



Method 6.5 – C-molasses: sucrose and reducing sugars by the Lane and Eynon method

1. Rationale

The method is applicable to C-molasses and relies upon the property of reducing sugars to reduce and precipitate copper from a known amount of Fehlings solution under standard conditions.

2. Principle

A sample of diluted molasses is titrated against mixed Fehlings reagents that contain a strongly alkaline cupric complex salt solution using methylene blue as an indicator. It has been found that calcium interferes with the determination and it is recommended that EDTA be used as a sequestrant. In most factory products EDTA will also reduce the colour significantly which aids in the detection of the end point.

3. Definitions

3.1 Reducing sugars

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

3.2 Invert: an equimolar mixture of glucose and fructose.

3.3 Total invert

An equimolar mixture of glucose and fructose after all of the sucrose in solution has been inverted by acid hydrolysis.

4. Apparatus

4.1 Light duty balance readable to 0.01 g

4.2 Pipette: 5 cm³

4.3 Volumetric flasks: 100, 200 and 1 000 cm³

4.4 Burette: 50 cm³

4.5 Conical flask: 500 cm³

4.6 Hot plate

4.7 Gauze or screen: 0.15 mm pore opening

4.8 Stop watch

4.9 Glass beads

4.10 Filter paper: Whatman No. 91 filter paper, S&S 3000 or equivalent: 185 mm ϕ

5. Reagents

5.1 Pumice powder to prevent over-boiling

5.2 Liquid paraffin to prevent foaming

5.3 Methylene blue indicator (1%)

Dissolve 1.0 g methylene blue in distilled water and dilute to 100 cm³ in a volumetric flask.

5.4 Ethylene diamine tetra acetic acid, disodium salt dihydrate (4%)

Ethylene diamine tetra acetic acid (EDTA), disodium salt dihydrate is mildly irritating to the skin, eyes and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

Weigh 4.00 g ethylene diamine tetra acetic acid, disodium salt dihydrate, dissolve in distilled water and dilute to 100 cm³ in a volumetric flask.

5.5 Copper sulphate pentahydrate

Copper sulphate pentahydrate (CuSO₄· 5H₂O) is highly irritating to the eyes, skin and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

5.6 Sodium potassium tartrate tetrahydrate (NaKC₄H₄O₆· 4H₂O)

5.7 Sodium hydroxide (4 M)

Sodium hydroxide (NaOH, also called caustic) is a corrosive base. Work in a fume cupboard while wearing gloves and safety glasses.

Dissolve 160.0 g sodium hydroxide pellets in distilled water. After cooling transfer to a 1000 cm³ volumetric flask and make to the mark.

5.8 Benzoic acid

Benzoic acid is a corrosive organic acid. Work in a fume cupboard while wearing gloves and safety glasses.

5.9 Hydrochloric acid (SG 1.1)

Hydrochloric acid (HCl) is a corrosive acid. Work in a fume cupboard while wearing gloves and safety glasses.

Carefully add 500 cm³ concentrated hydrochloric acid to 357 cm³ distilled water. Always add the acid to the base and not the other way around. The dilution is exothermic and the solution will heat. Allow cooling to room temperature.

5.10 Standard invert solution (1%)

Dissolve 9.500 g sucrose (first boiling refined sugar) in 70 cm³ of distilled water in a 250 cm³ beaker. Add 10 cm³ hydrochloric acid and stand at room temperature for 5 days. Add sufficient 4 M sodium hydroxide, (ca. 16 cm³), to raise the pH to about 3, using a pH meter and transfer to a 1000 cm³ volumetric flask. Dissolve 2 g benzoic acid in distilled

water (800 cm³) by boiling. Cool and add to the volumetric flask. Make to volume with distilled water. This solution contains 1 g invert sugar per 100 cm³.

5.11 Fehlings Solutions (A and B)

Solution A: Dissolve 69.278 g copper sulphate pentahydrate in distilled water and dilute to 1000 cm³ in a volumetric flask.

Solution B: Dissolve 346 g sodium potassium tartrate tetrahydrate in 500 cm³ distilled water. Dissolve 100 g sodium hydroxide in 200 cm³ of distilled water and cool to room temperature. Transfer both these solutions quantitatively to a 1000 cm³ volumetric flask. Mix thoroughly and make to the mark with distilled water. Filter the solution through a Whatman No. 91 filter paper. (It has been found that some suspended matter develops in this solution within two weeks. It is therefore advised to let this solution stand for 2 weeks before filtering).

Note: It is necessary to keep solutions A and B separate.

6. Procedure

6.1 Standardisation of the Fehlings solutions

Pipette 50 cm³ standard invert solution into a 250 cm³ volumetric flask and dilute to volume with distilled water. Fill the burette with the dilute standard invert solution. Pipette 5 cm³ Fehlings A solution and 5 cm³ Fehlings B solution into a 500 cm³ Erlenmeyer flask. Add a little pumice powder, three glass beads and four drops of liquid paraffin.

Titrate this solution against the dilute standard invert solution in the burette according to the titration procedure in 6.3.

6.2 Sample preparation

Weigh accurately *ca.* 4 g of the well mixed sample into the pol dish. Since a relatively small sample is taken for analysis, extreme care must be taken that a representative sample is weighed. Therefore, if the molasses is too viscous for thorough mixing at room temperature, the sample may be heated in a closed container on a water bath at approximately 50°C. Mix with approximately 50 cm³ distilled water by stirring with the glass rod. Transfer quantitatively into a 250 cm³ volumetric flask using the funnel and distilled water. Pipette 16 cm³ of the EDTA solution into the flask, swirl to mix and make to volume with distilled water. Stopper and mix thoroughly.

6.2.1 Reducing sugars

Pipette 100 cm³ of the prepared sample solution into a 200 cm³ volumetric flask, make to the mark with distilled water and mix. Fill the 50 cm³ burette with this solution.

Pipette 5 cm³ of Fehlings solution A and 5 cm³ of Fehlings solution B into a 500 cm³ Erlenmeyer flask. Add a little pumice powder, three glass beads and four drops of liquid paraffin.

Titrate this mixture against the solution in the burette according to the titration procedure in 6.3.

6.2.2 Total invert

Pipette 50 cm³ of the prepared sample solution into a 200 cm³ volumetric flask. Add *ca.* 30 cm³ distilled water, mix and heat in a water bath set at 65 ± 1°C for 10 minutes. Add

10 cm³ dilute hydrochloric acid and mix. Set aside for approximately 30 minutes. Add 1 drop of phenolphthalein solution and add the sodium hydroxide solution from a burette until the solution just turns pink. About 16.5 cm³ of the sodium hydroxide solution are required. Add 0.5 M hydrochloric acid drop wise until the pink colour of the indicator disappears (*ca.* 0.5 cm³ or less). Make to the mark with distilled water, stopper and mix. Fill the burette with this prepared solution.

Pipette 5 cm³ of Fehlings A solution and 5 cm³ of Fehlings B solution into the Erlenmeyer flask, add a little pumice powder, three glass beads and four drops of liquid paraffin.

Titrate this mixture against the solution in the burette according to the titration procedure in 6.3.

6.3 Titration procedure

Add 15 cm³ of the contents of the burette to the Erlenmeyer flask and note the colour of the mixture. Place the flask on a hot plate and bring the mixture to a boil in not more than 2¼ minutes. From this point on the flask may not be swirled or disturbed in any way. If the original colour persists after boiling for 10-15 seconds it indicates that most of the Fehlings solution has not yet been reduced. If this is the case hold the burette by hand and make further additions of 5 cm³ at a time with a few seconds of boiling after each addition, until the original colour of the reagent fades to bright orange.

Add three or four drops of methylene blue and continue the addition of solution from the burette until the indicator is completely decolourised. The boiling liquid resumes the bright orange appearance which it had before the addition of the indicator. If less than 10 cm³ of the burette volume is used, dilute the juice solution 1:1 with water and repeat the titration.

Take a reading from the burette as an approximate titration. Do a second titration by adding all but 1 cm³ of the approximate titre of the sample solution at once. Heat the liquid and start the stopwatch as soon as the boiling point is reached. Boil the liquid for 2 minutes. Add three or four drops of the methylene blue indicator and complete the titration drop wise until the indicator becomes colourless. The titration should be completed within 3 minutes and during this time the mixture must be kept boiling continuously to exclude air.

Repeat the last step of the titration. Duplicate titres should agree to within 0.1 cm³.

7. Calculations

7.1 Standardisation of the Fehlings solutions

5 cm³ of Fehlings solution A and 5 cm³ Fehlings solution B should result in a standard invert solution titre of exactly 25.64 cm³. If the titre is higher than 25.64 cm³ Fehlings solution A should be diluted until the desired titre is obtained. If the titre is lower than 25.64 cm³ copper sulphate should be added to Fehlings solution A until the desired titre is obtained.

7.2 Reducing sugars

The amount of reducing sugars in the sample solution is calculated using the equation below. Sucrose affects the titration in a predictable way. The equation assumes a sucrose content of 33% or 0.25 g/100 cm³ after dilution.

$$\text{mass reducing sugars (mg/100 cm}^3\text{)} = 4667.3932 \times \text{titre}^{-0.97226}$$

The percentage reducing sugars in the sample is calculated as indicated below.

$$\text{reducing sugars (\%)} = \frac{M_{RS} \times 0.5}{M_M} \times 100$$

where M_{RS} \equiv mass reducing sugars (mg) in 100 cm³
 M_M \equiv mass molasses (g) in 100 cm³
 0.5 \equiv dilution factor

7.3 Total invert

The amount of total invert in the solution is calculated using the equation below. Sucrose affects the titration in a predictable way. The equation assumes that all of the sucrose was inverted (0% or 0 g/100 cm³ sucrose).

$$\text{mass total invert (mg/100 cm}^3\text{)} = 4640.62824 \times \text{titre}^{-0.96933}$$

The percentage reducing sugars in the sample is calculated as indicated below.

$$\text{total invert (\%)} = \frac{M_{\Pi}}{M_M} \times 100$$

where M_{Π} \equiv mass total invert (mg) in 100 cm³
 M_M \equiv mass molasses (g) in 100 cm³

7.4 Sucrose

Total invert is the natural reducing sugars in the sample plus the reducing sugars due to sucrose inversion. The sucrose can therefore be calculated by applying a factor as indicated below.

$$\text{Sucrose \% molasses} = [\text{Total invert (\%)} - \text{reducing sugars (\%)}] \times 0.95$$

8. Example

$$\begin{aligned} \text{Mass of molasses in 200 cm}^3 &= 8.2468 \text{ g} \\ \text{Mass of molasses in 100 cm}^3 &= 4.1234 \text{ g} \end{aligned}$$

8.1 Reducing sugars

$$\begin{aligned} \text{Titre obtained} &= 37.40 \text{ cm}^3 \\ \text{Mass reducing sugars} &= 138.0 \text{ mg} \\ \text{Reducing sugars} &= \frac{138.0 \text{ mg} \times 0.5}{100 \times 4.1234 \text{ g}} \times 100 \\ &= 16.73\% \end{aligned}$$

8.2 Total invert

$$\begin{aligned} \text{Titre obtained} &= 24.50 \text{ cm}^3 \\ \text{Mass reducing sugars} &= 208.9 \text{ mg} \\ \text{Reducing sugars} &= \frac{208.9 \text{ mg}}{100 \times 4.1234 \text{ g}} \times 100 \\ &= 50.66\% \end{aligned}$$

8.3 Sucrose

$$\begin{aligned} \text{Sucrose} &= (50.66\% - 16.73\%) \times 0.95 \\ &= 32.23\% \end{aligned}$$

9. References

SASTA (1985). *Laboratory Manual for South African Sugar Factories*. 3rd Edition: 177, 304 - 306.

SMRI (1997). Determination of the reducing sugars in molasses by the Lane and Eynon method. *SMRI Test Methods*, TM048.

SMRI (1997). Determination of the sucrose in molasses by the Lane and Eynon method. *SMRI Test Methods*, TM049.