Experiment No. - 6

Estimation of total ash

Total ash was estimated in the sample by weighing about 5 g of dried samples into a crucible. The crucible was placed on a wire gauge and heated over a low flame till the material was completely carried and then the crucible was heated in the middle furnace for about 4 hours at 600^0 C. It was then cooled in a desiccator and weighed. To ensure the completion of ashing, the crucible was again heated in the furnace. For one hour cooled and weighed. This was repeatedly done till two consecutive weights were the same and the ash was almost white or greyish colour. The estimation was done in duplicate.

Calculation

Weight of the crucible – W g

Weight of the crucible + sample – W1 g

Weight of the crucible + ash – W2g

∴ Weight of sample (W1 – W) g

Weight of the ash (W2 – W)

$$\therefore \text{ \% Total ash} = \frac{(W2 - W)\ g}{(W1 - W)\ g} \times 100$$

$$\text{Per cent of Total ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample taken}} \times 100$$

Experiment No. – 7

Estimation of calcium

Reagents

1. Ammonium oxalate
2. Glaceal acetic acid
3. 2N sulphuric acid
4. 0.01N KMnO4

Procedure

An aliquot (2ml) of mineral solution was diluted to about 150ml with distilled water. Few drops of methyl red indicator were added and neutralized with ammonia till the pink colour change to yellow. The solution was heated to boiling followed by the addition of 10ml ammonium oxalate. Again the solution was allowed to boil for a few minutes and glacial acetic acid added till the colour was distinctly pink and kept aside in a warm place till the precipitate settles down. The precipitate was then filtered through Whatman No.40 filter paper and washed with warm water till free of oxalate. The precipitate along with filter paper was transferred to a beaker and 10ml, 2N sulphuric acid poured over it. Finally, the solution was hot titrated against N/100 potassium permanganate solution.

1 ml of N/100 KMnO4 = 0.2004 mg of Ca.

$$\text{Per cent calcium (mg)} = \frac{\text{N/100 KMnO4 X 0.2004 X Vol. of H2S04}}{\text{Weight of sample used for ashing x aliquot taken}} \times 100$$

Study questions:

1. Write the Importance of calcium for human body
2. List the rich sources of calcium along with values (minimum 15)

3. Explain the mechanism /formation of kidneys stones in the body

Experiment No. - 8

Estimation of phosphorus

Principle

Determination of phosphorus was carried out by measuring colorimetrically the blue colour formed when the ash solution is treated with ammonium molybdate and thus phosphomolybdate formed is reduced.

Reagents

1. Ammonium molybdate
2. Sodium thiosulphate
3. Hydroquinone

Procedure

To an aliquot, 0.1 ml of mineral solution was added with 1 ml of ammonium molybdate, 1ml of hydroquinone and 1ml of sodium thiosulphate solutions in this order, mixing well after each addition. The volume was then made up to 15 ml with water and the solution mixed thoroughly. After 30 minutes the optical density of this solution was measured in a spectrophotometer at 660 nm, against a reagent blank prepared in the same way as the test, except that the test solution was omitted. The phosphorus content of the sample was obtained from a standard curve prepared with standard phosphate solution (range 0.01 to 0.1 mg phosphorus).

Study questions:

1. Write the Importance of phosphorus for human body
2. List the rich sources of phosphorus along with values (minimum 15)

3. List the hormones/enzymes which require phosphorus for their function

Experiment No. - 9

Estimation of Iron

Iron was estimated in samples using chemito atomic absorption spectrophotometer 201.

Standard:

0.07 g ferrous ammonium sulphate was dissolved in 1000ml distilled water for atomic absorption spectrophotometer solution and appropriate dilutions were made to get standard solution ranging from 15 to 3o ppm. These standards were fed to atomic absorption spectrophotometer as that of sample to get standard curve and a

standard graph was fit. To this standard curve the sample readings were compared.

Protocol:

Suitable dilutions were made from the extractant with deionized triple distilled water so as to fit their absorbance with the range of standard curve.

Study questions:

1. Write the Importance of iron for human body and different forms
2. List the rich sources of iron along with values (minimum 15)
3. List the hormones/enzymes which require iron for their function

Experiment No. - 10

Estimation of zinc

Zinc was estimated in samples using chemito atomic absorption spectrophotometer 201.

Standard

100 ppm standard Zn2+ solution was prepared using 1000 ppm Zn2+ atomic absorption spectrophotometer solution and appropriate dilution were made to get standards solution ranging from 0 to 0.6 ppm. These standards were fed to atomic absorption spectrophotometer as that of sample to get standard curve and a graph was fit. Against this standard curve the sample readings were compared and corresponding readings were recorded.

Protocol

Suitable dilutions were made from the extract with deionized triple distilled water so as to fit their absorbance with the range of standard curve.

Study questions:

1. Write the Importance of Zinc for human body
2. List the rich sources of Zinc along with values (minimum 15)
3. List the hormones/enzymes which require zinc for their function

ANNEXURE–I

ESTIMATION OF PROTEIN

Principle

Organic nitrogen digested with sulphuric acid in the presence of catalyst is converted to ammonium sulphate. Ammonium liberated by making the solution alkaline is distilled into a known volume of standard acid, which is then back titrated. Protein per cent was calculated by multiplying the nitrogen presented the factor 6.25.

Reagents

1. 2% boric acid solution: 20g of boric acid was dissolved in some distilled water. The solution was then transferred to a 1000 ml volumetric flask and made up to the mark.
2. 40% NaOH (W/V).
3. 0.1 N HCl: 8.33 ml of fuming HCl was dissolved in 1000ml distilled water.
4. Mixed indicator: Was made by mixing methyl red (0.2%) and Bromocresol green (0.2%) in a 1:2 ratio (v/v) respectively.
5. Digestion mixture: Anhydrous sodium sulphate and copper sulphate
6. Concentrated sulphuric acid (H_2SO4).

Procedure

Digestion: 0.5 g of the sample was weighed into the digestion tubes of the Gerhardt digester in duplicate and two heaped spatulas each of sodium and copper sulphate were added to each tube. 25 ml of concentrated sulphuric was also added and samples digested until the contents of the tubes were sea green in color. Each of the digested materials was dissolved in distilled water and transferred into a 100 ml volumetric flask and then brought to the mark.

Distillation: 10ml of each samples was transferred into the distillation tube of the automatic Gerhardt unit and 20ml of 2 per cent boric acid to which was added 3-4 drops of the mixed indicator was placed in the collecting conical flask to trap the liberated ammonia. The unit was furnished with 40 per cent NaOH and distilled water to facilitate operation. Distillation was done for 5 minutes and the ammonia collected and trapped by the boric acid. In between the distillation of samples, the unit was rinsed with distilled water for 2.5 minutes. The boric acid turned from reddish pink to green as it collected the ammonia.

Titration: The green colored boric acid was titrated against the 0.1NHCl until its color turned to pink. A blank was run simultaneously. The titer values obtained were incorporated in the equation below to obtain the per cent nitrogen present in the sample which, in turn, was multiplied by the factor 6.25 to obtain the per cent protein.

$$\text{Per cent nitrogen (\% N)} = (V_a - V_b) \times 0.0014 \times \frac{V_1}{V_2} \times \frac{100}{W}$$

Where:

V_a = Titre value of sample

V_b = Titre value of blank

V1 = Volume to which digested sample was made up to 100 ml

V2 = Volume to aliquot used in distillation

W = Weight of samples taken for digestion

ANNEXURE-II

ESTIMATION OF FAT

Principle

The extraction of fat from substances is often tedious and requires thorough contact and heating with the solvent. This is done in the soxhlet apparatus in which fresh solvent continuously comes into contact with the material to be extracted over a relatively long period of time.

Procedure

Five gram of sample was weighed into a thimble and closed with fat free cotton wool. The thimble was placed in the soxhlet apparatus attached to a pre-weighed flask and extracted for about 14-26 hours. Thereafter, the flask was retrieved from the apparatus with as little solvent in it as was possible. It was then transferred into an oven to evaporate the remaining solvent, leaving behind only the residue or extract. The flask was then cooled in desiccators after which it was weighed to estimate the fat.

$$\text{Percent Fat content (g/100g)} = \frac{\text{Weight of the ether extract (g)}}{\text{Weight of the sample taken (g)}} \times 100$$

ANNEXURE-III

ESTIMATION OF CRUDE FIBRE

Principle

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight is the crude fiber content.

Reagents

Standard H_2SO4 (0.255 ± 0.005 N)

Standard NaOH (0.313 ± 0.005 N)

Method

Weighed amount of (2.5-5g) of moisture and fat free sample was transferred to a fibre bag which was pre heated and weighed. These bags were inserted into tubes and placed in a beaker provided in the instrument. The sample was boiled with 300ml 0.255 ± 0.005N H_2SO_4 for 30 minutes. Then the residue was washed with boiling water until acid free. Then residue was boiled with 300ml of 0.313 ± 0.005 N NaOH for 30 minutes. Again the residue was washed with boiling water followed by alcohol wash. The residue was transferred to pre weighed crucibles(W1) and it was dried to 2 hours at 130± 2 °C, cooled in a desiccators then weighed (W2). The dried desiccators containing samples were then ignited for 30 minutes at 600 ± 15°C. Finally the sample was cooled and weighed again.

$$\text{Crude fibre (g/100g sample)} = \frac{[100-(\text{moisture}^*+\text{fat})] \times (W_e - W_a)}{\text{Wt. of sample taken (moisture and fat free)}}$$

W_e preweighed ashing dish W_a weight of the dish after ashing

ANNEXURE-IV

PREPARATION OF MINERAL SOLUTION

To the ash that was obtained after burning followed by ashing was added 5 ml of a 1:1 solution of distilled water and fuming HCl. This mixture was then heated over a water bath to dryness before another 5 ml of the solution was added. It was heated further over the water bath until it started fuming and at this point, the crucible was retrieved and its contents filtered into a 100 ml volumetric flask using Whatman No.4 filter paper. After thorough rinsing of the crucible and the filter paper, the volume was made up to the mark with distilled water. Aliquots of this mineral solution were taken for the estimation of all the minerals in this study.

Practical No : 2: Sample preparation

Introduction

Sample preparation is the way in which a sample is treated to prepare for analysis. Careful sample preparation is critical in analytical chemistry to accurately generate either a standard or unknown sample for a chemical measurement. Errors in analytical chemistry methods are categorized as random or systematic. Random errors are errors due to change and are often due to noise in instrument. Systematic errors are due to investigator or instrumental bias, which introduces an offset in the measured value. Errors in sample preparation are systematic errors, which will propagate through analysis, causing uncertainty or inaccuracies through improper calibration curves. Systematic errors can be eliminated through correct sample preparation and proper use of the instrument. Poor sample preparation can also sometimes cause harm to the instrument.

Principles:

To make a solution, one must consider the solubility of the substance that is being measured. The compound of interest must dissolve in the solvent in order to make a solution. Solubility is a factor of intermolecular interactions of the analyte with the solvent and can often be manipulated by changing the type of the solvent or the pH.

Important points to be considered for Sample preparation :

- The first step in making a sample is choosing proper glassware and making a solution. Most samples in the liquid phase are made in volumetric flasks. Volumetric flasks are made to contain a certain volume of liquid at a given temperature (normally 20 °C), and are calibrated to be accurate less than 0.02% if they are class A glassware. Volumetric flasks are much more accurate for measuring liquids than graduated cylinders.
- To make a solution of a solid, the solid must first be accurately massed with a calibrated scale. However, the mass of some reagents and precipitates can change because they are hygroscopic and adsorb water. If the reagent has adsorbed water it is impossible to use the nonhydrated molecular weight to obtain the correct number of moles.
- To remove adsorbed water, solids that are thermally stable are dried in an oven at ~110 °C. Solid reagents and precipitates are then stored in a desiccator containing a desiccant that adsorbs any water present.
- If the sample to be diluted is a liquid a pipette is normally used to measure it. A glass transfer pipette is typically calibrated to deliver one accurate volume and the last drop stays in the pipette and should not be blown out.
- A measuring pipette will have multiple markings on it similar to a burette and is less accurate but more versatile than a transfer pipette. Smaller volumes can be measured using variable micropipetters, with disposable plastic tips, and these are available in volumes from 1–5,000 µL.
- Micropipetters should be calibrated every 6 months in order for them to maintain accuracy. If plastic is an issue, small microsyringes can also be used to measure out volumes in the microliter range. After a solution is made there are other elements of sample preparation that may be pertinent. Any sample with solid remaining in the liquid should be filtered.
- Traditional filtration uses a setup with a filter paper that sits in a fritted glass funnel on top of a filter flask with an arm where vacuum can be pulled. This type of filtering is used to collect a precipitate in experiments such as gravimetric analysis.

Smaller samples that are to be analyzed can be cleaned up via syringe filtering where the sample is loaded in a syringe and then passed through a polymer filter with down to 0.2-nm resolution.

Additionally, spin filters are available where the sample is loaded in a microcentrifuge tube with a filter, the tube is placed in a centrifuge, and the filtered liquid is at the bottom after centrifugation. Spin filters are also used to concentrate larger analytes, such as proteins. Syringe and spin filters are useful to filter out contaminants and other solids that might interfere with the instrument or measurement. The type of filtration used depends on the amount of sample and the size of the solid that needs to be filtered out. Sample preparation can also involve extracting or preconcentrating a sample.

Making a Solution from a Solid

1. Choose the correct glassware to make solution.

2. Clean the glassware thoroughly via an acid bath of 1% HCl or HNO3 along with soap to remove any impurities (safety warning: with any strong acid use gloves, goggles, and other appropriate personal protective equipment).

3. Rinse the glassware several times with distilled water. Dry in an oven if needed.

4. To make a solution from a solid, mass out the correct amount of solid.

5. Put the solid in the volumetric flask and then fill about ¾ full with solvent.

6. Swirl to fully dissolve the solid before filling the volumetric flask fully.

7. Fill the volumetric flask to the line. The meniscus should just touch the fill line. Then invert the flask several times with the cap on to further mix if necessary.

Making a Solution from a Liquid

1. Choose the correct glassware to make solution. To deliver a liquid using a transfer pipette, fill the pipette to the line using a pipette bulb.

2. Release the liquid into the volumetric flask for making the solution. Do not blow out the last drop.

3. Fill the volumetric flask to the line so the meniscus touches the line. Mix the solution by inverting several times.

Filtering

1. For a filter flask setup, place a piece of filter paper on the fritted glass filter.

2. Attach the fritted glass filter to a filter flask.

3. Attach a vacuum to the arm of the filter flask. A trap can also be used to prevent any liquid from going into the vacuum.

4. Turn on the vacuum and pour the sample through the filter paper.

5. Filter until a dry powder is left. Continue to dry the sample in an oven if a dry precipitate is desired.

6. To syringe filter, add the sample to a clean syringe with a Luer lock end.

7. Screw the syringe filter into the Luer lock. Push the plunger on the syringe and collect the liquid after the filter.

8. For a spin filter, pre-rinse the filter with buffer or ultrapure water.

9. Insert the spin filter into a microcentrifuge tube.

10. Load the sample on top of the filter and cap the tube.

11. Put the tube into a centrifuge, making sure to balance it properly with another tube on the other side, and centrifuge for 10–30 min, depending

on the type of spin filter.

12. Remove the filter and the liquid in the bottom is the filtered solution.

13. If the sample cannot go through the membrane — such as a big protein — it will remain at the top of the filter. In this case, turn the filter over,

put it in a new tube, and spin again. This will produce a concentrated sample.

Masking and Chelating

1. For masking and chelating, adjust the pH to an appropriate value depending on the formation constants of the masking agent and thechelating agent.

2. Add the masking agent to the solution and allow it to react for at least 10 min with the metal ion of choice.

3. Add the chelating reagent. For EDTA, it typically forms a 1:1 complex with the metal ion, so add as many moles of EDTA as metal that will be chelated.

4. After chelation, demask by adding a chemical that will react with the masked metal ion. The masked substance can then be analyzed or recovered by precipitation.

PREPARATION AND STANDARDIZATION OF SOLUTIONS

Soma Maji

Department of Dairy Technology

INTRODUCTION: Standard solutions

- The solutions with known strength.
- Calibration of other solutions and reagents depends upon the accurate strength of these solutions.
- Prepared by using certain substances (known as standard substances) having typical characteristics.

- Two Types of substances which are generally employed for preparing standard solutions:
  - Primary standard substances
  - Secondary standard substances

Primary standard substances

- Substances which can easily be obtained in pure and crystalline form e.g. Oxalic acid, sodium carbonate etc

Characteristics of primary standard substances

- It should be easy to obtain, to purify and to preserve.
- It must not be hygroscopic.
- It should not decompose at ordinary temperature.
- It should be readily soluble under the conditions in which it is used.
- Its reaction with other reagents should be quantitative and practically quick.
- It should have high eq. wt. so that the error due to weighing is minimized.
- It should be fairly cheap.

Sodium Carbonate

Primary standard substances used for Acid- Alkali titration

- *Sodium carbonate (eq. wt. 53 for anhydrous)*
  - Can be easily obtained in pure state
  - Its standard solution is prepared by directly dissolving a known weight of it in water and making the solution to a known volume.

Normality of solution	N/10	N/20
Sodium carbonate (anhydrous)	5.3g/l of solution	2.65g/l of solution

  - Used for finding strength of HCl, H_2SO_4 etc whose standard solutions cannot be prepared directly

- *Oxalic acid $(COOH)_2$ (eq. wt. 63 as it has two molecules of water of hydration)*
  - available in pure state

Normality of solution	N/10	N/20
Oxalic acid (hydrated)	6.3g/l of solution	3.15g/l of solution

  - Employed to find the strength of solutions of alkalies (NaOH and KOH) whose standard solutions cannot be prepared by the direct method

Preparation of some primary standard solutions

- Prepared by using standard substances.
- Known quantity of standard substance is dissolved in distilled water and desired volume is made.
- These substances have

Constant weight | High purity | Non hygroscopic

- Therefore, solution obtained is of

Known and definite concentration

The examples of such solutions:

Standard N/10 oxalic acid solution (Primary standard)

- Weigh 6.3g Oxalic acid (Hydrated salt)
- Dissolve in distilled water
- Make up the volume to one liter in volumetric flask
- used to find the strength of solutions of alkalies like NaOH, KOH (Secondary standards) whose standard solutions can not be made by direct weighing.

Standard N/10 sodium carbonate solution (Primary standard)

- Weigh 5.3g pure anhydrous salt
- Dissolve in distilled water
- Make up the volume to one liter in volumetric flask
- is used to find out the strength of solutions of acids like HCl, H_2SO_4, HNO_3 etc whose standard solutions cannot be prepared directly.

Secondary standard substances

- Those substances or reagents which cannot be obtained in a sufficient pure state
- e.g. NaOH, KOH, HCl, H_2SO_4, HNO_3

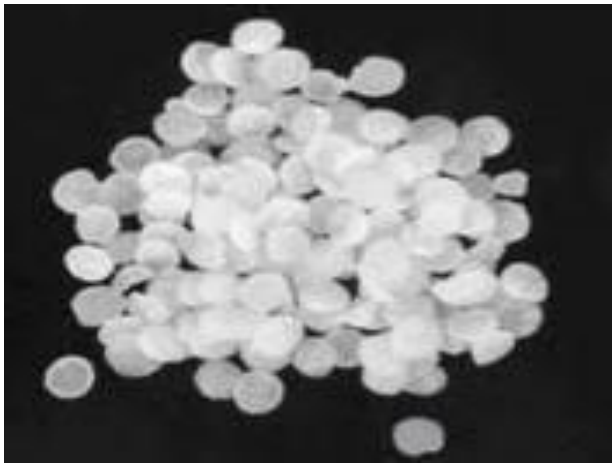


Fig. Sodium Hydroxide pellets

Preparation of some secondary standard solutions

For example: 1. Standard alkalies
2. Standard acids

Preparation of standard alkalies
N/10 NaOH

- Prepare concentrated stock solution
  - Say, 50% of NaOH by dissolving equal parts of NaOH pellets (50 gm) & water (50 gm) in a flask
- Keep it tightly stoppered for 3-4 days
- Use the clean, supernatant liquid for preparing N/10 solution
- Approximately 8 ml of this stock solution (50%) is required per litre of distilled water.

- This will give ***approximate solution***
- For standardization, take this solution i.e. approximate N/10 NaOH solution in burette

- Take 10 ml of standard N/10 oxalic acid (primary standard) solution in conical flask
- Add 2- 3 drops of phenolphthalein indicator to it.
- Add approximate N/10 NaOH solution from burette to the conical flask containing standard oxalic acid solution by continuous mixing by swirling the flask

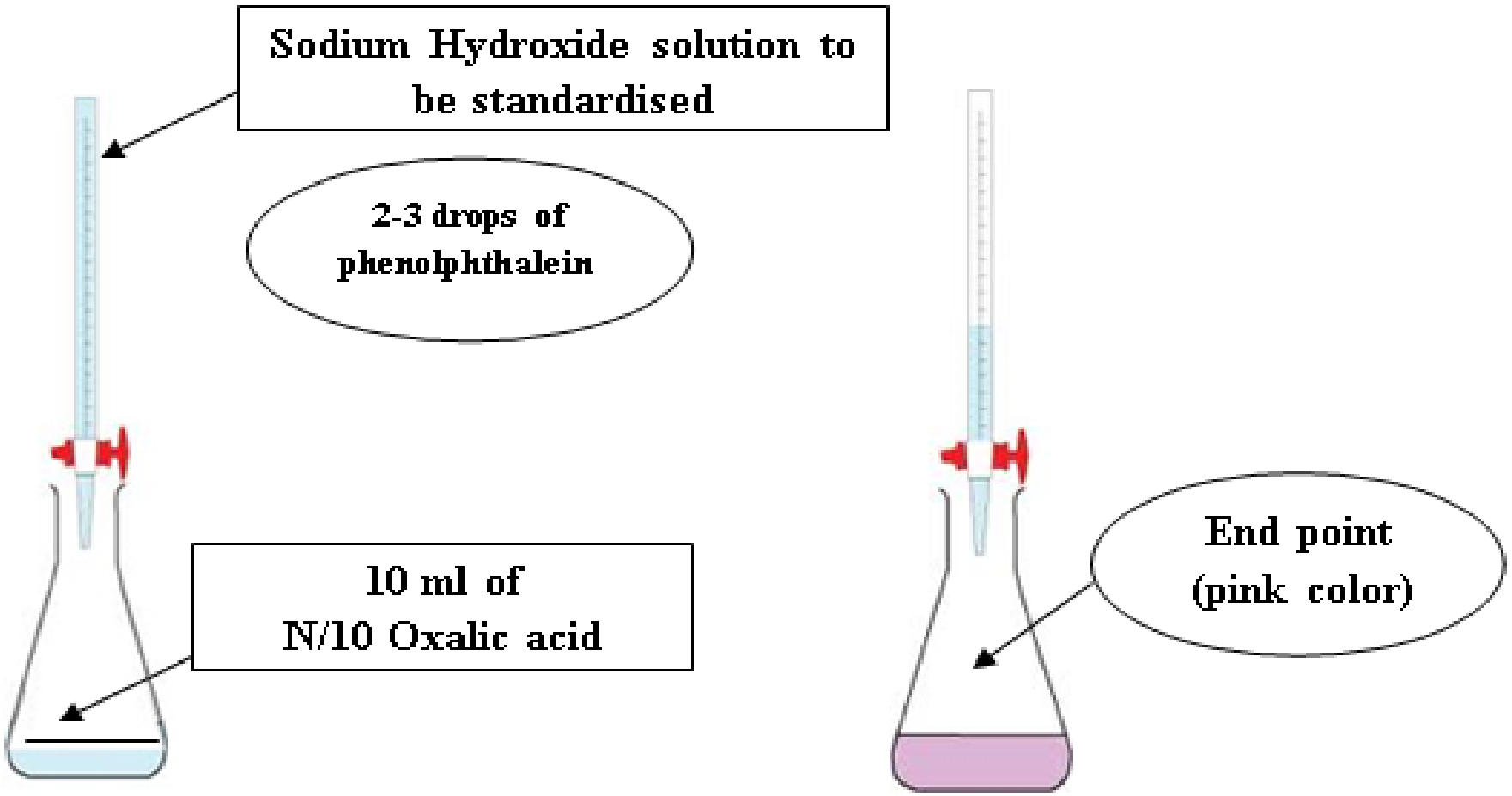
Till apearance of pink colour

- Note down the volume of approximate N/10 NaOH solution used in the titration of 10 ml of standard oxalic acid.
- Calculate the normality of the unknown sodium hydroxide solution by using Normality equation

$$N_1V_1 = N_2V_2$$

(Base) (Acid)

Note: Ideally for 10 ml of N/10 oxalic acid solution, exact 10 ml of N/10 NaOH solution should be consumed.



Standard Solutions

If volume of approx. NaOH used in the titration is less than 10 ml, means solution is strong and its normality is not N/10, so dilute the basic solution and again standardize with standard oxalic acid solution till normality of approximate solution is same as that of standard solution.

$$N1 = \frac{N2V2}{V1}$$

N_1 = Normality of NaOH solution. (?)

V_1 = Volume of NaOH solution used. (ml)

N_2 = Normality of standard oxalic acid solution. (0.1 N)

V_2 = Volume of standard oxalic acid solution. (10 ml)

If volume of approx. NaOH used in the titration < 10 ml

Solution is strong and its normality is not N/10

dilute the basic solution

and

again standardize with standard oxalic acid solution

Till

Normality of approximate solution is same as that of standard solution

Preparation of standard acids: before going for the preparation of standard acids, first let us see the strength of concentrated acids generally available

Approximate Strength of Concentrated Acids Generally Available

Sr. No.	Acids	Sp. Gr.	Approx. Strength
1	Acetic acid glacial CH_3COOH	1.05	16N
2	Hydrochloric acid HCl	1.16	12N
3	Nitric acid HNO_3	1.42	16N
4	**Sulphuric acid H_2SO_4**	**1.84**	**36N**

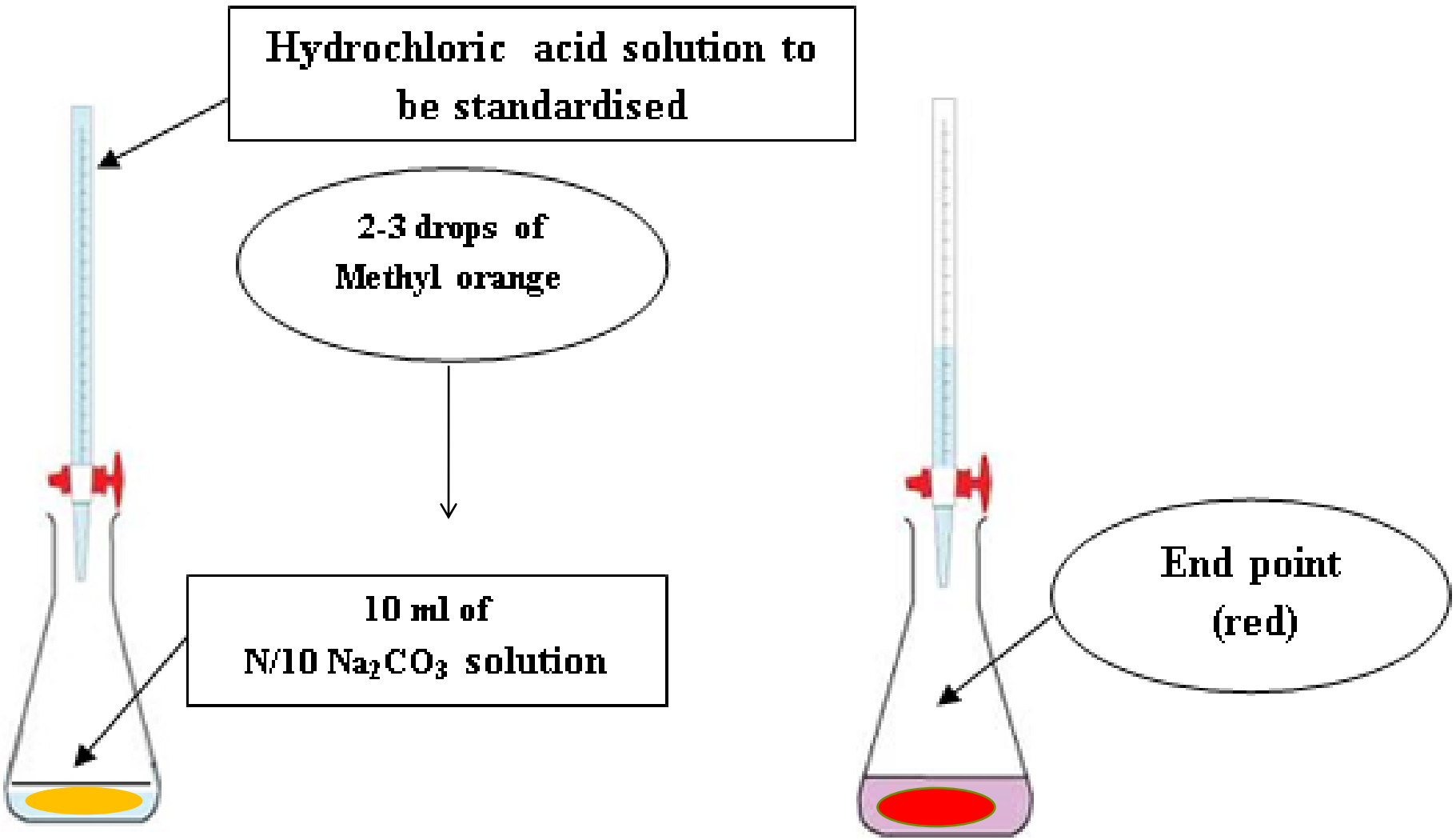
Preparation of N/10 HCl: following two ways can be used:

- First, prepare approximately 0.1 N solution on the basis of the strength given on the label (usually 12 N) by diluting it 120 times with distilled water

- Standardize it against standard N/10 Na_2CO_3 using methyl orange as an indicator.

- Firstly, prepare approximately 0.1 N solution on the basis of the strength given on the label (usually 12 N) by diluting it 120 times with distilled water

- Standardize it against standard N/10 NaOH which is already standardized against N/10 oxalic acid, using Phenolphthalein indicator



Standard Solutions

Preparation of N/10 H_2SO_4 : before its preparation, one must observe the cautions for handling H_2SO_4

- **Precautions :**
  - **Concentrated H_2SO_4 is very corrosive in nature, therefore, it should be handled carefully.**
  - **<u>Always remember</u>: "ADD ACID TO WATER" under cold conditions. This is done to avoid bumping due to the heat generated.**

- For preparation of N/10 H_2SO_4, take 10 ml of concentrated H_2SO_4 (usually about 36 N)
- Dilute 36 times by adding acid in small quantity to distilled water in a cold water bath to make it 1N
- Dilute this 1N solution further 10 times to make it N/10
- Standardize against standard N/10 NaOH or N/10 KOH using phenolphthalein indicator

Preparation of N/10 HNO_3

- Take 10 ml of concentrated HNO_3 (usually about 16 N)
- Dilute 16 times by adding acid to distilled water to make it 1N
- Dilute this 1N solution further 10 times to make it N/10.
- Standardize against standard N/10 NaOH or N/10 KOH using phenolphthalein indicator.

Preparation of Some Other Reagents

1 Preparation of chromic acid (Cleaning solution) H_2CrO_4

- Dissolve 50 g of $K_2Cr_2O_7$ in 50 ml of water in a beaker kept in cold water.
- Add slowly 500 ml of concentration H_2SO_4 and cool.

- It is a very corrosive solution and care should be taken to avoid its coming in contact with the skin.
- It is almost a saturated solution of $K_2Cr_2O_7$ in concentrated H_2SO_4.
- Here also, during preparation of chromic acid, observe the precaution for handling of conc. H_2SO_4 i.e. 'ADD ACID TO WATER', under cold conditions (in a cold water bath)

4. Preparation of 0.1 N sodium thiosulphate solution ($Na_2S_2O_3.5H_2O$)

1. Dissolve approximately 24.8 g of sodium thiosulphate crystals in previously boiled and cooled distilled water.
2. Make the volume to 1000 ml.
3. Store the solution in a cool place in a dark colored bottle.
4. After storing the solution for about two weeks, filter if necessary and standardize.

Standardization of sodium thiosulphate

- Weigh accurately about 5.0 g of finely ground potassium dichromate which has been previously dried to a constant weight at 105 ± 2° in to a clean 1.0 litre volumetric flask.
- Dissolve in water make up to the mark; shake thoroughly and keep the solution in dark place.
- Pipette 25.0 ml of this solution into a clean glass stoppered 250 ml conical flask.
- Add 5.0 ml of concentrated hydrochloric acid and 15.0 ml of 10% potassium iodide solution.
- Allow to stand in dark for 5 minutes and titrate the mixture with the solution of sodium thiosulphate using starch solution as an indicator towards the end.

End point: Blue color to Green color

- The normality (N) of the sodium thiosulphate can be calculated as:

- $N = 25W \div 49.03V$

Where,

- W is weight in g of the potassium dichromate
- V is volume in ml of sodium thiosulphate solution required for the titration.

5. Preparation of 0.1 N standard solution of silver nitrate

1. Dissolve slightly more than the required quantity (17.2 g instead of 16.989 g) of reagent grade silver nitrate in distilled water.
2. Dilute to one liter in a volumetric flask.
3. Weigh accurately 0.5844 g of NaCl (dried at 110°C before weighing) and transfer to a 100 ml volumetric flask and add 50 ml of halogen free water to dissolve the material. Make up the volume with distilled water to the mark and mix the contents.
4. Pipette out 10 ml of the prepared standard sodium chloride solution in 100 ml conical flask and add 2-3 drops of potassium chromate indicator (5% solution in water).
5. Titrate with silver nitrate solution until a perceptible reddish brown color appears.

- Carry out a blank titration using 10 ml of distilled water instead of sodium chloride solution.
- Deduct the blank reading from the reading for the standard sodium chloride solution.

Normality of the silver nitrate solution is calculated using normality equation:

- $N_1V_1 = N_2V_2$

Where,

N_1 = Normality of standard sodium chloride solution (0.1N)

V_1 = Volume in ml of sodium chloride used for titration (10 ml).

N_2 = Normality of prepared silver nitrate solution.

V_2 = Volume in ml of prepared silver nitrate solution used for titration.

6. Preparation of EDTA solutions

1. **Preparation of 0.01 M EDTA solution**:

   Dissolve 3.8 g of disodium ethylene diamine dihydrogen tetra acetate (EDTA, M.Wt. 372.25) in distilled water

   Volume made to 1 liter with distilled water

   Mix well and store in polyethylene reagent bottle

   It is standardized against 0.01 M $CaCO_3$ or $CaCl_2$

2. **Preparation of 0.01 M $CaCl_2$ solution:**

Prepare standard Ca solution i.e. 1ml = 1mg $CaCO_3$, M.wt. 100

Weigh 1g $CaCO_3$ into 500ml conical flask or beaker

Add dilute HCl through funnel until $CaCO_3$ is dissolved

Add 20 ml water, boil to expel CO_2 and cool.

Add few drops of methyl red indicator

[adjust colour intermediate orange (brownish red) with dilute NH_4OH or HCl as required].

Transfer quantitatively to 1 L volumetric flask and make up volume to the mark.

Shake it well and store it well and store in air-tight reagent bottle.

3. **Erichrome Black T indicator**:

Dissolve 0.5 g of Erichrome black T in 100 ml of triethanolamine

OR

0.4 g in 100 ml methanol

4. **Buffer solution**:

Dissolve 16.9 g NH_4Cl in 143 ml NH_4OH

Dilute to 250 ml with water.

Store in tightly stoppered Pyrex of plastic bottle

Dispense from bulb-operated pipette

Discard after 1 month or when 1-2 ml added to sample fails to produce pH 10.0±0.1 at end point titration.

7. Standardization of EDTA solution:

- Rinse and then fill burette with prepared EDTA solution.
- Pipette 25 ml of standard $CaCO_3$ solution into 250 ml Erlenmeyer flask
- **Add** 1 ml ammonia buffer

  (to raise the pH as reaction takes place at high pH)
- **Add** 3-4 drops of Erichrome black T indicator
- Titrate the EDTA solution until colour changes from wine red to dark blue with no reddish tinge remaining.
- Calculate the molarity of EDTA ($M_1V_1 = M_2V_2$),

[if excess, follow the procedure for the standardization, recheck the molarity and it should be 0.01 M]

8. Preparation of Fehling solution

- Used for the estimation of reducing sugars
- Generally prepared fresh by mixing equal quantities of Fehling's A and Fehling's B which are prepared separately as follows:

a) Fehling's A

- Dissolve 34.639 g $Cu(SO)_4.5H_2O$ in distilled water
  - Add 0.5 ml concentrated H_2SO_4, mix and
  - Make the volume to 500 ml. Filter if necessary.

b) Fehling's B

- Dissolve 173 g of Rochelle salt (Na K tartarate) and 50 g of NaOH in distilled water.
- Allow to stand for two days.
- Filter if necessary.

Standardisation of Fehling's solution:

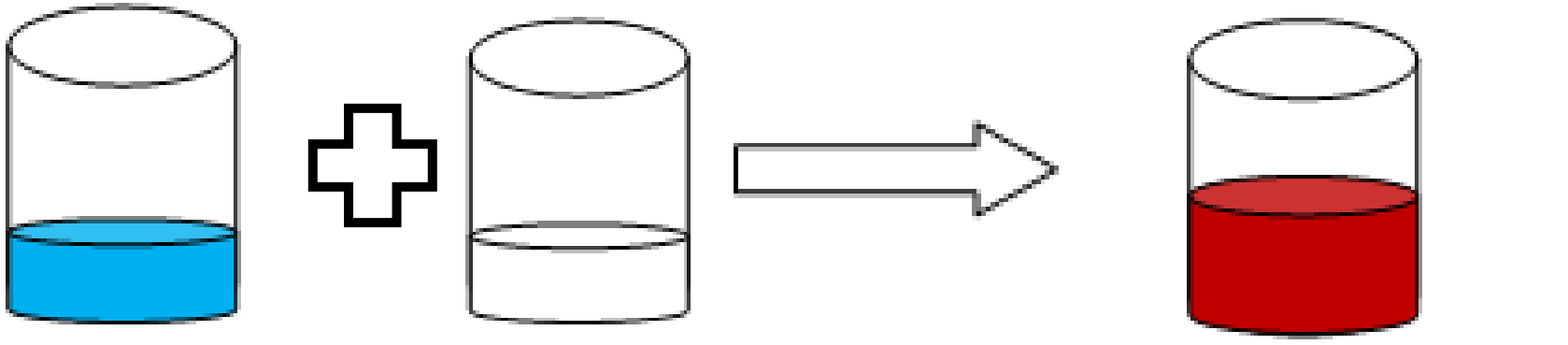
1. 5 ml of Fehling's solution A + 5 ml of Fehling's solution B

using two separate pipettes in a 250 ml Erlenmeyer flask

2. Fill up a burette with the standard lactose solution
3. Heat the content of the flask to boiling over burner or heater and maintain moderate boiling for 2 min (add some inert boiling chips to prevent bumping)
4. Add 3 to 4 drops of methylene blue indicator (0.2% in water) without removing from the flame.
5. Titrate the content of the flask against standard lactose solution (0.5%) from the burette until the blue colour disappears and the bright brick-red colour of precipitated Cu_2O appears.

- 6. At the end point the Cu_2O suddenly settles down giving a clear supernatant.
- 7. Note the volume of lactose solution required for the standardization of Fehling's solution.
- 8. After this preliminary titration, further titration or titrations should be carried out, adding practically the whole of the standard lactose solution volume (one ml less than required as observed in first titration) required for the titration before commencing the heating.
- 9. Let the contents boil for 2 minutes. Now, add 3-4 drops of methylene blue indicator, continue heating and complete the titration within 3 min from the commencement of boiling.
- 10. Let V_1 ml be the titre for this experiment.

- 11. Multiply the titre value by mg/ml lactose of the standard solution to obtain total lactose required to reduce the copper and term the value a "Factor F".



Fehling A solution Fehling B solution brick-red Precipitates of Cu_2O

Note: Carefully note the first disappearance of blue colour. Once missed, it is difficult to ascertain the end point. Maintain the boiling at a uniform rate during the titration.

9.Preparation of pH indicator solutions

- **Phenolphthalein indicator solution:**

Weigh 1.0 g phenolphthalein

place the powder in a 100 ml volumetric flask containing about 50ml of 95% ethanol

Stopper and shake vigorously for a few minutes

add 20ml more ethanol and shake until a clear solution is formed

make the volume to 100 ml

- **Methyl orange indicator solution**

1. Dissolve 1.0 g of methyl orange powder in distilled water
2. dilute to one litre
3. Filter, if necessary


Thank You

2. Preparation of Gerber sulphuric acid

- In Gerber fat test, Gerber Sulphuric acid is used to dissolve casein in milk.
- **concentrated H_2SO_4** → Causes → Charring of organic matter

  Dilute H_2SO_4 → Causes → Casein is precipitated & not dissolved

- Therefore, the concentration of sulphuric acid → So adjusted to just strong enough to dissolve the casein without charring the fat.
- Also, acid produces necessary heat to keep the fat in the liquid state

Gerber H_2SO_4

- Strength → 90-91%
- Sp. Gr. → 1.807 to 1.812

Concentrated H_2SO_4

- Strength → 97-99%
- Sp. Gr. → 1.835

For practical purposes:

900ml conc. H_2SO_4 + 100ml Distilled water

1000ml Gerber acid

Precaution for handling of concentrated H_2SO_4 and other acids

ADD ACID TO WATER under cold conditions

Preparation

1. Take required vol. of water (say 100 ml) in a flask
2. Keep the flask in a basin of ice-cold water
3. Carefully, add the required quantity of concentrated H_2SO_4 (say 900 ml) in small quantities at a time keeping the container sufficiently cold.
4. Mix gently.

3. Testing the amyl alcohol (a by product of fuel oil refinery) used for fat determination

- Iso-amyl alcohol (also called Iso-butyl carbinol) used in Gerber fat test

Should be clear, colorless

and

Free from impurities particularly fatty mater.

Perform various tests to know purity of iso-amyl alcohol:

- **Density**: At 27°C, Density shall be between 0.803 to 0.805 g/ml (using specific gravity bottle).
- **Boiling point**: Boiling point shall be 128-129°C (can be checked using boiling point apparatus).
- 95% of the liquid shall get distilled between 130-132°C.
- **Test for absence of furfural and other impurities**: 5 ml iso-amyl alcohol + 5 ml H_2SO_4 (97%) → Observe the color → shall not show more than a yellow or light brown color.
- **Test for absence of fatty matter**: Carry out a blank Gerber fat test using distilled water in place of milk. If any fat separation is observed → indicates impurities of some fatty matter.