APPENDIX C

USING A SPECTROPHOTOMETER

A spectrophotometer is an instrument designed to measure the amount of light of a given frequency absorbed by a solution. A diagram of a model spectrophotometer is shown in Figure C-1. This may differ from the design you use in the laboratory, but all such instruments are fundamentally the same.

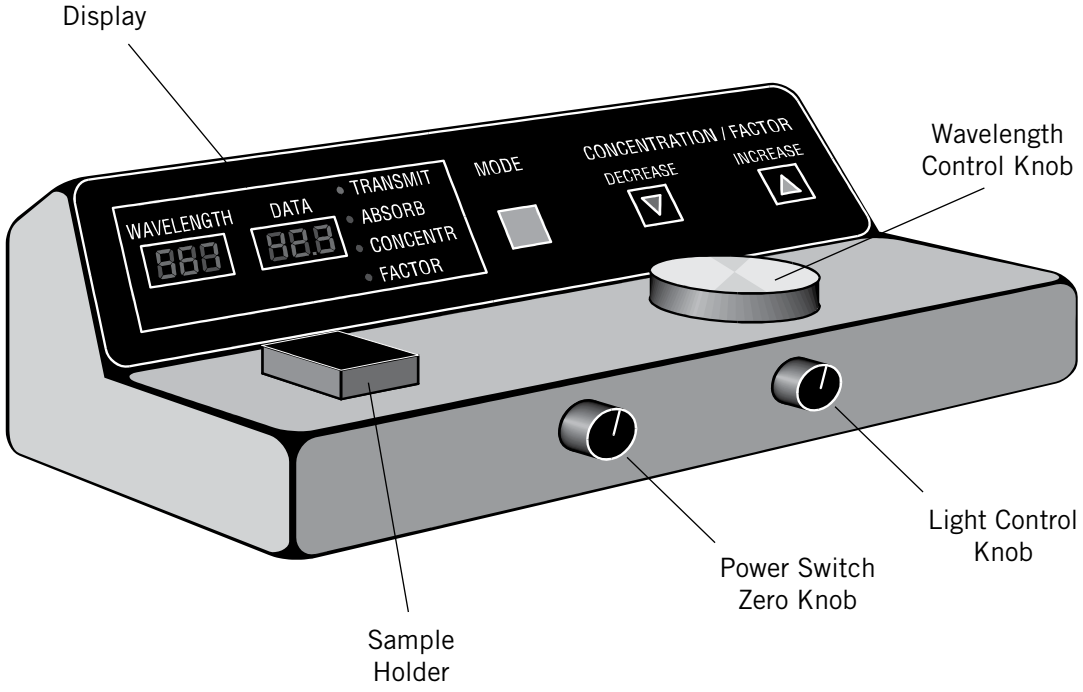

FIGURE C-1

Before starting to use the instrument, you will need at least two *cuvettes*, special, optically pure sample tubes designed to be placed in the instrument. Your instructor will provide these. Make sure each one is clean (if they aren't, clean them). Fill one cuvette with the solution you wish to study, and the other with a "blank" reference liquid. The blank is usually the solvent in which the molecule

that absorbs light (the "chromophore") is dissolved. Having the blank will make it possible for you to adjust the instrument so that it ignores any light absorbed by the solvent and measures only the light absorbed by the chromophore.

Note: Handle the cuvette only by its upper rim. Smudges from your fingers on the sides of the cuvette, where light passes through it, will scatter light and affect your data.

The spectrophotometer is used as follows:

1. **Make sure the instrument is on.** If it is not, check with your instructor to confirm that you should be using the instrument, and turn it on. It will likely take some time to warm up; ask your instructor.
2. **Set the wavelength for the light source.** This has likely been done for you, but you should check to make sure the wavelength in the instrument matches the one in your laboratory manual (or the one your instructor specified). The wavelength control knob, and the wavelength scale, are shown in Figure A-1. Check them, and (if your instructor confirms that they should be changed) adjust accordingly.
3. **Zero the spectrophotometer.** Open the sample cover and make sure there is no cuvette in the instrument. Close the sample cover. Adjust the zero knob until the instrument shows 0%T (0% transmittance of light through the sample). When no cuvette is in the instrument, a shutter closes so that no light reaches the detector. The instrument should, therefore, be calibrated to 0%T, so that it reads correctly when all light is absorbed by the sample.
4. **Insert the blank.** Open the sample cover and insert the cuvette containing the blank solution. You will notice that the cuvette has a vertical white line on it; make sure that line is aligned with the notch or groove on the rim of the sample container. This keeps the cuvette in the same orientation every time, so any imperfections in the glass will not affect your results. Close the sample cover.
5. **Adjust the light control (100%T control).** Now that the blank is in place, you want to calibrate the instrument so that it will ignore any light absorbed by the solution. Adjust the light control knob so that the readout shows 100%T/0A (100% light transmitted, 0 light absorbed). In reality, some small fraction of the light is scattered or absorbed by the blank, but by calibrating the instrument in this way you can ignore that. When you insert your sample, the instrument will report only the fraction of the light that is absorbed by your chromophore.
6. **Remove the blank, and insert the sample.** Open the sample cover and take out the blank; avoid smudging it if possible, as you may need it again. Insert your sample, as you did in Step 4 with the blank, aligning the cuvette in the same way. Close the cover and check the readout. Record both the percent transmittance and the absorbance.
7. **Remove the sample.** Take the sample out and close the cover.

To make use of Beer's Law, you must take measurements of multiple solutions of known concentration in your chromophore, and then take a measurement of your unknown solution. You may only be given two cuvettes. In that case, you will have to measure your first solution and return to your bench space. There you should empty your sample cuvette (not the blank—you will need it!), clean, rinse, and dry the cuvette carefully, and fill it with your second solution. Then you should take the new solution to the instrument and take another measurement. You should try to use the same

instrument, and make sure the wavelength has not been changed. Also, you should probably repeat Steps 3–7 to make sure the calibration has not drifted or been changed by another user.

If you have enough cuvettes to simultaneously prepare all of your samples, it is likely quickest and easiest to prepare them all at the bench and take them to the instrument together (in a test tube rack to avoid spilling or scratching). Then, after you have done Steps 1–7 for the first tube, you can immediately insert the next sample and only repeat Steps 6–7 for each subsequent measurement.

One final note: The Beer-Lambert Law links the concentration of a chromophore to *absorbance.* Absorbance is defined by

$$A = -\log[\frac{I_s}{I_o}]$$

where I_o is the intensity of the light that enters the sample, I_s is the intensity of the light leaving the sample, and A is the absorbance. The percent transmittance is just

$$\%T = \frac{I_s}{I_o} \times 100\%.$$

In other words, Beer's law says that the amount of light absorbed varies logarithmically with the amount of light absorbed.

Don't worry if you don't understand the details of that. Just understand that you need to use the absorbance (not transmittance) when you use the Beer-Lambert Law!