Experiment 7
pKa of Methyl Red
by UV-vis Spectroscopy

UV-vis spectroscopy

Figure 1 shows the electromagnetic spectrum in which ultraviolet and visible radiation comprise only a small part of it.

The energy (E) associated with electromagnetic radiation is defined by the following equation:

$$E = h\nu$$

where: h = 6.22 10^{-34} Js is Planck's constant, and ν is frequency (in Hertz).

Electromagnetic radiation can be considered as a combination of alternating electric and magnetic fields that travel through space with a wave motion. Because radiation acts as a wave, it can be described in terms of either wavelength or frequency, which is related by the following equation:

$$\nu = \frac{c}{\lambda}$$

where: ν is frequency (in Hertz), c = 3·10^8 ms is the speed of light, and λ is wavelength (in meters). In ultraviolet–visible (UV–vis) spectroscopy, wavelength usually is expressed in nanometers (1nm=10^{-9}m).

It follows from the above equations that radiation with shorter wavelength has higher energy. In some cases, this energy is sufficient to cause unwanted photochemical reactions when measuring sample spectra (remember, it is the UV component of light that causes sunburn).

When radiation interacts with matter, a number of processes can occur, including reflection, scattering, absorption, fluorescence/phosphorescence (absorption and reemission), and photochemical reaction (absorbance and bond breaking). In general, when measuring UV-visible spectra, we are only interested in the absorption of the light by a sample material.

Because light is a form of energy, absorption of light by matter causes the energy content of the molecule (or atoms) to increase. The total potential energy of a molecule generally is represented as a sum of its electronic, vibrational, and rotational energies:

$$E_{\text{total}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}}$$

The amount of energy a molecule possesses in each form is not continuum but a series of discrete levels or states. The differences in energy among the different states are in the order:

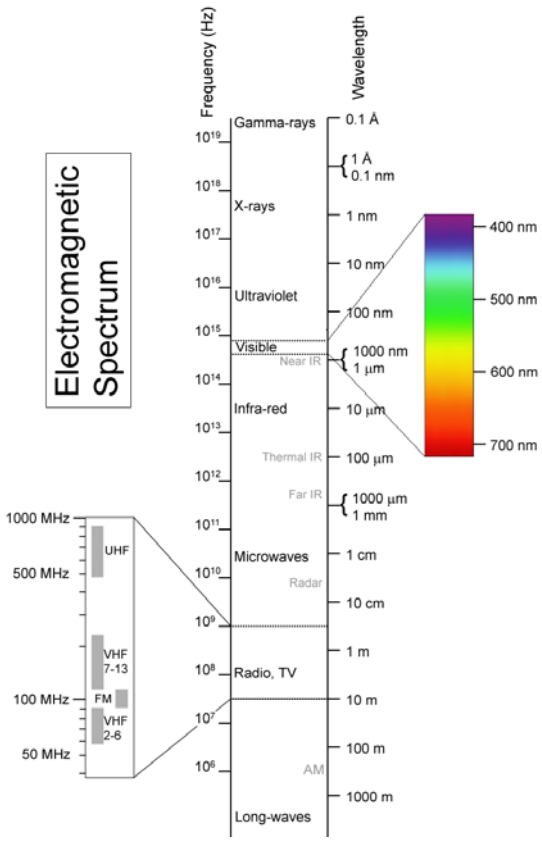

Figure 1. The electromagnetic spectrum.

$$E_{\text{electronic}} > E_{\text{vibrational}} > E_{\text{rotational}}$$

In some molecules and atoms, photons of UV and visible light have enough energy to cause transition between the different electronic energy levels. The wavelength of light absorbed corresponds to the energy difference between the two levels involved in a transition.

When light passes through or is reflected from a sample, the amount of light absorbed is the difference between the incident radiation (I_0) and the transmitted radiation (I). The amount of light absorbed is expressed as either transmittance or absorbance. Transmittance usually is given in terms of a fraction of 1 or as a percentage and is defined as follows:

$$T = \frac{I}{I_0} \quad or \quad \%T = \left(\frac{I}{I_0}\right) \times 100$$

Absorbance is defined as follows:

$$A = -\log T$$

For most applications, absorbance values are used since the relationship between absorbance and both concentration and path length normally is linear.

Acid dissociation constant from absorption spectra

Methyl red (4-dimethylaminobenzene-2'-carboxylic acid) is a commonly used indicator for acid-base titrations. In this experiment you will measure the absorption spectra of the acidic and basic forms of this compound. Next, you will prepare a series of buffered solutions of methyl red at known pH. By following the change in absorbance as a function of pH it is possible to determine the acid dissociation constant, or pKa. This technique 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 and the basic form is designated as MR^- (Figure 2).

If the equilibrium is written as

$$HMR + H_2O \leftrightarrows MR^- + H_3O^+ \qquad 1$$

the equilibrium constant is the acid dissociation constant

$$K_a' = \frac{[H_3O^+][MR^-]}{[HMR]} \qquad 2$$

The K_a' is the effective or conditional equilibrium constant since the concentrations rather than activities

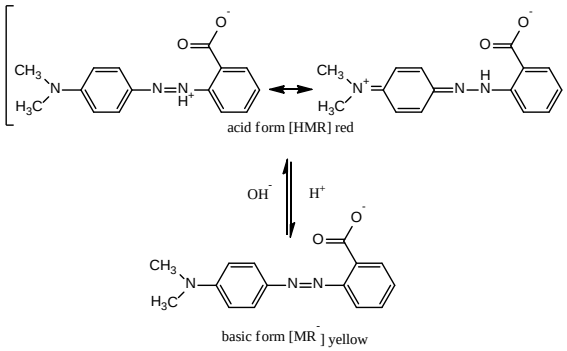

Figure 2. Acid and base forms of methyl red.

have been used in equation 2. Taking the –log of both sides of equation 2 gives:

$$pK_a' = pH - \log\frac{[MR^-]}{[HMR]} \qquad 3$$

From the above equation (3) it can be seen that the pK_a' can be determined by varying the pH and measuring the ratio $\frac{[MR^-]}{[HMR]}$.

The absorption of light is govern by the Beer-Lambert law

$$A = abc \qquad 4$$

where: A is the absorbance, c is the concentration of the absorbing species in moles per liter, b is the path length of the cell in centimeters, and a is the molar absorption coefficient. Since absorbance is dimensionless. It follows that the units of absorption coefficient are liters per moles per centimeter. 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

$$A = A_{[MR^-]} + A_{[HMR]} \qquad 5$$

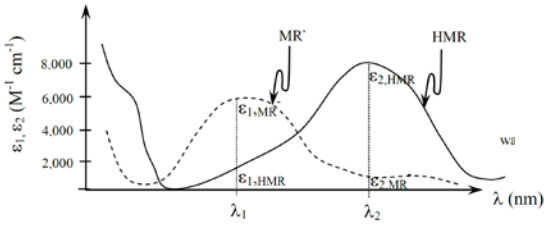

Figure 3. Absorbance of a solution is the sum of the absorbances of the constituents. Measurements at two wavelengths are necessary to determine the composition of a two-constituent solution if the absorbance bands overlap. The first subscript indexes the wavelength and the second subscript indexes the constituent.

The absorption spectra of HMR and MR^- are given schematically in Figure 3. 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 3 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:

$$A_1 = a_{1,[MR^-]}b[MR^-] + a_{1,[HMR]}b[HMR]$$
$$A_2 = a_{2,[MR^-]}b[MR^-] + a_{2,[HMR]}b[HMR] \quad (6)$$

The molar absorption coefficients are illustrated in Figure 3. The molar absorbance coefficients are determined from standard solutions that contain one component alone. Equation 6 provides two equations in two unknowns. For an unknown solution, the absorbances at the two wavelengths, A_1 and A_2, are determined and then Equation 6 are solved for the unknown concentrations [MR^-] and [HMR] at each given pH.

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 3 shows two isosbestic points. You will use your spectra to determine if there are only two absorbing species in this experiment.

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 pKa' 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

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 2 mL of ~0.1 M NaOH and mix thoroughly, add more drop-by-drop until all the solid dissolves, and then dilute to the mark with distilled water. 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. 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 0.1 M acetic acid solution (17.4 M glacial acetic acid will be provided with which to make the dilute 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.

Buffer Solutions. Prepare five buffer solutions with a total volume of 20 mL each, by mixing X mL of the basic solution with (20-X) mL of the acidic solution in small beakers. A range from 10 mL to 18 mL for X 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. 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 HP 8453 diode array spectrophotometer. Instructions how to use this spectrophotometer are given in the Appendix A. 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. The spectra will automatically overlay to make the isosbestic point easier to see. Scan your spectra from 350-850 nm.

1. Use distilled water for the reference.
2. 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.
3. 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.
4. 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 solution, and then fill. Measure the spectrum. Annotate the peaks to get the absorbance values for each buffer at both of the maximum absorbance wavelengths of the acid and base.

Calculations

Calculate the concentration of methyl red in your solutions. Use the spectrum in just sodium acetate to calculate $a_{1,[MR^-]}$ and $a_{2,[MR^-]}$. Use the spectrum in just acetic acid to calculate $a_{1,[HMR]}$ and $a_{2,[HMR]}$. Use equation 6 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 corresponds to equal concentrations of the basic and acidic forms of the indicator. From the pH at the x intercept, determine the pKa'. Also use the least-square fit to determine the pKa' from the y intercept (hint: Use the Henderson-Hasselbalch relationship $pH = \log\frac{[MR^-]}{[HMR]} + pK_a$)

Discussion

Did you find an isosbestic point? Discuss the importance of finding an isosbestic point. If the molar absorptivities of MR^- and HMR were determined at λ corresponding to an isosbestic point, how would they compare in value? What factors could contribute to the inability of the operator to obtain an isosbestic point in a study of a system of this nature? Comment on the number of absorbing species in the solutions with different pH.

Compare your final pKa' with the literature value of 5.1 for methyl red.

Additional Reading

Refer to text, **Principles of Instrumental Analysis** by Douglas Skoog, 5th ed. Saunders, Philadelphia (1998), Chapters 13 & 14, for more details on the theory of UV/Vis technique and its applications.

Appendix A

Follow the steps below to acquire spectra on the HP Agilent 8453 UV-vis spectrophotometer (Figure 4). The optical diagram of the spectrophotometer is shown below (Figure 5). If you have any questions or doubts, contact your TA or lab coordinator.

1. Power up the HP Vectra computer if it is off.
2. Power up the HP Agilent 8453 UV-vis spectrophotometer.
3. Wait until the green light appears on the HP Agilent 8453 UV-vis spectrophotometer, then double click the **"UV-VIS Instrument 2 online"** icon. There is no password, just click "cancel". Make sure the lamps are turned on (as shown by the lit up lamp figures in the spectrometer picture on the bottom-left corner of the screen), if not, click the lamps and click "lamp on".
4. Click **"method"** on the top menu bar and select **"Spectrum/Peaks Parameters"**, uncheck both **"find and annotate up to 3 peaks"** and **"find and annotate up to 3 valleys"**, check **"prompt for sample information"**, select data type **absorption**, and enter the desired wavelength range. Consider using **350-850 nm** initially. Then click **"OK"**.
5. Fill the UV cell with reference solution (distilled water in this experiment), wipe the cell with kimwipes, and place in the clamp (UV instrument).
6. Click on the **"Blank"** button in the software program to collect a background spectrum.
7. Replace the blank solution with the sample solution and click the **"Sample"** button in the software to collect the sample spectrum. Always wipe the UV cell before placing into the clamp.
8. Type in a brief description of the sample when the sample information window appears.
9. If you need to collect spectra of other solutions and your reference (blank) solution remains the same just replace the solution with new one and click on **"Sample"**. Remember to rinse the cuvette with the new solution and to wipe it with kimwipes.
10. You can rescale the displayed window by left clicking and holding while you drag the mouse pointer over the window. Double left click to reset the display if needed.
11. Click on **"Config"** on the top menu bar, then **"Report"**. Type in a brief description of your sample and approximate concentration and click **"OK"**, leaving the page set up parameters as they appear.
12. Click **"Method"** on the top menu bar and select **"Fixed Wavelengths"**. Type in the wavelengths of max absorption for the pure acid and base spectra and click **"OK".**
13. Left click once in the **"Sample Spectra"** window to be sure it is highlighted. Use **"File"**, then **"Print"**, then **"Results"** to get a hardcopy of selected window.

Once finished, be sure to do the following:

1. Exit the UV instrument online software (must be done before switching the instrument off).
2. Turn off the instrument.
3. Do not switch off the computer.

Figure 4. UV-vis spectrophotometer.

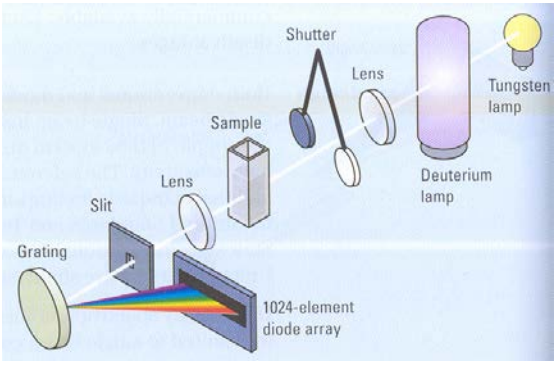

Figure 5. Optical diagram of the HP 8453 diode array spectrophotometer.