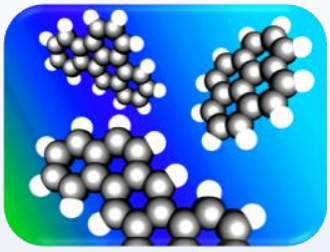
Laboratory Note Book (CH1202)

Department of Chemical Sciences



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IISER KOLKATA

Contents

GENERAL INSTRUCTIONS

1. **Attendance** is mandatory. In case of illness etc. the student must contact the instructor and fix a schedule for making up the missed lab. All labs must be completed in order to get a passing grade.
2. **All data and results** should be recorded directly in the lab notebook. The recording should include, title of the experiment, date of experiment, working formula, data in tabulated forms, results and calculations.
3. The instructor **should sign the data** before the student leaves the lab.
4. **Graph papers and computer print-outs may be directly pasted** on the lab notebook.

Grading:

The marking scheme in the lab will be as follows:

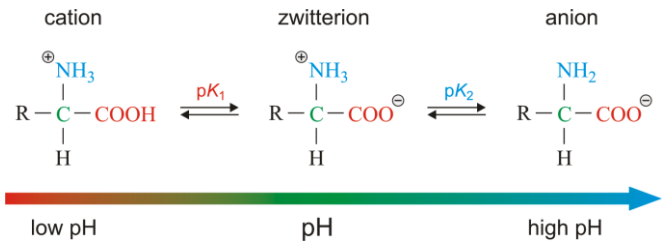
1. Mid-semester examination (Quiz) 30
2. Lab notebook 10
3. Attendance 10
4. Continuous assessment by teacher 10
5. Final examination 40

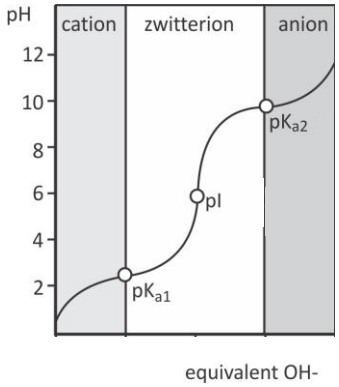
Mandatory!!!

Wear appropriate footwear!

EXPERIMENT 1

DETERMINATION OF ISOELECTRIC POINT OF AN AMINO ACID





PRINCIPLE: Amino acids are molecules that contain both a base site (an -NH_2 group) and an acid site (a -COOH group). Individual amino acids differ only in the identity of the group, -R. On dissolution of an amino acid in water, the proton from the -COOH group gets transferred to the -NH_2 end of the molecule as the NH_2 group is a stronger base than -COO^- resulting a *zwitterion*. Depending on the pH of the solution the amino acids will be either in cationic form (low pH) or in anionic form (high pH).

- The pH at which the presence of these two types of ions in the same concentration is called the isoelectric point (**pI**). **At this pH, the amino acid does not migrate in an electric field**. (gel electrophoresis)
- **pI** is the pH at which the amino acid is neutral, *i.e.* the zwitterion form is dominant.

INTRODUCTION:

$$HA(aq) + H_2O = H_3O^+(aq) + A^-(aq)$$

The extent of this reaction is indicated quantitatively using the equilibrium constant, K_{eq}. The equilibrium constant is given as

$$K_{eq} = K_a = \frac{[H_3O^+(aq)][A^-(aq)]}{[HA(aq)]} \qquad \text{..... (1)}$$

The equilibrium constant for reaction of an acid with water is usually symbolized as K_a to remind us the type of reaction being dealt with.

- The concentration of water, since present in high concentration and thus essentially a pure liquid, is not included in eq. (1).
- The strength of an acid in aqueous solution is defined in terms of the magnitude of K_a. Strong acids have K_a values larger than 1 and that of weak acids is less than 1.

The equilibrium established when a weak acid reacts with water can be explored using the following pH titration: The pH of the solution must change as the titration proceeds.

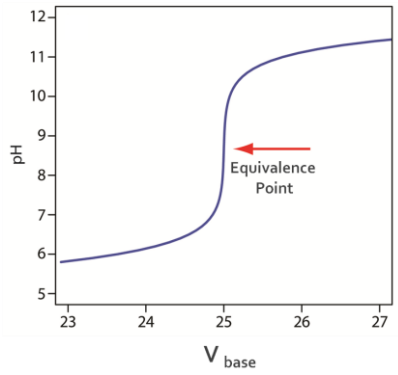
1. At the beginning of the process, before base is added, the pH of the solution is fairly low because it contains acid.
2. As titration proceeds, acid is neutralized by the added base, and pH rises.
3. Addition of base after all of the acid has been neutralized produces a basic solution, with a high pH.
4. The pH of the solution at each interval is monitored by a pH meter.

A plot of pH versus the volume of titrant added to the solution gives the so-called titration curve.

- The curve is shaped like "S". All titration curves have this characteristic shapes.

This provides the method for determining the equivalence point:

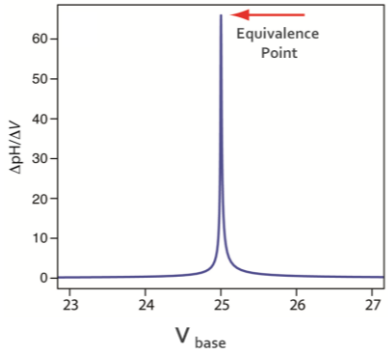
We successively add small volumes of base, measure pH after each addition, and plot the titration curve, from which we may find V_{base} at the inflection point (the equivalence point).



Moles of acid in the original aliquot is calculated as : Moles of acid = V_{base} at inflection point x M_{base}

A derivative plot needs to be created to determine the pK_a values accurately. The steps are as follows:

1. Calculate $\Delta pH/\Delta V$ from the collected pH data for each addition of the titrant.
2. Plot $\Delta pH/\Delta V$ against V (volume of titrant added).
3. The plot gives sharp peaks at the equivalence points corresponding to the sharp jumps in the titration plot. Calculate the respective half equivalence points, where pH = pK_a according to the Henderson-Hasselbach equation. Find out pI = $(pK_{a1}+pK_{a2})/2$ for the amino acid.



In the case of amino acids, titration of the zwitterion with standard NaOH would provide the K_a value for the $-NH_3^+$ acid, which is expected to be similar to that of NH_4^+ ($pK_a = 9.25$). However, K_a value for the -COOH group could also be determined. It is possible to generate the acid form in solution by adding a strong acid to the zwitterion. The strong acid transfers a proton to the $-COO^-$ group of the zwitterion, resulting into a cation. Titration of a solution of this cation with standard NaOH should then yield two equivalence points, one for each acid. It should thus be possible to measure both the K_a values.

APPARATUS: pH meter, beaker, burette, pipette, glass rod, spatula.

REAGENT AND MATERIALS: Potassium hydrogen phthalate, glycine, alanine, HCl, NaOH, phenolphthalein.

EXPERIMENTAL PROCEDURE:

i) Standardisation of NaOH solution

a) Prepare 100 mL 0.1 *M* KHP (Potassium hydrogen phthalate) solution.
b) Standardize the supplied ~0.1*M* NaOH solution against KHP solution using phenolphthalein indicator (three results).

ii) Amino acid titration and estimation of equivalence point

a) Transfer exactly 10 mL of the supplied protonated amino acid solution to a clean 100 mL beaker.
b) Add 15 mL of distilled water to the beaker so that the total volume of the amino acid solution is 25 mL.
c) To homogenize the solution, place the beaker on the top plate of a magnetic stirrer and place a 1-inch stir bar in the beaker. Rinse the pH electrode and submerge it in the solution containing protonated amino acid. Make sure that the tip of the electrode is clear of the magnetic stir bar in the beaker before starting the stirrer. The rotation rate should be reasonably fast, but not so vigorous that splashing of the solution occurs.
d) Record the initial pH of the solution. Initiate the pH titration by adding 0.5 mL of NaOH solution from burette.
e) On each addition of base solution, note the pH of the solution. Continue this addition until you find larger gaps between two subsequent pH values. This indicates approach of the equivalence point. Reduce the volume of addition of the alkali solution to 0.1 mL until you comfortably cross the sudden jump in pH, indicating the equivalence point. After the equivalence point is passed, increase each volume of addition to 0.5 mL. Repeat this process if you expect more than one equivalence points.
f) Discard the solution on completion. Rinse the pH electrode with distilled water till pH meter reading is approximately equal to that of distilled water. Leave the pH electrode in beaker of distilled water and turn the meter off.

RESULTS:

Table 1. Preparation of 100 mL standard 0.1 *N* KHP solution

Weight taken (g)	Weight to be taken (g)	Strength of KHP solution

Table 2. Standardization of NaOH solution using standard KHP solution

Sl. No.	Volume of KHP (mL)	Burette reading (mL)			Average volume (mL)	Strength of NaOH solution
		Initial	Final	Difference		

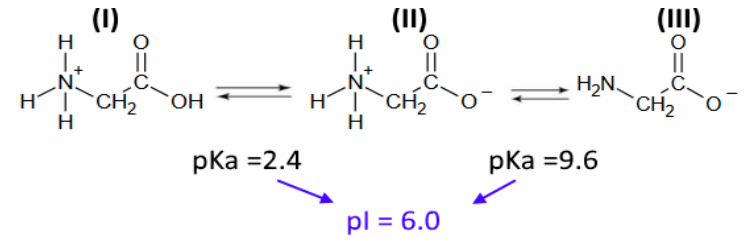
Table 3. Titration of amino acid solution using standard NaOH solution

Volume of amino acid (mL) =

Sl. No.	Volume of NaOH (mL)	pH	ΔV (mL)	ΔpH	ΔpH/ΔV

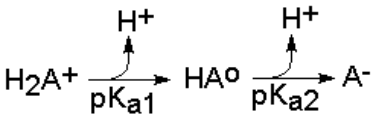
DISCUSSION:

Amino acids are more complicated than simple weak acids since amino acids have at least 2 ionizing groups. Glycine, for example, has both a carboxylic acid and an amino group that can ionize: If we dissolve the free base of glycine in pure water (ie neutral pH), it will ionize by protonating itself. The equilibrium is far to the right so most of the glycine is in the charged form called the zwitterion and glycine is still neutral because the +ve charge is netualized by the -ve charge. Glycine is always in the zwitterion form at neutral pH.



Glycine

Now if we put Glycine at an acid pH where it is fully protonated (i.e., it has all the protons bound to it which it bind), we can titrate it to reveal its 2 pK values for the alpha-carboxylic acid group and the alpha-amino group.

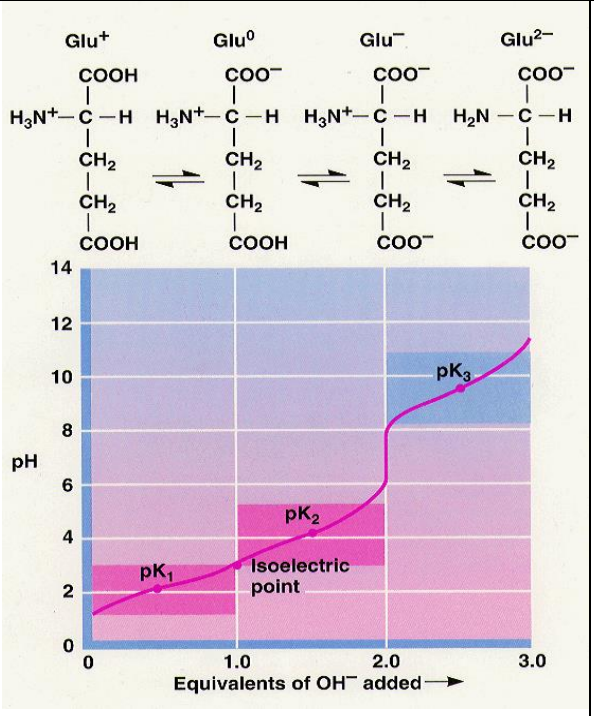
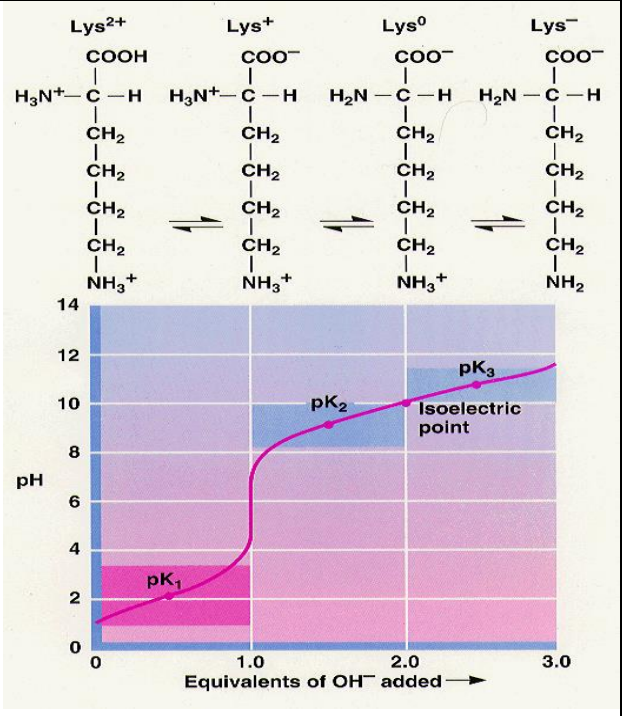


From the pK values, the pI (called the electric point or the place where Glycine has no net charge) can be calculated:

$pI = (2.4 + 9.6)/2 \approx 6$; ($pK_1 = 2.4$, $pK_2 = 9.6$)

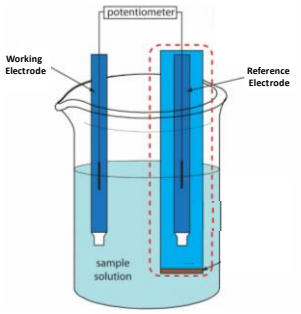
Glycine is neutral at pH 6; it has no net charge here.

Some amino acids are classified as triprotic. This is because, in addition to the ionizable protons of the α-COOH and α-NH_3 groups, they also have a dissociable proton in their R group. Although triprotic amino acids can exist as zwitterions, under physiological conditions these amino acids will be charged. If the net charge under physiological conditions is **negative**, the amino acid is classified as an **acidic** amino acid because the R group has a proton that dissociates at a pH significantly below pH 7. The remaining triprotic amino acids are classified as **basic** amino acids due to a) their having a net **positive** charge under physiological conditions and b) an R group dissociable proton with a pKa near or greater than pH 7. Titration curves for triprotic amino acids generate the same information as those for the diprotic amino acids. The pI for a triprotic amino acid can be determined graphically, although this is somewhat more challenging.

Glutamic Acid	Lysine
	

EXPERIMENT 2

Determination of the Degree of Hydrolysis and the Hydrolysis Constant by Potentiometry



PRINCIPLE: A potentiometer is used to determine the difference between the potential of two electrodes. The potential of one electrode—the working electrode—responds to the analyte's activity, and the other electrode—the reference electrode—has a known, fixed potential.

INTRODUCTION:

Anilinium hydrochloride, $C_6H_5NH_3^+Cl^-$ when dissolved in water, ionizes to form $C_6H_5NH_3^+$ and Cl^- ions, and the cation establishes the following hydrolytic equilibrium.

$$C_6H_5NH_3^+ + H_2O \rightleftharpoons C_6H_5NH_2 + H_3O^+$$

The equilibrium constant for this hydrolytic process is called the hydrolysis constant for the salt and is given by,

$$K_h = \frac{\left(a_{H^+} \times a_B\right)}{a_{BH^+}}$$

where, a_{H^+} is the activity of the free acid (H_3O^+); a_B is the activity of the free base ($C_6H_5NH_2$)· and a_{BH^+} is the activity of the unhydrolysed salt ($C_6H_5NH_3^+Cl^-$). However, for dilute solutions, we may replace activities by concentration terms;
Hence,

$$K_h = [H^+][B] / [BH^+] \quad (1)$$

Hydrolysis constant can also be related to the dissociation constant, K_b, of the base through the ionic product of water, K_w as

$$K_h = K_w / K_b \quad (2)$$

If c equivalents of the salt is dissolved in a litre of water, $c\alpha$ equivalents each of free base and free acid will be formed due to hydrolysis (α is the degree of hydrolysis). Thus, the pH of the solution may be related to the degree of hydrolysis as,

$$pH = - \log [H^+] = - \log (c\alpha)$$

Hence, by measuring the pH of the solution, $c\alpha$ can be calculated from which the degree of dissociation α can be obtained at a given concentration.

Also, expressing K, in terms α, using $K_h = c\alpha^2/(1-\alpha)$, the hydrolysis constant can be calculated. Substituting for K_h in equation (2) and taking $K_w = 1.0 \times 10^{-14}$ at 25°C the dissociation constant of the base, K_b can be evaluated.

Apparatus : Potentiometer, Platinum electrode and calomel electrode.

Chemicals : Anilinium hydrochloride, quinhydrone,

PROCEDURE:

- Prepare an N/10 aniline hydrochloride solution by dissolving appropriate quantity of the substance in distilled water (100 mL).
- From this stock solution, dilute appropriately and get 25 mL of M/20, M/50 and M/100 solutions. Then construct the following cell:
- Transfer the 25 mL solution to 100 mL beaker; add a pinch of Quinhydrone, stir properly to dissolve it, dip the electrodes (Pt and Calomel electrodes) in to the solution.

$$\oplus \text{ Pt|0.1 M Aniline Hydrochloride, Quinydrone // Calomel } \ominus$$

- Determine the potential of the cell. Repeat the experiment with each of the other solutions.

RESULTS:

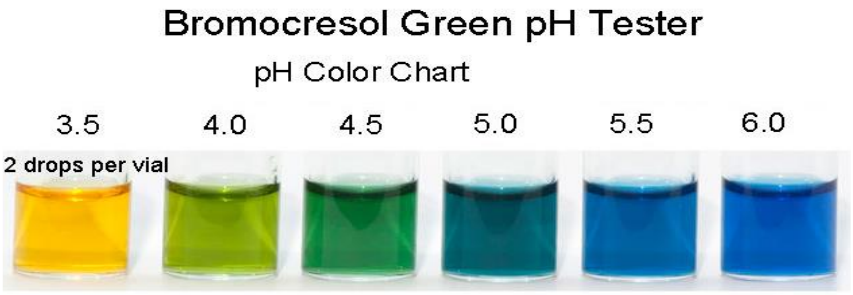
1. pH is given by $pH = (-E_{obs} + E_{QH} + E_{cal}) / 0.0591$ where $E_{QH} = 0.6996$ V and $E_{cal} = -0.242$ V (Oxidation Potential). From this relation, pH of the solution can be calculated.
2. As $pH = -\log [H^+] = -\log (c\alpha)$, $pH = -\log c - \log \alpha$, the degree of hydrolysis α can be calculated at every given concentration.
3. From α, calculate the hydrolysis constant using the relation, $K_h = c\alpha^2/(1-\alpha)$
4. The dissociation constant, K_b can be calculated from the relation $K_b = K_w/K_h$

$C_6H_5NH_3^+Cl^-$	E_{obs}(V)	pH	α	K_h	K_b
N/10					
N/20					
N/50					
N/100					

Mean K_h = - … , Mean K_b = …

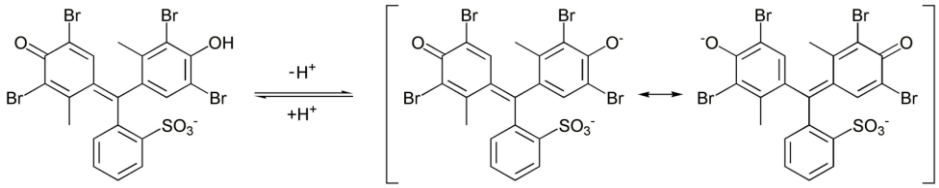
EXPERIMENT 4

DETERMINATION OF THE pK_{In} VALUE OF AN ACID-BASE INDICATOR BY SPECTROPHOTOMETRIC METHOD



PRINCIPLE: Spectrophotometric methods will be used to determine the acid dissociation constant of an acid-base indicator (Bromocresol green), the light absorption characteristics of its acid and base form. This experiment will provide you with opportunities to refine your understanding of absorption process while providing an opportunity to apply many aspects of acid-base chemistry.

INTRODUCTION: Acid–base indictaors are weak acids or bases having distinctly different colours in acidic and alkaline solution, and by virtue of change of colour they indicate the end points of acid-base titrations. To illustrate this point, consider the case for Bromocresol green (an organic acid):



As shown above, this proton can be donated/or received to water to obtain a hydronium ion. If we represent the acidic form of the bromocresol green as (HIn) and the conjugate base as (In-) then the dissociation reaction looks like:

$$HIn \leftrightarrow H^+ + In^- \Longrightarrow H_3O^+ + In^-$$

The acid dissociation constant (Equilibrium constant) can be represented as:

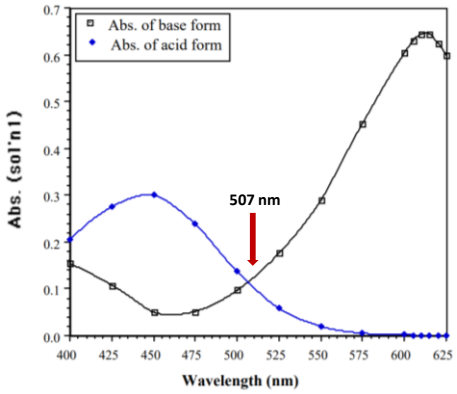
$$K_{In} = \frac{[H^+][In^-]}{[HIn]}$$

The strategy of this experiment is to adjust [H3O+] to known values using a buffer and then to measure the ratio [In-]/[HIn] spectrophotometrically. Knowing this ratio and the value of [H_3O^+/pH] will allow us to calculate Ka using the above equation.

- The trick then is knowing how to determine the ratio [In-]/[HIn] using light absorption measurements .

ABSORPTION SPECTRAL CHARACTERISTICS OF BROMOCRESOL GREEN:

The acid form of bromocresol green (HIn) absorbs light in a different region of the spectrum than the basic form of bromocresol green (In-). From the figure below, you may see that the two species have distinctly different values for λ_{max}. Acid-base indicators are useful for determining pH and indicating end-points because they change color as the pH of the solution changes. It is important to note that the two solutions used to measure these absorption spectra have the same concentration of the bromocresol green indicator. We want to select a wavelength that will allow us to determine the relative concentration of each species present. One very poor choice occurs at about 507 nm, because at this wavelength both the species absorbs equally well. The best choice is the wavelength which has the largest difference in absorbance for the two species. This may be at > 565 nm where the acid form hardly absorbs light.



THEORY: The ionization equilibria of a weak acid indicator (HIn) may be represented according to,

$$HIn \leftrightarrow H^+ + In^- \qquad \text{..... (1)}$$

Acidic form *Alkaline form*

for which the ionization constant (K_{In}) in dilute solution may be defined as the concentration quotient (2)

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

where, [ ]'s represent the molar concentrations of the respective species. Transforming the equation (2) in logarithmic form one obtains,

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

(where, $pK_{In} = -\log_{10} K_{In}$ and $pH = -\log_{10} [H^+]$ in dilute solution).

Thus, if a fixed amount of the indicator is placed in the same volume of a series of buffer solutions of different known pH values, the ratio, $[In^-]/[H_{In}]$, will increase with increase of pH. If the values of the ratio at different pH are determined by measuring the colour intensity of the indicator solutions then the pK_{In} value of the indicator can be found out if the pH of the buffer solutions is known.

If the alkaline form of the indicator (In^-) absorbs at a selected wavelength and Beer's law is obeyed in the range of concentration of the indicator used, then the absorbance (A) of the indicator

solution at a particular pH will be proportional to its concentration, provided the acid form (H_{In}) does not absorb at this wavelength.

$$A = \varepsilon [In^-] l \qquad (4)$$

In a strongly alkaline solution, H_{In} is practically absent, and the absorbance (A) will correspond to the total concentration (T_{In}) of the indicator.

$$A' = \varepsilon [T_{In}] l \qquad (5)$$

Where, ε = molar extinction coefficient of In^- and l = optical path length in cm.

Mass balance equation of the indicator is,

$$T_{In} = [H_{In}] + [In^-] \qquad (6)$$

$$\therefore \quad [H_{In}] = T_{In} - [In^-] \qquad (7)$$

From (5) – (4) one obtains, $\dfrac{(A' - A)}{\varepsilon\, l} = [H_{In}]$ (8)

$$\frac{(A)}{\varepsilon\, l} = [In^-] \qquad (9)$$

Substituting these values of HIn and In^- in equation (3) one obtains,

$$pH = pK_{In} + \log_{10}\left(\frac{A}{A' - A}\right) \qquad (10)$$

A and A′ may be measured colourimetrically. Therefore, by plotting $\log_{10}$ [A/(A′ - A)] against pH of the buffer solutions a straight line of slope =1 will be obtained, of which the intercept on the pH axis will give pK_{In} .

PROCEDURE:

You will be provided with ~0.5 N NaOH and~0.5 N acetic acid. Prepare 0.5 N oxalic acid (50 mL) and follow the procedure.

1. Standardisation of NaOH (~ 0.5 N) using oxalic acid. Than standardise the acetic acid.
2. Prepare 50 mL of exact 0.4 N acetic acid (pK^H= 4.74 at 25°C) and 50 mL of exact 0.4 N NaOH solutions separately by usual procedure.
3. Take 6 hard glass test tubes of uniform dimensions and label them from 1 to 6. Prepare the following series of solutions by proper mixing (experimental pH values may be obtained from chart below, or, may be determined using a pH meter).

Test tube	Vol. of 0.4 N acetic acid (mL)	Volume of 0.4 N NaOH (mL)	Volume of Water (mL)	pH (Expt.)	A	A/(A′-A)
1	5.0	0.5	4.5	3.72		
2	5.0	1.5	3.5	4.27		
3	5.0	2.5	2.5	4.63		
4	5.0	3.5	1.5	4.99		
5	5.0	4.5	0.5	5.57		
6	0	2.5	7.5		A′ =	

4. Add a few drops of bromocresol green indicator to test tube number 6 using a dropper.
5. Set spectrophotometer at 570 nm, adjust the transmittance of water to 100%.
6. Measure the transmittance of the solution in test tube 6. If the transmittance is below 15% (i.e. Absorbance is above 0.82), take test tube 7 and add fewer number drops of the indicator to it and measure the transmittance. In this way by adjusting the number of drops of the indicator, adjust the transmittance of the alkaline form between 25 to 15 % (absorbance is above 0.60 but below 0.82) using test tube numbers 6 to 8 as required.
7. Add the same number of drops of the indicator as adjusted in step 5 to each of test tubes 1 – 5 and measure their transmittance.
8. Calculate the absorbance (A) values of solutions 1 - 5 and the absorbance (A′) of the alkaline solution of the indicator (6, 7 or 8) using the relation:
   $A = \log (100/T\ \%) = 2 - \log T$
9. Plot pH against $\log_{10}$ [A/(A′ - A)] and draw the best straight line of unit slope passing through the experimental points, using the same scale for pH and $\log_{10}$ [A/(A′ - A)] axis. Find pK_{In} from the intercept on the pH axis.

Table 1: Standardisation of NaOH

Sl. No	Vol. of Oxalic acid (mL)	Burette reading			Avg. Vol(mL)	Strength of NaOH
		Initial	Final	Diff.		

Table 2: Standardisation of Acetic Acid

Sl. No	Vol. of Acetic acid (mL)	Burette reading			Avg. Vol(mL)	Strength of Acetic Acid
		Initial	Final	Diff.		

CONCLUSION: pK_{In} of bromocresol green is .....

EXPERIMENT 5

Determination of the Strength of a Solution of a Strong Acid by Conductometric Titration.

PRINCIPLE:

The strength of a solution of a strong acid, namely, hydrochloric acid (HCl) will be determined using a solution of a strong base, namely, sodium hydroxide (NaOH) from the change in the conductance of the solution mixture.

INTRODUCTION:

Suppose an acid is taken in a beaker and NaOH solution is gradually added to it from a burette. The reaction occurring during neutralisation is given by

$$HCl + NaOH \rightarrow NaCl + H_2O$$

$$Or, H^+ + Cl^- + Na^+ + OH^- \rightarrow Na^+ + Cl^- + H_2O$$

It is evident from the above equations that as NaOH solution is gradually added, the H^+ ions, having high ionic conductance, are replaced by Na^+ having lower (ionic) conductance and hence the conductivity of the solution in the beaker gradually decreases. At the equivalence point the conductivity would be the minimum. After the equivalence point the Na^+ and OH^- ions will be accumulated in the solution that increases the conductance of the solution. If the conductances corresponding to the volume of the NaOH solution added be plotted, two straight lines having opposite slopes will be obtained. The point of intersection of the two straight lines will give the equivalence point.

The strength of the NaOH solution should be at least 5 times greater than that of the HCl solution so that the effect of the volume change on the conductance be negligible.

APPARATUS:

Conductivity bridge, conductivity cell, beaker, burette, pipette.

CHEMICALS: Hydrochloric acid solution (0.05 N); Sodium hydrochloride solution (0.2 N); Oxalic acid (0.1 N); Phenolphthalein indicator; Water.

EXPERIMENTAL PROCEDURE:

- Prepare 250 ml of approximately 0.2 N NaOH solution and standardise it by a standard solution of 0.1 N oxalic acid using phenolphthalein indicator.
- Take NaOH in a burette.
- Take 25 ml of the supplied acid by a pipette into a 250 ml beaker and add 125 ml water to it.
- Place the conductivity cell in the beaker so that the electrodes are completely immersed in the acid solution.
- Connect the cell to the conductivity bridge and measure the conductance of the solution.

- Add NaOH solution from the burette 0.5 ml at a time in the beginning, 0.2 ml at a time near the end point point and again 0.1 ml at a time after the end point.
- Measure the conductance of the solution after each addition of the NaOH solution.
- Plot the conductance values against the corresponding titre values, draw the straight lines and obtain the point of intersection.

RESULTS:

Strength of NaOH solution S_1 = N

Volume of the supplied acid solution taken V_2 = 25 ml

Table 1. Titration of the supplied acid with the standardised NaOH solution.

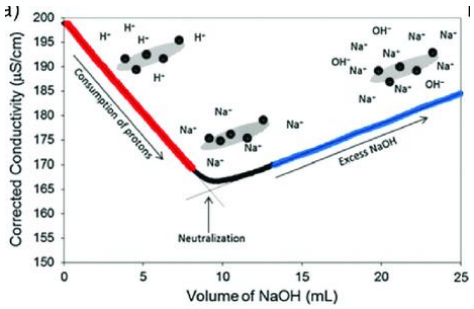
Volume of NaOH solution added (ml)	Observed conductance (ohm^{-1})	Volume of NaOH solution added (ml)	Observed conductance (ohm^{-1})

GRAPH AND CALCULATIONS:

Let the point of intersection of the straight lines obtained by plotting conductances against the titre values correspond to V_1 ml. This gives the volume of NaOH solution required to neutralise 25 ml (V_2) of the acid. Let S_2 be the strength of the supplied acid solution.

$V_1S_1 = 25 \times S_2$

$S_2 = V_1S_1/25$ (N)



EXPERIMENT 9

Molecular Modelling of a Few Organic/Inorganic Molecules Using Computational Calculation

This experiment will give students an idea of visualizing molecules and show how to obtain optimized ground state structures of these molecules. A very basic theoretical knowledge will be provided before the hands on session.

EXPERIMENT 10

HOMO-LUMO Energy Optimization of a Few Organic/ Inorganic Molecules Using Computational Calculation

This experiment will discuss very brief what are the levels of theory available in Modern Quantum Chemistry Package. As such discussion needs knowledge of advanced quantum chemistry mostly we will discuss very elementary quantum chemistry. We will show how Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) energy levels can be calculated for some molecules.

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