Shiksha Mandal's

JANKIDEVI BAJAJ COLLEGE OF SCIENCE, WARDHA

DEPARTMENT OF CHEMISTRY

A

Laboratory Manual

'Star College Scheme'

DBT, Ministry of Science and Technology, Govt. of India, New Delhi




College with Potential for Excellence

Star College Status by DBT Govt. of India

Affiliated to Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur

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PREFACE

I am extremely happy in presenting this **Laboratory manual of Chemistry** of **Star College Scheme** of Department of Biotechnology, Ministry of Science and technology, Government of India, New Delhi.

Attempts have been made to make the book free of errors and omissions. However, critical comments and concrete suggestions will be warmly and thankfully received which will helpful to improve the contents of this book in future. During the preparation of this book, authors/editor have referred to available books written by eminent authorities in the field, therefore I express our indebtedness to them.

This book is an attempt to provide a single chemistry manual to enhance laboratory skills of students. Some of the prescribed experiments could not be performed in department due to lack of instruments or costly consumables, so only theoretical aspects of such topics are mentioned in this book.

The most important feature of this book is that it is written in a very simple and lucid language. We hope that this book will be helpful to students.

I am grateful to **Department of Biotechnology**, **Ministry of Science and technology, Government of India, New Delhi** for financial support.

I acknowledge with deep sense of gratitude and respect to **Principal Dr. Om Mahodaya** for his constant encouragement and motivation. It is my duty to express the sincere thanks to programme coordinator **Dr. K.G. Dube** and **Dr. M.R. Chandrakar** , departmental programme co-ordinator **Dr. S. V. Bawankar**, members of advisory committee and colleagues of Jankidevi Bajaj College of Science, Wardha, for their valuable guidance and co-operation. This Manual would not have been prepared without combined efforts of the team at Chemistry Department especially, Incharge of department **Shri. V. B. Patil**, **Dr. Mrs. N. P. Mohabansi, Mr. M.D. Bansinge**, **Mr. N.A. Barwat, miss. Bhagyashri Tale** and **miss. Rajani Kewate,** so I thanks to them for giving valuable inputs for this book**.**

I would also like to thanks laboratory staff of department. I am deeply thankful to the students of 'Star College Scheme'.

A downloadable pdf version of this book is available on the College website *www.jbsw.shikshamandal.org* under the link DBT Science Centre.

Dr. P. V. Tekade

Editor

CONTENTS

A) Theory & Practicals related to biochemical analysis (For B.Sc. I):

- Determination of refractive index, purity percentage of given edible oil/solvents
- Determination of lactose present in milk samples
- Estimation of Food colours by colorimetric method
- Detection of adulterants in food stuffs.
- Detection of adulterants in petrol and diesel.

B) Theory & Practicals related to Electroanalytical Chemotechniques (For B.Sc. II):

- Analysis of commercial vinegar by conductometric method.
- Determination of Fe (II) by potentiometric titration.
- Determination of three dissociation constants of H_3PO_4 by pH metric titration.
- Determination of pKa of indicator by colorimetry.
- Use of MS-excel in statistical analysis of data and curve fitting.

C) Theory & Practicals related to Functional groups from Biomaterials (For B.Sc. III):

- Semimicro determination of various functional groups in organic compounds : hydroxyl, amino, nitro, nitroso, azo, N-acetyl, O-acetyl, methyl, aldehydes, ketones, thio,disulphide, sulphonamide, unsaturation

A) Theory & Practicals related to biochemical analysis (For B.Sc. I)

Experiment No. 1

TO FIND OUT THE REFRACTIVE INDEX OF THE GIVEN LIQUID AND ALSO FIND ITS MOLECULAR REFRACTIVITY

APPARATUS: Abbe refractometer, pyknometer, ordinary light lamp, thermometer etc.

THEORY: The molecular refractivity of the liquid is given by:

$$R_M = \frac{M}{d} \frac{n2-1}{n2+2},$$

M = Mol. Wt., **n= Refractive index,** **d= density**

PROCEDURE: The method consists of two steps.

First step consists of determining the refractive index of the liquid by means of Abbe's Refractometer

A) Setting of Instrument

- Place the instrument (Abbe's Refractometer) in front of a suitable light source or a window.
- Adjust Zero by rotating scale knob by looking into the scale eye-piece
- Now look into the other eye-piece and focus the cross by adjusting reflector (mirror)

  Now instrument is ready for testing the samples

B) Measurement of Refractive index

- Place 2-3 drops of a given liquid into the inlet hole by using a dropper
- Look into the other eye-piece, some rainbow like shades are seen. To eliminate rainbow rotate the prism drum (scale below eye-piece) clockwise. Now rotate the scale knob anticlockwise till the half black portion appears in the field with cross centre

- Now look into the scale eye-piece and note the reading of refractive index
- Open the prism box and clean the prism surfaces by cotton wool. Then close the prism box. Set the instrument and repeat the same procedure by taking other given liquid.

Second step consist of determination of density of the liquid by means of a density bottle method.

- Wash density bottle by distilled water and then rinse it with acetone and dry it.
- Measure the weight of empty density bottle on weighing machine. Note it as W_1 gm
- Now fill it with distilled water and measure its weight as W_2 gm
- Now again dry the density bottle. Add given liquid to it measure its weight as W_3 gm

OBSERVATIONS: Room temperature = ^{0}C

1. Refractive index (a) --------
   of the liquid, (b) --------
   (c) --------

2. Weight of empty density bottle = W_1 gm.

   Weight of density bottle + distilled water = W_2 gm.

   Weight of density bottle + liquid = W_3 gm

3. Molecular weight of the liquid = M (say)

Calculations:

The density (d) of the liquid = $\frac{Wa-W1}{Wa-W1}$

$$\therefore \ R_M = \frac{M}{d} \ \frac{n2-1}{n2+2}$$

Knowing all the values, we can calculate the value of R_M.

Result. The refractive index and molecular refractivity of the liquid are … and … respectively.

PRECAUTIONS:

(i) The polished surface of the prisms of Abbe's Refractometer should not be scratched.

(ii) Only 2- 3 drops of the liquid are sufficient.

Experiment No. 2

ISOLATION OF LACTOSE FROM MILK POWDER

Dissolve dry milk powder (10 g) in distilled warm water (50 ml) in a beaker and place it over a water bath at 50^{o} C. Add acetic acid (10 ml, 10 % v/v) solution and stir the mixture to coagulate. Filter the precipitate (casein) by a passing through a funnel containing cotton; collect the filtrate in a beaker. Add anhydrous calcium carbonate (about 1 gm) to the filtrate. Boil the filtrate with stirring for about 10 mins. Add decolorizing carbon (about 2 g), boil the mixture thoriughly, Filter under suction using a Buchner funnel Concentrate the filtrate to about 15 ml on a hot plate. Add ethnol (50 ml,95%) to the concentreted solution. Crystallize lactose by keeping it for 24 hrs. Collect the crystals of lactose by filtration or decantation.

Experiment No. 3

ESTIMATION OF FOOD COLOURS BY COLORIMETRIC METHOD

THEORY: Beer Lamberts law can be stated as when a beam of monochromatic light is passed through a transparent homogeneous medium, the decrease in intensity of light with thickness of the medium is directly proportional to the intensity of incident light and concentration of medium.

$$A = Є.c.t$$

Where A is the absorbance of the medium. Є is the molar absorptivity, t is the thickness (path length) of medium and c is the conc.in $mol.lit^{-1}$

If thickness of the medium is 1 cm, the equation reduces to A=Єc When graph is plotted between absorbance and conc., a straight line passing through origin and having slope Є is obtained. Then we can say that Beer Lambert's law is obeyed by the solution.

APPARATUS: Colorimeter with filters, cuvettes, ten well-labeled test tubes, two burettes.

CHEMICALS: 0.001 M Food colour solution, distilled water.

PROCEDURE:

1) Fill up the first burette with 0.001M Food colour solution and the other with distilled water.

2) Prepare a set of 10 well-labeled test tubes containing varying volumes of 0.001 M Food colour solution and distilled water as shown in the next table.

Test tube no.	1	2	3	4	5	6	7	8	9	10
Vol.of0.001 Food colour(ml)	1	2	3	4	5	6	7	8	9	10
Vol. of distilled water(ml)	9	8	7	6	5	4	3	2	1	0

3) **Determination of λ_{max}:**

a) Take distilled water in the cuvette and insert it in a colorimeter. Put one of the filters in a colorimeter and adjust the absorbance zero i.e. transmittance 100%. Remove the cuvette. Take and record the absorbance for this particular filter.

b) Repeat the process of adjusting absorbance zero with distilled water and measure the absorbance of the same system (i.e. solution from test tube no.1) for all other remaining filters of different wavelength. Select the filter which gives maximum absorbance. The wavelength corresponding to maximum absorbance is called λmax.

4) **Determination of conc. of unknown sample of Food colour solution**:

a) Now put the selected filter in a colorimeter. Adjust the absorbance zero with distilled water and measure the absorbance of all the 10 system using that selected filter.

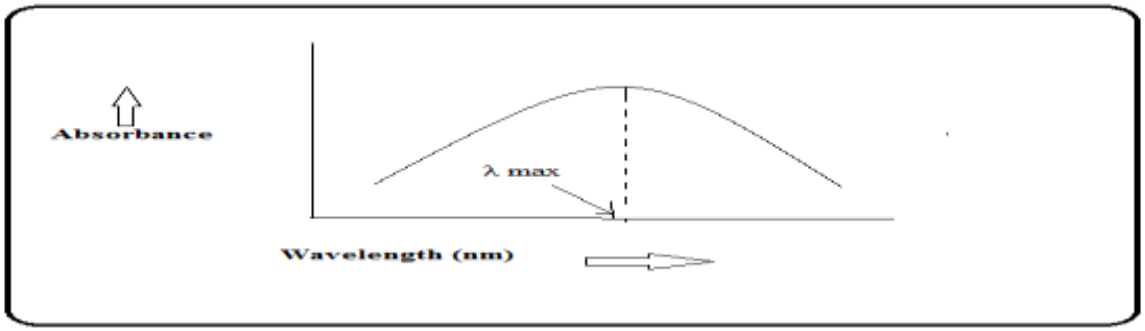
b) Finally take a given unknown sample solution of Food colour whose conc. is to be determined and record its absorbance by using selected filter.

OBSERVATIONS AND CALCULATION:

Determination of λ_{max}:

Wavelength λ (nm)	Absorbance A

Plot the graph of absorbance A as a function of wavelength λ.

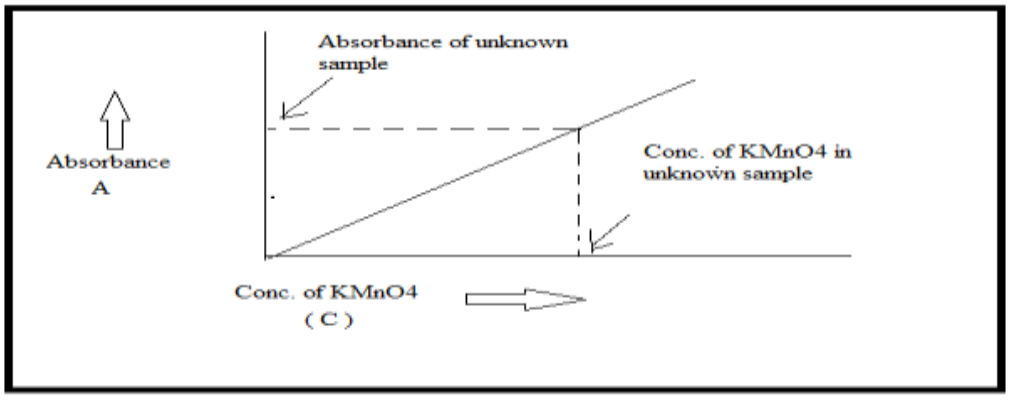


Wavelength corresponding to maximum absorbance λ max = _____nm

Determination of conc. of unknown sample of Food colour solution:

Test tube no.	Volume of 0.001M Food colour.	Volume of distilled water.	Absorbance. A
1	1ml	9 ml	_____
2	2 ml	8 ml	_____
3	3 ml	7 ml	_____
4	4 ml	6ml	_____
5	5 ml	5ml	_____
6	6 ml	4ml	_____
7	7 ml	4 ml	_____
8	8 ml	3ml	_____
9	9 ml	2ml	_____
10	10 ml	1ml	_____
Unknown sample	0ml	--	_____

Plot the graph of absorbance A as a function of conc. of Food colour solution.



RESULT:

1) The straight line graph between conc. and absorbance passing through origin shows that Beer-Lamberts law is verified.

2) Concentration of Food colour in a given unknown sample is found to be _____ mol lit^{-1}.

Experiment No. 4

DETECTION OF ADULTERANTS IN FOOD STUFF

THEORY: The objective of this practical is to study some of the common food adulterants present in different food stuffs.

Adulteration in food is normally present in its most crude form; prohibited substances are either added or partly or wholly substituted. In India normally the contamination/adulteration in food is done either for financial gain or due to carelessness and lack in proper hygienic condition of processing, storing, transportation and marketing. This ultimately results that the consumer is either cheated or often become victim of diseases. Such types of adulteration are quite common in developing countries or backward countries. However, adequate precautions taken by the consumer at the time of purchase of such produce can make him alert to avoid procurement of such food. It is equally important for the consumer to know the common adulterants and their effect on health.

PROCEDURE:

A. Vegetable oil

Adulteration: Castor oil & Argemone oil

Test:

1. Take 1 ml. of oil in a clean dry test tube. Add 10 ml. of acidified petroleum ether. Shake vigorously for 2 minutes. Add 1 drop of Ammonium Molybdate reagent. The formation of turbidity indicates presence of Castor oil in the sample
2. Add 5 ml conc. HNO_3 to 5 ml sample. Shake carefully. Allow the layer to separate. Yellow, orange yellow, crimson colour in the lower acid layer indicates adulteration.

B. Ghee

Adulteration: Mashed potato, sweet potato, vanaspati, rancid stuff (old ghee), synthetic colouring matter

Test:

1. Boil 5 ml of the sample in a test tube. Cool and a drop of iodine solution. Blue colour indicates presence of Starch. Colour disappears on boiling & reappears on cooling
2. Take 5 ml. of the sample in a test tube. Add 5 ml. of Hydrochloric acid and 0.4 ml of 2% furfural solution or sugar crystals. Insert the glass stopper and shake for 2 minutes. Development of a pink or red colour indicates presence of Vanaspati in Ghee.
3. Take one teaspoon of melted sample and 5 ml. of HCl in a stopper glass tube. Shake vigorously for 30 seconds. Add 5 ml. of 0.1% of ether solution of Phloroglucinol. Restopper & shake for 30 seconds and allow it to stand for 10 minutes. A pink or red colour in the lower (acid layer) indicates rancidity.
4. Take 2 g of filtered fat and dissolved it in ether. Divide it into two portions. Add 1 ml of HCl to one tube. Add 1 ml of 10% NaOH to the other tube. Shake well and allow it to stand. Presence of pink colour in acidic solution and yellow colour in alkaline solution indicates added colouring matter.

C. Honey

Adulteration: Invert sugar/jaggery

Test:

1. Fiehe's Test: Add 5 ml of solvent ether to 5 ml. of honey. Shake well and decant the ether layer in a petri dish. Evaporate completely by blowing the ether layer. Add 2 to 3 ml of resorcinol (1 g of resorcinol resublimed in 5 ml of conc. HCl) Appearance of cherry red colour indicates presence of sugar/jaggery.
2. Aniline Chloride Test: Take 5 ml of honey in a porcelain dish. Add Aniline Chloride solution (3 ml of Aniline and 7 ml of 1:3 HCl) and stir well. Orange red colour indicates presence of sugar.

D. Pulses/Besan

Adulteration: Kesari dal (Lathyrus sativus), Metanil Yellow (dye) Lead Chromate

Test:

1. Add 50 ml of dil. HCl to a small quantity of dal and keep on simmering water for about 15 minutes. If the pink colour is developed indicates the presence of Kesari dal.
2. Add conc.HCl to a small quantity of dal in a little amount of water. Immediate development of pink colour indicates the presence of metanil yellow and similar colour dyes.
3. Shake 5 g of pulse with 5 ml of water and add a few drops of HCl. Pink colour indicate Lead Chromate.

E. Bajra

Adulteration: Ergot infested Bajra

Test:

Swollen and black Ergot infested grains will turn light in weight and will float also in water

F. Wheat flour

Adulteration: Excessive sand & dirt, Excessive bran & Chalk powder

Test:

1. Shake a little quantity of sample with about 10 ml of carbon tetra chloride and allow it to stand. Grit and sandy matter will collect at the bottom.
2. Sprinkle on water surface. Bran will float on the surface.
3. Shake sample with dil.HCl Effervescence indicates chalk.

G. Common spices like turmeric, chilly, curry powder, etc

Adulteration: Colour

Test:

Extract the sample with Petroleum ether and add 13N H_2SO_4 to the extract. Appearance of red colour (which persists even upon adding little distilled water) indicates the presence of added colours. However, if the colour disappears upon adding distilled water the sample is not adulterated.

H. Black Pepper

Adulteration: Papaya seeds/light berries, etc.

Test:

Pour the seeds in a beaker containing Carbon tetra-chloride. Black papaya seeds float on the top while the pure black pepper seeds settle down.

I. Spices (Ground)

Adulteration: Powdered bran and saw dust

Test: Sprinkle on water surface. Powdered bran and sawdust float on the surface.

J. Food: Coriander powder

Adulteration: Dung powder, Common salt

Test:

1. Soak in water. Dung will float and can be easily detected by its foul smell.
2. To 5 ml of sample, add few drops of silver nitrate. White precipitate indicates adulteration.

K. Chillies

Adulteration: Brick powder grit, sand, dirt, filth, etc.

Test:

Pour the sample in a beaker containing a mixture of chloroform and carbon tetrachloride. Brick powder and grit will settle at the bottom.

L. Turmeric Powder

Adulteration: Starch of maize, wheat, tapioca, rice

Test: A microscopic study reveals that only pure turmeric is yellow coloured, big in size and has an angular structure. While foreign/added starches are colourless and small in size as compared to pure turmeric starch.

M. Food: Turmeric

Adulteration: Lead Chromate & Metanil Yellow

Test:

1. Ash the sample. Dissolve it in 1:7 Sulphuric acid (H_2SO_4) and filter. Add 1 or 2 drops of 0.1% dipenylcarbazide. A pink colour indicates presence of Lead Chromate.
2. Add few drops of conc. hydrochloric acid (HCl) to sample. Instant appearance of violet colour, which disappears on dilution with water, indicates pure turmeric. If colour persists metanil yellow is present.

N. Cumin seeds (Black jeera)

Adulteration: Grass seeds coloured with charcoal dust

Test: Rub the cumin seeds on palms. If palms turn black adulteration is indicated.

O. Food: Asafoetida (Heeng)

Adulteration: Soap stone, other earthy matter Chalk

Test:

1. Shake a little quantity of powdered sample with water. Soap stone or other earthy matter will settle at the bottom.
2. Shake sample with Carbon tetrachloride (CCl4). Asafoetida will settle down. Decant the top layer and add dil.HCl to the residue. Effervescence shows presence of chalk.

P. Food grains

Adulteration: Hidden insect infestation

Test:

Take a filter paper impregnated with Ninhydrin (1% in alcohol.) Put some grains on it and then fold the filter paper and crush the grains with hammer. Spots of bluish purple colour indicate presence of hidden insects' infestation.

Q. Sugar

Adulteration: Water insoluble impurities

Test:

Take 5 ml of water in test tube and 1 g sugar & shake it. Allow to stand for some time. If undissolved substance settles at bottom indicates adulteration.

RESULT & DISCUSSION

S. N.	Foods / Products	Adulteration (Test) Positive/ Negative
1		
2		
3		
4		
5		

CONCLUSION: Food is one of the essentials to sustain life. Access to pure, nutritious food, free from any type of adulteration is the genuine expectation of every human being. Selection of wholesome and non-adulterated food is essential for daily life to make sure that such foods do not cause any health hazard. It is not possible to ensure wholesome food only on visual examination when the toxic contaminants are present in ppm level. However, visual examination of the food before purchase makes sure to ensure absence of insects, visual fungus, foreign matters, etc. Therefore, due care taken by the consumer at the time of purchase of food after thoroughly examining can be of great help. Secondly, label declaration on packed food is very important for knowing the ingredients and nutritional value. It also helps in checking the freshness of the food and the period of best before use. The consumer should avoid taking food from an unhygienic place and food being prepared under unhygienic conditions. Such types of food may cause various diseases. Consumption of cut fruits being sold in unhygienic conditions should be avoided. It is always better to buy certified food from reputed shop.

Experiment No. 5

DETECTION OF ADULTERANTS IN PETROL AND DIESEL. (FILTER PAPER TEST)

APPARATUS AND CHEMICALS: Filter paper, density bottle petrol, diesel etc.

Filter Paper Test

PROCEDURE:

- Firstly take a filter paper.
- Then, a drop of petrol is put on the filter paper from the nozzle.
- The petrol dropped on the filter paper is allowed to evaporate for 2 minutes.
- The petrol should evaporate without leaving any stain on the filter paper. If the colour left on the paper, then it is the colour of MS and indicates that fuel i.e. petrol is adulterated.

Specific gravity measurement

PROCEDURE:

- Wash density bottle by distilled water and then rinse it with acetone and dry it.
- Measure the weight of empty density bottle on weighing machine. Note it as W_1 gm
- Now fill it with distilled water and measure its weight as W_2 gm
- Now again dry the density bottle. Add given liquid(petrol/ Diesel) to it measure its weight as W_3 gm

OBSERVATIONS: Room temperature = ^{0}C

Weight of empty density bottle = W_1 gm.

Weight of density bottle + distilled water = W_2 gm.

Weight of distilled water = W_2- W_1 gm.

Weight of density bottle + liquid (Petrol/ Diesel) = W_3 gm

Weight of given liquid (Petrol/ Diesel) = W_3- W_1 gm

Calculations

Density of given liquid (Petrol/ Diesel) (ϱ_2) = $\frac{W3-W1}{W2-W1}$ x density of distilled water (ϱ_1)

Specific gravity of given liquid (Petrol/ Diesel) = $\frac{\text{Density of given liquid } (\varrho 2)}{\text{Density of distilled water } (\varrho 1)}$

B) Theory & Practicals related to Electroanalytical Chemotechniques (For B.Sc. II):

Experiment No. 1

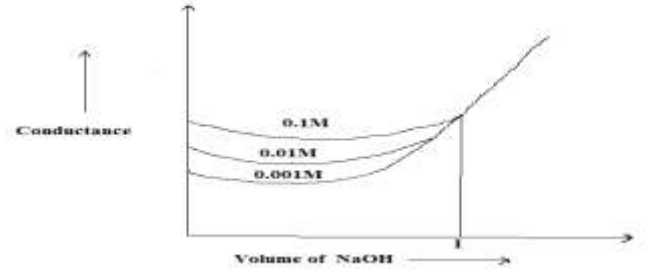
ANALYSIS OF COMMERCIAL VINEGAR BY CONDUCTOMETRIC METHOD

APPARATUS AND CHEMICALS: 1) 0.1 M NaOH solution which was standardized with std. oxalic acid.

2) Vinegar solution: 3ml of vinegar sample from market is taken in std. calibrated flask and diluted to 100 ml with distilled water.

THEORY: Vinegar is a sour liquid consisting mainly of acetic acid and water. Acetic acid (ethanoic acid) is an organic acid (carboxylic acid) and is classified as a weak acid. In conductometric titration, when acetic acid is titrated with a strong base like NaOH, initially conductance is low due to the low ionization of acetic acid. On addition of base, there is a decrease in conductance, not only due to the replacement of H^+ by Na^+ but also suppress ion of dissociation of acetic acid due to common ion acetate. But very soon, the conductance increases on adding NaOH as NaOH neutralizes the undissociated CH_3COOH to CH_3COONa which is strong electrolyte. This increase in conductance continues up to equivalence point. The graph near equivalence point is curved due to hydrolysis of salt CH_3COONa.

Beyond equivalence point, conductance increases more rapidly with the addition of NaOH due to highly conducting OH^- ions. The point of intersection of two lines gives the point of neutralization i.e. equivalence point.



Procedure:

1. Pipette out 30 ml of vinegar solution from flask in a beaker and dip conductivity cell into it.

2. Take NaOH solution into burette.

3. Calibrate the conductometer.

4. Make addition of 0.5 ml NaOH each time. After each addition, stir the solution well and note the conductance.

5. Plot the graph of conductance verses volume of NaOH solution.

OBSERVATIONS AND CALCULATIONS:

Table 1: Standardization of NaOH

Sr.No.	Volume of oxalic acid	Volume of NaOH	End point
1	10ml		
2	10ml		
3	10ml		

Oxalic acid verses NaOH

$$M_1V_1 = M_2V_2$$

$$M_2= M_1V_1/V_2$$

Table 2: Conductometric titration of a given vinegar sample with strong base

Volume of NaOH (ml)	Conductance
0ml	
0.5 ml	
1 ml	
1.5 ml	
-	

-	
-	
30 ml	

Estimation of strength of diluted vinegar solution

Calculation from graph:

Molarity of NaOH= M_1=----------M

Volume of NaOH solution used (from graph) =V_1=----------ml

Volume of diluted vinegar solution taken = V_3= 30 ml

Molarity of vinegar solution = M_3

Diluted vinegar solution vs NaOH

$M_3V_3 = M_1V_1$

$M_3 = M_1V_1/V_3$

$M_1V_1/30$

=---------M

Since 3 ml of commercial vinegar solution /sample is taken and diluted to 100 ml.

Molarity of commercial vinegar

$M_4 = \frac{M_3 \text{ X } 100}{3}$

M_4= ----------Mol/dm3

Mol.wt/formula of acetic acid= 60

Strength of commercial vinegar = M_4 X 60

= ----------g/dm^3

RESULTS: Strength of commercial vinegar is----------g/dm^3.

PRECAUTIONS:

1. After switching on the instrument (conductometer) it should be allowed to stabilize prior starting experiment.
2. The conductance cell must always be dipped either in solution or in distilled water.
3. The platinum electrode of conductance cell must be completely immersed in solution during measurement of the conductance.
4. There should be no air bubble between two electrodes.
5. The titrant must be at least ten times more concentrated than analyte.

Experiment No. 2

DETERMINATION OF Fe (II) BY POTENTIOMETRIC TITRATION

AIM: To determine the strength of Fe(II) from the given FAS solution by titrating potentiometrically with potassium dichromate solution and to calculate redox potential of Fe(II)/Fe(III) system on hydrogen scale.

APPARATUS AND CHEMICALS: Potentiometer, Saturated calomel electrode platinum electrode, burette, H_2SO_4, $K_2Cr_2O_7$ etc.

THEORY: Oxidizing agent $K_2Cr_2O_7$ in the presence of an acid oxidizes Fe^{++} (Ferrous ion) to Fe^{+++}(Ferric ion). $Fe^{++} \leftrightarrow Fe^{+++} + e^-$

In this redox titration of FAS (ferrous ammonium sulphate) against $K_2Cr_2O_7$, two electrodes viz. Platinum electrode (Indicator electrode) and Calomel electrode (reference electrode) are used. The emf at platinum electrode is given by Nernst equation i.e.

$$E_{Fe2+,\ Fe3+} = E^0_{Fe2+,\ Fe3+} - \frac{2.303\ RT}{nF} \log \frac{[Fe^{+++}]}{[Fe^{++}]}$$

$$E_{Fe2+,\ Fe3+} = E^0_{Fe2+,\ Fe3+} - \frac{0.0591}{nF} \log \frac{[Fe^{+++}]}{[Fe^{++}]}$$

Where, $E^0_{Fe2+,\ Fe3+}$ is standard electrode potential of Fe^{2+} - Fe^{3+}.

Thus emf depends upon the ratio $[Fe^{+++}]/[Fe^{++}]$.

At an equivalence point (end point), this ratio changes rapidly due to which there is a large increase in e.m.f. at exactly half equivalence point, half of Fe^{2+} ions are converted into equivalence amount of - Fe^{3+} ions. So at half the equivalence point, $Fe^{2+} = Fe^{3+.}$

Hence, $E_{Fe2+,\ Fe3+} = E^0_{Fe2+,\ Fe3+}$

In this titration, platinum electrode in Fe^{2+} - Fe^{3+} system is coupled with saturated calomel electrode. The electrochemical cell is represented as:

$$Hg|Hg_2Cl_{2(S)}, KCl\ (sat.)\ ||Fe^{3+}, Fe^{2+}|Pt$$

Emf of this cell is the difference between oxidation potentials of two electrodes.

Thus $E_{cell} = E_{calomel} - E_{Fe2+, Fe3+}$

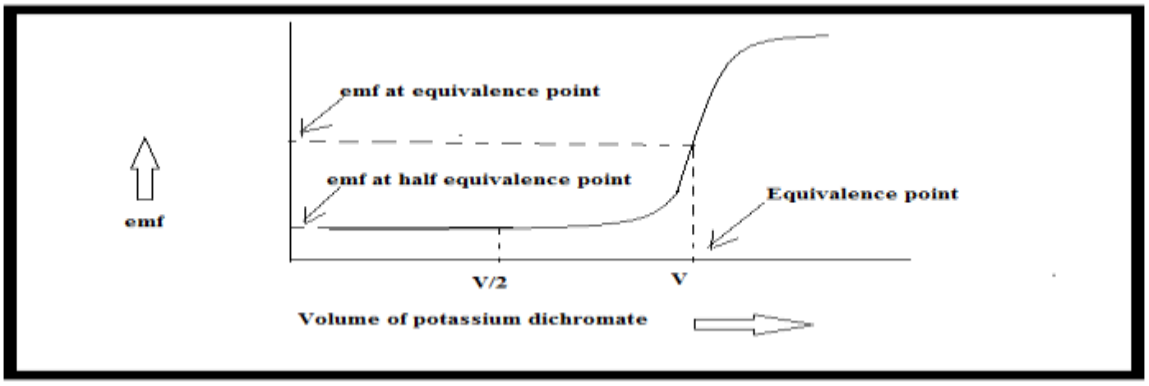
Where $E_{calomel}$ is oxidation potential of calomel electrode

And $E_{Fe2+, Fe3+}$ is oxidation potential of platinum electrode.

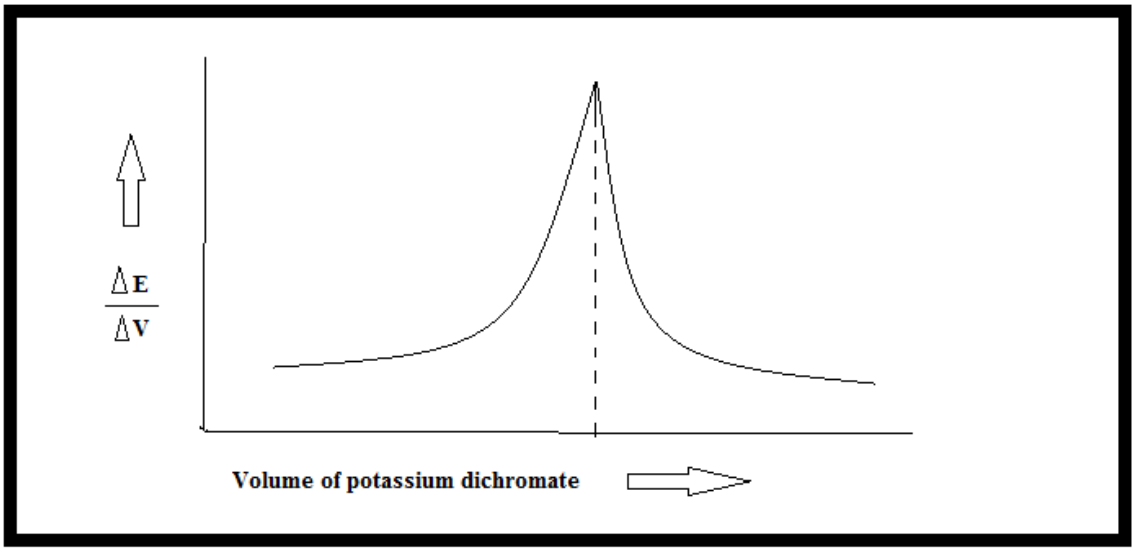
At half equivalence point, $E_{cell} = E_{calomel} - E_{Fe2+, Fe3+}$

Hence, Redox potential of $Fe^{2+, Fe3+}$system is $E_{cell} = E_{calomel} - E_{cell} = -0.2415 - E_{cell}$

In redox titration, when $K_2Cr_2O_7$ is added from burette, emf increases slowly in beginning. At half equivalence point, it increases rapidly. Beyond end point, it again increases slowly. Hence, if emf is plotted against volume of $K_2Cr_2O_7$ solution added, nature of graph is obtained as shown below. The point of inflexion (mid point of steep line) indicates the end point.



Sometime it is rather difficult to locate the exact point of inflexion. Therefore it is useful to plot $\Delta E/\Delta V$ against volume of $K_2Cr_2O_7$. The maxima of curve correspond to the end point as shown below:



In potentiometric titration, the solution in two compartments is connected by salt bridge. The salt bridge is U shaped tube containing saturated solution of an electrolyte like KCl, KNO_3, K_2SO_4 etc. or solidified solution of such electrolytein agar-agar or gelatin. The function of salt bridge is to allow the movement of ions from one solution to other without mixing of these two solutions. With this flow of ions, the circuit is complete and electrons pass freely through the wire to keep the net charge zero in the two compartments.

PROCEDURE:

1. Method of calibration of potentiometer

a) Switch on the instrument and allow it to warm up for 15 minutes.

b) Dip the calomel electrode in saturated KCl solution and platinum electrodes in conductivity water connect the calomel electrode to negative terminal and platinum electrode to positive terminal of the potentiometer.

c) Throw the switch down and turn the calibration shaft with the help of screw driver till the display reads 1.018

d) Throw the switch up .The instrument is now standardized and ready for use.

2. Measurement of emf of solution

a) Fill up the burette with standard 0.1 N $K_2Cr_2O_7$solution

b) Pipette out 10 ml unknown FAS solution in 100 ml beaker. Add half test tube dil. H_2SO_4. Then add some conductivity water. Dip platinum electrode in it. Connect this platinum electrode to positive terminal of potentiometer.

c) Take some saturated KCl in another beaker. Dip calomel electrode in it. Connect this calomel electrode to negative terminal of potentiometer.

d) Connect salt bridge between two solutions and note down emf. This is reading for 0 ml Potassium dichromate.

e) Now add 1 ml Potassium dichromate solution from burette to beaker containing FAS solution. Stir the solution and note the reading. This is reading for 1 ml Potassium dichromate.

f) Repeat the process of addition of 1 ml Potassium dichromate and measure the emf till emf increases little faster. Then add 0.5 ml $K_2Cr_2O_7$near end point (i.e. from 8 ml to 12ml, total $K_2Cr_2O_7$ solution added). Beyond end point, add Potassium dichromate in installment of 1 ml each. Take the readings up to 16 ml $K_2Cr_2O_7$ solution

OBSERVATION AND CALCULATION:

Volume of unknown FAS solution taken = 10 ml

Normality of standard $K_2Cr_2O_7$ solution = 0.1 N

Volume of $K_2Cr_2O_7$ i.e. (V)	e.m.f. (E)	ΔE	ΔV	$\Delta E/\Delta V$
0	E_0	-	-	-
1	E_1	E_1-E_0	1-0 =1	
2	E_2	E_2-E_1	2-1 =1	
3	E_3	E_3-E_2	3-2 =1	
4	E_4		1	
5			1	

6	--	--	1	
7			1	
8			1	
8.5			8.5-8=0.5	
9			9-8.5=0.5	
9.5			0.5	
10			0.5	
10.5			0.5	
11			0.5	
11.5			0.5	
12			0.5	
13			1	
14			1	
15			1	
16			1	

Plot graph of emf against volume of potassium dichromate added. Also plot graph of

$\Delta E/\Delta V$ against volume of $K_2Cr_2O_7$. And find out end point.

CALCULATION:

Strength of Fe(II) from given 10 ml FAS solution**:**

1000 ml 1 N $K_2Cr_2O_7$ is equivalent to 55.847 g of Fe (II)

So V_2 ml 0.1 N $K_2Cr_2O_7$ = $\frac{55.847 \times 0.1 \times V_2}{1000}$ = -------- g

This is weight of Fe(II) from the given 10 ml FAS solution.

Calculation of Redox potential of Fe(II) - Fe(III) system:

From graph find out emf corresponding to half equivalence point. This is value of E_{cell}.

At half equivalence point, E_{cell} = - volts

The oxidation potential of saturated calomel electrode, $E_{calomel}$ = - 0.2415 V at 25^0C.

Now redox potential of Fe(II) - Fe(III) system

= $E_{calomel}$ - E_{cell} = - 0.2415 - E_{cell} = ----- Volts

RESULT:

1. The amount of Fe(II) present in given 10 ml FAS solution = ------- g

2. Redox potential of Fe(II)- Fe(III) system at 25^0C is found to be ---- volts.

Experiment No. 3

DETERMINATION OF THREE DISSOCIATION CONSTANTS OF PHOSPHORIC ACID BY pH -METRIC TITRATION

APPARATUS AND CHEMICALS: Phosphoric acid solution, 0.25 M Sodium hydroxide solution, Calcium chloride dihydrate, pH meter, burette, pipette, stirrer etc.

THEORY: A polybasic acid contains two or more dissociable hydrogens. As a result, it ionizes in stages, each stage having its own characteristic dissociation constant. Phosphoric acid is tribasic acid (H_3A). It ionizes in 3 stages as:

1. $H_3A + H_2O \leftrightarrow H_3O^+ + H_2A^-$ or $H_3A^- \leftrightarrow H^+ + H_2A^-$

2. $H_2A^- + H_2O \leftrightarrow H_3O^+ + HA^-$ or $H_2A^{--} \leftrightarrow H^+ + HA^{--}$

3. $HA^{--} + H_2O \leftrightarrow H_3O^+ + A^{---}$ or $HA^{--} \leftrightarrow H^+ + A^{---}$

The corresponding dissociation constant values for the steps are:

$$K_1 = \frac{[H^+][H_2A^-]}{[H_3A]}$$

$$K_2 = \frac{[H^+][HA^{--}]}{[H_2A^-]}$$

$$K_3 = \frac{[H^+][A^{---}]}{[HA^{--}]}$$

The fact that ionization occurs in these 3 stages successively with increasing dilution shows that $K_1 > K_2 > K_3$. This is always true because of presence of a negative charge on H_2A^- and of two charges on HA^{--} makes it increasingly difficult for a proton to be lost.

The third inflection point can not be located directly because the solution becomes too alkaline for NaOH to show any difference in emf. This difficulty can be removed if excess of $CaCl_2$ solution is added in the acid solution after second neutralization step is complete. $CaCl_2$ added reacts with disodium phosphate present in the solution to precipitate calcium phosphate: HCl

equivalent to acid content of disodium phosphate is formed. The emf of solution thus increases (pH decreases), further titration can now carried out up to third neutralization point.

PROCEDURE:

1. Set up and standardize the pH –meter using buffer solutions of pH 4.00 and 10.00

2. Dilute the given phosphoric acid solution to 250 ml in standard measuring flask with distilled water

3. Pipette out 50 ml of diluted solution in 250 ml clean beaker.

4. Immerse the electrodes and stir the solution for one minutes. Note down the pH as initial pH

5. Wash and fill the burette with 0.25 N NaOH solution.

6 Add 0.5 ml of NaOH solution from burette, stir well and measure the pH of solution.

7. Repeat step no.6 and determine pH after each addition. If the relative change in pH is higher,(near the equivalence point),then, the volume of NaOH solution added should be decreased to 0.1 ml

8. Continue the addition of NaOH solution from burette till the pH of titrated solution reaches the value of about 9.

9. Add 1 g of calcium chloride (dihydrate) to the solution at this stage. Stir the solution well and note down new pH.

10. Continue the addition of NaOH in similar manner till pH finally reaches the value of 10.

11. Correct the recorded pH of solution after the addition of calcium chloride taking into consideration the decrease in pH.

(Where, corrected pH = observed pH + pH difference before and after addition of $CaCl_2$_)

OBSERVATION AND CALCULATION:

S.No.	Volume of NaOH solution added in ml	pH	Corrected pH
1	0.5		-
2	1.0		-
-	-		-
-	-		-
-	-		-
-	-		-
-	-		-
-	-		-
-	-	10.0	

Plot the graph of

1. pH vs. Volume of NaOH solution added

2. d/dV (pH) vs. volume of NaOH solution added

From the 3 breaks (V_1, V2, V3respectively), find out the volume at which 3 dissociation constants of the Phosphoric acid using the Henderson equation

pH = pKa + log [salt/acid]

pH = pKa at half neutralization change.

Hence, [salt] = [acid]

RESULT:

1. pK1 =

2. pK_2 =

3. pK_3 =

4. K_1 =

5. K_2 =

6. K_3 =

Experiment No. 4

DETERMINATION OF pKa OF INDICATOR BY COLORIMETRY

APPARATUS AND CHEMICALS: 0.2M NaOH, 0.2M Boric acid, sodium carbonate, a saturated solution of indicator (phenolphthalein 0.01 g in 50ml rectified spirit, 5ml of this solution diluted with water to 50ml), colorimeter, filter etc.

THEORY-: Acid-base indicators are known to have different colours in acidic and alkaline solutions. These for 3 common indicators are as follows-

Indicator	In acidic solution	In alkaline solution	pK_{In}
Phenolphthalein	Colourless	Pink	9.7
Methyl orange	Red	Yellow	3.7
Methyl red	Red	Yellow	5.1

The change in colour is due to an exchange of H^+ ions between two forms. If acid form of indicator is represented by HIn, the dissociation can be represented as

$$HIn \rightleftharpoons H^+ + In^-$$

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

Where K_{In}=dissociation constant of an indicator

If concentration of indicator in a solution is C moles per litre and its degree of dissociation is α.

The concentration factor will be

$[HIn]= C(1-\alpha)$

$[H^+]= C\alpha$

$[In^-] = C\alpha$

The degree of dissociation can be shifted not only by changing the concentration of indicator but also by changing hydrogen ion concentration or pH of solution. pH adjustments are possible by using appropriate buffer solutions.

A very important point in use of indicator is that changes in colour are visually perceptible from 10% dissociation to a 90% dissociation of indicator.

The whole range is controlled by a change in pH of solution $pK_{ln} = \pm 1$. Thus an experiment for study of dissociation of an indicator needs buffers of range of pH range $pK_{ln} = \pm 1$. When hydrogen ion concentration is controlled, we can write

$$K_{In} = \frac{[H+] \times C\alpha}{(1-\alpha)C}$$

$$K_{In} = \frac{[H+] \times \alpha}{(1-\alpha)}$$

Taking reciprocal, $\frac{1}{KIn} = \frac{(1-\alpha)}{\alpha} \times \frac{1}{[H+]}$

$$pK_{In} = pH - \log\left(\frac{\alpha}{1-\alpha}\right)$$

$$\log\left(\frac{\alpha}{1-\alpha}\right) = pH - pK_{In}$$

The second term on right (pK_{ln}) is constant.

As pH of solution increases, α also increases.

The evaluation of K_{ln} involves known adjustment in $[H^+]$ and a measurement of either $[ln^-]$ or [Hln], one or both of which may be coloured.

If we can have a filter which acts as an absorbent of light of colour corresponding to only one form of indicators, the study can be made colorimetrically.

It is possible for phenolphthalein and is usually possible for other indicators also.

PROCEDURE:

(1) Buffer solutions in pH range 8 to 10.5 made from NaOH and Boric acid(H_3BO_3) solutions as follows-

Test tube No.	0.2 M NaOH(ml)	0.2 M Boric acid(ml)	pH
1	0	10	6.90
2	1	9	7.94
3	2	8	8.55
4	3	7	8.99
5	3.5	6.5	9.12
6	4	6	9.46

(2) In seventh test tube, place 10 ml saturated sodium carbonate solution. This has high enough pH to cause almost complete dissociation of indicator. (phenolphthalein)

(3) Add 3 drops of indicator solution to each of seven test tubes.

(4) Maximum colour will be developed in tube no.7.

(5) Use this solution to find the appropriate filter for maximum absorption and lowest optical density.

(6) Using this filter, measure transmittance and optical density for all seven solutions.

OBSERVATION AND CALCULATIONS:

(1) For saturated solution of sodium carbonate-

Sr. no.	Wavelength(nm)	Optical density(D) or Extinction(E) or Absorbance(A)
1	400 (410-UV)	
2	420 (450-410)	
3	470 (480-450)	
4	500 (520-480)	
5	530 (580-520)	
6	620 (640-580)	
7	660 (670-640)	
8	700 (670-IR)	

(1) Choose the appropriate filter for maximum absorption and lowest optical density.

Sr. No.	0.2 M NaOH	0.2 M Boric acid (ml)	Saturated Na_2CO_3 (ml)	Phenolphthalein indicator(drops)	pH	Optical Density (Ai)
1	0	10	0	3	6.90	A_1 =
2	1	9	0	3	7.94	A_2 =
3	2	8	0	3	8.55	A_3 =
4	3	7	0	3	8.99	A_4 =
5	3.5	6.5	0	3	9.12	A_5 =
6	4	6	0	3	9.46	A_6 =
7	0	0	10	3	--	A_7 =

(2) C is concentration of phenolphthalein in each tube,

Concentration of ionized phenolphthalein = αC

For each tube, $\alpha i\ C\ \alpha\ A_i$

For tube no. 7, $C\ \alpha\ A_7$

Because α=1 in tube no.7

For any tube, $\alpha i = \frac{Ai}{A7}$ and $Ai = \log\frac{Io}{Ii} = \varepsilon(\alpha iC)x$

For tube no.7, $A_7 = \log\frac{I0}{I7} = \epsilon Cx$ (since α=1)

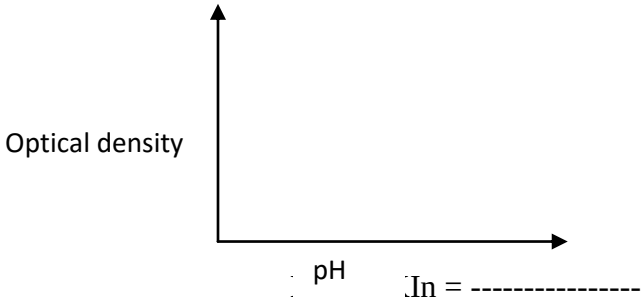
$$A_i = \frac{\log(\frac{I0}{Ii})}{\log(\frac{I0}{I7})} = \frac{\log(\frac{1}{Ti})}{\log(\frac{1}{T7})} = \frac{-\log Ti}{-\log T7} = \frac{\log Ti}{\log T7}$$

Calculate value of α for each tube and using value of pH, pKa can be calculated.

Formula- $pK_{In} = pH - \log(\frac{\alpha}{1-\alpha})$

Test tube no.	$\alpha_i = \frac{Ai}{A7}$	$pK_{In} = pH - \log(\frac{\alpha}{1-\alpha})$
1	$\alpha_1 = \frac{A1}{A7}$ =	
2	α_2=	
3	α_3=	
4	α_4=	
5	α_5=	
6	α_6=	

Plot a graph of pH against optical density, which is used to calculate pH of tube no.7.



In = ----------

Hence, $pK = \log_{10}(\frac{1}{K})$

RESULT: Dissociation constant of an indicator K is found to be ----------.

C) Theory & Practicals related to Functional groups from Biomaterials (For B.Sc. III):

- Semimicro determination of various functional groups in organic compounds : hydroxyl, amino, nitro, nitroso, azo, N-acetyl, O-acetyl, methyl, aldehydes, ketones, thio,disulphide, sulphonamide, unsaturation

B.Sc. III

SEMIMICRO DETERMINATION OF VARIOUS FUNCTIONAL GROUPS IN ORGANIC COMPOUNDS

THEORY: Microscale technique is being followed in many parts of the world for the last twenty-five years or so in the form of kits for individual experiments, especially in organic chemistry. Use of microscale laboratory techniques has many benefits:

• The chemical waste generation in the colleges is reduced markedly.

• Students learn waste minimization techniques that they will ultimately practice throughout their lives.

• Colleges will continue to offer hands-on laboratory experience to their students, a practice that has been threatened by the increasing costs and difficulty of waste disposal, it will introduce

laboratory work even in the institutions which are too poorly equipped.

• Health risks to students and teachers in the colleges laboratories are minimized.

• The cost of materials and equipment needed to provide students with an excellent science education is reduced to a significant extent.

• Educational laboratories are safer environmentally for both students and teachers when microscale methods are used.

• Saves time for preparation.

• Reduces waste at the source.

• Ensures more safety.

• Lowers the costs for chemical substances and equipment.

• Requires smaller storage area.

• Reduces reliance on intensive ventilation systems with a significant reduction in electricity consumption.

• Provides pleasant working atmosphere.

• Decreases reaction time.

TEST OF UNSATURATION

APPARATUS:

Well plate, micro test tubes, burner and W-tube, spatula.

CHEMICALS:

(a) Solid reagents- Calcium carbide

(b) Liquid reagents-Alkaline solution of KMnO4.

PROCEDURE:

Baeyer's Test-

1. Take a spatula of CaC_2 in one side of W-tube and in the other side a very dilute alkaline KMnO4 solution.

2. Add a few drops of distilled water to the side containing CaC2 and close the mouth by your thumb. The produced gas acetylene goes into other side and reacts with KMnO4 solution.

3. Dil. KMnO4 solution gets decolourised within few seconds.

TEST FOR SULPHONAMIDES

APPARATUS: Conical flask, magnetic stirrer, burette, etc.

CHEMICALS: Sodium methoxide (o.1N) in benzene-methanol, Dimethylformamide(DMF), Thymol blue indicator. (0.3g thymol blue in 100ml of methanol)

PROCEDURE:

(1) Place 25 ml of dimethylformamide in a 250 ml conical flask, add 2 or 3 drops of thymol blue indicator, and titrate with the 0.1N sodium methoxide solution to the first appearance of a blue colour. This will neutralize any acidic impurities in the solvent.

(2) Weigh out accurately 0.5-0.6 g. of the sulphonamide (eg. Sulphapyridine, sulphathiazole or sulphadiazine), and stir by means of a magnetic stirrer, until dissolved. Continue the titration with the standard 0.1N sodium methoxide solution to the first appearance of a permanent blue colour.

CALCULATIONS:

The percentage purity of the sulphonamide is given by the formula-

$$\% \text{ of purity} = \frac{V1 \times N1 \times M \times 100}{W \times 100}$$

Where, V_1= volume (ml) of the sodium methoxide solution used

N_1= normality of the sodium methoxide solution

M= molecular weight of the sulphonamide and

W= weight (g) of the sample

Result

The % purity of the Sulphonamide =

TEST FOR DISULPHIDES

APPARATUS: Conical flask, burette, ampoule, measuring cylinder, etc.

CHEMICALS:

1. **Potassium bromated-bromide solution(0.1N)-**

Dissolve 2.78 g of dry potassium bromate and 10 g of potassium bromide in water and dilute to 1 litre in a volumetric flask.

2. Glacial acetic acid
3. Conc.HCl
4. Di-n-butylsulphide or di-n-octylsulphide

PROCEDURE:

(1) Weigh out accurately 0.2-0.3 g of the thioether (di-n-butylsulphide or di-n-octyl sulphide) in an ampule.

(2) Place the ampule and a few glass beads in a 250 ml iodine flask containing 40 ml of glacial acetic acid. Stopper the flask and shake vigorously to break the ampoule.

(3) Now add 3 ml of concentrated hydrochloric acid, followed by 10 ml water, use less water if the sample is thrown out of solution.

(4) Titrate the solution with the bromated-bromide reagent until the first yellow colour due to excess of bromine is observed. The colour fades slowly (about 1 minute) due to oxidation of the sulphoxide to the sulphone.

(5) Run a blank on the solvents alone to correct for the excess of bromine needed to detect the end point, the blank is usually about 0.2-0.3 ml.

CALCULATION:

The percentage of sulphide sulphur is given by the formula-

$$\% S = \frac{V1 \times N1 \times 32.06 \times 100}{W \times 1000}$$

Where, V1= volume of bromated-bromide solution used for sample and corrected for blank,

N1= normality of bromated-bromide solution,

W= weight of sample

TEST FOR ALDEHYDE

(I). Schiff's cc Test -

(1) Take 3-4 drops of the liquid or 50 mg of solid organic compound in a micro test tube or well plate.

(2) Add 2-3 drops of the Schiff's reagent.

(3) Appearance of pink colour indicates the presence of an aldehyde.

(II). Fehling's solution test-

(1) Take 1 mL of both Fehling solution A and 1 mL of Fehling solution B in micro test tube.

(2) Add 2-3 drops of organic compound

(3) Heat the content for about 2 minutes on a water bath.

(4) Formation of a brick red ppt. of cuprous oxide indicates the presence of aldehyde.

(5) This test is not given by the aromatic aldehydes.

(III). Tollen's reagent test-

(1) Take 1 mL of freshly prepared silver nitrate (2 %) in a test tube. Add 1-2 drops of sodium hydroxide solution to it and shake. A dark brown ppt. of silver oxide appears. Dissolve the ppt. by adding ammonium hydroxide solution dropwise.

(2) Now, add an aqueous or alchoholic solution of about 50 mg of organic compound.

(3) Heat the reaction mixture on a water bath for 5 minutes.

(4) Formation of a deposit of silver, on the inner side of the test tube indicates the presence of aldehyde.

TEST FOR KETONE

Rapid method (5min). This method is most useful as a semi-quantitative test. A 0.2ml volume (or less) of sample (containing 25-500nmol of carbonyl groups) is allowed to react with 0.2ml of reagent (5mM-2, 4-dinitrophenylhydrazine in 2M-HCI) for 5min. The mixture is then diluted to 5ml with M-HCI and the extinction at 370nm determined. A blank is also prepared and may be used in the spectrophotometer to give a difference reading directly. As the blank extinction is near unity accurate dilution is required for determining small concentrations of carbonyl group

TEST FOR METHYL GROUP

CHEMICALS:

1. Sulphuric acid –chromic acid mixture (oxidizing mixture): Dissolve 16.8 g of A.R. chromic anhydride in 100 ml of water and add cautiously 20 ml of conc. Sulphuric acid .Cool

2. Barium hydroxide solution 0.05 N: Dissolve 8g of A.R. crystallized barium hydroxide in distilled water and dilute with boiled distilled water up to 1 litre and protect this solution using guard tube containing soda lime. Standardized with A.R. Potassium hydrogen phthalate or with standard acid solution.

3. Phenolphthalein indicator: Dissolve 0.1 g of phenolphthalein in 50 ml ethyl alcohol and then dilute with equal amount of water.

APPARATUS:

50 ml Pyrex flask, Liebig condenser, Connecting tube, condenser

PROCEDURE:

1. Clean the apparatus by fitting it with (or immersing it in) Sodium dichromate-sulphuric acid cleaning mixture, rinse thoroughly with tap water and finally with distilled water. Dry in oven at 120^0C
2. Weigh accurately about 25 mg sample (e.g. anhydrous sodium acetate or colonic acid) from weighing bottle into the flask A, introduce 2 or 3 small grains of Carborundum and 5 ml cold oxidizing mixture. Insert the reflux condenser E into neck of flask and reflux mixture for 90 min: surround the flask with small air bath and heat with semi micro burner. Allow to cool remove condenser E, wash it with water and collect the washings into flask. Add 7 g of crystalline magnesium sulphate to oxidation mixture insert tube D and set up apparatus for steam distillation.
3. Steams distill the mixture the mixture until 50 ml of distillate are collected in a measuring cylinder (not shown in fig.)
4. Heat the flask by means of small flame during distillation to prevent the volume of liquid in flask from becoming to large. Remove the flame temporarily if mixture tends to become viscous.

5. Titrate the distillate with standard 0.05 N Barium hydroxide solutions to a phenolphthalein end point: Use Semimicro burette. An additional 5 ml of distillate should not change the end point appreciably.
6. Carry out a blank titration, omitting the sample and apply a correction to original titration, if necessary.
7. Barium hydroxide solution is most convenient for titration because any sulphuric acid that might have been carried over is detected immediately. If sulphuric acid is found in distillate, the determination must be repeated.

NOTE:

It is essential to have complete control over the rate of passage of steam into flask, if steam is inadvertently passed at too rapid a rate, some acetic acid may be lost owing to incomplete condensation and low result will be obtained. A simple apparatus consists of a distillation flask fitted with a two holed rubber stopper in the neck: a long tube (acting as a safety tube) passes through one hole and extends almost to the bottom of flask, whilst a short tube, terminating in a glass stopcock, passes through the other hole. By adjusting the glass stopcock, the supply of steam to the reaction flask can be controlled. The side arm of distilling flask is connected to the apparatus.

CALCULATION:

Calculate the percentage of carbon linked methyl in the sample from relationship :

1 ml 0.05N $Ba(OH)_2$ = 0.752 mg CH_3

Alternatively, calculate the C-methyl number from formula:

$$\text{C-Methyl number} = \frac{(V_1 - V_2)\, X N_1\, X\, M}{W}$$

Where, V_1 = Volume of byrata solution used in analysis

V_2 = Volume of byrata solution used in blank

N_1 = Normality of Barium hydroxide solution

M = Molecular weight of sample

W = Weight (mg) of sample

TEST FOR THIO GROUP

Nitroprusside test: Dissolve 10 mg of sulphur containing unknown compound in minimum amount of sodium hydroxide solution and then add 2-3 drops of freshly prepared sodium nitroprusside solution. Appearance of red or purple colour is a positive test for sulphides.

Ferric chloride test: Dissolve 20 mg of sulphur containing unknown compound in a solvent which contain 0.5 ml water, 0.5 ml alcohol and 3 drops of ammonium hydroxide. Now add 3-4 drops of ferric chloride solution. Appearance of blood red colour or red brown colour is a positive test for sulphide.

TEST FOR AMINO GROUPS (PRIMARY AROMATIC AMINE): AZO DYE TEST

(1) Take one drop of aniline in small test tube and add $NaNO_2$ solution and dil. HCI.

(2) Cool the contents of the test tube in ice while maintaining the temperature of the mixture to about 0–5°C.

(3) In another test tube, take β- napthol solution and dissolve it in the dil. NaOH solution.

(4) Add diazonium chloride solution slowly with shaking.

(5) Formation of an orange dye confirms the presence of an aromatic primary amino compound

TEST FOR PHENOLIC HYDROXYL FUNCTIONAL GROUP

Take one drop of resorcinol in small test tube and add 1 drop of fresh aq.$FeCl_3$ solution, formation of green, blue or violet colour confirms presence of phenol.

TEST FOR NITRO FUNCTIONAL GROUP

(1) Take 0.1ml of nitrobenzene add 2ml. conc. HCl and pinch of Zn dust. Boil for 2 minutes; cool thoroughly, preferably in ice-cold water and filter.

(2) Add a few drops of $NaNO_2$ solution

(3) Cool the contents of the test tube in ice while maintaining the temperature of the mixture to about 0–5°C.

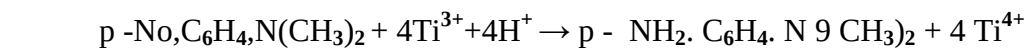
(4) In another test tube, take β- napthol solution and dissolve it in the dil. NaOH solution.

(5) Add diazonium chloride solution slowly with shaking.

(6) Formation of an orange dye confirms the presence of nitro group.

DETERMINATION OF NITROSO GROUPS

THEORY: Practice in this determination may be obtained by evaluation of the purity of a sample of p – nitrosodimethylaniline. Either titannous chloride or titanous sulphate may be used.



$$p\text{-}No,C_6H_4,N(CH_3)_2 + 4Ti^{3+} + 4H^+ \rightarrow p\text{-}\ NH_2.\ C_6H_4.\ N\ 9\ CH_3)_2 + 4\ Ti^{4+} + H_2O$$

Reduction occurs quantitatively at 45 – 50^{o}, and the end point is easily detected by the disappearance of the yellow colour of the solution.

PROCEDURE: Weigh out accurately about 0. 50g. of the sample of p-nitrosodimethylaniline, dissolve it in dilute hydrochloric acid. And dilute to 100 ml. in a volumetric flask. Transfer 25.0 ml. of this solution to a titration flask. Pass carbon dioxide into the flask for 5 minutes, warm to 40 – 50^{o}C., and titrate with standard 0.1N titanous chloride or sulphate until the yellow colour is just completely destroyed.

CALCULATION:

Calculate the purity of the sample.

$$-\quad NO + 4Ti^{3+}$$

$$-\quad 1\ Ml.\ 0.1N\ TiCl_3 = 0.7503\ mg.\ NO$$

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DETERMINATION OF AZO GROUPS

THEORY: Azo compounds are quantitatively reduced by titanous salts:

$RN = NR' + 4Ti^{3+} + 4H \rightarrow RNH_2 + R'NH_2 + 4Ti^{4+}$

This reaction occurs provided that the intermediate hydrazo compound does not undergo a benzidine or similar transformation. The azo linkage is more frequently encountered in dyes and dye intermediates. Soluble azo dyes (e.g., methyl orange and orange II) may be dissolved in water, excess of the titanous solution added, the solution boiled in a stream of carbon dioxide for 3 – 5 minutes and the excess of titanous salt evaluated by titration with standard 0.1N ferric ammonium sulphate solution using ammonium thiocyanate solution as indicator. Highly coloured dyestuffs, such as crystal violet 6R (dyestuff from α–naphthylamine and β –naphthol-6 8-disuphonic acid : $C_{20}H_{12}O_7N_2S_2Na.7H_2O$), if soluble in water may be titrated directly with the titanous solution, the dyestuff acting as its own indicator. Simple water-in solution dyes (e.g.,phenylazo-β-naphthol) may be sulphonated in the cold with fuming sulphuric acid, and the aqueous solution titrated directly with a titanous salt until decolourised. For more complex azo dyes excess of titanous salt is assed to a boiling solution of the dyestuff (dilute alcohol, alcohol, acetic acid, etc.) whilst a slow stream of carbon dioxide is passed through the flask; the excess of titanous salt is titrated with standard ferric ammonium sulphate solution, using either the dyestuff itself or ammonium thiocyanate solution as indicator.

PROCEDURE: Experience in the titration of azo groups may be acquired by determining the purity of a sample of methyl orange. Weigh out accurately about 0.25g. of methyl orange * into the titration flask, add 25 ml. of water and 25 ml. of glacial acetic acid, shake until dissolved, add 25 ml. of dilute sulphuric acid (1 : 2,v/v), pass carbon dioxide for 5 minutes to displace air, run in 50.0 ml. of 0 . 1N titanous sulphate solution, and boil for 5 minutes. Whilst

The sample should be dried to constant weight at 100^{o}; the water of crystallisation ($3H_2O$) is thus lost.

Maintaining the current of nitrogen, cool add 10 ml. of 10 per cent. Ammonium thiocyanate solution, and titrate with standard 0 .1 N ferric ammonium sulphate solution.

Run a blank on 50.0 ml. of the titranous sulphate solution.

CALCULATIONS:

Calculate the purity of the sample of methyl orange.

- N = N - Ξ $4Ti^{3+}$
- 1 Ml.0.1 N Ti^{3+} Ξ 0.7005 mg, N_2 (azo)

Ξ 8.185 mg. anhydrous methyl orage

$$\% \text{ Purity} = \frac{(V1 - V2) \times N1 \times M \times 100}{W \times 4 \times 1000}$$

Where V_1 = volume (ml.) of ferric solution used in the blank;

V_2 = volume (ml.) of ferric solution used for sample;

N_1 = normality of ferric ammonium sulphate solution;

M = molecular weight of sample; and

W = weight (g.) of sample.

SEMIMICRO DETERMINATION OF O – ACETYL GROUPS REAGENTS

THEORY: Alcoholic patassium hydroride solution, 1 N. Dissolve 5.6 g. of A. R. potassium hydroxide pellets in a mixture of 50 ml. of ethyl alcohol and 50ml. of water. Magnesium sulphate reagent. Dissolve 50g. of crystallised magnesium sulphate and 0.75 ml of concentrated sulphuric acid in water and dilute to 90 ml.

Barium hydroxide solution, 0.02 N or 0.05N. Prepare and standardise it with a standard acid solution.

PROCEDURE:

Weigh out accurately about 25mg. of the sample* from a weighing bottle (by difference) into the flask A. Introduco 3.0 ml. of the N ethanolic potassium hydroxide solution together with 2 or 3 minute fragments of carborundum. Insert the reflux condenser and heat the liquid to boiling or until the sample has dissolved Cool, remove the condenser wash it with a little water and collect the washings in the flask. Add 20 ml. of the magnesium sulphate reagent, and set up the apparatus for steam distillation, Steam distil the mixture. Heat, the flask with a semimicro burner in such a manner that the liquid in the flask distils at a fairly rapid rate and is concentrated to about 15 ml. during the collection of 50 ml. of distillate (use a Measuring cylinder as receiver). Titrate the distillate with 0.05 N (or 0.02N) baryta (contained in a semimicro burette) to a phenolPhthalein end point. An additional 5 ml. of distillate should not change the end point appreciably.

Carry out a blank determination, omitting the sample, and apply a correction to the original titration, if necessary.

*(Any of the following compounds may be used to acquire experience in the determination : phenl acetate, glucose pente – acetate, triacetin (triacetyul glycerol), acetoacetanilide, acety salicylic acid, and hydroquinone diacetate.)

CALCULATION:

Calculate the percentage of acetyl in the sample from the relationship :

$$1 \text{ Ml. } 0.05\text{N Ba (0H)}_2 = 2.152 \text{ mg. } CH_3CO$$

Alternatively, calculate the percentage of acetyl from the formula:

$$\% \text{ Acetyl} = \frac{(V1 - V2) \times N1 \times 43.04 \times 100}{W \times 1000}$$

Where V_1 = volume (ml.) of baryta solution used in the analyais.

V_2 = volume (ml.) of baryta solution used for blank;

N_1 = normality of barim hydroxide solution; and

W = weight (g.) of sample.

SEMIMICRO DETERMINATION OF N – ACETYL GROUPS

PROCEDURE: Weigh out accurately about 40 mg. of the sample (e.g., phenacetin) into the flask (And add either 5- 7 ml. of dilute sulphuric acid (1: 2, v/v) or 5 ml. of a 25 per cent. Aqueous solution of tolucne p-sulphonic acid. follwed by 2 or 3 small fragments of carborundum. Insert the reflux condenser into the neck of the flask, and reflux the mixture gently

for 90 minutes: surround the flask with a small air bath and heat with a semimicro burner. Allow to cool, remove the condenser wash it with a little water and collect the washings in the flask. Insert the steam distillation tube D, and steam distil the mixture slowly until 30-40 ml. of liquid have been collected (45-60 minutes; use a 50 ml. measuring cylinder as receive). Titrate the distillate with standard 0.05N $Ba(OH)_2$ a semimicro burette an additional 5 ml. of distillate should not change the end point appreciably.

Carry out a blank determination, omitting the sample, and apply a correction if necessary.

CALCULATION:

Calculate the percentage of acetyl in the sample from the relationship:

1 ml of 0.05N $Ba(OH)_2$ Ξ 2.152 mg CH_3CO

$$\% \text{ acetyl} = \frac{(v1 - v2) \times N1 \times 43.04 \times 100}{w \times 1000}$$

V_1= volume (ml) of $Ba(OH)_2$ solution used in the analysis

V_2= volume (ml) of $Ba(OH)_2$ solution used for blank

N1= Normality of $Ba(OH)_2$ solution

W= wt.(gm) of sample