you have selected. After repeating step 3, double-click on 'Custom Boundaries' again so that they are active.
   c. Your y-axis should show the peaks of interest (between 300 and 600 nm), with room at the top for the peak labels (more on that in step 8). Click 'Apply' to see the changes applied.
6. Go to Process, then click Advanced Baseline
   a. This will bring up two additional plots on your spectrum. You only want the baseline corrected ones.
7. Go to Plots/Views, then click Hide Spectra
   a. Deselect the original spectra, so you are only left with the baseline corrected spectra
   b. Click on Hide Spectra again to return the legend to normal
8. Next, you will want to label your peaks. This will help you in your post lab analysis.
   a. Go to Plots/Views, then click on Peak Labels.
   b. Be sure to select "all spectra" in the top bar to the right
   c. Adjust threshold and prominence so that enough peaks are labeled. You want at least three peaks in both spectra (absorption and emission) for both compounds.

Lab D: Electronic Absorption and Fluorescence

Student: XXX

Question 1: (x / 10 pts)

Question 2: (x / 10 pts)

Question 3: (x / 10 pts)

Question 4: (x / 5 pts)

Question 5: (x / 5 pts)

Question 6: (x / 5 pts)

Question 7: (x / 10 pts)

Question 8: (x / 15 pts)

Question 9 + 10: (x/ 20 pts)

Question 11: (x / 5 pts)

Question 12: (x / 5 pts)

Report Total: (x / 100)

General Comments: