

## Experiment 1: The Electronic Spectrum of $\beta$ -Carotene

Report due on Friday, February 15

### Logistical Basics for Chemistry 312 Lab Work:

- You will work in groups of two or three (of your own choosing).
- Note that you are not required to keep a notebook for your experiments this semester.
- You will follow the five Macalester Chemistry cardinal lab safety rules:
  1. Always wear goggles whenever you are inside a “wet” or “instrumental” lab.
  2. Always wear shoes that cover your feet.
  3. Never consume any food or drink in the lab.
  4. Always wash your hands whenever you leave the lab.
  5. Never work alone in a “wet” or “instrumental” lab, even when “just” using an instrument.
- Each group will turn in one lab report, and all of the members of a group will share the same grade for each lab.

### Introduction

In this laboratory exercise, you will measure the electronic spectrum of  $\beta$ -carotene, which is the plant pigment that gives rise to the distinctive color of the carrot. This polyene is used as a yellow coloring agent in foods and is a precursor of vitamin A in all species except cats. (It is not surprising that this most excellent of mammals has evolved its own biochemical pathway.) You will then rationalize the nature of the spectrum by applying the particle-in-a-box Hamiltonian to the pi electrons in this molecule. Computer modeling using the Schrodinger equation will enable you to predict accurate geometries for  $\beta$ -carotene and related molecules.

### Experimental Procedure

1. Shave a few pieces off the exterior of the carrot. (Avoid using the light yellow center portion, which contains higher concentrations of xanthophyll, another plant pigment.) Put the carrot shavings in a clean test tube and add 3-4 cm of heptane. Extract some of the yellow  $\beta$ -carotene by gently grinding the shavings against the inside of the test tube with a stirring rod. A few seconds should produce a pale yellow solution that is concentrated enough for you obtain a good UV-visible absorption spectrum.
2. Acquire the UV-visible spectrum (300–800 nm) of your yellow solution against a neat heptane reference. Use the following procedure:
  - a. Sign log book.
  - b. Turn on the spectrophotometer (the power switch is at the back right corner), monitor, and printer.
  - c. After the instrument has (successfully) completed its power-up diagnostics, use the mouse to click on **Quit** and then on **WAVELENGTH SCAN** (at the upper left of the screen).
  - d. Click on **VIS OFF** and **UV OFF** (at the bottom left of the new screen) to turn on the visible and ultraviolet light sources.

- e. In the upper panel, click next to Start  $\lambda$  to set it to 300 (nm) and click next to End  $\lambda$  to set it to 800 (it may already be at 800).
- f. Set the maximum [Abs] value on the y-axis of the spectrum panel to be 1.0.
- g. Fill a plastic cuvet about halfway with pure heptane, wipe the smooth sides of the cuvet with a Kimwipe, and place the cuvet in the back of the instrument's sample tray (that is, in Slot 1). Be sure to hold the cuvet by the ribbed sides, and orient the cuvet with the smooth sides exposed to the slits in the side of the tray.
- h. Click on **BLANK** in the lower left of your screen. This will store the absorbance in the instrument's memory. Now all subsequent readings will be automatically corrected!
- i. Take the blank cuvet out of the sample tray, and replace it with a cuvet filled (again about halfway) with your  $\beta$ -carotene solution.
- j. Click on **ReadSamples** (at the upper left of the screen).
- k. Click on **Print** (upper right) to print out a copy of the spectrum. **This should be included with your group's lab report.**
- l. Click on **Tabulate** (upper left). Scroll down to find the wavelength of maximum absorbance **for the longest wavelength feature in your spectrum.** Write down this  $\lambda_{\max}$  on your printed spectrum. Then click on **Exit**.
- m. Click on **Quit** in the upper right of the screen, then **OK**.
- n. In the log book, note if there were any instrumental problems. (Hopefully there weren't!)
- o. Turn off the spectrophotometer, monitor, and printer.
- p. Dispose of the heptane solutions in an organic waste container
- q. Dispose of the cuvetts and any Pasteur pipets you used. Rinse the other glassware several times with acetone and water, and leave them to dry next to the sink.

## Data Analysis and Computer Modeling

### I. The Free Electron Molecular Orbital Model

1. Look up the structure of  $\beta$ -carotene and determine how many  $\pi$  electrons this molecule has. (Be sure to cite the source of this information, and all other sources you refer to, in your report.) Assume (rather crudely) that the  $\pi$  electrons in the molecule are particles confined to a one-dimensional infinite well, with the energy levels given by the result we learned in Chapter 9. Further assume that the aufbau and Pauli exclusion principles determine the ground electronic state of  $\beta$ -carotene (that is, two  $\pi$  electrons fill each of the lowest available energy levels of the box). This is the so-called Free Electron Molecular Orbital Model. The first excited state of  $\beta$ -carotene involves promotion of one electron from the highest occupied molecular orbital (the HOMO) to the lowest unoccupied molecular orbital (the LUMO). Derive an expression for the separation in energy between the HOMO and the LUMO. This energy will depend on  $L$ , the length of the box. Then derive an expression (in terms of  $L$ ) for the wavelength of light required to excite this transition. Using these results, derive a "spectroscopic" value of  $L$  from the value of  $\lambda_{\max}$  you observed for  $\beta$ -carotene.

2. Next, calculate a “structural” value of  $L$  by assuming the  $\pi$  electrons are required to “travel” along the backbone of the molecule; use the known average values for the lengths of carbon-carbon single and double bonds in your calculation. Compare the structural and spectroscopic values by computing a percent difference. (Note: You may find some relevant information in Chapter 9 of Atkins and de Paula. Far be it from me to deprecate your textbook, but chances are that you will find a value of  $L$  in Chapter 9 that is around a factor of 10 too small.)
3. Try at least one alternate way of determining the structural value of  $L$  that may improve the agreement with the spectroscopic value. For example, use trigonometry to calculate the “through space” length of the box (“as the crow flies”). Again, compute a percent difference between the structural and spectroscopic values.

## II. A More Accurate Determination of Box Length Using Quantum Mechanics

The most accurate way to interpret the electronic spectrum of  $\beta$ -carotene is to solve the Schrodinger equation for the molecule. Such a calculation takes into account both  $\sigma$  and  $\pi$  electrons. This cannot be done analytically, but software programs like Spartan 06 can do an excellent job of solving the Schrodinger equation numerically. We will use this program (which is loaded on the iMacs in the department computer lab) to “optimize” the geometry of  $\beta$ -carotene—that is, we will have MacSpartan predict all the bond lengths and angles in the molecule. This should give us an accurate value for  $L$ .

### Spartan 06 Instructions

1. Find the program icon in the bottom toolbar and run it.
2. Select **New** from the **File** menu. The Model Kit window will appear.
3. Note the following tools for manipulating your structure on the screen:
  - Molecule Representation: The way in which a molecule is displayed can be changed by switching between the “wire”, “ball and wire”, “tube”, “ball and spoke”, and “space filing” options under the **Model** menu.
  - Translate the Molecule: Depress the mouse and apple key simultaneously while moving the mouse.
  - Rotate the Molecule Arbitrarily: Click on the background and drag the mouse in the desired direction.
  - Rotate the Molecule in the Plane of the Screen: Depress the mouse and shift key simultaneously while dragging the mouse in the desired direction.
  - Enlarge/Reduce the Molecule: Depress the shift and left side mouse keys simultaneously while dragging the mouse in the appropriate direction to achieve the desired effect.
4. Use the Organic Model Kit window on the right to build  $\beta$ -carotene. Click on desired atom/hybridization states on the right, and then click on the green area. (I suggest you build any rings in your molecule manually, instead of using the pre-defined ring structures.) The yellow “half-bonds” indicate sites where new fragments can be added. Modify your structure (*e.g* by deleting atoms or making bonds) via the **Build** menu. Note that a yellow half-bond is assumed to be H unless you put another atom there.

5. You can control the conformation around bonds (*e.g.*, do you want the substituents about a double bond to be *cis* or *trans* to each other?) by choosing **Measure Dihedral** under the **Geometry** menu, clicking on the four consecutive atoms whose conformation you wish to set, and then entering the desired dihedral angle in the text box at the bottom right corner. When you are done tweaking angles, choose **Add Fragment** under the **Build** menu to resume building your structure.
6. When you feel in the mood to save your work, choose **Save As** from the file menu and save a file to the Desktop. If you close out of Spartan 06, you can re-open your  $\beta$ -carotene file when you run the program again. Choose **Add Fragment** under the **Build** menu if you need to alter your saved structure.
7. When you are all done building the molecule, choose **Minimize** under the **Build** menu. This will start a so-called molecular mechanics force field calculation that adjusts bond lengths and angles to match typical experimental values. This minimization does not solve the Schrodinger equation.
8. Select **Calculations...** from the **Setup** menu. The resulting window can be used to establish appropriate parameters for the calculation. In the "Calculate" section select "**Equilibrium Geometry**" at "**Ground state**" with "**Semi-Empirical**" and "**AM1**". The latter two terms refer to the method by which we will truly solve the Schrodinger equation. "**Initial geometry**" should be indicated under the "Start from" heading. Click OK once your selections in this window are complete. Then choose **Submit** under the **Setup** menu.
9. The calculations will take a little while. (For the heck of it, you can monitor the progress of your calculations by choosing **Output** under the **Display** menu.) When they are over, measure the length of the  $\beta$ -carotene box by using **Measure Distance** under the **Build** menu. Compare this with the spectroscopic value. Is the agreement better than with the "structural" values you estimated above?
10. Repeat the above calculations for either  $\alpha$ -carotene or  $\gamma$ -carotene. Based on the  $L$  value predicted by Spartan 06, and your experimental value of  $\lambda_{\max}$  for  $\beta$ -carotene, predict a  $\lambda_{\max}$  for either  $\alpha$ -carotene or  $\gamma$ -carotene. Explain your reasoning.

### Final Product

You will write a paper with the same sections (Title, Your Names, Abstract, Introduction, Procedure, Results and Discussion, Conclusions, References, Appendix) as your Chemistry 311 written lab reports. However, note that your Procedure should be extremely short (two to three sentences will suffice). The focus of your paper should be addressing the questions posed in the **Data Analysis and Computer Modeling** section of the instructions.

The paper is due by Friday, February 15, at 4:00 p.m.