LABORATORY SESSION 2

Spectrophotometric determination of the pK of an indicator

Material

2 x 500 mL volumetric flasks
5 x 25 mL volumetric flasks
3 x 250 mL Erlenmeyer flasks
2 x 100 mL beakers
1 x 25 mL graduated pipette
1 x 10 mL volumetric pipette
1 x 10 mL graduated pipette
1 x 2 mL graduated pipette
1 x 50 mL burette
1 spectrophotometer
1 glass rod / 1 weight funnel / 1 dropper / 1 funnel
4 spectrophotometer cuvettes
1 dropping pipette /1 wash-bottle

Products

Sodium hydroxide
Sodium hydroxide solution, 2M
Methyl orange solution, 0.002% (m/V)
Hydrochloric acid
Formic acid
Phenolphthalein indicator

Objectives

1. To obtain the absorption spectrum of methyl orange at different pHs.
2. To validate the Lambert-Beer law (Absorbance vs [Methyl orange])
3. To determine the molar absorption coefficients of Methyl orange.
4. To locate the isosbestic point.
5. To prepare buffer solutions from formic acid and NaOH and obtain their pHs.
6. To determine the pK_a of methyl orange from the absorbance measurements.

Theoretical basics

In general, acid-base indicators can be considered compounds (**weak** acids or bases) whose acidic form (protonated) in solution has a different color from its basic form (deprotonated). The change in structure, which causes the change in color, takes place in a small pH range (1–2 units of pK around the pK of the indicator), which is called the "*pH turning range*". In this interval, both forms of the indicator (acidic and basic) are simultaneously present.

The ionization balance of an indicator can be expressed by the equation:

$$HIn + H_2O \xleftrightarrow{K_{HIn}} In^- + H_3O^+ \qquad (1)$$

where HIn represents the acidic form of the indicator molecule and In^- represents its basic form. The apparent ionization constant (as a function of concentration) is expressed as:

$$K_{HIn} = \frac{[H_3O^+][In^-]}{[HIn]} \qquad (2)$$

The solutions are assumed to be sufficiently diluted that all the activity coefficients are very close to unity. Under these conditions, the **Henderson-Hasselbalch** equation is applicable. Using logarithms in equation 2:

$$pH = pK_{HIn} + \log\frac{[In^-]}{[HIn]} \qquad (3)$$

If we know the [In -] / [HIn] ratio for a given pH, we can determine the pK of the indicator.

In our case, i.e. determination of the pK of methyl orange, the concentration quotient is calculated from the absorbances obtained by recording the electronic absorption spectrum of a series of solutions utilizing molecular absorption spectroscopy (UV-Vis spectroscopy) in the visible region.

When monochromatic light (photons with a determined wave length) passes through a solution which contains a substance with a chromophore functional group, part of the radiation can be absorbed for the atoms of this functional group. This process (absorption of monochromatic electromagnetic radiation) is regulated by the Lambert-Beer law.

According to the lambert law, the intensity of light beam that goes through the solution decreases as passes through due to the fact that the photons are absorbed by the chromophore groups that the beam encounters on its way. Therefore, the absorbance is proportional to the length of the sample that the light beam passes through (optic path).

According to the Beer law, the intensity of light beam that goes through the solution decreases as the quantity of the absorbent substance (in solution) increases due to the fact that the probability of the phonon absorption by the chromophore groups is higher. Therefore, the absorbance is proportional to the concentration of the sample.

The combination of both laws results in the Lambert-Beer law:

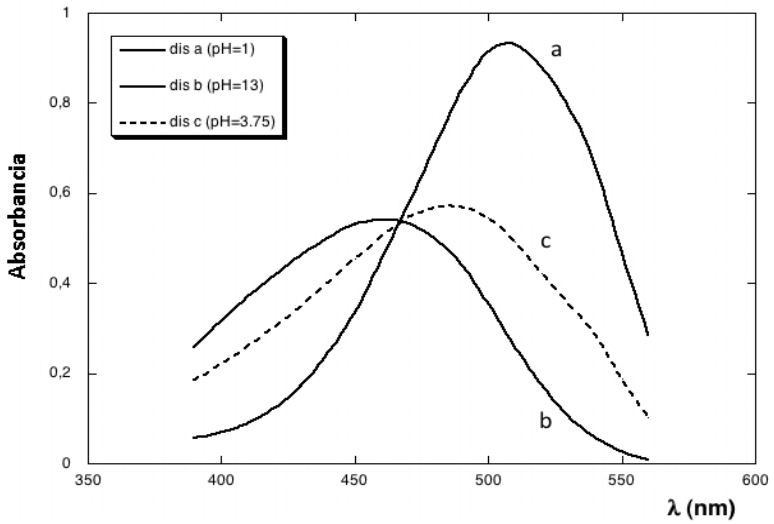
$$A^{\lambda} = \varepsilon^{\lambda} l \; c \qquad (4)$$

Where, A^{λ}, is the average of the absorbance that the equipment measures at one particular wave length (λ), ε^{λ} is the coefficient of molar absorption at that particular wave length, which indicates the phonon absorption probability of the substance (in $M^{-1}cm^{-1}$), l is the optic path (distance that the light beam goes across the sample) delimited by the cuvette width and c is the concentration of the sample.

The figure shows an example of the absorbance spectra that can be obtained in this experiment

- **a)** The absorbance spectrum of a solution exclusively containing the indicator in its red acidic form, HIn, for which the pH of the solution must be very low, i.e. pH≈1.
- **b)** The absorbance spectrum of a solution exclusively containing the indicator in its yellow basic form, In^-, for which the pH of the solution must be very basic, i.e. pH≈13.
- **c)** The absorbance spectrum of solutions simultaneously containing both forms of the indicator, which is achieved with buffer solutions whose pH lies in the interval 3.2-4.4, where the color of the methyl orange indicator changes.

The figure below shows an example of the recorded spectra for these solutions.



Using Beer-Lambert's law, we can express the ratio of concentrations in equation 3 in terms of the methyl orange absorbance for each solution. For the same wavelength, λ:

pH = 1 $$A_{HIn} = \varepsilon_{HIn} \, l \, c_0 \quad (5)$$

pH = 13 $$A_{In^-} = \varepsilon_{In^-} \, l \, c_0 \quad (6)$$

pH = 3.8 $$A = \varepsilon_{HIn} \, l \, [HIn] + \varepsilon_{In^-} \, l \, [In^-] \quad (7)$$

where A_{HIn} and A_{In^-} represent, respectively, the absorbance of a solution in which only one form of the indicator (acidic or basic) is present; "A" is the absorbance of the solution in which both forms of the indicator coexist (in the buffer solution); ε_{HIn} and ε_{In^-} represent the molar absorption coefficients of the acidic and basic forms of the indicator, respectively; and l is the optical path (width of the cuvette). Also, in the buffer solutions it is true that $c_0 = [In^-] + [HIn]$.

Finally, from equations (5) to (7) we can deduce:

$$pH = pK_{HIn} + \log\left(\frac{A_{HIn} - A}{A - A_{In^-}}\right) \quad (8)$$

Solutions

1. 500 mL of 0.1 M formic acid solution, from the commercial product (in fume hoods).
2. 500 mL of 0.1 M NaOH solution, from solid NaOH.

Experimental procedure

1) Connect the spectrophotometer as soon as the session begins, or at least 15 minutes before measuring.

2) Prepare the formic acid and sodium hydroxide solutions.

3) Titrate 25 mL of 0.1 M formic acid solution with NaOH 0.1 M, using phenolphthalein as the indicator. Repeat the titration at least three times.

Note: Please remember that there are containers to throw away the waste.

4) Record the absorption spectra of the methyl orange solutions:

- Mostly the acid form of the indicator
- Mostly the basic form of the indicator
- Both forms, acid and basic, in similar proportions; this is obtained using buffer solutions.

A) RECORDING OF THE SPECTRA IN WHICH ONLY IS PRESENT (MOSTLY) ONE THE FORMS OF THE INDICATOR (ACID OR BASIC)

It will be recorded the absorbance of the acid form, $\mathbf{A_{Hin}}$, and basic form, $\mathbf{A_{In^-}}$, as a function of the concentration of the indicator. This will allow us to validate the Lambert-Beer law and to determine the molar absorption coefficients of both forms, acid and basic.

To do this, several solutions will be prepared at different concentrations of the indicator in which the predominant form will be the acid form (pH ~1) or the basic form (pH ~13) and the absorbance of these solutions will be recorded.

Preparation of the methyl orange and blank solutions

a) Blank preparation

Only one blank will be prepared (acid or basic) to record the absorbance of all the solutions where the indicator is present (mostly) in the acid form (or basic).

Pour into a 25 mL volumetric flask the following components in the order that appears below:

1. A small amount of deionized water.
2. 4 drops of concentrated HCl (acid form) or 24 drops of NaOH 2M (basic form).
3. Water up to the etched line.

With this solution **rinse** and fill the spectrophotometer cuvette.

b)Problem methyl orange solution

Pour into a 25 mL volumetric flask, the following components in the order that appears below:

1. The volume of the 0.002% methyl orange solution indicated in table 1 (measured with a pipette.
2. 4 drops of concentrated HCl (acid form) or 24 drops of NaOH 2M (basic form).
3. Water up to the etched line.

With this solution **rinse** and fill the spectrophotometer cuvette.

Table 1. Volume of methyl orange, NaOH, HCl and water utilized in the preparation of the methyl orange solutions when mostly it is present the acid and basic forms of the indicator.

Form of MO	V(MO) mL	V(HCl conc.) drops	V(NaOH 2M) drops	V(H_2O)
HIn	**10**	**4**	**--**	**Make the volume**
HIn	**8**	**4**	**--**	**Make the volume**
HIn	**6**	**4**	**--**	**Make the volume**
HIn	**4**	**4**	**--**	**Make the volume**
In^-	**10**	**--**	**24**	**Make the volume**
In^-	**8**	**--**	**24**	**Make the volume**
In^-	**6**	**---**	**24**	**Make the volume**
In^-	**4**	**---**	**24**	**Make the volume**

Reordering of the absorbance

After preparing all the solutions (methyl orange and the blanks), the absorbance is recorded as a function of the wavelength following the guidelines of the spectrophotometer **Jemway73**. The initial and final wavelength is **350 nm and 600nm** respectively.

The name of the files should be saved in a clear and systematic way. As an example you can follow the following rule: NAME_A_V and NAME_B_V, where NAME means the name of the members of the group, A and B mean acid or basic and V is the volume of methyl orange utilized in the preparation of the solutions listed in Table 1.

Notes and precautions

- The spectrophotometer cell must be clean: do not touch its walls with your fingers. Also, fill it to ¾ of its capacity only (do not fill it to the brim).
- For each media, acid or basic, the base line is the same. Therefore, once the absorbance of the first solution of each media is recorded, introduce the next sample in the spectrophotometer and press repeat in the software of the apparatus Jenway73.

B) RECORDING OF THE SPECTRA OF THE SOLUTIONS WITH SIMILAR QUATITIES OF BOTH FOMS OF THE INDICATOR.

To make sure that both forms of the indicator are in similar quantities, the solutions should be prepared in a media in which the pH is close to the pK of the indicator. These pHs can be obtained preparing the solutions in a buffer a media. In this case the buffer will be based on a weak acid (formic acid) that the pK is very close to the methyl orange. Therefore, $pH \sim pK_{HFor} \sim pK_{HIn.}$

It will be prepared 5 buffer solutions recording the spectrum of each solution.

a) Preparation of the buffer solutions

The Buffer solutions are prepared in a 250 mL Erlenmeyer flask neutralizing (partially) the volume of formic acid partially titrated.

Pour into a 250 mL Erlenmeyer flask 25 mL of formic acid, **measured with a pipette** and add from the **burette** the volume of NaOH for each buffer solution shown in table 2.

Prior to the preparation of the buffer solutions determine the volumes of NaOH shown in table 2 that correspond to the percentages of the equivalence volume found in the titration of formic acid. Complete this table in the laboratory notebook. Please calculate the theoretical pH of each solution.

b) Preparation of the methyl orange and blank solutions

For each buffer solution, pour into a 25mL flask the following components for the blank and problem solutions in the order indicated below.

a) Blank

- 10 mL of water (measured with a pipette)
- Make the volume of the 25 mL volumetric flask with the buffer solution.

With this solution rinse and fill the spectrophotometer cuvette.

b) Problem methyl orange solution

- Pipette 10 mL of 0.002 % methyl orange solution into the 25 mL volumetric flask,
- Make the volume of the 25 mL volumetric flask with the buffer solution.

With this solution rinse and fill the spectrophotometer cuvette.

c) Reordering of the spectrum

After preparing all the solutions (methyl orange and the blank), the absorbance is recorded as a function of the wave length following the guidelines of the spectrophotometer Jemway73 (steps 5-11)

The name of the files should be saved in a clear and systematic way. As an example you can follow the following rule: NAME_B_N (1-5) where N is related to the buffer used (table 2)

Note: Once the experience is finished, make sure that you have saved all the data and turn off the equipment following the instructions.

Table 2. Percentage of the volume of NaOH at the equivalence point. Volume of NaOH utilized in the preparation of the buffer solution and pH of the buffer solution.

Buffer	Percentage V_{Equi}	$V_{NaOH\ (mL)}$	pH
1	20		3.15
2	30		3.38
3	40		3.57
4	50		3.75
5	60		3.93

Experimental results: presentation of data

1. Tabulate the data (masses or volumes) needed to prepare solutions 1 and 2 (both the calculated and the actual amounts).
2. In another table tabulate the results of the titration of formic acid with sodium hydroxide (volume of NaOH). Also calculate its average value (together with its random error).
3. Create a table with the volumes of the indicator highlighted in table 1 and their molar concentration.
4. Include in the notebook the table 2 with each NaOH volume that corresponds to each percentage of the equivalence point.
5. Include in tables, the reordered absorbance of the acid and basic form, as a function of the wavelength for the different concentrations of the indicator.
6. Include in tables the absorbance recorded as a function of the wavelength for the buffer solutions (same concentration of indicator).

Treatment and discussion of results

1. Create a table with the absorbance as a function of the wavelength of the basic form (A_{In^-}; pH = 13) y acid form (A_{HIn}; pH = 1) for the solutions with different concentration of indicator.

   Plot and analyze the spectra.
2. Create a table with the absorbance as function of the wavelength of the basic form (AIn-; pH = 13) y acid form (AHIn; pH = 1) for the buffer solutions, A, with the same concentration of indicator V(NM) = 10 mL. Plot and analyse the spectra.

3. Find the wavelengh of the isosbestic point, λ_{Isosb} and demostrate that in this particular point the coefficient of molar absorption of both forms of the indicator has the same value.

4. Validate the Lambert-Beer law.

   If the Lambert-Beer law is correct, it should be observed a linear behaviour between the absorbabce and the concentration, the slope for an optic path of 1 cm and the concentration expressed in molarity gives the molar absorption coefficient at one particular wavelength, ε^{λ}.

$$A^{\lambda} = \varepsilon^{\lambda}\, l\, c$$

   For both forms of the indicator, acid and basic, create a table with its absorbance, at the wavelength of its maximum of absorption, $A_{HIn}^{\lambda \max HIn}$ and $A_{In^-}^{\lambda \max In^-}$, as a function of the molar concentration of the indicator.

   To determine the molar concentration of the indicator, please take into account that its solutions are prepared in a 25 mL volumetric flask and the volume of methyl orange (0.002% (m / V)) is shown in table 1; Mr (NM) = 327.33.

   Plot the absorbance as a function of the molar concentration of the indicator.

   Analyze if a liner behavior is observed.

5. Determine the molar absorption coefficients.

From the equation obtained in the linear fit of the plot absorbance vs the molar concentration of the indicator, determine the molar absorption coefficients of the acid and basic form of the indicator at the maximun abosorption wavelenghts. $\varepsilon_{HIn}^{\lambda \max HIn}$ *and* $\varepsilon_{In^-}^{\lambda \max In^-}$

6. Determine the pK of the indicator.

The pK of methyl orange can be determined by analytical or graphical procedures utilizing the recorded spectra of the solutions with the same indicator concentration (V (NM) = 10 mL) and different pH.

1. Analytical procedure

If we know the absorbance of the indicator solutions with the same concetration at one particular wavelengh , in which these solutions mostly contains the acid form A_{HIn} (pH=1), the basic form A_{In^-} (pH=13) or both formr A(buffer pH), from the equation (7) it is possible to determine the pK of the indicator.

$$pH = pK_{HIn} + \log\left(\frac{A_{HIn} - A}{A - A_{In^-}}\right)$$

Take into account the analysis of the spectra carried out in the section 2 of the pK calculation.

Present in a table, the pK values obtained for each buffer solution at different wavelengths. Compare, and discuss, the pKs values obtained at wavelengths near to the maximum of

absorbance of the acid solution with the pKs values obtained in other regions of the spectrum, for example, near to the isosbestic point.

From this analysis, express the average value and the error of the pK

2. Graphical procedure 1

From the equation 3,

$$pH = pK_{HIn} + \log\frac{[In^-]}{[HIn]}$$

When [HIn] = [In^-], the pK is the same that the pH of the solution therefore, pK_{HIn} = pH.

The application of this scenario to the equation 7

$$pH = pK_{HIn} + \log\left(\frac{A_{HIn} - A}{A - A_{In^-}}\right)$$

involves that $log\frac{(A_{HIn}-A)}{(A-A_{In^-})}$ =0

The analytical determination of the pK of methyl orange will have allowed us to conclude the optimal region to determine the pK which is close to the maximum absorbance of the acid form of the indicator, (λ_{HIn}^{max}).

From the table of the recorded spectra (see section 2) get, for λ_{HIn}^{max}, the absorbaces as a function of the pH: A_{HIn} (pH=1), A_{In^-} (pH=13) and A(buffer 1 a 5) and create a tabe also including the values for $log\frac{(A_{HIn}-A)}{(A-A_{In^-})}$.

Plot the $log\frac{(A_{HIn}-A)}{(A-A_{In^-})}$ as a fuction of the pH and with a linear fit (y = a x + b), obtain the value of the pH which correspond with y = 0 = a x + b.

3. Graphical procedure 2 (optional)

At the pH where the recorded absorbance at the wavelengths of the maximums of the acid and the basic form of the indicator is the same : A(λ_{HIn}^{max}) = A($\lambda_{In^-}^{max}$) it is true that pH = pK, which allows determining the pK of the indicator.

From the table of the recorded spectra (see point 2), extract, for λ_{HIn}^{max} and $\lambda_{In^-}^{max}$, the absorbance data as a function of the pH for the different buffer solutions A (buffer 1 to 5) and create a table with them.

Plot A(λ_{HIn}^{max}) and A($\lambda_{In^-}^{max}$) as a function of pH and carry out (for each wavelength) a linear fit with the data showing a linear trend in the crossing area.

Match these equations and determine x (pH) of the intersection point of the lines that corresponds to pK = pH.

7. Compare and analyze the pK values obtained analytically and graphically in the previous question. Compare these values with the theoretical pK of methyl orange.
8. Calculate the pHs of the buffer solutions and check that they coincide with those indicated in the text. To do this, search the bibliographic value of the pKa of formic acid at 25 ºC.
9. What conditions it must be met in order to be applicable to equation (3) ? Indicate the approximations done in its deduction.
10. Prove the equation (8).