Method 7.14 – Raw sugar: phosphates (P_2O_5)

1. Rationale

The method is applicable to raw sugars and determines both the soluble and total phosphates present in raw sugar in mg/litre P_2O_5.

2. Principle

A standard graph of phosphate concentration *versus* absorbance is plotted. The same standard graph may be used for juice phosphates (Method 3.8) and boiler water phosphates (Method 9.6). For soluble phosphates the sample is simply diluted. For total phosphates the sample is first decomposed and dissolved in hydrochloric acid before dilution. In either case the resulting sample solution is reacted with ammonium molybdate and the absorbance of the colour of the resulting molybdenum blue complex is determined at 700 nm. The phosphate concentration is calculated from the standard graph.

3. Apparatus

3.1 **Spectrophotometer** with a wavelength of 700 nm

3.2 **Optical glass cell**: 10 mm

3.3 **Pipettes** with pipette stand: 5, 10, 20, 25 and 50 cm^3

3.4 **Volumetric flasks**: 100, 2 × 1 000 and 2 000 cm^3

3.5 **Stopwatch**

3.6 **Platinum dish**: 70-80 mm ϕ

3.7 **Muffle furnace** operating at 650 ± 25°C

3.8 **Analytical balance** readable to 0.0001 g

3.9 **Top pan balance** readable to 0.01 g

3.10 **Funnel**: 50-60 mm ϕ

3.11 **Glass wool** or Whatman No. 91 filter paper or equivalent

3.12 **Beaker**: 1 litre

4. Reagents

4.1 **Sulphuric acid** (concentrated)

Sulphuric acid (H_2SO_4) is a corrosive acid and should only be handled with gloves while wearing safety glasses.

4.2 Phosphate stock solution (400 mg/litre P_2O_5)

Potassium dihydrogen orthophosphate (KH_2PO_4) is corrosive and should be handled with gloves and safety glasses. Avoid swallowing and inhalation of the fumes by working in a fume cupboard.

Dissolve 0.7668 g potassium dihydrogen orthophosphate in distilled water and transfer to a 1 000 cm^3 volumetric flask. Add 10 cm^3 concentrated sulphuric acid and dilute to volume to make a 400 mg/litre P_2O_5 solution. This standard will keep indefinitely.

4.3 Phosphate standard solution (10 mg/litre P_2O_5)

Dilute 25 cm^3 of the 400 mg/litre P_2O_5 standard to 1 000 cm^3. This standard is used for the calibration graph.

4.4 Hydrochloric acid (concentrated, 32%)

Hydrochloric acid (HCl) is a corrosive acid and should only be handled with gloves while wearing safety glasses.

4.5 Ammonium molybdate solution (1.5% m/m)

Ammonium molybdate tetrahydrate [$(NH_4)_2MoO_4 \cdot 4H_2O$] is an irritant and contact with the skin or through inhalation or swallowing must be avoided. Work in a fume cupboard while wearing gloves and safety glasses.

Dissolve 15.0 g powered ammonium molybdate tetrahydrate in 300 cm^3 distilled water at 50°C in a 1 litre beaker and cool. Carefully add 310 cm^3 concentrated hydrochloric acid. This dilution is exothermic and the solution will heat. Cool to room temperature and transfer to a 1 000 cm^3 volumetric flask and make to the mark.

4.6 Reducing solution

Sodium metabisulphite ($Na_2S_2O_5$), sodium sulphite (Na_2SO_3) and 1-amino-2-naphthol-4-sulphonic acid ($C_6H_4C_4HNH_2OHSO_3H$) are irritants and contact with the skin or eyes and through swallowing should be avoided. Sodium metabisulphite is also toxic to humans.

Solution A: dissolve 180.0 g sodium metabisulphite in 1400 cm^3 distilled water.

Solution B dissolve 14.0 g anhydrous sodium sulphite and 3 g 1-amino-2-naphthol-4-sulphonic acid in 200 cm^3 distilled water.

Mix solutions A and B in a 2000 cm^3 volumetric flask and make to the mark with distilled water. Stand overnight and filter through glass wool or a Whatman No. 91 filter paper or equivalent. Store in a dark bottle and in a refrigerator. This solution has a maximum shelf life of 2 months. Remove the required amount of reducing solution from the refrigerator about 1 hour before use and allow warming to room temperature.

4.7 Magnesium nitrate solution

Magnesium nitrate hexahydrate [$Mg(NO_3)_2 \cdot 6H_2O$] is an irritant and toxic and contact with the skin or eyes and through swallowing must be avoided.

Saturate a solution of 50% ethanol in distilled water with magnesium nitrate hexahydrate (about 125 g per 100 cm^3).

4.8 Hydrochloric acid (1:1)

Add one part of concentrated hydrochloric acid (32%) to one part of distilled water and mix. Always add the acid to the water and not the other way around. The dilution is exothermic and the solution will heat.

5. Procedure

5.1 Preparation of standard graph

Pipette aliquots of the 10 mg/litre phosphate standard solution into 100 cm^3 volumetric flasks according to the amounts indicated in Table 1.

Table 1: Calibration graph solutions

Flask no	Aliquot (cm^3)	P_2O_5 concentration (mg/litre)
1	5	0.5
2	10	1.0
3	15	1.5
4	20	2.0
5	25	2.5
6	30	3.0
7	35	3.5
8	40	4.0
9	45	4.5
10	50	5.0

$$P_2O_5 \text{ concentration (mg/litre)} = \frac{\text{aliquot} \times \text{concentration}}{\text{volume}} = \frac{\text{aliquot} \times 10 \text{ mg/litre}}{100 \text{ cm}^3}$$

To each flask add 10 cm^3 of the ammonium molybdate solution and sufficient distilled water to bring the total volume in the flask to about 85 cm^3. Add 10 cm^3 reducing solution to the first flask and start the stopwatch. Quickly make to volume with distilled water and mix. Measure the absorbance exactly 10 minutes after addition of the reducing solution at 700 nm in a 10 mm cell using distilled water as the reference. Repeat these steps for all the other flasks using distilled water as the blank. Plot a graph of absorbance (AU) against P_2O_5 concentration (mg/litre).

5.2 Soluble phosphates

Weigh 20.0 ± 0.1 g of sugar into a 100 cm^3 volumetric flask. Dissolve in distilled water and make to the mark. Pipette 25 cm^3 (aliquot) of this solution into two 100 cm^3 volumetric flasks, one of which will be a blank. To the first flask, add approximately 50 cm^3 water, 10 cm^3 of the ammonium molybdate solution and 10 cm^3 of the reducing solution. Start the stopwatch. Mix and dilute to volume. Measure the absorbance exactly 10 minutes after addition of the reducing solution at 700 nm in a 10 mm cell using distilled water as the reference. Make the second flask to volume (blank). Determine the absorbance of the blank at 700 nm in a 10 mm cell using water as a reference. Subtract the blank from the absorbance and read the corresponding P_2O_5 concentration from the standard graph.

5.3 Total phosphates

Weigh 10.0 ± 0.1 g of sugar into the platinum dish. Moisten with 1.5 cm^3 alcoholic magnesium nitrate. Working in a fume cupboard, carefully heat the dish over a bunsen burner until the sample is dry. Switch off the fan of the fume cupboard and continue

heating until the sample is carbonised. Place the platinum dish in the muffle furnace until the ash is completely white (approximately 1 hour). Cool and moisten carefully with water. Add 5 cm^3 of the 1:1 hydrochloric acid solution and warm to dissolve. Filter any insoluble matter, collecting the filtrate in a 100 cm^3 volumetric flask. Make to volume. Pipette 25 cm^3 of this solution (aliquot) into another volumetric flask, add approximately 50 cm^3 water, 10 cm^3 ammonium molybdate and 10 cm^3 reducing solution. Start the stopwatch. Mix and dilute to volume. Measure the absorbance at 700 nm in a 10 mm cell exactly 10 minutes after the reducing solution was added using distilled water as the reference. A blank determination is not necessary. Read the corresponding P_2O_5 concentration from the standard graph.

6. Calculations

6.1 Soluble phosphates

$$\text{Soluble } P_2O_5 \text{ (mg/kg on sample)} = P_2O_5 \text{ conc} \times \left(\frac{100\ cm^3}{20\ g} \times \frac{100\ cm^3}{25\ cm^3} \right) = P_2O_5 \text{ conc} \times 20$$

where the P_2O_5 concentration is obtained from the calibration graph

6.2 Total phosphate

$$\text{Total } P_2O_5 \text{ (mg/kg on sample)} = P_2O_5 \text{ conc} \times \left(\frac{100\ cm^3}{10\ g} \times \frac{100\ cm^3}{25\ cm^3} \right) = P_2O_5 \text{ conc} \times 40$$

where the P_2O_5 concentration is obtained from the calibration graph

Report results to the nearest unit in mg/kg on sample.

7. Example

7.1 Standard graph

Actual mass KH_2PO_4 weighed = 0.7720 g
Actual concentration P_2O_5 standard = 10.07 mg/litre

Table 2: Example standard graph

Flask no	Aliquot (cm^3)	P_2O_5 (mg/litre)	Absorbance (AU)
1	5	0.5	0.035
2	10	1.0	0.070
3	15	1.5	0.105
4	20	2.0	0.140
5	25	2.5	0.175
6	30	3.0	0.210
7	35	3.5	0.245
8	40	4.0	0.280
9	45	4.5	0.315
10	50	5.0	0.350

$$P_2O_5 \text{ concentration (mg/litre)} = \frac{\text{aliquot} \times 10.07\ \text{mg/litre}}{100\ cm^3}$$

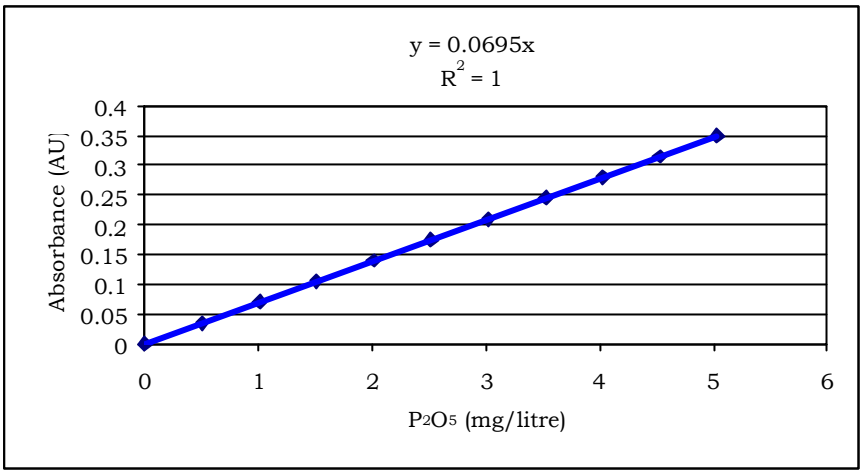

Figure 1: Standard phosphate graph

7.2 Sample

Soluble phosphates

Absorbance of blank	=	0.034 AU
Absorbance of sample	=	0.156 AU
P_2O_5 in solution	=	2.25 mg/litre
P_2O_5 in sample	=	2.25 × 20 mg/kg
	=	44.89 mg/kg

Report as 45 mg/litre on sample

Total phosphates

Absorbance of blank	=	0.106 AU
Absorbance of sample	=	0.156 AU
P_2O_5 in solution	=	1.53 mg/litre
P_2O_5 in sample	=	2.25 × 40 mg/kg
	=	61.01 mg/kg

Report as 61 mg/kg on sample

8. Precision

The tolerance associated with the analysis is ± 7 mg/kg for soluble phosphates and ± 11 mg/kg for total phosphates.

9. References

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: 191 - 192, 327 - 329.

SMRI (2004). Determination of the inorganic and total phosphate in raw sugar. *SMRI Test Methods*, TM041.