

BC367: Measuring Absorbance with the Ocean Optics Spectrophotometers

Introduction:

The absorbance of a sample is given as $A = \log\left(\frac{I_0}{I}\right)$,

where I_0 is the intensity without sample present and I is the intensity with the sample in the light path of the spectrophotometer. This calculation is done at each wavelength in the spectrum. The value of I_0 depends on wavelength and must be determined by placing a reference or blank sample in the instrument. The terms reference and blank are used interchangeably. The reference is usually a cuvette filled with only the solvent or the buffer for the experiment. The process of acquiring the reference spectrum is called calibration in Logger Pro. Every time you switch solvents or buffers, a new reference spectrum must be taken, so the instrument must be recalibrated.

I. Getting Started and Calibration

1. First, open the *Logger Pro 3* software. Then, in order, plug the spec power cord into an outlet, plug the other end into the spectrometer where it is labeled “POWER,” and plug in the USB port.
2. The software should recognize the spectrophotometer automatically, showing the colors of the visible spectrum in the data window with Wavelength as the horizontal axis. If the visible spectrum is not displayed, first check your connections. If this doesn't work, select Connect Interface → Spectrometer → Scan for Spectrometers from the Experiment menu. If you find the visible spectrum display annoying, turn it off by clicking on the graph and the going to “options” → “graph options.” Unselect “Draw visible spectrum.”
3. Select Set Up Sensors → Spectrometer: from the Experiment menu. The Spectrometer dialog box will be displayed. Set Wavelength Smoothing to 0 and Samples to Average to 30. (Use more Samples for more precise absorbance measurements.) If you are using regular plastic cuvettes, set the wavelength range to 350 – 850 nm; UV plastic cuvettes, set the wavelength range to 260-850 nm; or UV quartz cuvettes, set the wavelength range to 200-850 nm. The Integration time will be set for you automatically when you do the calibration in the next step. Close the window.

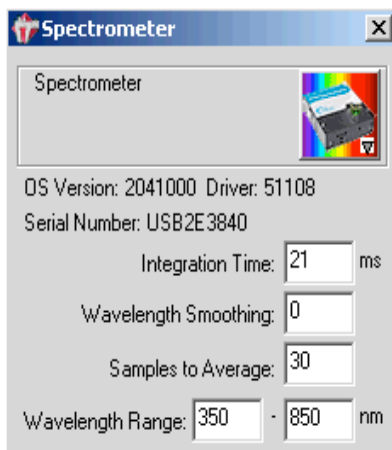


Figure 1. Settings for fast analysis with regular plastic cuvettes or work in the visible range.

4. To calibrate the Spectrometer, choose Calibrate → Spectrometer from the Experiment menu. The calibration dialog box will display the message: “Waiting...60 seconds for lamp to warm up.” (see Figure 2) The minimum warm up time is one minute. **NOTE: For best results, allow**

the spectrometer to warm up for at least five minutes. Following the instructions in the dialog box to complete the calibration, use a cuvette filled about $\frac{3}{4}$ full with the solvent or buffer for your reference, as instructed. Check to make sure the non-frosted, clear sides are in the light path. The cuvette should be inserted all the way through the cell holder. You should feel that the cuvette is gently, but firmly, held in place so that you cannot twist the cuvette. Click Finish Calibration and then click OK. This step “blanks” the spectrophotometer with your reference solution.

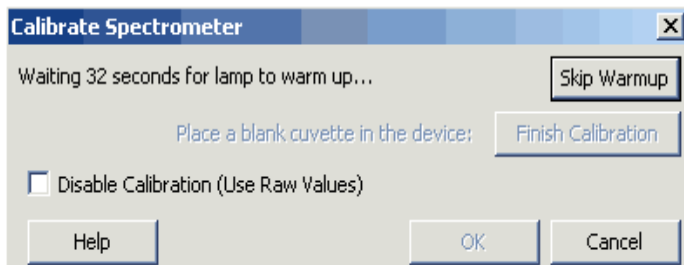





Figure 2. Calibration stores a reference spectrum.

After the first calibration, the lamp should be warmed up enough that you can skip the warm-up period on subsequent calibrations. The process of calibration sets the integration time for maximum sensitivity and also stores a reference spectrum.

II. Measuring Absorbance Spectra

1. Replace the reference cuvette with your sample. Click on  Collect and then  Stop.
2. You can read the absorbance using the Examine tool, by clicking on . Then move the cursor along the spectrum. The wavelength and absorbance will be displayed in the new dialog box in the data window, Figure 3. Determine the wavelength of maximum absorbance. Use this wavelength throughout your experiment.

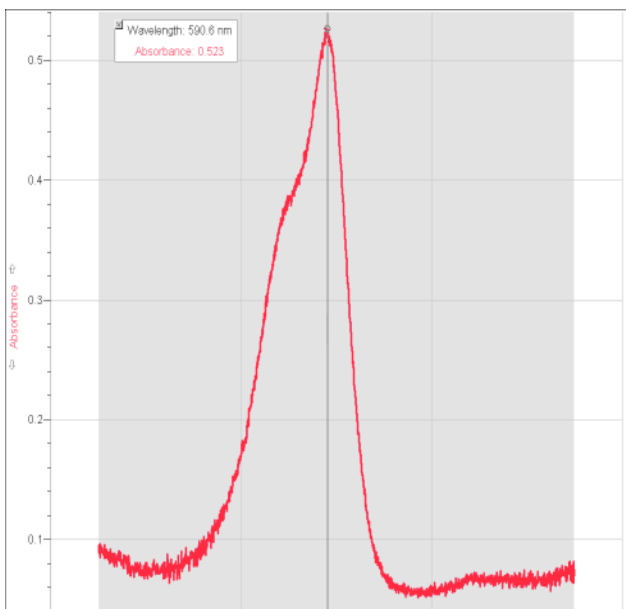



Figure 3. Spectrum of Crystal Violet with the Examine Tool.

3. **Expanding the Axes:** If you need to expand the x or y axis to see your spectrum better, use one of three methods:

Automatic scaling: Click on the Autoscale icon .

Using the cursor: Position the cursor over the axis you want to expand. The cursor will change shape, Figure 4. Drag the mouse to change the scale expansion.

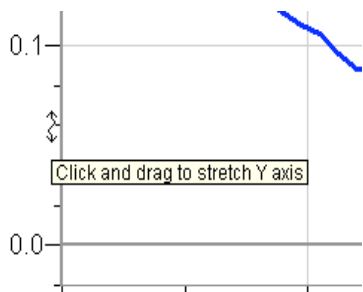


Figure 4. Move the cursor over the axis to change the axis scale.

Direct input: Click near the maximum or minimum of the axis you want to change. A dialog box will appear, Figure 5, and you can type in the value that you want for the scale limit.

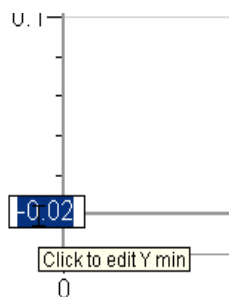


Figure 5. Click near the axis maximum or minimum to show the dialog box.

4. When working with multiple samples, you may choose to plot each spectrum separately or you can overlay successive spectra. Normally you plot each spectrum separately.

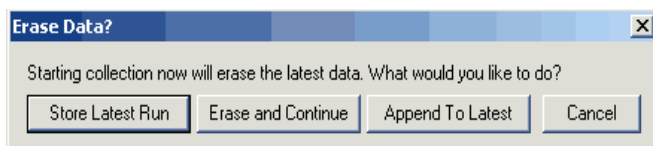
Plotting each spectrum separately:

- Save the data file to the disk by pulling down the file menu and choosing Save As... Save your data files to the Documents directory.
- Pull down the Data menu and choose Clear All Data.
- Replace the cuvette with another sample. You may need to calibrate again if there is a different reference solvent or buffer for the new sample. Return to step 1.

Overlaid Spectra

- If you wish to overlay several spectra, choose Store Latest Run from the Experiment menu. This step allows you to overlay the next spectrum on top of the current spectra, with all showing. However, this step does not save the data file to the disk.
- Replace the cuvette with another sample.

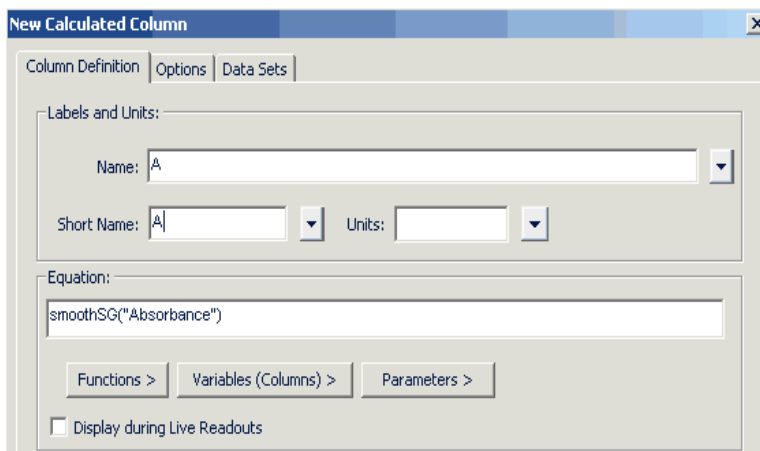
5. When you click on Collect this time, if you did not store the latest run to set-up for overlaid spectra, you will get a dialog box:



To overlay the next spectrum on top of the last spectra, click on Store Latest Run.

6. If you overlay your spectra, remember to save your combined spectra to disk. To save the data file to the disk pull down the file menu and choose Save As... Save your data files to the Documents directory.

7. If your spectrum is noisy, you can increase the setting for the number of Samples to Average in step I.3 and retake your spectrum. Alternatively, you can use spectral smoothing. Spectral smoothing averages together adjacent data points. To apply spectral smoothing, choose New Calculated Column from the Data menu. Enter "A" as the Name, "A" as the Short Name, and leave the units blank. Absorbance is unitless. Enter the formula for the column into the Equation edit box, by choosing smoothSg from the functions menu and "Absorbance" from the Variables menu, as shown below. Click .



Click on the y-axis label. Choose "A." A graph of smoothed absorbance will be displayed.

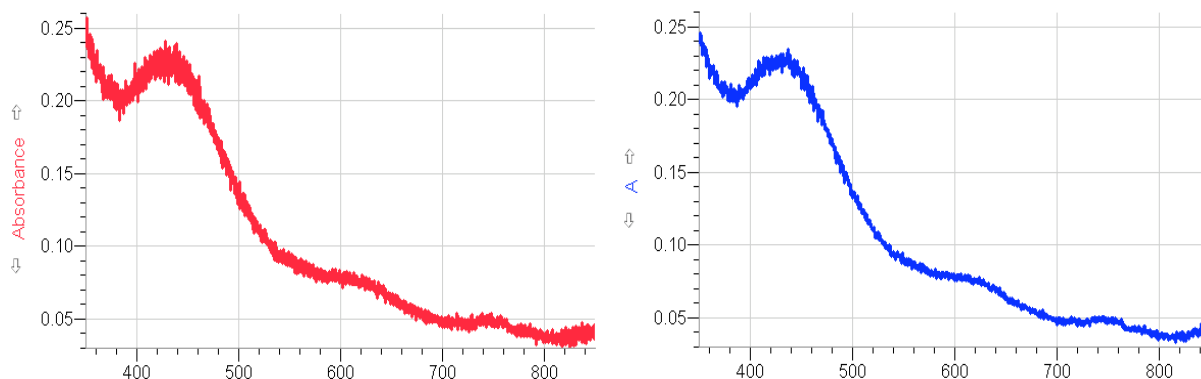


Figure 6. Spectral smoothing decreases the appearance of noise.

8. When you are done, please make sure that you unplug the instrument. The deuterium lamp in the spectrometer has a limited lifetime and replacements are very expensive.