CHEM 335: Physical Biochemistry Lab
pKa of a dye: UV-VIS Spectroscopy
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ABSTRACT

Using UV-VIS spectroscopy, absorbance of different pH solutions around pH 4 were taken while using pH 2 and pH 10 as a baseline for correction. The aim for the experiment was to solve for the pKa of the indicator that was used for the solutions and to do so using two different methods to compare their reliability. One method was plotting the absorbance vs. the pH and to find the inflection point that will point to the pKa value. The second method was to plot log[In-/HIn] vs. pH to find the intercepts, which will be equal to the pKa value. The pKa value that was obtained using the first method was 4.2 and 4.02 using wavelength 435nm and 590nm respectively. The pKa value that was obtained using the second method was 4.25. Overall, it seemed that the second method was more reliable. Further experiments should be done to see if there are more reliable or easier ways to solve for the pKa of an indicator.

INTRODUCTION

UV-VIS spectroscopy is a method which uses light with wavelengths ranging from 400 to 780nm. It is in this region of energy space that molecules undergo electronic transitions.[1] By bombarding atoms or molecules with radiation, it causes the "redirection of the radiation and/or the transition between energy levels of the atoms or molecules."[2] The transition with transfer of energy is absorption. Absorption is proportional to the concentration of a compound in a solution. The Beer-Lambert Law gives the relationship between absorbance and concentration. "The analytical utilization of atomic absorption rests on the principle that the total absorption of light, expressed as log I_0 / I**,** is directly proportional to the concentration and absorptivity."[3]

The Beer-Lambert Law states that the log of the ratio of the intensities is equal to the negative of the absorptivity coefficient (-ε) times the concentration (C) multiplied by the path length (b).

$$\log [I / I_0] = - \varepsilon C b \quad (1)$$

This equation is due to the fact that if light is passed through a sample, some of it will be absorbed and some will be let through (transmitted). Therefore, there are 2 different intensities. I_0 is the original intensity, meaning the one that was sent into the solution. I is the intensity of the light exiting the sample. This is often referred to as transmittance. Therefore, the equation for transmittance is

$$T = [I / I_0] \quad (2)$$

This is what defines absorption, which is the following equation:

$$A = -\log[T] = \varepsilon C b \quad (3)$$

By using UV-VIS spectroscopy, we can determine the pKa of an indicator, which are weak acids or bases. Our indicator has a protonated and deprotonated form, which absorbs differently at different wavelengths. Since they absorb differently, the absorbance spectra can be used to determine the value of the pKa. "The pK, values can be calculated in terms of the overall [H+]…[or in terms of the absorbance in the presence of the protonated or deprotonated form]."[4] Spectras of solutions at pH 2 and 10 were to be taken

to determine where the peaks will be. Those will be the absorbance wavelength that will be used in this experiment.

Our experiment revolves around trying to solve for the pKa of an indicator by using UV-VIS spectroscopy. Solutions with slight pH differences were to be made and have the indicator added into the solution. Using the absorbance, there are two methods to go solve for the pKa. The first is graphically plot the absorbance of the indicator at a wavelength (λ_1) against the pH solutions that will be prepared. After correcting the absorbance values that were obtained, the inflection point was to be found. The corresponding pH at that point would be the pKa value. This is also done with the absorbance of the indicator at another wavelength (λ_2).

The second method would be to solve for the ratio of the [In-] : [HIn] and then apply using Beer-Lambert's Law in conjunction with the Henderson-Hasselbalch equation to solve for the pKa. The Henderson-Hasselbalch equation can be written in this form:

$$\text{Log [In- / HIn]} = \text{pH - pKa} \quad (4)$$

Thus, by graphing log of the concentrations of the protonated and deprotonated forms of the indicator, we can find the value of the pKa using the intercepts on the graph.

The aim for this experiment is to find the pKa of the indicator used in a pH buffered solution and to see which of the two methods is more reliable.

MATERIALS AND METHOD

Stock solutions of pH 4, pH 2, and pH 10 were obtained. Using a pH indicator, 4 individual solutions that are slightly more acidic and 4 solutions that are slightly more basic than pH 4 were made. This was done by adding drops of acid or base to a portion of the pH 4 stock solution. The difference should not be greater than 1. From these solutions, 3mL was taken and placed into a small test tube. Also, in addition to the solutions, 1mL of an indicator was added. From each of those test tubes with different solutions, around 2mL was taken and placed into a cuvette. Using a UV-VIS spectrophotometer, the absorbance was measured for each solution. The blank that was used was a buffer solution. The data was recorded for the two peaks in absorbance from the spectra obtained. What were recorded were the two maximum absorbance values and their corresponding wavelength (λ), which was around 435nm and 590nm.

RESULTS

Data from UV-VIS Spectroscopy:

Table 1. Data table of λ values and their corresponding absorbance.

pH	λ (nm)	Absorbance
2	435.469	0.054
	590	0
3.2	435.4	0.059
	590.3073	0.029
3.6	435.2349	0.0545616
	590.1566	0.06799
3.4	435.4	0.0585

3.8	435.2359	0.04931
	590.1566	0.09462058
4	435.2349	0.03577
	590.1566	0.129184
4.25	435.2349	0.02738643
	590.1566	0.148853
4.32	435.2349	0.02215453
	590.1566	0.1613375
4.5	435.2349	0.01443309
	590.1566	0.18025143
4.66	435.2349	0.00869336
	590.1566	0.1915218
~10	435.2349	-0.0033752
	590.1566	0.2649229

Table 2. Corrected data for absorbance taken at 435nm by subtracting the absorbance from the minimum (background) absorbance measured.

pH	λ (nm)	Absorbance
2	435.469	0.0573752
3.2	435.4	0.0623752
3.6	435.2349	0.0579368
3.4	435.4	0.0618752
3.8	435.2359	0.0526852
4	435.2349	0.0391452
4.25	435.2349	0.03076163
4.32	435.2349	0.02552973
4.5	435.2349	0.01780829
4.66	435.2349	0.01206856
10	435.2349	0

Table 3. Corrected data for absorbance taken at 590nm by subtracting the absorbance from the minimum (background) absorbance measured.

pH	λ (nm)	Absorbance
2	590	0
3.2	590.3073	0.029
3.6	590.1566	0.06799
3.4	590.3	0.0477
3.8	590.1566	0.09462058
4	590.1566	0.129184
4.25	590.1566	0.148853
4.32	590.1566	0.1613375
4.5	590.1566	0.18025143
4.66	590.1566	0.1915218
10	590.1566	0.2649229

pKa of Indicator:
435nm:

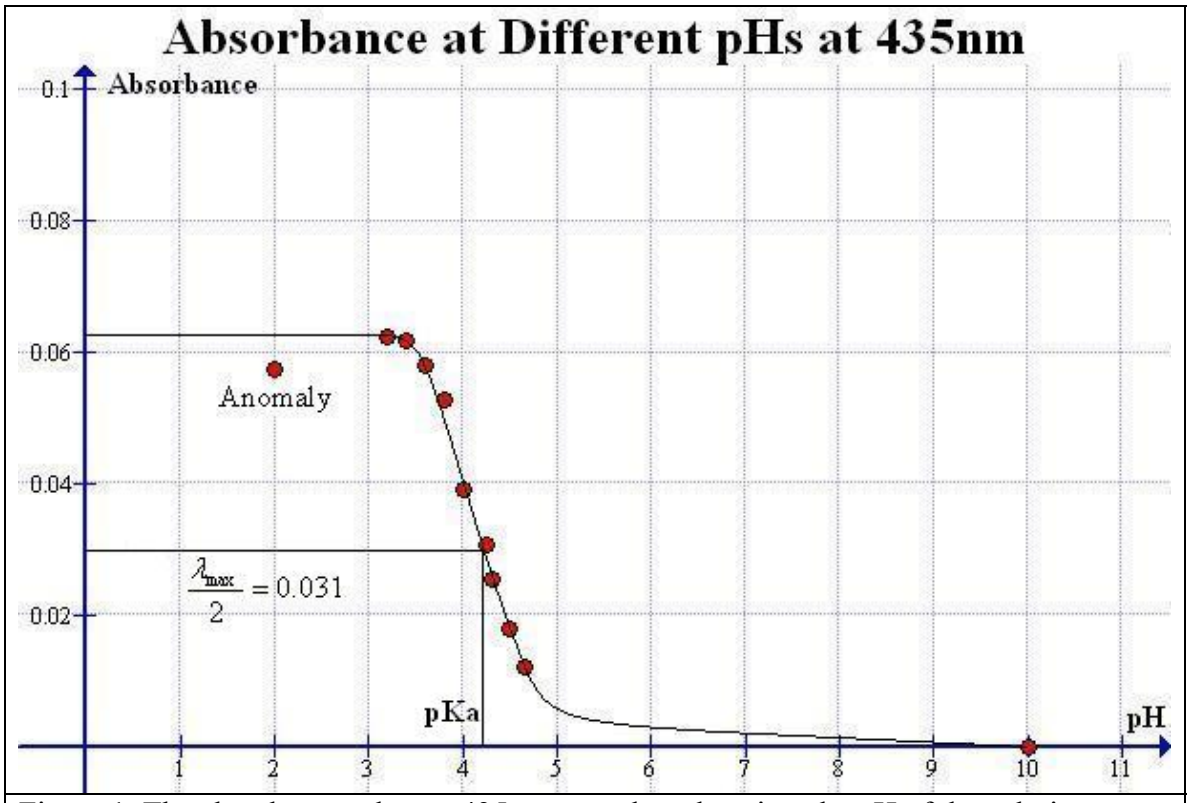

Figure 1. The absorbance taken at 435nm was plotted against the pH of the solution. From this, we can calculate pKa, which corresponds to the pH value at half the maximum absorbance. The pKa calculated from this graph is 4.2.

$$\frac{\lambda_{max}}{2} = 0.031$$

The pKa of the Indicator was found to be 4.2 by using Figure.

590nm:

$$\frac{\lambda_{max}}{2} = 0.132$$

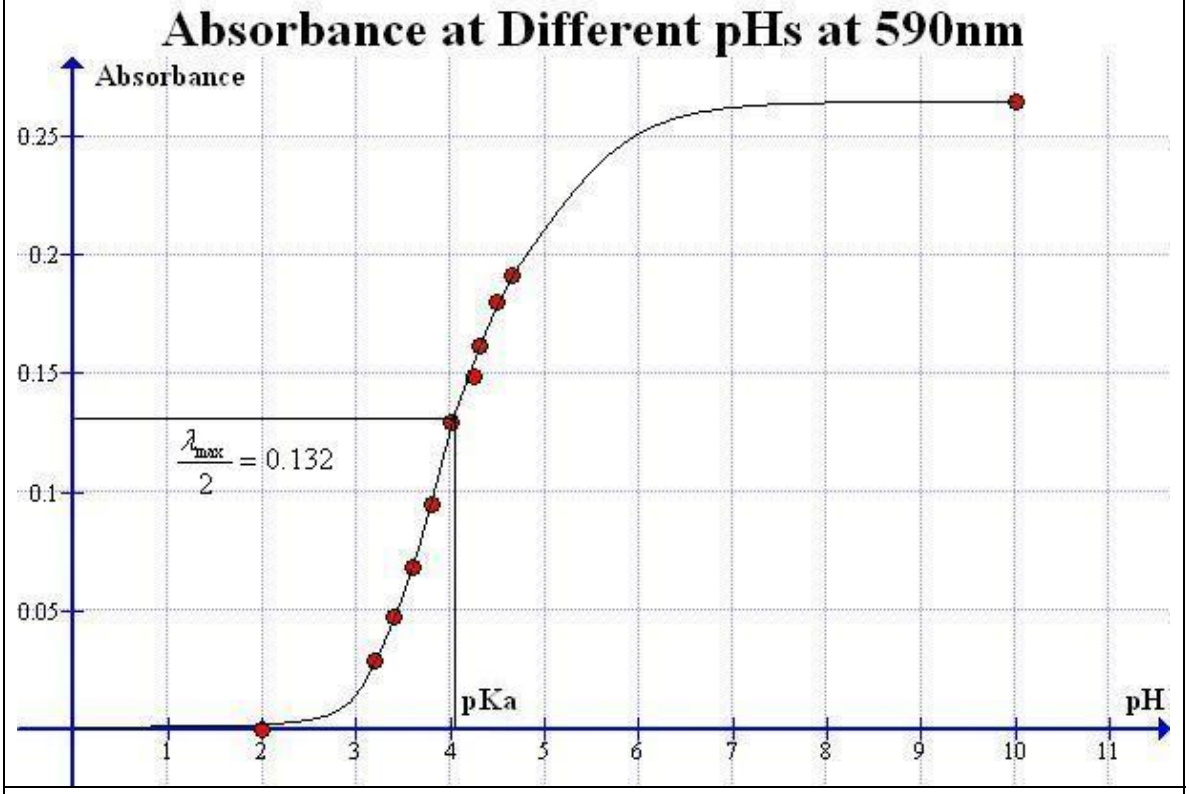

Figure 2. The absorbance taken at 590nm was plotted against the pH of the solution. From this, we can calculate pKa, which corresponds to the pH value at half the maximum absorbance. The pKa calculated from this graph is 4.05.

The pKa of the Indicator was found to be 4.05 by using Figure.

Logarithm of ratio of In^- and HIn in solution:

Table 4. Logarithm of the ratio of In^- to HIn in solution for solutions at different pHs.

pH	[In-]/[Hin]	log([In-]/[Hin])
3.2	0.10018905	-0.999179754
3.4	0.1662022	-0.779363228
3.6	0.25400656	-0.595155061
3.8	0.37806847	-0.422429546
4	0.73612336	-0.133049401
4.25	1.10790685	0.044503247
4.32	1.48441757	0.171556085
4.5	2.54563029	0.405795331
4.66	4.49063552	0.652307807

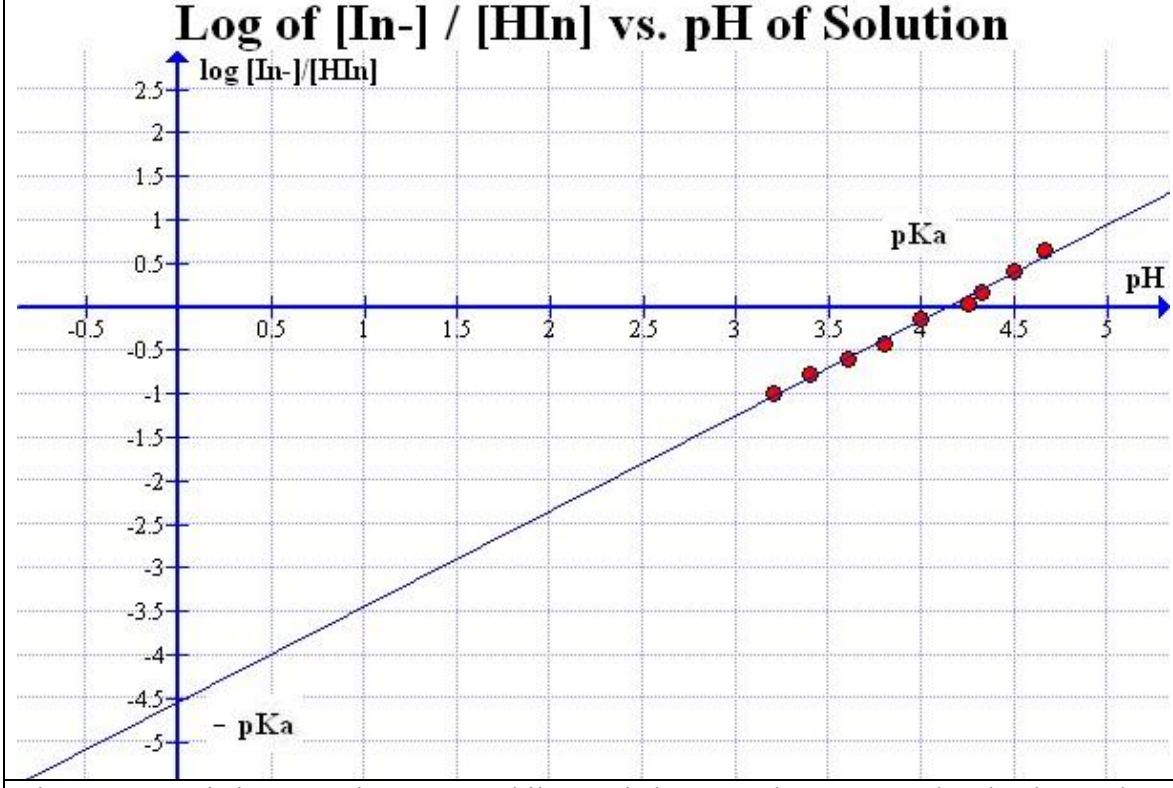

Figure 3. X-axis intercept is at 4.25, while y-axis intercept is at -4.25. The absolute value of these numbers is the pKa of the indicator.

DISCUSSION

In this experiment, we obtained spectra at two different wavelengths. At 435nm, the absorbance was greatest at pH 2. This meant that at this wavelength, the protonated form is dominant in the solution. At 590nm, the absorbance was the greatest at pH 10, meaning that the deprotonated was dominant in the solution. The values that were obtained were recorded in table 1. The corrected values were recorded in table 2 and 3. The corrected values were obtained by subtraction of the acidic minimum from the basic spectra, and vice versa.

Using the absorbance we obtained from UV-VIS spectroscopy, we were able to determine the pKa of the Indicator. Using the absorbance spectra obtained at 435nm (figure 1), the pKa was determined graphically to be 4.2. Using the spectra obtained at 590nm (figure 2), the pKa was determined graphically to be 4.02. The λ_{95} value (error) for those values is ±0.127.

An alternate method to determine the pKa was to plot the logarithm of the ratio of [In-]:[HIn] against the pH of the solution. The ratio of the concentrations of In- and HIn is determined by Beer-Lambert's Law. The pKa obtained from the graph 4.25 and -4.25, which is different from the values obtained from the absorbance spectra in figure 3.

These values The pKa is the absolute value of the x or y intercept, in the case that the y intercept is negative. Therefore, the absolute pKa value obtained from the x intercept should be equal to the absolute value of the pKa value obtained from the y intercept. Because of this, the pKa of the indicator that was calcaluated using figure 3 is 4.25. This differs from values calculated using figure 1 and 2.

The average pKa value of the indicator is 4.166. The λ_{95} value is equal to ±0.104. Thus, the results that were obtained in this lab seem to be accurate and plausible.

In conclusion, it seems that both methods to determine the pKa of the indicator rely on the absorbance of the different pH solutions. When comparing the results, the second method seems to be the better choice. The first method has a problem, where the baseline has to be subtracted and the graph needs to be arranged in such a way as to be able to see the full shape. Also, sometimes the inflection point is not very easy to find. The second method, however, uses Beer-Lambert's Law and calculates the ration of the concentrations of In- to HIn using the absorbance. The log of the calculated values can be plotted against the pH to obtain the pKa using Henderson-Hasselbalch equation. Therefore, you can check to see if the values that were obtained were incorrect because if the x intercept is not equal to the negative value of the y intercept, then the data is unreliable. Future applications and directions of experimentation may to experiment on other ways to obtain the pKa using more effective and reliable means and to diminish the possibility of error in the experiments.

Bibliography

[1] "UV-Visible Spectroscopy" URL: http://en.wikipedia.org/wiki/UV/VIS_spectroscopy> (Retrieved Apr 19 2007)

[2] MERCURY'S HELP DESK | UV-VIS SPECTROMETRY < http://jr.stryker.tripod.com/physchem/pka.html> (Retrieved Apr 19 2007)

[3] Keiichiro Fuwa, B. L. Valle. "The Physical Basis of Analytical Atomic Absorption Spectrometry. The Pertinence of the Beer-Lambert Law." Anal. Chem.; 1963; 35(8); 942-946.

[4] Mukerjee, Pasupati and Banerjee, Kalyan. "A Study of the Surface pH of Micelles Using Solubilized Indicator Dyes" *J. Phys. Chem.*, 68, 12, 3567 - 3574, 1964

APPENDIX: Baseline Spectra

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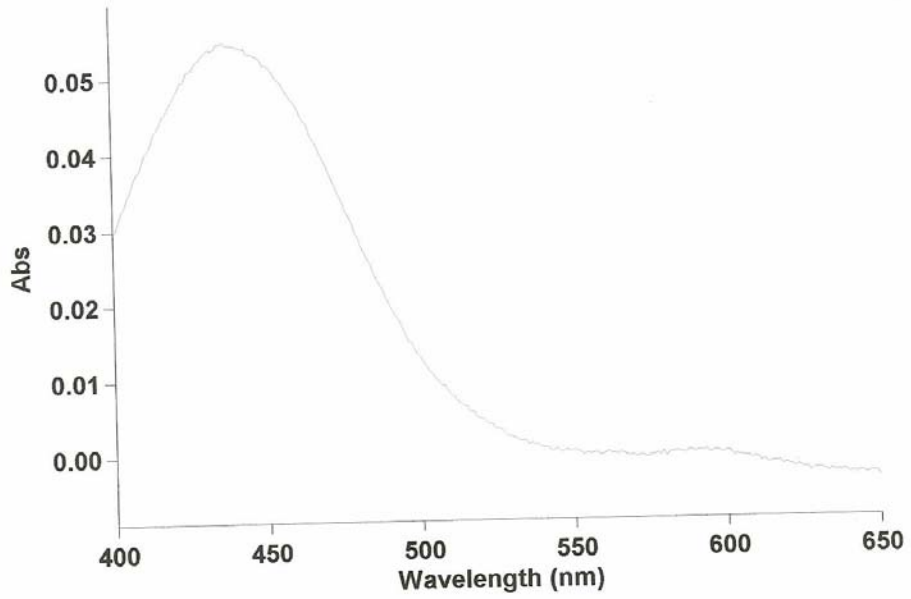
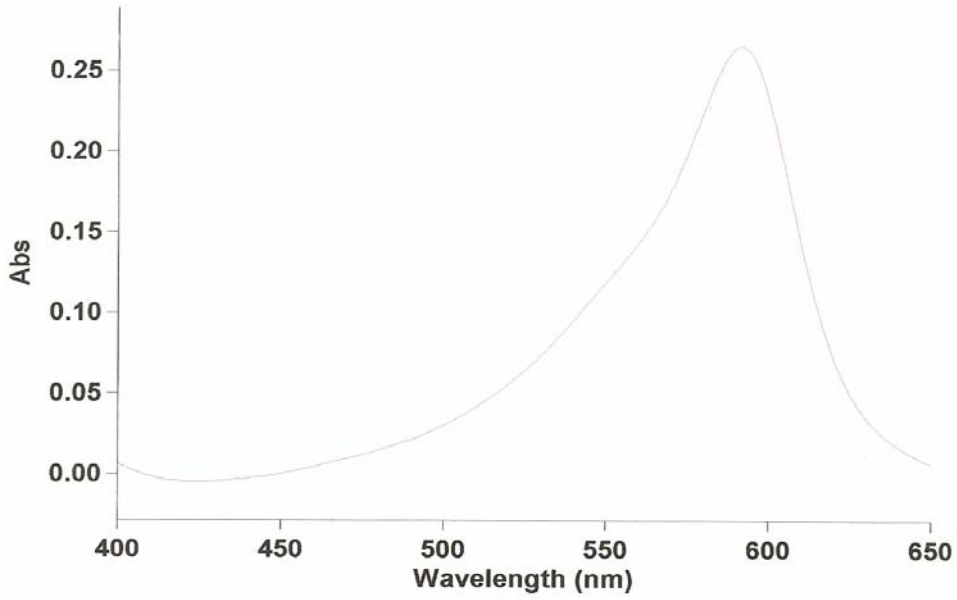


pKa of a Dye

Sample:
pH 2 solution

Instrument Serial Number EL01023883
Instrument Serial Number EL01023883



pKa of a Dye

Sample:
pH 10 solution

RAW DATA

Spectra Taken at 435nm and 590nm.
The following absorbances were taken at those points:

pH	λ (nm)	Absorbance
2	435.469	0.054
	590	0
3.2	435.4	0.059
	590.3073	0.029
3.6	435.2349	0.0545616
	590.1566	0.06799
3.4	435.4	0.0585
	590.3	0.0477
3.8	435.2359	0.04931
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