Method 8.7 – Refined sugar: reducing sugars by the Knight and Allen method

1. Rationale

The method is applicable to all refined and white speciality sugars in which the reducing sugar content does not exceed 0.02 % and is used to determine the amount of reducing sugars (mainly fructose and glucose) in the sample.

2. Principle

A solution of the sugar is heated in a boiling water bath with an alkaline copper reagent. The cupric ions are reduced to cuprous oxide by the reducing sugars present. After cooling the residual cupric ions are titrated with EDTA using murexide as an indicator.

3. Definitions

3.1 Reducing sugars

Reducing sugars consists primarily, but not exclusively, of glucose and fructose and is obtained through the hydrolysis of sucrose.

3.2 **Invert**: an equimolar mixture of glucose and fructose.

4. Apparatus

4.1 **Balance** readable to 0.0001 g

4.2 **Test tubes**: 150 mm × 20 mm

4.3 **Beakers**: 100 cm^3

4.4 **Water bath** maintained at 100°C with test tubes clamps

4.5 **Burettes**: 50 cm^3

4.6 **Pipettes**: 2 and 5 cm^3

4.7 **Magnetic stirrer** with stirrer bar

4.8 **Vortex mixer**

4.9 **Mortar and pestle**

4.10 **Small spatula**

5. Reagents

5.1 Sodium hydroxide pellets

Sodium hydroxide (NaOH, also called caustic) is a corrosive base. Wear gloves and safety glasses during handling.

5.2 Sodium hydroxide solution (1 M)

Weigh 4.0 g sodium hydroxide pellets and dissolve in some distilled water. This dissolution is exothermic and the solution will therefore heat. Allow the solution to cool and dilute to 100 cm^3 in a volumetric flask.

5.3 Sodium carbonate (anhydrous)

Sodium carbonate (Na_2CO_3) is a corrosive base. Wear gloves and safety glasses during handling.

5.4 Sodium potassium tartrate tetrahydrate ($NaKC_4H_4O_6 \cdot 4H_2O$)

5.5 Copper sulphate pentahydrate

Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) is highly irritating to the eyes, skin and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

5.6 Alkaline copper reagent

Add 8 cm^3 of the 1 M sodium hydroxide solution to about 120 cm^3 distilled water in a beaker. Dissolve 5 g anhydrous sodium carbonate and 5 g sodium potassium tartrate tetrahydrate in this caustic solution. In a separate beaker dissolve 1.200 g copper sulphate pentahydrate in 20 cm^3 distilled water and add to the caustic solution. Dilute to 200 cm^3 in a volumetric flask.

5.7 EDTA solution (0.0025 M)

Ethylenediamine tetraacetic acid (EDTA) is mildly irritating to the skin, eyes and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

Dissolve 0.9306 g EDTA in distilled water and dilute to 1 000 cm^3 in a volumetric flask.

5.8 Methylene blue

5.9 Sodium chloride (NaCl)

5.10 Murexide indicator

Mix 0.5 g murexide, 0.15 g methylene blue and 40.0 g sodium chloride and grind with a mortar and pestle. Store in a desiccator or air-tight container.

6. Procedure

Weigh 5.0 ± 0.1 g of white sugar into a test tube and dissolve in 5 cm^3 of distilled water on a vortex mixer without warming. Add exactly 2 cm^3 of the alkaline copper solution and thoroughly mix the contents of the tube. Immerse the tube in a boiling water bath for 5 minutes and cool in a cold water bath.

Transfer the contents of the tube into a 100 cm^3 beaker with distilled water and add approximately 0.1 g of the indicator with a small spatula.

Titrate this solution rapidly with the EDTA solution with continuous stirring using the a magnetic stirrer. The colour will change from green to grey to purple. Titrate until the purple colour is sustained. Record the titre.

7. Calculations

The concentration of reducing sugars expressed as percentage on sample can be calculated from the following standard equation:

$$\text{Reducing sugars (\%)} = \frac{15.297\ \text{cm}^3 - \text{titre}(\text{cm}^3)}{666\ \text{cm}^3/\%}$$

The equation was obtained from actual data using a 0.0025 M EDTA solution (SMRI, 1997).

Express results to three decimal places in % on sample.

8. Example

titre of solution = 10.3 cm^3

$$\text{reducing sugars \% sugar} = \frac{15.297\ \text{cm}^3 - 10.3\ \text{cm}^3}{666\ \text{cm}^3/\%}$$

$$= 0.008\%$$

9. Precision

The tolerance associated with the analysis is ± 0.001 unit.

10. References

ICUMSA (1994). Reducing sugars in white sugar by the Knight and Allen EDTA method. *ICUMSA Methods Book,* Method GS2/3-5.

Mellet P, Lionnet GRE, Kimmeling ZJ and Bennett PJ (1982). Standards for the Analytical Precision of Sugar and Molasses Analyses. *Proc S Afr Sug Technol Ass*, **56**: 55-57.

SASTA (1985). *Laboratory Manual for South African Sugar Factories*. 3rd Edition: 193 - 194, 341.

SMRI (1997). Determination of the reducing sugars in white sugar by the Knight and Allen method. *SMRI Test Methods,* TM051.