**ESTIMATION OF REDUCING AND NON-REDUCING SUGAR**

**A. REDUCING SUGAR**

**INTRODUCTION:**

Several methods are available for estimation of reducing sugars. They include chemical, polarimetric and chromatographic methods. However, for routine analysis of food products, Lane and Eynon chemical method is most widely used. Non-reducing sugars and starch are first converted into reducing sugars for estimation.

**AIM AND OBJECTIVES**:

To determine reducing sugars and non- reducing sugars (total sugars) in food products by Lane and Eynon method.

**PRINCIPLE**

Lane and Eynon method is based on the principle of reduction of Fehling’s solution by reducing sugars. Fehling’s solution is a mixture of copper sulphate and alkaline Rochelle salt (sodium potassium tartarate). Rochelle salt complexes with the cupric hydroxide formed in alkaline solution and prevent it from precipitation. Reducing sugars reduces the complexed cupric hydroxide to red, insoluble cuprous oxide under the experimental conditions. An oxidation-reduction indicator, usually methylene blue, detects the end point of the reaction. The first step in the estimation of reducing sugars by Lane and Eynon method is the determination of Factor for Fehling’s solution. Fehling factor is the quantity of invert sugar in grams required to completely reduce the Fehling’s solution (usually 5 ml each of Fehling’s A and B solutions). Total sugars include reducing sugars and non-reducing di - and oligosaccharides like sucrose, which on mild acid hydrolysis are converted into reducing sugars. Starch is hydrolysed by strong acids into glucose.

**2.A.2 REQUIREMENTS EQUIPMENT AND APPARATUS**

Chemical balance, 1mg sensitivity Hot plate Burette (50 ml cap.) with an off-set tip Volumetric flask, 250 ml Pipette, 5 ml and 25 ml Conical flask, 250 ml Weighing bottle Funnel (small) Whatman No. 1 filter circles

**Chemicals and Reagents**

Fehling’s solution A: Dissolve 69.28 g copper sulphate (CuSO4.5H2O) in distilled water and dilute to 1000 ml. Filter and store in amber color bottle.

Fehling’s solution B: Dissolve 346 g Rochelle salt (Potassium sodium tartrate: KNa C4H4O6. 4H2 O) and 100 g NaOH in distilled water. Dilute to 1000 ml. Filter and store in amber color bottle.

Neutral lead acetate solution: Prepare 20% neutral lead acetate solution.

Potassium oxalate solution: Prepare 10% potassium oxalate (K2C2O4. H2O) solution.

Methylene blue indicator: Prepare 1% methylene blue solution in distilled water

**PROCEDURE**

**i) Standardization of the Fehling’s Solution for Invert Sugar**

Accurately weigh 4.75g of AR grade sucrose. Transfer to 500 ml volumetric flask with 50 ml distilled water. Add 5 ml conc. HCl and allow to stand for 24 hr. Neutralise the solution with NaOH using phenolphthalein as end point indicator and make up to volume. Mix well and transfer 25 ml to a 100 ml volmetric flask and make up to volume (1 ml = 2.5 mg of invert sugar). Transfer to a burette having an off-set tip and titrate against Fehling’s solution as described below for sample

**OBSERVATIONS**

Titre = V1 = ------ m

**CALCULATIONS**

Factor for Fehling’s solution (g of invert sugar) = Titre × 2.5/1000

**ii) Determination of Reducing Sugars**

**Preparation of sample**

Weigh accurately 10-50 g sample as such (juices, beverages etc.) or homogenized sample (jams, preserves etc.) and transfer to 500 ml volumetric flask. Add about 100 ml water and neutralize with NaOH solution to phenolphthalein end point. Add 10 ml neutral lead acetate solution, shake and let stand for 10 min. Add potassium oxalate solution in small amounts until there is no further precipitation. Make up to volume, mix the solution well and filter through Whatman No. 1 filter circle. Transfer the filtrate to a 50 ml burette having an off-set tip.

**Preliminary titration**:

Pipette out 5 ml each of Fehling A and B solutions into 250 ml conical flask. Mix and add about 10 ml water and a few pumice stone or glass beads. Dispense the sugar solution from the burette. Heat the solution to boiling. Add 3 drops of methylene blue indicator. Continue the addition of the sugar solution drop wise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 to 50 ml). Maintain a total boiling peiod of 3 min. Note down the titre value.

**Final titration:**

Pipette out 5 ml each of Fehling A and B solutions into a 250 ml conical flask. Add sample solution about 0.05 to 1.0 ml less than titre value of the preliminary titration. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Complete the titration within 1 min by adding 2 to 3 drops of sugar solution at a time, until the indicator is decolourized. At the end point, the boiling liquid assumes the brick red colour. Note down the titre value. Perform the titration in duplicate and take the average.

Note: i) Preliminary titration must be finished within 3 min. 20 ii) Conical flask should not be disturbed or removed from the burner before the titration is finished.

**OBSERVATIONS**

Weight of the sample = W = ------- g

Dilution volume for the sample = V2 = ------ ml

Volume of clarified sample solution required for Fehling’s reaction (titre) = V3 = ------- ml

**CALCULATIONS**

Based on the factor for Fehling’s solution, V3 ml sample solution contains:

0.0025 V1 g reducing sugar (as invert sugar)

Therefore, % Reducing Sugars in the sample = 0.0025 × V1 × V2 × 100/ V3 × W

0.25 × V1 × V2/ V3 × W = X %

**RESULTS**

Reducing sugars (as invert sugar) = % by wt.

**B. ESTIMATION OF TOTAL REDUCING SUGARS**

**PRINCIPLE**

Total reducing sugars represent reducing sugars and non-reducing di and oligo saccharides, which can be hydrolysed into reducing sugars with dilute acids.

**REQUIREMENTS**

Same as for experiment 2a

**PROCEDURE**

Pipette an aliquot of 50 ml of the clarified, de-leaded filtrate to a 100 ml volumetric flask. Add 5 ml of conc. HCl and allow to stand at room temperature for 24 hours. Neutralise with concentrated NaOH solution followed by 0.1N NaOH using phenolphthalein as end point indicator. Make up to volume and transfer to 50 ml burette having an off-set tip. Perform the titration against Fehling's solution similar to the procedure described for reducing sugars, and determine the total sugars as invert sugars.

**OBSERVATIONS**

Volume of the acid hydrolysed sample solution required for Fehling’s solution (titre) = V4 = ------ ml

**CALCULATIONS**

Based on the factor for Fehling’s solution, total reducing sugars in V4 ml = 0.0025 × V1 g

As 50 ml of the clarified and de-leaded solution is diluted twice (50 ml to 100 ml) after hydrolysis, dilution volume of the sample = (2 × V2).

Therefore, % Total reducing sugars (as invert sugars) = 0.0025 × V1 × 2 × V2 × 100/ V4 × W

0.5 × V1 × V2/ V4 × W = = Y %

Total reducing sugars comprises of reducing sugars and non-reducing sugars, which can be hydrolysed into reducing sugars under the experimental conditions. This non-reducing sugar is usually expressed in terms of sucrose. As 0.95 g sucrose on hydrolysis yields 1 g invert sugar (glucose + fructose):

% Sucrose in the sample = (Total reducing sugars − % Reducing sugars originally present) × 0.95

= (Y – X) × 0.95

[% Total sugars = (% Reducing sugars + % Sucrose)]

Results Sucrose content in the sample = % by weight