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Apparent Dissociation Constant of a Porphyrin

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Apparent dissociation constant (K_a) of a Porphyrin

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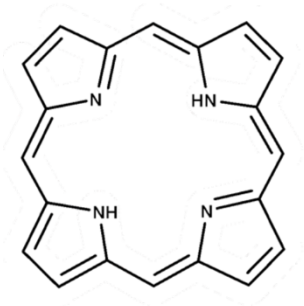
1 Abstract

The purpose of this laboratory is to determine the apparent acid dissociation constant, K_a, by estimating the pK_a value of a porphyrin using visible spectrophotometry. This apparent pK_a value is measured by graphic interpolating the absorbance changes obtained at varied wavelengths for different acid concentrations using the Henderson–Hasselbach equation.

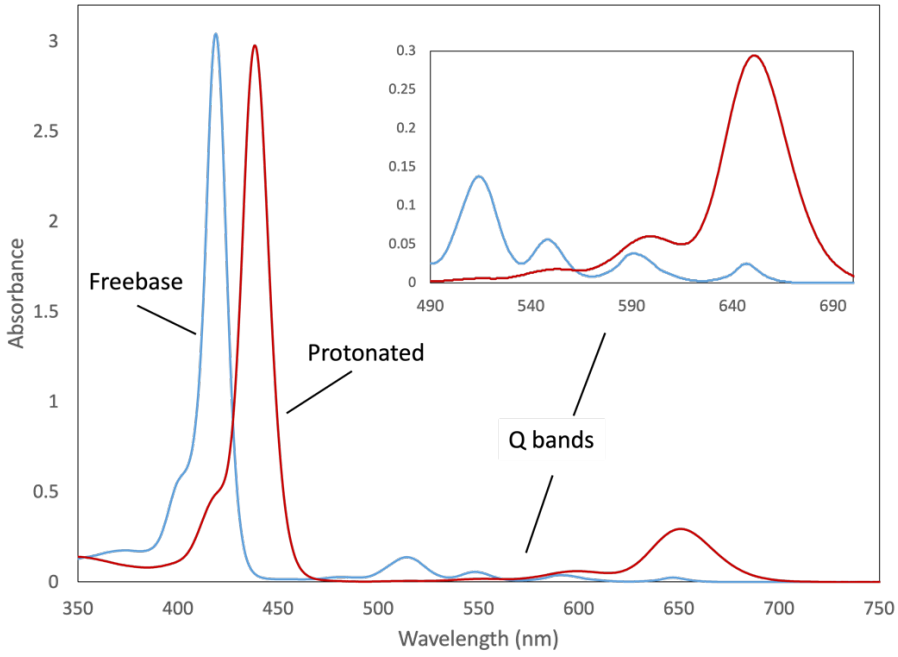
2 Introduction

2.1 The porphyrin Spectrum

A porphyrin is a large ring molecule consisting of four pyrroles, which are smaller rings made from four carbons and one nitrogen. These pyrrole molecules are connected together through a series of single and double bonds which forms the molecule into a large ring. Some porphyrins occur in Nature while others are made in the laboratory through chemical synthesis. Figure 1a shows a free base porphin, characterized by having two hydrogen atoms in the center of the ring. Figure 1b shows the visible absorption spectra, at room temperature, of a freebase porphyrin (blue) and its protonated state (red). The spectrum is characterized by a main peak (soret) followed by a series of smaller peaks (Q bands). These peaks are mainly due to electronic transition and molecular vibrations. They are also dependent on the orientation (polarization) of the electric vector of the absorbed light.



(a)



(b)

Figure 1. Structure of a freebase porphyrin and its spectrum.

2.2 Dissociation Constant

In the Brønsted Acid-Base theory, all acid-base reactions involve the transfer of an H^+ ion (or proton). For example, weak acids can experience a process in which they dissociate into a conjugate base and a proton

$$HA \rightleftharpoons A^- + H^+ \qquad \text{Eq. 1}$$

in which HA represents the acid, H^+ the proton, and A^- the base. A measure of the strength of an acid in a solution is the **dissociation** or **equilibrium constant** (K_a). The dissociation constant can be calculated by the equation:

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad \text{Eq. 2}$$

Thus, at the half equivalence point, when $[A^-]$ is equal $[HA]$, the H^+ molarity is equal to K_a.

Another measure of the strength of an acid was proposed by Sørenson in 1909. It consists in calculating the negative of the logarithm of a quantity. Using this approach, pK_a is the negative of the logarithm of the acid-dissociation equilibrium constant, $pK_a = -\log K_a$. Hence, the "p" stands for "*take the negative of the logarithm of …*". For example, the "p" in pH stands for "take the negative of the logarithm of H^+". Using this approach, Eq. 2 becomes:

$$pK_a = pH - \log_{10}\frac{[A^-]}{[HA]} \qquad \text{Eq. 3}$$

Thus, when $[A^-]$ equals $[HA]$ the solution's pH is equal to pK_a of the HA species.

2.3 Porphyrins

In this experiment you will be using a porphyrin. As a freebase, the un-protonated form of a porphyrin solution (porphyrin + solvent) (P) is more stable. But when acid is added, a new equilibrium is established and the diprotonated state is preferred (P^{2+}). The relation between the concentration of the system components, including the freebase porphyrin, its diprotonated form, and any excessive amount of acid, can been described in terms of *acid–base equilibrium*:

$$P^{2+} \rightleftarrows 2H^+ + P \qquad \text{Eq. 4}$$

We can find the dissociation constant for the protonation of a porphyrin by

$$K_a = \frac{[2H^+]\,[P]}{[P^{2+}]} \qquad \text{Eq. 5}$$

Our goal is to find this *stoichiometric equilibrium* constant, defined in terms of the concentration ratio $[P]/[P^{2+}]$, through a UV-vis spectra analysis.

2.4 Beer's Law

Beer's Law states that the absorption of light in a solution is given by:

$$A = \epsilon \ell [X] \qquad \text{Eq. 6}$$

where A is the *absorbance*, ϵ is the *molar absorption coefficient* ($M^{-1}cm^{-1}$) of the solution, ℓ is the *path length* of the cell (cm) containing the solution, and $[X]$ is the *molar concentration of the solution*. In the case of mixtures, the absorbance is the sum of the separate absorbencies of each species: $A = \epsilon_1 \ell\,[X_1] + \epsilon_2 \ell\,[X_2] + \cdots$.

Consider the case of a freebase porphyrin of concentration $[X]$ that has been protonated by the process of adding an acid gradually. Keep in mind that although the concentration of the overall solution changes, the concentration of the porphyrin remains the same, $[X]$. In Fig. 2 we can see the three snapshots of the process: the blue curve is the spectrum of the freebase porphyrin, the green curve is the spectrum of an arbitrary intermediate stage, and finally the red curve is the spectrum of the protonated stage. From the figure, we can see that the protonated curve has a maximum peak at a wavelength of λ_p. The absorbance of the solution at this point is given by

$$A_p = \epsilon_p\, \ell\, [X] \qquad \text{Eq. 7}$$

where ϵ_p is the molar absorptivity of P^{2+}. At this same wavelength, the freebase porphyrin and the intermediate stage (a combination of the freebase and protonated species) have absorbances of

$$A_{fb} = \epsilon_{fb}\, \ell\, [X] \qquad \text{Eq. 8}$$

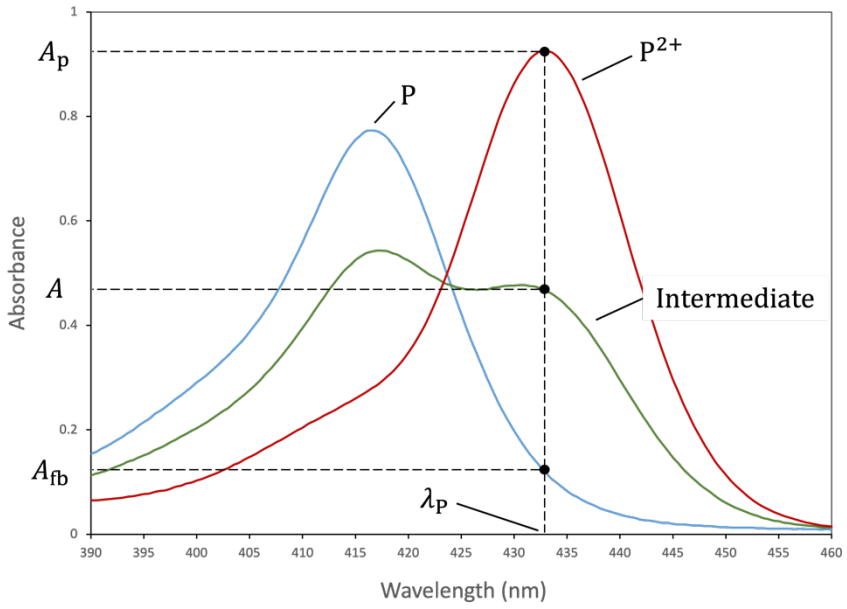


Figure 2. The blue curve represents the spectrum of the unprotonated porphyrin (freebase). The green curve is an intermediate state of protonationn, while the red curve represents the protonated form of the porphyrin.

and

$$A = \epsilon_p \, \ell \, [P^{2+}] + \epsilon_{fb} \, \ell \, [P] \qquad \text{Eq. 9}$$

respectively. In this case, ϵ_{fb} is the molar absorptivity of P. $[P^{2+}]$ and [P] are the concentrations of both species in the intermediate stage, such that the total concentration is given by

$$[X] = [P^{2+}] + [P] \qquad \text{Eq. 10}$$

These equations can be combined to obtain

$$\frac{[P]}{[P^{2+}]} = \frac{A - A_p}{A_{fb} - A} \qquad \text{Eq. 11}$$

3 Method: pK_a calculation

The *acid dissociation constant*, K_a, can be evaluated graphically by converting Eq. (5) into a logarithmic form, known as the Henderson–Hasselbalch equation:

$$\log_{10} K_a = \log_{10}[2H^+] + \log_{10} \frac{[P]}{[P^{2+}]} \qquad \text{Eq. 12}$$

We can know use the negative base-10 logarithm of the acid dissociation constant (K_a) of a solution to define of pK_a as

$$pK_a = -\log_{10} K_a \qquad \text{Eq. 13}$$

Thus

$$-pK_a = \log_{10}[2H^+] + \log_{10} \frac{[P]}{[P^{2+}]}$$

$$\log_{10} \frac{[HP]}{[HP^{2+}]} = -pK_a - \log_{10}[2H^+]$$

Finally, using Eq. (11), we get

$$\log_{10}\left(\frac{A-A_{\mathrm{p}}}{A_{\mathrm{fb}}-A}\right)=-\mathrm{p}K_a-\log_{10}[2\mathrm{H}^+] \qquad \text{Eq. 14}$$

Since we cannot measure $[2\mathrm{H}^+]$ directly, we are only able to find an *apparent* (observed) dissociation constant, $K_{a,\mathrm{obs}}$, for the porphyrin, using

$$\log_{10}\left(\frac{A-A_{\mathrm{p}}}{A_{\mathrm{fb}}-A}\right)=-\mathrm{p}K_{a,\mathrm{obs}}-\log_{10}[\mathrm{Acid}] \qquad \text{Eq. 15}$$

were [Acid] is the concentration of the acid added to the solution, which is only proportional, but not equal, to the proton concentration in solution ($[\mathrm{Acid}]\propto[2\mathrm{H}^+]$). Although it appears that we could get $-\mathrm{p}K_a$ directly by using Eq. 15 and then finding where the straight line intercepts the vertical axis, experimentally that is not the case.

Figure 3 shows a plot of $\log_{10}(A-A_{\mathrm{p}})/(A_{\mathrm{fb}}-A)$ vs. $-\log_{10}[\mathrm{Acid}]$ used to find the apparent value of $\mathrm{p}K_a$. First, the experimental data is fitted to a straight line by using the least-squared linear method. We can now use the fact that when the straight line intercepts the horizontal axis, the value of $\log_{10}(A-A_{\mathrm{p}})/(A_{\mathrm{fb}}-A)$ is zero. Hence, by using Eq. 15 we get $\mathrm{p}K_{a,\mathrm{obs}}=-\log_{10}[\mathrm{Acid}]$. It is interesting to note that at this intercept point $[\mathrm{P}]/[\mathrm{P}^{2+}]=1$, indicating that the concentrations of the two species are equal; this is called the **isosbestic point**.

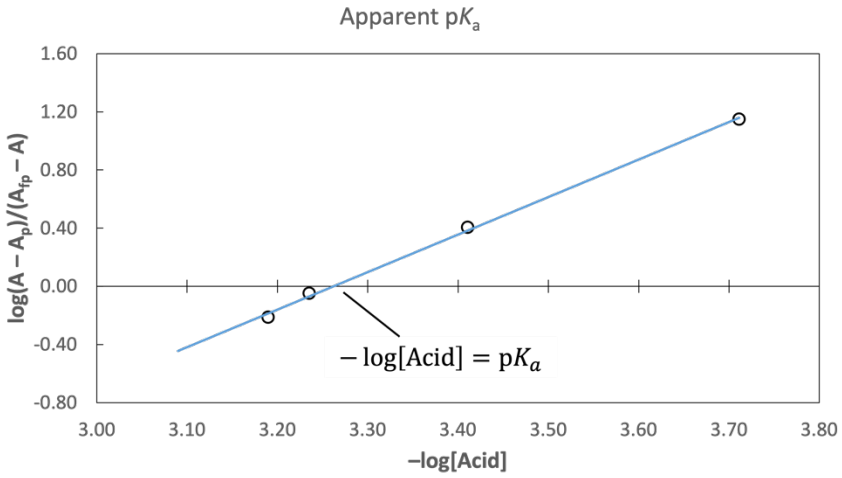


Figure 3. Plot of $\log_{10}(A-A_{\mathrm{p}})/(A_{\mathrm{fb}}-A)$ vs. $-\log_{10}[\mathrm{Acid}]$ leads to the value of the *apparent* or *observed* $\mathrm{p}K_a$.

4 Experimental Procedure

4.1 Before the experiment

1. Set up the spectrometer to a temperature of 25°C. Make all measurements at the same temperature.
2. You should have the following stock solutions available:
   a. A porphyrin in solution with toluene. Write down the name of the porphyrin and its concentration (**about 10^{-5} M**).
   b. TFA solution (**about 10^{-2} M**). Write down its concentration.
   c. HCl solution (**about 10^{-2} M**). Write down its concentration.

4.2 During the experiment

1. Follow the procedures in the annex below to set the zero value and baseline of the spectrometer. Use toluene as the blank.

2. Create a main folder following the format "`porphyrin-solvent-date`". For example, `oetpp-toluene-20220913`. Inside create two folders to store the data: one for `TFA` and one for `HCl`.
3. Prepare a cuvette with 2.0 mL of the porphyrin. This is your freebase. Put it in the spectrometer and get its spectrum. Save it in the TFA folder when it's done with a name such as `tfa-0ul`.
4. Use a pipette to deliver 2 µL of acid (TFA) into the cuvette. Put the sample in the spectrometer and get its spectrum. Save the file when it's done with a name such as `tfa-2ul`.
5. Repeat step #4 until the absorbance of the protonated soret does not change, or there is no more space in the cuvette. Name the files consecutively: `tfa-4ul`, `tfa-6ul`, `tfa-8ul`, etc.
6. Clean the cuvette (or use a new one) and repeat from steps #3 through 5, this time using HCl.

5 Calculations and Analysis.

You should perform the analysis of your data using a spreadsheet, such as MS Excel.

1. Ask your instructor to help you export the data to MS Excel. You should have two data sets of the same porphyrin, one for TFA and one for HCl. The data has information of absorbance vs. wavelength for all the different concentrations of the acid.
2. Make a plot of $\log_{10}(A - A_{\mathrm{p}})/(A_{\mathrm{fb}} - A)$ vs. $-\log_{10}[\mathrm{Acid}]$, as shown in Fig. 2 above.
3. Find the intercept with the horizontal axes and calculate the $\mathrm{p}K_a$ value.
4. When calculating the values of K_a and $\mathrm{p}K_a$ be sure to perform a propagation of errors for each value you calculate. Assume a nominal error of ± 0.5 for each absorbance measurement.
5. 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.

6 References

1. R. J. Sime, Physical Chemistry, Saunders, Philadelphia, PA, 1990; and CH341 Lab Manual, Colby College, Waterville, ME., 2011
2. R. Chang, Physical Chemistry, 2nd edition, Macmillan, 1981, Chapter 12.
3. S. W. Tobey, J. Chem. Ed., 1958, 35, 514.
4. 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.
5. Ramett, R. W., "Equilibrium Constants from Spectrophotometric Data," J. Chem. Ed., 1967, 44, 647.

Introduction

Module Configuration

You will be using the `UVProbe` software to take the data. Of the three measurement modules available, you will be using the `Spectrum module`. You can select the module from the `[Window]` menu (Fig. 1).

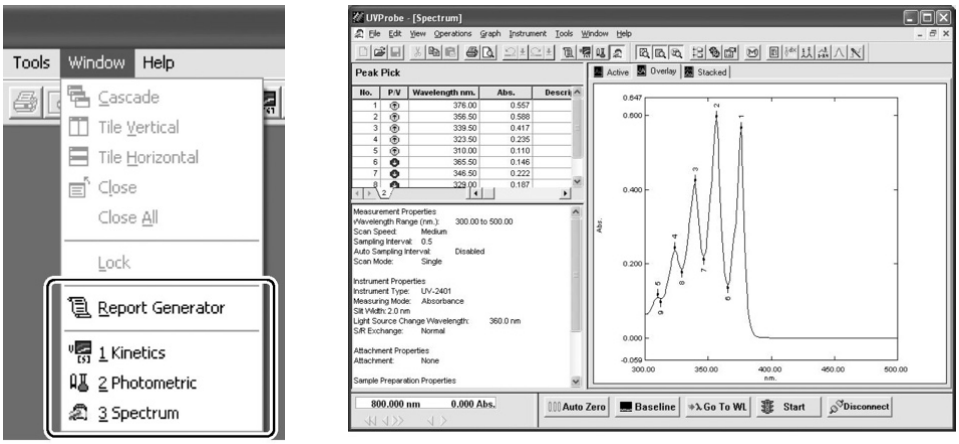
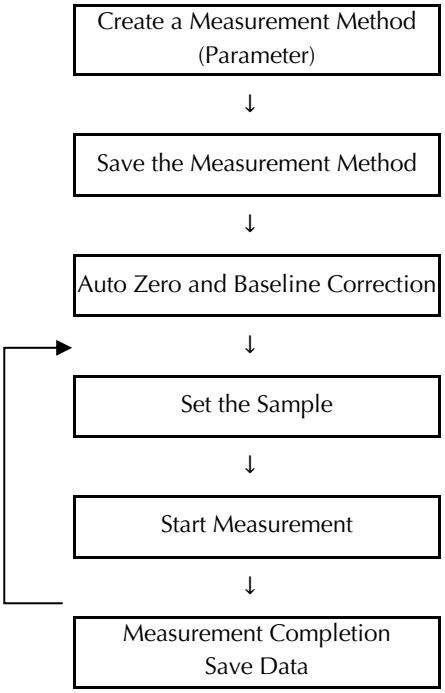


Figure 1. Selecting the Spectrum Module.

Measurement Procedure

When measuring samples in the spectrum module, follow the steps below.



Creating the Measurement Method (Parameter)

1. **Click** [Method] in the [Edit] menu and the [Spectrum Method] window appears.

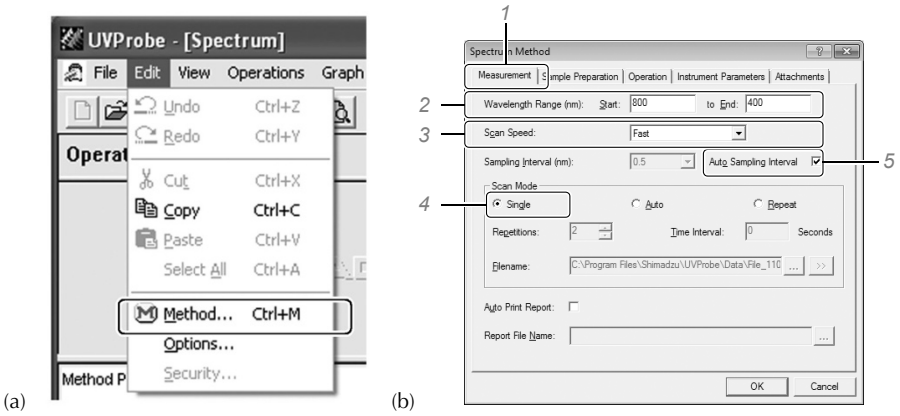


Figure 2. Setting the Measurement Method (Parameter)

No.	Name	Description
1	Wavelength Range	Sets up the wavelength range (nm).
2	Scan Speed	Sets up the wavelength scanning speed. The slower the speed, the more noise can be reduced.
3	Sampling Interval	Sets up the interval between measurement data. You can set it to one of the following intervals: 0.01 nm. 0.05 nm. 0.1 nm. 0.2 nm. 0.5 nm. 1.0 nm. 2.0 nm. or 5.0 nm. **Auto sampling interval**: Automatically sets the interval to the maximum not exceeding 1.800 points within the specified wavelength range.
4	Scan Mode	**Single**: Enter a file name. etc., for each measurement. **Auto**: Measurement takes place using prescribed file names. Use this feature to omit file name input operation. **Repeat**: Measurement takes place repeatedly using prescribed file names. Repetitions: 2 to 100. Time Interval: 0 seconds to 9999 seconds.

Table 1 – Measurement tab

2. **Set the** [Measurement] **tab**

   (1) Click the [Measurement] tab (Fig. 2b).

   (2) Enter the wavelength range (nm). We will be working between 750 nm to 350 nm.

   (3) Use the [▼] key to select [Scan Speed]. Select Fast.

   (4) Select [Single]. The spectrum measurement is performed only once.

   (5) Select a sampling interval of 0.5 nm in the [Sampling Interval] box. Make sure the [Auto Sampling Interval] box is unchecked.

3. **Set the** [Instrument Parameters] **tab**

   (1) Click the [Instrument Parameters] tab (Fig.3).

   (2) Use the [▼] key to select the appropriate measuring mode.

   (3) Use the [▼] key to select the appropriate slit width.

   (4) Click [OK]

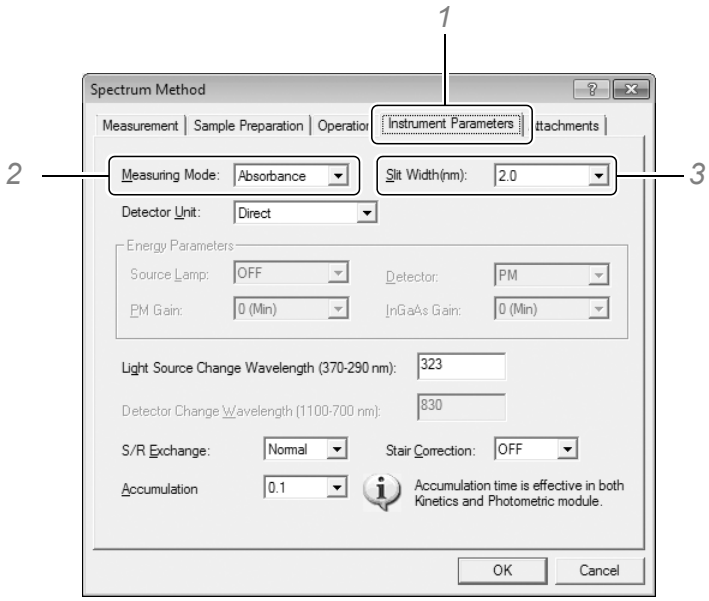


Figure 3. Instrument Parameter.

4. Saving the Measurement Method

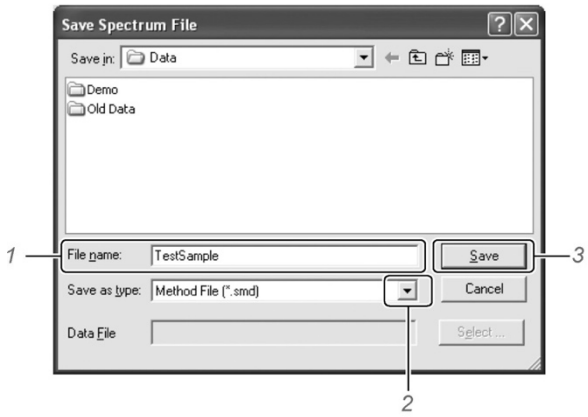


Figure 4. Saving the method.

Click `[Save As]` from the [File] menu. The `[Save Spectrum File]` window appears (Fig.4).

(1) Enter the file name. Make sure you save it in your **external USB stick**.

(2) Click `[▼]` to select `[Method File (*.smd)]` for the file type.

(3) Click `[Save]`.

5. Auto zero and Baseline Correction

The system corrects the baseline so that the 0 (zero) Abs line (100 % line for transmittance or reflectance) is leveled in the specified wavelength range under the current conditions of the sample compartment.

- When correcting the baseline after the instrument has not been used for a long period of time, turn on the power and wait for about one hour until the spectrophotometer enters a stable status.
- When measurement conditions are changed, be sure to perform the baseline correction.
- The default correction wavelength range that appears for baseline correction is the measurement wavelength range set in the Measurement Method window.

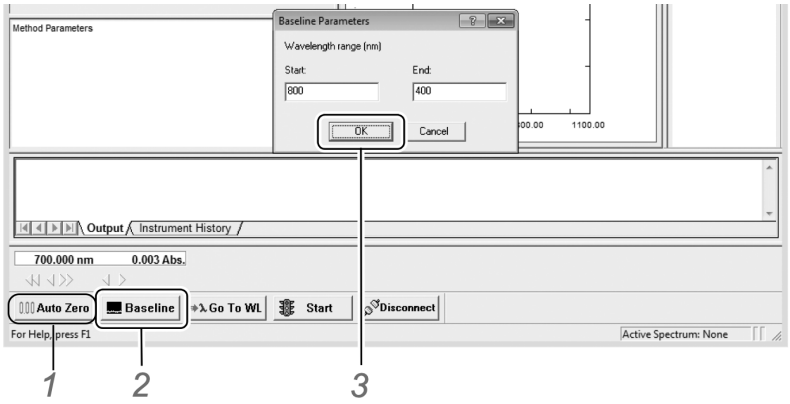


Figure 5. Auto zero and baseline.

(1) Open the sample compartment cover of the spectrophotometer and insert two cuvettes of solvent (blanks) in the cell holders (Fig.6). Click on `[Auto zero]` to zero the instrument at the starting wavelength (Fig.5).

(2) Click `[Baseline]` on the `[Instrument Control Button]` bar to zero the baseline over the spectrum region of interest. The `[Baseline Parameters]` window appears.

(3) Verify that the displayed correction range is the same as the wavelength range specified in the `[Spectrum Method]` and click the `[OK]` button.

- Baseline correction starts. Do not open the sample compartment cover until it is completed.
- The spectrophotometer's status is displayed in the Instrument Status window.
- After the baseline correction is finished, it returns to the baseline start wavelength and is ready to measure.
- Open the sample compartment cover and remove the sample cuvette.

6. Set the Sample

(1) Prepare the sample. Fill out the cuvette up to ¾ of its volume.

(2) Open the sample compartment cover of the spectrophotometer and insert the cell containing the sample into the cell holder on the sample side.

(3) Close the sample compartment cover.

(4) The **rear holder** is the reference compartment, and the **front holder** is for the sample. The optical sides of the cuvettes need to be facing the sides.

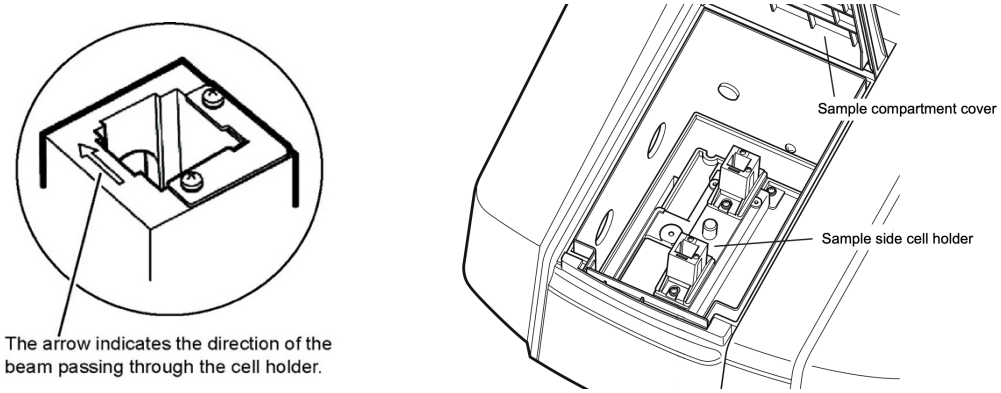


Figure 6. Setting the sample.

7. Start Measurement

- Click `[Start]` on the `[Instrument Control Button]` bar. The Measurement begins.

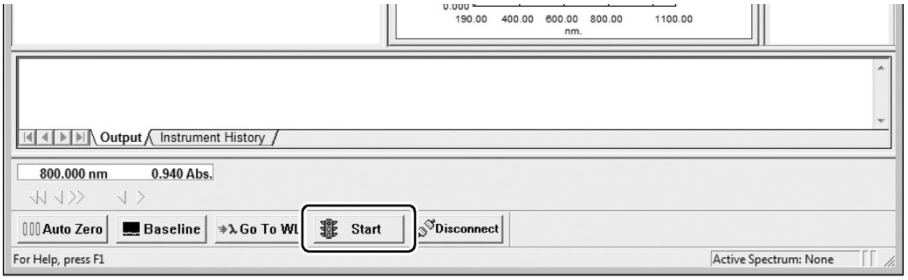
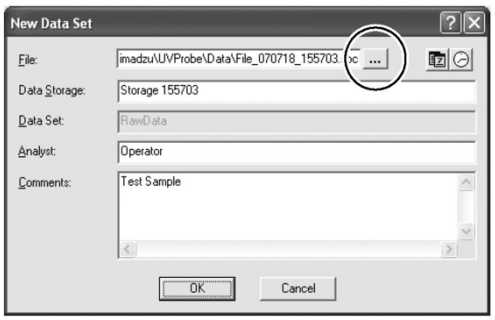


Figure 7. Start a measurement.

8. Measurement Completion/Save Data

(a)



(b)

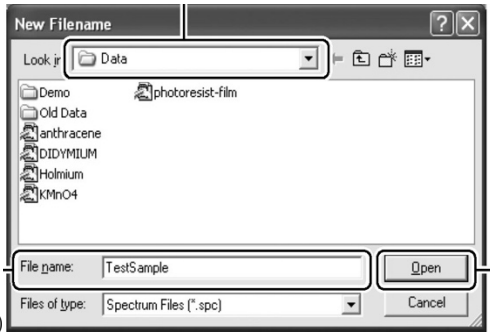


Figure 8. Saving data.

When measurement is complete, the `[New Data Set]` window appears (Fig. 8a).

a) Click the `Browse` (`...`) button (Fig. 8a) and the `[New Filename]` window appears (Fig. 8b).

b) If this is the first measurement of a sequence (the freebase porphyrin spectrum), create a folder on your external USB stick following the format "**`porphyrin-solvent-date`**". For example, say you used OETPP in water on September 13, 2022, then your folder should be named: `OETPP-water-20220913`.

c) For subsequent measurements, select the destination folder where you want to save the data file. Use the "**`acid name-concentration`**" format for these. For example, for TFA, starting with the freebase (concentration 0 μL) and making 2 μL increments the file names will be (note that we use ul for μL):

```
tfa-00ul
tfa-02ul
tfa-04ul ...
```

d) After entering the filename, click `[Open]`. The `[New Filename]` window closes, and then the folder path name and the file name appear in the `[File]` field of the `[New Data Set]` window.

e) Confirm that the correct path name and file name are displayed in the `[File]` field of the `[New Data Set]` window and click the `[OK]` button.

f) At this point, data has been collected and named, but the data is stored in memory only; it is not saved to disk. If you close UVProbe at this point the **data would be lost**.

g) Click `[Save]` from the `[File]` menu to save the data.

h) Select the appropriate data directory in the `[Save In]` box at the top of the dialog box.

i) Enter the same name you used in step (d) and select "`.spc`" in the `[Save As Type]` list.

j) Click `[Save]`.

Graph Settings

Change Graph Scales

You can change graph axis scales directly on the graph. Click the upper (or lower) limit value of the axis scale.

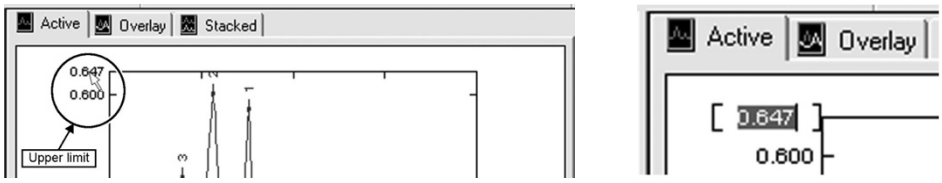


Figure 9. Graph scales.

- When the upper (or lower) limit value is highlighted, input the desired value, and press the key.
- Using a similar procedure, change the upper (or lower) limit of the other axis scale.

Change Graph Display Setting

From `[Customize]` on the graph shortcut menu, you can control the width and colors of graph lines as well as the format and size of label fonts.

- Right-click in the graph area and click `[Customize]` from the shown menu. The `[Customize Graph]` window appears.
- You can change the Graph Line Colors, Graph Line Width, and the Axis Label Font.

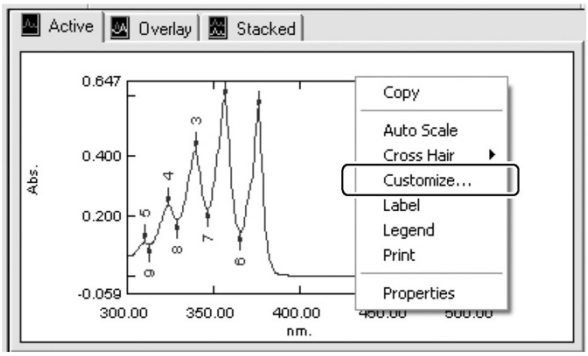


Figure 10. Customize display settings.

Quick Guide - Shimadzu UV-2600 Spectrometer

1. Turn on the UV-2600 spectrometer as soon as you get in the lab. This will give it enough time to warm it up.
2. Clean the cells; rinse with distilled water and let dry. Handle cells by the fogged sides, not the clear sides. Always use the same cell for the reference.
3. Open the UVProbe software. Click on the Connect icon on the bottom menu of the initial screen. The system will go through a series of instrument checks. Wait until this is completed. All bullets should be green.
4. Select the Spectrum Mode and click on the Method Manager (or Ctrl+M).
   - Click the [Measurement] tab.
     - Enter a wavelength range (nm) of 750 nm to 350 nm.
     - Select Fast in [Scan Speed].
     - Select [Single].
     - Select 0.5 nm in [Sampling Interval]. Make sure the [Auto Sampling Interval] box is unchecked.
   - Click the [Instrument Parameters] tab.
     - Select Absorbance in the measuring mode.
   - Click [OK]
   - You can save this method for later use.
5. Insert two cuvettes filled with solvent into the spectrometer. Make sure that there are no air bubbles and wipe the optical faces of the cuvettes with a tissue. The **rear holder** is the reference compartment, and the **front holder** is for the sample. The optical sides of the cuvettes need to be facing the sides.
6. Click on [Auto zero] and then select [Baseline]. Ensure the wavelengths are correct, then click [OK]. A baseline will be recorded.
7. Remove the sample cuvette of solvent.
8. Insert a sample to measure. Press [Start] and the spectrum will be recorded. You should see the line being plotted from right to left.
9. When the scan is completed, the [File Name Dialog] box will appear. In the [Enter File Name] box type in the file name (use the convention). In the [Enter Comment] box write the name of the porphyrin, the solvent used, and the name an amount of acid used.
10. Click (Browse ...) button to open the [New Filename] window. If this is the first measurement of a sequence, create a folder on your external USB stick and name it or select the destination folder for subsequent measurements.
11. Click [Open]. The [New Filename] window closes, and then the folder path name and the file name appear in the [File] field of the [New Data Set] window. Click the [OK] button.
12. Click [Save] from the [File] menu to save the data and select the appropriate data directory in the [Save In] box at the top of the dialog box. Enter the same name you used in step (10) and select ".spc". Click [Save].
13. You will repeat steps 9 to 13 for all the other samples.
14. Remove your sample and reference. Rinse the cells and let them dry
15. Turn off the spectrometer and quit the software. Don't forget to save any files left.