Appendix E - Calibrating and Operating the Vernier SpectroVis Plus Spectrophotometer

Important Notes

1. When taking measurements, make sure the clear (not ridged) sides of the cuvette are aligned in the slot so that the light shines through them. Between the ► and ☼.
2. These are *NOT* disposable cuvettes. Replace the cuvette in the spectrometer when you are done.
3. If the software is not already open, or if the computer goes to sleep and the software stops working, you will need to perform the following steps:
    a. Making sure the spectrophotometer is NOT connected to the computer, open the Logger Pro 3 software by clicking on the desktop icon.
    b. Connect the spectrophotometer to the computer via the USB cable.

A - Collecting absorbance Data at a Single Wavelength for a Calibration Curve

1. Set the mode and wavelength
    a. Select the  icon and select Absorbance vs. Concentration.
    b. Select the desired wavelength from the list.
    c. Click OK.
2. Calibrate with blank.
    a. Place a cuvette ¾ full of the blank solution in the cuvette slot.
    b. Choose from the **EXPERIMENT** menu:
        → Calibrate
        → Spectrometer
    c. After calibrating you should record the absorbency of the blank. This measurement should be very close to 0.000, if it is not you should calibrate again.

Follow the prompts to complete the calibration. If the spectrometer has been on for several minutes already you may skip the "warm-up" step

3. Collect Data

The SpectroVis Plus should already be collecting data. You do not need to do anything further with the computer – just follow the directions below to insert your samples and read the absorbance.

4. Measure the standards and unknown
    a. Rinse the cuvette with a small amount of the first solution you will measure. Discard the rinsing in the waste container and fill the cuvette ¾ full of the solution.

b. Place the cuvette in the cuvette slot of the SpectroVis Plus. When a steady absorbance reading appears in the lower left corner of the screen, write it in your data table.

c. Return your sample to its test tube, rinse the cuvette with water, shake it out and repeat steps 1 and 2 for each standard and your unknown(s).

d. When you are finished, rinse the cuvette, refill it with water and replace it in the SpectroVis Plus.

B - Collecting Absorbance Spectra

1. Set the mode and wavelength

   a. Select the  icon and select Absorbance vs. Wavelength is selected.

   b. Click OK.

2. Calibrate with blank.

   a. Place a cuvette ¾ full of the blank solution in the cuvette slot.

   b. Choose from the **EXPERIMENT** menu:

   → Calibrate

   → Spectrometer

Follow the prompts to complete the calibration. If the spectrometer has been on for several minutes already you may skip the "warm-up" step

3. Measure the Absorbance Spectrum

   a. Pour the blank out of the cuvette and shake it to dry out as much as possible.

   b. Rinse the cuvette with a small amount of the solution to be measured. Discard the rinsing in the waste container and fill the cuvette ¾ full of solution.

   c. Take a spectrum of your solution by clicking ▶ Collect and then end the collection of data once you see an absorbance spectrum on the graph (usually takes a few seconds) by clicking ■ Stop .

   d. Return your sample to its test tube, rinse the cuvette with water, shake it out and repeat steps 2 and 3 with the other solutions to be measured. *Important*: When you click ▶ Collect for the second solution it will ask if you want to store the latest run. Choose to store the latest run and continue.

   e. Once you have stopped collection of your last spectrum, rinse out the cuvette with DI water, refill it with DI water and leave it in the spectrometer for the next group.

4. Finding the Wavelength of Maximum Absorbance and Printing

   a. Make sure the curve(s) are fully on the graph by clicking  to autoscale the graph. Then click  to add statistics. Select the check boxes for all runs (if more than one present) and click OK.

   b. A box will display for each curve stating, among other things, the wavelength (reported in nm) where maximum absorbance was recorded. You can move these boxes so that it is clear which goes with which spectrum.

   c. Select **FILE** →*Page Setup* and set it to landscape (if it is not already set this way).

   d. Select **FILE** →*Print*, in the '*Printing Options*' dialog box be sure to complete or check the only the following boxes/features:

      i. In the Footer: Type you and your partners names

      ii. Check the '*Date*' box

      iii. Check the '*Print grid lines black*' box

      iv. Leave all other boxes un-checked

   e. In the print menu - check the print preview to make sure the graph fits on one page and looks the way you want it to look.

      i. Print one copy first and make sure it prints the way you want it to - look at all the titles and labels etc.

      ii. Then print the other copies you need for the rest of your group.

   f. When you are done, select **FILE** → *New* to reset it for the next group.