

pK_a of Methyl Red¹⁻³

Purpose By measuring absorbance spectra as a function of pH, the pK_a of methyl red will be determined.

Introduction

Methyl red (4-dimethylaminobenzene-2'-carboxylic acid) is a commonly used indicator for acid-base titrations. We will measure the visible absorption spectra of the acidic and basic forms of this compound. Next we will prepare a series of buffered solutions of methyl red at known pH. By following the change in absorbance as a function of pH we will determine the acid dissociation constant, or pK_a. This technique is not restricted to indicators, and can be used with any substance whose absorption spectrum changes with pH.

The acid form of the indicator, which we will designate as HMR, is zwitter ionic, Figure 1. The basic form is designated as MR⁻.

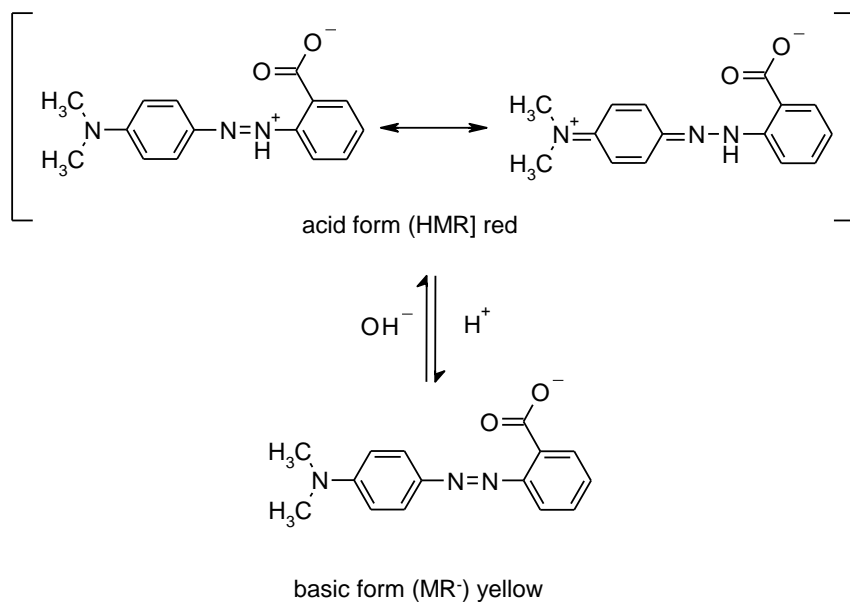
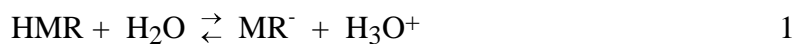


Figure 1. Acid and base forms of methyl red.

The equilibrium of interest is



The equilibrium constant is the acid dissociation constant:

$$K_a' = \frac{[\text{H}_3\text{O}^+][\text{MR}^-]}{[\text{HMR}]} \quad 2$$

The prime indicates that we have used concentrations rather than activities. Activities are necessary in true thermodynamic equilibrium constants. Using concentrations, instead, gives the effective or conditional equilibrium constant. By definition $\text{pH} = -\log [\text{H}^+]$ and $\text{pK}_a = -\log K_a$. Taking the $(-\log)$ of both sides of Eq. 2 gives:

$$\text{pK}_a' = \text{pH} - \log \frac{[\text{MR}^-]}{[\text{HMR}]} \quad 3$$

In this experiment we will determine this equilibrium constant, pK_a' , by varying the pH and measuring the ratio $[\text{MR}^-]/[\text{HMR}]$. We will use acetic acid-acetate buffers to control the pH, since the K_a value for acetic acid is in the same range as the K_a' value for methyl red. The pH of these buffers force methyl red to distribute itself somewhat evenly between the two colored forms.

The absorption of light is governed by the Beer-Lambert Law:

$$A = \epsilon \ell [X] \quad 4$$

where A is the absorbance, ϵ is the molar absorption coefficient, ℓ is the path length of the cell in centimeters, and $[X]$ is the concentration of the absorbing species in moles per liter. The absorbance of mixtures is the sum of the separate absorbencies. In mixtures of the acid and base forms of methyl red the total absorbance is

$$A = A_{\text{MR}^-} + A_{\text{HMR}} \quad 5$$

The absorption spectra of HMR and MR^- are given schematically in Figure 2. For two components in solution, the absorbance must be measured at two different wavelengths. The best wavelengths to choose for the analysis are where one form absorbs strongly and the absorbance of the other form is negligible. Examination of Figure 2 reveals that there are no wavelengths where one form, acid or base, absorbs exclusively. For this case, we need to set up two equations in two unknowns, one equation for each wavelength. Call the two wavelengths λ_1 and λ_2 . The absorbance at λ_1 is A_1 and at λ_2 is A_2 . The two measurements then provide two simultaneous equations with two unknowns:

$$A_1 = \epsilon_{1,\text{MR}^-} \ell [\text{MR}^-] + \epsilon_{1,\text{HMR}} \ell [\text{HMR}] \quad 6$$

$$A_2 = \epsilon_{2,\text{MR}^-} \ell [\text{MR}^-] + \epsilon_{2,\text{HMR}} \ell [\text{HMR}] \quad 7$$

The molar absorbance coefficients are illustrated in Figure 2. The molar absorbance coefficients are determined from standard solutions that contain one component alone. Eqs. 6 and 7 provide two equations in two unknowns. For an unknown solution, the absorbances at the two wavelengths, A_1 and A_2 , are determined and then Eqs. 6 and 7 are solved for the unknown concentrations $[\text{MR}^-]$ and $[\text{HMR}]$ at each given pH.

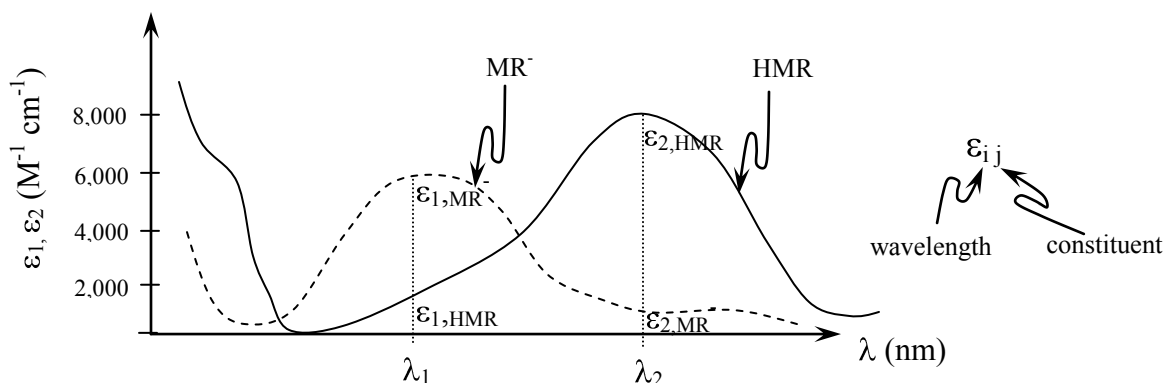


Figure 2: Absorbance of a solution is the sum of the absorbances of the constituents. Measurements at two wavelengths are necessary to determine the composition of a two-constituent solution if the absorbance bands overlap. The first subscript indexes the wavelength and the second subscript indexes the constituent.

An isosbestic point is defined as the wavelength where two species have the same molar absorptivity. At the isosbestic point the total absorbance of a solution of the two ions is independent of their relative concentrations but is dependent only upon the total dye concentration. The appearance of an isosbestic point is evidence that only two species are involved. Figure 2 shows two isosbestic points. You will use your spectra to determine if there are only two absorbing species in this experiment.

Ionic Strength Dependence

Equilibrium constants involving ionic species are especially sensitive to ionic strength. The ionic strength is a measure of the total ion concentration in solution. The activity of all the species in solution are a function of the ionic strength. In this experiment we are neglecting the difference between activity and concentration, so the pK_a' applies to only one specific ionic strength. The ionic strength is defined as

$$I = \frac{1}{2} \sum c_i z_i^2 \quad 7$$

where c_i is the concentration of ion i and z_i is the charge on ion i . The sum is taken over all ions in solution. For a 1:1 salt of singly charged ions, such as NaCl, KCl, and sodium acetate, the concentration of the salt is equal to the ionic strength. KCl is added to the solution in this experiment to maintain a constant ionic strength.

Procedure

Equipment and Solutions

2 x 100-mL volumetric flasks
3 x 10-mL volumetric pipets
1 x 10-mL graduated cylinder
7 x 30-mL beakers
2 x 50-mL burets
2 x plastic buret funnels
1 x stirring rod
2 x Pasteur pipets
1 x box Chem wipes
1 x buret stand
3 x 150 mL beakers
1 x plastic cuvette
pH meter and electrodes, pH 4 and 7 buffers for standardization
0.10 M acetic acid, 0.10 M sodium acetate, and 1.0 M KCl

Stock Solution of Methyl Red

Prepare a 0.05% solution of methyl red by dissolving 0.025 g in 20 mL of 95% ethanol in a 50 mL in a volumetric flask. Add water to within a few mL of the mark. Add ~0.1 M NaOH drop-by-drop until all the solid dissolves and then dilute to the mark. Transfer 20 mL of this solution into 50 mL of 95% ethanol in a 200 mL volumetric flask. Dilute to the mark with water. This solution should be orange colored. (This solution may be made up for you; check with your instructor.) Make sure to record the actual weight of methyl red used to make up this solution.

Basic Solution of Methyl Red

Prepare a basic solution of methyl red by adding to a 100-ml volumetric flask the following: 10.0 ml of 0.100 M sodium acetate, 10.0 ml of the stock methyl red solution, and using a graduated cylinder 9 ml of 1.0 M potassium chloride. Dilute to the mark with distilled water and mix thoroughly.

The ionic strength of all solutions will be kept at 0.1M using KCl.

The molarity of methyl red in this solution is negligible, compared to the KCl concentration.

Acid Solution of Methyl red

Prepare an acidic solution of the indicator by adding to a 100-ml volumetric flask the following: 10.0 ml of 0.100 M acetic acid solution, 10.0 ml of the stock methyl red solution, and 10 ml of the 1.00 M potassium chloride solution. Dilute to the mark with distilled water and mix thoroughly.

This solution requires more potassium chloride solution to maintain an ionic strength of 0.1 M because the acetic acid is not strongly ionized and therefore does not contribute ions to the solution.

Preparation of Buffer Solutions

Fill two burets with the acidic and basic methyl red solutions. Prepare five buffer solutions, by

mixing V ml of the basic solution with (20-V) ml of the acidic solution in small beakers. A range from 10 ml to 18 ml for V will give optimum results.

Measurement of the pH of the Methyl red-Buffer Solutions

Calibrate the pH meter using pH 7 and pH 4 buffers. Measure the pH of each of the five buffer solutions by inserting the measuring electrodes directly into each of the beakers you prepared in the last step. Be sure to rinse and dry off the electrodes (with a stream of air from an empty wash bottle) before inserting them into the next solution, to avoid cross contamination and dilution. Instructions on the use of the pH meter is in the Instrument Instructions section of this lab manual. You can do these measurements after you determine the absorbance spectra, if you are careful in keeping sufficient volume of your solutions to submerge the pH electrode past the reference junction.

Absorbance Measurements

In this experiment you will use an Ocean Optics diode array spectrophotometer. This instrument will allow you to scan the full spectrum of the solution at each pH. You will then be able to verify the existence of an isosbestic point. Instructions for use of the Ocean Optics diode array spectrophotometers are given in the Appendix. You should overlay the spectra to make the isosbestic point easier to see. Scan your spectra from 350-850 nm.

(a) Calibrate with distilled water.

(b) Rinse the cuvette with two small portions of the basic methyl red solution, that is in just sodium acetate, then fill the cuvette and measure its absorbance. This spectrum will be used to calculate ϵ_{1,MR^-} and ϵ_{2,MR^-} .

(c) Rinse the cuvette with two small portions of the acidic methyl red solution, that is in just acetic acid, then fill cuvette and measure its absorbance. This spectrum will be used to calculate $\epsilon_{1,HMR}$ and $\epsilon_{2,HMR}$.

(d) Use the above spectra to pick the two absorbance wavelengths. Measure the absorbance of each of the prepared buffered solutions: rinse the cuvette twice with small amounts of each new each solution, then fill. Change to a new file between each spectrum to overlay the spectra. Measure the spectrum.

Calculations

Calculate the concentration of methyl red in your solutions. Use the spectrum in just sodium acetate to calculate ϵ_{1,MR^-} and ϵ_{2,MR^-} . Use the spectrum in just acetic acid to calculate $\epsilon_{1,HMR}$ and $\epsilon_{2,HMR}$. Use Excel and Eqs. 6 and 7 to determine the concentrations of $[MR^-]$ and $[HMR]$ at each pH. Plot $\log([MR^-]/[HMR])$ versus pH. Fit a straight line to the plot. The intercept of this line with the x axis (not the y axis as usual) corresponds to equal concentrations of the basic and acidic forms of the indicator. From the pH at the x intercept, determine the pK_a' . Also determine the pK_a' from the y intercept. In your report rearrange Eq. 3 to give straight line form with $y = \log([MR^-]/[HMR])$ and prove that the x intercept is pK_a' and the y intercept is $-pK_a'$.

Report

Include an Introduction, Theory, Procedure, Results, and Discussion. In the Introduction, describe the experiment and the expected result in a few sentences. For the Theory section, just reference the write-up, but also do the requested derivation from the Calculations section. In other words, the Theory section is just a reference (e.g.: please see " **pK_a of Methyl Red** " in the CH341 Lab Manual for the theory and procedure) and the short derivation.

For the Procedure section, describe enough of your procedure so that another student could easily repeat your experiments. Tell exactly what you did using explicit volumes, weight, and temperature. Give the manufacturer and model of any major instrumentation (UV-Vis spectrophotometer in this experiment). Use past tense to describe your procedure. Don't copy the procedure from the write-up; state exactly what *you* did.

For the Results section, provide the data in a tabular format, including **all information necessary to repeat your calculations**. Please format your tables in a fashion similar to the literature (i.e. don't attach an Excel spreadsheet). Attach your graph. Graphs should be at least one-third page in size with axes labeled and with units. Slopes and intercepts from curve fitting should always be given with uncertainties. Include the uncertainty for the the pK_a' values propagated from the curve fit values (see the Error Analysis handout for instructions for representing uncertainties). (You do not need to propagate the uncertainties of the volume measurements through to the final results. Just start with the uncertainties in the fit coefficients).

In the Discussion section, comment on the uncertainty of the final results:

1. What is the predominate random experimental error? Note that correctible student mistakes are not random experimental errors. For example, spills or not following the instructions produce systematic errors, so you should not report them as random errors.
2. Which pK_a' is more accurate, and why?
3. Compare your final results to the literature value.
4. Estimate the expected error in the final result based on the measurement errors. Based on your estimate answer the following question. Is the difference between your equilibrium constant and the literature value larger than the technique is capable of? In other words, is there some unaccountable source of error? To help you answer this question, please note the following helpful hint on error propagation for this experiment.

Did you find an isosbestic point? Discuss the importance of finding an isosbestic point.

Finally in the Discussion section, also discuss the chemical significance of the results. The chemical significance can be addressed in several alternate ways:

- State why these results are useful and important, or
- State how this experiment and technique fit into the larger world of chemistry, or
- Discuss why someone might need to do a study of this type.

Estimating the Expected Error in the Final Result Based on the Measurement Errors: You can estimate an upper bound for the expected error in the result by using just one data point and Eq. 3. Least squares curve fitting will give a smaller error, since the result is based on multiple trials, but doing the calculation with just one data point will give an upper bound for the final error. The uncertainty in absorbance measurements is ± 0.002 at best. Since using the Beer-Lambert Law for calculating concentrations involves multiplication and division, the errors in the concentrations

from the absorbances propagate as relative errors (even though we used some matrix tricks in the process). The uncertainty in pH measurements is ± 0.03 , unless extra care is taken. For answering the question “is the difference between your equilibrium constant and the literature value larger than the technique is capable of?” only a rough estimation of the expected error is necessary. A complete and precise propagation of errors treatment is not necessary. You will need to use propagation of errors rules, but focusing only on the major errors with approximate calculations is sufficient.

Literature Cited

1. S. W. Tobey, *J. Chem. Ed.*, **1958**, 35, 514.
2. F. Daniels, J. W. Williams, P. Bender, R. A. Alberty, C. D. Cornwell, J. E. Harriman, “Acid Dissociation Constant of Methyl Red,” in *Experimental Physical Chemistry*, McGraw-Hill, New York, NY, 1970, pp. 113-115.
3. Ramett, R. W., “The Dissociation Quotient of Bromocresol Green,” *J. Chem. Ed.*, **1963**, 40, 252.
4. Ramett, R. W., “Equilibrium Constants from Spectrophotometric Data,” *J. Chem. Ed.*, **1967**, 44, 647.

Appendix: Ocean Optics Spectrophotometers with Vernier Data Acquisition Software Instructions

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. Start the Logger *Pro* 3 software.
2. The spectrophotometer should be automatically recognized by the software showing the colors of the visible spectrum in the data window with Wavelength as the horizontal axis. If the visible spectrum is not displayed, select Connect Interface → Spectrometer → Scan for Spectrometers from the Experiment menu.
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 in the 30 to 2000 range. Use more Samples for more precise absorbances. 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. Click on Close.

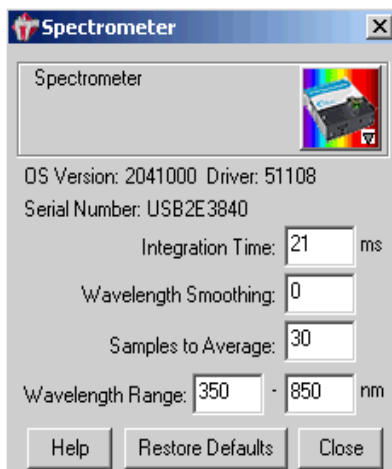


Figure 1. Settings for fast analysis with regular plastic cuvettes or work in the visible range.

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.

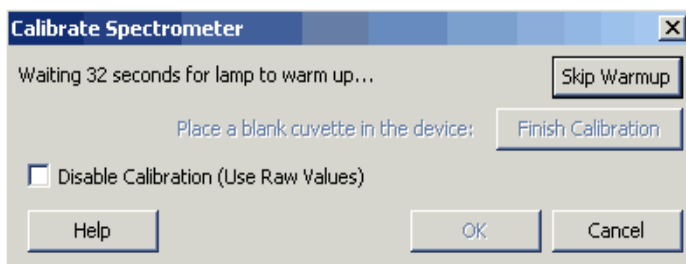





Figure 2. Calibration stores a reference spectrum.

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.

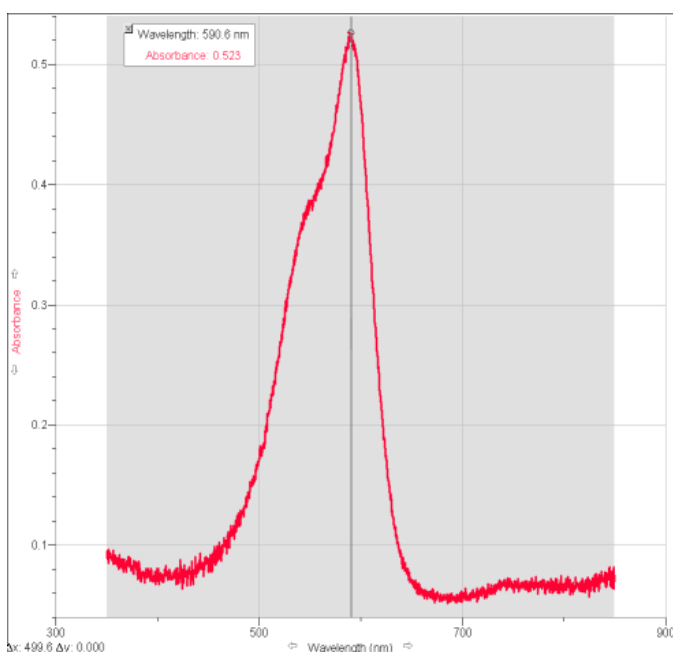



Figure 3. Spectrum of Crystal Violet with the Examine Tool.

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.

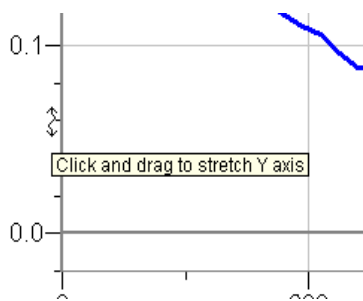


Figure 4. Move the cursor over the axis to change the axis scale.

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.

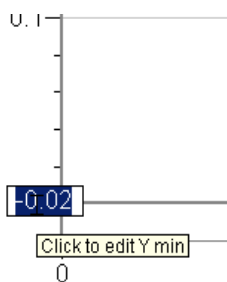


Figure 5. Click near the axis maximum or minimum to show the dialog box.

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:

4. 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.

5. Pull down the Data menu and choose Clear All Data.

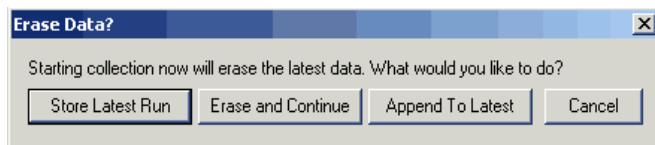
6. Replace the cuvette with another sample. You may need to calibrate again if there is a different reference solvent or buffer for the new sample. Return to step 1.

Overlaid Spectra

4. 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.

5. Replace the cuvette with another sample.

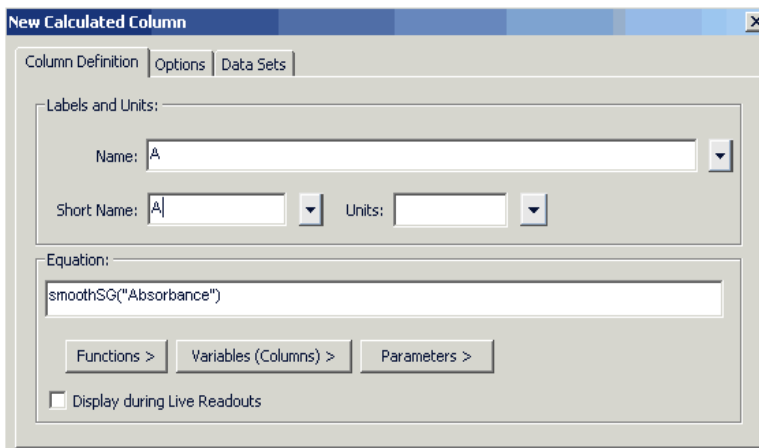
6. When you click on Collect this time, if you did not store the latest run to set-up for overlaid spectra in step 3, you will get a dialog box:



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

7. 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.

8. 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.

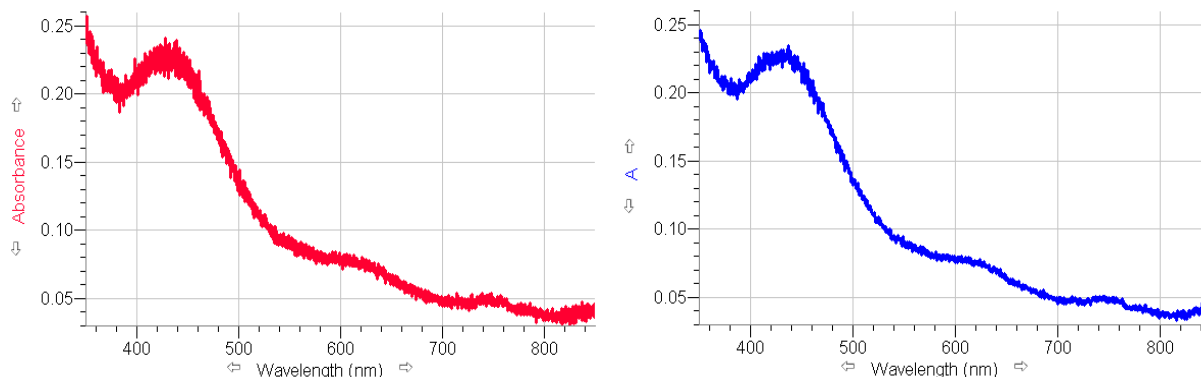


Figure 6. Spectral smoothing decreases the appearance of noise.

III. Finishing up

1. Make sure to rinse your cuvettes three times with reagent grade water. Remember not to use paper towels to clean the glass or plastic surfaces, only use ChemWipes. Don't stick anything sharp into the cuvettes, including a test tube brush.
2. Make sure the area around the spectrometer is clean and dry.
3. Please unplug the spectrometer's transformer. The deuterium lamp in the spectrometer has a limited lifetime and replacements are very expensive.