Experiment 11
ESTIMATION OF SUGARS

Structure.

11.1 INTRODUCTION

Two methods are in common use for the estimation of sugars.

(i) Chemical, depending upon the reducing properties of certain sugars

(ii) Polarimetric, depending upon the optical activity of the sugars concerned.

The second method is most accurate and rapid method, and is of considerable technical importance. The chemical method, although less accurate than the polarimetric method, is of great value for the estimation of sugars in biological fluids.

Using chemical methods, the reducing sugars like glucose and fructose may be estimated quantitatively be oxidising agents like

(i) Benedict's solution

ii) Fehling's solution

Now, we will perform two experiments using Benedict's solution and Fehling's solution.

Objectives

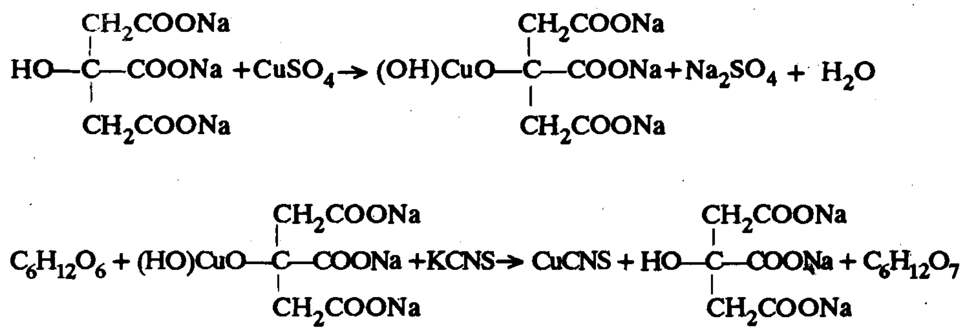
After studying and performing this experiment, you should be able to

- determine the amount of glucose in the given sample,
- describe oxidation reactions of reducing sugars with Benedicts and Fehling's solutions, and
- perform redox titrations using standard solution of Benedict's and Fehling's Solutions.

11.2 EXPERIMENT 11a: DETERMINATION OF GLUCOSE BY BENEDICT'S SOLUTION

11.2.1 Principle

Glucose readily reduces Benedict's solution, which is an alkaline solution of cupric ions. It is prepared by dissolving copper sulphate, sodium carbonate, sodium citrate, potassium thiocyanate and potassium ferrocyanide in proper proportions in distilled water. Sodium citrate prevents the precipitation of cupric hydroxide by forming a complex, while potassium thiocyanate is used to precipitate copper ions as copper thiocyanate.



Glucose

Cooper thiosulphate (white ppt.)

Gluconic acid

11.2.2 Requirements

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Glucose (AR)
Pipette (25 cm^3)	- 1	Sodium carbonate
Conical flask (250 cm^3)	- 1	Sodium citrate
Vol. flasks (250 cm^3)	- 1	Copper sulphate (AR)
Beaker (250 cm^3)	- 1	Potassium thiocyanate
Weighing bottle	- 1	Potassium ferrocyanide
Funnel (small)	- 1	
Wash-bottle for distilled water	- 1	
Burette stand	- 1	

Solution Provided:

Benedict's solution: i) Dissolve 4.5 g of copper sulphate in 20 cm^3 of distilled water.

ii) Dissolve 50 g of crystalline sodium carbonate in 175 cm^3 boiling distilled water. To the clear boiling solution add 50 g of crystalline sodium citrate, when sodium citrate has been completely dissolved then add 32 g of potassium thiocyanate and boil till a clear solution is obtained.

iii) Mix the solutions (i) and (ii) in a 250 cm^3 volumetric flask and add 2 cm^3 of 5% potassium ferrocyanide solution and make up the total volume to 250 cm^3.

If the above quantities are weighed accurately then, 25 cm^3 of the Benedict's a solution = 0.05 g of glucose

11.2.3 Procedure

i) **Preparation of Standard glucose solution:** Weigh accurately about 1.25 g of glucose and transfer to a 250 cm^3 volumetricflask. Dissolve it in small quantity of distilled water and make up to the mark.

ii) **Titration with standard glucose solution:** Fill the burette with the standard glucose solution. Now pipette out 25 cm^3 of Benedict's solution in a 250 cm^3 conical flask and add 3 g of anhydrous sodium carbonate to it. Boil the contents of the conical flask and add gradually the glucose solution with continuous shaking till the blue colour of the solution just disappears and white precipitate of CuCNS begins to appear. Keep the conical flask on burner during the addition of glucose solution. Repeat the process to get atleast two concordent. Record reading the observation in Observations Table I.

iii) **Titration with unknown solution:** Repeat the above procedure with unknown glucose solution. Records the observation in Observation Table II.

11.2.4 Observations

Mass of the weighing bottle	$= m_1$	=.................. g
Mass of the bottle + glucose	$= m_2$	=.................. g
Mass of the bottle		
(after transferring the compound)	$= m_3$	=.................. g
Mass of glucose transferred	$= m_2 - m_3 = m$	=.................. g

Observation Table I
Standard Glucose Solution Vs. Benedict's Solution

Sl. No.	Volume of Benedict's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final-Initial)
		Initial	Final	
1	25			
2	25			
3	25			

Observation Table II
Unknown Glucose Solution Vs. Benedict's Solution

Sl. No.	Volume of Benedict's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final-Initial)
		Initial	Final	
1	25			
2	25			
3	25			

11.2.5 Calculations

The volume of standard glucose used for titrating 25 cm^3 of Benedict's solution $= V_1\ cm^3$

The volume of unknown glucose used for titrating 25 cm^3 of Benedict's solution $= V_2\ cm^3$

The amount of glucose in the unknown glucose solution $= \dfrac{m \times V_1}{V_2} = \ldots\ldots\ldots$ g

The strength of the unknown glucose solution

$$= \frac{\text{strength of standard glucose solution} \times V_1}{V_2}$$

$$= \frac{4 \times m \times V_1}{V_2} = \text{g dm}^{-3}$$

11.2.6 Result

The amount of glucose in the given solution $= \ldots\ldots\ldots\ldots = $ g

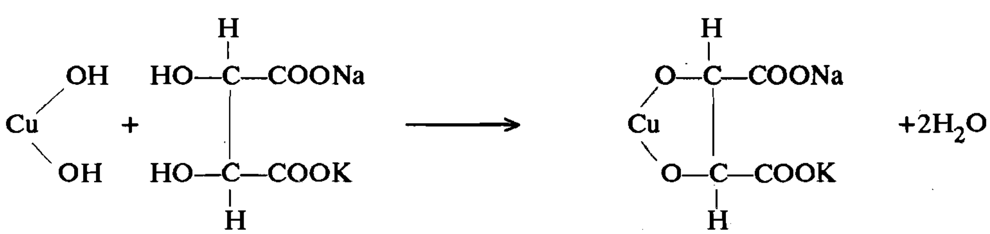
The strength of the unknown glucose solution $= \ldots\ldots\ldots\ldots = $ g dm^{-3}

11.3 EXPERIMENT 11b: DETERMINATION OF GLUCOSE WITH THE HELP OF FEHLING'S SOLUTION

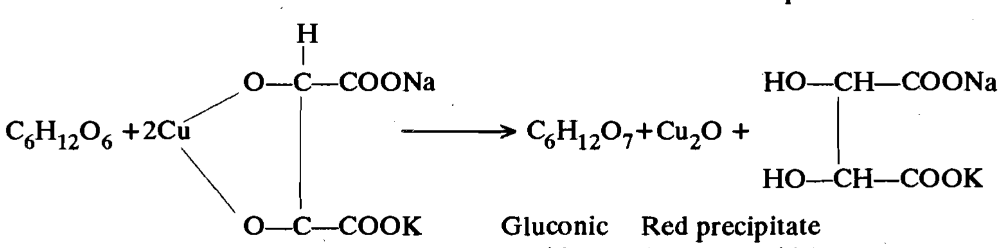
11.3.1 Principle

Glucose also reduces Fehling's solution quickly, the latter is obtained by mixing an aqueous solution of copper sulphate with alkaline solution of sodium potassium tartarate (Rochelle salt). The latter prevents the precipitation of cupric hydroxide by forming a complex. A freshly prepared Fehling's solution is first standardised by titration against a standard glucose solution. The standardised Fehling's solution is then used to determine the amount of glucose in an unknown sample.

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$



Rochelle salt — Soluble complex



Gluconic acid — Red precipitate (cuprous oxide)

11.3.2 Requirements

Apparatus		Chemicals
Burette (50 cm^3)	- 1	Glucose
Pipette (10 cm^3)	-1	Copper sulphate
Conical flask (250 cm^3)	- 1	Sodium hydroxide
Vol. flasks (250 cm^3)	- 1	Sodium Potassium

Beaker (250 cm^3)	- 1	tartarate
Weighing bottle	- 1	Methylene blue
Funnel (small)	- 1	
Wash-bottle for distilled water	- 1	
Burette stand	- 1	

Solution Provided:

i) **Fehling's solutlon A:** Dissolve 17.32 g of crystalling copper sulphate in distilled water. Transfer the solution in 250 cm^3 volumetric flask, wash the container with distilled water, transfer the washing to volumetric flask and make up to the mark.

ii) **Fehling's solution B:**
Dissolve 86.5 g of sodium potassium tartarate (Rochelle salt) and 30 g of sodium hydroxide in warm water, cool and transfer the solution in 250 cm^3 volumetric flask and make up to the mark.

iii) **Methylene blue indicator:** Prepare 1% aqueous solution of methylene blue.

11.3.3 Procedure

i) **Preparation of Standard solution of glucose:** i) Similar to Experiment 11a, dissolve accurately weighed quantity of glucose about 1.25 g in distilled water and make up to 250 cm^3 in a volumetric flask.

ii) **Titration with standard glucose solution:** Fill the burette with standard glucose solution. Pipette out 10 cm^3 each of Fehling's solution A and B in a 250 cm^3 conical flask and dilute it with 25 cm^3 water. Boil the solution gently over a wire gauze and titrate with standard glucose solution, (taken in burette) adding 1 cm^3 of the solution at a time till the blue colour of the solution just disappears and red precipitate of Cu_2O begins to appear. Continue the heating of the Fehling's solution during the titration. Repeat the process to get atleast two concordant readings. Record the observations in Observation Table I.

iii) **Titration with unknown glucose solution:** Now take unknown glucose solution in burette and repeat the titration exactly in the same manner as described for standard glucose solution. To detect the end point more accurately add 4-5 drops of methylene blue indicator to the Fehling's solution just before the end point. The disappearance of the colour will give exact and point. Record the Observations in Observation Table II.

11.3.4 Observations

Mass of the weighing bottle	$= m_1$	= .... g
Mass of the bottle + glucose	$= m_2$	= .... g
Mass of the bottle (after transferring the compound)	$= m_3$	= .... g
Mass of glucose transferred	$= m_2 - m_3 = m$	= .... g

Observation Table I
Standard Glucose Solution vs. Fehling's Solution

Sl. No.	Volume of Fehling's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final–Initial)
		Initial	Final	
1	20			
2	20			
3	20			

Observation Table II
Unknown Glucose Solution vs. Fehling's Solution

Sl. No.	Volume of Fehling's solution in cm^3	Burette reading		Volume of glucose solution in cm^3 (Final–Initial)
		Initial	Final	
1	20			
2	20			
3	20			

11.3.5 Calculations

The volume of standard glucose solution used for titrating 20 cm^3 of Fehling's solution $= V_1\ cm^3$

The volume of unknown glucose solution used for titrating 20 cm^3 of Fehling's solution $= V_2\ cm^3$

The amount of glucose in the unknown glucose solution $= m \times \dfrac{V_1}{V_2} =$ .... g

$$\text{The strength of the unknown glucose solution} = \frac{\text{Strength of standard glucose solution} \times V_1}{V_2}$$

$$= \frac{4 \times m \times V_1}{V_2} = \text{......} \ \text{g dm}^{-3}$$

11.3.6 Result

The amount of glucose in the given solution = ...g

The strength of the unknown glucose solution = ... g dm^3