

N.B. : (i) Compounds containing nitrogen in oxidised state such as nitroazo etc. cannot be used for nitrogen estimation by Kjeldahl's method. However, the use of glucose during digestion reduces nitrogen function and thus estimation of nitrogen is possible.

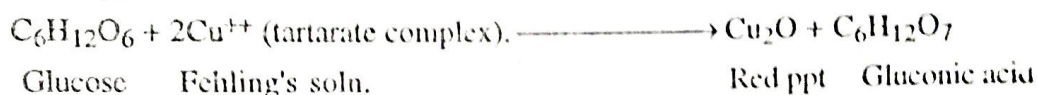
(ii) The titration may be carried out by 0.02 (N) HCl solution with methyl red-methylene blue mixed indicator (the first appearance of violet colour indicate the end point) in case boric acid solution is used as the absorbent.

(iii) A blank test should be performed with analytical glucose and the titre value of the original experiment should be corrected.

(iv) 0.02 (N) NaOH may be used for titration of excess acid using phenolphthalein as indicator and saturated 0.02 (N) oxalic acid solution have to be used for determination of strength of sodium hydroxide solution.

2. To determine the strength of a given glucose solution using Fehling's solution :

Principle : A known volume of Fehling's solution is reduced to cupric oxide by glucose solution of known and unknown strengths. The strength of the supplied glucose solution in g/l is then calculated from the volume of the sugar solution consumed.



Materials required and their preparation :

Fehling A : 17.32 gm. of hydrated copper sulphate is dissolved in 250 ml. of distilled water.

Fehling B : 86.5 gm. of sodium potassium tartarate and 25 gm. of sodium hydroxide is dissolved in 250 ml. of distilled water.

Standard glucose solution : 1.25 gm. of pure anhydrous glucose (A.R) is weighed accurately from a weighing bottle and volume is made up to the mark.

Methylene blue indicator : 0.5 gm. of methylene blue dissolved in 250 ml. of distilled water.

Unknown solution : Will be supplied [Approx 5 gm. dissolved in 1 lit.]

Procedure : Fehling's solution is prepared by thorough mixing of equal volumes of Fehling A and Fehling B in a clean dry bottle or conical flask. 25 ml of it is pipetted out for each titration into a 250 ml conical flask, 25 ml. of distilled water is added. boiled over a wire gauze and standard glucose solution is added from a burette, first in 1 ml. portion until the solution assumes a faint blue colour and then drop by drop until the blue colour just disappears keeping the mixture boiling throughout the titration. This gives an approximate value. Addition of methylene blue indicator just before the end point (only 0.5--1 ml. of glucose solution is left for reaching end point) sharpens end point. The process is repeated until a concordant result has been reached, Similar titrations are performed at least three times with supplied solution when concordant readings are expected.

Experimental Result :

Table I. Weighing of glucose for standard solution of 250 ml :

Initial weight	Final wt.	Weight of glucose
w ₁ gm	w ₂ gm.	= w ₁ - w ₂ = w gm.

Table II. Standardisation of Fehling's solution :

No. of observations	Vol. of Fehling. soln. taken.	Vol. of glucose solution required	Mean volume of glucose soln.
1.	25 ml.		
2.	25 ml.		
3.	25 ml.		

N.B. : In case of concordant readings average volume must not be taken.

Table III

Determination of strength of unknown glucose solution by standard Fehling's solution :

Same as Table II.

Calculation : Weight of glucose dissolved in 250 ml. = w gm.

Vol. of Fehling's solution taken for each titration = 25 ml.

Mean volume of standard (known) glucose solution = V_k .

Mean volume of supplied (unknown) glucose solution = V_u .

250 ml. of standard glucose solution \equiv w gm. glucose.

V_k ml. of " " " $\equiv \frac{w \times V_k}{250}$ gm. glucose.

Again V_u of unknown glucose solution $\equiv V_k$ ml. of standard glucose solution $\equiv \frac{w \times V_k}{250}$ gm. of glucose.

\therefore 1,000 ml. of unknown glucose solution $\equiv \frac{w \times V_k \times 1,000}{250 \times V_u} = \frac{w \times V_k \times 4}{V_u}$ gm. of glucose.

\therefore Strength of unknown glucose solution $\frac{w \times V_k \times 4}{V_u}$ gm./litre.

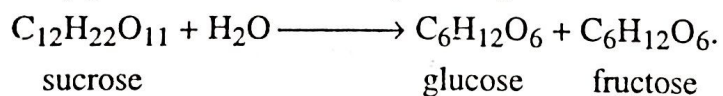
N.B. : Percentage of purity of glucose sample may be checked by preparing both the glucose solution-standard one using A.R. grade anhydrous glucose and the other from given glucose sample. Titrations are performed using same Fehling's solution for each titrations.

The working formula may be expressed as % of glucose $= \frac{V_k \times w}{V_u} \times \frac{100}{s}$

Where s = weight of glucose sample dissolved in 250 ml. of distilled water and w, V_k , V_u are of usual meaning in the above experiment.

3. Determination of strength of Sucrose solution by Fehling's solution.

Principle : The non-reducing sugar sucrose is estimated by converting it into a mixture of two reducing sugars, namely glucose and fructose by boiling with dilute acids.



i.e. 342 gm. of sucrose = 360 gm. of 'invert sugars'

The 'invert sugar' so produced is titrated by standard Fehling's solution using methylene blue as indicator.

Materials required and their preparation :

Unknown Solution : Will be supplied. (approx 4.5 gm. dissolved in 1 litre).

Preparation of standard glucose solution, Fehling's solution etc. are performed as described under estimation of glucose.

Procedure : Standardisation of Fehling solution :

As described under estimation of glucose.

Estimation of unknown sucrose solution with standard Fehling's solution :

25 ml. of given (supplied) sucrose solution is pipetted out into a 250 ml. conical flask and boiled with 25 ml. of 4(N) HCl [8 ml. of 12(N) HCl diluted to 25 ml. with water] at 60° – 70°C for 20 minutes on a water-bath. The solution is cooled and neutralised with 30% sodium hydroxide solution using methyl red as indicator. The resulting solution is transferred quantitatively to a 250 ml. volumetric flask; volume is made up to the mark with distilled water and the solution is titrated with standard Fehling's solution following the procedure given for estimation of glucose.

Experimental Results :

Results are given in tabular form as shown under estimation of glucose.

Calculations :

Let us suppose w gm. of glucose has been taken for preparation of 250 ml. of standard glucose solution.

Volume of st^d. glucose solutions required for 25 ml. Fehling solⁿ = V_k ml.

Volume of 'invert sugar' solⁿ required for 25 ml. Fehling solⁿ = V_u ml.

$\therefore V_k$ ml. of st^d. Glucose solⁿ \equiv V_u ml. of 'invert sugar' solⁿ.

250 ml. of st^d. glucose solⁿ \equiv w gm. of glucose.

$\therefore V_k$ ml. of st^d glucose solⁿ \equiv $\frac{w \times V_k}{250}$ gm. glucose.

$\therefore V_u$ ml. of 'invert sugar' solⁿ \equiv $\frac{w \times V_k}{250}$ gm. of glucose.

\therefore 250 ml. of 'invert sugar' solⁿ \equiv $\frac{w \times V_k \times 250}{250 \times u}$
 \equiv $\frac{w \times V_k}{V_u}$ gm. of glucose.

According to equation of hydrolysis,

360 gm. of 'invert sugar' \equiv 342 gm. of sucrose.

or, 360 gm. of glucose \equiv 342 gm. of sucrose.

or, $\frac{w \times V_k}{V_u}$ gm. of glucose \equiv $\frac{342 \times w \times V_k}{360 \times V_u}$ gm of sucrose which is present in 25 ml. of supplied solution.

\therefore Strength of supplied solution = $\frac{342 \times w \times V_k \times 1000}{360 \times V_u \times 25}$ gm/litre.

= $\frac{342 \times w \times V_k \times 40}{360 \times V_u}$ gm/litre.

4. Estimation of glucose and sucrose in a mixture :**Principle :**

(i) Glucose is estimated directly by standard Fehling solution, following the procedure given in Experiment No. 2.

(ii) 25 ml. of supplied solution is hydrolysed and total amount of glucose and 'invert sugar' are estimated following the procedure given in Experiment No. 3 using standard solution.

(iii) The amount of glucose and sucrose in the supplied solution can be determined by the calculation given below.

Calculation : Let wt of glucose taken for preparing 250 ml. of standard solution = w gm.

Now, 25 ml. of Fehling solution \equiv V_k ml. of std glucose solⁿ.

\equiv V_u ml. of unknown glucose solⁿ.

\equiv V_m ml. of glucose and invert sugar solⁿ.

$\therefore V_u$ ml. of unknown glucose solⁿ \equiv $\frac{w \times V_k}{250}$ gm. of glucose

[calculation as in Exp. 2]

$$\begin{aligned} \therefore 1,000 \text{ ml. of unknown glucose sol}^n &\equiv \frac{w \times V_k \times 1000}{250 \times V_u} \text{ gm. of glucose.} \\ &\equiv \frac{w \times V_k \times 4}{V_u} \text{ gm. of glucose.} \end{aligned}$$

$$\text{Again, } V_m \text{ ml. of glucose and inverted sugar sol}^n \equiv \frac{w \times V_k}{250} \text{ gm. of glucose.}$$

$$\begin{aligned} \therefore 250 \text{ ml. of glucose and inverted sugar sol}^n &\equiv \frac{w \times V_k \times 250}{250 \times m} \text{ gm. of glucose.} \\ &\equiv \frac{w \times V_k}{V_m} \text{ gm. of glucose.} \end{aligned}$$

$$\therefore 25 \text{ ml. of supplied sol}^n \equiv \frac{w \times V_k}{V_m} \text{ gm. of glucose.}$$

$$\therefore 1,000 \text{ ml. of supplied sol}^n \equiv \frac{w \times V_k \times 40}{V_m} \text{ gm. of glucose.}$$

\therefore Amount of invert sugar present in 1,000 ml. of supplied solution =

$$\left(\frac{w \times V_k \times 40}{V_m} - \frac{w \times V_k \times 4}{V_u} \right)$$

According to hydrolysis equation 360 gm. of invert sugar \equiv 342 gm. of sucrose

$$\therefore \left(\frac{w \times V_k \times 40}{V_m} - \frac{w \times V_k \times 4}{V_u} \right) \text{ gm. of invert sugar}$$

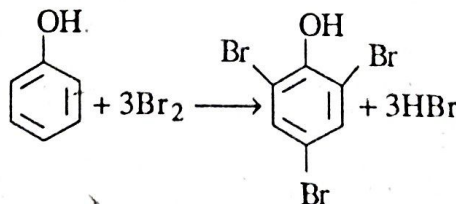
$$\equiv \frac{342}{360} \left(\frac{w \times V_k \times 40}{V_m} - \frac{w \times V_k \times 4}{V_u} \right) \text{ gm. of sucrose}$$

$$\therefore \text{Amount of glucose in supplied sol}^n = \frac{w \times V_k \times 4}{V_u} \text{ gm./litre.}$$

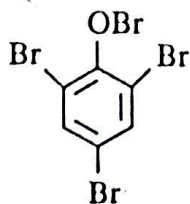
$$\text{and amount of sucrose} = \frac{342}{360} \left(\frac{w \times V_k \times 40}{V_m} - \frac{w \times V_k \times 4}{V_u} \right) \text{ gm./litre.}$$

5. Estimation of phenol by bromination method :

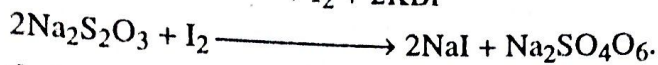
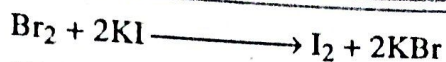
Principle : Aqueous solution of phenol gives 2,4,6 tribromophenol in quantitative yield when treated with excess potassium bromate and potassium bromide solution in presence of hydrochloric acid. The excess of bromine is determined iodometrically.



Some, tribromophenol bromide,



may also be formed but can be converted to tribromophenol on addition of potassium iodide during titration.



Materials required and their preparation :

Brominating mixture (0.2N) : 1.392 gm. of anhydrous potassium bromate is weighed accurately by method of difference; transferred into a 250 ml. volumetric flask, dissolved in little distilled water; 15 gm. of potassium bromide is then added, it is dissolved and volume is made up to the mark.

Sodium thiosulphate solution (0.2 N approx.) :

12.5 gm. of sodium thiosulphate is dissolved in 250 ml. of distilled water, in a volumetric flask.

Preparation of 1% starch solution (indicator) :

1 gm. of soluble starch is converted to paste with a little cold water, added to 100 ml. of boiling water, boiled for 2 minutes 3 gm. of potassium iodide is added and finally 2-3 drops of chloroform is added to render the solution more stable.

Unknown sample solution : Will be supplied (approx. of 0.2 N).

[Ca 3.2 gm. of phenol dissolved in 1 litre of distilled water or in 10-12 ml. of 10% sodium hydroxide and volume is made 1 litre by distilled water].

Procedure : 10 ml. of unknown sample solution is pipetted out into 250 ml. stoppered conical flask. 20 ml. of standard 0.2 N brominating mixture and then 5 ml. conc. HCl are added to it. The flask is stoppered immediately. The mixture is shaken for a minute and allowed to stand for 20-25 minutes at room temperature with occasional shaking for completion of the reaction. 2 gm of potassium iodide is then added by opening the stopper for a short time, shaken well and allowed to stand for another 5 minutes. The stopper and inner side of flask are washed down into the flask with distilled water and the liberated iodine is titrated with standard sodium thiosulphate solution until the solution assumes straw colour. A few drops of starch solution is added to obtain blue colour. Dropwise addition of thiosulphate is continued with shaking until sharp disappearance of blue colour occurs. The process is repeated three times. Three blank titrations are also performed with 10 ml. of distilled water instead of phenol solution.

Experiment Results : Students are advised to write the experimental results in tabular forms. Specimen tables are given in previous experiments.

Calculation : Let, wt. of KBrO_3 taken for 250 ml. of bromine solution = w gm.

$$\therefore \text{Strength of bromine solution} = \frac{w \times 0.2}{1.392} \text{ (N)}.$$

Vol., of thio. solution required for 20 ml. bromine solution (blank titration) = V_1 ml.

Vol. of thio. solution required for excess bromine solⁿ. = V_2 ml.

\therefore Vol. of thio. solution equivalent to bromine solⁿ consumed by phenol = $(V_1 - V_2)$ ml.

Again, V_1 ml. thio. solⁿ \equiv 20 ml. of $\frac{w \times 0.2}{1.392}$ (N) bromine solⁿ.

$$\therefore (V_1 - V_2) \text{ ml. of thio. sol}^n \equiv \frac{20 \times (V_1 - V_2) \times w \times 0.2}{V_1 \times 1.392} \text{ ml. of (N) of bromine sol}^n.$$

$$\equiv x \text{ ml. of (N). Bromine sol}^n.$$

Now, 6,000 ml. of (N) bromine solution \equiv 1 gm. mol of phenol.

$$\equiv 94 \text{ gm. of phenol.}$$

\therefore 1 ml. of (N) bromine solution $\equiv \frac{94}{6000}$ gm. of phenol.

$$\therefore \frac{20 \times (V_1 - V_2) \times w \times 0.2}{V_1 \times 1.392} \text{ ml. (N) bromine solution} \equiv \frac{94 \times 20 (V_1 - V_2) \times w \times 0.2}{6,000 \times V_1 \times 1.392} \text{ gm.}$$

of phenol.

$$\therefore 10 \text{ ml. of phenol sol}^n \text{ contains } \frac{94 \times 20 \times (V_1 - V_2) \times w \times 0.2}{6000 \times V_1 \times 1.392} \text{ gm. phenol.}$$

$$\text{So 1,000 ml. phenol sol}^n \text{ contains } \frac{94 \times (V_1 - V_2) \times w \times 0.2}{6 \times V_1 \times 1.392} \text{ gm. of phenol.}$$

$$= \frac{4.502 \times (V_1 - V_2) \times w}{V_1} \text{ gm.}$$

$$\therefore \text{Strength of phenol sol}^n = \frac{4.502 \times (V_1 - V_2) \times w}{V_1} \text{ gm/litre.}$$

N.B : (i) Equivalent wt. of $\text{KBrO}_3 = \frac{\text{mol wt}}{6}$ because Br^{+5} is changing to Br^- [$\text{Br}^{+5} + 6e = \text{Br}^-$] where

change of electron is 6. $\text{Eq wt} = \frac{\text{mol wt}}{6} = \frac{167.04}{6} = 27.84.$

(ii) Equivalent wt. of $\text{Na}_2\text{S}_2\text{O}_3 = \text{Molecular wt}$ because two moles of $\text{S}_2\text{O}_3^{2-}$ changes to $\text{S}_4\text{O}_6^{2-}$ ($2\text{S}_2\text{O}_3^{2-} \rightarrow \text{S}_4\text{O}_6^{2-} + 2e$) where change of electron is 2 for two moles of $\text{S}_2\text{O}_3^{2-}$ Eq wt. = Mol wt. 248.20.

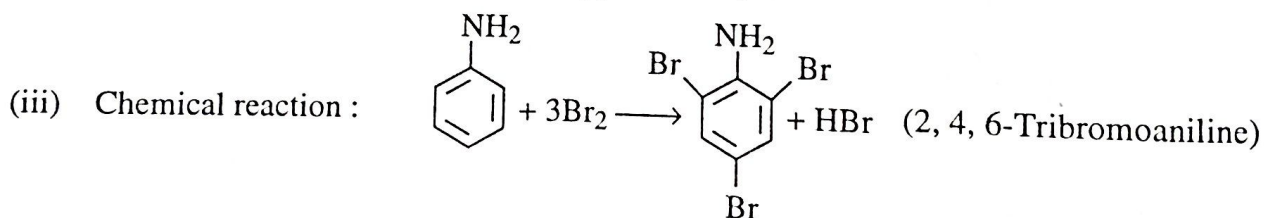
(iii) Equivalent wt of phenol = $\frac{\text{ml wt}}{6}$ because 3 moles (six equivalent) of bromine reacts with phenol to give 2,4,6 - tribromophenol.

$$\text{Eq.wt.} = \frac{\text{mol wt}}{6} = \frac{94}{6} = 15.67.$$

6. Estimation of aniline by bromination method.

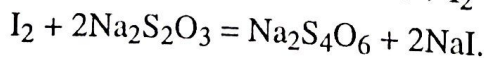
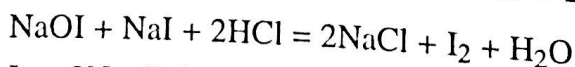
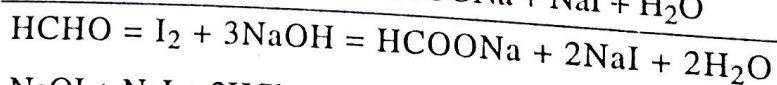
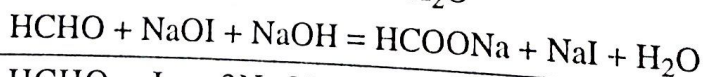
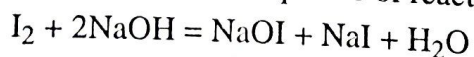
The principle and procedure are the same as given that for phenol (Expt. No. 5). Exception : (i) 6,000 ml. of (N) bromine solution = 93 gm. of aniline [See stoichiometric eqn below].

(ii) Aniline solution (sample soln) is prepared by dissolving 3.1 g of aniline in minimum volume of dilute hydrochloric acid and diluted to 1 litre (approx 0.2 N).



7. Estimation of formaldehyde by iodometry :

Dilute aqueous formaldehyde solution is oxidised quantitatively by alkaline sodium hypiodide solution to sodium formate, excess hypiodide is decomposed by hydrochloric acid to iodine which is titrated with standard sodium thiosulphate solution. The sequence of reactions are given below :



This method is only applicable to dilute formaldehyde solⁿ (concentration less than 1%) free from other carbonyl compounds.

Materials required and their preparation :

Preparation of formalin solution : About 4.0 gm. of formalin (35% - 40%) is dissolved in 250 ml. of distilled water.